Synthesis and Structure–Activity Relationships of Novel Naphthalenic and Bioisosteric Related Amidic Derivatives as Melatonin Receptor Ligands

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A series of *N*-naphthylethyl amide derivatives were synthesized and evaluated as melatonin receptor ligands. The affinity of each compound for the melatonin receptor was determined by binding studies using [2-^{125}I]iodomelatonin on ovine pars tuberalis membrane homogenates. Structure–activity relationships led to the conclusion that naphthalene is a bioisostere of the indole moiety of melatonin. Moreover it appears that the affinity is strongly affected by the size of the substituent of the nitrogen of the amidic function. Many of these ligands give biphasic dose–response curves which suggests that there may be two melatonin receptor subtypes within the ovine pars tuberalis cells. The replacement of naphthalene by benzofuran or benzothiophene did not strongly alter the affinity for the melatonin receptor. In contrast, the benzimidazole analogue was a poor ligand. Compound **7**, the naphthalenic analogue of melatonin, a selective ligand of the melatonin receptor and an agonist derivative, has been selected for clinical development.

Introduction

Melatonin (**1**) (*N*-acetyl-5-methoxytryptamine) is a neurohormone. Its role as a mediator of photoperiod in the regulation of seasonal reproduction is well known,[1] whereas in man its exact functions are not fully elucidated. However, it appears that it may have a role as a chronobiotic. Recent work has suggested its potential usefulness to a number of therapeutic areas such as those related to the desynchronization of biological rhythms, such as jet-lag, disturbed sleep–wake cycles,[2,3] seasonal disorders,[3] and depression.[3] Melatonin may also play a role in the cardiovascular system. This is supported by recent findings which show that [2-^{125}I]iodomelatonin-binding sites are localized in both the caudal and cerebral arteries of the rat.[5,6] A functional role has been demonstrated since melatonin is able to constrict the caudal artery of juvenile rats[7] and modulate the activity of norepinephrine.[6] From these preliminary data, it has been suggested that melatonin could play a role in the regulation of core body temperature or blood pressure and therefore be important to human conditions, such as migraine. In addition, melatonin binding has been reported at many other sites including the retina[8] (probably related to a resynchronization role) and peripheral tissues such as the spleen[9] (related to a role in the immune system), the gastrointestinal tract, blood platelets,[10] and the Harderian gland.[11] These binding data have been shown in several animal species, but their presence and functional role have yet to be demonstrated in humans. Perhaps more contentiously, it has been proposed that melatonin could have a role in degenerative processes, as the synthesis of melatonin is known to diminish with age[12] and the administration of melatonin has been suggested to prolong the life span of mice.[13] Furthermore antioxidant properties of melatonin have recently been proposed.[14] Despite its potential involvement in the regulation in many possible physiological processes, two problems limit its use therapeutically at the present time. The first is its very short biological half-life[15] (15–30 min), due to its rapid catabolism to 6-hydroxymelatonin and *N*-acetylkynurenamines. The second problem is the lack of selectivity of melatonin at its target sites. The development of novel analogues provides a strategic approach to overcome both of these limitations. It is hoped to design new compounds which are not only metabolically more stable but which will also provide basic pharmacological tools with which to define melatonin receptor subtype pharmacology and thereby improve our knowledge of the functions, sites, and mechanisms of action of these compounds. With this double purpose in mind, the synthesis of analogues was carried out in which the indole ring, one of the main targets of the metabolic degradation, was replaced by various bioisosteric modifications. The recent development of a radioligand-binding assay for melatonin receptors using ovine pars tuberalis membranes of the pituitary has enabled the rapid screening of melatonin analogues for their receptor-binding affinity.[16] A preliminary communication disclosed bioequivalency between naphthalene and indole and the excellent affinity of some naphthalene derivatives for the ovine par tuberalis.[17] In the original communication, the binding affinities of all compounds were reported after analysis using a single-site binding model; although for some compounds, it was noted that this did not provide the best mathematical fit of the data. Thus in this study, the binding data of these compounds together with some new compounds have been reanalyzed by 4-parameter logistic regression and/or a 5-parameter regression[18] model which describes biphasic inhibition.

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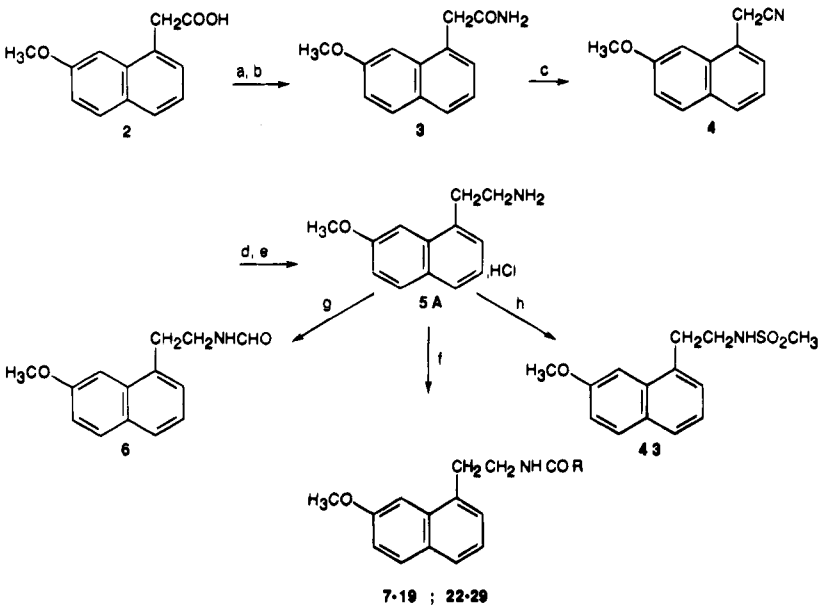
Scheme 1[a]



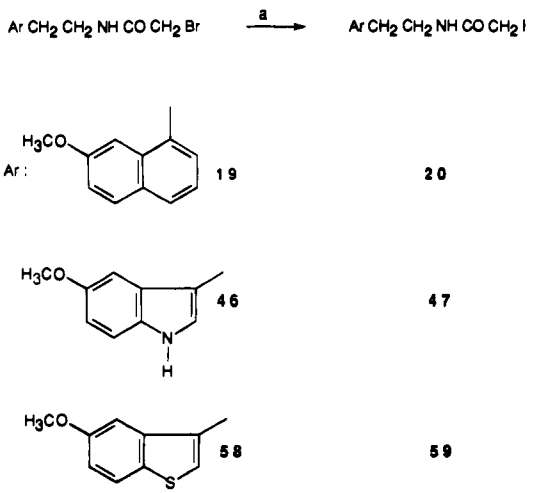
[a] Reagents: (a) $SOCl_2$, $CHCl_3$; (b) NH_4OH, ether; (c) $(CF_3CO)_2O$, THF; (d) H_2, Ni; (e) HCl, ether; (f) RCOCl, K_2CO_3, H_2O, $CHCl_3$; (g) HCOOH; (h) CH_3SO_2Cl, K_2CO_3, H_2O, $CHCl_3$.

In this paper, we report the synthesis of 60 derivatives among which were 38 naphthalenic bioisosteres, most of them including variations on the *N*-acylamino group (**6**–**43**). This group was first selected for chemical modification as it has been reported that the *N*-acetyl substituent of melatonin has a primary importance for the efficacy of binding,[19] whereas modifications of the methoxy group are thought to be of primary importance to biological activity.[19] Moreover, we introduced several variations on the ethylene group of the side chain and replaced the naphthalenic moiety by other bicyclic systems such as benzofuran, benzothiophene, and benzimidazole. Finally some indolic compounds with the most representative substituents introduced in the naphthalenic family were studied to check the equivalence for the melatonin receptor between these two systems. For these families of bicyclic compounds, the synthesis, the binding data for the melatonin receptor, and the potency of the agonistic character are reported.

Chemistry

2-(7-Methoxy-1-naphthyl)ethylamine (**5A**) was prepared from (7-methoxy-1-naphthyl)acetic acid (**2**) according to the procedures previously described[17,20,21] for the corresponding amine. Formamide **6** was conveniently obtained by reaction of **5A** with formic acid. The N-acylated derivatives (**7**–**19**, **22**–**29**) and the sulfonamide **43** were prepared from **5A** (Scheme 1) by treatment with the appropriate acid chloride in the presence of K_2CO_3 as base and a biphasic medium (according to a variant of the Schotten–Baumann procedure[22]). In a similar manner, amides **36**–**38**, **41**, **42**, **44**, **45**, **49**–**58**, and **60** from the previously described corresponding amines[23–27] were obtained. Reaction of the above obtained **19** and **58** or the previously described[28] bromoacetyl compound (**46**) with sodium iodide in acetone afforded (Scheme 2) the corresponding iodo derivatives (**20**, **47**, **59**) with good yields (75–87%). Tertiary amines **30** and **31** were prepared from **19** by treatment with the appropriate amine in acetone and in the presence of triethylamine (Scheme 3). Cyclization of **22** in DMF

Scheme 2[a]



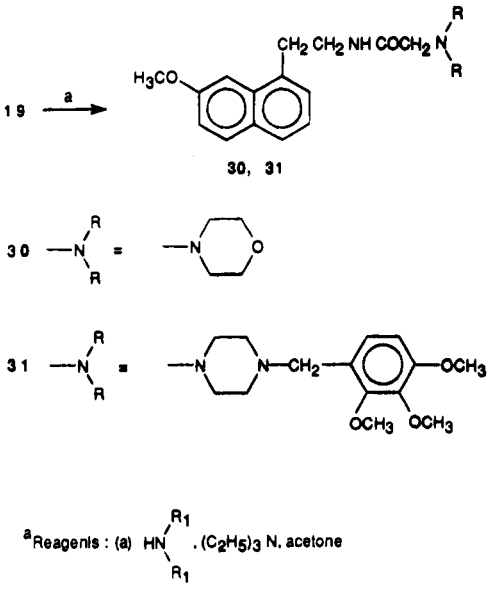
[a] Reagents: (a) NaI, acetone.

and in the presence of EtONa afforded the pyrrolidinone **32** (Scheme 4) with a moderate yield (32%). The trifluoroacetamido compounds (**21**, **39**, **48**) were obtained by treatment of the corresponding amines (**5A**, **5C**, **5F**) with trifluoroacetic anhydride[29] in pyridine (Scheme 5). N-Alkylation[30] of **21** with methyl iodide in DMF and in the presence of K_2CO_3 gave **33** which was hydrolyzed by K_2CO_3 in a methanol–water mixture to afford the secondary amine **5B**. The N-acylated derivatives of **5B** were obtained according to the procedure described for amide **7**. Treatment of amide **7** with HBr gave (Scheme 6), contrary to a previous report,[31] the hydrobromide salt of the amino phenol **5D**, which was acylated at 25 °C in a water–ethyl acetate mixture to afford the phenolic analogue of **7** (**40**).

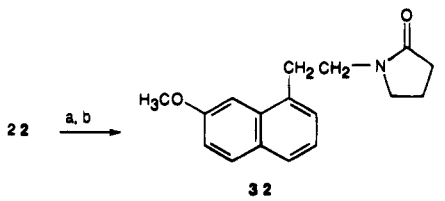
Results and Discussion

For all compounds, the chemical structures and their binding characteristics to melatonin receptors, as well as the biological activity expressed as a cAMP index, obtained by studying the melatonin-mediated inhibition

Scheme 3[a]

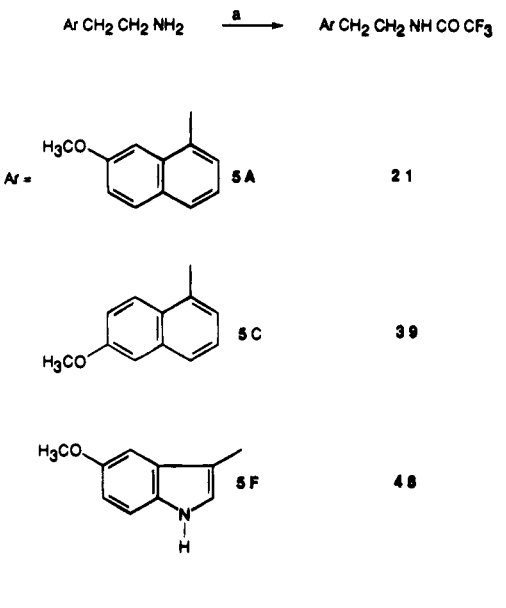


Scheme 4[a]



[a] Reagents: (a) Na, ethanol; (b) DMF, heat.

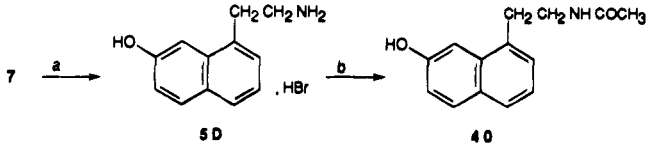
Scheme 5[a]



[a] Reagents: (a) $(CF_3CO)_2O$, pyridine; (b) CH_3I, K_2CO_3, DMF; (c) H_2O, CH_3OH, K_2CO_3; (d) HCl, ethanol.

of forskolin-stimulated cAMP production are shown in Table 1. Binding data are presented in two forms. Firstly after primary analysis using a single-site binding model as described previously:[17] these results are expressed as K_D values. Secondly after analysis of binding data by nonlinear monophasic and biphasic regression models which permitted distinction of the

Scheme 6[a]



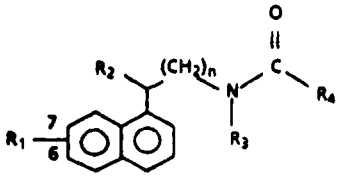
[a] Reagents: (a) HBr; (b) CH_3COCl, K_2CO_3, H_2O, ethyl acetate.

compounds into two categories: (1) compounds that give monophasic inhibition for which IC_{50} values are reported in Table 1 and (2) compounds that give biphasic inhibition for which Table 1 reports $\log_{10} K_H$ (high affinity) and $\log_{10} K_L$ (low affinity).

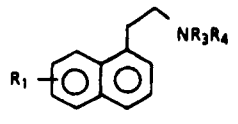
If biphasic curves provide the best fits to the data, then the simplest prediction is that either two melatonin receptor subtypes exist in the ovine pars tuberalis cells or the same receptor has been measured in two different affinity states. As it has been shown in recent studies that melatonin receptors are coupled through two independent and distinct G-proteins, both of which mediate the inhibition of cyclic AMP in pars tuberalis cells,[32] the former of these two possibilities seems the more likely to pertain. Furthermore the large differences in magnitude of the affinities would seem more compatible with distinct sites than with a single receptor in different affinity states. While it is possible for there to be only a single population of receptors which can interact with different G-proteins, it seems more probable that distinct receptors couple to distinct G-proteins. Nevertheless, in either case, the nature of the receptor–G-protein coupling is an important determinant of the efficacy of a ligand as a full or partial agonist.[33] Therefore in this study, the efficacy of a biphasic agonist will represent the combined effects of the drug acting as either a partial or full agonist through each receptor–G-protein-coupled pathway. This would explain why cAMP indices ranging from 0.2 to 1 were obtained. However, to dissect the specific differences in efficacy which are occurring will require full dose–response curves to be made rather than the single-dose bioassays used in this study. It appears from examination of the data in Table 1 that there is little correlation between the structure of a compound and its property as a biphasic ligand for the melatonin receptor, as both poor ligands (such as **11**) as well as compounds having affinity for the melatonin receptor (such as **15**) can give biphasic fits. This is also not dependent on the steric bulk of the substituents since the formamide naphthalenic analogue of melatonin (**6**), the least bulky compound of Table 1, and the bulky *n*-pentyl derivative **11** give biphasic fits.

From study of the structure–affinity relationships, the first important point to note is that the indole nucleus in compound **1** can be substituted by naphthalene without loss of binding potency. However structural variations of the acylamino group of the naphthalene family causes major changes in the affinity for the melatonin receptor. Increasing the chain length of the linear acyl substituent (ethyl (**8**), *n*-propyl (**9**), *n*-butyl (**10**)) results in a corresponding increase in binding affinity. Similarly substitution of the lower alkyl C_1 or C_3 groups by one (**19**, **20**, **22**) or several (**21**) halogen atoms slightly increases the affinity for the melatonin receptor. However further homologation as seen with **11** (*n*-pentyl) or **12** (*n*-hexyl) results in lowered affinity. When cycloalkyl substituents were used, the best af-

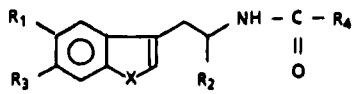
Table 1. Binding and Agonistic Properties of Analogues of Melatonin[a]



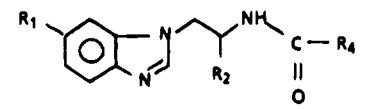
6-42



43



1; 44-59



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compd	R_1	R_2	n or x	R_3	R_4	K_D, mean ± SD	monophasic IC_{50} ± SD (M)	biphasic $\log_{10} K_H$ ± SD	biphasic $\log_{10} K_L$ ± SD	cAMP ± SEM, activity index
1	OCH_3	H	NH	H	CH_3	(9.15 ± 3.98) E-11	(1.57 ± 0.22) E-10			1.00
6	7-OCH_3	H	1	H	H	(4.05 ± 1.93) E-11		−13.14 ± 0.61	−8.11 ± 0.52	0.98 ± 0.32
7	7-OCH_3	H	1	H	CH_3	(1.00 ± 0.35) E-10	(7.61 ± 3.13) E-11			1.01 ± 0.22
8	7-OCH_3	H	1	H	C_2H_5	(2.19 ± 0.90) E-11		−11.54 ± 0.05	−8.71 ± 0.07	1.02 ± 0.22
9	7-OCH_3	H	1	H	n-C_3H_7	(6.15 ± 0.18) E-12	(3.45 ± 2.43) E-12			1.02 ± 0.35
10	7-OCH_3	H	1	H	n-C_4H_9	(3.43 ± 2.43) E-12		−11.22 ± 0.26	−7.03 ± 0.27	1.03 ± 0.23
11	7-OCH_3	H	1	H	n-C_5H_{11}	(2.80 ± 0.20) E-07		−9.09 ± 0.22	−5.69 ± 0.09	0.58 ± 0.07
12	7-OCH_3	H	1	H	n-C_6H_{13}	(2.60 ± 1.05) E-06	>E-05			0.18 ± 0.03
13	7-OCH_3	H	1	H	i-C_3H_7	(2.29 ± 0.75) E-09		−12.20 ± 0.18	−7.90 ± 0.11	1.01 ± 0.19
14	7-OCH_3	H	1	H	$CH{=}CHCH_3$	(8.70 ± 0.34) E-09		−14.49 ± 0.03	−7.15 ± 0.03	1.00 ± 0.19
15	7-OCH_3	H	1	H	C_3H_5	(4.18 ± 0.87) E-13		−13.17 ± 0.07	10.36 ± 0.23	1.02 ± 0.18
16	7-OCH_3	H	1	H	C_4H_7	(2.42 ± 0.54) E-08		−12.38 ± 0.23	−6.91 ± 0.17	0.52 ± 0.11
17	7-OCH_3	H	1	H	C_5H_9	(1.90 ± 1.16) E-07	(3.07 ± 1.04) E-07			0.48 ± 0.12
18	7-OCH_3	H	1	H	C_6H_{11}	(1.59 ± 0.79) E-07	(4.38 ± 0.47) E-07			0.39 ± 0.10
19	7-OCH_3	H	1	H	CH_2Br	(8.00 ± 0.74) E-13	(1.18 ± 1.72) E-12			1.05 ± 0.30
20	7-OCH_3	H	1	H	CH_2I	(1.55 ± 1.03) E-11		−12.56 ± 0.04	−8.93 ± 0.15	1.00 ± 0.33
21	7-OCH_3	H	1	H	CF_3	(2.70 ± 0.76) E-13	<E-12			0.99 ± 0.22
22	7-OCH_3	H	1	H	n-C_3H_6Cl	(1.64 ± 0.06) E-11	(1.53 ± 0.10) E-11			1.03 ± 0.34
23	7-OCH_3	H	1	H	C_6H_5	(4.99 ± 4.36) E-06	(2.51 ± 0.34) E-06			0.04 ± 0.02
24	7-OCH_3	H	1	H	(3,5-diClC_6H_3)	(1.13 ± 0.86) E-05	(9.94 ± 1.58) E-06			0.43 ± 0.17
25	7-OCH_3	H	1	H	(2-indolyl)	(1.17 ± 0.78) E-06	(2.69 ± 0.64) E-06			0.28 ± 0.11
26	7-OCH_3	H	1	H	$CH_2C_6H_5$	(1.96 ± 0.04) E-06	(9.79 ± 3.12) E-06			0.03 ± 0.01
27	7-OCH_3	H	1	H	$CH(C_6H_5)_2$	(3.76 ± 2.90) E-06	(1.02 ± 1.09) E-05			NC
28	7-OCH_3	H	1	H	$(CH_2)_2C_6H_5$	(9.62 ± 1.00) E-07	(2.61 ± 0.48) E-06			0.00 ± 0.12
29	7-OCH_3	H	1	H	$(CH_2)_3C_6H_5$	(1.20 ± 0.28) E-07	(2.44 ± 1.72) E-06			0.11 ± 0.05
30	7-OCH_3	H	1	H	CH_2-4-morph	(1.61 ± 0.10) E-07	(3.13 ± 2.52) E-07			0.63 ± 0.20
31	7-OCH_3	H	1	H	CH_2-TMZ	(1.05 ± 0.10) E-06	(2.99 ± 0.33) E-06			0.30 ± 0.07
32	7-OCH_3	H	1	NR_3R_4 = 2-pyrr		(0.53 ± 2.13) E-07	(9.89 ± 0.27) E-08			
33	7-OCH_3	H	1	CH_3	CF_3	(7.13 ± 1.30) E-08		−12.58 ± 0.35	−6.71 ± 0.09	0.68 ± 0.21
34	7-OCH_3	H	1	CH_3	CH_3	(2.00 ± 0.65) E-08	(2.58 ± 1.78) E-08			0.97 ± 2.00
35	7-OCH_3	H	1	CH_3	C_4H_7	(1.73 ± 0.95) E-07	(1.31 ± 0.56) E-06			0.04 ± 0.01
36	6-OCH_3	H	1	H	CH_3	(4.87 ± 0.19) E-08	(1.23 ± 0.16) E-07			0.92 ± 0.26
37	6-OCH_3	H	1	H	C_3H_5	(9.14 ± 0.07) E-08		−12.35 ± 0.29	−6.76 ± 0.09	0.68 ± 0.24
38	6-OCH_3	H	1	H	C_4H_7	(5.88 ± 0.19) E-08	(1.86 ± 0.05) E-06			0.11 ± 0.04
39	6-OCH_3	H	1	H	CF_3	(1.05 ± 0.62) E-06	(1.88 ± 1.53) E-06			0.62 ± 0.30
40	7-OH	H	1	H	CH_3	(6.46 ± 2.35) E-08	(2.43 ± 1.88) E-07			0.89 ± 0.54
41	7-OCH_3	$COOCH_3$	1	H	CH_3	(7.10 ± 1.81) E-09	(3.14 ± 0.93) E-08			0.43 ± 0.15
42	7-OCH_3	$COOCH_3$	1	H	C_3H_5	(7.98 ± 3.10) E-08		−11.25 ± 2.76	6.41 ± 0.31	0.20 ± 0.05
43	7-OCH_3			H	SO_2CH_3	(1.35 ± 0.48) E-08		−13.19 ± 0.45	−7.21 ± 0.12	0.72 ± 0.41
44	OCH_3	H	NH	H	C_3H_5	(4.53 ± 0.80) E-13	(5.00 ± 0.00) E-14			1.00 ± 0.15
45	OCH_3	H	NH	H	C_4H_7	(7.30 ± 3.10) E-09		−10.37 ± 0.02	−6.94 ± 0.06	0.98 ± 0.17
47	OCH_3	H	NH	H	CH_2I	(5.20 ± 3.20) E-10	(9.80 ± 13.50) E-10			0.97 ± 0.34
48	OCH_3	H	NH	H	CF_3	(2.01 ± 0.99) E-10		−13.30 ± 0.29	9.13 ± 0.21	1.05 ± 0.40
49	OCH_3	H	NH	F	n-C_3H_7	(4.10 ± 1.80) E-11		−13.29 ± 0.03	−10.05 ± 0.10	0.97 ± 0.31
50	OCH_3	H	NH	F	C_3H_5	(2.30 ± 1.60) E-09	(1.89 ± 0.85) E-09			1.00 ± 0.22
51	OCH_3	H	O	H	CH_3	(8.59 ± 4.18) E-10	(1.70 ± 1.04) E-09			1.45 ± 0.49
52	OCH_3	H	O	H	n-C_4H_9	(1.69 ± 2.35) E-08	(5.82 ± 3.60) E-09			1.31 ± 0.31
53	OCH_3	H	O	H	C_3H_5	(4.11 ± 3.59) E-09	(5.45 ± 3.09) E-09			1.45 ± 0.39
54	OCH_3	H	S	H	CH_3	(5.60 ± 2.30) E-10	(1.20 ± 1.57) E-09			0.98 ± 0.35
56	OCH_3	H	S	H	C_3H_5	(1.94 ± 0.33) E-09	(4.01 ± 0.65) E-09			1.01 ± 0.27
57	OCH_3	H	S	H	C_4H_7	(4.58 ± 0.99) E-08	(4.69 ± 3.26) E-08			0.00 ± 0.04
59	OCH_3	H	S	H	CH_2I	(2.33 ± 1.11) E-09	(3.87 ± 1.85) E-09			0.81 ± 0.57
60	OCH_3	H		H	CH_3	(1.70 ± 5.26) E-06	(5.26 ± 1.45) E-06			0.52 ± 0.20

[a] TMZ: 1-(2′,3′,4′-trimethoxybenzyl)piperazine. 2-pyrr: 2-pyrrolidinone. morph: morpholino. NC: not carried out.

finity was obtained with the cyclopropyl derivative **15**, whereas replacement by the bulkier cyclobutyl (**16**), cyclopentyl (**17**), and cyclohexyl (**18**) substituents decreased the affinity for the melatonin receptor. No strong modification in affinity results from branching of the side chain substitution (**13**).

Moreover it appears that unsaturated acyl substituents increase the affinity for the melatonin receptor (compound **14**, the most potent $\log_{10} K_H$ value: −14.49). The replacement of the amidic functionality by a sulfamide group bioisostere (**43**) also results in an increase of affinity ($\log_{10} K_H$: −13.19). On the contrary, introduction of larger aromatic groups (**23**, **25**) or optionally substituted (**24**), phenylalkyl (**26**, **28**, **29**), diphenylalkyl (**27**), or tertiary aminoalkyl (**30**, **31**) groups strongly decreases affinity. These results clearly confirm the crucial role played by the *N*-acyl side chain on the binding affinity for the melatonin receptors. The enhanced affinity displayed by compounds **9**, **14**, **15**, and **24** and the decreased affinity shown by compounds **11**,

12, **17**, and **18** suggest that there is an optimal size for the acyl groups. This predicts that the receptor site has a hydrophobic pocket of relatively small size which is important to ligand binding.

Another modification concerns the introduction of a methyl group on the nitrogen of the amidic function (**33**–**35**). In all the cases, this *N*-methylation decreases the binding for the melatonin receptor. The loss of affinity can be considerable: **34** has an IC_{50} value of 2.58 E-08 instead of 7.61 E-11 for the nonmethylated analogue **7**.

The displacement of the methoxy groups from the 7 to the 6 position also results in a decrease of affinity. The loss of affinity depends on the substituent of the amidic function and can be dramatic; for instance, the 6-OCH_3 derivative **39** has a 1.88 E-06 IC_{50} instead of an IC_{50} value < E-12 for the corresponding 7-OCH_3 analogue. This decrease in affinity can be less significant: $\log_{10} K_H = -12.35$ for the 6-OCH_3 cyclopropyl derivative instead of $\log_{10} K_H = -13.17$ for the 7-OCH_3 analogue. The replacement of the 7-OCH_3 by a 7-OH also strongly decreases the affinity: the IC_{50} of **40** has the 2.43 E-07 value instead of 7.61 E-11 for the 7-OCH_3 compound.

The last modification carried out in this work was the ramification of the ethylene moiety of the side chain that also resulted in a decreased affinity. Compound **41** has an IC_{50} value of 3.14 E-08 instead of 7.61 E-11 for the analogue **7** without a substituent on the ethylene moiety of the side chain. For **42**, $\log_{10} K_H = -11.25$, and analogue **15** without substitution on the ethylene moiety of the side chain has a $\log_{10} K_H = -13.17$.

These results indicate the particular importance of some structural features in this family of molecules. Actually the secondary amide site as well as the methoxy group fixed on the heterocyclic system seems to have an important part in the binding of the molecule to the melatonin receptor. Moreover it appears that a supplementary bulk due to the ramification of the side chain is unfavorable to the binding of the corresponding molecules to the melatonin receptor.

The most significant structure–affinity relationship results previously reported for the naphthalene family can be compared with others we obtained, where the indole ring of melatonin was replaced by other bicyclic systems. The benzofuran (**51**) and benzothiophene (**54**) analogues of melatonin are good ligands for the melatonin receptor even if they have slightly lower affinity for the melatonin receptor than melatonin itself and the naphthalene bioisostere. In contrast, the benzimidazole analogue (**60**) is a poor ligand of the melatonin receptor. In the naphthalene series, among the most potent compounds was that obtained for the cyclopropyl derivative (**15**). However, this substituent did not increase the potency of the corresponding compounds in either the benzothiophene (**56**) or benzofuran (**53**) series when compared with the methyl compound. On the contrary, in the indole family, replacement of the methyl substituent of the amidic function by a cyclopropyl (**44**) increases the affinity for the melatonin receptor 100-fold, just as with the cyclopropyl naphthalene derivative (**15**). This observation further emphasizes the ability of naphthalene to fully substitute for the indole nucleus.

The number of convenient biological systems available for the routine analysis of novel drugs for either agonist or antagonist activity at the melatonin receptor are few.[34] The cAMP activity index (Table 1) is a biological index which has been designed around the well-documented inhibition of forskolin-stimulated cAMP by melatonin that can be measured in ovine pars tuberalis cells.[35] The cAMP index is calculated from the following formula:

$$\text{cAMP index} = ([F]-[F/D])/([F]-[F/M]), \text{ where}$$

$[F]$ = cAMP levels in cells stimulated with 1 μM forskolin

$[F/D]$ = cAMP levels in cells stimulated with 1 μM forskolin in the presence of 1 or 10 μM drug

$[F/M]$ = cAMP levels in cells stimulated with 1 μM forskolin in the presence of 1 or 10 nM melatonin

As the index is calculated from the activity of the drug at only a single concentration, it provides only a semi-quantitative, yet nonetheless reliable, estimate of the biological activity of a given drug. A compound which fully mimics the maximal activity of melatonin has an index of 1, the same as melatonin, and thus the compound can be considered a full agonist of the melatonin receptor. Of the 60 compounds tested, 24 displayed an activity index of 0.8–1 and are therefore unambiguously full agonists. These compounds include compound **7**, the naphthalenic analogue of melatonin, and also compound **54**, the benzothiophene analogue.

In contrast to these compounds, some analogues display activities of 0 or somewhere intermediate between 0 and 1. Those compounds with an index of 0 have no intrinsic activity at the melatonin receptor at the concentration tested (10 μM). This could be due to a number of reasons. Firstly they have low affinity for the melatonin receptor, secondly they are antagonists, and thirdly they recognize a receptor subtype whose activity is not mediated via cAMP. As none of the compounds tested were able to block the inhibitory effect of 1 nM melatonin, despite a 10^4 molar excess of drug, it seems unlikely that any of these drugs displaying low intrinsic activity are antagonists. However, the two other alternative explanations cannot be excluded. For those compounds with intermediate indices, between 0 and 1, they are either full agonists with low affinity for the melatonin receptor or, alternatively, partial agonists with lower efficacy than melatonin. An example of the latter is compound **16**, which has moderate affinity for the receptor (24 nM) but only an index of 0.52, whereas the benzimidazole analogue **60** has the same cAMP index but much poorer affinity (5 μM) and therefore may be a full agonist.

Three of the analogues, each benzofuran derivatives, produced cAMP indices of greater than 1. The reason for this effect is unclear but could indicate an activity by a mechanism other than, or in addition to, that through the melatonin receptor. Another hypothesis would be that these compounds have greater intrinsic activity at the melatonin receptor than melatonin itself dispite lower affinity.

In conclusion, it has been demonstrated that the indole ring of melatonin is not an essential characteristic of the molecule for either its affinity for the melatonin receptor or its biological activity, as it can be replaced by a naphthalene bioisostere. While substitution of the

amide in the indolic ring by either S (benzothiophene) or O (benzofuran) can be tolerated, they both reduce binding affinities to some extent, and the latter substitution elicits effects which cannot be presently explained. Homologous extension of the *N*-acetyl side chain of the naphthalenic analogue together with other modifications can increase the affinity of the compounds for the melatonin receptor over that of melatonin itself. Furthermore some of these modifications have produced analogues which show biphasic rather than monophasic binding curves. Such data would be consistent with either the presence of two distinct receptor subtypes in ovine pars tuberalis cells or the detection of the receptor in two different affinity states. In preclinical studies, two of these analogues have been used for the reentrainment of sleep–wake cycles[36,37] (compound **7**) and for the restoration of the body core temperature rhythms in old rats[38] (compound **8**). Compound **7** is the naphthalenic analogue of melatonin and is currently under clinical development.

Experimental Section

Chemistry. Melting points were determined on a Buchi 510 capillary apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 297 spectrophometer. ^{1}H NMR spectra were recorded on a WP 80-54 or AC 300 Brucker spectrometer. Chemical shifts are reported in δ units (parts per million) relative to $(CH_3)_4Si$. Elemental analyses for new substances were performed by CNRS Laboratories (Vernaison, France). Obtained results were within 0.4% of the theoretical values.

2-(7-Methoxy-1-naphthyl)ethylamine, Hydrochloride (5A). An NH_3-oversaturated solution of 2 g (0.01 mol) of **4** in 80 mL of ethanol was hydrogenated over Raney nickel under pressure (50 bars) at 60 °C for 15 h. After filtration and evaporation, the oil was dissolved in dry ether and treated with HCl gas to give, after filtration and crystallization from ethanol, 2.1 g (88%) of **5A**, hydrochloride: mp 215 °C; ^{1}H NMR (80 MHz, DMSO-d_6) δ 8.5 (s, 3H), 7.9–7.1 (m, 6H), 4 (s, 3H), 3.45 (m, 2H), 3.1 (m, 2H). Anal. ($C_{13}H_{16}ClNO$) C, H, N, O.

General Procedure for the Synthesis of the N-Acylated Derivatives (7–19, 22–29, 34–38, 41, 42, 44, 45, 49–58, 60). The method adopted for the synthesis of *N*-[2-(7-methoxy-1-naphthyl)ethyl]acetamide (**7**) is described. Potassium carbonate (0.02 mol) was added to a solution of 2.38 g (0.01 mol) of **5A** in 40 mL of water and 60 mL of chloroform. The mixture was cooled to 0 °C; 0.78 g (0.01 mol) of acetyl chloride was added dropwise at this temperature. The reaction mixture was then stirred at room temperature for 30 min. The organic phase was separated, washed with water, dried, and evaporated under reduced pressure. The residue was crystallized from toluene/hexane, 2:1, affording 2.16 g (89%) of **7**: mp 109–110 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 7.8–7.1 (m, 6H), 5.7 (s, 1H), 3.95 (s, 3H), 3.6 (m, 2H), 3.25 (t, 2H, 1.9 (s, 3H). Anal. $C_{15}H_{17}NO_2$) C, H, N.

***N*-[2-(7-Methoxy-1-naphthyl)ethyl]formamide (6).** **5A** (2.37 g, 0.01 mol) and 0.92 g (0.02 mol) of formic acid were heated at 120 °C until the obtainment of a solid residue, which was then recrystallized from toluene/hexane, 3:1, giving 2.05 g (89%) of **6**: mp 93 °C; ^{1}H NMR (80 MHz, DMSO-d_6) δ 8.15 (s, 1H), 7.80–7.10 (m, 6H), 4.05 (s, 3H) 3.65 (m, 2H), 3.25 (m, 2H), 3.25 (m, 2H). Anal. ($C_{14}H_{15}NO_2$) C, H, N.

***N*-[2-(7-Methoxy-1-naphthyl)ethyl]methanesulfonamide (43).** Methanesulfonyl chloride (2.55 g, 0.022 mol) was added dropwise at 0 °C to a vigorously stirred mixture of 4.75 g (0.02 mol) of **5A**, hydrochloride, 100 mL of chloroform, 5.50 g (0.04 mol) of potassium carbonate, and 80 mL of water. After 1 h of stirring at room temperature, the organic layer was separated, washed with water, dried, and evaporated under reduced pressure to afford a solid residue, which was recrystallized from toluene, giving 4.6 g (82%) of **43**: mp 97–98 °C; 1H NMR (80 MHz, DMSO-d_6) δ 7.9–7.2 (m, 6H), 3.95 (s, 3H), 3.30 (m, 4H), 2.90 (s, 3H). Anal. ($C_{14}H_{17}NO_3S$) C, H, N.

General Procedure for the Synthesis of the (Iodoacyl)amino Derivatives (20, 47, 59). The method adopted for the synthesis of *N*-[2-(7-methoxy-1-naphthyl)ethyl]-2-iodoacetamide (**20**) is described. A solution of 3.22 g (0.01 mol) of the bromo compound **19** in 50 mL of anhydrous acetone was treated with 1.5 g (0.01 mol) of sodium iodide, and the whole was heated at reflux for 1 h. After cooling, the reaction mixture was filtered and evaporated. The residue was then crystallized from ethanol, affording 3.2 g (87%) of **20**: mp 110–112 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 7.8–7.1 (m, 6H), 6.2 (s, 1H), 3.95 (s, 3H), 3.65 (m, 4H), 3.2 (t, 2H). Anal. ($C_{15}H_{16}ONO_2$) C, H, N.

General Procedure for the Synthesis of the 2-Tertiary [(Aminoacyl)amino]alkyl Derivatives (30, 31). The method adopted for the synthesis of *N*-[2-(7-methoxy-1-naphthyl)-ethyl]-2-morpholin-1′-ylacetamide (**30**) is described. A mixture of 0.87 g (0.01 mol) of morpholine, 1.21 g (0.012 mol) of triethylamine, and 3.22 g (0.01 mol) of **19** was refluxed for 1 h. After cooling, the reaction mixture was filtered and evaporated. The residue was taken off with 10% NaOH until pH 8–9, filtered, washed with water, dried, and crystallized from toluene/cyclohexane, 2:1, affording 2.98 g (91%) of **30**: mp 114–115 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 7.8–7.1 (m, 6H), 3.95 (s, 3H), 3.6 (m, 2H), 3.5 (t, 4H), 3.25 (t, 2H), 2.90 (s, 2H), 2.4 (t, 4H). Anal. ($C_{19}H_{24}N_2O_3$) C, H, N.

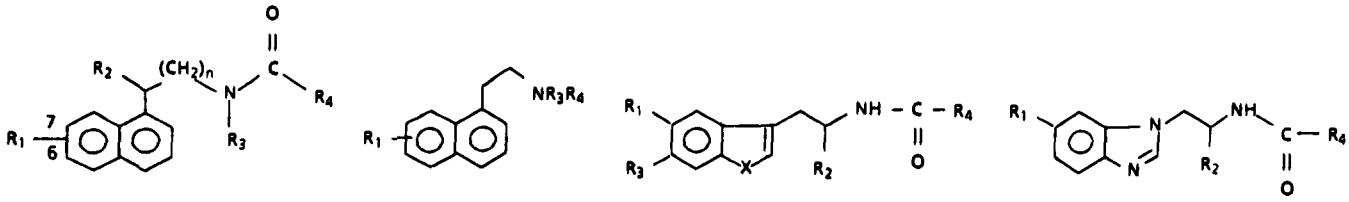
***N*-[2-(7-Methoxy-1-naphthyl)ethyl]-2-oxopyrrolidine (32).** **22** (3.05 g, 0.011 mol) was added to a solution of 0.23 g (0.01 g-atom) of sodium in 50 mL of absolute ethanol. The mixture was stirred for 20 min and then evaporated. The obtained residue was dissolved in 40 mL of anhydrous dimethylformamide and the solution refluxed for 7 h. After filtration and evaporation of the filtrate, the residue was taken off with water and extracted with ether. The organic phase, dried and evaporated under reduced pressure, afforded a solid residue that was recrystallized from petroleum ether, giving 0.86 g (32%) of **32**: mp 60–61 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 7.80–7.10 (m, 6H), 4.00 (s, 3H), 3.60 (m, 2H), 3.25 (m, 4H), 2.35 (t, 2H), 2.00 (m, 2H). Anal. ($C_{17}H_{19}NO_2$) C, H, N.

General Procedure for the Synthesis of the 2-[*N*-(Trifluoroacetyl)amino]ethyl Derivatives (21, 39, 48). The method adopted for the synthesis of *N*-[2-(7-methoxy-1-naphthyl)ethyl]trifluoroacetamide (**21**) is described. A suspension of 2.38 g (0.01 mol) of **5A** in 6 mL of pyridine was cooled to 5 °C. Trifluoroacetic anhydride (2.1 g, 0.01 mol) was then added dropwise at this temperature. After stirring for 30 min at room temperature, the solution was poured into ice water. The resulting precipitate was filtered, washed with water, and crystallized from cyclohexane/toluene, affording 2.58 g (87%) of **21**: mp 91–92 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 7–7.8 (m, 6H), 6.4 (s, 1H), 3.9 (s, 3H), 3.6 (m, 2H), 3.25 (t, 2H). Anal. ($C_{15}H_{14}F_3NO_2$) C, H, N.

***N*-Methyl-*N*-[2-(7-methoxy-1-naphthyl)ethyl]trifluoroacetamide (33).** Potassium carbonate (5.52 g, 0.04 mol) was added to a solution of 2.97 g (0.01 mol) of **21** in 60 mL of DMF. The mixture was refluxed for 1 h, and then 1.56 g (0.011 mol) of methyl iodide was added. Reflux was continued for 2 h. After cooling, the reaction mixture was filtered and evaporated and the residue extracted with ether. After drying, the organic phase was evaporated to afford crude **33** which was purified first by trituration with petroleum ether and then, after filtration and evaporation, by column chromatography (SiO_2, ethyl acetate) to give 2.5 g (80%) of pure **33**: ^{1}H NMR (80 MHz, DMSO-d_6) δ 7.9–7.05 (m, 6H), 4–3.95 (m, 3H), 3.7–3.6 (m, 2H), 3.3–3.2 (m, 2H), 3.0 (m, 3H). Anal. ($C_{16}H_{16}F_3NO_2$) C, H, N.

2-(7-Methoxy-1-naphthyl)-*N*-methylethylamine, Hydrochloride (5B). **33** (3.11 g, 0.01 mol) was dissolved in 50 mL of methanol; 4.14 g (0.03 mol) of K_2CO_3 dissolved in 30 mL of water was added, and the mixture was heated at reflux for 1 h. Methanol was then evaporated and the residue extracted with ethyl acetate. The organic phase was concentrated by evaporation. The residue was dissolved in absolute ethanol and treated with dry HCl gas. The hydrochloride salt was filtered and crystallized from absolute ethanol, affording 2.01 g (80%) of **5B**, HCl: mp 165–167 °C; ^{1}H NMR (80 MHz,

Table 2. Physical Properties of Analogues of Melatonin



	6-42			43		1; 44-59			60
compd	R_1	R_2	*n* or x	R_3	R_4	mp °C	recrys solvent	% yield	formula
6	7-OCH_3	H	1	H	H	93	toluene–hexane	89	$C_{14}H_{15}NO_2$
7	7-OCH_3	H	1	H	CH_3	109–110	toluene–hexane	92	$C_{15}H_{17}NO_2$
8	7-OCH_3	H	1	H	C_2H_5	103	toluene–hexane	89	$C_{16}H_{19}NO_2$
9	7-OCH_3	H	1	H	*n*-C_3H_7	99	toluene–hexane	87	$C_{17}H_{21}NO_2$
10	7-OCH_3	H	1	H	*n*-C_4H_9	90	cyclohexane	92	$C_{18}H_{23}NO_2$
11	7-OCH_3	H	1	H	*n*-C_5H_{11}	84–85	toluene–hexane	91	$C_{19}H_{25}NO_2$
12	7-OCH_3	H	1	H	*n*-C_5H_{13}	68–70	toluene–hexane	91	$C_{20}H_{27}NO_2$
13	7-OCH_3	H	1	H	*i*-C_3H_7	77–78	diisopropyl ether	91	$C_{17}H_{21}NO_2$
14	7-OCH_3	H	1	H	$CH{=}CHCH_3$	119–120	toluene–cyclohexane	86	$C_{17}H_{19}NO_2$
15	7-OCH_3	H	1	H	C_3H_5	91–92	cyclohexane	90	$C_{17}H_{19}NO_2$
16	7-OCH_3	H	1	H	C_4H_7	75–76	toluene–hexane	86	$C_{18}H_{21}NO_2$
17	7-OCH_3	H	1	H	C_5H_9	88–90	toluene–cyclohexane	87	$C_{19}H_{23}NO_2$
18	7-OCH_3	H	1	H	C_6H_{11}	105–106	cyclohexane	90	$C_{20}H_{25}NO_2$
19	7-OCH_3	H	1	H	CH_2Br	100–101	toluene–cyclohexane	93	$C_{15}H_{16}BrNO_2$
20	7-OCH_3	H	1	H	CH_2I	110–112	95 °C ethanol	87	$C_{15}H_{16}INO_2$
21	7-OCH_3	H	1	H	CF_3	91–92	cyclohexane–toluene	87	$C_{15}H_{14}F_3NO_2$
22	7-OCH_3	H	1	H	*n*-C_3H_6Cl	98–99	toluene–cyclohexane	94	$C_{17}H_{20}ClNO_2$
23	7-OCH_3	H	1	H	C_6H_5	128–130	ethanol	94	$C_{20}H_{19}NO_2$
24	7-OCH_3	H	1	H	(3,5-diClC_6H_3)	138	ethanol	93	$C_{20}H_{17}Cl_2NO_2$
25	7-OCH_3	H	1	H	(2-indolyl)	198–199	toluene	87	$C_{22}H_{20}N_2O_2$
26	7-OCH_3	H	1	H	$CH_2C_6H_5$	114–115	toluene–cyclohexane	93	$C_{21}H_{21}NO_2$
27	7-OCH_3	H	1	H	$CH(C_6H_5)_2$	130–131	95 °C ethanol	86	$C_{27}H_{25}NO_2$
28	7-OCH_3	H	1	H	$(CH_2)_2C_6H_5$	101–102	cyclohexane	90	$C_{22}H_{23}NO_2$
29	7-OCH_3	H	1	H	$(CH_2)_3C_6H_5$	80–81	cyclohexane	72	$C_{23}H_{25}NO_2$
30	7-OCH_3	H	1	H	CH_2-4-morph	114–115	toluene–cyclohexane	91	$C_{19}H_{24}N_2O_3$
31	7-OCH_3	H	1	H	CH_2-TMZ	207–209[a]	ethanol	90	$C_{28}H_{35}N_3O_5{\cdot}2HCl$
32	7-OCH_3	H	1	NR_3R_4=2-pyrr		60–61	petroleum ether	35	$C_{17}H_{19}NO_2$
33	7-OCH_3	H	1	CH_3	CF_3	oil	*b*	80	$C_{16}H_{16}F_3NO_2$
34	7-OCH_3	H	1	CH_3	CH_3	oil	*b*	86	$C_{16}H_{19}NO_2$
35	7-OCH_3	H	1	CH_3	C_4H_7	oil	*b*	84	$C_{19}H_{23}NO_2$
36	6-OCH_3	H	1	H	CH_3	129–130	toluene	86	$C_{15}H_{17}NO_2$
37	6-OCH_3	H	1	H	C_3H_5	138–139	toluene	88	$C_{17}H_{19}NO_2$
38	6-OCH_3	H	1	H	C_4H_7	91–92	cyclohexane–toluene	85	$C_{18}H_{21}NO_2$
39	6-OCH_3	H	1	H	CF_3	116–117	cyclohexane	85	$C_{15}H_{14}F_3NO_2$
40	7-OH	H	1	H	CH_3	125–126	water	40	$C_{14}H_{15}NO_2$
41	7-OCH_3	$COOCH_3$	1	H	CH_3	118–120	toluene–hexane	85	$C_{17}H_{19}NO_4$
42	7-OCH_3	$COOCH_3$	1	H	C_3H_5	95–96	toluene–cyclohexane	76	$C_{19}H_{21}NO_4$
43	7-OCH_3			H	SO_2CH_3	97–98	toluene	82	$C_{14}H_{17}NO_3S$
44	OCH_3	H	NH	H	C_3H_5	101–102	toluene	80	$C_{15}H_{18}N_2O_2$
45	OCH_3	H	NH	H	C_4H_7	111–112	toluene	86	$C_{16}H_{20}N_2O_2$
47	OCH_3	H	NH	H	CH_2I	159	toluene	75	$C_{13}H_{15}IN_2O_2$
48	OCH_3	H	NH	H	CF_3	135–136	toluene	40	$C_{13}H_{13}F_3N_2O_2$
49	OCH_3	H	Nh	F	*n*-C_3H_7	135	ethanol–water	78	$C_{15}H_{19}FN_2O_2$
50	OCH_3	H	NH	F	C_3H_5	125–126	dichloromethane–ether	83	$C_{15}H_{17}FN_2O_2$
51	OCH_3	H	O	H	CH_3	112–113[c]	ethanol–water	33	$C_{13}H_{15}NO_3$
52	OCH_3	H	O	H	*n*-C_4H_9	58–60	ether	69	$C_{16}H_{21}NO_3$
53	OCH_3	H	O	H	C_3H_5	98–102	ethanol–water	88	$C_{15}H_{17}NO_3$
54	OCH_3	H	S	H	CH_3	98–99[d]	petroleum ether–ethyl acetate	70	$C_{13}H_{15}NO_2S$
55	OCH_3	H	S	H	*n*-C_4H_9	99–101	*n*-hexane–ether	56	$C_{16}H_{21}NO_2S$
56	OCH_3	H	S	H	C_3H_5	124–126	cyclohexane	80	$C_{15}H_{17}NO_2S$
57	OCH_3	H	S	H	C_4H_7	98–101	*n*-hexane	69	$C_{16}H_{19}NO_2S$
58	OCH_3	H	S	H	CH_2Br	119–121	toluene	80	$C_{13}H_{14}BrNO_2S$
59	OCH_3	H	S	H	CH_2I	133–135	acetone	75	$C_{13}H_{14}INO_2S$
60	OCH_3	H		H	CH_3	173–175	toluene	54	$C_{12}H_{15}N_3O_2$

[a] Melting point of the dihydrochloride salt. [b] Purified by column chromatography: SiO_2, ethyl acetate. TMZ: 1-(2′,3′,4′-trimethoxybenzyl)piperazine. morph: morpholino. 2-pyrr: 2-pyrrolidinone. [c] Reference 25; mp 116 °C. [d] Reference 24; mp 99–100 °C.

DMSO-d_6) δ 10.0 (s, 2H), 7.8–7 (m, 6H), 4.0 (s, 3H), 3.9–3.6 (m, 2H), 3.5–3.1 (m, 2H), 2.75 (t, 3H). Anal. ($C_{14}H_{18}ClNO$) C, H, N.

2-(7-Hydroxy-1-naphthyl)ethylamine, Hydrobromide (5D). A mixture of 13.8 g (0.058 mol) of **5A**, hydrochloride and 46 mL of 47% HBr solution was refluxed for 7 h. After cooling, the resulting precipitate was filtered, washed with *n*-hexane, and then recrystallized from ethyl acetate/hexane, affording 12.5 g (80%) of **5D**, HBr: mp 174–175 °C; ^1H NMR (80 MHz, DMSO-d_6) δ 10.0 (s, 1H), 8.1 (s, 3H), 7.0–7.9 (m, 6H), 3.0–3.4 (m, 4H). Anal. ($C_{12}H_{14}BrNO$) C, H, N.

***N*-[2-(7-Hydroxy-1-naphthyl)ethyl]acetamide (40). 5D**, HBr (1.02 g, 0.0038 mol) was added to a solution of 0.9 g (0.062 mol) of potassium carbonate in 5 mL of water. Ethyl acetate

(20 mL) was added to the resulting suspension and then, slowly, 0.3 g (0.039 mol) of acetyl chloride. Stirring was maintained until obtainment of a clear solution. The organic phase was separated, washed with water, dried, and evaporated under reduced pressure. The residue was crystallized from water, affording 0.35 g (40%) of **40**: mp 125–126 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 9.8 (s, 1H) 7.9–7.0 (m, 6H), 5.8 (s, 1H), 3.6 (q, 2H), 3.2 (t, 2H), 2 (s, 3H). Anal. ($C_{14}H_{15}NO_2$) C, H, N.

Pharmacology. Reagents and Chemicals. 2-[^{125}I]-Iodomelatonin was synthesized using Iodogen and purified by HPLC to a specific activity of approximately 2000 Ci/mmol as previously described.[39] [^{125}I]AMP was also prepared as described.[40] Stock chemicals and media were purchased from Sigma Chemical Co. and Gibco BRL Life Technologies. Compounds were synthesized as described.

Cell and Membrane Preparations. Fresh ovine pars tuberalis tissue was obtained at a local abattoir from animals of mixed sex, breed, and age. Preparation of crude membranes[39] and primary culture of pars tuberalis cells[40] have been extensively described. For binding studies, washed membranes were resuspended in ice-cold assay buffer (0.025 M Tris-HCl, 1 mM EGTA, pH 7.5) at a final concentration of 0.1 pars tuberalis equiv/100 μL (ca. 15–30 μg of protein by the method of Bradford[41]). For cAMP inhibition studies, cells were harvested after a 24 h culture in Dulbecco's modified Eagle's medium (25 mM HEPES, 4500 g of glucose/L, penicillin (100 units/mL), streptomycin (100 μg/mL), fungizone (0.25 μg/mL)) at 37 °C, 95%/5% air/CO_2. Single-cell suspensions were washed and resuspended at a concentration of 1.0×10^6 cells/mL of medium for experimental use.

Binding Studies. In competitive binding experiments, a 100 μL membrane suspension was added to tubes containing 400 μL of buffer with approximately 65 pM [^{125}I]iodomelatonin tracer and varying concentrations of competing ligand (0, 10^{-14}–10^{-4} M final concentration). Reactions (2 h, 37 °C) were stopped by removing tubes to an ice bath and immediately adding 500 μL of ice-cold 1% (w/v buffer) sheep γ-globulin plus 1 mL of ice-cold poly(ethylene glycol) (PEG 8000, 24%, w/v, distilled water) followed by centrifugation (1800g, 4° C, 25 min). Supernatant was discarded, and the protein pellet was washed and recentrifuged once before final counting of the dried pellet on a Packard Instument Co. Model 5010 auto-gamma counter at 74% efficiency. Initially, binding parameters for a 1-site mass-action model were determined without transformation from raw data by microcomputer analysis using the LIGAND program.[42] Estimates of the binding affinity of [^{125}I]iodomelatonin (3.5×10^{10} L/mol), used as a constant in the computerized analysis of competition studies, were determined by saturation analysis using a similar protocol but employing 10^{-6} M melatonin to assess nonspecific binding as previously published.[16] A single-site binding model was inadequate for describing the behavior of several of the compounds reported here; more complex models could not be directly fitted to the heterologous competition data as no *a priori* knowledge of the competitor agonist-binding properties at putative additional binding sites was available. Subsequently, data from individual experiments were normalized, pooled, and analyzed by 4-parameter logistic (4PL) regression[18] to obtain IC_{50} and slope factor estimates or by a 5-parameter regression model which describes biphasic inhibition in terms of maximum, midpoint, and minimum response levels and two "inhibition" constants (K_H and K_L) for each component of the biphasic curve. For the compound having an IC_{50} value higher than 10^{-5} M, the cAMP index was not measured.

Cyclic AMP Studies. For cAMP inhibition studies, 200 μL cell suspensions (200 000 cells) were added to microtubes containing 50 μL of medium alone or with appropriately concentrated drug solution. Reactions (15 min, 37 °C) were stopped in a boiling water bath (2 min), and the tubes were sonicated and then frozen (−20 °C) until cAMP was measured by radioimmunoassay using a highly specific antibody (anti-cAMP serum No. 338; Drs. D. C. Klein and A. K. Ho, National Institutes of Health, Bethesda, MA) and [^{125}I]cAMP. Sodium acetate (0.05 M, pH 6.0) was used as the diluent buffer. Samples and standards (100 μL) were acetylated by addition of 10 μL of triethylamine/acetic anhydride (2:1) prior to the addition of iodinated tracer (100 μL, 15 000 cpm) and antibody (100 μL). Antibody-binding reactions were carried out overnight at 4 °C and separation of the bound fraction was accomplished by precipitation of carrier protein (100 μL of 10% (w/v buffer) bovine serum albumin) with 2 mL of ice-cold ethanol. Data were analyzed on-line (Packard Instrument Co. Model 5010 auto-gamma counter cobra system software) using 4PL regression of calibration curve standards. Both inter- and intra-assay coefficients of variation of this assay are less than 15%, and the sensitivity (lowest amount statistically distinguishable from zero dose) is 4 fmol/tube. The biological activity of each compound was assessed relative to the effect of melatonin inhibition (activity index = 1.0) of forskolin-stimulated cAMP production.

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