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, 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 N₃-N₇ methylene bridged derivative of the mother compound and a hydrolyzed product of the bridged compound. To confirm their structures, we synthesized them and examined their Na⁺ current blocking activity in guinea pig ventricular myocytes.

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) was esterified under standard conditions to give the ester (8). Compound 8 was treated with isobutene in the presence of a catalytic amount of H₂SO₄ according to the method of Beyerman and Bontekoe 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 Sieglicb, 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) was esterified under standard conditions to give 12. Compound 12 was treated with methoxymethyl chloride and disopropyl ethyl amine according to the method of Stork and Takahashi to give the methoxymethyl ether (13). Compound 13 was treated

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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.\(^9\) followed by deprotection of the methoxymethyl ether with HCl in dioxane.

**Chart 2**

\[
\begin{align*}
11 & \xrightarrow{\text{HCl, CH}_3\text{OH}} 12 \\
13 & \xrightarrow{1) \text{NaOH}} 14 \xrightarrow{2) \text{H}^+} 3 \\
1) \text{TsCl, 6, pyridine} & \xrightarrow{1) \text{TsCl, 6, pyridine}} 15 \xrightarrow{2) \text{HCl in dioxane}} 16
\end{align*}
\]

**Chart 3**

\[
\begin{align*}
1 & \xrightarrow{1) \text{K}_2\text{CO}_3} 15 \xrightarrow{2) \text{K}_2\text{CO}_3} 17 \xrightarrow{\text{HBr in AcOH}} 16 \\
15 & \xrightarrow{\text{HBr in AcOH}} 17 \xrightarrow{\text{CH}_3\text{I}, \text{KHCO}_3} 5
\end{align*}
\]

Compound 1 was changed to the free base form by treatment with K\(_2\)CO\(_3\) and then treated with benzylxycarbonyl chloride and K\(_2\)CO\(_3\) 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\(_2\)CO\(_3\) to give the secondary amine (16). Compound 4 was prepared from 16 by treatment with CH\(_2\)I\(_2\) and KHCO\(_3\) according to the method of Settimi et al.\(^10\).

**Chart 4**

\[
\begin{align*}
15 & \xrightarrow{\text{HBr in AcOH}} 17 \xrightarrow{\text{CH}_3\text{I}, \text{KHCO}_3} 5
\end{align*}
\]

\[
\begin{align*}
\text{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\(_2\)CO\(_3\) and then treated with benzylxycarbonyl chloride and K\(_2\)CO\(_3\) 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\(_2\)CO\(_3\) to give the secondary amine (16). Compound 4 was prepared from 16 by treatment with CH\(_2\)I\(_2\) and KHCO\(_3\) according to the method of Settimi 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 I and Its Metabolites in Guinea Pig Ventricular Myocytes

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Concentration (µM)a</th>
<th>IC50 (µM) (95% C.L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.7 ± 0.2% (45)</td>
<td></td>
</tr>
<tr>
<td>2-2HCl</td>
<td>11.1 ± 1.2% (5)</td>
<td>2.0 (1.6—2.3)</td>
</tr>
<tr>
<td>3-2HCl</td>
<td>12.7 ± 0.7% (5)</td>
<td>7.0 ± 0.9% (5)</td>
</tr>
<tr>
<td>4-1</td>
<td>7.0 ± 0.9% (5)</td>
<td></td>
</tr>
<tr>
<td>5-1-HI</td>
<td>3.4 ± 0.9% (5)</td>
<td></td>
</tr>
<tr>
<td>6-2HCl</td>
<td>4.5 ± 0.6% (5)</td>
<td></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Concentration (µM)a</th>
<th>IC50 (µM) (95% C.L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.3 ± 2.8% (5)</td>
<td>1.4 (1.1—1.7)</td>
</tr>
<tr>
<td>2-2HCl</td>
<td>45.0 ± 4.7% (5)</td>
<td>13.0 (9.0—16.8)</td>
</tr>
<tr>
<td>3-2HCl</td>
<td>21.2 ± 5.3% (5)</td>
<td>5.8 (4.5—7.7)</td>
</tr>
</tbody>
</table>

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; 1H-NMR spectra with a JEOL JNM-FX 100 spectrometer (100 MHz) or a JEOL GX-400 spectrometer (400 MHz) (using tetramethylsilane as the internal standard). Elemental analysis was carried out using a Yanagimoto MT-3 CN Chom C. 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 I₂ vapor.

3-methyl-2,7,7-triazacyclo[3.3.1]nonan-9-yl 4-chlorobenzene carboxylic acid (YUTAC) (I) was obtained from Gedeon Richter Ltd. (Budapest, Hungary). syn-3-Ethyl-7-methyl-3,7-diazabicyclo[3.3.1]nonan-9-yl 4-chlorobenzene carboxylic acid (YUTAC) (I) was obtained from 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 Et₂O. The organic layer was washed with water, dried over MgSO₄ and concentrated to give 8 (7.50 g, 90%) as a brown solid.

3-tetra-Butoxy-4-chlorobenzene (9) Isobutene (20.0 g, 0.357 mol) was absorbed into a solution of 8 (7.50 g, 0.0402 mol) and concentrated H₂SO₄ (0.2 ml) in dry dichloromethane (CH₂Cl₂) (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 CHCl₃ (60 ml) was added under ice cooling. The organic layer was separated and concentrated. The residue was chromatographed on a silica gel column (eluent, CHCl₃). The eluate was concentrated to give 9 (8.66 g, 89%) as a pale orange oil. 1H-NMR (100 MHz, CDCl₃) δ: 1.45 (9H, s, C(CH₃)₃), 3.91 (3H, s, CH₃), 7.2—7.8 (3H, m, ArH). 3-tetra-Butoxy-4-chlorobenzene (10) A 2N NaOH solution (5.00 ml, 0.0100 mol) was added to a solution of 9 (2.00 g, 0.00284 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 Et₂O (20 ml) was added to the residue under ice-water cooling. The organic layer was separated, washed over MgSO₄ and concentrated. Recrystallization of the product from CH₂Cl₂ gave 10 (1.67 g, 89%) as a colorless powder, mp 135—137 °C. 1H-NMR (100 MHz, CDCl₃) δ: 1.46 (9H, s, C(CH₃)₃), 7.4—7.9 (3H, m, ArH), 9.5 (1H, brs, COOH). FAB-MS m/z: 229 (M⁺ + 1). Anal. Calcd for C₃₅H₂₃O₄C₃: 57.78; H, 5.73. Found: C, 57.70; H, 5.89.
Methyl 4-Chloro-2,5-dihydroxybenzoate (12) Hydrogen chloride gas was used to convert 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 EtOAc. The organic layer was dried over MgSO4 and concentrated. The solid obtained was dried under reduced pressure for 1 h, then used without further purification (8.74 g).

Methyl 4-chloro-2-hydroxybenzoate (13) Diisopropyl ethylcarbamate (13.7 g, 0.0800 mol) was added to a solution of crude 12 (5.00 g) in dry CH2Cl2 (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 EtOAc. The organic layer was washed with 5% NaOH, dried over MgSO4 and concentrated. The residue was chromatographed on a silica gel column (eluent, CHCl3).

The eluate was concentrated to give an off-white solid (2.25 g). 1H-NMR (CDCl3, δ): 3.50 (6H, s, OCH3), 3.85 (3H, s, COOCH3), 5.15 (2H, s, OCH2O), 5.52 (2H, s, OCH2O), 7.24 (3H, s, ArH). 1H-NMR (CDCl3, δ): 4.05 (3H, s, OCH3), 3.76 (3H, s, COOCH3), 1.25 (9H, s, CH3) (Eluent, 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 mol) 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 dilute HCl under ice cooling. The resulting solution was added with MeOH and precipitated with EtOAc. The organic layer was dried over MgSO4 and concentrated to give 14 (1.75 g, 85%) as a white solid, mp 110−112°C. 1H-NMR (DMSO-d6, δ): 3.41 (6H, s, OCH3), 5.21 (2H, s, OCH2O), 5.26 (2H, s, OCH2O), 7.33 (1H, s, ArH), 7.53 (1H, s, ArH). Anal. Calcd for C10H16O6: 47.75; H, 4.74. Found: C, 47.81; H, 4.80.

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at the final concentrations indicated in the tables.

**Recording Techniques** The I_{na} was recorded by a whole-cell patch-clamp technique 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. 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 I 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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**References**