A Novel Anti-Ischemic ATP-Sensitive Potassium Channel (K_{ATP}) Opener without Vasorelaxation: *N*-(6-Aminobenzopyranyl)-*N′*-benzyl-*N″*-cyanoguanidine Analogue

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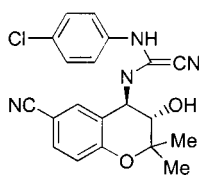
This paper describes the design, synthesis, and biological evaluation of a novel anti-ischemic compound, (2*S*,3*S*,4*R*)-*N*-(6-amino-3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-2*H*-benzopyranyl)-*N′*-benzyl-*N″*-cyanoguanidine (**33**), and the structure−activity relationships leading to the discovery of this compound. Compound **33** significantly reduced the myocardial infarct zone to area at risk (IZ/AAR) in the ischemic myocardium rat model with high cardioselectivity. Since the cardioprotective effect of compound **33** is reversed by ATP-sensitive potassium channel (K_{ATP}) blockers, its anti-ischemic effect appears to be at least mediated by K_{ATP} opening. In addition, compound **33** shows good protective activity on neuronal cells against oxidative stress, and therefore it is suggested that compound **33** may have therapeutic potential both in cardio- and in neuroprotection.

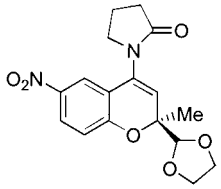
Introduction

Ischemic cell injury may arise from complex interactions which include disturbances in energy metabolism[1] and modifications in synaptic transmission.[2] Several studies have shown that the membrane depolarization is a trigger signal for the induction of neuronal hyperexcitability, irreversible cellular dysfunction, and cell death, which is mainly mediated by activation of the glutamate receptor.[3−5] Like brain ischemia, myocardial ischemia initiates various cellular changes progressively, ultimately leading to irreversible myocardial injury, cell death, and tissue necrosis.[6] Since complex mechanisms are involved in the cellular changes caused by ischemia, several strategies for protection against ischemic injury have been proposed,[7] including oxygen free radical scavengers, adenosine agonists, calcium channel blockers, and ATP-sensitive potassium channel (K_{ATP}) openers.[8]

K_{ATP} openers[9] have shown protective properties on ischemia-reperfusion injury both in heart and brain, presumably through "ischemic preconditioning", an endogenous protective mechanism.[10,11] Certain similarities[12] for preconditioning between heart and brain mediated by K_{ATP} channel have been reported,[13] raising the possibility of K_{ATP} openers having therapeutic potential as cardio and neuroprotective agents. Since the cardioprotective and vasorelaxant potencies of K_{ATP} openers follow distinct and separable structure−activity relationships,[14−18] substantial efforts have been devoted to find safe, cardioselective agents. In such an attempt, scientists at Bristol-Myers Squibb discovered BMS-180448 (**1**)[19] and BMS-191095[17] with enhanced selectivity toward the ischemic myocardium over vasorelaxation, which improved a narrow therapeutic window of the first-generation K_{ATP} openers (e.g., cromakalim).[20] Despite these efforts, more cardioselective compounds are needed. Thus, we set out to find agents having protective effects on both brain and cardiac ischemia without vasorelaxant activity.



BMS-180448 (1)



SKP-450 (2)

Previously, we discovered SKP-450 (**2**),[21−23] a new K-channel activator as a potential anti-hypertensive drug. Structurally SKP-450 is different from cromakalim in a number of aspects, including an acetal moiety at the 2-position which imparted a new chiral center and resulted in improvement of efficacy and pharmacokinetic parameters as well.[24,25] SKP-450 was orally well absorbed in rats, of which bioavailablity calculated on the basis of radioactivity was 68−97%.[24] Since then, we have turned our attention to discovering novel compounds with protective properties for ischemic diseases based on the K_{ATP} activation mechanism. For this purpose, we have designed various chemical structures based upon the benzopyran scaffold having an acetal moiety at the 2-position as in SKP-450. Onto this backbone, we introduced a cyanoguanidine portion at

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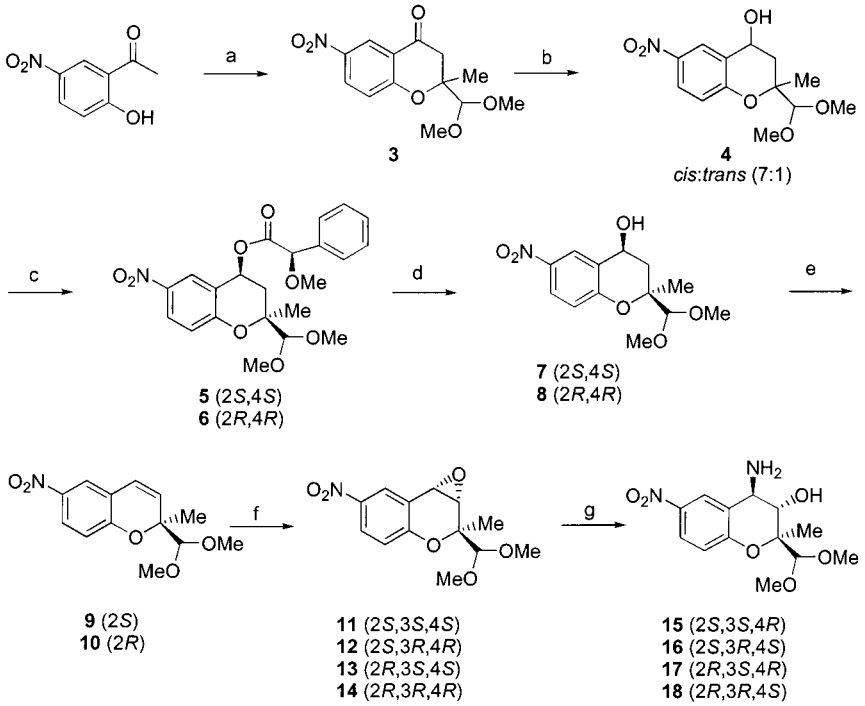
Scheme 1[a]



[a] Reagents and conditions: (a) pyruvic aldehyde dimethylacetal, pyrrolidine, toluene, reflux; (b) DIBAL-H, toluene, −78 °C; (c) (*R*)-(−)-α-methoxyphenylacetic acid, DCC, DMAP, EtOAc; (d) (1) separation by recrystallization (Et_2O−hexane), (2) LiOH, MeOH−H_2O, 0 °C; (e) (1) CH_3SO_2Cl, *i*-Pr_2NEt, CH_2Cl_2; (2) DBU, toluene, reflux; (f) Mn(III)-Salen, aq NaOCl, CH_2Cl_2; (g) aq NH_3, EtOH, 50 °C.

the 4-position, which seemed to be a crucial moiety for cardioprotective activity. Then we identified a structurally distinct analogue, (2*S*,3*S*,4*R*)-*N*-(6-amino-3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-2*H*-benzopyranyl)-*N*′-benzyl-*N*″-cyanoguanidine (**33**), which has both neuroprotective and cardioprotective effects with negligible vasorelaxant activity. In this paper, we describe the anti-ischemic and anti-oxidant effects of this series of compounds and discuss the structure−activity relationships leading to compound **33** (KR-31378)

Chemistry

The stereochemistry of benzopyrans has been reported to be critical for selectivity and cardioprotective potency.[17,18] Therefore, we prepared optically pure benzopyranyl cyanoguanidine analogues via four stereoisomers of amino alcohols **15**−**18** as described in Schemes 1−4. First, the reaction of 6-hydroxy-3-nitroacetophenone with pyruvic aldehyde dimethylacetal in the presence of pyrrolidine provided the cyclized 4-chromanone compound **3** in good yield. Next, we converted the keto group to a hydroxyl group which will serve as a handle for attaching an optically active auxiliary. The reduction of chromanone **3** was examined with various reducing agents to obtain preferentially one diastereoisomer which would make a subsequent resolution step much easier and effective. We found that the reduction with diisobutylaluminum hydride (DIBAL-H) at −78 °C in toluene was optimal to give a cis diastereoisomer between the C4-hydroxy and C2-acetal groups as a major product (cis:trans = 7:1 ratio) in 96% yield. The cis isomer of **4** was esterified with (*R*)-(−)-α-methoxyphenylacetic acid using 1,3-dicyclohexylcarbodiimide (DCC). One stereoisomer (2*S*,4*S*) **5** was crystallized out first in 28% yield, followed by the other stereoisomer (2*R*,4*R*) **6** in 15% yield. The absolute stereochemistry of the compounds **5** and **6** was determined by X-ray crystallographic analysis.

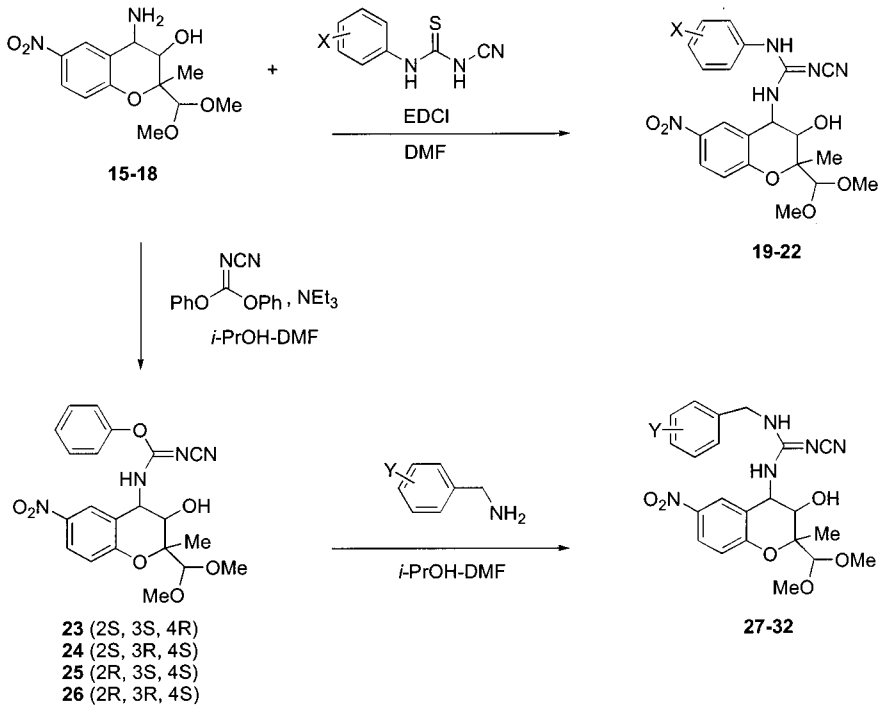
Alkaline hydrolysis of compounds **5** and **6** using LiOH gave the corresponding alcohols **7** and **8**, which were readily converted to the mesylated derivatives (CH_3SO_2-Cl, *i*-Pr_2NEt, CH_2Cl_2). The elimination of C4-mesylate by heating with 1,8-diazabicyclo[5.4.0]undec-7ene (DBU) provided the olefin compounds **9** (2*S*) and **10** (2*R*). The epoxidation of **9** and **10** with Jacobsen's reagent [(*S*,*S*)-Mn(III)-salen complex] and NaOCl afforded two optically active epoxides **11** (2*S*,3*S*,4*S*) and **13** (2*R*,3*S*,4*S*) in 83% and 85% yield, respectively.[26] Similarly, the other two chiral epoxides **12** (2*S*,3*R*,4*R*) and **14** (2*R*,3*R*,-4*R*) were obtained from **9** and **10** using (*R*,*R*)-Jacobsen's catalyst. Treatment of the four stereoisomeric epoxides with aqueous ammonia in ethanol at 50 °C provided the optically pure amino alcohols **15**−**18** (Scheme 1).

The cyanoguanidine derivatives with *N*-aryl groups **19**−**22** were prepared by the treatment of amino alcohols **15** or **17** with *N*-cyano-*N*-aryl-thioureas,[27] prepared from the corresponding isothiocyanates and preformed monosodium cyanamide using a water soluble coupling reagent (WSC), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) in DMF (Scheme 2).

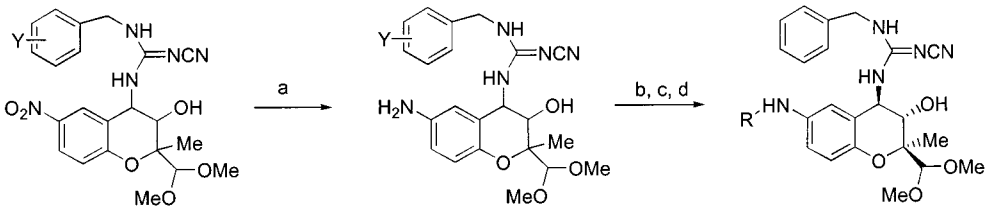
Similarly, the *N*-benzyl cyanoguanidine derivatives **27**−**32** were prepared with the amino alcohols **15**−**18** and diphenylcyanocarbonimidate to give intermediates **23**−**26**, which were subsequently reacted with appropriate benzylamines (Scheme 2).[28]

Reduction of the 6-nitro group was carried out either by catalytic hydrogenation or by sodium borohydride-copper(II) acetate in aqueous methanol to provide the corresponding 6-amino compounds **33**−**37** (Scheme 3).

Scheme 2

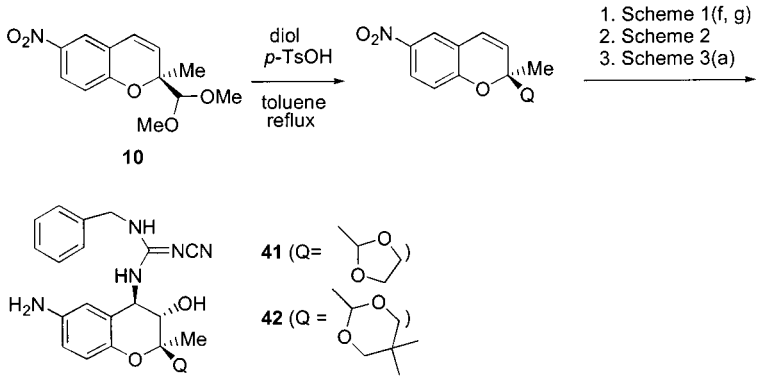


Scheme 3[a]



[a] Reagents and conditions: (a) H_2, 10% Pd/C, MeOH or $NaBH_4$, $Cu(OAc)_2$, MeOH−H_2O; (b) for **38**, Ac_2O, Et_3N, 4-DMAP, CH_2Cl_2; (c) for **39**, $MeSO_2Cl$, Et_3N, CH_2Cl_2; (d) for **40**, PhNCO, CH_2Cl_2.

Scheme 4



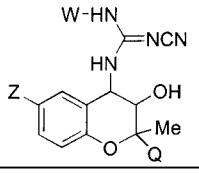
The amine **33** was elaborated to the acetamide **38**, sulfonamide **39**, and urea **40** using straightforward methods (Scheme 3).

To determine the pharmacological and pharmacokinetical effects on acetal analogues at C2, cyclic acetal compounds **41** and **42** were prepared by transacetalizations of olefin **10** with appropriate diols in the presence of catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) followed by the same procedures described for **33** (Scheme 4).

Result and Discussion

We employed functional tests to determine cardioprotective, neuroprotective, and vasorelaxant potencies of new compounds: Vasorelaxant potencies were determined by measuring IC_{50} values for relaxation of the methoxamine contracted rat aorta.[15] Cardioprotective effects were determined by measuring a ratio of myocardial infarction zone to area at risk (IZ/AAR),[29] using an ischemic myocardium damage rat model. Neuroprotective effects of compounds against the iron-induced

Table 1. Vasorelaxant Potencies and Cardio- and Neuroprotective Effects of Benzopyranyl Cyanoguanidine Analogues



Compounds	Z	W	Q[a]	Vasorelaxant Potency[b] IC_{50} (μM) or % at 30 μM[c]	Cardioprotection[d] IZ/AAR (%)/ AAR/LV (%) at 0.3 mg/Kg	Neuroprotection[e] IC_{50} (μM) or % at 30 μM
Vehicle				14%	61/40	
BMS-180448				3.2	39/39	20%
19 (2*R*,3*S*,4*R*)	NO_2	4-Cl-Ph		17.5	52/37	61%
20 (2*R*,3*S*,4*R*)	NO_2	3-Cl-Ph		30.5	40/47	70%
21 (2*S*,3*S*,4*R*)	NO_2	4-Cl-Ph		18%	46/46	58%
22 (2*S*,3*S*,4*R*)	NO_2	4-OMe-Ph		38%	43/38	45%
27 (2*S*,3*S*,4*R*)	NO_2	Bn		37%	49/37	7%
28 (2*S*,3*R*,4*S*)	NO_2	Bn		9.8	49/38	5%
29 (2*R*,3*S*,4*R*)	NO_2	Bn		12.6	nd[f]	37%
30 (2*R*,3*R*,4*S*)	NO_2	Bn		7.3	nd[f]	70%
32 (2*R*,3*R*,4*S*)	NO_2	4-OMe-Bn		16.1	42/32	nd[f]
33 (2*S*,3*S*,4*R*)	NH_2	Bn		14%[c]	37/35	2.2
34 (2*S*,3*R*,4*S*)	NH_2	Bn		15%	51/33	9.2
35 (2*R*,3*S*,4*R*)	NH_2	Bn		12%	46/39	8.0
36 (2*R*,3*R*,4*S*)	NH_2	Bn		11%	51/38	4.1
37 (2*S*,3*S*,4*R*)	NH_2	4-OMe-Bn		28%	42/37	4.1
38 (2*S*,3*S*,4*R*)	$NHCOCH_3$	Bn		37%	45/41	25%
39 (2*S*,3*S*,4*R*)	$NHSO_2CH_3$	Bn		16%	43/35	10%
40 (2*S*,3*S*,4*R*)	NHCONHPh	Bn				
41 (2*S*,3*S*,4*R*)	NH_2	Bn		12%	52/32	3.6
42 (2*S*,3*S*,4*R*)	NH_2	Bn		11%	56/42	60%

[a] Q = $CH(OCH_3)_2$ for compounds **19**−**40**. [b] Vasorelaxant potency was assessed by measurement of IC_{50} for inhibition of methoxamine-contracted rat aorta. IC_{50} value is presented as a mean with 95% confidence interval in parentheses, $n = 3$. If IC_{50} is not available, inhibition percentage at 30 μM (except **33**) was given. Each value is an average of three determinations and within ±10%. [c] Inhibition percentage at 300 μM. [d] Cardioprotective effect was determined by measuring a ratio of myocardial infarct zone to area at risk (IZ/AAR) in ischemic myocardium damage rat model, $n = 3$ or higher. Each value given is an average and within ±10%. [e] Neuroprotective effect was evaluated by the reduction rate of LDH release from neuronal cells injured by iron at 30 μM. Each value is an average of four determinations and within ±10%. IC_{50} value was determined on the compounds showing >70% reduction rate of LDH at 30 μM and presented as a mean of 3 experiments ($n = 4$ for each experiment) with 95% confidence interval in parentheses. [f] Not determined.

injury of neuronal cells were evaluated by the reduction of lactate dehydrogenase (LDH) release into the medium,[30] primarily at 30 μM concentration. The compounds showing greater than 70% neuroprotection at 30 μM were subjected to concentration−response studies to determine IC_{50} values.

Initially, we synthesized a series of *N*-phenylcyanoguanidine analogues (**19**−**22**) with the same (3*S*,4*R*)-stereochemistry as BMS-180448[15] and evaluated their biological activities. Compounds **20** and **22** showed comparable cardioprotective effects to BMS-180448 but with 10 times weaker vasorelaxation potencies (Table

1), indicating better cardioselectivity. Compound **21** with (2*S*,3*S*,4*R*)-stereochemistry showed weaker vasorelaxation effect than the stereoisomer **19** with (2*R*,3*S*,4*R*)-stereochemistry (74% vs 18% at 30 μM), with somewhat improved anti-ischemic potency. Thus, the stereochemistry at C2 seems to play a contribution to the pharmacological profile.

N-Arylcyanoguanidine analogues have been studied extensively for cardioselectivity,[17] but not with *N*-benzylcyanoguanidines. Thus, we prepared a number of optically pure *N*-benzylcyanoguanidinyl derivatives (**27**−**32**). Replacement of the phenyl ring with a benzyl group did not significantly change the cardioprotective effects (Table 1), and likely the *N*-phenylcyanoguanidine derivatives, compound **27** with (2*S*,3*S*,4*R*)-stereochemistry, showed the lowest vasorelaxant activity among four stereoisomers, although these stereoisomers showed only marginal neuroprotective activity.

Further work was concentrated on the modification at the 6-position of the compound **27**, since the modification at this position was reported to have significant effects on the pharmacological profiles.[31] Compound **33** with an amino group at C6 showed significantly improved anti-ischemic activity with much improved cardioselectivity (Table 1). While its anti-ischemic effect (36.6%, IZ/AAR) was comparable with that of BMS-180448 (39.1%, IZ/AAR), its vasorelaxant effect was dramatically reduced (negligible vasorelaxation even at 300 μM). It was rather an unexpected result to us because it has been reported that an electron withdrawing group at C6 of the benzopyran ring seems to be essential for the activity of K_{ATP} openers.[15] Furthermore, compound **33** showed a potent neuroprotective effect (IC_{50}, 2.2 μM) as well. Further modifications of amino group at C6 to amide **38**, sulfonamide **39**, and urea **40** analogues, resulted in the decrease of both cardio- and neuroprotective effects and selectivity, suggesting that the amino group is optimal for cardioprotective and neuroprotective potency.

With these results, we synthesized three other stereoisomers of compound **33** and evaluated their biological properties as shown in Table 1. The isomers with (3*S*,4*R*)-stereochemistry showed consistently better cardioprotective activity than the isomers with (3*R*,4*S*)-stereochemistry as in the case of BMS-180448. Among four stereoisomers, compound **33** was confirmed to have the best cardio- and neuroprotective properties.

Additionally we have modified the acetal moiety at C2 of compound **33** to examine the possibility of further improvement for pharmacological and particularly for physicochemical properties. But, as seen in Table 1, those modifications resulted in a loss of cardioprotective effects while their neuroprotective effects were retained, suggesting that structure−activity relationships for cardioprotective effects and neuroprotective effects may be different.

With compound **33** in hand, we carried out experiments to determine whether the cardioprotective effect of compound **33** was due to K_{ATP} activation using known K_{ATP} blockers, glibenclamide and sodium 5-hydroxydecanoate (5-HD). As shown in Table 2, both glibenclamide and 5-HD significantly blocked the cardioprotective effect of compound **33**, which suggests that at least the anti-ischemic effect is mediated via K_{ATP} opening.

Table 2. Dose Dependency of Cardioprotective Effect and the Effects of K_{ATP} Blockers

	cardioprotection,[a] IZ/AAR (%)/AAR/LV (%)		
	0.1 mg/kg (*n*)	0.3 mg/kg (*n*)	1 mg/kg (*n*)
vehicle		61/40 (6)	
BMS-180448	50/41 (5)	39/39 (5)	32/37 (4)
33	42/34 (5)	37/35 (7)	34/35 (7)
33 + Gliben (1 mg/kg)	nd[b]	nd[b]	51/33 (3)
33 + 5-HD (10 mg/kg)	nd[b]	nd[b]	46/35 (3)

[a] Cardioprotective effect was determined by measuring a ratio of myocardial infarct zone to area at risk (IZ/AAR) in ischemic myocardium damage rat model with or without a K_{ATP} blocker (glibenclamide (Gliben) or sodium 5-hydroxydecanoate (5-HD)). Each value is given as an average and within ±10%. [b] Not determined.

However, the neuroprotective effect of compound **33** against $FeSO_4$ toxicity was not reduced significantly by glibenclamide (data not shown), suggesting that its neuroprotective mechanism is not related to the activation of the K_{ATP} channel, as has been also demonstrated with other K_{ATP} openers such as diazoxide and levcromakalim.[32] Further studies will be needed to elucidate its mechanism of action for neuroprotection as well as cardioprotection. However, since ischemic cascades proceed by complex interactions, it may be a useful strategy to develop the compound acting at more than one target site in ischemic cascade.

Conclusion

We discovered a novel cardioprotective agent, (2*S*,3*S*,-4*R*)-*N*-(6-amino-3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-2*H*-benzopyranyl)-*N*′-benzyl-*N*″-cyanoguanidine (**33**), which protected ischemic rat heart in a dose-dependent manner by the mechanism of cardiac K_{ATP} channel activation. Anti-ischemic effect of compound **33** on ischemic rat heart was comparable with that of BMS-180448, but with much improved cardioselectivity than BMS-180448. In addition to the cardioprotective effect, compound **33** showed good neuroprotective effect against oxidative stress. These activities together make compound **33** (KR-31378) a new effective protecting agent for brain and cardiac ischemia.

Experimental Section

Chemistry. Melting points were determined on a capillary melting point apparatus and are uncorrected. Anhydrous solvents were dried by conventional methods. Reagents of commercial quality were used from freshly opened containers unless otherwise stated. ^{1}H NMR spectra were recorded on a Bruker AM-500 (500 MHz) or a Bruker AM-300 (300 MHz) with TMS as an internal standard. Chemical shifts are reported in δ (ppm) downfield from TMS. Mass spectra were obtained with a JEOL JMS-DX 303 instrument by using electron impact or chemical ionization techniques. Elemental analyses were performed by Korea Research Institute of Chemical Technology's Analytical Department and C, H, and N values are within ±0.4% of the calculated values.

3,4-Dihydro-2-dimethoxymethyl-2-methyl-6-nitro-4-oxo-2*H*-1-benzopyran (3). To a solution containing a mixture of 3- and 5-nito-6-hydroxyphenone (200 g, 1.10 mol; prepared by nitration of 2-hydroxyacetophenone and used without purification) in toluene (800 mL) were added pyruvic aldehyde dimethylacetal (147 mL, 1.21 mol) and pyrrolidine (22.5 mL, 0.27 mol). The mixture was heated at reflux for 4 h using Dean−Stark apparatus and cooled to room temperature, then silica gel (100 g) was added to the reaction, which was stirred

for 10 min and filtered through a short silica gel column. The filtrate was concentrated in vacuo, and the residue was recrystallized from ethyl acetate−hexane to give **3** (120 g, 39% based on 2-hydroxyacetophenone) as a yellow solid: mp 108 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 3H), 2.70 (d, J = 16.7 Hz, 1H), 3.10 (d, J = 16.9 Hz, 1H), 3.44 (s, 3H), 3.52 (s, 3H), 4.31 (s, 1H), 7.07 (d, J = 9.2 Hz, 1H), 8.31 (dd, J = 9.4, 2.8 Hz, 1H), 8.71 (d, J = 2.8 Hz, 1H); HRMS (M^+) 281.0899 calcd for $C_{13}H_{15}N_1O_6$, found 281.0904.

3,4-Dihydro-2-dimethoxymethyl-4-hydroxy-2-methyl-6-nitro-2*H*-1-benzopyran (4). The keto compound **3** (80 g, 285 mmol) was dissolved in toluene (800 mL) and cooled to −78 °C. To the solution was added diisobutylaluminum hydride (247 mL, 1.5 M in toluene) slowly over 2 h, and the mixture was continuously stirred for an additional 1 h at −78 °C. An aqueous solution of 1 N HCl (1 L) was added to the reaction, and the layers were separated. Water layer was extracted with ethyl acetate (500 mL) and was combined with the organic layer. The combined organic layers were washed with water (500 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give **4** (77 g, 96%) with 7:1 ratio of cis and trans, which was carried to the next step without further purification. Only small amounts of regioisomers were separated by silica gel column chromatography for analysis. Cis-compound: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H), 2.15 (dd, J = 14.1, 5.8 Hz, 1H), 2.23 (dd, J = 14.1, 7.1 Hz, 1H), 3.46 (s, 3H), 3.54 (s, 3H), 4.28 (s, 1H), 4.38 (d, 1H), 4.77 (m, 1H), 6.87 (d, J = 9.1 Hz, 1H), 8.00 (dd, J = 9.1, 2.7 Hz, 1H), 8.32 (d, J = 2.7 Hz, 1H); HRMS (M^+) 283.1055 calcd for $C_{13}H_{17}N_1O_6$, found 283.1046. Trans-compound: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 3H), 1.78 (dd, J = 14.1, 5.8 Hz, 1H), 2.59 (dd, J = 14.1, 5.8 Hz, 1H), 3.39 (s, 3H), 3.49 (s, 3H), 4.01 (d, 1H), 4.18 (s, 1H), 5.03 (m, 1H), 6.85 (d, J = 9.2 Hz, 1H), 7.98 (dd, J = 9.2, 2.7 Hz, 1H), 8.34 (d, J = 2.7 Hz, 1H).

(2*S*,4*S*)-3,4-Dihydro-2-dimethoxymethyl-4-(*R*-(−)-α-methoxyphenylacetoxy)-2-methyl-6-nitro-2*H*-1-benzopyran (5). To a solution of **4** (320.0 g, 1.13 mol) in ethyl acetate (2 L) were added *R*-(−)-α-methoxyphenylacetic acid (191.4 g, 1.15 mol), 1,3-dicyclohexylcarbodiimide (237.6 g, 1.15 mol), and 4-(dimethylamino)pyridine (6.9 g, 0.06 mol) slowly at 0 °C, then the mixture was stirred at room temperature for 1 h. After the completion of reaction, the reaction was filtered to remove byproduct, mainly urea, and the filtrate was concentrated under reduced pressure. The residue was purified by short silica gel column chromatography, followed by recrystallization (ethyl ether−hexane) twice to give **5** as a white solid (136 g, 28%): mp 111 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.34 (s, 3H), 1.99 (dd, J = 13.4, 6.3 Hz, 1H), 2.23 (dd, J = 14.4, 6.3 Hz, 1H), 3.47 (s, 3H), 3.48 (s, 3H), 3.53 (s, 3H), 4.20 (s, 1H), 4.91 (s, 1H), 6.06 (dd, J = 6.3, 6.3 Hz, 1H), 6.90 (d, 1H), 7.37−7.50 (m, 5H), 8.03−8.10 (m, 2H); HRMS (M^+) 432.1658 calcd for $C_{22}H_{26}N_1O_8$, found 432.1648.

(2*R*,4*R*)-3,4-Dihydro-4-(*R*-(−)-α-methoxyphenylacetoxy)-2-methyl-6-nitro-2*H*-1-benzopyran (6). Further crystallization of mother liquor gave **6** as a 15% yield: mp 78 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.32 (s, 3H), 2.10 (dd, J = 13.4, 9.9 Hz, 1H), 2.23 (dd, J = 13.4, 6.3 Hz, 1H), 3.43 (s, 3H), 3.51 (s, 6H), 4.21 (s, 1H), 4.81 (s, 1H), 5.96 (dd, J = 9.9, 6.3 Hz, 1H), 6.84 (d, 1H), 7.37−7.48 (m, 5H), 7.59 (d, 1H), 7.98−8.02 (m, 1H); HRMS (M^+) 432.1658 calcd for $C_{22}H_{26}N_1O_8$, found 432.1659.

(2*S*,4*S*)-3,4-Dihydro-2-dimethoxymethyl-4-hydroxy-2-methyl-6-nitro-2*H*-1-benzopyran (7). To a solution of **5** (156 g, 0.36 mol) in THF (870 mL) was added an aqueous solution of 1 N LiOH (434 mL) at 0 °C, and the mixture was stirred for 30 min. After the completion of reaction, the mixture was extracted with ethyl acetate (500 mL × 3). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give **7** (101 g, 99%) as a solid: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H), 2.15 (dd, J = 14.1, 5.7 Hz, 1H), 2.23 (dd, J = 14.1, 7.1 Hz, 1H), 3.47 (s, 3H), 3.55 (s, 3H), 4.28 (s, 1H), 4.42 (brs, 1H), 4.77 (m, 1H), 6.87 (d, J = 9.0 Hz, 1H), 8.01 (dd, J = 9.0, 2.8 Hz, 1H), 8.34 (d, J = 2.8 Hz, 1H); HRMS (M^+) 283.1055 calcd for $C_{13}H_{17}N_1O_6$, found 283.1049.

(2*S*)-2-Dimethoxymethyl-2-methyl-6-nitro-2*H*-1-benzopyran (9). To a solution of **5** (95 g, 0.34 mol) in dichloromethane (500 mL) was added *N*,*N*-diisopropylethylamine (100.7 mL, 0.58 mol) followed by methansulfonyl chloride (37 mL, 0.47 mol). The mixture was stirred at room temperature for 15 h, diluted with dichloromethane (300 mL), and then washed with water (500 mL) and brine (500 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give **9** (75 g, 84%): mp 66−68 °C; ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3H), 3.40 (s, 3H), 3.46 (s, 3H), 4.19 (s, 1H), 5.74 (d, J = 10.1 Hz, 1H), 6.39 (d, J = 10.1 Hz, 1H), 6.74 (d, J = 8.9 Hz, 1H), 7.75 (d, J = 2.8 Hz, 1H), 7.89 (dd, J = 8.9, 2.8 Hz, 1H); HRMS (M^+) 265.0950 calcd for $C_{13}H_{15}N_1O_5$, found 265.0957.

General Procedure for the Synthesis of Epoxide 11−14. To a solution of olefin (30 g, 113 mmol) and Jacobson's reagent (3.59 g, 5.65 mol) in dichloromethane (150 mL) was added a mixture of 0.55 M NaOCl (822 mL) and 0.05 M Na_2HPO_4 (342 mL) dropwise through an additional funnel at 0 °C. After vigorous stirring at room temperature overnight, the reaction was passed through a pad of Celite and washed with dichloromethane and water 3−4 times. The filtrate was extracted with dichloromethane, and the organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give an oil, which was solidified with standing.

(2*S*,3*S*,4*S*)-3,4-Dihydro-2-dimethoxymethyl-3,4-epoxy-2-methyl-6-nitro-2*H*-1-benzopyran (11). Epoxide **11** was obtained from **9** and (*S*,*S*)-Jacobson's reagent: mp 67−69 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.54 (s, 3H), 3.25 (s, 3H), 3.47 (s, 3H), 3.80 (d, J = 4.8 Hz, 1H), 3.97 (d, J = 4.8 Hz, 1H), 4.90 (s, 1H), 6.83 (d, J = 9.9 Hz, 1H), 8.11 (dd, J = 9.9, 3.0 Hz, 1H), 8.25 (d, J = 3.0 Hz, 1H); HRMS (M^+) 282.0977 calcd for $C_{13}H_{16}N_1O_6$, found 282.0967.

(2*S*,3*R*,4*R*)-3,4-Dihydro-2-dimethoxymethyl-3,4-epoxy-2-methyl-6-nitro-2*H*-1-benzopyran (12). Epoxide **12** was obtained from **9** and (*R*,*R*)-Jacobson's reagent: mp 61−63 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (s, 3H), 3.57 (s, 3H), 3.65 (s, 3H), 3.78 (d, J = 4.5 Hz, 1H), 3.94 (d, J = 4.5 Hz, 1H), 4.44 (s, 1H), 6.92 (d, J = 8.9 Hz, 1H), 8.13 (dd, J = 8.9, 2.8 Hz, 1H), 8.28 (d, J = 2.8 Hz, 1H); HRMS (M^+) 282.0977 calcd for $C_{13}H_{16}N_1O_6$, found 282.0976.

General Procedure for the Synthesis of Amino Alcohol 15−18. To a solution of epoxide (29 g, 103 mmol) in ethanol was added 25% NH_4OH (288 mL, 2.06 mol), and the mixture was stirred at 50 °C overnight. After evaporation of ethanol under reduced pressure, the residue was treated with NH_4Cl and extracted with ethyl acetate. The organic layer was washed with water, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a foam (26.4 g, 86%).

(2*S*,3*S*,4*R*)-4-Amino-3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2*H*-1-benzopyran (15): mp 108−113 °C; ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 3H), 3.52 (s, 3H), 3.53 (s, 3H), 3.71 (d, J = 9.75 Hz, 1H), 3.74 (d, J = 9.75 Hz, 1H), 4.34 (s, 1H), 6.78 (d, J = 9.10 Hz, 1H), 7.93 (dd, J = 9.10, 2.75 Hz, 1H), 8.43 (d, J = 2.75 Hz, 1H); HRMS (M^+) 298.1165 calcd for $C_{13}H_{19}N_2O_6$, found 298.1165.

(2*S*,3*R*,4*S*)-4-Amino-3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2*H*-1-benzopyran (16): mp 101−104 °C; ^{1}H NMR ($CDCl_3$) δ 1.56 (s, 3H), 3.38 (m, 4H), 3.45 (s, 3H), 3.93 (d, J = 9.8 Hz, 1H), 3.74 (d, J = 9.75 Hz, 1H), 4.36 (s, 1H), 6.85 (d, J = 9.0 Hz, 1H), 8.02 (dd, J = 9.1, 2.8 Hz, 1H), 8.49 (s, 1H); HRMS (M^+) 298.1165 calcd for $C_{13}H_{19}N_2O_6$, found 298.1148.

General Procedure for the Synthesis of *N*-Phenyl Cyanoguanidine 19−22. To a solution of of an appropriate *N*-cyano-*N*′-phenylthiourea sodium salt (1.68 mmol) and amino alcohol **15−18** (500 mg, 1.68 mmol) in DMF (5 mL) was added 1-[3-(dimethylamino)propyl]-2-ethylcarbodiimide hydrochloride (418 mg). The mixture was stirred for 5 h at room temperature and acidified with 10 mL of 1 N HCl, then extracted with ethyl acetate (30 mL × 2). The organic layer was washed with water and brine, dried ($MgSO_4$), filtered, and

concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane:ethyl acetate = 1:1) to afford the phenylcyanoguanidine **19**−**22** as a foam.

(2*R*,3*S*,4*R*)-*N*′-(4-Chlorophenyl)-*N*″-cyano-*N*-(3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2*H*-benzopyran-4-yl)guanidine (19): mp 117−121 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.38 (s, 3H), 3.45 (s, 3H), 3.51 (s, 3H), 4.15 (d, J = 7.6 Hz, 1H), 4.35 (s, 1H), 5.04 (m, 1H), 6.24 (br, 1H), 6.90 (d, J = 9.0 Hz, 1H), 7.26−7.36 (m, 4H), 8.06 (dd, J = 2.2, 9.0 Hz, 1H), 8.31 (br, 1H), 8.44 (br, 1H); HRMS $(M+1)^+$ calcd 476.1337, found 476.1343; Anal. ($C_{21}H_{22}ClN_5O_6$) C, H, N.

(2*R*,3*S*,4*R*)-*N*′-(3-Chlorophenyl)-*N*″-cyano-*N*-(3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2*H*-benzopyran-4-yl)guanidine (20): mp 118−121 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 3H), 3.48 (s, 3H), 3.54 (s, 3H), 4.21 (d, J = 8.1 Hz, 1H), 4.37 (s, 1H), 5.02 (m, 1H), 6.23 (br, 1H), 6.92 (d, J = 9.0 Hz, 1H), 7.18−7.40 (m, 4H), 8.10 (dd, J = 2.4, 9.0 Hz, 1H), 8.39 (br, 2H); HRMS $(M+1)^+$ calcd 476.1337, found 476.1333; Anal. ($C_{21}H_{22}ClN_5O_6$) C, H, N.

(2*S*,3*S*,4*R*)-*N*′-(4-Chlorophenyl)-*N*″-cyano-*N*-(3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2*H*-benzopyran-4-yl)guanidine (21): mp 129−132 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.29 (s, 3H), 3.40 (s, 3H), 3.41 (s, 3H), 4.08 (m, 1H), 4.40 (s, 1H), 5.04 (dd, J = 8.1, 8.4 Hz, 1H), 5.48 (br, 1H), 6.85 (d, J = 9.0 Hz, 1H), 7.23−7.40 (m, 3H), 7.95 (dd, J = 2.4, 9.0 Hz, 1H), 8.09 (br, 1H), 9.22 (br, 1H); HRMS $(M+1)^+$ calcd 476.1337, found 476.1339; Anal. ($C_{21}H_{22}ClN_5O_6$) C, H, N.

(2*S*,3*S*,4*R*)-*N*″-Cyano-*N*-(3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2*H*-benzopyran-4-yl)-*N*′-(4-methoxyphenyl)guanidine (22): mp 105−108 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.37 (s, 3H), 3.47 (s, 3H), 3.49 (s, 3H), 3.77 (s, 3H), 4.09 (d, J = 7.8 Hz, 1H), 4.16 (br, 1H), 4.34 (s, 1H), 5.10 (dd, J = 8.1, 9.3 Hz, 1H), 5.69 (br, 1H), 6.89 (m, 3H), 7.26 (m, 2H), 7.95 (br, 1H), 8.01 (dd, J = 2.0, 4.5 Hz, 1H), 8.16 (s, 1H); HRMS $(M+1)^+$ calcd 472.1832, found 472.1833; Anal. ($C_{22}H_{25}N_5O_7$) C, H, N.

General Procedure for the Synthesis of *N*-Benzyl Cyanoguanidine 27−32. To a solution of an appropriate amino alcohol **15**−**18** (26 g, 87 mmol) in 2-propanol (400 mL) and DMF (100 mL) were added triethylamine (16 mL, 113 mmol) and diphenylcyanocarbonimidate (22.8 g, 95.7 mmol) portionwise. After the mixture was stirred at room temperature overnight, all volatiles were removed under reduced pressure. To the residue was added water, which was extracted with ethyl acetate. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane:ethyl acetate = 1:2) to give a pale yellow foam (24.7 g, 64%) **23**−**26**. Phenoxymethyl imine **23**−**26** (24.7 g, 55.8 mmol) was dissolved in hot 2-propanol (100 mL), to which was added an appropriate benzylamine (139.5 mmol). After the mixture was stirred overnight, the volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 1:1) to give benzyl cyanoguanidine **27**−**32** as a pale yellow foam.

(2*S*,3*S*,4*R*)-*N*′-Benzyl-*N*″-cyano-*N*-(3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2*H*-benzopyran-4-yl)guanidine (27): mp 117−120 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.33 (s, 3H), 3.53 (s, 3H), 3.58 (s, 3H), 4.16 (d, J = 8.4 Hz, 1H), 4.38−4.52 (m, 3H), 4.82 (br, 1H), 5.85 (br, 1H), 6.25 (br, 1H), 6.72 (br, 1H), 6.90 (d, J = 8.7 Hz, 1H), 7.30 (m, 5H), 8.06 (d, J = 8.7 Hz, 1H), 8.29 (br, 1H); HRMS (M^+) calcd 455.1805, found 455.1802; Anal. ($C_{22}H_{25}N_5O_6$) C, H, N.

(2*S*,3*R*,4*S*)-*N*′-Benzyl-*N*″-cyano-*N*-(3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2*H*-benzopyran-4-yl)guanidine (28): mp 99−100 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 3H), 3.41 (s, 3H), 3.43 (s, 3H), 3.80 (dd, J = 7.8, 8.0 Hz, 1H), 4.30 (d, J = 5.7 Hz, 1H), 5.12 (m, 1H), 5.57 (br, 1H), 6.61 (dd, J = 5.5, 5.5 Hz, 1H), 6.84 (d, J = 9.0 Hz, 1H), 7.21−7.35 (m, 5H), 7.98 (dd, J = 2.4, 7.2 Hz, 1H), 8.03 (br, 1H); HRMS (M^+) calcd 455.1805, found 455.1827; Anal. ($C_{22}H_{25}N_5O_6$) C, H, N.

(2*R*,3*S*,4*R*)-*N*′-Benzyl-*N*″-cyano-*N*-(3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-6-nitro-2*H*-benzopyran-4-yl)guanidine (29): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.43 (s, 3H), 3.43 (s, 3H), 3.46 (s, 3H), 3.60 (m, 1H), 3.74 (d, J = 8.6 Hz, 1H), 4.47 (m, 3H), 5.17 (m, 1H), 5.30 (br, 1H), 6.57 (m, 1H), 6.87 (d, J = 9.3 Hz, 1H), 7.26−7.38 (m, 5H), 8.01 (m, 2H); HRMS $(M+1)^+$ calcd 456.1883, found 456.1868; Anal. ($C_{22}H_{25}N_5O_6$) C, H, N.

General Procedure for the Synthesis of 6-Aminobenzopyrans 33−37. To a solution of 6-nitrobenzopyrans **27**−**31** (43.7 mmol) in methanol (300 mL) was added 61 mL of 0.36 M $Cu(OAc)_2$ (21.8 mmol). After portionwise addition of $NaBH_4$ during 1 h at room temperature, the reaction was stirred for an additional 1 h, then filtered through a pad of Celite to remove black precipitate. Saturated $NaHCO_3$ (50 mL) was added to the filtrate, which was extracted with ethyl acetate (500 mL). The organic layer was washed with brine, dried ($MgSO_4$), filtered through a pad of silica gel, and concentrated under reduced pressure. The residue was recrystallized from hexanes−ethyl acetate (1:3) to give a solid.

(2*S*,3*S*,4*R*)-*N*-(6-Amino-3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-2*H*-benzopyran-4-yl)-*N*′-benzyl-*N*″-cyanoguanidine (33): mp 160−162 °C; ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 3H), 3.56 (s, 6H), 4.09 (d, 1H), 4.31 (s, 1H), 4.42 (dd, J = 15.1, 5.8 Hz, 1H), 4.50 (dd, J = 15.1, 5.8 Hz, 1H), 5.64 (d, 1H), 6.50 (d, J = 8.7 Hz, 1H), 6.63 (d, J = 8.7 Hz, 1H), 7.18 (br, 1H), 7.32 (m, 6H); HRMS (M^+) calcd 425.2063, found 425.2059; Anal. ($C_{22}H_{27}N_5O_4$) C, H, N.

(2*R*,3*S*,4*R*)-*N*-(6-Amino-3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-2*H*-benzopyran-4-yl)-*N*′-benzyl-*N*″-cyanoguanidine (35): mp 184−186 °C; ^{1}H NMR (DMSO-d_6) δ 1.13 (s, 3H), 3.38 (s, 3H), 3.39 (s, 3H), 4.72 (m, 3H), 4.59 (s, 2H), 4.82 (br, 1H), 4.98 (br, 1H), 6.35 (br, 1H), 6.45 (m, 2H), 7.11 (br, 1H), 7.26−7.38 (m, 5H), 7.51 (br, 1H); HRMS (M^+) calcd 425.2063, found 425.2059; Anal. ($C_{22}H_{27}N_5O_4$) C, H, N.

(2*R*,3*R*,4*S*)-*N*-(6-Amino-3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-2*H*-benzopyran-4-yl)-*N*′-benzyl-*N*″-cyanoguanidine (36): ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 3H), 3.55 (s, 6H), 4.08 (d, 1H), 4.31 (s, 1H), 4.42 (d, J = 15.2, 5.3 Hz, 1H), 4.50 (d, J = 15.2, 5.3 Hz, 1H), 5.78 (br, 1H), 6.51 (br, 1H), 6.61 (d, 1H), 7.14 (br, 1H), 7.29 (m, 6H); HRMS (M^+) calcd 425.2063, found 425.2057; Anal. ($C_{22}H_{27}N_5O_4$) C, H, N.

(2*S*,3*S*,4*R*)-*N*-(6-Amino-3,4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-2*H*-benzopyran-4-yl)-*N*″-cyano-*N*′-(4-methoxybenzyl)guanidine (37): mp 159−162 °C; ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3H), 3.57 (s, 6H), 3.80 (s, 3H), 4.10 (d, 1H), 4.15 (br, 1H), 4.30 (s, 1H), 4.41 (m, 3H), 5.53 (br, 1H), 6.69 (m, 2H), 6.88 (d, J = 8.3 Hz, 3H), 7.10 (br, 1H), 7.24 (d, J = 8.37 Hz, 2H); HRMS (M+) calcd 455.2171, found 455.2171; Anal. ($C_{23}H_{29}N_5O_5$) C, H, N.

(2*S*,3*S*,4*R*)-*N*′-Benzyl-*N*″-cyano-*N*-(3,4-dihydro-2-dimethoxymethyl-3-hydroxy-6-methanesulfonylamino-2-methyl-2*H*-benzopyran-4-yl)guanidine (39). To a solution of **33** (91 mg) in dichloromethane (2 mL) were added 45 μL of triethylamine and 20 μL of methanesulfonyl chloride. The reaction mixture was stirred for 2 h at room temperature and diluted with 10 mL of water and 20 mL of ethyl acetate. The layers were separated, and the organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (*n*-hexane:ethyl acetate = 1:2) to afford **39** (90 mg, 85%): mp 128−130 °C;^{1}H NMR ($CDCl_3$) δ 1.23 (s, 3H), 2.91 (s, 3H), 3.56 (s, 6H), 4.13 (m, 1H), 4.23 (br, 1H), 4.32 (s, 1H), 4.39 (m, 1H), 4.54 (m, 2H), 5.73 (br, 1H), 6.78 (d, 1H), 7.30 (m, 7H), 8.15 (br, 1H); HRMS (M^+) calcd 503.1839, found 503.1834; Anal. ($C_{23}H_{29}N_5O_6S$) C, H, N, S.

(2*S*,3*S*,4*R*)-*N*-[6-Amino-3,4-dihydro-2-([1,3]dioxolan-2-yl)-3-hydroxy-2-methyl-2*H*-benzopyran-4-yl]-*N*′-benzyl-*N*″-cyanoguanidine (41). The compound **41** was prepared from an appropriate epoxide by the general procedure for **27**−**32**, followed by the reduction using the general procedure for **33**−**37**: ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 3H), 3.54 (br, 3H), 4.08 (m, 5H), 4.48 (m, 2H), 4.96 (s, 1H), 5.48 (br, 1H), 6.71 (br, 1H),

6.74 (m, 1H), 7.00 (br, 1H), 7.36 (m, 6H); HRMS (M^+) calcd 423.1907, found 423.1905; Anal. ($C_{22}H_{25}N_5O_4$) C, H, N.

(2*S*,3*S*,4*R*)-*N*-[6-Amino-3,4-dihydro-2-([1,3]-5,5-dimethyldioxan-2-yl)-3-hydroxy-2-methyl-2*H*-benzopyran-4-yl]-*N*′-benzyl-*N*″-cyanoguanidine (42). The compound **42** was prepared from an appropriate epoxide by the general procedure for **27**−**32**, followed by the reduction using the general procedure for **33**−**37**. ^{1}H NMR ($CDCl_3$) δ 0.74 (s, 3H), 1.19 (s, 3H), 1.24 (s, 3H), 3.52 (t, J = 11.4 Hz, 2H), 3.72 (t, J = 11.4 Hz, 2H), 4.14 (d, 3H), 4.45 (m, 2H), 4.55 (br, 1H), 4.56 (s, 1H), 5.77 (d, 1H), 6.48 (d, J = 8.7 Hz, 1H), 6.53 (br, 1H), 6.64 (d, J = 8.7 Hz, 1H), 7.28 (m, 6H); HRMS (M^+) calcd 465.2376, found 465.2369; Anal. ($C_{25}H_{31}N_5O_4$) C, H, N.

Biology. Relaxation of Methoxamine Contracted Rat Aorta. Sprague−Dawley rats (350−450 g) were sacrificed by cervical dislocation and underwent thoracotomy. The thoracic aorta was deprived of the adipose tissue and cut into aortic rings of 3 mm width. The aorta was mounted in an organ bath containing an oxygenated physiological salt solution (PSS, a modified Krebs Henseleit buffer) at 37 °C, and was allowed to equilibrate under a resting tension of 2 g, then stand for 1 h for stabilization. The vascular tissue was constricted with 10^{-5} M of phenylephrine and washed several times with PSS, and this procedure was repeated again to ensure the stable reactivity of vascular smooth muscle on repetitive constriction/dilatation. Then, 3×10^{-6} M of methoxamine was added to induce an intensive constriction in the vascular smooth muscle. When the vasoconstriction induced by methoxamine reached and maintained a maximum, test compound was cumulatively added to the organ bath in concentrations of 1, 3, 10, and 30 μM. Following the addition of the test compounds, the change in the maximal constriction induced by methoxamine was calculated to plot a concentration−relaxation response curve. Through a linear regression analysis, IC_{50}, the drug concentration at which the methoamine induced vasoconstriction is 50% relaxed, was obtained for each compound.

Cardioprotective Activity in Ischemic Myocardium Rat Model. Male Sprague−Dawley rats (350−450 g) were anesthetized with pentobarbital (75 mg/kg, i.p.). Artificial respiration was managed at a rate of 60/min with a stroke volume of 10 mL/kg. A catheter for drug administration was inserted into the femoral vein and another was inserted into the femoral artery for blood pressure measurement. Body temperature was maintained at 37 °C using a homeothermic blanket control unit. The left coronary artery was occluded according to the Selye H. method.[33] The rats underwent a left thoracotomy operation. Immediately after the left anterior descending coronary artery (LAD) was carefully stitched with silk ligature, the heart was then repositioned in the thoracic cavity with the ligature ends exteriorized. The opposite ligature ends were passed through a PE tube (PE100, 2.5 cm), and the rats were allowed to stabilize for 20 min. Via the catheter inserted into the femoral vein, vehicles or test compounds were administered into the rats for 30 min. The PE tube was pressed on the surface of the heart directly above the coronary artery, and a hemostatic pincette was applied to clamp the tube and ligature for 45 min, resulting in coronary artery occlusion. Reperfusion was allowed for 90 min by the removal of the hemostatic pincette. The coronary artery was reoccluded, and 2 mL of a 1% Evans blue was injected intravenously to negatively mark the risk zone. Rats were then sacrificed by i.v. injection of pentobarbital, and the heart was removed. The left ventricle (LV) was cut horizontally to the heart apex into five or six slices, which were weighed. These slices were analyzed using a Hi-scope installed with an image analyzing program (Image Pro Plus). The area of the normal blood stream tissue region appeared blue in a computer monitor, while the ischemic area appeared colorless. The percentage of the colorless area to the total area of each slice was calculated and multiplied by the weight of each slice to determine the area at risk (AAR) of each slice. AARs obtained from each slice were summed for all slices, and the total AAR was divided by the total weight of the LV to yield % AAR in the entire LV. The AAR/LV (%) ranged from 31% to 47%. Then, the infarct zone (IZ) was evaluated by tetrazolium staining, and its size was calculated as a percent of AAR. The heart slices were incubated for 15 min in 2,3,5-triphenyltetrazolium chloride (TTC) phosphate buffer (pH 7.4) at 37 °C and fixed for 20−24 h in a 10% formalin solution. The viable portion of the slice is stained red by TTC, whereas the TTC stain is absent in the area of infarct. The infarct zone area of each slice was summed for all slices, and the resulting summed infarct zone area was divided by total AAR weight to yield % IZ to AAR (IZ/AAR, %).

Protective Activity in Neuronal Cells. From the brains of 17−18-day-old rat embryos, cerebral cortical neurons were isolated and cultured at 37 °C for 7−9 days in a 5% CO_2 incubator. The cortical cell cultures were washed twice with a minimum essential medium (MEM) to reduce the serum concentration to 0.2% and pretreated for 30 min with varying concentrations of compound. The test compounds were dissolved in DMSO and diluted in a medium, of which final concentration of DMSO was not allowed to exceed 0.2%. After the pretreatment with test compounds or vehicle, $FeSO_4$ was added to a final concentration of 50 μM, and the cultures were maintained for 24 h in a CO_2 incubator. During incubation, lactate dehydrogenase (LDH) was released into the medium upon neuronal death by the oxidative toxicity of iron. The extent of neuronal damage was assessed by measuring the amount of LDH secreted into the media. The protective effect of the compounds on neurons was evaluated by calculating the reduction rate of LDH release in the treatment group compared with the control group.

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Supporting Information Available: Crystallographic data for compound **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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