Mapping the Melatonin Receptor. 7. Subtype Selective Ligands Based on β-Substituted *N*-Acyl-5-methoxytryptamines and β-Substituted *N*-Acyl-5-methoxy-1-methyltryptamines

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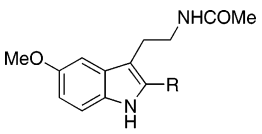
A series of β-substituted and β,β-disubstituted *N*-acyl 5-methoxy-1-methyltryptamines and 5-methoxytryptamines have been prepared as melatonin analogues to investigate the nature of the binding site of the melatonin receptor. The affinity of analogues was determined in a radioligand binding assay using cloned human MT_1 and MT_2 receptor subtypes expressed in NIH 3T3 cells. Agonist and antagonist potency of all analogues was measured using the pigment aggregation response of a clonal line of *Xenopus laevis* melanophores. β-Methylmelatonin (**17a**) and β,β-dimethylmelatonin (**17b**), though showing a slight decrease in binding at human receptors, show an increase in potency on *Xenopus*. *N*-Butanoyl 5-methoxy-1-methyl-β,β-trimethylenetryptamine (**12c**) is an antagonist at human MT_1 receptors but an agonist at MT_2, while *N*-butanoyl 5-methoxy-1-methyl-β,β-tetramethylenetryptamine (**13c**) is an antagonist at MT_1 but had no action at MT_2 and is one of the first examples of an MT_1 selective antagonist.

Introduction

The pineal hormone melatonin (*N*-acetyl 5-methoxytryptamine, **1a**)[1,2] is an important component in the regulation of seasonal and circadian rhythms. Its action is believed to be mediated through a family of specific, high-affinity, G-protein-coupled cell membrane receptors,[3] and radioligand binding studies using 2-[^{125}I]-iodomelatonin (**1b**) have revealed a widespread, heterogeneous distribution of binding sites throughout the central nervous system.[4] Two receptor subtypes (MT_1 and MT_2) have been cloned in mammals[5,6] which, when expressed in host cells, show the general pharmacological characteristics of native melatonin receptors. A third subtype, Mel_{1c}, has been cloned from chicken, *Xenopus*, and zebrafish[7] but has not been detected in mammals. A fourth melatonin binding site, MT_3, has been identified as the enzyme quinone reductase.[8]



1a R = H

1b R = I

A large number of studies have been directed toward understanding how melatonin binds to and activates these receptors using both indole[9−15] and non-indole[16−20] derivatives. The 5-methoxy group of melatonin has been shown to be important for binding to the receptor,[9,21] but it is not an essential requirement for agonist activity.[10] The active conformation of the 3-ethanamide side chain has been established from studies with conformationally restricted indole[10,13,22] and non-indole analogues.[15−18] Models for the binding of melatonin with its receptor have been formulated on the basis of structure−activity data, analogies with other G-protein-coupled receptors for ligands of low molecular mass, comparative molecular field analysis, and site-directed and chimeric receptor mutagenesis studies.[12−14,17,23−29] The conformational preferences of jet-cooled melatonin have recently been explored, and the active conformation is one of the four lowest minima.[30] There has been considerable interest in developing receptor subtype selective compounds. MT_2 subtype selective agonists and antagonists have been reported,[14,25−28,30−37] and MT_1 selective agonists and antagonists have recently been obtained.[34,38,39]

We have now examined a series of β-substituted *N*-acyl 5-methoxytryptamines and β-substituted *N*-acyl 5-methoxy-1-methyltryptamines on binding at the recombinant MT_1 and MT_2 subtypes and receptor activation using the pigment aggregation assay on a clonal *Xenopus* melanophore line.[40] For compounds that proved of interest in these assays, we have investigated the agonist and antagonist behavior in a functional assay on recombinant MT_1 and MT_2 melatonin receptors expressed in the NIH 3T3 cell line.

Chemistry

1,β-Dimethylmelatonin (**6a**), 1,β,β-trimethylmelatonin (**6d**), 1-methyl-β,β-tetramethylenemelatonin (**13h**), and their analogues were prepared through the Bischler reaction by the route shown in Scheme 1. *N*-Methyl-*p*-anisidine (**2**) was treated with the appropriate α-bromoketone **3a**−**c** to give the corresponding 3-indolylethanoic ester, which was hydrolyzed to the acid **4a**−**c** and then converted to the amide **5a**−**c**. Reduction of the amide and acylation of the resulting amine gave **6a**−**g** and **13a**−**e**. This route proved unsuccessful in attempts to prepare the

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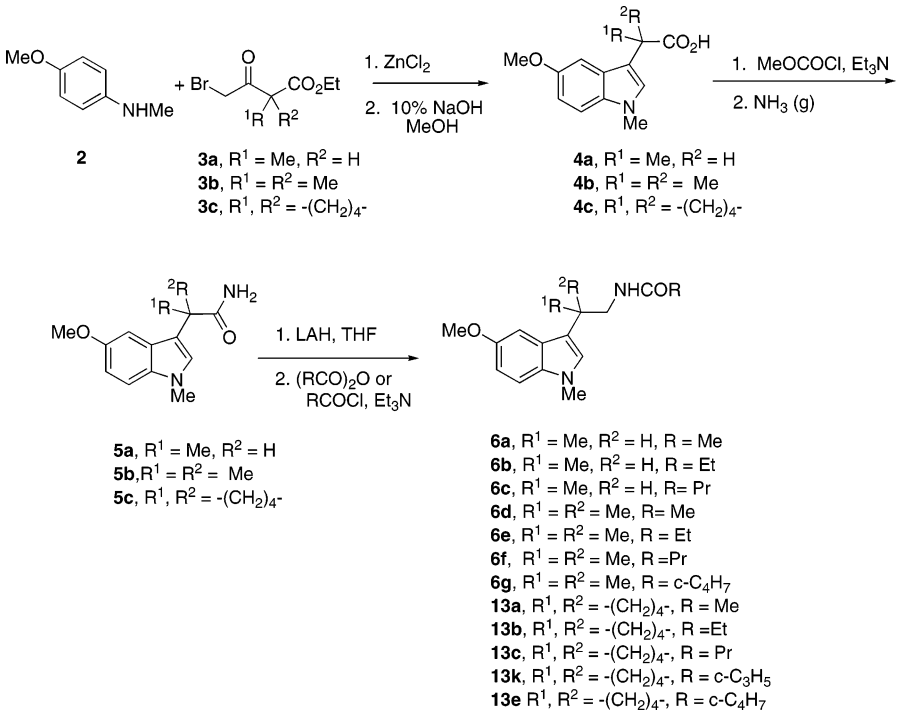
Scheme 1



2

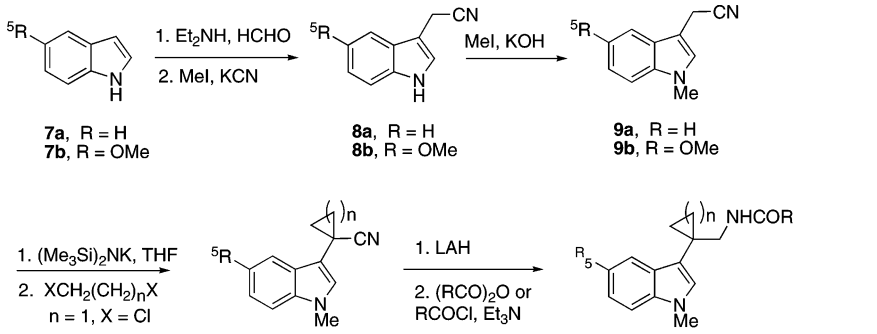
3a, R^1 = Me, R^2 = H
3b, R^1 = R^2 = Me
3c, R^1, R^2 = -$(CH_2)_4$-

4a, R^1 = Me, R^2 = H
4b, R^1 = R^2 = Me
4c, R^1, R^2 = -$(CH_2)_4$-

5a, R^1 = Me, R^2 = H
5b,R^1 = R^2 = Me
5c, R^1, R^2 = -$(CH_2)_4$-

6a, R^1 = Me, R^2 = H, R = Me
6b, R^1 = Me, R^2 = H, R = Et
6c, R^1 = Me, R^2 = H, R= Pr
6d, R^1 = R^2 = Me, R= Me
6e, R^1 = R^2 = Me, R = Et
6f, R^1 = R^2 = Me, R =Pr
6g, R^1 = R^2 = Me, R = c-C_4H_7
13a, R^1, R^2 = -$(CH_2)_4$-, R = Me
13b, R^1, R^2 = -$(CH_2)_4$-, R =Et
13c, R^1, R^2 = -$(CH_2)_4$-, R = Pr
13k, R^1, R^2 = -$(CH_2)_4$-, R = c-C_3H_5
13e R^1, R^2 = -$(CH_2)_4$-, R = c-C_4H_7

Scheme 2



7a, R = H
7b, R = OMe

8a, R = H
8b, R = OMe

9a, R = H
9b, R = OMe

10a, R^5 = H, n = 2
10b, R^5 = OMe, n = 1
10c, R^5 = OMe, n = 2

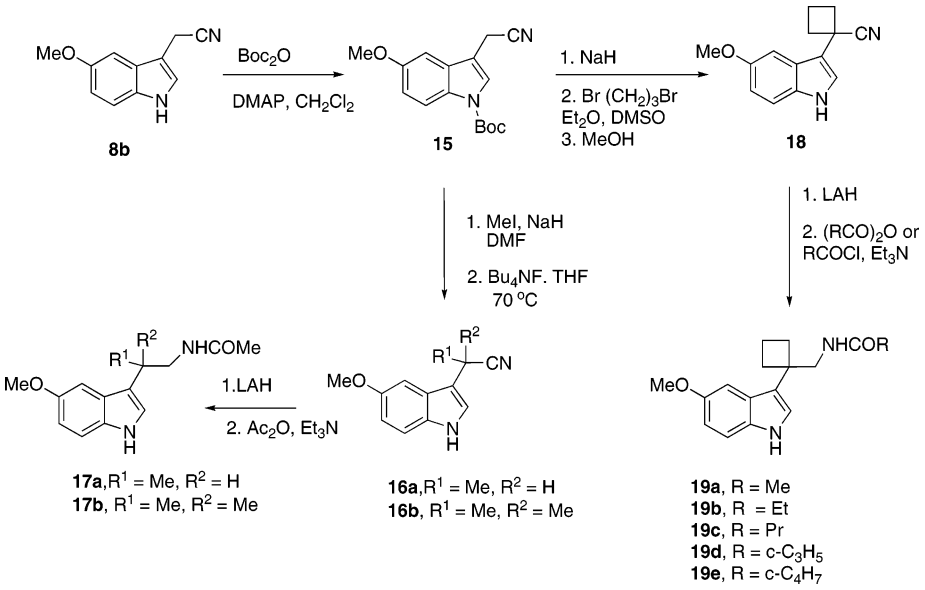
11a, R^5 = OMe, n = 1, R = Me
11b, R^5 = OMe, n = 1, R = Et
11c, R^5 = OMe, n = 1, R = Pr
12a, R^5 = OMe, n = 2, R = Me
12b, R^5 = OMe, n = 2, R =Et
12c, R^5 = OMe, n = 2, R = Pr
12d, R^5 = OMe, n = 2, R = c-C_3H_5
12e, R^5 = OMe, n = 2, R = c-C_4H_7
14a, R^5 = H, n = 2, R = Me
14b, R^5 = H, n = 2, R = Et
14c, R^5 = H, n = 2, R = Pr
14d, R^5 = H, n = 2, R = c-C_3H_5
14e, R^5 = H, n = 2, R = c-C_4H_7

analogues **11a**−**12e**, and the alternative route shown in Scheme 2 was used to obtain these and related systems. Indole (**7a**) or 5-methoxyindole (**7b**) was converted to the corresponding nitrile by the method of Pérez-Alvarez et al.,[41] and the nitrile was then *N*-methylated to **9a**,**b**. Treatment of **9a**,**b** with potassium bis(trimethylsilyl)amide followed by the appropriate α,ω-dihaloalkane gave **10a**−**c** which were reduced to the corresponding amine and acylated to give **11a**−**12e** and **14a**−**e**. To determine the effect of the *N*-1 methyl group, the cyanide **7b** was *N*1-Boc-protected and then methylated with MeI and NaH to give either **16a** or **16b** depending on the amount of MeI used. The nitriles **16a**,**b** were deprotected with (*n*-Bu)$_4$NF, reduced with LAH, and acetylated to give **17a** and **17b**. The *N*1-Boc-protected cyanide **15** was also treated with sodium hydride followed by 1,3-dibromopropane and MeOH to give **18** (Scheme 3). Reduction of **18** with LAH and acylation of the resulting amine gave **19a**−**e**.

Pharmacology

The affinity of the analogues was determined in competition radioligand binding assays using 2-[^{125}I]-iodomelatonin (specific activity 2200 Ci/mol, DuPont, Stevenage, U.K.), as described previously,[42] on the recombinant human MT_1 and MT_2 subtypes expressed in NIH 3T3 cells. The biological activity of the analogues was assessed in a well-established, specific model of melatonin action, the pigment aggregation response of

Scheme 3



Xenopus laevis melanophores. In these cells, melatonin triggers a translocation of pigment, normally distributed throughout the cell, toward the cell center. This response is termed pigment aggregation and is quantified by measuring the change in light (630 nm) absorbance of the cells as the pigment concentrates near the cell center. In the present study, a clonal melanophore line, generously provided by Dr. Michael Lerner (Department of Dermatology, University of Texas) was used.

For specific analogues whose properties in the affinity and potency tests were of interest, the agonist and antagonist potencies were determined on human MT_1 and MT_2 receptors expressed in NIH 3T3 cells by measuring their ability to inhibit forskolin stimulation of intracellular cyclic AMP.

Results and Discussion

The generally accepted active conformation of melatonin at its receptor site has the methyl group of the 5-methoxy moiety and the 3-ethylamino side chain orientated above the indole ring (Figure 1).[26] Substituents at C-2 of the indole ring increase the binding affinity of the compounds probably by a combination of an increase in the population of the active conformation and from binding to a specific pocket available to C-2 substituents.[9,10] For many C-2 substituted analogues, affinity is greater at MT_2 than at the MT_1 receptor, indicating that the MT_2 receptor cavity is larger and/or more flexible than that of the MT_1 receptor. This suggested to us that a combination of suitably placed substituents might provide compounds that were agonists at the MT_2 receptor but inactive or antagonists at the MT_1 receptor. In solution, the 3-*N*-acetyl ethylamine side chain of melatonin is probably fairly evenly distributed between the staggered conformations at C-3−βC, with those in which the side chain is away from the ring predominating. Substituents on the 3-side-chain, particularly at the β position, should initially increase the preference for the active conformation but, as the size of the substituents is increased, the population of the preferred conformation may decline. With sufficiently large substituents, for example the cyclopentane derivatives **13**, the preferred conformation may be with the ring and 3-side-chain orthogonal to the plane of the indole ring. In the active site, rotation of the side chain into the active conformation will orientate the β-substituents away from the indole ring. Introduction of a substituent on the indole nitrogen could further affect the binding space required around the *N*-1 to C-3 regions of the molecule, which again might be more readily tolerated by the MT_2 receptor. We had previously examined β- and α-substituted compounds having a phenylethyl group at *N*-1 which had a promising profile,[43] and we therefore prepared a series of 5-methoxy-1-methyltryptamines substituted at the β-position by one or two methyl groups or by a three-, four-, or five-membered ring. We also prepared 5-methoxytryptamines substituted at the β-position by a four-membered ring in order to determine the effect of the methyl substituent at *N*-1. The compounds were characterized by standard methods as described in the Experimental Section.

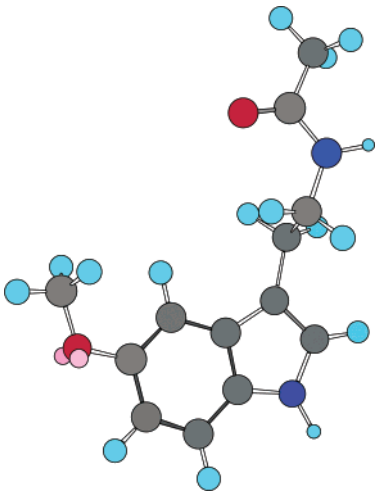


Figure 1. Probable active conformation of melatonin at its receptor. Displacements of the C-3 side chain require little energy.[30]

The cloning of the melatonin receptor[4] and the identification of high-affinity melatonin subtypes[2,5] have allowed the comparison of agonists and antagonists at the two sites. As we have previously reported,[14] the potency results obtained on pigment aggregation in *X. laevis* melanophores do not correlate well with either the MT_1 or MT_2 receptor subtypes. The results of the binding and melanophore assays for the three sets of compounds are shown in Tables 1, 2, and 3, respectively.

From Table 1 it can be seen that the introduction of methyl groups at the β-position of melatonin (**17a**,**b**) reduces the binding affinity to both the MT_1 and MT_2 receptors, but that a small

Table 1[a]

compd	R^1	R^2	R^3	R	receptor binding (K_i, nM) human MT_1	receptor binding (K_i, nM) human MT_2	MT_2/MT_1	*Xenopus* melanophores agonist (EC_{50}, nM)	*Xenopus* melanophores antagonist (IC_{50}, nM)
melato nin	H	H	H	Me	0.39 ± 0.08	0.35 ± 0.03	1.1	0.063 ± 0.004	NA
17a	H	Me	H	Me	1.67 ± 0.17	2.94 ± 0.88	0.6	0.049 ± 0.001	NA
17b	H	Me	Me	Me	1.12 ± 0.38	2.75 ± 0.33	0.4	0.0072 ± 0.0001	NA
6a	Me	Me	H	Me	5.48 ± 1.70	0.41 ± 0.12	13	1.72 ± 0.08	NA
(+)**6a**	Me	Me	H	Me	1.22 ± 0.36	0.50 ± 0.05	2	0.98 ± 0.01	NA
(−)**6a**	Me	Me	H	Me	7.71 ± 0.71	0.27 ± 0.07	28.5	10.8 ± 0.08	NA
6b	Me	Me	H	Et	4.90 ± 0.83	1.02 ± 0.07	5	3.72 ± 0.17	NA
6c	Me	Me	H	Pr	4.27 ± 0.29	0.47 ± 0.04	9	1.00 ± 0.24	NA
6d	Me	Me	Me	Me	11.5 ± 2.2	1.29 ± 0.24	9	6.31 ± 0.94	NA
6e	Me	Me	Me	Et	6.03 ± 0.66	1.15 ± 0.13	5	1.26 ± 0.11	NA
6f	Me	Me	Me	Pr	8.51 ± 0.92	0.74 ± 0.08	11.5	1.00 ± 0.37	NA
6g	Me	Me	Me	c-C_4H_7	170 ± 11	33.1 ± 2.2	5	57.5 ± 5.1	NA

[a] MT_1 and MT_2 data are the mean of quadruplicate determinations. Agonist and antagonist data on melanophores are the mean of triplicate experiments. NA = no antagonist effect detected at 10 μM.

Table 2[a]

compd	n	R	receptor binding (K_i nM) human MT_1	receptor binding (K_i nM) human MT_2	MT_2/MT_1	*Xenopus* melanophore s agonist (EC_{50}, nM)	*Xenopus* melanophore s antagonist (IC_{50}, nM)
melatonin			0.39 ± 0.08	0.35 ± 0.03	1.1	0.063 ± 0.004	NA
11a	1	Me	176 ± 26	90 ± 11	2	10.5 ± 3.0	NA
11b	1	Et	86 ± 20	39 ± 9	2	6.8 ± 2.7	NA
11c	1	Pr	212 ± 39	7.5 ± 2.8	28	1.7 ± 0.3	NA
12a	2	Me	483 ± 16	72 ± 3	7	1010 ± 210[b]	637 ± 17
12b	2	Et	34.0 ± 2.0	4.40 ± 0.15	8	980 ± 160[b]	826 ± 6
12c	2	Pr	10.6 ± 1.8	0.86 ± 0.13	12	2.00 ± 0.8	NA
12d	2	c-C_3H_5	307 ± 53	138 ± 12	2	NA	2066 ± 126
12e	2	c-C_4H_7	714 ± 14	3767 ± 569	0.2	NA	>10000
13a	3	Me	148 ± 33	66.1 ± 4.4	2	NA	628 ± 34
13b	3	Et	302 ± 27	102 ± 46	3	NA	811 ± 60
13c	3	Pr	589 ± 76	85.1 ± 33.8	7	NA	1635 ± 13
13d	3	c-C_3H_5	468 ± 21	257 ± 28	2	NA	1175 ± 16
13e	3	c-C_4H_7	479 ± 124	407 ± 105	1	NA	1766 ± 165

[a] See footnote to Table 1 for methods. NA = no agonist or antagonist effect detected at 10 μM. [b] Partial agonist with very low efficacy (see Figure 2).

preference for the MT_1 site occurs. β-Methylmelatonin (**17a**) is chiral and, from studies on the corresponding 1-methyl derivative described below, it is likely that the enantiomers exhibit different binding affinities. Introducing methyl groups at positions 1 and β (**6a**−**c**) also causes a drop in binding affinity with the effect being greater on the MT_1 site. Increasing the size of the alkyl chain on the amide group from methyl (**6a**) to propyl (**6c**) leads to only a small increase in binding. Introduction of a second methyl group at the β-position (**6d**−**g**) reduces the binding affinity a little further with the effect most pronounced at the MT_1 receptor to give compounds showing a small preference for MT_2. As the alkyl chain is increased (**6e**−**f**), there is a small increase in MT_2 affinity, with the butanoyl derivative **6f** having the maximal affinity for MT_2 and the propanoyl derivative **6e** for MT_1. For both receptors, cyclobutanecarbonyl as the *N*-acylating group dramatically reduces affinity. Most of theses compounds show a small selectivity (up to 13-fold) for the MT_2 receptor.

All the compounds are agonists in the melanophore assay; **17b** (EC_{50} = 7.2 pM) was 9-fold more potent than melatonin. Compounds **6a**−**c** are chiral, and since it is known that enantiomers can have different affinities and potencies at the receptor, the enantiomers of **6a** were separated by chiral chromatography and tested. Enantiomer (+)-**6a** has a 10-fold higher potency for pigment aggregation than (−)-**6a**. In previous studies,[14,17] it was found that in compounds that were chiral at the equivalent β-position, the (−)-enantiomers had higher potency on melanophores; however, in these compounds the chiral atom was in a six-membered ring and the compounds had much greater magnitudes of rotation. The (+)-enantiomer shows a similar binding affinity at the two receptors, whereas the (−)-enantiomer binds more strongly to the MT_2 receptor, giving (−)-**6a** 28-fold selectivity for MT_2. Interestingly, (+)-**6a** has a higher affinity than (−)-**6a** at MT_1 receptors (and a higher potency on melanophores), but at the MT_2 subtype it is the (−)-enantiomer which has the higher affinity (~2-fold). The absolute configuration of the enantiomers of **6a** is not known, and in order to gain more insight into the receptor stereochemistry we are now examining the affinities of a range of chiral compounds.

Table 2 shows the binding and potency results for melatonin analogues with a ring attached at the β-position of the side chain and a methyl group at *N*-1. The compounds **11a**−**c** with a cyclopropane ring are all agonists in the melanophore assay but show a reduced potency compared to melatonin. Potency increases on changing the amine acylating group from methyl through propyl, with **11c** the most potent. Increasing the length of the acylating group also improves binding affinity at MT_2 but not MT_1, **11c** having a 28-fold selectivity. The cyclobutane derivatives **12a**−**c** showed a remarkable (45−84-fold) increase in affinity at both melatonin receptors on changing the acyl group from methyl through propyl. On melanophores, the methyl (**12a**) and ethyl (**12b**) compounds were partial agonists giving only modest aggregation (8% and 20%, respectively, of the maximum response to melatonin) at 10 μM. However, the propyl derivative (**12c**) was essentially a full agonist (92% of the response of melatonin) with an EC_{50} of 2.00 nM. Changing the acyl group to cyclopropanecarbonyl (**12d**) or cyclobutanecar-

Table 3[a]

compd	R^1, R^2	R	receptor binding (K_i, nM) human MT_1	receptor binding (K_i, nM) human MT_2	MT_2/MT_1	*Xenopus* melanophores agonist (EC_{50}, nM)	*Xenopus* melanophores antagonist (IC_{50}, nM)
melatonin	H	Me	0.39 ± 0.08	0.35 ± 0.03	1.1	0.063 ± 0.004	NA
19a	$-(CH_2)_3-$	Me	8.20 ± 1.93	3.30 ± 0.17	2.5	1.67 ± 0.12	NA
19b	$-(CH_2)_3-$	Et	65.7 ± 10.7	32 ± 2	2	0.99 ± 0.05	NA
19c	$-(CH_2)_3-$	Pr	116 ± 10	57.4 ± 24.0	2	2.56 ± 0.20	NA
19d	$-(CH_2)_3-$	c-C_3H_5	57.8 ± 10.0	28.9 ± 1.7	2	NA	2498 ± 116
19e	$-(CH_2)_3-$	c-C_4H_7	493 ± 37	62.2 ± 13.5	8	NA	>10000

[a] See footnote to Table 1 for methods. NA = no agonist or antagonist effect detected at 10 μM.

Table 4[a]

compd	R	receptor binding (K_i nM) human MT_1	receptor binding (K_i nM) human MT_2	MT_2/MT_1	*Xenopus* melanophores agonist (EC_{50}, nM)	*Xenopus* melanophores antagonist (IC_{50}, nM)
14a	Me	2188 ± 1276	1259 ± 378	2	NA	1248 ± 153
14b	Et	1514 ± 790	1047 ± 24	1	NA	1149 ± 56
14c	Pr	724 ± 79	1738 ± 40	0.4	NA	3031 ± 432
14d	c-C_3H_5	617 ± 13	1445 ± 215	0.4	NA	3443 ± 321
14e	c-C_4H_7	>10000	2344 ± 899	>4	NA	2006 ± 293

[a] See footnote to Table 1 for methods. NA = no agonist effect detected.

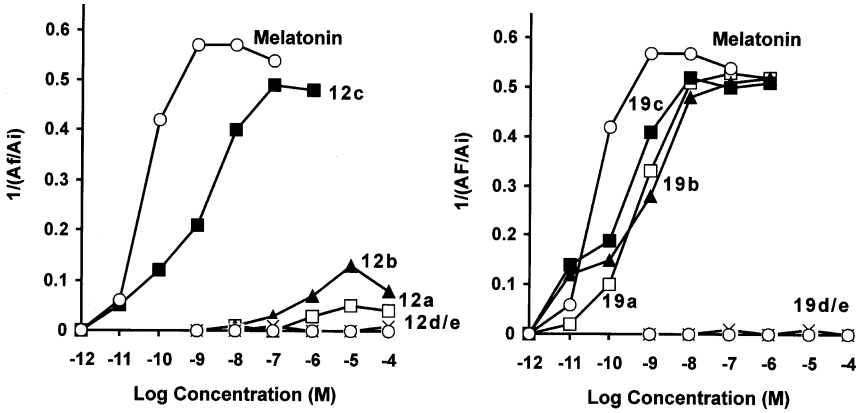


Figure 2. Concentration−response curves for pigment aggregation by melatonin analogues in melanophores. Cells were grown in 96-well plates, and growth medium was replaced by 0.7 × L-15 culture medium 18 h before testing. Initial absorbance (A_i, 630 nm) of the cells was measured in each well, and cells were then treated with the concentrations of the analogue indicated. The final absorbance (A_f) was measured after 60 min, and the fractional change [$1 - (A_f/A_i)$] was calculated. This figure shows the effect on agonist potency of changing the R group of the *N*-(2-[5-methoxy-1-methyl-1*H*-indol-3-yl]-2,2-trimethylene-ethyl)alkanamides (**12**) and *N*-acyl 5-methoxy-β,β-trimethylenetryptamines (**19**) shown in Tables 2 and 3. Each point represents the mean response of duplicate wells of melanophores at each concentration in a single experiment. Results for a single experiment are shown, but similar results were obtained in a second experiment.

bonyl (**12e**) dramatically reduced affinity at both receptor subtypes and gave weak antagonists on the melanophore response. Compound **13a** with a cyclopentane ring had a similar affinity to the corresponding cyclopropyl analogue **11a**, but it was a weak antagonist at the melanophore receptor rather than an agonist. In contrast to the cyclopropyl (**11a**−**c**) and cyclobutyl (**12a**−**c**) series, lengthening the acyl group (**13a**−**c**) tended to have little effect (MT_2) or reduced (MT_1) affinity. In this series, cyclopropanecarbonyl (**13d**) and cyclobutanecarbonyl (**13e**) acylating groups had little influence on affinity. All compounds with cyclopentane rings (**13a**−**e**) were weak antagonists on melanophores.

The compounds in Table 3 illustrate the effect of replacing the *N*-methyl group by hydrogen for the series of compounds with a four-membered ring. The derivatives **19a**,**b** are agonists in the melanophore assay, unlike their *N*-methyl analogues **12a**,**b**. Binding affinity at MT_1 and MT_2 receptors declines 15−17-fold as the acyl group lengthens from methyl through propyl (**19a**−**c**) rather than increasing dramatically as is seen for the *N*-methyl analogues (**12a**−**c**). Again, all show a small preference for the MT_2 receptor. The *N*-cyclopropanecarbonyl **19d** and *N*-cyclobutanecarbonyl **19e** derivatives are both antagonists in the melanophore assay, and both have a somewhat higher affinity at MT_1 and MT_2 receptors than their *N*-methyl analogues.

Table 4 shows the binding potency data for a series of cyclobutane derivatives with H rather than OMe at position 5 of the indole ring. All of the compounds are antagonists in the melanophore assay, and they all bind weakly at the human MT_1 and MT_2 receptors.

The dramatic change in activity on melanophores, illustrated in Figure 2, between **19a**−**c** (all agonists) and **12a**−**c** (**12a**,**b** very weak, low-efficacy partial agonists, **12c** agonist) by substituting a methyl group for hydrogen at *N*-1 in the cyclobutane series, led us to investigate their functional response at the MT_1 and MT_2 human receptors. While all of the compounds bind reasonably strongly to the human receptors, **12a**−**c** show an increase in binding with increasing length of side chain (Me, Et, Pr) whereas the NH series **19a**−**c** show a decrease with increasing side chain length. Compound **12c** is of particular interest in that increasing the side chain length from Me to Et to Pr (**12a** to **b** to **c**) has converted the cyclobutane

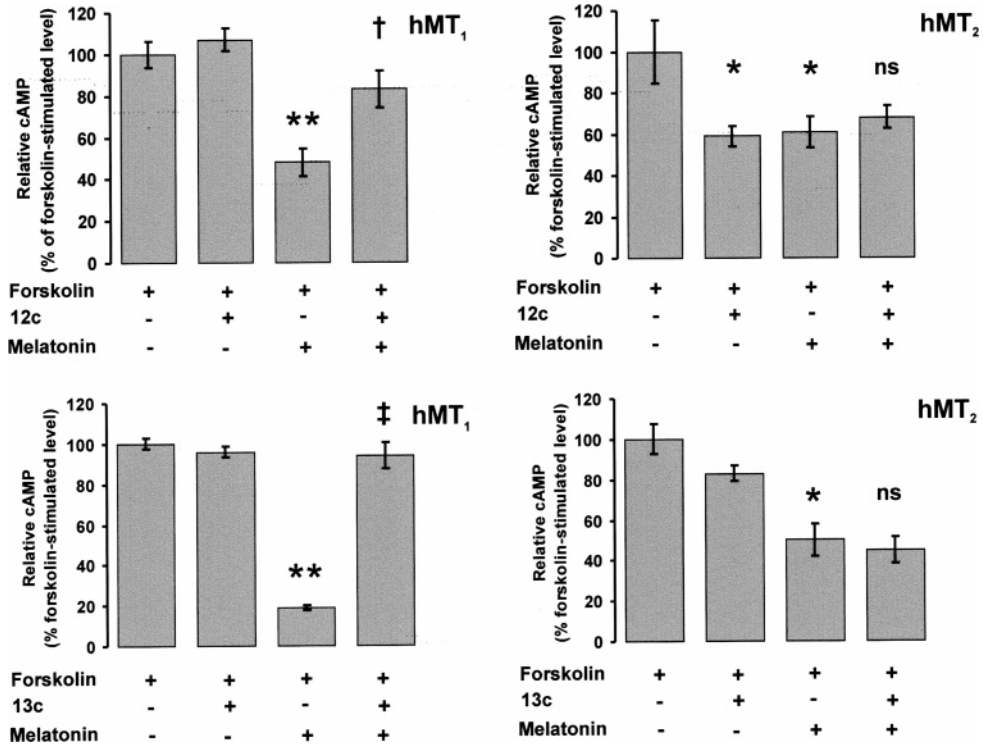


Figure 3. Inhibition of forskolin-induced cyclic AMP synthesis by melatonin, **12c**, and **13c** in NIH 3T3 cells expressing human MT_1 or MT_2 receptors. Cells were treated with 3-isobutyl-1-methylxanthine (IBMX, 25 μM, 10 min) then forskolin (1 μM, 10 min)[14] in the absence or presence of the compounds indicated: melatonin (1 nM), **12c** (1 μM), or **13c** (10 μM). For each cell sample, cyclic AMP was measured in duplicate by radioimmunoassay. Data are expressed as mean ± SEM of quadruplicate cell samples. *$p < 0.05$ compared to forskolin alone. **$p < 0.01$ compared to forskolin alone. †$p < 0.02$ compared to forskolin + melatonin. ‡$p < 0.0001$ compared to forskolin + melatonin.

derivatives from weak ($EC_{50} \sim 1000$ nM) partial agonists (**12a**,**b**) with very low efficacy (8−20%) to a potent ($EC_{50} = 2$ nM) agonist with virtually full efficacy. In two independent experiments, maximal aggregation with **12c** was 88% and 96% of that achieved with melatonin (1 nM). This may indicate that in the cyclobutane series (**12**) the methoxy group is not well accommodated in its binding site, perhaps because of the indole *N*-methyl group, and so cannot readily induce the receptor conformation needed for agonist action. Extra binding affinity obtained at the *N*-acyl bonding site by increasing the length of the alkyl group may allow full receptor activation.

We have examined the potency of **12c** and, initially for comparison, the cyclopentane analogue **13c** at the human MT_1 and MT_2 human receptors using the inhibition of forskolin-induced formation of cyclic AMP as a marker of receptor activation. The results are shown in Figure 3.

In NIH 3T3 cells expressing MT_1 receptors, melatonin (1 nM) inhibited forskolin-stimulated cyclic AMP synthesis but **12c** (1 μM) did not, indicating that **12c** does not have an agonist activity at MT_1 receptors. However, **12c** did block melatonin-induced suppression of cyclic AMP (Figure 3), indicating that it acts as an MT_1 antagonist. At the MT_2 receptor, **12c** (1 μM) acted as an agonist inhibiting cyclic AMP synthesis as effectively as melatonin. Compound **13c** (10 μM) also had no agonist action at MT_1 receptors even at this higher concentration, but it did act as an MT_1 antagonist. Unlike **12c**, **13c** had no significant MT_2 agonist action, nor did it block melatonin inhibition of cyclic AMP. Compounds **6f**, **11c**, **17b**, and **19c** also acted as MT_1 and MT_2 receptor agonists inhibiting forskolin stimulation of cyclic AMP synthesis (data not shown).

Taking *N*-methylmelatonin as the basic structure, these results illustrate the effect of adding methyl and cycloalkyl groups at the β-position. A single β-methyl group (**6a**) decreases the affinity for both human receptors, particularly at MT_1. This effect can largely be attributed to the (−)-enantiomer and illustrates the much greater steric requirement of the MT_1 receptor in this region. The introduction of a second β-methyl group (**6d**) further decreases the affinity, but mainly at the MT_1 receptor. The changes in potency at the *Xenopus* receptor reflect the change in affinity at the MT_1 receptor. The introduction of a three-membered (**11a**) ring drastically reduces the affinity at both human receptors and also the potency on *Xenopus* melanophores. Affinity is also decreased with four- and five-membered rings (**12a**, **13a**), but in these cases the compounds are antagonists on melanophores.

Comparison of the *N*-methyl derivative **6a** with its NH analogue **17a** shows that the methyl group increases affinity ~5-fold at MT_2 while decreasing it ~5-fold at MT_1. The same pattern of effects is observed on comparing **6d** and **17b**. In the case of the cyclobutyl derivatives, introducing the methyl group on the indole nitrogen (**13a**) greatly reduces the affinity to both human receptors from that found in the NH analogue **19a** and converts a melanophore agonist into an antagonist. The related analogue **13c** also acted as an antagonist on human MT_1 receptors (Figure 3) though not at MT_2 receptors despite its ~10-fold higher affinity at the MT_2 site. Compound **13c** is one of the first examples of a MT_1 selective antagonist and warrants further investigation. Further, whereas extending the *N*-acyl side chain (Me < Et < Pr) decreases the affinity of the indole NH compounds **19a**−**c**, extending the side chain with the NMe compounds (**12a**−**c**) dramatically increases affinity. In the melanophore assay, the indole NH compounds (**19a**−**c**) are all agonists while **12a** and **b** show only very weak partial agonist activity with only **12c** having near full efficacy as an agonist. Indeed, **12c** has different effects on the two mammalian

receptors, acting as an antagonist at MT_1 but as an agonist at the MT_2 site.

The four compounds, **11c**, **19c**, **12c,** and **13c**, nicely illustrate the subtle differences between the three receptors, with the small incremental changes in size and possibly shape affecting first the human MT_1 receptor, then the melanophore receptor, while the human MT_2 receptor remains much more tolerant to change. Thus, while all four compounds show a higher binding for MT_2 over MT_1, the cyclopentane derivative **13c** is an antagonist at melanophores while the cyclopropane derivative **11c** and the cyclobutane NH derivative **19c** are agonists.

Some of these compounds, particularly **12c** and **13c**, should be valuable tools for determining whether specific melatonin effects are mediated by the MT_1 or MT_2 receptors. We are currently examining the effects of **12c** and **13c** in a study on the sleep behavior of rats, hoping to establish the role of melatonin in sleep onset and to discover whether it acts through a melatonin receptor.

Experimental Section

Melting points were determined on a Reichert melting point apparatus or in glass capillary tubes on an Electrothermal 9100 apparatus and are uncorrected. EI mass spectra were recorded on a VG ZAB-2F mass spectrometer, CI mass spectra on a VG 12-250 mass spectrometer, and FAB mass spectra on a M550 mass spectrometer. Only molecular ions (M^+) or $M^+ + 1$ ions and base peaks are reported. IR spectra were recorded on Perkin-Elmer 883, PE-983, or 1650 FTIR spectrometers, using KBr pellets unless otherwise stated. NMR spectra were taken in $CDCl_3$ unless otherwise stated. ^{1}H NMR spectra were taken on either a Varian VXR-400 or a Bruker AC 300 spectrometer, and the spectra are reported in δ. ^{13}C NMR spectra were recorded on either a Varian VXR-400 or Bruker AC200 spectrometer and are reported in δ.

DC-Alufolien plates (Kieselgel 60 F_{254}, Schichtdicke 0.2 mm, Merck) were used for analytical TLC and were visualized with ultraviolet light or developed with *p*-anisidine, iodine, or ninhydrin. Flash chromatography was performed using Sorbsil c60-A silica as the stationary phase. Spinning plate chromatography (SPC) was performed in a Chromatotron apparatus (Model 7924), using plates of 4 mm thickness coated with Merck Kieselgel GF_{254} silica gel. Chiral HPLC was performed on a Varian PrepStar 218 instrument using Chiracel AD analytical and semipreparative columns with hexane/ethyl acetate as eluant. Optical rotations were recorded on a Polaar 2000 instrument as solutions in EtOAc. Circular dichroism spectra were determined at the Department of Pharmacy, King's College London, on a Jasco J720 spectropolarimeter as solutions in methanol. Recombinant human MT_1 and MT_2 subtypes expressed in NIH 3T3 cells were kindly provided by Dr. S. M. Reppert (Harvard Medical School, Boston, MA). Microanalyses were carried out by either the Microanalytical Section of the Institute of Organic and Pharmaceutical Chemistry, NHRF, or the Microanalytical Section, Department of Chemistry, UCL.

General Procedure for the Bischler Reaction. A mixture of *N*-methyl-*p*-anisidine (2 equiv) and the α-bromoketone (1 equiv) was stirred under nitrogen at 50 °C for 3 h. The resulting dark mixture was dissolved in propan-2-ol (100 mL/0.1 mol aniline), and $ZnCl_2$ (3 equiv, dried at 25 °C/1 mmHg for 2 d) was added. The mixture was refluxed under N_2 for 16 h. The solvent was removed by evaporation and the residue extracted with a mixture of 2 M HCl and EtOAc (3 × 100 mL HCl/150 mL EtOAc). The red organic layer was washed with water (2 × 100 mL) and saturated Na_2CO_3 solution (2 × 100 mL) and dried ($MgSO_4$). Evaporation of the solvent gave the crude product of sufficient purity for use in subsequent reactions.

General Procedure for Saponification of Esters. The crude product from the Bischler reaction was dissolved in hot 90% aqueous MeOH or EtOH, NaOH (10 equiv) was added, and the mixture refluxed for 6 h. The alcohol was removed by evaporation under reduced pressure and the remaining alkaline solution washed with CH_2Cl_2 (2 × 50 mL). The alkaline mixture was then poured into an excess of ice-cold HCl (10%) when the product precipitated. It was removed by filtration under reduced pressure, washed with water, and then dried at 25 °C/1 mmHg.

General Procedure for the Conversion of Acids to Amides. The carboxylic acid (1 equiv) was dissolved in CH_2Cl_2 (10 mL/g), Et_3N (1.1 equiv) was added, and the mixture was cooled to 0 °C and stirred. After 10 min methyl chloroformate (1.1 equiv) was added dropwise, and stirring was then continued for 30 min at room temperature. Ammonia was then bubbled through the solution for 2 min when a white precipitate formed. The mixture was stirred for 1 h and was then washed with water (20 mL), 2 M HCl (2 × 20 mL), and 2 M NaOH (2 × 20 mL) and dried ($MgSO_4$). The solvent was removed to leave the crude product which was of sufficient purity for subsequent reactions.

General Procedure for the Preparation of Amides. A solution of amine in dry CH_2Cl_2 (1 mL) was treated with triethylamine at 0 °C. The appropriate anhydride or acid chloride was then added dropwise, and the resulting mixture was left stirring at room temperature for 30−60 min (the reaction was monitored by TLC). The reaction mixture was then poured into water, and CH_2Cl_2 was added. The organic layer was washed with H_2O (15 mL) and brine and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to give the crude product, which was purified either by trituration with ethyl acetate or by SPC.

General Procedure for Reduction of Nitriles. A solution of the nitrile in benzene (3 mL) was added dropwise to a well-stirred suspension of LAH in dry ether (15 mL) at 0 °C. After the addition was completed, the mixture was allowed to thaw and then stirred at room temperature for 1 h. The resulting suspension was cooled to 0 °C, H_2O (2 mL) was added cautiously, and stirring continued for 30 min. The reaction mixture was filtered, and the filter cake was washed with ethyl acetate (3 × 30 mL). The filtrate collected was washed with H_2O (15 mL) and brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and the resulting amine then used without further purification.

General Procedure for Reduction of Amides. The amide (1 equiv) was dissolved in anhydrous THF (10 mL/g) and added dropwise to a suspension of LAH (10 equiv) in anhydrous THF (20 mL/g). After completion of addition the mixture was refluxed for 2 h and allowed to cool. Water (2 mL) was then added dropwise, the mixture was filtered, and the residue was washed with EtOAc. The filtrate was washed with water (20 mL) and extracted with 2 M HCl (2 × 20 mL). The aqueous solution was then washed with EtOAc (20 mL), and 2 M NaOH was then added until the reaction mixture was basic. The mixture was extracted with EtOAc (2 × 20 mL) and the organic extracts dried ($MgSO_4$). Removal of the solvent gave the amine as a colorless oil in sufficient purity for subsequent reactions.

2-(5-Methoxy-1-methyl-1*H*-indol-3-yl)propanoic Acid (4a). *N*-Methyl-*p*-anisidine (**2**) (15.50 g, 0.11 mol) was treated with ethyl 4-bromo-2-methyl-3-oxobutyrate (**3a**) (12.10 g, 0.055 mol) and $ZnCl_2$ (22.5 g) according to the general procedure to give a brown oil. This was dissolved in MeOH (50 mL) and saponified with 10% NaOH according to the general procedure to give **4a** (5.20 g, 20 mmol, 36%) as a yellow solid, mp 118−120 °C (lit.[44] 123−124 °C).

2-(5-Methoxy-1-methyl-1*H*-indol-3-yl)-2-methylpropanoic Acid (4b). *N*-Methyl-*p*-anisidine (**2**) (21.90 g, 0.16 mol) was treated with ethyl 4-bromo-2,2-dimethyl-3-oxobutyrate (**3b**) (19.20 g, 0.08 mol) and $ZnCl_2$ (25 g) according to the general procedure to give a brown oil. This was dissolved in MeOH (50 mL) and saponified with 10% NaOH according to the general procedure to give **4b** (5.90 g, 24 mmol, 30%) as a pale brown solid, mp 78−81 °C (lit.[44] 80.5−82 °C).

1-(5-Methoxy-1-methyl-1*H*-indol-3-yl)cyclopentanecarboxylic Acid (4c). *N*-Methyl-*p*-anisidine (**2**) (13.70 g, 0.10 mol) was treated with ethyl 1-bromoacetyl cyclopentane-1-carboxylate (**3c**) (13.10 g, 0.05 mol) and $ZnCl_2$ (15 g) according to the general procedure to give a brown oil. This was dissolved in MeOH (100 mL) and saponified with 10% NaOH according to the general

procedure to give **4c** (7.10 g, 26 mmol, 54%) as a pale brown solid, mp 60−70 °C. ^{1}H NMR δ 1.75 (m, 4H), 2.06−2.09 (m, 2H), 2.63−2.67 (m, 2H), 3.70 (s, 3H), 3.80 (s, 3H), 6.86−6.89 (m, 1H), 6.93 (s, 1H), 7.15−7.17 (m, 2H). ^{13}C NMR δ 23.9, 32.9, 35.8, 53.3, 55.8, 102.7, 110.0, 111.8, 116.0, 126.7, 126.7, 132.8, 153.5, 182.4. IR 3430, 2929, 1715, 1218 cm^{-1}. MS *m*/*e* 273, 228 (100). Anal. ($C_{16}H_{19}NO_3$) C, H, N.

2-(5-Methoxy-1-methyl-1*H*-indol-3-yl)propanamide (5a). Compound **4a** (5.10 g, 22 mmol) was treated with Et_3N (2.20 g, 22 mmol) and methyl chloroformate (2.10 g, 22 mmol) in CH_2Cl_2 by the general procedure to give **5a** (3.50 g, 15 mmol, 68%) as an amorphous solid, mp 80−89 °C. ^{1}H NMR δ 1.63 (d, J = 7.3 Hz, 3H), 3.74 (s, 3H), 3.82 (m, 1H), 3,84 (s, 3H), 5.62−5.68 (br s, 2H), 6.91 (dd, J = 2.4, 8.9 Hz, 1H), 6.98 (s, 1H), 7.02 (d, J = 2.3 Hz, 1H), 7.20 (d, J = 8.8 Hz, 1H). ^{13}C NMR δ 17.7, 32.9, 38.1, 55.9, 100.7, 110.3, 112.4, 113.9, 126.9, 127.0, 132.5, 154.0, 177.8. IR 3410, 2939, 1650, 1492, 1228 cm^{-1}. MS *m*/*e* 232, 188 (100), 97. Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

2-(5-Methoxy-1-methyl-1*H*-indol-3-yl)-2-methylpropanamide (5b). From **4b**, mp 43−51 °C.

1-(5-Methoxy-1-methyl-1*H*-indol-3-yl)cyclopentanecarboxamide (5c). From 4**c**, mp 63−74 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]propyl)acetamide (6a).** Compound **5a** (2.50 g, 11 mmol) was reduced with LAH (3.0 g) as described in the general procedure to give the amine (1.00 g, 4.6 mmol, 42%) as a yellow oil. The oil (0.40 g, 1.8 mmol) was treated with acetic anhydride (0.20 g) by the general procedure to give **6a** (0.25 g, 1.0 mmol, 54%), mp 45−47 °C. ^{1}H NMR δ 1.31 (d, J = 7.0 Hz, 3H), 1.86 (s, 3H), 3.18−3.22 (m, 1H), 3.41−3.48 (m, 2H), 3.62 (s, 3H), 3.80 (s, 3H), 6.39 br t, 1H), 6.78 (s, 1H), 6.83 (dd, J = 2.2, 9.0 Hz, 1H), 7.09 (d, J = 2.3 Hz, 1H), 7.10 (d, J = 8.7 Hz, 1H). ^{13}C NMR δ 18.4, 22.6, 30,5, 32.3, 45.5, 55.4, 100.7, 109.7, 111.3, 116.4, 125.5, 127.0, 132.1, 153.2, 170.3. IR 3305, 2943, 1639, 1498, 1218 cm^{-1}. MS *m*/*e* 260, 201 (100). Anal. ($C_{15}H_{20}N_2O_2$) C, H, N

The racemic mixture (40 mg of **6a**) was dissolved in hexane:2-propanol (9:1, 2 mg/mL) and injected in 0.5 mL aliquots onto a 25 cm × 1 cm Chiral AD semipreparative HPLC column, eluting with hexane:2-propanol (9:1) (**6a**, 20.5 min; **6b**, 22.0 min). Removal of the solvent gave the separate solid enantiomers, each of which was then examined by analytical HPLC for purity.

(+)-**6a**: 17.2 mg; 100%. $[\alpha]_D$ +9.0° (c = 0.0086). (−)-**6a**: 14.0 mg, 97%. $[\alpha]_D$ −8.3° (c = 0.0070).

UV and CD spectra of the enantiomers were acquired on a Jasco J720 spectropolarimeter in the range 400−185 nm as solutions (0.40 mg/mL) in methanol. A 0.02 cm cell path length was used, and all spectra were corrected for solvent baseline and normalized for ϵ and $\Delta\epsilon$. The UV spectra are virtually identical, and the CD spectra are mirror images

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]propyl)propanamide (6b).** From **5a**, 60%, yellow oil.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]propyl)butanamide (6c).** From **5a**, 70%, yellow oil.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2-methylpropyl)acetamide (6d).** From **5b**, 83%, mp 32−35 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2-methylpropyl)-propanamide (6e).** From **5b**, 55%, yellow oil.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2-methylpropyl)butanamide (6f).** From **5b**, 40%, yellow oil.

***N*-(2-(5-Methoxy-1-methyl-1*H*-indol-3-yl)-2-methylpropyl)cyclobutanecarboxamide (6g).** From **5b**, 62%, mp 135−137 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-tetramethyleneethyl)acetamide (13a).** From **5c**, 53%, mp 97−98 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-tetramethyleneethyl)propanamide (13b).** From **4c**, 72%, mp 118−119 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-tetramethyleneethyl)butanamide (13c).** From **5c**, 68%, mp 108−108.5 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-tetramethyleneethyl)cyclopropanecarboxamide (13d).** From **5c**, 84%, mp 134−135 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-tetramethyleneethyl)cyclobutanecarboxamide (13e).** From **5c**, 66%, mp 121−123 °C.

Preparation of 5-Methoxy-1*H*-indole-3-acetonitrile (8b).[41,43] A mixture of diethylamine (0.70 mL, 6.8 mmol), acetic acid (0.90 mL, 16 mmol), aqueous formaldehyde (37%, 0.70 mL, 24.5 mmol), and water (2 mL) was added in one portion to either indole or 5-methoxyindole (6.80 mmol). The mixture was stirred at room temperature for 3 h and was then made alkaline (pH 8) by addition of 10% aqueous NaOH. The mixture was extracted with EtOAc (3 × 5 mL), and the combined organic extracts were washed with brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue used directly without further purification. The residue (1.42 g) was dissolved in MeOH (23 mL) and stirred, and a solution of KCN (1.12 g, 17.3 mmol) in water (2 mL) and then iodomethane (1.30 mL, 20.7 mmol) were added at such a rate that the temperature did not rise above 35 °C. After completion of addition the mixture was stirred at room temperature for 10 h when TLC indicated that the reaction was complete. The solvent was removed under reduced pressure, and the residue was extracted with EtOAc. The extracts were washed with water then brine and dried (Na_2SO_4). The solvent was removed under reduced pressure to give the products as yellow oils. **8b**: 1.08 g, 95%. ^{1}H NMR δ 3.64 (s, 2H), 3.78 (s, 3H), 6.84 (dd, J = 2.2, 8.8 Hz, 1H), 6.95 (m, 2H), 7.16 (d, J = 8.8 Hz, 1H), 8.53 (bs, 1H). ^{13}C NMR δ 13.8, 55.5, 99.4, 103.3, 112.2, 112.5, 118.4, 123.5, 126.0, 131.1, 153.9.

Methylation of 8a,b. Potassium hydroxide 85% (5.45 mmol) was added at 0 °C to a stirred solution of nitrile **8a** or **8b** (5.45 mmol) in DMF (30 mL). Iodomethane (5.98 mmol) was then added dropwise at the same temperature, and the mixture was left stirring for 30 min at ambient temperature. The resulting suspension was then poured into water and extracted with EtOAc. The organic layer was washed with water and brine and dried (Na_2SO_4), and the solvent was removed under reduced pressure to give the crude product, which was triturated with EtOAc.

(1-Methyl-1*H*-indol-3-yl)acetonitrile (9a): 0.86 g, 93%, mp 58−60 °C (lit.[45] 59−60 °C).

(5-Methoxy-1-methyl-1*H*-indol-3-yl)acetonitrile (9b): 0.53 g, 88%, mp 105−106 °C (lit.[45] 104−106 °C).

General Method for the Synthesis of 10a−c. Potassium bis-(trimethylsilyl)amide (3 equiv for **10a**,**c**, 5 equiv for **10b**) was added to a solution of the nitrile **9a**,**b** (2.94 mmol) in dry tetrahydrofuran (23 mL) at 0 °C, under an argon atmosphere. The reaction mixture was then stirred for 3 min at 0 °C, and a solution of either 1,3-dibromopropane (1.2 equiv) or 1,2-dichloroethane (3 equiv) in dry THF (6 mL) was added dropwise. The reaction was stirred until completion (TLC) and then poured into a saturated solution of NH_4-Cl. The aqueous phase was extracted with EtOAc, and the combined organic layers were washed with water and brine and dried (Na_2-SO_4). The solvent was removed under reduced pressure and the product purified by flash column chromatography (diethyl ether/petroleum ether 40−60 °C) (25:75) to give the desired nitriles as off-white solids.

1-Cyano-1-(1-methyl-1*H*-indol-3-yl)]cyclobutane (10a): 58%, mp 80−81 °C. ^{1}H NMR δ 2.19 (m, 1H), 2.40 (m, 1H), 2.75 (m, 2H), 2.90 (m, 2H), 3.77 (s, 3H), 7.02 (s, 1H), 7.39 (m, 3H), 7.72 (d, J = 9.0 Hz, 1H). ^{13}C NMR δ 17.6, 32.7, 33.9, 64.9, 109.6, 113.3, 119.3, 119.6, 121.0, 122.3, 124.2, 125.3, 125.8, 137.6. Anal. ($C_{14}H_{14}N_2$) C, H, N.

1-Cyano-1-(5-methoxy-1-methyl-1*H*-indol-3-yl)cyclopropane (10b): 42%, mp 105−107 °C. ^{1}H NMR δ 1.26−1.36 (m, 2H), 1.60−1.68 (m, 2H), 3.55 (s, 3H), 3.91 (s, 3H), 6.91−6.97 (m, 2H), 7.17−7.25 (m, 2H). ^{13}C NMR δ 18.0, 32.8, 38.8, 55.2, 102.0, 110.3, 112.4, 112.6, 124.1, 125.6, 126.4, 133.2, 154.3. Anal. ($C_{14}H_{14}N_2O$) C, H, N.

1-Cyano-1-(5-methoxy-1-methyl-1*H*-indol-3-yl)cyclobutane (10c): 56%, mp 114−116 °C. ^{1}H NMR δ 2.18 (m, 1H), 2.40 (m, 1H), 2.70 (m, 2H), 2.90 (m, 2H), 3.72 (s, 3H), 3.87 (s, 3H), 6.95 (dd, J = 2.3, 9.0 Hz, 1H) 6.98 (s, 1H), 7.11 (d, J = 2.3 Hz, 1H), 7.21 (d, J = 9.0 Hz, 1H). ^{13}C NMR δ 17.6, 32.9, 33.6, 33.7, 56.0,

101.3, 110.5, 112.4, 112.6, 124.1, 125.6, 126.4, 133.0, 154.1. Anal. ($C_{15}H_{16}N_2O$) C, H, N.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-dimethyleneethyl)acetamide (11a).** The cyanide **10b**[46] was reduced to the corresponding amine by the general method for the reduction of nitriles, and the amine was then acetylated by the general method for the acylation of amines to give **11a**: 48%, mp 114−116 °C. ^{1}H NMR δ 0.77−0.94 (m, 4H), 1.90 (s, 3H), 3.43 (d, J = 5.8 Hz, 2H), 3.72 (s, 3H), 3.88, (s, 3H), 5.22 (bs, 1H), 6.86−6.96 (m, 2H), 7.13−7.28 (m, 2H). ^{13}C NMR δ 12.4, 22.9, 32.5, 46.5, 46.8, 55.5, 102.5, 110.0, 111.2, 118.7, 126.1, 126.6, 133.0, 153.1, 172.8. Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-dimethyleneethyl)propanamide (11b):** 52%, mp 104−105 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-dimethyleneethyl)butanamide (11c):** 62%, mp 110−112 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-trimethyleneethyl)acetamide (12a).** From **10c**,[46] 48%, mp 88−89 °C. ^{1}H NMR δ 1.86 (s, 3H), 1.90−2.46 (m, 6H), 3.70−3.74 (m, 5H), 3.81 (s, 3H), 5.45 (bs, 1H), 6.78−6.95 (m, 2H), 7.18−7.28 (m, 2H). ^{13}C NMR δ 15.7, 23.0, 30.0, 32.5, 46.6, 46.8, 55.5, 102.5, 109.9, 111.5, 118.6, 126.0, 126.6, 132.9, 153.0, 170.0. Anal. ($C_{17}H_{22}N_2O_2$) C, H, N.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-trimethyleneethyl)propanamide (12b):** 46%, mp 93−95 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-trimethyleneethyl)butanamide (12c):** 52%, mp 106−107 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-trimethyleneethyl)cyclopropanecarboxamide (12d):** 46%, mp 120−122 °C.

***N*-(2-[5-Methoxy-1-methyl-1*H*-indol-3-yl]-2,2-trimethyleneethyl)cyclobutanecarboxamide (12e):** 42%, mp 121−123 °C.

***N*-(2-[1-Methyl-1*H*-indol-3-yl]-2,2-trimethylene-ethyl)acetamide (14a).** Compound **10a**[46] was reduced by LAH according to the general procedure and then acetylated according to the general procedure to give **14a**: 52%, mp 89−90 °C. ^{1}H NMR δ 1.89 (s, 3H), 1.96−2.08 (m, 1H), 2.14−2.46 (m, 5H.), 3.79 (d, J = 5.8 Hz, 2H), 3.81 (s, 3H), 5.20 (bs, 1H), 6.85 (s, 1H), 7.06−7.12 (m, 1H), 7.22−7.35 (m, 2H) 7.48−7.54 (m, 1H). ^{13}C NMR δ 15.8, 22.3, 29.8, 33.5, 46.8, 47.8, 101.5, 110.1, 111.4, 118.9, 125.8, 126.4, 133.0, 140.9, 170.9. Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

***N*-(2-[1-Methyl-1*H*-indol-3-yl]-2,2-trimethylene-ethyl)propanamide (14b):** 46%, mp 96−97 °C.

***N*-(2-[1-Methyl-1*H*-indol-3-yl]-2,2-trimethylene-ethyl)butanamide (14c):** 62%, mp 109−110 °C.

***N*-(2-[1-Methyl-1*H*-indol-3-yl]-2,2-trimethylene-ethyl)cyclopropanecarboxamide (14d):** 42%, mp 123−124 °C.

***N*-(2-[1-Methyl-1*H*-indol-3-yl]-2,2-trimethylene-ethyl)cyclobutanecarboxamide (14e):** 48%, mp 125−127 °C.

(5-Methoxy-1-*t*-butoxycarbonyl-1*H*-indol-3-yl)acetonitrile (15). A solution of **8b** (0.37 g, 2.0 mmol) in CH_2Cl_2 (5 mL) was treated with 4-(dimethylamino)pyridine (0.24 g, 2 mmol) and di-*tert*-butyl dicarbonate (0.46 mL, 2 mmol). The mixture was stirred at ambient temperature for 3 h, and the resulting suspension was then poured into water and extracted with EtOAc. The organic layer was washed with water and brine and dried (Na_2SO_4). The solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography to give **15** as a beige solid (0.59 g, 95%), mp 118−120 °C. ^{1}H NMR δ 1.66 (s, 9H), 3.74 (s, 2H), 3.87 (s, 3H,), 6.93 (d, J = 2.4 Hz, 1H), 6.97 (dd, J = 2.4, 9.2 Hz, 1H), 7.60 (s, 1H), 8.05 (d, J = 8.6 Hz, 1H). Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

2-(5-Methoxy-1*H*-indol-3-yl)-2-methylpropanonitrile (16b). A mixture of the acetonitrile **15** (1.22 g, 4.50 mmol) and iodomethane (0.53 mL, 11 mmol) in DMF (9.2 mL) was added dropwise to a stirred slurry of NaH (0.420 g, 10.5 mmol) in DMF (9.2 mL) at 0 °C. The mixture was then allowed to warm to room temperature and stirred for 4 h. The mixture was then treated with saturated aqueous NH_4Cl and extracted with EtOAc, and the organic extract was washed with water and brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue purified by flash chromatography, eluting with cyclohexane/EtOAc (96:4) to give the methylated Boc-derivative as a brown oil (1.02 g, 76%). A solution of (n-Bu)$_4$NF in THF (7.4 mL, 7.4 mmol) was added to a stirred portion of the oil (0.47 g, 1.5 mmol) in THF (10 mL). The mixture was stirred and heated to 65 °C for 8 h and the resulting solution poured onto water (20 mL) and extracted with EtOAc. The organic layer was washed with water and brine and then dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue purified by flash chromatography to give **16b** as an oil (0.31 g, 1.45 mmol). ^{1}H NMR δ 1.78 (s, 6H), 3.84 (s, 3H), 6.88 (dd, J = 2.2, 8.8 Hz, 1H), 7.22 (d, J = 8.8 Hz, 1H), 7.28 (s, 1H), 8.55 (bs, 1H).

2-(5-Methoxy-1*H*-indol-3-yl)propanonitrile (16a). Prepared as for **16b** except that only a slight molar excess of iodomethane was added. The yield was substantially less (10%) than that for **16b**. ^{1}H NMR δ 1.72 (d, J = 6 0.9 Hz, 3H), 3.86 (3H), 3.99 (q, J = 6.9 Hz, 1H), 6.89 (dd, J = 2.2, 8.8 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 7.27 (s, 1H), 8.37 (bs, 1H).

β,β-Dimethylmelatonin (17b). A solution of the nitrile **16b** (0.33 g, 1.55 mmol) in benzene (1.0 mL) was added dropwise to a stirred suspension of LAH (0.18 g, 4.7 mmol) in dry ether (8.0 mL) at 0 °C. The mixture was allowed to warm to room temperature, and stirring continued for a further 45 min. The resulting mixture was cooled to 0 °C and stirred, and water (1.0 mL) was added dropwise followed by EtOAc (10 mL). Stirring was continued for 30 min, and the mixture was then filtered. The residue was washed with EtOAc (3 × 30 mL), and the combined filtrates were washed with water and brine and dried (Na_2SO_4). The solvent was removed under reduced pressure to give the amine as a pale yellow oil that was used without further purification. The amine (0.29 g, 1.33 mmol) was dissolved in dry CH_2Cl_2 (4.0 mL), and the solution was treated with Et_3N (0.30 mL, 2.2 mmol) at 0 °C and stirred. Ac_2O (0.17 mL, 1.2 mmol) was then added dropwise, the mixture allowed to come to room temperature, and stirring continued for 1 h. The reaction mixture was poured into water (20 mL), and CH_2Cl_2 (20 mL) was added. The organic layer was separated, washed with water and brine, and dried (Na_2SO_4), The solvent was removed under reduced pressure and the residue purified by column chromatography, eluting with EtOAc:cyclohexane (95:5) to give **17b** as a buff solid (0.22 g, 62%), mp 120−122 °C. ^{1}H NMR δ 1.40 (s, 6H), 1.84 (s, 3H), 3.63 (d, J = 5.8 Hz, 2H), 3.83 (s, 3H), 5.32 (br t, 5.5 Hz, 1H), 6.85 (dd, J = 2.4, 8.8 Hz, 1H), 6.97 (d, J = 2.4 Hz, 1H), 7.18 (d, J = 2.4 Hz), 7.27 (d, J = 8.8 Hz, 1H), 8.54 (bs, 1H). ^{13}C NMR δ 23.1, 26.1, 35.4, 48.6, 55.7, 103.1, 111.6, 112.2, 120.7, 122.5, 125.6, 132.5, 153.4, 170.4. Anal. ($C_{15}H_{20}N_2O_2$) C, H, N.

β-Methylmelatonin (17a). Prepared from **16a** as for **17b**, pale yellow oil (78%). ^{1}H NMR δ 1.35 (d, J = 7.1 Hz, 3H), 1.87 (s, 3H), 3.23 (m, 1H), 3.42 (m, 1H), 3.61 (m, 1H), 3.84 (s, 3H), 5.44 (bs, 1H), 6.86 (dd, J = 2.4, 8.8 Hz, 1H), 6.99 (d, J = 2.4 Hz, 1H), 7.07 (d, J = 2.4 Hz, 1H), 7.25 (d, J = 8.8 Hz, 1H), 8.05 (bs, 1H). ^{13}C NMR δ 18.7, 23.3, 30.9, 45.6, 55.9, 100.8, 112.1, 112.2, 118.2, 121.5. 126.9, 131.7, 153.8, 170.3. Anal. ($C_{14}H_{18}N_2O_2$) C, H, N

1-(5-Methoxy-1*H*-indol-3-yl)cyclobutanecarbonitrile (18). A solution of 1,3-dibromopropane (0.1 mL, 0.95 mmol) and **15** (0.27 g, 0.95 mmol) in a mixture of DMSO (3.5 mL) and ether (3.5 mL) was added dropwise to a slurry of NaH (2.8 mmol) in DMSO (2.5 mL) at ambient temperature). The resulting mixture was stirred at ambient temperature for 2 h, cooled to 0 °C, and treated with MeOH (3 mL). The cooling bath was removed, and the reaction was stirred at ambient temperature for 20 min. The mixture was extracted with EtOAc, and the organic layer was washed with water and brine and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (petroleum ether 40−60 °C/ethyl acetate) (75:25) to give **18** (0.15 g, 70%) as a brown viscous oil; ^{1}H NMR δ 2.12−2.18 (m, 1H), 2.28−2.44 (m, 1H), 2.62−2.73 (m, 2H), 2.81−2.95 (m, 2H), 3.86 (s, 3H), 6.89 (dd, J = 2.4, 9.2 Hz, 1H), 7.08−7.12 (m, 2H), 7.26 (d, J = 9.0 Hz, 1H), 8.15 (br s, 1H). Anal. ($C_{14}H_{14}N_2O$) C, H, N.

***N*-(2-[5-Methoxy-1*H*-indol-3-yl]-2,2-trimethyleneethyl)acetamide (19a).** Compound **18** was reduced with LAH following the general procedure and acetylated by the general procedure to give

19a, 65%, light yellow viscous oil. ^{1}H NMR δ 1.86 (s, 3H), 1.96−2.44 (m, 6H), 3.75 (d, J = 5.5 Hz, 2H), 3.81 (s, 3H), 5.29 (br s, 1H), 6.86 (dd, J = 2.3, 8.7 Hz, 1H), 6.93 (d, J = 2.2 Hz, 1H), 6.97 (d, J = 2.3 Hz, 1H), 7.23 (d, J = 8.7 Hz, 1H), 8.53 (br s, 1H). ^{13}C NMR δ 16.5, 23.3, 30.8, 41.5, 47.5, 55.9, 101.9, 112.0, 112.2, 120.9, 122.1, 125.7, 132.3, 153.6, 170.5. Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

***N*-(2-[5-Methoxy-1*H*-indol-3-yl]-2,2-trimethylene-ethyl)propanamide (19b):** 55%, light brown viscous oil.

***N*-(2-[5-Methoxy-1*H*-indol-3-yl]-2,2-trimethylene-ethyl)butanamide (19c):** 57%, viscous oil.

***N*-(2-[5-Methoxy-1*H*-indol-3-yl]-2,2-trimethylene-ethyl)cyclopropanecarboxamide (19d):** 40%, light brown viscous oil.

***N*-(2-[5-Methoxy-1*H*-indol-3-yl]-2,2-trimethylene-ethyl)cyclobutanecarboxamide (19e):** 44%, light brown viscous oil.

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Supporting Information Available: Experimental details and ^{1}H NMR and MS spectral data for listed compounds, elemental analysis results, and CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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