# Studies of Cardiotonic Agents. 8. Synthesis and Biological Activities of Optically Active 6-(4-(Benzylamino)-7-quinazolinyl)-4,5-dihydro-5-methyl-3(2*H*)-pyridazinone (KF15232)

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We previously reported that (±)-6-(4-(benzylamino)-7-quinazolinyl)-4,5-dihydro-5-methyl-3(2*H*)-pyridazinone ((±)-**1**, KF15232) showed potent cardiotonic activity with a strong myofibrillar $Ca^{2+}$-sensitizing effect. As an extension of our work, we attempted to synthesize optically active **1**. (±)-4-(4-(Benzylamino)-7-quinazolinyl)-3-methyl-4-oxobutyric acid (−)-menthyl ester (**6**) was separated into both diastereoisomers, and each was converted to optically pure **1** (>99% ee) in an enantioselective manner. In order to determine the absolute configuration of the isomers, an alternative synthesis of optically active **1** was employed. The precursor of (−)-**1** ((+)-**9**) was obtained by enantioselective synthesis from (*R*)-D-alanine. Consequently, we concluded that the absolute configuration of (−)-**1** at the 5-position of the pyridazinone ring was *R*. The cardiotonic effects and inhibitory activities to PDE III and V of racemic **1** and (−)-**1** were more potent than those of (+)-**1**. These compounds also demonstrated greater vasorelaxant effects in guinea pig aorta. In contrast, (+)-**1** showed only weak cardiotonic and vasodilating effects, although the compound displayed potent $Ca^{2+}$-sensitizing activity. Racemic and (−)-**1** attracted our interest for the treatment of congestive heart failure.

## Introduction

The extensive search to find a non-glycoside, non-catecholamine replacement for digitalis led to the discovery of several new cardiotonic drugs.[1] We have been investigating novel pyridazinones as cardiotonic agents, and we previously reported the synthesis and structure−activity relationships for the cardiotonic and myofibrillar $Ca^{2+}$-sensitizing activities of (±)-6-(4-(benzylamino)-7-quinazolinyl)-4,5-dihydro-5-methyl-3(2*H*)-pyridazinone ((±)-**1**, KF15232) and related compounds.[2,3] In these studies, we found that (±)-**1** showed the most potent cardiotonic and myofibrillar $Ca^{2+}$-sensitizing activities in the series, but structural requirements for cardiotonic activity were different from those for myofibrillar $Ca^{2+}$-sensitizing activities on the basis of the structure−activity relationship study of the related compounds.[3]

Previously, the myofibrillar $Ca^{2+}$-sensitizing effects of some 4,5-dihydro-3(2*H*)-pyridazinone cardiotonics, such as pimobendan, and MCI-154 were reported.[4,5] It can be speculated that the $Ca^{2+}$-sensitizing effect is responsible, at least in part, for the mechanism of the inotropic action, and the risk of arrhythmogenic action of compounds which show a $Ca^{2+}$-sensitizing effect in addition to a cardiac PDE III inhibitory activity that might be lower than that of pure PDE III inhibitors.[6−8]

Zaprinast, a PDE V (cGMP-PDE) inhibitor, was reported to have depressor and natriuretic effects[9] and was also known as an enhancer of the natriuretic activity of atriopeptin by inhibition of cGMP degradation.[10] Tradiational therapy for congestive heart failure includes a combination of diuretics and digitalis. We are interested in drug therapy for congestive heart failure using dual PDE III and V inhibitors, because such compounds may be expected to have combined inotropic-vasodilatory and diuretic action.

In a previous paper, we described (±)-**1** as having inhibitory properties on bovine aortic PDE III and PDE V.[11] The present paper describes the synthesis and biological activities of the racemic and optically active **1**.

## Chemistry

**Resolution of the Menthyl Ester 6 and Cyclization of Its Diastereomers to Optically Active 1.** Optically active **1** were synthesized by the route shown in Scheme 1. (±)-4-(4-Bromo-3-nitrophenyl)-3-methyl-4-oxobutyric acid (**2**)[12] was converted to the diastereomeric mixture of (−)-menthyl ester **3**. Without separation of the mixture, **3** was treated with CuCN to give cyanide **4**, which was reduced with $TiCl_3$ to anthranilonitrile **5**. Treatment of **5** with triethyl orthoformate in the presence of pyridinium hydrochloride followed by heating with benzylamine afforded quinazoline **6**. The diastereomixture (**6**) was separated by flash column chromatography to give **6a** and **6b**, which were purified by recrystallization to >99% de. Compound **6a**, which was formerly eluated with hexane−AcOEt, was cyclized by treatment with a mixture of hydrazine hydrate and acetic acid (1:2 v/v) in EtOH at 10 °C to afford optically pure (+)-**1** (>99% ee). The other diastereomer (**6b**) was converted to (−)-**1** (>99% ee) under similar conditions.

**Determination of Absolute Configuration of Optically Active 1.** We attempted to synthesize optically active **9** from the chiral amino acid in order to determine the absolute configuration of **1**.

Friedel−Crafts acylation of bromobenzene with (*R*)-(−)-2-chloropropionyl chloride (**7**)[13] derived from D-

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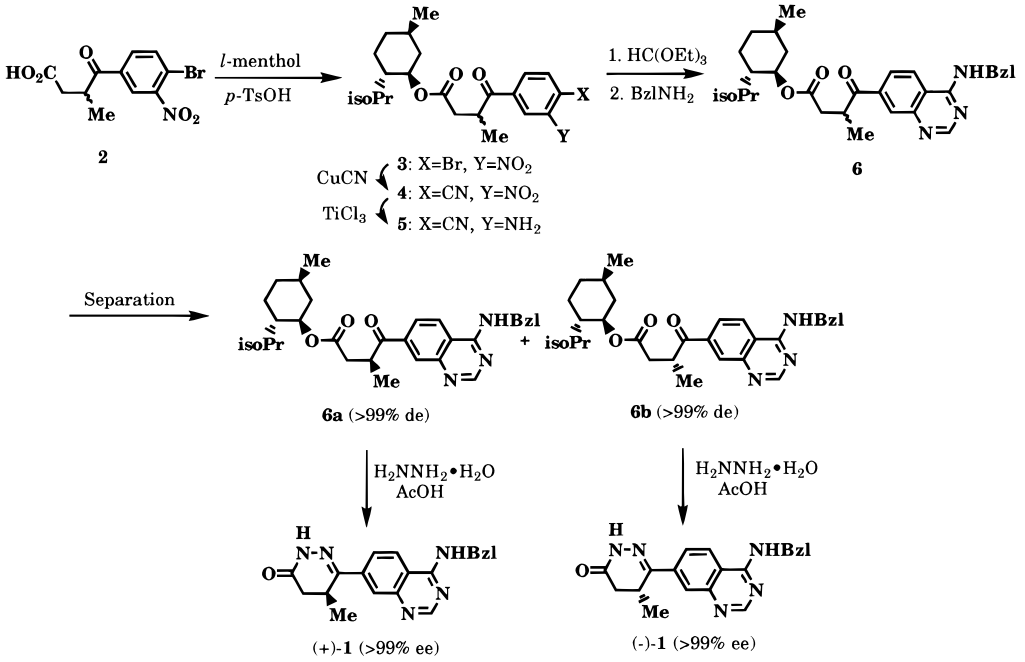
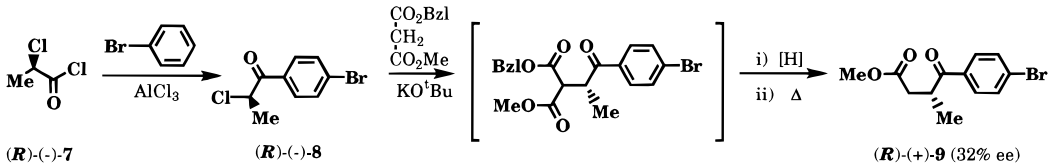
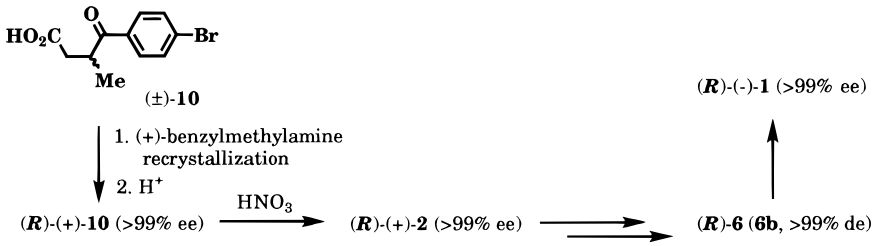
**Scheme 1**




**Scheme 2**




**Scheme 3**




alanine afforded (*R*)-(−)-4-bromo-α-chloropropiophenone (**8**) (>99% ee). Compound (*R*)-(−)-**8** was converted to the 4-oxobutyric acid methyl ester ((*R*)-(+)-**9**) (32% ee) using the method described by Owings et al.,[14] although a significant loss of enantiomeric purity occurred (Scheme 2).

On the other hand, the 4-oxobutyric acid ((+)-**10** (>99% ee)) was obtained by recrystallization of the diastereomeric salt formed by treatment of racemic **10**[22] with (+)-benzylmethylamine followed by hydrolysis. After esterification of (+)-**10** with $TMSCH_2N_2$, the chiral HPLC behavior of the product was identical with that of the methyl ester ((*R*)-(+)-**9**), and not identical with (−)-**9**. Therefore, the absolute configuration of (+)-**10** was *R*. The nitration of (*R*)-(+)-**10** afforded (*R*)-(+)-**2** (>99% ee). This compound was converted to **6b** ((*R*)-**6**, >99% de), which is the precursor of (−)-**1**, in several steps using a similar method as described for (*RS*)-**6** without racemization (Scheme 3).

On the basis of these findings, we concluded that the absolute configuration of (−)-**1** at the 5-position of the pyridazinone ring was *R*.

## Biological Results

**Cardiotonic Activities.** The in vitro properties of racemic and optically active **1** were evaluated in isolated guinea pig papillary muscles (Figure 1). All the test compounds produced concentration-dependent increases in developed tension. Racemic and (−)-**1** produced about 30% increases at a concentration of 30 μM, which were significantly more potent than (+)-**1**.

We next evaluated the compounds for cardiotonic activities in anesthetized guinea pigs. The effects of the compounds were determined by measuring the percent increase in the maximum d*P*/d*t* left ventricular pressure (LV $dP/dt_{max}$, Δ%) after iv administration. The results are expressed as absolute percentage increases in $dP/dt_{max}$ as shown in Figure 2. The administration of 0.001−0.1 mg/kg of racemic **1** and (−)-**1** and 0.01−1.0 mg/kg of (+)-**1** showed a dose-dependent increase in $dP/dt_{max}$. The positive inotropic effects of the compounds were probably not due to reflex but direct effects, because there were minor increases in heart rate (less than 16% even at maximum dose, data not shown).

Racemic **1** and (−)-**1** also demonstrated potent and

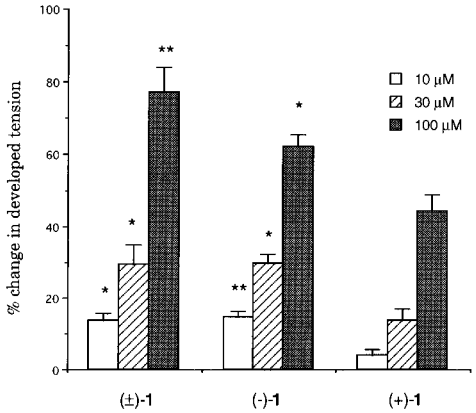



**Figure 1.** Positive inotropic effects of **1** in electrically driven papillary muscles from guinea pigs. Each point represents the mean ± SEM of seven preparations. *$p$ < 0.05, **$p$ < 0.01 vs (*S*)-(+)-**1**.

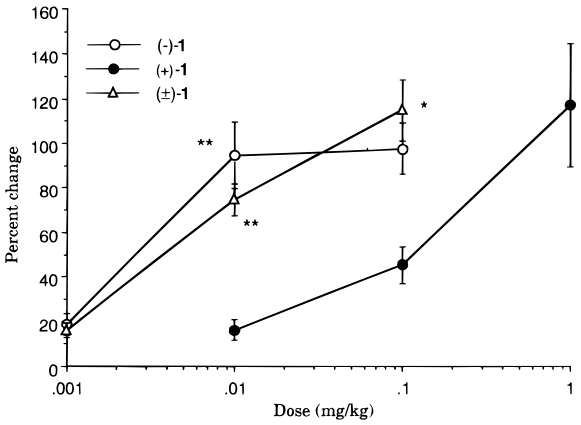



**Figure 2.** Effects of intravenously injected **1** on LV d$P$/d$t_{max}$ in anesthetized guinea pigs. Each point represents the mean ± SEM of four experiments. *$p$ < 0.05, **$p$ < 0.01 vs (*S*)-(+)-**1**.

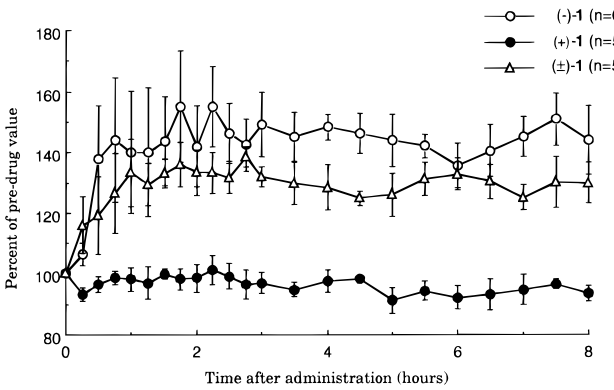



**Figure 3.** Effects of orally administered **1** (0.1 mg/kg) on LV d$p$/d$t_{max}$ in conscious dogs.

long-lasting cardiotonic activity in conscious dogs following oral administration at a dose of 0.1 mg/kg, while (+)-**1** showed no activity at the same dose (Figure 3).

**Inhibitory Activities for PDEs.** We investigated the ability of the compounds to inhibit canine heart PDE III[17] and bovine arterial PDE III, IV, and V[18] (Table 1).

Racemic **1** and (−)-**1** potently inhibited bovine arterial PDE III and V and canine heart PDE III but not PDE IV. The $IC_{50}$ values of (−)-**1** for these enzymes were 5, 10, and 4.9 nM and >10 μM, respectively, and were lower than those of (+)- and (±)-**1**. The inhibitory effect of (+)-**1** on PDE III was not detected even at 1000 nM.

**Table 1.** Inhibitory Effects of **1** on Various PDEs

| | inhibn activities ($IC_{50}$, nM) | | | |
|---|---|---|---|---|
| compd | canine heart III | bovine arterial III | bovine arterial IV | bovine arterial V |
| rolipram | | | 100 | >10000 |
| cGMP | 2400 | 100 | | |
| milrinone | 3000 | 1000 | 10000 | 5000 |
| (±)-**1** | 47 | 50 | >10000 | 46 |
| (−)-**1** | 4.9 | 5 | >10000 | 10 |
| (+)-**1** | >1000 | 300 | >10000 | 1000 |

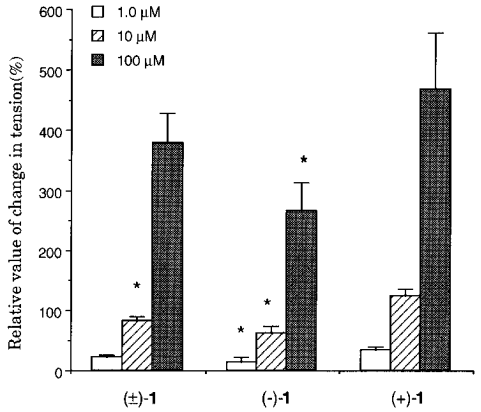



**Figure 4.** Effects of **1** on calcium-activated force in skinned papillary muscle fibers from guinea pigs. Data are expressed as percentage of the force development induced by 10 μM trifluoperazine. Each point represents the mean ± SEM of six preparations. *$p$ < 0.05 vs (*S*)-(+)-**1**.

**Myofibrillar $Ca^{2+}$ Sensitizing Activity.** We examined the myofibrillar $Ca^{2+}$-sensitizing effect in skinned muscle fibers from guinea pig papillary muscles on the tension development induced by pCa (−log[$Ca^{2+}$] M) 5.8.[3,15] The potency of $Ca^{2+}$-sensitizing activity of the test compounds was compared with trifluoperazine ($5 \times 10^{-5}$ M). Relative potency was calculated as the change in tension of each compound to that of trifluoperazine (TFP)[16] (TFP = 100%). This protocol compensates for any variation in fiber and facilitates comparison of activity among the test compounds. The relative tension developement was significantly increased by all the test compounds ($1 \times 10^{-6}$ to $1 \times 10^{-4}$ M) in a concentration-related manner (Figure 4). In contrast to the cardiotonic activity, (+)-**1** showed the most potent $Ca^{2+}$-sensitizing effect of these compounds.

**Vasorelaxant Activity.** Compounds (−)-**1** and (+)-**1** relaxed the aortic rings, which were contracted by 10 μM of *l*-phenylephrine (PHE), in a concentration-dependent manner (Figure 5). The vasorelaxant effect of (−)-**1** was more potent than that of (+)-**1** and was significant at 1 μM.

**Vascular Cyclic Nucleotide Levels.** The aortic rings treated with either (−)-**1** or milrinone showed higher cAMP and cGMP levels than those treated with DMSO (control). Cyclic AMP levels increased with 1 and 10 μM of (−)-**1** and were almost equal to those with 10 and 100 μM milrinone, respectively. In contrast, (−)-**1** increased cGMP levels more potently than milrinone (Figure 6).

## Discussion

The inhibition of cardiac PDE III represents the mechanism of action of cardiotonic activity of milrinone[19] and pyridazinones such as imazodan[20] and

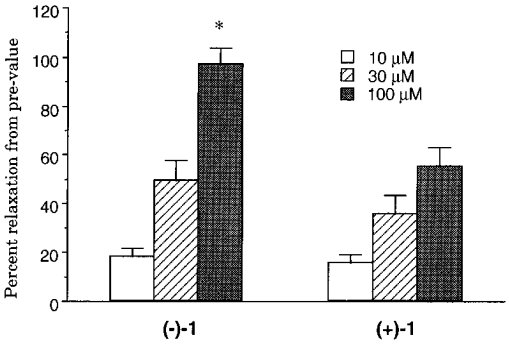



**Figure 5.** Vasorelaxant effects of **1** on the phenylephrine-induced contraction in guinea pig aorta. Each point represents the mean ± SEM of five or six preparations. *$p$ < 0.01 vs (*S*)-(+)-**1**.

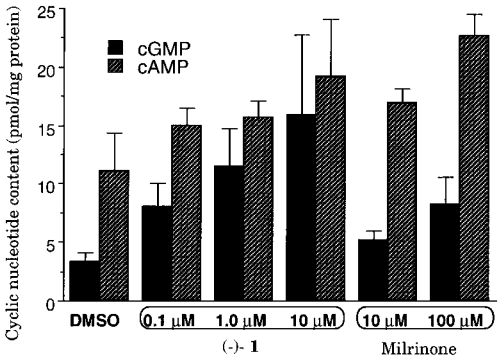



**Figure 6.** Increases in cyclic nucleotide levels (cAMP and cGMP) by (−)-**1** and milrinone in guinea pig aortic rings. Each column is the mean of three or four experiments and vertical bar indicates SEM.

indolidan[21] and is believed to be the principal component of the inotropic-vasodilatory action. The in vitro and in vivo positive inotropic effects of (−)-**1** in both dogs and guinea pigs were more potent than those of (+)-**1**. In addition, the order of the positive inotropic activity of the compounds was consistent with that of the inhibitory activity of heart PDE III. These results indicate that the PDE III inhibitory activity of (−)-**1** plays an important role in the cardiotonic activity of racemic **1**. It had been earlier demonstrated at Smith-Kline Beecham that (*R*)-(−)-4,5-dihydro-6-[4-(1,4-dihydro-4-oxo-1-pyridyl)phenyl]-5-methyl-3(2*H*)-pyridazinone (SK&F 95654) is about 80−100 times more active toward inhibition of PDE III than the corresponding (*S*)-isomer.[14] The (*R*)-configuration may, therefore, be essential for the inhibition of PDE III in the pyridazinone series.

In contrast to PDE III inhibitory and cardiotonic activities, (+)-**1** showed the most potent $Ca^{2+}$-sensitizing activity of these compounds. This result corresponds well with our finding[3] that the structural requirements for cardiotonic activity were different from those for myofibrillar $Ca^{2+}$-sensitizing activity. In this study, we found that the $Ca^{2+}$-sensitizing activity of the optical isomers was not consistent with the cardiotonic activities of them, although it was previously reported that both the positive inotropic and $Ca^{2+}$-sensitizing effects of (−)-pimobendan were more potent than those of its (+)-isomer.[7] This finding indicates that the $Ca^{2+}$ sensitizing effect is not a major mechanism of cardiotonic action of (−)-**1** and racemic **1**.

PDE III inhibitors generally dilate blood vessels by increasing cAMP levels. In this study, (−)-**1** and milrinone increased cAMP levels in guinea pig aorta. Increase in guinea pig aortic cAMP levels of (−)-**1** and milrinone showed only 10-fold difference, whereas $IC_{50}$ values of them for inhibition of bovine aortic PDE III showed 200-fold difference. We cannot clearly explain this discrepancy. However, milrinone inhibited PDE IV, which also modulates vascular cAMP level, with $IC_{50}$ value of 10 $\mu$M. Not only PDE III but also PDE IV dependent mechanism may be involved in the increasing effects of milrinone on guinea pig aortic cAMP.

Zaprinast, a PDE V specific inhibitor, also decreases vascular tone by elevation of the cGMP level.[9] In both bovine aortic PDE III and PDE V the inhibitory activities of (−)-**1** were more potent than those of (+)-**1**. The PDE inhibitory activities probably account for the difference in vasorelaxant activities between (−)-**1** and (+)-**1**. The cAMP-dependent vasodilating effects of PDE III inhibitors are beneficial for treatment of congestive heart failure by reducing preload and/or afterload. A guanylate cyclase activator isosorbide dinitrate also improves congestive heart failure.[23] Furthermore, the depressor and natriuretic actions of zaprinast appear to be mediated by an increase in cGMP. We, therefore, consider that a dual inhibitor of PDE III and PDE V may be a more effective agent for congestive heart failure than currently available pure PDE III inhibitors. Few compounds are known which have potent inhibitory activities on both PDE III and V. Racemic **1** and (−)-**1** demonstrated potent inhibitory activity on vascular PDE V in addition to both cardiac PDE III and vascular PDE III. Compound (−)-**1** actually increased cGMP levels in guinea pig aorta more potently than milrinone. Consequently, the inhibition of PDE V by racemic **1** and (−)-**1** might play an additional beneficial role in the treatment of congestive heart failure. Further biological and pharmacological studies of these compounds will be reported elsewhere.

In summary, we synthesized optically active **1** by the resolution of precursor **6** followed by enantioselective cyclization and determined the absolute configuration of (−)-**1**. Compound (−)-**1** was more potent in its cardiotonic and inhibitory activities on PDE III and V than (+)-**1**. Compound (+)-**1** showed only weak cardiotonic activities although the compound demonstrated potent $Ca^{2+}$ sensitizing activity. Racemic **1** and (−)-**1** attracted our interest for the treatment of congestive heart failure.

## Experimental Section

All melting points were determined on a Büchi 510 micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a Shimadzu IR-27G spectrophotometer. $^1$H-NMR spectra were measured on a Varian EM390, a Hitachi R-90H, and a JEOL JNM-GX-270 spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were run on a JEOL-JMS-01SG-2 and a JMS-SX102 spectrometer. Optical purity was determined by HPLC (CHIRALCEL OD, 0.46 cm i.d. × 25 cm; eluent, EtOH−hexane) (Daicel Chemistry Co., Ltd.). Carboxylic acids were analyzed after esterification by treatment with $TMSCH_2N_2$. Diastereomeric purity was determined by normal phase HPLC (YMC Pack SIL, 1.0 cm i.d. × 30 cm; eluent, AcOEt−hexane).

**(±)-4-(4-Bromo-3-nitrophenyl)-3-methyl-4-oxobutyric Acid (−)-Menthyl Ester (3).** A mixture of (±)-4-(4-bromo-3-nitrophenyl)-3-methyl-4-oxobutyric acid ((±)-**2**) (75.1 g, 238 mmol), (−)-menthol (225 g, 1.4 mol), and *p*-TsOH·$H_2O$ (15 g) was heated to 110 °C for 6 h. AcOEt and $H_2O$ were added, and the mixture was partitioned. The organic layer

was dried over $MgSO_4$ and concentrated, and then (−)-menthol was removed by distillation at 170 °C under reduced pressure to afford residual crude **3** as an oil. The crude product was used in the next reaction without further purification. An analytical sample was prepared by silica gel column chromatography purification (hexane−AcOEt = 3:1). HRMS: calcd for $C_{21}H_{29}BrNO_5$ 454.1230, found 454.1223.

**(±)-4-(4-Cyano-3-nitrophenyl)-3-methyl-4-oxobutyric Acid (−)-Menthyl Ester (4).** A mixture of **3** (11.8 g, 26 mmol) and CuCN (3.0 g, 31 mmol) in DMF (50 mL) was stirred at 130 °C for 30 min. AcOEt (50 mL) and hexane (200 mL) were added to the mixture. After removal of the precipitated copper salt, the mixture was washed with $H_2O$, dried over $MgSO_4$, and concentrated under reduced pressure to give crude **4** (10.0 g) as an oil. The crude **4** was used in the next reaction without further purification. An analytical sample was obtained by silica gel column chromatography purification (hexane−AcOEt = 2:1). HRMS: calcd for $C_{22}H_{29}N_2O_5$ 401.2077, found 401.2083.

**(±)-4-(3-Amino-4-cyanophenyl)-3-methyl-4-oxobutyric Acid (−)-Menthyl Ester (5).** $TiCl_3$ (20% aqueous solution) (85 mL) was added to a solution of **4** (10.0 g) in acetone (350 mL) portionwise. The mixture was concentrated to half volume and partitioned between AcOEt and $H_2O$. The organic layer was washed with saturated $NaHCO_3$ and $H_2O$, dried over $MgSO_4$, and concentrated to dryness to afford crude **5** (11.0 g, 84%). The crude product was used in the next reaction without further purification. An analytical sample was obtained by silica gel column chromatography purification (hexane−AcOEt = 2:1). HRMS: calcd for $C_{22}H_{30}N_2O_3$ 370.2256, found 370.2262.

**(1*R*,2*S*,5*R*)-Menthyl (*S*)-4-(4-(Benzylamino)-7-quinazolinyl)-3-methyl-4-oxobutyrate (6a) and (1*R*,2*S*,5*R*)-Menthyl (*R*)-4-(4-(Benzylamino)-7-quinazolinyl)-3-methyl-4-oxobutyrate (6b).** A mixture of **5** (41.0 g, 111 mmol), triethyl orthoformate (50 mL), and pyridinium hydrochloride (1.5 g) in DMF (250 mL) was stirred for 30 min at room temperature. Benzylamine (90 mL) was added to the mixture, and the resultant mixture was stirred at 110 °C for 2 h and then concentrated to dryness under reduced pressure. The residue was separated by silica gel flash column chromatography (2.5 kg, hexane−AcOEt = 3:1−1:1) to give 14.4 g (90% de) of **6a**, 15 g of **6**, and 18.7 g (95% de) of **6b**. Crude **6a** (90% de) was recrystallized four times from $Et_2O$−hexane to afford 3.8 g (7.0%) of pure **6a** (>99% de), and crude **6b** (95% de) was recrystallized from $Et_2O$−hexane to give 12.5 g (23%) of pure **6b** (>99% de).

**6a:** mp 69−72 °C; $[\alpha]^{25}_D = -28.1°$ ($c$ = 1.0, DMF). Anal. ($C_{30}H_{37}N_3O_3$) C, H, N. **6b:** mp 112−120 °C. $[\alpha]^{25}_D = -29.7°$ ($c$ = 1.0, DMF). Anal. ($C_{30}H_{37}N_3O_3$) C, H, N.

**(*R*)-(−)-4,5-Dihydro-5-methyl-6-(4-(benzylamino)-7-quinazolinyl)-3(2*H*)-pyridazinone ((*R*)-(−)-1).** A mixture of hydrazine monohydrate−acetic acid (1:2 v/v) (60 mL) was added to a solution of **6b** (10.0 g, 21 mmol) in EtOH (300 mL) at 10 °C and stirred for 60 h. AcOEt (1 L) was added, and the mixture was washed with $H_2O$, saturated $NaHCO_3$, and $H_2O$. The organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was crystallized from MeOH−$H_2O$ to give 3.63 g (51%) of (−)-**1** (>99% ee). An analytical sample was obtained by recrystallization from EtOH−$CH_2Cl_2$: $[\alpha]^{25}_D = -371°$ ($c$ = 1.0, DMF).

**(*S*)-(+)-4,5-Dihydro-5-methyl-6-(4-(benzylamino)-7-quinazolinyl)-3(2*H*)-pyridazinone ((*S*)-(+)-1).** Compound (*S*-)-(+)-**1** (>99% ee) (0.47 g, 66%) was obtained from **6a** (1.0 g, 2.1 mmol) using same procedure described above: $[\alpha]^{25}_D = 375°$ ($c$ = 1.0, DMF).

**(*R*)-(−)-4-Bromo-α-chloropropiophenone ((*R*)-(−)-8).** (*R*)-(−)-2-Chloropropionyl chloride (**7**), bromobenzene (7.6 mL, 72 mmol), finely powdered $AlCl_3$ (13.8 g, 100 mmol), and $CH_2Cl_2$ were mixed at 10 °C, and then the mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was poured onto a mixture of 3 N HCl (100 mL) and ice (500 mL) and then extracted with $CH_2Cl_2$. The organic layer was dried over $MgSO_4$ and concentrated to dryness to afford crude (*R*)-(−)-**8** (12.1 g, 49%) as a solid. The crude product was used in the next reaction without further purification. An analytical sample was obtained by recrystallization from hexane: >99% ee; mp 66 °C; $[\alpha]^{25}_D = -40.4°$ ($c$ = 1.0, MeOH). Anal. ($C_9H_8BrClO$) C, H, N.

**(*R*)-(+)-Methyl 4-(4-Bromophenyl)-3-methyl-4-oxobutyrate ((*R*)-(+)-9).** Potassium *tert*-butoxide (0.4 g) was added to a solution of diethyl malonate (0.46 mL) in DMF (10 mL), and the mixture was stirred for 30 min at room temperature. After addition of (*R*)-(−)-**8** (>99% ee, 0.25 g, 1.0 mmol), the reaction mixture was stirred for 15 min at same temperature, and then the reaction was quenched with phosphate buffer (pH 7). After stirring for 15 min, the mixture was partitioned between AcOEt and $H_2O$. The water layer was adjusted to pH 4 with 1 N HCl and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated to dryness. The residue was purified by silica gel column chromatography to give 0.05 g (18%) of oily (*R*)-(+)-**9**: 32% ee; $[\alpha]^{25}_D = 3.3°$ ($c$ = 1.0, MeOH); HRMS calcd for $C_{12}H_{13}{}^{79}BrO_3$ 284.0048, found 284.0033.

The chiral HPLC behavior of this compound was identical with that of the methyl ester ((+)-**9**) derived from (+)-**10** which was obtained by optical resolution (see below).

**Resolution of (±)-4-(4-Bromophenyl)-3-methyl-4-oxobutyric Acid ((±)-10).** A mixture of (±)-**10** (271 mg, 1.0 mmol) and (±)-α-methylbenzylamine (0.13 mL, 1.0 mmol) was suspended in EtOH (3 mL) and stirred for 2 h with ice cooling. Precipitated crystals were collected by filtration and were recrystallized from EtOH to give 76 mg (20%) of ((+)-α-methylbenzyl)ammonium salt of crude (*R*)-(+)-**10** (85% ee). The product was recrystallized two times from EtOH to afford pure (*R*)-(+)-**10** (>99% ee): mp 134−136 °C; $[\alpha]^{25}D = 8.7°$ ($c$ = 1.0, DMF). This compound (0.70 g, 1.8 mmol) was dissolved in MeOH (5 mL) and saturated $NaHCO_3$ (50 mL) and partitioned with $CHCl_3$. The water layer was adjusted to pH 4 with 1 N HCl and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and concentrated. The residue was crystallized from hexane to give 0.33 g of (*R*)-(+)-**10** (68%): >99% ee; $[\alpha]^{25}_D = 13.5°$ ($c$ = 1.0, MeOH).

**Enantioselective Conversion of (*R*)-(+)-10 to (*R*)-6. Nitration of (*R*)-(+)-10.** Compound (*R*)-(+)-**10** (>99% ee) (0.20 g, 0.74 mmol) was added portionwise to fuming $HNO_3$ (2 mL), and the reaction mixture was stirred at room temperature for 15 min. $H_2O$ was added to the mixture and then extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was crystallized from $Et_2O$−hexane to give 0.16 g of (*R*)-(+)-**2** (68%): >99% ee; $[\alpha]^{25}_D = 17.1°$ ($c$ = 1.0, MeOH).

**Conversion of (*R*)-(+)-2 to (*R*)-6.** Compound (*R*)-(+)-**2** (>99% ee) was converted to (*R*)-**6** (>99% de) in several steps using the method described for (*RS*)-**6**.

**Pharmacological Methods. Material.** The optical purities of the test compounds used in pharmacological tests were 99% ee for (*R*)-(−)-**1** and 96% ee for (*S*)-(+)-**1**.

**Statistics.** Intergroup differences were statistically analyzed using Mann−Whitney's *U*-test (two groups) or Tukey's test (three groups). Differences were considered significant at $p < 0.05$.

**In Vitro Studies. Positive Inotropic Activity.** Male guinea pigs, weighing 250−400 g, were anesthetized with sodium pentobarbital (50 mg/kg iv) and exsanguinated. The hearts were rapidly excised. Right ventricular papillary muscles were dissected and suspended isometrically in organ baths containing Krebs−Henseleit solution (composition in mM: NaCl, 133.5; KCl, 4.96; $CaCl_2$, 2.5; $NaH_2PO_4$, 1.35; $NaHCO_3$, 16.3; $MgSO_4$, 0.61; glucose, 7.77 (pH 7.4)) maintained at 35 °C, gassed with 95% $O_2$−5% $CO_2$. Isometric contractile force was measured with a force transducer (Nihon Kohden, TB-611T, Tokyo, Japan) under a resting tension of 0.5 g and recorded on a chart recorder (Yokogawa, Type 3066). The papillary muscles were placed between two parallel platinum plates and stimulated to contract by square-wave pulses of 2 ms at 50% above threshold voltage (Nihon Kohden, SEN-2201). After a 90-min equilibration period, increasing concentrations of racemic **1**, (*R*)-(−)-**1**, or (*S*)-(+)-**1** dissolved in dimethyl sulfoxide (DMSO) were added cumulatively to baths at 10-min intervals to provide concentration−response curves. Increases in developed tension are expressed as percent of the predrug-developed tension.

**$Ca^{2+}$-Sensitizing Activity.** Experiments were performed at 24 °C. Male guinea pigs (Hartley) weighing 300−400 g were killed by a blow on the head, and the hearts were excised. The papillary muscles were isolated from the right ventricle in the relaxing solution (RS) of the following composition (in mM): $KSO_3CH_3$, 129; $Mg(SO_3CH_3)_2$, 5.1; PIPES, 20; $Na_2ATP$, 4.2; EGTA, 2 (adjusted to pH 7.00 with KOH at 24 °C). A small bundle (about 0.1−0.2 mm in diameter and 2−3 mm in length) of muscle was prepared from the papillary muscles. One end of the bundle was connected to a strain gauge transducer (Nihon Koden, TB-611T) for measurement of isometric tension, and the other end was fixed in an organ bath. Developed tension was recorded on a chart recorder (Yokogawa). To obtain a skinned fiber, the small bundle was treated with RS containing 250 $\mu$g/mL saponin for 30 min.

At the beginning of the experiment, the fiber stretched in RS until resting tension was 1−2 mg. After RS was removed, the saponin-treated fiber was contracted with the activating solution (AS, pCa(−log[$Ca^{2+}$], M) = 6.00) of the following composition (in mM): $KSO_3CH_3$, 90; $Mg(SO_3CH_3)_2$, 5.2; PIPES, 20; $Ca(SO_3CH_3)_2$, 7.2; $Na_2ATP$, 4.2; EGTA, 10; (adjusted to pH 7.00 with KOH at 24 °C) and then relaxed with RS. For subsequent experiments, we used the fibers, which were immediately relaxed to the basal level by the RS treatment, as the skinned fibers.

The skinned fiber was contracted with AS. When the contraction reached a stable plateau (predrug value), 10 $\mu$M TFP·2HCl (Boeringer Mannheim) was added to the bath. The skinned fiber was washed with RS and contracted again with RS. The contacted fiber was treated with racemic or optically active **1** dissolved in DMSO. The calcium-sensitizing effects induced by the drugs were expressed as a percentage against of the TFP-induced contraction.

**Isolation of cAMP Phosphodiesterases.** Canine cardiac cAMP PDE (PDE III and IV) were prepared by the procedure of Weishaar and colleagues.[17] Bovine arterial PDE III, IV, and V were prepared by the method of Torphy and Cieslinski.[18a]

**Assay of Canine Cardiac PDE Activity.** The reaction buffer contained 50 mM BES−NaOH (pH 7.2) and 20 mM $MgCl_2$. The peak III fraction (40 $\mu$L) diluted with 60 $\mu$L of diluent (50 mM BES−NaOH, 0.1 mg/mL soybean tryprin inhibitor, pH 7.2) was added to the buffer in a final volume of 300 $\mu$L. The PDE activity of PDE III and PDE IV was measured with 1 $\mu$M substrate ([$^3$H]cAMP) in the presence of 25 $\mu$M cGMP and 10 $\mu$M rolipram, respectively.

After incubation at 30 °C for 10 min, the reaction was stopped by the addition of 100 $\mu$L of HCl solution (0.25 M) and then neutralized in an ice bath. The reaction mixture was incubated at 30 °C for additional 10 min with 100 $\mu$L of 100 $\mu$g/mL 5′-nucleotidase and subsequently applied to a 1 mL DEAE-Sephadex A-25 column. The radioactivity of the eluate containing [$^3$H]adenosine was determined by a liquid scintillation counter.

**Assay of Bovine Aortic PDE Activity.** The reaction buffer contained 40 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM dithiothreitol, and 20 mM NaCl. The PDE V fraction or the PDE III and IV mixture fraction (40 $\mu$L) was added to the buffer in a final volume of 300 $\mu$L. The PDE V activity was measured with 1 $\mu$M [$^3$H]cGMP in the presence of 3 mM EGTA. The PDE activity of PDE III and PDE IV was assayed with 1 $\mu$M [$^3$H]cAMP in the presence of 20 $\mu$M cGMP and 20 $\mu$M rolipram, respectively. After incubation at 30 °C for 30 min, the reaction was stopped by the addition of 100 $\mu$L of HCl solution (0.25 M) and then neutralized in an ice bath. The reaction mixture was incubated at 30 °C for additional 30 min with 100 $\mu$L of 100 $\mu$g/mL 5′-nucleotidase and subsequently applied to a 1 mL DEAE-Sephadex A-25 column. The radioactivity of the eluate containing [$^3$H]guanosine or [$^3$H]adenosine was determined by a liquid scintillation counter.

**Measurement of PDE Inhibitory Activity.** The PDE inhibitors tested were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was 1% in the reaction mixture and inhibited PDE activity by less than 10%. In the presence of the drugs, PDE activities of the four PDE fractions were measured in duplicate. The concentrations of the drugs that caused 50% inhibition of substrate hydrolysis ($IC_{50}$) were calculated from the concentration−inhibition curves.

**Vasorelaxant Activity.** Thoracic aortas were isolated from male Hartley guinea pigs (500−600 g) and cut into 3−4-mm rings. The aortic rings were mounted on metal holders and suspended in Krebs-Henseleit solution (KHS) maintained at 35 °C, gassed with 95% $O_2$−5% $CO_2$. Isometric contraction was measured under a resting tension of 1.5 g.

The rings were contracted with 10 $\mu$M PHE. The contraction reached a plateau, after which cumulatively increasing concentrations of the drugs tested, dissolved in DMSO, were added to the bath. The vasorelaxation induced by the drugs was measured.

**Measurement of Vascular cAMP and cGMP Contents.** Aortic rings from guinea pigs were contracted with 10 $\mu$M PHE. Subsequently, each concentration of (−)-**1** or milrinone, dissolved in DMSO, was added to the organ bath. After the maximum vasorelaxant effects were measured, the tissues were immediately frozen in liquid nitrogen. The frozen tissues were homogenized in ice-cold 6% trichloroacetic acid (TCA). After centrifugation, TCA in the supernatant was extracted with water-saturated ether. The content of cyclic nucleotides in the TCA-removed supernatant was assayed by a radioimmunoassay method.

**In Vivo Studies. Anesthetized Guinea Pigs.** Male Hartley guinea pigs (460−570 g) were anesthetized with halothane−nitrous oxide and were artificially ventilated (60 breaths/min with a tidal volume of 10 mL/kg) with a respirator pump. The left jugular vein was cannulated with a polyethylene tube for the administration of the drugs. To measure left ventricular pressure (LVP), a heparin-filled polyethylene catheter was inserted into the left ventricle through the left carotid artery and coupled to a pressure transducer (MPC-500, Millar Instruments). LV $dp/dt_{max}$ was derived with an electric differentiator. Increasing doses of racemic and optically active **1** were administered intravenously at 5-min intervals. The maximal increase in LV $dp/dt_{max}$ for each concentration was recorded.

**Conscious Dogs.** Adult mongrel dogs of either sex weighing 10−15 kg were anesthetized with pentobarbital sodium (35 mg/kg iv). A Millar Micro-Tip pressure transducer (MPC-500, Millar Instruments) was tunneled subcutaneously from the back to the neck and implanted into the left ventricle through the right carotid artery. After 2 days for recovery, the concious dogs were placed consciously in a small cage. The pressure transducer was coupled to a connector, and LVP and LV $dp/dt_{max}$ were measured. Racemic and optically active **1**, diluted 100 times with lactose, were administered orally in gelatin capsules. The parameters were recorded for 8 h after administration of the drugs.

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