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

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Abstract—A previous paper reported the synthesis of melatonin receptor ligands. In order to complete the structure–activity relationships and to obtain antagonists to the melatonin receptor, a new series of naphthalenic analogues of melatonin have been synthesized. Modifications include deletion of the 7-methoxy group, replacement of the ethylene moiety, replacement of the amidic function by bioisosteres, and replacement of the naphthalenic nucleus by other bicyclic rings. Almost all the structural modifications lead to decreased affinity for the melatonin receptor. However, the *N-n* propyl urea derivative (**27**) is a very potent ligand at this receptor ($pK_i = 14.3$). Most interestingly deletion of the methoxy group resulted in the first antagonist in this series. This molecule, compound **12**, or *N*-[2-(1-naphthyl)-ethyl]cyclobutyl carboxamide has been selected for preclinical development.

Introduction

In a previous paper[1] we reported that although the exact functions of melatonin in man were not fully elucidated, recent work had suggested its potential usefulness in a number of therapeutic areas. However the very short half-life and lack of selectivity of this natural molecule justified the development of novel analogues that would overcome these limitations.

For these reasons, we initiated and have reported work on the synthesis of bicyclic analogues of melatonin. These studies described very potent agonists for the melatonin receptor and among these, the naphthalenic analogue of melatonin has been selected for clinical development.

To continue this work, we have further developed new compounds in order to gain improved structure–activity relationships for melatonin analogues and better characterize the melatonin receptor. We additionally hoped to obtain potent antagonist derivatives as the previous work only obtained agonist derivatives. This second aim is fully justified as compound **12**, in this paper has been the subject of extensive studies that confirmed its antagonist activity. This compound restored the neuronal response of surprachiasmatic neurones to a light flash in presence of melatonin leading to the possibility that such compounds may be of use in the treatment of seasonal affective disorders.[2] Moreover, this compound was able to prevent weight gain in an animal model of

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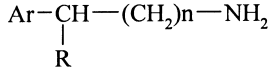
seasonal obesity indicating that a melatonin antagonist could be useful in the treatment of obesity.[3]

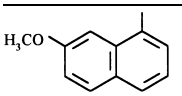
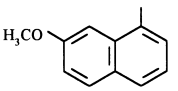
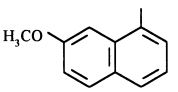
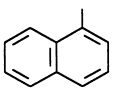
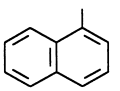
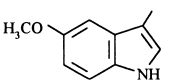
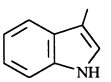
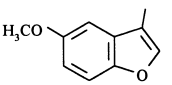
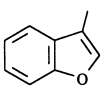
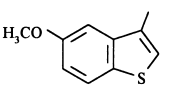
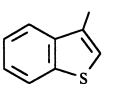
We have synthesized 50 new derivatives, among which are thirty six naphthalenic bioisosteres. Following our previous report we studied direct naphthalenic analogues of melatonin by deleting the 7-methoxy group to check its importance for biological activity as previously hypothesized.[4] We have also extended our study related to the importance of the amidic function by replacing it with various bioisosteric systems: ureas, thioureas, carbamates, esters, ethers and retroamides. It has been reported that this part of the molecule has a primary importance for binding affinity.[4] Thirdly, in studies to investigate the role of the side chain, we replaced the ethylene moiety by lower and higher homologues and also added a ramification. Finally, 13 indolic, benzofuranic and benzothiophenic derivatives with the most representative structural modifications introduced in the naphthalenic family were studied to verify the equivalence of these variations in these series. The organic synthesis, melatonin receptor binding data and agonist activity properties for these bicyclic compounds are described in this paper.

Chemistry

Key intermediates in the synthesis of the target compounds described here are the corresponding primary amines, alcohols or carboxylic acids (Tables 1 and 2). Some of the primary amines (Table 1) such as **3b**, **3f**, **3g**, **3h**, **3i**, **3j**, and **3k** are commercially available or previously described.[1,5–8] Primary amine (**3a**) was prepared (Scheme 1) through Hoffman's degradation[9] of the previously described[8] 7-methoxy-1-naphthylacetamide. To form[10] (Scheme 2) the homologous derivative (**3c**), the acid precursor (**2a**) was esterified with methanol and thionyl chloride. The ester (**4a**) was reduced with $LiAlH_4$ to the alcohol (**5a**), that reacted with methane sulfonyl chloride (94% yield) to give the mesylate (**6a**). Nucleophilic displacement[11] of (**6a**) with potassium cyanide in DMSO provided (95% yield) the nitrile (**7a**), which was catalytically reduced to the amine (**3c**). Compound **3d** was obtained using the same procedure as for the attainment of its 7-methoxy analogue (**3c**).[1] Amino-ester (**3e**) was synthesized (Scheme 3) from commercially available 1-naphthylacetonitrile via methyl-2-cyano(1-naphthyl) acetate (**7c**) according to a previously described procedure.[8] Alkaline hydrolysis of the nitrile (**7a**) gave (Scheme 4) the propanoic acid derivative (**2b**), which was transformed by the same route to the butyryl homologue (**2c**). Etherification of the alcohols (**5a** and **5b**) with various dialkylsulfates under phase transfer catalysis conditions[12] with benzyltriethylammonium chloride (BTEAC) as catalyst gave (Scheme 5) compounds (**39**–**41**). The ester (**42**) was obtained by heating the alcohol (**5a**) with acetic anhydride. Treatment (Scheme 6) of the acids (**2b** and **2c**) with thionyl chloride in chloroform yielded in situ the corresponding acyl chlorides, which were then reacted without further purification with ammonia or primary amines to give the carboxamides (**43**–**46**). Monosubstituted ureas (**24** and **30**) could be synthezised by reaction of amines (**3b** and **3d**) with a large excess of potassium cyanate in acidic aqueous medium, or more efficiently, with a slight excess of potassium cyanate

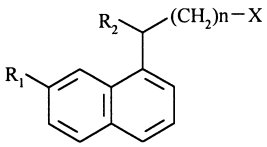
Table 1. Structure of starting primary amines



Ar	R	*n*	
	H	0	**3a**
	H	1	**3b**
	H	2	**3c**
	H	1	**3d**
	$COOCH_3$	1	**3e**
	H	1	**3f**
	H	1	**3g**
	H	1	**3h**
	H	1	**3i**
	H	1	**3j**
	H	1	**3k**

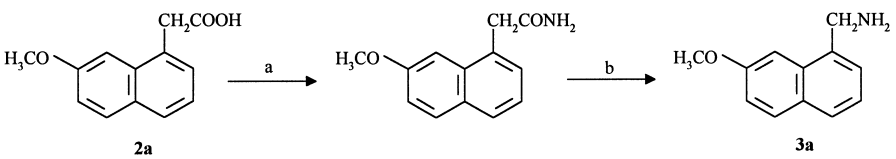
alone in water. Dissymmetric disubstituted analogues (**25**–**28**; **31**–**38**; **52**; **54**–**56**; **58**) were obtained (Scheme 7) by reaction with various alkylisocyanates of the corresponding primary amines in ether or pyridine. Symmetric disubstituted ureas (**29** and **53**) were obtained (Scheme 7) by coupling two equivalents of the suitable amines with carbonyldiimidazole as reactant. Carbamates (**22** and **23**) were synthezised from **3b** in THF by action of an appropriate alkylchloroformate in the presence of triethylamine. The *N*-acylated derivatives (**10**–**12**; **14**–**21**;

Table 2. Physical properties of key intermediates

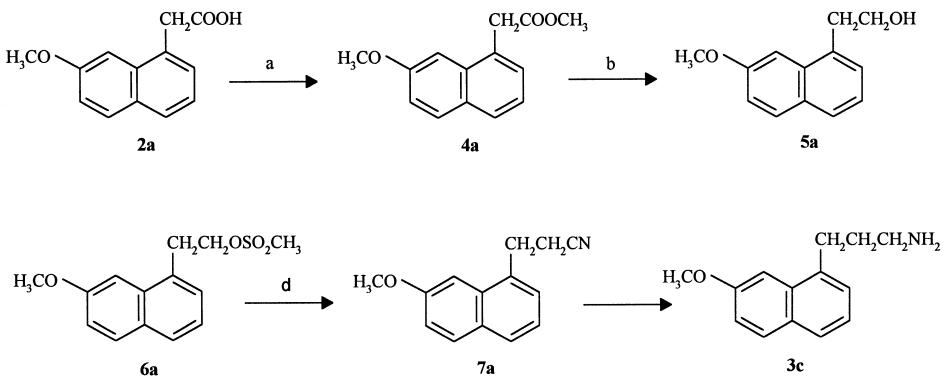


Compd	R_1	R_2	*n*	X	Mp (°C)	Recrys solvent	Formula
2b	OCH_3	H	1	COOH	154–155	toluene	$C_{14}H_{14}O_3$
2c	OCH_3	H	2	COOH	104–106	toluene-cyclohexane	$C_{15}H_{16}O_3$
3a	OCH_3	H	0	NH_2,HCl	>260	ethanol	$C_{12}H_{14}ClNO$
3c	OCH_3	H	2	NH_2,HCl	197–200	cyclohexane-ethanol	$C_{14}H_{18}ClNO$
3d	H	H	1	NH_2,HCl	230–233	ethanol	$C_{12}H_{14}ClN$
3e	H	$COOCH_3$	1	NH_2,HCl	217–219	acetonitrile	$C_{14}H_{16}ClNO_2$
4a	OCH_3	H	0	$COOCH_2$	51–52	hexane	$C_{14}H_{14}O_3$
4b	OCH_3	H	1	$COOCH_2$	Oil	—[a]	$C_{15}H_{16}O_3$
5b	OCH_4	H	1	OH	80–82	cyclohexane	$C_{13}H_{14}O_2$
5b	OCH_3	H	2	OH	38–39	cyclohexane	$C_{14}H_{16}O_2$
6a	OCH_3	H	1	OSO_2CH_3	62–63	cyclohexane	$C_{14}H_{16}O_4S$
6b	OCH_3	H	2	OSO_2CH_2	52–54	toluene-cyclohexane	$C_{15}H_{18}O_4S$
7a	OCH_3	H	1	CN	62–64	hexane	$C_{14}H_{13}NO$
7b	OCH_3	H	2	CN	52–54	ethanol-water	$C_{15}H_{15}NO$
7c	H	$COOCH_3$	1	CN	89–90	cyclohexane	$C_{14}H_{11}NO_2$

[a]Purified by column chromatography: SiO_2, acetone/toulene/cyclohexane (4/2/2).



Scheme 1. Reagents: (a) $SOCl_2$, NH_3; (b) Br_2, NaOH.

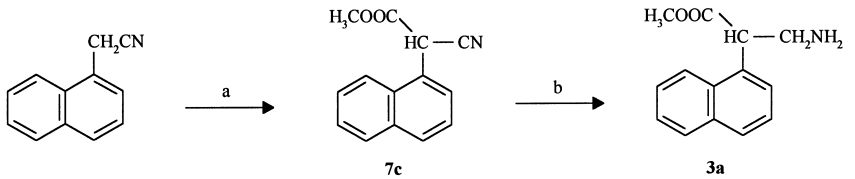


Scheme 2. Reagents: (a) $SOCl_2$, CH_3OH; (b) $LiALH_4$, ether; (c) CH_3SO_2Cl, $(C_2H_5)_3N$, CH_2Cl_2; (d) KCN, DMSO; (e) H_2, Ni, EtOH, NH_3.

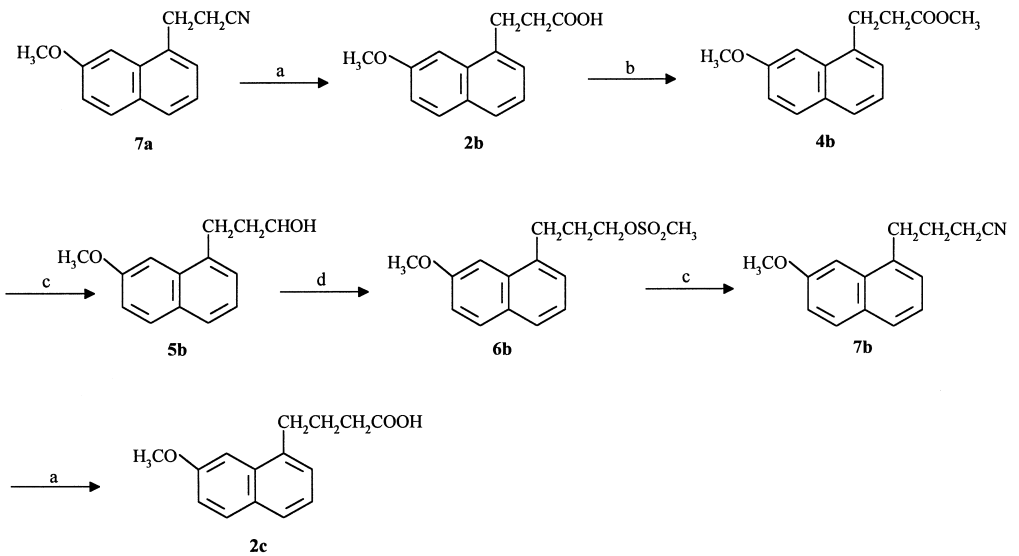
47–51; **57**; **59–60**) were prepared (Scheme 7) from the appropriate primary amine (**3**) by treatment with the suitable acid chloride in the presence of K_2CO_3 as base and a biphasic medium according to a variant[1] of the Schotten–Baumann reaction. The trifluoroacetamido compound **13** was obtained by treatment of the corresponding amine (**3d**) with trifluoroacetic anhydride[13] in pyridine.

Results and Discussion

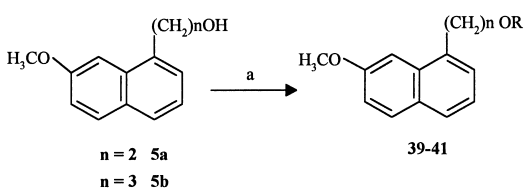
For all compounds, the chemical structures, melatonin receptor binding characteristics and biological activities (expressed as a cAMP index determined by the degree of melatonin mediated inhibition of forskolin-stimulated cAMP production) under single dose conditions[14] are shown in Table 4.



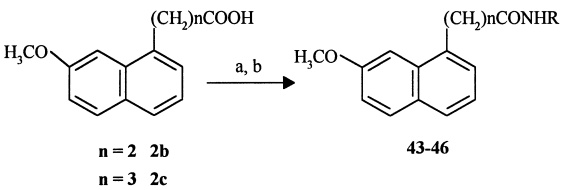
Scheme 3. Reagents: (a) $(CH_3O)_2CO$, Na; (b) $NaBH_4$, $CoCl_2$.



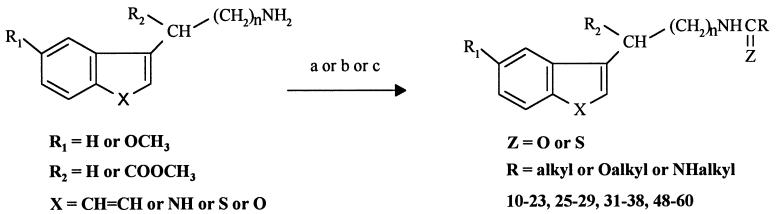
Scheme 4. Reagents: (a) 1. NaOH, MeOH; 2. HCl; (b) $SOCl_2$, CH_3OH; (c) $LIAlH_4$, ether; (d) CH_3SO_2Cl, $(C_2H_5)_3N$, CH_2Cl_2; (e) KCN, DMSO.



Scheme 5. Reagents: (a) BTEAC, 50% NaOH, toluene, $(CH_3O)_2SO_2$ or $(C_2H_5O)SO_2$.



Scheme 6. Reagents: (a) $SOCl_2$, $CHCl_3$; (b) NH_3 or RNH_3, ether.



Scheme 7. Reagents: (a) R′COCl, K_2CO_3, H_2O, $CHCl_3$; (b) R′NCO or R′NCS, ether or pyridine; (c) carbonyldiimidazole, $CHCl_3$.

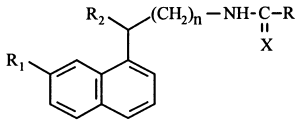
Binding data were analyzed by non-linear regression to curves for one or two-state receptor models[14,17] to determine best least-squares fit based on an *F*-ratio test for relative reduction in the Sum of Squares for each fit.[15] All binding experiments were carried out under Zone A conditions[16] whereby free ligand concentration approximates total concentration in the reaction. Fitted mono or biphasic binding affinity parameter estimates for compounds are given as the negative log (pK_i) of molar inhibition constants, K_i, calculated by application of Cheng–Prusoff correction factors to observed IC_{50} values. As for some G-protein coupled receptors, the 2-state estimates may provide an indication of the ability of different agonists to descriminate different conformational states of the recepto.[18] The binding method however does not exclude the possibility of binding to distinct sites on a multivalent receptor as has been described for other G-protein coupled receptors[19] or to distinct receptor subtypes.

The binding data are complemented by in vitro screening experiments using ovine pars tuberalis primary cultures to characterize the activity of the reported compounds on forskolin stimulated cAMP production. The bioassay is described elsewhere in detail,[14] but briefly, forskolin (1 μM) stimulates a robust increase in cAMP production (typically 20- to 50-fold) in these cells which is inhibited (typically 80%) by 1 nM melatonin. The effect of 10 μM drug (D) treatment alone (a high dose chosen to maximize the likelihood of observing a significant response in the screening assay), with forskolin (F/D), and with the forskolin/melatonin (F/M/D) is compared to the effect of forskolin/melatonin (F/M) in order to derive an 'agonist' (F/D) and an 'antagonist' (F/M/D) activity index. For each experiment, the cAMP responses are normalized against the response induced by forskolin alone which is taken as the 100% effect. The indexed activity of melatonin (M) is defined from the normalized response ratio [F–F/M]/[F–F/M] = 1 and the indexed activity of the various treatments are calculated as the ratio [F–treatment]/[F–F/M] where treatments are: basal response melatonin alone, drug alone, drug/forskolin or drug/forskolin/melatonin at final concentrations indicated above. The index values reported are averages taken from three separate experiments and error estimates are derived from standard statistical formulae for error propogation through sums and quotients.[24] As these experiments are performed at only a single dose, these indices provide only semi-quantitative information. In some cases, rather than mimicking the inhibition activity of melatonin, drug induced potentiation of the forskolin response is seen resulting in a negative activity value (e.g. [F–F/D] = 1.0–1.5) in the numerator of the ratio giving rise to a negative activity index value. A compound that has no agonist activity or that blocks the effect of melatonin will have indices approaching 0, while one that fully mimics the activity of melatonin will have an agonist activity index approaching 1. There are also cases where a compound has a negative 'agonist' index and a corresponding positive 'antagonist' index showing that the potentiating effect of the compound is blocked by melatonin. At present, we have no experimental information which allows us to explain the potentiation of forskolin stimulated cAMP by these compounds but we suggest these observations may possibly reflect inverse agonism. The criteria used to used to categorize the biological properties of the compounds are as follows:

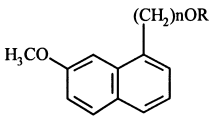
AGONIST INDEX (Drug Alon cf. Melatonin)	ANTAGONIST INDEX (Drug/Melatonin cf. Melatonin)	Activity*
> 0.8	> 0.8	Full agonist
0.4 < < 0.8	> 0.8	Weak agonist
0.4 < < 0.8	< 0.8	} Partial agonist/antag.
< 0.4	0.2 < < 0.8	
< 0.4	< 0.2	Antagonist
< 0.4	> 0.8	No activity

*Activity on the melatonin receptor coupled to cAMP transduction pathway.

Table 3. Physical properties of melatonin analogues



10-38



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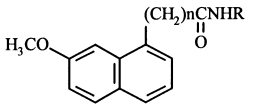
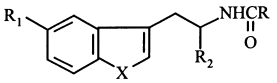


43-47

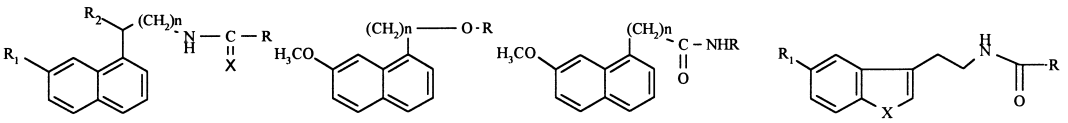


48-60

Compd	R_1	R_2	*n*	X	R	Mp (°C)	Recryst solvent	Yield (%)	Formula
10	H	H	1	O	CH_3	85–87	toluene-cyclohexane	90	$C_{15}H_{13}NO$
11	H	H	1	O	C_3H_5	117–118	toluene-cyclohexane	88	$C_{17}H_{15}NO$
12	H	H	1	O	C_4H_7	88–89	toluene-cyclohexane	89	$C_{17}H_{19}NO$
13	H	H	1	O	CF_3	79–80	cyclohexane	85	$C_{14}H_{12}F_3NO$
14	H	H	1	O	$CH_2C_6H_5$	79–80	cyclohexane	70	$C_{20}H_{19}NO$
15	H	H	1	O	$(CH_2)_3C_6H_5$	51–53	cyclohexane	78	$C_{22}H_{23}NO$
16	H	$COOCH_3$	1	O	C_3H_5	140–142	toluene-cyclohexane	80	$C_{18}H_{19}NO_3$
17	H	$COOCH_3$	1	O	C_4H_7	88–90	toluene-cyclohexane	79	$C_{19}H_{21}NO_3$
18	OCH_3	H	0	O	CH_3	165–166	toluene	94	$C_{14}H_{15}NO_2$
19	OCH_3	H	2	O	CH_3	66–68	cyclohexane	56	$C_{16}H_{19}NO_2$
20	OCH_3	H	2	O	nC_4H_9	75–76	cyclohexane	56	$C_{19}H_{25}NO_2$
21	OCH_3	H	2	O	C_3H_5	121–123	cyclohexane	92	$C_{18}H_{21}NO_2$
22	OCH_3	H	1	O	OCH_3	70–71	toluene-cyclohexane	35	$C_{15}H_{17}NO_3$
23	OCH_3	H	1	O	$O\text{-}nC_3H_7$	87–88	cyclohexane	90	$C_{17}H_{21}NO_3$
24	OCH_3	H	1	O	NH_2	187–188	ethylacetate	65	$C_{14}H_{16}N_2O_2$
25	OCH_3	H	1	O	$NHCH_3$	139–141	toluene-cyclohexane	80	$C_{15}H_{18}N_2O_2$
26	OCH_3	H	1	O	NHC_2H_5	134–135	ethanol-water	69	$C_{16}H_{16}N_2O_2$
27	OCH_3	H	1	O	$NHnC_3H_7$	104–105	toluene-cyclohexane	93	$C_{17}H_{22}N_2O_2$
28	OCH_3	H	1	O	$NHnC_4H_9$	106–107	toluene-cyclohexane	80	$C_{18}H_{24}N_2O_2$
29	OCH_3	H	1	O	$NHCH_2CH_2Naph$[a]	187–189	toluene	56	$C_{27}H_{28}N_2O_3$
30	H	H	1	O	NH_2	141–143	toluene	44	$C_{13}H_{14}N_2O$
31	H	H	1	O	$NHCH_3$	104–106	toluene-cyclohexane	72	$C_{14}H_{16}N_2O$
32	OCH_3	H	0	O	$NHnC_3H_7$	161–162	95 ethanol	77	$C_{16}H_{20}N_2O_2$
33	OCH_3	H	0	O	$NHnC_4H_9$	154–156	95 ethanol	99	$C_{17}H_{22}N_2O_2$
34	OCH_3	H	2	O	$NHnC_3H_7$	120–121	toluene	70	$C_{18}H_{24}N_2O_2$
35	OCH_3	H	1	S	$NHCH_3$	105–107	toluene-cyclohexane	80	$C_{15}H_{18}N_2O_S$
36	OCH_3	H	1	S	NHC_2H_5	114–115	toluene-cyclohexane	81	$C_{16}H_{20}N_2OS$
37	OCH_3	H	1	S	$NHnC_3H_7$	95–97	toluene-n.hexane	80	$C_{17}H_{22}N_2O_2S$
38	OCH_3	H	1	S	$NHnC_4H_9$	65–68	toluene-cyclohexane	80	$C_{18}H_{24}N_2OS$
39			2		CH_3	Oil	—[b]	53	$C_{14}H_{16}O_2$
40			2		C_2H_5	Oil	—[c]	50	$C_{15}H_{18}O_2$
41			3		CH_3	Oil	—[b]	38	$C_{15}H_{18}O_2$
42			2		$COCH_3$	51–52	hexane	96	$C_{15}H_{16}O_3$
43			2		H	125–126	95 ethanol	43	$C_{14}H_{15}NO_2$
44			2		CH_3	98–100	cyclohexane	78	$C_{15}H_{17}NO_2$
45			3		CH_3	96–98	cyclohexane	45	$C_{16}H_{19}NO_2$
46			3		nC_3H_7	109–112	water-ethanol	38	$C_{18}H_{23}NO_2$
47	H		1		$(CH_2)_3C_6H_5$	156–157	cyclohexane	75	$C_{22}H_{23}NO_2$
48	H	H	-	NH	nC_4H_9	91–92	toluene-cyclohexane	78	$C_{15}H_{20}N_2O$
49	H	H	-	NH	C_3H_5	99–100	toluene	86	$C_{14}H_{16}N_2O$
50	H	H	-	NH	C_4H_7	102–103	toluene	87	$C_{15}H_{18}N_2O$
51	H	H	-	NH	C_5H_9	99–100	toluene	76	$C_{16}H_{20}N_2O$
52	OCH_3	H	-	NH	$NHnC_3H_7$	79–80	toluene	85	$C_{15}H_{21}N_3O_2$
53	OCH_3	H	-	NH	$NHCH_2CH_2Ind$[d]	125–127	toluene	59	$C_{13}H_{16}N_2O_3$
54	OCH_3	H	-	O	$NHCH_3$	163–165	toluene	59	$C_{13}H_{16}N_2O_3$
55	OCH_3	H	-	O	NHC_2H_5	153–155	toluene	87	$C_{14}H_{18}N_2O_3$
56	OCH_3	H	-	O	$NHnC_3H_7$	126–127	cyclohexane-toluene	30	$C_{15}H_{20}N_2O_3$
57	H	H	-	O	C_4H_7	64–67	ethanol-water	88	$C_{15}H_{17}NO_2$
58	OCH_3	H	-	S	NHC_3H_7	103–105	cyclohexane	65	$C_{15}H_{20}N_2SO_2$
59	H	H	-	S	C_3H_5	98–99	cyclohexane	78	$C_{14}H_{15}NOS$
60	H	H	-	S	C_4H_7	80–82	n.hexane-toluene	75	$C_{15}H_{17}NOS$

[a]Naph: 7-methoxy-1-naphthyl.
[b]Purified by column chromatography: SiO_2, cyclohexane-ethyl acetate (4/1).
[c]Purified by column chromatography: SiO_2, dichloromethane.
[d]Ind: 5-methoxy-3-indolyl.

Table 4. Binding and biological data for new melatonin analogues



10-38 **39-42** **43-47** **1 ; 48-60**

Compd	R_1	R_2	*n*	X	R	1-State (site) Model	2- State (site) Model		Agonist Index (± sd)	Antagonist Index (± sd)
						pK_i ± est. sd	pK_i (H_i) ± est. sd	pK_i (Lo) ± est. sd		
1	OCH_3	H		NH	CH_3	9.57 ± 0.13			1.00	
10	H	H	1	O	CH_3	8.40 ± 0.49			0.75 ± 0.31	0.85 ± 0.36
11	H	H	1	O	C_3H_5	7.38 ± 0.19			0.33 ± 0.13	0.28 ± 0.11
12	H	H	1	O	C_4H_7	7.40 ± 1.77			−0.10 ± 0.03	0.01 ± 0.00
13	H	H	1	O	CF_3	7.18 ± 0.14			0.38 ± 0.15	0.37 ± 0.14
14	H	H	1	O	CH_2-C_6H_5	5.67 ± 1.09			−0.13 ± 0.02	0.63 ± 0.13
15	H	H	1	O	$(CH_2)_3C_6H_5$	5.84 ± 0.26			0.12 ± 0.09	0.85 ± 0.36
16	H	$COOCH_3$	1	O	C_3H_5	6.04 ± 0.11	−0.10 ± 0.03	0.16 ± 0.05		
17	H	$COOCH_3$	1	O	C_4H_7	5.16 ± 0.13			N.D.	N.D.
18	OCH_3	H	0	O	CH_3	6.34 ± 0.28			0.26 ± 0.15	1.00 ± 0.75
19	OCH_3	H	2	O	CH_3	8.34 ± 0.16			−1.28 ± 0.42	−1.07 ± 0.41
20	OCH_3	H	2	O	nC_4H_9	6.71 ± 0.10			−6.10 ± 2.29	−5.39 ± 1.83
21	OCH_3	H	2	O	C_3H_5	7.62 ± 0.15			−10.00 ± 3.39	−4.75 ± 1.74
22	OCH_3	H	1	O	OCH_3	7.72 ± 0.21			1.16 ± 0.33	1.00 ± 0.33
23	OCH_3	H	1	O	O-nC_3H_7		12.99 ± 0.68	7.29 ± 0.16	1.01 ± 0.28	1.00 ± 0.30
24	OCH_3	H	1	O	NH_2	8.45 ± 0.31			1.15 ± 1.41	1.19 ± 0.42
25	OCH_3	H	1	O	$NHCH_3$	8.66 ± 0.46			1.27 ± 0.34	1.18 ± 0.38
26	OCH_3	H	1	O	NHC_2H_5	8.17 ± 0.14			1.33 ± 0.26	1.29 ± 0.36
27	OCH_3	H	1	O	$NHnC_3H_7$		14.31 ± 0.09	7.62 ± 0.33	0.95 ± 0.22	0.99 ± 0.30
28	OCH_3	H	1	O	$NHnC_4H_9$	5.61 ± 0.10			−1.19 ± 0.06	0.75 ± 0.22
29	OCH_3	H	1	O	$NHCH_2CH_2MN$	5.92 ± 0.55			0.61 ± 0.13	1.00 ± 0.24
30	H	H	1	O	NH_2	6.62 ± 0.12			0.47 ± 0.17	0.91 ± 0.33
31	H	H	1	O	$NHCH_3$	6.97 ± 0.13			0.55 ± 0.13	0.92 ± 0.25
32	OCH_3	H	0	O	$NHnC_3H_7$	7.28 ± 0.16			0.12 ± 0.02	0.52 ± 0.10
33	OCH_3	H	0	O	$NHnC_4H_9$	5.70 ± 0.15			−1.43 ± 0.23	0.83 ± 0.16
34	OCH_3	H	2	O	$NHnC_3H_7$	6.79 ± 0.05			0.47 ± 0.16	0.82 ± 0.29
35	OCH_3	H	1	S	$NHCH_3$	8.05 ± 0.05			0.98 ± 0.29	0.98 ± 0.34
36	OCH_3	H	1	S	NHC_2H_5		12.43 ± 0.40	7.37 ± 0.06	0.99 ± 0.23	1.01 ± 0.34
37	OCH_3	H	1	S	$NHnC_3H_7$		12.48 ± 0.63	7.38 ± 0.50	0.96 ± 0.22	0.96 ± 0.50
38	OCH_3	H	1	S	$NHnC_4H_9$	5.44 ± 1.08			N.D.	N.D.
39			2		CH_3	5.94 ± 0.13			−1.63 ± 0.19	0.19 ± 0.08
40			2		C_2H_5	6.09 ± 0.09			−0.40 ± 0.09	0.03 ± 0.01
41			3		$COCH_3$	6.70 ± 0.19			−0.93 ± 0.45	−0.45 ± 0.19
42			2		$COCH_3$	6.16 ± 0.11			0.52 ± 0.19	0.70 ± 0.23
43			2		H	6.36 ± 0.13			0.89 ± 0.33	1.04 ± 0.38
44			2		CH_3	8.15 ± 0.14			1.14 ± 0.47	1.05 ± 0.41
45			3		CH	8.29 ± 0.02			−0.05 ± 0.01	−0.09 ± 0.03
46			3		nC_3H_7	7.37 ± 0.04			0.02 ± 0.01	0.09 ± 0.05
47			1		$CH_2CH_2CH_2C_6H_5$	7.16 ± 0.86			0.95 ± 0.34	1.02 ± 0.34
48	H		-	NH	nC_4H_9	5.60 ± 0.60			N.D.	N.D.
49	H		-	NH	C_3H_5	6.81 ± 0.07			0.40 ± 0.16	0.50 ± 0.20
50	H		-	NH	C_4H_7	5.65 ± 0.34			N.D.	N.D.
51	H		-	NH	C_5H_9	5.57 ± 0.67			N.D.	N.D.
52	OCH_3		-	NH	$NHnC_3H_7$		11.50 ± 0.64	7.92 ± 0.14	1.03 ± 0.48	1.08 ± 0.52

(continued)

Table 4—*contd*

Compd	R_1	R_2	*n*	X	R	1-State (site) Model	2- State (site) Model		Agonist Index (± sd)	Antagonist Index (± sd)
						pK_i ± est. sd	pK_i (H_i) ± est. sd	pK_i (Lo) ± est. sd		
53	OCH_3		-	NH	$NHCH_2CH_2MI$	6.40 ± 0.55			0.89 ± 0.25	0.98 ± 0.23
54	OCH_3		-	O	$NHCH_3$	8.22 ± 0.03			1.01 ± 0.19	0.98 ± 0.22
55	OCH_3		-	O	NHC_2H_5	8.37 ± 0.16			0.95 ± 0.24	0.99 ± 0.20
56	OCH_3		-	O	$NHnC_3H_7$	7.33 ± 0.28			1.32 ± 0.32	0.98 ± 0.23
57	H		-	O	C_4H_7	5.80 ± 0.07			−2.16 ± 0.79	0.62 ± 0.19
58	OCH_3		-	S	NHC_3H_7		12.44 ± 0.68	7.43 ± 0.22	−0.53 ± 0.28	0.77 ± 0.37
59	H		-	S	C_3H_5	7.53 ± 0.08			−1.13 ± 0.58	0.65 ± 0.33
60	H		-	S	C_4H_7	6.27 ± 0.24			0.48 ± 0.16	1.00 ± 0.33

MN: 7-Methoxy-1-naphthyl.
MI: 5-Methoxy-3-indolyl.

Binding data

The structure–affinity relationships for the naphthalenic analogues of melatonin from this study confirms our previous finding that the 7-methoxy group is essential for high affinity binding to the melatonin receptor. For example, **10** has a K_{i50} of 4.0×10^{-9} as compared to 8.9×10^{-11} for its methoxylated counterpart.[1] Some modifications to the acetyl side chain have been shown to improve the affinity of 7-methoxy analogues for the melatonin receptor.[1] However similar modifications to the non-methoxylated analogues, such as replacement of the methyl group of the amidic function by a cyclopropyl group (**11**) or a trifluoromethyl group (**13**), failed to improve the affinity. Instead, a slight decrease in affinity was observed. It is therefore not surprising that the introduction of a bulky arylalkylgroup, which is detrimental for binding of 7-methoxylated analogues[1] to melatonin receptors, also had a negative impact on the affinity of the nonmethoxylated analogues (**14** and **15**). Increasing **19** or decreasing **18** the length of the side chain between the naphthalenic system and the amidic function results in a loss of binding affinity. This is also the result of the introduction of a ramification of the side chain: **16** and **17** are only poor ligands, which is consistent with the results of our previous study where the introduction of a methyl group on the side chain reduced the affinity.

Substitution of the amidic function by a urethane (**22**), a urea (**25**), a thiourea (**35**), an alkoxy group (**39**), a carboxy (**42**) or a retroamide function (**44**) dramatically reduces the affinity for the melatonin receptor when compared to the amidic analogue.[1]

Introduction of an *n*-propyl group (**27**) as a urea substituent increases the affinity binding to the melatonin receptor as in the amidic series.[1] In the urea family we observed a decrease in the affinity following deletion of the 7-methoxy group (**30**, **31** v/v **24**, **25**) and by increasing (**34** v/v **27**) or decreasing (**32** v/v **27**) in the length of the side chain between the naphthalenic system and the urea function similar to the amidic series. However, that is not the case when comparing **33** to **28** but for both compounds, affinities are very low and this result cannot be considered as significant.

In the indolic, benzofuranic and benzothiophenic families, deletion of the methoxy group as well as replacement of the amidic function by urea also decreased affinity (**48**–**60**) for the melatonin receptor.

Some of the compounds studied gave biphasic binding curves. These include carbamate, ureas or thioureas substituted by ethyl (**36**) or bulky *n*-propyl groups (**23**, **27**, **37**, **52**, and **58**). The lack of correlation between biphasic binding curves and nonsterically hindered derivatives contrast with our previous study.[1] In addition, most of the bulky substituents introduced in the present study onto amidic, urea, and thiourea functions in the naphthalenic, indolic, benzofuranic or benzothiophene series failed to show biphasic binding curves under the conditions employed here. Thus at present there is no structural clue for the basis of biphasic binding curves.

Biological data

From the two last columns of Table 4 it first appears that the 7-methoxy group is of primary importance to biological activity as seen previously.[20] Deletion of this group permitted us to obtain our first antagonist: compound **12**. Moreover most of compounds lacking this group are poor agonists with only derivative **10** having

any intrinsic activity. However, its affinity for the melatonin receptors is approximately 4500 times lower than that of the corresponding methoxylated analogue.[1] This result confirms the importance of the 7-methoxy group for biological activity as previously hypothesized.[4] Others are either weak agonists (**30**, **31**, and **49**) or partial agonists (**11** and **13**). The properties of compounds (**14**, **15**, **57** and **59**) in this group are less clear. While compound **15** has no measurable activity, others potentiate the effect of forskolin raising the possibility that they may be acting as inverse agonists in this system.

Deletion of the 7-methoxy group from the full agonists (**24** and **25**) gives the weak agonists (**30** and **31**) while introduction of a carboxymethyl group lowers the binding affinity for melatonin receptors (**16** and **17**).

Replacement of the amidic group by an urethane (**22**), a urea (**25**), a thiourea (**35**), or a retroamide (**44**) does not modify the biological activity. The carboxy derivative (**42**) is only a partial agonist while the alkoxy compound **39** is an antagonist.

In the urea family we note that the *n*-propyl derivative (**32**) is a weak antagonist but its higher homologue, the *n*-butyl derivative (**33**) may be an inverse agonist. All the urea derivatives studied are full agonists except compounds **32** and **33**, which have a shortened methylene side chain and the *n*-propyl benzothiophene compound **58**, which surprisingly has no agonist properties even though the naphthalenic, indolic and benzofuranic analogues are full agonists.

Changing the length of the side chain strongly decreases the biological activity of the corresponding compounds. For instance amides (**19**, **20**, and **21**) with a propylene side chain are not full agonists in contrast to the ethylene homologues. These compounds also potentiate forskolin induced cAMP production. The same result is seen when retroamidic compound **44** is compared to its lengthened side chain analogue **45** and when the side chain is shortened in urea compound **27** to give derivative **32**.

We note that the retroamide with lengthened side chain **46** is an antagonist but that its affinity for the receptor is quite low. A similar result was obtained after shortening the side chain; **32** is a weak antagonist and no activity was seen for **18** and **33**.

If we take into account the above mentioned Table 4 it appears that reasoning strictly according to agonist and antagonist indexes we have obtained several antagonist derivatives, especially compounds **12**, **16**, **19**, **20**, **21**, **39**, **40**, **41**, **45**, and **46**. Weak antagonists are compounds **11**, **13**, **14**, **32**, **49**, **57**, **58** and **59**. Lastly, replacement of the naphthalenic ring by indole, benzothiophene or benzofuran ring has little effect on the previously described structure–activity relationships.

Among the potential antagonists we note that we have:

- the 7-demethoxy compound **12** with a bulky cyclobutyl group as substituent of the carbonyl of the amidic function (The corresponding cyclopropyl derivative is a weak antagonist and the corresponding methyl derivative is a weak agonist);
- a compound having a ramification on the side chain **16** and a cyclopropyl substituent on the carbonyl of the amidic function (The analogue (**11**) without a ramification is an agonist);
- the three amidic compounds (**19**, **20**, and **21**) we made with an extended side chain, a propyl link replacing the ethyl one (Analogues with an ethyl side chain are agonists);
- the two ether compounds (**39** and **40**);
- an ester compound (**41**) with an extended propyl side chain (The analogue **42** with the ethyl side chain is a partial agonist);
- the two retroamidic derivatives (**45** and **46**) having an extended propyl side chain. The analogue of the derivative **45** having an ethyl side chain (**44**) is a full agonist;

Lastly, it is important to compare the binding values obtained with compounds **46** and **27**. In **46** the NH group of the original amidic function (which is also one of the two NH groups of the urea function of compound **27** is replaced by a CH_2 group. Compound **46** is a retroamidic derivative with a propylene side chain and the binding inhibition profiles are different.

Derivative **27** gives a biphasic binding curve with a very high affinity ($pK_i(H_i) = 14.3$) component and is an agonist whereas compound **46** gives a much lower affinity monophasic response ($pK_i = 7.37$) and is an antagonist. This provides evidence for the importance of the NH group of the amidic function, which could provide a hydrogen bond, for full functional activation of the receptor response. It appears that the presence of this group, separated from the aromatic ring by an ethylene side chain is necessary to obtain ligands with full agonist activity and with good affinity whereas compounds, which do not possess this NH group (**39**–**42**), are poor ligands.

These results confirm the importance of the secondary amide site as well as the methoxy group fixed on the heterocyclic system for binding of the molecule to the melatonin receptor as was previously reported.[1] It also appears that the respective position of these two structural features is similarly important since any modification

of the length of the ethylene moiety of the side chain dramatically reduces binding affinity and modifies the biological activity of the compounds. For full agonists, a 7-methoxy group as well as an ethyl side chain between the NH group of the amidic function and an aromatic ring are necessary. Deletion of this 7-methoxy group, an increase in the global steric size of the molecule, modifications concerning the amidic function itself and its position relative to the aromatic ring can result in full or partial agonist. For these antagonists, we have yet to compare the binding data with Schild analyses of the full functional responses curves. However, compound **12** appears to be the best antagonist from this series and it has been selected for preclinical development.

Experimental

Chemistry

Melting points were determined on a BUCHI 510 capillary apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 297 spectrophotometer. ^{1}H NMR spectra were recorded on a WP 80-54 or a 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.

(7-Methoxy-1-naphthyl) methylamine hydrochloride (3a). To a 10% NaOH solution (30 mL), 0.65 mL of bromine and 2.15 g (0.01 mol) of 7-methoxy-1-naphthylacetamide were slowly added, under cooling and stirring. The mixture was stirred for 30 min at room temperature and then for a further 16 h at 80 °C. After cooling, the mixture was ether extracted and 1 N HCl was added until pH 2–3. Aqueous phases were neutralized with NaOH and extracted with ether. Evaporation of the organic layer yielded an oil that, by treatment with HCl gas in anhydrous ether, afforded a solid, that was recrystallized from ethanol to give 1.61 g (72%) of (**3a**): mp > 260 °C, ^{1}H NMR (80 MHz, DMSO d_6) 8.75 (s, 3H), 8.00–7.00, (m, 6H), 4.50 (s, 2H), 4.00 (s, 3H). Anal. ($C_{12}H_{14}$Cl NO) C, H, N.

General procedure for the synthesis of the methyl (7-methoxy-1-naphthyl)alkylcarboxylates derivatives (4a–4b). The method adopted for the synthesis of methyl (7-methoxy-1-naphthyl)acetate (**4a**) is described. A solution of 5 g (0.023 mol) of the acid (**2a**) in 150 mL of methanol was cooled to −10 °C. Under stirring, 6.75 mL (0.092 mol) of $SOCl_2$ were added dropwise and the mixture was stirred for 30 min at room temperature. After evaporation, the oil was dissolved in ethylacetate and the solution washed with 10% potassium carbonate aqueous solution and then with water. After drying, the organic layer was evaporated yielding a residue, that was crystallized from *n*-hexane affording 4.98 g (94%) of **4a**: mp 51–52 °C, ^{1}H NMR (80 MHz, $CDCl_3$) 7.90–7.60 (m, 2H), 7.45–7.10 (m, 4H), 4.00 (s, 2H), 3.90 (s, 3H), 3.65 (s, 3H). Anal. ($C_{14}H_{14}O_3$) C, H, O.

General procedure for the synthesis of the (7-methoxy-1-naphthyl)alkanols derivatives (5a–5b). The method adopted for the synthesis of 2-(7-methoxy-1-naphthyl) ethanol (**5a**) is described. To a stirred suspension of 3.35 g (0.088 mol) of $LiAlH_4$ in 50 mL of dry ether was added a solution of 5 g (0.022 mol) of (**4a**) in 50 mL of dry ether and the reaction was stirred at room temperature for 30 min. Addition of water, filtration, ether extraction of the filtrate and evaporation of the organic layer gave a solid residue, that was crystallized from cyclohexane affording 3.65 g (82%) of **5a**: mp 80–82 °C, ^{1}H NMR (80 MHz, $CDCl_3$) 7.80–7.60 (m, 2H) 7.40–7.00 (m, 4H), 4.00 (t, 2H), 3.90 (1, 3H), 3.30 (t, 2H), 1.60 (s, 1H). Anal. ($C_{13}H_{14}O_2$), C, H, O.

General procedure for the synthesis of the (7-methoxy-1-naphthyl)alkanol mesylates derivatives (6a–6b). The method adopted for the synthesis of 2-(7-methoxy-1-naphthyl)ethanol mesylate (**6a**) is described. A solution of 4 g (0.02 mol) of (**5a**) in 100 mL of dichloromethane and 3.3 mL (0.024 mol) of triethylamine at −10 °C was treated with 2.75 g (0.024 mol) of mesyl chloride and, the mixture was stirred at 0 °C for 30 min. The reaction mixture was washed with H_2O, 1 N HCl and the organic layer dried. Concentration afforded the crude mesylate which was crystallised from cyclohexane to give 5.27 g (94%) of **6a**: mp 62–63 °C, ^{1}H NMR (80 MHz, $CDCl_3$) 7.80–7.60 (m, 2H), 7.40–7.10 (m, 4H), 4.55 (t, 2H), 3.95 (s, 3H), 3.50 (t, 2H), 2.85 (s, 3H). Anal. ($C_{14}H_{16}O_4$) C, H, O.

General procedure for the synthesis of the (7-methoxy-1-naphthyl)alkyl nitriles derivatives (7a–7b). The method adopted for the synthesis of 3-(7-methoxy-1-naphthyl) propionitrile (**7a**) is described. 4.2 g (0.015 mole) of the mesylate (**6a**) was dissolved in 20 mL of DMSO and treated under reflux for 2 h with 2.90 g (0.045 mol) of KCN. The reaction mixture was cooled and poured into ice/H_2O precipitating the crude nitrile which was crystallised from *n*-hexane to give 3 g (95%) of **7a**: mp 62–64 °C, ^{1}H NMR (80 MHz, $CDCl_3$) 7.90–7.70 (m, 2H), 7.40–7.20 (m, 4H), 4.00 (s, 3H), 3.40 (t, 2H), 2.75 (t, 2H). Anal. ($C_{14}H_{13}NO$) C, H, O.

General procedure for the synthesis of the (7-methoxy-1-naphthyl)alkylamine derivatives (3c and 3d). The method adopted for the synthesis of 3-(7-methoxy-1-naphthyl)-propylamine hydrochloride (**3c**) is described. An over NH_3 saturated solution of 1.50 g (0.006 mol) of (**7a**) in 150 mL of ethanol was hydrogenated over Raney nickel

under pressure (50 bars) at 60 °C for 12 h. After filtration and evaporation, the oil was dissolved in dry ether and treated with gazeous HCl to give after filtration and crystallisation from cyclohexane/ethanol (1:1) 1.37 g (91%) of **3c**: mp 197–200 °C, ^{1}H NMR (300 MHz, DMSO-d_6) 8.10 (s, 3H), 7.85 (d, 1H), 7.75 (d, 1H), 7.40–7.15 (m, 4H), 3.95 (s, 3H), 3.10 (t, 2H), 2.90 (t, 2H), 2.00 (m, 2H). Anal ($C_{14}H_{18}ClNO$) C, H, N, O.

Methyl-2-cyano(1-naphthyl) acetate (7c). To a hot (110 °C) solution of 16.7 g (0.1 mol) of 1-naphthylacetonitrile in 120 mL of dimethylcarbonate, 2.3 g (0.1 atg) of sodium was added slowly during 30 min. The mixture was maintained at this temperature for 1 h and then evaporated. After extraction with ethylacetate, the organic layer was dried and evaporated. The solid residue was recrystallised from cyclohexane to give 19.8 g (88%) of **7c**: mp 89–90 °C, ^{1}H NMR (80 MHz, DMSO-d_6) 8.10–7.40,(m, 7H), 5.40 (s, 1H), 3.75 (s, 3H). Anal. ($C_{14}H_{11}NO_2$) C, H, N.

Methyl-3-amino-2-(1-naphthyl)propionate (3e) hydrochloride. To a solution of 4.5 g (0.02 mol) of the nitrile (**7c**) in 250 mL of methanol, 9.5 g (0.04 mol) of $CoCl_2$ was added, followed by slow addition of 5.29 g (0.14 mol) of $NaBH_4$ at room temperature. The mixture was stirred at this temperature for 2 h and then made acidic with 3 N HCl. Solvent was evaporated and the solution extracted with ethylacetate. The aqueous solution was made basic with aqueous ammonia and extracted several times with ethylacetate. The organic layers were evaporated and the obtained residue dissolved in ethanol. Gazeous HCl was bubbled into the solution to afford a solid, that was recrystallized from acetonitrile to give 2.38 g (42%) of **3e**: mp 217–219 °C, ^{1}H NMR (80 MHz, DMSO-d_6) 8.40 (s, 3H), 8.25–7.40, (m, 7H), 5.10 (dd, 1H), 3.60 (s, 3H), 3.60–3.00 (m, 4H). Anal. ($C_{14}H_{16}ClNO_2$, H_2O) C, H, N.

General procedure for the synthesis of the (7-methoxy-1-naphthyl)alkanoic acids derivatives (2b and 2c). The method adopted for the synthesis of 3-(7-methoxy-1-naphthyl)propionic acid (**2b**) is described. One gramme (0.0047 mol) of **7a** was dissolved in 10 mL of methanol and 10 ml (0.06 mol) of a 6 N aqueous solution of sodium hydroxyde was then added. The mixture was refluxed for 15 h. After cooling and acidification with 6 N HCl, the precipitate was separated and then crystallized from toluene affording 0.97 g (90%) of **2b**: mp 154–155 °C, ^{1}H NMR (80 MHz, DMSO-d_6) 12.20 (s, 1H), 7.95–7.60 (m, 2H), 7.10–7.40 (m, 4H), 3.90 (s, 3H), 3.30 (t, 2H), 2.65 (t, 2H). Anal. ($C_{14}H_{14}O_3$) C, H, O.

General procedure for the synthesis of the (7-methoxy-1-naphthyl)alkyl ethers derivatives (39–41). The method adopted for the synthesis of 2-(7-methoxy-1-naphthyl)-ethyl methyl ether (**39**) is described. 5 mL (0.05 mol) of dimethylsulfate and 0.04 g (0.00014 mol) of benzyltriethylammonium chloride were added to a mixture of 0.6 g (0.003 mol) of the alcohol (**5a**) in 1.2 mL of 50% aqueous sodium hydroxyde solution and 5 ml of toluene. The reaction mixture was vigorously stirred for 24 h. Toluene was separated and the aqueous layer extracted with toluene. The organic layer was separated, washed with water, dried and evaporated under reduced pressure to afford crude (**39**), which was purified by column chromatography (SiO_2, cyclohexane/ethylacetate, 4/1) to give 0.34 g (53%) of pure **39**: ^{1}H NMR (300 MHz, $CDCl_3$) 7.73 (d, 1H), 7.65 (d, 1H), 7.44–7.13 (m, 4H), 3.93 (s, 3H), 3.73 (t, 2H), 3.40 (s, 3H), 3.30 (t, 2H). Anal. ($C_{14}H_{16}O_2$) C, H, N.

(7-Methoxy-1-naphthyl) ethyl acetate (42). A solution of 2 g (0.01 mol) of the alcohol (**5a**) in 20 g (0.2 mol) of acetic anhydride was refluxed for 2 h. After cooling and evaporation under reduced pressure, the oily residue was purified by column chromatography (SiO_2, cyclohexane/ethylacetate, 4/1) to give an oil, which then afforded a solid that was crystallized from hexane to give 0.235 g (96%) of pure **42**: mp 51–52 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 7.77 (d, 1H), 7.67 (d, 1H), 7.47–7.13 (m, 4H), 4.41 (t, 2H), 3.98 (s, 3H), 3.35 (t, 2H), 2.07 (s, 3H). Anal. ($C_{15}H_{16}O_3$) C, H, N.

General procedure for the synthesis of the (7-methoxy-1-naphthyl)alkyl carboxamides derivatives (43–46). The method adopted for the synthesis of 2-(7-methoxy-1-naphthyl)ethyl carboxamide (**43**) is described. To a solution of 1.1 g (0.0048 mol) of the acid (**2b**) in 20 mL of chloroform, 5.3 mL (0.072 mol) of $SOCl_2$ were added dropwise under stirring and the mixture was then refluxed for 2 h. After cooling and evaporation, the residue was taken off with anhydrous ether and filtrated. To the cooled filtrate, 8.8 mL (0.096 mol) of 20% aqueous solution of NH_3 was added in one portion. Stirring was continued for 1 h. After filtration, the crude amide was crystallized from 95 °C ethanol affording 0.47 g (43%) of **43**: mp 125–126 °C, ^{1}H NMR (80 MHz, $CDCl_3$) 7.80–7.60 (m, 2H), 7.40–7.00 (m, 4H), 5.40 (s, 2H), 3.95 (s, 3H), 3.40 (t, 2H), 2.60 (t, 2H). Anal. ($C_{14}H_{15}NO_2$) C, H, N.

General procedure for the synthesis of the *N*-monosubstituted ureas derivatives (24 and 30). The method adopted for the synthesis of *N*-[2-(7-methoxy-1-naphthyl)ethyl] urea (**24**) is described. 0.55 g (0.0067 mol) of potassium cyanate was added to a solution of 1.5 g (0.006 mol) of **3b** hydrochloride in 8 mL of water. The reaction mixture was then stirred at room temperature for 30 min. The solid formed was separated and then crystallized from ethylacetate, affording 0.79 g (65%) of **24**: mp 187–188 °C; ^{1}H NMR (300 MHz, DMSO-d_6)

7.82 (d, 1H), 7.69 (m, 2H), 7.29 (m, 2H), 7.15 (dd, 1H), 6.14 (m, 1H), 5.52 (s, 2H), 3.94 (s, 3H), 3.28 (m, 2H), 3.10 (t, 2H). Anal. ($C_{14}H_{16}N_2O_2$) C, H, N.

General procedure for the synthesis of the dissymetric *N*,*N*′-disubstituted ureas derivatives (25–28; 31–38; 52; 54–56; 58). The method adopted for the synthesis of *N*-[2-(7-methoxy-1-naphthyl)ethyl]-*N*′-methyl urea (**25**) is described. 0.23 mL (0.0055 mol) of methyl isocyanate was added to a solution of 1 g (0.005 mol) of **3b** in 8 mL of ether. The reaction mixture was then stirred at room temperature for 30 min. The solid formed was separated and then crystallized from toluene/cyclohexane (9/1) affording 1.03 g (80%) of **25**: mp 139–141 °C; ^{1}H NMR (80 MHz, DMSO-d_6) 7.85–7.05 (m, 6H), 4.55 (t, 1H), 3.95 (s, 3H), 3.55 (m, 2H), 3.20 (t, 2H), 2.65 (d, 3H). Anal. ($C_{15}H_{18}N_2O_2$) C, H, N.

General procedure for the synthesis of the symetric *N*,*N*′-disubstituted ureas derivatives (29 and 53). The method adopted for the synthesis of bis *N*,*N*′-[2-(7-methoxy-1-naphthyl)ethyl urea (**29**) is described. 0.8 g (0.005 mol) of *N*,*N*′-carbonyl diimidazole was added to a solution of 2 g (0.01 mol) of **3b** in 100 mL of chloroform. The reaction mixture was then refluxed for 4 h. The organic layer was separated, washed with 2 N HCl, then with water, dried and evaporated under reduced pressure. The residue was crystallized from toluene, affording 1.20 g (56%) of **29**: mp 187–189 °C; ^{1}H NMR (80 MHz, $CDCl_3$) (7.80–7.10 (m, 12H), 7.95 (s, 6H), 3.50 (m, 4H), 3.20 (m, 4H). Anal. ($C_{27}H_{28}N_2O_3$) C, H, N.

General procedure for the synthesis of the alkyl *N*-2-(7-methoxy-1-naphthyl)ethyl carbamates derivatives (22–23). The method adopted for the synthesis of methyl *N*-2-(7-methoxy-1-naphthyl)ethyl carbamate (**22**) is described. Methyl chloroformate (0.0055 mol) was added dropwise to a cooled solution of 1.25 g (0.005 mol) of **3b** hydrochloride in 10 mL of THF and 1.1 mL (0.015 mol) of triethylamine. The reaction mixture was then stirred at room temperature for 1 h and filtered. The filtrate was evaporated under reduced pressure and the residue crystallized from toluene/cyclohexane (9/1) affording 0.45 g (35%) of **22**: mp 70–71 °C; ^{1}H NMR (80 MHz, DMSO-d_6) 7.90–7.05 (m, 6H), 4.75 (m, 1H), 3.80 (s, 3H), 3.70 (s, 3H), 3.55 (m, 2H), 3.30 (t, 2H). Anal. ($C_{15}H_{17}NO_3$) C, H, N.

General procedure for the synthesis of the *N*-acylated derivatives (10–12; 14–21; 47–51; 57; 59–60). The method adopted for the synthesis of *N*-(7-methoxy-1-naphthyl)methyl acetamide (**18**) is described. Potassium carbonate (0.003 mol) was added to a solution of 0.24 g (0.001 mol) of **3a** hydrochloride in 15 mL of water and 50 mL of chloroform. The mixture was cooled to 0 °C. 0.12 mL (0.0016 mol) of acetyl chloride was added dropwise at this temperature. The reaction mixture was then stirred at room temperature for 1 h. After acidification with 2 N HCl and stirring for 15 min, the aqueous layer was extracted three times with chloroform. The organic layers were separated, washed with water, dried and evaporated under reduced pressure. The residue was crystallized from toluene affording 0.21 g (94%) of **18**: mp 165–166 °C; ^{1}H NMR (80 MHz, $CDCl_3$) 7.80–7.00 (m, 6H), 5.70 (s, 1H), 4.80 (d, 2H), 3.90 (s, 3H), 2.00 (s, 3H). Anal. ($C_{14}H_{15}NO_2$) C, H, N.

***N*-[2-(1-naphthyl)ethyl] trifluoroacetamide (13).** A suspension of 2.10 g (0.01 mol) of **3d** hydrochloride in 6 mL of pyridine was cooled to 5 °C. 2.1 g (0.01 mol) of trifluoroacetic anhydride 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 crystallised from cyclohexane affording 0.23 g (85%) of **13**: mp 79–80 °C ^{1}H NMR (80 MHz, $CDCl_3$) 8.15–7.30 (m, 7H), 6.50 (s, 1H), 3.70 (m, 2H), 3.32 (t, 2H). Anal. ($C_{14}H_{12}F_3NO$), C, H, N.

Pharmacology. Reagents and chemicals. 2-[^{125}I]-Iodomelatonin was purchased from a commercial source (Dupont-NEN) at a specific activity of approximately 2000 Ci/mmol. [^{125}I]AMP was also prepared as described.[21] Stock chemicals and media were purchased from Sigma Chemical Co. and BRL Life Technologies. Novel compounds were synthesized as described in the present paper.

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[22] and primary culture of pars tuberalis cells[21] 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 0.04 pars tuberalis equiv/50 µL (ca. 6–12 µg of protein by the method of Bradford[23]). 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. For competitive binding experiments, 50 µL membrane suspension was added to wells of specially designed 96-well plates (Milipore Multiscreen Assay System, Millipore Inc, Bedford, MA, USA) containing 150 µL buffer with approximately 50 pM 2-[^{125}I]-iodomelatonin and varying concentrations of competing ligand (0, 10^{-14}–10^{-4} M final concentration). Reactions

(2 h, 37 °C) were stopped by rapid filtration (ca. 15 sec/plate) on a vacuum manifold and the wells were washed once with 200 μL cold buffer. Plates were then dried and the filter disks, with trapped membrane bound radioligand, were punched from the bottom of each well directly into tubes which were then counted on a Packard Instrument Co Model 5010 autogamma counter at 82% efficiency. Count data were analyzed by non-linear regressions as described earlier. Individual drugs were assayed at least three times and the values reported are the means and estimated standard deviations of logarithms of the calculated inhibition constants.

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 radioimunnoassay 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 analysed on-line (Packard Instrument Co. Model 5010 auto-gamma counter cobra system software) using 4-parameter logistic 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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