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Design and Synthesis of 3-Phenyl Tetrahydronaphthalenic Derivatives as New Selective MT_2 Melatoninergic Ligands

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Abstract—Tetrahydronaphthalenic analogues of melatonin have been synthesized and evaluated as melatonin receptor ligands. Introduction of a phenyl substituent in the 3-position of the tetraline ring allows to obtain MT_2 selective ligands. Activity and MT_2 selectivity can be modulated with suitable modifications of the *N*-acyl substituent. The (+)-(*RR*)-*cis* enantiomer of the *N*-[2-(7-methoxy-3-phenyl-1,2,3,4-tetrahydro-naphthalen-1-yl)ethyl]cyclobutyl carboxamide (**14**) is one of the most MT_2 selective ligands described until now and behaves as an antagonist.

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) (**1**) (Chart 1) is a neurohormone secreted by the pineal gland during the dark period.[1] Its activity is mediated through two high-affinity G-protein coupled receptors named MT_1 and MT_2[2–4] and a low-affinity site termed MT_3 that was recently identified as the quinone reductase 2 (QR2).[5] MT_1 receptors are expressed in several areas of the brain, more particularly in the suprachiasmatic nuclei and in the *Pars tuberalis* of the pituitary, and might be implicated in the sleep promoting effect of melatonin and in the control of reproductive function.[2] MT_2 receptors are essentially localized in the suprachiasmatic nuclei and in retina and might be implicated in the resynchronizing activity of melatonin.[6] The design of new compounds which selectively bind on each MT_1 or MT_2 receptor subtype, would provide a better understanding of melatonin receptors functions and probably could open new therapeutic perspectives beside the resynchronizing properties of melatonin clearly demonstrated in humans.[7,8]

To date, only a few selective melatonin receptor ligands (i.e., agonists and antagonists) have been reported (Chart 1). They principally concern the MT_2 subtype and are connected to diverse chemical families. The naphthalene and indole derivatives **3** and **4**[9,10] have been respectively described as agonist and antagonist with MT_1/MT_2 affinity ratio about 80 and 25. The most interesting compounds belong to the tetralinic series; compound **5**[10] behaves as a selective MT_2 antagonist, nevertheless there is no report about the stereochemistry of this compound.

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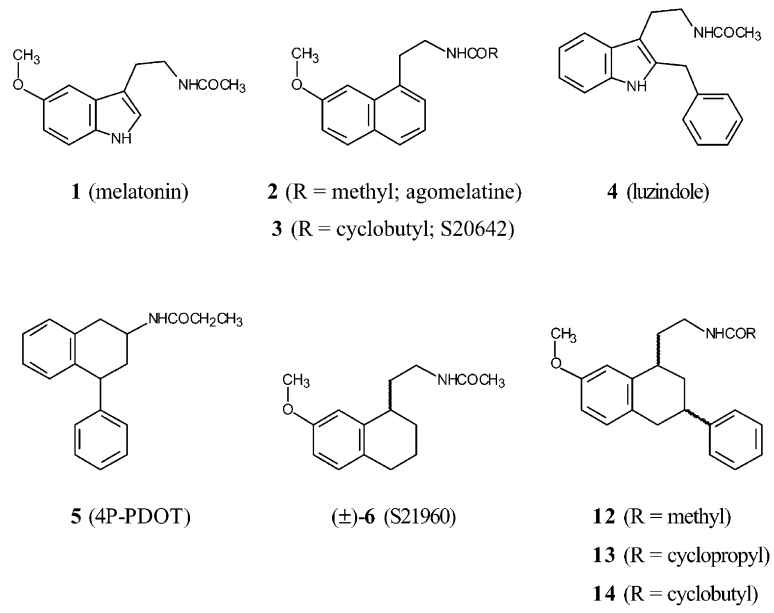


1 (melatonin) **2** (R = methyl; agomelatine) **3** (R = cyclobutyl; S20642) **4** (luzindole)

5 (4P-PDOT) (±)-**6** (S21960) **12** (R = methyl) **13** (R = cyclopropyl) **14** (R = cyclobutyl)

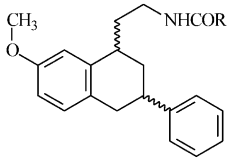
Chart 1. Chemical structures of melatonin ligands.

Our earlier studies[11,12] have shown that bioisosteric replacement of the indole heterocycle of melatonin by some rings such as naphthalene, benzofuran or benzothiophen leads to agonist ligands that possess the same level of binding affinity and selectivity as melatonin on both MT_1 and MT_2 receptor subtypes. The naphthalenic bioisostere (**2**, agomelatine) has been selected for clinical development and is, at the present time, in phase III clinical trials. Further structural modifications of **2** were undertaken by replacing the naphthalene ring by its tetrahydronaphthalenic analogue **6**[13] which has the same affinity as **2** for the MT_2 subtype but a 10 times lower affinity for the MT_1 receptor, leading to a slight degree of selectivity (Table 1).

We therefore selected **6** as a lead compound in the search for MT_2 selective ligands. Considering the potential role of: (i) a phenyl substituent (**5**) and (ii) a cyclobutyl ring (**3**) we synthesized the tetrahydronaphthalenic derivatives **12**–**14** bearing a phenyl group in the 3-position and a methyl or a cycloalkyl amidoethyl side chain in the 1-position.

Herein, we report the synthesis and the structure–selectivity relationships, which have led us to the finding of a novel series of potent selective MT_2 antagonists.

Table 1. MT_1 and MT_2 receptor binding affinities of tetralinic compounds



Compd	R	$K_i \pm$SEM (nM) MT_1	$K_i \pm$SEM (nM) MT_2	MT_1/MT_2
1 (melatonin)	—	0.12±0.02	0.31±0.05	0.4
5 (4P-PDOT)	—	108±15	0.96±0.23	112
(±)-**6** (S21960)	—	0.96±0.11	0.103±0.003	9
(±)-*cis*-**12**	Methyl	20.5±8.70	0.31±0.094	66
(±)-*cis*-**13**	Cyclopropyl	132±18.0	1.14±0.683	116
(±)-*cis*-**14a**	Cyclobutyl	439±124	2.28±0.521	193
(+)-(*RR*)-*cis*-**14a**	Cyclobutyl	797±79.0	0.905±0.465	880
(−)-(*SS*)-*cis*-**14a**	Cyclobutyl	557±70.5	1.21±0.141	460
(±)-*trans*-**14b**	Cyclobutyl	109±6.00	4.53±0.78	24
(+)-*trans*-**14b**	Cyclobutyl	660±27.5	0.902±0.399	730
(−)-*trans*-**14b**	Cyclobutyl	248±6.52	2.08±0.445	120

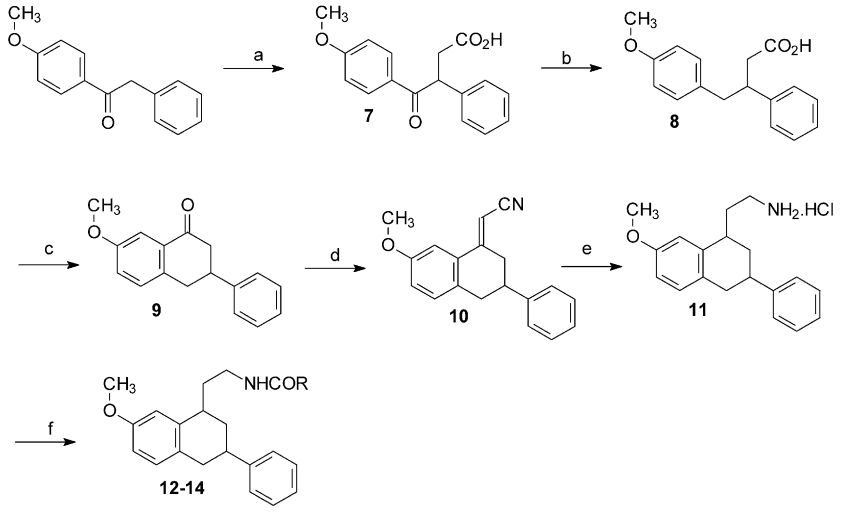
Concentration–response curves were analyzed by non-linear regression comparing a one-site and a two sites analysis. All the curves were found to be monophasic with a Hill number close to unity (not shown). Binding affinities (nM) are expressed as mean $K_i \pm$SEM of at least three independent experiments.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

Results and Discussion

Chemistry

The synthetic pathway for compounds **12**–**14** is outlined in Scheme 1.

1-(4-Methoxyphenyl)-2-phenylethanone[14] was reacted with methyl bromoacetate in the presence of sodium hydride in DMF and the crude ester was saponified by heating in a 3 M NaOH solution to produce the acid **7**. Selective reduction of the ketonic group was achieved by treatment with the triethylsilane/trifluoroacetic acid reagent[15] to afford compound **8** which was cyclized to the tetralone **9** by heating in polyphosphoric acid. A Horner–Emmons reaction[16] with diethyl cyanomethylphosphonate gave a *Z*/*E* mixture of nitrile **10**. After recrystallisation we obtain only the *E* isomer as shown by a proton correlation (COSY) data. This was hydrogenated in the presence of Raney nickel in ethanol to afford the primary amine **11** which was identified as a mixture of 2/3(±)-*cis* and 1/3(±)-*trans* as measured by



Scheme 1. Synthesis of compounds **12–14**. Reagents: (a) (i) $BrCH_2COOCH_3$, NaH, DMF; (ii) NaOH, methanol, water; (b) $(C_2H_5)_3SiH$, CF_3COOH; (c) Polyphosphoric acid; (d) $(C_2H_5O)_2P(O)CH_2CN$, NaH, anhydrous THF; (e) H_2, Raney nickel, ethanol, NH_3 (g); (f) RCOCl, K_2CO_3, chloroform, water.

^{1}H NMR. The acylated compounds **12–14** were then prepared from this mixture by treatment with the appropriate acid chloride in the presence of K_2CO_3.[17] Crude amides **12–14** were obtained as mixtures of 2/3(±)-*cis* and 1/3(±)-*trans* as measured by HPLC and ^{1}H NMR.

The relative configuration of the tetralinic cyclic carbons (C1 and C3) was assigned through ROESY (mixing time: 1 s). The observed cross-peaks between H1 and H3 and the lack of cross-peaks between H3 and the CH_2 of the ethyl side chain were unambiguously indicative of the *cis* relationship.

Isolation of *cis* racemates **11–14** was achieved by fractional crystallization while *trans* racemate isolation was only successful for compound **14**. The four stereoisomers of compound **14** were resolved using chiral preparative HPLC after optimisation of chiral analytical HPLC.[18] They were obtained with a high enantiomeric purity (>99%) (except for compound (−)-*trans*-**14b**: ee 96%) and were characterized by their mass and ^{1}H NMR spectra which were identical to their racemic mixtures and by their melting point and specific optical rotation (α_D).

An X-ray crystallographic determination was carried out on the two *cis* enantiomers (+)-(*cis*)-**14a** and (−)-(*cis*)-**14a** which were respectively found to have the (*RR*) and the (*SS*) configuration (for experimental procedure, see Supplementary Material).

Pharmacology

The compounds were evaluated for their binding affinity for human MT_1 and MT_2 receptors stably transfected in Human embryonic kidney (HEK 293) cells, using 2-[^{125}I]-iodomelatonin as radioligand.[19]

The [^{35}S]GTPγS binding assay used to determine the functional activity of the compounds was difficult to handle using the transfected HEK 293 cell lines, while reliable results were obtained using Chinese hamster ovarian (CHO) cell lines stably expressing the human MT_1 or MT_2 receptors. At each receptor, binding affinities were verified for more than 50-selective and non selective molecules, either using the transfected HEK 293 or the CHO cell lines. Indeed, the correlations between affinities in HEK 293 and CHO cells are highly significant ($r = 0.98$) (unpublished data), for both MT_1 and MT_2 receptors.

The chemical structures, binding affinities and MT_1/MT_2 selectivity ratios of compounds **12–14** are reported in Table 1. Their agonist (EC_{50}) or antagonist (K_B) potencies and efficacies (expressed relatively to that of melatonin taken at 100%) in the [^{35}S]GTPγS binding assay are shown in Table 2.

Introduction of a phenyl group in the 3-position of the tetraline ring [(±)-**6** vs (±)-*cis*-**12**] causes a 20-fold decrease in the MT_1 but maintains the MT_2 binding affinity, leading to an enhancement of the MT_1/MT_2 selectivity ratio from 9 to 66. This result confirms the importance of a phenyl substituent to achieve MT_2 selectivity. Moreover compound (±)-*cis*-**12** behaves as a MT_1 antagonist and a MT_2 partial agonist. Replacement of the methyl group of the acetamido side chain by cycloalkyl ones such as cyclopropyl [(±)-*cis*-**13**] or cyclobutyl [(±)-*cis*-**14a**], leads to a higher decrease in MT_1 than in MT_2 binding affinity enhancing the MT_1/MT_2 selectivity ratios. This may be related to the substituent size since the best selectivity ratio is obtained with the bulkier cyclobutyl group. The *cis* racemates (±)-*cis*-**13** and (±)-*cis*-**14a** respectively show 2 and 3-fold higher selectivity ratio toward the MT_2 subtype than (±)-*cis*-**12**, a finding that parallels previous result about the role of a cycloalkyl substituent on the acylamino side chain.[9] Moreover, compounds (±)-*cis*-**13** and (±)-*cis*-**14a** retain a high (nM) MT_2 affinity and behave as MT_2 and MT_1 antagonists. Comparison between the two racemates (±)-*cis*-**14a** and (±)-*trans*-**14b** shows that the *cis* racemate (±)-*cis*-**14a** is 8-fold more MT_2 selective

Table 2. Activity values

Compd	MT_1		MT_2		
	$EC_{50}\pm$SEM (nM)	$E_{max}\pm$SEM (%)	$E_{max}\pm$SEM (%)	$EC_{50}\pm$SEM (nM)	$K_B\pm$SEM (nM)
1 (melatonin)	2.24±0.35	100	0.49±0.05	100	nd
5 (4P-PDOT)	Inactive	<10	1.33±0.28	19.1±2.4	1.78±0.8
(±)-*cis*-**12**	Inactive	<10	1.28±0.39	37.2±2.63	0.68±0.3
(±)-*cis*-**13**	Inactive	<10	Inactive	<10	1.87±0.4
(±)-*cis*-**14a**	Inactive	<10	Inactive	<10	3.53±1.5
(+)-*cis*-**14a**	Inactive	<10	Inactive	<10	3.1±1.8
(−)-*cis*-**14a**	Inactive	<10	Inactive	<10	26.6±1.3
(±)-*trans*-**14b**	Inactive	<10	Inactive	<10	2.01±0.1
(+)-*trans*-**14b**	Inactive	<10	Inactive	<10	7.18±2.1
(−)-*trans*-**14b**	Inactive	<10	Inactive	<10	nd

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

than the *trans* one, principally because of a worse MT_1 binding affinity. It seems therefore that the relative spatial orientation of the phenyl group and the acylamino side chain is more important in the case of the MT_1 subtype. This supposition is partially ascertained by the results obtained with the four isomers of **14**: (+)-*cis*-**14a** and (+)-*trans*-**14b** show the same and greatest MT_2 affinity (0.90 nM) and slightly differ by their low MT_1 affinity, which result in the best selectivity ratios (880 and 730, respectively). The (−)-*cis* enantiomer of **14a** shows a comparable MT_2 affinity (1.2 nM) but a slightly higher MT_1 affinity leading to a lower selectivity ratio (460). On the other hand, the (−)-*trans* enantiomer of **14b** shows the less MT_2 selective (ratio = 120) principally because of its 2- to 3-fold better MT_1 affinity (248 nM). Concisely, these four isomers show the same range (nM) of MT_2 affinity but their selectivity ratio toward this MT_2 subtype varies from 120 to 880 according to the following rank of order: (−)-*trans* <(−)-*cis* <(+)-*trans* <(+)-*cis*. The stereochemistry in this tetrahydronaphthalenic series does not seem therefore relevant for the MT_2 binding affinity whereas it allows to modulate the MT_1/MT_2 selectivity ratios.

Experimental

Chemistry

Melting points were determined on a Buchi SMP-20 capillary apparatus and are uncorrected. IR spectra were recorded on a Vector 22 Bruker spectrophotometer. ^{1}H NMR spectra were recorded on a AC 300 Bruker spectrometer. Chemical shifts are reported in δ units (parts per million) relative to $(Me)_4Si$. Mass spectra were performed on a Ribermag 10-106 Finnigan mass spectrometer in fast atom bombardment (FAB) mode (with 2-hydroxyethyl disulfide as matrix) or by electron impact (EI). Optical rotations (α_D) at the sodium D-line were determined in CH_2Cl_2 solutions of the indicated concentrations using a Perkin-Elmer 241 polarimeter. Elemental analyses for final substances were performed by CNRS Laboratories (Vernaison, France). Obtained results were within 0.4% of the theoretical values.

4-(4-Methoxyphenyl)-4-oxo-3-phenylbutyric acid (7). NaH (60% in mineral oil) (5.2 g, 0.13 mol) was added portionwise to a cooled solution of methyl bromoacetate (12.35 mL, 0.13 mol) and 1-(4-methoxyphenyl)-2-phenylethanone (22.6 g, 0.1 mol) in dry DMF (150 mL). The reaction mixture was stirred at room temperature for 16 h and then poured into ice-water. The aqueous phase was extracted with ether and the organic layer was washed with water, aqueous potassium bicarbonate solution, and concentrated under reduced pressure. The residue was dissolved in methanol (100 mL) and an aqueous sodium hydroxide solution (20 g, 0.5 mol) was added. The solution was heated at reflux for 1 h and then extracted twice with diethylether. The aqueous phase was acidified with concentrated HCl and the precipitate was filtered and crystallized from toluene to give 20 g (70% yield) of **7**; mp 147–148 °C; ^{1}H NMR (DMSO-d_6) δ: 2.58 (dd, 1H, J = 4.2, 16.9 Hz), 3.14 (dd, 1H, J = 10.5, 16.9 Hz), 3.80 (s, 3H), 5.15 (dd, 1H, J = 4.2, 10.5 Hz), 6.98 (d, 2H, J = 8.8 Hz), 7.17–7.20 (m, 2H), 7.25–7.35 (m, 3H), 8.00 (d, 2H, J = 8.8 Hz), 12.25 (s, 1H).

4-(4-Methoxyphenyl)-3-phenylbutyric acid (8). Triethylsilane (21 mL, 0.132 mol) was added to a solution of **7** (17.05 g, 0.06 mol) in TFA (80 mL). The reaction mixture was stirred vigorously at room temperature for 48 h. TFA was then evaporated under reduced pressure and the residue was taken off with petroleum ether. The resulting precipitate was filtered and crystallized from toluene to give 14 g (86% yield) of **8**; mp 152–153 °C; ^{1}H NMR (DMSO-d_6) δ: 2.47–2.55 (m, 2H), 2.74–2.87 (m, 2H), 3.26 (m, 1H), 3.68 (s, 3H), 6.78 (d, 2H, J = 8.5 Hz), 7.00 (d, 2H, J = 8.5 Hz), 7.13–7.27 (m, 5H), 12.00 (s, 1H).

7-Methoxy-3-phenyl-3,4-dihydro-2*H*-naphthalen-1-one (9). A mixture of **8** (8.1 g, 0.03 mol) in polyphosphoric acid (80 g) was stirred at 70 °C for 4 h. The reaction mixture was poured into ice-water (200 mL) and the precipitate was filtered, washed with water and dried. Recrystallization from cyclohexane gave 5.7 g (75% yield) of **9**; mp 103–104 °C; ^{1}H NMR (DMSO-d_6) δ: 2.73 (dd, 1H, J = 16.4, 2.44 Hz), 2.91–3.18 (m, 3H), 3.38 (m, 1H), 3.80 (s, 3H), 7.18 (dd, 1H, J = 8.6, 2.8 Hz), 7.26 (d, 1H, J = 2.8 Hz), 7.30–7.39 (m, 6H).

(7-Methoxy-3-phenyl-3,4-dihydro-2*H*-naphthalen-1-ylidene) acetonitrile (10). Under stirring and N_2, a solution of 6.3 mL (0.04 mol) of diethyl cyanomethylphosphonate in 30 mL of anhydrous THF was added dropwise in a mixture of 1.60 g (0.04 mol) of NaH (60% in mineral oil) and 50 mL of anhydrous THF cooled to −10 °C. After 1 h, a solution of 5.04 g (0.02 mol) of **9** in 60 mL of anhydrous THF was added dropwise and the mixture was stirred at room temperature for 16 h. The mixture was then poured into cold water and the solid was collected by filtration, washed with water, and diethyl ether. Recrystallization from ethanol gave 4.0 g (72%) of pure **10**; mp 137–138 °C; ^{1}H NMR: (300 MHz, DMSO-d_6) δ: 2.86 (dd, 1H, J = 14.6, 2.4 Hz), 3.0–3.16 (m, 3H), 3.39 (dd, 1H, J = 15.3, 1.7 Hz), 3.85 (s, 3H), 5.79 (d, 1H, J = 2.0 Hz), 6.97 (dd, 1H, J = 8.5, 2.4 Hz), 7.11 (d, 1H, J = 2.4 Hz), 7.15 (d, 1H, J = 8.5 Hz), 7.27–7.38 (m, 5H).

(±)-*cis*-2-(7-Methoxy-3-phenyl-1,2,3,4-tetrahydro-naphthalen-1-yl)ethylamine hydrochloride ((±)-*cis*-11). A NH_3-saturated solution of **10** (3.3 g, 0.012 mol) in ethanol (150 mL) was hydrogenated over Raney nickel under pressure (60 bars) at 60 °C for 12 h. After filtration and evaporation, the residual oil was dissolved in dry diethyl ether and treated with gaseous HCl. The obtained solid was filtered to give 3.2 g (84%) of **11** [mixture of 2/3(±)-*cis* and 1/3(±)-*trans*].

A sample of this mixture was recrystallized from ethanol to give pure (±)-*cis* **11**: mp 203–205 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.55–170 (m, 2H), 2.05–2.32 (m, 2H), 2.70–2.95 (m, 3H), 3.05 (m, 1H), 3.05–3.15 (m, 2H), 3.77 (s, 3H), 6.76 (dd, 1H, J = 8.4, 2.5 Hz), 6.93 (d, 1H, J = 2.5 Hz), 7.05 (d, 1H, J = 8.4 Hz), 7.23 (m, 1H), 7.28–7.37 (m, 4H), 8.18 (br s, 3H).

General procedure for the preparation of amides (12–14)

Potassium carbonate (1.45 g, 0.0105 mol) was added to a solution of crude **11** (1.1 g, 0.0035 mol), in 60 mL of water and 80 mL of methylene chloride. After stirring for 10 min at 0 °C, 0.0040 mol of the appropriate acid chloride was added dropwise at this temperature. The reaction mixture was stirred at room temperature for 2 h. The organic phase was separated, washed with a 1 M HCl solution and water, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the desired amide as a mixture of 2/3(±)-*cis* and 1/3(±)-*trans*.

(±)-*cis*-*N*-[2-(7-Methoxy-3-phenyl-1,2,3,4-tetrahydro-naphthalen-1-yl)ethyl]acetamide [(±)-*cis*-12]. Recrystallization from isopropyl ether gave 0.4 g (35%) of pure (±)-*cis*-**12**: mp 126–127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.70 (m, 1H), 1.85 (m, 1H), 1.95 (s, 3H), 2.15–2.25 (m, 2H), 2.85–2.90 (m, 3H), 3.09 (m, 1H), 3.37 (m, 2H), 3.82 (s, 3H), 5.41 (br s, 1H), 6.72 (dd, 1H, J = 8.4, 2.5 Hz), 6.87 (d, 1H, J = 2.5 Hz), 7.03 (d, 1H, J = 8.4 Hz), 7.20–7.40 (m, 5H). Anal. calcd for $C_{21}H_{25}NO_2$: C, 77.98; H, 7.79; N, 4.33. Found: C, 77.88; H, 7.41; N, 4.36.

(±)-*cis*-*N*-[2-(7-Methoxy-3-phenyl-1,2,3,4-tetrahydro-naphthalen-yl)ethyl]cyclopropyl carboxamide [(±)-*cis*-13]. Recrystallisation from isopropyl ether gave 0.45 g (36%) of pure (±)-*cis*-**13**: mp 145–146 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.72 (m, 2H), 0.95 (m, 2H), 1.27 (m, 1H), 1.68 (m, 1H), 2.25 (m, 2H), 2.89 (m, 3H), 3.12 (m, 1H), 3.38 (m, 2H), 3.81 (s, 3H), 5.61 (br s, 1H), 6.72 (dd, 1H, J = 8.3, 2.3 Hz), 6.89 (d, 1H, J = 2.3 Hz), 7.02 (d, 1H, J = 8.3 Hz), 7.30–7.40 (m, 5H). Anal. calcd for $C_{23}H_{27}NO_2$: C, 79.04; H, 7.78; N, 4.00. Found: C, 79.15; H, 7.70; N, 4.16.

(±)-*cis*-*N*-[2-(7-Methoxy-3-phenyl-1,2,3,4-tetrahydro-naphthalen-1-yl)ethyl]cyclobutyl carboxamide ((±)-*cis*-14a). Recrystallization from isopropyl ether gave 0.42 g (33%) of pure (±)-*cis*-**14a**: mp 160–162 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.70–2.17 (m, 11H), 2.86–2.96 (m, 3H), 3.11 (m, 1H), 3.37 (m, 2H), 3.80 (s, 3H), 5.30 (br s,1H), 6.72 (dd, 1H, J = 8.5, 2.5 Hz), 6.88 (d, 1H, J = 2.5 Hz), 7.02 (d, 1H, J = 8.5 Hz), 7.28–7.34 (m, 5H). Anal. calcd for $C_{24}H_{29}NO_2$: C, 79.30; H, 8.04; N, 3.86. Found: C, 79.03; H, 8.15; N, 3.70.

(±)-*trans*-*N*-[2-(7-Methoxy-3-phenyl-1,2,3,4-tetrahydro-naphthalen-1-yl)ethyl]cyclobutyl carboxamide ((±)-*trans*–14b). Fractional crystallization of the mother liquors of compound (±)-*cis*-**14a** gave 0.08 g (7%) of pure (±)-*trans*-**14b**: mp 111–112 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.70–2.25 (m, 11H), 2.81 (m, 1H), 3.11 (m, 1H), 3.89–3.00 (m, 2H), 3.40–3.51 (m, 2H), 3.81 (s, 3H), 5.33 (br s,1H), 6.71 (m, 1H), 6.75 (d, 1H, J = 2.3 Hz), 7.02 (d, 1H, J = 8.2 Hz), 7.26–742 (m, 5H). Anal. calcd for $C_{24}H_{29}NO_2$: C, 79.30; H, 8.04; N, 3.86. Found: C, 79.35; H, 8.12; N, 3.75.

Resolution of (±)-*cis*-14a and (±)-*trans*-14b. (For experimental procedure see Supplementary Material.)

(+)-*cis*-14a. Mp 165 °C; MS: M^+ = 363.2 (42%), 113.1 (88%), 91.1 (64%), 55.1 (100%); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.70–2.18 (m, 11H), 2.88–2.98 (m, 3H), 3.11 (m, 1H), 3.37 (m, 2H), 3.81 (s, 3H), 5.31 (br s,1H), 6.72 (dd, 1H, J = 8.5, 2.5 Hz), 6.87 (d, 1H, J = 2.5 Hz), 7.02 (d, 1H, J = 8.5 Hz), 7.27–7.36 (m, 5H); $[\alpha]_D$ = 53.20 (CH_2Cl_2); ee = 99% (HPLC, Chiralcel OD-H).

(−)-*cis*-14a. Mp 163 °C; MS: M^+ = 363.2 (38%), 113.1 (58%), 69.2 (74%), 55.1 (100%); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.71–2.17 (m, 11H), 2.88–2.97 (m, 3H), 3.11 (m, 1H), 3.38 (m, 2H), 3.81 (s, 3H), 5.30 (br s,1H), 6.72 (dd, 1H, J = 8.5, 2.5 Hz), 6.87 (d, 1H, J = 2.5 Hz), 7.02 (d, 1H, J = 8.5 Hz), 7.27–7.35 (m, 5H); $[\alpha]_D$ = −52.33 (CH_2Cl_2); ee = 99% (HPLC, Chiralcel OD-H).

(+)-*trans*-14b. Mp 111–112 °C; MS: $M^{+\cdot}$ = 363.2 (64%), 264.1 (76%), 113.1 (71%), 55.1 (100%); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.72–2.24 (m, 11H), 2.82 (m, 1H), 3.11 (m, 1H), 3.88–3.00 (m, 2H), 3.42–3.50 (m, 2H), 3.81 (s, 3H), 5.33 (br s,1H), 6.71 (m, 1H), 6.75 (d, 1H, J = 2.3 Hz), 7.02 (d, 1H, J = 8.2 Hz), 7.30–7.42 (m, 5H); $[\alpha]_D$ = 43.0 (CH_2Cl_2); ee = 99% (HPLC, Chiralcel OD-H).

(−)-*trans*-14b. Mp 97–98 °C; MS: $M^{+\cdot}$ = 363.1 (35%), 113.1 (73%), 91.1 (84%), 55.1 (100%); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.72–2.25 (m, 11H), 2.81 (m, 1H),

3.12 (m, 1H), 3.88–3.00 (m, 2H), 3.42–3.50 (m, 2H), 3.80 (s, 3H), 5.33 (br s,1H), 6.71 (m, 1H), 6.75 (d, 1H, $J=2.3$ Hz), 7.02 (d, 1H, $J=8.2$ Hz), 7.30–7.41 (m, 5H); $[\alpha]_D=-39.53$ (CH_2Cl_2); ee = 96% (HPLC, Chiralcel OD-H).

Pharmacology

Reagents and chemicals. 2-[^{125}I]-Iodomelatonin (2200 Ci/mmol) was purchased from NEN (Boston, MA, USA). Other drugs and chemicals were purchased from Sigma-Aldrich (Saint Quentin, France).

Cell culture. HEK (provided by A.D. Strosberg, Paris, France) and CHO cell lines stably expressing the human melatonin MT_1 or MT_2 receptors were grown in DMEM medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 IU/mL penicillin and 100 μg/mL streptomycin. Grown at confluence at 37 °C (95%O_2/5%CO_2), they were harvested in PBS containing EDTA 2 mM and centrifuged at 1000*g* for 5 min (4 °C). The resulting pellet was suspended in Tris 5 mM (pH 7.5), containing EDTA 2 mM and homogenized using a Kinematica polytron. The homogenate was then centrifuged (95,000*g*, 30 min, 4 °C) and the resulting pellet suspended in 75 mM Tris (pH 7.5), 12.5 mM $MgCl_2$ and 2 mM EDTA. Aliquots of membrane preparations were stored at −80 °C until use.

Binding assays. 2-[^{125}I]iodomelatonin binding assay conditions were essentially as previously described.[19] Briefly, binding was initiated by addition of membrane preparations from stable transfected HEK cells (40 μg/mL) diluted in binding buffer (50 mM Tris–HCl buffer, pH 7.4 containing 5 mM $MgCl_2$) to 2-[^{125}I]-iodomelatonin (0.025 and 0.2 nM respectively for MT_1 and MT_2 receptors due to a MT_1/MT_2 ratio of approximately 0.125 for cold 2-iodo-melatonin) and the tested drug. Nonspecific binding was defined in the presence of 1 μM melatonin. After a 120-min incubation at 37 °C, reaction was stopped by rapid filtration through GF/B filters presoaked in 0.5% (v/v) polyethylenimine. Filters were washed three times with 1 mL of ice-cold 50 mM Tris–HCl buffer, pH 7.4.

Data from the dose–response curves (seven concentrations in duplicate) were analysed using the program PRISM (Graph Pad Software Inc., San Diego, CA, USA) to yield IC_{50} (inhibitory concentration 50). Results are expressed as $K_i=IC_{50}/1+([L]/K_D)$, where $[L]$ is the concentration of radioligand used in the assay and K_D, the dissociation constant of the radioligand characterising the membrane preparation.

[^{35}S] GTPγS binding assay was performed according to published methodology.[20] Briefly, membranes from transfected CHO cells and compounds were diluted in binding buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 3 μM GDP, 3 mM $MgCl_2$, and 20 μg/mL saponin). Incubation was started by the addition of 0.2 nM [^{35}S]GTPγS to membranes (20 μg/mL) and drugs, and further followed for 1 h at room temperature. For experiments with antagonists on MT_2 receptors, membranes were preincubated with both the melatonin (3 nM) and the antagonist for 30 min prior the addition of [^{35}S] GTPγS. Non specific binding was defined using cold GTPγS (10 μM). Reaction was stopped by rapid filtration through GF/B filters followed by three successive washes with ice-cold buffer.

Usual levels of [^{35}S]GTPγs binding (expressed in dpm) were respectively for CHO-MT_1 and CHO-MT_2 membranes: 1000 and 2000 for basal activity, 4800 and 8000 in the presence of melatonin 1 μM and 160 and 180 in the presence of GTPγS 10 μM which defined the non specific binding. Data from the dose–response curves (seven concentrations in duplicate) were analysed by using the program PRISM (Graph Pad Software Inc., San Diego, CA, USA) to yield EC_{50} (Effective concentration 50%) and E_{max} (maximal effect) for agonists. Antagonist potencies are expressed as $K_B=IC_{50}/1+([Ago]/EC_{50}$ ago), where IC_{50} is the inhibitory concentration of antagonist that gives 50% inhibition of [^{35}S] GTPγS binding in the presence of a fixed concentration of melatonin ([Ago]) and EC_{50} ago is the EC_{50} of the molecule when tested alone.

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