Design and Synthesis of Naphthalenic Dimers as Selective MT_1 Melatoninergic Ligands

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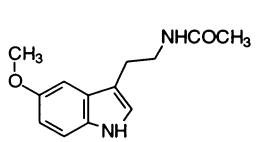
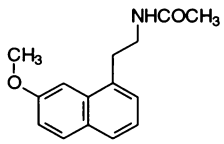
Abstract: We report the synthesis and binding properties at MT_1 and MT_2 receptors of the first example of agomelatine (*N*-[2-(7-methoxynaphth-1-yl)ethyl]acetamide) dimers in which two agomelatine moieties are linked together through their methoxy substituent by a polymethylene side chain according to the "bivalent ligand" approach. Some of these compounds behave as MT_1-selective ligands. The most selective one (**5**) behaves as an antagonist.

Introduction. Melatonin (*N*-acetyl-5-methoxytryptamine, **1**; Chart 1) is synthesized and released by the pineal gland during the dark period. Indeed, its synthesis is regulated by the day−night alternation and by the way melatonin transmits information about the photoperiod to the organism. This neurohormone plays an important role in the regulation of mammalian circadian rhythms and reproductive functions,[1] and it has been implicated in a number of pathological states suggesting its therapeutic application in several disorders.[2,3] These effects are mediated through high-affinity G-protein-coupled receptors, two of which (MT_1 and MT_2) have been cloned in mammals, including humans.[4−6] These two receptors are present in humans in different parts of the brain (suprachiasmatic nuclei, cortex, *pars tuberalis*, etc.) and at the periphery (kidney, adipocytes, retina, blood vessels, etc.).[7−13] The functional physiological roles of these receptors are not well-known, and current research goals include the design of subtype-selective melatonin receptor agonists and antagonists, which will provide pharmacological tools to assess and better characterize the role of each receptor subtype. To date, only a few MT_2-selective ligands (agonists and antagonists) have been described[14−20] and some of them have allowed the identification of several physiological responses mediated through activation of MT_2 receptors.[19] But there is no selective agonist or antagonist for MT_1 receptors, and this is the reason that the physiological role of this subtype is still unknown. The aim of this study was to obtain such selective MT_1 ligands. Among the different methods currently available for medicinal chemists to design potent and selective receptor subtype ligands, the "bivalent ligands" approach appears very promising. Since its first application by Portoghese[21,22] in the field of opioid research, this concept has been successfully applied to numerous research areas and particularly to monoamine neurotransmitters such as norepinephrine,[23] dopamine,[24] and serotonin.[25] During our efforts to discover selective melatonin receptor subtype ligands, we decided to apply this bivalent ligand approach to agomelatine (**2**, Chart 1), the naphthalenic bioisostere of melatonin, which we have previously described[26] and which is, at the present time, in phase III clinical trials.

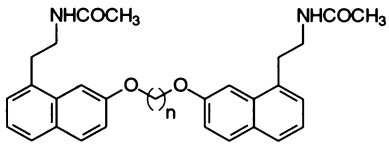
Chart 1. Chemical Structures of Melatonin Ligands



1 (melatonin)

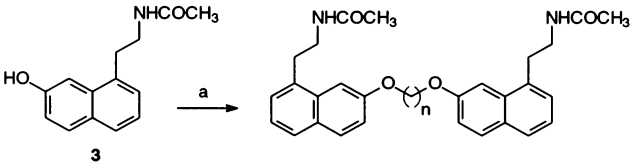


2 (agomelatine)



4-10

Scheme 1. Preparation of Dimeric Compounds **4**−**10**[a]



3

compd	n	Yield (%)
4	2	62
5	3	56
6	4	74
7	5	55
8	6	60
9	8	74
10	10	76

[a] Reagents: (a) $Br(CH_2)_nBr$, K_2CO_3, acetonitrile.

Chemistry. Dimers **4**−**10** (Chart 1) were obtained in one step by condensation of 2 equiv of *N*-[2-(7-hydroxynaphth-1-yl)ethyl]acetamide (**3**)[27] with the appropriate dibromoalkane in the presence of potassium carbonate in acetonitrile (Scheme 1).

Pharmacology. The compounds were evaluated for their binding affinity for human MT_1 and MT_2 receptors stably transfected in human embryonic kidney (HEK 293) cells, using 2-[^{125}I]iodomelatonin as radioligand.

The [^{35}S]GTPγS binding assay used to determine the functional activity of the compounds was difficult to handle using the transfected HEK 293 cell lines, while reliable results were obtained using Chinese hamster

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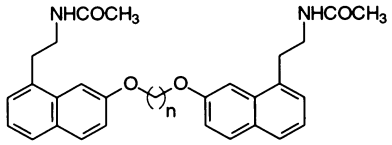
Table 1. MT_1 and MT_2 Receptor Binding Affinities of Compounds **1**, **2**, and **4**−**10**[a]



4-10

compd	*n*	$K_i \pm$ SEM (nM) MT_1	$K_i \pm$ SEM (nM) MT_2	ratio MT_2/MT_1
1		0.14 ± 0.03	0.41 ± 0.04	3
2		0.06 ± 0.01	0.27 ± 0.04	4
4	2	2.05 ± 0.76	182 ± 48.20	88
5	3	0.50 ± 0.03	112 ± 16.70	224
6	4	0.60 ± 0.12	72.70 ± 22.20	120
7	5	0.05 ± 0.01	1.74 ± 0.26	35
8	6	0.08 ± 0.01	2.33 ± 0.02	30
9	8	0.05 ± 0.01	0.18 ± 0.01	36
10	10	0.03 ± 0.00	0.04 ± 0.01	1.3

[a] Concentration−response curves were analyzed by nonlinear regression comparing a one-site and a two-site analysis. All the curves were found to be monophasic with a Hill number close to unity (not shown). Binding affinities (nM) are expressed as the mean $K_i \pm$ SEM of at least three independent experiments. The selectivity ratio of MT_1 to MT_2 receptors is calculated for the compounds.

ovarian (CHO) cell lines stably expressing the human MT_1 or MT_2 receptors. At each receptor, binding affinities were verified for more than 50 selective and nonselective molecules, using either the transfected HEK 293 or the CHO cell lines. Indeed, the correlations between affinities in HEK 293 and CHO cells are highly significant ($r = 0.98$) (unpublished data) for both MT_1 and MT_2 receptors.

Results and Discussion. The chemical structures, binding affinities, and MT_1/MT_2 selectivity ratios of the new compounds **4**−**10** are reported in Table 1. The agonist (EC_{50}) or antagonist (K_B) potencies and efficacies (expressed relative to that of melatonin taken at 100%) in the [^{35}S]GTPγS binding assay are shown in Table 2.

Comparison of the relative MT_1 and MT_2 binding affinities of both melatonin (**1**) and agomelatine (**2**) shows that the MT_1 affinity of **2** is about 2-fold lower than that of melatonin, leading to respective MT_2/MT_1 selectivity ratios of 4 versus 3. These results justify our choice of agomelatine as the starting monomer for the assembly of bivalent ligands. On the other hand, we decided to link the two agomelatine moieties through their 7-methoxy rather than their 3-acetamido substituent according to previously described[28] structure−affinity-relationships, which showed that homologation of the former was more favorable than that of the latter.

Comparison of the relative MT_1 and MT_2 binding affinities of the bivalent ligands (**4**−**10**) shows that as the number of methylene groups in the linking chain increases from 2 to 10, the MT_1 binding affinities increase from 2.05 to 0.03 nM whereas the MT_2 affinities increase from 182 to 0.04 nM. Compound **10**, with 10 methylene groups, is the most potent with MT_1 and MT_2 affinities respectively 2-fold and 15-fold better than agomelatine, but this compound is not selective ($MT_2/MT_1 = 1.3$). These results suggest that for both MT_1 and MT_2 receptors compound **10** binds at two binding sites located on two neighboring receptors and that the distance between these sites should coincide with the optimal distance (10 methylene units) between the dimer headgroups. Recently homodimerization of melatonin receptors has been reported.[29] Consequently, another hypothesis could be that this molecule binds at each binding site of the homodimerized receptors.

Compounds **7**−**9**, which respectively possess five, six, and eight methylene groups, have the same MT_1 affinity and the same (**9**) or a slightly higher MT_2 affinity as agomelatine. We can therefore assume that in these cases, the headgroups can still bind to both sites, but the fit is reduced as a result of a less favorable connecting chain conformation that is more sensitive in the case of the MT_2 than the MT_1 subtype. This difference in sensitivity could explain the appearance of weak MT_1 selectivity of these compounds ($MT_2/MT_1 = 30$).

Compounds **4**−**6** with less than five methylene groups in the linker show a slightly lower (10- to 30-fold) MT_1 affinity but a strongly decreased (300- to 500-fold) MT_2 affinity compared to agomelatine. In these cases, the dimer is too short to bridge both MT_2 sites located on two separate or homodimerized receptors. This hypothesis is probably the same for MT_1 binding sites. Nevertheless, these compounds retain good (nM) MT_1 affinity and behave as potent MT_1-selective ligands. The optimal distance for MT_1 selectivity between the dimer headgroups is obtained with three methylene units ($MT_2/MT_1 = 224$), but the two homologous derivatives with two and four methylene units also show good selectivity ratios (88 and 120, respectively). These results suggest that in these cases steric factors could be predominant: the voluminous seven-substituent of the monomer is able to take place in or near the MT_1 but not in or near the MT_2 receptor pocket. The MT_1 selectivity could be due to the choice of the position of the spacer attachment to agomelatine and to the bulkiness of the substituent.

The functional activity of the most selective MT_1 ligand (**5**) has been evaluated on both receptors in comparison with agomelatine. The full agonist activity is confirmed for agomelatine, whereas compound **5** behaves as an antagonist on both receptors.

Table 2. Activity Values of Compounds **1**, **2**, and **5**[a]

	MT_1			MT_2		
compd	$EC_{50} \pm$ SEM (nM)	$E_{max} \pm$ SEM (%)	$K_B \pm$ SEM (nM)	$EC_{50} \pm$ SEM (nM)	$E_{max} \pm$ SEM (%)	$K_B \pm$ SEM (nM)
1 (melatonin)	2.24 ± 0.35	110 ± 2	nd	0.49 ± 0.04	104 ± 6	nd
2	1.56 ± 0.44	101.3 ± 5.7	nd	0.1 ± 0.04	91 ± 7	nd
5	inactive	<10	5.32 ± 0.95	inactive	<10	143 ± 25

[a] Concentration−response curves were analyzed by nonlinear regression. Agonist potency was expressed as $EC_{50} \pm$ SEM (nM), while the maximal efficacy $E_{max} \pm$ SEM was expressed as a percentage of that observed with 1 μM (=100%) melatonin. Antagonist potency to inhibit the effect of melatonin (30 and 3 nM, respectively, for MT_1 and MT_2 receptors) was expressed as $K_B \pm$ SEM. Data are the mean of at least three independent experiments. Inactive means no dose−response effect, and nd means not determined.

The respective binding (affinities) and functional (potencies) profiles of agonists are conserved for each receptor. However, for hMT_1, a shift in the order of potency (5- to 10-fold) is seen for reference agonists including melatonin (**1**) and agomelatine (**2**), probably due to less efficient coupling at this receptor. This shift is also observed for the antagonist, compound **5**. This explains why the ratio of selectivity is lower in the [^{35}S]-GTPγS assay compared to the binding assay.

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Supporting Information Available: Experimental section, including chemistry and pharmacology information. This material is available free of charge via the Internet at http://pubs.acs.org.

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