Novel Potassium Channel Opener Prodrugs with a Slow Onset and Prolonged Duration of Action

Haruhiko Horino,* Tetsuya Mimura, Syozo Kobayashi, Masahiro Ohta, Hideo Kubo, Kuniko Ito, Mitsuyoshi Tsumura, and Masayuki Kitagawa

New Product Research Laboratories II, Daiichi Pharmaceutical Company, Ltd., 1–16–13 Kita-kasai, Edogawa-ku, Tokyo 134–8630, Japan. Received September 30, 1999; accepted December 6, 1999

(−)-(3*S*,4*R*,1′*R*,6′*S*)-4-(4-Benzyl-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yloxy)-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile and its derivatives with a modified benzyl group were synthesized with the objective of discovering novel ATP-sensitive potassium channel openers (PCOs) with a slow onset of action and a reduced tendency to induce tachycardia. Among the compounds synthesized, 4-(2-chlorobenzyl) derivative 5bB had potent hypotensive activity in spontaneously hypertensive rats (SHRs). In addition, compound 5bB showed the desired pharmacological profile with a slow onset and long duration of action and induction of only mild tachycardia. Compound 5bB was found to be quantitatively metabolized in rats to give active des-2-chlorobenzyl derivative 6B. These results suggest that the incorporation of an *N*-benzyl group is a useful method for the preparation of prodrugs, the function of which is to delay the onset and prolong the duration of action of the active substance.

Key words diazabicyclo[4.1.0]heptene; potassium channel opener; prodrug; hypotensive activity; 1-benzopyran

The adenosine triphosphate (ATP) sensitive potassium channel is involved in the regulation of smooth muscle tone *via* an efflux of potassium ions from cells. Potassium channel openers (PCOs) such as levcromakalim (**II**), accelerate the efflux of potassium ions and then induce the dilation of smooth muscles of blood vessels.[1] Therefore, PCOs are expected to be useful agents for the treatment of cardiovascular diseases such as hypertension and angina pectoris. In fact, compound **II** showed potent antihypertensive activity in clinical trials. However, adverse reactions (ADRs) such as reflex tachycardia, headache and edema were frequently encountered.[2] These results led to preliminary conclusions that PCOs have no clinical advantage over conventional antihypertensive agents such as calcium channel blockers and angiotensin converting enzyme inhibitors.

It has been suggested that ADRs such as reflex tachycardia and headache might be compensatory reactions in response to the potent hypotensive effect induced immediately following the administration of PCOs, and we therefore expected that a slow onset of hypotensive action would probably decrease these ADRs. Based on this assumption, our investigation targeted PCOs showing a slow onset of action.

We have previously reported the synthesis and activity of a series of PCOs with a 4-[4-alkyl-3,4-diazabicyclo[4.1.0]heptenyloxy]-1-benzopyran pharmacophore (**III**).[3] In that study, we reported structure–activity relationships (SARs) focusing on variations of the 4-substituent of the 3,4-diazabicyclo[4.1.0]heptenyl group: straight-chain alkyl substituents gave compounds which showed potent hypotensive activity, but branched chain substituents such as an isobutyl group resulted in a loss of activity. We have systematically modified the 4-substituent of the 3,4-diazabicyclo[4.1.0]heptenyl group, and found that the incorporation of a 4-benzyl moiety such as compound **IV** produced PCOs with the desired pharmacological profiles. These compounds showed potent activity *in vivo* and, furthermore, they also had the desired profile *i.e.* a slow onset and long-duration of action.

In this paper, we describe the synthesis and biological activity of a series of 3,4-dihydro-2*H*-1-benzopyrans with 4-substituted-3,4-diazabicyclo[4.1.0]heptenyl groups.

Chemistry Chart 2 shows the general method for the synthesis of the 4-benzyl-3,4-diazabicyclo[4.1.0]heptenyl-1-benzopyran derivatives **5**. Condensation of 1,2-cyclopropanedicarboxylic anhydride[4] (**1**) with benzylhydrazines **2a**—**j** and phenethylhydrazine **2k** gave (±)-3-benzyl-3,4-diazabicyclo[4.1.0]heptane-2,5-diones **3a**—**j** and 3-phenethyl congener **3k**, respectively, in 7—34% yields (Table 1). Compounds **3a**—**k** reacted with (3*S*,4*S*)-3,4-epoxy-2*H*-1-benzopyran-6-carbonitrile[5] (**4**) in the presence of pyridine in ethanol at reflux to afford a mixture of two diastereomers **5a**—**k**A (less polar isomer, (3*S*,4*R*,1′*S*,6′*R*)) and **5a**—**k**B (more polar isomer, (3*S*,4*R*,1′*R*,6′*S*)), which were separated by silica gel column chromatography (Table 2).

Oxidative cleavage of the 4-methoxybenzyl group[6] of compounds **5i**A and **5i**B by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in refluxing 1,2-dichloroethane afforded 4-unsubstituted diazabicyclo[4.1.0]heptenyl derivatives **6**A and **6**B, respectively, in good yield (Chart 3).

Alkylation of diazabicyclo[4.1.0]heptenyl derivative **6**B with 4-cyanobenzyl bromide and 4-pyridylmethyl chloride in the presence of potassium carbonate produced 4-substituted diazabicyclo[4.1.0]heptenyl derivatives **5l**B and **5m**B, respectively, in good yield (Chart 3).

The 3D structures of compounds **5a**B and **6**B were determined by X-ray crystallography and are shown in Fig. 1. Their configurations were determined to be (3*S*,4*R*,1′*R*,6′*S*) from correlation of the relative configurations of **5a**B and **6**B obtained by X-ray crystallographic analysis and the known absolute configuration (3*S*,4*S*) of intermediate **4**.[5]

The NMR δ values of the proton at the 4-position (H4) of the benzopyran nucleus of compounds **5** and **6** are listed in Table 2, respectively. In all diazabicyclo[4.1.0]heptenyl derivatives synthesized, the δ value of H4 in every isomer B was larger than the value for the corresponding isomer A. The difference between δ values of H4 in isomers A and B was in the range 0.04 to 0.26 ppm. Correlations between the configuration, chemical shift and polarity were demonstrated in the series of compounds reported previously.[3] Based on

* To whom correspondence should be addressed.

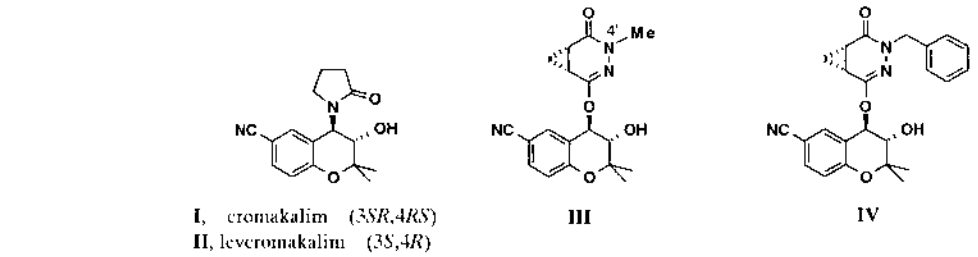


Chart 1

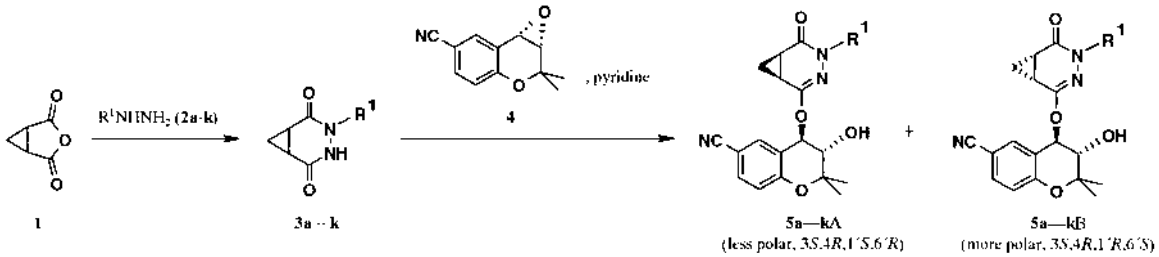
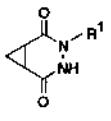


Chart 2

Table 1. 3,4-Diazabicyclo[4.1.0]heptane-2,5-diones (**3a**—**k**)



3a—k

No.	R^1	Reaction solvent[a)]	Yield (%)	Recrystallization solvent[a)]	mp[b)] (°C)	Formula[c)]
3a	$CH_2C_6H_5$	E	30	EA	186—188	$C_{12}H_{12}N_2O_2$
3b	$CH_2C_6H_4$(2-Cl)	A	16	E–H	162—163	$C_{12}H_{11}ClN_2O_2$
3c	$CH_2C_6H_4$(3-Cl)	A	15	E–H	132—133	$C_{12}H_{11}ClN_2O_2$
3d	$CH_2C_6H_4$(4-Cl)	E	20	M–IPE	187—188	$C_{12}H_{11}ClN_2O_2$
3e	$CH_2C_6H_3$(2,6-Cl_2)	E	7	M–IPE	172—174	$C_{12}H_{10}Cl_2N_2O_2$
3f	$CH_2C_6H_3$(2,4-Cl_2)	A	18	M	199—202	$C_{12}H_{10}Cl_2N_2O_2$
3g	$CH_2C_6H_4$(2-CH_3)	A	33	M	177—190	$C_{13}H_{14}N_2O_2$
3h	$CH_2C_6H_4$(4-CH_3)	A	27	M	203—209	$C_{13}H_{14}N_2O_2$
3i	$CH_2C_6H_4$(4-OCH_3)	A	34	M–H	180—183	$C_{13}H_{14}N_2O_3$
3j	$CH_2C_6H_4$(4-SO_2NH_2)	A	11	M	250—253	$C_{12}H_{13}N_3O_4S \cdot 0.25H_2O$
3k	$CH_2CH_2C_6H_5$	A	25	E–H	160—162	$C_{13}H_{14}N_2O_2$

a) Solvent: A, acetonitrile; E, ethanol; EA, ethyl acetate; H, hexane; IPE, diisopropyl ether; M, methanol. *b*) Uncorrected. *c*) All compounds exhibited satisfactory (±0.4%) elemental analyses for C, H, N.

the observed relationships, all of the compounds **5b**—**m**B were confirmed to have a (3*S*,4*R*,1′*R*,6′*S*)-configuration, the same as that of **5a**B and **6**B.

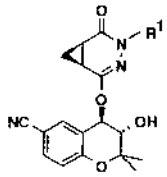
Results and Discussion

The *in vivo* antihypertensive activity ($ED_{50\,mmHg}$) of the compounds synthesized in this study was evaluated by oral administration to SHRs. The *in vitro* potassium channel opening activity of selected compounds was evaluated by their effects on K^+ efflux ($EC_{AUC0.2}$) in the rat isolated aorta using $^{86}Rb^+$ as a surrogate marker for K^+ (Table 3).[7)]

Most of the compounds tested exhibited potent antihypertensive activity *in vivo*. In particular, 4-benzyl, 4-(2-chlorobenzyl), 4-(4-chlorobenzyl), 4-(2-methylbenzyl), 4-(2,4-dichlorobenzyl), 4-(4-cyanobenzyl), 4-(4-pyridinylmethyl) and 4-phenethyl derivatives (**5a**,**b**,**d**,**f**,**g**,**k**,**l**,**m**B) showed high potency. The activities of the remaining compounds were comparable to that of levcromakalim (**II**) or less.

Although 4-benzyl and 4-(2-chlorobenzyl) derivatives **5a**,**b**B had high potency *in vivo*, they showed no activity in the *in vitro* assay. Similarly, the 4-phenethyl derivative **5k**B was inactive *in vitro*. Generation of an active metabolite seemed to be the most plausible explanation for this anomaly. It is known that an amide *N*-alkyl group, in particular an *N*-benzyl group, is cleaved by cytochrome P-450.[8)] Therefore, the common metabolite **6**B may well be produced from compounds such as **5a**—**m**B *via* cleavage of the 4-substituent on the diazabicyclo[4.1.0]heptenyl group. Therefore, we synthesized the suspected metabolite **6**B to confirm our hypothesis. HPLC analysis of serum samples (Fig. 2) showed that compound **5b**B was quantitatively converted into **6**B after oral

Table 2. (3*S*,4*R*)-4-(5-Oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yloxy)-1-benzopyran Derivatives **5**



5a—kA (less polar, (3*S*,4*R*,1′*S*,6′*R*)) 5a—kB (more polar, (3*S*,4*R*,1′*R*,6′*S*))

No.	R^1	Yield (%)	Solvent[a)]	mp[b)] (°C)	*Rf* value[c)]	$[\alpha]_D$[d)]	Formula[e)]	δ value[f)] (ppm)
5aA	$CH_2C_6H_5$	35	—	Amorphous	0.64 (B)	−185.7	$C_{24}H_{23}N_3O_4$	5.44
5aB		32	IPE	118—120	0.50 (B)	−212.8	$C_{24}H_{23}N_3O_4$	5.60
5bA	$CH_2C_6H_4$(2-Cl)	30	E–H	199—201	0.48 (B)	−207.0	$C_{24}H_{22}ClN_3O_4$	5.56
5bB		33	E–H	188—190	0.41 (B)	−266.6	$C_{24}H_{22}ClN_3O_4$	5.60
5cA	$CH_2C_6H_4$(3-Cl)	25	—	Amorphous	0.64 (A)	−183.6	$C_{24}H_{22}ClN_3O_4$	5.47
5cB		25	E–H	165—167	0.58 (A)	−182.6	$C_{24}H_{22}ClN_3O_4$	5.64
5dA	$CH_2C_6H_4$(4-Cl)	32	—	Amorphous	0.48 (B)	−165.4	$C_{24}H_{22}ClN_3O_4\cdot 0.5H_2O$	5.44
5dB		29	E–H	163—164	0.40 (B)	−181.0	$C_{24}H_{22}ClN_3O_4$	5.61
5eA	$CH_2C_6H_3$(2,6-Cl_2)	26	E	208	0.52 (B)	−211.8	$C_{24}H_{21}Cl_2N_3O_4$	5.57
5eB		34	E	255—256	0.38 (B)	−411.2	$C_{24}H_{21}Cl_2N_3O_4$	5.62
5fA	$CH_2C_6H_3$(2,4-Cl_2)	38	—	Amorphous	0.53 (B)	−186.8	$C_{24}H_{21}Cl_2N_3O_4\cdot 0.5H_2O$	5.45
5fB		32	—	Amorphous	0.44 (B)	−233.7	$C_{24}H_{21}Cl_2N_3O_4\cdot 0.5H_2O$	5.57
5gA	$CH_2C_6H_4$(2-CH_3)	16	E–H	192—193	0.24 (C)	−208.5	$C_{25}H_{25}N_3O_4$	5.47
5gB		29	—	Amorphous	0.18 (C)	−249.5	$C_{25}H_{25}N_3O_4$	5.53
5hA	$CH_2C_6H_4$(4-CH_3)	30	—	Amorphous	0.31 (C)	−225.9	$C_{25}H_{25}N_3O_4\cdot 0.5H_2O$	5.47
5hB		44	—	Amorphous	0.26 (C)	−226.1	$C_{25}H_{25}N_3O_4$	5.65
5iA	$CH_2C_6H_4$(4-OCH_3)	38	—	Amorphous	0.23 (C)	−195.8	$C_{25}H_{25}N_3O_5\cdot 0.25H_2O$	5.46
5iB		42	—	Amorphous	0.17 (C)	−205.1	$C_{25}H_{25}N_3O_5\cdot 0.25H_2O$	5.63
5jA	$CH_2C_6H_4$(4-SO_2NH_2)	23	—	Amorphous	0.39 (A)	−137.5	$C_{24}H_{24}N_4O_6S\cdot 0.25H_2O$	5.67
5jB		17	—	Amorphous	0.37 (A)	−149.3	$C_{24}H_{24}N_4O_6S\cdot 0.5H_2O$	5.73
5kA	$CH_2CH_2C_6H_5$	22	—	Amorphous	0.60 (A)	−105.4	$C_{25}H_{25}N_3O_4\cdot 0.25H_2O$	5.24
5kB		25	—	Amorphous	0.47 (A)	−107.4	$C_{25}H_{25}N_3O_4\cdot 0.25H_2O$	5.50

a) Recrystallization solvent: E, ethanol; H, hexane; IPE, diisopropyl ether. *b*) Uncorrected. *c*) *Rf* values on silica gel TLC: solvent system: A, chloroform : methanol = 10 : 1 (v/v); B, chloroform : methanol = 20 : 1 (v/v); C, chloroform : methanol = 40 : 1 (v/v). *d*) $c = 1.0$ in methanol at 25 °C. *e*) All compounds exhibited satisfactory (±0.4%) elemental analyses for C, H, N. *f*) Chemical shifts of the proton at the 4-position of the benzopyran ring observed in $CDCl_3$.

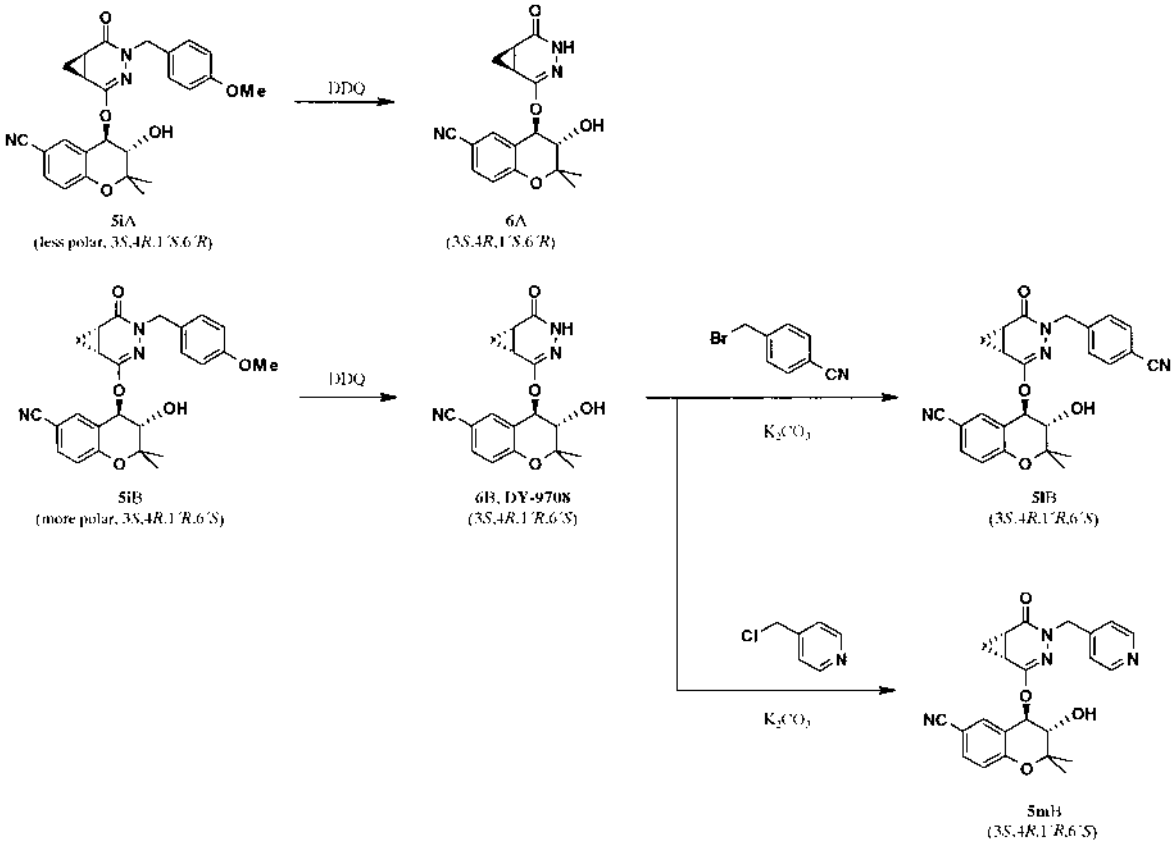
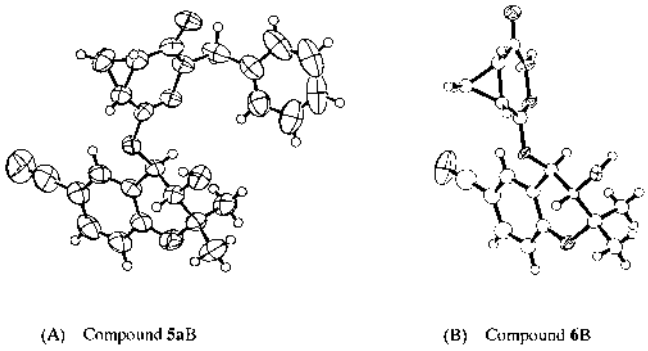


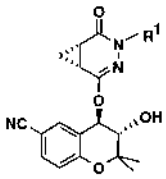
Chart 3



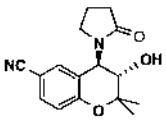
(A) Compound 5aB (B) Compound 6B

Fig. 1. X-Ray Crystal Structures of Compounds **5a**B and **6**B

Table 3. Biological Data for 4-(5-Oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yloxy)-1-benzopyran Derivatives



5a--mB, 6B, III



II

Compound	R^1	Rb efflux $EC_{AUC0.2}$, μM[a)]	Antihypertensive activity $ED_{50\,mmHg}$, mg/kg, *p.o.*[b)]
5aB	$CH_2C_6H_5$	NA	0.053
5bB	$CH_2C_6H_4$(2-Cl)	NA	0.031
5cB	$CH_2C_6H_4$(3-Cl)	NT	0.20
5dB	$CH_2C_6H_4$(4-Cl)	NT	0.060
5eB	$CH_2C_6H_3$(2,6-Cl_2)	NT	0.40
5fB	$CH_2C_6H_3$(2,4-Cl_2)	NT	0.035
5gB	$CH_2C_6H_4$(2-CH_3)	NT	0.085
5hB	$CH_2C_6H_4$(4-CH_3)	NT	(>0.3)
5iB	$CH_2C_6H_4$(4-OCH_3)	NT	1.6
5jB	$CH_2C_6H_4$(4-SO_2NH_2)	NT	(>0.1)
5kB	$CH_2CH_2C_6H_5$	NA	0.083
5lB	$CH_2C_6H_4$(4-CN)	NT	0.063
5mB	CH_2(4-pyridyl)	NT	0.021
6B	H	0.15	0.0093
II	—	1.6	0.14
III	CH_3	0.18	0.029

a) NT, not tested; NA, not active. *b*) Blood pressure lowering activity was less than 50 mmHg at the dose shown in parentheses.

dosing. The administration of 0.5 mg/kg of **5b**B to rats gave **6**B with a t_{max} of 12 h and C_{max} of 0.377 μg/ml.

Since compounds **5a**,**c**—**m**B are metabolized to the common active metabolite **6**B, the *in vivo* SARs in this series of compounds may reflect relative susceptibility to metabolism of the 4-substituent of the 3,4-diazabicyclo[4.1.0]heptenyl group. Thus, 4-benzyl, 4-(2-chlorobenzyl), 4-(4-chlorobenzyl), 4-(2-methylbenzyl), 4-(2,4-dichlorobenzyl), 4-(4-cyanobenzyl), 4-(4-pyridinylmethyl) and 4-phenethyl groups are all probably easily removed by metabolism, since compounds **5a**,**b**,**d**,**f**,**g**,**k**,**l**,**m**B, which have these substituents, show high potency *in vivo*. In contrast, 4-(3-chlorobenzyl), 4-(2,6-dichlorobenzyl), 4-(4-methybenzyl), 4-(4-sulfamoylbenzyl) and 4-(4-methoxybenzyl) groups seem to resist metabolism, as shown by the lower potency of compounds **5c**,**e**,**h**,**i**,**j**B (Table 3).

Figure 3 shows the changes in systolic blood pressure (SBP) and heart rate (HR) after the oral administration of 4-(2-chlorobenzyl) derivative **5b**B (0.1 mg/kg), 4-unsubstituted derivative **6**B (0.03 mg/kg) and levcromakalim (**II**, 1 mg/kg) to SHRs. Compound **5b**B exhibited a maximal decrease in blood pressure more than 8 h after administration, and the hypotensive effect lasted more than 24 h. Compound **6**B also had long-lasting activity, but showed a maximal decrease in blood pressure at 1 h following administration. Compound **II** also had a rapid onset of action, and the hypotensive effect disappeared within 24 h. Moreover, compound **II** showed acute elevation of heart rate, while compound **5b**B exhibited a gradual and less pronounced increase in heart rate. Thus, **5b**B had a desired pharmacological profile with a slow onset, long duration of action, and less acute induced tachycardia.

The above results are consistent with the report that Y-27152 (**Va**, Chart 4) is a slow-onset and long-acting PCO with reduced tachycardia.[2,9)] This compound is also metabolized to an active metabolite, Y-26763 (**Vb**), through *O*-debenzylation in the liver. Taken together, these results indicate that a slow onset of action can partially overcome some of the adverse effects of first generation PCOs.

In conclusion, we prepared 4-(4-substituted-3,4-diazabicyclo[4.1.0]heptenyl)-2*H*-1-benzopyrans, and demonstrated that 4-(2-chlorobenzyl) derivative **5b**B (DY-9804) is a slow-onset and long-acting PCO which causes only mild tachycardia. Compound **5b**B was metabolized to give active des-2-

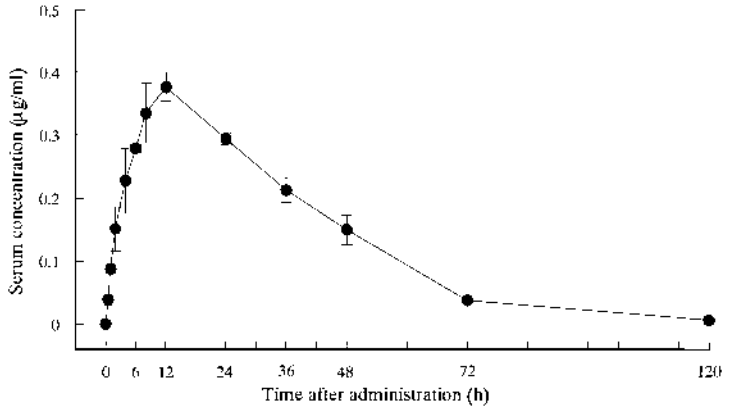


Fig. 2. Serum Concentration of Compound **6**B in Wistar Rats after Oral Administration of **5b**B

Male Wistar rats (n=3) were treated with compound **5b**B (0.6 mg/kg, *p.o.*). The serum concentration of **6**B was determined by HPLC. The data are means±S.E. for 3 rats. Compound **5b**B was not detected at any sampling time following dosing.

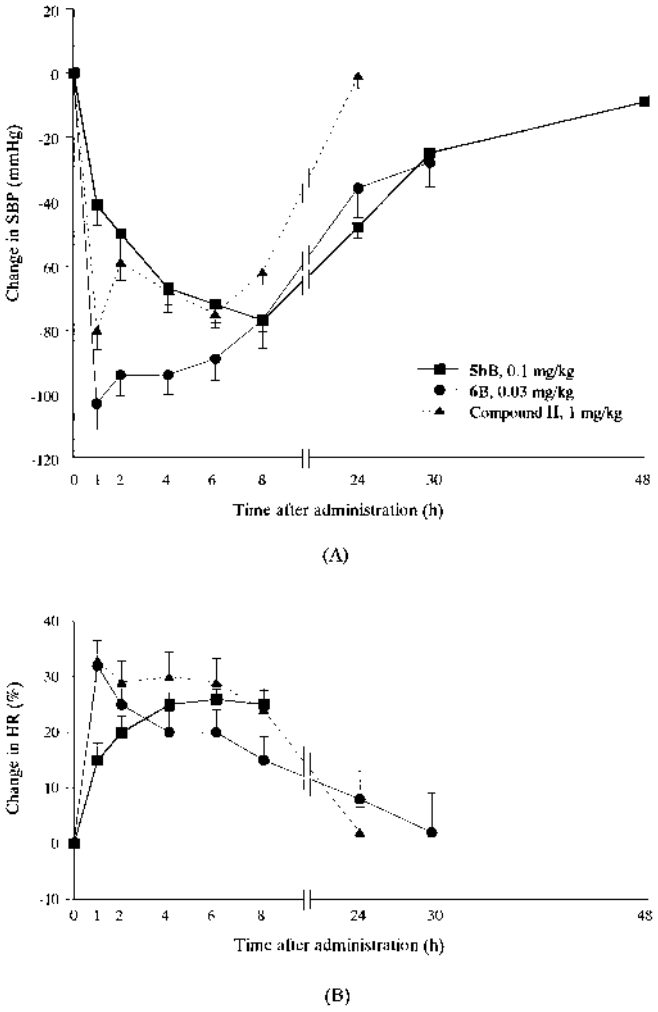
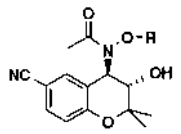


Fig. 3. Hypotensive Activity (A) and Change of Heart Rate (B) of PCOs

Male spontaneously hypertensive rats were treated with compound **5b**B (0.1 mg/kg, *p.o.*, ■), **6**B (0.03 mg/kg, *p.o.*, ●) and **II** (levcromakalim, 1 mg/kg, *p.o.*, ▲). The systolic blood pressure (SBP) and the heart rate (HR) were measured by a tail-cuff method, and the changes of SBP and HR are expressed *vs.* initial. The data are means±S.E. for 5 rats.



Va, Y-27152 (R = Bn)
Vb, Y-26763 (R = H)

Chart 4

chlorobenzyl derivative **6**B (DY-9708). These results suggest that *N*-benzyl substitution on amides or heterocycles may be a useful method for the preparation of prodrugs which display delayed onset and prolonged duration of action.

Further work is in progress to investigate the metabolism of *N*-debenzylation by cytochrome P450 in rats, and to determine the extent of metabolic activation in other mammals.

Experimental

Chemistry Melting points were determined on a Büchi 535 melting point apparatus and are uncorrected. Optical rotations were measured on a Horiba SEPA200 digital polarimeter. The ^{1}H-NMR spectra were recorded on a JEOL JNM-EX400 (400 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard. Signal multiplicities are represented by s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Chemical shifts are expressed in δ values and the coupling constants in Hz. For column chromatography, silica gel (Kieselgel 60, 70—230 mesh, E. Merck) was used. Mass spectra (MS) were recorded on JEOL JMS-HX110 and JMS-AX505W instruments. Precoated Silica gel 60 F_{254} plates with a layer thickness of 0.25 mm (E. Merck, Darmstadt, Germany) were used for thin-layer chromatography (TLC) to determine *Rf* values.

Starting Materials 1,2-Cyclopropanedicarboxylic anhydride (**1**) was prepared according to McCoy's method.[4] Benzylhydrazines (**2a**—**k**) were prepared from alkyl halide and hydrazine monohydrate according to the usual procedures.[10] Epoxide **4** was prepared according to the methods previously reported.[5]

(±)-3-Benzyl-3,4-diazabicyclo[4.1.0]heptane-2,5-dione (3a) A solution of benzylhydrazine (**2a**, 45.8 g, 0.374 mol) in ethanol (50 ml) was added dropwise to a solution of **1** (42.0 g, 0.374 mol) in ethanol (150 ml) with stirring. The reaction mixture was heated under reflux for 15 h. After evaporation of the solvent *in vacuo*, the resulting residue was purified by silica gel column chromatography with ethyl acetate (EtOAc) as an eluent, followed by recrystallization from EtOAc to give **3a** (24.1 g, 30%), mp 186—188 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.14 (1H, m), 1.69 (1H, m), 2.11 (1H, m), 2.28 (1H, m), 4.67 (1H, d, J=15.1 Hz), 4.83 (1H, d, J=15.1 Hz), 7.32 (5H, s). *Anal*. Calcd for $C_{12}H_{12}N_2O_2$: C, 66.65; H, 5.59; N, 12.95. Found: C, 66.29; H, 5.55; N, 12.75.

Compounds **3b**—**k** were prepared in an analogous manner (see Table 1) from **1** and the appropriate corresponding alkylhydrazines (**2b**—**k**).

(−)-(3*S*,4*R*,1′*S*,6′*R*)-4-(4-Benzyl-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yloxy)-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (5aA) and (−)-(3*S*,4*R*,1′*R*,6′*S*)-4-(4-Benzyl-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yloxy)-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (5aB) A mixture of (−)-(3*S*,4*S*)-3,4-epoxy-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**4**, 744 mg, 3.7 mmol), **3a** (800 mg, 3.7 mmol) and pyridine (0.4 ml, 5.0 mmol) in EtOH (20 ml) was heated under reflux for 16 h. After evaporation of the solvent *in vacuo*, the resulting residue was purified by silica gel column chromatography with $CHCl_3$: MeOH=50 : 1 (v/v) as an eluent to give **5a**A (less polar) and **5a**B (polar). **5a**A: Yield 540 mg (35%) as a colorless amorphous solid, $[\alpha]_D^{25}$=−185.7° (c=1, MeOH). *Rf* value=0.64 ($CHCl_3$: MeOH=20 : 1). ^{1}H-NMR ($CDCl_3$) δ: 1.00 (1H, m), 1.24 (3H, s), 1.48 (3H, s), 1.74 (1H, m), 2.28 (1H, m), 2.39 (1H, m), 3.81 (1H, dd, J=2.9, 7.8 Hz), 4.34 (1H, d, J=2.9 Hz, OH), 4.63 (1H, d, J=14.1 Hz), 4.93 (1H, d, J=14.1 Hz), 5.44 (1H, d, J=7.8 Hz), 6.87 (1H, d, J=8.3 Hz), 7.32 (5H, m), 7.48 (1H, dd, J=2.0, 8.3 Hz), 7.53 (1H, d, J=2.0 Hz). *Anal*. Calcd for $C_{24}H_{23}N_3O_4$: C, 69.05; H, 5.55; N, 10.06. Found: C, 68.89; H, 5.81; N, 9.60. **5a**B: Yield 490 mg (32%) as colorless needles (from diisopropyl ether), mp 118—120 °C, $[\alpha]_D^{25}$=−212.8° (c=1, MeOH). *Rf* value=0.50 ($CHCl_3$: MeOH=20 : 1). ^{1}H-NMR ($CDCl_3$) δ: 1.01 (1H, m), 1.25 (3H, s), 1.44 (3H, s), 1.72 (1H, m), 2.17 (1H, m), 2.33 (1H, m), 3.01 (1H, d, J=4.4 Hz, OH), 3.76 (1H, dd, J=4.4, 7.3 Hz), 4.74 (1H, d, J=14.1 Hz), 4.84 (1H, d, J=14.1 Hz), 5.60 (1H, d, J=7.3 Hz), 6.88 (1H, d, J=8.3 Hz), 7.31 (5H, m), 7.48 (1H, dd, J=2.0, 8.3 Hz), 7.53 (1H, d, J=2.0 Hz). *Anal*. Calcd for $C_{24}H_{23}N_3O_4$: C, 69.05; H, 5.55; N, 10.06. Found: C, 69.42; H, 5.43; N, 10.24. Compounds **5b**—**k**A and **5b**—**k**B were prepared in an analogous manner (see Table 2) from **4** and corresponding (±)-3-alkyl-3,4-diazabicyclo[4.1.0]heptane-2,5-diones (**3b**—**k**).

(−)-(3*S*,4*R*,1′*R*,6′*S*)-4-[4-(4-Cyanobenzyl)-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yloxy]-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (5lB) To a solution of **6**B (200 mg, 0.61 mmol) in acetone (10 ml) were added 4-cyanobenzyl bromide (130 mg, 0.66 mmol) and potassium carbonate (90 mg, 0.65 mmol). The mixture was heated with stirring under reflux for 40 h. The reaction mixture was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography with $CHCl_3$ as an eluent to give **5l**B (170 mg, 63%) as colorless crystals, mp 185—187 °C, $[\alpha]_D^{25}$=−198.9° (c=1, MeOH). *Rf* value=0.32 ($CHCl_3$: MeOH=20 : 1, v/v). ^{1}H-NMR (DMSO-d_6) δ: 1.07 (1H, m), 1.22 (3H, s), 1.39 (3H, s), 1.71 (1H, m), 2.27 (2H, m), 3.77 (1H, dd, J=5.4, 6.8 Hz), 4.69 (1H, d, J=15.6 Hz), 4.93 (1H, d, J=15.6 Hz), 5.58 (1H, d, J=6.8 Hz), 5.89 (1H, d, J=5.4 Hz, OH), 6.95 (1H, d, J=8.8 Hz), 7.44 (2H, d, J=8.3 Hz), 7.54 (1H, d, J=2.0 Hz), 7.64 (1H, dd, J=2.0, 8.8 Hz), 7.77 (2H, d, J=8.3 Hz). *Anal*. Calcd for $C_{25}H_{22}N_4O_4$: C, 67.86; H, 5.01; N, 12.66. Found: C, 67.79; H, 5.18; N, 12.58.

(−)-(3*S*,4*R*,1′*R*,6′*S*)-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-[5-oxo-4-(4-pyridylmethyl)-3,4-diazabicyclo[4.1.0]hept-2-en-2-yloxy]-2*H*-1-benzopyran-6-carbonitrile (5mB) Compound **6**B was reacted with 4-

chloromethylpyridine using a similar procedure to that described above to give **5m**B (61%) as a colorless amorphous solid, $[\alpha]_D^{25}=-173.4°$ ($c=1$, MeOH). *Rf* value=0.64 ($CHCl_3$: MeOH=5 : 1, v/v). ^{1}H-NMR ($CDCl_3$) δ: 1.07 (1H, m), 1.27 (3H, s), 1.47 (3H, s), 1.78 (1H, m), 2.21 (1H, m), 2.32 (1H, m), 3.52 (1H, br s, OH), 3.84 (1H, d, J=7.3 Hz), 4.74 (1H, d, J=15.1 Hz), 4.84 (1H, d, J=15.1 Hz), 5.64 (1H, d, J=7.3 Hz), 6.89 (1H, d, J=8.3 Hz), 7.21 (2H, br s), 7.48 (1H, dd, J=2.0, 8.3 Hz), 7.55 (1H, d, J=2.0 Hz), 8.56 (2H, br s). *Anal*. Calcd for $C_{23}H_{22}N_4O_4$: C, 66.02; H, 5.30; N, 13.39. Found: C, 65.88; H, 5.46; N, 13.00.

(−)-(3*S*,4*R*,1′*R*,6′*S*)-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-(5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yloxy)-2*H*-1-benzopyran-6-carbonitrile (6B) A mixture of **5i**B (5.91 g, 13.2 mmol), DDQ (17.7 g, 78 mmol) and H_2O (0.9 ml, 50 mmol) in 1,2-dichloroethane (450 ml) was heated under reflux for 8 h. After removal of the insoluble product by filtration, the filtrate was evaporated *in vacuo*. The resulting residue was purified by silica gel column chromatography with $CHCl_3$: MeOH=50 : 1 (v/v) as eluent, followed by recrystallization from EtOH–H_2O (1 : 2, v/v) to yield **6**B (2.81 g, 65%), mp 199—201 °C, $[\alpha]_D^{25}=-173.8°$ ($c=1$, MeOH). *Rf* value=0.32 (EtOAc). ^{1}H-NMR ($CDCl_3$) δ: 1.18 (1H, m), 1.33 (3H, s), 1.52 (3H, s), 1.60—2.10 (1H, br s, OH), 1.81 (1H, m), 2.21 (2H, m), 3.92 (1H, d, J=7.3 Hz), 5.71 (1H, d, J=7.3 Hz), 6.91 (1H, d, J=8.3 Hz), 7.50 (1H, dd, J=2.0, 8.3 Hz), 7.59 (1H, d, J=2.0 Hz), 7.71 (1H, s, NH). *Anal*. Calcd for $C_{17}H_{17}N_3O_4$: C, 62.37; H, 5.23; N, 12.83. Found: C, 62.51; H, 5.50; N, 12.48. Compound **6**A was prepared in an analogous manner from the corresponding 4′-methoxybenzyl derivative **5i**A. **6**A: Yield 40%, mp 126—129 °C (from CH_2Cl_2–hexane), $[\alpha]_D^{25}=-63.7°$ ($c=1$, MeOH). *Rf* value=0.34 (EtOAc). ^{1}H-NMR ($CDCl_3$) δ: 1.13 (1H, m), 1.31 (3H, s), 1.51 (3H, s), 1.79 (1H, m), 2.30 (2H, m), 3.91 (1H, dd, J=2.9, 7.3 Hz), 4.28 (1H, d, J=2.9 Hz, OH), 5.62 (1H, d, J=7.3 Hz), 6.91 (1H, d, J=8.3 Hz), 7.51 (1H, dd, J=2.0, 8.3 Hz), 7.53 (1H, s, NH), 7.59 (1H, d, J=2.0 Hz). *Anal*. Calcd for $C_{17}H_{17}N_3O_4$: C, 62.37; H, 5.23; N, 12.83. Found: C, 62.02; H, 5.21; N, 12.74.

X-Ray Crystallography of Compound 5aB Crystals of **5a**B were grown from diisopropyl ether as colorless prisms. Diffraction intensities were collected from a crystal which had dimensions of 0.50×0.20×0.15 mm on a Rigaku AFC5R diffractometer with graphite monochromated CuK_α radiation (λ=1.54178 Å) at 23 °C. Crystal data: $C_{24}H_{23}N_3O_4\cdot 0.25C_6H_{14}O$, M.W.=443.01, a=12.142(1) Å, b=8.098(2) Å, c=13.664(1) Å, β=106.896(9)°, V=1285.5(3) Å^3, space group $P2_1$ (#4), Z=2, Dc=1.144 g/cm^3, F_{000}=469. For structural determination, 1735 observed reflections were used (I>3.00s(I)). The structure was solved by direct methods using SHELXS-86[11] and refined by full-matrix least-squares refinement with anisotropic temperature factors for non-hydrogen atoms. The final R value was 0.052 (R_w=0.066).

X-Ray Crystallography of Compound 6B Crystals of **6**B were grown from ethanol–water as colorless rhomboids. Diffraction intensities were collected from a crystal which had dimensions of 0.30×0.07×0.07 mm on a Rigaku AFC7R diffractometer with graphite monochromated CuK_α radiation (λ=1.54178 Å) at −60 °C. Crystal data: $C_{17}H_{17}N_3O_4$, M.W.=327.34, a=6.422(2) Å, b=10.045(3) Å, c=12.720(1) Å, β=102.27(1)°, V=801.9(2) Å^3, space group $P2_1$ (#4), Z=2, D_c=1.356 g/cm^3, F_{000}=344. For structural determination, 713 observed reflections were used (I>3.00s(I)). The structure was solved by direct methods using SHELXS-86[11] and refined by full-matrix least-squares refinement with anisotropic temperature factors for non-hydrogen atoms. The final R value was 0.049 (R_w=0.044).

Potassium Channel Opening Activity The potassium channel opening activity of the test compounds shown in Table 4 was determined according to Quast's test method.[7] ^{86}Rb was incorporated into a segment of excised aorta from a Wistar rat, and the segment was surface-perfused with a solution containing a test compound for 10 min. The potassium channel opening activity of the test compound was expressed in terms of an effective concentration at which the area under the peak of the ^{86}Rb release rate reached 0.2 ($EC_{AUC0.2}$).

Antihypertensive Activity in SHRs Male SHRs (16- to 20-week-old, body weight, 300—400 g) fed *ad lib*. were dosed orally with test compound suspended in 0.5% (w/v) carboxymethylcellulose aqueous solution. At 1, 2, 4, 6, 8, 24, 30, and 48 h after the administration, the systolic blood pressure and the heart rate were measured by the tail-cuff method.[12] The antihypertensive activity of the test compound was determined as the effective dose for reducing blood pressure by 50 mmHg ($ED_{50\,mmHg}$).

Serum Concentration of Compounds after Oral Administration of 5bB in Rats Unfasted male Wistar rats (9-week-old, body weight, 205—229 g) were dosed orally with compound **5b**B suspended in 0.5% (w/v) carboxymethylcellulose aqueous solution (0.6 mg/5 ml/kg). At 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72 and 120 h, respectively, after the administration, three rats were killed and their blood samples were taken. Blood samples were centrifuged (3000 rpm, 15 min) to separate plasma. Serum sample (1 ml) and water (6 ml) was added to compound **5f**B (2 μg, as an internal standard), and absorbed in a Sep-pak C_{18} Plus cartridge (360 mg, Waters). After the cartridge was washed with water (10 ml) and eluted using methanol (5 ml), the eluent was concentrated *in vacuo* and the resulting residue was diluted with a mobile phase for HPLC (H_2O : MeCN=4 : 6, 200 μl). Concentrations of compound **5b**B and **6**B were determined by HPLC analysis.

Acknowledgments We thank Dr. Ken-ichi Yamazaki and Mr. Makoto Suzuki (Daiichi Pharmaceutical Co., Ltd.) for X-ray crystal analysis.

References

1) Ashwood V. A., Buckingham R. E., Cassidy F., Evans J. M., Faruk E. A., Hamilton T. C., Nash D. J., Stemp G., Willcocks K., *J. Med. Chem*., **29**, 2194—2201 (1986).
2) Nakajima T., *Cardiovasc. Drug. Rev*., **9**, 372—384 (1991).
3) Horino H., Mimura T., Kagechika K., Ohta M., Kubo H., Kitagawa M., *Chem. Pharm. Bull*., **46**, 602—609 (1997).
4) McCoy L. L., *J. Am. Chem. Soc*., **80**, 6568—6572 (1958).
5) Jacobsen E. N., Zhang W., Muci A. R., Ecker J. R., Deng L., *J. Am. Chem. Soc*., **113**, 7063—7064 (1991).
6) Oikawa Y., Yoshioka T., Yonemitsu O., *Tetrahedron Lett*., **23**, 885—888 (1982); Oikawa Y., Tanaka T., Hirota K., Yoshioka T., Yonemitsu O., *Tetrahedron Lett*., **25**, 5393—5396 (1984).
7) Quast U., Baumlin Y., *Naunyn-Schmiedeberg's Arch. Pharmacol*., **338**, 319—326 (1988).
8) Hall L. R., Hanzlik R. P., *Xenobiotica*, **21**, 1127—1138 (1991).
9) Nakajima T., Shinohara T., Yaoka O., Fukunari A., Shinagawa K., Aoki K., Katoh A., Yamanaka T., Setoguchi M., Tahara T., *J. Pharmacol. Exp. Ther*., **261**, 730—736 (1992).
10) Biel J. H., Drukker A. E., Mitchell T. F., Sprengeler E. P., Nuhfer P. A., Conway A. C., Horita A., *J. Am. Chem. Soc*., **81**, 2805—2813 (1959).
11) Sheldrick G. M., "Crystallographic Computing 3," ed. by Sheldrick G. M., Kruger C., Goddard R., Oxford University Press, Oxford, 1985, pp. 175—189.
12) Heptner W., Kellner H.-M., Christ O., Weihrauch D., *Arzneim.-Forsch*., **19**, 1400—1404 (1969).