Polycyclic *N*-Heterocyclic Compounds. Part 64[1]: Synthesis of 5-Amino-1,2,6,7-tetrahydrobenzo[*f*]furo[2,3-*c*]isoquinolines and Related Compounds. Evaluation of Their Bronchodilator Activity and Effects on Lipoprotein Lipase mRNA Expression

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Reaction of 1-(3-cyanopropoxy)-3,4-dihydronaphthalene-2-carbonitriles with potassium *tert*-butoxide gave 5-amino-1,2,6,7-tetrahydrobenzo[*f*]furo[2,3-*c*]isoquinolines *via* a Truce–Smiles rearrangement. The 5-amino group was transformed to the bromo derivatives which were allowed to react with aliphatic cyclic amines to produce amino derivatives. In contrast, a combination of imidazole and NaH gave a dihydrofuran ring cleaved product, the structure of which was confirmed by X-ray crystallographic analysis. Effects of the newly synthesized compounds on carbamylcholine chloride-induced contractions of trachea and lipoprotein lipase mRNA expression were also evaluated and found one promising bronchodilator.

Key words Truce–Smiles rearrangement; bronchodilator activity; lipoprotein lipase; ring cleavage; furo[2,3-*b*]pyridine; cyclization

The Truce–Smiles rearrangement features an intramolecular aromatic substitution reaction by a C-nucleophile making it a particularly useful rearrangement reaction because of the formation of a new C–C bonds.[2—6] Recently, we found that this rearrangement could be extended to aliphatic electron-deficient alkenes as well as aromatic substrates.[7] Thus, treatment of 2-(3-cyanopropoxy)cyclohexene-1-carbonitrile (**1**) with base gives a modest yield (15—43%) of 5-amino-1,2,6,7,8,9-hexahydrofuro[2,3-*c*]isoquinoline (**2**) by a sequence that includes a Truce–Smiles rearrangement followed by cyclization (Chart 1). This sequence was initiated by Michael addition of a carbanion nucleophile adjacent to nitrile group, followed by β-elimination and intramolecular trapping of the resulting oxyanion by the nitrile group. We then decided to investigate application of this unique procedure to derivatives of tetralin and its seven-membered ring homologue (3,4-dihydro-1-(3-cyanopropoxy)naphthalene-2-carbonitrile (**3a**) and 9-(3-cyanopropoxy)-6,7-dihydro-5*H*-benzo[*f*]cycloheptane-8-carbonitrile (**3b**), respectively). Like **1**, these compounds would be susceptible to Michael addition. Moreover the conjugated benzene ring should facilitate this attack, leading to improved yields that would make the method synthetically more practical. Herein we report the details of the Truce–Smiles type rearrangement reaction for a series of **3**. We also report a dihydrofuran ring cleavage reaction by an amide nucleophile. In addition, since current bronchodilator drugs are known to have certain detrimental side effects (*e.g.* increased heart rate) and reduced efficacy, we tested these new analogues for bronchodilator activity. The effect on lipoprotein lipase (LPL) mRNA expression, which is one of key targets for diabetes drug, was also evaluated.

Results and Discussion

Chemistry The starting compounds were readily obtained from commercially available 1-tetralone and/or benzocycloheptan-5-one, which were transformed to cyclic ketonitriles (**4a**, **4b**) in three steps.[8,9] Reaction of **4a** with 4-bromobutyronitrile in the presence of potassium carbonate in dioxane gave the desired **3a** (75%) along with a C-alkylated minor by-product **3′a** (18%) (Chart 2). In the IR spectrum of **3a**, the carbonyl band disappeared, strongly indicating that **3a** is an O-alkyl derivative, not a C-alkyl derivative. A similar reaction of **4b** in *N*,*N*-dimethylformamide (DMF) afforded the desired **3b** (71%) as the sole product.

When the conditions for the Truce–Smiles rearrangement were applied to compound **3a** using potassium *tert*-butoxide in dry dioxane, the expected 5-amino-1,2,6,7-tetrahydrobenzo-[*f*]furo[2,3-*c*]isoquinoline (**5a**) was obtained in 68% yield as the sole product. In the IR spectrum of **5a**, the cyano band disappeared and amino bands were observed at 3300 and 3180 cm^{-1}. In the ^{1}H-NMR spectrum of **5a**, two dihydrofuran methylene resonances appeared at 3.48 and 4.59 ppm, respectively. The two deuterium oxide exchangeable protons of the amino group appeared at 4.29 ppm. These data are consistent with the structure of **5a** and assignment was further

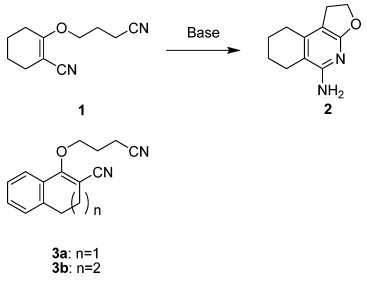

Chart 1. Substrates (**1**, **3**) with Base and Rearranged Product (**2**) from **1**

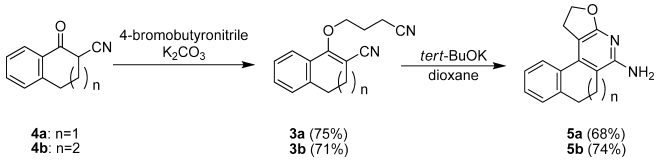

Chart 2. Synthesis of **5**

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supported by FAB-MS and elemental analysis. The improved yield compared to the yield we obtained using substrate **1** was probably due to that the presence of the conjugated benzene ring that facilitates Michael addition of the carbanion nucleophile to the benzyl position. Similar results were obtained for **3b** to give **5b** (74%).

With the goal of synthesizing additional derivatives for biological evaluation, the 5-amino groups of **5a** and **5b** were transformed to 5-chloro derivatives (**6a**, **6b**) as potential intermediates for coupling with amines. The 5-amino derivatives **5a** and **5b** were treated with sodium nitrite and conc. hydrochloric acid to give **6a** in 48% and **6b** in 51% yield, respectively (Chart 3). However, reaction of **6a** and **6b** with excess ethanolamine and ethylene glycol in the presence of potassium carbonate gave complex mixtures. Many spots were detected on TLC and no product could be isolated in either case. Since we observed unwanted side products from 5-chloro derivatives (**6a**, **6b**) with the above nucleophile reagents, we next prepared the more robust 5-bromo derivatives (**7a**, **7b**). Diazotization of **5a** with sodium nitrite, 48% hydrobromic acid and molecular bromine, using a method reported by Allen and Thirtle,[10] gave **7a** in a modest 27% yield. Disappearance of amino band and appearance of bromine atom were confirmed in the IR and MS spectral data of **7a**.

Because treatment of bromine seemed to be problematic with our substrate, we decided to see if potassium bromide would work as an effective bromide source in this reaction. This adjustment of the reaction conditions was fruitful and the yield of **7a** was increased to 66%. The 5-hydroxy (lactam) derivative (**8a**) was isolated as a by-product in 7% yield. These new conditions were also used to convert **5b** to the bromo derivative **7b** (36%) and hydroxy (lactam) **8b** (11%) derivatives.

We next explored reactions of **7a** and **7b** with ethanolamine and ethylene glycol as done for **6a** and **6b**. However we again observed many spots on TLC and no products could be isolated in either case. We then tried similar reactions with cyclic amines (Chart 4). First, reaction of **7a** with excess pyrrolidine at 80 °C gave a normal 5-substituted product (**9a**, 63%). Likewise, **7b** was converted to **9b** in 61% yield using similar conditions. Using morpholine as another cyclic amine gave a similar reaction with **7a** and **7b** to give the expected 5-substituted compounds (**10a**, **10b**) in 53% and 44% yield, respectively.

Finally, a similar reaction of **7a** with imidazole was carried out in the presence of potassium carbonate under refluxing dioxane. In this case, no reaction occurred. We therefore prepared the imidazole anion using an equivalent of sodium hydride with imidazole in dry dioxane. This was allowed to react with **7a** at 90 °C for 72 h to produce an unexpected furan ring opened compound (**11**) in 52% yield (Chart 5). In the ^{1}H-NMR spectrum of **11**, the imidazole protons were observed and MS spectrum clearly showed that the bromo substituent remained. The spectral and analytical data suggested that furan ring cleavage had occurred. The structure of **11** was confirmed unambiguously with an X-ray analysis as shown in Fig. 1, in which hydroxy group of C-2 position exists not as the lactam but as in enol because of intermolecular hydrogen bonding. In the IR spectrum of **11**, broad absorption around 2550 cm^{-1} is further indication of the hydrogen bonded hydroxy group.

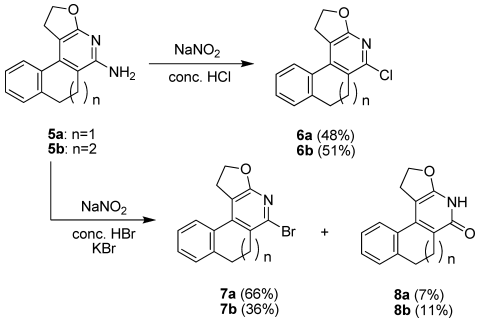

Chart 3. Preparation of **6** and **7**

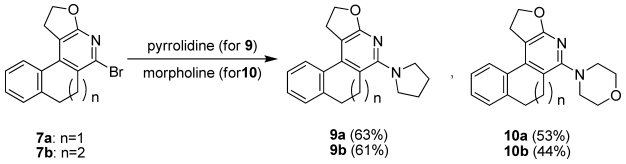

Chart 4. Preparation of **9** and **10**

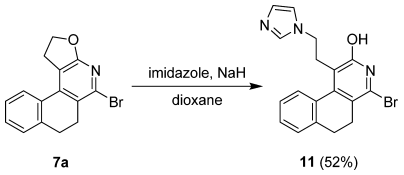

Chart 5. Synthesis of **11**

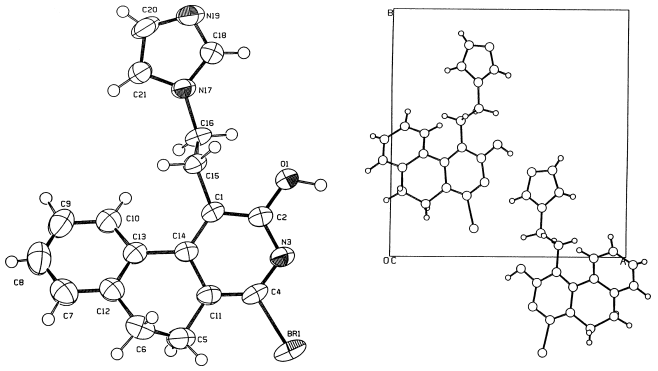

Fig. 1. ORTEP Representation of **11**

Biology The bronchodilator activities of these compounds were evaluated as part of our continuing program[11] to develop bronchodilating agents. The primary *in vitro* assay was based on the ability of test compounds to relax tracheal contraction induced by carbamylcholine. For this test, the inhibition of carbamylcholine chloride-induced contraction in trachea isolated from guinea pigs was employed. Compounds which produced more than 30% relaxation at 10.0 μg/ml, a value calculated from the percent of maximum relaxation by papaverine, were regarded as active. Their IC_{30} value then were obtained by a cumulative method. 3-Isobutyl-1-methylxanthine (IBMX)[12,13] was employed as a reference compound.

Data from these assays are shown in Table 1. Of the compounds tested, **11** showed the best activity, with potency comparable to that of IBMX.

Table 1. IC_{30} Value on Carbamylcholine Chloride-Induced Tracheal Response *in Vitro*

Compound	IC_{30} (μg/ml)	IC_{30} (μM)
5a	5.38	22.8
5b	3.18	12.6
6a	>10.0	—
6b	2.31	8.5
7a	3.72	12.3
7b	N.D.[a]	—
9a	4.60	15.7
9b	4.15	22.4
10a	6.91	13.5
10b	>10.0	—
11	1.36	3.67
IBMX	0.59	2.79

IC_{30} value shows the concentration of each compound which gives 30% relaxation to tracheal contraction by carbamylcholine chloride (1.00 μM). It was calculated from the percent of maximum relaxation produced by 100 μM papaverine. *a*) Not determined because of insolubility.

Next, effects of these compounds on lipoprotein lipase (LPL) mRNA expression in 3T3-L1 preadipocytes were examined using a new random *in vitro* screening test for hyperlipemia. Troglitazone[14] was employed as a reference compound and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen for the house keeping gene. The LPL/GAPDH mRNA ratio was evaluated as the relative values of LPL/GAPDH ratio from vehicle control group and tests were done in triplicate. None of the compounds showed activity significantly different from the vehicle control group by Dunnett's one-tailed multiple comparison test under 0.01 critical region (data not shown).

In summary, we have developed a method for the synthesis of benzo-fused 5-aminocycloalkeno[1,2-*d*]furo[2,3-*b*]pyridines (**5**) *via* a Truce–Smiles rearrangement. The 5-amino group was transformed to the bromo derivative, which was allowed to react with cyclic amines to give amino-substituted derivatives. In contrast, reaction with the imidazolate anion form by treatment of imidazole with sodium hydride gave a dihydrofuran ring cleaved product. Its structure was confirmed by X-ray crystallographic analysis. Compound **11** showed bronchodilator activity comparable to IBMX. On the other hand, none showed meaningful effects on lipoprotein lipase mRNA expression. We are currently exploring their structure–activity relationships for further development of bronchodilator active compounds.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus, and are uncorrected. Elemental analyses were performed on a Yanagimoto MT-5 CHN Corder elemental analyzer. The FAB-mass and EI-mass spectra were obtained on a VG 70 mass or a Micromass Autospec-OA-Tof spectrometer and *m*-nitrobenzyl alcohol or glycerol was used as the matrix. The IR spectra were recorded on a Japan Spectroscopic FT/IR-200 spectrophotometer and frequencies are expressed in cm^{-1}. The ^{1}H-NMR spectra were recorded on a Varian VXR-200 instrument operating at 200 MHz with tetramethylsilane as an internal standard. Chemical shifts are given in ppm (δ) and *J* values in Hz, and the signals are designated as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; quin, quintet; br, broad; m, multiplet. Column chromatography was performed on silica gel (IR-60-63-210-W, Daiso). TLC was carried out on Kieselgel 60F254 (Merck) or silica gel 70FM (Wako). 1-Oxo-1,2,3,4-tetrahydronaphthalene-2-carbonitrile (**4a**)[8] and 5-oxo-6,7,8,9-tetrahydro-5*H*-benzocycloheptene-6-carbonitrile (**4b**)[9] were synthesized according to the literature.

The structure on X-ray analysis was solved by direct methods with MITHRIL[15] and DIRDIF[16] and refined by the full-matrix least squares method by using TEXSAN.[17] H atoms were found by difference synthesis and refined isotopically. The displacement ellipsoids were drawn with the aid of ORTEP II.[18] Most of the calculations were performed on a VAX 3100 computer using TEXSAN at the X-ray Laboratory of Okayama University.

1-(3-Cyanopropoxy)-3,4-dihydronaphthalene-2-carbonitrile (3a) and 2-(3-Cyanopropyl)-1-oxo-1,2,3,4-tetrahydronaphthalene-2-carbonitrile (3′a) To a solution of **4a** (20.0 g, 117 mmol) and 4-bromobutyronitrile (26.0 g, 176 mmol) in dry dioxane (75 ml) was added K_2CO_3 (57.2 g, 414 mmol) and the mixture was then refluxed for 48 h. Ice water (500 ml) was added and the aqueous mixture was extracted with benzene (500 ml×2). The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and then evaporated *in vacuo*. The residue was chromatographed on silica gel. The eluate of petroleum ether–diethyl ether (1 : 1) was evaporated to give **3a** (20.8 g, 75%) as a colorless viscous oil. ^{1}H-NMR ($CDCl_3$) δ: 2.20 (2H, quin, *J*=6.2 Hz, $OCH_2C\underline{H}_2$), 2.57 (2H, t, *J*=7.8 Hz, H-3), 2.67 (2H, t, *J*=6.2 Hz, CH_2CN), 2.85 (2H, t, *J*=7.8 Hz, H-4), 4.47 (2H, t, *J*=6.2 Hz, OCH_2), 7.27 (3H, m, H-5, 6, 7), 7.38 (1H, m, H-8). IR ($CHCl_3$) cm^{-1}: 2220 (shoulder), 2200 (CN). FAB-MS *m/z*: 239 (MH^+). FAB-HR-MS *m/z*: 239.1183 (Calcd for $C_{15}H_{15}N_2O$: 239.1184). The eluate of petroleum ether–diethyl ether (1 : 3) was evaporated and the residue was recrystallized from diethyl ether to give **3′a** (5.07 g, 18%) as colorless prisms, mp 95 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.98, 2.24, 2.54, 3.09, 3.26 (3H, 2H, 3H, 1H, 1H, each m, H-3, 4, $CH_2CH_2CH_2CN$), 7.29 (1H, br d, *J*=7.6 Hz, H-5), 7.38 (1H, br t, *J*=7.6 Hz, H-7), 7.58 (1H, td, *J*=7.6, 1.5 Hz, H-6), 8.12 (1H, dd, *J*=7.6, 1.5 Hz, H-8). IR (KBr) cm^{-1}: 2250, 2230 (CN), 1700 (CO). FAB-MS *m/z*: 239 (MH^+). *Anal.* Calcd for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.74; H, 6.03; N, 11.68.

9-(3-Cyanopropoxy)-6,7-dihydro-5*H*-benzocycloheptene-8-carbonitrile (3b) To a solution of **4b** (10.0 g, 54.0 mmol), 4-bromobutyronitrile (9.61 g, 64.9 mmol), and KI (269 mg, 1.62 mmol) in dry DMF (50 ml) was added K_2CO_3 (22.4 g, 162 mmol) and the reaction mixture was stirred at 80 °C for 70 min. After evaporation of DMF *in vacuo*, water (300 ml) was poured into the reaction mixture which was then acidified with 6 N HCl. The mixture was extracted with ethyl acetate (300 ml×3) and the combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and then evaporated *in vacuo*. The residue was chromatographed on silica gel. The eluate of petroleum ether–diethyl ether (3 : 2) was evaporated and the residue was recrystallized from diethyl ether to give **3b** (9.63 g, 71%) as colorless needles, mp 80—81 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.80—2.45 (6H, m, H-6, 7, $OCH_2C\underline{H}_2$), 2.68, 2.71 (each 2H, each t, *J*=6.7, 6.4 Hz, H-5, CH_2CN), 4.02 (2H, t, *J*=6.4 Hz, OCH_2), 7.34 (4H, m, H-1, 2, 3, 4). IR (KBr) cm^{-1}: 2250, 2210 (CN). FAB-MS *m/z*: 253 (MH^+). *Anal.* Calcd for $C_{16}H_{16}N_2$: C, 76.16; H, 6.39; N, 11.10. Found: C, 76.50; H, 6.51; N. 11.02.

5-Amino-1,2,6,7-tetrahydrobenzo[*f*]furo[2,3-*c*]isoquinoline (5a) To a preheated solution of **3a** (16.7 g, 70.1 mmol) in dry dioxane (50 ml) at *ca.* 95 °C was added *tert*-BuOK (11.8 g, 105 mmol) in one portion and the mixture was refluxed for 1 h. After evaporation of dioxane *in vacuo*, ice water (500 ml) was added, the aqueous mixture was then extracted with ethyl acetate (500 ml×3). The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and then evaporated *in vacuo*. The residue was chromatographed on silica gel. The eluate of benzene–ethyl acetate (4 : 1) was evaporated and the residue was recrystallized from ethyl acetate to give **5a** (11.4 g, 68%) as brown prisms, mp 235—237 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.56, 2.78 (each 2H, each m, H-6, 7), 3.48 (2H, t, *J*=8.4 Hz, H-1), 4.29 (2H, br s, D_2O exchangeable, NH_2), 4.59 (2H, t, *J*=8.4 Hz, H-2), 7.31 (3H, m, H-8, 9, 10), 7.64 (1H, m, H-11). IR (KBr) cm^{-1}: 3300, 3180 (NH_2). FAB-MS *m/z*: 239 (MH^+). *Anal.* Calcd for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.54; H, 5.88; N, 11.77.

5-Amino-1,2,7,8-tetrahydro-6*H*-benzo[6,7]cyclohepta[1,2-*d*]furo[2,3-*b*]pyridine (5b) To a preheated solution of **3b** (11.3 g, 44.8 mmol) in dry dioxane (50 ml) at *ca.* 95 °C was added *tert*-BuOK (6.04 g, 53.8 mmol) in one portion and the mixture was refluxed for 1 h. After evaporation of dioxane, ice water (500 ml) was added, the mixture was extracted with ethyl acetate (500 ml×3). The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and then evaporated *in vacuo*. The residue was chromatographed on silica gel. The eluate of benzene–ethyl acetate (3 : 2) was evaporated and the residue was recrystallized from ethyl acetate to give **5b** (7.85 g, 74%) as brown prisms, mp 222—225 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.06, 2.49, 2.88, 3.44 (3H, 3H, 1H, 1H, each m, H-1, 6, 7, 8), 4.32 (2H, br s, D_2O exchangeable, NH_2), 4.56 (2H, m, H-2), 7.30 (4H, m, H-9, 10, 11, 12). IR (KBr) cm^{-1}: 3280, 3170 (NH_2). FAB-MS *m/z*: 253 (MH^+). *Anal.* Calcd for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10. Found: C, 75.81; H, 6.43; N, 10.95.

5-Chloro-1,2,6,7-tetrahydrobenzo[*f*]furo[2,3-*c*]isoquinoline (6a) To a

suspension of **5a** (10.0 g, 42.0 mmol) in conc. HCl (150 ml) under 5 °C was added dropwise $NaNO_2$ (20.3 g, 294 mmol) in water (58.3 ml) during 1 h. Water (300 ml) was added to the reaction mixture which was then basified with $NaHCO_3$. The precipitate was collected by filtration and chromatographed on silica gel. The eluate of benzene–ethyl acetate (9 : 1) was evaporated and the residue was recrystallized from cyclohexane–benzene to give **6a** (5.18 g, 48%) as colorless needles, mp 163 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.70 (4H, m, H-6, 7), 3.56 (2H, t, J=8.5 Hz, H-1), 4.68 (2H, t, J=8.5 Hz, H-2), 7.32 (3H, m, H-8, 9, 10), 7.64 (1H, m, H-11). FAB-MS *m/z*: 258 (MH^+), 260 (MH^++2). *Anal.* Calcd for $C_{15}H_{12}ClNO$: C, 69.91; H, 4.69; N, 5.43. Found: C, 70.01; H, 4.89; N, 5.43.

5-Chloro-1,2,7,8-tetrahydro-6*H*-benzo[6,7]cyclohepta[1,2-*d*]furo[2,3-*b*]pyridine (6b) To a suspension of **5b** (5.00 g, 19.8 mmol) in conc. HCl (150 ml) under 5 °C was added dropwise $NaNO_2$ (9.59 g, 139 mmol) in water (27.4 ml) during 0.5 h. Water (200 ml) was added to the reaction mixture which was then basified with $NaHCO_3$. The precipitate was collected by filtration and then chromatographed on silica gel. The eluate of benzene–ethyl acetate (9 : 1) was evaporated and the residue was recrystallized from cyclohexane–benzene to give **6b** (2.75 g, 51%) as colorless needles, mp 137—138 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.10, 2.35, 2.62, 3.00, 3.53 (3H, 1H, 1H, 2H, 1H, each m, H-1, 6, 7, 8), 4.65 (2H, m, H-2), 7.31 (4H, m, H-9, 10, 11, 12). FAB-MS *m/z*: 272 (MH^+), 274 (MH^++2). *Anal*. Calcd for $C_{16}H_{14}ClNO$: C, 70.72; H, 5.19; N, 5.15. Found: C, 70.96; H, 5.37; N, 5.18.

5-Bromo-1,2,6,7-tetrahydrobenzo[*f*]furo[2,3-*c*]isoquinoline (7a) and 1,2,6,7-Tetrahydrobenzo[*f*]furo[2,3-*c*]isoquinolin-5(4*H*)-one (8a) To a suspension of **5a** (3.00 g, 12.6 mmol) and KBr (15.0 g, 126 mmol) in 48% HBr aq. (50 ml) was added dropwise $NaNO_2$ (6.09 g, 88.3 mmol) in water (17.4 ml) during 1 h under 5 °C. Ice water (300 ml) was added to the reaction mixture which was then neutralized with Na_2CO_3 and extracted with ethyl acetate (300 ml×3). The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and then evaporated *in vacuo*. The residue was chromatographed on silica gel. The eluate of benzene–ethyl acetate (19 : 1) was evaporated and the residue was recrystallized from cyclohexane–benzene to give **7a** (2.51 g, 66%) as colorless needles. A further eluant of ethyl acetate was evaporated and the residue was recrystallized from benzene to give **8a** (217 mg, 7%) as colorless prisms. **7a**: mp 188 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.89 (4H, m, H-6, 7), 3.53 (2H, t, J=8.5 Hz, H-1), 4.68 (2H, t, J=8.5 Hz, H-2), 7.34 (3H, m, H-8, 9, 10), 7.64 (1H, m, H-11). FAB-MS *m/z*: 302 (MH^+), 304 (MH^++2). *Anal*. Calcd for $C_{15}H_{12}BrNO$: C, 59.62; H, 4.00; N, 4.64. Found: C, 59.72; H, 4.17; N, 4.59. **8a**; mp 256—258 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.85 (4H, s, H-6, 7), 3.51 (2H, t, J=8.5 Hz, H-1), 4.70 (2H, t, J=8.5 Hz, H-2), 7.31 (3H, m, H-8, 9, 10), 7.66 (1H, m, H-11). IR (KBr) cm^{-1}: 3100—2500 (br, NH), 1620 (CO). FAB-MS *m/z*: 240 (MH^+). *Anal.* Calcd for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85. Found: C, 75.69; H, 5.77; N, 5.93.

5-Bromo-1,2,7,8-tetrahydro-6*H*-benzo[6,7]cyclohepta[1,2-*d*]furo[2,3-*b*]pyridine (7b) and 1,2,7,8-Tetrahydro-6*H*-benzo[6,7]cyclohepta[1,2-*d*]furo[2,3-*b*]pyridin-5(4*H*)-one (8b) To a suspension of **5b** (5.00 g, 19.7 mmol) and KBr (16.5 g, 139 mmol) in 48% HBr aq. (60 ml) was added dropwise $NaNO_2$ (9.56 g, 139 mmol) in water (26.7 ml) during 1 h under 5 °C. Ice water (500 ml) was added to the reaction mixture which was then neutralized with Na_2CO_3. The precipitate was collected by filtration and then chromatographed on silica gel. The eluate of benzene–ethyl acetate (37 : 3) was evaporated and the residue was recrystallized from cyclohexane–benzene to give **7b** (2.26 g, 36%) as colorless needles. A further eluant of acetone was evaporated and the residue was recrystallized from ethanol to give **8b** (566 mg, 11%) as colorless prisms. **7b**: mp 144 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.10, 2.32, 2.62, 2.98, 3.51 (3H, 1H, 1H, 2H, 1H, each m, H-1, 6, 7, 8), 4.65 (2H, m, H-2), 7.33 (4H, m, H-9, 10, 11, 12). FAB-MS *m/z*: 316 (MH^+), 318 (MH^++2). *Anal.* Calcd for $C_{16}H_{14}BrNO$: C, 60.78; H, 4.46; N, 4.43. Found: C, 60.82; H, 4.56; N, 4.23. **8b**: mp 295 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.66—1.82, 1.90—2.06, 2.16—2.38, 2,61, 2.70—2.84, 3.59 (1H, 2H, 1H, 1H, 2H, 1H, each m, H-1, 6, 7, 8), 4.37—4.65 (2H, m, H-2), 7.32—7.47 (4H, m, H-9, 10, 11, 12). IR (KBr) cm^{-1}: 3100—2500 (br, NH), 1625 (CO). FAB-MS *m/z*: 254 (MH^+). *Anal.* Calcd for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 76.07; H, 6.23; N, 5.57.

5-(Pyrrolidin-1-yl)-1,2,6,7-tetrahydrobenzo[*f*]furo[2,3-*c*]isoquinoline (9a) A solution of **7a** (300 mg, 0.993 mmol) in pyrrolidine (2.0 ml) was heated at 80 °C for 37 h under stirring. Ice water (150 ml) was added to the reaction mixture which was then extracted with ethyl acetate (100 ml×3). The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and then evaporated *in vacuo*. The residue was chromatographed on silica gel and the eluate of benzene–ethyl acetate (19 : 1) was evaporated and the residue was recrystallized from diluted ethanol to give **9a** (183 mg, 63%) as colorless prisms, mp 125—126 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.90 (4H, m, H-3′, 4′), 2.76 (4H, br s, H-6, 7), 3.46 (6H, m, H-1, H-2′, 5′), 4.59 (2H, t, J=8.4 Hz, H-2), 7.30 (3H, m, H-8, 9, 10), 7.58 (1H, m, H-11). EI-MS *m/z*: 292 (M^+). *Anal.* Calcd for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58. Found: C, 78.11; H, 6.99; N, 9.63.

5-(Pyrrolidin-1-yl)-1,2,7,8-trahydro-6*H*-benzo[6,7]cyclohepta[1,2-*d*]furo[2,3-*b*]pyridine (9b) A solution of **7b** (200 mg, 0.633 mmol) in pyrrolidine (2.0 ml) was heated at 80 °C for 24 h under stirring. Ice water (100 ml) was poured into the reaction mixture which was then extracted with ethyl acetate (100 ml×3). The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and then evaporated *in vacuo*. The residue was chromatographed on silica gel. The eluate of benzene–ethyl acetate (19 : 1) was evaporated and the residue was recrystallized from diluted ethanol to give **9b** (119 mg, 61%) as colorless prisms, mp 128—129 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.68—2.24, 2.57, 2.73—2.99, 3.41—3.58 (7H, 2H, 2H, 1H, each m, H-1, 6, 7, 8, H-3′, 4′), 3.32, 3.72 (each 2H, each m, H-2′, 5′), 4.40—4.69 (2H, m, H-2), 7.32 (4H, m, H-9, 10, 11, 12). FAB-MS *m/z*: 307 (MH^+). *Anal.* Calcd for $C_{20}H_{22}N_2O$: C, 78.40; H, 7.24; N, 9.14. Found: C, 78.37; H, 7.27; N, 9.03.

5-Morpholino-1,2,6,7-tetrahydrobenzo[*f*]furo[2,3-*c*]isoquinoline (10a) A solution of **7a** (300 mg, 0.993 mmol) in morpholine (2.0 ml) was heated at 100 °C for 48 h. Ice water (200 ml) was added to the reaction mixture and the resulting precipitate was collected by filtration and chromatographed on silica gel. The eluate of benzene–ethyl acetate (4 : 1) was evaporated and the residue was recrystallized from ethanol to give **10a** (163 mg, 53%) as colorless prisms, mp 191—192 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.76 (4H, m, H-6, 7), 3.13 (4H, t, J=4.7 Hz, H-3′, 5′), 3.50 (2H, t, J=8.5 Hz, H-1), 3.85 (2H, t, J=4.7 Hz, H-2′, 6′), 4.62 (2H, t, J=8.5 Hz, H-2), 7.30 (3H, m, H-8, 9, 10), 7.60 (1H, m, H-11). FAB-MS *m/z*: 309 (MH^+). *Anal.* Calcd for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N 9.08. Found: C, 74.11; H, 6.63; N, 8.97.

5-Morpholino-1,2,7,8-tetrahydro-6*H*-benzo[6,7]cyclohepta[1,2-*d*]furo[2,3-*b*]pyridine (10b) A solution of **7b** (200 mg, 0.633 mmol) in morpholine (2.0 ml) was heated at 80 °C for 169 h under stirring. Ice water (150 ml) was poured into the reaction mixture which was then extracted with ethyl acetate (100 ml×3). The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and then evaporated *in vacuo*. The residue was chromatographed on silica gel. The eluate of benzene–ethyl acetate (4 : 1) was evaporated and the residue was recrystallized from diluted methanol to give **10b** (90.0 mg, 44%) as colorless prisms, mp 168—169 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.15, 2.44, 2.60, 2.88—3.34 (3H, 1H, 1H, 3H, each m, H-1, 6, 7, 8), 3.21 (4H, m, H-3′, 5′), 3.90 (4H, m, H-2′, 6′), 4.60 (2H, m, H-2), 7.32 (4H, m, H-9, 10, 11, 12). FAB-MS *m/z*: 323 (MH^+). *Anal.* Calcd for $C_{20}H_{22}N_2O_2$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.45; H, 6.91; N, 8.59.

4-Bromo-2-hydroxy-1-[2-(imidazol-1-yl)ethyl]-5,6-dihydrobenzo[*f*]isoquinoline (11) To a solution of imidazole (675 mg, 9.92 mmol) in dry dioxane (3.0 ml) was added NaH (238 mg, 9.92 mmol) until generation of H_2 gas stopped. Compound **7a** (300 mg, 0.993 mmol) was added to the mixture and heated at 90 °C for 72 h under stirring. After evaporation of solvent, ice water (100 ml) was poured into the mixture which was then extracted with ethyl acetate (100 ml×3). The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and then evaporated *in vacuo*. The residue was chromatographed on silica gel. The eluate of ethyl acetate–acetone (1 : 2) was evaporated and the residue was recrystallized from ethanol to give **11** (190 mg, 52%) as colorless needles, mp 234—235 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.70 (4H, br s, H-5, 6), 3.40 (2H, t, J= 7.3 Hz, H-1′), 4.39 (2H, t, J=7.3 Hz, H-2′), 6.82, 6.97 (each 1H, each s, imidazole-H-4′, 5′), 7.33 (4H, m, H-7, 8, 9, 10), 7.42 (1H, s, imidazole-H-2′). IR (KBr) cm^{-1}: 2550 (br, OH). FAB-MS *m/z*: 370 (MH^+), 372 (MH^++2). *Anal.* Calcd for $C_{18}H_{16}BrN_3O$: C, 58.39; H, 4.36; N, 11.35. Found: C, 58.62; H, 4.55; N, 11.31.

Crystal Structure Analysis of **11**[19]: Crystal data: $C_{18}H_{16}N_3OBr$; M= 370.25; monoclinic, space group $P2_1/c$, a=13.233(2), b=14.022(7), c= 8.551(5) Å, β= 92.98(2)°, V=1584(2) Å^3; Z=4; Dc=1.552 g cm^{-3}. A crystal of size 0.450×0.380×0.530 mm was examined by the ω–2θ scan technique using graphite-monochromated Mo$K\alpha$ radiation (λ=0.71069 Å). Cell dimensions were obtained from 25 reflections (21.0<2θ<22.0°). A total of 3125 unique data points were obtained and 2038 of these having I>2.00σ(I) were used in the refinement; R=0.044, Rw=0.031, S=2.43.

Carbamylcholine Chloride-Induced Tracheal Response *in Vitro* This assay was performed according to the literature procedure.[11]

Lipoprotein Lipase (LPL) mRNA Expression in 3T3-L1 Preadipocytes Dulbecco's Modified Eagle Medium (DMEM, phenol red free) was from Sigma, fetal calf serum (FCS) was from Boehringer manheim, dexamethasone from Wako, reverse transcriptase (SuperScript™ II) form

Gibco Brl, Taq DNA polymerase (TaKaRa Ex Taq™) was obtained from Takara, Isogen® from Nippongene, multigel 10/20 and 2D-Silver Staining Reagent II (Daiichi) from Daiichi Kagaku Yakuhin. Mouse derived cloned 3T3-L1 cells as preadipocyte from Japanese Cancer Research Resources Bank. The remaining chemicals and buffers obtained from various sources were molecular biology grade. Samples were dissolved in DMSO at the concentration of 10 or 50 mM, then sterized by filtration to add medium. The final concentration of DMSO was 0.2 or 1.0%.

Isolation of mRNA 3T3-L1 preadipocytes were plated in 60 mm diameter dish and incubated in DMEM/10% FCS medium until they reach confluent. Then cells were treated with samples (100 μM) in the presence of 0.1 μM dexamethasone and incubated for 2 d. Total RNA was isolated with an ISOGEN®.

Reverse Transcription (RT) of RNA RT-polymerase chain reaction (PCR) was performed for 1.0 μg of total RNA. For LPL, the sense primer 5′-TGG GAT CCA GAA ACC AGT GGG GCA-3′ and antisense primer 5′-TTA AGC TTC ATC ATG AGC AG-3′ were used to amplify a 560 bp product. RNA levels were standardized by quantification of the GAPDH as the housekeeping gene with the sense primer 5′-ATG GAT CCC TTC ATT GAC CTC AAC TA-3′ and antisense primer 5′-ATA AGC TTG TCA TAC CAG GAA ATG AG-3′ were used to amplify a 842 bp product. These primers were designed from mouse LPL cDNA[20] and rat GAPDH cDNA[21] base sequences. *Bam*HI recognition sequences were added to GAPDH antisense primer and *Hin*dIII sequences were added to sense primer.

An aliquot of the solution of RNA (1.0 μg) from the above procedure was placed in a sterile tube, along with 1.25 μM oligo(dT)$_{15}$, which total volume was 25 μl. The mixture was incubated at 70 °C for 10 min, then ice-cooled for 3 min. The above solution (4.0 μl) was mixed with first strand buffer (with 3.0 mM $MgCl_2$), 10 mM dithiothreitol, 0.5 mM deoxyribo nucleotide triphosphate (dNTP) mixtures, 2.0 U/μl RNase inhibitor, and 10 U/μl reverse transcriptase (SuperScript™). The mixture was incubated for 10 min at 25 °C, 59 min at 42 °C, 15 min at 70 °C. Storage of the RT products was at 4 °C.

PCR Conditions The cDNA solution from the previous procedure was mixed with PCR buffer (with 2.0 mM $MgCl_2$), 0.2 mM dNTP mixture, 0.1 μM Antisense primer, 0.1 μM Sense primer, 0.025 U/μl Taq DNA polymerase (Ex Taq™), which total volume was 25 μl. Mineral oil was placed on top of the aqueous layer. Then the tube was heated for 5 min at 94 °C in the thermocycler. The PCR cycles were 1 min at 94 °C, 1 min at 50 or 54 °C, and 1 min at 72 °C. After completion of 20 or 25 PCR cycles, storage of the PCR products was done at 4 °C. (LPL: 50 °C, 25 cycles, GAPDH: 54 °C, 20 cycles).

Quantitation of the Transcription Product The transcription mixture was electrophoresed on a polyacrylamide gel. The gel was stained with silver staining method, and the band was quantified with image analyzer. LPL mRNA expression was calculated by LPL/GAPDH ratio and this ratio was further evaluated with solvent blank ratio. The results were expressed as the mean value with standard error and Dunnett's one-tailed multiple comparison test was employed for judging significant difference from the vehicle control group at $p<0.01$.

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References and Notes

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