# Synthesis and Calcium Antagonistic Activity of (+)-(*R*)- and (−)-(*S*)-3-Acetyl-2-[5-methoxy-2-[4-[*N*-methyl-*N*-(3,4,5-trimethoxyphenethyl)amino]butoxy]phenyl]benzothiazoline Hydrochloride

Masanobu FUJITA,* Atsutoshi OTA, Susumu ITO, Koji YAMAMOTO, Yoichi KAWASHIMA, Tadashi ISO and Jun-ichi IWAO

*Central Research Laboratories, Santen Pharmaceutical Co., Ltd., 9–19, Shimoshinjo 3-chome, Higashiyodogawa-ku, Osaka 533, Japan.*
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**SA2572 ((±)-1), 3-acetyl-2-[5-methoxy-2-[4-[*N*-methyl-*N*-(3,4,5-trimethoxyphenethyl)amino]butoxy]phenyl]-benzothiazoline hydrochloride is a newly synthesized $Ca^{2+}$ antagonist having a inhibitory effect on the fast $Na^+$ inward channel. In order to clarify the absolute configurations and the pharmacological properties of both enantiomers, compounds ((+)-1 and (−)-1) were synthesized. The configurations of these compounds were assigned on the basis of an X-ray crystallographic analysis of synthetic precursor (5). The *in vitro* $Ca^{2+}$ channel blocking activities of (+)-1 and (−)-1 were evaluated in terms of the inhibitory activities on depolarization-induced contraction of guinea pig taenia cecum and rabbit aorta. The *in vivo* efficacy of the enantiomers was evaluated with their hypotensive effects in spontaneously hypertensive rats. Compound (−)-1 showed more potent $Ca^{2+}$ antagonistic activities on guinea pig taenia cecum and rabbit aorta and the hypotensive effect than those activities of (+)-1. In the electrophysiological study of Langendorff perfused rabbit hearts, compound (+)-1 showed more potent inhibitory effect on the fast $Na^+$ inward channel than that of compound (−)-1, and an approximately equal potent inhibitory effect on the slow $Ca^{2+}$ inward channel as compared with compound (−)-1. Stereoselectivity of the pharmacological activity was found.**

**Keywords** calcium antagonist; SA2572; synthesis; enantiomer; stereoselectivity; hypotensive effect; electrophysiological study; X-ray crystallography

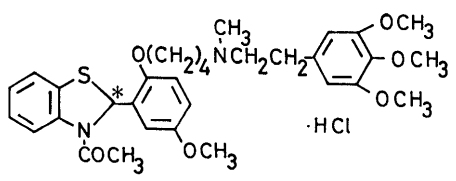
The structure–activity relationship study with respect to a $Ca^{2+}$ antagonistic activity in a series of benzothiazolines revealed compound ((±)-**1**: SA2572) having dual inhibitory effects on the slow $Ca^{2+}$ and the fast $Na^+$ inward channels.[1]

In general, $Ca^{2+}$ antagonists, known to be extremely useful for treatment of angina pectoris, hypertension and certain cardiac arrhythmias, are classified pharmacologically in two groups, specific $Ca^{2+}$ antagonists and non-specific $Ca^{2+}$ antagonists (dual inhibitors on the slow inward channel and the fast inward channel).[2]

The pharmacological activities of specific $Ca^{2+}$ antagonists, verapamil,[3] diltiazem[4] and 1,4-dihydropyridine derivatives,[5] have been found stereoselective. The differences between the activities of the enantiomers were surprisingly variable, *i.e.*, two- to 20-fold differences between the enantiomers. However, as far as we know, there are very few studies on the enantiomers of dual inhibitors.[6] Therefore, it is of interest to characterize the enantiomers of (±)-**1** in respect to the slow $Ca^{2+}$ and the fast $Na^+$ inward channel inhibitory effects. The present paper describes the synthetic and pharmacological study of (+)-**1** and (−)-**1**.

## Chemistry

Initially, we focused our efforts on the resolution of the free base of (±)-**1** itself, using various resolving agents ((+)-camphor-10-sulfonic acid, *etc.*), but none of the salts were crystallized. Then, we made an attempt to separate a racemic phenol compound ((±)-**2**) *via* the diastereomeric ester of (±)-**2**. Various esters were synthesized by the condensation of (±)-**2** and many asymmetric carboxylic acids ((−)-menthoxyacetic acid, *etc.*) and tried to separate the diastereomers using chromatography or a crystallization method. It was found that the separation of the diastereomeric mixture (**4** and **5**) obtained by condensation of (±)-**2** and a 3-acyl-2-aryl-4-thiazolidinecarboxylic acid derivative,[7] especially (−)-(2*S*,4*S*)-3-acetyl-5,5-dimethyl-2-phenyl-4-thiazolidinecarboxylic acid (**3**), was effected by column chromatography on silica gel. This result might be accounted for by the rigid conformation of thiazolidinecarboxylic acid that had two asymmetric centers at the 2- and 4- positions on the five membered ring. The optically pure phenol compound ((+)-**2**) obtained by hydrolysis of **4** was alkylated with 1,4-dibromobutane to give the corresponding bromide ((+)-**6**), which was treated with *N*-methyl-*N*-3,4,5-trimethoxyphenethylamine to afford desir-




(±)-**1** (SA2572)

Chart 1

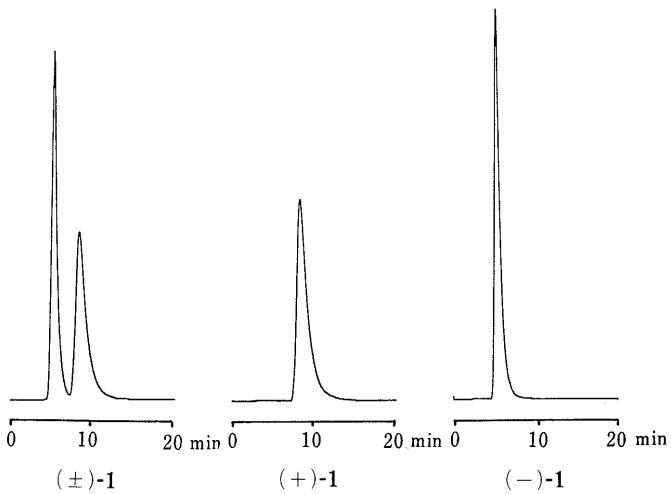



Fig. 1. Chromatogram of (±)-3-Acetyl-2-[5-methoxy-2-[4-[*N*-methyl-*N*-(3,4,5-trimethoxyphenethyl)amino]butoxy]phenyl]benzothiazoline Hydrochloride ((±)-**1**) and Its Enantiomers ((+)-**1** and (−)-**1**)

Column: CHIRALCEL OC (Daicel Chemical Industries), 4.6 × 250 mm. Eluent: hexane–EtOH–MeOH–$Et_2NH$ (40:20:40:0.2), 1.5 ml·min$^{-1}$. Detection: UV 300 nm.

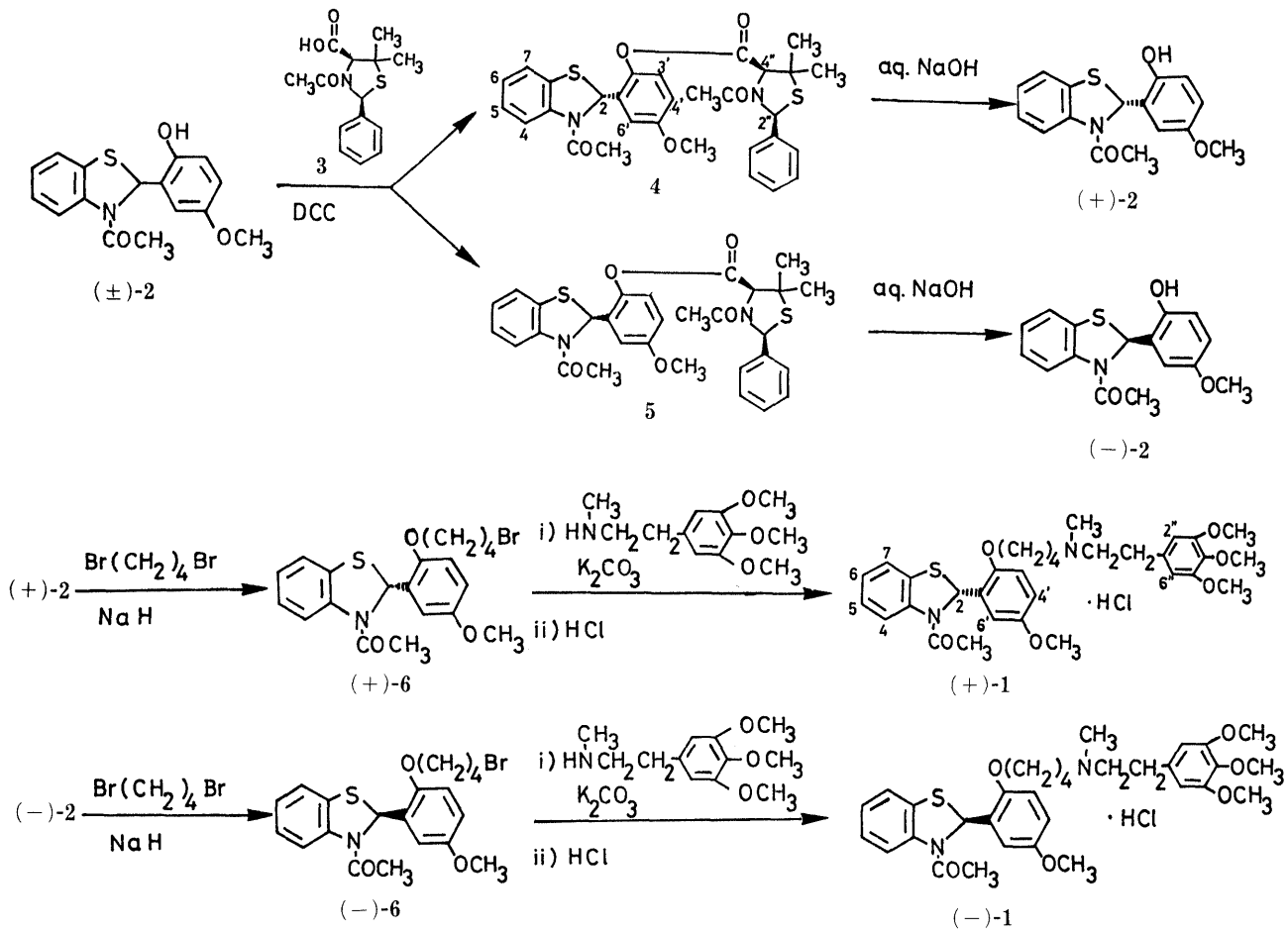



Chart 2

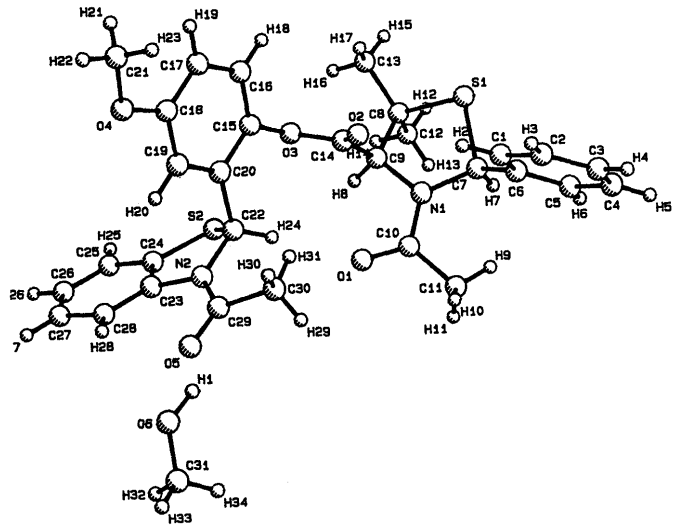


Fig. 2. Molecular Structure of (2*S*)-3-Acetyl-2-[2-[(2*S*,4*S*)-3-acetyl-5,5-dimethyl-2-phenylthiazolidin-4-ylcarbonyloxy]-5-methoxyphenyl]benzothiazoline (**5**) Drawn by the PLUTO Program

ed (+)-**1**. By the same procedure, (−)-**1** was obtained from **5** (Chart 1). The enantiomeric purity of these compounds was determined by a high performance liquid chromatography (HPLC) using a chiral column[8] which comprised cellulose phenylcarbamate (Fig. 1).

**X-Ray Analysis** The absolute configuration of the enantiomers ((+)-**1** and (−)-**1**) was determined by a single-crystal X-ray analysis of the synthetic precursor (**5**). The X-ray crystal structure of **5**, shown in Fig. 2, revealed that the absolute configuration of the 2-position (C22) is *S*.

TABLE I. $Ca^{2+}$ Antagonistic Activity of (+)-**1**, (−)-**1** and (±)-**1**

| Compd. | Guinea pig taenia cecum | Rabbit aorta |
|---|---|---|
| | $IC_{50}$ [a] | $IC_{50}$ [b] |
| (+)-**1** | $(5.4\pm0.4)\times10^{-7}$ | $(5.5\pm1.3)\times10^{-7}$ |
| (−)-**1** | $(1.1\pm0.3)\times10^{-7}$ | $(5.8\pm0.6)\times10^{-8}$ |
| (±)-**1** | $(1.4\pm0.2)\times10^{-7}$ | $(1.3\pm0.2)\times10^{-7}$ |

*a*) Molar concentration required to block $Ca^{2+}$-induced contraction of $K^+$-depolarized taenia cecum by 50%. Data represent the mean±S.E.M. of three experiments. *b*) Molar concentration required to block $Ca^{2+}$-induced concentration of $K^+$-depolarized rabbit aorta by 50%. Data represent the mean±S.E.M. of three experiments.

## Biological Activity and Discussion

**$Ca^{2+}$ Antagonistic Activity** $Ca^{2+}$ antagonistic activities of (+)-**1** and (−)-**1** were measured in comparison with (±)-**1** by using isolated depolarized guinea pig taenia cecum and rabbit aorta. Compound (−)-**1** is 5—10 times more potent than (+)-**1** (Table I).

The time courses of hypotensive effects of (+)-**1**, (−)-**1** and (±)-**1** in conscious spontaneously hypertensive rats (SHR) after an oral dose of 100 mg/kg are shown in Fig. 3. Compounds (−)-**1** and (±)-**1** lowered the systolic blood pressure (SBP). On the other hand, (+)-**1** did not significantly affect at 100 mg/kg. The hypotensive effect of (−)-**1** persisted up to 24 h after administration. The hypotensive effects of these compounds reflected the $Ca^{2+}$ antagonistic activities *in vitro*.

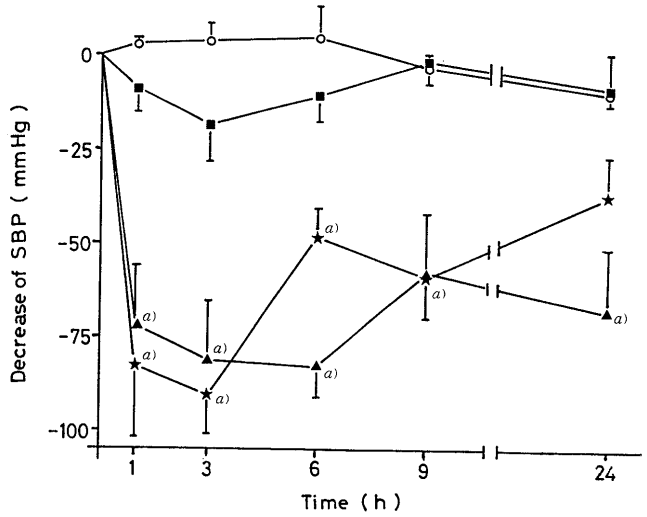



Fig. 3. Effects of Compound (+)-**1** (■), (−)-**1** (▲), (±)-**1** (★) and Methylcellulose (Control) (○) on Systolic Blood Pressure (SBP) in Conscious SHR (100 mg/kg, *p.o.*)

Data indicate the mean±S.E.M. of 4—5 animals. Test groups were compared statistically to control group values by Dunnett's multiple comparison test. *a*) $p \leq 0.01$.

TABLE II. Electrophysiological Effects of (+)-**1**, (−)-**1** and (±)-**1** in Isolated Rabbit Hearts

| Compd. | A-H interval ($EC_{30}$)[a] | H-V interval ($EC_{10}$)[b] | H-V($EC_{10}$)/ A-H($EC_{30}$)[c] |
|---|---|---|---|
| (+)-**1** | $(3.0\pm0.2)\times10^{-7}$ | $(1.5\pm0.2)\times10^{-7}$ | 0.5±0.1 |
| (−)-**1** | $(3.4\pm0.5)\times10^{-7}$ | $(9.5\pm1.1)\times10^{-7}$ | 3.1±1.2 |
| (±)-**1** | $(3.0\pm0.7)\times10^{-7}$ | $(2.0\pm0.2)\times10^{-7}$ | 1.0±0.3 |

*a*) Molar concentration that prolongs the atrio-His bundle (A-H) conduction time by 30%. Data represent mean±S.E.M. of three to five experiments. *b*) Molar concentration that prolongs the His bundle ventricular (H-V) conduction time by 10%. Data represent the mean±S.E.M. of three to five experiments. *c*) H-V($EC_{10}$) value was divided by A-H($EC_{30}$) value in each experiment. The value represents the mean±S.E.M. of three to five experiments.

**Electrophysiological Study** In Langendorff perfused rabbit hearts, both enantiomers prolonged an atrio-His bundle conduction time (A-H interval; mediated through the slow $Ca^{2+}$ inward current[2]) in the same degree (Table II). This result did not correspond to the $Ca^{2+}$ antagonistic activities examined by using the isolated depolarized guinea pig taenia cecum and rabbit aorta. This phenomenon could be explained by the difference between a smooth muscle and a cardiac muscle. In respect to the effects on a His bundle ventricular conduction time (H-V interval; mediated through the fast $Na^+$ inward current[6a]), (+)-**1** showed clearly greater inhibition than (−)-**1**. The dual inhibitory effects on the cardiac muscle of (±)-**1** were mainly due to the action of (+)-isomer.

In conclusion, the experiments characterized (+)-**1** and (−)-**1** as a potent dual inhibitor and a $Ca^{2+}$ antagonist, respectively. Since the antiarrythmic actions of some $Ca^{2+}$ antagonists[9] and $Na^+$ channel blockers[10] have been revealed, the possibility of (+)-**1** as an antiarrythmic agent is under inyestigation. On the other hand, the possibility of (−)-**1** as an antihypertensive agent is under investigation too.

## Experimental

Melting points were determined in open glass capillaries with a YAMATO MP-21 melting point apparatus and are uncorrected. Elemental analyses were performed by a Yanagimoto MT-3 CHN Corder elemental analyzer. Infrared (IR) spectra were recorded on a JASCO A-302 infrared spectrophotometer. Mass (MS) spectra were obtained on a HITACHI M-80B spectrometer in the electron impact (EI) mode with samples introduced directly into the ion source for spectra determination. Nuclear magnetic resonance (NMR) spectra were measured by a JEOL PMX-60 spectrometer using tetramethylsilane as an internal standard. Numberings of the compounds ((±)-**1** and **4**) in NMR spectra is indicated in Chart 2. Merck Silica gel 60 (70—230 mesh) was used for column chromatography. All observed rotations at the sodium D line were determined at 25 °C with a JASCO DIP-140 Digital polarimeter (1-dm cells).

**(−)-(2*S*,4*S*)-3-Acetyl-5,5-dimethyl-2-phenyl-4-thiazolidinecarboxylic Acid (3)** A solution of benzaldehyde (106 g, 1.0 mol) in EtOH (670 ml) was added to a stirred aqueous solution of D-penicillamine (149 g, 1.0 mol) at room temperature and stirring was continued at the same temperature for 1 h and at 0 °C for 3 h. Precipitated crystals were filtered, washed with $H_2O$–EtOH (8 : 2) and dried to give 210 g of (4*S*)-5,5-dimethyl-2-phenyl-4-thiazolidinecarboxylic acid that was pure enough for the following reaction. To a stirred suspension of this compound (210 g, 0.88 mol) in $H_2O$ (500 ml), acetic anhydride (500 ml, 5.28 mol) was added at 80 °C, and stirred at the same temperature for 10 min, at room temperature for 30 min and at 0 °C for 1 h. Precipitated solid was collected by filtration, washed with water and dried. Recrystallization from MeOH gave 157 g (total yield 56%) of **3**: mp 212—213.5 °C (dec.). $[\alpha]_D^{25}$ −117° ($c$=1.0, MeOH). IR (KBr): 3392, 2920, 1730, 1618, 1411 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.30 (3H, s, 5-$CH_3$), 1.57 (3H, s, 5-$CH_3$), 1.73 (3H, s, $COCH_3$), 4.50 (1H, s, 4-H), 6.31 (1H, s, 2-H), 6.9—8.0 (5H, m, aromatic H), 12.4—13.3 (1H, br, $CO_2H$). *Anal.* Calcd for $C_{14}H_{17}NO_3S$; C, 60.19; H, 6.13; N, 5.10. Found: C, 60.13; H, 6.11; N, 5.02.

**(+)-(2*R*)- and (−)-(2*S*)-3-Acetyl-2-[2-[(2*S*,4*S*)-3-acetyl-5,5-dimethyl-2-phenylthiazolidin-4-ylcarbonyloxy]-5-methoxyphenyl]benzothiazoline (4 and 5)** To a stirred solution of compound **2**[1] (79.9 g, 0.27 mol) and (−)-(2*S*,4*S*)-3-acetyl-5,5-dimethyl-2-phenyl-4-thiazolidinecarboxylic acid (**3**) (111 g, 0.40 mol) in dimethylformamide (DMF) (500 ml), a solution of *N*,*N*′-dicyclohexylcarbodiimide (81.9 g, 0.40 mol) in DMF (150 ml) and 4,4-dimethylaminopyridine (6.6 g, 54 mmol) were added while ice-cooled, and stirred at the same temperature for 30 min and at room temperature for 10 h. The reaction mixture was poured into $H_2O$–AcOEt (2.5 l–1 l) and then oxalic acid (23.9 g, 0.27 mol) was added with stirring at room temperature for 15 min. The precipitated solid was filtered. The filtrate was separated into two layers, and the aqueous layer was extracted with AcOEt. The pooled organic phases were washed with $H_2O$, brine and dried over $MgSO_4$, and evaporated to give a brown oil. After dissolving of this oil in hot MeOH, crystals were precipitated and collected by filtration to give 57.5 g (36%) of **5** (MeOH of crystallization was included): mp 125—127 °C (dec.). $[\alpha]_D^{25}$ +497° ($c$=1.0, $CHCl_3$). IR (KBr): 3424, 1766, 1647, 1461 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.54 (3H, s, 5′′-$CH_3$), 1.76 (3H, s, 5′′-$CH_3$), 1.83 (3H, s, $COCH_3$), 2.17 (3H, s, $COCH_3$), 3.41 (3H, s, $CH_3OH$), 3.62 (3H, s, $OCH_3$), 4.94 (1H, s, 4′′-H), 6.11 (1H, s, 2′′-H), 6.6—7.8 (12H, m, aromatic H and 2-H), 7.8—8.3 (1H, br m, 4-H). *Anal.* Calcd for $C_{30}H_{30}N_2O_5S_2\cdot CH_4O$; C, 62.60; H, 5.76; N, 4.71. Found: C, 62.73; H, 5.75; N, 4.78. The mother liquor was chromatographed on silica gel with hexane–AcOEt (2 : 1) to give 70.5 g (48%) of **4** as an amorphous powder: $[\alpha]_D^{25}$ +260° ($c$=1.0, $CHCl_3$). IR (KBr): 3412, 1753, 1652, 1463 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.52 (3H, s, 5′′-$CH_3$), 1.72 (3H, s, 5′′-$CH_3$), 1.93 (3H, s, $COCH_3$), 2.23 (3H, s, $COCH_3$), 3.59 (3H, s, $OCH_3$), 4.87 (1H, s, 4′′-H), 6.10 (1H, s, 2′′-H), 6.5—7.8 (12H, m, aromatic H and 2-H), 7.8—8.3 (1H, br m, 4-H). EIMS $m/z$: 562.1589 (calcd. 562.1594).

**(+)-(*R*)-3-Acetyl-2-(2-hydroxy-5-methoxyphenyl)benzothiazoline ((+)-2)** To a stirred solution of **4** (70.1 g, 0.13 mol) in DMF (1.25 l), 1 N NaOH (374 ml, 0.37 mol) was added at 0 °C and stirred at the same temperature for 15 min. The reaction mixture was acidified with 0.3 N HCl (3.6 l) and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated at a reduced pressure. The residual oil was dissolved in $CHCl_3$ (100 ml) and then hot cyclohexane (50 ml) was added. After cooling, precipitated crystals were filtered and washed with isopropylether to give 14.2 g (38%) of (+)-**2**. An analytical sample was recrystallized from isopropylether, mp 130—131.5 °C. $[\alpha]_D^{25}$ +418° ($c$=1.0, $CHCl_3$). IR (KBr): 3332, 1637, 1502, 1464 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.17 (3H, s, $COCH_3$), 3.50 (3H, s, $OCH_3$), 6.4—7.4 (7H, m, aromatic H and 2-H), 7.7—8.2 (1H, br s, 4-H), 9.58 (1H, s, OH). *Anal.* Calcd for $C_{16}H_{15}NO_3S$; C, 63.77; H, 5.02; N, 4.65. Found: C, 63.62; H, 5.06; N, 4.61.

**(−)-(*S*)-3-Acetyl-2-(2-hydroxy-5-methoxyphenyl)benzothiazoline ((−)-**

2) This compound was obtained from **5** (MeOH of crystallization was included) (39.8 g, 67 mmol) in the same manner as above: yield 12.2 g (60%) of (−)-**2**. An analytical sample was recrystallized from isopropyl-ether, mp 130—131.5 °C. $[\alpha]_D^{25}$ −418° ($c$=1.0, $CHCl_3$). The IR and the $^1$H-NMR spectra were identical with those of the enantiomer. *Anal.* Calcd for $C_{16}H_{15}NO_3S$; C, 63.77; H, 5.02; N, 4.65. Found: C, 63.86; H, 5.03; N, 4.68.

**(+)-(*R*)-3-Acetyl-2-[2-(4-bromobutoxy)-5-methoxyphenyl]benzothiazoline ((+)-6)** To a stirred suspension of NaH (60% oil dispersion; 0.44 g, 11 mmol) in dry DMF (10 ml), was added dropwise a solution of (+)-**2** (3.0 g, 10 mmol) in dry DMF (6 ml) while ice-cooled and then stirred at 5—8 °C for 5 min. To the resultant mixture, 1,4-dibromobutane (21.6 g, 0.10 mol) was added at 0 °C, and stirred at room temperature for 30 min. The reaction mixture was poured into $H_2O$ (60 ml) and extracted with ether. The organic layer was washed with $H_2O$, brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residual oil was chromatographed on silica gel with $CH_2Cl_2$ to give 3.8 g (87%) of (+)-**6**. An analytical sample was recrystallized from EtOH, mp 84.5—85.5 °C. $[\alpha]_D^{25}$ +649° ($c$=1.0, $CHCl_3$). IR (KBr): 1674, 1499, 1463, 1378 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.7—2.3 (4H, m, $OCH_2C\underline{H}_2C\underline{H}_2$), 2.20 (3H, s, $COCH_3$), 3.49 (2H, t, $J$=6.0 Hz, $CH_2Br$), 3.59 (3H, s, $OCH_3$), 4.01 (2H, t, $J$=5.0 Hz, $OCH_2$), 6.50 (1H, d, $J$=2.2 Hz, 6′-H), 6.6—7.2 (6H, m, aromatic H and 2-H), 7.6—8.3 (1H, br, 4-H). *Anal.* Calcd for $C_{20}H_{22}BrNO_3S$; C, 55.05; H, 5.08; N, 3.21. Found: C, 55.06; H, 5.10; N, 3.22.

**(−)-(*S*)-3-Acetyl-2-[2-(4-bromobutoxy)-5-methoxyphenyl]benzothiazoline ((−)-6)** This compound was obtained from (−)-**2** (9.0 g, 30 mmol) in the same manner as above: yield 11.7 g (89%) of (−)-**6**. An analytical sample was recrystallized from EtOH, mp 83.5—85 °C. $[\alpha]_D^{25}$ −654° ($c$=1.0, $CHCl_3$). *Anal.* Calcd for $C_{20}H_{22}BrNO_3S$; C, 55.05; H, 5.08; N, 3.21. Found: C, 55.18; H, 5.12; N, 3.23.

**(+)-(*R*)-3-Acetyl-2-[5-methoxy-2-[4-[*N*-methyl-*N*-(3,4,5-trimethoxyphenethyl)amino]butoxy]phenyl]benzothiazoline Hydrochloride ((+)-1)** To a solution of (+)-**6** (2.1 g, 4.8 mmol) and *N*-methyl-3,4,5-trimethoxyphenethylamine (1.2 g, 5.3 mmol) in dry DMF (7.2 ml), $K_2CO_3$ (1.3 g, 9.6 mmol) was added and stirred at 60 °C for 2 h. The reaction mixture was poured into $H_2O$ (50 ml) and extracted with toluene (30 ml). The organic extract was washed with $H_2O$ and then 1 N HCl (20 ml) was added with vigorous stirring. A very dense oil was precipitated and supernatant liquid was decanted off. Residual oil was dissolved in $CHCl_3$ (30 ml). The organic solution was washed with $H_2O$, 1 N NaOH, brine, dried over $MgSO_4$, and concentrated at a reduced pressure. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (50 : 1) to give free base of (+)-**1** as oil. The oil was dissolved in $CHCl_3$ (30 ml) and stirred with 1 N HCl (20 ml) to give a hydrochloride form. The organic solution was washed with brine and dried over $MgSO_4$. Solvent was removed and the residue was lyophilized to give 2.3 g (77%) of (+)-**1** as amorphous powders. Enantiomeric excess (100%) was determined with a chiral column (CHIRALCEL OC[8]). HPLC conditions are as follows, eluent: hexane–EtOH–MeOH–$Et_2NH$ (40 : 20 : 40 : 0.2); flow rate: 1.5 ml min$^{-1}$; column temperature 35 °C; detective wavelength 300 nm. The retention time was 9.28 min. $[\alpha]_D^{25}$ +447° ($c$=1.1, $CHCl_3$). IR (KBr): 3400, 2930, 2580, 1664, 1589, 1496, 1462 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.6—2.5 (4H, m, $OCH_2C\underline{H}_2C\underline{H}_2$), 2.22 (3H, s, $COCH_3$), 2.7—3.0 (3H, m, $NCH_3$), 3.0—3.6 (6H, m, $CH_2NCH_2CH_2Ar$), 3.59 (3H, s, 5′-$OCH_3$), 3.75 (9H, s, 3″, 4″ and 5″-$OCH_3$), 4.03 (2H, br t, $J$=5.0 Hz, $OCH_2$), 6.42 (2H, s, 2″ and 6″-H), 6.4—7.2 (7H, m, aromatic H and 2-H), 7.5—8.1 (1H, m, 4-H), 12.0—12.7 (1H, br, HCl). EIMS (free base of (+)-**1**) $m/z$: 580.2601 (calcd. 580.2605).

**(−)-(*S*)-3-Acetyl-2-[5-methoxy-2-[4-[*N*-methyl-*N*-(3,4,5-trimethoxyphenethyl)amino]butoxy]phenyl]benzothiazoline Hydrochloride ((−)-1)** This was obtained from (−)-**6** (10.9 g, 25 mmol) in the same manner as above: yield 12 g (78%) of (−)-**1** as amorphous powders: $[\alpha]_D^{25}$ −446° ($c$=1.0, $CHCl_3$). The IR and the $^1$H-NMR spectra were identical with those of enantiomer. The enantiomeric excess (100%) was determined as above. The retention time was 6.42 min. EIMS (free base of (−)-**1**) $m/z$: 580.2628 (calcd. 580.2605).

**X-Ray Analysis** Compound **5** crystallized from the methanol solution as colorless prisms. The crystals belong to a monoclinic system with space group $P2_1$. Crystal data are shown in Table III. The intensities of 2423 independent reflections up to $2\theta$=120.1° were collected on a Rigaku AFC5R diffractometer with graphite-monochromated Cu$K_\alpha$ radiation. The data were corrected for Lorentz and polarization effects. A correction for secondary extinction was applied.

**Structure Determination and Refinement** The structure was solved by direct methods[11] using the TEXSAN program,[12] and the positions of all the non-hydrogen atoms in the molecule were successfully assigned. The structure was refined by full-matrix least-squares and difference Fourier method. The final $R$ value was 0.043 for 2048 non-zero reflections. Throughout the refinements, a unit weight was given to the intensity of each reflection. The atomic scattering factors cited in International Tables for X-Ray Crystallography Vol. IV[13] were used. The absolute configuration was determined to be *S* at the 2-position (C22) as shown in Fig. 2. The final parameters are listed in Table IV, and bond lengths and bond angles are listed in Tables V and VI, respectively, along with their standard deviations.

TABLE III. Crystal Data for Compound **5**

| | |
|---|---|
| Chemical formular | $C_{31}H_{34}N_2O_6S_2$ |
| Formular weight | 594.74 |
| Crystal system | monoclinic |
| Space group | $P2_1$ |
| $Z$ | 2 |
| $a$ | 8.606 (2) Å |
| $b$ | 12.130 (3) Å |
| $c$ | 14.850 (2) Å |
| $\beta$ | 96.25 (2)° |
| $V$ | 1541.0 (5) Å$^3$ |
| $\mu$(Cu$K\alpha$) | 1.891 mm$^{-1}$ |
| $\lambda$ | 1.54178 Å |
| $D_{calc}$ | 1.282 Mgm$^{-3}$ |

TABLE IV. Final Positional Parameters ($\times 10^4$) and Equivalent Isotropic Thermal Parameters with Estimated Standard Deviations in Parenthesis for Compound (**5**)

| Atom | $x$ | $y$ | $z$ | $B_{eq}$ (Å$^2$) |
|---|---|---|---|---|
| S(1) | 4033 (2) | 803 (1) | 6867 (1) | 5.58 (6) |
| S(2) | −1307 (2) | 5126 (2) | 7038 (1) | 5.41 (6) |
| O(1) | 2010 (5) | 3672 (3) | 8609 (3) | 5.7 (2) |
| O(2) | 4013 (4) | 3818 (4) | 6869 (3) | 5.8 (2) |
| O(3) | 1523 (4) | 3976 (3) | 6249 (2) | 4.9 (2) |
| O(4) | 2642 (5) | 8227 (4) | 5165 (3) | 6.8 (2) |
| O(5) | 1291 (7) | 8035 (5) | 8797 (3) | 9.1 (3) |
| O(6) | 2710 (1) | 9790 (6) | 9749 (4) | 11.6 (4) |
| N(1) | 3056 (4) | 2193 (4) | 8022 (2) | 4.5 (2) |
| N(2) | 404 (5) | 6720 (4) | 7809 (3) | 4.6 (2) |
| C(1) | 6650 (6) | 2188 (6) | 8169 (4) | 5.5 (2) |
| C(2) | 8242 (6) | 2247 (6) | 8490 (4) | 6.5 (3) |
| C(3) | 8900 (7) | 1442 (7) | 9055 (5) | 7.1 (3) |
| C(4) | 8026 (7) | 593 (6) | 9317 (4) | 6.7 (3) |
| C(5) | 6399 (6) | 530 (5) | 9012 (4) | 5.6 (3) |
| C(6) | 5755 (6) | 1329 (5) | 8430 (3) | 4.7 (2) |
| C(7) | 4065 (6) | 1190 (4) | 8052 (4) | 4.8 (2) |
| C(8) | 2084 (6) | 1381 (5) | 6578 (4) | 5.0 (2) |
| C(9) | 2123 (5) | 2412 (5) | 7169 (3) | 4.6 (2) |
| C(10) | 2842 (6) | 2868 (5) | 8730 (3) | 4.8 (2) |
| C(11) | 3644 (7) | 2592 (5) | 9654 (4) | 5.7 (3) |
| C(12) | 842 (7) | 575 (6) | 6859 (5) | 7.1 (3) |
| C(13) | 1840 (7) | 1596 (6) | 5559 (4) | 6.2 (3) |
| C(14) | 2711 (6) | 3449 (5) | 6758 (3) | 4.5 (2) |
| C(15) | 1828 (5) | 5034 (5) | 5936 (3) | 4.7 (2) |
| C(16) | 2530 (6) | 5191 (6) | 5152 (3) | 5.6 (3) |
| C(17) | 2795 (7) | 6248 (6) | 4865 (4) | 5.9 (3) |
| C(18) | 2413 (6) | 7138 (6) | 5365 (4) | 5.3 (2) |
| C(19) | 1684 (6) | 6975 (5) | 6146 (3) | 4.7 (2) |
| C(20) | 1404 (5) | 5917 (5) | 6447 (3) | 4.3 (2) |
| C(21) | 3687 (9) | 8455 (8) | 4515 (6) | 9.2 (5) |
| C(22) | 646 (5) | 5710 (4) | 7305 (3) | 4.4 (2) |
| C(23) | −1092 (6) | 7200 (5) | 7551 (3) | 4.7 (2) |
| C(24) | −2124 (6) | 6432 (5) | 7118 (4) | 4.9 (2) |
| C(25) | −3639 (7) | 6749 (7) | 6800 (5) | 6.7 (3) |
| C(26) | −4079 (8) | 7817 (8) | 6905 (5) | 8.0 (4) |
| C(27) | −3048 (9) | 8575 (7) | 7320 (5) | 7.7 (4) |
| C(28) | −1548 (8) | 8266 (6) | 7657 (4) | 6.3 (3) |
| C(29) | 1528 (7) | 7178 (6) | 8411 (4) | 6.1 (3) |
| C(30) | 3063 (8) | 6584 (8) | 8556 (5) | 8.2 (4) |
| C(31) | 2420 (2) | 9924 (9) | 10599 (6) | 12.0 (6) |

TABLE V. Bond Lengths (Å) with Standard Deviations in Parentheses for Compound (**5**)

| Atom 1 | Atom 2 | Distance (Å) | Atom 1 | Atom 2 | Distance (Å) |
|---|---|---|---|---|---|
| S(1) | C(7) | 1.818 (5) | C(4) | C(5) | 1.426 (8) |
| S(1) | C(8) | 1.825 (5) | C(5) | C(6) | 1.376 (7) |
| S(2) | C(22) | 1.828 (5) | C(6) | C(7) | 1.510 (7) |
| S(2) | C(24) | 1.743 (6) | C(8) | C(9) | 1.526 (8) |
| O(1) | C(10) | 1.212 (6) | C(8) | C(12) | 1.539 (8) |
| O(2) | C(14) | 1.201 (6) | C(8) | C(13) | 1.528 (8) |
| O(3) | C(14) | 1.363 (6) | C(9) | C(14) | 1.509 (8) |
| O(3) | C(15) | 1.399 (7) | C(10) | C(11) | 1.504 (8) |
| O(4) | C(18) | 1.373 (8) | C(15) | C(16) | 1.383 (7) |
| O(4) | C(21) | 1.416 (8) | C(15) | C(20) | 1.384 (7) |
| O(5) | C(29) | 1.215 (8) | C(16) | C(17) | 1.38 (1) |
| O(6) | C(31) | 1.32 (1) | C(17) | C(18) | 1.371 (9) |
| N(1) | C(7) | 1.492 (6) | C(18) | C(19) | 1.391 (7) |
| N(1) | C(9) | 1.449 (6) | C(19) | C(20) | 1.390 (8) |
| N(1) | C(10) | 1.362 (6) | C(20) | C(22) | 1.515 (7) |
| N(2) | C(22) | 1.462 (7) | C(23) | C(24) | 1.395 (7) |
| N(2) | C(23) | 1.427 (6) | C(23) | C(28) | 1.365 (8) |
| N(2) | C(29) | 1.361 (7) | C(24) | C(25) | 1.392 (8) |
| C(1) | C(2) | 1.403 (8) | C(25) | C(26) | 1.36 (1) |
| C(1) | C(6) | 1.376 (8) | C(26) | C(27) | 1.38 (1) |
| C(2) | C(3) | 1.37 (1) | C(27) | C(28) | 1.38 (1) |
| C(3) | C(4) | 1.36 (1) | C(29) | C(30) | 1.50 (1) |

TABLE VI. Bond Angles (°) with Standard Deviations in Parentheses for Compound (**5**)

| Atom 1 | Atom 2 | Atom 3 | Angle (°) | Atom 1 | Atom 2 | Atom 3 | Angle (°) |
|---|---|---|---|---|---|---|---|
| C(7) | S(1) | C(8) | 92.6 (2) | C(12) | C(8) | C(13) | 110.6 (5) |
| C(22) | S(2) | C(24) | 90.1 (2) | N(1) | C(9) | C(8) | 109.1 (4) |
| C(14) | O(3) | C(15) | 117.2 (4) | N(1) | C(9) | C(14) | 109.1 (4) |
| C(18) | O(4) | C(21) | 116.9 (6) | C(8) | C(9) | C(14) | 116.0 (4) |
| C(7) | N(1) | C(9) | 116.2 (4) | O(1) | C(10) | N(1) | 119.8 (5) |
| C(7) | N(1) | C(10) | 126.5 (4) | O(1) | C(10) | C(11) | 121.3 (5) |
| C(9) | N(1) | C(10) | 117.2 (4) | N(1) | C(10) | C(11) | 118.9 (5) |
| C(22) | N(2) | C(23) | 112.2 (4) | O(2) | C(14) | O(3) | 122.3 (5) |
| C(22) | N(2) | C(29) | 123.2 (5) | O(2) | C(14) | C(9) | 127.1 (4) |
| C(23) | N(2) | C(29) | 124.4 (5) | O(3) | C(14) | C(9) | 110.5 (4) |
| C(2) | C(1) | C(6) | 120.0 (5) | O(3) | C(15) | C(16) | 121.4 (5) |
| C(1) | C(2) | C(3) | 119.7 (6) | O(3) | C(15) | C(20) | 117.2 (4) |
| C(2) | C(3) | C(4) | 120.8 (5) | C(16) | C(15) | C(20) | 121.3 (6) |
| C(3) | C(4) | C(5) | 120.3 (6) | C(15) | C(16) | C(17) | 119.4 (6) |
| C(4) | C(5) | C(6) | 118.5 (5) | C(16) | C(17) | C(18) | 120.5 (5) |
| C(1) | C(6) | C(5) | 120.7 (5) | O(4) | C(18) | C(17) | 126.3 (5) |
| C(1) | C(6) | C(7) | 121.7 (5) | O(4) | C(18) | C(19) | 113.9 (6) |
| C(5) | C(6) | C(7) | 117.5 (5) | C(17) | C(18) | C(19) | 119.8 (6) |
| S(1) | C(7) | N(1) | 103.6 (3) | C(18) | C(19) | C(20) | 120.6 (5) |
| S(1) | C(7) | C(6) | 107.5 (4) | C(15) | C(20) | C(19) | 118.3 (5) |
| N(1) | C(7) | C(6) | 117.0 (4) | C(15) | C(20) | C(22) | 119.8 (5) |
| S(1) | C(8) | C(9) | 102.6 (3) | C(19) | C(20) | C(22) | 122.0 (5) |
| S(1) | C(8) | C(12) | 109.9 (4) | S(2) | C(22) | N(2) | 104.9 (3) |
| S(1) | C(8) | C(13) | 108.8 (4) | S(2) | C(22) | C(20) | 110.6 (3) |
| C(9) | C(8) | C(12) | 109.7 (5) | N(2) | C(22) | C(20) | 113.0 (4) |
| C(9) | C(8) | C(13) | 114.9 (5) | N(2) | C(23) | C(24) | 111.2 (5) |
| N(2) | C(23) | C(28) | 128.1 (5) | C(24) | C(23) | C(28) | 120.6 (5) |
| S(2) | C(24) | C(23) | 113.4 (4) | S(2) | C(24) | C(25) | 126.7 (5) |
| C(23) | C(24) | C(25) | 119.5 (5) | C(24) | C(25) | C(26) | 119.0 (6) |
| C(25) | C(26) | C(27) | 120.9 (6) | C(26) | C(27) | C(28) | 120.7 (7) |
| C(23) | C(28) | C(27) | 118.9 (6) | O(5) | C(29) | N(2) | 121.0 (6) |
| O(5) | C(29) | C(30) | 122.3 (6) | N(2) | C(29) | C(30) | 116.7 (6) |

**$Ca^{2+}$ Antagonistic Activity *in Vitro*** **A. Guinea Pig Taenia Cecum** Isolated taenia cecum (from male Hartley guinea pig weighing 300 to 450 g) was cut into strips in 2 mm width and 1.5 cm length. The strip was suspended in a 20 ml organ bath containing Krebs-Hensleit solution, which was maintained at $31 \pm 1$ °C and bubbled with 5% carbon dioxide in oxygen. After equilibration, the strip was washed with $Ca^{2+}$-free high-$K^+$ Krebs solution. The strip was exposed to test compounds for 5 min before addition of $CaCl_2$, and the contraction evoked by $CaCl_2$ ($2 \times 10^{-3}$ M) was recorded isotonically. The $Ca^{2+}$ antagonistic activity was represented by the concentration of the test compound that elicited 50% inhibition of $Ca^{2+}$-evoked contraction ($IC_{50}$).

**B. Rabbit Aorta** Isolated rabbit aorta (from male rabbit weighing 2 to 3 kg) was cut into helical strips in 2 mm width and 2 cm length. The strip was suspended in a 20 ml organ bath containing Krebs-Hensleit solution, which was maintained at $37 \pm 0.5$ °C and bubbled with 5% carbon dioxide in oxygen. After equilibration, the strip was washed with $Ca^{2+}$-free high-$K^+$ Krebs solution. The strip was exposed to test compounds for 30 min before addition of $CaCl_2$, and the contraction evoked by $CaCl_2$ ($2 \times 10^{-3}$ M) was recorded isotonically. The $Ca^{2+}$ antagonistic activity was represented by the concentration of the test compound that elicited 50% inhibition of $Ca^{2+}$-evoked contraction ($IC_{50}$).

**Electrophysiological Study (Langendorff Perfused Rabbit Hearts)** The hearts were rapidly removed, and cannula were inserted into the aorta for Langendorff perfusion. The preparations were perfused at a constant flow rate (20 ml·min$^{-1}$) with Krebs bicarbonate solution equilibrated with 5% carbon dioxide in oxygen. The temperature of the perfusate entering the heart was maintained at $33 \pm 0.5$ °C. Bipolar silver wire electrodes (200 $\mu$m in diameter) with an interpolar distance of 1.0 mm were inserted through a small incision made in the atria so as to record His-bundle electrograms (HBE). The signal was amplified at a frequency response from 100 to 500 Hz with a time constant of 3 ms and displayed on the oscilloscope (Tektronix 5113A). The same electrodes as for HBE recording were placed on the atrium close to the coronary sinus region. The hearts were electrically driven at a constant rate of 2.0 Hz through the stimulating electrodes on the right atrium. The pulses for stimulation were 5 ms in duration and twice the diastolic threshold in intensity. The atrio-His bundle conduction time (A-H interval) was defined as the period from the onset of the first rapid atrial deflection (A) to the first rapid His bundle deflection (H) on HBE, and the His bundle-ventricular conduction time (H-V interval) from H to the beginning of the ventricular activity (V). The preparations were allowed to equilibrate for at least 40 min. The actions of the drugs were evaluated after 30 min of exposure to the preparations at each concentration.

**Hypotensive Effect in Conscious SHR** The experiments were performed in 4—5 male SHR weighing 400—500 g. (SHR had been given courtesy of Professor K. Okamoto, Department of Pathology, Kinki University School of Medicine. They were inbred thereafter in our laboratory.) Systolic blood pressure (SBP) was measured in a conscious state by a tail cuff plethysmographic method with an electrosphygmomanometer (Narco, PE-300) at 0, 1, 3, 6, 9 and 24 h after administration (initial SBP: 219—231 mmHg). Test compounds were orally administered as a 0.5% methylcellulose suspension.

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