Tricyclic Alkylamides as Melatonin Receptor Ligands with Antagonist or Inverse Agonist Activity

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This work reports the design and synthesis of novel alkylamides, characterized by a dibenzo[*a,d*]cycloheptene nucleus, as melatonin (MLT) receptor ligands. The tricyclic scaffold was chosen on the basis of previous quantitative structure−activity studies on MT_1 and MT_2 antagonists, relating selective MT_2 antagonism to the presence of an aromatic substituent out of the plane of the MLT indole ring. Some dibenzo seven-membered structures were thus selected because of the noncoplanar arrangement of their benzene rings, and an alkylamide chain was introduced to fit the requirements for MLT receptor binding, namely, dibenzocycloheptenes with an acylaminoalkyl side chain at position 10 and dibenzoazepines with this side chain originating from the nitrogen atom bridging the two phenyl rings. Binding affinity at human cloned MT_1 and MT_2 receptors was measured by 2-[^{125}I]iodomelatonin displacement assay and intrinsic activity by the GTPγS test. The majority of the compounds were characterized by higher affinity at the MT_2 than at the MT_1 receptor and by very low intrinsic activity values, thus confirming the importance of the noncoplanar arrangement of the two aromatic rings for selective MT_2 antagonism. Dibenzocycloheptenes generally displayed higher MT_1 and MT_2 affinity than dibenzoazepines. *N*-(8-Methoxy-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-10-ylmethyl)propionamide (**4c**) and -butyramide (**4d**) were the most selective MT_2 receptor antagonists of the series, with MT_2 receptor affinity comparable to that of melatonin and as such among the highest reported in the literature for MLT receptor antagonists. The acetamide derivative **4b** produced a noticeable reduction of GTPγS binding at MT_2 receptor, thus being among the few inverse agonists described.

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, MLT), a neurohormone primarily produced in the pineal gland at night, is known to have a central role in the regulation of daily and seasonal rhythms in vertebrates. In mammals, the exact role of MLT in the coordination of circadian rhythms remains to be clarified.[1] Accumulating evidence indicates that exogenously administered MLT restores some circadian rhythms disturbed by jet lag, shift work, blindness, and aging.[2−5] MLT, in addition to its chronobiologic effects, has important immune regulatory,[6] antioxidant,[7] and oncostatic[8] properties. It has been suggested that MLT might be useful in some degenerative pathologies, like Alzheimer's disease,[9] and in reducing edema formation in ischemic animals.[10]

Most physiological MLT effects are mediated through the activation of at least two high-affinity G-protein-coupled receptors (named MT_1 and MT_2),[11−13] localized both in the central nervous system and in peripheral tissues. In addition to these high-affinity MLT receptors, another low-affinity melatonin binding site, termed (MT_3), has recently been characterized as the hamster homologue of the human enzyme quinone reductase 2.[14]

The physiological role of these receptors has yet to be clarified. Early data suggested that these receptor subtypes have distinct biological roles in mediating the circadian[15] and vascular[16] functions of MLT, as well as its neuroprotective[17] and antitumor[18] effects and the enhancement of humoral and cellular immunity.[19] While these findings have provided some understanding of the roles of MT_1 and MT_2 receptors, the availability of high-affinity subtype-selective ligands would greatly assist in probing the pharmacology associated with the two receptor subtypes. Therefore, medicinal chemistry research in the melatonin area is being directed toward the discovery of subtype-selective melatonin ligands, both as pharmacological tools and as potential therapeutic agents.[20]

Numerous studies have focused on the discovery of high-affinity ligands for melatonin receptors, and several have been described.[21−24] However, only a few selective melatonin antagonists/partial agonists have been reported to date (Figure 1),[25−33] and very few agonists, displaying some selectivity for the MT_2 receptor, have appeared in the literature.[34,35]

In the course of our studies, various modifications of the melatonin structure have been examined[36−39] in

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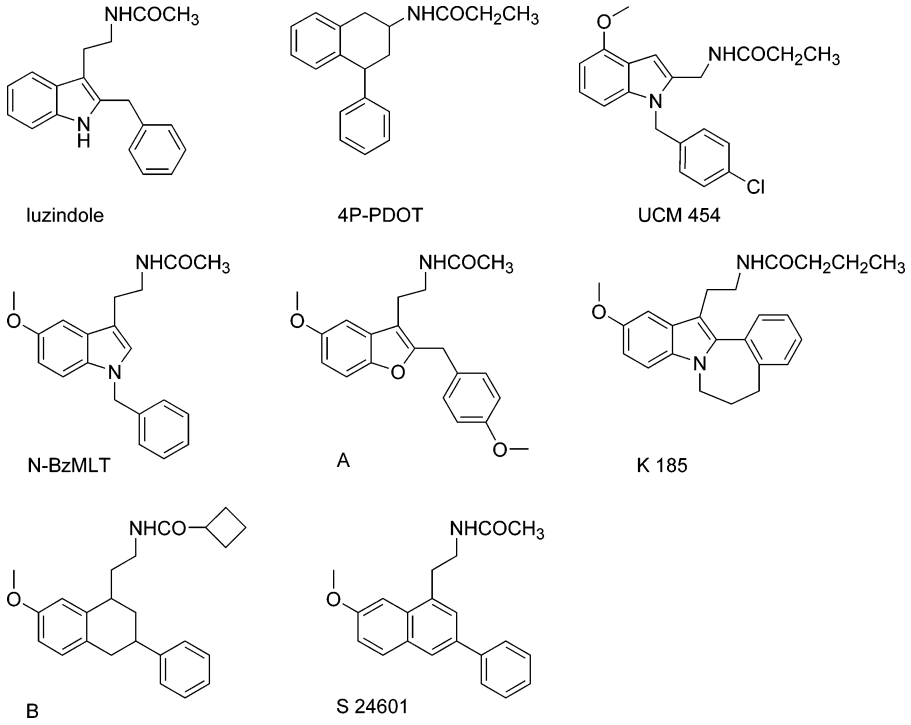


Figure 1. Chemical structures of selective MT_2 receptor antagonists or partial agonists.

order to determine which structural features are required for receptor affinity, intrinsic activity, and/or subtype selectivity. We have discovered, for example, a new series of melatonin receptor antagonists by translocation of the MLT side chain from the C_3 to the C_2 position of the indole nucleus.[40] Further modifications aimed at developing more potent and selective MT_2 ligands have revealed the necessity of shortening the length of the side chain by one C unit and of introducing a benzyl substituent into the N_1-indole[29] (UCM 454 in Figure 1).

A comparison of the molecular models of the potent and selective UCM 454 and of other MT_2 selective antagonists reported in the literature (e.g., 4P-PDOT,[25,41] K185,[28] luzindole[25] in Figure 1) has allowed us to formulate a hypothesis for the structural requirements for receptor subtype selectivity.[29] This hypothesis has been tested by performing three-dimensional quantitative structure−affinity and structure−intrinsic activity relationship (3D-QSAR) studies by means of the CoMFA approach.[42] 3D-QSAR models for MLT antagonists have shown the presence of a bulky group in the region corresponding to positions 1 and 2 of MLT, located out of the plane of the melatonin indole ring, to be the key feature conferring MT_2 selective antagonism. The occupation of this region by a substituent was tolerated at the MT_2 receptor only and it was correlated with a limited intrinsic activity of the compounds.

On the basis of these findings, we looked for scaffolds fulfilling the requirements for selective antagonism and other than the tetralin, indole, naphthalene, and isosteric structures reported in Figure 1. We focused on compounds having a short *N*-acylaminoalkyl chain and a tricyclic scaffold; in particular, we observed that dibenzo seven-membered ring systems held the two benzene rings in a skewed out-of-plane conformation. We thus designed a small series of dibenzocycloheptene and dibenzoazepine derivatives, their dihydro or oxo analogues, bearing an acylaminoalkyl side chain of different length and in different positions, to reproduce the spatial disposition of the pharmacophore elements of known MT_2 selective antagonists. In this work the synthesis of these compounds, their binding affinity at human MT_1 and MT_2 receptors, and their intrinsic activity are reported.

Chemistry

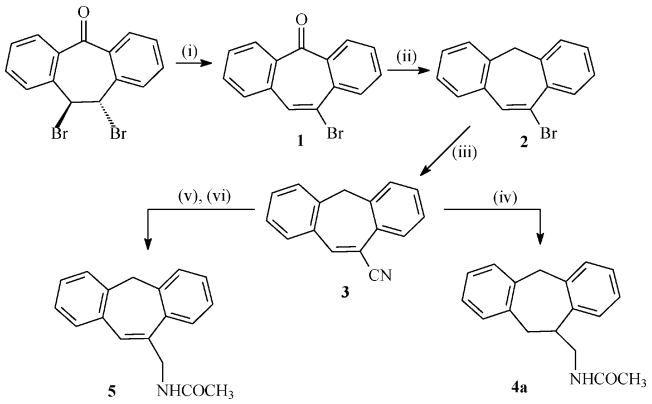
The synthetic strategies (Schemes 1−5) for the target compounds are different according to the seven-membered central ring, the length of the acylaminoalkyl side chain, and the nature of the substituent on the phenyl ring.

Useful intermediates for the synthesis of the new tricarbocyclic compounds were dibenzocycloheptenes bearing functional groups suitable for further conversion.

We synthesized (Scheme 1) the 10-bromoketone **1**[43] by dehydrobromination of *trans*-10,11-dibromodibenzosuberone by potassium *tert*-butoxide and converted it by classical methods ($LiAlH_4/AlCl_3$ reduction of the keto group, bromo substitution with cuprous cyanide) into the dibenzocycloheptencarbonitrile **3**.[44] Raney nickel hydrogenation of the nitrile and concomitant N-acylation with acetic anhydride gave the saturated derivative **4a**; reduction with lithium aluminum hydride/$AlCl_3$, followed by N-acylation with acetic anhydride gave compound **5**.

A more complex procedure was required for the preparation of derivatives **4b**−**d** bearing a methoxy substituent on the phenyl ring (Scheme 2). In accordance with a previously reported procedure for similar compounds,[45] condensation of 3-methoxyphenylacetonitrile and 2-carboxybenzaldehyde gave exclusively (*Z*)-2-[2-cyano-2-*m*-methoxyphenyl)vinyl]benzoic acid, which was converted into the dibenzocycloheptanone deriva-

Scheme 1[a]



[a] Reagents: (i) *t*-BuO$^-$K$^+$, *t*-BuOH; (ii) $LiAlH_4$, $AlCl_3$, THF; (iii) CuCN, *N*-methylpyrrolidone; (iv) H_2, Raney-Ni, 4 atm, Ac_2O, THF; (v) $LiAlH_4$, $AlCl_3$, Et_2O; (vi) Ac_2O, TEA, THF.

tive **6** by double bond reduction with $NaBH_4$ and Friedel−Crafts cyclization. Reduction of the cyanoketone **6** by successive treatment with $NaBH_4$ and then Me_2SiCl_2/NaI[46] led to a mixture of dibenzocycloheptencarbonitrile **7** and dibenzocycloheptencarboxamide **8**. Reduction of **8** with $LiAlH_4/AlCl_3$ or hydrogenation of **7** over Raney nickel in the presence of NH_3/EtOH gave crude (8-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-10-yl)methylamine, which was subsequently acylated with the suitable anhydride in the presence of triethylamine (TEA) to yield the desired tricyclic derivatives **4b**−**d**. The 5-oxo analogue **9** was synthesized by hydrogenation of the cyanoketone **6** over Raney nickel in the presence of NH_3/EtOH and successive N-acylation of the intermediate amine with acetic anhydride.

Key intermediates in the synthesis of the new tricyclic compounds **18a** and **18b** were the dibenzocycloheptanones **16a**[47] and **16b**,[48] which were prepared as outlined in Scheme 3 in accordance with previously reported procedures. Briefly, reaction of phthalic anhydride with the Grignard reagent from *p*-bromoanisole gave the 2-benzoylbenzoic acid derivative **10b**. The ketones **10a**,**b** were reduced to the corresponding hydrocarbons by heating an alkaline solution of the ketoacid with excess Zn dust. The carboxylic acid moieties were transformed by ester formation and $LiAlH_4$ reduction to the benzyl alcohol derivatives **12a**,**b**. These were converted to the corresponding bromides **13a**,**b** by treatment with hydrogen bromide; displacement of bromine with potassium cyanide yielded the cyanomethyl derivatives **14a**,**b**, which were then hydrolyzed to the carboxylic acids **15a**,**b** by refluxing in a mixture of sulfuric and acetic acid. The dibenzocycloheptanones **16a**[47] and **16b**[48] were formed by polyphosphoric acid (PPA) internal Friedel−Crafts acylation of the acids **15a**,**b**. The cycloheptanone underwent a two-carbon homologation by the Wittig−Horner reaction with diethyl (cyanomethyl)phosphonate to give the nitriles **17a**,**b**; they were directly transformed into the saturated acetamidoethyl derivatives **18a**,**b** by hydrogenation over Raney nickel in the presence of acetic anhydride.

The dibenzo[*b*,*f*]azepine derivatives **21** and **25a**,**b** were synthesized by alkylation of the suitable dibenzoazepine **19** or **23a**,**b** with bromoacetonitrile. Hydrogenation of the *N*-cyanomethyldibenzoazepine **20** in the presence of Raney nickel and propionic anhydride gave the saturated derivative **21** (Scheme 4), whereas reduction of dibenzoazepines **24a**,**b** with lithium aluminum hydride followed by N-acylation with acetic or propionic anhydride gave the unsaturated compounds **25a**,**b** (Scheme 5). Synthesis of the starting unsymmetrical 3-methoxydibenzoazepine **23b** was obtained by PPA rearrangement of 6-methoxy-1-phenylindole[29] to dibenzoazepine, in accordance with a previously described procedure for similar compounds.[49] The *N*-acetylaminoacetyl derivative **28** was synthesized by acylation of **23a**[50] with bromoacetyl bromide, treatment with potassium phthalimide, N-deprotection, and N-acylation with acetic anhydride (Scheme 5).

Pharmacology

Compounds were tested by measuring their binding affinity for human MT_1 and MT_2 receptors and their in vitro functional activity.

Binding affinity was assessed in competition experiments, using 2-[^{125}I]iodomelatonin as the labeled ligand, on cloned human MT_1 and MT_2 receptors expressed in NIH3T3 rat fibroblast cells.

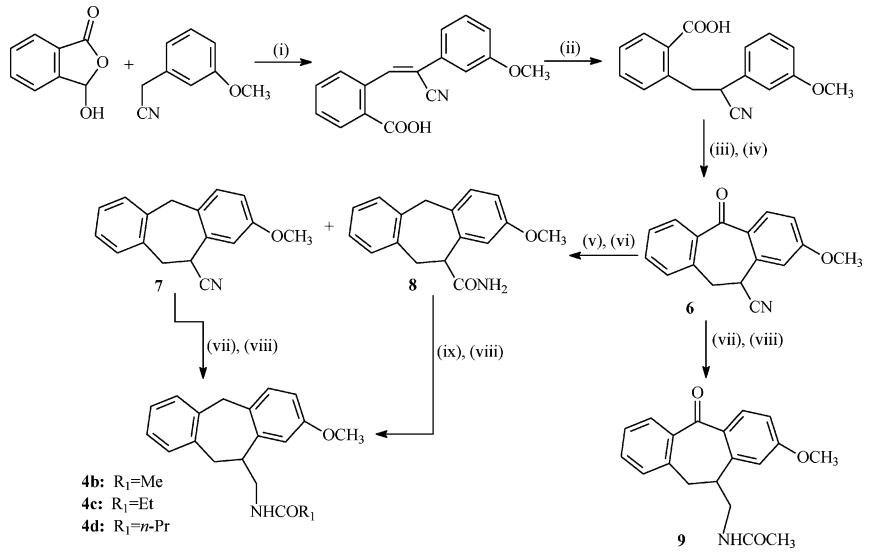
The relative intrinsic activity (IA_r) was measured by means of the GTPγS test by measuring the direct activation of the G protein after binding of the tested compound to the cloned human MT_1 or MT_2 receptor. Full agonists increase the binding of [^{35}S]GTPγS in a concentration-dependent manner, similar to the natural ligand MLT, partial agonists increase the binding to a lesser extent than MLT, and antagonists are without effect, whereas inverse agonists reduce the basal [^{35}S]GTPγS binding. The 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. The interaction between ligands and MLT was investigated by competition experiments in which increasing concentrations of the antagonist inhibit the maximum MLT-induced stimulation of [^{35}S]GTPγS binding.

Results and Discussion

Table 1 reports the pharmacological results obtained with compounds synthesized to investigate the effect of a dibenzo seven-membered cyclic scaffold on human melatonin receptor binding and intrinsic activity. These derivatives are also characterized by the presence of an acylaminoalkyl side chain.[51]

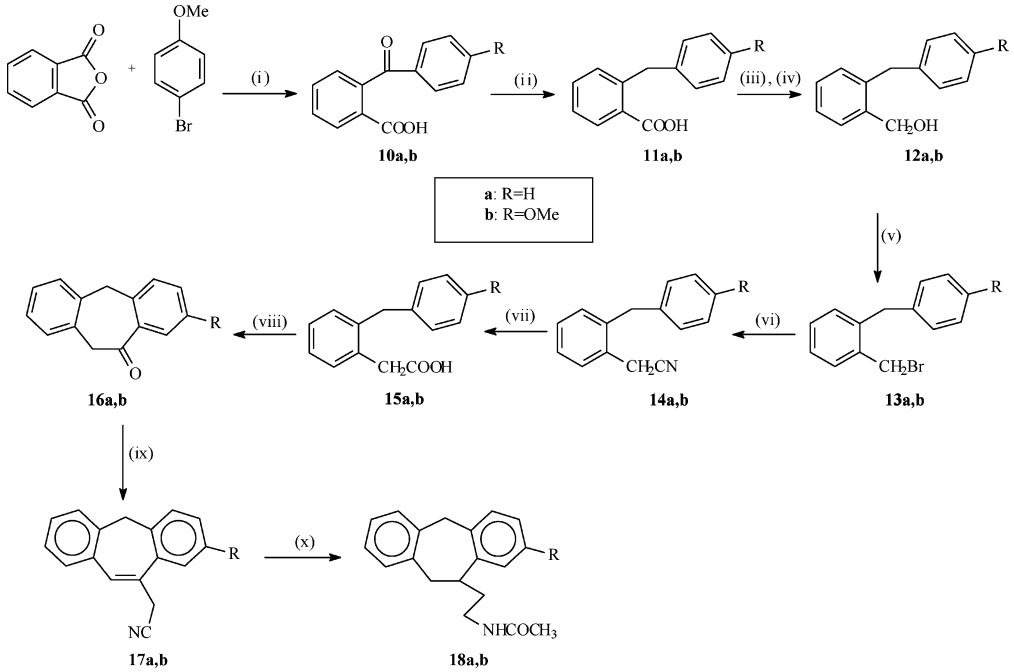
The nature of the tricyclic ring and the position and the length of the side chain were varied by selecting those combinations that gave good superposition to known MT_2 selective antagonists (e.g., UCM 454 and 4P-PDOT in Figure 1) in the conformations chosen from a previous 3D-QSAR model.[42] Both dibenzocycloheptene (**4a**−**d**, **5**, **9**, **18a**,**b** in Table 1) and dibenzoazepine (**21**, **25a**,**b**, **28**) derivatives were prepared. The acylaminoalkyl chain originated from position 10 in the first series and from the nitrogen atom in the second one. Mono- or dimethylene spacers connected the amide function to the tricyclic scaffold directly or through a carbonyl (**28**). The effect of different geometries of the tricyclic nucleus was evaluated by employing the aromatic scaffolds, their 10,11-dihydro analogues, or the

Scheme 2[a]



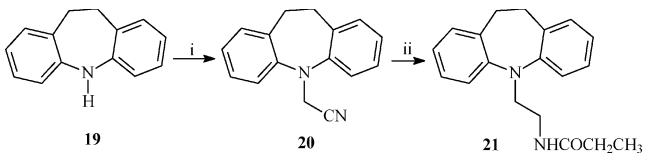
[a] Reagents: (i) NaOMe, MeOH; (ii) $NaBH_4$, *i*-PrOH; (iii) $SOCl_2$, $ClCH_2CH_2Cl$; (iv) $SnCl_4$, $ClCH_2CH_2Cl$; (v) $NaBH_4$, dry MeOH; (vi) NaI, $(CH_3)_2SiCl_2$, CH_3CN; (vii) H_2, Raney-Ni, NH_3/EtOH, THF, 4 atm, 60 °C; (viii) $(R_1CO)_2O$, TEA, THF; (ix) $LiAlH_4$, $AlCl_3$, THF, reflux 4 h.

Scheme 3[a]



[a] Reagents: (i) Mg, Et_2O, I_2, toluene; (ii) Zn, NaOH; (iii) CH_2N_2, Et_2O; (iv) $LiAlH_4$, Et_2O; (v) HBr 48%; (vi) KCN, EtOH; (vii) H_2SO_4, CH_3COOH; (viii) PPA, 90 °C; (ix) $(EtO)_2P(O)CH_2CN$, NaH, THF; (x) H_2, Raney-Ni, 4 atm, Ac_2O, THF, 60 °C.

Scheme 4[a]

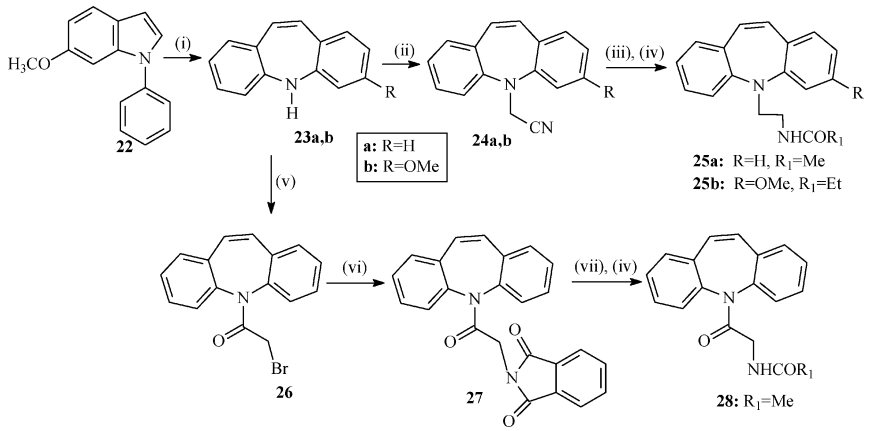


[a] Reagents: (i) $BrCH_2CN$, Δ; (ii) H_2, Raney-Ni, 4 atm, $(EtCO)_2O$, THF.

oxo derivative (**9**); the role of the methoxy group, placed in a position topographically corresponding to position 5 of the indole ring of MLT, was also considered. Different acylating groups were selected for the amino function to investigate the importance of steric effects on biological activity.

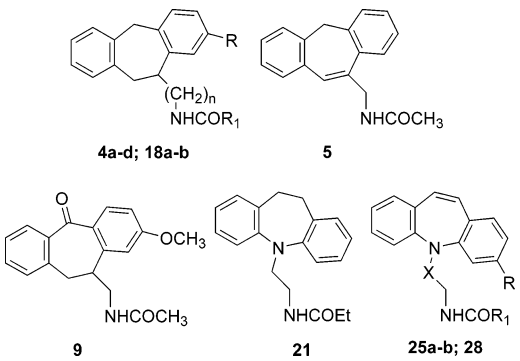
Our design strategy yielded compounds with remarkable affinity, particularly at MT_2 receptors. Almost all compounds showed higher affinity at the MT_2 receptor, with compounds **4a**−**c** characterized by the highest selectivity. Dibenzocycloheptenes were more potent than dibenzoazepines, and, within the first series, the monomethylene spacer conferred higher affinity at both receptor subtypes (**4a** versus **18a**; **4b** versus **18b**). A methoxy group at position 8 led to an increase in binding affinity at both MT_1 and MT_2 receptors with a more relevant effect for the monomethylene derivatives, enhancing their potency by about 30 times (**4a** versus **4b**). On the other hand, it did not influence subtype selectivity. The *N*-propionyl and *N*-butyryl derivatives showed higher affinity at both receptor subtypes compared to the

Scheme 5[a]



[a] Reagents: (i) PPA, 80 °C; (ii) Na_2CO_3, $BrCH_2CN$, CH_2Cl_2; (iii) $LiAlH_4$, THF; (iv) $(R_1CO)_2O$, TEA, THF; (vi) potassium phthalimide, DMF; (vii) NH_2-NH_2, Δ.

Table 1. Binding Affinity[a] and Intrinsic Activity (IA_r)[b] of New Tricyclic Derivatives for the Human MT_1 and MT_2 Melatonin Receptors Stably Expressed in NIH3T3 Cells



					human MT_1		human MT_2	
compd	R	R_1	X	*n*	pK_i	$IA_r \pm$ SEM	pK_i	$IA_r \pm$ SEM
MLT					9.54	1 ± 0.01	9.55	1 ± 0.02
4a	H	Me		1	6.62	0.03 ± 0.02	7.52	−0.32 ± 0.07
4b	OCH_3	Me		1	8.21	−0.18 ± 0.04	9.18	−0.55 ± 0.08
4c	OCH_3	Et		1	8.87	−0.10 ± 0.02	9.79	−0.15 ± 0.08
4d	OCH_3	*n*-Pr		1	8.89	−0.09 ± 0.06	9.61	−0.17 ± 0.05
5					6.26	0.02 ± 0.03	7.07	−0.09 ± 0.03
9					8.52	−0.14 ± 0.02	9.05	−0.08 ± 0.03
18a	H	Me		2	6.41	0.21 ± 0.03	7.05	0.69 ± 0.02
18b	OCH_3	Me		2	6.79	−0.07 ± 0.02	7.40	0.01 ± 0.01
21					6.26	0.03 ± 0.01	6.70	0.13 ± 0.01
25a	H	Me	CH_2		6.95	0.01 ± 0.01	6.90	−0.03 ± 0.06
25b	OCH_3	Et	CH_2		7.24	−0.04 ± 0.02	7.42	−0.10 ± 0.02
28	H	Me	C=O		4.69	nd[c]	4.96	nd[c]

[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] Not determined.

N-acetyl one (**4c**,**d** versus **4b**), confirming the positive effect of longer side chains observed in the case of other MLT receptor antagonists.[28,42] The presence of a 10−11 double bond in compound **5** slightly reduced the binding affinity at both receptor subtypes compared with the corresponding compound **4a** whereas the carbonyl function at position 5 was tolerated, leading to affinity values for compound **9** comparable to those of **4b**.

Longer side chains were only considered for dibenzoazepine derivatives (**21**, **25a**,**b**, **28**), following the aforementioned superposition models, and these compounds showed lower affinity and subtype selectivity. Within this series, hydrogenation of the 10−11 double bond was not favorable, and the positive effect of the ethyl substituent in the amide side chain and of the methoxy group was less marked than in the dibenzocycloheptenes; the presence of a carbonyl function in the side chain of **28** was not tolerated and produced a huge drop in affinity at both receptors.

Concerning intrinsic activity, dibenzoazepine derivatives **21** and **25a**,**b** did not significantly influence GTPγS binding, showing IA_r values around zero. The same was observed for the majority of dibenzocycloheptenes, with some notable exceptions. Compound **18a**, with a dimethylene spacer, evidenced a partial agonist behavior, particularly marked at the MT_2 receptor. In this case the methoxy group, which had been related to an increase in intrinsic activity in other series of ligands,[28,29,37] had an opposite effect, lowering IA_r and leading to an antagonist behavior for **18b**. Compounds **4a** and **4b**, having a monomethylene spacer, behaved as inverse agonists at the MT_2 receptor, being characterized by relative intrinsic activity values of −0.32 and −0.55, respectively. These compounds can be considered among the few inverse agonists reported so far[40,52] that could be interesting as pharmacological tools or in the adjustment of the circadian clock.[52] On the other hand, the *N*-propionyl and *N*-butyryl derivatives (**4c** and **4d**) showed a very low effect on GTPγS binding at the MT_2 receptor, practically behaving as antagonists. Both a double bond between atoms 10 and 11 and a carbonyl group in position 5 of the tricyclic scaffold shifted intrinsic activity toward antagonist behavior, although the resulting compounds were less potent (**5**) or less selective (**9**) than the corresponding parent compounds (**4a** and **4b**, respectively).

Our previous investigations into MLT receptor ligands led to a set of 3D-QSAR models[42] supporting the hypothesis that occupation of the out-of-the-plane space corresponding to positions 1 and 2 of MLT was a key feature for obtaining selective MT_2 antagonists. The novel compounds here described fulfilled this requirement. They are in fact characterized by a tricyclic scaffold in which the two benzene rings are maintained in a noncoplanar arrangement and at a distance similar to those of other selective antagonists. In fact, the centroids of the benzene portion of the tetralin ring and

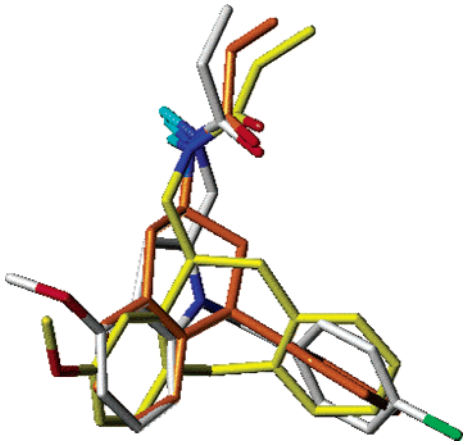


Figure 2. Superposition of *cis*-4P-PDOT (orange carbons), UCM 454 (white carbons), and **4c** (yellow carbons).

Table 2. Root Mean Square Distances (RMSD) of the Pharmacophore Elements (Four Amide Atoms and Two Aromatic Centroids) of the Newly Synthesized Compounds from Those of *cis*-(*S*,*S*)-4P-PDOT in Their Best-Fitting Conformations

compd	chirality	RMSD
4a−**d**	*R*	0.50
	S	0.37
5		0.52
9	*R*	0.42
	S	0.51
18a,**b**	*R*	0.70
	S	0.93
21		0.62
25		0.42
28		0.27

of the phenyl substituent in *cis*-4P-PDOT are at a distance of 4.82 Å, similar to that of 4.81 Å between the centroids of the two benzene rings of **4c**. Moreover, the amide function of **4c** can adopt the same arrangement as in known selective MT_2 antagonists, as depicted in Figure 2. Similar superpositions could be obtained for the other compounds here described, with the weighted Root Mean Square Distances (RMSD) from the pharmacophoric elements of *cis*-4P-PDOT reported in Table 2. For compounds containing a chiral center (**4a**−**d**, **9**, **18a**,**b**), similar RMSD values were observed when different conformations of the two enantiomers were fitted on the same enantiomer of *cis*-4P-PDOT, suggesting poor enantioselectivity. For this reason, these compounds were only tested as a racemic mixture.

The steric features of the tricyclic compounds of this study are compatible with those predicted to be suitable for MT_2 selectivity, as illustrated by the comparison of the CoMFA coefficients for MT_1/MT_2 selectivity with the structure of **4c** depicted in Figure 3. It can be observed that the two red regions, corresponding to positive correlation between steric field and MT_2 selectivity, are occupied by a portion of the bent tricyclic nucleus and the methoxy group, respectively. It is noted, however, that in the present series of compounds the methoxy group does not seem to significantly influence receptor selectivity (see above). On the basis of the 3D-QSAR selectivity model reported in ref 42, a selectivity ratio of between 10 and 100 times was predicted for the tricyclic compounds here reported. Indeed, the most interesting dibenzocycloheptene derivatives displayed a selectivity ratio of about 10 times, which can be

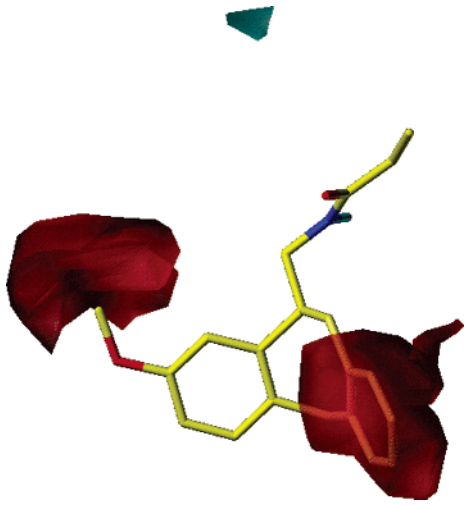


Figure 3. Compound **4c** surrounded by the CoMFA coefficients for MT_1 (cyan) or MT_2 (red) selectivity, in accordance with the 3D-QSAR models described in ref 42.

considered a promising result if associated with the MT_2 binding affinity observed for compounds **4b**−**d**, among the highest ever reported for melatonin receptor antagonists.

A CoMFA model based on intrinsic activity data, homogeneously evaluated by means of the GTPγS test, had shown that steric occupancy of the out-of-plane region led toward antagonist behavior.[42] Tricyclic derivatives generally confirmed this trend, although their intrinsic activity was quantitatively predicted to be slightly higher than observed when an 8-methoxy group was present. In fact, while the 5-methoxy group in MLT derivatives had a significantly positive effect on intrinsic activity, the corresponding 8-methoxy group in the dibenzocycloheptene series showed a negative effect, if any (see **4a** versus **4b**).

Considering the results obtained for the present series of compounds, the reported CoMFA models revealed limited ability to quantitatively predict the observed values of receptor affinity and intrinsic activity, as generally happens with extrapolations of QSARs. On the other hand, the qualitative information provided by them proved to be useful for the design of this new series, which comprises some valuable compounds.

These indications cannot be applied to all the MLT receptor ligands reported so far. For example, S24601 (Figure 1) is a potent and selective MT_2 antagonist whose phenyl substituent in position 3 is coplanar with the naphthalene ring. However, it is possible to superpose the amide function, the phenyl substituent, and the methoxy oxygen of S24601 onto those of the other selective antagonists fulfilling the cited pharmacophore, resulting in a slightly different orientation of the naphthalene ring with respect to the aromatic scaffold of the other compounds. The 3D-QSAR model of ref 42 for S24601 predicted an MT_2 selectivity 46 times that of MLT, in accordance with experimental data.[31]

In conclusion, tricyclic compounds belonging to the dibenzocycloheptene series represent a novel class of selective MT_2 antagonists, having the core scaffold fulfilling a key feature outlined by our previous 3D-QSAR models, i.e., two aromatic rings in a noncoplanar arrangement. In particular, compounds **4c** and **4d** displayed binding affinities among the highest reported

so far for MT_2 antagonists and comparable to those of compound **A**, K185, and S24601 in Figure 1.

Experimental Section

Melting points were determined on Buchi SMP-510 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer; 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 of the tested compounds 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.

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-Methoxyphenylacetonitrile, 2-carboxybenzaldehyde, *trans*-10,11-dibromodibenzosuberone, **10a**, **19**, and **23a** were commercially available (Aldrich).

(*Z*)-2-[2-Cyano-2-(3-methoxyphenyl)vinyl]benzoic Acid. 3-Methoxyphenylacetonitrile (0.845 g, 5.74 mmol) was added to an ice-cooled solution of sodium (0.124 g, 5.4 mmol) in dry MeOH (4 mL) under nitrogen. After the mixture was stirred for 20 min, 2-carboxybenzaldehyde (0.735 g, 4.9 mmol) was added at room temperature, and the mixture was refluxed for 1 h, cooled to room temperature, and poured into ice/water (50 mL) containing concentrated HCl (0.74 mL). After the mixture was stirred for 10 min, the solid was collected by filtration, washed with water, and after dissolution in EtOAc, washed once with brine and dried (Na_2SO_4). Removal of the solvent afforded a yellowish solid that was purified by crystallization from EtOAc (82% yield), mp 173−174 °C. MS (EI): *m*/*z* 279 (M^+), 133 (100). ^{1}H NMR ($CDCl_3$): δ 3.87 (s, 3H), 6.97 (m, 1H), 7.25−7.41 (m, 3H), 7.56 (dd, 1H), 7.73 (dd, 1H), 7.91 (d, 1H), 8.22 (d, 1H), 8.35 (s, 1H).

2-[2-Cyano-2-(3-methoxyphenyl)ethyl]benzoic Acid. Solid sodium borohydride (2 g, 53 mmol) was added portionwise in 1 h to a boiling suspension of (*Z*)-2-[2-cyano-2-(3-methoxyphenyl)vinyl]benzoic acid (6.23 g, 22.3 mmol) in dry *i*-PrOH (92 mL) under nitrogen, and the mixture was refluxed for 6 h. The reaction mixture was cooled to room temperature, poured into ice/water, acidified with 2 N HCl, and extracted with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and evaporated to afford a white solid that was purified by trituration with diethyl ether (72% yield), mp 145 °C. MS (EI): *m*/*z* 281 (M^+), 135 (100). ^{1}H NMR ($CDCl_3$): δ 3.31 (dd, 1H, J = 12.9, 10.2), 3.76 (dd, 1H, J = 12.9, 5.3), 3.81 (s, 3H), 4.33 (dd, 1H, J = 10.1, 5.3), 6.88 (dd, 1H, J = 7.8, 1.9), 7.02 (m, 2H), 7.30−7.63 (m, 4H), 8.18 (d, 1H). IR (cm^{-1}, Nujol): 3417, 2241, 1678, 1598.

8-Methoxy-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-10-carbonitrile (6). Thionyl chloride (2.4 mL, 33 mmol) was added to a solution of 2-[2-cyano-2-(3-methoxyphenyl)ethyl]benzoic acid (6.2 g, 22.3 mmol) in dry 1,2-dichloroethane (30 mL), and the mixture was refluxed for 1 h. Thionyl chloride and the solvent were removed under vacuo, the residue was dissolved in dry 1,2-dichloroethane (30 mL), the mixture was cooled to 0 °C, and then tin tetrachloride (3.45 mL, 29.5 mmol) was added. The reaction mixture was stirred for 1 h at room temperature, poured into ice/water, and extracted with EtOAc. The combined organic phases were washed once with brine and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (silica gel; cyclohexane/EtOAc, 7:3, as eluent) and crystallization from diethyl ether/petroleum ether (82% yield), mp 79−80 °C. MS (EI): *m*/*z* 263 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 3.47 (dd, 1H, J = 15.3, 7.9), 3.60 (dd, 1H, J = 15.3, 2.2), 3.92 (s, 3H), 4.44 (dd, 1H, J = 7.9, 2.2), 6.97−7.57 (m, 5H), 7.02 (s, 1H), 7.28−7.56 (m, 3H), 8.04 (dd, 1H, J = 7.26, 1.34), 8.21 (d, 1H, J = 8.34). IR (cm^{-1}, Nujol): 2237, 1632, 1602, 1584.

8-Methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-10-carbonitrile (7) and 8-Methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-10-carboxylic Acid Amide (8). Solid sodium borohydride (0.289 g, 7.65 mmol) was added to a stirred solution of **6** (3 g, 11.4 mmol) in dry MeOH (60 mL) under nitrogen. After being stirred for 30 min, the reaction mixture was diluted with EtOAc, washed once with water and once with brine, and then dried (Na_2SO_4). The solvent was evaporated and the residue was dissolved in acetonitrile (40 mL) to give a solution to which were added NaI (4.6 g, 30.6 mmol) and dichlorodimethylsilane (2 mL, 16.5 mmol). The reaction mixture was stirred at room temperature for 1 h and then diluted with EtOAc and washed with water, aqueous $NaHCO_3$, aqueous $Na_2S_2O_3$, and brine. After the mixture was dried over Na_2SO_4, the solvent was evaporated under reduced pressure to give a mixture of crude compounds **7** and **8** that were separated by flash chromatography (silica gel; cyclohexane/EtOAc 8:2 and then EtOAc as eluent).

8-Methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-10-carbonitrile (7). 10% yield, white solid, mp 115 °C. MS (EI): *m*/*z* 249 (M^+), 222 (100). ^{1}H NMR ($CDCl_3$): δ 3.41 (dd, 1H, J = 15.0, 9.7), 3.57 (dd, 1H, J = 15.0, 4.0), 3.79 (s, 3H), 3.95 (d, 1H, J = 15.4), 4.18 (d, 1H, J = 15.4), 4.51 (dd, 1H, J = 9.7, 4.0), 6.77 (dd, 1H, J = 8.34, 2.7), 7.99 (d, 1H, J = 2.7), 7.13−7.19 (m, 5H). IR (cm^{-1}, Nujol): 2237, 1615, 1571.

8-Methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-10-carboxylic Acid Amide (8). 56% yield, white solid, mp 202 °C. MS (EI): *m*/*z* 267 (M^+), 223 (100). ^{1}H NMR ($CDCl_3$): δ 3.44 (dd, 1H, J = 14.5, 7.2), 3.55 (dd, 1H, J = 14.5, 5.0), 3.75 (s, 3H), 3.98 (d, 1H, J = 15.2), 4.08 (dd, 1H, J = 7.2, 5.0), 4.18 (d, 1H, J = 15.2), 4.93 (brs, 1H), 5.20 (brs, 1H), 6.68 (d, 1H, J = 2.8), 6.75 (dd, 1H, J = 8.1, 2.8), 7.15−7.19 (m, 5H). IR (cm^{-1}, Nujol): 3429, 3394, 1645.

(5*H*-Dibenzo[*a*,*d*]cyclohepten-10-yl)acetonitrile (17a). Diethyl (cyanomethyl)phosphonate (0.35 mL, 2.16 mmol) was added dropwise under a nitrogen atmosphere to a stirred suspension of NaH (80% in mineral oil, 0.065 g, 2.16 mmol) in dry THF (3 mL), and the mixture was stirred for 40 min at room temperature. A solution of the ketone **16a**[47] (0.15 g, 0.72 mmol) in dry THF (2 mL) was added, and the resulting mixture was stirred at room temperature for 24 h. To the reaction mixture was added water, and the mixture was extracted with diethyl ether. The combined organic phases were dried (Na_2SO_4) and the solvent was evaporated to give a crude residue that was purified by flash chromatography (silica gel; cyclohexane/EtOAc 8:2 as eluent): oil (60% yield). MS (EI): *m*/*z* 231 (M^+), 191 (100). ^{1}H NMR ($CDCl_3$): δ 3.71 (s, 2H), 3.86 (s, 2H), 7.17−7.36 (m, 9H).

(8-Methoxy-5*H*-dibenzo[*a*,*d*]cyclohepten-10-yl)acetonitrile (17b). 17b was obtained following the above procedure by using the ketone **16b**[48] instead of **16a**: oil (48% yield). MS (EI): *m*/*z* 261 (M^+, 100). ^{1}H NMR ($CDCl_3$): δ 3.65 (s, 2H), 3.79 (s, 3H), 3.83 (s, 2H), 6.90 (m, 2H), 7.18−7.31 (m, 6H). IR (cm^{-1}, neat): 2250, 1606, 1565.

(10,11-Dihydrodibenzo[*b*,*f*]azepin-5-yl)acetonitrile (20). A solution of iminodibenzyl (0.5 g, 2.56 mmol) in bromoacetonitrile (1.4 mL) was stirred at 80 °C for 24 h. The excess bromoacetonitrile was removed by bulb to bulb distillation, and the residue was purified by flash chromatography (silica gel; cyclohexane/EtOAc, 9:1 as eluent) and crystallization from diethyl ether/petroleum ether: white solid (27% yield), mp 92 °C. MS (EI): *m*/*z* 234 (M^+), 194 (100). ^{1}H NMR ($CDCl_3$): δ 3.16 (s, 4H), 4.58 (s, 2H), 6.99−7.31 (m, 8H). IR (cm^{-1}, Nujol): 2256, 1586.

Synthesis of Tricyclic Alkylamides (4a−d, 5, 9, 18a,b, 21) by Reduction of Suitable Nitriles or Amides. Procedure A. Raney Nickel Hydrogenation of Nitriles and Concomitant N-Acylation with Suitable Anhydride. A solution of a suitable nitrile (**3**),[44] **17a**,**b**, and **20** (1 mmol) in

THF (10 mL) and acetic or propionic anhydride (3 mL) was hydrogenated over Raney nickel at 4 atm of H_2 for 5 h at 60 °C. The catalyst was filtered on Celite, the filtrate was concentrated in vacuo, and the residue was partitioned between ethyl acetate and 2 N NaOH. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. Purification by flash chromatography (silica gel; EtOAc as eluent) and crystallization gave the desired tricyclic amide **4a**, **18a**,**b**, and **21**.

***N*-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-10-ylmethyl)acetamide (4a).** White crystalline solid, 69% yield, mp 147−8 °C (diethyl ether). MS (EI): *m*/*z* 265 (M^+), 206 (100). ^{1}H NMR ($CDCl_3$): δ 1.94 (s, 3H), 3.00 (dd, 1H, J = 14.6, 9.0), 3.31−3.64 (m, 4H), 3.94 (d, 1H, J = 15.0), 4.25 (d, 1H, J = 15.0), 5.40 (brs, 1H), 7.10−7.23 (m, 8H). IR (cm^{-1}, Nujol): 3274, 1642, 1564. Anal. ($C_{18}H_{19}NO$) C, H, N.

***N*-[2-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-10-yl)ethyl]acetamide (18a).** White crystalline solid, 60% yield, mp 98 °C (diethyl ether/petroleum ether). MS (EI): *m*/*z* 279 (M^+), 73 (100). ^{1}H NMR ($CDCl_3$): δ 1.84 (m, 2H), 1.95 (s, 3H), 2.99 (dd, 1H, J = 15.4, 10.3), 3.38 (m, 4H), 3.95 (d, 1H, J = 15.0), 4.25 (d, 1H, J = 15.0), 5.38 (brs, 1H), 7.08−7.20 (m, 8H). IR (cm^{-1}, Nujol): 3259, 1638, 1561. Anal. ($C_{19}H_{21}NO$) C, H, N.

***N*-[2-(8-Methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-10-yl)ethyl]acetamide (18b).** Oil, 67% yield. MS (EI): *m*/*z* 309 (M^+), 73 (100). ^{1}H NMR ($CDCl_3$): δ 2.0 (m, 2H), 2.10 (s, 3H), 3.12 (dd, 1H, J = 15.8, 6.2), 3.52 (m, 4H), 3.91 (s, 3H), 4.03 (d, 1H, J = 15.0), 4.33 (d, 1H, J = 15.0), 5.61 (brs, 1H), 6.80 (dd, 1H, J = 8.3, 2.5), 6.85 (d, 1H, J = 2.5), 7.22−7.41 (m, 5H). IR (cm^{-1}, Nujol): 3281, 1649, 1551. Anal. ($C_{20}H_{23}NO_2$) C, H, N.

***N*-[2-(10,11-Dihydrodibenzo[*b*,*f*]azepin-5-yl)ethyl]propionamide (21).** White crystalline solid. Purification by flash chromatography (silica gel; cyclohexane/EtOAc 1:1 as eluent), 64% yield, mp 111−2 °C (diethyl ether). MS (EI): *m*/*z* 294 (M^+), 208 (100). ^{1}H NMR ($CDCl_3$): δ 1.10 (t, 3H, J = 7.54), 2.15 (q, 2H, J = 7.54), 3.18 (s, 4H), 3.45 (m, 2H), 3.90 (t, 2H), 5.59 (brs, 1H), 6.92−7.20 (m, 8H). IR (cm^{-1}, Nujol): 3289, 1637, 1572. Anal. ($C_{19}H_{22}N_2O$) C, H, N.

Procedure B. Raney Nickel Hydrogenation of Nitriles Followed by N-Acylation of Crude Amine with Suitable Anhydride. A solution of the suitable cyano derivative **6** or **7** (1.6 mmol) in THF (7 mL) and 2 N NH_3 in EtOH (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 that was purified by flash chromatography (silica gel; CH_2Cl_2/MeOH 95:5 as eluent) and then used without any further purification (60−65% yields). TEA (1.1 equiv) and the appropriate anhydride (1.1 equiv) were added to a cold solution of the above amine (1 mmol) in THF (4 mL), and the resulting reaction mixture was left stirring at room temperature for 6 h. The solvent was evaporated under reduced pressure, and the residue was taken up in ethyl acetate and washed with a saturated aqueous solution of $NaHCO_3$ followed by brine. After the mixture was dried over Na_2SO_4, the solvent was distilled off in vacuo to give a crude product, which was purified by flash chromatography on silica gel (cyclohexane/EtOAc 3:7 as eluent) and crystallization.

***N*-(8-Methoxy-5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-10-ylmethyl)acetamide (9).** Mp 97−98 °C (EtOAc/diethyl ether), 30% yield. MS (EI): *m*/*z* 309 (M^+), 237 (100). ^{1}H NMR ($CDCl_3$): δ 1.96 (s, 3H), 3.1 (dd, 1H, J = 15.1, 6.0), 3.25 (m, 2H), 3.54 (m, 2H), 3.88 (s, 3H), 5.42 (brs, 1H), 6.78 (d, 1H, J = 2.3), 6.91 (dd, 1H, J = 8.9, 2.3), 7.21−7.51 (m, 3H), 8.0 (dd, 1H), 8.24 (d, 1H, J = 8.9). IR (cm^{-1}, Nujol): 3268, 1637, 1597. Anal. ($C_{19}H_{19}NO_3$) C, H, N.

***N*-(8-Methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-10-ylmethyl)acetamide (4b).** Mp 124−125 °C (diethyl ether/petroleum ether), 75% yield. MS (EI): *m*/*z* 295 (M^+), 236 (100). ^{1}H NMR ($CDCl_3$): δ 1.94 (s, 3H), 3.00 (dd, 1H, J = 15.3, 8.9), 3.28−3.62 (m, 4H), 3.76 (s, 3H), 3.9 (d, 1H, J = 15.0), 4.17 (d, 1H, J = 15.0), 5.4 (brs, 1H), 6.68 (dd, 1H, J = 8.3, 2.4), 6.75 (d, 1H, J = 2.4), 7.1−7.2 (m, 5H). IR (cm^{-1}, Nujol): 3298, 1645, 1559. Anal. ($C_{19}H_{21}NO_2$) C, H, N.

***N*-(8-Methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-10-ylmethyl)propionamide (4c).** Mp 114 °C (diethyl ether/petroleum ether), 74% yield. MS (EI): *m*/*z* 309 (M^+), 236 (100). ^{1}H NMR ($CDCl_3$): δ 1.11 (t, 3H, J = 7.6), 2.17 (q, 2H, J = 7.6), 3.00 (dd, 1H, J = 15.1, 8.9), 3.29−3.62 (m, 4H), 3.76 (s, 3H), 3.89 (d, 1H, J = 15.0), 4.17 (d, 1H, J = 15.0), 5.4 (brs, 1H), 6.68 (dd, 1H, J = 8.5, 2.6), 6.75 (d, 1H, J = 2.6), 7.1−7.2 (m, 5 H). IR (cm^{-1}, Nujol): 3292, 1644, 1561. Anal. ($C_{20}H_{23}NO_2$) C, H, N.

***N*-(8-Methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-10-ylmethyl)butyramide (4d).** Mp 102−103 °C (diethyl ether/cyclohexane), 57% yield. MS (EI): *m*/*z* 323 (M^+), 71 (100). ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 3H, J = 7.26), 1.62 (m, 2H), 2.12 (t, 2H, J = 7.3), 3.00 (dd, 1H, J = 14.9, 8.5), 3.28−3.66 (m, 4H), 3.76 (s, 3H), 3.89 (d, 1H, J = 15.2), 4.16 (d, 1H, J = 15.2), 5.44 (brs, 1H), 6.68 (dd, 1H, J = 8.3, 2.7), 6.74 (d, 1H, J = 2.7), 7.1−7.21 (m, 5H). IR (cm^{-1}, Nujol): 3283, 1640, 1561. Anal. ($C_{21}H_{25}NO_2$) C, H, N.

Tricyclic alkylamides 4b−d were prepared following Procedure B or Procedure C depending on the starting nitrile (**7**) or amide (**8**) employed.

Procedure C. $LiAlH_4$/$AlCl_3$ Reduction of the Amide 8 Followed by N-Acylation of Crude Amine with Suitable Anhydride. A solution of aluminum trichloride (0.198 g, 1.5 mmol) in dry THF (6 mL) was added dropwise to an ice-cooled suspension of $LiAlH_4$ (0.164 g, 4.3 mmol) in dry THF (6 mL). After the mixture was stirred for 10 min, a solution of the amide **8** (0.75 mmol) in dry THF (6 mL) was added dropwise, and the resulting mixture was heated at reflux for 4 h. The reaction mixture was cooled to 0 °C, and the excess hydride was cautiously destroyed with water. The resulting mixture was filtered on Celite, and the filtrate was concentrated in vacuo and partitioned between EtOAc and 2 N NaOH (pH 10). The combined organic phases were washed once with brine, dried (Na_2SO_4), and evaporated to afford a crude oily amine (80% yield) that was N-acylated, without any further purification, using the above-described procedure B.

***N*-(5*H*-Dibenzo[*a*,*d*]cyclohepten-10-ylmethyl)acetamide (5).** A solution of aluminum trichloride (0.305 g, 2.27 mmol) in dry Et_2O (2 mL) was added dropwise to a stirred ice-cooled suspension of $LiAlH_4$ (0.085 g, 2.22 mmol) in dry Et_2O (2 mL) under nitrogen, and the mixture was stirred for 10 min. A solution of the nitrile (**3**)[44] (0.2 g, 0.92 mmol) in dry Et_2O (2 mL) was added dropwise to the above mixture, and the resulting mixture was stirred for 1 h at room temperature. Standard workup and N-acetylation (see procedure C) gave the desired title compound. Mp 146 °C (diethyl ether/petroleum ether), 7% yield. MS (EI): *m*/*z* 263 (M^+), 204 (100). ^{1}H NMR ($CDCl_3$): δ 1.99 (s, 3H), 3.68 (brs, 2H), 4.65 (brd, 2H), 5.58 (brt, 1H), 7.13−7.46 (m, 9H). IR (cm^{-1}, Nujol): 3272, 1642, 1561. Anal. ($C_{18}H_{17}NO$) C, H, N.

3-Methoxy-5*H*-dibenzo[*b*,*f*]azepine (23b). PPA (17 g) was brought to 100 °C under N_2 and mechanically stirred for 1 h. The temperature was set to 80 °C, and *N*-phenyl-6-methoxyindole (0.5 g, 2.24 mmol) was added in small portions in 30 min. After being stirred at 80 °C for 20 h, the reaction mixture was cooled and poured into crushed ice and $NaHCO_3$ solution to set the pH at 6. The aqueous solution was extracted three times with CH_2Cl_2, and the emulsion was broken by filtration. The combined organic phases were dried (Na_2SO_4) and evaporated to give a residue that was purified by flash chromatography (silica gel; cyclohexane/EtOAc 9:1 as eluent) and crystallization from diethyl ether/petroleum ether. Yellow solid (10% yield), mp 182 °C. MS (EI): *m*/*z* 223 (M^+, 100). ^{1}H NMR (DMSO-d_6): δ 3.64 (s, 3H), 5.85 (d, 1H, J = 11.8), 5.96 (d, 1H, J = 11.8), 6.23 (m, 2H), 6.51−6.69 (m, 3H), 6.90 (m, 2H). IR (cm^{-1}, Nujol): 3330, 1616, 1595.

(Dibenzo[*b*,*f*]azepin-5-yl)acetonitrile (24a). Bromoacetonitrile (1.5 mL, 21.5 mmol) and Na_2CO_3 (0.587 g, 5.53 mmol)

were added to a solution of iminostilbene (0.147 g, 0.76 mmol) in CH_2Cl_2 (6 mL), and the resulting mixture was heated at reflux for a week. The reaction mixture was poured into ice/water, extracted twice with CH_2Cl_2, and dried (Na_2SO_4). After evaporation of the solvent we obtained a crude residue that was purified by flash chromatography (silica gel, cyclohexane/EtOAc, 85:15 as eluent): oil (52% yield). MS (EI): *m*/*z* 232 (M^+), 192 (100). ^{1}H NMR ($CDCl_3$): δ 4.47 (s, 2H), 6.77 (s, 2H), 7.09−7.39 (m, 8H). IR (cm^{-1}, neat): 2250, 1708, 1595.

(3-Methoxydibenzo[*b,f*]azepin-5-yl)acetonitrile (24b). This product was obtained following the above procedure by using the dibenzoazepine **23b** instead of iminostilbene: oil (50% yield). MS (EI): *m*/*z* 262 (M^+), 222 (100). ^{1}H NMR ($CDCl_3$): δ 3.82 (s, 3H), 4.44 (s, 2H), 6.64 (d, 1H, J = 11.5), 6.69 (dd, 1H, J = 8.3, 2.4), 6.71 (d, 1H, J = 11.5), 6.81 (d, 1H, J = 2.4), 7.05−7.32 (m, 6H). IR (cm^{-1}, neat): 2260, 1605.

***N*-(2-(Dibenzo[*b,f*]azepin-5-ylethyl)acetamide (25a).** A solution of **24a** (0.27 g, 1.16 mmol) in dry THF (6 mL) was added dropwise to a stirred ice-cooled suspension of $LiAlH_4$ (0.088 g, 2.3 mmol) in dry THF (11 mL) under nitrogen. Upon completion of the addition, the mixture was refluxed for 30 min. Standard workup (see procedure C) and N-acetylation of the crude amine (see procedure B) gave the desired title compound. Mp 127−8 °C (EtOAc/petroleum ether), 40% yield. MS (EI): *m*/*z* 278 (M^+), 206 (100). ^{1}H NMR ($CDCl_3$): δ 1.89 (s, 3H), 3.38 (m, 2H), 3.89 (t, 2H), 6.05 (brs, 1H), 6.81 (s, 2H), 7.00−7.25 (m, 8H). IR (cm^{-1}, Nujol): 3262, 1635, 1570. Anal. ($C_{18}H_{18}N_2O$) C, H, N.

***N*-[2-(3-Methoxydibenzo[*b,f*]azepin-5-yl)ethyl]propionamide (25b). 25b** was obtained following the above procedure by using **24b** instead of **24a**. Purification by flash chromatography (cyclohexane/EtOAc 7:3 as eluent): oil (47% yield). MS (EI): *m*/*z* 322 (M^+), 236 (100). ^{1}H NMR ($CDCl_3$): δ 1.08 (t, 3H, J = 7.52), 2.13 (q, 2H, J = 7.52), 3.4 (m, 2H), 3.80 (s, 3H), 3.87 (t, 2H), 6.10 (brs, 1H), 6.61 (m, 2H), 6.67 (d, 1H, J = 11.5), 6.75 (d, 1H, J = 11.5), 7.00−7.11 (m, 4H), 7.23−7.30 (m, 1H). Anal. ($C_{20}H_{22}N_2O_2$) C, H, N.

2-(2-Dibenzo[*b,f*]azepin-5-yl-2-oxoethyl)isoindole-1,3-dione (27). Potassium phthalimide (0.17 g, 0.91 mmol) was added to a stirred solution of 2-bromo-1-dibenzo[*b,f*]azepin-5-ylethanone (**26**)[50] (0.28 g, 0.89 mmol) in dry DMF (5 mL), and the mixture was heated at reflux for 30 min. The mixture was cooled to room temperature, H_2O was added, and the aqueous solution was extracted twice with EtOAc. The combined organic phases were dried (Na_2SO_4), and the solvent was partially evaporated. Hexane was added, and the precipitated solid was filtered, dried, and used without any further purification (75% yield), mp 262−265 °C. MS (EI): *m*/*z* 380 (M^+), 192 (100). H NMR (acetone-d_6): δ 3.78 (d, 1H, J = 16.6), 4.50 (d, 1H, J = 16.6), 7.08 (d, 1H, J = 11.5), 7.16 (d, 1H, J = 11.5), 7.36−7.88 (m, 12H).

***N*-(2-(Dibenzo[*b,f*]azepin-5-yl-2-oxoethyl)acetamide (28).** Hydrazine hydrate (30 μL) was added to a suspension of 2-(2-dibenzo[*b,f*]azepin-5-yl-2-oxoethyl)isoindole-1,3-dione (0.1 g, 0.26 mmol) in 2 mL of EtOH, and the resulting mixture was refluxed for 30 min. The solvent was removed by distillation under reduced pressure to give a residue that was dissolved in EtOAc and washed twice with water. The organic phase was dried (Na_2SO_4) and evaporated to give the intermediate oily amine that was directly used in the next step without any further purification (76% yield). MS (EI): *m*/*z* 250 (M^+), 193 (100).

TEA (0.134 mL, 0.95 mmol) and acetic anhydride (0.09 mL, 0.95 mmol) were added to a solution of the above amine (0.2 g, 0.8 mmol) in THF (3 mL), and the mixture was stirred at room temperature for 6 h. After evaporation of the solvent under reduced pressure, we obtained a crude residue that was purified by flash chromatography (silica gel; EtOAc as eluent) and crystallization from $CHCl_3$/hexane (47% yield), mp 207−208 °C. MS (EI): *m*/*z* 193 (100). ^{1}H NMR ($CDCl_3$): δ 1.97 (s, 3H), 3.38 (dd, 1H, J = 18.3, 4.2), 4.13 (dd, 1H, J = 18.3, 4.2), 6.36 (brs, 1H), 6.92 (dd, 1H, J = 11.5), 6.99 (dd, 1H, J = 11.5), 7.32−7.53 (m, 8H). IR (cm^{-1}, Nujol): 3308, 1657, 1545. Anal. ($C_{18}H_{16}N_2O_2$) C, H, N.

Pharmacology. Tricyclic MLT derivatives were characterized by evaluating their binding affinity at h-MT_1 and h-MT_2 receptors and their in vitro functional activity.

Binding affinities of compounds at each receptor 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 had already been described in detail.[53,54] Membranes were incubated for 2 h 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. 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 melatonin. IC_{50} values were determined by nonlinear fitting strategies with the program PRISM (GraphPad SoftWare Inc., San Diego, CA). pK_i values were calculated from the IC_{50} values in accordance with the Cheng−Prusoff equation:[56] $K_i = IC_{50}/[1 + (L/K_D)]$ where IC_{50} is the 50% inhibitory concentration, K_D is the dissociation constant of the radioligand, and L is the concentration of 2-[^{125}I]iodomelatonin. pK_i values are the mean of at least three independent determinations performed in duplicate; SEM of pK_i values was lower than 0.06.

To define the functional activity of the new compounds at each melatonin receptor subtype, [^{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 have been reported elsewhere.[40,53,54] Membranes (15−25 μg of protein, final incubation volume of 100 μL) were incubated at 30 °C for 30 min, in the presence and in the absence of melatonin 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 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% for MT_1 and MT_2 receptors, respectively. Basal stimulation 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). Compounds were added at three different concentrations (one concentration equivalent to 100 nM MLT, a second one 10 times smaller, and a third one 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 to 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 measurements were performed in triplicate.

Molecular Modeling. Molecular modeling studies were performed with Sybyl 6.8 [57] running on a Silicon Graphics O2 workstation. Molecules were built using the standard sketch option of Sybyl, and their geometries were optimized using the Tripos force field[58] with the Powell method[59] to an energy gradient of 0.01 kcal mol^{-1} $Å^{-1}$, ignoring the electrostatic contribution. Only minimum energy conformations were considered for compound superposition.

The final conformations of the tricyclic scaffolds accurately reproduced the geometry found in the Cambridge Structural Database;[60] for example, the root mean square distance value, derived from the superposition of heavy atoms, for 10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene (reference code HBZCHP) was 0.06 Å and for 5*H*-dibenzo[*b,f*]azepine (reference code BZAZPO) it was 0.11 Å.

The superposition of molecules was performed by means of a rigid-fit procedure, considering the four atoms of the amide function, the centroid of the benzene portion of the indole or of the tricyclic ring, and the centroid of the substituent

positioned out of the plane of the molecule (for tricyclic compounds the centroid of the second benzene ring), to those of *cis*-4P-PDOT.

Selectivity and intrinsic activity predictions were performed by means of the QSAR/CoMFA module of Sybyl, applying PLS models previously described (models 5, 9, and 10 in Table 4 of ref 42) and following the alignment rules employed to derive the models.

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