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ARTICLE

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1,6-Dihydro-2*H*-indeno[5,4-*b*]furan Derivatives: Design, Synthesis, and Pharmacological Characterization of a Novel Class of Highly Potent MT_2-Selective Agonists

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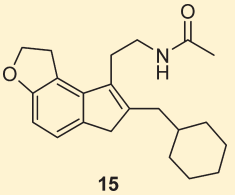
Supporting Information

ABSTRACT: A novel series of 1,6-dihydro-2*H*-indeno[5,4-*b*]furan derivatives were designed and synthesized as MT_2-selective ligands. This scaffold was identified as a potent mimic of the 5-methoxy indole core of melatonin, and introduction of a cyclohexylmethyl group at the 7-position of this scaffold afforded an MT_2-selective ligand **15** (K_i = 0.012 nM) with high MT_1/MT_2 selectivity (799). Compound **15** was identified as a potent full agonist for the MT_2 subtype and exhibited reentrainment effects to a new light/dark cycle in ICR mice at 3−30 mg/kg. This result demonstrated the involvement of the MT_2 receptors in chronobiotic activity.



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INTRODUCTION

Melatonin (5-methoxy-*N*-acetyltryptamine, Figure 1) is a neurohormone synthesized and secreted from the pineal gland in a circadian manner, and it reaches its peak concentration at night.[1] Melatonin plays an important role in the circadian aspects of human physiology[2] such as regulation of body temperature, control of seasonal cycles, and regulation of the sleep/wake cycle. In humans, it has been suggested that melatonin might have a variety of clinical applications such as the delayed sleep phase syndrome,[3] jet lag,[4] and seasonal affective disorders[5] and as a hypnotic agent.[6] Moreover, melatonin is reported to have neuroprotective,[7] cardiovascular,[8] antioxidant,[9] and antitumor properties.[10] In mammals, melatonin activates two high-affinity G-protein-coupled receptors (MT_1 and MT_2).[11] MT_1 receptors are expressed in several areas of the brain, especially in the suprachiasmatic nuclei (SCN) and the pars tuberalis of the pituitary, whereas MT_2 receptors are essentially localized in the SCN and retina. Another melatonin receptor binding site, MT_3, has lower affinity than do MT_1 and MT_2 and has been characterized as the hamster homologue of quinone reductase 2.[12]

Over the last two decades, many MT_1/MT_2 agonists have been reported, and some have been evaluated in clinical trials.[13] Ramelteon,[14,15] designed and synthesized in the course of our investigation on melatonin-mimic MT_1/MT_2 agonists, reduces sleep latency and increases the total sleep time and has been marketed for the treatment of insomnia (in the USA in 2005, and in Japan in 2010).[16] Several MT_2-selective ligands have also been reported recently.[17] The representative ligands luzindole[18] and 4-phenyl-2-propionamidotetralin (4P-PDOT)[19] act as antagonists for the MT_2 receptor and block the melatonin-mediated phase advances of circadian rhythms in mice.[20] This result suggests the involvement of the MT_2 receptor in the entrainment of circadian rhythms.[21] MT_1 knockout studies on mice,[22] in which melatonin causes phase-shifting effects, also support the involvement of this receptor on chronobiotic activity. However, most of these MT_2-selective ligands behave as antagonists, and only a few MT_2-selective agonists[23] have been reported to date. To clarify the distinct functions of MT_2 subtype, MT_2 full agonist with high potency and sufficient selectivity is still required.

In our previous studies aimed at identifying MT_1/MT_2 agonists, we reported potent 1,6,7,8-tetrahydro-2*H*-indeno[5,4-*b*]furan derivatives,[14] including ramelteon. A key element contributing to the high binding affinity of ramelteon (MT_1, K_i = 0.014 nM; MT_2, K_i = 0.112 nM)[15] is its angular furan-fused tricyclic ring. We also designed and synthesized indene derivative **1**,[24] a potent mimic of the indole core of melatonin (Figure 1). For the exploration of potent MT_2-selective ligands, we designed a novel 1,6-dihydro-2*H*-indeno[5,4-*b*]furan core, an indene-type of tricyclic indeno[5,4-*b*]furans (Figure 2). Substitution at the 7-position of this scaffold may enhance selectivity toward MT_2 subtype in preference to the MT_1 because the common structural feature in the most of the reported MT_2-selective ligands[17] bear bulky substituent at the 2-position of the indole core of melatonin, like luzindole. In this paper, we report the synthesis and pharmacological characterization of MT_2-selective 1,6-dihydro-2*H*-indeno[5,4-*b*]furan derivatives.

CHEMISTRY

7-Alkyl derivatives **8**−**16** were synthesized from the previously reported 1,2,6,7-tetrahydro-8*H*-indeno[5,4-*b*]furan-8-one **2**[14] (Scheme 1). Aldol reaction of **2** with various aldehydes or ketones under basic conditions, followed by hydrogenation in the presence of 10% Pd/C, gave the α-alkylated tricyclic ketones

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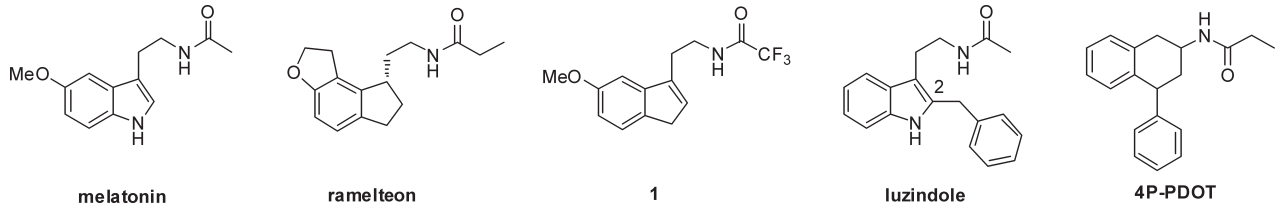

Figure 1. Chemical structures of melatonin, MT_1/MT_2 agonists (ramelteon and indene derivative **1**) and representative MT_2-selective ligands (luzindole and 4P-PDOT).

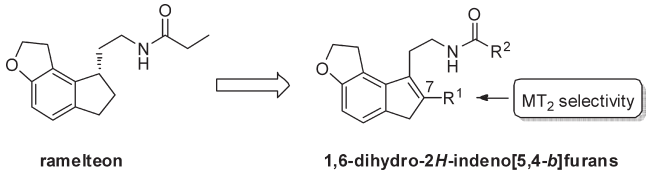

Figure 2. Design of 1,6-dihydro-2*H*-indeno[5,4-*b*]furan derivatives as MT_2-selective ligands.

4b−**e**. Compounds **2** and **4b**−**e** were subjected to the Horner−Emmons reaction in the presence of diethyl cyanomethylphosphonate to give unsaturated nitriles **5a**−**e**, which were converted to amines **6a**−**e** by hydrogenation over Raney cobalt (ODHT-60). Isomerization of *exo* olefins **6a**−**e** with hydrogen chloride in ethyl acetate gave the 1,6-dihydro-2*H*-indeno[5,4-*b*]furan derivatives **7a**−**e**. *N*-acylated derivatives **8**−**12** and **14**−**16** were prepared by treatment with the appropriate acyl chlorides, and the *N*-ureide derivative **13** was prepared by treatment with ethyl isocyanate.

Derivatives **18**−**27**, which have an aromatic ring group at the 7-position, were synthesized as described in Scheme 2. Regioselective bromination of **8** with 1.0 equiv of Br_2 in dichloromethane provided the desired compound **17** in 41% yield. Aromatic ring groups were introduced by Suzuki coupling in the presence of $Pd(PPh_3)_4$, Na_2CO_3, and the corresponding boronic acids.

RESULTS AND DISCUSSION

1. Binding Affinity for MT_1 and MT_2. The binding affinities of **8**−**27** for human MT_1 and MT_2 melatonin receptors were evaluated by using 2-[^{125}I]iodomelatonin as a radioligand in Chinese hamster ovary (CHO) cells expressing these receptors.[15] The chemical structures of **8**−**27** and their binding affinities for MT_1 and MT_2 are shown in Table 1.

Compound **8**, which has no substituent at the 7-position of the 1,6-dihydro-2*H*-indeno[5,4-*b*]furan core, showed strong binding affinity both for MT_1 (K_i = 0.045 nM) and MT_2 (K_i = 0.022 nM). The affinities of **8** were 5−10 times those of melatonin, which indicated that the 1,6-dihydro-2*H*-indeno[5,4-*b*]furan core is a potent mimic of the 5-methoxy indole core of melatonin. Introduction of an *i*-propyl group at the 7-position of the core (R^1) was tolerated, and the binding affinity of *i*-propyl derivative **9** for MT_1 and MT_2 was comparable to that of **8**. Before further exploration of the 7-position of the scaffold, the acetamido group (R^2) of compound **9** was investigated. Replacement of the acetamido group by a propionylamido, cyclopropylcarboxamido, or trifluoroacetamido group (corresponding derivatives: **10**, **11**, and **12**, respectively) cause only slight change in the binding affinity and MT_1/MT_2 selectivity. On the other hand, replacement of the acetamido group by an ethylureido group (compound **13**) resulted in moderate MT_1/MT_2 selectivity (13), however, this replacement also resulted in a 4.6-fold decrease in the binding affinity for the MT_2 subtype. Because sufficient MT_1/MT_2 selectivity was not achieved by replacement of the amide side chain, the effect of various substituents at the 7-position of the scaffold was investigated, keeping the amido moiety as acetamido group.

Introduction of a benzyl group (compound **14**) resulted in a drastic decrease in the binding affinity for MT_1 (K_i = 2.68 nM) but no notable change in the affinity for MT_2 (K_i = 0.0085 nM), and this led to enhanced MT_1/MT_2 selectivity (316). This result suggested that the MT_2 receptor has a larger hydrophobic pocket and wider structural diversity than the MT_1 receptor at the 7-position of this scaffold. Furthermore, this result agreed with the result that luzindole,[18] which has a benzyl group at the 2-position of the indole core, shows MT_2 selectivity. The high MT_2 binding affinity of **14** was maintained when the benzyl group of **14** was converted to a cyclohexylmethyl (compound **15**) or 3-thienylmethyl (compound **16**) group. **15** showed an MT_2 binding affinity 18 times higher (K_i = 0.012 nM) than that of melatonin and exhibited enhanced MT_1/MT_2 selectivity (799).

Introduction of aromatic ring groups was also explored. Phenyl and furyl derivatives (**18** and **19**) showed enhanced binding affinities for both MT_1 and MT_2, while the pyridyl derivative **20** maintained its affinity. This result suggested that the substituents whose sizes were similar to those of phenyl and furyl were tolerated for the hydrophobic pockets around the 7-position for both MT_1 and MT_2 receptors. Then, bulky aromatic ring groups were investigated. As in the case of benzyl derivative **14**, the MT_1 receptor was less tolerant to structural diversity at the 7-position of the scaffold, and introduction of a methoxy group at the *ortho*, *meta*, or *para* positions of the 7-phenyl ring of **18** (derivatives **21**, **22**, and **23**) led to enhanced MT_1/MT_2 selectivity ratios of 55, 6.3, and 81, respectively. Bulkier substituents such as *ortho*-O-*i*-propylphenyl and *meta*-*i*-propyl-phenyl (compounds **24** and **25**) helped enhance the MT_1/MT_2 selectivity ratios (>292 and 1200, respectively), whereas the *para*-*t*-butyl-phenyl substituent (compound **26**) resulted in the poor MT_1/MT_2 ratio (1.8). The presence of bulky heteroaromatic groups such as 3-benzothienyl (compound **27**) also resulted in enhanced MT_1/MT_2 selectivity (220). **25** was the most selective; it showed high MT_1/MT_2 selectivity (1200), and its MT_2 binding affinity (K_i = 0.011 nM) was 19 times that of melatonin itself.

The structure−activity relationship at the 7-position of this 1,6-dihydro-2*H*-indeno[5,4-*b*]furan core was consistent with that of the reported melatonin-mimic ligands[17] which bear bulky substituents at the topologically equivalent position to the 2-position of the indole core of melatonin. The formation of angular furan-fused tricyclic ring system might contribute to the high binding affinity of this series as observed in the derivatives of ramelteon,[14] thus the introduction of bulky substituents at the 7-position of this tricyclic core provided the MT_2-selective ligands with high binding affinity.

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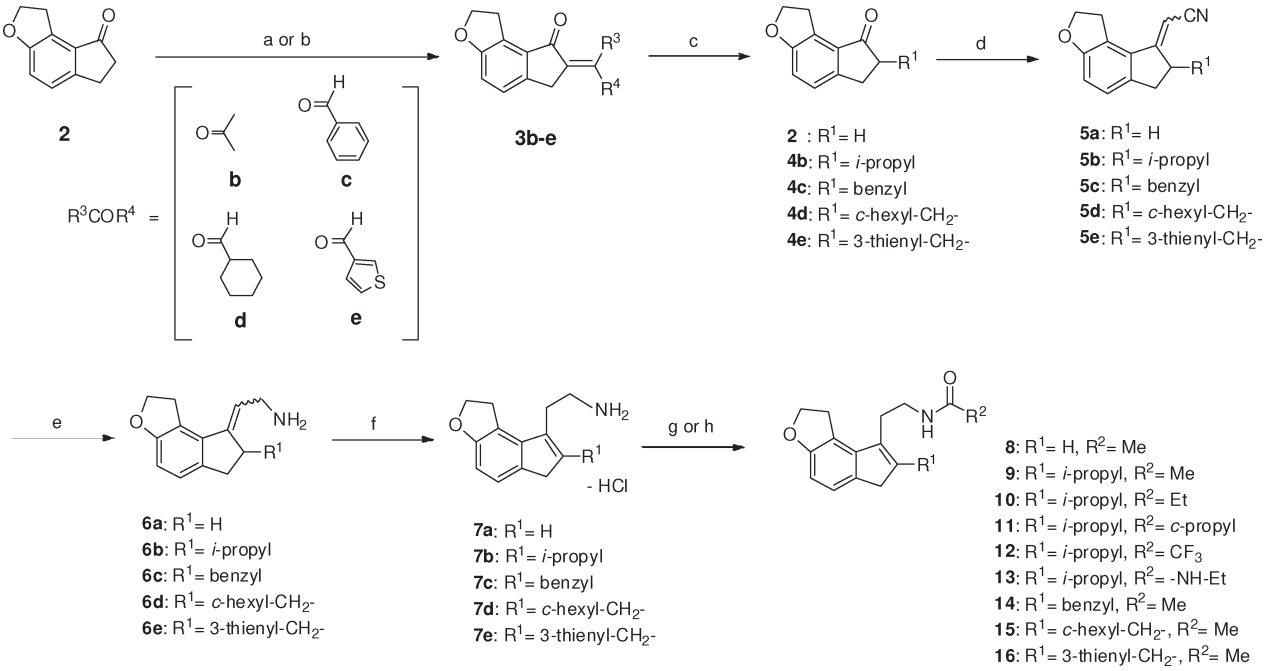
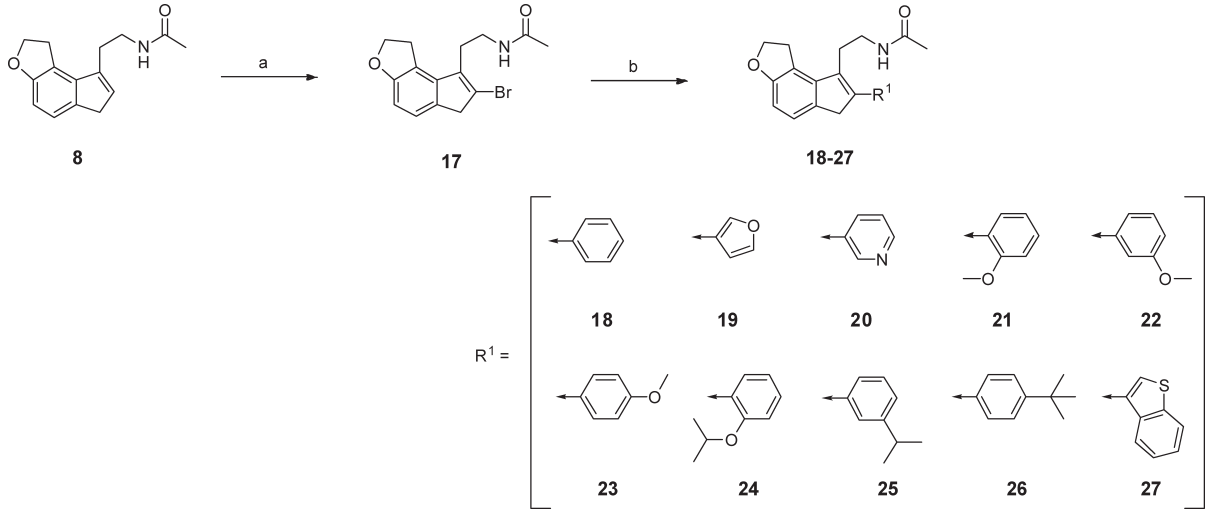
Scheme 1[a]



[a] Reagents and conditions: (a) R^3COR^4, NaOH, H_2O, THF, 60 °C; (b) R^3COR^4, $A1_2O_3$, CH_2Cl_2; (c) H_2, Pd/C, MeOH or EtOH; (d) NaH, $(EtO)_2POCH_2CN$, THF, 40 °C; (e) H_2, Raney Co, NH_3, EtOH; (f) HCl, EtOAc, 60 °C; (g) R^2COCl, Et_3N, CH_2Cl_2; (h) EtNCO, pyridine.

Scheme 2[a]



[a] Reagents and conditions: (a) Br_2, CH_2Cl_2; (b) $R^1B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, EtOH, toluene, H_2O, 80 °C.

2. Functional Activity of 14, 15, and 25. The functional activities of the most selective compounds (**14**, **15**, and **25**) were evaluated for the forskolin-induced cAMP accumulation in CHO cells expressing human MT_1 or MT_2 receptors, based on the method of Kato et al.,[15] with some modifications. The agonist potency was expressed as EC_{50} (nM), while the maximal efficacy (E_{max}) was expressed as a percentage of the efficacy observed with 1 μM melatonin.

Compounds **14**, **15**, and **25** exhibited moderate agonistic activity for the MT_1 subtype, which corresponds to their low binding affinities compared to melatonin (Figure 3). On the other hand, these compounds exhibited potent agonistic activity for the MT_2 subtype. Interestingly, their maximal efficacy (E_{max}) was observed in the different manner. While the benzyl (**14**, E_{max} = 62) and *meta-i*-propyl-phenyl (**25**, E_{max} = 73) derivatives behaved as partial agonists for MT_2 receptors, the cyclohexylmethyl derivative (**15**, E_{max} = 99) behaved as a full agonist. This result indicated that the hydrophobic substituents at the 7-position of the scaffold can contribute not only to the selectivity for MT_2 receptors, but also to the intrinsic activity. Because the aromaticity and electronic density of the cyclohexylmethyl substituent of **15** differ greatly from those of the benzyl and *meta-i*-propyl-phenyl substituents (derivatives **14** and **25**), these

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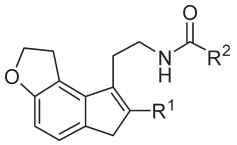
Table 1. Binding Affinities of 8−27 for Human MT_1 and MT_2 Obtained in Competition Radioligand Binding Assays Using 2-[^{125}I]Iodomelatonin



			K_i[a] (nM)		
compd	R^1	R^2	MT_1	MT_2	MT_1/MT_2
melatonin			0.24 ± 0.06	0.21 ± 0.03	1.1
8	−H	Me	0.045 ± 0.005	0.022 ± 0.002	2.1
9	−*i*-Pr	Me	0.024 ± 0.003	0.018 ± 0.002	1.3
10	−*i*-Pr	Et	0.013 ± 0.001	0.011 ± 0.002	1.3
11	−*i*-Pr	*c*-Pr	0.11 ± 0.03	0.035 ± 0.005	3.2
12	−*i*-Pr	CF_3	0.032 ± 0.003	0.010 ± 0.002	3.1
13	−*i*-Pr	-NH-Et	1.1 ± 0.2	0.084 ± 0.016	13
14	$-CH_2-Ph$	Me	2.7 ± 1.4	0.0085 ± 0.0018	316
15	$-CH_2$-*c*-Hex	Me	9.2 ± 1.6	0.012 ± 0.002	799
16	$-CH_2$-3-thienyl	Me	0.88 ± 0.12	0.0067 ± 0.0014	132
17	−Br	Me	0.0087 ± 0.0013	0.014 ± 0.004	0.61
18	−Ph	Me	0.0082 ± 0.0020	0.0065 ± 0.0015	1.3
19	−3-furyl	Me	0.0065 ± 0.0014	0.0096 ± 0.0021	0.68
20	−3-pyridyl	Me	0.069 ± 0.009	0.034 ± 0.005	2.1
21	−Ph(OMe) (*o*)	Me	1.1 ± 0.3	0.020 ± 0.005	53
22	−Ph(OMe) (*m*)	Me	0.057 ± 0.007	0.0090 ± 0.0023	6.3
23	−Ph(OMe) (*p*)	Me	3.0 ± 0.5	0.037 ± 0.011	81
24	−Ph(O-*i*-Pr) (*o*)	Me	>100	0.34 ± 0.16	>292
25	−Ph(*i*-Pr) (*m*)	Me	13 ± 4	0.011 ± 0.003	1200
26	−Ph(*t*-Bu) (*p*)	Me	1.4 ± 0.2	0.77 ± 0.18	1.8
27	−3-benzothienyl	Me	1.3 ± 0.4	0.0060 ± 0.0014	220

[a] K_i values are the mean of at least three measurements.

parameters may strongly influence the intrinsic activity. To the best of our knowledge, the cyclohexylmethyl derivative **15** is the most potent MT_2-selective ligand (K_i = 0.012 nM), with high MT_1/MT_2 selectivity (799) and full agonistic activity.

3. Chronobiotic Activity of 15. The selective MT_2 agonist **15** was assessed in terms of its effect on reentrainment to a new light/dark cycle in ICR mice, based on the method of Hirai et al.,[25] with some modifications (Figure 4). The mice were maintained in a 12 h light/dark cycle for at least 2 weeks and then subjected to 8 h advances of the cycle. Individual actograms showed that daily treatment of the mice with 30 mg/kg of **15** accelerated reentrainment to the new light/dark cycle. Daily treatment with 3 mg/kg (mean ± SEM; 5.80 ± 0.95, n = 10) and 30 mg/kg (4.20 ± 0.57, n = 10) of **15** accelerated days to reentrainment, while it was slow in the case of treatment with the vehicle (6.67 ± 1.07, n = 9). This result demonstrates that MT_2 receptors might mediate the chronobiotic activity and is in agreement with the studies using MT_2-selective antagonists[20] or MT_1 knockout mice.[22] Ramelteon, the MT_1/MT_2 agonists as a hypnotic agent, also showed chronobiotic activity in rats[25] as one of the feature of its pharmacological effects. This result suggests that the chronobiotic activity of ramelteon might be mediated through MT_2 receptors.

CONCLUSION

The results of this study confirm that the tricyclic 1,6-dihydro-2*H*-indeno[5,4-*b*]furan skeleton is a potent mimic of the 5-methoxy indole core of melatonin. Introduction of a cyclohexylmethyl group at the 7-position of the core afforded a potent MT_2-selective agonist (K_i = 0.012 nM, E_{max} = 99) with high MT_1/MT_2 selectivity (799). Treatment with 3−30 mg/kg of **15** accelerated reentrainment to a new light/dark cycle in ICR mice, thus indicating the involvement of the MT_2 receptor in chronobiotic activity.

EXPERIMENTAL SECTION

Melting points were determined on a Buchi melting point apparatus and were not corrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian Gemini-200 (200 MHz), Varian Gemini-300 (300 MHz), or Bruker DPX300 (300 MHz) instruments. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane of the indicated organic solution. Peak multiplicities are expressed as follows. Abbreviations are used as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, doublet of doublet of doublets; dt, doublet of triplet; brs, broad singlet; m, multiplet. Coupling constants (J values) are given in hertz (Hz). LC/MS (ESI^+) was performed on a ZQ-2000 apparatus with acetonitrile/water mobile phase. Preparative HPLC were performed an automated Gilson HPLC system using a YMC C-18

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column (S-5 μM 50 mm × 20 mm I.D.) with 10−100% gradient water−acetonitrile containing 0.1% TFA. Reaction progress was determined by thin layer chromatography (TLC) analysis on silica gel 60 F254 plate (Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.). The purities (>95%) of all compounds tested in biological systems were established by elemental analyses. Element analyses were carried out by Takeda Analytical Laboratories, and the results were within 0.4% of theoretical values.

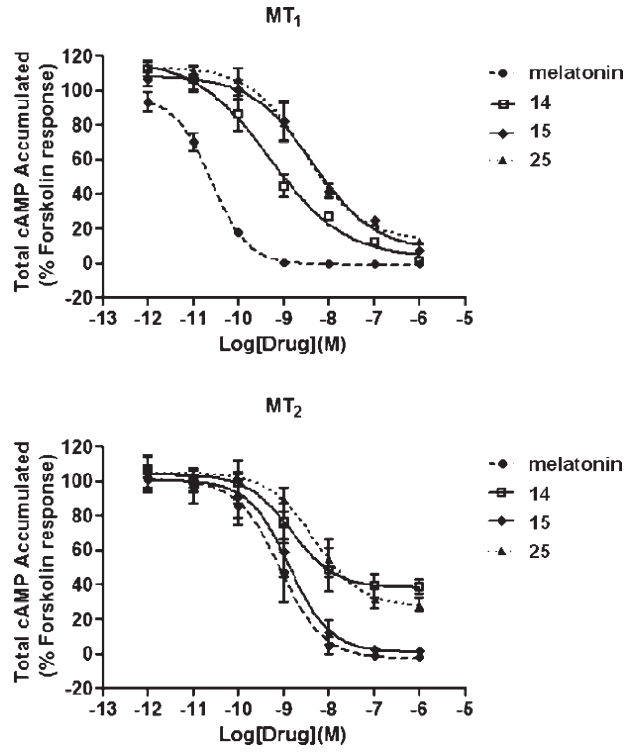

Figure 3. Functional analysis of melatonin, **14**, **15**, and **25** on forskolin-stimulated cAMP formation in CHO cells expressing human MT_1 or human MT_2 receptor. Each data point represents the mean ± standard error of two dependent experiments performed in triplicate. Curves are fit using nonlinear regression analysis with a variable slope (four parameters) to obtain potency (EC_{50}) values; GraphPad Prism software is used for this purpose. The $LogEC_{50}$ values for MT_1 are −10.59 ± 0.03 (melatonin), −9.29 ± 0.09 (**14**), −8.35 ± 0.09 (**15**), and −8.52 ± 0.06 (**25**). The $LogEC_{50}$ values for MT_2 are −9.07 ± 0.07 (melatonin), and −8.88 ± 0.08 (**15**). E_{max} (%) values for MT_2 are 61.7 ± 3.1 (**14**), 99.0 ± 2.9 (**15**), and 72.8 ± 2.7 (**25**).

7-(1-Methylethylidene)-1,2,6,7-tetrahydro-8*H*-indeno[5,4-*b*]-furan-8-one (3b). To a solution of **2** (10.0 g, 57.4 mmol) and acetone (42.1 mL, 574 mmol) in THF (100 mL), a solution of sodium hydroxide (2.52 g, 63.0 mmol) in water (100 mL) was added and stirred at 60 °C for 3 days. After being cooled to room temperature, saturated aqueous sodium hydrogen carbonate was added, and the mixture was extracted with EtOAc. The extract was washed with brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated and then washed with diethyl ether to give **3b** (5.69 g, 46%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.99 (3H, s), 2.42 (3H, s), 3,54 (2H, t, *J* = 8.9 Hz), 3.58 (2H, s), 4.66 (2H, t, *J* = 8.9 Hz), 6.98 (1H, d, *J* = 8.1 Hz), 7.19 (1H, d, *J* = 8.1 Hz). MS (ESI) *m*/*z* 215 $(M + H)^+$.

7-Benzylidene-1,2,6,7-tetrahydro-8*H*-indeno[5,4-*b*]furan-8-one (3c). Benzaldehyde (7.00 mL, 68.9 mmol) and activated basic aluminum oxide (150 g) were added to a vigorously stirred solution of **2** (10.0 g, 57.4 mmol) in methylene chloride (300 mL). After 1 h of stirring at room temperature, aluminum oxide was filtered and washed abundantly with methylene chloride. The filtrate was concentrated and then recrystallized from a mixture of EtOAc and hexane to give **3c** (9.65 g, 64%, single isomer) as a white solid. ^{1}H NMR ($CDCl_3$) δ 3.58 (2H, t, *J* = 8.9 Hz), 4.00 (2H, d, *J* = 1.7 Hz), 4.69 (2H, t, *J* = 8.9 Hz), 7.04 (1H, d, *J* = 8.1 Hz), 7.28 (1H, d, *J* = 8.1 Hz), 7.37−7.53 (3H, m), 7.60−7.71 (3H, m). MS (ESI) *m*/*z* 263 $(M + H)^+$.

7-(Cyclohexylmethylene)-1,2,6,7-tetrahydro-8*H*-indeno[5,4-*b*]furan-8-one (3d). By a similar procedure that described for **3b**, **3g** (71%) was obtained as a white solid (single isomer). ^{1}H NMR ($CDCl_3$) δ 1.18−1.43 (5H, m), 1.65−1.86 (5H, m), 2.25−2.43 (1H, m), 3.54 (2H, t, *J* = 8.9 Hz), 3.63 (2H, s), 4.67 (2H, t, *J* = 8.9 Hz), 6.68 (1H, dt, *J* = 9.8, 2.1 Hz), 7.01 (1H, d, *J* = 7.9 Hz), 7.22 (1H, d, *J* = 8.3 Hz). MS (ESI) *m*/*z* 269 $(M + H)^+$.

7-(3-Thienylmethylene)-1,2,6,7-tetrahydro-8*H*-indeno[5,4-*b*]furan-8-one (3e). By a similar procedure that described for **3c**, **3e** (52%) was obtained as a colorless solid (single isomer). ^{1}H NMR ($CDCl_3$) δ 3.56 (2H, t, *J* = 8.9 Hz), 3.91 (2H, d, *J* = 1.5 Hz), 4.68 (2H, t, *J* = 8.9 Hz), 7.03 (1H, d, *J* = 8.1 Hz), 7.27 (1H, d, *J* = 8.1 Hz), 7.41 (2H, d, *J* = 2.1 Hz), 7.58−7.71 (2H, m). MS (ESI) *m*/*z* 269 $(M + H)^+$.

7-Isopropyl-1,2,6,7-tetrahydro-8*H*-indeno[5,4-*b*]furan-8-one (4b). A solution of **3b** (5.69 g, 26.6 mmol) in EtOH (1000 mL) was hydrogenated in the presence of 10% palladium on carbon (3 g, containing 50% water) at room temperature for 30 min. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane) to give **4b** (4.84 g, 84%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.20 (6H, d, *J* = 6.7 Hz), 2.63−2.91 (2H, m), 3.06−3.25 (1H, m), 3.45 (2H, t, *J* = 8.8 Hz), 4.65 (2H, t, *J* = 8.8 Hz), 6.99 (1H, d, *J* = 8.0 Hz), 7.16 (1H, d, *J* = 8.0 Hz). MS (ESI) *m*/*z* 217 $(M + H)^+$.

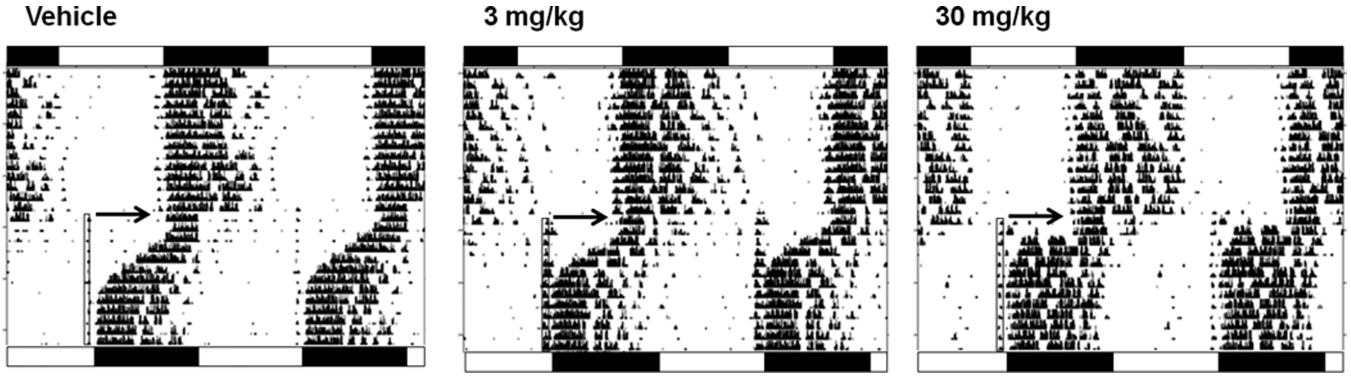

Figure 4. Effect of **15** on running-wheel activity in ICR mice after an 8 h advance of the light/dark cycle. Representative double-plotted actograms are shown for the vehicle, as well as for 3 and 30 mg/kg of **15**. The arrows indicate the day during the shifting phase, and the vertical bar represents the time of injection. The upper horizontal bar indicates the light (white) and dark (black) periods during preshifting, and the lower bar shows those for postshifting.

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7-Benzyl-1,2,6,7-tetrahydro-8*H*-indeno[5,4-*b*]furan-8-one (4c). By a similar procedure that described for **4b**, **4c** (84%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.64 (1H, dd, J = 14.0, 10.3 Hz), 2.79 (1H, dd, J = 16.1, 3.4 Hz), 2.90−3.17 (2H, m), 3.38 (1H, dd, J = 14.0, 4.1 Hz), 3.50 (2H, t, J = 8.9 Hz), 4.66 (2H, t, J = 8.9 Hz), 6.99 (1H, d, J = 8.1 Hz), 7.13 (1H, d, J = 8.0 Hz), 7.18−7.38 (5H, m). MS (ESI) m/z 265 $(M + H)^+$.

7-(Cyclohexylmethyl)-1,2,6,7-tetrahydro-8*H*-indeno[5,4-*b*]-furan-8-one (4d). A solution of **3d** (8.00 g, 29.8 mmol) in MeOH (200 mL) was hydrogenated in the presence of 10% palladium on carbon (500 mg) at room temperature for 3 h. THF (200 mL) was added to the mixture, the catalyst was removed by filtration, and the filtrate was concentrated. The residue was washed with MeOH to give **4d** (6.23 g, 77%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.85−1.13 (2H, m), 1.15−1.39 (4H, m), 1.39−1.58 (1H, m), 1.62−1.94 (6H, m), 2.66−2.81 (2H, m), 3.28 (1H, dd, J = 17.6, 8.6 Hz), 3.48 (2H, t, J = 8.9 Hz), 4.66 (2H, t, J = 8.9 Hz), 7.01 (1H, d, J = 8.2 Hz), 7.18 (1H, d, J = 8.0 Hz). MS (ESI) m/z 271 $(M + H)^+$.

7-(3-Thienylmethyl)-1,2,6,7-tetrahydro-8*H*-indeno[5,4-*b*]-furan-8-one (4e). By a similar procedure that described for **4b**, **4e** (78%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.70−2.87 (2H, m), 2.89−3.04 (1H, m), 3.09−3.24 (1H, m), 3.30 (1H, dd, J = 14.3, 4.0 Hz), 3.48 (2H, t, J = 8.9 Hz), 4.66 (2H, t, J = 8.9 Hz), 6.85−7.07 (3H, m), 7.14 (1H, d, J = 8.1 Hz), 7.20−7.30 (1H, m). MS (ESI) m/z 271 $(M + H)^+$.

2-(1,6-Dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)ethanamine hydrochloride (7a). A 65% suspension of sodium hydride in mineral oil (3.18 g, 86.1 mmol) was added to a solution of diethyl cyanomethylphosphonate (16.8 g, 94.7 mmol) in THF (100 mL). After stirred at room temperature for 30 min, the mixture was added dropwise to a stirred solution of **2** (5.00 g, 28.7 mmol) in THF (100 mL) at 40 °C and then stirred at 40 °C for 1 h. Water was added, and the mixture was extracted with EtOAc. The extract was washed with brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (EtOAc/hexane) to give **5a** (5.03 g, 89%) as a mixture of *cis*−*trans* isomers. A solution of **5a** (19.4 g, 98.4 mmol) in EtOH (saturated with NH_3, 500 mL) and EtOH (500 mL) was hydrogenated in the presence of Raney cobalt ODHT-60 (50 g, washed with EtOH two times before use) at room temperature for 3 days. The catalyst was removed by filtration. The filtrate was concentrated to afford **6a** as a crude oil. To a solution of crude **6a** in MeOH (300 mL), 4 M hydrogen chloride in EtOAc (300 mL) was added and stirred at 60 °C for 3 h. After being cooled to room temperature, the resulting solid was collected by filtration and washed with EtOAc to give **7a** (16.2 g, 69%) as a white solid; mp 246−248 °C (recrystallized from MeOH). ^{1}H NMR (methanol-d_4) δ 2.92−3.06 (2H, m), 3.16−3.34 (4H, m), 3.42 (2H, t, J = 8.6 Hz), 4.56 (2H, t, J = 8.6 Hz), 6.40 (1H, d, J = 1.5 Hz), 6.59 (1H, d, J = 8.1 Hz), 7.15 (1H, d, J = 8.1 Hz). MS (ESI) m/z 202 $(M + H)^+$. Anal. ($C_{13}H_{16}NOCl$) C, H, N, Cl.

2-(7-Isopropyl-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)ethanamine hydrochloride (7b). By a similar procedure that described for **7a**, **7b** (69%) was obtained as a white solid; mp 212−215 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 1.14 (6H, d, J = 6.6 Hz), 2.85 (4H, brs), 3.00−3.13 (1H, m), 3.23 (2H, s), 3.40 (2H, t, J = 8.6 Hz), 4.54 (2H, t, J = 8.6 Hz), 6.51 (1H, d, J = 8.0 Hz), 7.09 (1H, d, J = 8.0 Hz), 8.15 (2H, brs). Anal. ($C_{16}H_{22}NOCl$) C, H, N, Cl.

2-(7-Benzyl-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)ethanamine hydrochloride (7c). By a similar procedure that described for **7a**, **7c** (48%) was obtained as a white solid. ^{1}H NMR (methanol-d_4) δ 3.05 (4H, s), 3.19 (2H, s), 3.43 (2H, t, J = 8.5 Hz), 3.86 (2H, s), 4.57 (2H, t, J = 8.5 Hz), 6.50 (1H, d, J = 8.0 Hz), 7.02 (1H, d, J = 8.0 Hz), 7.10−7.40 (5H, m). MS (ESI) m/z 292 $(M + H)^+$.

2-[7-(Cyclohexylmethyl)-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl]ethanamine hydrochloride (7d). By a similar procedure that described for **7a**, **7d** (55%) was obtained as a white solid. ^{1}H NMR (methanol-d_4) δ 0.90−1.10 (2H, m), 1.12−1.40 (3H, m), 1.44−1.83 (6H, m), 2.39 (2H, d, J = 7.2 Hz), 2.82−3.09 (4H, m), 3.27 (2H, s), 3.40 (2H, t, J = 8.5 Hz), 4.56 (2H, t, J = 8.5 Hz), 6.52 (1H, d, J = 7.9 Hz), 7.08 (1H, d, J = 7.9 Hz). MS (ESI) m/z 298 $(M + H)^+$.

2-[7-(3-Thienylmethyl)-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl]ethanamine hydrochloride (7e). By a similar procedure that described for **7a**, **7e** (63%) was obtained as a white solid. ^{1}H NMR (methanol-d_4) δ 2.97−3.09 (4H, m), 3.24 (2H, s), 3.43 (2H, t, J = 8.6 Hz), 3.87 (2H, s), 4.57 (2H, t, J = 8.6 Hz), 6.52 (1H, d, J = 7.9 Hz), 6.97 (1H, dd, J = 5.0, 1.2 Hz), 7.01−7.13 (2H, m), 7.35 (1H, dd, J = 5.0, 2.8 Hz). MS (ESI) m/z 298 $(M + H)^+$.

***N*-[2-(1,6-Dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)ethyl]acetamide (8).** To a solution of **7a** (1.20 g, 5.05 mmol) in methylene chloride (50 mL) was added triethylamine (1.41 mL, 10.1 mmol) and acetyl chloride (431 μL, 6.06 mmol). After the mixture was stirred at room temperature for 10 min, water and EtOAc were added to the mixture. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (EtOAc/hexane) and then recrystallized from a mixture of EtOAc and hexane to give **8** (0.95 g, 77%) as a white solid; mp 157−158 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.98 (3H, s), 2.68−2.87 (2H, m), 3.31 (2H, d, J = 1.5 Hz), 3.43 (2H, t, J = 8.7 Hz), 3.57 (2H, q, J = 6.8 Hz), 4.60 (2H, t, J = 8.7 Hz), 5.63 (1H, s), 6.29 (1H, s), 6.67 (1H, d, J = 7.9 Hz), 7.19 (1H, d, J = 7.9 Hz). MS (ESI) m/z 244 $(M + H)^+$. Anal. ($C_{15}H_{17}NO_2$) C, H, N.

***N*-[2-(7-Isopropyl-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethyl]acetamide (9).** By a similar procedure that described for **8**, **9** (57%) was obtained as a white solid; mp 132−134 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.16 (6H, d, J = 6.9 Hz), 1.96 (3H, s), 2.76 (2H, d, J = 7.2 Hz), 2.97−3.10 (1H, m), 3.28 (2H, s), 3.35−3.48 (4H, m), 4.58 (2H, t, J = 8.5 Hz), 5.62 (1H, brs), 6.60 (1H, d, J = 8.0 Hz), 7.13 (1H, d, J = 8.0 Hz). MS (ESI) m/z 286 $(M + H)^+$. Anal. ($C_{18}H_{23}NO_2$) C, H, N.

***N*-[2-(7-Isopropyl-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethyl]propanamide (10).** By a similar procedure that described for **8**, **10** (69%) was obtained as a white solid; mp 144−146 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.15 (3H,t, J = 7.7 Hz), 1.16 (6H, d, J = 6.6 Hz), 2.18 (2H, q, J = 7.7 Hz), 2.76 (2H, t, J = 7.3 Hz), 2.97−3.08 (1H, m), 3.27 (2H, s), 3.37−3.47 (4H, m), 4.58 (2H, t, J = 8.5 Hz), 5.62 (1H, brs), 6.60 (1H, d, J = 7.7 Hz), 7.13 (1H, d, J = 7.7 Hz). MS (ESI) m/z 300 $(M + H)^+$. Anal. ($C_{19}H_{25}NO_2$) C, H, N.

***N*-[2-(7-Isopropyl-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethyl]cyclopropanecarboxamide (11).** By a similar procedure that described for **8**, **11** (75%) was obtained as a white solid; mp 197−200 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 0.70−0.79 (2H, m), 0.93−1.03 (2H, m), 1.17 (6H, d, J = 6.9 Hz), 1.17−1.33 (1H, m), 2.77 (2H, t, J = 7.1 Hz), 2.97−3.10 (1H, m), 3.28 (2H, s), 3.37−3.50 (4H, m), 4.58 (2H, t, J = 8.5 Hz), 5.78 (1H, brs), 6.60 (1H, d, J = 8.0 Hz), 7.13 (1H, d, J = 8.0 Hz). MS (ESI) m/z 312 $(M + H)^+$. Anal. ($C_{20}H_{25}NO_2$) C, H, N.

2,2,2-Trifluoro-*N*-[2-(7-isopropyl-1,6-dihydro-2*H*-indeno-[5,4-*b*]furan-8-yl)ethyl]acetamide (12). By a similar procedure that described for **8**, **12** (38%) was obtained as a white solid; mp 162−164 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.16 (6H, d, J = 6.9 Hz), 2.84 (2H, t, J = 7.3 Hz), 2.93−3.06 (1H, m), 3.30 (2H, s), 3.41 (2H, t, J = 8.5 Hz), 3.53 (2H, q, J = 7.3 Hz), 4.59 (2H, t, J = 8.5 Hz), 6.48 (1H, brs), 6.62 (1H, d, J = 7.8 Hz), 7.15 (1H, d, J = 7.8 Hz). MS (ESI) m/z 340 $(M + H)^+$. Anal. ($C_{18}H_{20}F_3NO_2$) C, H, N.

***N*-Ethyl-*N*′-[2-(7-isopropyl-1,6-dihydro-2*H*-indeno[5,4-*b*]-furan-8-yl)ethyl]urea (13).** Ethyl isocyanate (165 mg, 2.32 mmol) was added to a solution of **7b** (500 mg, 1.79 mmol) in pyridine (5 mL) at room temperature, and the reaction mixture was stirred at 60 °C for 2 h. The solvent was evaporated, and aqueous hydrogen chloride was added and extracted with methylene chloride. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (MeOH/chloroform) to give **13** (499 mg, 89%) as a white solid; mp 167−169 °C (EtOAc). ^{1}H NMR ($CDCl_3$) δ 1.10 (3H, t, J = 7.3 Hz),

1.15 (6H, d, J = 7.0 Hz), 2.76 (2H, t, J = 7.1 Hz), 2.97−3.23 (3H, m), 3.26 (2H, s), 3.33 (2H, q, J = 7.0 Hz), 3.43 (2H, t, J = 8.5 Hz), 4.17 (1H, brs), 4.41 (1H, brs), 4.57 (2H, t, J = 8.5 Hz), 6.58 (1H, d, J = 7.7 Hz), 7.11 (1H, d, J = 7.7 Hz). MS (ESI) m/z 315 $(M + H)^+$. Anal. ($C_{19}H_{26}N_2O_2$) C, H, N.

***N*-[2-(7-Benzyl-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethyl]acetamide (14).** By a similar procedure that described for **8**, **14** (38%) was obtained as a white solid; mp 132−133 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.89 (3H, s), 2.86 (2H, t, J = 7.3 Hz), 3.23 (2H, s), 3.35−3.56 (4H, m), 3.81 (2H, s), 4.60 (2H, t, J = 8.6 Hz), 5.58 (1H, s), 6.60 (1H, d, J = 7.7 Hz), 7.07 (1H, d, J = 7.7 Hz), 7.13−7.35 (5H, m). MS (ESI) m/z 334 $(M + H)^+$. Anal. ($C_{22}H_{23}NO_2$) C, H, N.

***N*-{2-[7-(Cyclohexylmethyl)-1,6-dihydro-2*H*-indeno[5,4-*b*]-furan-8-yl]ethyl}acetamide (15).** By a similar procedure that described for **8**, **15** (79%) was obtained as a white solid; mp 133−134 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 0.82−1.03 (2H, m), 1.06−1.32 (3H, m), 1.42−1.78 (6H, m), 1.96 (3H, s), 2.32 (2H, d, J = 7.2 Hz), 2.74 (2H, t, J = 7.2 Hz), 3.26 (2H, s), 3.32−3.52 (4H, m), 4.59 (2H, t, J = 8.6 Hz), 5.60 (1H, s), 6.59 (1H, d, J = 7.9 Hz), 7.11 (1H, d, J = 7.9 Hz). MS (ESI) m/z 340 $(M + H)^+$. Anal. ($C_{22}H_{29}NO_2$) C, H, N.

***N*-{2-[7-(3-Thienylmethyl)-1,6-dihydro-2*H*-indeno[5,4-*b*]-furan-8-yl]ethyl}acetamide (16).** By a similar procedure that described for **8**, **16** (34%) was obtained as a pale-yellow solid; mp 125−127 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.87 (3H, s), 2.84 (2H, t, J = 7.3 Hz), 3.29 (2H, s), 3.34−3.55 (4H, m), 3.81 (2H, s), 4.60 (2H, t, J = 8.6 Hz), 5.58 (1H, s), 6.61 (1H, d, J = 7.7 Hz), 6.88−6.98 (2H, m), 7.09 (1H, d, J = 7.7 Hz), 7.21−7.31 (1H, m). MS (ESI) m/z 340 $(M + H)^+$. Anal. ($C_{20}H_{21}NO_2S$) C, H, N.

***N*-[2-(7-Bromo-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethyl]acetamide (17).** To a solution of **8** (8.48 g, 34.9 mmol) in methylene chloride (50 mL), bromine (1.79 mL, 34.9 mmol) was added dropwise and stirred at room temperature for 30 min. Then 1 M aqueous $Na_2S_2O_3$ solution and EtOAc were added to the mixture. The organic layer was separated, washed with saturated aqueous sodium hydrogen carbonate and brine, and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (MeOH/EtOAc) and preparative HPLC to give **17** (4.56 g, 41%) as a white solid; mp 145−146 °C (EtOAc). ^{1}H NMR ($CDCl_3$) δ 1.98 (3H, s), 2.83 (2H, t, J = 7.2 Hz), 3.34−3.50 (4H, m), 3.56 (2H, s), 4.61 (2H, t, J = 8.7 Hz), 5.72 (1H, s), 6.65 (1H, d, J = 7.9 Hz), 7.10 (1H, d, J = 7.9 Hz). MS (ESI) m/z 323 $(M + H)^+$. Anal. ($C_{15}H_{16}BrNO_2$) C, H, N, Br.

***N*-[2-(7-Phenyl-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethyl]acetamide (18).** A mixture of **17** (200 mg, 0.621 mmol), phenyl boronic acid (189 mg, 1.55 mmol), $Pd(PPh_3)_4$ (72 mg, 0.062 mmol), and 2 M aqueous Na_2CO_3 (4 mL) in EtOH (3 mL) and toluene (3 mL) was stirred at 80 °C for 30 min. Water and EtOAc were added to the mixture. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (EtOAc/hexane) and preparative HPLC and then recrystallized from a mixture of EtOAc and hexane to give **18** (124 mg, 63%) as a white solid; mp 149−151 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.78 (3H, s), 2.96 (2H, t, J = 7.3 Hz), 3.35−3.47 (2H, m), 3.53 (2H, t, J = 8.6 Hz), 3.70 (2H, s), 4.63 (2H, t, J = 8.6 Hz), 5.43 (1H, s), 6.70 (1H, d, J = 7.9 Hz), 7.21 (1H, d, J = 7.9 Hz), 7.28−7.37 (1H, m), 7.38−7.48 (4H, m). MS (ESI) m/z 320 $(M + H)^+$. Anal. ($C_{21}H_{21}NO_2$) C, H, N.

***N*-{2-[7-(3-Furyl)-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl]-ethyl}acetamide (19).** By a similar procedure that described for **18**, **19** (33%) was obtained as a pale-yellow solid; mp 156−157 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.90 (3H, s), 3.00 (2H, t, J = 7.4 Hz), 3.40−3.55 (4H, m), 3.61 (2H, s), 4.62 (2H, t, J = 8.6 Hz), 5.67 (1H, s), 6.65 (1H, d, J = 7.9 Hz), 6.75 (1H, dd, J = 1.9, 0.9 Hz), 7.16 (1H, d, J = 7.9 Hz), 7.49 (1H, t, J = 1.9 Hz), 7.76 (1H, s). MS (ESI) m/z 310 $(M + H)^+$. Anal. ($C_{19}H_{19}NO_3$) C, H, N.

***N*-[2-(7-Pyridin-3-yl-1,6-dihydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethyl]acetamide (20).** By a similar procedure that described for **18**, **20** (26%) was obtained as a yellow solid; mp 168−170 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.87 (3H, s), 2.95 (2H, t, J = 7.8 Hz), 3.36−3.50 (2H, m), 3.58 (2H, t, J = 8.6 Hz), 3.71 (2H, s), 4.65 (2H, t, J = 8.6 Hz), 5.59 (1H, s), 6.73 (1H, t, J = 4.0 Hz), 7.23 (1H, d, J = 4.9 Hz), 7.38 (1H, dd, J = 7.7, 4.9 Hz), 7.72−7.82 (1H, m), 8.55 (1H, dd, J = 4.9, 1.6 Hz), 8.70 (1H, d, J = 1.6 Hz). MS (ESI) m/z 321 $(M + H)^+$. Anal. ($C_{20}H_{20}N_2O_2$) C, H, N.

***N*-{2-[7-(2-Methoxyphenyl)-1,6-dihydro-2*H*-indeno[5,4-*b*]-furan-8-yl]ethyl}acetamide (21).** By a similar procedure that described for **18**, **21** (49%) was obtained as a white solid; mp 137−138 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.74 (3H, s), 2.75 (2H, t, J = 6.5 Hz), 3.36 (2H, q, J = 6.5 Hz), 3.47 (2H, t, J = 8.6 Hz), 3.65 (2H, s), 3.83 (2H, s), 4.62 (2H, t, J = 8.6 Hz), 5.57 (1H, s), 6.69 (1H, d, J = 7.9 Hz), 6.94−7.07 (2H, m), 7.12−7.22 (2H, m), 7.28−7.40 (1H, m). MS (ESI) m/z 350 $(M + H)^+$. Anal. ($C_{22}H_{23}NO_3$) C, H, N.

***N*-{2-[7-(3-Methoxyphenyl)-1,6-dihydro-2*H*-indeno[5,4-*b*]-furan-8-yl]ethyl}acetamide (22).** By a similar procedure that described for **18**, **22** (40%) was obtained as a white solid; mp 132−134 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.80 (3H, s), 2.96 (2H, t, J = 7.3 Hz), 3.35−3.47 (2H, m), 3.53 (2H, t, J = 8.6 Hz), 3.68 (2H, s), 3.85 (3H, s), 4.63 (2H, t, J = 8.6 Hz), 5.42 (1H, s), 6.70 (1H, d, J = 7.9 Hz), 6.83−7.04 (3H, m), 7.21 (1H, d, J = 7.9 Hz), 7.35 (1H, t, J = 7.9 Hz). MS (ESI) m/z 350 $(M + H)^+$. Anal. ($C_{22}H_{23}NO_3$) C, H, N.

***N*-{2-[7-(4-Methoxyphenyl)-1,6-dihydro-2*H*-indeno[5,4-*b*]-furan-8-yl]ethyl}acetamide (23).** By a similar procedure that described for **18**, **23** (28%) was obtained as a white solid; mp 204−205 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.81 (3H, s), 2.95 (2H, t, J = 7.3 Hz), 3.34−3.58 (4H, m), 3.66 (2H, s), 3.85 (3H, s), 4.63 (2H, t, J = 8.6 Hz), 5.45 (1H, s), 6.68 (1H, d, J = 7.9 Hz), 6.92−7.00 (2H, m), 7.20 (1H, d, J = 7.9 Hz), 7.32−7.43 (2H, m). MS (ESI) m/z 350 $(M + H)^+$. Anal. ($C_{22}H_{23}NO_3$) C, H, N.

***N*-{2-[7-(2-Isopropoxyphenyl)-1,6-dihydro-2*H*-indeno-[5,4-*b*]furan-8-yl]ethyl}acetamide (24).** By a similar procedure that described for **18**, **24** (47%) was obtained as a white solid; mp 135−136 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.30 (6H, d, J = 6.0 Hz), 1.74 (3H, s), 2.76 (2H, t, J = 6.5 Hz), 3.36 (2H, q, J = 6.5 Hz), 3.48 (2H, t, J = 8.6 Hz), 3.66 (2H, s), 4.53−4.69 (3H, m), 5.57 (1H, s), 6.70 (1H, d, J = 7.9 Hz), 6.92−7.04 (2H, m), 7.11−7.36 (3H, m). MS (ESI) m/z 378 $(M + H)^+$. Anal. ($C_{24}H_{27}NO_3$) C, H, N.

***N*-{2-[7-(3-Isopropylphenyl)-1,6-dihydro-2*H*-indeno[5,4-*b*]-furan-8-yl]ethyl}acetamide (25).** By a similar procedure that described for **18**, **25** (53%) was obtained as a white solid; mp 176−178 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.29 (6H, d, J = 7.0 Hz), 1.76 (3H, s), 2.89−3.03 (3H, m), 3.43 (2H, q, J = 6.7 Hz), 3.52 (2H, t, J = 8.6 Hz), 3.71 (2H, s), 4.63 (2H, t, J = 8.6 Hz), 5.37 (1H, s), 6.70 (1H, d, J = 7.9 Hz), 7.15−7.28 (4H, m), 7.36 (1H, t, J = 7.5 Hz). MS (ESI) m/z 362 $(M + H)^+$. Anal. ($C_{24}H_{27}NO_2$) C, H, N.

***N*-{2-[7-(4-*tert*-Butylphenyl)-1,6-dihydro-2*H*-indeno[5,4-*b*]-furan-8-yl]ethyl}acetamide (26).** By a similar procedure that described for **18**, **26** (47%) was obtained as a white solid; mp 212−213 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.36 (9H, s), 1.77 (3H, s), 2.98 (2H, t, J = 7.2 Hz), 3.29−3.58 (4H, m), 3.69 (2H, s), 4.63 (2H, t, J = 8.7 Hz), 5.43 (1H, s), 6.69 (1H, d, J = 7.9 Hz), 7.20 (1H, d, J = 7.9 Hz), 7.35−7.49 (4H, m). MS (ESI) m/z 376 $(M + H)^+$. Anal. ($C_{25}H_{29}NO_2$) C, H, N.

***N*-{2-[7-(1-Benzothien-3-yl)-1,6-dihydro-2*H*-indeno[5,4-*b*]-furan-8-yl]ethyl}acetamide (27).** By a similar procedure that described for **18**, **27** (11%) was obtained as a pale-brown solid; mp 208−210 °C (EtOAc/hexane). ^{1}H NMR ($CDCl_3$) δ 1.60 (3H, s), 2.81 (2H, t, J = 7.1 Hz), 3.25−3.36 (2H, m), 3.55 (2H, t, J = 8.6 Hz), 3.73 (2H, s), 4.66 (2H, t, J = 8.6 Hz), 5.26 (1H, s), 6.74 (1H, d, J = 7.9 Hz), 7.24 (1H, d, J = 7.9 Hz), 7.32 (1H, s), 7.35−7.45 (2H, m), 7.58−7.73 (1H, m), 7.83−8.00 (1H, m). MS (ESI) m/z 376 $(M + H)^+$. Anal. ($C_{23}H_{21}NO_2S$) C, H, N.

Preparation of CHO Membrane for Melatonin Receptor (MT_1 or MT_2) Binding Assays. Cell lines stably expressing human MT_1 receptor (hMT_1-CHO/binding, cell line: TPCCB0117) or human MT_2 receptor (hMT_2-CHO, cell line: TPCCB0098) were used for the experiments. hMT_1-CHO/binding cells and hMT_2-CHO cells were cultured in F-12 nutrient mixture (Ham) supplemented with 10% fetal bovine serum (FBS) and 500 μg/mL of Geneticin, 100 U/mL penicillin, and 100 μg/mL streptomycin in a 5% CO_2/95% air atmosphere. Cells were harvested at confluence in Ca^{2+}-Mg^{2+}-free phosphate buffered saline (PBS) containing 0.5 mM ethylenediaminetetraacetic acid (EDTA) and collected by centrifugation. Cells were washed with PBS, pelleted, and homogenized in ice-cold 10 mM $NaHCO_3$ buffer (pH 7.4 at 25 °C) containing 5 mM EDTA and 1× Complete proteinase inhibitor (Roche). Cell homogenates were centrifuged (1000g, 10 min, 4 °C). Supernatant was collected by centrifugation (140000g, 60 min, 4 °C). Pellets were resuspended in ice-cold Tris-HCl buffer (pH 7.4 at 25 °C) containing 1 mM EDTA and 1× Complete proteinase inhibitor and stored at −80 °C until binding assays.

Affinity for the Human MT_1/MT_2 Receptors. The frozen homogenate was thawed, suspended in ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C), and used for the binding assay. For the assay using CHO cell membrane homogenate, 2 μL of DMSO solution of a test compound was mixed with homogenate and 40 (MT_1) or 80 pM (MT_2) 2-[^{125}I]iodomelatonin in a total volume of 200 μL and incubated at 25 °C for 150 min. The binding reaction was terminated by rapid filtration using a cell harvestor (PerkinElmer), followed by four 300 μL washes with ice-cold incubation buffer. Nonspecific binding was defined in the presence of 1 μM melatonin. GF/C filter plates were dried and radioactivity determined after addition of 25 μL of Microscint-0 using a TopCount (PerkinElmer). The 50% inhibitory concentration (IC_{50}) was calculated using nonlinear regression analysis (a three-parameter dose−response curve) in GraphPad Prism software (GraphPad Software). The dissociation constant of the compound for the receptor (K_i) was calculated using the following equation:

$$K_i = IC_{50}/(1 + L/K_d)$$

where L and K_d represent the concentration and the affinity constant of 2-[^{125}I]iodomelatonin in the binding assay, respectively. The specific binding of 2-[^{125}I]iodomelatonin for human MT_1 receptors in hMT_1-CHO/binding was saturable. The one site specific binding plot in GraphPad Prism software (GraphPad Software) of saturation isotherm using ligand concentrations ranging from 5 to 160 pM revealed affinity binding sites with a K_d value of 33.9 ± 4.2 pM and a B_{max} of 33.8 ± 1.6 fmol/mg protein (one experiment). Human MT_2 receptors expressed in hMT_2-CHO showed lower affinities than those of human MT_1 receptors, and its K_d value and B_{max} were 72.6 ± 9.4 pM and 72.1 ± 4.4 fmol/mg protein (one experiment), respectively, determined with ligand concentrations ranging from 5 to 160 pM.

Inhibition of Forskolin-Induced cAMP Formation in CHO Cells Expressing Human Melatonin Receptor (MT_1 or MT_2). CHO cell lines stably expressing human MT_1 receptor (hMT_1-CHO/cAMP, cell line: TPCCB229) or human MT_2 receptor (hMT_2-CHO, cell line: TPCCB0098) were used for cAMP assays. Cells were cultured in F-12 nutrient mixture (Ham) supplemented with 10% fetal bovine serum (FBS) and 500 μg/mL of Geneticin, 100 U/mL penicillin, and 100 μg/mL streptomycin in a 5% CO_2/95% air atmosphere. hMT_1-CHO/cAMP cells or hMT_2-CHO cells were suspended with assay buffer (modified Hanks' balanced salt solution (HBSS) containing 5 mM HEPES-buffer [pH 7.4], 0.1% BSA, 100 μM 3-isobutyl-1-methylxanthine (IBMX), and 100 μM 4-(3-butoxy-4-methoxyphenyl) methyl-2-imidazolidone (Ro20-1724) after cells were harvested at confluence in Ca^{2+}-Mg^{2+}-free PBS containing 0.5 mM EDTA and collected by centrifugation. Ten μL of hMT_1-CHO/cAMP cells and hMT_2-CHO cells were plated at a density of 2×10^4 and 6×10^4 cells/well on 384-well plates, respectively. After 10 μL of a test compound or melatonin solution in the assay buffer were added, 10 μL of forskolin solution in the assay buffer was added (final concentration 2 μM), and the cells were incubated for 30 min at room temperature. The reaction was terminated by the addition of 10 μL of anti-cAMP acceptor beads solution and 10 μL of lysis/detection buffer (the assay buffer containing 0.3% Tween-20, biotinylated cAMP, and donor bead) of AlphaScreen cAMP Assay Kit (PerkinElmer). The plates were mixed for 30 s on an orbital shaker, covered, and incubated in the dark for 12 h at room temperature and then read on EnVision Multilabel plate reader (PerkinElmer). The 50% effective concentration (EC_{50}) and maximal effect for agonists (E_{max}) were calculated using nonlinear regression analysis (a three-parameter dose−response curve) in GraphPad Prism software (GraphPad Software). E_{max} was expressed as a percentage of that observed with 1 μM melatonin (= 100%).

Reentrainment for 8 h Advance of the Dark Phase in Mice Running Wheel Activity. Male ICR mice (6 weeks old) were purchased from CLEA Japan, Inc. (Tokyo, Japan). Mice were housed individually in cages equipped with 20 cm diameter running wheels under 12/12 light/dark cycle (lights-on at 6 a.m., lights-off at 6 p.m.) with food and water ad libitum. A data acquisition system (ClockLab software, Actimetrics, Wilmette, IL, USA) was used to record the number of wheel rotations in 1 min bins. After the data were downloaded, the ClockLab software package (MATLAB 6.5, MathWorks, Natick, MA, USA) was used to calculate the activity onset and display the double-plotted actograms. Animals were habituated in the running wheel cages until a reliable activity pattern was established (at least for 2 weeks). Following habituation period, the dark cycle was advanced by 8 h and animals were kept in a new 12/12 light/dark cycle for 14 days. Compound **15** (3 and 30 mg/kg) was suspended in 0.5% methylcellulose saline and administered in a volume of 10 mL/kg intraperitoneally at 1 h before dark period once daily from the day of phase shifting. Days to reentrainment was defined as the day which activity onset was advanced more than 7 h from the day before phase shifting and maintained for at least 3 consecutive days. In case animals did not meet this criterion, days to reentrainment of these animals were defined as 14 days. All animal handling and procedures were carried out according to the guidelines of the Takeda Experimental Animal Care and Use Committee (Takeda Pharmaceutical Company Ltd., Osaka, Japan).

■ ASSOCIATED CONTENT

Supporting Information. Elemental analyses for compounds **8**−**27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

MLT, melatonin; SCN, suprachiasmatic nuclei; 4P-PDOT, 4-phenyl-2-propionamidotetralin; CHO, Chinese hamster ovary; PBS, phosphate buffered saline

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