Synthesis and Biological Activity of the Metabolites of *syn*-3-Ethyl-7-methyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-Chlorobenzoate Hydrochloride

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Five metabolites of *syn*-3-ethyl-7-methyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-chlorobenzoate hydrochloride (YUTAC) (1) were prepared and examined for Na^+ current blocking activity in guinea pig ventricular myocytes. These metabolites showed lower inhibitory activities than the parent compound or were inactive.

Keywords metabolite; anti-arrhythmic agent; sodium current blocking activity; *syn*-3-ethyl-7-methyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-chlorobenzoate

syn-3-Ethyl-7-methyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-chlorobenzoate hydrochloride (YUTAC) (**1**), which was synthesized by Gedeon Richter Ltd. as an anti-arrhythmic agent,[1] is a new derivatives of bispidine and is being examined for possible clinical application as an anti-arrhythmic agent. In metabolic studies, five metabolites were isolated from rats and suggested to be mono- and di-hydroxylated compounds on the phenyl ring, a hydrolyzed compound, the N_3–N_7 methylene bridged derivative of the mother compound and a hydrolyzed product of the bridged compound.[2] To confirm their structures, we synthesized them and examined their Na^+ current blocking activity in guinea pig ventricular myocytes.

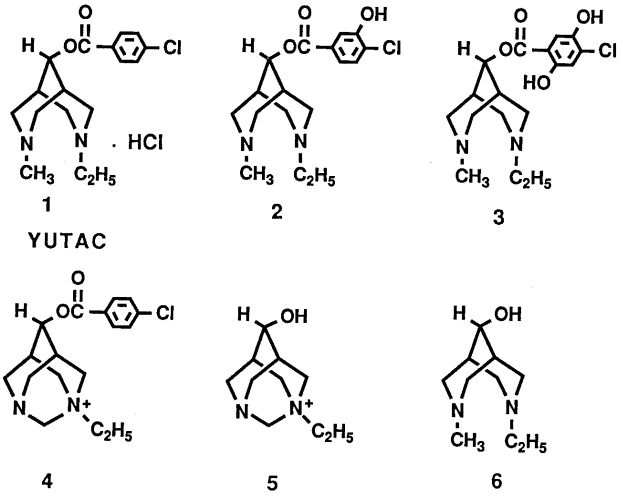

Fig. 1

Synthesis

syn-3-Ethyl-7-methyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-chloro-3-hydroxybenzoate (**2**) was synthesized according to the method shown in Chart 1. 4-Chloro-3-hydroxybenzoic acid (**7**)[3] was esterified under standard conditions to give the ester (**8**). Compound **8** was treated with isobutene in the presence of a catalytic amount of H_2SO_4 according to the method of Beyerman and Bontekoe[4] to give the *tert*-butyl ether (**9**). Compound **9** was hydrolyzed using NaOH, followed by treatment with citric acid to give **10**. Compound **2** was prepared from **10** by treatment with *syn*-3-ethyl-7-methyl-3,7-diazabicyclo[3.3.1]nonan-9-ol (**6**) and 1,3-dicyclohexylcarbodiimide (DCC) according to a modification of the method of Neises and Steglich,[5] followed by deprotection of the *tert*-butyl ether with HCl in dioxane.

syn-3-Ethyl-7-methyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-chloro-2,5-dihydroxybenzoate (**3**) was prepared according to the method shown in Chart 2. 4-Chloro-2,5-dihydroxybenzoic acid (**11**)[6] was esterified under standard conditions to give **12**. Compound **12** was treated with methoxymethyl chloride and diisopropyl ethyl amine according to the method of Stork and Takahashi[7] to give the methoxymethyl ether (**13**). Compound **13** was treated

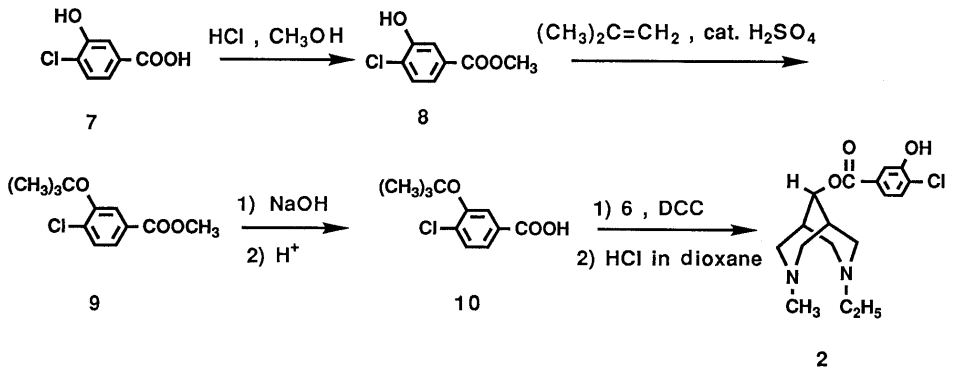

Chart 1

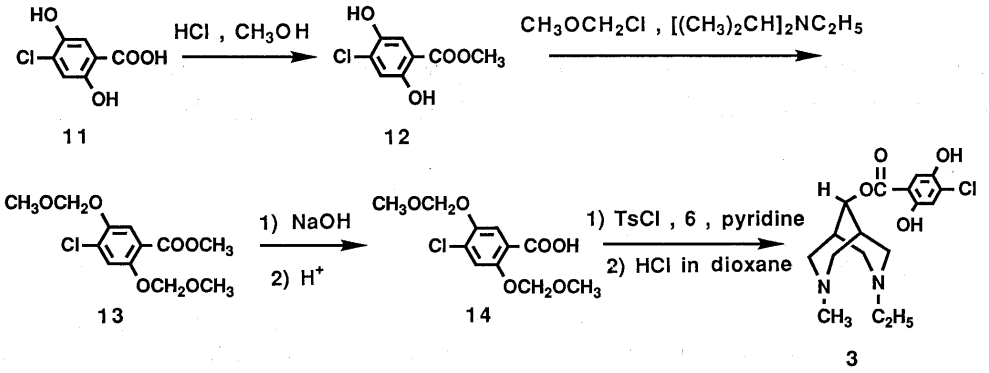

Chart 2

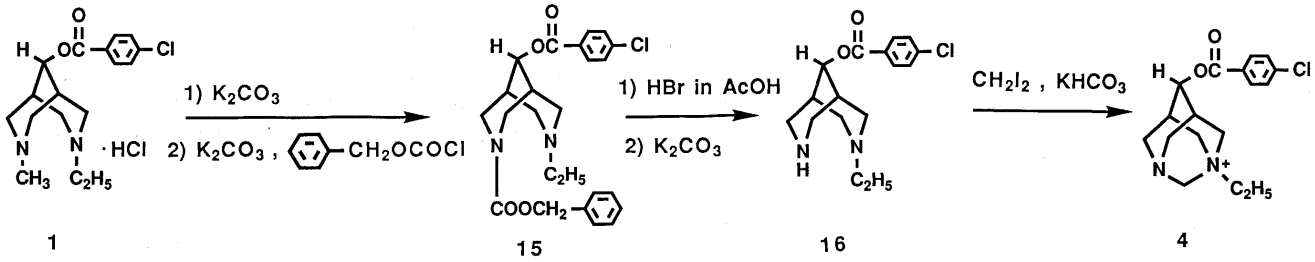

Chart 3

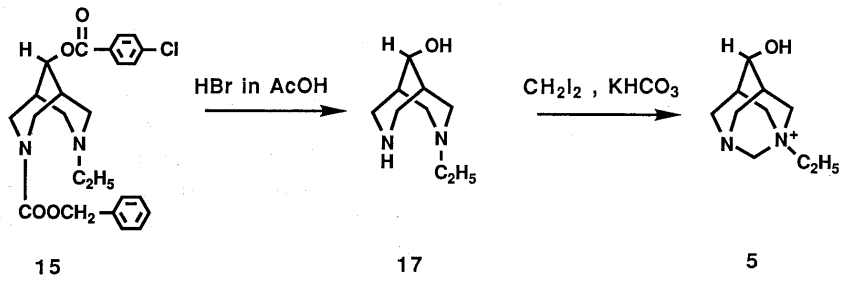

Chart 4

with NaOH, followed by neutralization with HCl to give **14**. Compound **3** was prepared from **14** by treatment with *syn*-3-ethyl-7-methyl-3,7-diazabicyclo[3.3.1]nonan-9-ol (**6**), *p*-toluenesulfonyl chloride (TsCl) and pyridine according to the method of Brewster and Ciotti, Jr.[8] followed by deprotection of the methoxymethyl ether with HCl in dioxane.

syn-9-(4-Chlorobenzoyloxy)-3-ethyl-3,7-diazatricyclo-[3.3.1.1^{3,7}]decanium (**4**) was synthesized according to the method shown in Chart 3. Compound **1** was changed to the free base form by treatment with K_2CO_3 and then treated with benzyloxycarbonyl chloride and K_2CO_3 according to the method of Flynn *et al.*[9] to give the carbamate (**15**). Compound **15** was treated with HBr in acetic acid (AcOH), followed by neutralization with K_2CO_3 to give the secondary amine (**16**). Compound **4** was prepared from **16** by treatment with CH_2I_2 and $KHCO_3$ according to the method of Settimj *et al.*[10]

syn-9-Hydroxy-3-ethyl-3,7-diazatricyclo[3.3.1.1^{3,7}]decanium (**5**) was synthesized according to the method shown in Chart 4. Compound **15** was hydrolyzed with HBr in AcOH under heating to give **17**. Compound **5** was prepared from **17** by treatment with CH_2I_2 and $KHCO_3$ according to the method of Settimj *et al.*[10]

Each of the metabolites (**2**—**5**) isolated from biological fluids in our laboratory was identical with the corresponding synthetic compound on comparison of the proton nuclear magnetic resonance (^{1}H-NMR) spectra, the molecular ion peak in the fast atom bombardment mass spectra (FAB-MS) and the retention time on reversed-phase liquid chromatography with an ultraviolet (UV) absorbance detector.

Biological Results

The Na^+ current blocking activity of the synthetic metabolites in guinea pig ventricular myocytes was examined and the results are summarized in Tables I and II. Metabolite **2**·2HCl was about 11 times and **3**·2HCl was about 4 times less potent than the mother compound in terms of use-dependent block activity. Compounds **4**·I, **5**·I·HI and **6**·2HCl showed no activity. On the other hand, metabolite **2**·2HCl showed about 9 times and **3**·2HCl about 4 times lower potency than the mother compound in terms of total block activity. Based on the pharmacological data, it is concluded that YUTAC itself contributes predominantly to the anti-arrhythmic action

TABLE I. Use-Dependent Block of Na Current by Compound **1** and Its Metabolites in Guinea Pig Ventricular Myocytes

Compound No.	Concentration (μM)[a]						IC_{30} (μM) (95% C.L.)
	1	2	5	10	20	50	
Control	2.7±0.2% (45)						
1	11.1±1.2% (5)	30.8±2.2% (5)	51.0±4.7% (5)	85.4±5.7% (6)			2.0 (1.6—2.3)
2·2HCl				11.4±1.4% (5)	27.7±2.4% (5)	51.7±5.4% (5)	21.3 (17.9—25.4)
3·2HCl			12.7±0.7% (5)	36.4±3.6% (5)	59.8±9.1% (5)		8.3 (6.5—10.1)
4·I						7.0±0.9% (5)	
5·I·HI						3.4±0.9% (5)	
6·2HCl						4.5±0.6% (5)	

Values are mean±S.E. *a*) Numbers in parentheses represent the number of experiments.

TABLE II. Total Block of Na Current by Compound **1** and Its Metabolites in Guinea Pig Ventricular Myocytes

Compound No.	Concentration (μM)[a]						IC_{30} (μM) (95% C.L.)
	1	2	5	10	20	50	
1	17.3±2.8% (5)	45.0±4.7% (5)	67.5±6.5% (5)	95.0±2.1% (6)			1.4 (1.1—1.7)
2·2HCl				15.4±3.6% (5)	54.5±6.1% (5)	72.3±7.7% (5)	13.0 (9.0—16.8)
3·2HCl			21.2±5.3% (5)	56.3±8.0% (5)	74.9±10.0% (5)		5.8 (4.5—7.7)

Values are mean±S.E. *a*) Numbers in parentheses represent the number of experiments.

caused by Na^+ channel blocking, and that the other metabolites have very little pharmacological effect compared to the mother compound.

Experimental

All melting points were recorded with a Yanagimoto micromelting point apparatus and are uncorrected. Spectral data were obtained as follows: electron impact mass spectra (EI-MS) and FAB-MS with a JEOL JMS-DX 303 spectrometer; ^{1}H-NMR spectra with a JEOL JNM-FX 100 spectrometer (100 MHz) or a JEOL GSX-400 spectrometer (400 MHz) (using tetramethylsilane as the internal standard). Elemental analysis was carried out with a Yanagimoto MT-3 CHN Corder. Column chromatography and thin layer chromatography were carried out on Kieselgel 60 (70—230 mesh) and Kieselgel 60 F-254 (E. Merck). Visualization was accomplished with UV light or I_2 vapor.

syn-3-Ethyl-7-methyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-chlorobenzoate hydrochloride (YUTAC) (**1**) was obtained from Gedeon Richter Ltd. (Budapest, Hungary). *syn*-3-Ethyl-7-methyl-3,7-diazabicyclo[3.3.1]nonan-9-ol (**6**) was obtained by acid hydrolysis followed by neutralization of **1**.

Methyl 4-Chloro-3-hydroxybenzoate (8) Hydrogen chloride was bubbled into the solution of 4-chloro-3-hydroxybenzoic acid (**7**, 7.73 g, 0.0448 mol) in MeOH (300 ml) under ice-water cooling, followed by heating under reflux for 1 h. The reaction mixture was concentrated and extracted with Et_2O. The organic layer was washed with water, dried over $MgSO_4$ and concentrated to give **8** (7.50 g, 90%) as a brown solid. An analytically pure sample was obtained by recrystallization from benzene after silica gel column separation (eluent, MeOH : $CHCl_3$ = 1 : 20), mp 100—101 °C. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 3.91 (3H, s, CH_3), 6.03 (1H, s, OH), 7.2—7.8 (3H, m, ArH). *Anal.* Calcd for $C_8H_7ClO_3 \cdot 1/8H_2O$: C, 50.88; H, 3.87. Found: C, 50.94; H, 3.92.

Methyl 3-*tert*-Butoxy-4-chlorobenzoate (9) Isobutene (20.0 g, 0.357 mol) was absorbed into a solution of **8** (7.50 g, 0.0402 mol) and concentrated H_2SO_4 (0.2 ml) in dry dichloromethane (CH_2Cl_2) (40 ml) under ice cooling, followed by stirring at room temperature for 3 d in a sealed tube. A mixture of sodium bicarbonate (3 g), water (40 ml) and $CHCl_3$ (60 ml) was added under ice cooling. The organic layer was separated and concentrated. The residue was chromatographed on a silica gel column (eluent, $CHCl_3$). The eluate was concentrated to give **9** (8.66 g, 89%) as a pale orange oil. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.45 (9H, s, $C(CH_3)_3$), 3.91 (3H, s, CH_3), 7.2—7.8 (3H, m, ArH).

3-*tert*-Butoxy-4-chlorobenzoic Acid (10) A 2 N NaOH solution (5.00 ml, 0.0100 mol) was added to a solution of **9** (2.00 g, 0.00824 mol) in MeOH (20 ml), followed by stirring overnight at room temperature. After removal of MeOH, a mixture of citric acid (2.3 g), water (10 ml) and Et_2O (20 ml) was added to the residue under ice-water cooling. The organic layer was separated, dried over $MgSO_4$ and concentrated. Recrystallization of the product from CH_3CN gave **10** (1.67 g, 89%) as a colorless powder, mp 135—137 °C. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.46 (9H, s, $C(CH_3)_3$), 7.4—7.9 (3H, m, ArH), 9.5 (1H, br s, COOH). FAB-MS *m/z*: 229 ($M^+ + 1$). *Anal.* Calcd for $C_{11}H_{13}ClO_3$; C, 57.78; H, 5.73. Found: C, 57.70; H, 5.89.

***syn*-3-Ethyl-7-methyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-Chloro-3-hydroxybenzoate (2)** A solution of DCC (1.41 g, 0.00683 mol) in dry CH_2Cl_2 (10 ml) was added dropwise to a solution of **10** (1.42 g, 0.00621 mol) in dry CH_2Cl_2 (20 ml) below 3 °C, followed by stirring for 30 min below 3 °C. A solution of **6** (1.15 g, 0.00624 mol) in dry CH_2Cl_2 (10 ml) was added to the mixture below 3 °C. The reaction mixture was stirred overnight at room temperature, then filtered. The filtrate was washed with water, dried over $MgSO_4$ and concentrated. The residue was taken up in dioxane (10 ml) then the solution was filtered and 6.97 M HCl in dioxane (10 ml) was added at room temperature. Stirring was continued for 4 h at the same temperature, then insoluble material was collected on a filter, washed with Et_2O and recrystallized from MeOH to give **2**·2HCl (0.758 g, 30%) as an off-white solid, mp 180—181 °C (dec., with foaming). ^{1}H-NMR (400 MHz, D_2O) δ: 1.31 (3H, t, J=7 Hz, CH_3), 2.54 (3H, s, CH_3), 2.58 (2H, br s, H-1(5)), 2.99 (2H, d, J=12 Hz, H-2$(4)_{ax}$), 3.10 (2H, q, J=7 Hz, CH_2), 3.40 (2H, d, J=13 Hz, H-6$(8)_{ax}$), 3.47 (2H, d, J=12 Hz, H-2$(4)_{eq}$), 3.59 (2H, d, J=13 Hz,

H-6(8$_{eq}$), 5.24 (1H, s, H-9), 7.54 (1H, d, J=8 Hz, ArH), 7.58 (1H, dd, J=8.2 Hz, ArH), 7.62 (1H, d, J=2 Hz, ArH). FAB-MS m/z: 339 (M^+1). *Anal.* Calcd for $C_{17}H_{25}Cl_3N_2O_3 \cdot 1/2H_2O$; C, 48.53; H, 6.23; N, 6.66. Found: C, 48.30; H, 6.60; N, 6.54.

Methyl 4-Chloro-2,5-dihydroxybenzoate (12) Hydrogen chloride gas was bubbled into a solution of the 4-chloro-2,5-dihydroxybenzoic acid (**11**, 11.0 g) in MeOH (200 ml) for 20 min under ice-water cooling. The mixture was heated under reflux for 75 min and concentrated. The residue was partitioned between water and Et_2O. The organic layer was dried over $MgSO_4$ and concentrated. The solid obtained was dried under reduced pressure for 16 h, then used without further purification (8.74 g).

Methyl 4-Chloro-2,5-bis(methoxymethoxy)benzoate (13) Diisopropylethylamine (13.7 g, 0.0800 mol) was added to a solution of crude **12** (5.00 g) in dry CH_2Cl_2 (100 ml). The mixture was stirred for 10 min under ice-water cooling then chloromethyl methyl ether (6.00 ml, 0.0800 mol) was added dropwise. The mixture was stirred for 1 h under ice-water cooling, then for 16 h at room temperature. After concentration, the residue was dissolved in water and extracted with Et_2O. The organic layer was washed with 5% NaOH, dried over $MgSO_4$ and concentrated. The residue was chromatographed on a silica gel column (eluent, $CHCl_3$). The eluate was concentrated to give an off-white solid (2.25 g). ^{1}H-NMR ($CDCl_3$) δ: 3.50 (6H, s, OCH_3), 3.85 (3H, s, $COOCH_3$), 5.15 (2H, s, OCH_2O), 5.20 (2H, s, OCH_2O), 7.24 (1H, s, ArH), 7.55 (1H, s, ArH).

4-Chloro-2,5-bis(methoxymethoxy)benzoic Acid (14) Aqueous 2 N NaOH (3.88 ml) was added to a solution of **13** (2.16 g, 0.00742 mol) in MeOH (16 ml). The mixture was stirred at room temperature for 18 h and then at 45 °C for 2 h. Further 2 N NaOH (1.29 ml) was added and the whole was stirred at 45 °C for 1 h. After concentration, the residue was dissolved in water and the solution was neutralized with diluted HCl under ice cooling. The resulting solution was extracted with Et_2O. The organic layer was dried over $MgSO_4$ and concentrated to give **14** (1.75 g, 85%) as a white solid, mp 110—112 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.41 (6H, s, OCH_3), 5.21 (2H, s, OCH_2O) , 5.26 (2H, s, OCH_2O), 7.33 (1H, s, ArH), 7.53 (1H, s, ArH). *Anal.* Calcd for $C_{11}H_{13}ClO_6$; C, 47.75; H, 4.74. Found: C, 47.81; H, 5.01.

***syn*-3-Ethyl-7-methyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-Chloro-2,5-dihydroxybenzoate (3)** *p*-Toluenesulfonyl chloride (4.20 g, 0.0223 mol) was added to a solution of **14** (3.0 g, 0.0108 mol) in pyridine (12 ml) and the mixture was stirred for 2 h under ice-water cooling. Compound **6** (1.98 g, 0.0108 mol) was added, and the reaction mixture was stirred for 2 h under ice-water cooling, then at room temperature for 16 h. A mixture of ethyl acetate (60 ml), water (15 ml) and saturated K_2CO_3 solution (10 ml) was added and the organic layer was separated. The aqueous layer was extracted again with EtOAc. The combined organic layers were washed with saturated K_2CO_3 solution and water, dried with $MgSO_4$ and concentrated. The residue was co-concentrated with xylene to give a crude solid (3.86 g, FAB-MS m/z: 443 (M^+1)). The solid was dissolved in dioxane (6 ml) and 6.84 M HCl in dioxane (15 ml) was added. The mixture was stirred very vigorously for 30 min and insoluble material was collected on a filter, washed with Et_2O and recrystallized from EtOH to give **3**·2HCl (1.35 g, 31%) as an off-white solid, mp 176—177 °C (dec.). ^{1}H-NMR (400 MHz, D_2O) δ: 1.35 (3H, t, J=7 Hz, CH_3), 2.87 (3H, br s, NCH_3), 2.87 (2H, br s, H-1(5)), 3.14 (2H, d, J=13 Hz, H-6(8$_{ax}$), 3.17 (2H, q, J=7 Hz, CH_2), 3.43 (2H, d, J=13 Hz, H-2(4$_{ax}$), 3.58 (2H, d, J=13 Hz, H-6(8$_{eq}$), 3.66 (2H, d, J=13 Hz , H-2(4$_{eq}$), 5.32 (1H, s, H-9), 7.05 (1H, s, ArH), 7.43 (1H, s, ArH). FAB-MS m/z: 355 (M^+1). *Anal.* Calcd for $C_{17}H_{25}Cl_3N_2O_4 \cdot 3/2H_2O$; C, 44.89; H, 6.21; N, 6.16. Found: C, 44.77; H, 6.38; N, 5.97.

***syn*-7-Benzyloxycarbonyl-3-ethyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-Chlorobenzoate (15)** Potassium carbonate (12.0 g, 0.0868 mol) was added to *syn*-3-ethyl-7-methyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-chlorobenzoate hydrochloride (**1**, 12.0 g, 0.0334 mol) in Et_2O (800 ml) and water (80 ml) and stirred at room temperature for 10 min. The organic layer was separated, washed with water, dried over $MgSO_4$ and concentrated. The residue was dried at room temperature overnight under reduced pressure. Benzyl chloroformate (8.52 ml, 0.0479 mol) was added dropwise to a solution of the dried residue (10.3 g, 0.0319 mol) and K_2CO_3 (2.21 g, 0.0160 mol) in dry benzene (260 ml) under ice-water cooling. The mixture was stirred at room temperature for 1 h, heated under reflux for 9 h, and then filtered. The filtrate was concentrated, and the residue was chromatographed on a silica gel column (eluent, EtOH : $CHCl_3$ = 1 : 20). The product obtained from the eluate was recrystallized from iso-Pr_2O to give **15** (5.39 g, 36%) as a white powder, mp 100—102 °C. ^{1}H-NMR (100 MHz, DMSO-d_6) δ: 0.89 (3H, t, J=7 Hz, CH_3), 2.00 (2H, br s, H-1(5)), 2.13 (2H, q, J=7 Hz, CH_2), 2.4 (2H, d, J=11 Hz, H-2(4$_{ax}$), 2.82 (2H, d, J=11 Hz, H-2(4$_{eq}$), 3.6 (2H, d, J=13 Hz, H-6(8$_{ax\ or\ eq}$), 4.5 (2H, d, J=13 Hz, H-6(8$_{ax\ or\ eq}$), 5.06 (2H, s, $COOCH_2$), 5.11 (1H, t, J=5 Hz, H-9), 7.1—7.4 (5H, m, ArH), 7.76 (2H, d, J=8 Hz, ArH), 8.00 (2H, d, J=8 Hz, ArH). FAB-MS m/z: 443 (M^+1). *Anal.* Calcd for $C_{24}H_{27}ClN_2O_4$; C, 65.08; H, 6.14; N, 6.32. Found: C, 65.48; H, 6.01; N, 6.36.

***syn*-3-Ethyl-3,7-diazabicyclo[3.3.1]non-9-yl 4-Chlorobenzoate (16)** A 25% solution of HBr in AcOH (13 ml) was added to **15** (1.27 g, 0.00287 mol) under ice-water cooling, followed by stirring at room temperature for 2 h. Ethyl ether was added to the reaction mixture and insoluble material was collected (mp 241—245 °C with foaming). Potassium carbonate (1.27 g, 0.00919 mol) was added to the collected white solid in Et_2O (130 ml) and water (10 ml) and the mixture was stirred at room temperature for 10 min. The organic layer was separated and washed with water (10 ml), dried over $MgSO_4$ and concentrated to give **16** as a pale yellow solid (0.789 g, 89%). ^{1}H-NMR (100 MHz, DMSO-d_6) δ: 1.03 (3H, t, J=7 Hz, CH_3), 1.88 (2H, br s, H-1(5)), 2.24 (2H, q, J=7 Hz, CH_2), 2.54 (2H, d, J=11 Hz, H-2(4$_{ax}$), 2.80 (2H, d, J=13 Hz, H-6(8$_{ax}$), 2.92 (2H, d, J=11 Hz, H-2(4$_{eq}$), 3.14 (2H, d, J=13 Hz, H-6(8$_{eq}$), 5.05 (1H, t, J=5 Hz, H-9), 7.63 (2H, d, J=9 Hz, ArH), 8.01 (2H, d, J=9 Hz, ArH).

***syn*-9-(4-Chlorobenzoyloxy)-3-ethyl-3,7-diazatricyclo[3.3.1.1^{3,7}]decanium (4)** A mixture of **16** (0.561 g, 0.00182 mol), CH_2I_2 (1.1 ml, 0.0137 mol) and $KHCO_3$ (3.63 g, 0.0363 mol) in dry *N*,*N*-dimethylformamide (55 ml) was stirred at room temperature for 3.5 d. The reaction mixture was filtered and the filtrate was concentrated. The residue was washed with Et_2O and poured into water. Insoluble material was collected on a filter and recrystallized from EtOH–iso-Pr_2O to give **4**·I (0.555 g, 68%) as white needles, mp 149—152 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.26 (3H, t, J=7 Hz, CH_3), 2.38 (2H, br s, H-1(5)), 3.20 (2H, q, J=7 Hz, CH_2), 3.21 (2H, d, J=14 Hz, H-2(4$_{ax}$), 3.41 (2H, d, J=14 Hz, H-2(4$_{eq}$), 3.68 (2H, d, J=12 Hz, H-6(8$_{ax}$), 3.78 (2H, d, J=12 Hz, H-6(8$_{eq}$), 4.61 (2H, s, N-CH_2-N), 5.37 (1H, t, J=3 Hz, H-9), 7.63 (2H, d, J=9 Hz, ArH), 8.13 (2H, d, J=9 Hz, ArH). FAB-MS m/z: 321 (M^+1). *Anal.* Calcd for $C_{17}H_{22}ClIN_2O_2 \cdot H_2O$; C, 43.75; H, 5.18; N, 6.00. Found : C, 43.40; H, 4.94; N, 5.90.

***syn*-3-Ethyl-3,7-diazabicyclo[3.3.1]nonan-9-ol (17)** Aqueous 30% HBr (35 ml) was added slowly to a solution of **15** (4.10 g, 0.00926 mol) in AcOH (35 ml). The mixture was heated under reflux for 6 h. After cooling on ice for 20 min, insoluble material was separated by filtration and the filtrate was concentrated. The residue was washed with Et_2O and recrystallized from MeOH to give **17**·2HBr (1.30 g, 40%) as an off-white solid, mp 233—236 °C (dec.). *Anal.* Calcd for $C_9H_{20}Br_2N_2O$: C, 32.55; H, 5.74; N, 8.44. Found: C, 32.86; H, 5.74; N, 8.42.

***syn*-9-Hydroxy-3-ethyl-3,7-diazatricyclo[3.3.1.1.^{3,7}]decanium (5)** Potassium carbonate (1.20 g, 0.00868 mol) was added to a solution of **17**·2HBr (1.20 g, 0.00361 mol) in water (15 ml). The mixture was stirred for 45 min and then lyophilized. The resulting solid was extracted with EtOH. The extract was concentrated and dried under reduced pressure for 18 h. The resulting solid (0.617 g) was used without further purification. Diiodomethane (2.20 ml, 0.0272 mol) was added to a solution of the solid and $KHCO_3$ (7.26 g, 0.0725 mol) in dry *N*,*N*-dimethylformamide (50 ml), and the mixture was stirred at room temperature for 5 d. It was then filtered and the filtrate was concentrated under reduced pressure. The residue was solidified with EtOH, followed by recrystallization from EtOH to give **5**·I·HI as an off-white solid (0.743 g, 47%), mp 194—200 °C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.20 (3H, t, J=7 Hz, CH_3), 1.98 (2H, s, H-1(5)), 2.99 (2H, d, J=13 Hz, H-6(8$_{ax}$), 3.06 (2H, q, J=7 Hz, CH_2), 3.30 (2H, d, J=13 Hz, H-6(8$_{eq}$), 3.47 (2H, d, J=12 Hz, H-2(4$_{ax}$), 3.66 (2H, d, J=12 Hz, H-2(4$_{eq}$), 3.93 (1H, s, OH), 4.51 (2H, S, NCH_2N), 5.59 (1H, S, H-9). FAB-MS m/z: 183 (M^+ − I − HI). *Anal.* Calcd for $C_{10}H_{20}I_2N_2O$: C, 27.42; H, 4.60; N, 6.39. Found: C, 27.39; H, 3.99; N, 6.36.

Biological Method; Myocyte Preparation Single ventricular myocytes from guinea pig hearts were prepared by an enzymatic procedure.[11]

Solutions For measuring sodium current (I_{Na}), the external solution contained NaCl 10.0 mM, tetramethylammonium chloride 130.0 mM, $CaCl_2$ 1.8 mM, $CoCl_2$ 1.0 mM, CsCl 5.0 mM, $MgCl_2$ 1.2 mM, glucose 11.0 mM and HEPES 20.0 mM (pH 7.3, adjusted by tetramethylammonium hydroxide). The pipette solution was composed of NaF 5.0 mM, CsF 125.0 mM, K_2ATP 5.0 mM, K_2 creatine phosphate 5.0 mM, EGTA 5.0 mM, and HEPES 5.0 mM (pH 7.2, adjusted with CsOH). Compound **1** and its metabolites were added to the external bath solution

at the final concentrations indicated in the tables.

Recording Techniques I_{Na} was recorded by a whole-cell patch-clamp technique[12] using an amplifier (AXOPATCH-1D, Axon Instruments, Foster City, CA, U.S.A.). Details of the recording technique have been described in a previous report.[13] To optimize voltage control, we selected small cells and used electrodes with large tips. When filled with internal solution, the pipettes had a tip resistance in the range from 300 to 600 kΩ. Current signals were monitored by a Hitachi VC-6050 storage oscilloscope and were recorded simultaneously by a Sony PC-108M PCM data recorder. The recorded analog signals were converted into digital signals with an AD converter (TL-1 DMA INTERFACE, Axon Instruments) and the latter were stored in a personal computer.

Experimental Protocol Experiments were performed at room temperature (22—25 °C). To assess the I_{Na} block by **1** and its metabolites, trains of 50 pulses of 10 ms were applied to −10 mV from a holding potential of −100 mV at an interpulse interval of 500 ms. The pulse trains were repeated in control and test solutions. In the drug-containing solutions, an interval of at least 3 min was allowed between pulse trains to permit full recovery from block. Here, the total I_{Na} block ("total block") consisted of tonic and use-dependent block, which were defined as the diminution in the peak I_{Na} of the first depolarizing pulse after exposure to a test solution compared to that in the control and the decrease in the peak I_{Na} at the 50th pulse relative to the first in control and test solutions, respectively.

Data Analysis All the values were expressed as mean ± S.E. The 30% inhibitory concentration (IC_{30}) values and 95% confidence limits were calculated by regression analysis of the concentration-response curves.

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