Synthesis and Antihypertensive Activity of 4-(Diazabicyclo[4.1.0]-heptenyloxy)benzopyran Derivatives and Their Analogues

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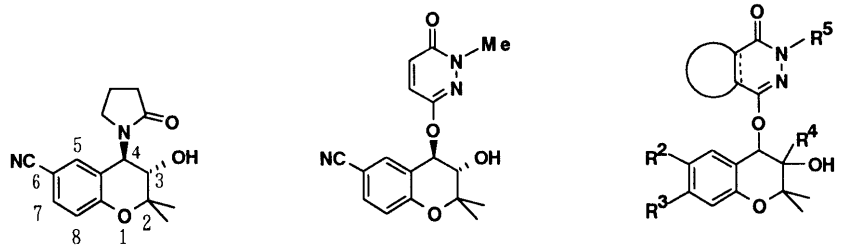
A series of 3,4-dihydro-3-hydroxy-4-[(5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy]-2*H*-1-benzopyrans and their analogues were synthesized and evaluated on potassium channel opening and hypotensive activities. Compound (−)-13B with a (4-methyl-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy group for the 4-position of the benzopyran ring was 3 times as potent as EMD 57283 (II), the lead compound, in hypotensive activity. The results would demonstrate that 5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yloxy moieties are effective as the substituents at the 4-position of benzopyran-type potassium channel openers.

Key words diazabicyclo[4.1.0]heptene; potassium channel opener; hypotensive activity; 2*H*-1-benzopyran

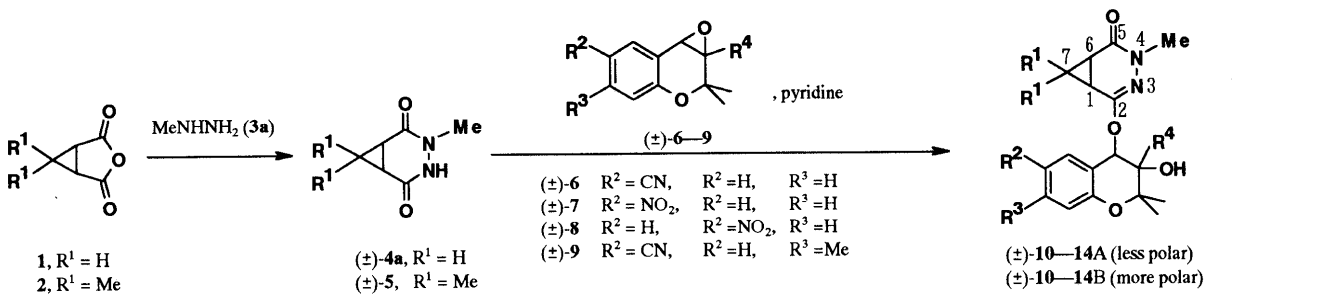
The ATP-sensitive potassium channel (K_{ATP}) is involved in regulation of smooth muscle tone in various organs, and activation of K_{ATP} evokes efflux of potassium ions, leading to relaxation of smooth muscles. Therefore, agents that open K_{ATP}, potassium channel openers (PCOs), have been expected to have therapeutic applications for certain diseases such as hypertension, angina pectoris and asthma.[1)]

A series of synthetic studies of PCOs originated in cromakalim[2)] and its (3*S*,4*R*)-isomer levcromakalim (I, Chart 1) having a 4-substituted 2*H*-1-benzopyran structure. These studies produced many congeners which mostly varied in the 4-substituent on the benzopyran nucleus. Those modification studies showed that the 4-substituent critically affected potency and increased knowledge of the structure–activity relationships (SARs) of that substituent, but were restricted within narrow limits: most of the studies dealt with monocyclic or acyclic 4-substituents. Therefore, we are interested in the synthesis of dihydrobenzopyrans with a bicyclic moiety at that position: such a study would contribute to further understanding of the structural requirements for the 4-substituent, and might lead to the discovery of improved agents. Recently, Bergmann *et al.* have reported a potent PCO, coded as EMD 57283 (symakalim, II), which has a unique monocyclic 6-oxo-3-pyridazinyloxy moiety at the 4-position of the benzopyran skeleton.[3)] We previously reported a study in which the monocyclic pyridazinyloxy



I, levcromakalim II, EMD 57283, symakalim III

Chart 1



compounds	R^1	R^2	R^3	R^4
(±)-10A,B	H	CN	H	H
(±)-11A,B	H	NO_2	H	H
(±)-12A,B	H	H	NO_2	H
(±)-13A,B	H	CN	H	Me
(±)-14A,B	Me	CN	H	H

Chart 2

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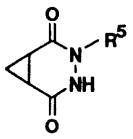
moiety of EMD 57283 was replaced with a bicyclic pyridazinyloxy group.[4] In this paper, we describe the synthesis and activity of benzopyran derivatives in which the 4-position is substituted with a series of bicyclic pyridazinyl groups, including various diazabicyclo[4.1.0]-hept-2-en-2-yloxy groups.

Chemistry

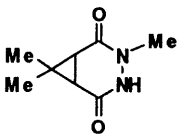
We first synthesized racemic *trans*-3,4-dihydro-3-hydroxy-4-[(4-methyl-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy]-2*H*-1-benzopyrans (±)-**10**—**14** (Chart 2). Cyclopropanedicarboxylic anhydride **1**[5] and **2**[6] reacted with methylhydrazine (**3a**) to produce 3-methyl-3,4-diazabicyclo[4.1.0]heptane-2,5-diones (±)-**4a** and (±)-**5**, respectively (Table 1). Reaction of (±)-**4a** or (±)-**5** with racemic 3,4-epoxybenzopyrans (±)-**6**—**8**[7] and (±)-**9**[8] gave a mixture of two racemates of *trans*-4-[(5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy]-3,4-dihydro-2*H*-1-benzopyran-3-ols **10**—**14**, which were separated by column chromatography to produce racemates (±)-**10**—**14A** (higher *Rf* value on TLC) and racemates (±)-**10**—**14B** (lower *Rf* value), respectively (Table 2).

Racemate (±)-**13B** was further resolved using chiral HPLC (Chiralpak™ AD) to give optically pure enan-

Table 1. Synthesis of Diazabicyclo[4.1.0]heptanediones **4**, **5**



(±)-**4a**—**i**

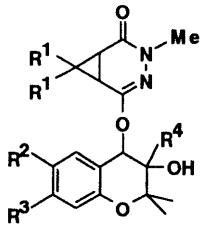


(±)-**5**

Compound	R^5	Reaction solvent[a]	Yield (%)	Recrystallization solvent[a]	mp[b] (°C)	Formula[c]
(±)-**4a**	CH_3	A	49	E	195—196	$C_6H_8N_2O_2$
(±)-**4b**	n-C_4H_9	A	44	EA–H	104—105	$C_9H_{14}N_2O_2$
(±)-**4c**	$CH_2CH=CH_2$	E	16	EA–H	147—148	$C_8H_{10}N_2O_2$
(±)-**4d**	$CH_2C(CH_3)=CH_2$	A	25	EA–H	140—142	$C_9H_{12}N_2O_2$
(±)-**4e**	$CH_2CH(CH_3)_2$	A	80[d]	EA–H	150—152	$C_9H_{14}N_2O_2$
(±)-**4f**	$CH_2CH=C(CH_3)_2$	A	19	EA–H	99—101	$C_{10}H_{14}N_2O_2$
(±)-**4g**	$CH_2C\equiv CH$	A	7	EA	147—149	$C_8H_8N_2O_2$
(±)-**4h**	CH_2CH_2OH	A	32	E	164—165	$C_7H_{10}N_2O_3$
(±)-**4i**	$CH_2CH_2OCH_3$	A	34	EA–H	79—82	$C_8H_{12}N_2O_3 \cdot 0.1H_2O$
(±)-**5**	—	E	27	EA–H	151—154	$C_8H_{12}N_2O_2$

a) Solvent, A: acetonitrile, E: ethanol, EA: ethyl acetate, H: hexane. *b*) Uncorrected. *c*) Analyses for C, H and N indicated were within ±0.4% of the theoretical values. *d*) Yield from compound (±)-**4d** by catalytic hydrogenation.

Table 2. Syntheses and Physical Properties of Racemic 4-Diazabicyclo[4.1.0]heptenyloxybenzopyrans **10**—**14**



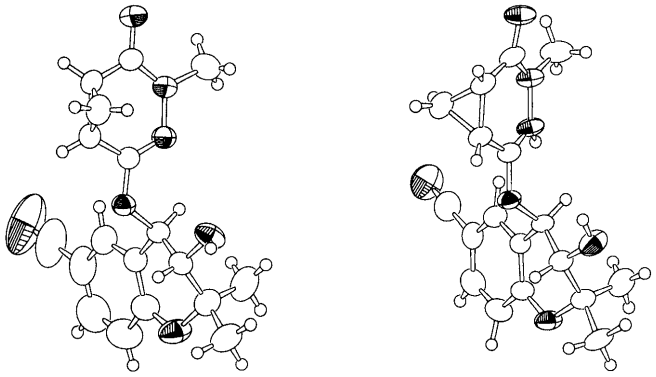
(±)-**10**—**14A** (less polar)
(±)-**10**—**14B** (more polar)

Compound	R^1	R^2	R^3	R^4	Epoxide	Yield (%)	Solvent[a]	mp[b] (°C)	Formula[c]	*Rf* value (solvent)[d]	δ value[e] (ppm)
(±)-**10A**	H	CN	H	H	(±)-**6**	19	EA–H	208—209	$C_{18}H_{19}N_3O_4$	0.43 (EA)	5.61
(±)-**10B**	H	CN	H	H	(±)-**6**	25	EA–H	190—191	$C_{18}H_{19}N_3O_4$	0.33 (EA)	5.73
(±)-**11A**	H	NO_2	H	H	(±)-**7**	9	E–H	231—232	$C_{17}H_{19}N_3O_6$	0.36 (CM20)	5.65
(±)-**11B**	H	NO_2	H	H	(±)-**7**	10	E–H	219—223	$C_{17}H_{19}N_3O_6$	0.25 (CM20)	5.79
(±)-**12A**	H	H	NO_2	H	(±)-**8**	11	EA–H	185—186	$C_{17}H_{19}N_3O_6$	0.38 (EA)	5.67
(±)-**12B**	H	H	NO_2	H	(±)-**8**	18	E	236—237	$C_{17}H_{19}N_3O_6$	0.29 (EA)	5.78
(±)-**13A**	H	CN	H	Me	(±)-**9**	27	EA–H	178—180	$C_{19}H_{21}N_3O_4$	0.33 (EA)	5.74
(±)-**13B**	H	CN	H	Me	(±)-**9**	28	EA	226—227	$C_{19}H_{21}N_3O_4$	0.21 (EA)	5.83
(±)-**14A**	Me	CN	H	H	(±)-**6**	23	—	Amorphous	$C_{20}H_{23}N_3O_4 \cdot 0.25EtOAc$	0.54 (EA)	5.62
(±)-**14B**	Me	CN	H	H	(±)-**6**	25	EA–H	190—197	$C_{20}H_{23}N_3O_4$	0.39 (EA)	5.76

a) Recrystallization solvent, EA: ethyl acetate, H: hexane, E: ethanol. *b*) Uncorrected. *c*) Analyses for C, H and N indicated were within ±0.4% of the theoretical values. *d*) Eluent, EA: ethyl acetate, CM20: chloroform/methanol = 20/1 (v/v). *e*) Chemical shift of the 4-proton on the benzopyran ring.

tiomers (+)-**13B** and (−)-**13B**.

X-Ray crystallographic analysis elucidated the configurations of several racemates A and B: the configurations of compounds (±)-**10A** (Fig. 1, left) and (±)-**11A** are (3*S*,4*R*,1′*S*,6′*R*)/(3*R*,4*S*,1′*R*,6′*S*), while those of (±)-**10B** (Fig. 1, right), (±)-**12B**, (±)-**13B** and (±)-**14B** are (3*S*,4*R*,1′*R*,6′*S*)/(3*R*,4*S*,1′*S*,6′*R*). In addition, the 4-protons of (±)-**10**—**14A**, observed at 5.61—5.74 ppm, were shifted to higher field than those of the corresponding racemates B by 0.09—0.14 ppm in the NMR spectra. Thus, racemates A are characterized by both the chemical shift of the 4-proton and *Rf* values higher than those of racemates B.



(A) Compound (±)-**10A** ((3*S*,4*R*,1′*S*,6′*R*)-isomer only)

(B) Compound (±)-**10B** ((3*S*,4*R*,1′*R*,6′*S*)-isomer only)

Fig. 1. X-Ray Crystal Structures Showing Configurations of Compounds (±)-**10A** and (±)-**10B**

Reaction of cyclopentenedicarboxylic anhydride **15** with methylhydrazine (**3a**) afforded 3,4-diazabicyclo[4.3.0]nona-1(6)-ene-2,5-dione **16**, which reacted with (±)-**6** to give *trans*-4-[5-oxo-3,4-diazabicyclo[4.3.0]nona-1(6),2-dien-2-yl)oxy]-2*H*-1-benzopyran (±)-**19** (Chart 3). Similar reaction of (±)-**6** with phthalazinedione **17** gave *trans*-4-[4-oxo-3,4-dihydrophthalazin-1-yl)oxy]-2*H*-1-benzopyran (±)-**19**.

We next synthesized a mixture of two enantiomers of (3*S*,4*R*)-3,4-dihydro-3-hydroxy-4-[(4-substituted-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy]-2*H*-1-benzopyran-6-carbonitriles ((−)-**10**, (−)-**20b**—**i**) (Chart 4). The anhydride **1** reacted with alkylhydrazine (**3b**—**d**, **f**—**i**) to produce corresponding 3-alkyl-3,4-diazabicyclo[4.1.0]-heptane-2,5-diones (±)-**4b**—**d**, **f**—**i** (Table 1). The isobutyl congener (±)-**4e** was prepared by catalytic hydrogenation of the 3-methallyl compound (±)-**4d**. Reaction of the optically active (3*S*,4*S*)-epoxide (−)-**6**[9] with (±)-**4a**—**i** gave less polar isomers (−)-**10A** and (−)-**20b**—**iA** and polar isomers (−)-**10B** and (−)-**20b**—**iB** (Chart 4, Table 3). Crystals of (−)-**20h**A suitable for X-ray crystallographic analysis were obtained, and the absolute configuration of (−)-**20h**A was characterized as (3*S*,4*R*,1′*S*,6′*R*); the (3*S*,4*R*)-configuration was determined by the

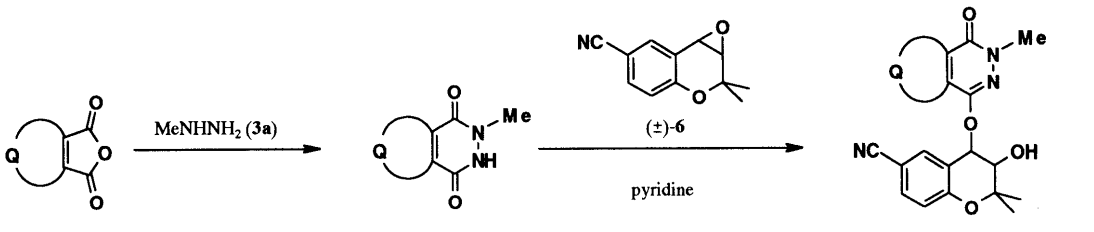


Chart 3

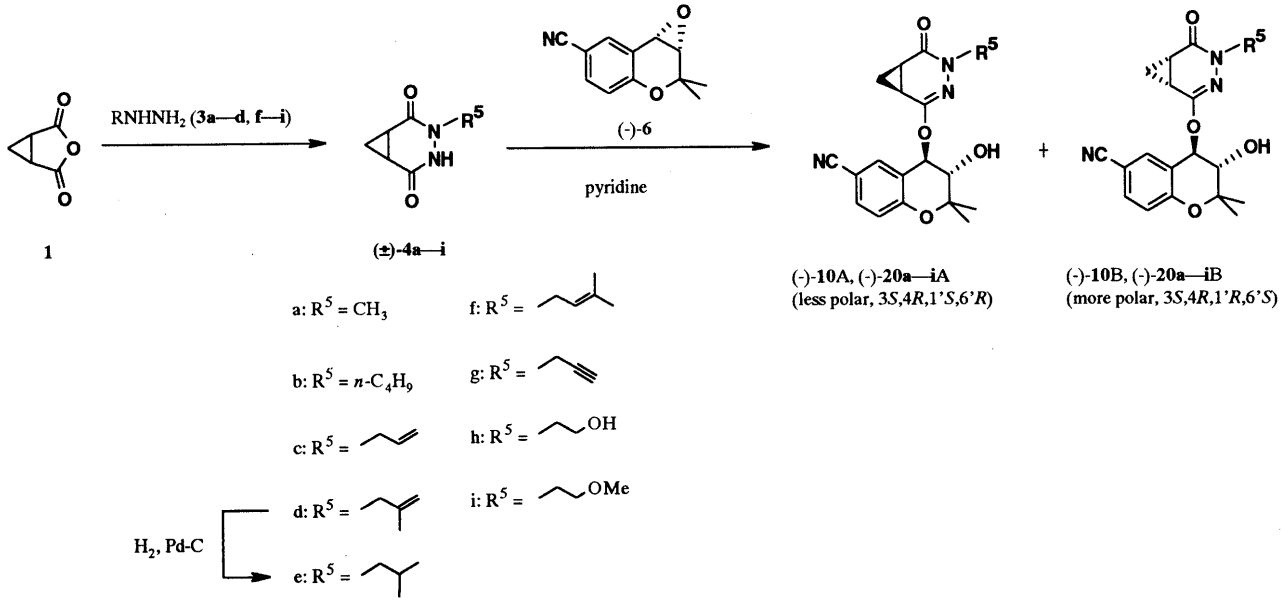
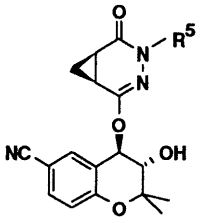
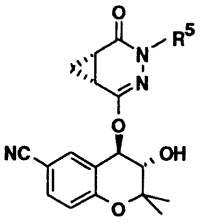


Chart 4

Table 3. Chiral 4-(4-Substituted Diazabicyclo[4.1.0]heptenyloxy)benzopyrans (−)-**10** and (−)-**20**



(−)-**10A**, (−)-**20b**—**iA**
(less polar, (3*S*,4*R*,1′*S*,6′*R*))



(−)-**10B**, (−)-**20b**—**iB**
(more polar, (3*S*,4*R*,1′*R*,6′*S*))

Compound	R^5	Configuration[a]	Yield (%)	mp[b] (°C)	$[\alpha]_D$[c] (deg)	Formula[d]	*Rf* value (solvent)[e]	δ value[f] (ppm)
(−)-**10A**	Me	(3*S*,4*R*,1′*S*,6′*R*)	32	Amorphous	−94.2	$C_{18}H_{19}N_3O_4$	0.45 (EA)	5.62
(−)-**10B**	Me	(3*S*,4*R*,1′*R*,6′*S*)	32	Amorphous	−150.4	$C_{18}H_{19}N_3O_4$	0.35 (EA)	5.75
(−)-**20bA**	*n*-C_4H_9	(3*S*,4*R*,1′*S*,6′*R*)	29	Amorphous	−124.7	$C_{21}H_{25}N_3O_4$	0.53 (EA)	5.59
(−)-**20bB**	*n*-C_4H_9	(3*S*,4*R*,1′*R*,6′*S*)	32	Amorphous	−173.4	$C_{21}H_{25}N_3O_4 \cdot 0.25H_2O$	0.37 (EA)	5.70
(−)-**20cA**	$CH_2CH=CH_2$	(3*S*,4*R*,1′*S*,6′*R*)	37	Amorphous	−171.3	$C_{20}H_{21}N_3O_4$	0.50 (CM)	5.60
(−)-**20cB**	$CH_2CH=CH_2$	(3*S*,4*R*,1′*R*,6′*S*)	32	87—89	−215.4	$C_{20}H_{21}N_3O_4$	0.42 (CM)	5.71
(−)-**20dA**	$CH_2C(CH_3)=CH_2$	(3*S*,4*R*,1′*S*,6′*R*)	18	Amorphous	−155.8	$C_{21}H_{23}N_3O_4 \cdot 0.5H_2O$	0.71 (CM)	5.60
(−)-**20dB**	$CH_2C(CH_3)=CH_2$	(3*S*,4*R*,1′*R*,6′*S*)	17	138—140	−238.6	$C_{21}H_{23}N_3O_4$	0.54 (CM)	5.70
(−)-**20eA**	$CH_2CH(CH_3)_2$	(3*S*,4*R*,1′*S*,6′*R*)	33	Amorphous	−109.6	$C_{21}H_{25}N_3O_4 \cdot 0.5H_2O$	0.72 (CM)	5.58
(−)-**20eB**	$CH_2CH(CH_3)_2$	(3*S*,4*R*,1′*R*,6′*S*)	38	177—180	−216.2	$C_{21}H_{25}N_3O_4$	0.53 (CM)	5.69
(−)-**20fA**	$CH_2CH=C(CH_3)_2$	(3*S*,4*R*,1′*S*,6′*R*)	41	Amorphous	−229.6	$C_{22}H_{25}N_3O_4 \cdot 0.5H_2O$	0.71 (CM)	5.61
(−)-**20fB**	$CH_2CH=C(CH_3)_2$	(3*S*,4*R*,1′*R*,6′*S*)	33	82—83	−247.6	$C_{22}H_{25}N_3O_4$	0.56 (CM)	5.70
(−)-**20gA**	$CH_2C\equiv CH$	(3*S*,4*R*,1′*S*,6′*R*)	14	Amorphous	−185.6	$C_{20}H_{19}N_3O_4$	0.54 (CM)	5.72
(−)-**20gB**	$CH_2C\equiv CH$	(3*S*,4*R*,1′*R*,6′*S*)	20	88—90	−232.2	$C_{20}H_{19}N_3O_4$	0.47 (CM)	5.80
(−)-**20hA**	CH_2CH_2OH	(3*S*,4*R*,1′*S*,6′*R*)	30	162—165	−93.6	$C_{19}H_{21}N_3O_5$	0.21 (EA)	5.65
(−)-**20hB**	CH_2CH_2OH	(3*S*,4*R*,1′*R*,6′*S*)	35	Amorphous	−142.2	$C_{19}H_{21}N_3O_5$	0.14 (EA)	5.78
(−)-**20iA**	$CH_2CH_2OCH_3$	(3*S*,4*R*,1′*S*,6′*R*)	32	Amorphous	−112.2	$C_{20}H_{23}N_3O_5 \cdot 0.25H_2O$	0.56 (CM)	5.66
(−)-**20iB**	$CH_2CH_2OCH_3$	(3*S*,4*R*,1′*R*,6′*S*)	35	Amorphous	−165.0	$C_{20}H_{23}N_3O_5$	0.50 (CM)	5.82

a) The absolute configurations are not determined except for (−)-**20hA**. *b*) Uncorrected. *c*) $c=1$ in methanol at 25 °C. *d*) Analyses for C, H and N indicated were within ±0.4% of the theoretical values. *e*) Eluent, EA: ethyl acetate. CM: chloroform/methanol = 10/1 (v/v). *f*) Chemical shift of the 4-proton on the benzopyran ring.

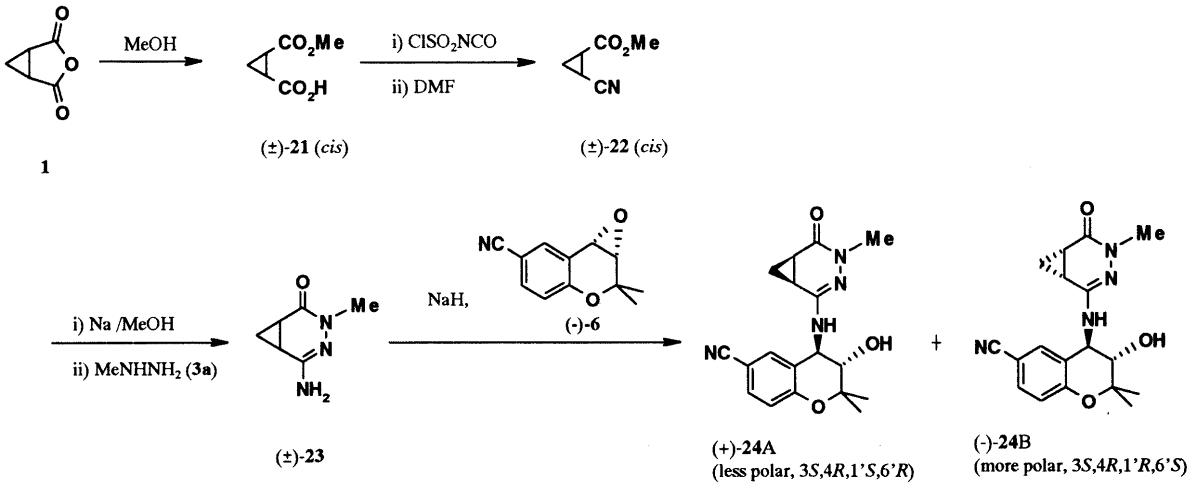


Chart 5

stereospecific synthetic pathway.

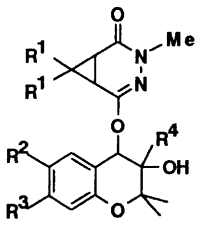
Reaction of racemic methyl *cis*-2-cyanocyclopropanecarboxylate ((±)-**22**) with **3a** gave 2-amino-3,4-diazabicyclo[4.1.0]hept-2-en-5-one ((±)-**23**), which reacted with (−)-**6** to produce chiral 4-[(5-oxo-3,4-diazabicyclo[4.1.0]-hept-2-en-2-yl)amino]-3,4-dihydro-2*H*-1-benzopyran-3-ols (+)-**24**A (3*S*,4*R*,1′*S*,6′*R*) and (−)-**24**B (3*S*,4*R*,1′*R*,6′*S*) (Chart 5).

The structures of (+)-**24**A and (−)-**24**B were assumed on the basis of their *Rf* values and the chemical shifts of the 4-proton by extrapolating the results for the *O*-bridged compounds to the *NH*-bridged analogues.

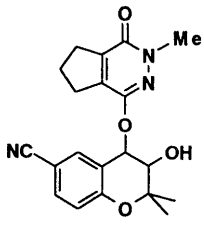
Results and Discussion

Potassium channel opening activity was assessed in both *in vitro* and *in vivo* assays, namely potentiation of ^{86}Rb efflux ($EC_{AUC0.2}$) and antihypertensive activity ($ED_{50\,mmHg}$) in spontaneously hypertensive rats (SHRs) after oral

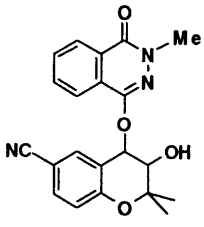
Table 4. Biological Activity of Diazabicyclo[4.1.0]heptenyloxybenzopyrans and their Analogues



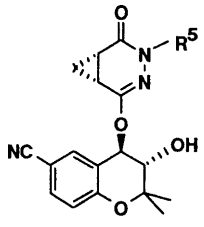
(±)-10A (less polar)
(±)-10—14B (more polar)



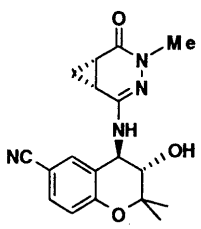
(±)-18



(±)-19



(-)-10B, (-)-20b—iB
(more polar, (3*S*,4*R*,1'*R*,6'*S*))



(-)-24B

Compound	R^1	R^2	R^3	R^4	R^5	$EC_{AUC0.2}$ (μM)	$ED_{50\,mmHg}$ (mg/kg)
(±)-**10A**	H	CN	H	H	—	NT	>1
(±)-**10B**	H	CN	H	H	—	0.39	0.047
(−)-**10B**	—	—	—	—	Me	0.18	0.029
(±)-**11B**	H	NO_2	H	H	—	NT	0.049
(±)-**12B**	H	H	NO_2	H	—	NT	>0.3
(±)-**13B**	H	CN	H	Me	—	NT	0.0042
(−)-**13B**	H	CN	H	Me	—	0.021	0.0023
(±)-**14B**	Me	CN	H	H	—	NT	>0.1
(±)-**18**	—	—	—	—	—	NT	0.86
(±)-**19**	—	—	—	—	—	NT	>10
(−)-**20bB**	—	—	—	—	n-C_4H_9	0.45	0.076
(−)-**20cB**	—	—	—	—	$CH_2CH=CH_2$	0.18	0.069
(−)-**20dB**	—	—	—	—	$CH_2C(CH_3)=CH_2$	NT	>1
(−)-**20eB**	—	—	—	—	$CH_2CH(CH_3)_2$	NT	0.36
(−)-**20fB**	—	—	—	—	$CH_2CH=C(CH_3)_2$	NT	0.36
(−)-**20gB**	—	—	—	—	$CH_2C\equiv CH$	0.27	0.020
(−)-**20hB**	—	—	—	—	CH_2CH_2OH	4.37	0.102
(−)-**20iB**	—	—	—	—	$CH_2CH_2OCH_3$	9.1	0.21
(−)-**24B**	—	—	—	—	—	NT	0.0065
I, levcromakalim	—	—	—	—	—	1.6	0.14
II, EMD 57283	—	—	—	—	—	0.16	0.0064

NT: not tested.

administration were determined according to the literature methods[10,11] (Table 4).

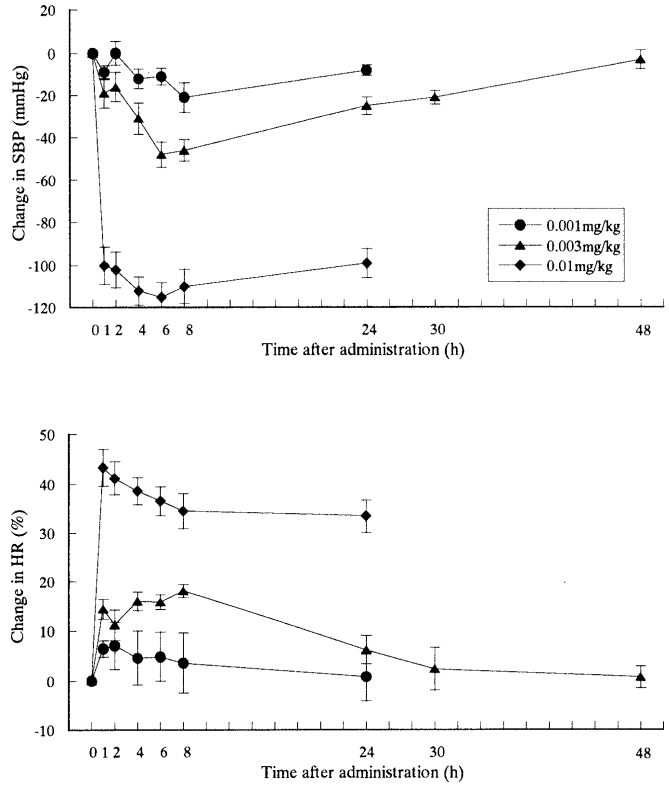
Compound (±)-**10B** showed potent antihypertensive activity, but another type of racemate (±)-**10A** did not, indicating that the racemates A and isomer A of the series of compounds **11**—**14**, **20** and **24** have little or no activity. Compounds (±)-**10B**, (±)-**11B** and (±)-**13B** with an electron-withdrawing group at the 6-position of the benzopyran ring exhibited potent activity, but (±)-**12B**, with such a substituent at the 7-position, did not. These results suggest that the electron-withdrawing group at the 6-position is crucial for potency. In addition, 6-cyano-3-methylbenzopyran (±)-**13B** was highly potent, as expected from the report that introduction of a 3-methyl moiety into EMD 57283 (II) increases the relaxing activity in arteries.[8] The optically active (−)-**13B** was the most potent PCO among the compounds synthesized here and the hypotensive activity was approximately three-fold more potent than that of II. Compounds (±)-**14B**, (±)-**18** and (±)-**19** had low potency. These results suggest that large size and/or coplanarity of the rings fused to the pyridazine component are deleterious for drug–receptor interaction.

Compound (−)-**10B** was approximately two-fold more potent than its racemate (±)-**10B** in both potassium channel opening and hypotensive activities, suggesting that the other enantiomer (+)-**10B** is weakly active or inactive. This result is consistent with the difference in potency between levorotatory I (levcromakalim) and the corresponding dextrorotatory enantiomer.[12]

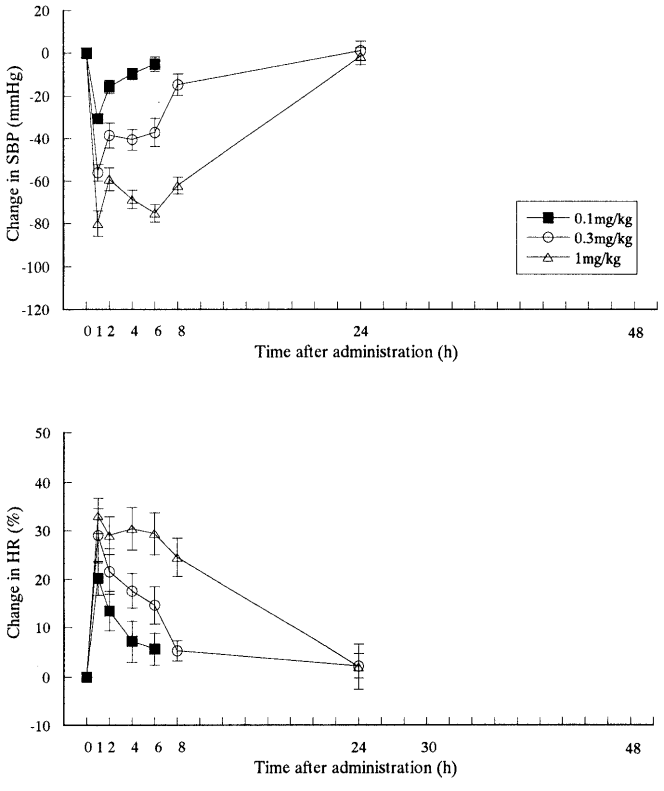
With respect to SARs, compounds (−)-**10B** and (−)-**20b, c, gB** having straight chain methyl, *n*-butyl, allyl and propargyl groups, respectively, had rather similar potencies in both ^{86}Rb efflux and antihypertensive activities. On the other hand, compounds (−)-**20d**—**fB** having branched methallyl, isobutyl and prenyl groups, respectively, showed one order less potency. Hydroxyethyl and methoxyethyl derivatives (−)-**20hB** and (−)-**20iB** also had low potency. These results imply that the target molecule K_{ATP} has a narrow and hydrophobic pocket which can accommodate the straight chain 4-substituent of the 5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yloxy group.

On the other hand, the *NH*-bridge compound (−)-**24B** was equipotent with II and 4.5 times more potent than the corresponding *O*-bridge congener (−)-**10B** in hypotensive activity.

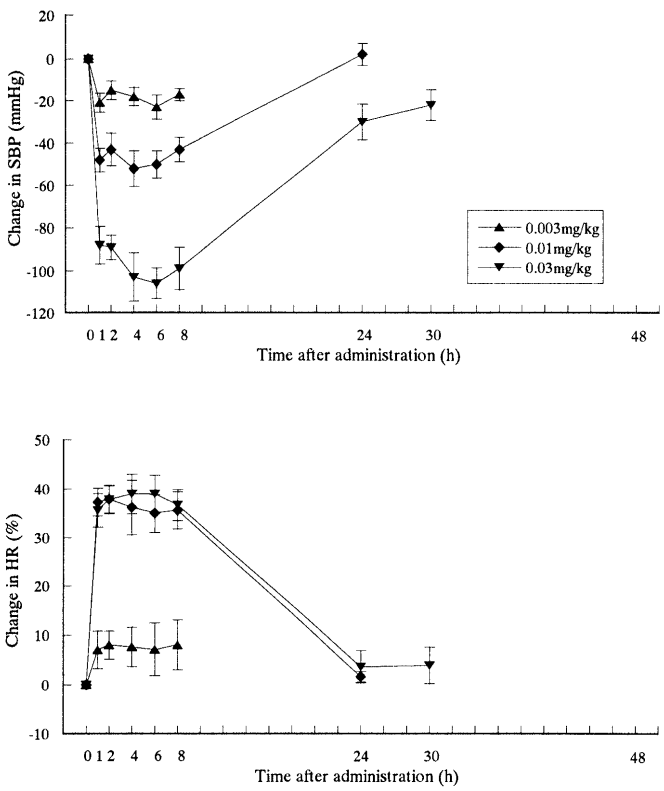
The effects on blood pressure and heart rate of the representative compounds (−)-**13B**, levcromakalim (I) and EMD 57283 (II) are shown in Fig. 2. Compound (−)-**13B** showed a longer duration of hypotensive activity than I. Compounds (−)-**13B** (0.003 mg/kg, *p.o.*) and II (0.01 mg/kg, *p.o.*) gradually lowered the blood pressure to



(A) Compound (-)-13B



(B) Levcromakalim (I)



(C) EMD 57283 (II)

Fig. 2. Hypotensive Activity and Change of Heart Rate (HR) of PCOs

Male spontaneously hypertensive rats fed *ab lib.* were treated with compound (−)-**13B** (0.001, 0.003, 0.01 mg/kg, *p.o.*, A), levcromakalim (0.1, 0.3, 1 mg/kg, *p.o.*, B) and EMD 57283 (0.003, 0.01, 0.03 mg/kg, *p.o.*, C). The systolic blood pressure (SBP) and the HR were measured by a tail-cuff method and the changes of SBP and HR are expressed as vs initial. The data are means ± S.E. of 5 rats.

reach maximal hypotension after 4—6 h and their action continued for more than 8 h.

In conclusion, we have synthesized PCOs such as (−)-**13B** having high potency and long duration of action and thereby demonstrated that 5-oxo-3,4-diazabicyclo[4.1.0]-hept-2-en-2-yloxy groups are effective as 4-substituents of benzopyran-type PCOs.

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 taken 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 taken 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.

(±)-3-Methyl-3,4-diazabicyclo[4.1.0]heptane-2,5-dione ((±)-4a) A solution of 1,2-cyclopropanedicarboxylic anhydride[5] (**1**, 30.0 g, 0.268 mol) in acetonitrile (100 ml) was added dropwise to a stirred solution of methylhydrazine (**3a**, 12.33 g, 0.268 mol) in acetonitrile (200 ml) under ice-cooling. The reaction mixture was heated under reflux for 12 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, followed by recrystallization from ethanol to give (±)-**4a**

(18.36 g, 49%). mp 195—196 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.21 (m, 1H), 1.72 (m, 1H), 2.13 (m, 1H), 2.25 (m, 1H), 3.22 (s, 1H), 10.90 (br s, 1H). *Anal.* Calcd for $C_6H_8N_2O_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.55; H, 5.58; N, 19.65.

Compounds (±)-**4b**—**d**, **f**—**i** were prepared in an analogous manner from **1** and the corresponding alkylhydrazines (**3b**—**d**, **f**—**i**) and compound (±)-**5** was also prepared in a similar manner from 3,3-dimethyl-1,2-cyclopropanedicarboxylic anhydride[6] (**2**) and **3a** (Table 1).

(±)-3-Isobutyl-3,4-diazabicyclo[4.1.0]heptane-2,5-dione ((±)-4e) A solution of (±)-**4d** (2.03 g, 11.28 mmol) in ethanol (40 ml) was catalytically hydrogenated on 5% (w/w) palladium carbon (230 mg) under atmospheric pressure at room temperature. The catalyst was filtered off, and the filtrate was evaporated *in vacuo*. The residual material was recrystallized from a mixture of ethyl acetate (EtOAc) and hexane to give (±)-**4e** (1.60 g, 80%) as colorless needles. mp 150—152 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (d, 3H, J=6.4 Hz), 0.95 (d, 3H, J=6.8 Hz), 1.17 (m, 1H), 1.71 (m, 1H), 2.05 (m, 1H), 2.13 (m, 1H), 2.25 (m, 1H), 3.23 (dd, 1H, J=6.8, 14.2 Hz), 3.68 (dd, 1H, J=7.8, 14.2 Hz), 10.14 (br s, 1H). *Anal.* Calcd for $C_9H_{14}N_2O_2$: C, 59.32; H, 7.74; N, 15.37. Found: C, 59.35; H, 7.87; N, 15.17.

(±)-3,4-*trans*-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-[(4-methyl-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy]-2*H*-1-benzopyran-6-carbonitrile ((±)-10A and (±)-10B) A mixture of (±)-3,4-epoxy-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile[7] ((±)-**6**, 3.142 g, 15.6 mmol), (±)-**4a** (2.188 g, 15.6 mmol) and pyridine (1.26 ml, 15.5 mmol) in ethanol (60 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 EtOAc as an eluent to give (±)-**10A** (racemate A) and (±)-**10B** (racemate B). Compound (±)-**10A**: Yield 1.036 g (19%). Colorless needles (from EtOAc–hexane). mp 208—209 °C. *Rf* value = 0.43 (EtOAc). ^{1}H-NMR ($CDCl_3$) δ: 0.99 (m, 1H), 1.31 (s, 3H), 1.53 (s, 3H), 1.72 (m, 1H), 2.2—2.4 (m, 2H), 3.25 (s, 3H), 3.92 (d, 1H, J=7.8 Hz), 4.78 (br, 1H), 5.61 (d, 1H, J=7.8 Hz), 6.92 (d, 1H, J=8.5 Hz), 7.51 (dd, 1H, J=2.0, 8.5 Hz), 7.64 (d, 1H, J=2.0 Hz). *Anal.* Calcd for $C_{18}H_{19}N_3O_4$: C, 63.33; H, 5.61; N, 12.31. Found: C, 63.08; H, 5.87; N, 12.25.

Compound (±)-**10B**: Yield 1.355 g (25%). Colorless needles (from EtOAc–hexane). mp 190—191 °C. *Rf* value = 0.33 (EtOAc). NMR ($CDCl_3$) δ: 1.03 (m, 1H), 1.33 (s, 3H), 1.53 (s, 3H), 1.73 (m, 1H), 2.20 (m, 1H), 2.25 (m, 1H), 3.24 (s, 3H), 3.93 (d, 1H, J=7.8 Hz), 4.21 (br, 1H), 5.73 (d, 1H, J=7.8 Hz), 6.91 (d, 1H, J=8.5 Hz), 7.51 (dd, 1H, J=2.0, 8.5 Hz), 7.61 (d, 1H, J=2.0 Hz). *Anal.* Calcd for $C_{18}H_{19}N_3O_4$: C, 63.33; H, 5.61; N, 12.31. Found: C, 62.96; H, 5.80; N, 12.19.

Compounds (±)-**11**—**13A** and (±)-**11**—**13B** were prepared in an analogous manner from (±)-**4a** and the corresponding (±)-3,4-epoxy-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans (±)-**7**—**9**, respectively (Table 2). Compounds (±)-**14A** and (±)-**14B** were also prepared in a similar manner from (±)-**5** and (±)-**6**.

(−)-(3*S*,4*R*,1′*S*,6′*R*)-3,4-Dihydro-3-hydroxy-2,2,3-trimethyl-4-[(4-methyl-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy]-2*H*-1-benzopyran-6-carbonitrile ((−)-13B) and (+)-(3*R*,4*S*,1′*R*,6′*S*)-3,4-Dihydro-3-hydroxy-2,2,3-trimethyl-4-[(4-methyl-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy]-2*H*-1-benzopyran-6-carbonitrile ((+)-13B) Racemate (±)-**13B** (300 mg, 0.85 mmol) was resolved by HPLC (column, Chiralpak™ AD (Daicel Chemical Industries Co., Ltd.) 20 mmϕ × 250 mm; eluent, 15% EtOH–hexane; flow rate, 4.0 ml/min; detection, UV 254 nm) to give each enantiomer. Compound (−)-**13B**: Yield 102 mg (34%). Colorless needles (from EtOAc–hexane). mp 192—193 °C. Retention time (HPLC) 33—41 min. $[\alpha]_D^{25} = -162.2°$ (c=1, EtOAc). NMR ($CDCl_3$) δ: 1.04 (m, 1H), 1.25 (s, 3H), 1.42 (s, 3H), 1.50 (s, 3H), 1.74 (m, 1H), 2.22—2.35 (m, 2H), 3.25 (s, 3H), 3.90 (s, 1H), 5.83 (s, 1H), 6.91 (d, 1H, J=8.3 Hz), 7.51 (dd, 1H, J=2.0, 8.3 Hz), 7.66 (d, 1H, J=2.0 Hz). *Anal.* Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82. Found: C, 64.30; H, 5.98; N, 11.69.

Compound (+)-**13B**: Yield 114 mg (38%). Colorless needles (from EtOAc–hexane). mp 185—188 °C. Retention time (HPLC) 44—74 min. $[\alpha]_D^{25} = +153.3°$ (c=1, EtOAc). NMR ($CDCl_3$) δ: 1.04 (m, 1H), 1.25 (s, 3H), 1.42 (s, 3H), 1.50 (s, 3H), 1.75 (m, 1H), 2.22—2.35 (m, 2H), 3.25 (s, 3H), 3.95 (s, 1H), 5.83 (s, 1H), 6.91 (d, 1H, J=8.3 Hz), 7.51 (dd, 1H, J=2.0, 8.3 Hz), 7.67 (d, 1H, J=2.0 Hz). *Anal.* Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82. Found: C, 64.33; H, 6.05; N, 11.70.

3-Methyl-3,4-diazabicyclo[4.3.0]nona-1(6)-en-2,5-dione (16) This compound was prepared in 60% yield from commercially available 1,2-cyclopentenedicarboxylic anhydride (**15**) and **3a** using a procedure similar to that described for (±)-**4a**. mp 212—213 °C (from EtOH). NMR ($CDCl_3$) δ: 1.77—2.12 (m, 2H), 2.45—2.75 (m, 4H), 3.47 (s, 3H), 11.01 (br s, 1H, NH). *Anal.* Calcd for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.06; N, 16.85. Found: C, 57.48; H, 6.00; N, 16.74.

(±)-3,4-*trans*-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-(3-methyl-2-oxo-3,4-diazabicyclo[4.3.0]nona-1(6),4-dien-5-yloxy)-2*H*-1-benzopyran-6-carbonitrile ((±)-18) A mixture of (±)-**6** (0.32 g, 1.94 mmol), **16** (0.32 g, 1.92 mmol) and pyridine (0.2 ml) in EtOH (10 ml) was refluxed for 15 h. After evaporation of the solvent *in vacuo*, the resulting residue was purified by silica gel column chromatography with $CHCl_3$: MeOH = 100 : 1 (v/v) as an eluent to give (±)-**18** (325 mg, 46%). mp 236—237 °C (from EtOH). *Rf* value = 0.23 ($CHCl_3$: MeOH = 20 : 1). NMR (DMSO-d_6) δ: 1.31 (s, 3H), 1.39 (s, 3H), 2.01—2.09 (m, 2H), 2.76 (t, 4H, J=7.3 Hz), 3.59 (s, 3H), 3.84 (dd, 1H, J=5.4, 5.8 Hz), 5.81 (d, 1H, J=5.8 Hz), 5.88 (d, 1H, OH, J=5.4 Hz), 6.98 (d, 1H, J=8.8 Hz), 7.67 (dd, 1H, J=2.0, 8.8 Hz), 7.78 (d, 1H, J=2.0 Hz). *Anal.* Calcd for $C_{20}H_{21}N_3O_4$: C, 65.38; H, 5.76; N, 11.43. Found: C, 65.17; H, 6.10; N, 11.63.

(±)-3,4-*trans*-3,4-Dihydro-4-(1,2-dihydro-2-methyl-1-oxophthalazin-4-yloxy)-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile ((±)-19) A mixture of (±)-**6** (1.00 g, 5 mmol), *N*-methylphthalhydrazide[13] (**17**, 1.32 g, 7.5 mmol) and pyridine (0.4 ml) in EtOH (35 ml) was refluxed for 14 h. After evaporation of the solvent *in vacuo*, the resulting residue was taken up in H_2O, and then the mixture was agitated vigorously. The resulting insoluble product was collected by filtration and dried *in vacuo*. The obtained solid was added to EtOH (50 ml), and then the mixture was stirred vigorously at 80 °C. The resulting precipitates were collected by filtration and washed with EtOH. The crude solid material was recrystallized from *N*,*N*-dimethylformamide (DMF)–EtOH to give (±)-**19** (367 mg, 20%). mp 268—269 °C (dec.). NMR (DMSO-d_6) δ: 1.34 (s, 3H), 1.44 (s, 3H), 3.68 (s, 3H), 3.99 (dd, 1H, J=5.4, 5.8 Hz), 5.93 (d, 1H, OH, J=5.4 Hz), 6.02 (d, 1H, J=5.8 Hz), 7.01 (d, 1H, J=8.8 Hz), 7.68 (dd, 1H, J=2.0, 8.8 Hz), 7.88 (d, 1H, J=2.0 Hz), 7.90 (m, 3H), 8.28 (m, 1H). *Anal.* Calcd for $C_{21}H_{19}N_3O_4$: C, 66.83; H, 5.07; N, 11.13. Found: C, 66.65; H, 5.25; N, 11.41.

(−)-(3*S*,4*R*,1′*S*,6′*R*)-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-[(4-substituted-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy]-2*H*-1-benzopyran-6-carbonitriles ((−)-10A, (−)-20b—iA) and (−)-(3*S*,4*R*,1′*R*,6′*S*)-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-[(4-substituted-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)oxy]-2*H*-1-benzopyran-6-carbonitriles ((−)-10B, (−)-20b—iB) The title compounds were prepared in an analogous manner from the levorotatory epoxide (−)-**6** and (±)-**4a**—**i** (Table 3).

Methyl *cis*-2-Cyanocyclopropanecarboxylate ((±)-22) Methyl hydrogen *cis*-1,2-cyclopropanedicarboxylate[14] ((±)-**21**, 12.70 g, 88 mmol) was added to a solution of chlorosulfonyl isocyanate (13.77 g, 97 mmol) in benzene (25 ml) and the mixture was stirred at 60 °C for 30 min, then cooled to room temperature. DMF (13.6 ml, 176 mmol) was added and the mixture was stirred at room temperature for 30 min. The reaction mixture was poured into water and extracted with benzene. The organic layer was washed with water and brine and then dried over anhydrous sodium sulfate. The solvent was evaporated off, and the resulting residue was distilled under reduced pressure (4 mmHg, 94—98 °C) to give (±)-**22** (6.14 g, 56%) as a colorless oil. NMR ($CDCl_3$) δ: 1.43 (m, 1H), 1.69 (m, 1H), 186 (m, 1H), 2.15 (m, 1H), 3.80 (s, 3H). IR (KBr) cm^{-1}: 2252 (CN), 1740 (C=O).

2-Amino-4-methyl-3,4-diazabicyclo[4.1.0]hept-2-en-5-one ((±)-23) Sodium (2.2 g, 96 mmol) was dissolved in methanol (150 ml), then methyl *cis*-2-cyanocyclopropanecarboxylate (6.01 g, 48 mmol) and **3a** (2.21 g, 48 mmol) were added and the whole was stirred for 67 h. After evaporation of the solvent *in vacuo*, the resulting residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (20 : 1 to 10 : 1, v/v) and recrystallized from ethanol to give (±)-**23** (577 mg, 9%). mp 197—200 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (m, 1H), 1.54 (m, 1H), 1.97 (m, 1H), 2.17 (m, 1H), 3.18 (s, 1H), 4.18 (br s, 2H). *Anal.* Calcd for $C_6H_9N_3O$: C, 51.78; H, 6.51; N, 30.19. Found: C, 51.69; H, 6.41; N, 29.95.

(+)-(3*S*,4*R*,1′*S*,6′*R*)-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-[(4-methyl-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)amino]-2*H*-1-benzopyran-6-carbonitrile ((+)-24A) and (−)-(3*S*,4*R*,1′*R*,6′*S*)-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-[(4-methyl-5-oxo-3,4-diazabicyclo[4.1.0]hept-2-en-2-yl)amino]-2*H*-1-benzopyran-6-carbonitrile ((−)-24B) Sodium hydride (60% w/w in oil, 160 mg, 4 mmol) and (−)-(3*S*,4*S*)-3,4-epoxy-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile ((−)-**6**, 885 mg, 4.4 mmol) were added to a solution of 2-amino-4-methyl-3,4-

diazabicyclo[4.1.0]hept-2-en-5-one ((±)-**23**, 557 mg, 4 mmol) in DMSO (25 ml) and the mixture was stirred at room temperature for 4 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with water and brine and then dried over anhydrous sodium sulfate. After evaporation of the solvent *in vacuo*, the resulting residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (20 : 1, v/v). The resulting product was subsequently purified with silica gel column chromatography with EtOAc to give (+)-**24A** and (−)-**24B**. Compound (+)-**24A**: Yield 270 mg (20%). Colorless amorphous solid. *Rf* value = 0.32 ($CHCl_3$–MeOH (20 : 1, v/v)). $[\alpha]_D^{25} = +52.8°$ ($c = 1$, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.96 (m, 1H), 1.28 (s, 3H), 1.50 (s, 3H), 1.66 (m, 1H), 2.15 (m, 1H), 2.31 (m, 1H), 3.23 (s, 3H), 3.72 (dd, 1H, J = 2.0, 8.8 Hz), 4.49 (d, 1H, J = 8.3 Hz), 4.85 (dd, 1H, J = 8.3, 8.8 Hz), 5.69 (d, 1H, J = 2.0 Hz), 6.91 (d, 1H, J = 8.3 Hz), 7.49 (dd, 1H, J = 2.0, 8.3 Hz), 7.72 (d, 1H, J = 2.0 Hz). FAB-MS *m/z*: 341 (M + 1). *Anal.* Calcd for $C_{18}H_{20}N_4O_3$: C, 63.51; H, 5.92; N, 16.46. Found: C, 63.12; H, 5.95; N, 15.94. Compound (−)-**24B**: Yield 152 mg (11%). Colorless needles (from EtOAc). mp 157—158 °C (dec.). *Rf* value = 0.24 ($CHCl_3$–MeOH (20 : 1, v/v)). $[\alpha]_D^{25} = -217.0°$ ($c = 1$, MeOH). NMR ($CDCl_3$) δ: 1.00 (m, 1H), 1.28 (s, 3H), 1.52 (s, 3H), 1.66 (m, 1H), 2.00 (m, 1H), 2.21 (m, 1H), 3.22 (s, 3H), 3.73 (dd, 1H, J = 2.0, 8.3 Hz), 4.39 (d, 1H, J = 7.8 Hz), 4.82 (d, 1H, J = 2.0 Hz), 4.91 (dd, 1H, J = 7.8, 8.3 Hz), 6.90 (d, 1H, J = 8.3 Hz), 7.49 (dd, 1H, J = 2.0, 8.3 Hz), 7.67 (d, 1H, J = 2.0 Hz). FAB-MS *m/z*: 341 (M + 1). *Anal.* Calcd for $C_{18}H_{20}N_4O_3$: C, 63.51; H, 5.92; N, 16.46. Found: C, 62.98; H, 6.00; N, 15.95.

Potassium Channel Opening Activity Potassium channel opening activity of the test compounds shown in Table 4 was determined according to the Quast's test method.[10] ^{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 *ab lib.* were given orally a test compound suspended in 0.5% (w/v) carboxymethylcellulose aqueous solution. At 1, 2, 4, 6, 8, 24, 30, or 48 h after the administration, the systolic blood pressure and the heart rate were measured by a tail-cuff method.[11] The antihypertensive activity of the test compound was obtained as an effective dose for reducing blood pressure by 50 mmHg ($ED_{50\,mmHg}$).

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