Synthesis of a Novel Series of Tricyclic Indan Derivatives as Melatonin Receptor Agonists

Osamu Uchikawa,* Kohji Fukatsu, Ryosuke Tokunoh, Mitsuru Kawada, Kiyoharu Matsumoto, Yumi Imai, Shuji Hinuma, Koki Kato, Hisao Nishikawa, Keisuke Hirai, Masaomi Miyamoto, and Shigenori Ohkawa

Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532-8686, Japan

Received March 12, 2002

To develop a new therapeutic agent for sleep disorders, we synthesized a novel series of tricyclic indan derivatives and evaluated them for their binding affinity to melatonin receptors. In our previous paper, we proposed a conformation of the methoxy group favorable for the binding of the MT_1 receptor. To fix the methoxy group in an active conformation, we decided to synthesize conformationally restricted tricyclic indan analogues with the oxygen atom in the 6-position incorporated into a furan, 1,3-dioxane, oxazole, pyran, morpholine, or 1,4-dioxane ring system. Among these compounds, indeno[5,4-*b*]furan analogues were found to be the most potent and selective MT_1 receptor ligands and to have superior metabolic stability. The optimization of substituents led to (*S*)-(−)-**22b**, which showed very strong affinity for human MT_1 (K_i = 0.014 nM), but no significant affinity for hamster MT_3 (K_i = 2600 nM) or other neurotransmitter receptors. The pharmacological effects of (*S*)-(−)-**22b** were studied in experimental animals, and it was found that a dose of 0.1 mg/kg, po promoted a sleep in freely moving cats, as demonstrated by a decrease in wakefulness and increases in slow wave sleep and rapid eye movement sleep, which lasted for 6 h after administration. Melatonin (1 mg/kg, po) also had a sleep-promoting effect, though it lasted only 2 h. A new chiral method for the synthesis of (*S*)-(−)-**22b** starting from **60**, which was prepared from **59** employing asymmetric hydrogenation with the (*S*)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl−Ru complex, was developed. (*S*)-(−)-**22b** (TAK-375) is currently under clinical trial for the treatment of insomnia and circadian rhythm disorders.

Introduction

The pineal hormone melatonin (**1**) is of considerable interest for its transduction of photoperiodic information,[1] control of mammalian circadian rhythms,[2] and modulation of retinal physiology.[3] The synthesis of melatonin and its secretion at night from the pineal gland are controlled by a circadian clock within the hypothalamic suprachiasmatic nucleus (SCN)[4] and are synchronized by environmental light.[5] Melatonin production in humans is concurrent with nocturnal sleep, and the increase in melatonin levels in the evening correlates with the onset of self-reported evening sleepiness or with the increase in the evening sleep propensity.[6]

In humans, it has been suggested that melatonin might have a variety of clinical applications such as the treatment of delayed sleep phase syndrome,[7] jet lag,[8] shift work disturbances,[9] seasonal affective disorders,[10] blindness, and aging[11] and as a hypnotic agent.[12] Over the past few years, numerous reports have described the effects of melatonin on the immune system suggesting that melatonin has immunomodulatory properties.[13] Additionally, melatonin was shown to inhibit the proliferation of various types of cancer cells and the human breast cancer cell line MCF-7.[14] However, the use of melatonin as a drug is limited by its short biological half-life (15−20 min),[15] its poor oral bioavailability, and its ubiquitous action. Melatonin receptors are classified as MT_1, MT_2, and MT_3 based on pharmacological profiles. The MT_1 receptor[16] is localized in the hypothalamic SCN and is thought to mediate the circadian and reproductive actions of melatonin, while the MT_2 receptor[17] is distributed in the SCN and neural retina and is thought to mediate the effects of melatonin on circadian rhythms and is implicated in the regulation of visual function. The binding site of the MT_3 receptor has been recently characterized as the hamster homologue of the human enzyme quinone reductase 2.[18] The physiological function of the MT_3 receptor in the brain is not yet completely clear; however, in consideration of therapy for circadian rhythm disorders in the elderly, effects on MT_3 receptors might induce unknown side effects. Because the MT_1 receptor was initially suggested to mediate circadian functions in mammals due to the high level of expression within brain and the MT_2 receptor was expressed in retina and to a lesser extent the brain, we have attempted to develop a MT_1 agonist, which has a longer half-life and is more potent than melatonin but has no affinity for the MT_3 receptor.

Previously, we synthesized a novel series of benzocycloalkene derivatives, with an *endo* double bond, an *exo* double bond (*E*- and *Z*-configurations), or a chiral center (*S*- and *R*-configurations[19]) at position C-1 to control the spatial position of the amide group, which is one of the most important pharmacophores. We found compounds with the *S*-configuration at C-1 to be the most promising in terms of potency and selectivity for the MT_1 receptor. Furthermore, we suggested that the methyl orientation of the 6-methoxy group on the indan nucleus is very

* To whom correspondence should be addressed. Tel: +81-6-6300-6546. Fax: +81-6-6300-6306. E-mail: uchikawa_osamu@takeda.co.jp.

10.1021/jm0201159 CCC: $22.00
Published on Web 08/15/2002

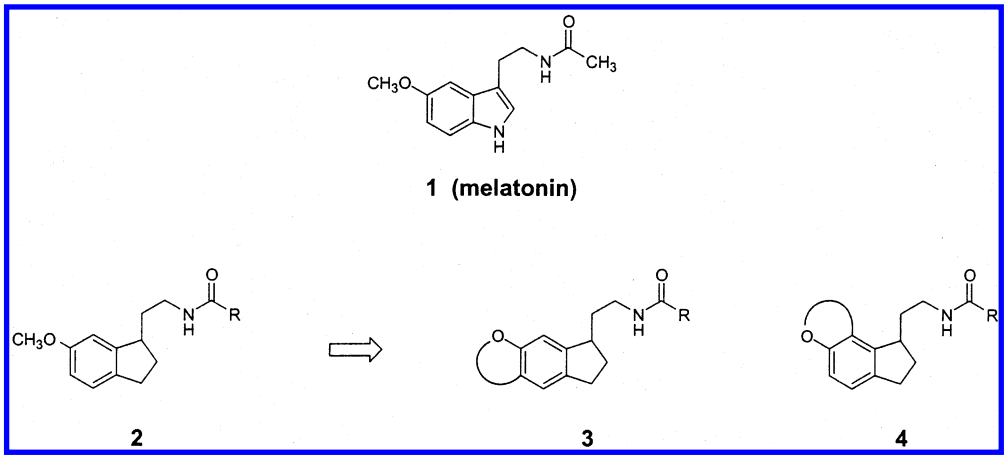
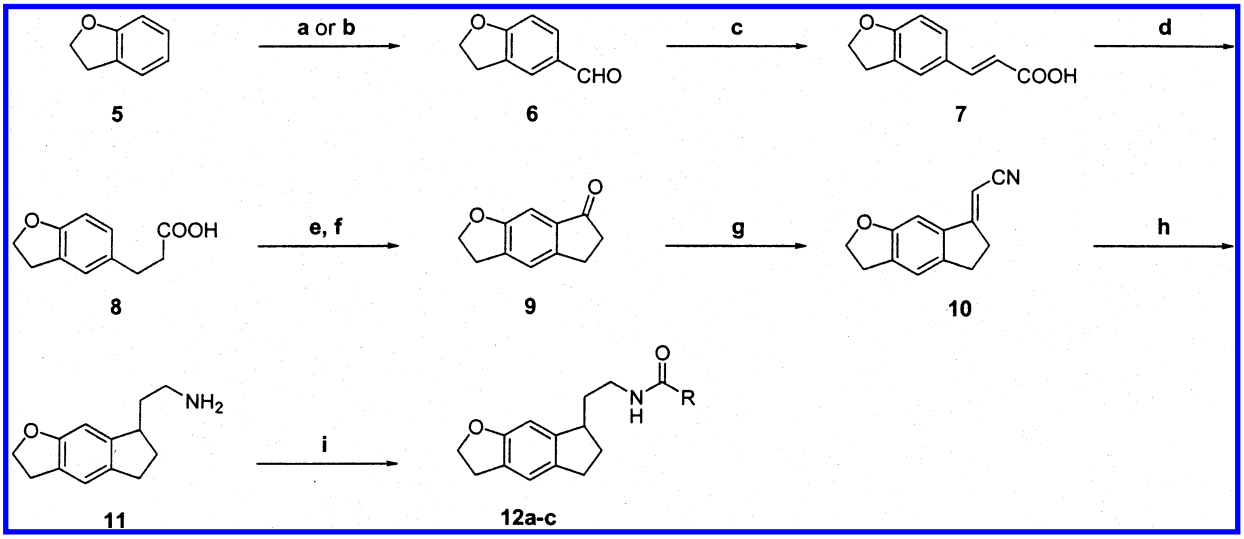


Figure 1. Progression from indan derivative **2** to tricyclic indan derivatives **3** and **4**.

Scheme 1. Synthesis of the Indeno[5,6-*b*]furan Compounds[a]



[a] Reagents: (a) $POCl_3$, DMF. (b) $TiCl_4$, Cl_2CHOCH_3, CH_2Cl_2. (c) Malonic acid, piperidine, pyridine. (d) H_2, Pd/C, AcOH. (e) $SOCl_2$. (f) $AlCl_3$, $ClCH_2CH_2Cl$. (g) $(EtO)_2P(O)CH_2CN$, NaH, THF. (h) H_2, Raney-Ni, NH_3−EtOH. (i) Ac_2O or RCOCl, NaOH, H_2O−THF.

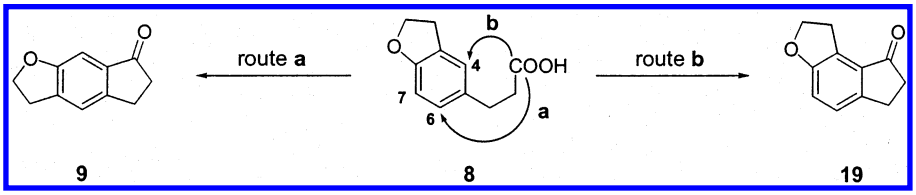


Figure 2. Construction of tricyclic indan analogues.

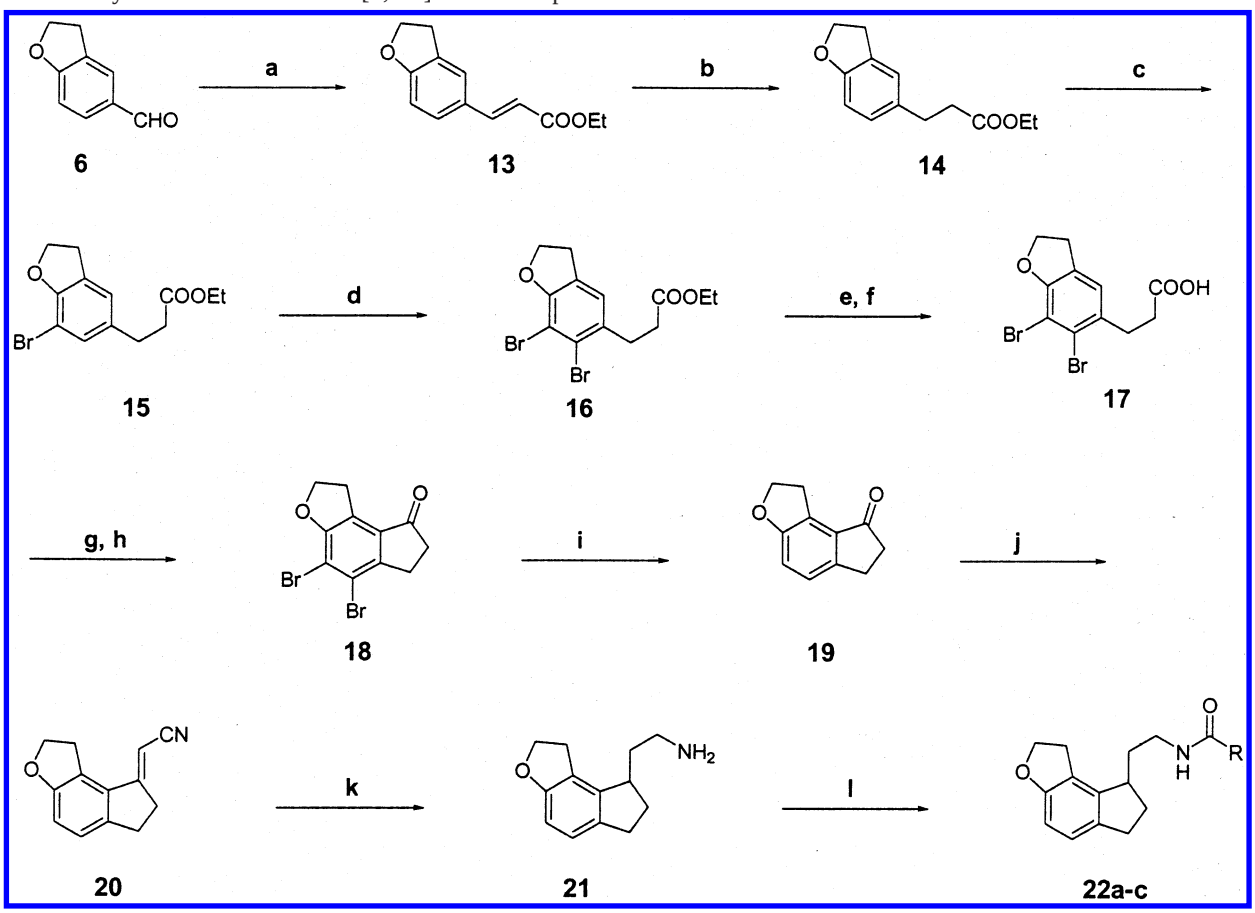
important for the optimal orientation of the oxygen lone pairs with a view to obtaining the best binding at the receptor level. Thus, in this study, we explored the possibility of limiting the conformational flexibility of the methoxy group. It appeared to us desirable to design and synthesize conformationally restricted tricyclic analogues[20] as the indanic bioisostere of melatonin, with the oxygen atom in the 6-position incorporated into a furan, 1,3-dioxolane, oxazole, pyran, morpholine, or 1,4-dioxane heterocyclic ring system.

This approach culminated in the discovery of indeno-[5,4-*b*]furan analogues, which are not only metabolically stable but also very potent and selective for the MT_1 receptor, suggesting a superior in vivo activity and safety profile. We report in this paper the synthesis and biological activities of tricyclic analogues **3** and **4** as shown in Figure 1.

Chemistry

The 2,3-dihydrobenzofuran (**5**) was chosen as the starting material for the preparation of several indenofuran derivatives (**12a**−**c**) as shown in Scheme 1. The formylation of **5** under Vilsmeier−Haack conditions[21] gave the known aldehyde **6**.[22] A reaction with malonic acid followed by a hydrogenation reaction in the presence of 10% Pd on charcoal as a catalyst gave the carboxylic acid analogue **8**. Friedel−Crafts cyclization[23] of **8** was accompanied by ring closure at C-6 of the benzofuran nucleus, to provide the linear tricyclic ketone **9** via the acyl chloride intermediate as shown in Figure 2 (route a). Compound **9** was subjected to a Horner−Emmons reaction[24] using diethyl cyanomethylphosphonate to give the intermediary unsaturated nitrile **10**, which was converted to the amine **11** by hydrogenation

Scheme 2. Synthesis of the Indeno[5,4-*b*]furan Compounds[a]



[a] Reagents: (a) $(EtO)_2P(O)CH_2COOEt$, NaH, THF. (b) H_2, Pd/C, EtOH. (c) Br_2, NaOAc, AcOH. (d) Br_2, Fe, AcOH. (e) KOH, EtOH. (f) HCl. (g) $SOCl_2$. (h) $AlCl_3$, $ClCH_2CH_2Cl$. (i) H_2, Pd/C, AcOH. (j) $(EtO)_2P(O)CH_2CN$, NaH, THF. (k) H_2, Raney-Ni, NH_3−EtOH. (l) Ac_2O or RCOCl, NaOH, H_2O−THF.

over Raney-nickel. The *N*-acylated derivatives (**12a**−**c**) were prepared from **11** by treatment with the appropriate acyl chloride in the presence of sodium hydroxide as base and a biphasic medium according to the Schotten−Baumann procedure.[25]

We turned our attention to the synthesis of angular indeno[5,4-*b*]furan derivatives (**22a**−**c**). Because the Friedel−Crafts reaction of **8** led to cyclization (route a) only at C-6 of the benzofuran nucleus as shown in Figure 2, we opted to explore another approach for the preparation of the angular tricyclic ketone **19** ring system (route b).

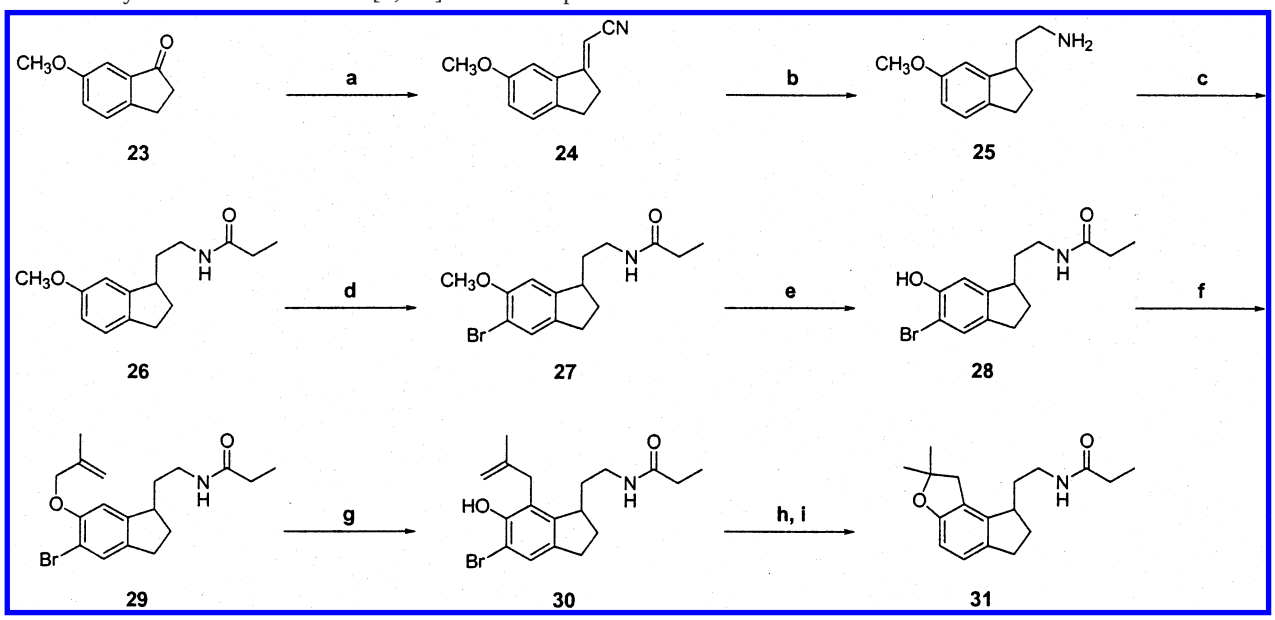
To avoid ring closure at C-6, we tried to block this position by introducing a bromine atom prior to cyclization. As outlined in Scheme 2, the aldehyde **6** was subjected to a Horner−Emmons reaction using triethyl phosphonoacetate, followed by hydrogenation in the presence of 10% Pd on charcoal to afford the objective, ester **14**, which was brominated selectively in C-7 by bromine in acetic acid to give the monobrominated ester **15**. The second bromination in C-6 was performed using 1.5 equiv of bromine in the presence of a catalytic amount of Fe.[26] The alkaline hydrolysis of **16** provided the corresponding carboxylic acid **17**. As expected, the Friedel−Crafts cyclization of **17** occurred at C-4 leading to the angular tricyclic ketone **18**. Debromination of **18** to **19** was readily achieved by hydrogenation in the presence of 10% Pd on charcoal. Compound **19** was transformed into the indeno[5,4-*b*]furan derivatives (**22a**−**c**) in three steps involving the reaction of the ketone **19** with diethyl cyanomethylphosphonate, hydrogenation over Raney-Ni, and *N*-acylation.

The 2,2-dimethyl-indeno[5,4-*b*]furan derivative (**31**) was prepared from commercially available 6-methoxyindanone (**23**) as shown in Scheme 3. Using the same conditions employed for the preparation of **22a**−**c**, the ketone **23** was converted in three steps into the corresponding indan derivative **26**. The bromide **27**, obtained by bromination of **26**, was allowed to react with boron tribromide to afford the alcohol **28**, which was converted to the alkylated derivative **29** by reaction with 3-chloro-2-methylpropene. The claisen rearrangement of **29** in *N*,*N*-diethylaniline afforded **30**. Acid-catalyzed cyclization of **30** with boron trifluoride diethyl etherate, followed by hydrogenation of the bromide, gave the desired product 2,2-dimethyl-indeno[5,4-*b*]furan (**31**).

The same strategy was used to prepare **37** bearing a 1,3-dioxolane ring condensed with the benzene ring (Scheme 4). The ketone **32**[27] was converted in three steps into the corresponding indan derivative **35** by the same procedure employed in the preparation of **22a**−**c**. Deprotection of the two methoxy groups of **35** with boron tribromide gave the diol **36**, which was in turn treated with diiodomethane and sodium hydride in hexamethyl phosphoramide[28] to afford indeno[4,5-*d*][1,3]dioxole (**37**).

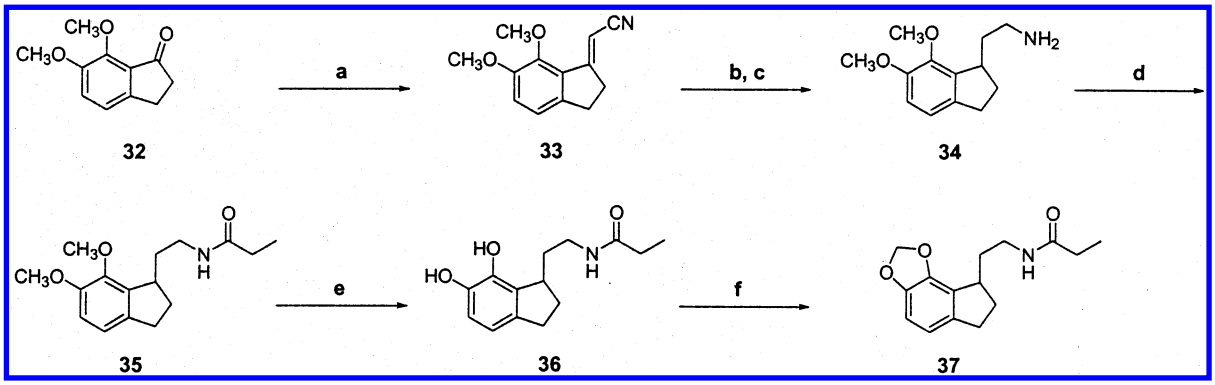
The 6-methoxyindanone (**23**) was also used for the preparation of the indeno[4,5-*d*][1,3]oxazole derivative (**43**) as shown in Scheme 5. Nitration of **23** with

Scheme 3. Synthesis of the Indeno[5,4-*b*]furan Compounds[a]



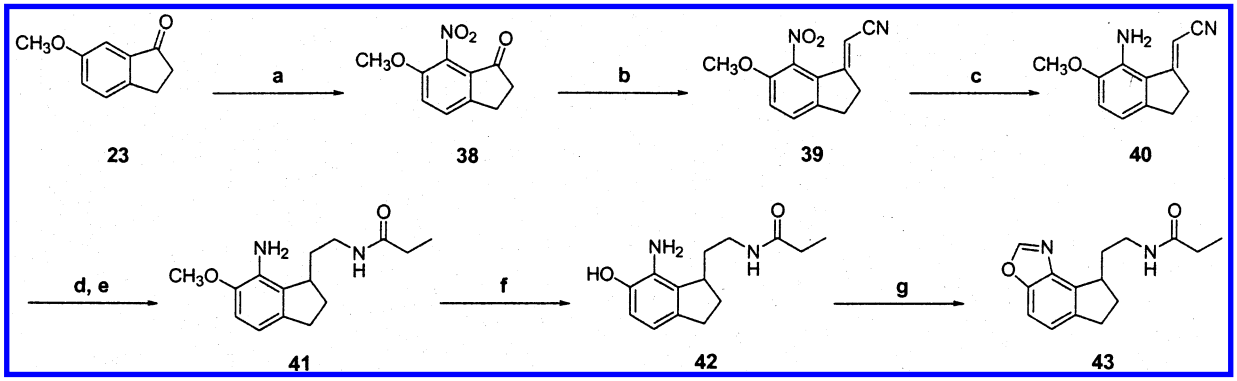
[a] Reagents: (a) $(EtO)_2P(O)CH_2CN$, NaH, THF. (b) H_2, Raney-Ni, NH_3−EtOH. (c) CH_3CH_2COCl, Et_3N, THF. (d) Br_2, NaOAc, AcOH. (e) BBr_3, CH_2Cl_2. (f) 3-Chloro-2-methylpropane, NaH, DMF. (g) *N*,*N*-Diethylaniline, 200 °C. (h) BF_3−Et_2O, CH_2Cl_2. (i) H_2, Pd/C, EtOH.

Scheme 4. Synthesis of the Indeno[4,5-*d*][1,3]dioxole Compounds[a]



[a] Reagents: (a) $(EtO)_2P(O)CH_2CN$, NaH, THF. (b) H_2, Raney-Co, NH_3−EtOH. (c) H_2, Pd/C, EtOH. (d) CH_3CH_2COCl, Et_3N, THF. (e) BBr_3, CH_2Cl_2. (f) CH_2I_2, NaH, HMPA.

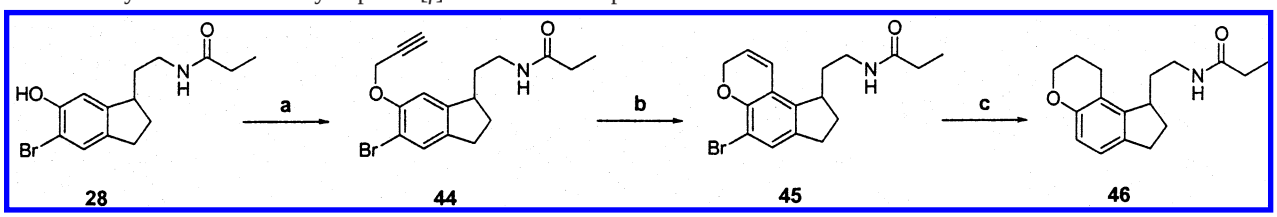
Scheme 5. Synthesis of the Indeno[4,5-*d*][1,3]oxazole Compounds[a]



[a] Reagents: (a) KNO_3, concentrated H_2SO_4. (b) $(EtO)_2P(O)CH_2CN$, NaH, THF. (c) H_2, Pd/C, EtOH. (d) H_2, Raney-Ni, NH_3−EtOH. (e) CH_3CH_2COOH, WSC, HOBt, DMF. (f) BBr_3, CH_2Cl_2. (g) $CH(OCH_3)_2$, HCl−MeOH.

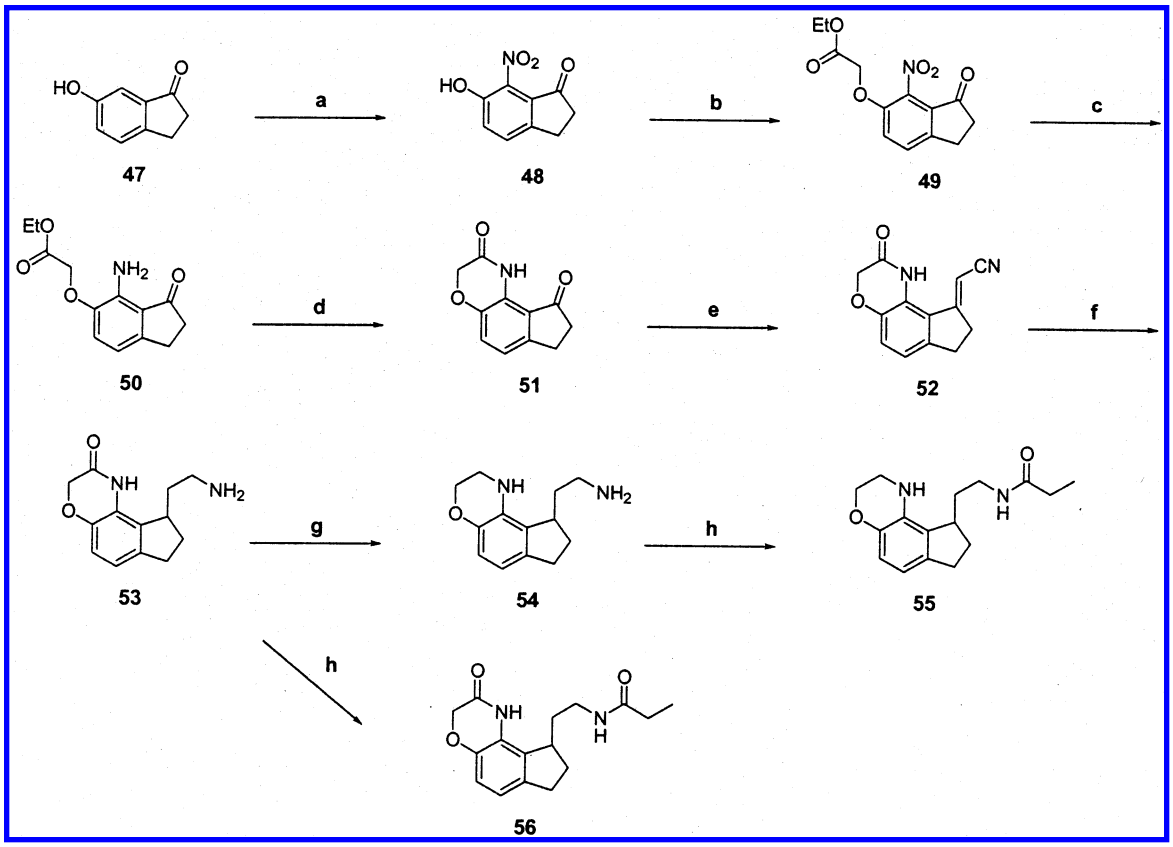
potassium nitrate in concentrated H_2SO_4 gave the nitro derivative **38**. Compound **38** was subjected to a Horner−Emmons reaction to give the unsaturated nitrile **39**, which was converted to the aniline **40** by catalytic hydrogenation. Hydrogenation over Raney-nickel of **40** and acylation with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSC)-mediated coupling of propionic acid with the primary amine in the presence of 1-hydroxybenzotriazole monohydrate (HOBt) gave the indan **41**. The indan **41** was converted to the phenol **42** by a reaction with boron tribromide, which was then transformed into the indeno[4,5-*d*][1,3]oxazole derivative (**43**) by heating in HCl−MeOH with methyl orthoformate.

Scheme 6. Synthesis of the Cyclopenta[*f*]chromene Compounds[a]



[a] Reagents: (a) Propargyl bromide, NaH, DMF. (b) Bromobenzene. (c) H_2, Pd/C, EtOH.

Scheme 7. Synthesis of the Indeno[5,4-*b*][1,4]oxazine Compounds[a]

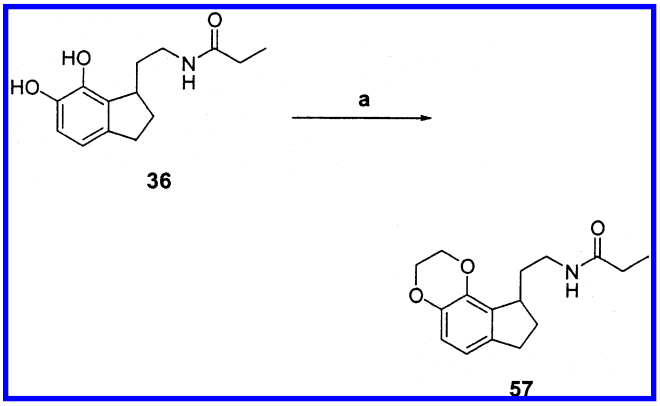


[a] Reagents: (a) KNO_3, concentrated H_2SO_4. (b) NaH, $BrCH_2COOEt$, DMF. (c) H_2, Pd/C, EtOH. (d) tBuOK, toluene. (e) $(EtO)_2P(O)CH_2CN$, NaH, THF. (f) H_2, Raney-Ni, NH_3−EtOH. (g) LAH, THF. (h) CH_3CH_2COOH, WSC, HOBt, DMF.

The indan **28**, obtained in the preceding experiment, was treated with propargyl bromide and sodium hydride in *N*,*N*-dimethylformamide (DMF) to give the corresponding ether **44** (Scheme 6). Thermal intramolecular cyclization of the aryl propargyl ether **44** led to **45**, and catalytic reduction gave the angular cyclopenta[*f*]-chromene derivative (**46**).

The amine **53** was the key material for the preparation of indeno[5,4-*b*][1,4]oxazine derivatives (Scheme 7). Nitration of 6-hydroxy-1-indanone (**47**) with potassium nitrate in concentrated H_2SO_4 followed by alkylation of the nitro compound **48** with ethyl bromoacetate gave the ester **49**, which was transformed into the key intermediate **53** in four straightforward steps, namely, reduction of the nitro group, cyclization of the obtained aminoester, condensation by the Horner−Emmons reaction, and reduction of the unsaturated nitrile **52**. After reduction of **53** with $LiAlH_4$, **54** was acylated to indeno-[5,4-*b*][1,4]oxazine (**55**) using the WSC−HOBt procedure. The *N*-acylated derivative **56** was prepared directly from **53**.

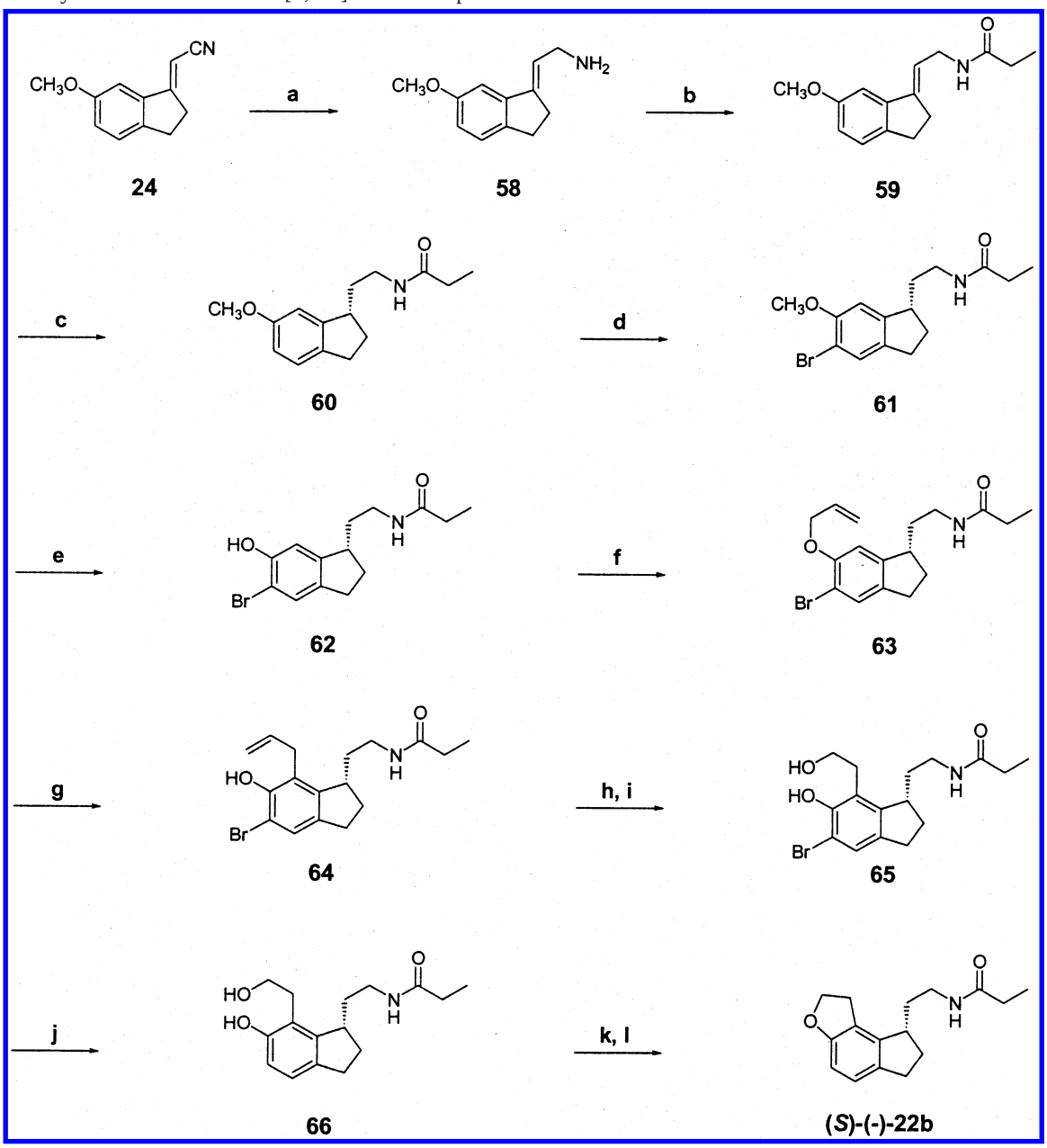
Scheme 8. Synthesis of the Indeno[4,5-*b*][1,4]dioxine Compounds[a]



[a] Reagents: (a) $Br(CH_2)_2Br$, CuO, K_2CO_3, DMF.

The indeno[4,5-*b*][1,4]dioxine (**57**) was prepared via the same diol **36** mentioned above by heating with dibromoethane, CuO, and K_2CO_3 in DMF. As with a related series of benzocycloalkene derivatives, the absolute configuration of the stereocenter had a significant

Scheme 9. Synthesis of the Indeno[5,4-*b*]furan Compounds[a]



[a] Reagents: (a) H_2, Raney-Co, NH_3−EtOH. (b) CH_3CH_2COCl, Et_3N, THF, 0 °C. (c) H_2, $Ru(OAc)_2$[(*S*)-binap], MeOH. (d) Br_2, NaOAc, MeOH, 0 °C. (e) BBr_3, CH_2Cl_2, −20 °C. (f) $CH{=}CHCH_2Br$, NaH, DMF, 0 °C. (g) *N*,*N*-Diethylaniline, 200 °C. (h) O_3, MeOH, −78 °C. (i) $NaBH_4$, MeOH, −78 °C. (j) H_2, Pd/C, Et_3N, MeOH. (k) MsCl, pyridine, −10 to 0 °C. (l) Et_3N, EtOAc, reflux.

effect on the selectivity toward the MT_1 receptor. We therefore focused on the asymmetric synthesis of the (*S*)-isomer of **22b** according to the results of our previous study on indan derivatives. The key step was the asymmetric reduction of the *exo* double bond in the intermediate **59**. This reaction proceeded nicely under 9.1 MPa of hydrogen at 70 °C using the (*S*)-2,2′-bis-(diphenylphosphino)-1,1′-binaphthyl (binap)−Ru complex as a catalyst to give the enantiomerically pure **60** as we previously reported (Scheme 9).

Having solved the problem of asymmetric hydrogenation, we turned our attention to the construction of a furan ring condensed with the indan nucleus. Because of the low reactivity at C-7 of the indan ring, our attempts to introduce substituents using the Fries rearrangement, the Friedel−Crafts reaction, nitration, or formylation for the construction of indeno[5,4-*b*]furan scaffolding were all unsuccessful. We finally attempted to introduce an allyl moiety into the C-7 position of the indan ring using a claisen rearrangement and then cyclized it to dihydrobenzofuran. To block the more reactive C-5 position, bromination was performed. Subsequent boron tribromide treatment provided the 6-hydroxy indan **62**. Alkylation of **62** with allyl bromide gave **63**, which was then subjected to a claisen rearrangement to afford **64**. Ozonolysis of the vinyl moiety followed by a reductive workup and deprotection of the C-5 bromide gave the diol **66**. The primary alcohol **66** was converted to mesylate and then cyclized to the desired (*S*)-(−)-**22b** by treatment with Et_3N in EtOAc at reflux.

Results and Discussion

MT_1 and MT_3 Receptor Binding Affinities. The affinities of the compounds for melatonin binding sites were evaluated by binding assays with 2-[^{125}I]iodomelatonin radioligand using Chinese hamster ovary (CHO) cells expressing human melatonin receptor (MT_1 site) and Syrian hamster brain and peripheral organs (MT_3 site).

The affinities of compounds for these receptors are shown in Table 1. From the structure−affinity relationships, the position of the third ring condensed with the indan nucleus seems to be critical for binding affinity. This is supported by the fact that the angular tricyclic derivatives **22a**−**c** showed a binding affinity for the MT_1 receptor that was ca. 3−4 orders of magnitude higher than that of the corresponding linear tricyclic derivatives **12a**−**c**. These results were consistent with the observation that the methyl orientation of the 6-methoxy group on the indan nucleus was very important for the optimal orientation of the oxygen lone pairs as discussed previously. Regarding the length of the acyl side chain, although compounds **22a**−**c** showed high affinity for the MT_1 receptors, there was a tendency for the affinity to improve with increasing chain length, the optimum group being the propionyl (**22b**). With respect to the MT_3 receptor, the increase of acyl chain length in the linear tricyclic series led to a decrease in the binding affinity whereas the corresponding angular tricyclic series gave the opposite results. As a consequence of these experiments, further optimization was pursued in the propionyl series. The introduction of dimethyl groups (**31**) into the furan ring was detrimental to the binding affinity for the MT_1 receptor. We therefore extensively modified the third ring part with substituents such as a 1,3-dioxolane (**37**), oxazole (**43**), pyran (**46**), morpholine (**55** and **56**), and 1,4-dioxane (**57**) heterocyclic ring. Replacement of the furan ring with a 1,3-dioxolane ring (**37**) preserved a moderate level of affinity for the MT_1 receptor, but further modification as seen with **43**, **46** and **55**−**57** resulted in less affinity as compared with that of **22b**. These results indicated that the angular unsubstituted dihydro furan ring condensed with the indan nucleus was the best structure. For a more rational explanation of the observed binding affinity, we carried out molecular orbital (MO) calculations of substrates **22b**, **37**, and **43** to compare the electron density on the oxygen atom. The calculations were made by the MNDO−PM$_3$ method with MOPAC version 6.00.[29] The net atomic charges of **22b** and **37** were −0.19 and −0.20, respectively, and these compounds exhibited a similar high binding affinity. However, the value (−0.11) of **43** is worth noting, suggesting that the parameter accounts for the weak potency.

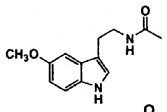
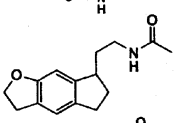
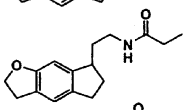
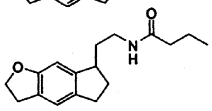
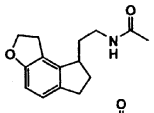
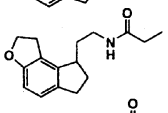
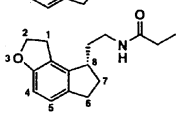
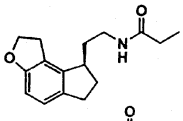
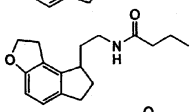
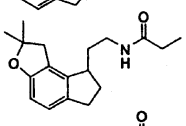
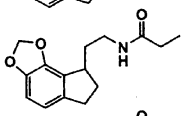
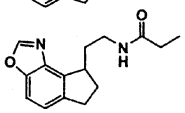
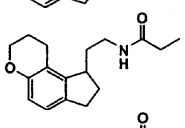
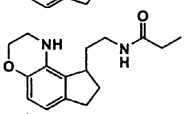
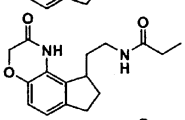
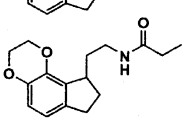
To investigate the stereochemical requirements, the two enantiomers, (*S*)-(−)-**22b** and (*R*)-(+)-**22b**, of compound **22b** were prepared and examined for binding affinity. As expected, significant differences were observed and (*S*)-(−)-**22b** was more than 500 times as potent as (*R*)-(+)-**22b** for the MT_1 receptor, confirming the favorable influence of the *S*-configuration. Compound (*S*)-(−)-**22b** had the most impressive binding profile for the MT_1 (K_i = 13.8 pM) receptor. (*S*)-(−)-**22b** showed very low affinity for the MT_3 receptor with a K_i value of 2.6 μM, which was 190 000-fold the value for the human MT_1 subtype. These results indicate that (*S*)-(−)-**22b** is a potent and selective MT_1 agonist and warrants further pharmacological study.

Adenosine Cyclic 3′,5′-Phosphate (cAMP) Production in the Rat Pituitary. This experiment was based on the method of Vanecek and Vollrath[30] with minor modifications. The stimulation of 9 day old rat pituitary with 1 μM forskolin raised the cAMP content to 10 times the basal level. Melatonin inhibited forskolin-stimulated cAMP production in a concentration-dependent manner, the effect of which was maximal at 1 nM reaching about 50−65% inhibition. (*S*)-(−)-**22b**, like melatonin, inhibited cAMP production, indicating an agonistic effect on the melatonin receptor in the pituitary, although its activity was slightly weaker than that of melatonin, as shown by the IC_{50} values in Table 2.

Effects of (*S*)-(−)-22b and Melatonin on Sleep and Wakefulness in Freely Moving Cats. The sleep-promoting effects were studied in freely moving cats. Administration of melatonin (1 mg/kg, po) reduced wakefulness and increased slow wave sleep (SWS) and rapid eye movement (REM) sleep (analysis of variance (ANOVA), both $p < 0.01$). However, the duration of sleep-inducing action was not very long and significant effects were observed only for 2 h after the administration, although the effect on REM was not significant (Figure 3). (*S*)-(−)-**22b** (0.1 mg/kg, po) had potent sleep-promoting activity, as indicated by the significant decrease in wakefulness and significant increases in SWS and REM sleep (ANOVA, $p < 0.05$ or $p < 0.01$). The effects lasted for about 6 h after the administration (Figure 4). Thus, the sleep-inducing action of (*S*)-(−)-**22b** and the duration of the effect were much greater than for melatonin.

Docking Study. To explain the potency of (*S*)-(−)-**22b**, we performed a docking experiment using a model of the human melatonin receptor (MT_1) constructed from the crystal structure of bovine rhodopsin.[31] Details of the model are given in the Experimental Section. Figure 5 shows the most reasonable mode of binding of (*S*)-(−)-**22b** in the ligand binding pocket. In this model, the oxygen atom in the furan ring forms a hydrogen bond with His195 and the adjacent carbon atom interacts with Val192. Both residues are located in transmembrane segment V and are suggested to be concerned with ligand recognition.[32] The orientation of the oxygen lone pairs incorporated into the furan ring would be important as can be seen from the data on the binding affinity of **12b** and (*S*)-(−)-**22b**. Actually, the orientation of the oxygen lone pairs of **12b** as compared to (*S*)-(−)-**22b** would be unfavorable for a strong hydrogen-bonding interaction, though the furan ring of **12b** can be accommodated around His195 (data not shown). Fixing the orientation of the methoxy moiety of melatonin by its incorporation into a furan ring seems to play a main role in reinforcing the interaction. The propanamide side chain is favorably surrounded by Val261 and Pro265, both in transmembrane segment VI and is simultaneously hydrogen-bonded to Tyr175 and Ser182 in the second extracellular loop, which is suggested to be important for ligand binding.[33] The interaction with Pro265, which is not available for melatonin, would

Table 1. Binding Affinity and Physical Properties of Tricyclic Analogues

compound		receptor binding (K_i^a, nM) MT_1^b	MT_3^c	mp (°C)	formula	anal.[d]
melatonin		0.0823 ± 0.0021	27.6 ± 0.3			
12a		483 ± 131	237 ± 69	127-128	$C_{15}H_{19}NO_2$	C, H, N
12b		255 ± 45	255 ± 101	125-126	$C_{16}H_{21}NO_2$	C, H, N
12c		240 ± 35	492 ± 199	120-121	$C_{17}H_{23}NO_2$	C, H, N
22a		0.126 ± 0.035	5960 ± 1330	78-79	$C_{15}H_{19}NO_2$	C, H, N
22b		0.0174 ± 0.0041	1250 ± 191	102-104	$C_{16}H_{21}NO_2$	C, H, N
(*S*)-(-)-22b		0.0138 ± 0.0006	2600 ± 190	113-115	$C_{16}H_{21}NO_2$	C, H, N
(*R*)-(+)-22b		7.31 ± 2.86	2270 ± 656	113-115	$C_{16}H_{21}NO_2$	C, H, N
22c		0.0214 ± 0.0050	1070 ± 271	55-57	$C_{17}H_{23}NO_2$	C, H, N
31		0.422 ± 0.070	4790 ± 1860	69-72	$C_{18}H_{25}NO_2$	C, H, N
37		0.0241 ± 0.0059	5830 ± 1740	102-104	$C_{15}H_{19}NO_3$	C, H, N
43		3.29 ± 0.63	52.8 ± 18.0	81-84	$C_{15}H_{18}N_2O_2$	C, H, N
46		0.242 ± 0.035	6220 ± 1920	85-88	$C_{17}H_{23}NO_2$	C, H, N
55		15.3 ± 5.10	> 10000	80-83	$C_{16}H_{22}N_2O_2$	C, H, N
56		326 ± 35	2560 ± 854	216-219	$C_{16}H_{20}N_2O_3$	C, H, N
57		0.423 ± 0.088	5390 ± 165	120-122	$C_{16}H_{21}NO_3$	C, H, N

[a] K_i values for binding are the means of three experiments. [b] Values for binding affinity in CHO cells expressing human melatonin (MT_1) receptor. [c] Values for binding affinity in syrian hamster brain and peripheral organs. [d] Analytical results are within ±0.4% of the theoretical value.

Table 2. Effects of (*S*)-(−)-**22b** on cAMP Production in the Neonatal Rat Pituitary[a]

	IC_{50} (pM)	K_i (pM)	
compd	cAMP production	human (MT_1)	cAMP/human
(*S*)-(−)-**22b**	20.8	13.8	1.51
melatonin	79.8	82.3	0.97

[a] IC_{50} values for cAMP production were calculated from the mean data of two experiments each done in triplicate. K_i values for binding are the means of three experiments.

reinforce the binding of (*S*)-(−)-**22b** in this moiety. The receptor has many hydrophobic residues at the bottom of the pocket. The hydrophobic interaction at this site caused by replacing the indole nitrogen atom in melatonin with a methylene unit would contribute to the high affinity of (*S*)-(−)-**22b**.

Conclusion

We synthesized a variety of tricyclic indan analogues to maximize affinity for the MT_1 receptor in consideration of the interaction between two valuable pharmacophores and MT_1 receptors based on the results of our previous study. The angular indeno[5,4-*b*]furan structure serves as an effective, conformationally restricted bioisostere of melatonin, and the orientation controlled by the furan ring and the propanamide side chain exemplified by (*S*)-(−)-**22b** most likely represents the active conformation during melatonin receptor activation.

(*S*)-(−)-**22b** showed remarkably high affinity for the human MT_1 receptor with a K_i value of 13.8 pM, whereas it showed very low affinity for the hamster MT_3 receptor with a K_i value of just 2.6 μM. The affinities for MT_1 and MT_3 receptors were 6-fold higher and 94-fold lower than those of melatonin, respectively. (*S*)-(−)-**22b** inhibited forskolin-stimulated cAMP production in the neonatal rat pituitary with an IC_{50} of 20.8 pM, indicating that this compound is a MT_1 agonist. The pharmacological effects of (*S*)-(−)-**22b** were studied in experimental animals. (*S*)-(−)-**22b** (0.1 mg/kg, po) showed a sleep-promoting action in freely moving cats, as demonstrated by a decrease in wakefulness and increases in SWS and REM sleep, which lasted for 6 h after the administration. These results indicate that (*S*)-(−)-**22b** is a potent and highly selective agonist for the MT_1 melatonin receptor, which is expected to be a useful therapeutic for sleep and/or circadian rhythm disorders, and a valuable tool in pharmacological investigations of the structure−activity relationships of melatonergic agents. (*S*)-(−)-**22b** also showed high affinity for the MT_2 receptor with a K_i value of 0.045 ± 0.018 nM, which was 4-fold higher than melatonin (K_i = 0.195 ± 0.051

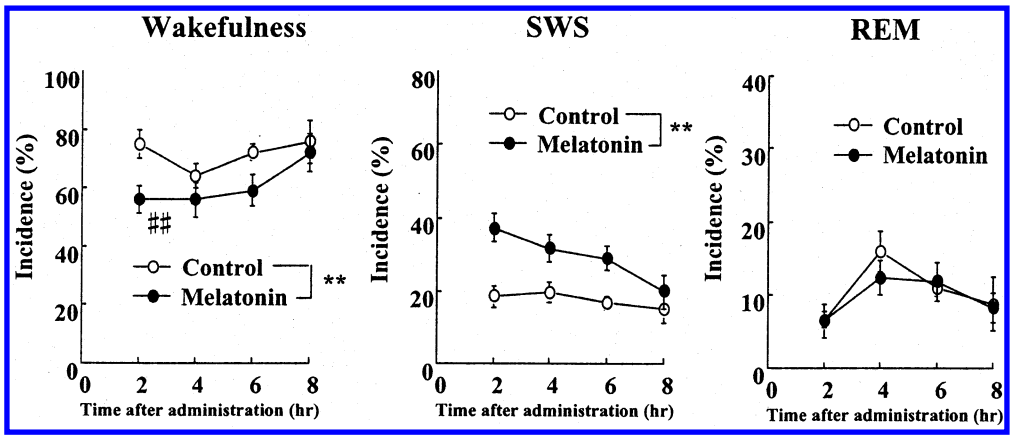


Figure 3. Effects of melatonin on sleep and wakefulness in freely moving cats. The experiment was based on the crossover design. Eight cats were treated with melatonin (1 mg/kg, po) or vehicle. Each value shows the mean percentage at each stage during each block of 2 h. $^{**}p < 0.01$, main group effect by ANOVA; $^{\#\#}p < 0.01$, as compared with the vehicle control (paired *t*-test with Holm's correction).

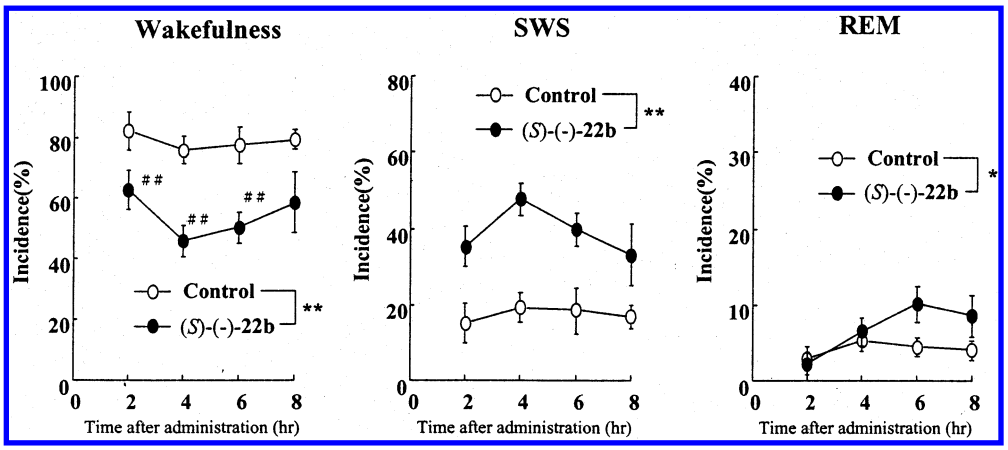


Figure 4. Effects of (*S*)-(−)-**22b** on sleep and wakefulness in freely moving cats. The experiment was based on the crossover design. Eight cats were treated with (*S*)-(−)-**22b** (0.1 mg/kg, po) or vehicle. Each value shows the mean percentage at each stage during each block of 2 h. $^{*}p < 0.05$ and $^{**}p < 0.01$, main group effect by ANOVA; $^{\#\#}p < 0.01$, as compared with the vehicle control (paired *t*-test with Holm's correction).

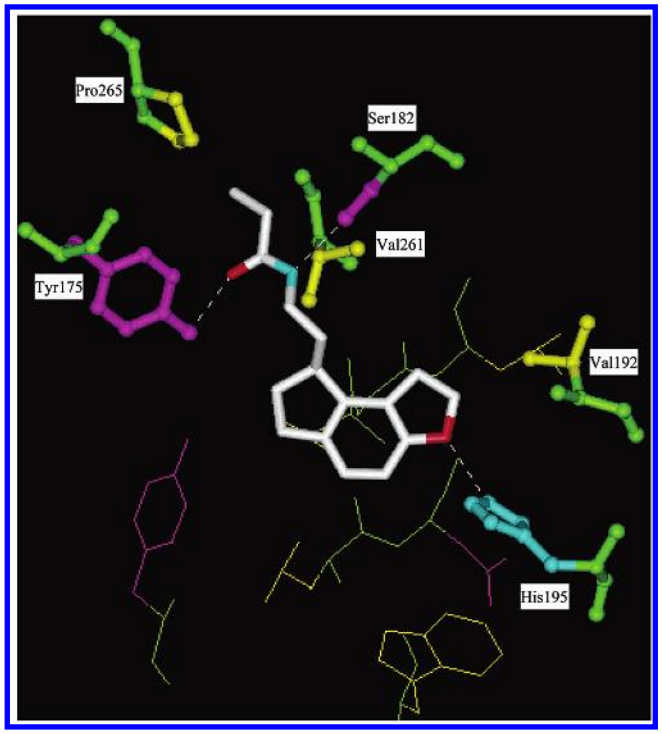


Figure 5. Schematic model for the binding of (*S*)-(−)-**22b** with the MT_1 receptor.

nM). This affinity for the MT_2 receptor may be related to the pharmacological effect of (*S*)-(−)-**22b**. Furthermore, we have developed a practical method for the stereospecific synthesis of optically active (*S*)-(−)-**22b** by transforming the indan ring into the angular tricyclic ring via catalytic asymmetric hydrogenation with the (*S*)-binap−Ru complex. (*S*)-(−)-**22b** is a promising drug candidate for the treatment of primary insomnia and circadian rhythm disorders and is now under clinical evaluation.

Experimental Section

A. Chemistry. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-370 digital polarimeter. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Gemini-200 spectrometer in the solvent indicated. Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard. Abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Coupling constants (*J* values) are given in hertz (Hz). Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ (Merck), and column chromatography was carried out on a silica gel column (Kieselgel 60, 0.063−0.200 mm, Merck). Evaporation was carried out in vacuo on a rotary evaporator. (*E*)-2-(6-Methoxy-2,3-dihydro-1*H*-inden-1-ylidene)-acetonitrile (**24**), 6-methoxy-2,3-dihydro-1*H*-inden-1-ethanamine (**25**), *N*-[2-(6-methoxy-2,3-dihydro-1*H*-inden-1-yl)ethyl]-propionamide (**26**), 2-(6-methoxy-2,3-dihydro-1*H*-inden-1-ylidene)ethylamine (**58**), *N*-[2-(6-methoxy-2,3-dihydro-1*H*-inden-1-ylidene)ethyl]propionamide (**59**), and (*S*)-*N*-[2-(6-methoxy-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (**60**) were synthesized according to a process as described in the previous paper.

2,3-Dihydro-1-benzofuran-5-carbaldehyde (6).[22] Method A. Phosphorus oxychloride (31.0 mL, 0.333 mol) was slowly added to a solution of DMF (28.4 mL, 0.366 mol) while the temperature was maintained below 50 °C. Then, 2,3-dihydrobenzofuran (**5**) (20.0 g, 0.166 mol) was added dropwise to this reaction mixture at below 50 °C, over a period of 10 min. The resulting mixture was warmed and stirred at 80−85 °C for 12 h. The reaction mixture was then poured into ice-water and carefully quenched with a solution of sodium hydroxide. The organic material was separated, and the aqueous layer was extracted with ethyl acetate. It was washed with brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with hexane/ethyl acetate (8:2) as eluent followed by distillation to yield 21.2 g (86%) of **6**; bp 93−96 °C/0.2−0.3 mm Hg. ^{1}H NMR ($CDCl_3$): δ 3.28 (t, 2H, J = 8.8), 4.70 (t, 2H, J = 8.8), 6.88 (d, 1H, J = 8.4), 7.67 (dd, 1H, J = 1.0, 8.4), 7.75 (d, 1H, J = 1.0), 9.83 (s, 1H). Anal. ($C_9H_8O_2$) C, H.

Method B. Titanium(IV) chloride (28 mL) was added to an ice-cooled solution of **5** (10.0 g, 83.2 mmol) and dichloromethyl methyl ether (11.3 mL, 0.125 mmol) in dichloromethane (100 mL). After 1 h, the ice-bath was removed and the dark mixture was quenched by the addition of ice-water. The aqueous layer was discarded, and the organic phase was washed with water and brine and then dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with hexane/ethyl acetate (1:1) as eluent to yield 11.4 g (92%) of **6** as an oil.

3-(2,3-Dihydro-1-benzofuran-5-yl)-2-propenoic Acid (7). A mixture of **6** (4.99 g, 33.7 mmol) and malonic acid (5.27 g, 50.6 mmol) in pyridine (30 mL) containing piperidine (0.192 g, 2.25 mmol) was heated at 100 °C for 4 h. The cooled reaction mixture was concentrated, and the residue was poured into water. It was acidified with 6 N HCl and extracted with methanol/chloroform (1:9). The extract was washed with brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with methanol/chloroform (5:95) as eluent to yield 3.98 g (62%) of **7**; mp 173−176 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 3.24 (t, 2H, J = 8.8), 4.64 (t, 2H, J = 8.8), 6.28 (d, 1H, J = 15.9), 6.55 (d, 1H, J = 8.2), 7.34 (d, 1H, J = 8.2), 7.44 (s, 1H), 7.74 (d, 1H, J = 15.9), hidden (1H). Anal. ($C_{11}H_{10}O_3$) C, H.

3-(2,3-Dihydro-1-benzofuran-5-yl)propanoic Acid (8). A solution of **7** (3.75 g, 19.7 mmol) in acetic acid (50 mL) was subjected to hydrogenation in the presence of 10% palladium on carbon (0.5 g, containing 50% water) in a hydrogen atmosphere at room temperature for 1 h. After absorption of the theoretical volume of hydrogen, the catalyst was removed by filtration. The filtrate was concentrated and washed with hexane to yield 3.41 g (90%) of **8**. The analytical sample was obtained by recrystallization from a mixture of ethyl acetate and hexane; mp 95−98 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 2.63 (t, 2H, J = 7.5), 2.89 (t, 2H, J = 7.5), 3.18 (t, 2H, J = 8.8), 4.55 (t, 2H, J = 8.8), 6.70 (d, 1H, J = 8.1), 6.94 (d, 1H, J = 8.1), 7.04 (s, 1H), hidden (1H). Anal. ($C_{11}H_{12}O_3$) C, H.

2,3,5,6-Tetrahydro-7*H*-indeno[5,6-*b*]furan-7-one (9). Compound **8** (5.84 g, 30.4 mmol) was added to thionyl chloride (6.64 mL, 19.9 mol) followed by stirring at 75 °C for 40 min. After excess reagent was removed, the residue was dissolved in 1,2-dichloroethane (10 mL). One-third of the solution of acid chloride in 1,2-dichloroethane was added to an ice-cooled suspension of anhydrous aluminum chloride (1.49 g, 11.2 mmol) in 1,2-dichloroethane (150 mL). After the mixture was stirred for 15 min, anhydrous aluminum chloride (2.97 g, 22.3 mmol) and the rest of the solution of acid chloride were added, followed by stirring at room temperature further for 15 min. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with 1 N HCl, 1 N NaOH, and brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with hexane/ethyl acetate (7:3) as eluent and recrystallized from a mixture of ethyl acetate and isopropyl ether to yield 4.09 g (77%) of **9**; mp 110−111 °C (from ethyl acetate/isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 2.66−2.74 (m, 2H), 2.99−3.07 (m, 2H), 3.27 (t, 2H, J = 8.6), 4.64 (t, 2H, J = 8.6), 7.07 (s, 1H), 7.27 (s, 1H).

2-(2,3,5,6-Tetrahydro-7*H*-indeno[5,6-*b*]furan-7-ylidene)-acetonitrile (10). A 60% suspension of sodium hydride in

mineral oil (0.744 g, 18.6 mmol) was added to a solution of diethyl cyanomethylphosphonate (3.30 g, 18.6 mmol) in tetrahydrofuran (40 mL). The mixture was stirred at room temperature for 30 min and was added dropwise at room temperature to a stirred mixture of **9** (2.95 g, 16.9 mmol) in tetrahydrofuran (20 mL). After the addition was complete, the reaction mixture was stirred at room temperature a further 2 h. Water was added, and the mixture was extracted with ethyl acetate. The extract was washed with brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with hexane/ethyl acetate (1:1) as eluent and recrystallized from a mixture of ethyl acetate and hexane to yield 2.20 g (66%) of **10**; mp 141−142 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 2.97−3.04 (m, 2H), 3.06−3.17 (m, 2H), 3.22 (t, 2H, J = 8.6), 4.61 (t, 2H, J = 8.6), 5.50−5.52 (m, 1H), 6.84 (s, 1H), 7.16 (s, 1H).

2-(3,5,6,7-Tetrahydro-2*H*-indeno[5,6-*b*]furan-7-yl)ethylamine (11). A solution of **10** (2.15 g, 10.9 mmol) in ethanol (50 mL) saturated with NH_3 gas was subjected to hydrogenation in the presence of Raney-nickel (2.0 g washed with ethanol two times before use) in a hydrogen atmosphere (480 kPa) at 40 °C for 5 h. After hydrogen absorption ceased, the catalyst was removed by filtration. The filtrate was concentrated to yield 2.10 g (95%) of **11** as an oil, which was used directly in the next step. ^{1}H NMR ($CDCl_3$): δ 1.39 (br s, 2H), 1.47−1.77 (m, 2H), 1.86−2.03 (m, 1H), 2.20−2.38 (m, 1H), 2.65−2.94 (m, 4H), 3.02−3.18 (m, 1H), 3.15 (t, 2H, J = 8.6), 4.55 (t, 2H, J = 8.6), 6.63 (s, 1H), 7.02 (s, 1H).

***N*-[2-(3,5,6,7-Tetrahydro-2*H*-indeno[5,6-*b*]furan-7-yl)ethyl]acetamide (12a).** Acetic anhydride (0.168 mL, 1.78 mmol) was added all at once to a vigorously stirred solution of **11** (0.301 g, 1.48 mmol) and aqueous 1 N NaOH (2.5 mL) in tetrahydrofuran (3 mL) at room temperature. The mixture was stirred for 30 min at room temperature, and ethyl acetate and water were added. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated to give a solid, which was recrystallized from a mixture of ethyl acetate and hexane to yield 0.280 g (77%) of **12a**; mp 127−128 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.48−1.80 (m, 2H), 1.94−2.10 (m, 1H), 1.97 (s, 3H), 2.23−2.41 (m, 1H), 2.66−2.92 (m, 2H), 3.00−3.19 (m, 3H), 3.31−3.42 (m, 2H), 4.55 (t, 2H, J = 8.6), 5.52 (br s, 1H), 6.61 (s, 1H), 7.02 (s, 1H). Anal. ($C_{15}H_{19}NO_2$) C, H, N.

***N*-[2-(3,5,6,7-Tetrahydro-2*H*-indeno[5,6-*b*]furan-7-yl)ethyl]propionamide (12b).** Compound **12b** was prepared in 71% yield from propionyl chloride and **11** by a method similar to that described for **12a**; mp 125−126 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, J = 7.6), 1.49−1.80 (m, 2H), 1.93−2.09 (m, 1H), 2.15−2.41 (m, 3H), 2.65−2.92 (m, 2H), 3.00−3.19 (m, 3H), 3.33−3.43 (m, 2H), 4.55 (t, 2H, J = 8.6), 5.46 (br s, 1H), 6.61 (s, 1H), 7.02 (s, 1H). Anal. ($C_{16}H_{21}NO_2$) C, H, N.

***N*-[2-(3,5,6,7-Tetrahydro-2*H*-indeno[5,6-*b*]furan-7-yl)ethyl]butanamide (12c).** Compound **12c** was prepared in 72% yield from butyryl chloride and **11** by a method similar to that described for **12a**; mp 120−121 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 0.94 (t, 3H, J = 7.2), 1.49−1.81 (m, 4H), 1.94−2.18 (m, 3H), 2.23−2.40 (m, 1H), 2.66−2.92 (m, 2H), 2.99−3.19 (m, 3H), 3.12−3.43 (m, 2H), 4.55 (t, 2H, J = 8.6), 5.45 (br s, 1H), 6.61 (s, 1H), 7.02 (s, 1H). Anal. ($C_{17}H_{23}NO_2$) C, H, N.

Ethyl (*E*)-3-(2,3-Dihydro-1-benzofuran-5-yl)-2-propenoate (13). A 60% suspension of sodium hydride in mineral oil (3.39 g, 84.6 mmol) was added to a solution of triethyl phosphonoacetate (19.0 g, 84.6 mmol) in tetrahydrofuran (150 mL). The mixture was stirred at room temperature for 20 min and was added dropwise at room temperature to a stirred mixture of **6** (11.4 g, 76.9 mmol) in tetrahydrofuran (15 mL). After the addition was complete, the reaction mixture was stirred at room temperature a further 1 h. Water was added, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate. The extract was washed with brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with hexane/ethyl acetate (from 95:5 to 9:1) as eluent to yield 14.7 g (88%) of **13** as an oil. ^{1}H NMR ($CDCl_3$): δ 1.33 (t, 3H, J = 7.2), 3.23 (t, 2H, J = 8.8), 4.25 (q, 2H, J = 7.2), 4.63 (t, 2H, J = 8.8), 6.28 (d, 1H, J = 16.0), 6.79 (d, 1H, J = 8.4), 7.31 (d, 1H, J = 8.4), 7.41 (s, 1H), 7.64 (d, 1H, J = 16.0).

Ethyl 3-(2,3-Dihydro-1-benzofuran-5-yl)propanoate (14). A solution of **13** (14.7 g, 66.7 mmol) in ethanol (150 mL) was subjected to hydrogenation in the presence of 10% palladium on carbon (1 g, containing 50% water) in a hydrogen atmosphere at room temperature for 2 h. After the theoretical volume of hydrogen was absorbed, the catalyst was removed by filtration. The filtrate was concentrated to yield 14.6 g (99%) of **14** as an oil, which was used directly in the next step. ^{1}H NMR ($CDCl_3$): δ 1.24 (t, 3H, J = 7.2), 2.57 (t, 2H, J = 7.8), 2.88 (t, 2H, J = 7.8), 3.18 (t, 2H, J = 8.6), 4.13 (q, 2H, J = 7.2), 4.55 (t, 2H, J = 8.6), 6.70 (d, 1H, J = 8.2), 6.94 (d, 1H, J = 8.2), 7.05 (s, 1H).

Ethyl 3-(7-Bromo-2,3-dihydro-1-benzofuran-5-yl)propanoate (15). A solution of **14** (14.5 g, 65.8 mmol) and sodium acetate (5.94 g, 72.4 mmol) in acetic acid (150 mL) was stirred at room temperature, and bromine (10.5 g, 65.8 mmol) was added dropwise over a period of 15 min. After 1 h at room temperature, the mixture was evaporated under reduced pressure. The residual oil was taken up in ethyl acetate and washed successively with aqueous 5% $NaHSO_3$, saturated $NaHCO_3$, and H_2O before it was dried over anhydrous magnesium sulfate. The filtrate was concentrated to yield 19.2 g (97%) of **15** as an oil, which was used directly in the next step. ^{1}H NMR ($CDCl_3$): δ 1.25 (t, 3H, J = 7.2), 2.57 (t, 2H, J = 7.6), 2.85 (t, 2H, J = 7.6), 3.28 (t, 2H, J = 8.8), 4.13 (q, 2H, J = 7.2), 4.65 (t, 2H, J = 8.8), 6.97 (s, 1H), 7.11 (s, 1H).

Ethyl 3-(6,7-Dibromo-2,3-dihydro-1-benzofuran-5-yl)propanoate (16).[26] A suspension of **15** (1.00 g, 3.34 mmol) and iron (10 mg) in acetic acid (10 mL) was stirred at room temperature, and bromine (0.801 g, 5.01 mmol) was added dropwise over a period of 15 min. After the mixture was stirred for 5 h at 50 °C, iron was removed by filtration and concentrated under reduced pressure. The residual oil was taken up in ethyl acetate, washed successively with aqueous 5% $NaHSO_3$, saturated $NaHCO_3$, and H_2O, and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with ethyl acetate/hexane (1:3) as eluent to yield 670 mg (53%) of **16**; mp 42−43 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.25 (t, 3H, J = 7.3), 2.60 (t, 2H, J = 7.7), 3.07 (t, 2H, J = 7.7), 3.27 (t, 2H, J = 8.8), 4.14 (q, 2H, J = 7.3), 4.68 (t, 2H, J = 8.8), 7.06 (s, 1H).

3-(6,7-Dibromo-2,3-dihydro-1-benzofuran-5-yl)propanoic Acid (17). Aqueous KOH (0.138 g, 2.46 mmol) was added slowly to an ice-cooled solution of **16** (0.620 g, 1.64 mmol) in EtOH (10 mL). The solution was heated at 90 °C for 1 h. The mixture was poured into 5 N HCl and extracted with ethyl acetate. The extract was washed with brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated to give a solid, which was recrystallized from a mixture of ethyl acetate and hexane to yield 0.53 g (93%) of **17**; mp 117−118 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 2.67 (t, 2H, J = 7.5), 3.08 (t, 2H, J = 7.5), 3.27 (t, 2H, J = 8.8), 4.68 (t, 2H, J = 8.8), 7.07 (s, 1H), hidden (1H).

4,5-Dibromo-1,2,6,7-tetrahydro-8*H*-indeno[5,4-*b*]furan-8-one (18). Compound **18** was prepared in 88% yield from **17** by a method similar to that described for **9**; mp 224−226 °C (from chloroform/isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 2.72 (t, 2H, J = 5.9), 3.05 (t, 2H, J = 5.9), 3.55 (t, 2H, J = 9.0), 4.79 (t, 2H, J = 9.0).

1,2,6,7-Tetrahydro-8*H*-indeno[5,4-*b*]furan-8-one (19). A solution of **18** (0.349 g, 1.05 mmol) in AcOH (150 mL) was subjected to hydrogenation in the presence of 10% palladium on carbon (0.45 g, containing 50% water) in a hydrogen atmosphere at room temperature for 1 h. After the theoretical volume of hydrogen was absorbed, the catalyst was removed by filtration. The filtrate was concentrated, and the residue

was dissolved in ethyl acetate. This solution was washed with water and saturated aqueous $NaHCO_3$ and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography to yield 0.163 g (89%) of **19**; mp 133−134 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 2.68 (t, 2H, J = 5.9), 3.08 (t, 2H, J = 5.9), 3.47 (t, 2H, J = 8.8), 4.65 (t, 2H, J = 8.8), 7.01 (d, 1H, J = 8.1), 7.21 (d, 1H, J = 8.1). Anal. ($C_{11}H_{10}O_2$) C, H.

2-(1,2,6,7-Tetrahydro-8*H*-indeno[5,4-*b*]furan-8-ylidene-)acetonitrile (20). Compound **20** was prepared in 60% yield from **19** by a method similar to that described for **10**; mp 149−151 °C (from methanol). ^{1}H NMR ($CDCl_3$): δ 3.00−3.20 (m, 4H), 3.31 (t, 2H, J = 8.8), 4.67 (t, 2H, J = 8.8), 5.45 (t, 1H, J = 2.4), 6.86 (d, 1H, J = 8.1), 7.11 (d, 1H, J = 8.1). Anal. ($C_{13}H_{11}$-NO) C, H, N.

2-(1,6,7,8-Tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl)ethylamine (21). Compound **21** was prepared in 81% yield from **20** by a method similar to that described for **11** as an oil. ^{1}H NMR ($CDCl_3$): δ 1.42−2.35 (m, 4H), 2.64−2.98 (m, 4H), 3.01−3.38 (m, 3H), 4.41−4.70 (m, 2H), 6.61 (d, 1H, J = 8.1), 6.95 (d, 1H, J = 8.1), hidden (2H).

***N*-[2-(1,6,7,8-Tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethyl]acetamide (22a).** Acetic anhydride (0.050 mL, 0.528 mmol) was added all at once to a vigorously stirred solution of **21** (71.6 mg, 0.352 mmol) and aqueous 1 N NaOH (1.5 mL) in tetrahydrofuran (1.5 mL) at room temperature. After the mixture was stirred for 30 min at room temperature, ethyl acetate and water were added. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. The filtrate was concentrated to give a solid, which was recrystallized from a mixture of ethyl acetate and hexane to yield 57.0 mg (66%) of **22a**; mp 78−79 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.53−2.12 (m, 3H), 1.96 (s, 3H), 2.20−2.38 (m, 1H), 2.70−2.96 (m, 2H), 3.02−3.40 (m, 5H), 4.45−4.68 (m, 2H), 5.46 (br s, 1H), 6.62 (d, 1H, J = 8.0), 6.96 (d, 1H, J = 8.0). Anal. ($C_{15}H_{19}NO_2$) C, H, N.

***N*-[2-(1,6,7,8-Tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethyl]propionamide (22b).** Compound **22b** was prepared in 78% yield from propionyl chloride and **21** by a method similar to that described for **22a**; mp 102−104 °C (from isopropyl ether/hexane). ^{1}H NMR ($CDCl_3$): δ 1.14 (t, 3H, J = 7.6), 1.55−2.38 (m, 4H), 2.18 (q, 2H, J = 7.6), 2.69−2.99 (m, 2H), 3.02−3.40 (m, 5H), 4.42−4.63 (m, 2H), 5.61 (br s, 1H), 6.62 (d, 1H, J = 7.8), 6.95 (d, 1H, J = 7.8). Anal. ($C_{16}H_{21}NO_2$) C, H, N.

***N*-[2-(1,6,7,8-Tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethyl]butanamide (22c).** Compound **22c** was prepared in 67% yield from butyryl chloride and **21** by a method similar to that described for **22a**; mp 55−57 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 0.94 (t, 3H, J = 7.3), 1.51−1.90 (m, 4H), 1.92−2.08 (m, 1H), 2.12 (t, 2H, J = 7.3), 2.17−2.38 (m, 1H), 2.68−2.98 (m, 2H), 3.00−3.40 (m, 5H), 4.41−4.68 (m, 2H), 5.43 (br s, 1H), 6.62 (d, 1H, J = 8.0), 6.96 (d, 1H, J = 8.0). Anal. ($C_{17}H_{23}NO_2$) C, H, N.

***N*-[2-(5-Bromo-6-methoxy-2,3-dihydro-1*H*-inden-1-yl)-ethyl]propionamide (27).** Compound **27** was prepared in 86% yield from bromine and *N*-[2-(6-methoxy-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (**26**) by a method similar to that described for **15**; mp 105−107 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3H, J = 7.6), 1.49−1.82 (m, 2H), 1.98−2.41 (m, 2H), 2.21 (q, 2H, J = 7.6), 2.71−2.90 (m, 2H), 3.00−3.20 (m, 1H), 3.39 (q, 2H, J = 7.1), 3.88 (s, 3H), 5.50 (br s, 1H), 6.78 (s, 1H), 7.37 (s, 1H). Anal. ($C_{15}H_{20}BrNO_2$) C, H, N.

***N*-[2-(5-Bromo-6-hydroxy-2,3-dihydro-1*H*-inden-1-yl)-ethyl]propionamide (28).** A solution of **27** (26.0 g, 79.7 mmol) in dichloromethane (400 mL) was cooled to −20 °C in a solid CO_2−CCl_4 bath, and BBr_3 (40.1 g, 0.160 mol) was then added slowly by syringe. The reaction was allowed to proceed for 1 h as the cooling bath gradually warmed to room temperature. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with ethyl acetate as eluent to yield 21.4 g (86%) of **28**; mp 149−151 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3H, J = 7.5), 1.50−1.80 (m, 2H), 1.90−2.12 (m, 1H), 2.20−2.40 (m, 1H), 2.24 (q, 2H, J = 7.5), 2.65−2.95 (m, 2H), 3.00−3.18 (m, 1H), 3.38 (q, 2H, J = 7.1), 5.82 (br s, 1H), 6.86 (s, 1H), 7.27 (s, 1H), hidden (1H). Anal. ($C_{14}H_{18}BrNO_2$) C, H, N, Br.

***N*-[2-(5-Bromo-6-(isopropenyloxy)-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (29).** A 60% suspension of sodium hydride in mineral oil (0.648 g, 16.2 mmol) was added slowly to an ice-cooled solution of **28** (4.21 g, 13.5 mmol) in DMF (50 mL). After 30 min, by which time gas evolution had ceased, 3-chloro-2-methylpropane (3.67 g, 40.5 mmol) was added cautiously, and stirring was continued at 0 °C for 90 min. The mixture was made slightly acidic by the careful addition of a few drops of dilute HCl and then partitioned between ethyl acetate and H_2O. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with hexane/ethyl acetate (1:2) as eluent to yield 4.15 g (84%) of **29**; mp 105−108 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3H, J = 7.6), 1.86 (s, 3H), 1.9−2.4 (m, 6H), 2.80 (m, 2H), 3.08 (m, 1H), 3.38 (q, 2H, J = 7.6), 4.47 (s, 2H), 5.00 (s, 1H), 5.17 (s, 1H), 5.40 (br s, 1H), 6.76 (s, 1H), 7.37 (s, 1H). Anal. ($C_{18}H_{24}BrNO_2$) C, H, N, Br.

***N*-[2-(5-Bromo-6-hydroxy-7-(2-methyl-2-propenyl)-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (30).** A suspension of **29** (4.29 g, 11.7 mmol) in *N*,*N*-diethylaniline (30 mL) was heated at 200−205 °C for 2.5 h under an atmosphere of argon. The excess *N*,*N*-diethylaniline was removed in vacuo. The residual oil was taken up in ethyl acetate, washed successively with a brine solution and H_2O, and then dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with hexane/ethyl acetate (2:1) as eluent to yield 3.90 g (91%) of **30**; mp 89−91 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.14 (t, 3H, J = 7.6), 1.40−1.80 (m, 2H), 1.80 (s, 3H), 1.90−2.10 (m, 2H), 2.17 (q, 2H, J = 7.6), 2.60−3.50 (m, 7H), 4.49 (s, 1H), 4.79 (s, 1H), 5.32 (br s, 1H), 5.47 (s, 1H), 7.21 (s, 1H). Anal. ($C_{18}H_{24}BrNO_2$) C, H, N, Br.

***N*-[2-(2,2-Dimethyl-1,6,7,8-tetrahydro-2*H*-indeno[5,4-*b*]-furan-8-yl)ethyl]propionamide (31).** Boron trifluoride diethyl etherate (4.12 mL, 32.5 mmol) was added slowly to an ice-cooled solution of **30** (2.38 g, 6.50 mmol) in dichloromethane (40 mL), and stirring was continued at 0 °C for 3 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with water and saturated aqueous $NaHCO_3$ and dried over anhydrous magnesium sulfate. The filtrate was concentrated to give a solid, which was recrystallized from a mixture of ethyl acetate and isopropyl ether to yield 2.12 g (89%) of *N*-[2-(4-bromo-2,2-dimethyl-1,6,7,8-tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl)ethyl]-propionamide; mp 98−101 °C (from ethyl acetate/isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, J = 7.5), 1.48 (s, 3H), 1.54 (s, 3H), 1.76−2.02 (m, 2H), 2.19 (q, 2H, J = 7.5), 2.25−2.38 (m, 1H), 2.62−3.16 (m, 6H), 3.32 (q, 2H, J = 5.3), 5.41 (br s, 1H), 7.11 (s, 1H). Anal. Calcd for $C_{18}H_{24}BrNO_2$: C, 59.02; H, 6.60; N, 3.82; Br, 21.81. Found: C, 58.94; H, 6.48; N, 3.98; Br, 21.97.

A solution of the propionamide (1.06 g, 2.90 mmol) and Et_3N (0.808 mL, 5.80 mmol) in ethanol (15 mL) was subjected to hydrogenation in the presence of 10% palladium on carbon (0.11 g, containing 50% water) in a hydrogen atmosphere at room temperature for 1 h and 50 °C for 30 min. After hydrogen absorption had ceased, the catalyst was removed by filtration. The filtrate was concentrated and recrystallized from a mixture of ethyl acetate and isopropyl ether to yield 0.635 g (76%) of **31**; mp 69−72 °C (from ethyl acetate/isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 1.14 (s, 3H), 1.43 (s, 3H), 1.50 (s, 3H), 1.60−2.10 (m, 2H), 2.13 (q, 2H, J = 7.5), 2.24−2.40 (m, 1H), 2.60−3.20 (s, 6H), 3.35 (q, 2H, J = 5.3), 5.39 (br s, 1H), 6.55 (d, 1H, J = 7.6), 6.95 (d, 1H, J = 7.6). Anal. ($C_{18}H_{25}NO_2$) C, H, N.

2-(6,7-Dimethoxy-2,3-dihydro-1*H*-inden-1-ylidene)-acetonitrile (33). Compound **33** was prepared in 81% yield

from 6,7-dimethoxy-1-indanone[34] (**32**) and diethyl cyanomethylphosphonate by a method similar to that described for **10**; mp 111−113 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 2.95−3.15 (m, 4H), 3.87 (s, 3H), 3.91 (s, 3H), 6.24 (t, 1H, J = 2.4), 6.95 (d, 1H, J = 8.6), 7.00 (d, 1H, J = 8.6). Anal. ($C_{13}H_{13}NO_2$) C, H, N.

2-(6,7-Dimethoxy-2,3-dihydro-1*H*-inden-1-yl)ethylamine (34). A solution of **33** (20.0 g, 92.9 mmol) in ethanol (760 mL) saturated with NH_3 gas was subjected to hydrogenation in the presence of Raney-cobalt (45 g washed with ethanol two times before use, ODHT-60, available through Kawaken Co.) in a hydrogen atmosphere (480 kPa) at room temperature for 7 h. After hydrogen absorption had ceased, the catalyst was removed by filtration. The filtrate was concentrated to yield 20.4 g (quant) of (*E*)-2-(6,7-dimethoxyindan-1-ylidene)-ethylamine as an oil. (*E*)-2-(6,7-Dimethoxyindan-1-ylidene)-ethylamine (10.0 g, 45.6 mmol) was dissolved in ethanol (100 mL), and the mixture was subjected to hydrogenation in the presence of 10% palladium on carbon (1.0 g, containing 50% water) in a hydrogen atmosphere at room temperature for 2 h. After absorption of the theoretical volume of hydrogen, the catalyst was removed by filtration. The filtrate was concentrated to yield 20.6 g (quant) of **34** as an oil, which was used directly in the next step. This oil was dissolved in ethanol saturated with HCl gas. The resulting solid was collected and recrystallized from ethanol to yield **34**·HCl (78%); mp 141−143 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.59−1.83 (m, 2H), 1.95−2.26 (m, 2H), 2.60−2.94 (m, 4H), 3.21−3.41 (m, 1H), 3.75 (s, 3H), 3.76 (s, 3H), 6.83 (d, 1H, J = 8.4), 6.89 (d, 1H, J = 8.4), 7.99 (br s, 2H). Anal. ($C_{13}H_{19}NO_2$·HCl) C, H, N, Cl.

***N*-[2-(6,7-Dimethoxy-2,3-dihydro-1*H*-inden-1-yl)ethyl]-propionamide (35).** A solution of propionyl chloride (3.89 g, 42.0 mmol) was added dropwise to an ice-cooled solution of **34** (7.15 g, 32.3 mmol) and triethylamine (6.54 g, 64.6 mmol) in tetrahydrofuran (80 mL). After the mixture was stirred for 3 h at room temperature, ethyl acetate and water were added. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with methanol/ethyl acetate (1:99) as eluent to yield 7.72 g (86%) of **35**; mp 90−92 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.14 (t, 3H, J = 7.7), 1.70−1.94 (m, 3H), 2.10−2.36 (m, 1H), 2.18 (q, 2H, J = 7.7), 2.65−3.20 (m, 3H), 3.25−3.55 (m, 2H), 3.85 (s, 3H), 3.87 (s, 3H), 5.90 (br s, 1H), 6.75 (d, 1H, J = 8.0), 6.90 (d, 1H, J = 8.0). Anal. ($C_{16}H_{23}NO_3$) C, H, N.

***N*-[2-(6,7-Dihydroxy-2,3-dihydro-1*H*-inden-1-yl)ethyl]-propionamide (36).** Compound **36** was prepared in 73% yield from **35** and BBr_3 by a method similar to that described for **28**; mp 98−101 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.21 (t, 3H, J = 7.5), 1.60−1.98 (m, 3H), 2.10−2.30 (m, 1H), 2.31 (q, 2H, J = 7.5), 2.60−3.15 (m, 3H), 3.22−3.40 (m, 1H), 3.52−3.75 (m, 1H), 5.95 (s, 1H), 6.01 (br s, 1H), 6.63 (d, 1H, J = 7.9), 6.74 (d, 1H, J = 7.9), 9.62 (s, 1H). Anal. ($C_{14}H_{19}NO_3$) C, H, N.

***N*-[2-(7,8-Dihydro-6*H*-indeno[4,5-*d*][1,3]dioxol-8-yl)ethyl]propionamide (37).** A 60% suspension of sodium hydride in mineral oil (0.300 g, 7.50 mmol) was added to an ice-cooled solution of hexamethyl phosphoramide (5 mL). This mixture was stirred for 10 min, and then, a solution of **36** (0.850 g, 3.41 mmol) in hexamethyl phosphoramide (5 mL) was added dropwise at room temperature over a period of 6 min. After gas evolution had subsided, diiodomethane (1.10 g, 4.10 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 2 h. Then, the mixture was made slightly acidic by the careful addition of a few drops of dilute HCl and partitioned between ethyl acetate and H_2O. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with ethyl acetate as eluent to yield 280 mg (31%) of **37**; mp 102−104 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3H, J = 7.7), 1.70−1.89 (m, 2H), 1.90−2.10 (m, 1H), 2.15−2.40 (m, 1H), 2.20 (q, 2H, J = 7.7), 2.68−3.00 (m, 2H), 3.13−3.36 (m, 2H), 3.40−3.59 (m, 1H), 3.68 (br s, 1H), 5.92 (dd, 2H, J = 1.5, 9.9), 6.67 (s, 2H). Anal. ($C_{15}H_{19}NO_3$) C, H, N.

6-Methoxy-7-nitro-1-indanone (38). A solution of potassium nitrate (24.3 g, 0.240 mol) in concentrated sulfuric acid (100 mL) was added slowly to a solution of **23** (30.0 g, 0.185 mol) in concentrated sulfuric acid (130 mL) at −10 °C. The reaction mixture was stirred at −10 °C for 20 min. It was then poured into ice-water and extracted with ethyl acetate. The organic layer was washed with water and saturated aqueous $NaHCO_3$ and dried over anhydrous magnesium sulfate. The filtrate was concentrated to give a solid, which was recrystallized from a mixture of ethyl acetate and hexane to yield 22.3 g (58%) of **38**; mp 155−158 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 2.78 (t, 2H, J = 5.6), 3.13 (t, 2H, J = 5.6), 3.94 (s, 3H), 7.34 (d, 1H, J = 8.4), 7.56 (d, 1H, J = 8.4). Anal. ($C_{10}H_9NO_4$) C, H, N.

2-(6-Methoxy-7-nitro-2,3-dihydro-1*H*-inden-1-ylidene)-acetonitrile (39). Compound **39** was prepared in 84% yield from **38** and diethyl cyanomethylphosphonate by a method similar to that described for **10**; mp 138−141 °C (from ethyl acetate/isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 3.00−3.20 (m, 4H), 3.92 (s, 3H), 5.42 (t, 1H, J = 2.6), 7.14 (d, 1H, J = 8.6), 7.43 (d, 1H, J = 8.6). Anal. ($C_{12}H_{10}N_2O_3$) C, H, N.

2-(7-Amino-6-methoxy-2,3-dihydro-1*H*-inden-1-ylidene)-acetonitrile (40). Compound **40** was prepared in 79% yield from **39** by a method similar to that described for **14**; mp 119−121 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 2.90−3.20 (m, 4H), 3.87 (s, 3H), 4.23 (br s, 2H), 5.60 (t, 1H, J = 2.2), 6.69 (d, 1H, J = 8.0), 6.84 (d, 1H, J = 8.0).

***N*-[2-(7-Amino-6-methoxy-2,3-dihydro-1*H*-inden-1-yl)-ethyl]propionamide (41).** 3-(2-Aminoethyl)-5-methoxy-2,3-dihydro-1*H*-inden-4-ylamine was prepared from **40** and Raney-nickel by a method similar to that described for **11**, which was used directly in the next step. Propionic acid (0.852 g, 11.5 mmol) was added slowly to an ice-cooled suspension of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (3.30 g, 17.2 mmol) and 1-hydroxybenzotriazole monohydrate (2.21 g, 14.4 mmol). The mixture was stirred at room temperature for 1 h and then recooled to 0 °C. A solution of 3-(2-aminoethyl)-5-methoxy-2,3-dihydro-1*H*-inden-4-ylamine (2.00 g, 9.70 mmol) in DMF (10 mL) was added, and the reaction temperature was raised to room temperature. After 30 min, the reaction mixture was poured into water and extracted with ethyl acetate. The extract was taken up in dilute HCl, and the water layer was basified with a 4 N aqueous solution of sodium hydroxide to pH 10. The mixture was then extracted several times with ethyl acetate. The combined extracts were dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with ethanol/ethyl acetate (1:10) as eluent to yield 1.02 g (40%) of **41**; mp 71−73 °C (from ethyl acetate/isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 1.09 (t, 3H, J = 7.5), 1.6−2.0 (m, 3H), 2.12 (q, 2H, J = 7.5), 2.25 (m, 1H), 2.7−3.2 (m, 3H), 3.34 (q, 2H, J = 5.0), 3.80 (br s, 2H), 3.83 (s, 3H), 5.67 (br s, 1H), 6.59 (d, 1H, J = 8.0), 6.66 (d, 1H, J = 8.0).

***N*-[2-(7-Amino-6-hydroxy-2,3-dihydro-1*H*-inden-1-yl)-ethyl]propionamide (42).** Compound **42** was prepared in 88% yield from **41** and BBr_3 by a method similar to that described for **28** as an oil. ^{1}H NMR ($CDCl_3$): δ 1.11 (t, 3H, J = 7.5), 1.60−2.00 (m, 3H), 2.14 (q, 2H, J = 7.5), 2.23 (m, 1H), 2.70−2.90 (m, 2H), 3.19 (m, 1H), 3.34 (q, 2H, J = 5.1), 4.10 (br s, 2H), 5.69 (br s, 1H), 6.52 (d, 1H, J = 7.6), 6.60 (d, 1H, J = 7.6), hidden (1H).

***N*-[2-(7,8-Dihydro-6*H*-indeno[4,5-*d*][1,3]oxazol-8-yl)ethyl]propionamide (43).** A solution of methyl orthoformate (7.36 mL, 67.3 mmol) and methanol (1.4 mL) saturated with HCl gas was added dropwise to an ice-cooled solution of **42** (670 mg, 2.70 mmol) in methanol (5 mL). The mixture was stirred at room temperature for 30 min and then at 60 °C for 1 h. The reaction mixture was poured into ice-water and extracted with chloroform. The organic layer was washed with water and saturated aqueous $NaHCO_3$ and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with

chloroform/methanol (20:1) as eluent to yield 425 mg (61%) of **43**; mp 81−84 °C (from ethyl acetate/isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3H, J = 7.5), 1.80−2.10 (m, 3H), 2.27 (q, 2H, J = 7.5), 2.37−2.53 (m, 1H), 2.80−3.20 (m, 3H), 3.55−3.80 (m, 2H), 6.93 (br s, 1H), 7.25 (d, 1H, J = 8.8), 7.40 (d, 1H, J = 8.8), 8.09 (s, 1H). Anal. ($C_{15}H_{18}N_2O_2$) C, H, N.

***N*-[2-(5-Bromo-6-(2-propynyloxy)-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (44).** Compound **44** was prepared in 99% yield from **28** and propargyl bromide by a method similar to that described for **29**; mp 104−107 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3H, J = 7.6), 1.50−2.40 (m, 6H), 2.55 (t, 1H, J = 2.3), 2.7−3.2 (m, 3H), 3.38 (t, 2H, J = 7.6), 4.76 (d, 2H, J = 2.3), 5.48 (br s, 1H), 6.93 (s, 1H), 7.38 (s, 1H). Anal. ($C_{17}H_{20}BrNO_2$) C, H, N, Br.

***N*-[2-(5-Bromo-3,7,8,9-tetrahydrocyclopenta[*f*]chromen-9-yl)ethyl]propionamide (45).** A mixture of **44** (2.94 g, 8.40 mmol) in bromobenzene (30 mL) was heated at 200 °C for 18 h in a stainless steel bomb. The reaction mixture was cooled to room temperature, and the solvent was evaporated to dryness in vacuo. The residue was purified by column chromatography with ethyl acetate as eluent to yield 2.59 g (88%) of **45**; mp 110−111 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.14 (t, 3H, J = 7.5), 1.50−2.50 (m, 5H), 2.60−3.10 (m, 3H), 3.15−3.25 (m, 1H), 3.32 (q, 2H, J = 7.5), 4.80−4.90 (m, 2H), 5.40 (br s, 1H), 5.88 (dt, 1H, J = 10, 3.8), 6.45 (dd, 1H, J = 1.6, 9.8), 7.18 (s, 1H). Anal. ($C_{17}H_{20}BrNO_2$) C, H, N, Br.

***N*-[2-(1,2,3,7,8,9-Hexahydrocyclopenta[*f*]chromen-9-yl)ethyl]propionamide (46).** A solution of **45** (210 mg, 0.600 mmol) in ethanol (5 mL) was subjected to hydrogenation in the presence of 10% palladium on carbon (200 mg, containing 50% water) in a hydrogen atmosphere at room temperature for 3 h. After hydrogen absorption had ceased, the catalyst was removed by filtration. The filtrate was concentrated, and the residue was purified by column chromatography with hexane/ethyl acetate (1:1) as eluent to yield 139 mg (85%) of **46**; mp 85−88 °C (from ethyl acetate/isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3H, J = 7.6), 1.80−2.10 (m, 6H), 2.15 (q, 2H, J = 7.6), 2.60−3.50 (m, 7H), 4.00−4.30 (m, 2H), 5.35 (br s, 1H), 6.63 (d, 1H, J = 8.2), 6.94 (d, 1H, J = 8.2). Anal. ($C_{17}H_{23}NO_2$) C, H, N.

6-Hydroxy-7-nitro-1-indanone (48). Compound **48** was prepared in 61% yield from **47** and potassium nitrate by a method similar to that described for **38**; mp 218−220 °C (from ethanol/hexane). ^{1}H NMR (CD_3OD): δ 2.37 (t, 2H, J = 5.5), 2.74 (t, 2H, J = 5.5), 2.95 (s, 1H), 6.95 (d, 1H, J = 8.4), 7.15 (d, 1H, J = 8.4).

Ethyl 2-[(4-Nitro-3-oxo-2,3-dihydro-1*H*-inden-5-yl)oxy]-acetate (49). Compound **49** was prepared in 94% yield from **48** and ethyl bromoacetate by a method similar to that described for **29**; mp 137−139 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.29 (t, 3H, J = 7.1), 2.79 (t, 2H, J = 6.0), 3.14 (t, 2H, J = 6.0), 4.25 (q, 2H, J = 7.1), 4.74 (s, 2H), 7.25 (d, 1H, J = 8.4), 7.55 (d, 1H, J = 8.4).

Ethyl 2-[(4-Amino-3-oxo-2,3-dihydro-1*H*-inden-5-yl)-oxy]acetate (50). Compound **50** was prepared in 98% yield from **49** by a method similar to that described for **14** and used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$): δ 1.29 (t, 3H, J = 7.1), 2.30−3.00 (m, 4H), 4.28 (q, 2H, J = 7.1), 4.61 (s, 2H), 5.89 (br s, 2H), 6.53 (d, 1H, J = 8.2), 6.87 (d, 1H, J = 8.2).

7,8-Dihydroindeno[5,4-*b*][1,4]oxazine-2,9(1*H*,3*H*)-dione (51). A mixture of **50** (8.70 g, 34.9 mmol) and potassium *tert*-butoxide (404 mg, 3.60 mmol) in toluene (200 mL) was heated at reflux for 12 h under an atmosphere of argon. The reaction mixture was cooled to room temperature and poured into water. It was then made slightly acidic by careful addition of a few drops of dilute HCl and partitioned between ethyl acetate and H_2O. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with ethyl acetate/hexane (1:1) as eluent to yield 4.8 g (66%) of **51**; mp 136−139 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 2.74 (t, 2H, J = 5.8), 3.10 (t, 2H, J = 5.8), 4.68 (s, 2H), 7.01 (d, 1H, J = 7.2), 7.17 (d, 1H, J = 7.2), 9.52 (br s, 1H).

2-(2-Oxo-2,3,7,8-tetrahydroindeno[5,4-*b*][1,4]oxazin-9(1*H*)-ylidene)acetonitrile (52). Compound **52** was prepared in 86% yield from **51** and diethyl cyanomethylphosphonate by a method similar to that described for **10**; mp 158−161 °C (from chloroform). ^{1}H NMR ($CDCl_3$): δ 3.00−3.20 (m, 4H), 4.62 (s, 2H), 5.62 (t, 1H, J = 2.3), 6.97 (d, 1H, J = 8.2), 7.06 (d, 1H, J = 8.2), 8.07 (br s, 1H). Anal. ($C_{13}H_{10}N_2O_2$) C, H, N.

9-(2-Aminoethyl)-1,7,8,9-tetrahydroindeno[5,4-*b*][1,4]-oxazin-2(3*H*)-one (53). A solution of **52** (3.00 g, 13.3 mmol) in ethanol (300 mL) saturated with NH_3 gas was subjected to hydrogenation in the presence of Raney-nickel (14 g washed with ethanol two times before use) in a hydrogen atmosphere (480 kPa) at 40 °C for 6 h. After hydrogen absorption had ceased, the catalyst was removed by filtration and the filtrate was concentrated. The residue was taken up into 50 mL of ethyl acetate, and the product was extracted with 50 mL of 2 N HCl. The aqueous extracts were combined and rendered strongly basic with the addition of a 4 N aqueous solution of sodium hydroxide to pH 10. The free base was then extracted several times with chloroform/methanol (10:1). The combined extracts were dried over anhydrous magnesium sulfate. The filtrate was concentrated to give a solid, which was recrystallized from a mixture of ethyl acetate and isopropyl ether to yield 1.95 g (62%) of **53**; mp 128−134 °C (from ethyl acetate/isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 1.40−1.90 (m, 6H), 2.20−2.50 (m, 2H), 2.70 (dd, 1H, J = 8.0, 15.4), 2.90−3.00 (m, 2H), 3.40 (q, 1H, J = 7.9), 4.44 (d, 1H, J = 15.0), 4.58 (d, 1H, J = 15.0), 6.75 (d, 1H, J = 8.0), 6.79 (d, 1H, J = 8.0). Anal. ($C_{13}H_{16}N_2O_2 \cdot 0.25H_2O$) C, H, N.

2-(1,2,3,7,8,9-Hexahydroindeno[5,4-*b*][1,4]oxazin-9-yl)-ethylamine (54). A solution of **53** (1.23 g, 5.30 mmol) was dissolved in dry tetrahydrofuran (30 mL) and added dropwise to a stirring ice-cooled suspension of $LiAlH_4$ (0.812 g, 21.4 mmol) in tetrahydrofuran (5 mL). After the addition was complete, the mixture was heated at reflux for 18 h under an atmosphere of argon. The mixture was then cooled in an ice bath, and the excess $LiAlH_4$ was decomposed by the careful addition of water (4 mL). The mixture was filtered through a Celite pad, the filter cake was rinsed well with ethyl acetate, and the solvent was removed by rotary vacuum evaporation. The residue was taken up into 50 mL of ethyl acetate, and the product was extracted with 50 mL of 2 N HCl. The aqueous extracts were combined and rendered strongly basic with the addition of a 4 N aqueous solution of sodium hydroxide to pH 10. The free base was then extracted several times with chloroform/methanol (10:1). The combined extracts were dried over anhydrous magnesium sulfate. The filtrate was concentrated to yield 0.93 g (80%) of **54** as an oil, which was used directly in the next step. ^{1}H NMR ($CDCl_3$): δ 1.10−3.20 (m, 12H), 3.41 (m, 2H), 4.20 (m, 2H), 6.49 (d, 1H, J = 8.0), 6.61 (d, 1H, J = 8.0).

***N*-[2-(1,2,3,7,8,9-Hexahydroindeno[5,4-*b*][1,4]oxazin-9-yl)ethyl]propionamide (55).** Propionic acid (0.134 mL, 1.80 mmol) was added slowly to an ice-cooled suspension of WSC (0.518 g, 2.70 mmol) and HOBt (0.352 g, 2.30 mmol). The mixture was stirred at room temperature for 1 h and then recooled to 0 °C. A solution of **54** (0.393 g, 1.80 mmol) in DMF (15 mL) was added, and the reaction temperature was raised to room temperature. After 30 min, the reaction mixture was poured into water and extracted with ethyl acetate. The combined extracts were dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with ethyl acetate/ethanol (10:1) as eluent to yield 252 mg (51%) of **55**; mp 80−83 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.11 (t, 3H, J = 7.5), 1.50−2.30 (m, 6H), 2.60−3.20 (m, 3H), 3.32 (q, 2H, J = 6.7), 3.43 (t, 2H, J = 4.4), 3.85 (br s, 1H), 4.20 (t, 2H, J = 4.4), 5.84 (br s, 1H), 6.50 (d, 1H, J = 8.0), 6.62 (d, 1H, J = 8.0). Anal. ($C_{16}H_{22}N_2O_2$) C, H, N.

***N*-[2-(2-Oxo-1,2,3,7,8,9-hexahydroindeno[5,4-*b*][1,4]-oxazin-9-yl)ethyl]propionamide (56).** Compound **56** was

prepared in 88% yield from **53** and propionic acid by a method similar to that described for **55**; mp 216−219 °C (from ethyl acetate/methanol). ^{1}H NMR ($CDCl_3$): δ 1.18 (d, 3H, J = 7.5), 1.50−2.00 (m, 3H), 2.10−2.30 (m, 3H), 2.70−3.10 (m, 2H), 3.30−3.50 (m, 3H), 4.59 (s, 2H), 5.97 (br s, 1H), 6.81 (s, 2H), 9.77 (br s, 1H). Anal. ($C_{16}H_{20}N_2O_3$) C, H, N.

***N*-[2-(2,3,8,9-Tetrahydro-7*H*-indeno[4,5-*b*][1,4]dioxin-9-yl)ethyl]propionamide (57).** A mixture of **36** (1.00 g, 4.01 mmol), dibromoethane (2.90 g, 15.4 mmol), potassium carbonate (1.63 g, 11.8 mmol), and copper(II) oxide (31.8 mg, 0.400 mmol) in DMF (15 mL) was heated at 140 °C for 6 h. The mixture was cooled to room temperature and poured into water. Then it was made slightly acidic by the careful addition of a few drops of dilute HCl and partitioned between ethyl acetate and H_2O. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with ethyl acetate as eluent to yield 718 mg (65%) of **57**; mp 120−122 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, J = 7.5), 1.60−2.00 (m, 3H), 2.10−2.32 (m, 1H), 2.19 (q, 2H, J = 7.5), 2.61−3.01 (m, 2H), 3.08−3.53 (m, 3H), 4.25 (br s, 4H), 5.67 (br s, 1H), 6.69 (s, 2H). Anal. ($C_{16}H_{21}NO_3$) C, H, N.

(*S*)-*N*-[2-(5-Bromo-6-methoxy-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (61). Compound **61** was prepared in 86% yield from **60** and bromine by a method similar to that described for **15**; mp 105−107 °C (from ethyl acetate); $[\alpha]_D^{20}$ +5.2° (*c* 1.000, ethanol). ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3H, J = 7.7), 1.49−1.81 (m, 2H), 1.98−2.41 (m, 2H), 2.21 (q, 2H, J = 7.7), 2.69−2.98 (m, 2H), 3.00−3.20 (m, 1H), 3.39 (q, 2H, J = 7.3), 3.88 (s, 3H), 5.48 (br s, 1H), 6.78 (s, 1H), 7.37 (s, 1H). Anal. ($C_{15}H_{20}BrNO_2$) C, H, N.

(*S*)-*N*-[2-(5-Bromo-6-hydroxy-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (62). A solution of **61** (56.7 g, 174 mmol) in dichloromethane (400 mL) was cooled to −30 °C in a solid CO_2−acetone bath. BBr_3 (95.8 g, 382 mmol) was then added slowly by syringe. The reaction was stirred at −20 to −15 °C for 30 min. The reaction mixture was poured into ice-water and stirred for a further 10 min at room temperature. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with ethyl acetate as eluent to yield 51.1 g (94%) of **62**; mp 146−148 °C (from ethyl acetate); $[\alpha]_D^{20}$ +2.7° (*c* 1.001, ethanol). ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3H, J = 7.5), 1.50−1.80 (m, 2H), 1.90−2.12 (m, 1H), 2.20−2.40 (m, 1H), 2.24 (q, 2H, J = 7.5), 2.65−2.95 (m, 2H), 3.00−3.18 (m, 1H), 3.38 (q, 2H, J = 7.1), 5.82 (br s, 1H), 6.86 (s, 1H), 7.27 (s, 1H), hidden (1H). Anal. ($C_{14}H_{18}BrNO_2$) C, H, N.

(*S*)-*N*-[2-(6-Allyloxy-5-bromo-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (63). Compound **63** was prepared in 96% yield from **62** and allyl bromide by a method similar to that described for **29**; mp 86−87 °C (from ethyl acetate/hexane); $[\alpha]_D^{20}$ +3.7° (*c* 1.003, ethanol). ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3H, J = 7.5), 1.48−1.80 (m, 2H), 1.90−2.40 (m, 2H), 2.20 (q, 2H, J = 7.5), 2.70−2.91 (m, 2H), 3.00−3.20 (m, 1H), 3.37 (q, 2H, J = 7.4), 4.59 (m, 2H), 5.25−5.60 (m, 3H), 5.97−6.20 (m, 1H), 6.76 (s, 1H), 7.37 (s, 1H). Anal. ($C_{17}H_{22}BrNO_2$) C, H, N.

(*S*)-*N*-[2-(7-Allyl-5-bromo-6-hydroxy-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (64). Compound **64** was prepared in 80% yield from **63** and *N*,*N*-diethylaniline by a method similar to that described for **30**; mp 85−87 °C (from ethyl acetate/hexane); $[\alpha]_D^{20}$ −51.3° (*c* 1.003, ethanol). ^{1}H NMR ($CDCl_3$): δ 1.14 (t, 3H, J = 7.6), 1.45−2.13 (m, 4H), 2.18 (q, 2H, J = 7.6), 2.68−3.65 (m, 7H), 4.93−5.13 (m, 2H), 5.41 (br s, 1H), 5.49 (s, 1H), 5.89−6.10 (m, 1H), 7.20 (s, 1H). Anal. ($C_{17}H_{22}BrNO_2$) C, H, N, Br.

(*S*)-*N*-[2-(5-Bromo-6-hydroxy-7-(2-hydroxyethyl)-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (65). A stream of O_3 was bubbled through a solution of **64** (588 mg, 1.67 mmol) in methanol (30 mL), which was cooled to −78 °C until a blue color persisted. The excess ozone was removed by first purging with oxygen and then with nitrogen. Sodium borohydride (510 mg, 13.4 mmol) was added at about −70 °C to decompose ozonide, and the mixture was allowed to warm to room temperature with stirring for 1 h. Then, the mixture was made slightly acidic by the careful addition of a few drops of dilute HCl and partitioned between ethyl acetate/butanol (1:1) and H_2O. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The filtrate was concentrated to yield 0.589 g (99%) of **65**, which was used directly in the next step. The analytical sample was obtained by recrystallization from a mixture of ethyl acetate and methanol; mp 85−87 °C (from ethyl acetate/methanol); $[\alpha]_D^{20}$ −43.7° (*c* 1.002, ethanol). ^{1}H NMR ($CDCl_3$): δ 1.13 (t, 3H, J = 7.5), 1.40−2.10 (m, 4H), 2.17 (q, 2H, J = 7.5), 2.62−3.01 (m, 4H), 3.07−3.22 (m, 1H), 3.28 (q, 2H, J = 6.8), 3.89 (br s, 2H), 5.47 (t, 1H, J = 3.7), 6.31 (br s, 1H), 7.20 (s, 1H), 9.07 (s, 1H). Anal. ($C_{16}H_{22}BrNO_3$) C, H, N, Br.

(*S*)-*N*-[2-(6-Hydroxy-7-(2-hydroxyethyl)-2,3-dihydro-1*H*-inden-1-yl)ethyl]propionamide (66). A mixture of **65** (590 mg, 1.66 mmol) and triethylamine (184 mg, 1.82 mmol) in methanol (5 mL) was subjected to hydrogenation in the presence of 10% palladium on carbon (100 mg, containing 50% water) in a hydrogen atmosphere at room temperature. After absorption of the theoretical volume of hydrogen, the catalyst was removed by filtration. Then, the mixture was made slightly acidic by the addition of a few drops of dilute HCl and partitioned between ethyl acetate/butanol (1:1) and H_2O. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The filtrate was concentrated to yield 0.419 g (91%) of **66**, which was used directly in the next step. The analytical sample was obtained by recrystallization from a mixture of ethyl acetate and methanol; mp 144−146 °C (from ethyl acetate/hexane); $[\alpha]_D^{20}$ −69.7° (*c* 1.002, ethanol). ^{1}H NMR ($CDCl_3$): δ 1.12 (t, 3H, J = 7.7), 1.45−2.10 (m, 4H), 2.16 (q, 2H, J = 7.7), 2.60−3.00 (m, 4H), 3.10−3.23 (m, 1H), 3.29 (q, 2H, J = 6.8), 3.86 (q, 2H, J = 5.5), 5.00 (t, 1H, J = 4.4), 6.41 (br s, 1H), 6.69 (d, 1H, J = 7.9), 6.91 (d, 1H, J = 7.9), 8.86 (s, 1H). Anal. ($C_{16}H_{23}NO_3$) C, H, N.

(*S*)-*N*-[2-(1,6,7,8-Tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl)ethyl]propionamide ((*S*)-(−)-22b). Methanesulfonyl chloride (1.39 mL, 18.0 mmol) was added dropwise to a stirred solution of the alcohol **66** (4.99 g, 18.0 mmol) in pyridine (14.6 mL, 180 mmol) at about −10 °C. After the mixture was stirred for 25 min at −10 to −5 °C, an additional quantity of methanesulfonyl chloride (0.697 mL, 9.00 mmol) was added and the solution was stirred at −10 to −5 °C for another 25 min. A mixture of ethyl acetate (10 mL) and saturated aqueous $NaHCO_3$ (10 mL) was added slowly to the above solution at 0 °C. The solution was allowed to warm to room temperature and stirred for an additional 30 min. It was poured into water and made slightly acidic by the careful addition of dilute HCl and then partitioned between ethyl acetate and H_2O. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The filtrate was concentrated, and the residue was purified by column chromatography with ethyl acetate as eluent to yield 4.02 g (86%) of **(*S*)-(−)-22b**; mp 113−115 °C (from ethyl acetate); $[\alpha]_D^{20}$ −57.8° (*c* 1.004, chloroform). ^{1}H NMR ($CDCl_3$): δ 1.14 (t, 3H, J = 7.7), 1.52−2.40 (m, 4H), 2.17 (q, 2H, J = 7.7), 2.69−3.00 (m, 2H), 3.01−3.40 (m, 5H), 4.42−4.64 (m, 2H), 5.40 (br s, 1H), 6.62 (d, 1H, J = 7.7), 6.95 (d, 1H, J = 7.7). Anal. ($C_{16}H_{21}NO_2$) C, H, N.

Separation of Enantiomers by Chiral High-Performance Liquid Chromatography (HPLC). The racemic mixture **22b** was optically resolved by high-performance column chromatography (apparatus, LC Module 1 (Nippon Millipore Ltd.); column, Ceramospher RU-1 (10 mm (id) × 250 mm, Shiseido); mobile phase, methanol; flow rate, 4.4 mL/min; column temperature, 50 °C; sample concentration, 17% (w/v); amount injected, 8.5 mg). Removal of the solvent gave the separate enantiomers, each of which was then examined by analytical chiral HPLC for purity. **(*R*)-(+)-22b**: $[\alpha]_D^{20}$ +57.8° (*c* 1.005, chloroform). Anal. ($C_{16}H_{21}NO_2$) C, H, N.

Docking Study. The three-dimensional (3D) model of the human melatonin receptor (MT_1) was constructed by comparative modeling techniques from the crystal structure of bovine

rhodopsin (Protein Data Bank access number 1F88).[31] Among the loop regions, only the second extracellular loop was included in the model, which is involved in ligand binding according to chimera receptor experiments.[33] In the initial model, the second extracellular loop occludes the ligand binding pocket and should be drastically moved to make favorable interactions with ligands. We applied a simulated annealing procedure with van der Waals parameter scaling to change the conformation of the loop. Comparative modeling was carried out on a SiliconGraphics O^2(C) workstation (Silicon Graphics Inc., Mountain View, CA) with the InsightII/Discover program package (Molecular Simulations Inc., San Diego, CA). The binding mode was analyzed by the program DOCK (version 4.01, UCSF, Kuntz, et al.) using standard parameters.

B. Pharmacology. Preparation of CHO Membranes for Receptor Binding Assays. cDNA encoding the human MT_1 gene was introduced into CHO cells. A cell line stably expressing the MT_1 receptor (CHO-hMelR7) was selected and cultured in Eagle's Minimum Essential Medium-α (MEM-α) supplemented with 10% dialyzed fetal bovine serum (dFBS) in a 5% CO_2/95% air atmosphere. Cells were harvested at confluence in $Ca^{2+}-Mg^{2+}$-free Hanks' balanced salt solution containing 5 mM EDTA and collected by centrifugation. Cells were homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C), washed twice, pelleted, and stored at −30 °C until binding assays.

The day before transfection, CHO cells were grown in MEM-α supplemented with 10% dFBS until the cells were 80% confluent. The cells were transfected with pCMV-human MT_2 melatonin receptor expression vector by using Lipofectamine reagent (Invitrogen). Two days after the transfection, the cells were harvested in $Ca^{2+}-Mg^{2+}$-free Hanks' balanced salt solution containing 5 mM EDTA and collected by centrifugation. Cells were homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C), washed twice, pelleted, and stored at −30 °C until binding assays.

Affinity for MT_1 and MT_2 Melatonin Receptor. The procedure of this binding assay was based on the method of Rivkees et al. with minor modifications.[35] 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-hMelR7 homogenate, 10 μL of DMSO solution of a test compound was mixed with homogenate and 40 (MT_1) or 200 pM (MT_2) 2-[^{125}I]iodomelatonin in a total volume of 1 mL and incubated at 25 °C for 150 min. The reaction was terminated by addition of 3 mL of ice-cold buffer followed by vacuum filtration on Whatman GF/B. The filter was further washed twice with 3 mL of ice-cold buffer, and the radioactivity was measured by a γ-counter. Nonspecific binding was defined in the presence of 10 μM melatonin. The 50% inhibitory concentration (IC_{50}) was calculated using log-probit analysis. 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]melatonin in the binding assay, respectively.

Affinity for Low Affinity Melatonin Binding Sites (MT_3 Site and Others) in Syrian Hamster Brain and Peripheral Organs. The procedure of this binding assay was based on the method of Niles et al.[36] with minor modifications. The specific binding of 2-[^{125}I]iodomelatonin to the hamster MT_3 receptor was saturable. The brain, liver, kidney, and spleen were dissected from male Syrian hamsters (7−8 weeks old) and homogenized in 50 mM Tris-HCl (pH 7.4 at 4 °C). After the removal of connective tissues by filtration using a quadruple layer of cotton gauze, the homogenate was centrifuged at 48 000*g* for 10 min. The resultant pellet was washed twice with ice-cold buffer by resuspension and recentrifugation, and the final pellet was suspended in the same buffer. An aliquot of the homogenate was incubated with 200 pM 2-[^{125}I]-iodomelatonin and a test compound at 4 °C for 60 min in a total volume of 200 μL. The reaction was terminated by addition of 3 mL of ice-cooled assay buffer followed by rapid vacuum filtration on Whatman GF/B. The filter was washed a further two times with 3 mL of ice-cooled buffer and placed in a polystylene tube. The radioactivity was measured with a γ-counter. Nonspecific binding was determined in the presence of 10^{-4} M melatonin. The IC_{50} value was calculated by log-probit analysis.

Inhibition of Forskolin-Induced cAMP Formation in CHO Cells Expressing Human Melatonin Receptor (MT_1 Site). CHO cells transfected with the human melatonin receptor (MT_1) gene were plated in MEM-α supplemented with 10% dFBS at a density of 5×10^4 cells/well (1.3×10^4 cells/cm^2) on Iwaki 12 well plates and cultured for 3 days. Cells were washed twice with 1 mL of modified Hanks' balanced salt solution (with 10 mM HEPES−Na, pH 7.3, without $NaHCO_3$) and then 0.5 mL of the same solution containing 100 μM 3-isobutyl-1-methyl xanthine (IBMX), and a test compound was added. After the cells were preincubated for 6 min at 37 °C, 10 μL of forskolin (final concentration of 1 μM) was added and the cells were incubated further for 15 min. The reaction was terminated by the addition of 50 μL of 55% perchloric acid. An aliquot (200 μL) of the reaction mixture was transferred to a test tube, neutralized with 100 μL of 1.6 M NaOH solution, and assayed for cAMP concentration using an Amersham cAMP radioimmunoassay system. The IC_{50} value was caluculated by log-probit analysis.

Effects of (*S*)-(−)-22b on Sleep and Wakefulness in Cats. Eight adult cats (three males and five females) weighing 2.5−5.5 kg at the time of surgery were used. The cats were housed individually in a room maintained at 24 ± 1 °C with a 12 h light/dark cycle (light on 7:00 a.m.). They were fed once daily (9:00 a.m.), and water was available ad libitum. On the day of the experiment, however, they were fed after completion of the experiment. Cats were mounted on a stereotaxic apparatus under pentobarbital (40 mg/kg, ip) anesthesia. Electrodes were implanted bilaterally in the frontal and parietal cortices and hippocampus according to the cat brain atlas of Snider and Niemer.[37] As cortical electrodes, stainless steel screws were used. The depth bipolar recording electrode was made up of twisted stainless steel wires (0.3 mm in diameter) insulated except at the tips (0.5 mm). Ocular movement was recorded from stainless steel screws fixed over the bony orbit. The effect of (*S*)-(−)-**22b** on sleep was investigated by electroencephalogram (EEG) recording. The cats were allowed to recover from surgery for at least 7 days before habituation to the test chamber and EEG recording. The test chamber (650 cm × 35 cm × 45 cm) was constructed entirely of metal except for one Plexiglass wall and was located in a constantly illuminated, ventilated, sound-proof, and electrically shielded room. The cats were well-accustomed to the test chamber and EEG recording before the test. A cat was transferred to the test chamber in the experimental room, and the cables were attached. EEGs, electrooculograms (EOG), and electromyograms (EMG) were recorded for 9 h (1 h before to 8 h after treatment with vehicle, (*S*)-(−)-**22b** or melatonin). During the recording, cats were observed from outside the experimental room on a video monitor and their behavioral and postural changes were recorded continuously throughout the experiment. Each potential amplified and filtered with a polygraph (Nihondenki-Sanei) was recorded by a magnetic pen recorder. An EEG power spectral analysis was also performed continuously using a fast Fourier transform (FFT) system equipped with a personal computer (NEC, PC-9801) and recorded on a MO disk. The state of the cat was classified into three stages based on the following criteria: wakefulness (marked tonic EMG activity, low-voltage fast cortical EEG with a low power spectrum of δ-waves, a regular hippocampal θ-rhythm, and slow EOG activity); SWS (markedly reduced EMG activity, spindles and slow waves of high-voltage cortical EEG with a high power spectrum of δ-waves, and reduced EOG activity); REM (an almost complete absence of EMG activity, low-voltage fast cortical EEG with a low power spectrum of δ-waves, an extremely regular hippocampal θ-rhythm, and a frequently observed high-voltage EOG caused

by REM). (*S*)-(−)-**22b** and melatonin were suspended in 0.5% methyl cellulose (MC). Each cat was administered orally with a capsule containing the (*S*)-(−)-**22b** or melatonin solution. In the control trial, the cat was given a capsule containing vehicle. Each dose of (*S*)-(−)-**22b** or melatonin was compared with the counterbalanced control trial conducted before or after the drug trial (dependent sample). The duration between the trials was more than 7 days in order to exclude any effect of the former trial. For statistical analysis, a paired *t*-test was used.

Acknowledgment. We acknowledge the contributions of Mr. Koji Onishi and Mr. Norio Tada for the plasma metabolite analysis, Mr. Toshimasa Tanaka for the MO calculations, and Mr. Nobuhiro Fujii and Mr. Masaki Setoh for technical assistance.

Note Added after ASAP Posting. This manuscript was released ASAP on 8/15/2002 with an error in the compound designation for the eighth entry of Table 1. The correct version was posted on 9/5/2002.

References

(1) (a) Goldman, B. D.; Darrow, J. M. The pineal gland and mammalian photoperiodism. *Neuroendocrinology* **1983**, *37*, 386-396. (b) Reiter, R. J. The melatonin message: Duration versus coincidence hypotheses. *Life Sci.* **1987**, *40*, 2119−2131. (c) Reiter, R. J. Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. *Endocr. Rev.* **1991**, *12*, 151−180. (d) Hagan, R. M.; Oakley, N. R. Melatonin comes of age? *Trends Pharmacol. Sci.* **1995**, *16*, 81−83.

(2) (a) Underwood, H.; Goldman, B. D. Vertebrate circadian and photoperiodic systems: role of the pineal gland and melatonin. *J. Biol. Rhythms* **1987**, *2*, 279−315. (b) Cassone, V. M. Effects of melatonin on vertebrate circadian systems. *Trends Neurosci.* **1990**, *13*, 457−464. (c) Armstrong, S. M. Melatonin and circadian control in mammals. *Experientia* **1989**, *45*, 932−938.

(3) Wiechmann, A. F. Melatonin: parallels in pineal gland and retina. *Exp. Eye Res.* **1986**, *42*, 507−527.

(4) (a) Moore, R. Y.; Klein, D. C. Visual pathways and the central neural control of a circadian rhythm in pineal serotonin *N*-acetyltransferase activity. *Brain Res.* **1974**, *71*, 17−33. (b) Reppert, S. M.; Perlow, M. J.; Ungerleider, L. G.; Mishkin, M.; Tamarkin, L.; Orloff, D. G.; Hoffman, H. J.; Klein, D. C. Effects of damage to the suprachiasmatic area of the anterior hypothalamus on the daily melatonin and cortisol rhythms in the rhesus monkey. *J. Neurosci.* **1981**, *1*, 1414−1425. (c) Moore, R. Y. Neural control of the pineal gland. *Behav. Brain Res.* **1996**, *73*, 125−130.

(5) (a) Lynch, H. J.; Ozaki, Y.; Shakal, D.; Wurtman, R. J. Melatonin excretion of man and rats: effect of time of day, sleep, pinealectomy and food consumption. *Int. J. Biometeorol.* **1975**, *19*, 267−279. (b) Jimerson, D. C.; Lynch, H. J.; Post, R. M.; Wurtman, R. J.; Bunney, W. E. Urinary melatonin rhythms during sleep deprivation in depressed patients and normals. *Life Sci.* **1977**, *20*, 1501−1508.

(6) (a) Aakerstedt, T.; Froeberg, J. E.; Friberg, Y.; Wetterberg, L. Melatonin excretion, body temperature and subjective arousal during 64 h of sleep deprivation. *Psychoneuroendocrinology* **1979**, *4*, 219−225. (b) Nakagawa, H.; Sack, R. L.; Lewy, A. J. Sleep propensity free-runs with the temperature, melatonin and cortisol rhythms in a totally blind person. *Sleep* **1992**, *15*, 330−336. (c) Tzischinsky, O.; Shlitner, A.; Lavie, P. The association between the nocturnal sleep gate and nocturnal onset of urinary 6-sulfatoxymelatonin. *J. Biol. Rhythms* **1993**, *8*, 199−209. (d) Zhdanova, I. V.; Wurtman, R. J. Efficacy of melatonin as a sleep-promoting agent. *J. Biol. Rhythms* **1997**, *12*, 644−650.

(7) (a) Wehr, T. A. The Durations of Human Melatonin Secretion and Sleep Respond to Changes in Daylength (Photoperiod). *J. Clin. Endocrinol. Metab.* **1991**, *73*, 1276−1280. (b) Oldani, A.; Ferini-Strambi, L.; Zucconi, M.; Stankov, B.; Fraschini, F.; Smirne, S. Melatonin and delayed sleep phase syndrome: ambulatory polygraphic evaluation. *NeuroReport* **1994**, *6*, 132−134.

(8) (a) Petrie, K.; Conaglen, J. V.; Thompson, L.; Chamberlain, K. Effect of melatonin on jet lag after long haul flights. *Br. Med. J.* **1989**, *298*, 705−707. (b) Arendt, J. Melatonin in humans: jet-lag and after. *Adv. Pineal Res.* **1991**, *5*, 299−302. (c) Nickelsen, T.; Lang, A.; Bergau, L. The effect of 6-, 9-, and 11-hour time shifts on circadian rhythms: adaptation of sleep parameters and hormonal patterns following the intake of melatonin or placebo. *Adv. Pineal Res.* **1991**, *5*, 303−306.

(9) Sack, R. L.; Blood, M. L.; Lewy, A. J. Melatonin rhythms in night shift workers. *Sleep* **1992**, *15*, 434−441.

(10) Rosenthal, N. E.; Sack, D. A.; Jacobsen, F. M.; James, S. P.; Parry, B. L.; Arendt, J.; Tamarkin, L.; Wehr, T. A. Melatonin in seasonal affective disorder and phototherapy. *J. Neural. Transm. Suppl.* **1986**, *21*, 257−267.

(11) (a) Nair, N. P. V.; Hariharasubramanian, N.; Pilapil, C.; Isaac, I.; Thavundayil, J. X. Plasma melatonin − an index of brain aging in humans? *Biol. Psychiatry* **1986**, *21*, 141−150. (b) Waldhauser, F.; Weiszenbacher, G.; Tatzer, E.; Gisinger, B.; Waldhauser, M.; Schemper, M.; Frisch, H. Alterations in nocturnal serum melatonin levels in humans with growth and aging. *J. Clin. Endocrinol. Metab.* **1988**, *66*, 648−652. (c) Reiter, R. J. The pineal grand and melatonin in relation to aging: a summary of the theories and of the data. *Exp. Gerontol.* **1995**, *30*, 199−212.

(12) (a) Dollins, A. B.; Zhdanova, I. V.; Wurtman, R. J.; Lynch, H. J.; Deng, M. H. Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1824−1828. (b) Sack, R. L.; Lewy, A. J.; Parrott, K.; Singer, C. M.; McArthur, A. J.; Blood, M. L.; Bauer, V. K. Melatonin analogues and circadian sleep disorders. *Eur. J. Med. Chem.* **1995**, *30*, 661s−669s. (c) Zhdanova, I. V.; Wurtman, R. J.; Lynch, H. J.; Ives, J. R.; Dollins, A. B.; Morabito, C.; Matheson, J. K.; Schomer, D. L. Sleep-inducing effects of low doses of melatonin ingested in the evening. *Clin. Pharmacol. Ther. (St. Louis)* **1995**, *57*, 552−558. (d) Attenburrow, M. E. J.; Cowen, P. J.; Sharpley, A. L. Low dose melatonin improves sleep in healthy middle-aged subjects. *Psychopharmacology (Berlin)* **1996**, *126*, 179−181. (e) Garfinkel, D.; Laudon, M.; Zisapel, N. Improvement of sleep quality by controlled-release melatonin in benzodiazepine-treated elderly insomniacs. *Arch. Gerontol. Geriatr.* **1997**, *24*, 223−231.

(13) (a) Liebmann, P. M.; Wölfler, A.; Felsner, P.; Hofer, D.; Schauenstein, K. Melatonin and the immune system. *Int. Arch. Allergy Immunol.* **1997**, *112*, 203-211. (b) Reiter, R. J.; Maestroni, G. J. M. Melatonin in relation to the antioxidative defense and immune systems: possible implications for cell and organ transplantation. *J. Mol. Med.* **1999**, *77*, 36−39.

(14) Hill, S. M.; Blask, D. E. Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. *Cancer Res.* **1988**, *48*, 6121−6126.

(15) (a) Claustrat, B.; Le Bars, D.; Brun, J.; Thivolle, P.; Mallo, C.; Arendt, J.; Chazot, G. Plasma and brain pharmacokinetic studies in humans after intravenous administration of cold or ^{11}C labeled melatonin. *Adv. Pineal Res.* **1989**, *3*, 305−310. (b) Le Bars, D.; Thivolle, P.; Vitte, P. A.; Bojkowski, C.; Chazot, G.; Arendt, J.; Frackowiak, R. S. J.; Claustrat, B. PET and plasma pharmacokinetic studies after bolus intravenous administration of [^{11}C]-melatonin in humans. *Nucl. Med. Biol.* **1991**, *18*, 357−362.

(16) Ebisawa, T.; Karne, S.; Lerner, M. R.; Reppert, S. M. Expression cloning of a high-affinity melatonin receptor from *Xenopus* dermal melanophores. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6133−6137.

(17) (a) Reppert, S. M.; Godson, C.; Mahle, C. D.; Weaver, D. R.; Slaugenhaupt, S. A.; Gusella, J. F. Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel_{1b} melatonin receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8734−8738. (b) Liu, C.; Weaver, D. R.; Jin, X.; Shearman, L. P.; Pieschl, R. L.; Gribkoff, V. K.; Reppert, S. M. Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. *Neuron* **1997**, *19*, 91−102. (c) Dubocovich, M. L.; Yun, K.; Al-ghoul, W. M.; Benloucif, S.; Masana, M. I. Selective MT_2 melatonin receptor antagonists block melatonin-mediated phase advances of circadian rhythms. *FASEB. J.* **1998**, *12*, 1212−1220.

(18) (a) Paul, P.; Lahaye, C.; Delagrange, P.; Nicolas, J.-P.; Canet, E.; Boutin, J. A. Characterization of 2-[^{125}I]iodomelatonin binding sites in Syrian hamster peripheral organs. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 334−340. (b) Nosjean, O.; Ferro, M.; Cogé, F.; Beauverger, P.; Henlin, J.-M.; Lefoulon, F.; Fauchère, J.-L.; Delagrange, P.; Canet, E.; Boutin, J. A. Identification of the melatonin-binding site MT_3 as the quinone reductase 2. *J. Biol. Chem.* **2000**, *275*, 31311−31317.

(19) (a) Fourmaintraux, E.; Depreux, P.; Lesieur, D.; Guardiola-Lemaître, B.; Bennejean, C.; Delagrange, P.; Howell, H. E. Tetrahydronaphthalenic derivatives as new agonist and antagonist ligands for melatonin receptors. *Bioorg. Med. Chem.* **1998**, *6*, 9−13. (b) Kloubert, S.; Mathé-Allainmat, M.; Andrieux, J.; Sicsic, S.; Langlois, M. Synthesis of benzocycloalkane derivatives as new conformationally restricted ligands for melatonin receptors. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3325−3330.

(20) (a) Leclerc, V.; Depreux, P.; Lesieur, D.; Caignard, D. H.; Renard, P.; Delagrange, P.; Guardiola-Lemaitre, B.; Morgan, P. Synthesis and boiological activity of conformationally restricted tricyclic analogues of the hormone melatonin. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1071−1076. (b) Spadoni, G.; Balsamini, C.; Diamantini, G.; Giacomo, B. D.; Tarzia, G. Conformationally restrained melatonin analogues: synthesis, binding affinity for the mela-

tonin receptor, evaluation of the biological activity, and molecular modeling study. *J. Med. Chem.* **1997**, *40*, 1990−2002. (c) Beresford, I. M.; Browning, C.; Starkey, S. J.; Brown, J.; Foord, S. M.; Coughlan, J.; North, P. C.; Dubocovich, M. L.; Hagan, R. M. GR196429: A nonindolic agonist at high-affinity melatonin receptors. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 1239−1245. (d) Davies, D. J.; Garratt, P. J.; Tocher, D. A.; Vonhoff, S. Mapping the melatonin receptor. 5. Melatonin. Agonists and antagonists derived from tetrahydrocyclopent[*b*]indoles, tetrahydrocarbazoles and hexahydrocyclohept[*b*]indoles. *J. Med. Chem.* **1998**, *41*, 451−467. (e) Marot, C.; Chavatte, P.; Morin-Allory, L.; Viaud, M. C.; Guillaumet, G.; Renard, P.; Lesieur, D.; Michel, A. Pharmacophoric search and 3D-QSAR comparative molecular field analysis studies on agonists of melatonin sheep receptors. *J. Med. Chem.* **1998**, *41*, 4453−4465. (f) Jellimann, C.; Mathé-Allainmat, M.; Andrieux, J.; Kloubert, S.; Boutin, J. A.; Nicolas, J.-P.; Bennejean, C.; Delagrange, P.; Langlois, M. Synthesis of phenalene and acenaphthene derivatives as new conformationally restricted ligands for melatonin receptors. *J. Med. Chem.* **2000**, *43*, 4051−4062. (g) Poël, H. V.; Guillaumet, G.; Viaud-Massuard, M.-C. Synthesis of 6,7,8,9-tetrahydropyrido[2,3-*b*]-indolizine and 3,4-dihydro-2*H*-pyrido[2′,3′:4,5]pyrrolo[2,1-*b*][1,3]-oxazine derivatives as new melatonin receptor ligands. *Tetrahedron Lett.* **2002**, *43*, 1205−1208.

(21) Paquette, Leo A. *Organic Reactions*; John Wiley & Sons: New York, 1997; pp 1−330.

(22) Monte, A. P.; Marona-Lewicka, D.; Cozzi, N. V.; Nichols, D. E. Synthesis and pharmacological examination of benzofuran, indan, and tetralin analogues of 3,4-(methylenedioxy)amphetamine. *J. Med. Chem.* **1993**, *36*, 3700−3706.

(23) House, H. O.; Hudson, C. B. Perhydroindan derivatives. XII. 6-Methoxyindanone and its derivatives. *J. Org. Chem.* **1970**, *35*, 647−651.

(24) (a) Wittig, G.; Schöllkopf, U. Triphenylphosphinemethylene as an olefin-forming reagent. I. *Chem. Ber.* **1954**, *87*, 1318−1330. (b) Wadsworth, W. S., Jr.; Emmons, W. D. The utility of phosphonate carbanions in olefin synthesis. *J. Am. Chem. Soc.* **1961**, *83*, 1733−1738.

(25) Lindberg, U. H.; Nylén, B.; Akerman, B. Potential local anaesthetics. I. Basic N-(alpha-cycloalkylbenzyl)acylamides and some related compounds. *Acta Pharm. Suec.* **1968**, *5*, 429−440.

(26) (a) Kubo, I.; Ochi, M.; Shibata, K.; Hanke, F. J.; Nakatsu, T.; Tan, K.-S.; Taniguchi, M.; Kamikawa, T.; Yamagiwa, Y.; Arizuka, M.; Wood, W. F. Effect of a marine algal constituent on the growth of lettuce and rice seedlings. *J. Nat. Prod.* **1990**, *53*, 50−56. (b) Ford, P. W.; Narbut, M. R.; Belli, J.; Davidson, B. S. Synthesis and structural properties of the benzopentathiepins varacin and isolissoclinotoxin A. *J. Org. Chem.* **1994**, *59*, 5955−5960.

(27) (a) De Silva, S. O.; Ahmad, I.; Snieckus, V. A convergent route to phthalideisoquinoline alkaloids via directed metalation of tertiary benzamides. *Can. J. Chem.* **1979**, *57*, 1598−1605. (b) Bellamy, F. D.; Chazan, J. B.; Ou, K. Synthesis of derivatives structurally related to glaziovine. *Tetrahedron* **1983**, *39*, 2803−2806.

(28) Castillo, P.; Rodriguez-Ubis, J. C.; A high-yield method for the methylenation of *o*-dihydroxyaromatic compounds: synthesis of methylenedioxycoumarins. *Synthesis* **1986**, 839−840.

(29) Stewart, J. J. Optimization of parameters for semiempirical methods. *J. Comput. Chem.* **1989**, *10*, 209−220.

(30) Vaněček, J.; Vollrath, L. Melatonin inhibits cyclic AMP and cyclic GMP accumulation in the rat pituitary. *Brain Res.* **1989**, *505*, 157−159.

(31) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Trong, I. L.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal Structure of Rhodopsin: A G Protein-coupled Receptor. *Science* **2000**, *289*, 739−745.

(32) Kokkola, T.; Watson, M.-A.; White, J.; Dowell, S.; Foord, S. M.; Laitinen, J. T. Mutagenesis of Human Mel_{1a} Melatonin Receptor Expressed in Yeast Reveals Domains Important for Receptor Function. *Biochem. Biophys. Res. Commun.* **1998**, *249*, 531−536.

(33) Conway, S.; Drew, J. E.; Mowat, E. S.; Barrett P.; Delagrange, P.; Morgan, P. J. Chimeric Melatonin mt1 and Melatonin-related Receptors. *J. Biol. Chem.* **2000**, *275*, 20602−20609.

(34) De Silva, S. O.; Ahmad, I.; Snieckus, V. A convergent route to phthalideisoquinoline alkaloids via directed metalation of tertiary benzamides. *Can. J. Chem.* **1979**, *57*, 1598−1605.

(35) Rivkees, S. A.; Cassone, V. M.; Weaver, D. R.; Reppert, S. M. Melatonin receptors in chick brain: characterization and localization. *Endocrinology* **1989**, *125*, 363−368.

(36) Niles, L. P.; Pickering, D. S.; Sayer, B. G. HPLC-purified 2-[^{125}I]-iodomelatonin labels multiple binding sites in hamster brain. *Biochem. Biophys. Res. Commun.* **1987**, *147*, 949−956.

(37) Snider, R. S.; Niemer, W. T. *A Stereotaxic Atlas of the Cat Brain*; University of Chicago Press: Chicago, IL, 1961.

JM0201159