Novel Potassium Channel Activators. III.[1] Synthesis and Pharmacological Evaluation of 3,4-Dihydro-2*H*-1,4-benzoxazine Derivatives: Modification at the 2 Position

Yuzo MATSUMOTO,*[,a,b] Wataru UCHIDA,[a] Hideaki NAKAHARA,[a] Isao YANAGISAWA,[a] Tadao SHIBANUMA,[a] and Hiroyuki NOHIRA[b]

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd.,[a] 21, Miyukigaoka, Tsukuba, Ibaraki 305–8585, Japan, and Graduate School of Science and Engineering, Saitama University,[b] Shimo-Ohkubo, Urawa, Saitama 338–8570, Japan. Received September 8, 1999; accepted November 8, 1999

A new series of 3,4-dihydro-2*H*-1,4-benzoxazine derivatives, where various substituents were introduced into one of the geminal dimethyl groups at the 2 position, were synthesized and their potassium channel-activating activity was evaluated. Introduction of a hydroxyl group, as in compound 5, resulted in good solubility in water and a long duration of action compared with the parent compound 1. Introduction of a nitrato group, as in compound 8, produced typical nitrate activity such as exhibited by nitroglycerine in addition to potassium channel-activating activity. X-ray structural analysis of compound 5 showed that the sum of the bond angles around the N atom at the 4 position was 357.8°, suggesting that the N atom had an approximately sp^2-like planar bond configuration.

Key words potassium channel activator; 1,4-benzoxazine; antihypertensive; cromakalim; nitroglycerine; nitrate activity

Potassium channels regulated by changes in the intracellular level of adenosine triphosphate (ATP) are called ATP-sensitive K^+ channels or K_{ATP} channels and are an important class of ionic channels. These channels are closed when intracellular ATP levels are elevated and opened when intracellular ATP levels decline, linking the membrane potential directly to the metabolic state of the cell.[2] Activation of these channels in the membrane of cells, such as smooth muscle cells, allows K^+ ions to move out, causing transmembrane hyperpolarization and repolarization. These effects reduce the intracellular calcium concentration by blocking voltage-dependent calcium channels and inhibiting intracellular calcium release, producing smooth muscle relaxation and antispasmodic action.[3] The use of potassium channel activators or openers[4] may, therefore, be valuable in the treatment of diseases caused by smooth muscle contraction, such as hypertension, angina pectoris, asthma,[5] and urinary incontinence,[6] as well as baldness.[7] Additionally, these agents are expected to afford cellular protection against ischemic attacks, independent of their vasodilating actions,[8] and exhibit antilipemic effects, lowering low density lipoprotein (LDL) cholesterol and triglycerides, while increasing high density lipoprotein (HDL) cholesterol.[9]

There are several prototypes of this class of compound, represented by cromakalim,[10] pinacidil,[11] nicorandil,[12] and aprikalim.[13] A great deal of research on structural modifications based on cromakalim, a benzopyran derivative, has been reported. As part of our chemical program based on cromakalim, we previously reported[1] the synthesis and biological activity of a new series of 3,4-dihydro-2*H*-1,4-benzoxazine derivatives, represented by compound **1**, which have potent ATP-sensitive K^+ channel-activating activity. Recently, several modifications of the 2 position of the benzopyran skeleton have been reported, such as JTV-506[14] with selective coronary vasorelaxant activity, KC-399[15] which reduces reflux tachycardia, SKP-450[16] with a more potent hypotensive activity than levcromakalim, and KP-30818[17] with an equivalent vasorelaxant activity to levcromakalim in the rat aorta, as shown in Chart 1. As an example of the benzoxazine skeleton, ZM-260384[18] has also been reported, where the geminal dimethyl group at the 2 position of compound **1** was changed to the bis-difluoromethyl group, resulting in more potent vasorelaxant activity than compound **1** in detrusor tissue. Thus, our attention has focused on further modifications of the 2 position of benzoxazine. We tried to introduce various substituents into one of the geminal dimethyl groups at the 2 position of compound **1** and obtained two characteristic potassium channel activators. Firstly, introduction of a hydroxyl group, as in compound **5**, was found to result in good solubility and a long duration of action compared with the parent compound **1**. Secondly, introduction of a nitrato group, as in compound **8**, produced typical nitrate activity such as exhibited by nitroglycerine in addition to potassium channel-activating activity.

Chemistry Preparation of the 1,4-benzoxazine derivatives modified at the 2 position followed the route outlined in Chart 2. Treatment of 2-amino-4-nitrophenol (**2**) with diethyl 2-bromo-2-methylmalonate and potassium fluoride in dimethylformamide (DMF) gave 2-ethoxycarbonyl-2-methyl-3-oxo-1,4-benzoxazine **3**, which was reduced with borane-tetrahydrofuran (BH_3-THF) complex to yield 2-hydroxymethyl-1,4-benzoxazine **4**. Pyridine *N*-oxide derivative **5** was prepared easily without heating by nucleophilic substitution of **4** with 2-bromopyridine *N*-oxide in the presence of sodium hydride (NaH) in DMF. In this case, selective substitution on the N atom at the 4 position occurred and substitution on the O atom at the 2 position did not. *O*-alkylation or acetylation of **5** with several alkyl halides or acetylchloride in the presence of NaH gave **6a**—**e** or **6f** respectively. Treatment of **5** with methylisocyanate yielded the carbamoyl derivative **7**. Selective nitration at the 2 position of compound **5** could be achieved with nitronium tetrafluoroborate at −35—−40 °C to give compound **8**, while nitration at −10—0 °C gave 2-nitratomethyl-6,7-dinitro-1,4-benzoxazine derivative **9**.

* To whom correspondence should be addressed.

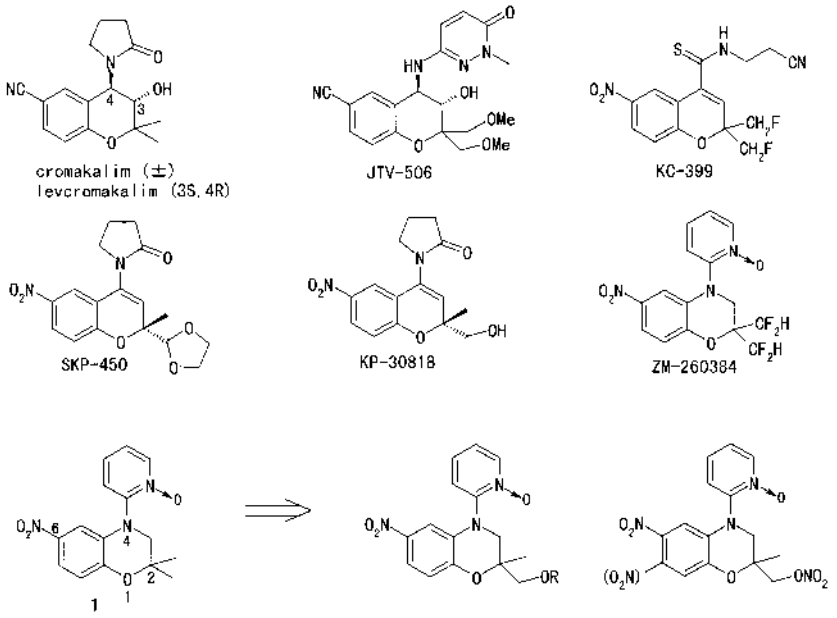


Chart 1

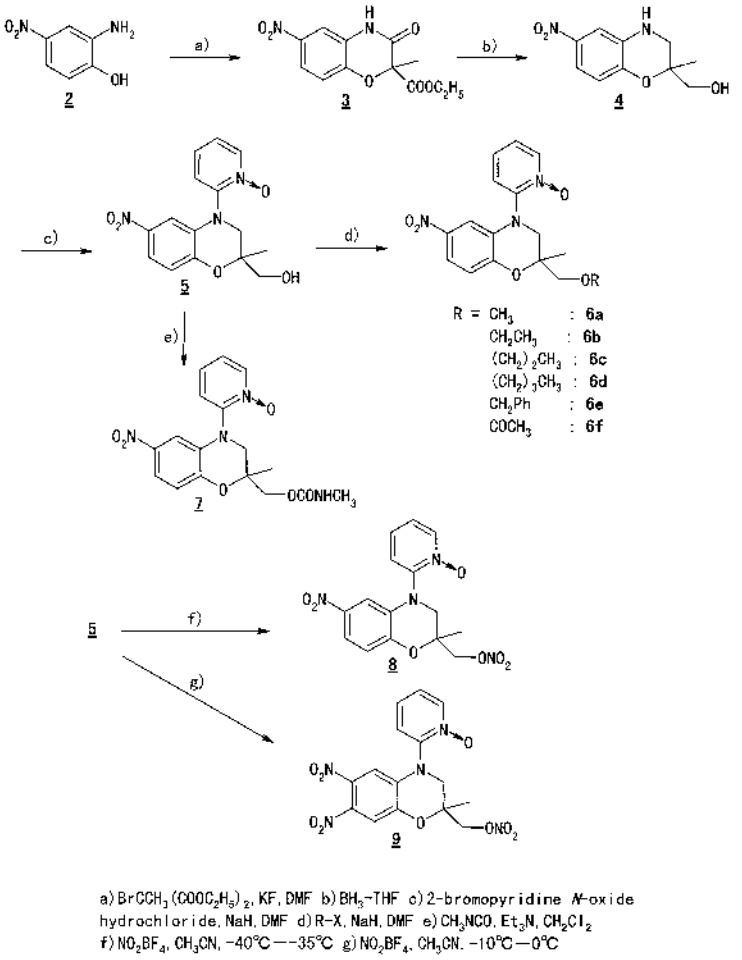
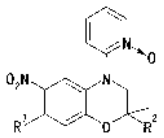


Chart 2

Results and Discussion

The potassium channel-activating effects of the compounds were evaluated *in vitro* in terms of their inhibitory effect (IC_{50}) on 3,4-diaminopyridine-induced rhythmic contraction[19] in isolated dog coronary artery (Table 1). Introduction of a hydroxyl group into one of the geminal methyl groups at the 2 position of the 1,4-benzoxazine derivative **1** afforded **5** which retained potent activity. Alkylation of the hydroxyl

Table 1. Modification at the 2 Position



Comp.	R^2	R^7	*In vitro* IC_{50}[a] (μM)
1	CH_3	H	0.014
5	CH_2OH	H	0.057
6a	CH_2OCH_3	H	0.083
6b	$CH_2OCH_2CH_3$	H	0.17
6c	$CH_2O(CH_2)_2CH_3$	H	0.55
6d	$CH_2O(CH_2)_3CH_3$	H	>10
6e	CH_2OCH_2Ph	H	>10
6f	CH_2OCOCH_3	H	0.069
7	$CH_2OCONHCH_3$	H	0.51
8	CH_2ONO_2	H	0.025
9	CH_2ONO_2	NO_2	1.4
Nicorandil			7.2
Cromakalim			0.39

a) Drug concentration required to inhibit 3,4-diaminopyridine-induced rhythmic contractions in dog coronary artery by 50% (n=3—6).

Table 2. Hemodynamic Effect in Dogs

Comp.	μg/kg i.v.	MBP[a]	CBF[a]	
		Δ%	Δ%	Duration (min)
1	3	−16	155	30
5	3	−24	204	>60

a) Measured in groups of 4—5 dogs.

group of **5** with a methyl group, as in **6a**, caused a slight reduction in potency. Alkylation with an ethyl group, as in **6b**, caused a further reduction and alkylation with a propyl group, as in compound **6c**, caused a much greater reduction. In the case of alkylation with *n*-butyl and benzyl groups, as in compound **6d** and **6e**, there was a loss of activity at 10 μM. From the above data, it was concluded that as the alkyl substituent introduced into the hydroxymethyl group at the 2 position of compound **5** became more bulky, the activity became weaker. Acetylation of **5** afforded **6f** with restored high activity, while the carbamate derivative **7** showed reduced activity. Surprisingly, the nitrato derivative **8** was found to be approximately 10 times more potent than cromakalim. Further introduction of a nitro group into the 7 position of **8** afforded 6,7-dinitro derivative **9** with reduced activity. Although the compounds mentioned above have a chiral center at the 2 position, we prepared them as racemic mixtures. So, the relationship between activity and chirality remains unknown.

Among these compounds, compound **5** possessing a hydroxyl group, was readily soluble in water compared with compound **1**. Its pharmacological properties were investigated, because we thought that high solubility might change the physicochemical properties of the parent compound **1** to produce a unique pharmacological profile. In anesthetized dogs, compound **5** exhibited a more potent mean blood pressure (MBP)-lowering effect and coronary artery blood flow

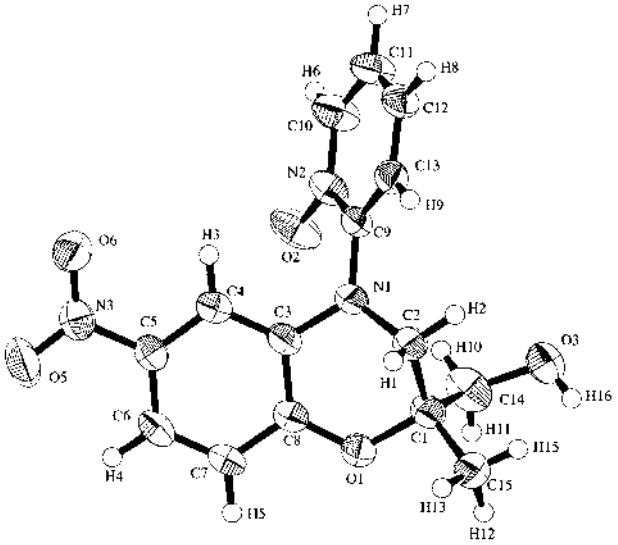


Fig. 1. X-Ray Structure of Compound **5**

Table 3. Vasodilating Effect in Rabbit Isolated Coronary Artery

Comp.	IC_{50} (nM)[a]	
	No treatment methylene blue	Pretreated with methylene blue
8	7	45

a) Drug concentration to inhibit phenylephrine-induced contraction by 50% (n=2).

(CBF)-increasing effect than compound **1** (Table 2). Surprisingly, there was a remarked difference between compound **1** and **5** in the duration of action on CBF. In the case of compound **1**, the CBF returned to baseline after 30 min, but in the case of compound **5**, there was no return to baseline even after 1 h, and the CBF-increasing effect persisted. Differences in the physicochemical properties and affinity for coronary artery tissue would account for the different duration of action. It has also been reported that N–K hybrids,[20] which have a dual vasodilatory mechanism of a nitrate and a K^+ channel activator, such as nicorandil and KRN2391,[21] are beneficial compared with the pure K^+ channel activators in the treatment of angina pectoris and hypertension. This is because N–K hybrids relax not only small coronary arteries by the K^+ channel activation but also large coronary arteries by the nitrate action, without causing a "coronary steal" phenomenon.[22] Thus, we investigated whether compound **8**, possessing a nitrato group at the 2 position, exhibited nitrate activity or not. In isolated rabbit coronary artery, compound **8** relaxed the phenylephrine-induced contraction in a dose-dependent manner, and the effect was clearly inhibited by pre-treatment with methylene blue, a c-GMP synthetase inhibitor (Table 3). This indicates that compound **8** exhibits nitrate activity, like nitroglycerine, in addition to potassium channel-activating activity. Details of the pharmacological profile of compound **8** are currently under investigation.

The X-ray crystal structure of compound **5** is shown in Fig. 1. The torsion angle formed from the four centers, C3/N1/C9/N2, amounted to 53.6°, indicating that the pyridine *N*-oxide ring was roughly orthogonal to the pseudoplane of 1,4-benzoxazine, like the relationship between the γ lactam

ring and the benzopyran skeleton of cromakalim.[23] The bond angle around N1 was 123.3° (C3/N1/C9), 112.7° (C3/N1/C2), 121.8° (C2/N1/C9) and the sum of the angles was 357.8°, suggesting that N1 had approximately an sp^2-like planar bond configuration, while the atom at the 4 position of the benzopyran in cromakalim had an sp^3 tetrahedral bond configuration. This result was similar to the result of our previous work[1b] and a feature of 1,4-benzoxazine derivatives. However, the relationship between activity and the bond angles is not yet clear.

In conclusion, we synthesized and evaluated pharmacologically a new series of 1,4-benzoxazine derivatives where one of the geminal dimethyl groups at the 2 position of compound **1** was modified. Among these compounds, compound **5** with a hydroxyl group exhibited potent hypotensive and coronary vasodilating effects in dogs, characterized by a long duration of action. Compound **8**, with a nitrato group, exhibited nitrate activity, like nitroglycerine, in addition to potassium channel-activating activity, suggesting a unique profile as a drug for the treatment of cardiovascular diseases.

Experimental

All melting points were determined on a Yanaco MP-500D micro melting point apparatus without correction. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL FX90Q, FX100, FX270 or FX400 spectrometer using tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=double doublet, br s=broad singlet. Mass spectra (MS) were recorded with a Hitachi M-80, JEOL JMS-DX300, or JMS-700T spectrometer. Infrared (IR) spectra were measured with a Hitachi 270-30 spectrophotometer. Elemental analyses were performed using Yanaco MT-5 equipment. HPLC was carried out using a Hitachi L-6000 pump, L-4000 UV-detector and D-2500 recorder. Thin-layer chromatography (TLC) plates was performed on silica-gel F_{254} (Merck), and column chromatography was performed on 100—200 mesh silica-gel from Wako. Anhydrous $MgSO_4$ or Na_2SO_4 was used as the drying agent for organic extraction. All solvent evaporation was performed under vacuum. Yields were not optimized.

Ethyl (3,4-Dihydro-2-methyl-6-nitro-3-oxo-2*H*-1,4-benzoxazin-2-yl)-carboxylate (3) A mixture of KF (6.0 g, 100 mmol), DMF (30 ml), diethyl 2-bromo-2-methylmalonate (10 g, 40 mmol), and 4-nitro-2-aminophenol (6.1 g, 40 mmol) was stirred for 16 h, and then, poured into water, and extracted with AcOEt. The extract was washed with brine, dried, and concentrated to give **3**, which was recrystallized from EtOH (8.7 g, 79%), mp 170—172 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, t, J=7 Hz), 1.95 (3H, s), 4.21 (2H, q, J=7 Hz), 7.17 (1H, d, J=9 Hz), 7.85 (1H, d, J=3 Hz), 7.96 (1H, dd, J=3, 9 Hz), 9.66 (1H, br s). *Anal*. Calcd for $C_{12}H_{12}N_2O_6$: C, 51.43; H, 4.32; N, 10.00. Found: C, 51.41; H,4.39; N, 10.04.

3,4-Dihydro-2-hydroxymethyl-2-methyl-6-nitro-2*H*-1,4-benzoxazine (4) Compound **3** (2.8 g, 10 mmol) was added to a solution of borane–THF complex in THF (1.0 M, 100 ml) and the mixture was refluxed for 4 h and then carefully diluted with MeOH (24 ml). After adding conc. HCl (24 ml), it was refluxed for 0.5 h. After concentration, the residue was made alkaline with NaOH solution to give **4** as a precipitate, which was recrystallized from EtOH–ether (1.7 g, 76%), mp 125—127 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.23 (3H, s), 3.01 (2H, ABq, J=12 Hz), 3.35—3.39 (1H, m), 3.42—3.46 (1H, m), 6.50 (1H, br s), 6.81 (1H, d, J=9 Hz), 7.41 (1H, dd, J=3, 9 Hz), 7.48 (1H, d, J=3 Hz). *Anal*. Calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.45; H,5.34; N, 12.42.

2-(3,4-Dihydro-2-hydroxymethyl-2-methyl-6-nitro-2*H*-1,4-benzoxazin-4-yl)pyridine 1-Oxide (5) To a mixture of **4** (1.7 g, 7.4 mmol) and 2-bromopyridine 1-oxide hydrochloride (1.6 g, 7.4 mmol) in DMF 10 ml, NaH (60% in oil, 0.74 g, 18.5 mmol) was slowly added under ice-cooling, followed by stirring at r.t. for 1 h. The whole was poured into ice water and concentrated to give **5** as a precipitate, which was collected and washed with ether and then recrystallized from MeOH–EtOH (1.3 g, 88%), mp 189—191 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.33 (3H, s), 3.46—3.81 (4H, m), 6.91 (1H, d, J=3 Hz), 7.03 (1H, d, J=9 Hz), 7.33—7.38 (1H, m), 7.45—7.49 (1H, m), 7.64 (1H, m), 7.71 (1H, dd, J=3, 9 Hz), 8.39 (1H, m). *Anal*. Calcd for $C_{15}H_{15}N_3O_5$: C, 56.78; H, 4.76; N, 13.24. Found: C, 56.55; H,4.72; N, 13.05.

2-(3,4-Dihydro-2-methoxymethyl-2-methyl-6-nitro-2*H*-1,4-benzoxazin-4-yl)pyridine 1-Oxide Monohydrochloride (6a) To a solution of **5** (0.2 g, 0.63 mmol) in DMF (2 ml), NaH (60% in oil, 0.05 g, 1.3 mmol) was added, followed by methyl iodide (0.27 g, 1.9 mmol). The whole was stirred for 15 min, poured into ice water, and extracted with AcOEt. The extract was washed with brine, dried, and concentrated. The residue was subjected to column chromatography with AcOEt–MeOH (20 : 1, v/v) and treated with HCl in EtOH to give **6a**, which was crystallized from EtOH–ether (0.22 g, 96%), mp 140—143 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.35 (3H, s), 3.24 (3H, s), 3.37—4.00 (4H, m), 7.08 (1H, d, J=9 Hz), 7.37—7.40 (1H, m), 7.53—7.56 (1H, m), 7.69—7.75 (2H, m), 8.44—8.45 (1H, m). *Anal*. Calcd for $C_{16}H_{17}N_3O_5 \cdot HCl$: C, 52.25; H, 4.93; N, 11.43; Cl, 9.64. Found: C, 52.25; H,4.88; N, 11.42; Cl, 9.73.

Compounds **6b**—**f** were prepared in the same way.

2-(3,4-Dihydro-2-ethoxymethyl-2-methyl-6-nitro-2*H*-1,4-benzoxazin-4-yl)pyridine 1-Oxide (**6b**): An amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (3H, t, J=7 Hz), 1.43 (3H, s), 3.32—4.10 (6H, m), 6.98 (1H, d, J=9 Hz), 7.2—7.4 (3H, m), 7.51 (1H, d, J=2 Hz), 7.80 (1H, dd, J=2, 9 Hz), 8.37 (1H, m). *Anal*. Calcd for $C_{17}H_{19}N_3O_5 \cdot 0.75H_2O$: C, 56.90; H, 5.76; N, 11.71. Found: C, 56.90; H, 5.83; N, 11.71.

2-(3,4-Dihydro-2-methyl-6-nitro-2-propoxymethyl-2*H*-1,4-benzoxazin-4-yl)pyridine 1-Oxide (**6c**): An amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 0.78 (3H, t, J=7 Hz), 1.25—1.58 (5H, m), 3.2—3.6 (4H, m), 3.81 (2H, ABq, J=14 Hz), 6.96 (1H, d, J=9 Hz), 7.12—7.36 (3H, m), 7.45 (1H, d, J=3 Hz), 7.79 (1H, dd, J=3, 9 Hz), 8.33 (1H, m). *Anal*. Calcd for $C_{18}H_{21}N_3O_5 \cdot 0.25H_2O$: C, 59.41; H, 5.96; N, 11.55. Found: C, 59.28; H, 5.94; N, 11.54.

2-(2-Butoxymethyl-3,4-dihydro-2-methyl-6-nitro-2*H*-1,4-benzoxazin-4-yl)pyridine 1-Oxide Monohydrochloride (**6d**): An amorphous powder. ^{1}H-NMR (DMSO-d_6) δ: 0.79 (3H, t, J=7 Hz), 1.1—1.4 (7H, m), 3.2—3.9 (6H, m), 7.0—7.1 (2H, m), 7.3—7.8 (4H, m), 8.4—8.5 (1H, m). *Anal*. Calcd for $C_{19}H_{23}N_3O_5 \cdot HCl$: C, 55.68; H, 5.90; N, 10.25; Cl, 8.65. Found: C, 55.63; H, 5.86; N, 10.25; Cl, 8.41.

2-(2-Benzyloxymethyl-3,4-dihydro-2-methyl-6-nitro-2*H*-1,4-benzoxazin-4-yl)pyridine 1-Oxide (**6e**): An amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (3H, s), 3.50—4.01 (4H, m), 4.45—4.47 (2H, m), 6.97 (1H, d, J=9 Hz), 7.12—7.31 (8H, m), 7.44 (1H, s), 7.78 (1H, dd, J=3, 9 Hz), 8.30—8.31 (1H, m). *Anal*. Calcd for $C_{22}H_{21}N_3O_5 \cdot 0.25H_2O$: C, 64.15; H, 5.26; N, 10.20. Found: C, 63.99; H, 5.33; N, 10.16.

2-(2-Acetyloxymethyl-3,4-dihydro-2-methyl-6-nitro-2*H*-1,4-benzoxazin-4-yl)pyridine 1-Oxide (**6f**): An amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (3H, s), 2.03 (3H, s), 3.81 (2H, ABq, J=14 Hz), 4.20 (2H, ABq, J=12 Hz), 6.99 (1H, d, J=9 Hz), 7.17—7.40 (3H, m), 7.48 (1H, d, J=3 Hz), 7.79 (1H, dd, J=3, 9 Hz), 8.30—8.36 (1H, m). *Anal*. Calcd for $C_{17}H_{17}N_3O_6 \cdot H_2O$: C, 54.11; H, 5.08; N, 11.14. Found: C, 54.02; H, 4.72; N, 10.97.

2-[3,4-Dihydro-2-methyl-2-(*N*-methylcarbamoyloxymethyl)-6-nitro-2*H*-1,4-benzoxazin-4-yl]pyridine 1-Oxide (7) To a solution of **5** (0.2 g, 0.63 mmol) and triethylamine (0.19 g, 1.9 mmol) in 4 ml CH_2Cl_2, methyl isocyanate (0.11 g, 1.9 mmol) was added. The mixture was stirred for 22 h and then poured into ice water, and extracted with $CHCl_3$. The extract was washed with brine, dried, and concentrated. The residue was subjected to column chromatography with $CHCl_3$–MeOH (50 : 1, v/v) to give **7** (0.14 g, 61%), as an amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (3H, s), 2.73 (3H, s), 3.69—4.18 (4H, m), 7.00 (1H, d, J=9 Hz), 7.20—7.49 (4H, m), 7.81 (1H, d, J=9 Hz), 8.33 (1H, m). *Anal*. Calcd for $C_{17}H_{18}N_4O_6 \cdot 0.5H_2O$: C, 53.26; H, 5.00; N, 14.61. Found: C, 53.22; H, 5.05; N, 14.27.

2-(3,4-Dihydro-2-methyl-6-nitro-2-nitrooxymethyl-2*H*-1,4-benzoxazin-4-yl)pyridine 1-Oxide (8) To a solution of **5** (2.0 g, 6.3 mmol) in acetonitrile (50 ml), nitronium tetrafluoroborate (1.26 g, 7.6 mmol) was slowly added at −35—−40 °C and then the mixture was poured into ice water, made alkaline with $NaHCO_3$ solution, and extracted with $CHCl_3$. The extract was washed with brine, dried, and concentrated. The residue was subjected to column chromatography with $CHCl_3$ to give **8**, which was recrystallized from ether (0.74 g, 32%), 156—158 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, s), 3.83 (2H, ABq, J=13 Hz), 4.67 (2H, ABq, J=12 Hz), 7.01 (1H, d, J=9 Hz), 7.24—7.27 (1H, m), 7.38—7.43 (3H, m), 7.79 (1H, dd, J=3, 9 Hz), 8.33—8.34 (1H, m). *Anal*. Calcd for $C_{15}H_{14}N_4O_7$: C, 49.73; H, 3.89; N, 15.46. Found: C, 49.61; H, 3.83; N, 15.31.

2-(3,4-Dihydro-6,7-dinitro-2-methyl-2-nitrooxymethyl-2*H*-1,4-benzoxazin-4-yl)pyridine 1-Oxide (9) To a solution of **5** (0.4 g, 1.3 mmol) in acetonitrile (10 ml), nitronium tetrafluoroborate (0.45 g, 3.4 mmol) was slowly added at −10—0 °C. The mixture was poured into ice water, made alkaline with $NaHCO_3$ solution, and extracted with $CHCl_3$. The extract was washed with brine, dried, and concentrated to give **9**, which was recrystallized from

AcOEt–EtOH (0.16 g, 31%), 156—159 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.45 (3H, s), 3.90 (2H, ABq, J=13 Hz), 4.77—4.87 (2H, m), 6.93 (1H, s), 7.41—7.52 (2H, m), 7.71—7.74 (2H, m), 8.40—8.42 (1H, m). *Anal*. Calcd for $C_{15}H_{13}N_5O_9$: C, 44.23; H, 3.22; N, 17.19. Found: C, 44.09; H, 3.23; N, 17.19.

Single-Crystal X-Ray Analysis of 5 Crystals of compound **5** were grown from AcOEt as yellow prisms. Data was collected from a crystal of dimensions 0.28×0.10×0.10 mm^3 on a Rigaku AFC5R diffractometer and corrected for Lorentz and polarization factors. The structure was solved by a direct method using the program SIR92 and refined using the program teXsan.[24] The final refinement was achieved by the full-matrix least-squares method with anisotropic thermal parameters for all non-hydrogen atoms and fixed isotropic thermal parameters for all hydrogen atoms. Crystal data: $C_{15}H_{15}N_3O_5$, M.W.=317.30, monoclinic, space group $P2_1/n$(#14), a=13.092 (1) Å, b=11.027 (1) Å, c=10.274 (1) Å, β=101.977 (7)°, V=1450.9 (3) Å^3, Z=4, Dc=1.452 g/cm^3, F_{000}=664.00, temperature 25.0 °C, Cu$K\alpha$ (λ=1.54178 Å), 2610 observed reflections (I>3.00σ (I)), 218 variable parameters, R=0.047, R_w=0.070.

Biological Testing i) Effects on 3,4-Diaminopyridine-Induced Rhythmic Contractions[19]: The left coronary circumflex branch or the anterior-descending branch of mongrel dogs of either sex was isolated in Krebs–Henseleit solution and cut into rings about 2 mm wide. A ring segment was fixed to a stainless-steel hook and suspended in a Krebs–Henseleit bath (37 °C) aerated with 95%O_2–5%CO_2 under a tension load of 1.0 g, and isometric contractions were recorded. The specimen was allowed to stabilize for 30 min, then rhythmic contractions were induced by the addition of 3,4-diaminopyridine (10 mM). When the amplitude and frequency of the rhythmic contractions stabilized, cumulative addition of the test compound to the organ bath was started. Concentration–response curves for the amplitude and frequency of contractions were constructed and efficacy was evaluated. The inhibitory effect (IC_{50}) on the frequency of contractions is shown in Table 1.

ii) Hemodynamic Effect in Dogs (i.v.): Mongrel dogs of either sex were anesthetized with pentobarbital (30 mg/kg, i.v.). The experiment was performed under artificial respiration after tracheal intubation. After a thoracotomy, blood pressure was measured with a pressure transducer and CBF was measured directly with an electromagnetic flowmeter *via* a probe. The test compound was administered through a cannula in the femoral vein. The MBP-lowering effect (Δ%), the CBF-increasing effect (Δ%) and the duration are shown in Table 2.

iii) Vasodilating Effect on Rabbit Isolated Artery: The aorta of rabbits of either sex was isolated and cut into rings about 3 mm wide. After removal of the endothelium, each ring was suspended on wire hooks in an organ bath containing Krebs–Henseleit solution maintained at 37 °C and aerated with 95%O_2–5%CO_2, under a tension of 1.0 g, and isometric contractions were measured. Contraction was induced by the addition of phenylephrine (10^{-7} M) and the contractile response was allowed to reach a plateau. The test compound was then added to the preparation cumulatively. As far as antagonism by methlene blue was concerned, after adding methylene blue (3×10^{-6} g/ml) and allowing the contractile response to reach a plateau, the test compound was added cumulatively. As an indicator of the vasodilative effect, the inhibitory effect (IC_{50}) on the response to papaverine (10^{-4} M) was taken as 100%, and is shown in Table 3.

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References and Notes

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