Synthesis and Pharmacological Effects of Optically Active 2-[4-(4-Benzhydryl-1-piperazinyl)phenyl]-ethyl Methyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate Hydrochloride

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Optically active 2-[4-(4-benzhydryl-1-piperazinyl)phenyl]ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate [(S)(++)-1 and (R)-(−)-1] hydrochlorides were synthesized with high optical purities from (R)-(−) and (S)(++)-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridinecarboxylic acids [(R)-(−)-6 and (S)(++)-6], which are available from (±)-6 by optical resolution using quinidine and cinchonidine, respectively. From pharmacological investigations of (S)(++)-1 and (R)-(−)-1 such as the antihypertensive effect on spontaneously hypertensive rats and inhibition of [3H]nimodipine binding to rat cardiac membrane homogenate, the active form of 1 was defined to be the (4S)-(++)-enantiomer of 1.

Keywords 1,4-dihydropyridine; calcium antagonist; antihypertensive effect; receptor binding assay; optically active compound; AE0047

Introduction

In a previous paper,1) we reported the synthesis and antihypertensive activity of 1,4-dihydropyridine derivatives with 3-[4-(substituted amino)phenylalkyl] ester, and that among them, 2-[4-(4-benzhydryl-1-piperazinyl)phenyl]ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate dihydrochloride (I·2HCl, AE0047) had long lasting antihypertensive activity and selective vasodilating activity on canine vertebral artery. AE0047 has been selected as a promising candidate from other pharmacological investigations also. Since 1 has an asymmetric center at C-4 of the dihydropyridine ring, it is a racemic mixture (Fig. 1). We have been interested in biological activities of each enantiomer of 1 because a number of reports described that one optical isomer of 1,4-dihydropyridine derivatives showed much more potent biological activities than the other.2~8) Therefore, we synthesized each enantiomer of 1, and investigated the difference of in vivo and in vitro biological activities, namely antihypertensive activity on spontaneously hypertensive rats (SHR) and the effect on the binding of [3H]nimodipine to rat cardiac membranes.

In this paper, we report the synthesis of each enantiomer of 1 and their biological activities.

Synthesis Method for synthesis of each enantiomer of 1 is shown in Charts 1 and 2.

Optically active nicardipine3) and other such 1,4-dihydropyridine derivatives5~8) were synthesized from (+)- and (−)-6 which were obtained by an optical resolution of 1-ethoxymethylated (±)-6 and subsequent removal of the ethoxymethyl group. Tamazawa et al.9) and Kajino et al.9) independently determined the absolute configuration of 6 by X-ray crystallographic analysis of its derivatives to assign unambiguously that (±)-6 and (−)-6 have S and R configurations at C-4, respectively.

We tried to ascertain the utility of the direct optical resolution of (±)-6 reported by Genain.10) Several hundreds of grams of (±)-6 was easily prepared from ethylene
cyanohydrin (2) via 1,4-dihydropyridine 5 in a ca. 60% overall yield without chromatographical purification (Chart 1). The compound (S)-(+)-6 was obtained in a 46% yield from a quinidine salt of 6 in pure form by recrystallizing it to a constant optical rotation. On the other hand, 6 recovered from the mother liquor gave (R)-(−)-6 by recrystallizing it as cinchonidine salt (yield 48%). Treatment of (R)-(−)-6 and (S)-(+)-6 with SOCl₂ and a subsequent reaction with 7 gave (S)-(+)-1 and (R)-(−)-1, respectively. They were treated with a solution of hydrogen chloride in 1,2-dimethoxyethane (DME) in Et₂O-CH₂Cl₂ (90:10, v/v) to afford monohydrochlorides, (S)-(+)-1·HCl, 62%, mp 143.5—147°C, [α]D²⁰ + 31.0° (c = 0.50, acetone) and (R)-(−)-1·HCl, 59%, mp 145—149°C, [α]D²⁰ −31.4° (c = 0.50, acetone) (Chart 2).

**Determination of Optical Purities of (S)-(+)-1 and (R)-(−)-1**

The optical purities of (S)-(+)-1·HCl and (R)-(−)-1·HCl were determined by high performance liquid chromatography (HPLC) analyses using chiral stationary phase columns, Chiralcel OF® (4.6 mm i.d. x 250 mm) [column temperature, 50°C; mobile phase, hexane–2-propanol–trifluoroacetic acid (50:50:0.05, v/v); flow rate, 1.0 ml/min; detection, ultraviolet (UV) at 254 nm] and Chiralpak AD® (4.6 mm i.d. x 250 mm) [column temperature, 40°C; mobile phase, hexane–ethanol (9:1, v/v); flow rate, 1.0 ml/min; detection, UV at 254 nm] purchased from Daicel Chemical Industries, Tokyo. Retention times of the compounds are as follows: in Chiralcel OF®, (S)-(+)-1, 22.5 min; (R)-(−)-1, 50.3 min; in Chiralpak AD®, (S)-(+)-1, 23.1 min; (R)-(−)-1, 27.0 min. Since in both cases the peaks of antipodal compounds were not detected at all, the optical purities of each compound were concluded to be almost 100% ee.

**Pharmacological Results and Discussion**

The antihypertensive effects of (±)-AE0047 and each enantiomer of AE0047 on SHR are shown in Table I. The compound (S)-(+)-1 showed a dose dependent potent antihypertensive effect, particularly at doses of 1 and 3 mg/kg, and the duration was estimated to be over 7 h, whereas at a dose of 30 mg/kg the antihypertensive effect of (R)-(−)-1 was barely comparable to the effect of (S)-(+)-1 at a dose of 0.3 mg/kg. Although the antihypertensive effect of (±)-1

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<tr>
<th>Drug and dose (mg/kg)</th>
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<th>Reduction of SBP (%) (mean±S.E.)</th>
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<td>(R)-(−)-1</td>
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<td>41 ± 2</td>
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<td>(S)-(+)-1</td>
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<td>(R)-(−)-1</td>
<td>6</td>
<td>19 ± 3</td>
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<td>(S)-(+)-1</td>
<td>3</td>
<td>43 ± 4</td>
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<tr>
<td>(R)-(−)-1</td>
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ᵃ 0.3% Tween 80 solution.ᵇ Time after administration.

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Table I. Effects of (±)-1, (S)-(+)-1, and (R)-(−)-1 on SBP in SHR (p.o. Administration)
was less than \((S)-(+)-1\), it had enough potency at doses 3 and 10 mg/kg. The doses necessary for a 30% reduction of systolic blood pressure (SBP) \(ED_{30}\) obtained from the dose–response curves were 1.1 mg/kg in \((S)-(+)-1\) and 2.6 mg/kg in \((\pm)-1\). These results indicate that the antihypertensive effect of \((\pm)-1\) depends mostly on \((S)-(+)-1\), which is supported by the fact that the \(ED_{30}\) value of \((S)-(+)-1\) is about a half of the value of \((\pm)-1\).

We performed a receptor binding assay to evaluate pharmacological profiles of these compounds in vitro. Drug concentrations required for 50% inhibition of \(^{3}H\)nimodipine binding to rat cardiac membrane homogenate (IC\(_{50}\)) of these compounds and \((\pm)-n$-nicardipine are shown in Table II. The compound \((S)-(+)-1\) shows the highest affinity to the receptor, and the affinity was decreased in the following order; \((\pm)-1\) (0.26 nm) > \((\pm)-n$-nicardipine (0.48 nm) > \((R)-(-)-1\) (18.2 nm). The affinity of \((S)-(+)-1\) was found to be 140-fold higher than \((R)-(-)-1\) and to be 2-fold higher than \((\pm)-1\). This result is consistent with the potency of the antihypertensive effects obtained in the \textit{in vivo} study.

Conclusion
We synthesized the compounds \((S)-(+)-1\) and \((R)-(-)-1\) with high optical purities from \((R)-(-)-6\) and \((S)-(+)-6\), respectively, which were obtained by the direct optical resolution of \((\pm)-6\) through its quinidine and cinchonidine salts. From pharmacological investigations in \textit{vivo} and \textit{in vitro}, the active form of \(1\) was defined to be the \((4S)-(+)-\) enantiomer of \(1\). The \(4S\) configuration of \(1\) was found to be important for interaction with the \(1,4\)-dihydropyridine receptor, as is found in other \(1,4\)-dihydropyridine enantiomers.

Experimental
Melting points were determined on a Yanagimoto micromelting point apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-420 spectrophotometer. \(^1\)H-Nuclear magnetic resonance (\(1\)H-NMR) spectra were determined on a BRUKER AC-200 spectrometer with tetramethylsilane (TMS) as an internal standard. Optical rotations were measured on a JASCO DIP-181 digital polarimeter. Extraction solvents were dried over anhydrous MgSO\(_4\). Silica gel 60, 230–400 mesh (Nacalai Tesque) was used for flash column chromatography, and Kieselgel 60, F\(_{254}\) (Merck) plates were used for thin layer chromatography (TLC).

\[\text{(\pm)-1,4-Dihydropyridine-5-methoxybenzyl-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridinethiocarboxylic Acid [(\pm)-6]}\]

\[\text{Diketide (106 ml, 1.36 mol) was added dropwise to ethylene cyanohydrin (2, 96.5 g, 1.36 mmol) preheated at about 80 °C at temperatures between 75 and 100 °C over 1.5 h. After the addition was completed, the mixture was stirred at 70–80 °C for 2.5 h. After dissolving the resulting mixture in 2-propanol (450 ml), m-nitrobenzaldehyde (3, 205.2 g, 1.36 mmol), methyl 3-aminoacetate (4, 156.3 g, 1.36 mmol) and 2-propanol (510 ml) were added to the solution, and then refluxed for 7 h, stirred at room temperature for 11 h, and with ice-water cooling for 3 h. A precipitated solid was collected by filtration, and washed with 2-propanol. After drying vacuum until the 0.9% remained, the solid was added as a yellow powder (332.4 g). A solution of 5 obtained above in acetonitrile (1300 ml) was added 1.5 NaOH (2600 ml) at a time with water cooling. After the mixture was stirred at 28 °C for 1 h, the resulting solution was diluted with water (2600 ml) and then washed with CH\(_{3}\)Cl (three times). With ice-water cooling, the aqueous layer was acidified with 35% HCl to pH 1–2, and stirred for 3 h to afford a precipitated solid. The sold collected by filtration was rinsed with water and then dried \textit{in vacuo} at 50 °C for 4 d to give the product \((\pm)-6\) as a slightly yellow powder (268.9 g, 60%). mp 199–200 °C. IR (KBr): 3325, 2925, 2700, 2600, 1670, 1655, 1605, 1450, 1435, 1370 cm\(^{-1}\). \(^{1}\)H-NMR (DMSO-d\(_{6}\), CDCI\(_{3}\)): (10.1, v/v) \[\delta 2.79, 2.30 (3 \times \text{CH}_3), 3.56 (\text{CH}_3), 5.00 (\text{H}, \delta 7.45–7.65 (2 \times \text{H}), 7.9–8.05 (2 \times \text{H}), 8.94 (\text{H}, \delta 7.18) (118.2, \text{H}, \delta 7.18)\]. Anal. Calc. For C\(_{22}\)H\(_{16}\)C\(_{6}\)O\(_{4}\) : C 78.3, H 4.85, N 8.43. Found: C 78.1, H 4.67, N 8.33. optical Rotational power of \((\pm)-1,4-Dihydropyridine-5-methoxybenzyl-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridinethiocarboxylic Acid [(\pm)-6]}\]

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rinsed with Et2O, and then dried in vacuo to give \( (S)^{(+)}-I \) HCl (83.69 g, 75%) as a yellow powder, mp 143.5-147°C, \( [\alpha]_{D}^{20} +31.0^\circ \) (c = 0.50, acetone). IR (KBr): 3375, 3050, 2925, 2550, 1690, 1610, 1520, 1485, 1455, 1350 cm\(^{-1}\). \(^{1}\)H-NMR (CDCl\(_3\)) \( \delta \) 2.25, 2.36 (each 3H, s), 2.82 (2H, t, \( J = 6.5 \text{ Hz} \)), 3.0-3.2 (2H, br), 3.35-3.6 (4H, br), 3.64 (3H, s), 3.75-4.05 (2H, br), 4.24 (2H, t, \( J = 6.5 \text{ Hz} \)), 4.88 (1H, br d, \( J = 7 \text{ Hz} \)), 5.06 (1H, s), 6.42 (1H, s), 6.76, 7.00 (4H, \( \text{Ar} \_\text{B} \_\text{B} \)), \( J = 8 \text{ Hz} \)), 7.2-7.65 (8H, m), 7.8-8.1 (6H, m), 13.10 (1H, br). Anal. Calc'd for \( \text{C}_2\_\text{H}_8\_\text{N}_2\_\text{O}_6 \) HCl: C, 68.09; H, 5.99; N, 7.75. Found: C, 68.43; H, 5.83; N, 7.65.

\( (4R)^{(-)}-2\-[4-(4\text{-Benzyldihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (}[R]-^{(-)}-I \) Hydrochloride The compound \( (4R)^{(-)}-I \) HCl was prepared from \( (S)^{(+)}-I \) HCl by the same method employed for the synthesis of \( (S)^{(+)}-I \) HCl; yield 59%, mp 145-149°C, \( [\alpha]_{D}^{20} -31.4^\circ \) (c = 0.50, acetone). Anal. Calc'd for \( \text{C}_2\_\text{H}_8\_\text{N}_2\_\text{O}_6 \) HCl: C, 68.09; H, 5.99; N, 7.75. Found: C, 68.07; H, 5.85; N, 7.74.

**Biological Tests** Antihypertensive Activity \(^{11}\): The experiments were performed in groups of 3-6 male SHR. SBP was measured in a conscious state by a tail cuff plethysmographic method with an electrophygmonometer (PS-200A, Riken-Kaibatsu) at 0, 1, 2, 4 and 7h after oral administration. The test compounds were prepared as follows: A compound was dissolved in EOH (0.3 ml) and Tween 80 (0.1 ml) and then diluted with distilled water for the volume of administration to be 10 ml/kg. Antihypertensive effects are shown as reductions in SBP (%) from 0h values.

**Receptor Binding Assay** Rat cardiac membrane for the assay was prepared by the same method reported by Isihi et al.\(^{12}\).

To solve a solution of test compound in 0.5 ml of 50 mM Tris buffer (pH 7.4) containing 0.1% albumin was added 0.05 ml of \( [\text{H}] \)-imidophen [4.729 TBq/mmol] in 10% ETOH (160000 dpm) and 0.5 ml of cardiac membrane homogenate. After incubation at 25°C for 3h in the dark, the incubation mixture was filtered under vacuum through a glass fiber filter (Whatman GF/F), and washed twice with 1 ml of 50 mM Tris buffer (pH 7.4) which was used for washing the test tube, and three times with another 5 ml of Tris buffer (pH 7.4). After the addition of 2 ml of Solutecne 350 (Packard) to the cardiac membrane homogenate, and standing overnight, 13 ml of Hionic-Fluor was added to the mixture, and kept in a cool and dark place for 24h. Subsequently, the radio activity was measured by liquid scintillation counter (Tri-carb Model 464CD, Packard). Non-specific binding was determined by the result of measurement in the presence of 100 ng/ml of \( (\text{±}) \)-I.

**References and Notes**