Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

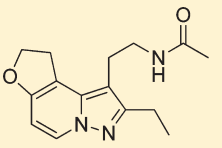
Synthesis of a Novel Series of Tricyclic Dihydrofuran Derivatives: Discovery of 8,9-Dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridines as Melatonin Receptor (MT_1/MT_2) Ligands

Tatsuki Koike,* Takafumi Takai, Yasutaka Hoashi, Masaharu Nakayama, Yohei Kosugi, Masato Nakashima, Shin-ichi Yoshikubo, Keisuke Hirai, and Osamu Uchikawa

Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, 17-85 Jusohonmachi, 2-Chome, Yodogawa-ku, Osaka 532-8686, Japan

Supporting Information

ABSTRACT: Novel tricyclic dihydrofuran derivatives were designed, synthesized, and evaluated as melatonin receptor (MT_1/MT_2) ligands based on the previously reported 1,6-dihydro-2*H*-indeno[5,4-*b*]furan **1a**. By screening the central tricyclic cores, we identified 8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridine as a potent scaffold with a high ligand-lipophilicity efficiency (LLE) value. Subsequent optimization of the side chains led to identification of the potent MT_1/MT_2 agonist **4d** (MT_1, K_i = 0.062 nM; MT_2, K_i = 0.420 nM) with good oral absorption and blood−brain barrier (BBB) penetration in rats. The oral administration of compound **4d** exhibited a sleep-promoting action in freely moving cats at 0.1 mg/kg.



4d

INTRODUCTION

Melatonin (5-methoxy-*N*-acetyltryptamine, Figure 1) is a tryptophan-derived neurohormone characterized by a circadian rhythm of secretion, with its peak concentration occurring at night.[1] It regulates the circadian rhythm,[2] and its chronobiotic effects have led to many therapeutic targets such as sleep disorders,[3] neurodegenerative diseases,[4,5] cancer,[6,7] and stroke.[8] However, the therapeutic use of melatonin[9] is limited by its short biological half-life (15−20 min), its poor oral bioavailability, and its ubiquitous action. It has been demonstrated that melatonin exerts its effects through the activation of the high-affinity G-protein-coupled receptors MT_1 and MT_2,[10,11] the distinct function of which has yet to be elucidated. However, knockout studies of MT_1 in mice[12] and a few MT_2-selective ligands have revealed some of the functions of these receptors.[13] MT_1 receptors are now thought to be involved in the sleep-promotion effects of melatonin, while MT_2 receptors mediate the effects of melatonin on circadian rhythms.[14]

During the past 2 decades, many melatonin receptor ligands,[15−17] including subtype-selective ligands, have been reported, and some of these have been developed in clinical trials.[18] Ramelteon,[19,20] which was designed and synthesized in the course of our exploration for a melatonin-mimicking agonist, shows a high binding affinity for MT_1 (K_i = 0.014 nM) and MT_2 (K_i = 0.112 nM). Ramelteon reduces sleep latency and increases total sleep time and has been successfully marketed for the treatment of insomnia (in the U.S. in 2005 and in Japan in 2010).[21]

In the further development of melatonin receptor ligands, we previously reported the potent 1,6-dihydro-2*H*-indeno[5,4-*b*]furan derivatives **1a**−**e** as MT_1/MT_2 agonists[22] (Figure 1). The activity was increased through the introduction of lipophilic substituents at the 7-position (R^1) of the core, and the most potent compound **1a** (MT_1, K_i = 0.0082 nM; MT_2, K_i = 0.0065 nM) showed a binding affinity 20 times higher than that of melatonin for both the MT_1 and MT_2 subtypes. In addition, we achieved good MT_2 selectivity over the MT_1 subtype by changing this R^1 substituent, which led to the identification of a potent MT_2-selective agonist.[22] Although this strategy provided potent melatonin receptor ligands with a wide range of MT_1/MT_2 selectivities, the introduction of lipophilic substituents (R^1) also resulted in the increased lipophilicity of the molecule, which might be correlated with the undesirable absorption, distribution, metabolism, and elimination (ADME) profiles.[23] For the further development of this tricyclic series, especially in terms of the exploration of the R^1 group, we planned to decrease the lipophilicity of the molecule by replacing the lipophilic central 1,6-dihydro-2*H*-indeno[5,4-*b*]furan core with a less lipophilic tricyclic nucleus.

We report here a lead generation of less lipophilic tricyclic cores and their optimization (R^1 and R^2) to identify an MT_1/MT_2 agonist with good ADME profiles and potent in vivo activity (Figure 2). The lead generation step involves the introduction of two nitrogen atoms (**2a**−**4a**) to the central tricyclic core of **1a**, maintaining the angular furan-fused tricyclic system, which seems to be the key element in its potent activity. One nitrogen atom is placed para to the oxygen, which corresponds to the original nitrogen position of the indole core of melatonin. Since the lipophilic indene moiety of **1a** is converted to the less lipophilic imidazo[1,2-*a*]pyridine (**2a**), benzimidazole

Received: April 1, 2011
Published: May 13, 2011

(**3a**), and pyrazolo[1,5-*a*]pyridine (**4a**) rings, it is expected that these molecules will exhibit lower lipophilicity.

CHEMISTRY

The synthesis of the 1,2-dihydrofuro[2,3-*e*]imidazo[1,2-*a*]pyridine derivative **2a** is illustrated in Scheme 1. 2,3-Dihydrofuro[3,2-*b*]pyridin-5-amine **5**[24] was coupled with α-bromoacetophenone to give the novel 1,2-dihydrofuro[2,3-*e*]imidazo[1,2-*a*]pyridine derivative **6** with 47% yield. An aminoethyl side chain was introduced on the basis of the reported procedure applied for the imidazo[1,2-*a*]pyridine ring.[25] The Mannich reaction, followed by quarternization of the Mannich base and substitution with a cyanide, afforded the nitrile **8**. The target compound **2a** was obtained through hydrogenation of **8**, followed by acetylation.

The synthesis of 7,8-dihydro-1*H*-furo[3,2-*e*]benzimidazole derivative **3a** is illustrated in Scheme 2. The synthesis of the tricyclic core, targeted at RNA binding, was reported recently.[26] We employed this synthetic method with some modification to synthesize compound **3a**. The commercially available 2,6-difluorophenylacetic acid **9** was converted to the alcohol **10** by esterification and reduction, followed by protection with an acetyl group to afford the acetylate **11**. Nitration of **11** using fuming nitric acid, then selective S_NAr displacement of the fluoro group of **12** with Boc-ethylenediamine afforded compound **13**. The acetyl group of compound **13** was deprotected using LiOH in H_2O−dioxane. Under these conditions, direct intramolecular cyclization proceeded well and the dihydrofuran **14** was obtained with 82% yield. The nitro group of **14** was hydrogenated, and the resultant aniline was treated with triethyl orthobenzoate to give 7,8-dihydro-1*H*-furo[3,2-*e*]benzimidazole **16**. The target compound **3a** was obtained by deprotection of **16** and subsequent acetylation.

The synthesis of 8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]-pyridines **4a**−**i** is illustrated in Scheme 3. The furopyridine

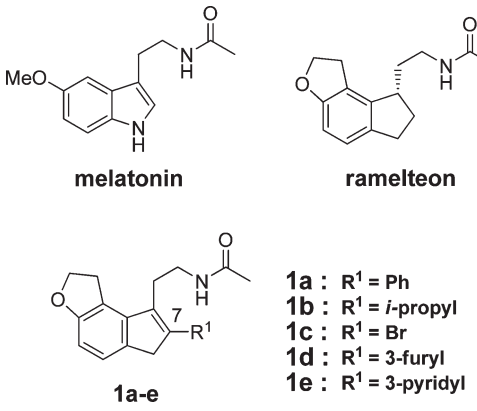


Figure 1. Chemical structures of melatonin, ramelteon, and 1,6-dihydro-2*H*-indeno[5,4-*b*]furan derivatives **1a**−**e**.

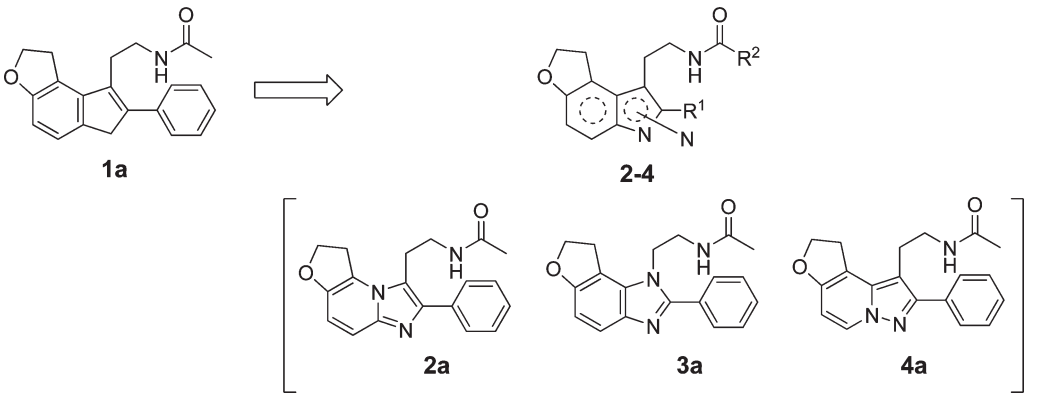
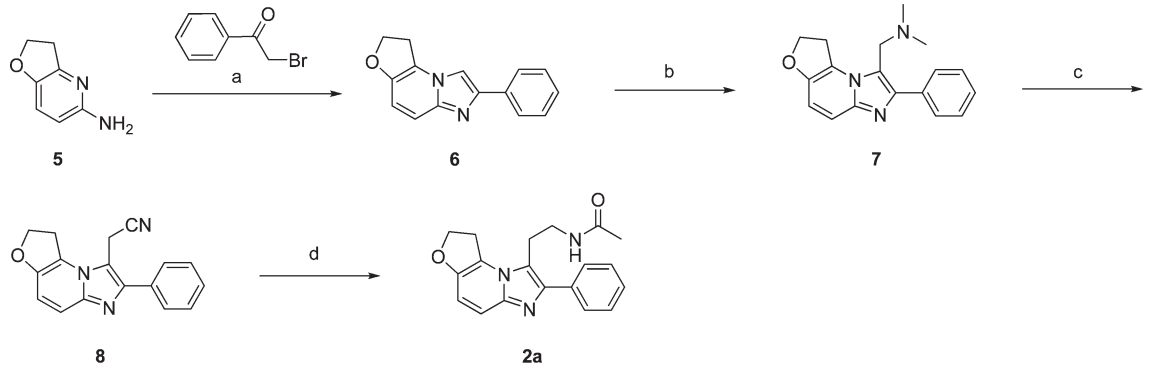


Figure 2. Design of novel tricyclic dihydrofurans.

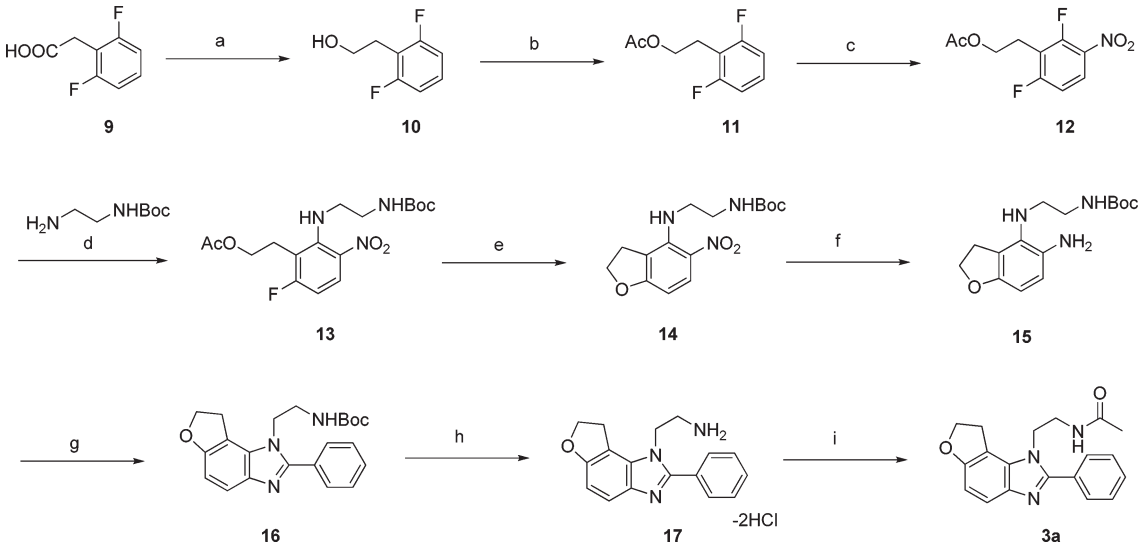
Scheme 1[a]



[a] Reagents and conditions: (a) TsOH·H_2O, toluene, reflux; (b) Me_2NH, HCHO, H_2O, MeCN, 70 °C; (c) (1) MeI, acetone, room temp; (2) NaCN, EtOH, H_2O, 80 °C; (d) (1) H_2, Raney Co, NH_3, EtOH, room temp; (2) AcCl, Et_3N, THF, room temp.

dx.doi.org/10.1021/jm200385u | *J. Med. Chem.* 2011, 54, 4207–4218

Scheme 2[a]



[a] Reagents and conditions: (a) (1) H_2SO_4, EtOH, reflux; (2) LAH, THF, 0 °C; (b) AcCl, Et_3N, THF, room temp; (c) HNO_3, 0 °C; (d) K_2CO_3, DMF, room temp; (e) $LiOH \cdot H_2O$, H_2O, dioxane, 60 °C; (f) H_2, Pd/C, MeOH, room temp; (g) $C(OEt)_3Ph$, $TsOH \cdot H_2O$, THF, 60 °C; (h) HCl, EtOAc, MeOH, room temp; (i) AcCl, Et_3N, THF, room temp.

18[27] was treated with *O*-(2,4-dinitrophenyl)hydroxylamine[28] to give its *N*-aminopyridinium salt **19**. Then a 1,3-dipolar cycloaddition reaction of **19** with various alkyne esters **20a**−**e** afforded the 8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridines **21a**−**e** in 12−38% isolated yield. The linear cyclized 2,3-dihydrofuro-[2,3-*d*]pyrazolo[1,5-*a*]pyridines **22a**−**e**, which were easily separated by silica gel column chromatography, were also obtained in an approximately 1:1 ratio (LCMS peak ratio) because of the poor regioselectivity of this cycloaddition reaction. Reduction of the esters **21a**−**e** and subsequent cyanation using trimethylsilyl cyanide and boron trifluoride etherate[29] afforded the nitriles **24a**−**e** directly. The target compounds **4a**−**i** were obtained through hydrogenation of **24a**−**e** followed by acylation.

RESULTS AND DISCUSSION

The binding affinities of compounds **1a**, **2a**, **3a**, and **4a**−**i** for human MT_1 and MT_2 melatonin receptors were evaluated using 2-[^{125}I]iodomelatonin as a radioligand in Chinese hamster ovary (CHO) cells expressing these receptors.[20] To evaluate the ADME profiles of the compounds, the in vitro metabolic clearance in rat hepatic microsomes was also investigated. The results are shown in Table 1, together with the chemical structures and their log *D* values. The log *D* values were measured at pH 7.4 with the relative retention time over the standard compounds of HPLC analysis.

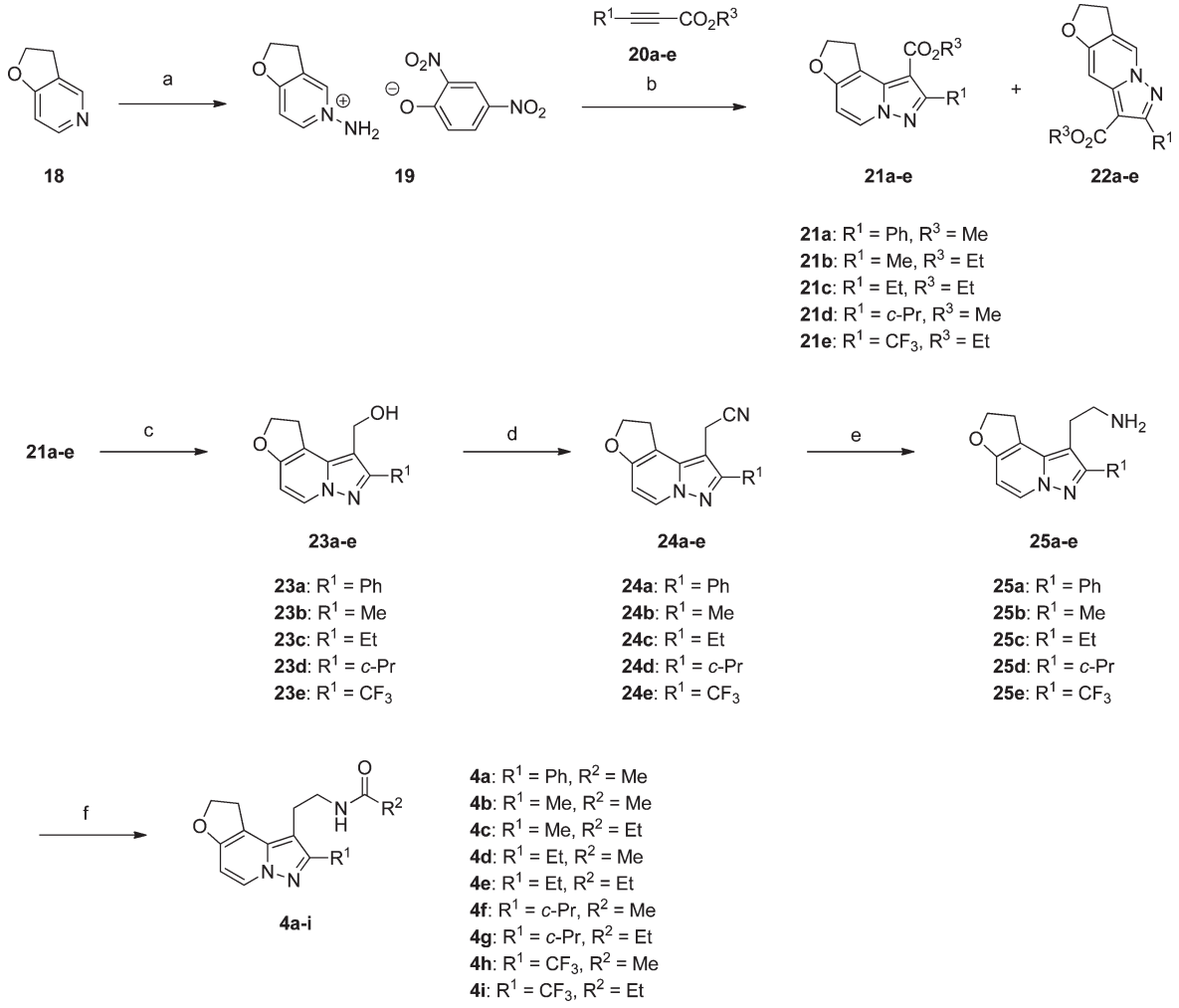
Lead Generation: Selection of the Central Tricyclic Core. For the selection of a lead central core among the newly synthesized tricyclic cores, the phenyl derivatives **1a**−**4a** were compared in terms of binding affinity, lipophilicity (log *D*, measured at pH 7.4), and ligand-lipophilicity efficiency (LLE)[30,31] (Table 1). LLE (=pK_i − log *D*) is an important parameter for estimating the potential of the binding interaction without the contribution of lipophilicity. Compounds with lower lipophilicity are expected to show better ADME properties.

Replacement of the indene moiety of 1,6-dihydro-2*H*-indeno-[5,4-*b*]furan **1a** with imidazo[1,2-*a*]pyridine (**2a**), benzimidazole (**3a**), and pyrazolo[1,5-*a*]pyridine (**4a**) resulted in compounds with lower log *D* values (**2a**, 1.66; **3a**, 1.39; **4a**, 2.04) than **1a** (3.49). This showed that less lipophilic melatonin receptor ligands were successfully prepared by this replacement. Regarding the in vitro metabolic clearance, the less lipophilic compounds **2a** (36 (μL/min)/mg) and **3a** (27 (μL/min)/mg) were much more stable than **1a** (250 (μL/min)/mg) against oxidative metabolism for rat hepatic microsomes. However, the binding affinities of **2a** and **3a** decreased drastically, and the LLE values of **2a** (MT_1, <5.3; MT_2, 6.6) and **3a** (MT_1, 6.3; MT_2, 7.0) were much lower than those of **1a** (MT_1, 7.6; MT_2, 7.7), suggesting that the binding affinities of **2a** and **3a** decreased not only because of the decreased lipophilicity but also as a result of other factors. On the other hand, compound **4a** showed only a small decrease in binding affinity (MT_1, K_i = 0.082 nM; MT_2, K_i = 0.085 nM) and exhibited a potency comparable to that of melatonin itself for both MT_1 and MT_2 subtypes. It was notable that the LLE values of **4a** (MT_1, 8.0; MT_2, 8.0) were as high as those for **1a**, which indicates that the 8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridine scaffold is the desired surrogate of the 1,6-dihydro-2*H*-indeno-[5,4-*b*]furan for the preparation of less lipophilic melatonin receptor ligands.

Optimization of 8,9-Dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridines. The 8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridine **4a** was selected as a lead compound. A further decrease of the lipophilicity of the molecule was required to improve the in vitro metabolic clearance of **4a** in rat hepatic microsomes (240 (μL/min)/mg). In a previous study,[22] we reported that the introduction of lower alkyl groups (for example, the isopropyl derivative **1b**, Figure 1) at the 7-position (R^1) of the 1,6-dihydro-2*H*-indeno[5,4-*b*]furan core resulted in a binding affinity comparable to that of the phenyl derivative **1a** for both MT_1 and MT_2. From this structure−activity relationship (SAR) information, the phenyl substituent at the 2-position (R^1) of **4a** was replaced with lower alkyl substituents (methyl, ethyl, cyclopropyl, trifluoromethyl) to give **4b**−**i**. These compounds were expected not only to show high binding affinities but also to

dx.doi.org/10.1021/jm200385u |*J. Med. Chem.* 2011, 54, 4207–4218

Scheme 3[a]



[a] Reagents and conditions: (a) *O*-(2,4-dinitrophenyl)hydroxylamine, MeCN, room temp; (b) K_2CO_3, DMF, room temp; (c) LAH, THF, room temp; (d) TMSCN, $BF_3 \cdot Et_2O$, CH_2Cl_2, room temp; (e) H_2, Raney Co, NH_3, MeOH, room temp; (f) $(MeCO)_2O$ or $(EtCO)_2O$, Et_3N, THF, room temp.

exhibit lower lipophilicities because of their less lipophilic alkyl groups. Regarding the amide side chain, propionylamides, which correspond to the amide moiety of ramelteon,[19] were also synthesized.

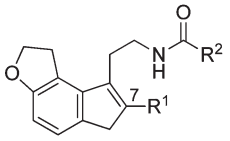
The SAR of these 8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]-pyridines **4a**−**i** was consistent with that of 1,6-dihydro-2*H*-indeno[5,4-*b*]furans **1a**−**e**, and their binding affinities exhibited potent subnanomolar values both for MT_1 and MT_2 (Table 1). Interestingly, the pK_i values of **4a**−**i** showed good correlation with the log *D* values of the molecule, and the LLE values of the alkyl derivatives **4b**−**i** (7.6−8.8 for MT_1, 7.4−8.3 for MT_2) indicated high values comparable to those of their lead phenyl compound **4a** (MT_1, 8.0; MT_2, 8.0). These results suggest that MT_1 and MT_2 receptors have hydrophobic pockets around this R^1 position and that the binding affinities are strongly affected by the lipophilicity of the molecule. The LLE values of the alkyl derivatives **4b**−**i** were also comparable to those of their original lead compound **1a** (MT_1, 7.6; MT_2, 7.7). This result allows us to conclude that we could successfully reduce the lipophilicity of the molecules (**4b**−**i**) while maintaining the high LLE value of the lead compound **1a** (Figure 3).

The in vitro metabolic clearances of **4b**−**i** were much lower than that of **4a** against oxidative metabolism for rat hepatic microsomes (16−100 (μL/min)/mg, Table 1). This stability might be because the lipophilicity of the molecules **4b**−**i** (log *D* = 1.0−1.9) is lower than that of **4a** (log *D* = 2.04). The ethyl derivative **4d** (MT_1, K_i = 0.062 nM; MT_2, K_i = 0.420 nM), which exhibited the highest metabolic stability against oxidative metabolism for rat hepatic microsomes (16 (μL/min)/mg), was selected for further evaluation of its pharmacokinetic and pharmacological properties. Compound **4d** was also stable against oxidative metabolism for human hepatic microsomes (3 (μL/min)/mg).

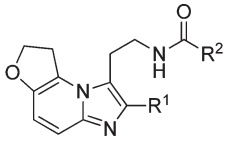
Pharmacokinetic Properties and Pharmacological Evaluation of 4d. The oral bioavailability of compound **4d** was 19.7% in rats (Table 2). This oral bioavailability is presumably explained by its good metabolic stability against oxidative metabolism for rat hepatic microsomes (16 (μL/min)/mg). The brain distribution 15 and 30 min after intravenous (iv) administration was also evaluated (Table 3), and compound **4d** was shown to penetrate the rat blood−brain barrier (BBB). These results confirm that 8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridine is a promising

dx.doi.org/10.1021/jm200385u | *J. Med. Chem.* 2011, 54, 4207–4218

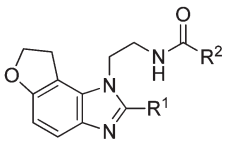
Table 1. Binding Affinities of 1a, 2a, 3a, and 4a−i for MT_1 and MT_2, Their log *D* and LLE Values, and Their in Vitro Metabolic Clearance in Rat Hepatic Microsomes



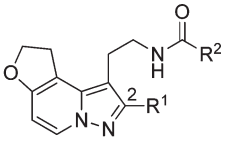
1a



2a



3a



4a-i

			K_i [a] (nM)			LLE [c]		
compd	R^1	R^2	MT_1	MT_2	log *D* [b]	MT_1	MT_2	clearance [d] rat
melatonin			0.24 ± 0.06	0.21 ± 0.03	0.50	9.5	9.6	35
1a	Ph	Me	0.0082 ± 0.0020	0.0065 ± 0.0015	3.49	7.6	7.7	250
2a	Ph	Me	>100	6.1 ± 1.4	1.66	<5.3	6.6	36
3a	Ph	Me	20 ± 5	4.5 ± 2.2	1.39	6.3	7.0	27
4a	Ph	Me	0.082 ± 0.007	0.085 ± 0.016	2.04	8.0	8.0	240
4b	Me	Me	0.16 ± 0.03	0.53 ± 0.13	1.04	8.8	8.2	21
4c	Me	Et	0.083 ± 0.014	0.19 ± 0.04	1.39	8.7	8.3	40
4d	Et	Me	0.062 ± 0.011	0.42 ± 0.08	1.48	8.7	7.9	16
4e	Et	Et	0.039 ± 0.008	0.22 ± 0.05	1.71	8.7	8.0	46
4f	*c*-Pr	Me	0.044 ± 0.005	0.28 ± 0.08	1.61	8.7	7.9	42
4g	*c*-Pr	Et	0.026 ± 0.003	0.14 ± 0.03	1.89	8.7	8.0	85
4h	CF_3	Me	0.48 ± 0.07	0.93 ± 0.53	1.64	7.7	7.4	59
4i	CF_3	Et	0.31 ± 0.06	0.33 ± 0.09	1.94	7.6	7.5	100

[a] Receptor binding, mean of at least three experiments. [b] Measured at pH 7.4. [c] LLE = pK_i − log *D*. [d] In vitro metabolic clearance in hepatic microsomes ((μL/min)/mg).

scaffold for melatonin receptor ligands with good oral bioavailability and BBB penetration.

A functional assay of **4d** was evaluated using the cyclic adenosine monophosphate (cAMP) production in CHO cells expressing human MT_1 or MT_2 receptor based on the method of Kato et al.[20] with some modifications (Figure 4). Melatonin induced a concentration-dependent decrease in forskolin-stimulated cAMP accumulation (MT_1, EC_{50} = 0.026 nM; MT_2, EC_{50} = 0.82 nM). Compound **4d** also exhibited full agonism and a similar potency for the MT_1 and MT_2 receptors (MT_1, EC_{50} = 0.021 nM; MT_2, EC_{50} = 0.53 nM).

The pharmacological effects of **4d** were also examined using experimental animals. In our previous study, we reported that the cat model is useful for studying the sleep-promoting action of melatonin or melatonin receptor agonists.[32] In this study, we examined the sleep-promoting effects of compound **4d** in freely moving cats. Oral administration of **4d** at 0.1 mg/kg significantly decreased the percentage of wakefulness and increased the percentage of slow wave sleep (SWS) compared to the vehicle-treated control (Figure 5). We confirmed that compound **4d** exerted sleep-promoting effects in cats.

CONCLUSION

In this report, we described the design and synthesis of the novel tricyclic dihydrofurans **2a**, **3a**, and **4a**−**i** for decreasing the lipophilicity of the previously reported tricyclic dihydrofuran **1a** (log *D* = 3.49). 8,9-Dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridine was identified as a less lipophilic central core with a high LLE value, and the optimization of this scaffold allowed us to identify compound **4d** as a potent MT_1/MT_2 agonist (MT_1, K_i = 0.062 nM; MT_2, K_i = 0.420 nM) with reduced lipophilicity (log *D* = 1.48). Compound **4d** exhibited good oral absorption and BBB permeability in rats, and its sleep-promoting effects were confirmed in cats. These results show that 8,9-dihydrofuro-[3,2-*c*]pyrazolo[1,5-*a*]pyridine is a promising scaffold for melatonin receptor ligands with potent activities and good ADME profiles. Further profiling of 8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]-pyridine derivatives is in progress and will be reported in due course.

EXPERIMENTAL SECTION

Melting points were determined on a Buchi melting point apparatus and were not corrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian Gemini-200 (200 MHz), Varian Gemini-300 (300 MHz), or Bruker DPX300 (300 MHz) instrument. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane of the indicated organic solution. Peak multiplicities are expressed as follows. Abbreviations are used as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, doublet of doublet of doublets; dt, doublet of triplet; brs, broad singlet; m, multiplet. Coupling constants (*J* values) are given in hertz (Hz). LC/MS (ESI^+) was performed on a ZQ-2000 apparatus with acetonitrile/water mobile phase. Preparative HPLC was performed with an automated Gilson HPLC system using a YMC C-18 column (S-5 μm, 50 mm × 20 mm i.d.) with 10−100% gradient water−acetonitrile containing 0.1% TFA. Reaction progress was determined by thin layer

dx.doi.org/10.1021/jm200385u |*J. Med. Chem.* 2011, 54, 4207–4218

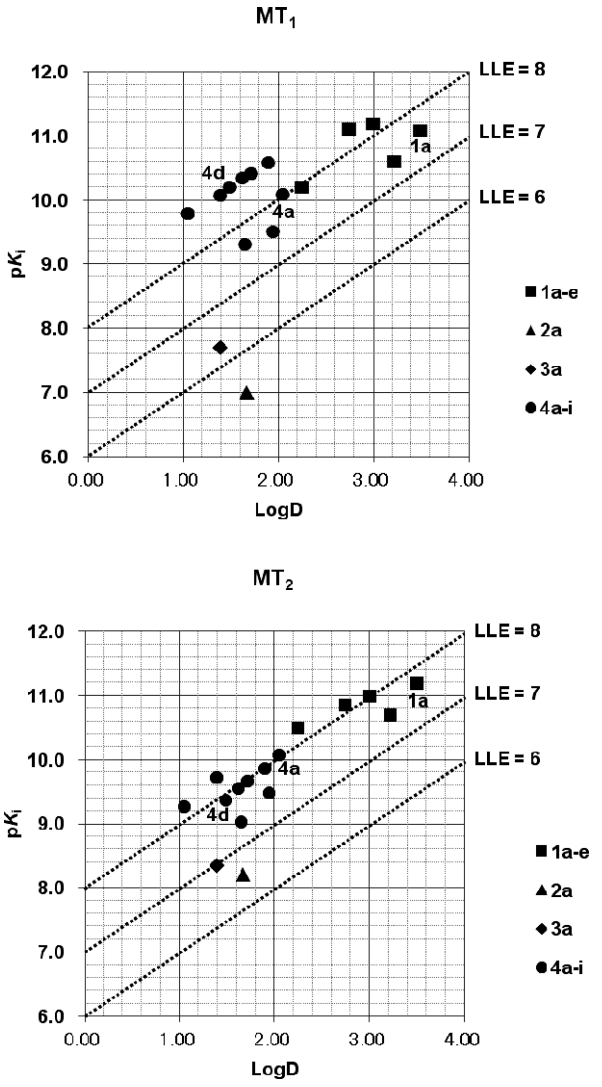


Figure 3. Plot of pK_i (MT_1 and MT_2) against log D for **1a**−**e**, **2a**, **3a**, and **4a**−**i**.

chromatography (TLC) analysis on silica gel 60 F254 plate (Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.). The purities (>95%) of all compounds tested in biological systems were established by elemental analyses. Element analyses were carried out by Takeda Analytical Laboratories, and the results were within 0.4% of theoretical values.

***N*-[2-(7-Phenyl-1,2-dihydrofuro[2,3-*e*]imidazo[1,2-*a*]pyridin-8-yl)ethyl]acetamide (2a).** A solution of **8** (17 mg, 0.062 mmol) in 2 M ammonia EtOH solution (5 mL) was stirred in the presence of Raney cobalt ODHT-60 (500 mg, washed with EtOH before use) under hydrogen atmosphere at room temperature for 2.5 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was dissolved in THF (2 mL). Triethylamine (17.3 μL, 0.124 mmol) and acetyl chloride (5.3 μL, 0.074 mmol) were added, and the mixture was stirred at room temperature for 20 min. Saturated aqueous sodium bicarbonate solution was added, extracted with EtOAc, washed with brine, dried over magnesium sulfate, filtered, and evaporated. Purification by silica gel column chromatography (MeOH/EtOAc), then recrystallization (EtOAc/Hexane) afforded **2a** (12.9 mg, 65%) as a white crystal. Mp 200−201 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.80 (3H, s), 3.32−3.47 (4H, m), 3.91 (2H, t, J = 8.9 Hz), 4.76 (2H, t, J = 8.9 Hz), 5.64 (1H, s), 6.96 (1H, d, J = 9.3 Hz), 7.29−7.50 (4H, m), 7.65−7.75 (2H, m); MS (ESI) m/z 322 $(M + H)^+$. Anal. ($C_{19}H_{19}N_3O_2$) C, H, N.

***N*-[2-(2-Phenyl-7,8-dihydro-1*H*-furo[3,2-*e*]benzimidazol-1-yl)ethyl]acetamide (3a).** Triethylamine (237 μL, 1.70 mmol) and acetyl chloride (36.3 μL, 0.51 mmol) were added to a suspension of **17** in THF (10 mL), and the mixture was stirred at room temperature for 24 h. Saturated aqueous sodium bicarbonate solution was added, extracted with EtOAc, washed with brine, dried over magnesium sulfate,

Table 2. Pharmacokinetic Parameters of 4d

parameter[a]	0.1 mg/kg
$Vd_{(SS)}$ (mL/kg)	664 ± 114
CL_{total} ((mL/h)/kg)	1506 ± 613
C_{max} (ng/mL)	12.4 ± 1.9
AUC[b] $_{po}$ (ng·h/mL)	14.4 ± 1.1
MRT_{po}[c] (h)	0.95 ± 0.31
F[d] (%)	19.7 ± 6.8

[a] Mean ± SD (n = 3). [b] Area under the concentration−time curve. [c] Mean residence time. [d] Bioavailability (oral).

Table 3. Brain and Plasma Concentrations after iv Administration of 4d to Rats[a]

time[b] (min)	plasma (ng/mL)	brain (ng/g)	B/P ratio
15	83.2	23.7	0.28
30	55.9	12.4	0.22

[a] Cassette dosing (0.2 mg/kg). [b] Time after iv administration.

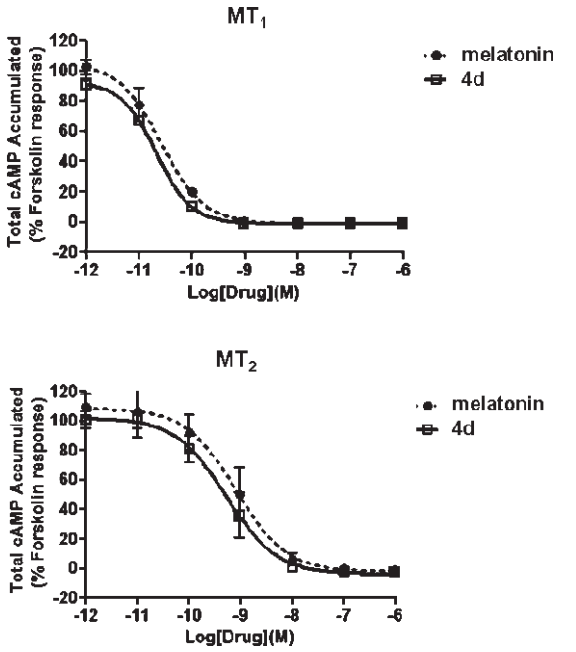


Figure 4. Functional analysis of melatonin and **4d** on forskolin-stimulated cAMP formation in CHO cells expressing either human MT_1 or human MT_2 receptor. Each data point represents the mean ± standard error of two dependent experiments performed in triplicate. Curves were fit by nonlinear regression analysis with a variable-slope (four parameters) fit to obtain potency (EC_{50}) values using the GraphPad Prism software. The log EC_{50} values for MT_1 are −10.58 ± 0.04 (melatonin) and −10.67 ± 0.01 (**4d**), and those for MT_2 are −9.09 ± 0.09 (melatonin) and −9.27 ± 0.06 (**4d**).

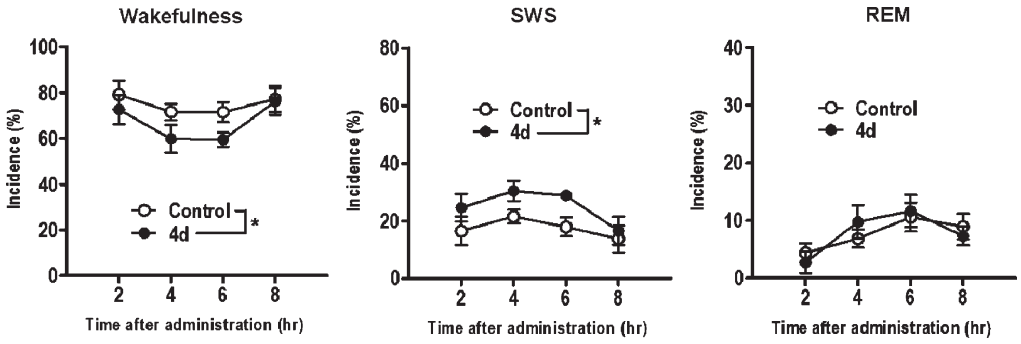


Figure 5. Effects of **4d** on amount of each sleep stage at sleep periods in freely moving cats. Six cats were treated with **4d** (0.1 mg/kg, po) or vehicle. Each value (Wakefulness, SWS, or REM) shows the mean percentage at each stage during each block of 2 h: *, $p < 0.05$ compared with the vehicle-treated control (ANOVA).

filtered, and evaporated. Purification by silica gel column chromatography (EtOAc/hexane) followed by recrystallization (MeOH/diisopropyl ether) afforded **3a** (96.5 mg, 70%) as a white crystal. Mp 256−257 °C (MeOH/diisopropyl ether); ^{1}H NMR (DMSO-d_6) δ 1.58 (3H, s), 3.20 (2H, q, J = 6.5 Hz), 3.60 (2H, t, J = 8.8 Hz), 4.31 (2H, t, J = 6.5 Hz), 4.65 (2H, t, J = 8.8 Hz), 6.76 (1H, d, J = 8.7 Hz), 7.41 (1H, d, J = 8.7 Hz), 7.48−7.60 (3H, m), 7.67−7.78 (2H, m), 7.97 (1H, t, J = 5.7 Hz). Anal. ($C_{19}H_{19}N_3O_2$) C, H, N.

***N*-[2-(2-Phenyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethyl]acetamide (4a).** By a similar procedure described for **4d**, **4a** (55%) was obtained from **25a** and acetic anhydride as a solid. Mp 195−196 °C (EtOAc/diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.78 (3H, s), 3.06 (2H, t, J = 7.1 Hz), 3.28−3.38 (2H, m), 3.57 (2H, t, J = 9.1 Hz), 4.76 (2H, t, J = 9.1 Hz), 5.43 (1H, brs), 6.48 (1H, d, J = 7.4 Hz), 7.35−7.51 (3H, m), 7.65−7.73 (2H, m), 8.22 (1H, d, J = 7.4 Hz); MS (ESI) m/z 322 (M + H)$^+$. Anal. ($C_{19}H_{19}N_3O_2$) C, H, N.

***N*-[2-(2-Methyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethyl]acetamide (4b).** By a similar procedure described for **4d**, **4b** (81%) was obtained from **25b** and acetic anhydride as a solid. Mp 158−159 °C (EtOAc/diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.95 (3H, s), 2.38 (3H, s), 2.84 (2H, t, J = 7.2 Hz), 3.32−3.52 (4H, m), 4.72 (2H, t, J = 9.1 Hz), 5.56 (1H, brs), 6.38 (1H, d, J = 7.4 Hz), 8.13 (1H, d, J = 7.4 Hz); MS (ESI) m/z 260 (M + H)$^+$. Anal. ($C_{14}H_{17}N_3O_2$) C, H, N.

***N*-[2-(2-Methyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethyl]propanamide (4c).** By a similar procedure described for **4d**, **4c** (46%) was obtained from **25b** and propionic anhydride as a solid. Mp 161−162 °C (from EtOAc/diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.13 (3H, t, J = 7.7 Hz), 2.17 (2H, q, J = 7.7 Hz), 2.38 (3H, s), 2.84 (2H, t, J = 7.1 Hz), 3.33−3.52 (4H, m), 4.71 (2H, t, J = 9.1 Hz), 5.53 (1H, brs), 6.37 (1H, d, J = 7.1 Hz), 8.10−8.14 (1H, m); MS (ESI) m/z 274 (M + H)$^+$. Anal. ($C_{15}H_{19}N_3O_2$) C, H, N.

***N*-[2-(2-Ethyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethyl]acetamide (4d).** Acetic anhydride (90.0 μL, 0.952 mmol) was added to a mixture of 2-(2-ethyl-8,9-dihydrofuro[3,2-*c*]pyrazolo [1,5- *a*]pyridin-1-yl)ethanamine **25c** (110 mg, 0.476 mmol) and triethylamine (133 μL, 0.954 mmol) in THF (5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After addition of saturated aqueous sodium bicarbonate solution (50 μL), the mixture was evaporated. Purification by silica gel column chromatography (EtOAc/hexane), then recrystallization (EtOAc/diisopropyl ether) afforded **4d** (67.8 mg, 52%) as a solid. Mp 161−162 °C (EtOAc/diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.34 (3H, t, J = 7.7 Hz), 1.95 (3H, s), 2.75 (2H, q, J = 7.7 Hz), 2.84 (2H, t, J = 7.3 Hz), 3.33−3.42 (2H, m), 3.48 (2H, t, J = 9.1 Hz), 4.71 (2H, t, J = 9.1 Hz), 5.57 (1H, brs), 6.38 (1H, d, J = 7.4 Hz), 8.15 (1H, d, J = 7.4 Hz); MS (ESI) m/z 274 (M + H)$^+$. Anal. ($C_{15}H_{19}N_3O_2$) C, H, N.

***N*-[2-(2-Ethyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethyl]propanamide (4e).** By a similar procedure described for **4d**, **4e** (35%) was obtained from **25c** and propionic anhydride as a solid. Mp 155−156 °C (EtOAc/diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.14 (3H, t, J = 7.7 Hz), 1.34 (3H, t, J = 7.7 Hz), 2.17 (2H, q, J = 7.7 Hz), 2.76 (2H, q, J = 7.7 Hz), 2.82−2.89 (2H, m), 3.34−3.43 (2H, m), 3.49 (2H, t, J = 9.1 Hz), 4.72 (2H, t, J = 9.1 Hz), 5.56 (1H, s), 6.39 (1H, d, J = 7.4 Hz), 8.13−8.19 (1H, m); MS (ESI) m/z 288 (M + H)$^+$. Anal. ($C_{16}H_{21}N_3O_2$) C, H, N.

***N*-[2-(2-Cyclopropyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethyl]acetamide (4f).** By a similar procedure described for **4d**, **4f** (52%) was obtained from **25d** and acetic anhydride as a solid. Mp 149−150 °C (EtOAc/diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 0.95−1.04 (4H, m), 1.87−2.02 (4H, m), 2.94 (2H, t, J = 7.0 Hz), 3.39−3.50 (4H, m), 4.71 (2H, t, J = 9.1 Hz), 5.61 (1H, brs), 6.35 (1H, d, J = 7.4 Hz), 8.06−8.10 (1H, m); MS (ESI) m/z 286 (M + H)$^+$. Anal. ($C_{16}H_{19}N_3O_2$) C, H, N.

***N*-[2-(2-Cyclopropyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethyl]propanamide (4g).** By a similar procedure described for **4d**, **4g** (33%) was obtained from **25d** and propionic anhydride as a solid. Mp 164−165 °C (EtOAc/diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 0.93−1.05 (4H, m), 1.13 (3H, t, J = 7.7 Hz), 1.87−1.99 (1H, m), 2.17 (2H, q, J = 7.7 Hz), 2.94 (2H, t, J = 7.0 Hz), 3.41−3.50 (4H, m), 4.70 (2H, t, J = 9.1 Hz), 5.59 (1H, brs), 6.35 (1H, d, J = 7.4 Hz), 8.05−8.10 (1H, m); MS (ESI) m/z 300 (M + H)$^+$. Anal. ($C_{17}H_{21}N_3O_2$) C, H, N.

***N*-{2-[2-(Trifluoromethyl)-8,9-dihydrofuro[3,2-*c*]pyrazolo [1,5-*a*]pyridin-1-yl]ethyl}acetamide (4h).** By a similar procedure described for **4d**, **4h** (76%) was obtained from **25e** and acethyl chloride as a solid. Mp 196−198 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.97 (3H, s), 2.92−3.07 (2H, m), 3.31−3.48 (2H, m), 3.62 (2H, t, J = 9.2 Hz), 4.79 (2H, t, J = 9.2 Hz), 5.71 (1H, s), 6.63 (1H, d, J = 7.7 Hz), 8.25 (1H, d, J = 7.7 Hz); MS (ESI) m/z 314 (M + H)$^+$. Anal. ($C_{14}H_{14}N_3F_3O_2$) C, H, N.

***N*-{2-[2-(Trifluoromethyl)-8,9-dihydrofuro[3,2-*c*]pyrazolo [1,5-*a*]pyridin-1-yl]ethyl}propanamide (4i).** By a similar procedure described for **4h**, **4i** (61%) was obtained from **25e** and propionyl chloride as a solid. Mp 186−188 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.14 (3H, t, J = 7.7 Hz), 2.19 (2H, q, J = 7.7 Hz), 2.91−3.06 (2H, m), 3.34−3.48 (2H, m), 3.64 (2H, t, J = 9.2 Hz), 4.78 (2H, t, J = 9.2 Hz), 5.68 (1H, s), 6.63 (1H, d, J = 7.4 Hz), 8.25 (1H, d, J = 7.4 Hz); MS (ESI) m/z 328 (M + H)$^+$. Anal. ($C_{15}H_{16}N_3F_3O_2$) C, H, N.

7-Phenyl-1,2-dihydrofuro[2,3-*e*]imidazo[1,2-*a*]pyridine (6). A mixture of 2,3-dihydrofuro[3,2-*b*]pyridin-5-amine **5** (500 mg, 3.67 mmol), 2-bromo-1-phenylethanone (1.10 g, 5.51 mmol), and *p*-toluenesulfonic acid monohydrate (70 mg, 0.37 mmol) in toluene (30 mL) was stirred at reflux for 12 h. After the mixture was cooled to room temperature, saturated aqueous sodium bicarbonate solution was added, extracted with EtOAc, washed with brine, dried over magnesium sulfate, filtered, and evaporated. Purification by silica gel column

chromatography (EtOAc/hexane) afforded **6** (405 mg, 47%) as a white crystal. Mp 164−165 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 3.46 (2H, t, J = 9.1 Hz), 4.80 (2H, t, J = 9.1 Hz), 6.97 (1H, d, J = 9.6 Hz), 7.27−7.36 (1H, m), 7.38−7.53 (3H, m), 7.60 (1H, s), 7.89−8.00 (2H, m); MS (ESI) m/z 237 $(M + H)^+$. Anal. ($C_{15}H_{12}N_2O$) C, H, N.

***N*,*N*-Dimethyl-1-(7-phenyl-1,2-dihydrofuro[2,3-*e*]imidazo[1,2-*a*]pyridin-8-yl)methanamine (7).** To a solution of **6** (40 mg, 0.17 mmol) in acetonitrile (1 mL) and water (400 μL), methylamine (50% solution in water, 15.1 mg, 0.17 mmol) and formalin (38% solution in water, 14.2 mg, 0.17 mmol) were added, and the mixture was stirred at 70 °C for 4 h. After the mixture was cooled to room temperature, the solvent was evaporated. Purification by silica gel column chromatography (EtOAc/hexane) afforded **7** (45 mg, 91%) as a white crystal. Mp 122−123 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 2.11 (6H, s), 3.79 (2H, s), 3.94 (2H, t, J = 9.1 Hz), 4.74 (2H, t, J = 9.1 Hz), 6.97 (1H, d, J = 9.3 Hz), 7.30−7.52 (4H, m), 7.60−7.75 (2H, m); MS (ESI) m/z 294 $(M + H)^+$. Anal. ($C_{18}H_{19}N_3O$) C, H, N.

(7-Phenyl-1,2-dihydrofuro[2,3-*e*]imidazo[1,2-*a*]pyridin-8-yl)acetonitrile (8). A mixture of **7** (293 mg, 1.00 mmol) and methyl iodide (568 mg, 4.00 mmol) in acetone (3 mL) was stirred at room temperature for 20 h. The precipitate was collected by filtration and dissolved in EtOH (5 mL) and water (5 mL). Sodium cyanide (245 mg, 5.00 mmol) was added, and the mixture was stirred at 80 °C for 5 h. After the mixture was cooled to room temperature, saturated aqueous sodium bicarbonate solution was added, extracted with EtOAc, washed with brine, dried over magnesium sulfate, filtered, and evaporated. Purification by silica gel column chromatography (EtOAc/hexane) afforded **8** (85 mg, 31%) as a white crystal. Mp 189−191 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 3.98 (2H, t, J = 8.8 Hz), 4.18 (2H, s), 4.82 (2H, t, J = 8.8 Hz), 7.05 (1H, d, J = 9.6 Hz), 7.37−7.57 (4H, m), 7.61−7.72 (2H, m); MS (ESI) m/z 276 $(M + H)^+$. Anal. ($C_{17}H_{13}N_3O$) C, H, N.

2-(2,6-Difluorophenyl)ethanol (10). A mixture of (2,6-difluorophenyl)acetic acid **9** (6.0 g, 34.9 mmol) and H_2SO_4 (2 mL) in EtOH (50 mL) was refluxed for 14 h. The solvent was removed. Water and EtOAc were added to the mixture. The organic layer was washed with saturated aqueous sodium bicarbonate solution and brine, dried over magnesium sulfate, filtered, and evaporated. The residue was dissolved in THF (10 mL), and a suspension of $LiAlH_4$ (1.79 g, 47.3 mmol) in THF (30 mL) was added at 0 °C. After being stirred at 0 °C for 1 h, the mixture was poured into aqueous saturated sodium bicarbonate solution. The solid was filtered off with EtOAc, and the filtrate was washed with water, dried over magnesium sulfate, filtered, and evaporated to afford **10** (4.4 g, 79%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.92 (1H, brs), 2.95 (2H, t, J = 6.9 Hz), 3.92 (2H, t, J = 6.9 Hz), 6.80−6.92 (2H, m), 7.10−7.22 (1H, m).

2-(2,6-Difluorophenyl)ethyl Acetate (11). Triethylamine (26.5 mL, 190 mmol) and acetyl chloride (6.48 mL, 91.1 mmol) were added to a solution of **10** (12.0 g, 75.9 mmol) in THF (500 mL) at 0 °C, and the mixture was stirred at room temperature for 3 h. Water was added, and the appropriate layer was extracted with EtOAc, washed with brine, dried over magnesium sulfate, filtered, and evaporated to afford **11** (15.1 g, 99%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.03 (3H, s), 3.02 (2H, t, J = 6.7 Hz), 4.26 (2H, t, J = 6.7 Hz), 6.72−6.93 (2H, m), 7.08−7.24 (1H, m).

2-(2,6-Difluoro-3-nitrophenyl)ethyl Acetate (12). Compound **11** (11.0 g, 55.0 mmol) was added dropwise to fuming nitric acid (30 mL) at 0 °C, and the mixture was stirred at 0 °C for 30 min. The mixture was neutralized with 8 M aqueous NaOH solution, extracted with EtOAc, washed with aqueous saturated sodium bicarbonate solution and brine, dried over sodium sulfate, filtered, and evaporated. Purification by silica gel column chromatography (EtOAc/hexane) afforded **12** (12.7 g, 94%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.04 (3H, s), 3.12 (2H, t, J = 6.4 Hz), 4.32 (2H, t, J = 6.4 Hz), 6.94−7.13 (2H, m), 7.93−8.16 (1H, m).

2-[2-({2-[(*tert*-Butoxycarbonyl)amino]ethyl}amino)-6-fluoro-3-nitrophenyl]ethyl Acetate (13). To a mixture of **12** (7.09 g, 28.9 mmol) and *tert*-butyl (2-aminoethyl)carbamate (5.10 g, 31.8 mmol) in DMF (80 mL), potassium carbonate (4.40 g, 31.8 mmol) was added. The mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with water, extracted with EtOAc, washed with brine, dried over sodium sulfate, filtered, and evaporated. Purification by silica gel column chromatography (EtOAc/hexane) afforded **13** (7.72 g, 69%) as a yellow crystal. Mp 89−91 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 2.06 (3H, s), 3.01−3.14 (2H, m), 3.24−3.44 (4H, m), 4.27 (2H, t, J = 7.3 Hz), 4.88 (1H, brs), 6.60 (1H, t, J = 9.0 Hz), 6.74 (1H, brs), 7.95 (1H, dd, J = 9.0, 6.0 Hz); MS (ESI) m/z 386 $(M + H)^+$. Anal. ($C_{17}H_{24}N_3O_6F$) C, H, N.

***tert*-Butyl {2-[(5-Nitro-2,3-dihydro-1-benzofuran-4-yl)amino]ethyl}carbamate (14).** To a solution of **13** (1.00 g, 2.59 mmol) in water (30 mL) and dioxane (30 mL), lithium hydroxide monohydrate (163 mg, 3.89 mmol) was added. The mixture was stirred at 60 °C for 16 h. After cooling to room temperature, the reaction mixture was diluted with aqueous saturated sodium bicarbonate solution, extracted with EtOAc, washed with brine, dried over sodium sulfate, filtered, and evaporated. Purification by silica gel column chromatography (EtOAc/hexane) afforded **14** (690 mg, 82%) as a yellow crystal. Mp 149−150 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 3.35 (2H, q, J = 6.0 Hz), 3.45 (2H, t, J = 8.9 Hz), 3.64 (2H, q, J = 6.0 Hz), 4.66 (2H, t, J = 8.9 Hz), 4.79 (1H, brs), 6.23 (1H, d, J = 9.2 Hz), 8.12 (1H, d, J = 9.2 Hz), 8.48 (1H, brs); MS (ESI) m/z 346 $(M + Na)^+$. Anal. ($C_{15}H_{21}N_3O_5$) C, H, N.

***tert*-Butyl {2-[(5-Amino-2,3-dihydro-1-benzofuran-4-yl)amino]ethyl}carbamate (15).** A solution of **14** (4.89 g, 15.1 mmol) in MeOH (150 mL) was stirred at room temperature in the presence of 10% Pd/C (300 mg, containing 50% water) under hydrogen atmosphere for 3 h. The catalyst was removed by filtration, and the solvent was evaporated. The residue was washed with diisopropyl ether to afford **15** (3.40 g, 77%) as a purple crystal. Mp 93−95 °C (diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 3.08−3.46 (8H, m), 4.50 (2H, t, J = 8.5 Hz), 4.87 (1H, brs), 6.23 (1H, d, J = 8.1 Hz), 6.54 (1H, d, J = 8.1 Hz); MS (ESI) m/z 294 $(M + H)^+$. Anal. ($C_{15}H_{23}N_3O_3$) C, H, N.

***tert*-Butyl [2-(2-Phenyl-7,8-dihydro-1*H*-furo[3,2-*e*]benzimidazol-1-yl)ethyl]carbamate (16).** To a solution of **15** (500 mg, 1.70 mmol) and triethyl orthobenzoate (2 mL) in THF (2 mL), *p*-toluenesulfonic acid monohydrate (32 mg, 0.17 mmol) was added. The mixture was stirred at 60 °C for 4 h. After the mixture was cooled to room temperature, the solvent was evaporated. Purification by silica gel column chromatography (EtOAc/hexane) afforded **16** (550 mg, 85%) as a white crystal. Mp 201−203 °C (MeOH/EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.33 (9H, s), 3.28 (2H, q, J = 6.2 Hz), 3.60 (2H, t, J = 8.6 Hz), 4.28−4.51 (3H, m), 4.71 (2H, t, J = 8.6 Hz), 6.85 (1H, d, J = 8.7 Hz), 7.44−7.60 (4H, m), 7.61−7.77 (2H, m); MS (ESI) m/z 380 $(M + H)^+$. Anal. ($C_{22}H_{25}N_3O_3$) C, H, N.

2-(2-Phenyl-7,8-dihydro-1*H*-furo[3,2-*e*]benzimidazol-1-yl)ethanamine Dihydrochloride (17). To a solution of **16** (500 mg, 1.32 mmol) in MeOH (20 mL), 4 M hydrogen chloride (EtOAc solution, 20 mL) was added. The mixture was stirred at room temperature for 20 min. The resulting solid was collected by filtration to afford **17** (454 mg, 98%) as a white crystal. Mp 234−237 °C (MeOH/EtOAc); ^{1}H NMR (DMSO-d_6) δ 2.94−3.24 (2H, m), 3.78 (2H, t, J = 8.8 Hz), 4.54−4.89 (4H, m), 7.13 (1H, d, J = 8.7 Hz), 7.53−7.84 (4H, m), 7.93 (2H, dd, J = 7.9, 1.5 Hz), 8.51 (3H, s). Anal. ($C_{17}H_{19}N_3OCl_2$) C, H, N, Cl.

5-Amino-2,3-dihydrofuro[3,2-*c*]pyridin-5-ium 2,4-Dinitrobenzenolate (19). *O*-(2,4-Dinitrophenyl)hydroxylamine (55.0 g, 240 mmol) was added to a solution of 2,3-dihydrofuro[3,2-*c*]pyridine **18** (23.8 g, 200 mmol) in MeCN (130 mL) at room temperature. The reaction mixture was stirred at room temperature for 3 days. After addition of Et_2O, the precipitate was collected by filtration to afford **19**

(57.0 g, 89%) as a solid. ^{1}H NMR (DMSO-d_6) δ 3.33−3.43 (2H, m), 4.94 (2H, t, J = 8.9 Hz), 6.33 (1H, d, J = 9.6 Hz), 7.37 (1H, d, J = 6.9 Hz), 7.65 (2H, s), 7.78 (1H, dd, J = 9.6, 3.2 Hz), 8.49−8.53 (1H, m), 8.56−8.61 (2H, m).

Methyl 2-Phenyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]-pyridine-1-carboxylate (21a). By a similar procedure described for **21c**, **21a** (16%) was obtained from **19** and ethyl 2-butynoate **20a** as a solid. Mp 174−175 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 3.69−3.80 (5H, m), 4.76 (2H, t, J = 9.2 Hz), 6.63 (1H, d, J = 7.1 Hz), 7.39−7.47 (3H, m), 7.63−7.69 (2H, m), 8.29−8.34 (1H, m); MS (ESI) m/z 295 $(M + H)^+$. Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

Ethyl 2-Methyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridine-1-carboxylate (21b). By a similar procedure described for **21c**, **21b** (12%) was obtained from **19** and ethyl 2-butynoate **20b** as a solid. Mp 132−133 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.39 (3H, t, J = 7.2 Hz), 2.60 (3H, s), 3.73 (2H, t, J = 9.2 Hz), 4.32 (2H, q, J = 7.2 Hz), 4.72 (2H, t, J = 9.2 Hz), 6.54 (1H, d, J = 7.4 Hz), 8.16−8.21 (1H, m); MS (ESI) m/z 247 $(M + H)^+$. Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

Ethyl 2-Ethyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridine-1-carboxylate (21c) and Ethyl 7-Ethyl-2,3-dihydrofuro[2,3-*d*]pyrazolo[1,5-*a*]pyridine-8-carboxylate (22c). Ethyl 2-pentynoate **20c** (0.91 mL, 6.87 mmol) was added to a mixture of **19** (2.00 g, 6.24 mmol) and potassium carbonate (1.21 g, 8.75 mmol) in DMF (40 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight. After addition of water, the mixture was extracted with EtOAc. The extract was dried over sodium sulfate and evaporated. Purification by silica gel column chromatography (EtOAc/hexane) afforded less polar **21c** (284 mg, 17%) as a solid and polar **22c** (244 mg, 15%) as a solid. **21c**: ^{1}H NMR ($CDCl_3$) δ 1.33 (3H, t, J = 7.4 Hz), 1.39 (3H, t, J = 7.1 Hz), 3.05 (2H, q, J = 7.4 Hz), 3.74 (2H, t, J = 9.2 Hz), 4.32 (2H, q, J = 7.1 Hz), 4.72 (2H, t, J = 9.2 Hz), 6.54 (1H, d, J = 7.1 Hz), 8.19−8.23 (1H, m). **22c**: ^{1}H NMR ($CDCl_3$) δ 1.32 (3H, t, J = 7.4 Hz), 1.40 (3H, t, J = 7.1 Hz), 3.04 (2H, q, J = 7.4 Hz), 3.24−3.34 (2H, m), 4.34 (2H, q, J = 7.1 Hz), 4.71 (2H, t, J = 8.2 Hz), 7.30 (1H, s), 8.14−8.20 (1H, m).

Methyl 2-Cyclopropyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridine-1-carboxylate (21d). By a similar procedure described for **21c**, **21d** (12%) was obtained from **19** and methyl 3-cyclopropylprop-2-ynoate **20d** as a solid. Mp 171−172 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.00−1.06 (4H, m), 2.71−2.82 (1H, m), 3.70 (2H, t, J = 9.2 Hz), 3.85 (3H, s), 4.71 (2H, t, J = 9.2 Hz), 6.51 (1H, d, J = 7.1 Hz), 8.10−8.15 (1H, m); MS (ESI) m/z 259 $(M + H)^+$. Anal. ($C_{14}H_{14}N_2O_3$) C, H, N.

Ethyl 2-(Trifluoromethyl)-8,9-dihydrofuro[3,2-*c*]pyrazolo-[1,5-*a*]pyridine-1-carboxylate (21e). By a similar procedure described for **21c**, **21e** (38%) was obtained from **19** and ethyl 4,4,4-trifluoro-2-butynoate **20e** as a solid. Mp 162−163 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.39 (3H, t, J = 7.2 Hz), 3.78 (2H, t, J = 9.3 Hz), 4.35 (2H, q, J = 7.2 Hz), 4.78 (2H, t, J = 9.3 Hz), 6.74 (1H, d, J = 7.5 Hz), 8.33 (1H, d, J = 7.5 Hz); MS (ESI) m/z 301 $(M + H)^+$. Anal. ($C_{13}H_{11}N_2F_3O_3$) C, H, N.

(2-Phenyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl) methanol (23a). By a similar procedure described for **23c**, **23a** (91%) was obtained from **21a** as a solid. Mp 188−190 °C (EtOH/diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 3.55 (2H, t, J = 9.1 Hz), 4.78 (2H, t, J = 9.1 Hz), 4.83 (2H, d, J = 4.9 Hz), 6.51 (1H, d, J = 7.1 Hz), 7.37−7.52 (3H, m), 7.80−7.85 (2H, m), 8.25−8.30 (1H, m); MS (ESI) m/z 267 $(M + H)^+$. Anal. ($C_{16}H_{14}N_2O_2$) C, H, N.

(2-Methyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)methanol (23b). By a similar procedure described for **23c**, **23b** (92%) was obtained from **21b** as a solid. Mp 173−174 °C (EtOH/diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.29 (1H, t, J = 5.1 Hz), 2.45 (3H, s), 3.48 (2H, t, J = 9.1 Hz), 4.68−4.81 (4H, m), 6.42 (1H, d, J = 7.4 Hz), 8.11−8.17 (1H, m). Anal. ($C_{11}H_{12}N_2O_2$) C, H, N.

(2-Ethyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)methanol (23c). A solution of ethyl 2-ethyl-8,9-dihydrofuro [3,2-*c*]pyrazolo[1,5-*a*]pyridine-1-carboxylate **21c** (770 mg, 2.96 mmol) in THF (30 mL) was added to a suspension of 80% $LiAlH_4$ (548 mg, 11.8 mmol) in THF (30 mL) at 0 °C. The reaction mixture was stirred at room temperature for 30 min. Sodium sulfate decahydrate (10 g) was added to the reaction mixture at 0 °C, and the mixture was filtered and evaporated. The residue was washed with diisopropyl ether to afford **23c** (485 mg, 75%) as a solid. Mp 119−120 °C (EtOAc/diisopropyl ether); ^{1}H NMR ($CDCl_3$) δ 1.35 (3H, t, J = 7.7 Hz), 2.83 (2H, q, J = 7.7 Hz), 3.48 (2H, t, J = 9.1 Hz), 4.70−4.79 (4H, m), 6.42 (1H, d, J = 7.4 Hz), 8.17 (1H, d, J = 7.4 Hz). Anal. ($C_{11}H_{14}N_2O_2$) C, H, N.

(2-Cyclopropyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)methanol (23d). By a similar procedure to that for **23c**, **23d** (93%) was obtained from **21d** as a solid. Mp 170−172 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.96−1.06 (4H, m), 1.43 (1H, brs), 1.99−2.12 (1H, m), 3.47 (2H, t, J = 9.1 Hz), 4.73 (2H, t, J = 9.1 Hz), 4.81 (2H, s), 6.38 (1H, d, J = 7.4 Hz), 8.06−8.13 (1H, m). Anal. ($C_{13}H_{14}N_2O_2$) C, H, N.

[2-(Trifluoromethyl)-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*] pyridin-1-yl]methanol (23e). By a similar procedure described for **23c**, **23e** (49%) was obtained from **21e** as a solid. Mp 144−145 °C (EtOH/hexane); ^{1}H NMR ($CDCl_3$) δ 3.56 (2H, t, J = 9.2 Hz), 4.73−4.89 (4H, m), 6.65 (1H, d, J = 7.4 Hz), 8.27 (1H, d, J = 7.4 Hz). Anal. ($C_{11}H_9N_2F_3O_2$) C, H, N.

(2-Phenyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl) acetonitrile (24a). By a similar procedure described for **24c**, **24a** (83%) was obtained from **23a** as a solid. Mp 188−189 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 3.66 (2H, t, J = 9.1 Hz), 3.85 (2H, s), 4.82 (2H, t, J = 9.1 Hz), 6.55 (1H, d, J = 7.4 Hz), 7.41−7.55 (3H, m), 7.60−7.67 (2H, m), 8.26−8.30 (1H, m); MS (ESI) m/z 276 $(M + H)^+$. Anal. ($C_{17}H_{13}N_3O$) C, H, N.

(2-Methyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)acetonitrile (24b). By a similar procedure described for **24c**, **24b** (46%) was obtained from **23b** as a solid. Mp 173−174 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 2.43 (3H, s), 3.55 (2H, t, J = 9.1 Hz), 3.72 (2H, s), 4.77 (2H, t, J = 9.1 Hz), 6.44 (1H, d, J = 7.4 Hz), 8.12−8.17 (1H, m); MS (ESI) m/z 214 $(M + H)^+$. Anal. ($C_{12}H_{11}N_3O$) C, H, N.

(2-Ethyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)acetonitrile (24c). A solution of (2-ethyl-8,9-dihydrofuro[3,2-*c*]-pyrazolo[1,5-*a*]pyridin-1-yl)methanol **23c** (480 mg, 2.20 mmol) in dichloromethane (10 mL) was added to a solution of $BF_3 \cdot Et_2O$ (836 μL, 6.60 mmol) and TMSCN (1.17 mL, 8.77 mmol) in dichloromethane (5 mL) at 0 °C under argon atmosphere. The reaction mixture was stirred at room temperature for 30 min. After addition of saturated aqueous sodium bicarbonate solution, the mixture was extracted with EtOAc. The extract was dried over sodium sulfate and evaporated. Purification by silica gel column chromatography (EtOAc/hexane) afforded **24c** (296 mg, 59%) as a solid. Mp 179−180 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.35 (3H, t, J = 7.7 Hz), 2.80 (2H, q, J = 7.7 Hz), 3.56 (2H, t, J = 9.1 Hz), 3.73 (2H, s), 4.76 (2H, t, J = 9.1 Hz), 6.44 (1H, d, J = 7.4 Hz), 8.14−8.19 (1H, m); MS (ESI) m/z 228 $(M + H)^+$. Anal. ($C_{13}H_{13}N_3O$) C, H, N.

(2-Cyclopropyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)acetonitrile (24d). By a similar procedure described for **24c**, **24d** (55%) was obtained from **23d** as a solid. Mp 139−140 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.95−1.08 (4H, m), 1.85−1.97 (1H, m), 3.55 (2H, t, J = 9.1 Hz), 3.83 (2H, s), 4.76 (2H, t, J = 9.1 Hz), 6.41 (1H, d, J = 7.4 Hz), 8.08−8.13 (1H, m); MS (ESI) m/z 240 $(M + H)^+$. Anal. ($C_{14}H_{13}N_3O$) C, H, N.

[2-(Trifluoromethyl)-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*] pyridin-1-yl]acetonitrile (24e). By a similar procedure described for **24c**, **24e** (96%) was obtained from **23e** as a solid. Mp 151−153 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 3.66 (2H, t, J = 9.3 Hz), 3.89 (2H, s), 4.84 (2H, t, J = 9.3 Hz), 6.70 (1H, d, J = 7.6 Hz), 8.29 (1H, d, J = 7.6 Hz); MS (ESI) m/z 268 $(M + H)^+$. Anal. ($C_{12}H_8N_3F_3O$) C, H, N.

2-(2-Phenyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethanamine (25a). By a similar procedure described for **25c**, **25a** (100%) was obtained from **24a** as a solid. Mp 126−127 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.01 (2H, brs), 2.81−2.93 (2H, m), 2.94−3.02 (2H, m), 3.49−3.59 (2H, m), 4.75 (2H, t, J = 9.1 Hz), 6.46 (1H, d, J = 7.1 Hz), 7.33−7.50 (3H, m), 7.65−7.74 (2H, m), 8.21−8.27 (1H, m); MS (ESI) m/z 280 $(M + H)^+$.

2-(2-Methyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethanamine (25b). By a similar procedure described for **25c**, **25b** (95%) was obtained from **24b** as an oil. ^{1}H NMR (methanol-d_4) δ 2.35 (2H, brs), 2.79 (3H, brs), 3.23−3.53 (4H, m), 4.62−4.77 (2H, m), 6.40−6.51 (1H, m), 8.03−8.18 (1H, m); MS (ESI) m/z 218 $(M + H)^+$.

2-(2-Ethyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethanamine (25c). A mixture of (2-ethyl-8,9-dihydrofuro[3,2-*c*]-pyrazolo[1,5-*a*]pyridin-1-yl)acetonitrile **24c** (120 mg, 0.528 mmol), 2 M ammonia MeOH solution (25 mL), and Raney cobalt ODHT-60 (1.2 g, washed with MeOH before use) was stirred under hydrogen atmosphere at room temperature for 12 h. After the catalyst was removed by filtration, the filtrate was evaporated to afford **25c** (120 mg, 98%) as an oil. ^{1}H NMR (methanol-d_4) δ 1.30 (3H, t, J = 7.6 Hz), 2.72−2.85 (6H, m), 3.47 (2H, t, J = 9.1 Hz), 4.71 (2H, t, J = 9.1 Hz), 6.46 (1H, d, J = 7.4 Hz), 8.15 (1H, d, J = 7.4 Hz); MS (ESI) m/z 232 $(M + H)^+$.

2-(2-Cyclopropyl-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl)ethanamine (25d). By a similar procedure described for **25c**, **25d** (97%) was obtained from **24d** as an oil. ^{1}H NMR (methanol-d_4) δ 0.87−1.06 (4H, m), 1.95−2.10 (1H, m), 2.76−2.98 (4H, m), 3.46 (2H, t, J = 9.1 Hz), 4.70 (2H, t, J = 9.1 Hz), 6.42 (1H, d, J = 7.4 Hz), 8.08 (1H, d, J = 7.4 Hz); MS (ESI) m/z 244 $(M + H)^+$.

2-[2-(Trifluoromethyl)-8,9-dihydrofuro[3,2-*c*]pyrazolo[1,5-*a*]pyridin-1-yl]ethanamine (25e). By a similar procedure described for **25c**, **25e** (95%) was obtained from **24e** as an oil. Mp 118−119 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 2.92 (4H, s), 3.54 (2H, t, J = 9.3 Hz), 4.78 (2H, t, J = 9.3 Hz), 6.61 (1H, d, J = 7.6 Hz), 8.25 (1H, d, J = 7.6 Hz); MS (ESI) m/z 272 $(M + H)^+$. Anal. ($C_{12}H_{12}N_3F_3O$) C, H, N.

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

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

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

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

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

Sleep-Promoting Action of 4d in Cats. Healthy male and female cats were purchased from Nisseiken Company, Ltd. (Tokyo, Japan) and housed individually in a room maintained at 22−24 °C with a 12 h light−dark cycle (light on at 7:00 a.m.). They were fed once daily (7:00 a.m.), and water was available ad libitum. Animals used in this study were cared in accordance with Principles and Guidelines on Animal Experimentation of Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd. (Osaka, Japan).

Surgery for electroencephalogram (EEG)/electromyogram (EMG) recordings were performed as previously described.[32] Cats were placed on a stereotaxic apparatus under general anesthesia. Electrodes for EEG recoding were implanted bilaterally in the frontal and parietal cortices and hippocampus according to the cat brain atlas of Snider and Niemer (1961). Stainless steel screws were used as cortical electrodes. The depth bipolar recording electrode consisted of insulated stainless steel wires (0.3 mm in diameter) except at the tips (0.5 mm). A pair of stainless steel wires was implanted into the back cervical muscles to record EMG data.

The cats were allowed to recover from surgery for at least 7 days before habituation to test chamber (an acrylic cage 75 cm × 65 cm × 40 cm located in a soundproof, electrically shielded room that was maintained under conditions similar to those of the home cage). The animals were connected to the recording equipment via a light flexible cable with a slip-ring connector that permitted them free movement. After we confirmed that animals slept sufficiently in the experimental room, polysomnographic recordings were conducted. The behavioral and postural changes of the animals were observed using a video camera continuously throughout the experiment. The EEG-EMG signals were acquired using an electroencephalograph (GE Yokogawa Medical System, Osaka, Japan). These recordings were performed for 8 h after drug administration. Compound **4d** (0.1 mg/kg) or vehicle was orally administered between 9:30 a.m. and 10:00 a.m. The signals were digitally filtered (EEG, 0.5−30 Hz; EMG, 20−200 Hz) and semiautomatically scored in 20 s epochs as wakefulness, SWS, or rapid eye movement sleep (REM) by a sleep scoring system (Sleep Sign, version 2; Kissei Comtec, Matsumoto, Japan). This preliminary scoring was visually inspected and corrected if necessary.

Compound **4d** was suspended in 0.5% (w/v) methylcellulose solution. The solution was administered orally to each cat in a gelatin capsule. In the control trial, each cat was given a capsule-containing vehicle. We used a crossover design with six cats per group and at least 7-day washout periods.

In Vitro Metabolic Clearance in Human and Rat Hepatic Microsomes. Hepatic microsomes from rats and humans were purchased from Xenotech, LLC (Lenexa, KS). An incubation mixture with a final volume of 0.1 mL consisted of microsomal protein in 50 mmol/L KH_2PO_4−K_2HPO_4 phosphate buffer (pH 7.4) and 1 μmol/L test compound. The concentration of hepatic microsomal protein was 0.2 mg/mL. An NADPH-generating system containing 50 mmol/L $MgCl_2$, 50 mmol/L glucose 6-phosphate, 5 mmol/L β-$NADP^+$, and 15 unit/mL glucose 6-phosphate dehydrogenase was added to the incubation mixture with a 10% volume of the reaction mixture to initiate the enzyme reaction. After the addition of the NADPH-generating system, the reaction mixture was incubated at 37 °C for 20 min. The reaction was terminated by the addition of acetonitrile equivalent to the volume of the reaction mixture. As a control, the mixture without 37 °C incubation was also prepared. All incubations were made in duplicate. Test compound in the reaction mixture was measured by an HPLC system equipped with a UV detector. For the determinations of the metabolic clearance, chromatograms were analyzed for parent compound disappearance from the reaction mixtures.

Pharmacokinetics. Test compounds were administered to nonfasted rats. After oral and iv administration, blood samples were collected and immediately centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted with 0.2% (v/v) formic acid in 10 mmol/L ammonium formate (pH 3) and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

Brain Distribution in Rats. Test compounds were administered as a cassette dosing to nonfasted rats. At 15 and 30 min after iv administration, blood and plasma samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The brain samples were homogenized in saline to obtain the brain homogenate. The plasma and brain homogenate samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted with 0.2% (v/v) formic acid in 10 mmol/L ammonium formate (pH 3) and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Elemental analysis results for compounds **2a**, **3a**, and **4a**−**i**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +81-6-6300-6546. Fax: +81-6-6300-6306. E-mail: Koike_Tatsuki@takeda.co.jp.

■ ACKNOWLEDGMENT

The authors thank Dr. Shigenori Ohkawa and Dr. Yuji Ishihara for their helpful discussions.

■ ABBREVIATIONS USED

LLE, ligand-lipophilicity efficiency; BBB, blood−brain barrier; ADME, absorption distribution metabolism, elimination; SAR, structure−activity relationship; iv, intravenous; cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; SWS, slow wave sleep; REM, rapid eye movement; NMR, nuclear magnetic resonance; TLC, thin layer chromatography; FBS, fetal bovine serum; PBS, phosphate buffered saline; EDTA, ethylenediaminetetraacetic acid; EEG, electroencephalogram; EMG, electromyogram

■ REFERENCES

(1) Reiter, R. J. Pineal Melatonin: Cell Biology of Its Synthesis and of Its Physiological Interactions. *Endocr. Rev.* **1991**, *12*, 151–180.

(2) Pevet, P.; Bothorel, B.; Slotten, H.; Saboureau, M. The Chronobiotic Properties of Melatonin. *Cell Tissue Res.* **2002**, *309*, 183–191.

(3) Scheer, F. A. J. L.; Czeisler, C. A. Melatonin, Sleep, and Circadian Rhythms. *Sleep Med. Rev.* **2005**, *9*, 5–9.

(4) Srinivasan, V.; Pandi-Perumal, S. R.; Cardinali, D. P.; Poeggeler, B.; Hardeland, R. Melatonin in Alzheimer's Disease and Other Neurodegenerative Disorders. *Behav. Brain Funct.* **2006**, *2*, 15.

(5) Medeiros, C. A.; Carvalhedo de Bruin, P. F.; Lopes, L. A.; Magalhaes, M. C.; de Lourdes Seabra, M.; de Bruin, V. M. Effect of Exogenous Melatonin on Sleep and Motor Dysfunction in Parkinson's Disease. A Randomized, Double Blind, Placebo-Controlled Study. *J. Neurol.* **2007**, *254*, 459–464.

(6) Blask, D. E.; Sauer, L. A.; Dauchy, R. T. Melatonin as a Chronobiotic/Anticancer Agent: Cellular, Biochemical, and Molecular Mechanisms of Action and Their Implications for Circadian-Based Cancer Therapy. *Curr. Top. Med. Chem.* **2002**, *2*, 113–132.

(7) Mills, E.; Wu, P.; Seely, D.; Guyatt, G. Melatonin in the Treatment of Cancer: A Systematic Review of Randomized Controlled Trials and Meta-Analysis. *J. Pineal Res.* **2005**, *39*, 360–366.

(8) Macleod, M. R.; O'Collins, T.; Horky, L. L.; Howells, D. W.; Donnan, G. A. Systematic Review and Meta-Analysis of the Efficacy of Melatonin in Experimental Stroke. *J. Pineal Res.* **2005**, *38*, 35–41.

(9) Claustrat, B.; Brun, J.; Chazot, G. The Basic Physiology and Pathophysiology of Melatonin. *Sleep Med. Rev.* **2005**, *9*, 11–24.

(10) Reppert, S. M.; Weaver, D. R.; Godson, C. Melatonin Receptors Step into the Light: Cloning and Classification of Subtypes. *Trends Pharmacol. Sci.* **1996**, *17*, 100–102.

(11) Dubocovich, M. L.; Cardinali, D. P.; Delagrange, P.; Krause, D. N.; Strosberg, A. D.; Sugden, D.; Yocca, F. D. *The IUPHAR Compendium of Receptor Characterization and Classification*; IUPHAR Media: London, 2000; pp 271−277.

(12) 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.

(13) 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*, 1211–1220.

(14) Dubocovich, M. L.; Markowska, M. Functional MT_1 and MT_2 Melatonin Receptors in Mammals. *Endocrine* **2005**, *27*, 101–110.
(15) Mor, M.; Rivara, S.; Pala, D.; Bedini, A.; Spadoni, G.; Tarzia, G. Recent Advances in the Development of Melatonin MT_1 and MT_2 Receptor Agonists. *Expert Opin. Ther. Pat.* **2010**, *20*, 1059–1077.
(16) Zlotos, D. P. Recent Advances in Melatonin Receptor Ligands. *Arch. Pharm. Chem. Life Sci.* **2005**, *338*, 229–247.
(17) Garratt, P. J.; Tsotinis, A. Synthesis of Compounds as Melatonin Agonists and Antagonists. *Mini-Rev. Med. Chem.* **2007**, *7*, 1075–1088.
(18) Hardeland, R. Investigational Melatonin Receptor Agonists. *Expert Opin. Invest. Drugs* **2010**, *19*, 747–764.
(19) Uchikawa, O.; Fukatsu, K.; Tokunoh, R.; Kawada, M.; Matsumoto, K.; Imai, Y.; Hinuma, S.; Kato, K.; Nishikawa, H.; Hirai, K.; Miyamoto, M.; Ohkawa, S. Synthesis of a Novel Series of Tricyclic Indan Derivatives as Melatonin Receptor Agonists. *J. Med. Chem.* **2002**, *45*, 4222–4239.
(20) Kato, K.; Hirai, K.; Nishiyama, K.; Uchikawa, O.; Fukatsu, K.; Ohkawa, S.; Kawamata, Y.; Hinuma, S.; Miyamoto, M. Neurochemical Properties of Ramelteon (TAK-375), a Selective MT_1/MT_2 Receptor Agonist. *Neuropharmacology* **2005**, *48*, 301–310.
(21) Miyamoto, M. Pharmacology of Ramelteon, a Selective MT_1/MT_2 Receptor Agonist: A Novel Therapeutic Drug for Sleep Disorders. *CNS Neurosci. Ther.* **2009**, *15*, 32–51.
(22) Koike, T.; Hoashi, Y.; Takai, T.; Nakayama, M.; Yukuhiro, N.; Ishikawa, T.; Hirai, K.; Uchikawa, O. 1,6-Dihydro-2*H*-indeno[5,4-*b*]furan Derivatives: Design, Synthesis and Pharmacological Characterization of a Novel Class of Highly Potent MT_2-Selective Agonists. *J. Med. Chem.* **2011**, *54*, 3436–3444.
(23) Waring, M. J. Lipophilicity in Drug Discovery. *Expert Opin. Drug Discovery* **2010**, *5*, 235–248.
(24) Hayakawa, I.; Suzuki, N.; Suzuki, K.; Tanaka, Y. Synthesis of Antimicrobial Agents. VI. Studies on the Synthesis of Furo[3,2-b]-[1,8]naphthyridine Derivatives. *Chem. Pharm. Bull.* **1984**, *32*, 4914–4922.
(25) George, P.; Rossey, G.; Depoortere, H.; Mompon, B.; Allen, J.; Wick, A. Imidazopyridines: Towards Novel Hypnotic and Anxiolytic Drugs. *Farmaco* **1991**, *46*, 277–288.
(26) Seth, P. P.; Miyaji, A.; Jefferson, E. A.; Sannes-Lowery, K. A.; Osgood, S. A.; Propp, S. S.; Ranken, R.; Massire, C.; Sampath, Ra.; Ecker, D. J.; Swayze, E. E.; Griffey, R. H. SAR by MS: Discovery of a New Class of RNA-Binding Small Molecules for the Hepatitis C Virus: Internal Ribosome Entry Site IIA Subdomain. *J. Med. Chem.* **2005**, *48*, 7099–7102.
(27) Shiotani, S.; Kurosaki, M.; Taniguchi, K.; Moriyama, M. Furopyridines. XXIV[1]. Nitration, Chlorination, Acetoxylation and Cyanation of 2,3-Dihydrofuro[2,3-*b*]-, -[3,2-*b*]-, -[2,3-*c*]- and -[3,2-*c*]pyridine *N*-Oxides. *J. Hetrocycl. Chem.* **1997**, *34*, 941–952.
(28) Legault, C.; Charette, A. B. Highly efficient synthesis of *O*-(2,4-Dinitrophenyl)hydroxylamine. Application to the Synthesis of Substituted *N*-Benzoyliminopyridinium Ylides. *J. Org. Chem.* **2003**, *68*, 7119–7122.
(29) Pawlak, J. M.; Khau, V. V.; Hutchison, D. R.; Martinelli, M. J. A Practical, Nenitzescu-Based Synthesis of LY311727, the First Potent and Selective s-PLA(2) Inhibitor. *J. Org. Chem.* **1996**, *61*, 9055–9059.
(30) Leeson, P. D.; Springthorpe, B. The Influence of Drug-like Concepts on Decision-Making in Medicinal Chemistry. *Nat. Rev. Drug Discovery* **2007**, *6*, 881–890.
(31) Wager, T. T.; Chandrasekaran, R. Y.; Hou, X.; Troutman, M. D.; Verhoest, P. R.; Villalobos, A.; Will, Y. Defining Desirable Central Nervous System Drug Space through the Alignment of Molecular Properties, in Vitro ADME, and Safety Attributes. *ACS Chem. Neurosci.* **2010**, *1*, 420–434.
(32) Miyamoto, M.; Nishikawa, H.; Doken, Y.; Hirai, K.; Uchikawa, O.; Ohkawa, S. The Sleep-Promoting Action of Ramelteon (TAK-375) in Freely Moving Cats. *Sleep* **2004**, *27*, 1319–1325.