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

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A new series of 3,4-dihydro-2H-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 sp²-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. 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 anti-spasmodic action. The use of potassium channel activators or openers may, therefore, be valuable in the treatment of diseases caused by smooth muscle contraction, such as hypertension, angina pectoris, asthma, and urinary incontinence, as well as baldness. Additionally, these agents are expected to afford cellular protection against ischemic attacks, independent of their vasodilating actions, and exhibit antilipemic effects, lowering low density lipoprotein (LDL) cholesterol and triglycerides, while increasing high density lipoprotein (HDL) cholesterol.

There are several prototypes of this class of compound, represented by cromakalim, pinacidil, nicorandil, and aprikalin. 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 the synthesis and biological activity of a new series of 3,4-dihydro-2H-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 with selective coronary vasorelaxant activity, KC-399 which reduces reflux tachycardia, SKP-450 with a more potent hypotensive activity than levomakalim, and KP-30818 with an equivalent vasorelaxant activity to levomakalim in the rat aorta, as shown in Chart 1. As an example of the benzoxazine skeleton, ZM-260384 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 nitrate 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 with diethyl bromoacetamide in dimethylformamide (DMF) gave 2-ethoxycarbonyl-2-methyl-3-oxo-1,4-benzoxazine 3, which was reduced with borane-tetrahydrofuran (BH₃·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 acetyl chloride 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.
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
The introduction of a nitro group into the 7 position of approximately 10 times more potent than cromakalim. Fur-}


tivity. Surprisingly, the nitrato derivative activity, while the carbamate derivative showed activity, like nitroglycerine, in addition to potassium channel-activating activity. Details of the pharmacological properties of the parent compound 1 were found to be 53.6 °, indicating that the pyridine oxide ring was roughly orthogonal to the pseudoplane of 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 1 possesses a nitrate group at the 2 position, exhibited nitrate 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 nitrate 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


Table 1. Modification at the 2 Position

<table>
<thead>
<tr>
<th>Comp.</th>
<th>R²</th>
<th>R²</th>
<th>In vitro</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IC₅₀ (µM)</td>
</tr>
<tr>
<td>1</td>
<td>CH₃ H</td>
<td></td>
<td>0.014</td>
</tr>
<tr>
<td>5</td>
<td>CH₂OH H</td>
<td></td>
<td>0.057</td>
</tr>
<tr>
<td>6a</td>
<td>CH₂OCH₃ H</td>
<td></td>
<td>0.083</td>
</tr>
<tr>
<td>6b</td>
<td>CH₂OCH₂CH₃ H</td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>6c</td>
<td>CH₂O(CH₂)₂CH₃ H</td>
<td></td>
<td>0.55</td>
</tr>
<tr>
<td>6d</td>
<td>CH₂O(CH₂)₃CH₃ H</td>
<td></td>
<td>&gt;10</td>
</tr>
<tr>
<td>6e</td>
<td>CH₂OCH₃Ph H</td>
<td></td>
<td>&gt;10</td>
</tr>
<tr>
<td>6f</td>
<td>CH₂OCH₂CH₃ H</td>
<td></td>
<td>0.069</td>
</tr>
<tr>
<td>7</td>
<td>CH₂OCONHCH₃ H</td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>8</td>
<td>CH₂ONO₂ NO₂</td>
<td></td>
<td>0.025</td>
</tr>
<tr>
<td>9</td>
<td>CH₂OCH₂Ph H</td>
<td></td>
<td>1.4</td>
</tr>
<tr>
<td>Nicorandil</td>
<td></td>
<td></td>
<td>7.2</td>
</tr>
<tr>
<td>Cromakalim</td>
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<td></td>
<td>0.39</td>
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Table 2. Hemodynamic Effect in Dogs

<table>
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<tr>
<th>Comp.</th>
<th>µg/kg i.v.</th>
<th>MBP¹</th>
<th>Δ%</th>
<th>CBF²</th>
<th>Δ%</th>
<th>Duration (min)</th>
</tr>
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<tr>
<td>1</td>
<td>3</td>
<td>-16</td>
<td></td>
<td>155</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>-24</td>
<td>204</td>
<td>&gt;60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Measured in groups of 4—5 dogs.

Table 3. Vasodilating Effect in Rabbit Isolated Coronary Artery

<table>
<thead>
<tr>
<th>Comp.</th>
<th>No treatment</th>
<th>Pretreated with methylene blue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ (µM)</td>
<td>IC₅₀ (µM)</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>45</td>
</tr>
</tbody>
</table>

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

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 (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 currently under investigation.

Fig. 1. X-Ray Structure of Compound 5
ring and the benzopyran skeleton of cromakalim. 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 sp²-like planar bond configuration, while the atom at the 4 position of the benzopyran in cromakalim had an sp² tetrahedral bond configuration. This result was similar to the result of our previous work[1] and a feature of 1,4-benzoxazin 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-benzoxazin 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 nitrate 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, bs=broad singlet. Mass spectra (MS) were recorded with a Hitachi M-80 spectrometer. Elemental analyses were performed using Yanaco MT-5 equipment. HPLC was carried out using a Hitachi L-6000 pump, L-4000 UV detector, and an LC-2010A column, a Hitachi M-80, JEOL JMS-DX300, or JMS-700T spectrometer using tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doubled, t=triplet, q=quartet, m=multiplet, dd=double doublet, br s=broad singlet.

Ethyl (3,4-Dihydro-2-methyl-6-nitro-3-oxo-2H-1,4-benzoxazin-2-yl)carboxylate (3) A mixture of KF (6.0 g, 100 mmol), DMF (30 ml), diethyl carboxylate (3) (1.45 g, 13.2 mmol) and triethylamine (0.19 g, 1.9 mmol) in 4 ml CH2Cl2, methyl iso

2-(3,4-Dihydro-2-methyl-6-nitro-2H-1,4-benzoxazin-4-yl)pyridine 1-Oxide (6b) An amorphous powder. 1H-NMR (CDCl3) δ: 6.07 (3H, t, J=7 Hz), 1.25—1.58 (5H, m), 3.2—3.6 (4H, m), 3.81 (2H, ABq, J=8 Hz), 1.83 (1H, m), 7.3—7.8 (4H, m), 8.30—8.45 (1H, m). Anal. Calcd for C17H17N3O6·H2O: C, 54.11; H, 5.08; N, 11.14. Found: C, 55.63; H, 5.86; N, 10.25. CI, 8.41.

2-(2-Benzoxymethyl-3,4-dihydro-2-methyl-6-nitro-1,4-benzazin-4-yl)pyridine 1-Oxide (6d) An amorphous powder. 1H-NMR (CDCl3) δ: 7.55 (1H, d, J=3 Hz), 8.33 (1H, m). Anal. Calcd for C17H16N4O7: C, 49.73; H, 3.89; N, 15.46. Found: C, 49.61; H, 3.72; N, 15.41.

2-(3,4-Dihydro-2-methyl-6-nitro-2H-1,4-benzazin-4-yl)pyridine 1-Oxide (7) To a solution of (0.2 g, 0.63 mmol) in 4 ml CH3Cl, 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 CHCl3, the extract was washed with brine, dried and concentrated. The residue was subjected to column chromatography with CHCl3–MeOH (50 : 1, v/v) and treated with 30% HCl to give 7 (0.14 g, 61%), as an amorphous powder. 1H-NMR (CDCl3) 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 C17H16N4O7: C, 52.36; H, 5.00; N, 14.61. Found: C, 52.32; H, 5.05; N, 14.27.

2-(4,6-Dihydro-2-methyl-6-nitro-1,4-benzazin-4-yl)pyridine 1-Oxide (8) To a solution of (5.0 g, 6.33 mmol) in acetonitrile (50 ml), nitronium tetrafluoroborate (1.26 g, 7.6 mmol) was slowly added at −35—−40 °C. After the mixture was poured into ice water, made alkaline with NaHCO3 solution, and extracted with CHCl3, the extract was washed with brine, dried, and concentrated. The residue was subjected to column chromatography with CHCl3, MeOH (50 : 1, v/v) to give 9 (0.14 g, 61%), as an amorphous powder. 1H-NMR (CDCl3) 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 C17H16N4O7: C, 52.36; H, 5.00; N, 14.61. Found: C, 52.32; H, 5.05; N, 14.27.

2-(3,4-Dihydro-2-methyl-6-nitro-2H-1,4-benzazin-4-yl)pyridine 1-Oxide (9) To a solution of (5.0 g, 6.33 mmol) in acetonitrile (50 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 NaHCO3 solution, and extracted with CHCl3, the extract was washed with brine, dried, and concentrated to give 9, which was recrystallized from
AcOEt–EtOH (0.16 g, 31%). 1H-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$_{15}$N$_3$O$_5$: 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$_3$O$_5$, M.W. 282.24 g/mol, monoclinic, space group P2$_1$/c(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, D$_c$=1.452 g/cm$^3$, CuKα ($λ$=1.54178 Å), 2610 observed reflections ($I$>3.0σ(I)), 218 variable parameters, R=0.047, R$_{w}$=0.070.

**Biological Testing** i) Effects on 3,4-Diaminopyridine-Induced Rhythmic Contractions$^{15}$: 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 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). Then 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 spectral measurements.

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**References and Notes**