N-(Substituted-anilinoethyl)amides: Design, Synthesis, and Pharmacological Characterization of a New Class of Melatonin Receptor Ligands

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A novel series of melatonin receptor ligands, characterized by a *N*-(substituted-anilinoethyl)amido scaffold, along with preliminary structure–activity relationships (SARs), is presented. MT_1 and MT_2 receptor binding affinity and intrinsic activity have been modulated by the introduction of different substituents on the aniline nitrogen, on the benzene ring, and on the amide side chain. Modulation of intrinsic activity and MT_2 selectivity of the newly synthesized compounds has been achieved by applying SAR models previously developed, providing compounds with different binding and intrinsic activity profiles. Compound **3d**, with a bulky ß-naphthyl group, behaves as an MT_2-selective antagonist with sub-nM affinity. Size reduction of the substituent enhances intrinsic activity, as in the nonselective *N*-methyl-anilino agonist **3i**. The phenyl derivative **3g** is an MT_2-selective partial agonist, with MT_2 binding affinity higher than melatonin, showing promising sleep-promoting and antianxiety properties in animal models.

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, MLT[a], Figure 1) is a tryptophan-derived hormone characterized by a circadian rhythm of secretion, with peak levels occurring during the period of darkness. It is mainly synthesized by the pineal gland, although other cells and organs, such as retina, gut, testes, bone marrow cells, and human lymphocytes, produce minor amounts of MLT, which may be of some local importance. Besides the well-known chronobiotic and sleep-inducing properties,[1] many other physiological and behavioral effects have been ascribed to MLT, such as the modulation of cardiovascular[2] and immune systems[3] and the influence on metabolism, bone formation,[4] and hormone secretion.[5] For this reason, the potential benefits of MLT administration have been evaluated in a variety of pathological conditions, such as sleep disturbances,[6] cancer,[7] neurodegenerative diseases,[8,9] headache disorders,[10] and stroke.[11]

MLT exerts its effects by multiple mechanisms. It acts as an antioxidant and radical scavenger and it regulates the activity of a series of enzymes affecting the redox state of the cell.[12] In mammals, MLT activates two G-protein-coupled receptors, named MT_1 and MT_2,[13,14] at nanomolar concentrations, and it binds with lower affinity to the so-called *MT3* binding site, recently characterized as a melatonin-sensitive form of the human enzyme quinone reductase 2.[15] Moreover, calmodulin has also been identified as an intracellular binding site for MLT.[16] MT_1 and MT_2 receptors are widely distributed in different areas of the central nervous system, as well as in peripheral organs.[17] The limited availability of subtype-selective ligands has hampered an exhaustive elucidation of their patho/physiological role. Indeed, the MT_1 receptor produces vasoconstriction, inhibits prolactin secretion from the hypophyseal pars tuberalis, and reduces neuronal firing of the hypothalamic suprachiasmatic nucleus, suggesting a role in the sleep-promoting effect of MLT; the MT_2 receptor induces vasodilation and produces a phase shift in circadian rhythms, probably sustaining the resynchronizing activity of MLT.[18] Irreversible melatonin receptor ligands, such as *N*-[2-(2-bromoacetyl-7-methoxynaphthyl)ethyl]propionamide (BMNEP) that permanently activates the MT_2 receptor, may help the elucidation of MLT receptor functions.[19]

During the past decades, many series of melatonin receptor ligands have been reported in the literature and in patents. Compounds with agonist activity have been mainly evaluated for their sleep inducing properties or for circadian phase shift action. Ramelteon is at present the only melatonin receptor agonist marketed for the treatment of insomnia[20] and other compounds,[21] such as LY-156735[22] or VEC-162,[23] have been tested in clinical trials for their hypnotic properties. Agomelatine, an MT_1/MT_2 agonist and $5HT_{2c}$ antagonist is under evaluation for the treatment of major depressive disorders.[24] Examples of subtype-selective agonists are still limited.[25] Evaluation of melatonin receptor antagonists has been limited to preclinical studies, for example, S22153 in circadian rhythm entrainment experiments,[26] luzindole for its antidepressant-like properties,[27] or ML-23 in the treatment and management of Parkinson's disease.[28] While only few examples of MT_1-selective antagonists are reported, several series of MT_2-selective antagonists have been discovered.[25,29] The main classes of MT_2-selective antagonists are represented in Figure 1; they comprise benzofuran[30]

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[a] Abbreviations: MLT, melatonin; MT_1, melatonin receptor subtype 1; MT_2, melatonin receptor subtype 2; MT_3, third binding site of melatonin; 4P-PDOT, 4-phenyl-propionamidotetralin; [^{35}S]GTPγS, [^{35}S]guanosine-5′-*O*-(3-thio-triphosphate); IA_r, relative intrinsic activity.

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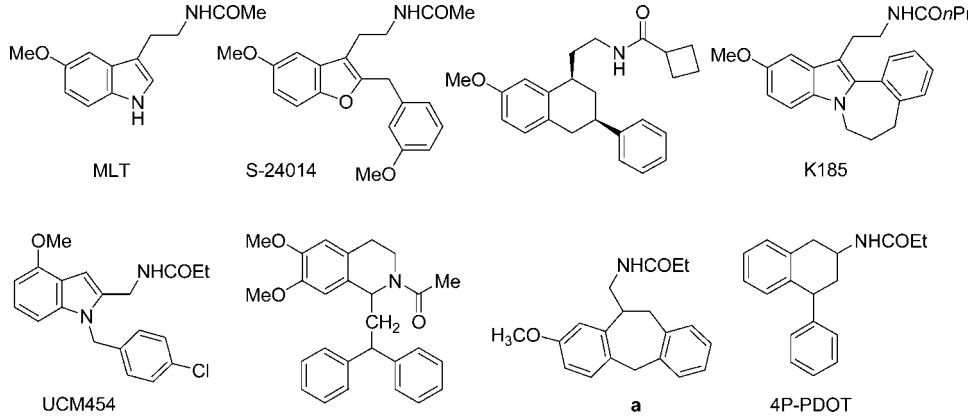


Figure 1. Melatonin and representative MT_2 selective antagonists.

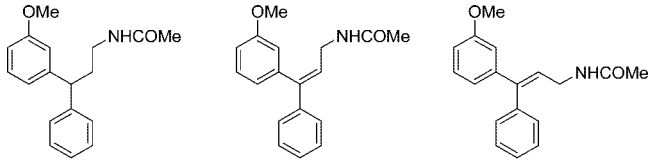
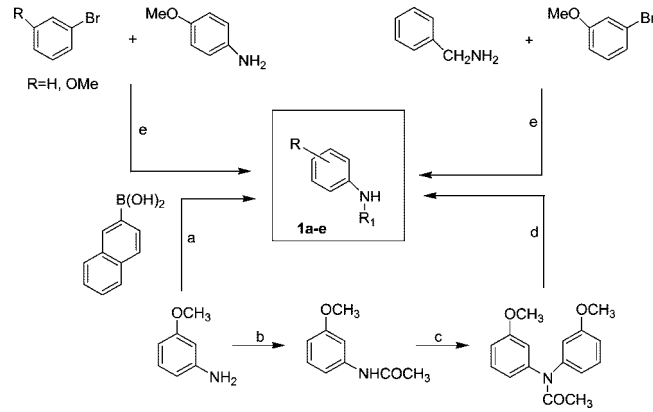


Figure 2. *N*-(3,3-Diphenylpropyl)acetamide and *N*-(3,3-diphenylpropenyl)acetamide derivatives; see ref 38.

and tetrahydronaphthalenic[31] derivatives, 6,7-dihydro-5*H*-benzo[*c*]azepino[2,1-*a*]indoles,[32] 2-*N*-acylaminoalkylindoles,[33] and tetrahydroisoquinoline derivatives.[34]

Our research in the field of melatonin receptor ligands is dedicated to the study of their structure–activity relationships and to the synthesis of new agonists and antagonists endowed with selectivity for each of the two receptor subtypes. Molecular modeling on representative ligands has led us to hypothesize that a bulky substituent in an area corresponding to positions 1–2 of the indole nucleus of MLT, and "out-of-plane" from the indole ring, relates to selectivity for the MT_2 receptor and reduces intrinsic activity at melatonin receptors.[33] The importance of this structural element has been confirmed by 3D-QSAR[35] and by docking within three-dimensional models of the MT_2 and MT_1 receptors; the putative receptor binding sites have a lipophilic pocket available, where the lipophilic "out-of-plane" substituents of MT_2 antagonists can be accommodated.[36] This information has been applied to the design of a novel series of tricyclic dihydrodibenzocycloheptene antagonists, characterized by a skewed scaffold, which fulfills the requirements for MT_2-selective antagonism (compound **a** in Figure 1).[37] An open chain analog approach has been subsequently applied to the rigid structures of compound **a** and of the tetralin antagonist 4-phenyl-propionamidotetralin (4P-PDOT, Figure 1), maintaining the pharmacophoric elements for MT_2 binding. This structural simplification has led to the new classes of *N*-(3,3-diphenylpropyl)alkanamides and *N*-(3,3-diphenylpropenyl)alkanamides (Figure 2), some of which have MT_2 binding affinity higher than MLT and a remarkable selectivity for the MT_2 receptor.[38] The two aromatic rings of these classes are linked to an alkyl or alkenyl portion, resulting in different enantiomers or *E*/*Z* diastereoisomers, respectively. The *N*-(substituted-anilinoethyl)amides obtained by replacing the benzhydryl carbon with a nitrogen atom fulfill the structural requirements for MLT receptor binding, while avoiding isomerism and likely leading to a desirable improvement in water solubility. We report here the synthesis, receptor binding characterization, and preliminary structure–activity relationships for a series of these compounds.

Scheme 1[a]



[a] Reagents and conditions: (a) $Cu(OAc)_2$, CH_2Cl_2, pyridine, room temperature; (b) Ac_2O, AcOH, room temperature; (c) 3-bromoanisole, CuI, K_2CO_3, 220 °C; (d) KOH, EtOH, reflux; (e) CuI, K_2CO_3, L-proline, DMSO, 90 °C.

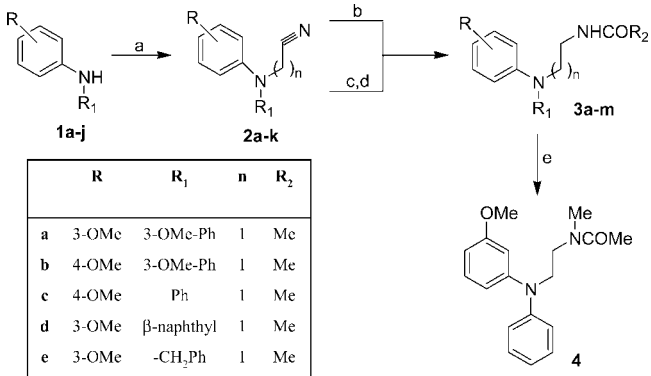
Starting from this scaffold, the acylating group has been slightly modulated, a methoxy or a bromine substituent has been introduced on the phenyl ring to mimic the 5-methoxy group of MLT, the effect of lengthening of the ethyl chain was examined, and the anilinoethyl moiety has been replaced by a phenoxyethyl group.

Moreover, different substituents have been added at the aniline nitrogen to try to reproduce the cited SARs for the out-of-plane group of MT_2-selective antagonists. The synthesized compounds have been tested for their binding affinity for MT_1 and MT_2 receptors and for their intrinsic activity. A representative compound has been preliminarily evaluated in animal models to investigate potential sleep-inducing properties.

Chemistry

The (anilinoalkyl)amido derivatives (**3a**−**m**) are prepared by *N*-cyanoalkylation of anilines (**1a**−**j**) with bromoacetonitrile (chloroacetonitrile for **2j**) or 3-bromoproprionitrile (for **2k**) in the presence of sodium hydride (for **2a**−**d** and **2f**−**h**) or in the absence of a base (for **2e**, **2i**−**k**), followed by reduction of the intermediate nitriles (**2a**−**k**) and *N*-acylation of the crude diamines with anhydrides or acid chlorides (Scheme 2). The cyano group of nitriles **2a**−**k** is easily reduced using standard procedures. Briefly, Raney nickel hydrogenation of nitriles **2a**−**d**, **2f**−**i**, or **2k** and concomitant *N*-acylation of the corresponding crude anilinoalkylamine with acetic or *n*-butyric

Scheme 2[a]



	R	R_1	n	R_2
a	3-OMe	3-OMe-Ph	1	Me
b	4-OMe	3-OMe-Ph	1	Me
c	4-OMe	Ph	1	Me
d	3-OMe	β-naphthyl	1	Me
e	3-OMe	$-CH_2Ph$	1	Me
f	H	Ph	1	Me
g	3-OMe	Ph	1	Me
h	3-Br	Ph	1	Me
i	3-OMe	Me	1	Me
j	3-OMe	H	1	Me
k	3-OMe	Me	2	Me
l	3-OMe	Ph	1	*n*-Pr
m	3-OMe	Ph	1	*c*-Bu

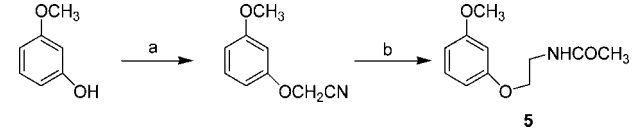
[a] Reagents and conditions: (a) $BrCH_2CN$ ($ClCH_2CN$) or $BrCH_2CH_2CN$, DMF, NaH, 100 °C (NaH is not necessary for **e**, **i**−**k**); (b) H_2, Ni/Raney, $(R_2CO)_2O$, THF, or H_2, Ni/Raney, NH_3/EtOH, then Ac_2O/TEA for **3j** or *c*-butanoyl chloride/TEA for **3m**; (c) $LiAlH_4$, THF for **3e**; (d) Ac_2O, TEA, THF; (e) MeI, NaH, DMF.

anhydride provides the desired melatonin ligands **3a**−**d**, **3f**−**i**, and **3k**,**l**. The cyclobutanecarboxamido derivative **3m** and the monosubstituted aniline derivative **3j** are prepared by hydrogenation of the nitriles **2g** and **2j** over Raney nickel in the presence of 1 M NH_3−EtOH, followed by *N*-acylation with *c*-butanoyl chloride or acetic anhydride, respectively, in the presence of triethylamine (TEA). To prepare the *N*-benzyl derivative **3e**, the corresponding nitrile **2e** is reduced with lithium aluminum hydride, and the resulting crude amine was *N*-acylated with acetic anhydride. Compound **4** is prepared by *N*-alkylation of **3g** with methyl iodide in the presence of NaH as a base.

The key starting anilines are commercially available (**1f**−**j**) or have been synthesized (**1a**−**e**, Scheme 1) using known procedures. Briefly, 3-methoxy-*N*-(3-methoxyphenyl)benzenamine **1a** is synthesized by reacting 3-bromoanisole with 3-methoxyacetanilide and subsequent alkaline hydrolysis of the intermediate *N*,*N*-bis(3-methoxyphenyl)acetamide.[39] The *N*-substituted-anilines **1b**, **c**, and **e** are prepared in good yield by CuI-catalyzed coupling reaction of the suitable amine with bromobenzene or 3-bromoanisole, in the presence of L-proline.[40] Alternatively, the new compound (3-methoxy)-*N*-(naphthalen-2-yl)aniline **1d** is obtained by coupling *m*-anisidine and 2-naphthaleneboronic acid in the presence of cupric acetate and pyridine, according to a previously reported procedure,[41] Scheme 1.

(3-Methoxyphenoxy)acetonitrile[42] is obtained by alkylation of 3-hydroxyanisole with chloroacetonitrile and then converted to the target compound **5** by hydrogenation over Raney nickel of the cyano group and concomitant *N*-acetylation with acetic anhydride, as depicted in Scheme 3.

Scheme 3[a]



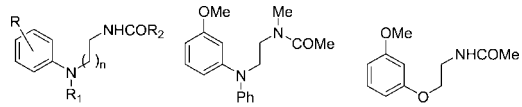
[a] Reagents and conditions: (a) $ClCH_2CN$, K_2CO_3, CH_3CN, reflux; (b) H_2, Ni/Raney, Ac_2O, THF, 60 °C.

Results and Discussion

N-(Substituted-anilinoethyl)amides have some advantages relative to the previously reported *N*-(3,3-diphenylpropyl)alkanamides, that is, a shorter and higher yielding reaction pathway, the absence of optical or geometrical isomers, a water solubility sufficient to perform the pharmacological tests on the free bases, and the possibility to prepare water-soluble salts, if necessary. The synthetic procedure allows insertion on the aniline nitrogen of groups of different size and shape and, in general, modification of the scaffold according to classical medicinal chemistry procedures.

The preliminary binding data for the *N*-phenyl-*N*-(3-methoxyphenyl) derivative **3g**, which showed partial agonist activity with good MT_2 selectivity, supported the bioisosteric role of nitrogen, leading us to further explore the SARs of this new class of compounds. Groups with different size and shape were thus inserted on the aniline nitrogen, and other more classical changes were also performed, such as the substitution of the methoxy with a bromine atom and the use of different acylating groups. Methylation of the amide nitrogen and lengthening of the ethyl spacer were also evaluated. The MT_1 and MT_2 binding affinity of the newly synthesized compounds and their effect on GTPγS binding (intrinsic activity) are reported in Table 1. Compound **3f**, having two unsubstituted phenyl rings, displays moderate receptor affinity, with some selectivity for the MT_2 receptor; it behaves as an MT_2 partial agonist, while it is an antagonist at the MT_1 receptor. This profile is similar to those observed for *N*-(3,3-diphenylpropyl)acetamide and *N*-(3,3-diphenylpropenyl)acetamide derivatives,[38] being closer to that of the unsaturated compounds and with higher intrinsic activity at the MT_2 receptor. The introduction of a methoxy group in the *meta*-position, superposable to the 5-methoxy group of MLT (Figure 3, left panel), induces a remarkable increase in binding affinity at both receptor subtypes, while maintaining MT_2 selectivity. Compound **3g** has one of the highest binding affinities ever reported for MT_2 receptor ligands ($pK_i = 10.18$). The *meta*-methoxy group influences intrinsic activity at the MT_1 receptor only, with a modest or null effect at the MT_2; compound **3g** thus behaves as a partial agonist at both receptor subtypes. It must be noted that the previously obtained 3D-QSAR models evidence a role for the methoxy group in both MT_2 intrinsic activity and selectivity, which is not observed in the present series (see **3f** and **3g**). Comparison with the corresponding propyl and propenyl derivatives (Figure 2) evidences highest resemblance with the binding profile of the (*E*)-unsaturated derivative, with the (*Z*)-isomer and the saturated analog showing lower affinity for both subtypes. Replacement of the methoxy group with bromine is tolerated, affording **3 h** with binding affinities and intrinsic activities only slightly lower than those of the methoxy analog **3g**. This behavior is consistent with what was observed for other series of melatonin receptor ligands.[43] Shift of the methoxy group to the *para*-position (**3c**) greatly reduces binding affinity at both receptor subtypes; the pK_i values obtained for **3c** are even lower than those of the unsubstituted **3f**; intrinsic activity at the MT_2 receptor is also abolished. The negative role of the *para*-methoxy group, seen in **3c**, is confirmed in the di-methoxyphenyl derivative **3b**. This behavior resembles what was observed for MLT derivatives, where moving the methoxy group from position 5 to position 6 led to micromolar binding affinities and to partial agonist behavior.[43] Reduction of binding affinity and intrinsic activity because of

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



3a-m **4** **5**

					MT_1		MT_2	
cmpd	R	R_1	*n*	R_2	pK_i[a]	$IA_r \pm SEM$[b]	pK_i[a]	$IA_r \pm SEM$[b]
MLT					9.85 ± 0.09	1.0 ± 0.01	9.62 ± 0.10	1.01 ± 0.01
3a	3-OMe	3-OMe-Ph	1	Me	7.72 ± 0.13	0.59 ± 0.05	10.56 ± 0.10	0.15 ± 0.01
3b	3-OMe	4-OMe-Ph	1	Me	7.00 ± 0.01	0.06 ± 0.03	9.06 ± 0.10	0.03 ± 0.01
3c	4-OMe	Ph	1	Me	6.11 ± 0.12	0.02 ± 0.01	7.56 ± 0.10	0.06 ± 0.03
3d	3-OMe	β-naphthyl	1	Me	6.88 ± 0.07	0.17 ± 0.03	9.95 ± 0.64	−0.20 ± 0.03
3e	3-OMe	-CH_2Ph	1	Me	7.30 ± 0.10	0.82 ± 0.06	9.12 ± 0.05	0.31 ± 0.02
3f	H	Ph	1	Me	6.90 ± 0.04	0.10 ± 0.03	8.41 ± 0.13	0.57 ± 0.04
3g	3-OMe	Ph	1	Me	8.38 ± 0.01	0.79 ± 0.03	10.18 ± 0.32	0.61 ± 0.04
3h	3-Br	Ph	1	Me	7.77 ± 0.19	0.43 ± 0.04	9.70 ± 0.43	0.37 ± 0.01
3i	3-OMe	Me	1	Me	9.09 ± 0.10	0.95 ± 0.08	9.19 ± 0.01	1.06 ± 0.05
3j	3-OMe	H	1	Me	8.28 ± 0.01	0.84 ± 0.01	8.13 ± 0.18	0.95 ± 0.03
3k	3-OMe	Me	2	Me	9.08 ± 0.04	0.87 ± 0.05	8.70 ± 0.26	1.07 ± 0.06
3l	3-OMe	Ph	1	*n*-Pr	8.38 ± 0.01	1.01 ± 0.04	9.98 ± 0.26	0.74 ± 0.02
3m	3-OMe	Ph	1	*c*-Bu	6.49 ± 0.50	0.22 ± 0.01	8.43 ± 0.40	0.29 ± 0.01
4					5.89 ± 0.07	−0.01 ± 0.03	7.28 ± 0.06	−0.01 ± 0.03
5					8.14 ± 0.03	0.83 ± 0.02	7.90 ± 0.05	0.97 ± 0.05

[a] pK_i values were calculated from IC_{50} values obtained from competition curves by the method of Cheng and Prusoff,[61] and are the mean of at least three determinations performed in duplicate. [b] The relative intrinsic activity values were obtained by dividing the maximum analogue-induced G-protein activation by that of MLT.

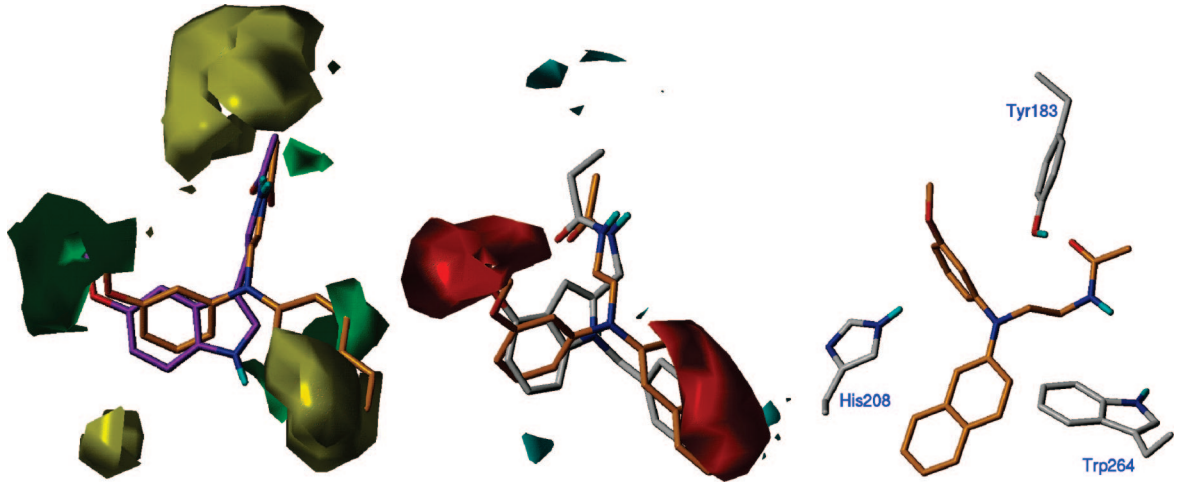


Figure 3. Left panel: superposition of MLT (purple carbons) and **3d** (orange carbons) within CoMFA regions related to MT_2 intrinsic activity.[35] Occupation of green regions is related to an increase of intrinsic activity, and occupation of yellow regions is related to a decrease of intrinsic activity. Middle panel: MT_2-selective antagonists **3d** (orange carbons) and UCM454 (Figure 1, white carbons) surrounded by the CoMFA regions, whose occupancy is related to MT_1 (cyan) or MT_2 (red) selectivity, in accordance with the 3D-QSAR models described in ref 35. Right panel: energy-minimized conformation of the last step of 1 ns MD simulation for the complex between MT_2 receptor model and **3d**.

the shifting of the methoxy group has also been observed in other series of MLT receptor ligands.[33,34] Insertion of an additional *meta*-methoxy group on the second phenyl ring of **3g** (i.e., compound **3a**) leads to the reduction of binding at MT_1 and to an increase in MT_2 binding affinity, thus enhancing MT_2 selectivity. Compound **3a** is characterized by the highest binding affinity for the MT_2 receptor of the whole series, about ten times greater than that of MLT, and by about seven hundred times selectivity over the MT_1 receptor. The second methoxy group decreases intrinsic activity, more at the MT_2 receptor than at the MT_1. The different behavior of **3g** and **3a** is consistent with the putative role of the out-of-plane group for MT_2-selective antagonism. As hypothesized on the basis of 3D models of melatonin receptor subtypes, the corresponding pocket within the MT_1 receptor is smaller, leading to a reduction of binding affinity for compounds with bulky substituents.[36] This is also consistent with the data observed for **3e** and **3d**, having a benzyl and a β-naphthyl group, respectively; the increase of substituent bulk parallels the decrease of MT_1 binding affinity and of MT_2 intrinsic activity. In Figure 3 (left panel), compound **3d** is surrounded by the CoMFA coefficients correlated with intrinsic activity at the MT_2 receptor, previously obtained from a set of ligands evaluated by the GTPγS binding assay.[35] The *N*-phenyl substituent of **3g** is halfway between the green region, corresponding to position 2 of MLT, which correlates with an increase in intrinsic activity, while the yellow one denotes a decrease in intrinsic activity; this alignment is consistent with the observed MT_2 partial agonist behavior. Conversely, compound **3d**, which possesses a naphthyl substituent deeply inserted into the yellow region, behaves as an antagonist. Moreover, the naphthyl substituent occupies the red region, which is related to MT_2 selectivity, as depicted in Figure 3

(middle panel). Indeed, the β-naphthyl derivative **3d** has a remarkable binding affinity for the MT_2 receptor, with more than 1000× selectivity over the MT_1 receptor.

When **3d** is docked within the MT_2 receptor model, by means of an automated docking procedure implemented in the program Glide,[44] the solution characterized by the best E-model score gives a complex that is stable during 1 ns MD simulation (Figure 3, right panel). Compound **3d** occupies the same region as other previously docked MT_2 antagonists and undertakes similar interactions.[36] In particular, the β-naphthyl substituent is deeply inserted into the lipophilic pocket, close to Trp264 of the CWXP motif in TM6, known to be involved in the process of receptor activation. No significant correlation is found between calculated E-model values and receptor affinity for this series of compounds. Even if this model provides only partial explanation for the observed SARs, its qualitative application has proved useful for the design and refinement of potent and selective MT_2 antagonists.

The effect of acyl chain modulation has been evaluated on the basis of compounds **3l** and **3m**. The propyl substituent is tolerated at both receptor subtypes and it increases MT_1 intrinsic activity; in fact, compound **3l** behaves as an MT_1 full agonist. The cyclobutyl group has a negative effect on both binding affinity and intrinsic activity, as already observed for MLT derivatives[45,46] and consistent with the results of a 3D-QSAR analysis performed on an extended set of melatonin receptor ligands.[35] Methylation of the amide nitrogen in compound **4** produces a huge drop in binding affinity at both receptors and abolishes intrinsic activity. This behavior underlines that the amide NH group is important for receptor binding and activation. On the other hand, this cannot be considered a general rule for MT_2-selective agonists, as a series of indanyl-piperazine derivatives, in which the amide nitrogen is part of the piperazine ring, are reported as nanomolar MT_2-receptor agonists.[47]

Chain elongation is tolerated at the MT_1 receptor, while it produces a limited reduction in MT_2 binding affinity, leading to a modest MT_1 selectivity for **3k**. As its shorter homologue, **3i**, compound **3k** behaves as a melatonin receptor agonist.

Replacement of one of the phenyl rings with the smaller methyl group (compound **3i**) mainly influences receptor intrinsic activity, while retaining good binding affinity. Once again, this effect is probably related to the size of the substituent on the nitrogen atom. The smaller group is preferred at the MT_1 receptor, giving a moderate increase of binding affinity; MT_2 binding affinity decreases about ten times, while intrinsic activity increases. Compound **3i** is, therefore, a potent nonselective melatonin receptor agonist. Removal of the methyl group has a negative effect on binding affinity, as the monosubstituted aniline derivative **3j** has lower pK_i at both receptor subtypes. Substitution of the NH group of **3j** with an oxygen atom provides the *N*-(2-phenoxyethyl)acetamide derivative **5**, with similar binding affinities and intrinsic activities to **3j**. A series of propyl-alkanamido melatonin receptor ligands, differing from our *N*-methyl-anilino derivative **3i** for a methylene group replacing the NCH_3 portion, is described in literature.[48] Their profile, with nanomolar binding affinities for the 3-methoxy derivatives and agonist activity on the melanophore pigment aggregation assay[49] is similar to that of the compounds of this study.

The respective roles of MT_1 and MT_2 receptors in the CNS as well as in other organs have not been clarified. Considering that ramelteon is a nonselective MT_1/MT_2 agonist, we have thought it could be useful to see if the moderately MT_2 selective partial agonist **3g** would have sleep promoting activity. Preliminary results indicate that this compound, when administered (40 mg/kg s.c.) to freely moving rats, promotes sleep, as demonstrated by the decrease in the latency and the increase in the duration of slow wave sleep, and by the increase of paradoxical sleep latency, without significantly altering its duration and quantity.[50] Moreover, **3g** displayed activity in the Elevated Plus Maze Test in Sprague–Dawley rats,[51] an experimental animal model of anxiety.[52] Additionally, pharmacological characterization of this compound, which may become a candidate hypnotic drug, is now ongoing.[53]

In conclusion, substitution of benzhydryl carbon atom of the previously reported *N*-(3,3-diphenylpropyl)alkanamido series of melatonin receptor ligands with a nitrogen atom results in a novel series of *N*-(substituted-anilinoalkyl)amido ligands, whose binding affinity and intrinsic activity can be modulated by application of SAR derived from earlier 3D-QSAR and molecular modeling studies. A methoxy group in the *meta*-position, corresponding to the position of the methoxy group in MLT, greatly improves receptor binding affinity. Intrinsic activity and MT_1/MT_2 subtype selectivity can be modulated by the introduction of substituents with different sizes on the aniline nitrogen. This chemical modification has afforded compounds **3a** and **3d**, carrying bulky 3-methoxyphenyl and β-naphthyl substituents, which are highly selective antagonists, with MT_2 binding affinities greater than MLT. Insertion of a phenyl ring on the nitrogen atom leads to the MT_2-selective partial agonist **3g**, while the smaller methyl substituent gives compound **3i**, behaving as a nonselective full agonist. The partial agonist **3g** shows sleep-inducing and antianxiety properties in animal models, which will be reported elsewhere.

Experimental Section

General Methods. Melting points were determined on a Büchi B-540 capillary melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker AVANCE 200 spectrometer, using $CDCl_3$ as solvent unless stated otherwise. 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). 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 FT-IR 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. Diphenylamine (**1f**), 3-methoxydiphenylamine (**1g**), 3-bromodiphenylamine (**1h**), 3-methoxy-*N*-methylaniline (**1i**), *m*-anisidine (**1j**), and all the others 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-thiotriphosphate); specific activity, 1000 Ci/mmol) were purchased from Amersham Pharmacia Biotech (Italy).

3-Methoxy-*N*-(3-methoxyphenyl)benzenamine (1a). Compound **1a** was prepared as previously described.[39]

3-Methoxy-*N*-(4-methoxyphenyl)benzenamine (1b). Compound **1b** was prepared as previously described.[40] ^1H NMR ($CDCl_3$): δ 3.77 (s, 3H), 3.81 (s, 3H), 6.39–6.52 (m, 3H), 6.85–6.91 (m, 2H), 7.08–7.17 (m, 3H).

4-Methoxy-*N*-phenylbenzenamine (1c). Compound **1c** was prepared as previously described.[40]

3-Methoxy-*N*-(naphthalen-2-yl)benzenamine (1d). Cupric acetate (2.54 g, 14 mmol) and dry pyridine (2 mL) were added to a vigorously stirred solution of *m*-anisidine (0.86 g, 7 mmol) and

2-naphthaleneboronic acid (2.41 g, 14 mmol) in dry CH_2Cl_2 (22 mL), under a nitrogen atmosphere. After stirring at room temperature for 72 h, the reaction mixture was filtered on Celite, and the filtrate was evaporated to give a residue that was partitioned between water and EtOAc. The combined organic phases were 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 8:2 as eluent). Amorphous solid, 51% yield. MS (EI): *m/z* 249 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 3.81 (s, 3H), 5.92 (br s, 1H), 6.53–6.58 (m, 1H), 6.74–6.79 (m, 2H), 7.20–7.52 (m, 8H). IR (cm^{-1}, Nujol): 3380, 1594.

***N*-(3-Methoxyphenyl)benzenemethanamine (1e).** Compound **1e** was prepared as previously described.[40] ^{1}H NMR ($CDCl_3$): δ 3.72 (s, 3H), 4.01 (br s, 1H), 4.29 (s, 2H), 6.15–6.30 (m, 3H), 7.02–7.20 (m, 6H).

General Procedure for the Synthesis of Nitriles 2a−d and 2f−h. A solution of the suitable *N*,*N*-diarylamine **1a−d** or **1f−h** (2 mmol) in dry DMF (5 mL) was added dropwise to a stirred ice-cooled suspension of sodium hydride (0.15 g of an 80% dispersion in mineral oil, 5 mmol) in dry DMF (5 mL) under a N_2 atmosphere. The mixture was stirred at 0 °C for 30 min, then bromoacetonitrile (0.65 mL, 9.3 mmol) was added dropwise, and the resulting mixture was heated at 100 °C for 24 h. After pouring the reaction mixture into ice/water, the aqueous solution was extracted with EtOAc, and the combined extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to give a crude residue of the desired product.

[(Bis-3-methoxyphenyl)amino]acetonitrile (2a). Purification by flash chromatography (silica gel; cyclohexane/EtOAc 7:3 as eluent). Oil, 37% yield. MS (EI): *m/z* 268 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 3.78 (s, 6H), 4.51 (s, 2H), 6.58–6.69 (m, 6H), 7.26 (t, 2H). IR (cm^{-1}, neat): 2236.

[(3-Methoxyphenyl)-4-methoxyphenylamino]acetonitrile (2b). Purification by flash chromatography (silica gel, cyclohexane/EtOAc 9:1 as eluent). Oil, 58% yield. MS (EI): *m/z* 268 (M^+), 131 (100). ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 3H), 3.84 (s, 3H), 4.46 (s, 2H), 6.31–6.52 (m, 3H), 6.92–6.97 (m, 2H), 7.15–7.23 (m, 3H).

[(4-Methoxyphenyl)phenylamino]acetonitrile (2c). Purification by flash chromatography (silica gel; cyclohexane/EtOAc 9:1 as eluent) and crystallization. White solid, 65% yield; mp 103–4 °C (diethyl ether/petroleum ether; lit.[54] mp 94 °C). MS (EI): *m/z* 238 (M^+), 154 (100).

[(3-Methoxyphenyl)-2-naphthylamino]acetonitrile (2d). Purification by flash chromatography (silica gel, cyclohexane/EtOAc 8:2 as eluent). Solid, 11% yield, mp 74 °C (subl.). MS (EI): *m/z* 288 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 3.77 (s, 3H), 4.64 (s, 2H), 6.62–6.72 (m, 3H), 7.18–7.81 (m, 8H).

***N*,*N*-Diphenylaminoacetonitrile (2f).** Purification by flash chromatography (silica gel; cyclohexane/EtOAc 9:1 as eluent) and crystallization. White solid, 36% yield; mp 44–5 °C (diethyl ether/petroleum ether; lit.[54] oil). MS (EI): *m/z* 208 (M^+), 167 (100).

[(3-Methoxyphenyl)phenylamino]acetonitrile (2g). Purification by flash chromatography (silica gel; cyclohexane/EtOAc 9:1 as eluent) and crystallization. Pink solid, 59% yield; mp 53–4 °C (petroleum ether). MS (EI): *m/z* 238 (M^+), 154 (100). ^{1}H NMR ($CDCl_3$): δ 3.78 (s, 3H), 4.52 (s, 2H), 6.55 (t, 1H), 6.59–6.65 (m, 2H), 7.08–7.41 (m, 6H). IR (cm^{-1}, neat): 2235.

[(3-Bromophenyl)phenylamino]acetonitrile (2h). Purification by flash chromatography (silica gel; cyclohexane/EtOAc 9:1 as eluent). Oil, 67% yield. MS (EI): *m/z* 286–288 (M^+), 167 (100). ^{1}H NMR ($CDCl_3$): δ 4.50 (s, 2H), 6.82–6.92 (m, 1H), 7.08–7.46 (m, 8H). IR (cm^{-1}, neat): 2235.

General Procedure for the Synthesis of Nitriles 2e and 2i−k. Bromoacetonitrile (0.21 mL, 3 mmol; chloroacetonitrile for **2j** or 3-bromopropionitrile for **2k**) was added dropwise to a solution of the suitable aniline **1e** or **1i**,**j** (1.5 mmol) in dry DMF (3 mL), and the resulting mixture was heated at 100 °C for 3 h (8 h for **2e** and **2j**). The mixture was poured into water and extracted with ethyl acetate. The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to give a crude residue that was purified by flash chromatography (silica gel; cyclohexane/EtOAc 8:2 as eluent).

[Benzyl-(3-methoxyphenyl)amino]acetonitrile (2e). Oil, 59% yield. MS (EI): *m/z* 252 (M^+), 91 (100). ^{1}H NMR ($CDCl_3$): δ 3.81 (s, 3H), 4.10 (s, 2H), 4.53 (s, 2H), 6.50–6.61 (m, 3H), 7.20–7.41 (m, 6H). IR (cm^{-1}, neat): 2230.

[(3-Methoxyphenyl)methylamino]acetonitrile (2i). White solid, 88% yield, mp 65–6 °C (petroleum ether; lit.[55] mp 67–68 °C). MS (EI): *m/z* 176 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 3.00 (s, 3H), 3.82 (s, 3H), 4.15 (s, 2H), 6.40–6.53 (m, 3H), 7.24 (t, 1H).

(3-Methoxyphenylamino)acetonitrile (2j). Oil (lit.[56] 137 °C/0.4 mmHg), 54% yield. MS (EI): *m/z* 162 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 3.80 (s, 3H), 4.05 (s, 2H), 6.25–6.48 (m, 3H), 7.18 (t, 1H). IR (cm^{-1}, neat): 3381, 2235.

3-[(3-Methoxyphenyl)methylamino]propionitrile (2k). Oil (lit.[57] 175 °C/5 mmHg), 48% yield. MS (EI): *m/z* 190 (M^+), 150 (100). ^{1}H NMR ($CDCl_3$): δ 2.57 (t, 2H), 3.02 (s, 3H), 3.70 (t, 2H), 3.80 (s, 3H), 6.24–6.3 (m, 3H), 7.18 (t, 1H). IR (cm^{-1}, neat): 2241.

General Procedure for Hydrogenation of Nitriles and Concomitant *N*-Acylation. A solution of the suitable nitrile **2a−d**, **2f−i**, **2k**, or (3-methoxyphenoxy)acetonitrile[42] (1 mmol), in THF (10 mL) and acetic anhydride (16 mmol, butyric anhydride for **3l**), was hydrogenated over Raney nickel at 4 atm of H_2 for 5 h at 60 °C (1 atm of H_2 for 3 h at room temperature for **3h**). 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; EtOAc as eluent, unless otherwise specified).

***N*-[2-(Bis-3-methoxyphenylamino)ethyl]acetamide (3a).** White solid, 53% yield; mp 84–5 °C (diethyl ether/petroleum ether). MS (EI): *m/z* 314 (M^+), 242 (100). ^{1}H NMR ($CDCl_3$): δ 1.94 (s, 3H), 3.50 (q, 2H, J = 6.3), 3.77 (s, 6H), 3.87 (t, 2H, J = 6.3), 5.63 (br s, 1H), 6.51–6.68 (m, 6H), 7.23 (t, 2H). IR (cm^{-1}, Nujol): 3304, 1646. Anal. ($C_{18}H_{22}N_2O_3$) C, H, N.

***N*-{2-[(4-Methoxyphenyl)-3-methoxyphenylamino]ethyl}acetamide (3b).** White solid, 84% yield; mp 115–6 °C (CH_2Cl_2/petroleum ether). MS (EI): *m/z* 314 (M^+), 242 (100). ^{1}H NMR ($CDCl_3$): δ 1.94 (s, 3H), 3.48 (q, 2H, J = 6.2), 3.74 (s, 3H), 3.76 (t, 2H, J = 6.2), 3.82 (s, 3H), 5.67 (br s, 1H), 6.33–6.41 (m, 3H), 6.88–6.93 (m, 2H), 7.05–7.14 (m, 3H). IR (cm^{-1}, Nujol): 3227, 1640. Anal. ($C_{18}H_{22}N_2O_3$) C, H, N.

***N*-{2-[(4-Methoxyphenyl)phenylamino]ethyl}acetamide (3c).** White solid, 30% yield; mp 85–6 °C (diethyl ether/petroleum ether). MS (EI): *m/z* 284 (M^+), 212 (100). ^{1}H NMR ($CDCl_3$): δ 1.94 (s, 3H), 3.49 (q, 2H, J = 6.2), 3.81 (t, 2H, J = 6.2), 3.82 (s, 3H), 5.70 (br s, 1H), 6.75–6.93 (m, 5H), 7.07–7.20 (m, 4H). IR (cm^{-1}, Nujol): 3221, 1636. Anal. ($C_{17}H_{20}N_2O_2$) C, H, N.

***N*-{2-[(3-Methoxyphenyl)-2-naphthylamino]ethyl}acetamide (3d).** Mp 92–3 °C, 48% yield. MS (EI): *m/z* 334 (M^+), 262 (100). ^{1}H NMR ($CDCl_3$): δ 1.92 (s, 3H), 3.54 (q, 2H, J = 6.2), 3.76 (s, 3H), 3.99 (t, 2H, J = 6.2), 5.89 (br t, 1H), 6.52–6.69 (m, 3H), 7.15–7.48 (m, 5H), 7.69–7.78 (m, 3H). IR (cm^{-1}, film): 3298, 1649. Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

***N*-[2-(Diphenylamino)ethyl]acetamide (3f).** White solid, 78% yield; mp 102–103 °C (diethyl ether/petroleum ether). MS (EI): *m/z* 254 (M^+), 182 (100). ^{1}H NMR ($CDCl_3$): δ 1.93 (s, 3H), 3.50 (q, 2H, J = 6.2), 3.90 (t, 2H, J = 6.2), 5.77 (br s, 1H), 6.95–7.07 (m, 6H), 7.25–7.33 (m, 4H). IR (cm^{-1}, Nujol): 3304, 1643. Anal. ($C_{16}H_{18}N_2O$) C, H, N.

***N*-{2-[(3-Methoxyphenyl)phenylamino]ethyl}acetamide (3g).** White solid, 85% yield; mp 73–74 °C (isopropyl ether). MS (EI): *m/z* 248 (M^+), 212 (100). ^{1}H NMR ($CDCl_3$): δ 1.93 (s, 3H), 3.50 (q, 2H, J = 6.2), 3.76 (s, 3H), 3.89 (t, 2H, J = 6.2), 5.77 (br s, 1H), 6.50–6.63 (m, 3H), 6.99–7.35 (m, 6H). IR (cm^{-1}, Nujol): 3298, 1650. Anal. ($C_{17}H_{20}N_2O_2$) C, H, N.

***N*-{2-[(3-Bromophenyl)phenylamino]ethyl}acetamide (3h).** Purification by flash chromatography (silica gel; CH_2Cl_2/acetone 95:5 as eluent), mp 73–74 °C, 43% yield. MS (EI): *m/z* 332–334 (M^+), 77 (100). ^{1}H NMR ($CDCl_3$): δ 1.94 (s, 3H), 3.48 (q, 2H, J = 6.2),

3.87 (t, 2H, $J = 6.2$), 5.65 (br s, 1H), 6.81–6.89 (m, 1H), 6.97–7.01 (m, 1H), 7.05–7.14 (m, 5H), 7.32–7.41 (m, 2H). IR (cm^{-1}, Nujol): 3292, 1638. Anal. ($C_{16}H_{17}BrN_2O$) C, H, N.

***N*-{2-[(3-Methoxyphenyl)methylamino]ethyl}acetamide (3i).** White solid, 49% yield, mp 70–71 °C (diethyl ether/petroleum ether). MS (EI): *m/z* 222 (M^+), 150 (100). ^1H NMR ($CDCl_3$): δ 1.97 (s, 3H), 3.01 (s, 3H), 3.45 (m, 4H), 3.81 (s, 3H), 5.73 (br s, 1H), 6.30–6.41 (m, 3H), 7.11–7.27 (m, 1H). IR (cm^{-1}, Nujol): 3243, 1654. Anal. ($C_{12}H_{18}N_2O_2$) C, H, N.

***N*-{3-[(3-Methoxyphenyl)methylamino]propyl}acetamide (3k).** Oil, 43% yield. MS (EI): *m/z* 236 (M^+), 150 (100). ^1H NMR ($CDCl_3$): δ 1.80 (m, 2H), 1.95 (s, 3H), 2.91 (s, 3H), 3.24–3.40 (m, 4H), 3.80 (s, 3H), 5.62 (br s, 1H), 6.24–6.37 (m, 3H), 7.15 (t, 1H). IR (cm^{-1}, neat): 3285, 1655. Anal. ($C_{13}H_{20}N_2O_2$) C, H, N.

***N*-{2-[(3-Methoxyphenyl)phenylamino]ethyl}butanamide (3l).** Purification by flash chromatography (silica gel; EtOAc/cyclohexane 1:1 as eluent). White solid, 80% yield, mp 50–2 °C (petroleum ether). MS (EI): *m/z* 312 (M^+), 212 (100). ^1H NMR ($CDCl_3$): δ 0.92 (t, 3H, $J = 7.3$), 1.52–1.71 (m, 2H), 2.10 (t, 2H, $J = 7.2$), 3.52 (q, 2H, $J = 6.2$), 3.76 (s, 3H), 3.89 (t, 2H, $J = 6.2$), 5.71 (br s, 1H), 6.55–6.61 (m, 3H), 7.02–7.31 (m, 6H). IR (cm^{-1}, Nujol): 3277, 1638. Anal. ($C_{19}H_{24}N_2O_2$) C, H, N.

***N*-[2-(3-Methoxyphenoxy)ethyl]acetamide (5).** Oil, 51% yield. MS (EI): *m/z* 209 (M^+), 86 (100). ^1H NMR ($CDCl_3$): δ 2.01 (s, 3H), 3.65 (q, 2H, $J = 5.4$), 3.79 (s, 3H), 4.02 (t, 2H, $J = 5.4$), 6.08 (br s, 1H), 6.45–6.56 (m, 3H), 7.20 (t, 1H). IR (cm^{-1}, neat): 3291, 1653. Anal. ($C_{11}H_{15}NO_3$) C, H, N.

***N*-[2-(3-Methoxyphenylamino)ethyl]acetamide (3j).** A solution of the nitrile **2j** (0.19 g, 1.1 mmol) in THF (7 mL) and 1 M NH_3 in EtOH (1.5 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 water. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to give a crude oily amine, which was used without any further purification.

TEA (0.14 mL, 1 mmol) and acetic anhydride (0.095 mL, 1 mmol) were added to a cold solution of the above crude amine in THF (4 mL) and the resulting mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, and the residue was taken up in EtOAc and washed with a saturated aqueous solution of $NaHCO_3$, followed by brine. After drying over Na_2SO_4, the solvent was removed by distillation in vacuo to give a crude product that was purified by flash chromatography (silica gel; EtOAc as eluent). Oil, 51% yield. MS (EI): *m/z* 208 (M^+), 136 (100). ^1H NMR ($CDCl_3$): δ 2.00 (s, 3H), 3.27 (t, 2H, $J = 6.2$), 3.50 (m, 2H), 3.78 (s, 3H), 5.84 (br s, 1H), 6.17–6.32 (m, 3H), 7.09 (t, 1H). IR (cm^{-1}, neat): 3402, 3314, 1655. Anal. ($C_{11}H_{16}N_2O_2$) C, H, N.

***N*-{2-[(3-Methoxyphenyl)-phenylamino]ethyl}cyclobutancarboxamide (3m).** This compound was prepared according to the above-described procedure for **3j**, starting from the nitrile **2g** and using cyclobutanecarbonyl chloride (1.0 mmol) instead of acetic anhydride to acylate the intermediate crude amine. Purification by flash chromatography (silica gel; cyclohexane/EtOAc 9:1 as eluent) and crystallization. White solid, 56% overall yield, mp 64–5 °C (diethyl ether/petroleum ether). MS (EI): *m/z* 324 (M^+), 55 (100). ^1H NMR ($CDCl_3$): δ 1.82–2.26 (m, 6H), 2.84–2.97 (m, 1H), 3.52 (q, 2H, $J = 6.2$), 3.76 (s, 3H), 3.90 (t, 2H, $J = 6.2$), 5.63 (br s, 1H), 6.50–6.67 (m, 3H), 7.03–7.35 (m, 6H). IR (cm^{-1}, Nujol): 3292, 1642. Anal. ($C_{20}H_{24}N_2O_2$) C, H, N.

***N*-{2-[(3-Methoxyphenyl)-benzylamino]ethyl}acetamide (3e).** A solution of the nitrile **2e** (0.15 g, 0.6 mmol) in dry THF (3 mL) was added dropwise to an ice-cooled suspension of $LiAlH_4$ (0.04 g, 1 mmol) in dry THF (3 mL), and the resulting mixture was stirred at room temperature for 5 h. The reaction mixture was cooled to 0 °C and water was added dropwise to destroy the excess of hydride. The resulting mixture was filtered on Celite, and the filtrate was concentrated in vacuo and partitioned between EtOAc and water. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated to afford the crude amine which was used without any further purification. Acetic anhydride (0.08 mL, 0.85 mmol) was added to a solution of the amine in THF (3 mL) and TEA (0.12 mL, 0.87 mmol), and the mixture was stirred at room temperature for 16 h. The solvent was evaporated in vacuo, and the residue was dissolved in EtOAc. The solution was washed with 2 N NaOH and then with brine and dried (Na_2SO_4). After distillation of the solvent, a crude residue was obtained that was purified by flash chromatography (silica gel; EtOAc as eluent). Oil, 20% yield. MS (EI): *m/z* 298 (M^+), 91 (100). ^1H NMR ($CDCl_3$): δ 1.84 (s, 3H), 3.52 (m, 4H), 3.78 (s, 3H), 4.57 (s, 2H), 5.63 (br t, 1H), 6.29–6.42 (m, 3H), 7.11–7.38 (m, 6H). IR (cm^{-1}, neat): 3282, 1655. Anal. ($C_{18}H_{22}N_2O_2$) C, H, N.

***N*-Methyl-*N*-{2-[(3-methoxyphenyl)-phenylamino]ethyl}acetamide (4).** A solution of **3g** (0.284 g, 1 mmol) in dry DMF (2.5 mL) was added dropwise to an ice-cooled stirred suspension of sodium hydride (0.075 g of an 80% dispersion in mineral oil, 2.5 mmol) in dry DMF (2.5 mL) under a N_2 atmosphere. The mixture was stirred at 0 °C for 40 min, then iodomethane (0.075 mL, 1.2 mmol) was added, and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was poured into ice/water and then extracted with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to give a crude residue that was purified by flash chromatography (silica gel; cyclohexane/EtOAc 9:1 as eluent); oil, 72% yield. MS (EI): *m/z* 298 (M^+), 212 (100). ^1H NMR ($CDCl_3$, registered at 55 °C): δ 2.04 (s, 3H), 2.96 (s, 3H), 3.61 (m, 2H), 3.77 (s, 3H), 3.93 (m, 2H), 6.49–6.64 (m, 3H), 6.99–7.33 (m, 6H). IR (cm^{-1}, neat): 3449, 1646. Anal. ($C_{18}H_{22}N_2O_2$) C, H, N.

Pharmacology. Binding affinities of compounds **3a**−**m**, **4**, and **5** 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.[58,59] 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.[60] 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.[61] 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.[58,62] 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 receptors, respectively. Basal stimulation is the amount of [^{35}S]GTPγS specifically bound in the absence of compounds, and it was taken as 100%. The maximal G-protein activation was measured in each experiment by using MLT (100 nM). Compounds were added at three different concentrations (one concentration was equivalent to 100 nM MLT, a second one was 10 times smaller, and a third one was 10 times larger), and the percent stimulation above basal was determined. 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 100 nM MLT. All of the measurements were performed in triplicate. The relative intrinsic activity (IA_r) values were obtained by dividing the maximum ligand-induced stimulation of [^{35}S]GTPγS binding by that of MLT, as measured in the same experiment. 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.[30]

Docking and Molecular Dynamics (MD) Simulations. Docking studies were performed with Glide 3.5,[44] employing our previously developed MT_2 receptor model.[36] Ligand geometry was optimized using the MMFF94s force field,[63] as implemented in Macromodel.[64] Docking experiments were performed starting from minimum energy conformations of the ligands placed in arbitrary position 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 the protein atoms were not scaled, while Van der Waals radii of the ligand atoms with partial atomic charges 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 0 kcal/mol were rejected, while the other docking solutions were ranked according to their E_{model} value.

The best scoring solution of **3d** 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), and with fixed protein backbone. A MD simulation was performed on the complex, with MMFF94 force field,[65] 1 fs time step, for 1 ns after 100 ps of equilibration, with a fixed protein backbone. The last step of MD was energy minimized, and it is depicted in Figure 3 (right panel).

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Supporting Information Available: Elemental analysis for compounds **3a**−**m**, **4**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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