Synthesis and Selective Coronary Vasodilatory Activity of 3,4-Dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-3-ol Derivatives: Novel Potassium Channel Openers

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A variety of compounds having a benzopyran such as levcromakalim generally exhibit potent antihypertensive activity. During extensive investigations aimed toward identifying K^+ channel openers having selective coronary vasodilation without potent hypotensive and tachycardiac effects, we synthesized a series of 3,4-dihydro-2*H*-1-benzopyran-3-ol derivatives modified at positions 2, 4, and 6 in the benzopyran ring. Initially, compounds having two methoxymethyl groups at position 2 were found to show a selective effect on coronary blood flow (CoBF) relative to mean arterial pressure (MAP) in anesthetized dogs. To find more potent vasodilators, various benzopyran derivatives modified at position 4 were synthesized and structure−activity relationships were examined by evaluation of the extent and duration of the increase in CoBF in anesthetized dogs. As a result, compounds having a (1,6-dihydro-6-oxopyridazin-3-yl)amino group at position 4, in addition to the two methoxymethyl groups at position 2, were found to be more potent and to have an improved duration of action. Among these compounds, JTV-506, (−)-(3*S*,4*R*)-6-cyano-3,4-dihydro-4-[(1,6-dihydro-1-methyl-6-oxopyridazin-3-yl)amino]-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-3-ol, exhibited good selectivity for its effect. Administration of this compound (0.03 mg/kg, po) elicited an increase of CoBF without a change of systemic blood pressure and heart rate (HR) in conscious dogs. Further evaluation was performed with respect to (i) the selectivity of its action on the coronary artery versus the aorta and (ii) its effects on MAP, HR, and electrocardiographic ST elevation. As a result, JTV-506 was selected as a potent and selective coronary vasodilator with various pharmacological features favoring clinical development.

Introduction

Since the discovery of cromakalim, a potent antihypertensive agent, a variety of related compounds possessing a benzopyran skeleton have been reported as K^+ channel openers.[1] Among them the well-known compounds include levcromakalim (the active enantiomer of cromakalim), bimakalim,[2] and Y-27152 (Figure 1).[3] These benzopyrans exert a hypotensive effect by relaxing peripheral vascular smooth muscle via opening the ATP-sensitive K^+ channels in the cell membrane. When peripheral vasodilators are used to treat ischemic heart disease, the dosage is limited by hemodynamic changes such as hypotension and tachycardia. In clinical studies, cromakalim was found to decrease the blood pressure, but it also caused reflex tachycardia.[4] The contribution of K_{ATP} channels to the regulation of blood pressure, coronary circulation, and myocardial contraction is unclear. It was recently reported that the myoprotective effect of K^+ channel openers (e.g., BMS-180448) on the ischemic heart was unrelated to the antihypertensive effect.[5] On the other hand, we hypothesized that it might be possible to find K_{ATP} channel openers selective for coronary vascular smooth muscle. Therefore, we investigated coronary-selective K^+ channel openers with minimal hypotensive effects for use as anti-ischemic agents.

During previous investigations, we found a potent coronary vasodilator (compound **1**, Figure 1) which had a slow onset of antihypertensive effect.[6] In the present study, our investigations were directed toward the discovery of more potent coronary vasodilators lacking hypotensive and tachycardiac activity. We therefore synthesized a series of 3,4-dihydro-2*H*-1-benzopyran-3-ol derivatives with modified substituents at positions 2, 4, and 6 and investigated the structure−activity relationships of these compounds by evaluation of their effects on coronary arterial blood flow (CoBF) and the mean arterial pressure (MAP) in anesthetized dogs. In addition, an extensive pharmacological evaluation was performed on compound **3**, which was selected for development.

Chemistry

(A) General Synthetic Methods. Synthesis of Key Intermediates 8 and 9 of the 3,4-Dihydro-2*H*-1-benzopyran-3-ol Derivatives. The key optically pure intermediate **8** was synthesized according to Scheme 1. Thus, cyclization of 5-cyano-2-hydroxyacetophenone[7] with 1,3-dimethoxypropan-2-one[8] in pyrrolidine-AcOH followed by reduction with $NaBH_4$, dehydration with *p*-TsOH, and chiral epoxidation with Jacobsen's reagent [(*S*,*S*)-Mn(III)−salen complex][9] and NaOCl in the presence of pyridine *N*-oxide or 4-phenylpyridine *N*-oxide afforded epoxide **8** in good yield and having an ee of 95%. The optical purity of **8** was determined by high-performance liquid chromatography (HPLC), with the racemate being prepared by epoxidation of compound **7** with *m*-chloroperbenzoic acid. Treatment of compound **8** with an aqueous solution of NH_3 in ethanol gave amino alcohol **9**. Similarly, the synthesis of compounds substituted with a halogen atom and a nitro group at position 6 was carried out using

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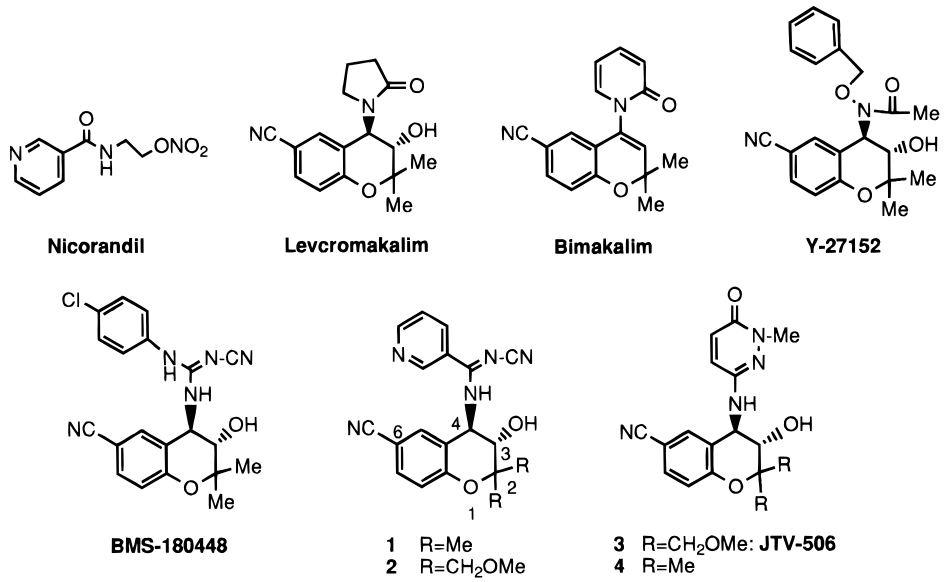
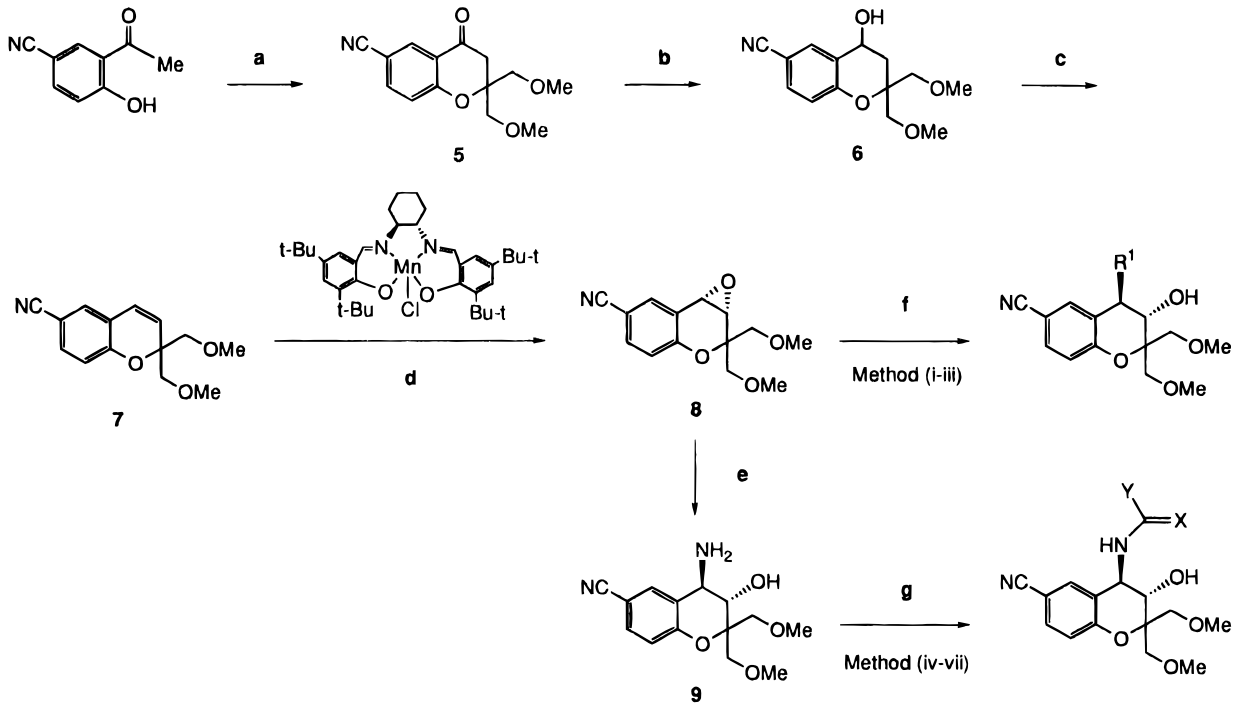


Figure 1. Representative K^+ channel openers and typical compounds **1**−**4**.

Scheme 1[a]



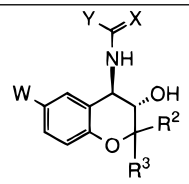
[a] Reagents: (a) $MeOCH_2COCH_2OMe$/pyrrolidine-AcOH/toluene/reflux/1 h; (b) $NaBH_4$/MeOH/0 °C/1.5 h; (c) *p*-TsOH/toluene/reflux/1 h; (d) $NaOCl/CH_2Cl_2$/pyridine *N*-oxide/0 °C/7 h; (e) aq NH_3/EtOH/rt/1 day; (f) method i, primary amine/NaH, 60 °C, 2 h, or *t*-BuOK, 0−5 °C, 1 h/anhyd DMF, method ii, heterocyclic secondary amine/NaH, 50 °C, or K_2CO_3, 80 °C, 2.5 h/DMF, method iii, phenol, or thiophenol/pyridine/EtOH/reflux/8.5 h, or Na_2CO_3/DMF; (g) method iv, imidate/MeOH/50−70 °C/1 day, method v, thiourea/WSC-HCl/DMF/rt/6 h, method vi, isocyanate/EtOH/80−90 °C/18 h, method vii, acyl chloride/Et_3N/CH_2Cl_2/rt/18 h.

5-bromo-2-hydroxyacetophenone and 5-nitro-2-hydroxyacetophenone as the respective starting materials instead of 5-cyano-2-hydroxyacetophenone.[7]

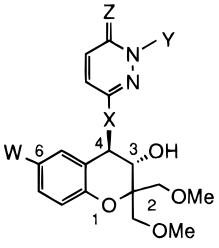
Modifications at Position 2 of the Benzopyran Skeleton. Employing essentially the same process shown in Scheme 1, compounds **10**−**13** were synthesized via five steps using 2*H*-benzopyran-4-ones derived from 5-cyano-2-hydroxyacetophenone and the following commercially available ketones: 1-methoxypropan-2-one, 1,3-diethoxypropan-2-one, 1-phenoxypropan-2-one, 1,1-dimethoxypropan-2-one, pentan-2-one, and cyclohexanone instead of 1,3-dimethoxypropan-2-one, respectively[10] (Table 1 and Supporting Information). In the case of compound **14**, additional procedures were required to construct the spiro-benzopyran skeleton; namely, 2,2-bis(*tert*-butoxymethyl)-6-cyano-2*H*-1-benzopyran was successively treated with trifluoroacetic acid and chloromethyl methyl ether in diisopropylamine to yield the corresponding spiro-benzopyran, which was converted via three steps to compound **14** (Experimental Section). In an attempt to investigate the stereochemistry at position 2 of compounds **10**, **12**, and **13**, stereoisomers of each compound were isolated, but the stereochemistry could not be determined.[11]

Modifications at Position 4 of the Benzopyran Skeleton. Compounds having modifications at position 4 of the benzopyran skeleton were prepared by a variety of methods.

Table 1. Extent and Duration of the Increase of Coronary Blood Flow in Anesthesized Dogs[a]



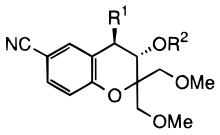
no.	R^2	R^3	W	X	Y	ED_{50}	$T_{1/2}$ (min)
1	Me	Me	CN	N-CN	3-pyridyl	1.3	4.8
2	CH_2OMe	CH_2OMe	CN	N-CN	3-pyridyl	14	2.8
10	CH_2OMe	Me	CN	N-CN	3-pyridyl	17	2.4
11	CH_2OEt	CH_2OEt	CN	N-CN	3-pyridyl	>30	
12	$CH_2OC_6H_5$	Me	CN	N-CN	3-pyridyl	>30	
13	CH_2CH_2Me	Me	CN	N-CN	3-pyridyl	20	1.0
14	$CH_2OCH_2OCH_2$		CN	N-CN	3-pyridyl	>30	
42	CH_2OMe	CH_2OMe	CN	N-CN	Me	6.0	1.9
43	CH_2OMe	CH_2OMe	CN	N-CN	C_6H_5	5.4	0.8
44	CH_2OMe	CH_2OMe	CN	N-CN	NHC_6H_5	>30	
45	CH_2OMe	CH_2OMe	CN	O	NHC_6H_5	>30	
46	CH_2OMe	CH_2OMe	CN	O	3-pyridyl	14	0.6
49	CH_2OMe	CH_2OMe	Br	N-CN	3-pyridyl	20	1.0
50	CH_2OMe	CH_2OMe	NO_2	N-CN	3-pyridyl	4.4	1.0



no.	W	X	Y	Z	ED_{50}	$T_{1/2}$ (min)
3	CN	NH	Me	O	0.58	3.2
16	CN	NH	H	O	6.2	2.6
18	CN	NH	Me	S	24	0.6
20	CN	NH	Et	O	0.57	5.2
21	CN	NH	*i*-Pr	O	1.3	5.5
22	CN	NH	Bz	O	28	1.0
23	CN	NH	CH_2COOMe	O	17	1.5
24	CN	NH	$CH_2CH{=}CH_2$	O	1.2	2.9
25	CN	NH	CH_2CH_2COOMe	O	2.3	2.3
26	CN	NH	C_7H_{15}	O	13	1.5
27	CN	NH	CH_2COOH	O	>30	
28	CN	NH	$CH_2CH_2NO_2$	O	1.6	4.9
29	CN	NH	C_6H_4-*p*-NO_2	O	>30	
30	CN	NH	CH_2CH_2OMe	O	4.1	1.6
31	CN	NH	CH_2CH_2SMe	O	3.3	1.8
32	CN	NH	CH_2-cyclopropyl	O	1.5	10.5
33	CN	NH	CH_2CH_2F	O	1.8	4.1
34	CN	NH	CH_2CN	O	5.0	3.1
35	CN	NH	CH_2CF_3	O	6.4	3.2
36	CN	NH	*n*-Pr	O	1.5	3.8
40	CN	O	Me	O	6.4	2.6
47	Br	NH	Me	O	2.3	3.5
48	NO_2	NH	Me	O	1.1	10.0

[a] After intracoronary administration, the ED_{50} was calculated as follows: ED_{50} is the dose (μg) of a compound causing a 50% increase of coronary blood flow relative to the flow increase caused by injection of 1 μg of nifedipine (100%); $n = 2$.

Method i: Compounds were obtained by nucleophilic cleavage of optically active epoxide **8** with primary amines, hydrazine, or hydroxylamine derivatives (Supporting Information). For example, epoxide **8** was treated with 3-amino-1-methyl-1,6-dihydropyridazin-6-one[12] in the presence of NaH or *t*-BuOK in anhydrous DMF to give compound **3** in 70−80% yield (99.5% ee). The optical purity of compound **3** was determined by comparison with the racemate using HPLC analysis.



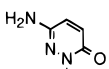
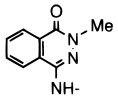
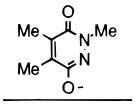
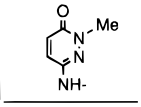
#	R^1	R^2	#	R^1	R^2
37		H	**38**		H
39		H	**15**		H
17		H	**19**		H
41		H	**51**		COC_6H_4Br-p

Figure 2. Compounds having modifications at position 4 of the benzopyran skeleton.

The racemate was prepared by nucleophilic addition of 3-amino-1-methyl-1,6-dihydropyridazin-6-one to the racemic epoxide.[12] Compound **16** was synthesized by successive chemical transformations via compounds **8**, **15**, and **17** (Figure 2 and Experimental Section).

Treatment of compound **3** with Lawesson's reagent furnished thiocarbonyl derivative **18**. Since the coronary vasodilatory activity of compound **3** was very potent, modifications on the nitrogen atom (N-1) of this compound were carried out (Table 1). Treatment of **16**[13] with alkyl halides, allyl halides, or benzyl halides in the presence of potassium carbonate in DMF yielded a series of compounds (**20**−**36**), and the Michael addition reaction of **16** with methyl acrylate provided compound **25**.

Method ii: Compounds **37** and **39** were obtained by nucleophilic cleavage of epoxide **8** with the heterocyclic secondary amines in the presence of NaH in DMF (Figure 2). Compounds **38**, **64**, and **65** were obtained by reaction of compound **8** with the amines in the presence of potassium carbonate. A nucleophilic substitution of the nitrogen atom at position 1 (not the amino group at position 3) in 3-amino-1,6-dihydropyridazin-6-one to epoxide **8** gave compound **39** without the formation of compound **16**.

Method iii: In the presence of pyridine or sodium carbonate, nucleophilic substitution of heterocyclic compounds with OH or SH group to epoxide **8** gave compounds **40**, **41**, and thioether derivatives, respectively (Supporting Information).

Method iv: Amidine derivatives **2**, **42**, and **54** were obtained by the reaction of amino alcohol **8** with the imidates in methanol (Table 1).

Method v: Guanidine derivatives **44** and **55** were obtained by the reaction of compound **9** with the thioureas in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC/HCl) in DMF.

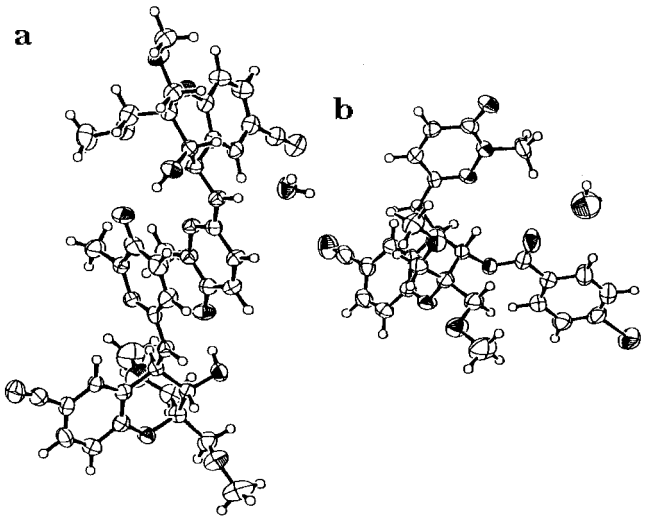


Figure 3. ORTEP drawings of (a) compound **3** ($^1/_2H_2O$) and (b) compound **51**.

Method vi: Urea derivatives **45** and **56** were obtained by the reaction of compound **9** with the isocyanates in ethanol.

Method vii: Amide derivatives **46** and **57** were obtained by acylation of compound **9** with the acyl chloride in triethylamine/methylene chloride.

(B) Absolute Structure. By analogy to the work of Jacobsen,[9] the optically active epoxide **8** should have the 3*S*,4*R*-stereochemistry as shown in Scheme 1. In order to confirm the stereochemistry of a series of benzopyran derivatives, X-ray crystallographic analysis of compound **3** was carried out. It revealed that two molecules of compound **3** and one molecule of water were arranged in a unit cell and that the relative configuration of the two substituents at positions 3 and 4 was trans (Figure 3a, the ORTEP drawings). To determine the absolute stereochemistry, compound **3** was quantitatively converted to heavy atom-containing compound **51** by benzoylation with *p*-bromobenzoyl chloride, and X-ray crystallographic analysis gave the absolute structure depicted in Figure 3b. Since no epimerization could occur through benzoylation, compound **3** was determined to have the expected 3*S*,4*R*-configuration.

Results and Discussion

During the course of synthetic modification of compound **1**, it was found that compounds **2** and **10**, with respectively one or two methoxymethyl groups introduced at position 2, selectively increased CoBF and decreased hypotensive effects in anesthetized dogs (Table 1, Figures 4 and 5, and Experimental Section for the pharmacological evaluation). In order to examine the effect of substitutions at position 2, we synthesized compounds **11**−**14** with other alkoxy or alkyl groups at this position.[11] Evaluation of the increase of CoBF produced by these derivatives demonstrated that substitution of groups other than the methoxymethyl group led to a weaker effect (Supporting Information), suggesting that bulk (**2** vs **11**, **2** vs **14**, and **10** vs **12**), flexibility of the methoxymethyl group (**2** vs **14**), and the existence of an oxygen atom (**10** vs **13**) in the group at position 2 might influence the extent and duration of the increase in CoBF. Thus, subsequent efforts to obtain more potent coronary vasodilators with minimal hypotensive activity were directed to modification of the substituents at position 4 while maintaining the methoxymethyl groups at position 2. As shown in Table 1, amidines **42** and **43** exhibited more potent coronary vasodilation than compound **2**, but the duration of action was shorter. Guanidine **44** and urea **45** did not increase CoBF even after the injection of 30 μg. Various compounds substituted with heterocyclic, phenolic, or thiophenolic groups at position 4 were synthesized (Figure 2 and Supporting Information), but, with the exception of compounds, these did not cause any increase in CoBF after injection at a dose of 30 μg.

Compound **3** having the (1,6-dihydro-6-oxopyridazin-3-yl)amino group elicited an extremely potent increase of CoBF (ED_{50} = 0.58 μg, $T_{1/2}$ = 3.2 min) (Table 1).[12,14] Following this result, derivatives of compound **3** (compounds **20**−**36**) were synthesized, which possessed a lower alkyl group or a lower alkyl substituted by an electron-withdrawing group at the N-1 atom of the 1,6-dihydro-6-oxopyridazin-3-yl ring. Among these compounds, **20**, **21**, **28**, **32**, **33**, **36**, and **48** had potent activity (ED_{50} = 0.57−1.8 μg) and a relatively long duration of action ($T_{1/2}$ > 3.2 min). Compound **18** with a thioxo group instead of an oxo group on the dihydropyridazine skeleton was 40-fold less active than compound **3**, while compound **40** with a (1,6-dihydro-6-oxopyridazin-3-yl)-oxy group at position 4 of the benzopyran ring maintained activity. Compound **19** with a (3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)amino residue did not increase CoBF at all (Figure 2). Position 6 was modified to obtain halogen derivatives **47** and **49** as well as nitro derivatives **48** and **50**. As shown in Table 1, the bromo atom of **47** and **49** proved to be less effective in increasing CoBF than the cyano group.

Further pharmacological evaluation of these potent coronary vasodilators to select compounds for possible clinical development was carried out by detailed examination of the selectivity of their effect on CoBF relative to that on MAP. In order to confirm the importance of the methoxymethyl groups at benzopyran position 2 for coronary selectivity, compound **3** was compared to compound **4**[15] (the corresponding dimethyl benzopyran) and levcromakalim (Figure 1). As shown in Figures 4 and 5, compound **3** revealed selectivity in comparison to compound **4** and also levcromakalim, as compound **2** did to compound **1**. Since compound **37**, which is a levcromakalim analog with two methoxymethyl groups at position 2 instead of dimethyl groups, caused a very weak increase of CoBF (ED_{50} > 30 μg, Figures 1 and 2), the potent and coronary-selective vasodilatory activity of compound **3** was due to the combination of two methoxymethyl groups at position 2 crucial for selectivity and a (1,6-dihydro-6-oxopyridazin-3-yl)amino group at position 4 that increased the potency. Next, a comparison was performed of compound **3** and the fairly potent coronary vasodilators **20**, **21**, **32**, and **48**, which caused a more prolonged increase of CoBF than **3** (Figure 5). Compound **32** was found to have almost the same selectivity as **3**, but compounds **20**, **21**, and **48** showed less selectivity.

Compound **3** was selected as the candidate for development because it was obtained as fine crystals, while compound **32** was amorphous. Oral administration of compound **3** (0.03 mg/kg, po) elicited an increase of CoBF without apparent changes of the systemic blood

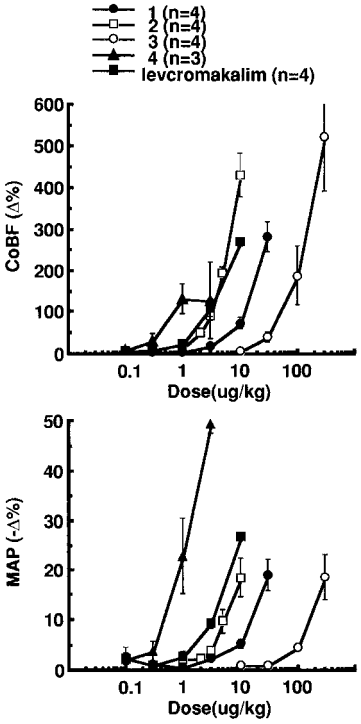


Figure 4. Effects of coronary vasodilators on coronary blood flow (CoBF) and mean arterial pressure (MAP) in anesthetized dogs. Each compound was administered intravenously.

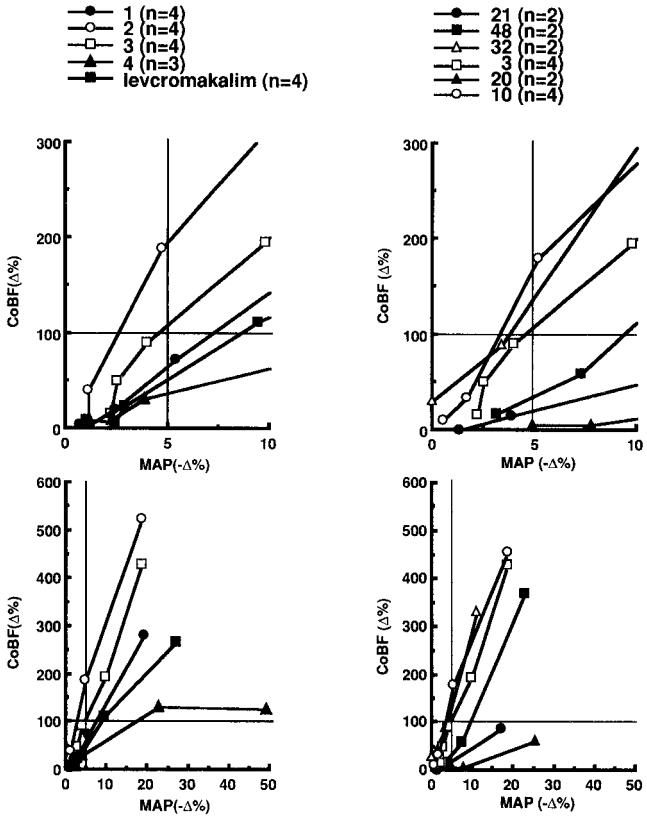


Figure 5. Correlation between the CoBF increase and MAP decrease after intravenous administration of various vasodilators. Abbreviations are as for Figure 4.

pressure and heart rate in conscious dogs (data not shown). The coronary artery selectivity of compound **3** was also compared to that of other vasodilators using isolated arteries. As shown in Table 2, the selectivity (coronary artery versus aorta) of compound **3** was superior to that of levcromakalim and nifedipine (calcium antagonist) but not to that of nicorandil.[16] These results demonstrated that the coronary selectivity of compound **3** with bis(methoxymethyl) groups at position 2 was superior to that of benzopyran compounds with dimethyl groups at position 2 and to that of typical calcium antagonists. As for the mechanism of action, compound **3** was proved to be a K^+ channel opener because glibenclamide (0.3, 1, and 3 μM), a selective blocker of K_{ATP} channels,[17] antagonized compound **3**-induced relaxation of isolated rat aorta precontracted with phenylephrine (1 μM) (Figure 6), and compound **3** caused slight or no relaxation in arteries contracted by 60 or 80 mM KCl. In an experimental angina model, intraduodenal administration of compound **3** inhibited the electrocardiographic ST elevation induced by intracoronary administration of methacholine to anesthetized rats without any apparent changes of heart rate and systemic blood pressure (Figure 7). This result suggests that compound **3** may be useful in the therapy of angina pectoris, especially variant angina pectoris. Further pharmacological results will be reported in due course.

In conclusion, a novel K^+ channel opener, compound **3** (JTV-506), appears to show promise as a potent and selective coronary vasodilator, and a clinical trial is now under way.[18]

Table 2. Vasorelaxant Effect on KCl-Induced Contraction of Porcine Coronary Artery and Rat Aorta[a]

	IC_{50} (μM)			
	compound **3**	levcromakalim	nicorandil	nifedipine
coronary artery (a)	0.11	0.36	3.2	0.0036
aorta (b)	0.3	0.29	13	0.0036
ratio (b/a)	2.73	0.81	4.06	1.0

[a] KCl-induced established contraction was taken as 100%. The mean IC_{50} (50% relaxation vs KCl-induced contraction) was determined from the concentration−relaxation curves.

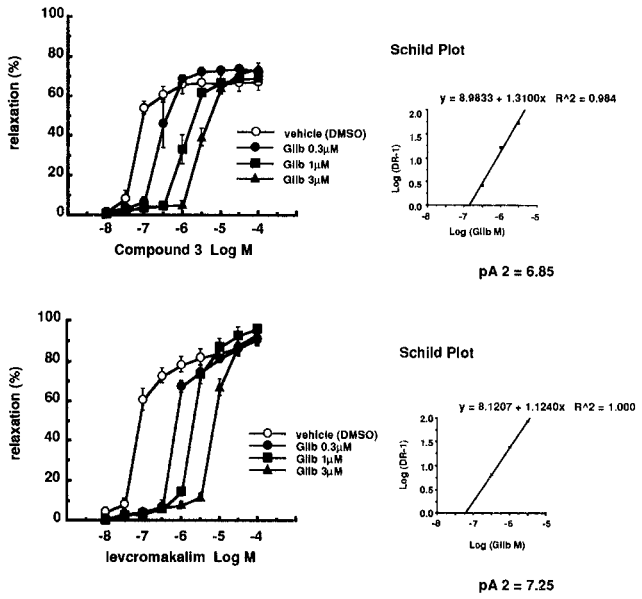


Figure 6. Effect of glibenclamide (Glib: 0.3, 1, and 3 mM) on compound **3**-, levcromakalim-, nicorandil-, or nifedipine-induced relaxation of rat aorta precontracted with phenylephrine (1 mM). Glib or DMSO was added to the bath 20 min before the addition of phenylephrine. Each point represents the mean ± SEM derived from five experiments. Inset: The corresponding Schild plots for Glib yield a slope of 1.31 and 1.12 and pA_2 values of 6.85 and 7.25 for **3**- and levcromakalim-induced relaxation, respectively.

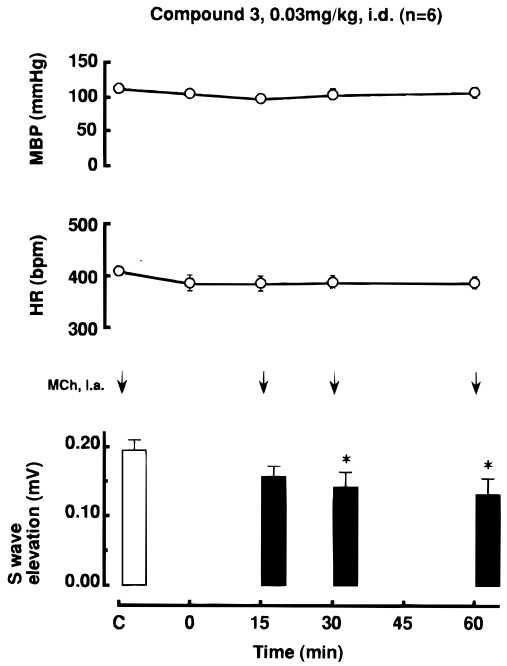


Figure 7. Effect of compound **3** on mean blood pressure (MBP), heart rate (HR), and S wave elevation of the ECG (lead II) induced by intracoronary administration of methacholine. Each value represents the mean ± SEM for six animals. Compound **3** was administered at time 0. S wave elevation occurred 30−60 s after methacholine administration. *p < 0.05 vs the control (c) by Dunnett's multiple comparison test.

Experimental Section

General Methods (Chemistry). Melting points were determined with a Yanagimoto (Yanako) micromelting point apparatus (HK-10D) and are uncorrected. ^{1}H NMR spectra (ppm, δ) were recorded using a JEOL JNMA 300 (300 MHz) spectrometer with tetramethylsilane as the internal standard. IR spectra (cm^{-1}) were obtained with a Perkin Elmer FT 1650 infrared spectrometer, and UV spectra were obtained with a Hitachi U-3000 spectrophotometer. Mass spectra (FAB$^+$) were recorded with a Finnigan TSQ 700 instrument. $[\alpha]_D$ values were obtained at 25 °C with a Perkin Elmer 241 polarimeter. HPLC was performed with a Shimazu LC-6A instrument, and thin-layer chromatography (TLC) was carried out on Merck silica gel plates (60F-254). Column chromatography was performed with Merck silica gel (70−230 mesh) using activated charcoal (or Shirasagi-A, Takeda Pharmaceutical Co.). After extraction, the extract was dried over anhydrous magnesium sulfate.

Syntheses of Compounds 5−9. (i) Modified Preparation of 1,3-Dimethoxypropan-2-one. To a stirred solution of 1,3-dimethoxypropan-2-ol (216 g, 1.80 mol) in methylene chloride (800 mL) were successively added 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical (TEMPO; 3.25 g, 0.02 mol), sodium bromide (10.75 g, 0.1 mol), and sodium bicarbonate (17.5 g, 0.2 mol) at 0 °C. To the mixture, 1.63 L of sodium hypochloride (NaOCl; Wako Ltd.; available chlorine min 5%) was added dropwise over 2 h at 0 °C. After 10 min, brine was added and the organic layer was separated. The aqueous layer was extracted with methylene chloride, and the organic layer was combined with the above solution, which was washed with potassium iodide (6.8 g, 0.04 mol) dissolved in a saturated aqueous solution of potassium hydrogen sulfate ($KHSO_4$). Then it was successively washed with a saturated aqueous solution of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$), dried, and evaporated to leave a residue. Distillation of the oily compound gave 1,3-dimethoxypropan-2-one (152.179 g, 72%): bp 56 °C/1 × 10^3 Pa.

(ii) Synthesis of 6-Cyano-3,4-dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-4-one (5). To a stirred solution of 5-cyano-2-hydroxyacetophenone (40.18 g, 248 mmol)[7] in toluene (320 mL) were added 1,3-dimethoxypropan-2-one[8] (40.87 g, 346 mmol), acetic acid (8.5 mL, 148 mmol), and pyrrolidine (10 mL, 120 mmol). Then the solution was refluxed for 1 h using a Dean−Stark apparatus. After cooling, the mixture was treated with activated charcoal (20 g) and filtered. The filtrate was washed successively with 1 N hydrochloric acid, 2 N sodium hydroxide, and brine. Then it was dried and evaporated under reduced pressure to give crystals, which were recrystallized from ethanol to yield 39.75 g (61%) of compound **5**: ^{1}H NMR ($CDCl_3$/ppm) δ 2.95 (2H, s), 3.35 (6H, s), 3.55 (2H, d, J = 10.3 Hz), 3.60 (2H, d, J = 10.3 Hz), 7.07 (1H, d, J = 8.6 Hz), 7.68 (1H, dd, J = 8.6, 2.2 Hz), 8.14 (1H, d, J = 2.2 Hz). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

Using the reaction mentioned above, 6-bromo-3,4-dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-4-one and 3,4-dihydro-2,2-bis(methoxymethyl)-6-nitro-2*H*-1-benzopyran-4-one were also synthesized (Supporting Information).

Synthesis of 6-Cyano-3,4-dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-4-ol (6). To a stirred solution of compound **5** (12.1 g, 46.4 mmol) in methanol (150 mL) was added sodium borohydride (1.96 g, 51.8 mmol) at 0 °C. After 1.5 h, the reaction was quenched with water and the mixture extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated to yield compound **6** (11.5 g, 95%): IR (neat) 3444, 2224; ^{1}H NMR ($CDCl_3$) 2.12 (1H, dd, J = 14.5, 5.3 Hz), 2.33 (1H, d, J = 14.5, 5.6 Hz), 3.36 (3H, s), 3.41 (3H, s), 3.40−3.70 (4H, m), 4.77 (1H, m), 6.92 (1H, d, J = 8.6 Hz), 7.44 (1H, dd, J = 8.6, 2.1 Hz), 7.72 (1H, d, J = 2.1 Hz). Anal. ($C_{14}H_{17}NO_4$) C, H, N.

The compounds 6-bromo-3,4-dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-4-ol and 3,4-dihydro-2,2-bis(methoxymethyl)-6-nitro-2*H*-1-benzopyran-4-ol were obtained in a similar manner.

Synthesis of 6-Cyano-2,2-bis(methoxymethyl)-2*H*-1-benzopyran (7). To a stirred solution of compound **6** (6.5 g, 24.7 mmol) in toluene (60 mL) was added *p*-toluenesulfonic acid (0.6 g, 3.15 mmol), and the solution was refluxed for 1 h with a Dean−Stark apparatus. After cooling the reaction was quenched with water and the mixture extracted with ethyl acetate. The organic layer was washed with an aqueous solution of sodium bicarbonate and brine, dried, and evaporated to yield compound **7** (4.91 g, 82%): IR (KBr) 2221; ^{1}H NMR ($CDCl_3$) 3.39 (6H, s), 3.55 (2H, d, J = 10.2 Hz), 3.60 (2H, d, J = 10.2 Hz), 5.76 (1H, d, J = 10.1 Hz), 6.49 (1H, d, J = 10.1 Hz), 6.87 (1H, d, J = 8.4 Hz), 7.25 (1H, d, J = 2.0 Hz), 7.39 (1H, dd, J = 8.4, 2.0 Hz). Anal. ($C_{14}H_{15}NO_3$) C, H, N.

The same procedure also gave 6-bromo-2,2-bis(methoxymethyl)-2*H*-1-benzopyran and 2,2-bis(methoxymethyl)-6-nitro-2*H*-1-benzopyran.

Synthesis of (−)-(3*S*,4*S*)-6-Cyano-3,4-dihydro-3,4-epoxy-2,2-bis(methoxymethyl)-2*H*-1-benzopyran (8). To a solution of compound **7** (14.72 g, 60 mmol) in methylene chloride (75 mL) were added (*S*,*S*)-mangane(III)-salen complex[9] (1.91 g, 3.0 mmol) and pyridine *N*-oxide (2.85 g, 30.0 mmol). Then a cooled solution of 0.05 M sodium dihydrogen phosphate (NaH_2PO_4) (150 mL) and fresh 0.6 M sodium hypochlorite solution (NaOCl) (375 mL, 225 mmol) were added to the mixture at 0 °C. Vigorous stirring was continued for 7 h at 0 °C, after which methylene chloride (100 mL) and Celite were added, and the mixture was filtered through a filter covered with Celite. The organic layer of the filtrate was separated from the aqueous layer, dried, and evaporated to leave a residue, which was purified by silica gel column chromatography (the residue was dissolved in toluene and developed with ethyl acetate:*n*-hexane = 1:4) to yield compound **8** [13.32 g, 85%, 95.1% ee; DAICEL Chemical Industries Ltd., HPLC Chiralcel OD column; *n*-hexane:ethanol = 19:1; flow rate, 0.5 mL/min; **8** was eluted as the earlier fraction than its enantiomer (k' = 2.31, α = 1.19)]: IR (film) 2222; ^{1}H NMR ($CDCl_3$) 3.27 (3H, s), 3.48 (3H, s), 3.57 (1H, d, J = 10.3 Hz), 3.70 (1H, d, J = 10.3 Hz), 3.71 (1H, s), 3.82 (1H, d, J = 4.4 Hz), 3.94 (1H, d, J = 4.4 Hz), 6.91 (1H, d, J = 8.5 Hz), 7.53 (1H, dd, J = 8.5, 2.0 Hz), 7.65 (1H, d, J = 2.0 Hz); $[\alpha]_D$ = −18.4° (c = 1.22, MeOH). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

In addition, (+)-(3*R*,4*R*)-6-cyano-3,4-dihydro-3,4-epoxy-2,2-bis(methoxymethyl)-2*H*-1-benzopyran was obtained with (*R*,*R*)-mangane(III)−salen complex by the procedure described above. In addition, the racemate was obtained from compound **7** and

m-chloroperbenzoic acid under the usual reaction conditions. 6-Bromo-3,4-epoxy-3,4-dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran and 3,4-epoxy-3,4-dihydro-2,2-bis(methoxymethyl)-6-nitro-2*H*-1-benzopyran were also obtained as mentioned above.

Synthesis of (−)-(3*S*,4*R*)-6-Cyano-3,4-dihydro-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)amino]-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-3-ol (3). To a stirred solution of compound **8** (4.18 g, 16 mmol) [dried by azeotropic distillation with anhydrous toluene (20 mL)] in anhydrous *N*,*N*-dimethylformamide (40 mL) was added 3-amino-1-methyl-1,6-dihydropyridazin-6-one[12] (1.92 g, 48 mmol) under an argon atmosphere. Then sodium hydride (60% dispersed in mineral oil; 1.92 g, 48 mmol) was added, and stirring was continued at 60 °C for 2 h, after which the reaction mixture was poured into ice water (200 mL) and extracted three times with chloroform (100 mL). The organic layer was washed with brine (5 mL), dried, and evaporated to leave a residue (15 g), which was purified by silica gel column chromatography [silica gel 100 g, ethyl acetate:ethanol = 9:1 (v/v)] to yield crystals (5.07 g). The crystals were dissolved in ethyl acetate, and the insoluble solid was filtered off. The filtrate was concentrated to give compound **3**, which was recrystallized from ethyl acetate (10 mL) to yield colorless crystals of this compound **3** (2.89 g, 47%, 99.5% ee) [HPLC column, Shinwakakou Ltd. ES-OVM, 4.6 × 150 mm; solvent, 20 mM K_2HPO_4 (pH 6.0); acetonitrile = 97:3; flow rate, 1 mL/min at 25 °C; **3** was eluted as the later fraction ($k' = 2.52$, $\alpha = 1.75$) than its enantiomer].

Alternatively compound **3** was prepared by the following procedure. To a stirred solution of compound **8** (1.0 g, 3.83 mmol) and 3-amino-1-methyl-1,6-dihydropyridazin-6-one[12] (528 mg, 4.21 mmol) in anhydrous *N*,*N*-dimethylformamide (17 mL) was added potassium *tert*-butoxide (1.29 g, 11.5 mmol). After stirring for 1 h at 0−5 °C, 6 N sulfuric acid (1.91 mL) was added (pH 6−7). Concentration of the reaction mixture at 60 °C reduced pressure gave a residue to which chloroform and 1 N hydrochloric acid (50 mL each) were added. The organic layer was separated, washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried, and evaporated to leave a solid that was dissolved in methanol. The solution was treated with activated charcoal (500 mg), filtered, and concentrated to yield pale yellow crystals of compound **3** (545 mg, 80%), which were purified by recrystallization from ethyl acetate or silica gel column chromatography [colorless crystals, prisms; mp 159.5−161.5 °C (AcOEt) or mp (160.0−161.0 °C (EtOH:H_2O = 1:2)]: $[\alpha]_D = -126.6°$ ($c = 0.99$, MeOH); IR (KBr) 2221, 1655, 1572; UV λ_{max} (MeOH) 205.6 (ϵ = 31 000), 245.6 (ϵ = 32 000), 350.0 nm (ϵ = 1800); ^{1}H NMR (CD_3OD) 3.32 (3H, s), 3.38 (3H, s), 3.59 (3H, s), 3.64−3.85 (4H, m), 4.16 (1H, d, J = 9 Hz), 4.36 (1H, d, J = 9 Hz), 5.09 (1H, t, J = 9 Hz), 6.80−7.00 (3H, m), 7.46 (1H, dd, J = 9, 1 Hz), 7.65 (1H, dd, J = 9, 1 Hz); MS *m*/*z* 387 $(M + H)^+$. Anal. ($C_{19}H_{22}N_4O_5 \cdot {}^1/_2H_2O$) C, H, N.

Compounds **47** and **48** were also obtained by the above procedure.

Synthesis of (−)-(3*S*,4*R*)-6-Cyano-3,4-dihydro-4-[(1,6-dihydro-6-oxo-1-*n*-propyl-3-pyridazinyl)amino]-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-3-ol (36). (i) Preparation of (−)-(3*S*,4*R*)-4-[(6-Chloro-3-pyridazinyl)amino]-6-cyano-3,4-dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-3-ol (15). Treatment of epoxide **8** (1.05 g, 4.02 mmol) with 3-amino-6-chloropyridazine (1.00 g, 7.68 mmol) instead of 3-amino-1-methyl-1,6-dihydropyridazin-6-one yielded compound **15** (1.56 g, 99%).

(ii) Preparation of (−)-(3*S*,4*R*)-4-[(6-Acetyloxy)-3-pyridazinyl)amino]-6-cyano-3,4-dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-3-ol (17) and (−)-(3*S*,4*R*)-6-Cyano-3,4-dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-3-ol (16). To a stirred solution of compound **15** (750 mg, 1.92 mmol) in acetic acid (6 mL) was added potassium acetate (380 mg, 3.87 mmol), and the mixture was refluxed for 5 h. After cooling, the reaction mixture was filtered off. The filtrate was concentrated under reduced pressure to give a residue, which was purified by SiO_2 chromatography (ethyl acetate:ethanol = 95:5) to yield compound **17** (180 mg, 23%) as the first fraction and compound **16** (500 mg, 70%) as the second fraction.

(iii) Preparation of Compound 36. To a stirred solution of compound **16** (60 mg, 0.16 mmol) in dimethylformamide (DMF) (1 mL) were added 1-bromopropane (0.017 mL, 0.19 mmol) and potassium carbonate (45 mg). The reaction mixture was heated for 3 h at 70 °C with stirring, after which the reaction was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated to leave a residue, which was purified by SiO_2 column chromatography (methanol:chloroform = 1:24) to yield compound **36** (33 mg, 50%).

Synthesis of (3*S*,4*R*)-6-Cyano-3,4-dihydro-4-[(1,6-dihydro-1-methyl-6-thioxo-3-pyridazinyl)amino]-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-3-ol (18). To a stirred solution of 3-amino-1-methyl-1,6-dihydropyridazin-6-one (500 mg, 4 mmol) in pyridine (5 mL) was added Lawesson's reagent (970 mg, 2.4 mmol), and the mixture was refluxed for 5 h. After concentration, the residue was purified by silica gel column chromatography (chloroform:methanol = 100:1) to give 3-amino-1-methyl-1,6-dihydropyridazine-6-thione (218 mg). Cleavage of epoxide **8** with this compound yielded compound **18** via the same synthetic procedure. Spectral data for compounds **15**−**36**, **47**, and **48** are available as Supporting Information.

Synthesis of (3*S*,4*R*)-4-Amino-6-cyano-3,4-dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-3-ol (9). To a stirred solution of compound **8** (3.88 g, 14.9 mmol) in ethanol (64 mL) was added an aqueous solution of 28% ammonium hydroxide (64 mL) at room temperature, and stirring was continued for 24 h at the same temperature. After evaporation, the residue was purified by silica gel column chromatography to give compound **9** (4.00 g, 96.5%): IR (KBr) 2220; ^{1}H NMR ($CDCl_3$) 3.33 (3H, s), 3.48 (3H, s), 3.60−3.90 (6H, m), 6.91 (1H, d, J = 8.5 Hz), 7.43 (1H, dd, J = 8.5, 1.9 Hz), 7.92 (1H, d, J = 1.9 Hz). Anal. ($C_{14}H_{18}N_2O_4$) C, H, N.

Syntheses of Compounds 10−14. All of the compounds shown in Table 1 were synthesized by the same procedure as that used for compound **2** (Supporting Information).

Synthesis of *N*′-Cyano-*N*-[*trans*-6-cyano-3,4-dihydro-3-hydroxy-2*H*-1-benzopyran-2-spiro-5′-(1′,3′-dioxacyclohexyl)-4-yl]-3-pyridinecarboxamidine (14). 6-Cyano-2,2-bis(*tert*-butoxymethyl)-2*H*-1-benzopyran (5.2 g; 19% yield over three steps) was obtained by cyclization of 5-cyano-2-hydroxyacetophenone (13.3 g, 82.6 mmol) and 1,3-di-*tert*-butoxypropan-2-one (19.9 g, 98.5 mmol), reduction with $NaBH_4$ in methanol, mesylation with methanesulfonylchloride, and dehydration with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). To a stirred solution of 6-cyano-2,2-bis(*tert*-butoxymethyl)-2*H*-1-benzopyran (4.8 g, 14.6 mmol) in methylene chloride (30 mL) was added trifluoroacetic acid (20 mL, 260 mmol) at 0 °C. After stirring for 5 h at 0 °C, the reaction mixture was concentrated under reduced pressure. Then the residue was dissolved in an aqueous solution of sodium bicarbonate, and the solution was extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated to leave 6-cyano-2,2-bis(hydroxymethyl)-2*H*-1-benzopyran (2.7 g, 85%): ^{1}H NMR (DMSO-d_6) 3.40−3.60 (4H, m), 4.98 (1H, d, J = 5.9 Hz), 5.00 (1H, d, J = 5.9 Hz), 5.79 (1H, d, J = 10.1 Hz), 6.57 (1H, d, J = 10.1 Hz), 6.85 (1H, d, J = 8.1 Hz), 7.50−7.60 (2H, m). MS *m*/*z* 218 $(M + H)^+$.

To a stirred solution of 6-cyano-2,2-bis(hydroxymethyl)-2*H*-1-benzopyran (1.0 g, 4.6 mmol) in methylene chloride (9 mL) were added diisopropylamine (3 mL, 22.9 mmol) and chloromethyl methyl ether (1.2 mL, 15.8 mmol) at 0 °C. Stirring was continued for 3 h at room temperature and then for another 3 h at 40 °C. The reaction mixture was concentrated to leave a residue, which was dissolved in 10% citric acid. The resulting solution was extracted with ethyl acetate. The organic layer was then washed with sodium bicarbonate solution and brine, dried, and evaporated to leave an oily compound. This was dissolved in benzene, and boron trifluoride etherate (1.4 mL, 11.4 mmol) was added at room temperature. After 2 h, the reaction mixture was poured into ice water, and the solution was extracted with methylene chloride. The organic layer was washed with sodium bicarbonate solution and brine, dried, and evaporated to leave a residue, which was triturated with methanol to give 6-cyanospiro[2*H*-1-benzopyran-2,5′-1′,3′-dioxacyclohexane] (0.74 g, 70%): ^{1}H NMR ($CDCl_3$) 3.83 (2H, d,

J = 11.8 Hz), 4.02 (2H, d, J = 11.8 Hz), 4.85 (1H, d, J = 6.2 Hz), 4.98 (1H, d, J = 6.2 Hz), 5.76 (1H, d, J = 10.0 Hz), 6.52 (1H, d, J = 10.0 Hz), 6.99 (1H, d, J = 8.5 Hz), 7.29 (1H, d, J = 1.9 Hz), 7.44 (1H, dd, J = 8.5, 1.9 Hz). Anal. ($C_{13}H_{11}NO_3$) C, H, N.

Successive reactions of compound **2** afforded compound **14** as a colorless powder: IR (KBr) 3428, 2226, 2182; ^{1}H NMR (DMSO-d_6) 3.90−4.20 (5H, m), 4.68 (1H, d, J = 6.0 Hz), 4.90−5.10 (2H, m), 6.21 (1H, d, J = 5.4 Hz), 7.12 (1H, d, J = 8.6 Hz), 7.63 (1H, dd, J = 7.9, 4.9 Hz), 7.70 (1H, d, J = 8.6 Hz), 7.99 (1H, s), 8.12 (1H, d, J = 7.9 Hz), 8.78 (1H, d, J = 4.9 Hz), 8.86 (1H, s), 9.57 (1H, d, J = 7.5 Hz); MS m/z 392 $(M + H)^+$. Anal. ($C_{20}H_{17}N_5O_4$) C, H, N.

Synthesis of (−)-(3*S*,4*R*)-3-[(4-Bromobenzoyl)oxy]-3,4-dihydro-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)-amino]-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-6-carbonitrile (51). *p*-Bromobenzoyl chloride (560 mg, 2.55 mmol) was added to a stirred solution of compound **3** (250 mg, 0.647 mmol) in pyridine (5 mL), and the mixture was stirred at 80 °C for 2 h. The solvent was evaporated under reduced pressure to leave a residue, which was diluted with chloroform. The organic layer was washed with water, dried, and evaporated to leave a residue, which was purified with silica gel column chromatography ($CHCl_3$:EtOH = 97:3, v/v) to give compound **51** (368 mg) quantitatively as a colorless crystalline solid: mp 184.0−186.5 °C (EtOH:H_2O = 2:1); IR (KBr) 2222, 1721, 1661, 1610; ^{1}H NMR ($CDCl_3$) 3.31 (3H, s), 3.37 (3H, s), 3.54 (3H, s), 3.69 (2H, s), 3.71 (1H, d, J = 10.5 Hz), 3.78 (1H, d, J = 10.5 Hz), 4.76 (1H, d, J = 8.5 Hz), 5.31 (1H, dd, J = 8.5, 7.8 Hz), 5.79 (1H, d, J = 7.8 Hz), 6.72 (1H, d, J = 9.7 Hz), 6.78 (1H, d, J = 9.7 Hz), 7.03 (1H, d, J = 8.6 Hz), 7.49 (1H, d, J = 8.7 Hz), 7.68 (2H, s), 7.79 (2H, d, J = 8.7 Hz); $[\alpha]_D$ = −82.6° (c = 1.05, MeOH). Anal. ($C_{26}H_{25}BrN_4O_6$) C, H, N.

Synthesis of (−)-(3*S*,4*R*)-4-(1*H*-3-Amino-6-oxopyridazin-1-yl)-6-cyano-3,4-dihydro-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-3-ol (39). To a stirred solution of compound **8** (212 mg, 0.81 mmol) and 3-amino-1,6-dihydropyridazin-6-one (178 mg, 1.60 mmol) in anhydrous *N*,*N*-dimethylformamide (4 mL) was added 60% NaH (58 mg, 1.45 mmol) at 0 °C, and stirring was continued at 50 °C for 36 h. Then the reaction mixtures was poured into ice water and extracted with chloroform. The organic layer was washed with brine, dried, and evaporated to leave a residue, which was purified by silica gel column chromatography (chloroform:methanol = 30:1) to give compound **39** (134 mg, 44%) as the sole product: colorless crystals; mp 186−189 °C ($CHCl_3$−*n*-hexane); IR (KBr) 3353, 2225, 1611, 1560; ^{1}H NMR (DMSO-d_6) 3.18 (3H, s), 3.34 (3H, s), 3.53−3.73 (4H, m), 4.53−4.60 (1H, m), 5.75 (1H, d, J = 6.0 Hz), 5.86 (2H, s), 6.08 (1H, brs), 6.84 (1H, d, J = 10.0 Hz), 6.94−6.99 (3H, m), 7.56 (1H, dd, J = 8.5, 1.5 Hz); MS m/z 373 $(M + H)^+$; $[\alpha]_D$ = −51.5° (c = 1.31, MeOH). Anal. ($C_{18}H_{20}N_4O_5$) C, H, N.

Compound **37** was also obtained by the above procedure.

Synthesis of (−)-(3*S*,4*R*)-6-Cyano-3,4-dihydro-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)oxy]-2,2-bis-(methoxymethyl)-2*H*-1-benzopyran-3-ol (40). To a stirred solution of compound **8** (280 mg 1.07 mmol) and 3-hydroxy-1-methyl-1,6-dihydropyridazin-6-one[19] (142 mg, 1.13 mmol) in ethanol (2.8 mL) was added pyridine (0.13 mL), and the solution was refluxed for 8.5 h with stirring. After cooling, evaporation of the solvent gave a residue, which was purified with silica gel column chromatography (methanol:chloroform = 1:99) to provide compound **40** (228 mg, 55%): mp 137.7−138.7 °C; IR (KBr) 2222, 1658, 1580; ^{1}H NMR (DMSO-d_6) 3.24 (3H, s), 3.28 (3H, s), 3.49 (1H, d, J = 10.3 Hz), 3.55−3.65 (2H, m), 3.58 (3H, s), 3.73 (1H, d, J = 10.3 Hz), 4.20 (1H, dd, J = 6.0, 5.6 Hz), 5.05 (1H, d, J = 6.0 Hz), 6.06 (1H, d, J = 5.6 Hz), 7.02 (1H, d, J = 9.8 Hz), 7.04 (1H, d, J = 8.5 Hz), 7.25 (1H, d, J = 9.8 Hz), 7.69 (1H, dd, J = 8.5, 1.8 Hz), 7.80 (1H, d, J = 1.8 Hz); MS m/z 388 $(M + H)^+$; $[\alpha]_D$ = −112.6° (c = 1.0, MeOH). Anal. ($C_{19}H_{21}N_3O_6$) C, H, N.

Synthesis of (−)-(3*S*,4*R*)-*N*′-Cyano-*N*-[6-cyano-3,4-dihydro-3-hydroxy-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-4-yl]-3-pyridinecarboxamidine (2). To a stirred solution of compound **9** (3.00 g, 10.8 mmol) in methanol (5 mL) was added a solution of methyl *N*-cyano-3-pyridinecarboximidate[20] (3.00 g, 18.6 mmol) in methanol (5 mL) at room temperature. Then stirring was continued at 50−70 °C for 1 day. After removal of the solvent, the residue was purified by silica gel column chromatography (3% methanol in chloroform) to yield compound **2** as a colorless powder (2.21 g, 50.3%): IR (KBr) 3428, 2226, 2182; ^{1}H NMR (DMSO-d_6) 3.20 (3H, s), 3.32 (3H, s), 3.50−3.80 (4H, m), 4.19 (1H, dd, J = 9.6, 5.8 Hz), 5.36 (1H, dd, J = 9.6, 8.5 Hz), 6.30 (1H, d, J = 5.8 Hz), 6.98 (1H, d, J = 8.5 Hz), 7.60−7.70 (2H, m), 7.78 (1H, s), 8.28 (1H, d, J = 8.0 Hz), 8.79 (1H, d, J = 4.9 Hz), 8.99 (1H, s), 9.54 (1H, d, J = 8.5 Hz); $[\alpha]_D$ = −139.6° (c = 1.01, MeOH). Anal. ($C_{21}H_{21}N_5O_4$) C, H, N.

Compounds **42**, **43**, **49**, and **50** were obtained in a similar manner.

Synthesis of (−)-(3*S*,4*R*)-*N*″-Cyano-*N*-[6-cyano-3,4-dihydro-3-hydroxy-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-4-yl]-*N*′-phenylguanidine (44). To a stirred solution of compound **9** (253 mg, 0.91 mmol) and *N*-cyano-*N*′-phenylthiourea (230 mg, 1.18 mmol) in DMF (1.2 mL) was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (226 mg, 1.18 mmol) at room temperature. Then stirring was continued for 6 h at room temperature, after which the reaction mixture was diluted with ethyl acetate and dilute hydrochloric acid. The organic layer was separated, washed successively with saturated sodium bicarbonate and brine, dried, and evaporated to leave a residue, which was purified by silica gel column chromatography (2% methanol in chloroform) to afford compound **44** as a colorless powder (200 mg, 52%): IR (KBr) 2224, 2184, 1607, 1582; ^{1}H NMR (DMSO-d_6) 3.17 (3H, s), 3.30 (3H, s), 3.50−3.70 (4H, m), 4.17 (1H, dd, J = 9.7, 6.0 Hz), 5.06 (1H, dd, J = 9.7, 8.5 Hz), 6.00 (1H, brs), 6.95 (1H, d, J = 8.5 Hz), 7.10−7.20 (1H, m), 7.30−7.40 (4H, m), 7.50−7.70 (3H, m), 9.28 (1H, s); $[\alpha]_D$ = −43.1° (c = 0.99, MeOH); MS m/z = 422 $(M + H)^+$. Anal. ($C_{22}H_{23}N_5O_4$) C, H, N.

Synthesis of *N*-[*trans*-6-Cyano-3,4-dihydro-3-hydroxy-2,2-bis(methoxymethyl)-2*H*-1-benzopyran-4-yl]-*N*′-phenylurea (45). To a suspension of compound **9** (racemate) (500 mg, 1.80 mmol) in ethanol (2 mL) was added phenyl isocyanate (0.32 mL, 2.92 mmol) at room temperature, and stirring was continued overnight at 80−90 °C. After evaporation, the residue was purified by silica gel column chromatography (chloroform and then 4% methanol in chloroform) to give compound **45** (697 mg), which was recrystallized from methanol to yield this compound as colorless needles (359.5 mg, 50.3%): mp 186.7−187.7 °C; IR (KBr) 2225, 1677; ^{1}H NMR (DMSO-d_6) 3.19 (3H, s), 3.23 (3H, s), 3.50−3.70 (4H, m), 4.01 (1H, dd, J = 9.6, 5.7 Hz), 4.84 (1H, dd, J = 9.6, 8.2 Hz), 5.74 (1H, d, J = 5.7 Hz), 6.57 (1H, d, J = 8.3 Hz), 6.90−7.00 (2H, s), 7.20−7.30 (2H, m), 7.40−7.50 (2H, m), 7.54−7.65 (2H, m), 8.52 (1H, s). Anal. ($C_{21}H_{23}N_3O_5$) C, H, N.

Synthesis of (−)-(3*S*,4*R*)-6-Cyano-3,4-dihydro-2,2-bis-(methoxymethyl)-4-(3-pyridinecarboxamido)-2*H*-1-benzopyran-3-ol (46). To a stirred mixture of compound **9** (306.5 mg, 1.10 mmol) in methylene chloride (4 mL) and triethylamine (0.4 mL, 2.88 mmol) was added nicotinoyl chloride hydrochloride (236.3 mg, 1.33 mmol) at room temperature, and stirring was continued overnight at the same temperature. Then the reaction was quenched with water and the mixture extracted with chloroform. The organic layer was washed with brine, dried, and evaporated to leave a residue (408 mg), which was purified by silica gel column chromatography to yield compound **46** as a colorless powder (240.5 mg, 56.9%): IR (KBr) 2225, 1644; ^{1}H NMR ($CDCl_3$) 3.36 (3H, s), 3.42 (3H, s), 3.73 (1H, d, J = 11.0 Hz), 3.74 (2H, s), 3.88 (1H, d, J = 11.0 Hz), 4.25 (1H, d, J = 8.7 Hz), 5.52 (1H, dd, J = 8.7, 8.5 Hz), 6.90 (1H, d, J = 8.5 Hz), 7.30 (1H, d, J = 8.5 Hz), 7.35−7.50 (2H, m), 7.59 (1H, s), 8.17 (1H, J = 8.0 Hz), 8.71 (1H, d, J = 4.9 Hz), 9.02 (1H, s); $[\alpha]_D$ = −46.0° (c = 0.50, methanol). Anal. ($C_{20}H_{21}N_3O_5$) C, H, N.

Pharmacological Studies. (1) Effect on Coronary Blood Flow. Mongrel dogs of both sexes were used. Under sodium pentobarbital anesthesia (30 mg/kg + 2−5 mg/kg/h, iv), the animals were ventilated with a positive pressure respirator (20 mL/kg × 18/min) after endotracheal intubation.

Measurement of Coronary Blood Flow following Intracoronary Administration. Coronary blood flow was

measured using an extracorporeal flow probe connected to an electromagnetic flowmeter (Nihon Kohden MFV-2100). To measure blood flow, the left coronary artery was cannulated with a metal tube through the right common carotid artery and autoperfused with blood from the right femoral artery, and the flow probe was placed in the perfusion circuit. The perfusion pressure and systemic blood pressure were measured with pressure transducers (Millar, MPC-500). Then each compound was dissolved in 30% DMF and diluted with saline, and the solution was injected into the coronary artery.

Measurement of Coronary Blood Flow following Intravenous Administration. After thoracotomy at the left fourth intercostal space, the left circumflex artery was exposed and blood flow was measured with an intracorporeal flow probe connected to an electromagnetic flowmeter (Nihon Kohden MFV 2100). Systemic blood pressure was measured from the right femoral artery with a pressure transducer. Both parameters were recorded continuously on a polygraph (NEC San-ei 360). All compounds were dissolved in DMF and diluted with saline below 10% of DMF, and the solutions were administered into the cannulated femoral vein.

(2) Vasorelaxant Effect. The thoracic aorta was isolated from exsanguinated rats, and coronary arteries were isolated from porcine hearts obtained from an abattoir. Helical or open ring strips were prepared, and the endothelium was removed mechanically. Each preparation was suspended in an organ bath containing 10 mL of Krebs−Henseleit solution at 37 °C and aerated with a mixture of 95% O_2 and 5% CO_2. Changes in tension were recorded under a resting load of 1 g using an isometric force transducer (Nihon Kohden TB-612T). The strips were contracted with 30 or 40 mM KCl and 1 μM phenylephrine. All test compounds were dissolved in DMSO and applied to the organ bath.

(3) Antianginal Effect. The antianginal effect was investigated in Sprague−Dawley rats under pentobarbital anesthesia as the inhibition of ST segment (T wave) elevation induced by intracoronary administration of methacholine (5 μg). Systemic blood pressure was measured with a pressure transducer in a femoral artery, and heart rate was measured with a heart rate counter. Standard limb lead II of the electrocardiogram was recorded on an ECG recorder. A poly(ethylene) tube was inserted into the coronary ostium through the right carotid artery to a point near the aortic valve in order to allow administration of methacholine into the coronary arteries. Rats showing S wave elevation of over 0.15 mV were used for this experiment. Compound **3** was dissolved in distilled water and administered intraduodenally.

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Supporting Information Available: IR spectra, ^{1}H NMR spectra, mass spectra, $[\alpha]_D$ values, and elementary analysis data for compounds **10**−**13**, **15**−**67**, 6-bromo derivatives, and 6-nitro derivatives and crystallographic data and table of positional parameters, bond distances, and bond angles for compounds **3** and **51** (55 pages). Ordering information is given on any current masthead page.

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