Design and Synthesis of *N*-(3,3-Diphenylpropenyl)alkanamides as a Novel Class of High-Affinity MT_2-Selective Melatonin Receptor Ligands

Annalida Bedini,[†] Gilberto Spadoni,*[,†] Giuseppe Gatti,[†] Simone Lucarini,[†] Giorgio Tarzia,[†] Silvia Rivara,[‡] Simone Lorenzi,[‡] Alessio Lodola,[‡] Marco Mor,[‡] Valeria Lucini,[§] Marilou Pannacci,[§] and Francesco Scaglione[§]

Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Urbino "Carlo Bo", Piazza Rinascimento 6, 61029 Urbino, Italy, Dipartimento Farmaceutico, Università degli Studi di Parma, V.le G. P. Usberti 27/A Campus Universitario, 43100 Parma, Italy, and Dipartimento di Farmacologia, Chemioterapia e Tossicologia Medica, Università degli Studi di Milano, Via Vanvitelli 32, 20129 Milano, Italy

Received July 20, 2006

A novel series of melatonin receptor ligands was discovered by opening the cyclic scaffolds of known classes of high affinity melatonin receptor antagonists, while retaining the pharmacophore elements postulated by previously described 3D-QSAR and receptor models. Compounds belonging to the classes of 2,3- and [3,3-diphenylprop(en)yl]alkanamides and of *o*- or [(*m*-benzyl)phenyl]ethyl-alkanamides were synthesized and tested on MT_1 and MT_2 receptors. The class of 3,3-diphenyl-propenyl-alkanamides was the most interesting one, with compounds having MT_2 receptor affinity similar to that of MLT, remarkable MT_2 selectivity, and partial agonist or antagonist behavior. In particular, the (*E*)-*m*-methoxy cyclobutanecarboxamido derivative **18f** and the di-(*m*-methoxy) acetamido one, **18g**, have sub-nM affinity for the MT_2 subtype, with more than 100-fold selectivity over MT_1, **18f** being an antagonist and **18g** a partial agonist on GTPγS test. Docking of **18g** into a previously developed MT_2 receptor model showed a binding scheme consistent with that of other antagonists. The MT_2 expected binding affinities of the new compounds were calculated by a previously developed 3D-QSAR CoMFA model, giving satisfactory predictions.

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, MLT, **1**) is a neurohormone primarily secreted by the pineal gland at night in all species.[1] The circadian pattern of MLT secretion, coupled with the localization of specific MLT binding sites in the brain region associated with the "biological clock", suggests that MLT may play an important role in modulation of the sleep−wake cycle and of circadian rhythms in humans.[2] Some MLT receptor agonists, with improved properties in comparison to MLT, are now in clinical trials for treatment of insomnia or circadian rhythm sleep disorders,[3−5] and ramelteon (TAK-375) has been recently approved for insomnia.[6] Other effects of MLT described in the literature include its anti-inflammatory,[7] pain modulatory,[8] retinal,[9]vascular,[10] antitumor,[11] and antioxidant[12] properties. A remarkable efficacy of MLT in animal models of focal cerebral ischemia had also been reported, suggesting the hormone as a candidate neuroprotective drug for human stroke.[13] However, the functions of MLT in mammals are still a matter of investigation, and more rigorous clinical studies are needed to demonstrate the potential benefits of MLT assumption and to rule out the possibility of toxic effects.

Most physiological MLT effects result from the activation of high affinity G protein-coupled receptors. MT_1 and MT_2 receptors[14−16] have been found in mammals, including humans, and subsequently cloned. A third subtype (Mel_{1c}), first cloned from *Xenopous laevis*, has been found only in nonmammalian species.[14] In addition to these high-affinity MLT receptors ($K_i \cong 0.1$ nM), another low-affinity MLT binding site, termed MT_3 ($K_i \cong 60$ nM), has recently been characterized as a melatonin-sensitive form of the human enzyme quinone reductase 2.[17] Whereas it is known that MT_1 and MT_2 receptors are expressed both centrally (suprachiasmatic nucleus, cortex, *pars tuberalis*, etc.) and peripherally (kidney, adipocytes, retina, blood vessels, etc.),[18] the physiological roles of these receptors are not as yet well defined. There is evidence that MT_1 receptors might be implicated in the sleep promoting effects of MLT[19,20] and in mediating vasoconstriction,[21] whereas MT_2 receptors appear to play a major role in the resynchronizing activity of MLT[19,22] and in mediating vasodilation. Moreover, an antidepressive effect has been reported for the antagonist luzindole in a mice model, being ascribed to its selective action at the MT_2 receptor.[23] However, an accurate characterization of MLT receptors-mediated functions in native tissues can only be made by using subtype-selective ligands. Therefore, a substantial share of current drug discovery efforts in the melatonin area is being directed toward the development of subtype-selective MLT receptor agonists and antagonists, which will be valuable tools in understanding the role of this enigmatic hormone in health and disease.

In the course of our studies, various modifications of the MLT structure had been examined to determine which structural features are required for receptor affinity, intrinsic activity, and/or subtype selectivity at MLT receptors.[24,25] However, marked subtype selectivity is still a challenge, and only recently this field has registered some important advances, leading to the identification of a small number of selective compounds.[26] While only a few examples of MT_1[27] and MT_3[28] ligands have been reported, the majority of subtype-selective compounds behave as MT_2 receptor antagonists. These compounds belong to different structural classes (Figure 1) and display various degrees of binding affinity and selectivity. A consistent structural motif found in most of these MLT ligands is the presence of a lipophilic substituent, which can be located out-of-the-plane of their *core* nucleus (i.e., the indole ring in luzindole or the tetralin scaffold in 4P-PDOT) in a position corresponding to positions 1 and 2 of the indole in MLT, and we had hypothesized that this arrangement confers selectivity for the MT_2 receptor and

* To whom correspondence should be addressed. Tel.: ++39 0722 303323. Fax: ++39 0722 303313. E-mail: gilberto@uniurb.it.
[†] Università degli Studi di Urbino "Carlo Bo".
[‡] Università degli Studi di Parma.
[§] Università degli Studi di Milano.

10.1021/jm060850a CCC: $33.50
Published on Web 11/15/2006

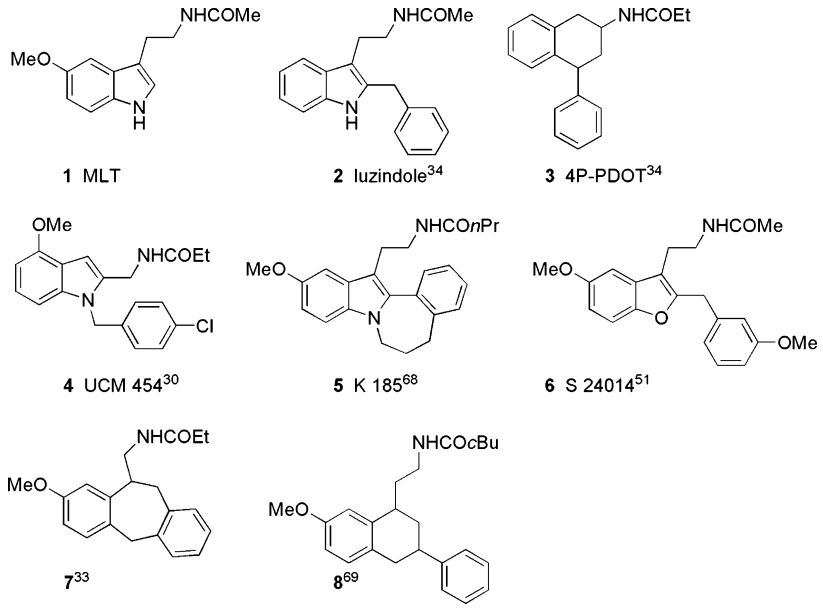


Figure 1. Chemical structures of MLT and representative MT_2-selective antagonists.

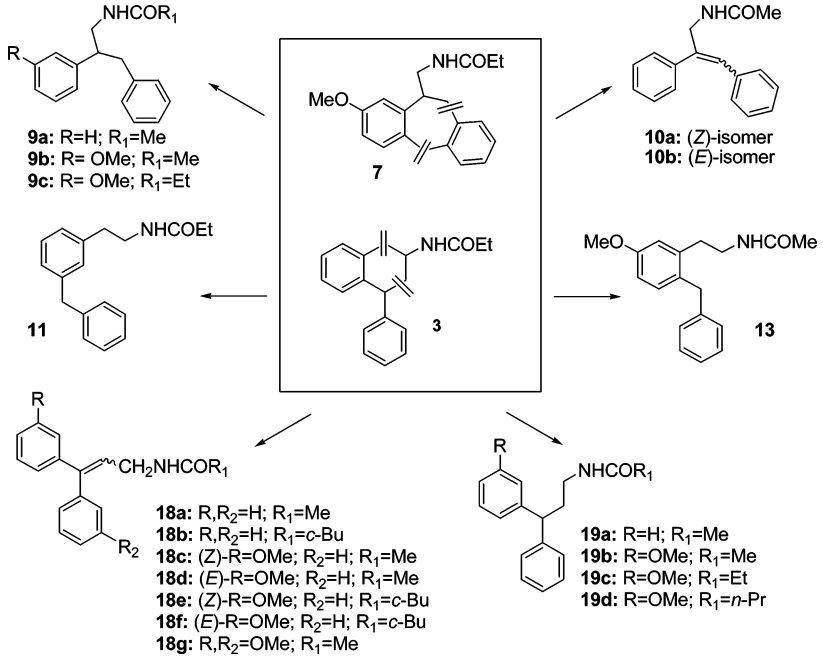
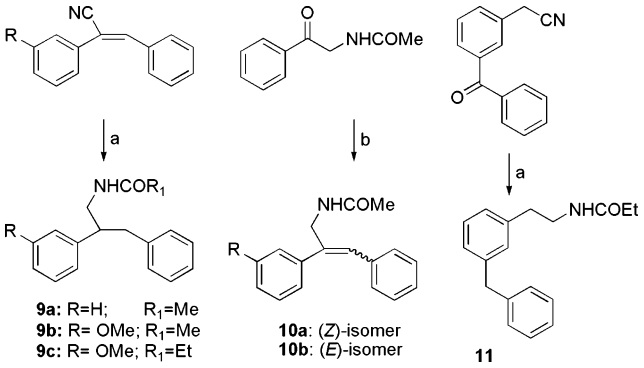


Figure 2. Application of the open-chain analog approach leading to the newly synthesized compounds.

leads to a reduction of intrinsic activity.[29,30] This hypothesis had been statistically validated by a three-dimensional quantitative structure−activity relationship (3D-QSAR) analysis on a series of structurally different MT_1 and MT_2 receptor ligands,[31] revealing a correlation between the occupation of the out-of-plane region and both MT_2 selectivity and antagonist behavior. Moreover, three-dimensional homology models of the MT_1 and MT_2 receptors allowed the rationalization, at the receptor level, of the structure−activity relationships (SARs) found for several ligands, providing a common interaction pattern and a possible explanation for their MT_2 selectivity and low intrinsic activity.[32] This information had been applied to the identification of a novel class of MT_2 antagonists, derived from 10,11-dihydrodibenzocycloheptene,[33] whose MT_2 selectivity was attributed to the skewed shape of the tricyclic scaffold, fulfilling the out-of-plane requirement previously defined. The dihydrodibenzocycloheptene derivative **7** (Figure 1) is endowed with one of the highest binding affinities reported to date, comparable to that of the tetralin derivative 4P-PDOT (**3**).[34]

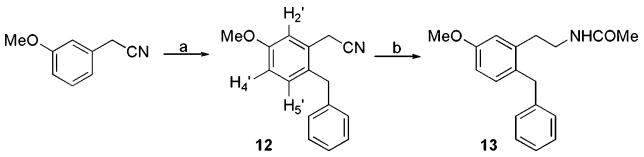
As a further step in the process of exploiting SAR information accumulated on MT_2-selective antagonism, we applied an "open-chain analog" approach[35] to this tricyclic scaffold and to the traditional tetralin scaffold of 4P-PDOT, looking for more flexible structures retaining the requirements described above. In the present paper, we report the design, synthesis, receptor binding characterization, and SAR analysis of novel classes of MLT receptor ligands of the *N*-[diphenylprop(en)yl]alkanamido type and benzyl-phenylethyl-alkanamides, whose structures are shown in Figure 2. These compounds were designed by structural simplification of the potent and selective cyclic antagonists **3** and **7**, and their structures appeared promising for MT_2 antagonism when superposed to the pharmacophore and 3D-QSAR-based models or docked into the MT_2 receptor binding site model.

Scheme 1[a]



[a] Reagents and conditions: (a) H_2, Raney-Ni, 4 atm, Ac_2O or $(EtCO)_2O$, THF, 60−80 °C; (b) *n*-BuLi, benzyltriphenylphosphonium chloride, Et_2O.

Scheme 2[a]



[a] Reagents and conditions: (a) $AlCl_3$, benzyl chloride, DCE; (b) H_2, Raney-Ni, 4 atm, Ac_2O, THF, 60 °C.

Chemistry

N-(3,3-Diphenylpropyl)acetamide (**19a**)[36] was prepared by *N*-acetylation of the commercially available 3,3-diphenylpropanamine with Ac_2O/NaOAc in AcOH. Synthetic routes to the other target compounds are described in Schemes 1−3. Briefly, melatonin receptor ligands **9a**−**c** and **11** were prepared by hydrogenation over Raney nickel of suitable nitriles (commercially available α-cinnamonitrile or its 3-methoxy analogue[37] for **9a**−**c** and 3-benzoyl-phenylacetonitrile[38] for **11**) and concomitant *N*-acylation with acetic or propionic anhydride (Scheme 1). *N*-(2-Oxo-2-phenylethyl)acetamide[39] was subjected to Wittig reaction conditions[40] using benzyltriphenylphosphonium chloride in the presence of *n*-BuLi to give an *E*/*Z* mixture of *N*-(2,3-diphenylpropenyl)acetamide (**10a**,**b**) from which each diastereoisomer was separated by flash chromatography (Scheme 1). Friedel−Crafts benzylation (benzyl chloride/$AlCl_3$) of 3-methoxyphenylacetonitrile gave a complex mixture from which 2-benzyl-5-methoxy-phenylacetonitrile (**12**) could be separated in low yield (6%) by flash chromatography. The structure of the intermediate **12** was confirmed by two NOE experiments (see Experimental Section). The nitrile **12** was then converted to the target compound **13** by hydrogenation over Raney nickel and concomitant *N*-acetylation with acetic anhydride (Scheme 2).

The *N*-(3,3-diphenylpropenyl)alkanamides **18a**−**g** were obtained by reduction of the suitable nitriles **14**−**17** with $LiAlH_4$/$AlCl_3$, followed by *N*-acylation of the crude intermediate amine with acetic anhydride or cyclobutanecarbonyl chloride; (*E*)- and (*Z*)-configurations were determined by ^{1}H NMR NOE experiments. Partial isomerization of the double bond was observed during the reduction step; the best diastereoselective reduction (retention of configuration >90%, determined by ^{1}H NMR) was achieved by using an 1:2 $LiAlH_4/AlCl_3$ molar ratio. 3,3-Diphenylacrylonitrile (**14**)[41] and 3,3-bis(3-methoxyphenyl)-acrylonitrile (**15**)[42] were prepared by submitting the appropriate benzophenone to Horner−Emmons reaction with diethyl (cyanomethyl)phosphonate, as previously described. The key (*Z*)-**16** and (*E*)-**17** nitriles were synthesized by diastereoselective Pd-catalyzed Heck reaction of (*E*)-3-(3-methoxyphenyl)acrilonitrile[43] with iodobenzene or of *trans*-cinnamonitrile with 3-iodoanisole, respectively (Scheme 3). The relative stereochemistry of the double bond was assigned through ^{1}H NMR NOE experiments. Whereas selective irradiation of the vinyl proton of compound **17** enhances H-2′ and H-6′ signals, the same irradiation in the case of compound **16** enhances *ortho* proton signals of the unsubstituted phenyl ring, showing unambiguously the (*E*)- and (*Z*)-configuration, respectively (see Experimental Section). Hydrogenation of the unsaturated nitriles **16** and **17** in the presence of Raney nickel and of the suitable anhydride gave the target *N*-(3,3-diphenylpropyl)alkanamides **19b**−**d** (Scheme 3).

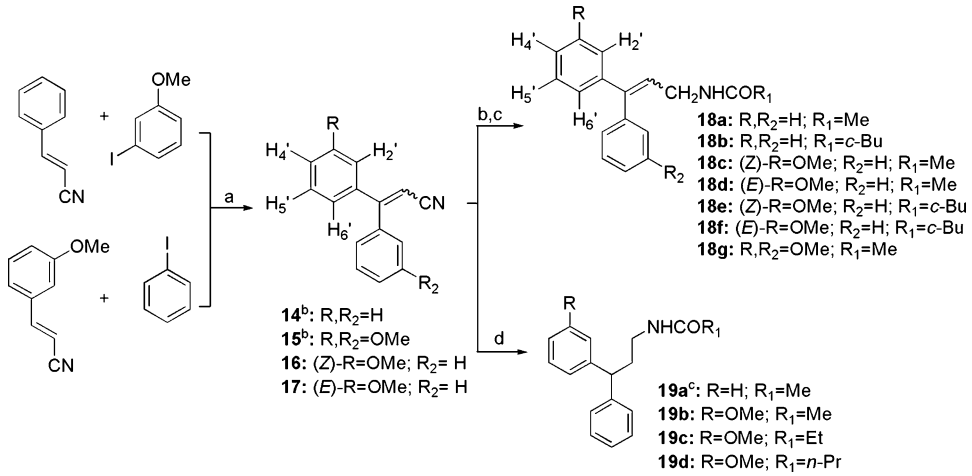
Results and Discussion

The tricyclic antagonist **7** and the tetralin derivative **3** were the starting structures used to generate novel ligands, according to the "open-chain analog" approach represented in Figure 2. Deletion of the lower methylene group in the tricycle of **7** gave the open structure **9**, while removal of the upper one afforded that of **13**, which can also be derived from the cyclic structure of **3** by deleting the lower methylene. On the other hand, structure **19** results from the deletion of the upper methylene from **3**. In our study, we also considered compound **11**, which could be superposed to the pharmacophore elements for MT_2 antagonism despite its different topology. Finally, the effect of a conformational constraint by a double bond (structures **10** and **18**) and of some groups typical of MLT receptor ligands was investigated for the most promising structures. We thus synthesized (2,3-diphenylpropyl)alkanamido (**9a**−**c**) and (3,3-diphenylpropyl)alkanamido derivatives (**19a**−**d**), the corresponding unsaturated derivatives **10a**,**b** and **18a**−**g**, and the two phenylethyl alkanamido derivatives carrying a benzyl substituent in different positions (**11** and **13**).

The spatial requirements for selective MT_2 receptor binding, as defined by our previous molecular modeling studies,[31,32] were checked for the new MLT receptor ligands here described. All the selected compounds were endowed with the three pharmacophore features previously identified, two aromatic rings and an amide function, connected through an acyclic scaffold that fulfilled the antagonist pharmacophore model and could be docked into the three-dimensional MT_2 receptor model in at least one accessible conformation.

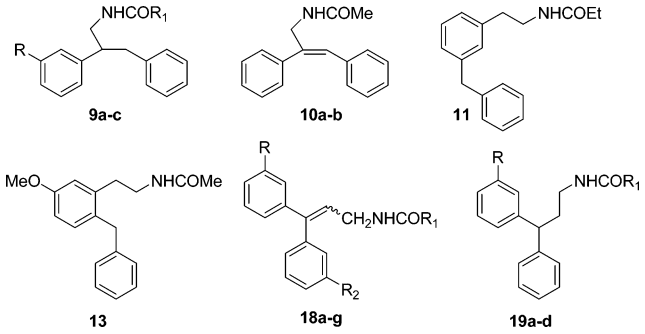
MT_1 and MT_2 binding affinity for compounds **9**−**11**, **13**, **18**, and **19** and their effect on GTPγS binding (intrinsic activity) are reported in Table 1. Chiral compounds **9** and **19b**−**d** were tested as racemic mixtures. The group of [2,3-diphenylprop-(en)yl]alkanamides (**9a**−**c** and **10a**,**b**) showed an antagonist/weak inverse agonist behavior, but they failed to demonstrate high affinity for MLT receptors (pK_i 5.44−6.83). The presence of the methoxy substituent did not improve the binding affinity in the saturated series, and its effect was therefore not further evaluated in the unsaturated one. In contrast, the isomeric [3,3-diphenylprop(en)yl]alkanamides (**18** and **19**), resembling the carbon framework of 4P-PDOT (**3**), had good to excellent affinity for the MT_2 receptor, with lower affinity for the MT_1 receptor. Within this series, the role of a methoxy group and changes in the *N*-acyl side chain were considered to investigate structure-affinity and structure-intrinsic activity relationships for MT_1 and MT_2 receptors. The effect of different geometries of the acylamino side chain was also evaluated by testing the (*E*)- and (*Z*)-propenyl derivatives (**18c**−**f**) and the dihydro analogues (**19a**−**d**). The binding affinities of compounds with these scaffolds showed that derivatives with a methoxy group (**18c**−**f** vs **18a**,**b** and **19b**−**d** vs **19a**) placed in a position that can be

Scheme 3[a]



[a] Reagents and conditions: (a) $Pd(OAc)_2$, AcO^-K^+, $(n\text{-}Bu)_4N^+Br^-$, DMF, 80 °C; (b) $AlCl_3$, $LiAlH_4$, dry Et_2O, room temperature, 1 h; (c) Ac_2O or cyclobutanecarbonyl chloride, TEA, THF, room temperature; (d) H_2, Raney-Ni, 4 atm, $(R_1CO)_2O$, THF, 60 °C. [b]Nitriles **14**[41] and **15**[42] were prepared as previously described. [c]**19a**[36] was prepared by *N*-acetylation of the commercially available 3,3-diphenylpropanamine.

Table 1. Experimental Binding Affinity and Intrinsic Activity (IA_r) of Newly Synthesized Compounds for Human MT_1 and MT_2 Melatonin Receptors Stably Expressed in NIH3T3 Cells and Calculated MT_2 pK_i Values



				human MT_1		human MT_2		
cmpd	**R**	**R_1**	**R_2**	**pK_i**[a]	**$IA_r \pm SEM$**[b]	**pK_i**[a]	**$IA_r \pm SEM$**[b]	**pK_i calcd**[c]
MLT				9.49	1.00 ± 0.01	9.59	1.01 ± 0.02	
9a	H	Me		6.15	−0.30 ± 0.04	6.38	−0.43 ± 0.2	7.49
9b	OMe	Me		6.16	−0.23 ± 0.08	6.69	−0.18 ± 0.04	7.49
9c	OMe	Et		6.28	−0.13 ± 0.03	6.72	−0.25 ± 0.2	7.90
10a-(*Z*)				5.92	0.11 ± 0.02	6.83	0.18 ± 0.01	6.81
10b-(*E*)				5.44	−0.23 ± 0.01	6.28	−0.15 ± 0.03	7.39
11				6.41	0.24 ± 0.01	6.18	0.48 ± 0.04	8.12
13				7.16	0.24 ± 0.04	8.22	0.06 ± 0.02	8.68
18a	H	Me	H	6.29	−0.03 ± 0.03	7.94	0.19 ± 0.14	7.81
18b	H	*c*-Bu	H	6.04	−0.18 ± 0.06	7.88	−0.20 ± 0.15	8.06
18c-(*Z*)	OMe	Me	H	6.64	0.45 ± 0.05	8.60	0.30 ± 0.18	7.94
18d-(*E*)	OMe	Me	H	8.57	0.54 ± 0.06	9.66	0.38 ± 0.06	8.45
18e-(*Z*)	OMe	*c*-Bu	H	6.59	−0.04 ± 0.01	8.81	−0.02 ± 0.01	7.76
18f-(*E*)	OMe	*c*-Bu	H	7.03	0.29 ± 0.04	9.28	0.13 ± 0.01	8.68
18g	OMe	Me	OMe	7.23	0.20 ± 0.02	9.56	0.34 ± 0.02	8.60
19a	H	Me	H	5.38	−0.19 ± 0.02	6.85	−0.23 ± 0.13	7.59
19b	OMe	Me	H	5.66	0.24 ± 0.05	8.27	0.04 ± 0.12	8.70
19c	OMe	Et	H	6.44	0.31 ± 0.05	8.30	0.28 ± 0.02	8.91
19d	OMe	*n*-Pr	H	6.26	0.21 ± 0.05	8.58	0.27 ± 0.01	8.69

[a] pK_i values were calculated from IC_{50} values, obtained from competition curves by the method of Cheng and Prusoff[56] and are the mean of at least three determinations performed in duplicate. SEM of pK_i values were lower than 0.06. [b] The relative intrinsic activity values were obtained by dividing the maximum analogue-induced G-protein activation by that of MLT. [c] MT_2 pK_i values, calculated with the CoMFA model described in the text (training set of 34 compounds), for the new compounds as an external test set.

easily superposed to position 5 of the indole ring of MLT, usually have the highest binding affinity. The methoxy group led to a higher increase in MT_2 than in MT_1 binding affinity (**18c** vs **18a** and **19b** vs **19a**), causing a MT_2/MT_1 selectivity ratio of more than two log units for compounds **18f**, **18g**, **19b**, and **19d**. The presence of a 2,3 double bond in compounds **18** increased the binding affinity at both receptor subtypes compared to that of the corresponding saturated compounds **19**. Analysis of the two diastereoisomers (*Z*)-**18c** and (*E*)-**18d** (or (*Z*)-**18e** and (*E*)-**18f**) revealed that the (*E*)-isomer has higher binding affinity at both receptor subtypes, suggesting that the relative spatial orientation of the phenyl group and the acylamino side chain affects optimal receptor binding, even if this difference is less evident, at the MT_2 receptor, for the cyclobutanamides **18e** and **18f**. Structural variations at the *N*-acylamino group were found to be less important than in other series of

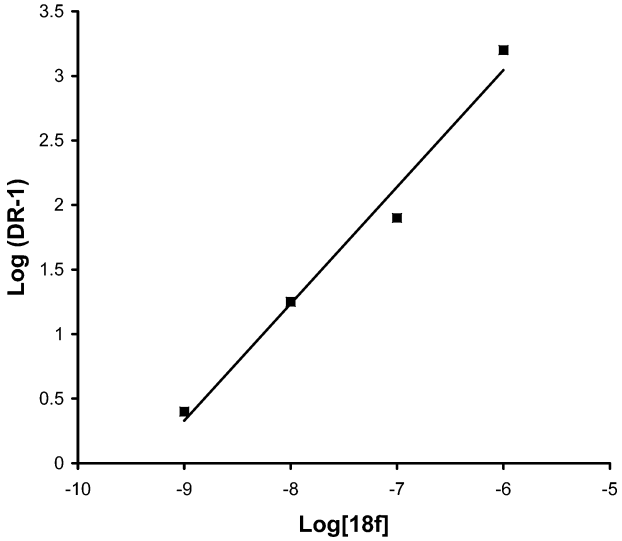


Figure 3. Schild plot for **18f** in the [^{35}S]GTPγS assay.

MLT analogues.[44] Homologation of the methyl group of the amido side chain to ethyl (**19c**) or propyl (**19d**) did not considerably modify the binding affinity and efficacy for each of the two receptor subtypes. Replacement of the methyl group of the acetamido side chain by cyclobutyl in (*Z*)-**18e** or (*E*)-**18f** retains a high MT_2 binding affinity but causes a loss of intrinsic activity, a finding that parallels previous results about the role of a cycloalkyl substituent on the acylamino side chain.[45,46] *N*-[3,3-Bis(3-methoxyphenyl)propenyl]acetamide (**18g**) retains the same MT_2 binding affinity of the monomethoxy derivative (*E*)-**18d**, but it shows lower MT_1 binding affinity (ca. 22-fold), leading to an enhancement of the MT_2/MT_1 selectivity ratio from 10 to 214. All the studied compounds behaved as antagonists or partial agonists. The presence of the methoxy group of the double bond and the nature of the *N*-acyl substituent allowed the modulation of MT_2 selectivity and intrinsic activity. Compound (*E*)-**18f** (MT_2/MT_1 ratio 178) is about 12-fold more selective for MT_2 than the lead **18d**; it presents an affinity for MT_2 similar to that of melatonin itself and acts as an MT_2 antagonist.

The ability of compound **18f** to antagonize MLT-stimulated [^{35}S]GTPγS binding was further investigated by carrying out a series of MLT concentration−response curves in the presence of four different concentrations of compound **18f** (1, 10, 100, and 1000 nM). Compound **18f** causes a dose-dependent parallel rightward shift of the concentration−response curve for MLT stimulation of [^{35}S]GTPγS binding. As can be seen from the Schild plot in Figure 3, the slope was not significantly different from unity (0.90 ± 0.09, $R^2 = 0.98$), being consistent with a competitive antagonism, with an X intercept corresponding to a pA_2 value of 9.41 and a pK_B value, obtained by forcing the intercept to 1, of 9.28, identical to the pK_i value from binding experiments.

A series of propyl-alkanamido derivatives with only one phenyl substituent linked to the propyl side chain had previously been described.[47,48] Unlike [3,3-diphenylprop(en)yl]alkanamides **18** and **19**, which are MT_2-selective, they showed no selectivity, having about the same binding affinity at MT_1 and MT_2 receptors.[47] Moreover, *N*-[3-(3-methoxyphenyl)propyl]acetamide had been described as an agonist in *Xenopus* melanophore assay,[49] while the presence of the second phenyl ring in **18** and **19** greatly affected intrinsic activity.

As for the 2-phenylethyl-alkanamides **11** and **13**, whereas the *ortho*-benzyl derivative **13** retained significant binding affinity and MT_2 selectivity, the *meta*-benzyl derivative **11** showed little affinity for both MT_1 and MT_2 receptor subtypes. Even if the reduced binding affinity of **11** could be, at least in part, explained by the lack of the methoxy substituent, these two compounds were considered less promising than **18** and **19**.

The binding mode for the potent and selective representative of the (3,3-diphenylpropenyl)alkanamido series, **18g**, was inferred by an automated docking procedure, implemented in the program Glide,[50] within our previously developed MT_2 receptor model,[32] and by evaluating the stability of receptor−ligand complexes by molecular dynamics (MD) simulations. The docking solution giving the best Emodel score was employed to build the complex, which proved to be stable during 1 ns of MD simulation (Figure 4, left). Compound **18g** occupied the same region of space as previously docked antagonists and undertook similar interactions with the putative MT_2 binding cavity. The amide oxygen is engaged in a hydrogen bond with Tyr183, belonging to the extracellular loop 2, and one phenyl ring is involved in a T-shaped interaction with His208 in TM5, while the other one is inserted into the lipophilic pocket close to Trp264 of the CWXP motif in TM6. One of the two symmetrical methoxy groups reinforced the T-shape interaction, occupying a region of space as the 5-methoxy group of MLT, while the second one could be accommodated within the lipophilic pocket also receiving the out-of-plane substituents of MT_2-selective antagonists.[32] In fact, this second methoxyl, even if not giving any affinity improvement (see **18g** vs **18d** in Table 1), is tolerated similarly to what was observed for *p*-methyl and *p*-methoxyluzindole,[34] and for some benzofuran[51] (e.g., **6**) and 2-acylaminomethylindole derivatives[30] (e.g., **4**), compared to their unsubstituted analogues. Contrary to expectations, the methoxy substituent was preferentially accommodated within the lipophilic pocket when the monomethoxy derivatives were docked into the model. Actually, the precise role of the 5-methoxy group of MLT-like ligands is still partially unexplained by this model.

As stated in the introduction, the design of the putative MT_2-selective antagonists of this study had been driven by pharmacophore and 3D-QSAR models, which showed a satisfactory predictive ability across structurally different classes of ligands.[25,33] Therefore, within this work, the binding affinity expected for the newly synthesized compounds was calculated applying a previously defined 3D-QSAR model[31] based on thirty-four in-house and literature antagonists. The former CoMFA model, however, was revised to be fully consistent with our MT_2 receptor model,[32] where the out-of-plane group and the acylamino chain had a different mutual orientation than in the original alignment (see Experimental Section). The statistical parameters of the new PLS analysis were very similar to those of the previous model: $Q^2 = 0.62$, SDEP = 0.52, $R^2 = 0.87$, and $s = 0.33$ for 34 compounds and 5 latent variables (previous model: $Q^2 = 0.59$, SDEP = 0.53, $R^2 = 0.87$, and $s = 0.34$, 5 LVs), and the coefficient contour plots, applied to the new alignment, carried the same information (Figure 4, right). The predicted MT_2 pK_i values for the new compounds **9**−**11**, **13**, **18**, and **19** are reported in Table 1. From a structural point of view, the CoMFA training set greatly differs from our test set, being mainly composed by indole-based antagonists. Despite this structural difference, binding affinities were generally adequately predicted, with the greatest over- or under-estimation being around 10 times the experimental values. An important exception was represented by compound **11**, whose binding affinity was greatly overestimated. This 2-phenylethyl-propi-

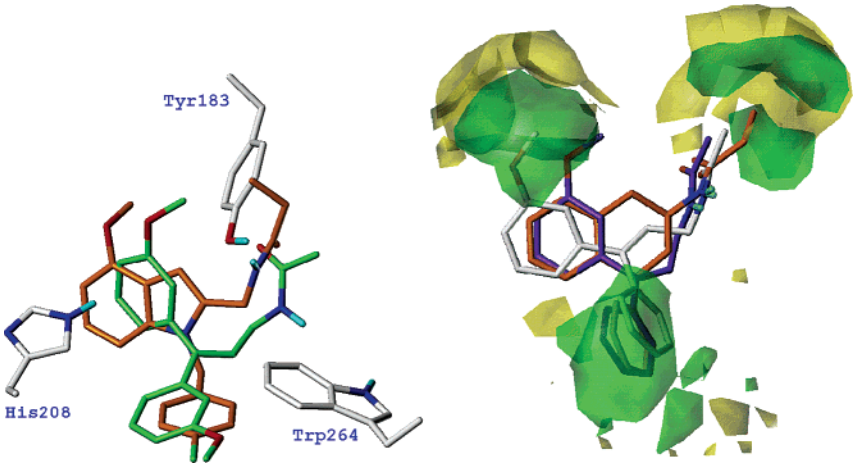


Figure 4. Left: energy-minimized conformation of the last step of 1 ns MD simulation for the complex between MT_2 receptor model and **18g** (green carbons); the reference compound **4** (orange carbons), docked as described in ref 32, is represented for comparison. Right: superposition of **3** (orange carbons), **18c** (purple carbons), and **18d** (white carbons) in the new CoMFA alignment; regions of positive (>0.0045) and negative (<−0.0045) standard deviation × coefficients for the model with five latent variables (see text) are illustrated in green and yellow, respectively.

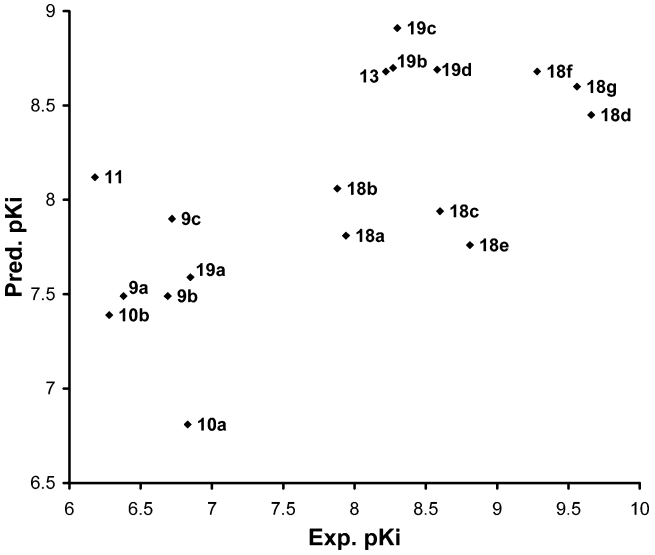


Figure 5. Experimental pK_i values at the MT_2 receptor vs those predicted by the CoMFA model described in the text (training set of 34 compounds).

onamido derivative has a unique structure within the newly synthesized compounds; its high conformational freedom may account for the poor binding affinity, despite its good superposition to the 3D-QSAR model and high predicted activity. Excluding the outlier **11**, the correlation between experimental and predicted pK_i values for the external test set gave $R^2 = 0.54$ (Figure 5), acceptable if compared to the predictive ability of the CoMFA model, estimated by leave-one-out cross-validation ($Q^2 = 0.62$). The limited accuracy of quantitative predictions can be attributed to some SAR information for the present series that was not fully addressed by the old training set. If a comprehensive model with all the 52 compounds included is built, a significant improvement in Q^2 was achieved ($Q^2 = 0.70$, SDEP = 0.53, $R^2 = 0.90$, and $s = 0.33$ with 5 LVs), even if the shape of regions with negative and positive coefficients was highly conserved (data not shown). More importantly, some relevant SAR elements for the new compounds were properly explained by the CoMFA model. Thus, among the 3,3-diphenyl-propenyl-alkanamido derivatives **18**, the (*E*)-isomers were predicted more potent than the (*Z*)-isomers, and the presence of the methoxy substituent led to a higher predicted binding affinity in both the **18** and **19** series (Table 1).

In conclusion, the constrained cycloalkyl scaffolds of the dihydrodibenzocycloheptene compound **7** and of the 4-phenyltetralin **3** were successfully replaced by a conformationally flexible di-phenyl-prop(en)yl side chain. This structural modification led to the discovery of a series of *N*-[3,3-diphenylprop-(en)yl]alkanamides (**18** and **19**), having a structural motif fulfilling the key features outlined by our previous 3D-QSAR models (i.e., two aromatic rings and an amide side chain in a suitable arrangement), as novel MT_2-selective ligands. Modulation of the selectivity and of the intrinsic activity can be obtained by proper modification on the *N*-acyl, phenyl substituents, and stereochemistry of the side chain. The most interesting results are obtained with the (*E*)-propenyl derivatives **18d** and **18f** and for the symmetrical propenyl derivative **18g**. They are very good MT_2 ligands, with affinities as strong as that of MLT itself, and the last two compounds show MT_2/MT_1 selectivity ratios higher than one hundred. Compounds **18d** and **18g** behave as MT_1 and MT_2 partial agonists, whereas the *N*-cyclobutyl analogue **18f** is an MT_2 antagonist. This class represents a successful example of molecular simplification based on a pharmacophore hypothesis.

Experimental Section

General Methods. Melting points were determined on a Buchi B-540 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AVANCE 200 spectrometer, using $CDCl_3$ as solvent unless otherwise noted. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to the central peak of the solvent. Coupling constants (*J* values) are given in hertz (Hz). NOE spectra were measured using the Bruker pulse program Selnogp, based on double pulsed field gradient spin echo nuclear Overhauser experiment (DPFGSE),[52] pre-irradiation time: 5 s. EI-MS spectra (70 eV) were taken on a Fisons Trio 1000 instrument. Only molecular ions (M^+) and base peaks are given. Infrared spectra were obtained on a Nicolet Avatar 360 FTIR spectrometer; absorbances are reported in ν (cm^{-1}). Elemental analyses for C, H, and N were performed on a Carlo Erba analyzer, and the results 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. All chemicals were purchased from commercial suppliers and used directly without any further purification. The two radioligands 2-[^{125}I]iodomelatonin (specific activity, 2000 Ci/mmol) and [^{35}S]GTPγS ([^{35}S]guanosine-5′-*O*-(3-thio-triphosphate); specific activity, 1000 Ci/mmol) were purchased from Amersham Pharmacia Biotech (Italy).

***N*-(2,3-Diphenylpropyl)acetamide (9a):** A solution of α-phenylcinnamonitrile (0.41 g, 2 mmol) and acetic anhydride (3 mL, 31 mmol) in THF (10 mL) was hydrogenated over Raney nickel at 4 atm of H_2 for 6 h at 60 °C. The catalyst was filtered on Celite, the filtrate was concentrated in vacuo, and the residue was partitioned between EtOAc and 2 N NaOH. The organic layer was washed twice with 2 N NaOH and once with brine, dried (Na_2SO_4), and evaporated under reduced pressure to give a residue that was purified by flash chromatography (silica gel; EtOAc/cyclohexane 1:1 as eluent) and crystallization (Et_2O/petroleum ether); 59% yield; mp = 68 °C. MS (EI): *m*/*z* 253 (M^+), 194 (100). ^1H NMR ($CDCl_3$): δ 1.83 (s, 3H), 2.92 (m, 2H), 3.09 (m, 1H), 3.33 (m, 1H), 3.77 (m, 1H), 5.19 (br s, 1H), 7.03−7.35 (m, 10H). IR (cm^{-1}, Nujol): 3272, 1635. Anal. ($C_{17}H_{19}NO$) C, H, N.

***N*-[2-(3-Methoxyphenyl)-3-phenylpropyl]acetamide (9b):** Compound **9b** was obtained following the procedure above-described by using 2-(3-methoxyphenyl)-3-phenyl-acrylonitrile[37] instead of α-phenylcinnamonitrile. Purification by flash chromatography (silica gel; EtOAc as eluent) gave a 27% yield of an oil. MS (EI): *m*/*z* 283 (M^+), 224 (100). ^1H NMR ($CDCl_3$): δ 1.83 (s, 3H), 2.91 (m, 2H), 3.07 (m, 1H), 3.30 (m, 1H), 3.74 (m, 1H), 3.77 (s, 3H), 5.21 (br s, 1H), 6.67−6.79 (m, 3H), 7.04−7.22 (m, 6H). IR (cm^{-1}, neat): 3292, 1651. Anal. ($C_{18}H_{21}NO_2$) C, H, N.

***N*-[2-(3-Methoxyphenyl)-3-phenylpropyl]propanamide (9c):** Compound **9c** was obtained following the above procedure described for **9b**, by using propionic anhydride instead of acetic anhydride. Purification by flash chromatography (silica gel; EtOAc/cyclohexane 1:1 as eluent) gave a 44% yield of an oil. MS (EI): *m*/*z* 297 (M^+), 57 (100). ^1H NMR ($CDCl_3$): δ 1.03 (t, 3H, J = 7.3), 2.04 (q, 2H, J = 7.3), 2.92 (m, 2H), 3.13 (m, 1H), 3.32 (m, 1H), 3.81 (m, 1H), 3.90 (s, 3H), 5.20 (br s, 1H), 6.73 (m, 3H), 7.05−7.22 (m, 6H). IR (cm^{-1}, neat): 3293, 1644. Anal. ($C_{19}H_{23}NO_2$) C, H, N.

***N*-(2,3-Diphenyl-2-propenyl)acetamides (10a and 10b):** *n*-BuLi (10 M in hexane, 0.157 mL, 1.57 mmol) was added dropwise to a stirred ice-cooled suspension of benzyltriphenylphosphonium chloride (0.61 g, 1.57 mmol) in diethyl ether (4 mL) under N_2. The mixture was stirred at 0 °C for 30 min, and a solution of *N*-(2-oxo-2-phenyl-ethyl)acetamide[39] (0.27 g, 1.52 mmol) in Et_2O (2.5 mL) and CH_2Cl_2 (2.5 mL) was then added dropwise. The resulting mixture was stirred at room temperature for 90 min and filtered, and the filter cake was washed with diethyl ether. The combined filtrates were evaporated to give a crude residue from which the two (*E*)- and (*Z*)-diastereoisomers were separated by flash chromatography (silica gel; cyclohexane/EtOAc, 1:1 as eluent).

***(Z)*-*N*-(2,3-Diphenyl-2-propenyl)acetamide (10a):** Compound **10a** was obtained in a 31% yield; mp 113 °C (Et_2O/hexane). MS (EI): *m*/*z* 251 (M^+, 100). ^1H NMR ($CDCl_3$): δ 1.96 (s, 3H), 4.30 (d, 2H), 5.54 (br s, 1H), 6.60 (s, 1H), 6.95−7.4 (m, 10H). Selective irradiation of the CH_2 allylic protons (δ = 4.30) resulted in 3.2% enhancement of the signal at δ = 6.60 due to the CH═ vinylic proton. IR (cm^{-1}, Nujol): 3289, 1649, 1544. Anal. ($C_{17}H_{17}NO$) C, H, N.

***(E)*-*N*-(2,3-Diphenyl-2-propenyl)acetamide (10b):** Compound **10b** was obtained in a 13% yield; mp 180 °C (trituration from Et_2O). MS (EI): *m*/*z* 251 (M^+, 100). ^1H NMR ($CDCl_3$): δ 1.89 (s, 3H), 4.57 (d, 2H), 5.37 (br s, 1H), 7.01 (s, 1H), 7.3−7.55 (m, 10H). Selective irradiation of the CH_2 allylic protons (δ = 4.57) did not enhance the signal at δ = 7.01 due to the CH═ vinylic proton. IR (cm^{-1}, Nujol): 3291, 1632, 1536. Anal. ($C_{17}H_{17}NO$) C, H, N.

***N*-{2-[(3-Benzyl)phenyl]ethyl}propanamide (11):** A solution of (3-benzoylphenyl)acetonitrile[38] (0.15 g, 0.68 mmol) and propionic anhydride (1 mL, 7.8 mmol) in THF (7.5 mL) was hydrogenated over Raney nickel at 4 atm of H_2 for 5 h at 80 °C. The catalyst was filtered on Celite, the filtrate was concentrated in vacuo, and the residue was partitioned between EtOAc and 2 N NaOH. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to give a crude residue that was purified by flash chromatography (silica gel; cyclohexane/EtOAc, 1:1 as eluent) and crystallization (diethyl ether/petroleum ether) to give a 38% yield; mp 47−48 °C. MS (EI): *m*/*z* 267 (M^+), 194 (100). ^1H NMR ($CDCl_3$): δ 1.09 (t, 3H, J = 7.5), 2.10 (q, 2H, J = 7.5), 2.77 (t, 2H, J = 6.7), 3.48 (q, 2H, J = 6.7), 3.95 (s, 2H), 5.4 (br s, 1H), 7.02−7.32 (m, 9H). IR (cm^{-1}, Nujol): 3302, 1639. Anal. ($C_{18}H_{21}NO$) C, H, N.

(2-Benzyl-5-methoxyphenyl)acetonitrile (12): Benzyl chloride (2 mL, 17.3 mmol) was added to an ice-cooled stirred solution of 3-methoxyphenylacetonitrile (1.47 g, 10 mmol) in dry DCE (20 mL). Aluminum trichloride (2.12 g, 16 mmol) was added portionwise (30 min), and the resulting mixture was stirred for 30 min at 0 °C and then at room temperature for 5 h. The reaction mixture was poured into an ice-cooled 2 N HCl aqueous solution, filtered on Celite, and the filtrate was extracted three times with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure, to give a residue that was purified by flash chromatography (silica gel; EtOAc/cyclohexane 1:9 as eluent) to give a 6% yield of an oil. MS (EI): *m*/*z* 237, (M^+), 165 (100). ^1H NMR ($CDCl_3$): δ 3.53 (s, 2H), 3.83 (s, 3H), 3.98 (s, 2H), 6.86 (dd, 1H, H-4′, J = 2.7 and J = 8.3), 6.99 (d, 1H, H-2′, J = 2.7), 7.17 (d, 1H, H-5′, J = 8.3), 7.09 (m, 2H, $phenyl_{ortho}$), 7.20−7.35 (m, 3H, phenyl). Selective irradiation of signal due to CH_2CN at δ = 3.53 resulted in enhancement of signal due to CH_2Ph (1%) and of signal due to H-2′ (1.4%). Moreover, irradiation of CH_2Ph (at δ = 3.98) resulted in enhancement of H-5′ (1.2%), CH_2CN (1%), and H_{ortho} of the unsubstituted phenyl ring (2.4%). IR (cm^{-1}, neat): 2241.

***N*-[2-(2-Benzyl-5-methoxyphenyl)ethyl]acetamide (13):** A solution of **12** (1 mmol) and acetic anhydride (1.5 mL, 15.5 mmol) in THF (6 mL) was hydrogenated over Raney nickel at 4 atm of H_2 for 6 h at 60 °C. Standard workup gave a residue that was purified by flash chromatography (silica gel; EtOAc as eluent) and crystallization from diethyl ether to give a 44% yield; mp 84−85 °C. MS (EI): *m*/*z* 283 (M^+), 209 (100). ^1H NMR ($CDCl_3$): δ 1.88 (s, 3H), 2.76 (m, 2H), 3.36 (m, 2H), 3.80 (s, 3H), 3.98 (s, 2H), 5.38 (br s, 1H), 6.76−7.31 (m, 8H). IR (cm^{-1}, Nujol): 3306, 1647. Anal. ($C_{18}H_{21}NO_2$) C, H, N.

(*Z*)-3-(3-Methoxyphenyl)-3-phenylacrylonitrile (16): (*E*)-3-Methoxyphenyl-acrylonitrile[43] (1.37 g, 8.6 mmol) was added to a mixture of iodobenzene (1.85 mL, 16.5 mmol), potassium acetate (2.11 g, 21.5 mmol), tetrabutylammonium bromide (3.03 g, 9.4 mmol), and palladium acetate (0.095 g, 0.42 mmol) in dry DMF (30 mL) under N_2. After stirring at 80 °C for 3 days, the reaction mixture was cooled to room temperature, poured into water, and extracted three times with diethyl ether. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to give a residue that was purified by flash chromatography (silica gel; cyclohexane/EtOAc, 95:5 as eluent) and crystallization from diethyl ether/petroleum ether to give a 79% yield; mp 79 °C. MS (EI): *m*/*z* 235 (M^+, 100). ^1H NMR (acetone-d_6): δ 3.83 (s, 3H), 6.13 (s, 1H), 6.96 (ddd, 1H, H-6′, J = 1.0, J = 1.7, and J = 7.6), 6.98 (dd, 1H, H-2′, J = 1.0 and J = 2.4), 7.09 (ddd, 1H, H-4′, J = 1.0, J = 2.4, and J = 8.3), 7.35−7.55 (m, 6H, H-5′ and unsubstituted phenyl). Selective irradiation of the vinylic proton (δ = 6.13) resulted in 3.0% enhancement of the signal at δ = 7.4 due to the *ortho* protons of the unsubstituted phenyl ring. IR (cm^{-1}, Nujol): 2211, 1569.

(*E*)-3-(3-Methoxyphenyl)-3-phenylacrylonitrile (17): *trans*-Cinnamonitrile (0.28 mL, 2.24 mmol) was added to a mixture of 3-iodoanisole (0.56 mL, 4.25 mmol), potassium acetate (0.55 g, 5.6 mmol), tetrabutylammonium bromide (0.79 g, 2.45 mmol), and palladium acetate (0.025 g, 0.11 mmol) in dry DMF (8 mL) under N_2. After stirring at 80 °C for 24 h, the reaction mixture was cooled to room temperature, poured into water, and extracted three times with diethyl ether. The organic combined phases were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to give a residue that was purified by flash chromatography (silica gel; cyclohexane/EtOAc, 95:5 as eluent) and crystallization from diethyl ether/petroleum ether to give a 62% yield; mp 60−61 °C. MS (EI): *m*/*z* 235 (M^+, 100). ^1H NMR (acetone-d_6): δ 3.80 (s, 3H), 6.14 (s, 1H), 6.89 (ddd, 1H, H-6′, J = 1.0, J = 1.7, and J = 7.6), 6.97 (dd, 1H, H-2′, J = 1.5 and J = 2.4), 7.06 (ddd, 1H, H-4′, J = 1.5, J = 2.7, and J = 8.3), 7.34 (dd, 1H, H-5′, J = 7.8

and $J = 8.1$), 7.40−7.55 (m, 5H, unsubstituted phenyl). Selective irradiation of the vinylic proton ($\delta = 6.14$) resulted in 3.3% enhancement of signals at $\delta = 6.89$ and $\delta = 6.97$ due to H-6′ and H-2′, respectively. IR (cm^{-1}, Nujol): 2212, 1583.

General Procedure for the Synthesis of *N*-(3,3-Diphenyl-2-propenyl)alkanamides 18a−g: A suspension of aluminum trichloride (0.293 g, 2.2 mmol) in dry Et_2O (2.8 mL) was added to a stirred ice-cooled suspension of $LiAlH_4$ (0.041 g, 1.1 mmol) in dry Et_2O (1.3 mL) under a nitrogen atmosphere. The mixture was stirred for 10 min, a solution of the suitable nitrile **14**−**17** (1.0 mmol) in dry Et_2O (0.9 mL) was then added dropwise, and the resulting mixture was stirred at room temperature for 1 h. Water was carefully added at 0 °C, and the resulting mixture was filtered through a Celite pad. The filtrate was concentrated in vacuo and partitioned between water and EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated to yield the crude (3,3-diphenyl)propenylamines, which were used without any further purification for the preparation of **10a**−**g**. TEA (0.15 mL, 1.07 mmol) and acetic anhydride (0.1 mL, 1.07 mmol for **18a**, **18c**,**d**, and **18g**), or cyclobutanecarbonyl chloride (0.114 mL, 1 mmol for **18b**, **18e**,**f**) were added to a stirred solution of the corresponding crude amine in dry THF (12 mL), and the mixture was stirred at room temperature for 6 h (1 h for **18b**, **18e**,**f**). The solvent was evaporated in vacuo, and the residue was dissolved in EtOAc. The solution was washed with 2 N NaOH and brine and then dried (Na_2SO_4). After distillation of the solvent, a crude residue was obtained, which was purified by crystallization or flash chromatography.

***N*-(3,3-Diphenyl-2-propenyl)acetamide (18a):** Compound **18a** was obtained with purification by crystallization from EtOAc/petroleum ether, giving a 39% yield; mp 139 °C. MS (EI): *m*/*z* 251 (M^+), 192 (100). ^1H NMR ($CDCl_3$): δ 1.98 (s, 3H), 3.95 (dd, 2H, $J = 6.0$ and $J = 6.8$), 5.48 (br s, 1H), 6.08 (t, 1H, $J = 6.8$), 7.15−7.43 (m, 10H). IR (cm^{-1}, Nujol): 3308, 1643, 1541. Anal. ($C_{17}H_{17}NO$) C, H, N.

***N*-(3,3-Diphenyl-2-propenyl)cyclobutanecarboxamide (18b):** Compound **18b** was obtained with purification by crystallization from EtOAc, giving a 53% yield; mp 131 °C. MS (EI): *m*/*z* 291 (M^+), 192 (100). ^1H NMR ($CDCl_3$): δ 1.83−2.37 (m, 6H), 2.95 (m, 1H), 3.96 (dd, 2H, $J = 5.9$ and $J = 6.8$), 5.36 (br t, 1H), 6.07 (t, 1H, $J = 7.0$), 7.16−7.38 (m, 10H). IR (cm^{-1}, Nujol): 3325, 1638, 1531. Anal. ($C_{20}H_{21}NO$) C, H, N.

(*Z*)-*N*-[3-(3-Methoxyphenyl)-3-phenyl-2-propenyl]acetamide (18c): Compound **18c** was obtained by reduction of the nitrile (*Z*)-**16**, followed by *N*-acylation of the corresponding crude amine with acetic anhydride, according to the above-described general procedure. Purification by flash chromatography (silica gel; EtOAc/cyclohexane 1:1 as eluent) gave 57% yield of an oil. MS (EI): *m*/*z* 281 (M^+), 222 (100). ^1H NMR (acetone-d_6): δ 1.88 (s, 3H), 3.80 (s, 3H), 3.84 (dd, 2H, $J = 5.6$ and $J = 7.1$), 6.10 (t, 1H, $J = 7.0$), 6.75 (m, 2H, H-2′ and H-6′), 6.93 (ddd, 1H, H-4′, $J = 1.5$, $J = 2.2$, and $J = 8.0$), 7.15−7.43 (m, 7H, NH, H-5′and unsubstituted phenyl). Selective irradiation of vinylic proton ($\delta = 6.10$) resulted in 2.0% enhancement of resonance due to *ortho* protons of the unsubstituted phenyl ring. IR (cm^{-1}, neat): 3281, 1651, 1550. Anal. ($C_{18}H_{19}NO_2$) C, H, N.

(*E*)-*N*-[3-(3-Methoxyphenyl)-3-phenyl-2-propenyl]acetamide (18d): Compound **18d** was obtained by reduction of the nitrile (*E*)-**17**, followed by *N*-acylation of the corresponding crude amine with acetic anhydride, according to the above-described procedure. Purification by flash chromatography (silica gel; EtOAc/cyclohexane 1:1 as eluent) and crystallization from diethyl ether gave a 60% yield; mp 128 °C. MS (EI): *m*/*z* 281 (M^+), 222 (100). ^1H NMR (acetone-d_6): δ 1.85 (s, 3H), 3.73 (s, 3H), 3.83 (dd, 2H, $J = 5.0$ and $J = 7.0$), 6.12 (t, 1H, $J = 7.0$), 6.76 (dd, 1H, H-2′, $J = 2.5$ and $J = 2.6$), 6.78 (ddd, 1H, H-6′, $J = 1.0$, $J = 1.6$, and $J = 7.0$), 6.84 (ddd, 1H, H-4′, $J = 2.0$, $J = 2.5$, and $J = 8.0$), 7.21 (t, 1H, H-5′, $J = 8.0$), 7.15−7.48 (m, 6H, NH and unsubstituted phenyl). Selective irradiation of vinylic proton ($\delta = 6.12$) resulted in 4.2% enhancement of signals at $\delta = 6.76$ and $\delta = 6.78$ due to H-2′ and H-6′ protons. IR (cm^{-1}, Nujol): 3269, 1644, 1557. Anal. ($C_{18}H_{19}NO_2$) C, H, N.

(*Z*)-*N*-[3-(3-Methoxyphenyl)-3-phenyl-2-propenyl]cyclobutancarboxamide (18e): Compound **18e** was obtained by reduction of the nitrile (*Z*)-**16**, followed by *N*-acylation of the corresponding crude amine with cyclobutanecarbonyl chloride, according to the above-described general procedure. Purification by flash chromatography (silica gel; EtOAc/cyclohexane 3:7 as eluent) and crystallization from diethyl ether/ petroleum ether gave a 61% yield; mp 121−122 °C. MS (EI): *m*/*z* 321 (M^+), 222 (100). ^1H NMR ($CDCl_3$): δ 1.81−2.37 (m, 6H), 2.98 (m, 1H), 3.80 (s, 3H), 3.95 (dd, 2H, $J = 5.9$ and $J = 6.7$), 5.38 (br t, 1H), 6.07 (t, 1H, $J = 7.0$), 6.74 (m, 2H, H-2′ and H-6′), 6.89 (ddd, 1H, H-4′, $J = 0.8$, $J = 2.4$, and $J = 8.3$), 7.14−7.43 (m, 6H, H-5′ and unsubstituted phenyl). IR (cm^{-1}, Nujol): 3315, 1635, 1532. Anal. ($C_{21}H_{23}NO_2$) C, H, N.

(*E*)-*N*-[3-(3-Methoxyphenyl)-3-phenyl-2-propenyl]cyclobutancarboxamide (18f): Compound **18f** was obtained by reduction of the nitrile (*E*)-**17**, followed by *N*-acylation of the corresponding crude amine with cyclobutanecarbonyl chloride, according to the above-described general procedure. Purification by flash chromatography (silica gel; EtOAc/cyclohexane 1:1 as eluent) and crystallization from diethyl ether/petroleum ether gave a 62% yield; mp 89−90 °C MS (EI): *m*/*z* 321 (M^+), 222 (100). ^1H NMR ($CDCl_3$): δ 1.82−2.32 (m, 6H), 2.98 (m, 1H), 3.76 (s, 3H), 3.95 (dd, 2H, $J = 5.9$ and $J = 6.7$), 5.36 (br t, 1H), 6.07 (t, 1H, $J = 7.0$), 6.74−6.85 (m, 3H, H-2′, H-4′, and H-6′), 7.14−7.45 (m, 6H, H-5′ and unsubstituted phenyl). IR (cm^{-1}, Nujol): 3308, 1636, 1540. Anal. ($C_{21}H_{23}NO_2$) C, H, N.

***N*-[3,3-Bis-(3-methoxyphenyl)-2-propenyl]acetamide (18g):** Compound **18g** was obtained by reduction of the nitrile **15**,[42] followed by *N*-acylation of the corresponding crude amine with acetic anhydride, according to the general procedure. Purification by flash chromatography (silica gel; EtOAc/cyclohexane 7:3 as eluent) gave a 70% yield of an oil. MS (EI): *m*/*z* 311 (M^+), 252 (100). ^1H NMR ($CDCl_3$): δ 1.99 (s, 3H), 3.77 (s, 3H), 3.80 (s, 3H), 3.95 (m, 2H), 5.54 (br t, 1H), 6.08 (t, 1H, $J = 6.9$), 6.70−6.91 (m, 6H), 7.17 (dd, 1H, $J = 7.8$ and $J = 1.0$), 7.30 (t, 1H, $J = 7.8$). IR (cm^{-1}, neat): 3281, 1651, 1577. Anal. ($C_{19}H_{21}NO_3$) C, H, N.

***N*-(3,3-Diphenyl-propyl)acetamide (19a):** Compound **19a** was prepared by *N*-acetylation of the commercially available 3,3-diphenylpropanamine with Ac_2O/NaOAc in AcOH, as previously described;[36] mp 103 °C (EtOAc/petroleum ether; lit.[36] mp 101−102 °C, from benzene−petroleum ether). ^1H NMR ($CDCl_3$): δ 1.87 (s, 3H), 2.28 (m, 2H), 3.23 (m, 2H), 3.95 (t, 1H, $J = 7.8$), 5.48 (br t, 1H), 7.15−7.34 (m, 10H).

General Procedure for the Synthesis of (±)-*N*-[3-(3-methoxyphenyl)-3-phenylpropyl]alkanamides 19b−d: A solution of the nitrile (*Z*)-**16** or (*E*)-**17** (0.3 g, 1.27 mmol) in THF (7 mL) and acetic, propionic, or butyric anhydride (21 mmol) was hydrogenated over Raney nickel at 4 atm of H_2 for 6 h at 60 °C. The catalyst was filtered on Celite, the filtrate was concentrated in vacuo, and the residue was partitioned between EtOAc and 2 N NaOH. The organic layer was washed twice with 2 N NaOH and once with brine, dried (Na_2SO_4), and evaporated under reduced pressure to give a crude residue, which was purified by flash chromatography (silica gel; EtOAc/cyclohexane 1:1 as eluent).

(±)-*N*-[3-(3-Methoxyphenyl)-3-phenylpropyl]acetamide (19b): Compound **19b** was obtained as an oil, 35% yield. MS (EI): *m*/*z* 283, (M^+), 73 (100). ^1H NMR ($CDCl_3$): δ 1.89 (s, 3H), 2.27 (m, 2H), 3.24 (m, 2H), 3.78 (s, 3H), 3.92 (t, 1H, $J = 7.8$), 5.36 (br t, 1H), 6.71−6.86 (m, 3H), 7.18−7.32 (m, 6H). IR (cm^{-1}, neat): 3296, 1642. Anal. ($C_{18}H_{21}NO_2$) C, H, N.

(±)-*N*-[3-(3-Methoxyphenyl)-3-phenylpropyl]propanamide (19c): Compound **19c** was obtained as an oil, 75% yield. MS (EI): *m*/*z* 297 (M^+), 87 (100). ^1H NMR ($CDCl_3$): δ 1.09 (t, 3H, $J = 7.6$), 2.10 (q, 2H, $J = 7.6$), 2.27 (m, 2H), 3.25 (m, 2H), 3.77 (s, 3H), 3.92 (t, 1H, $J = 7.8$), 5.38 (br t, 1H), 6.69−6.90 (m, 3H), 7.14−7.35 (m, 6H). IR (cm^{-1}, neat): 3293, 1644. Anal. ($C_{19}H_{23}NO_2$) C, H, N.

(±)-*N*-[3-(3-Methoxyphenyl)-3-phenylpropyl)butanamide (19d): Compound **19d** was obtained as an oil, 70% yield. MS (EI): *m*/*z* 311 (M^+), 101 (100). ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 3H, $J = 7.5$), 1.62 (m, 2H), 2.05 (dd, 2H, $J = 7.0$ and $J = 7.8$), 2.29 (m, 2H), 3.25 (m, 2H), 3.77 (s, 3H), 3.92 (t, 1H, $J = 7.8$), 5.38 (br s, 1H), 6.73 (dd, 1H, $J = 2.4$ and $J = 8.0$), 6.79 (t, 1H, $J = 2.1$), 6.85 (d, 1H, $J = 7.8$), 7.14−7.35 (m, 6H). IR (cm^{-1}, neat): 3294, 1643. Anal. ($C_{20}H_{25}NO_2$) C, H, N.

Pharmacology

Binding affinities of compounds **9**−**11**, **13**, **18**, and **19** 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 was already described in detail.[53,54] 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.[55] 2-[^{125}I]Iodomelatonin (100 pM) and different 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). The pK_i values were calculated from the IC_{50} values in accordance with the Cheng−Prusoff equation.[56] The pK_i values are the mean of at least three independent determinations performed in duplicate.

To define the functional activity of the new compounds at MT_1 and MT_2 receptor subtypes, [^{35}S]GTPγS binding assays in NIH3T3 cells expressing human-cloned MT_1 or MT_2 receptors were performed. The amount of bound [^{35}S]GTPγS is proportional to the level of the analogue-induced G-protein activation and is related to the intrinsic activity of the compound under study. The detailed description and validation of this method were reported elsewhere.[53,57] Membranes (15−25 μg of protein, final incubation volume 100 μL) were incubated at 30 °C for 30 min in the presence and in the absence of MLT analogues in an assay buffer consisting of [^{35}S]GTPγS (0.3−0.5 nM), GDP (50 μM), NaCl (100 mM), and $MgCl_2$ (3 mM). Nonspecific binding was defined using [^{35}S]GTPγS (10 μM). In cell lines expressing human MT_1 or MT_2 receptors, MLT 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. Data from [^{35}S]GTPγS binding experiments are given as percentage of basal binding, where the basal binding is the amount of [^{35}S]-GTPγS specifically bound in the absence of compounds and was taken as 100%. The maximal G-protein activation was measured in each experiment by using MLT (100 nM). The relative intrinsic activity (IA_r) values of the new compounds were obtained by dividing the maximum ligand-induced stimulation of [^{35}S]GTPγS binding by that of MLT, as measured in the same experiment. All of the measurements were performed in triplicate. Compounds were added at a concentration equivalent to 100 nM MLT; 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 this concentration the test compound occupies the same number of receptors as 100 nM MLT. Concentrations 10 times smaller and 10 times higher were also tested to verify that a plateau in [^{35}S]GTPγS binding had been reached.

Molecular Modeling

Docking and Molecular Dynamics (MD) Simulations. Docking studies were performed with Glide,[50] employing our previously developed MT_2 receptor model.[32] Ligand geometries were optimized using the MMFF94s force field,[58] as implemented in Macromodel.[59] Docking experiments were performed starting from the minimum energy conformations of the ligands placed in arbitrary positions, within a region centered on amino acids His208, Trp264, and Tyr183, using enclosing and bounding boxes of 46 and 14 Å on each side, respectively. Van der Waals radii of protein and ligand atoms were not scaled. The amide bond of the ligand was maintained fixed in *anti* disposition. Docking solutions were ranked according to their Emodel value.

The best scoring solution of **18g** was merged into the MT_2 receptor model, and the complex was submitted to geometry optimization, with MMFF94s force field to an energy gradient of 0.01 kJ/(mol·Å), with fixed protein backbone. A MD simulation was performed on the complex, with MMFF94[60] force field, 1 fs time step, for 1 ns after 100 ps of equilibration, with fixed protein backbone.

3D-QSAR

3D-QSAR analysis was performed with the CoMFA[61] module of Sybyl.[62] A former 3D-QSAR model built from a set of 34 MT_2 antagonists (model 7 in Table 4 of ref 31) was rebuilt, changing ligand conformations and orientations, to get an alignment consistent with docking into the previously described receptor model.[32] Thus, the side chain of acylaminoethyl-indole antagonists (e.g., luzindole), adopted a $\tau 1$ (C3a−C3−Cβ−Cα) $\approx$ 270° (instead of the previous ≈90°), with unmodified $\tau 2$ (C3−Cβ−Cα−N) $\approx$ 180° and $\tau 3$ (Cβ−Cα−N−C) $\approx$ 180°. The new CoMFA models were calculated within the same grid region and applying the parameters previously described. Leave-one-out cross-validation was applied to PLS analysis[63] to select the model with the number of latent variables giving the first maximum of Q^2 value, calculated with the SAMPLS algorithm.[64] Standard deviation of error in prediction (SDEP)[65] was calculated as $SDEP = [\sum(y - y_{PRED})^2/N]^{1/2}$.

The newly synthesized compounds were aligned on the reference 4P-PDOT (**3**) by means of a rigid fit procedure, superposing the four atoms of the amide group and the two centroids of the phenyl rings. The minimum energy conformation giving the best fit in terms of root-mean-square distances (rmsd) was selected for binding affinity prediction. Although for chiral compounds only affinity data for racemic mixtures were available, the *R*-isomers of compounds **9** and the *S*-isomers of **19b**−**d** were chosen, as they gave better superpositions. Geometry optimization was achieved applying the Tripos force field[66] with the Powell method[67] to an energy gradient of 0.01 kcal/mol·Å, without the electrostatic contribution.

Acknowledgment. This work was supported by the Italian M.I.U.R. (Ministero dell'Istruzione, dell'Università e della Ricerca). The C.I.M. (Centro Interdipartimentale Misure) and C.C.E. (Centro di Calcolo Elettronico) of the University of Parma are gratefully acknowledged for providing the Sybyl software license.

Supporting Information Available: Elemental analyses for compounds **9a**−**c**, **10a**,**b**, **11**, **13**, **18a**−**g**, and **19b**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Barrenetxe, J.; Delagrange, P.; Martinez, J. A. Physiological and metabolic functions of melatonin. *J. Physiol. Biochem.* **2004**, *60*, 61−72.
(2) Pevet, P.; Bothorel, B.; Slotten, H.; Saboureau, M. The chronobiotic properties of melatonin. *Cell Tissue Res.* **2002**, *309*, 183−191.

(3) Turek, F. W.; Gillette, M. U. Melatonin, sleep, and circadian rhythms: rationale for development of specific melatonin agonists. *Sleep Med.* **2004**, *5*, 523−532.

(4) Zemlan, F. P.; Mulchahey, J. J.; Scharf, M. B.; Mayleben, D. W.; Rosenberg, R.; Lankford, A. The efficacy and safety of the melatonin agonist β-methyl-6-chloromelatonin in primary insomnia: A randomized, placebo-controlled, crossover clinical trial. *J. Clin. Psychiatry* **2005**, *66*, 384−390.

(5) Fitzgerald, L. R.; Reed, J. E. Melatonin agonists for the treatment of sleep disorders and major depression. *Ann. Rep. Med. Chem.* **2004**, *39*, 25−37.

(6) Chilman-Blair, K.; Castañer, J.; Bayes, M.; Silvestre, J. S.; Bayés, M. TAK-375: Treatment of insomnia, treatment of circadian rhythm disorders Melatonin MT1/MT2. agonist. *Drug Future* **2003**, *28*, 950−958.

(7) Genovese, T.; Mazzon, E.; Muia, C.; Bramanti, P.; De Sarro, A.; Cuzzocrea, S. Attenuation in the evolution of experimental spinal cord trauma by treatment with melatonin. *J. Pineal Res.* **2005**, *38*, 198−208.

(8) Peres, M. F. P. Melatonin, the pineal gland and their implications for headache disorders. *Cephalalgia* **2005**, *25*, 403−411.

(9) Iuvone, P. M.; Tosini, G.; Pozdeyev, N.; Haque, R.; Klein, D. C.; Chaurasia, S. S. Circadian clocks, clock networks, arylalkylamine *N*-acetyltransferase, and melatonin in the retina. *Prog. Retinal Eye Res.* **2005**, *24*, 433−456.

(10) Sewerynek, E. Melatonin and the cardiovascular system. *Neuroendocrinol. Lett.* **2002**, *23* (S.1), 79−83.

(11) (a) Blask, D. E.; Sauer, L. A.; Dauchy, R. T. Melatonin as a chronobiotic/anticancer agent: cellular, biochemical, and molecular mechanisms of action and their implications for circadian-based cancer therapy. *Curr. Top. Med. Chem.* **2002**, *2*, 113−132. (b) Sauer, L. A.; Dauchy, R. T.; Blask, D. E. Melatonin inhibits fatty acid transport in inguinal fat pads of hepatoma 7288CTC-bearing and normal Buffalo rats via receptor-mediated signal transduction. *Life Sci.* **2001**, *68*, 2835−2844. (c) Collins, A.; Yuan, L.; Kiefer, T. L.; Cheng, Q.; Lai, L.; Hill, S. M. Overexpression of the MT_1 melatonin receptor in MCF-7 human breast cancer cells inhibits mammary tumor formation in nude mice. *Cancer Lett.* **2003**, *189*, 49−57.

(12) Sofic, E.; Rimpapa, Z.; Kundurovic, Z.; Sapcanin, A.; Tahirovic, I.; Rustembegovic, A.; Cao, G. Antioxidant capacity of the neurohormone melatonin. *J. Neural Transm.* **2005**, *112*, 349−358.

(13) Macleod, M. R.; O'Collins, T.; Horky, L. L.; Howells, D. W.; Donnan, G. A. Systematic review and meta-analysis of the efficacy of melatonin in experimental stroke. *J. Pineal Res.* **2005**, *38*, 35−41.

(14) Reppert, S. M.; Weaver, D. R.; Godson, C. Melatonin receptors step into the light: Cloning and classification of subtypes. *Trends Pharmacol. Sci.* **1996**, *17*, 100−102.

(15) Dubocovich, M. L.; Cardinali, D. P.; Delagrange, P.; Krause, D. N.; Strosberg, A. D.; Sugden, D.; Yocca, F. D. In *The IUPHAR compendium of receptor characterization and classification*, 2nd ed.; Girdlestone, D., Ed.; IUPHAR Media: London, 2000; pp 271−277.

(16) Von Gall, C.; Stehle, J. H.; Weaver, D. R. Mammalian melatonin receptors: Molecular biology and signal transduction. *Cell Tissue Res.* **2002**, *309*, 151−162.

(17) Nosjean, O.; Ferro, M.; Cogé, F.; Beauverger, P.; Henlin, J.-M.; Lefoulon, F.; Fauchère, J.-L.; Delagrange, P.; Canet, E.; Boutin, J. A. Identification of the melatonin-binding site MT_3 as the quinone reductase 2. *J. Biol. Chem.* **2000**, *275*, 31311−31317.

(18) Li, P.-K.; Witt-Enderby, P. A. Melatonin receptors as potential targets for drug discovery. *Drug Future* **2000**, *25*, 945−957.

(19) Dubocovich, M. L.; Yun, K.; Al-Ghoul, W. M.; Benloucif, S.; Masana, M. I. Selective MT_2 melatonin receptor antagonists block melatonin-mediated phase advances of circadian rhythms. *FASEB J.* **1998**, *12*, 1211−1220.

(20) Liu, C.; Weaver, D. R.; Jin, X.; Shearman, L. P.; Pieschl, R. L.; Gribkoff, V. K.; Reppert, S. M. Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. *Neuron* **1997**, *19*, 91−102.

(21) Doolen, S.; Krause, D. N.; Dubocovich, M. L.; Duckles, S. P. Melatonin mediates two distinct responses in vascular smooth muscle. *Eur. J. Pharmacol.* **1998**, *345*, 67−69.

(22) Hunt, A. E.; Al-Ghoul, W. M.; Gillette, M. U.; Dubocovich, M. L. Activation of MT(2) melatonin receptors in rat suprachiasmatic nucleus phase advances the circadian clock. *Am. J. Physiol.* **2001**, *280*, C110−C118.

(23) Sumaya, I. C.; Masana, M. I.; Dubocovich, M. L. The antidepressant-like effect of the melatonin receptor ligand luzindole in mice during force swimming requires expression of MT_2 but not MT_1 melatonin receptors. *J. Pineal Res.* **2005**, *39*, 170−177.

(24) Mor, M.; Plazzi, P. V.; Spadoni, G.; Tarzia, G. Melatonin. *Curr. Med. Chem.* **1999**, *6*, 501−518.

(25) Mor, M.; Rivara, S.; Lodola, A.; Lorenzi, S.; Bordi, F.; Plazzi, P. V.; Spadoni, G.; Bedini, A.; Duranti, A.; Tontini, A.; Tarzia, G. Application of 3D-QSAR in the rational design of receptor ligands and enzyme inhibitors. *Chem. Biodiversity* **2005**, *2*, 1438−1451.

(26) Zlotos, D. P. Recent advances in melatonin receptor ligands. *Arch. Pharm. Chem. Life Sci.* **2005**, *338*, 229−247.

(27) (a) Sun, L.-Q.; Chen, J.; Bruce, M.; Deskus, J. A.; Epperson, J. R.; Takaki, K.; Johnson, G.; Iben, L.; Mahle, C. D.; Ryan, E.; Xu, C. Synthesis and structure−activity relationship of novel benzoxazole derivatives as melatonin receptor agonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3799−3802. (b) Audinot, V.; Mailliet, F.; Lahaye-Brasseur, C.; Bonnaud, A.; Le Gall, A.; Amossé, C.; Dromaint, S.; Rodriguez, M.; Nagel, N.; Galizzi, J.-P.; Malpaux, B.; Guillaumet, G.; Lesieur, D.; Lefoulon, F.; Renard, P.; Delagrange, P.; Boutin, J. A. New selective ligands of human cloned melatonin MT_1 and MT_2 receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2003**, *367*, 553−561. (c) Descamps-François, C.; Yous, S.; Chavatte, P.; Audinot, V.; Bonnaud, A.; Boutin, J. A.; Delagrange, P.; Bennejan, C.; Renard, P.; Lesieur, D. Design and synthesis of naphthalenic dimers as selective MT_1 melatoninergic ligands. *J. Med. Chem.* **2003**, *46*, 1127−1129.

(28) (a) Leclerc, V.; Yous, S.; Delagrange, P.; Boutin, J. A.; Renard, P.; Lesieur, D. Synthesis of nitroindole derivatives with high affinity and selectivity for melatoninergic binding sites MT_3. *J. Med. Chem.* **2002**, *45*, 1853−1859. (b) Boussard, M.-F.; Truche, S.; Rousseau-Rojas, A.; Briss, S.; Descamps, S.; Droual, M.; Wierzbicki, M.; Ferry, G.; Audinot, V.; Delagrange, P.; Boutin, J. A. New ligands at the melatonin binding site MT_3. *Eur. J. Med. Chem.* **2006**, *41*, 306−320.

(29) Mor, M.; Spadoni, G.; Di Giacomo, B.; Diamantini, G.; Bedini, A.; Tarzia, G.; Plazzi, P. V.; Rivara, S.; Nonno, R.; Lucini, V.; Pannacci, M.; Fraschini, F.; Stankov, B. M. Synthesis, pharmacological characterization and QSAR studies on 2-substituted indole melatonin receptor ligands. *Bioorg. Med. Chem.* **2001**, *9*, 1045−1057.

(30) Spadoni, G.; Balsamini, C.; Diamantini, G.; Tontini, A.; Tarzia, G.; Mor, M.; Rivara, S.; Plazzi, P. V.; Nonno, R.; Lucini, V.; Pannacci. M.; Fraschini, F.; Stankov, B. M. 2-*N*-Acylaminoalkylindoles: Design and quantitative structure−activity relationship studies leading to MT_2-selective melatonin antagonists. *J. Med. Chem.* **2001**, *44*, 2900−2912.

(31) Rivara, S.; Mor, M; Silva, C.; Zuliani, V.; Vacondio, F.; Spadoni, G.; Bedini, A.; Tarzia, G.; Lucini, V.; Pannacci, M.; Fraschini, F.; Plazzi, P. V. Three-dimensional quantitative structure-activity relationship studies on selected MT_1 and MT_2 melatonin receptor ligands: Requirements for subtype selectivity and intrinsic activity modulation. *J. Med. Chem.* **2003**, *46*, 1429−1439.

(32) Rivara, S.; Lorenzi, S.; Mor, M.; Plazzi, P. V.; Spadoni, G.; Bedini, A.; Tarzia, G. Analysis of structure−activity relationships for MT_2 selective antagonists by melatonin MT_1 and MT_2 receptor models. *J. Med. Chem.* **2005**, *48*, 4049−4060.

(33) Lucini, V.; Pannacci, M.; Scaglione, F.; Fraschini, F., Rivara, S.; Mor, M.; Bordi, F.; Plazzi, P. V.; Spadoni, G.; Bedini, A.; Piersanti, G.; Diamantini, G.; Tarzia, G. Tricyclic alkylamides as melatonin receptor ligands with antagonist or inverse agonist activity. *J. Med. Chem.* **2004**, *47*, 4202−4212.

(34) Dubocovich, M. L.; Masana, M. I.; Iacob, S.; Sauri, D. M. 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.* **1997**, *355*, 365−375.

(35) Wermuth C. G. In *The Practice of Medicinal Chemistry II*; Wermuth C. G., Ed.; Elsevier Academic Press: London, 2003; p 215.

(36) Blank, B.; Zuccarello, W. A.; Cohen, S. R.; Frishmuth, G. J.; Scaricaciottoli, D. Synthesis and adrenocortical inhibiting activity of substituted diphenylalkylamines. *J. Med. Chem.* **1969**, *12*, 271−276.

(37) Zupancic, B.; Kokalj, M. Aromatic α,β-unsaturated nitriles via polyethylene glycol-catalyzed two-phase aldol-type condensation. *Synthesis* **1981**, 913−915.

(38) Schlegel, D. C.; Zenitz, B. L.; Fellows, C. A.; Laskowski, S. C.; Behn, D. C.; Phillips, D. K.; Botton, I.; Speight, P. T. Bulky amine analogues of ketoprofen: Potent anti-inflammatory agents. *J. Med. Chem.* **1984**, *27*, 1682−1690.

(39) Widler, L.; Green, J.; Missbach, M.; Susa, M.; Altmann, E. 7-Alkyl- and 7-cycloalkyl-5-aryl-pyrrolo[2,3-*d*]pyrimidines, potent inhibitors of the tyrosine kinase c-Src. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 849−852.

(40) Wittig, G.; Schollkopf, U. Triphenylphosphinemethylene as an olefin-forming reagent. I. *Chem. Ber.* **1954**, *97*, 1318−1330.

(41) van der Bent, A.; Blommaert, A. G. S.; Melman, C. T. M.; IJzerman, A. P.; van Wijngaarden, I.; Soudijn, W. Hybrid cholecystokinin-A antagonists based on molecular modeling of lorglumide and L-364,-718. *J. Med. Chem.* **1992**, *35*, 1042−1049.

(42) Groundwater, P. W.; Sharp, J. T. Electrocyclic aromatic substitution by nitrile ylides to give 3*H*-2-benzoazepines: Substituent effects and mechanism. *Tetrahedron* **1992**, *48*, 7951−7964.
(43) Happer, D. A. R.; Steenson, B. E. Side-chain ^{13}C nuclear magnetic resonance shifts in ring-substituted styrenes. The effect of β-substituents on β-carbon shifts. *J. Chem. Soc., Perkin Trans. 2* **1988**, 19−24.
(44) Depreux, P.; Lesieur, D.; Mansour, H. A.; Morgan, P.; Howell, H. E.; Renard, P.; Caignard, D.-H.; Pfeiffer, B.; Delagrange, P.; Guardiola, B.; Yous, S.; Demarque, A.; Adam, G.; Andrieux, J. Synthesis and structure−activity relationships of novel naphthalenic and bioisosteric-related amidic derivatives as melatonin receptor ligands. *J. Med. Chem.* **1994**, *37*, 3231−3239.
(45) Garratt, P. J.; Jones, R.; Tocher, D. A.; Sugden, D. Mapping the melatonin receptor. 3. Design and synthesis of melatonin agonists and antagonists derived from 2-phenyltryptamines. *J. Med. Chem.* **1995**, *38*, 1132−1139.
(46) Conway, S.; Canning, S. J.; Howell, H. E.; Mowat, E. S.; Barrett, P.; Drew, J. E.; Delagrange, P.; Lesieur, D.; Morgan, P. J. Characterization of human melatonin MT(1) and MT(2) receptors by CRE-luciferase reporter assay. *Eur. J. Pharmacol.* **2000**, *390*, 15−24.
(47) Garratt, P. J.; Travard, S.; Vonhoff, S.; Tsotinis, A.; Sugden, D. Mapping the melatonin receptor. 4. Comparison of the binding affinities of a series of substituted phenylalkyl amides. *J. Med. Chem.* **1996**, *39*, 1797−1805.
(48) Pégurier, C.; Curtet, S.; Nicolas, J.-P.; Boutin, J. A.; Delagrange, P.; Renard, P.; Langlois, M. Synthesis of a small library of phenylalkylamido derivatives as melatoninergic ligands for human MT_1 and MT_2 receptors. *Bioorg. Med. Chem.* **2000**, *8*, 163−171.
(49) Pickering, H.; Sword, S.; Vonhoff, S.; Jones, R.; Sugden, D. Analogues of diverse structure are unable to differentiate native melatonin receptors in the chicken retina, sheep pars tuberalis and Xenopus melanophores. *Br. J. Pharmacol.* **1996**, *119*, 379−387.
(50) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **2004**, *47*, 1739−1749.
(51) Wallez, V.; Durieux-Poissonnier, S.; Chavatte, P.; Boutin, J. A.; Audinot, V.; Nicolas, J.-P.; Bennejean, C.; Delagrange, P.; Renard, P.; Lesieur, D. Synthesis and structure−affinity−activity relationships of novel benzofuran derivatives as MT_2 melatonin receptor selective ligands. *J. Med. Chem.* **2002**, *45*, 2788−2800.
(52) Stott, K.; Stonehouse, J.; Keeler, J.; Hwang, T. L.; Shaka, A. J. Excitation sculpting in high-resolution nuclear magnetic resonance spectroscopy: Application to selective NOE experiments. *J. Am. Chem. Soc.* **1995**, *117*, 4199−4200.
(53) Nonno, R.; Lucini, V.; Pannacci, M.; Mazzucchelli, C.; Angeloni, D.; Fraschini, F.; Stankov, B. M. Pharmacological characterization of the human melatonin Mel1a receptor following stable transfection into NIH3T3 cells. *Br. J. Pharmacol.* **1998**, *124*, 485−492.
(54) Nonno, R.; Pannacci, M.; Lucini, V.; Angeloni, D.; Fraschini, F.; Stankov, B. M. Ligand efficacy and potency at recombinant human MT_2 melatonin receptors: Evidence for agonist activity of some MT_1-antagonists. *Br. J. Pharmacol.* **1999**, *127*, 1288−1294.
(55) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(56) Cheng, Y. C.; Prusoff, W. H. Relation between the inhibition constant (K_i) and the concentration of inhibitor which causes fifty percent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(57) Spadoni, G.; Balsamini, C.; Bedini, A.; Diamantini, G.; Di Giacomo, B.; Tontini, A.; Tarzia, G.; Mor, M.; Plazzi, P. V.; Rivara, S.; Nonno, R.; Panacci, M.; Lucini, V.; Fraschini, F.; Stankov, B. M. 2-[*N*-Acylamino(C1−C3.)alkyl]indoles as MT1 melatonin receptor partial agonists, antagonists, and putative inverse agonists. *J. Med. Chem.* **1998**, *41*, 3624−3634.
(58) Halgren, T. A. MMFF VI. MMFF94s option for energy minimization studies. *J. Comput. Chem.* **1999**, *20*, 720−729.
(59) *Macromodel 8.5*; Schrödinger, L.L.C.: New York.
(60) Halgren, T. A. Merck molecular force field. I. Basis, form, scope, parameterization and performance of MMFF94. *J. Comput. Chem.* **1996**, *17*, 490−519.
(61) Cramer, R. D., III; Patterson, D. E.; Bunce, J. D. Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. *J. Am. Chem. Soc.* **1988**, *110*, 5959−5967.
(62) *Sybyl 7.1*; Tripos, Inc.: St. Louis, MO.
(63) Wold, S.; Ruhe, A.; Wold, H.; Dunn, W. J. The covariance problem in linear regression. The partial least squares (PLS) approach to generalized inverses. *SIAM J. Sci. Stat. Comput.* **1984**, *5*, 735−743.
(64) Bush, B. L.; Nachbar, R. B. Sample-distance partial least squares: PLS optimized for many variables, with application to CoMFA. *J. Comput.-Aided Mol. Des.* **1993**, *7*, 587−619.
(65) Cruciani, G.; Clementi, S.; Baroni, M. Variable selection in PLS analysis. In *3D QSAR in Drug Design. Theory Methods and Applications*; Kubinyi, H., Ed.; ESCOM: Leiden, 1993; p 552.
(66) Clark, M.; Cramer, R. D., III; Van Opdenbosch, N. Validation of the general purpose Tripos 5.2 force field. *J. Comput. Chem.* **1989**, *10*, 982−1012.
(67) Powell, M. J. D. Restart procedures for the conjugate gradient method. *Math. Prog.* **1977**, *12*, 241−254.

JM060850A