Novel Potassium-Channel Openers: Preparation and Pharmacological Evaluation of Racemic and Optically Active *N*-(6-Amino-3-pyridyl)-*N'*-bicycloalkyl-*N''*-cyanoguanidine Derivatives

Masahiro Eda, Tadahiro Takemoto, Shin-ichiro Ono, Takehiro Okada, Keigo Kosaka, Maki Gohda, Sumio Matzno, Norifumi Nakamura, and Chikara Fukaya*

Research Division, The Green Cross Corporation, 2-25-1 Shodai-Ohtani, Hirakata, Osaka 573, Japan

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The previous paper reported on the synthesis and pharmacological evaluation of *N*-(6-amino-3-pyridyl)-*N'*-bicycloalkyl-*N''*-cyanoguanidine derivatives, from among which three compounds were selected as potent potassium-channel openers. In the present study, selected compounds were tested for antagonism of potassium-induced contraction of rat aorta, hypotensive activity in normotensive rats, and diuretic activity in spontaneously hypertensive rats. This led to further evaluation of compound (±)-10 and selection of (+)-*N*-(6-amino-3-pyridyl)-*N'*-[(*1S,2R,4R*)-bicyclo-[2.2.1]hept-2-yl]-*N''*-cyanoguanidine ((+)-10) (AL0670) for development as an antihypertensive agent. Although AL0670 is regarded as a pinacidil-type K^+-channel opener, it showed different pharmacological and conformational profiles from pinacidil.

Introduction

Potassium-channel openers have a function of vasorelaxation through hyperpolarization of the cell membrane in vascular smooth muscle.[1] They also relax a variety of nonvascular tissues including the vas deferens, uterus, urinary bladder, trachea, and intestinal smooth muscle.[2-5] They therefore have possible applications in the treatment of, for example, hypertension, irritable bladder, coronary artery and peripheral vascular diseases, and obstructive airway disease.[6,7] A number of the compounds have shown effectiveness in models of hypertension. There are several structural groups in this class of compounds, represented by cromakalim (1), pinacidil (2), diazoxide (3), aprikalim (4), nicorandil (5), and minoxidil sulfate (6) (Figure 1). Although it is controversial in some instances, the ATP-sensitive potassium channel (K_{ATP} channel) has generally been suggested to be the target of these compounds. Despite this structural diversity, synthetic elaborations have focused mainly on modification of the cromakalim-type structure with few based on structural modification of the pinacidil-type structure. The present approach to the latter type, which involved structural modification of 3,4-diaminopyridine (7), a nonspecific potassium-channel blocker, successfully generated novel potassium-channel openers.[8] From among these were selected three *N*-(6-amino-3-pyridyl)-*N'*-bicycloalkyl-*N''*-cyanoguanidine derivatives (8) for the purpose of development of an antihypertensive agent (Figure 2). This paper reports on further pharmacological evaluations of these three compounds and their corresponding enantiomers for antagonism of potassium-induced contraction of de-endothelialized rat aorta, hypotensive activity in normotensive rats (NTR), and diuretic activity in spontaneously hypertensive rats (SHR). It also discusses differences in pharmacological and structural profiles between the final candidate and pinacidil.

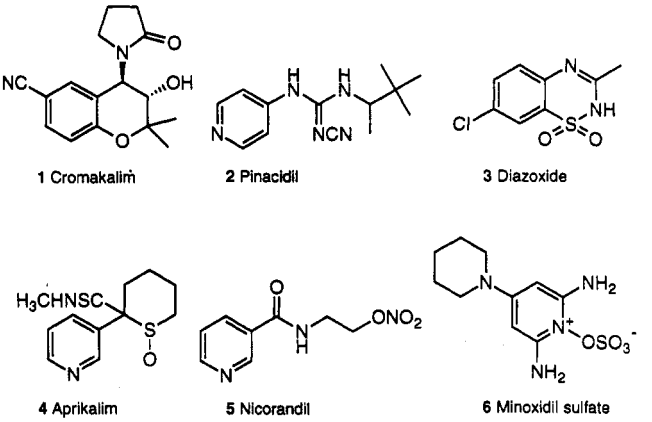

Figure 1. Six structurally different potassium-channel openers.

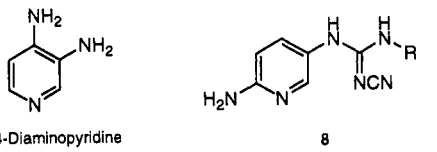

Figure 2.

Chemistry

The racemic compounds 9 and 10 listed in Table 2 were prepared using the method reported in the previous paper.[8] The enantiomerically pure compounds (+)-9, (−)-9, (+)-10, and (−)-10 were synthesized via the route outlined in Scheme 1. The requisite enantiomerically pure *endo*-5-norbornene-2-carboxylic acids 13 and 16 were prepared by asymmetric Diels–Alder reactions described by Poll.[9,10] (*R*)-Pantolactone and (*S*)-2-hydroxy-*N*-methylsuccinimide are highly efficient chiral auxiliaries for compounds 13 and 16. Preparation of *exo*-5-norbornene-2-carboxylic acids 14 and 17 was performed according to the method of Benson.[11] The endo acids (13, 16) were thus converted to their corresponding methyl esters and equilibrated. The resulting mixture of esters was hydrolyzed and each of the desired exo acids (14, 17) obtained by removing the endo acids as the corresponding lactones formed by iodolactonization. After catalytic hydrogenation of the enantiomerically pure acids 13, 14, 16, and 17, the obtained carboxylic acids 18a–d were converted to the amines 19a–d according to a modification of Weinstock's method[12] without epimerization. Reaction of the amines 19a–d and *N*-(2-amino-5-pyridyl)-*N'*-cyano-*O*-phenylisourea (20) pre-

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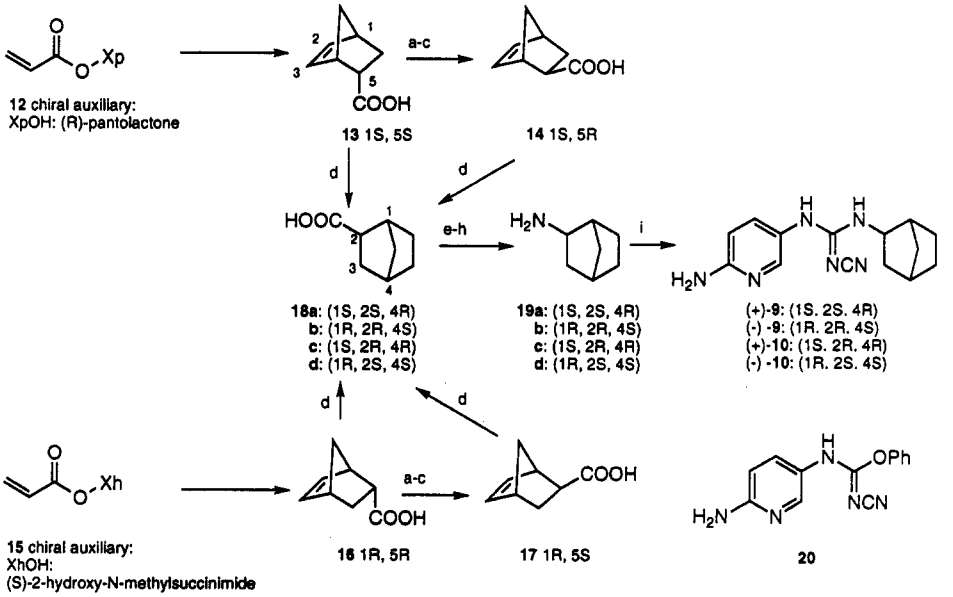
Scheme 1[a]



[a] Reagents: (a) catalytic TsOH/MeOH; (b) NaOMe/MeOH; (c) I_2, Na_2CO_3/H_2O; (d) H_2, 10% Pd/C; (e) $ClCO_2Et$, TEA/hexane; (f) NaN_3/H_2O/acetone; (g) hexane, Δ; (h) 12 N HCl/xylene; (i) 20/AcOEt.

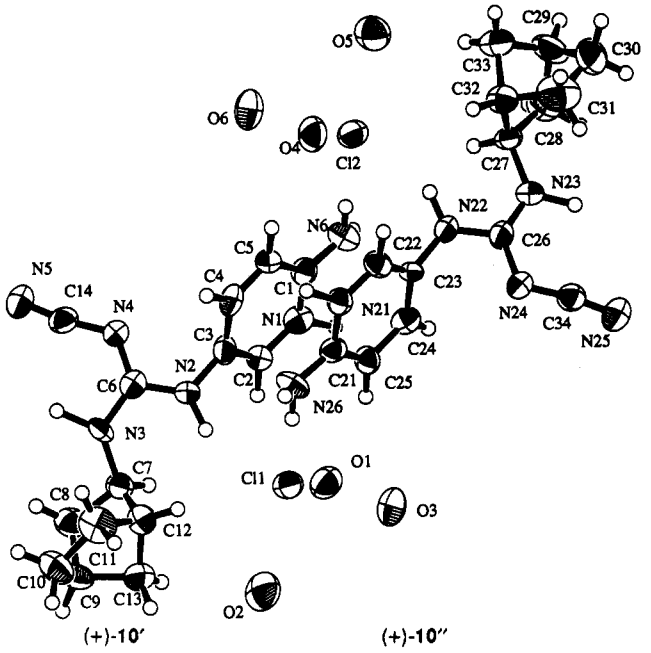
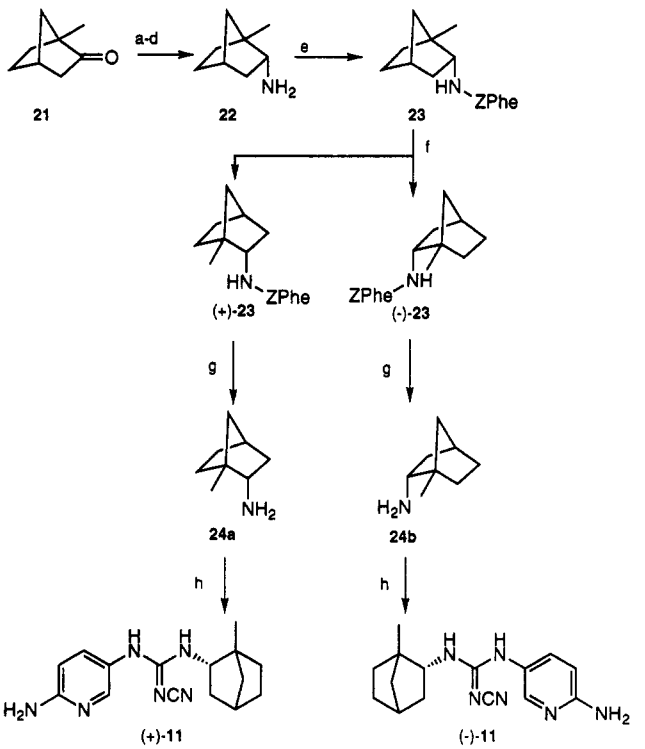


Figure 3. Two crystallographically independent molecules ((+)-10′ and (+)-10″) of (+)-10 in asymmetric unit.

pared from *N*-cyanodiphenoxyimidocarbonate[13] and 2,5-diaminopyridine gave the corresponding cyanoguanidines (+)-9, (−)-9, (+)-10, and (−)-10, respectively. Single-crystal X-ray analysis of (+)-10 hydrochloride confirmed the absolute configuration to be *1S,2R,4R* (Figure 3).

The enantiomerically pure (+)-11 and (−)-11 were synthesized via the route outlined in Scheme 2. *endo*-(±)-2-Amino-1-methylnorbornane (**22**) was prepared using a combination of methods reported by Ingersoll[14] and Brown[15] with a minor modification. 1-Methylnorbornanone (21) was converted to an exo:endo (13:87) mixture of the amine **22** by the Leuckart reaction. Preparation of pure (±)-*endo*-**22** (exo:endo = 1:99) was found in recrystallization of the fumalic acid salts. Several attempts to produce the enantiomers of the endo amine **24a,b** via fractional crystallization of their diastereomeric salts were

Scheme 2[a]

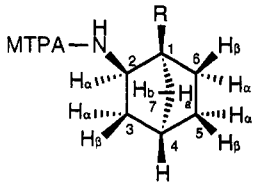


[a] Reagents: (a) HCOOH, $HCONH_2$; (b) 6 N HCl, Δ; (c) fumalic acid/MeOH; (d) NaOH/H_2O; (e) Z-Phe, HBT, WSC/THF; (f) separate diastereomers by HPLC; (g) 12 N HCl, Δ; (h) 21/AcOEt.

unsuccessful. Ultimately, (±)-*endo*-**22** was converted to a mixture of diastereomeric amides of *N*-(benzyloxy)-carbonyl-protected phenylalanine (±)-**23**, which was then separated by HPLC to give pure (+)- and (−)-**23**. Subsequent hydrolysis with concentrated HCl gave the enantiomerically pure *endo*-2-amino-1-methylnorbornanes **24a,b**. Reactions of the amines **24a,b** with **20** gave the cyanoguanidines (+)-11 and (−)-11, respectively.

To determine the absolute configuration of **24a,b**, a modification of Mosher's method using α-methoxy-α-(trifluoromethyl)phenylacetic acid amides (MTPA amides)

Table 1[a]



25 R = H
26 R = Me

no.	R	2α	3α, 3β	4	5α, 5β	6α, 6β	7a, 7b
25	−31	6	20, 25	2	−14, −11	−48, −92	−9, 0
26	−36	−5	39, 24	4	8, −2	−24, −64	−22, −4

[a] Values are chemical shift differences (Δδ) given in hertz.

was applied.[16] Its appropriate application to these amines was demonstrated with the amide **25** (R = H) of the amine **19c**, whose absolute configuration was confirmed by X-ray analysis of (+)-**10** (vide supra). Chemical shift differences ($\Delta\delta = \delta_S - \delta_R$; Δδ values given in hertz) obtained by subtracting the chemical shift (δ_R) of protons of the (*R*)-MTPA amide from those (δ_S) of the (*S*)-MTPA amide are shown in Table 1. As is apparent from the table, all protons of **25** having large positive Δδ values are located at carbon 3 and those having large negative Δδ values at carbons 1 and 6. The *1S,2R,4R* configuration observed was identical with that seen in the X-ray study. The MTPA amide **26** (R = Me) showed exactly the same profile as **25** with regard to Δδ values. The absolute configuration of **26** derived from **24b** was unambiguously determined as *1S,2R,4R*.

Results and Discussion

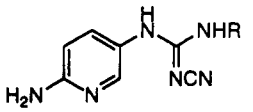
The candidate compounds *exo*-(±)-**9**, *endo*-(±)-**10**, and *endo*-(±)-**11** were selected on the basis of studies on the inhibition of spontaneous mechanical activity on rat portal vein and iv hypotensive activity in NTR.[8] It was previously reported that antihypertensive activity following oral administration to conscious SHR was in keeping with that observed in rat portal vein. Vasorelaxant activity of (±)-**9**–(±)-**11**, pinacidil, and cromakalim was evaluated on the basis of ability to relax endothelium-denuded rat aortic strips toned with 20 mM KCl (Table 2). From the data in Table 2, these compounds are seen to show potent vasorelaxant activity in rat aorta, which was also consistent with the results obtained in the study using rat portal vein.

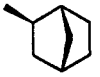
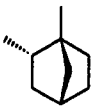
Resolution of the racemates of pinacidil and cromakalim demonstrated that the (−)-enantiomers of both compounds were more potent than their corresponding (+)-enantiomers in in vitro potassium-channel-opening activity. For pinacidil, it was found that potassium-channel-opening activity was stereoselective with (*R*)-(−)-pinacidil being 12 times more potent than (*S*)-(+)-pinacidil in inhibition of spontaneous mechanical activity and stimulation of ^{86}Rb efflux in rat portal vein,[17] while cromakalim enantiomers were found in these studies to possess an eudismic ratio of 100 to 200.[18] Attention was therefore turned to evaluation of the individual enantiomers of **9**–**11**. As shown in Table 2, similar stereoselectivity was found for (±)-**10** but not for (±)-**9** and (±)-**11**. These enantiomers showed good correlation between in vitro study and iv hypotensive activity. Thus, (+)-**10** was approximately 7 times more potent than (−)-**10** in its ability to relax rat aortic strips and equipotent to cromakalim. The eutomer (+)-(*1S,2R,4R*)-**10** had a reverse optical rotation to the eutomer (*R*)-(−)-pinacidil but the same *R* configuration at the carbon atom attached to the nitrogen in the guanidine moiety. Despite these compounds having potent activities, the lack of stereoselectivity of **9** and **11** and the relatively small eudismic ratio of **10** seem unusual in that they are counter to Pfeiffer's rule.[19,20]

Pinacidil is the most clinically evaluated of all the potassium-channel openers.[21] While patients given pinacidil in a placebo-controlled monotherapy study have shown significant decreases in total cholesterol and triglyceride with effective antihypertensive activity, dose-related edema, a typical phenomenon of most general vasodilators, has been observed as a side effect.[22] Cotreatment with diuretic agents to control edema seems to diminish the beneficial effect of pinacidil on blood lipids.[23] The attention of this study was therefore focused on further evaluation through observation of diuretic activity. Oral diuretic activity on SHR was examined by measuring urinary volume and urinary excretion of Na^+ and K^+. As shown in Table 3, the test compounds at a dose of 1 mg/kg did not produce significant changes in urinary volume or urinary excretion of Na^+ and K^+. At a dose of 5 mg/kg of (±)-**9** and (±)-**11**, however, Na^+ excretion was suppressed and the urinary Na^+/K^+ ratio was significantly decreased. Surprisingly, (±)-**10** at the same dose, which is sufficient to produce antihypertensive activity (reduces systolic blood pressure of SHR by approximately 30% in oral administration), increased urinary volume without changing excretion of Na^+ and K^+ or the urinary ratio of Na^+/K^+, whereas pinacidil significantly decreased both. The results obtained in this study indicate that (±)-**10** possesses a diuretic activity which does not affect urinary balance of electrolytes. A study of the diuretic effect of the eutomer (+)-**10** (data presented elsewhere) found that it increased urinary flow, urinary excretion of electrolytes, and fractional excretion of electrolytes following intrarenal arterial infusion to anesthetized dogs.[24] These results show that (+)-**10** has the potent hypotensive and diuretic activity which are necessary criteria for an antihypertensive agent.

K_{ATP} is known to be present in a variety of tissues. The potency of different types of compounds in potassium-channel-opening activity may vary considerably with tissue localization of the K_{ATP} channels. Relative order of potency of several potassium-channel openers in smooth muscle is significantly different from that in pancreatic β-cells. In the latter, diazoxide stimulates the opening of K_{ATP}, inducing membrane hyperpolarization and inhibiting insulin secretion.[25] Thus, diazoxide produces 93% inhibition of insulin release at a concentration of 100 μM, whereas a higher concentration of cromakalim (500 μM) produces only 35% inhibition. Pinacidil inhibits release by 36% and 72% at 100 and 500 μM, respectively. Accordingly, the order of potency for inhibition of insulin release is diazoxide > pinacidil > cromakalim.[26] However, the order of potency for inhibition of spontaneous activity in vascular smooth muscle is the reverse: cromakalim > pinacidil > diazoxide.[27,28] This suggests that the channels in vascular smooth muscle and pancreatic β-cells have quite different pharmacological profiles. Recently, Pirotte synthesized derivatives of pyrido[4,3-*e*]-1,2,4-thiazine 1,1-dioxides **27** bearing different aminoalkyl side chains at the 3-position (Figure 4).[29] A study of insulin release from rat pancreatic islets showed that **28** (BPDZ 44) was more active than diazoxide. Pirotte's speculation that the sulfonylguanidine of **27** can be regarded as a bioisostere of the cyanoguanidine of pinacidil-type derivatives is also

Table 2



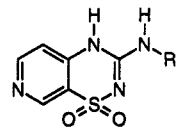
no.	R	formula	analysis[a]	relaxation of rat aorta, 20 mM K^+, IC_{50} (nM)[b]	hypotensive activity, ED_{30} (mg/kg)[c]
exo-(±)-9		$C_{14}H_{18}N_6 \cdot HCl \cdot {}^1/_2H_2O$	C, H, N	223 ± 16	0.51 ± 0.21
(+)-9		$C_{14}H_{18}N_6 \cdot {}^3/_5H_2O$	C, H, N	267 ± 30	0.40 ± 0.13
(−)-9		$C_{14}H_{18}N_6$	C, H, N	278 ± 33	0.57 ± 0.21
endo-(±)-10		$C_{14}H_{18}N_6 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N	392 ± 66	0.17 ± 0.05
(+)-10		$C_{14}H_{18}N_6$	C, H, N	90 ± 22	0.10 ± 0.01
(−)-10		$C_{14}H_{18}N_6 \cdot {}^1/_2H_2O$	C, H, N	593 ± 52	0.74 ± 0.15
endo-(±)-11		$C_{15}H_{20}N_6 \cdot HCl \cdot {}^1/_2H_2O$	C, H, N	79 ± 6	0.20 ± 0.05
(+)-11		$C_{15}H_{20}N_6$	C, H, N	118 ± 24	0.18 ± 0.05
(−)-11		$C_{15}H_{20}N_6$	C, H, N	71 ± 7	0.28 ± 0.18
pinacidil				380 ± 19	0.15 ± 0.03
cromakalim				110 ± 10	0.11 ± 0.01

[a] Analyses for elements indicated were within ±0.4% of theoretical values. [b] Values are means ± standard error of the mean (SEM). Number of determinations, ≥2. [c] Hypotensive activity in anesthetized normotensive rats by iv injection. Systolic blood pressure (SBP) measured 30 min after injection; all values expressed as ED_{30} values (dose of drug producing 30% fall in SBP) (n = 4).

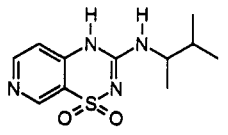
Table 3. Diuretic Effects of Compounds 9–11[a]

no.	dose (mg/kg)	urinary volume (mL/kg/5 h)	Na^+ excretion (mequiv/kg/5 h)	K^+ excretion (mequiv/kg/5 h)	Na^+/K^+ ratio
(±)-9	vehicle	5.15 ± 0.82	1.357 ± 0.209	1.078 ± 0.148	1.28 ± 0.15
	1	8.77 ± 2.97	1.523 ± 0.436	1.350 ± 0.297	1.08 ± 0.15
	5	4.68 ± 0.79	0.694 ± 0.248	1.249 ± 0.138	0.50 ± 0.14**
(±)-10	vehicle	6.84 ± 1.64	1.072 ± 0.260	0.800 ± 0.218	1.56 ± 0.24
	1	5.53 ± 1.10	0.890 ± 0.187	0.646 ± 0.160	1.54 ± 0.28
	5	18.37 ± 2.88**	1.865 ± 0.325	1.460 ± 0.216	1.27 ± 0.05
(±)-11	vehicle	6.84 ± 1.64	1.072 ± 0.260	0.800 ± 0.218	1.56 ± 0.24
	1	6.92 ± 1.21	0.800 ● 0.091	0.747 ± 0.085	1.08 ± 0.08
	5	7.31 ± 2.46	0.311 ± 0.057	0.515 ± 0.030	0.59 ± 0.08**
pinacidil	vehicle	5.15 ± 0.82	1.357 ± 0.209	1.078 ± 0.148	1.28 ± 0.15
	1	7.62 ± 0.98	1.270 ± 0.306	1.546 ± 0.153	0.78 ± 0.17
	5	2.56 ± 0.81*	0.078 ± 0.032***	0.584 ± 0.135*	0.12 ● 0.02***

[a] Compounds administered orally to spontaneously hypertensive rats (n = 5 or 6). Values are means ± SEM. Asterisks (*, **, or ***) represent significant difference from value in control group at $p < 0.05$, 0.01, or 0.001, respectively.



27



28 BPDZ 44

Figure 4.

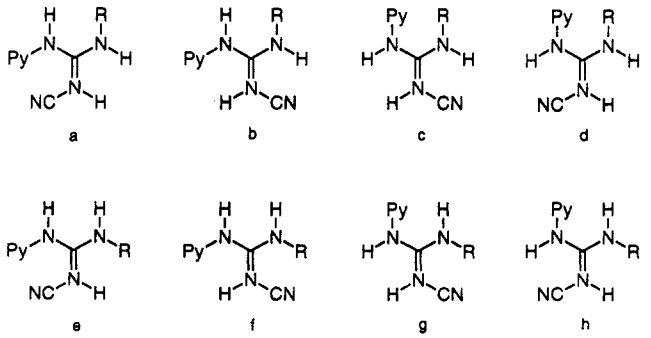


Figure 5. Eight different conformations of cyanoguanidine derivatives.

supported by crystallographic data for pinacidil.[30] It is known that *N*-alkyl-*N*″-pyridyl-*N*′-cyanoguanidines can exist theoretically in eight different conformations due to the restricted rotation around their carbon–nitrogen bonds (see a–h, Figure 5). The staggered conformations b and h seem energetically the most favorable; pinacidil, however, assumes conformation e in the crystal form. In contrast, Manley suggested that the staggered conformation b in derivatives with a nitroethanediamine moiety, which is another bioisostere of cyanoguanidine, could correspond to the active conformation at the binding site of the channel.[31] The present X-ray analysis of the hydrochloric acid salt of (+)-10 (Figure 3) showed that the crystal was composed of asymmetric crystal units formed by two independent molecules, (+)-10′ and (+)-10″, which were generated by rotation around the N_2–C_3 and N_{22}–C_{23} bonds. In rotamer (+)-10′, the torsion angle is 58° around the atoms C_4–C_3–N_2–C_6 and the bond distances between C_6–N_4, C_6–N_2, and C_6–N_3 are the same (1.33 Å), while rotamer (+)-10″ has the N_{22}–C_{23} bond twisted 120° relative to that of the rotamer (+)-10′. The guanidine moiety has a nearly planar geometry. The analysis confirmed that (+)-10 assumes conformation b, which is identical to the active conformation suggested by Manley. Further pharmacological investigations using rat aorta have been presented demonstrating that at high concentration (10 μM), AL0671 (the hydrochloride of (+)-10), in common with cromakalim, does not inhibit contraction induced by 60 mM KCl whereas pinacidil almost totally suppresses the latter.[32] It has been reported that, at high concentrations, diazoxide also has the ability to inhibit contractions produced by 80 mM KCl.[33] Considering the conformational differences among them, this discrepancy may be speculated to come from the direction of the alkyl–NH bond in the guanidine moiety (conformation b versus e in Figure 5). The fact that the vasorelaxant effects of AL0671 are competitively

inhibited by the potassium-channel blocker glibenclamide indicates that vasorelaxation caused by AL0671 is due to K^+ hyperpolarization through an ATP-sensitive potassium channel.[32] Further evidence of this mode of action comes from whole-cell current measurements in cultured rat aortic smooth muscle cells using a patch clamp technique, which have clearly shown that AL0671 opens an ATP-modulated channel.[33] AL0671 also possesses dose-related serum lipid-lowering action in obese Zucker rats.[34]

Conclusion

The above describes the synthesis and biological activity of aminopyridine derivatives structurally related to pinacidil-type potassium-channel openers. From the study, (+)-10 (AL0670) was found to be a potent potassium-channel opener. Interestingly, it showed different pharmacological and conformational profiles from pinacidil. AL0670 appeared to fulfill the criteria of vasorelaxant potency and diuretic effect suitable for an antihypertensive agent.

Experimental Section

Chemistry. Reagents were purchased from commercial suppliers and used without further purification. Reaction solvents were distilled from an appropriate drying agent before use. Melting points were measured on a Yanaco micromelting point apparatus and are uncorrected. Optical rotations, unless otherwise indicated, were measured at 25 °C in 99% EtOH with a JASCO DIP-181 digital polarimeter. IR and NMR spectra, which were in agreement with the structures cited, were recorded on a Shimadzu IR-420 instrument for IR and a Brucker AM-500 spectrometer (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) and a Brucker AC-200 spectrometer (200 MHz for ^1H NMR and 50 MHz for ^{13}C NMR) for NMR using TMS as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Coupling constants (J) are shown in Hz. EI-MS and SI-MS were taken on a Shimadzu QP-1000 mass spectrometer and a Hitachi M-2000 mass spectrometer, respectively. Optical purity of selected final compounds was determined by HPLC using an Ultron ES-OVM column (150 mm × 6 mm o.d.; Shinwa Chemical Ind. Ltd.): mobile phase, 20 mM phosphate buffer (pH 7.85)/CH_3CN, 200:5 (v/v); flow rate, 1 mL/min; temperature, 5 °C; sample size, 2 $\mu g/\mu L$; injection volume, 1 μL; detection, 230 nm.

(+)-(*1R,4R,5R*)-Bicyclo[2.2.1]hept-2-ene-5-carboxylic acid (16) was prepared according to Poll[10] as a colorless crystal: mp 42–44 °C; $[\alpha]_D$ = +146.2° (c = 3.0) (lit.[35] $[\alpha]_D$ = +149° (20 °C, c = 2.4, 95% EtOH)).

(−)-(*1S,4S,5S*)-Bicyclo[2.2.1]hept-2-ene-5-carboxylic acid (13) was prepared according to Poll[9] as a colorless crystal: mp 44.5–45.5 °C; $[\alpha]_D$ = −147.1° (c = 2.7) (lit.[9] $[\alpha]_D$ = −147° (20 °C, c = 3.0, 95% EtOH)).

(−)-(*1S,4S,5R*)-Bicyclo[2.2.1]hept-2-ene-5-carboxylic Acid (14). To a solution of 13 (4.87 g, 35.2 mmol) in 180 mL of MeOH was added toluenesulfonic acid monohydrate (0.67 g, 3.5 mmol) at room temperature. After refluxing for 16 h, the reaction mixture was concentrated under reduced pressure (>20 mmHg). The residue was diluted with Et_2O and washed successively with 10% aqueous Na_2CO_3 and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give the methyl ester (4.41 g) as a colorless oil. To a solution of sodium methoxide (from 266 mg of sodium) in 48 mL of MeOH was added the methyl ester (4.41 g, 29.0 mmol) in 10 mL of MeOH at room temperature, and the mixture was heated at reflux for 40 h. MeOH (48 mL) was then distilled off, and to the residue was added 6 N NaOH. After refluxing for 2 h, the resulting solution was cooled to room temperature, acidified (pH 1–2) with concentrated HCl, and extracted with Et_2O. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give 3.94 g of a mixture of acids (endo/exo, 1/1). The acid mixture was dissolved in a solution of 8.33 g of Na_2CO_3 in 132 mL of water and treated for 25 min with dropwise addition of a solution (65 mL) of iodine (7.3 g) in 87 mL of water. At this point, there was a permanent excess of iodine. The precipitated brown oil was decolorized with 10% sodium thiosulfate and washed with Et_2O. The aqueous layer was carefully acidified to pH 3 with concentrated HCl and extracted with Et_2O. The extract was washed with brine and dried over magnesium sulfate to give the crude product (1.88 g, 39%). Further purification was accomplished by distillation (bp 77–81 °C, 0.3 mmHg) to give the title compound (1.57 g, 32%) as a colorless oil: $[\alpha]_D$ = −13.9° (c = 3.6); ^1H NMR (200 MHz, $CDCl_3$) δ 6.12 (m, 2 H), 3.09 (br s, 1 H), 2.92 (br s, 1 H), 2.25 (dd, J = 4.4, 5.8 Hz, 1 H), 1.93 (dt, J = 4.0, 12.1 Hz, 1 H), 1.52 (d, J = 8.3 Hz, 1 H), 1.45–1.33 (m, 2 H); IR ($CHCl_3$) 3000, 1700 cm^{-1}.

(+)-(*1R,4R,5S*)-Bicyclo[2.2.1]hept-2-ene-5-carboxylic acid (17) was prepared as a colorless oil using a method similar to that described above (10%): $[\alpha]_D$ = +14.0° (c = 3.6) (lit.[36] $[\alpha]_D$ = +14.3° (20 °C, c = 3.7, 95% EtOH)). The spectral data were identical to those of **14**.

(+)-(*1S,2R,4R*)-Bicyclo[2.2.1]heptane-2-carboxylic Acid (18c). To a solution of 16 (56.9 g) in 550 mL of AcOEt was added 10% Pd on charcoal (2.0 g). After the mixture had been stirred for 3 h under a hydrogen atmosphere, the catalyst was removed by filtration through Celite and the filtrate concentrated in vacuo to give quantitatively the title compound as a colorless oil: $[\alpha]_D$ = +31.8° (c = 1.1) (lit.[37] $[\alpha]_D$ = +33.9 (20 °C, c = 1.06, 95% EtOH)); ^1H NMR (200 MHz, $CDCl_3$) δ 2.83 (dt, J = 5.5, 10.3 Hz, 1 H), 2.59 (m, 1 H), 2.28 (m, 1 H), 1.70–1.32 (m, 8 H); IR ($CHCl_3$) 3300–2200, 1670 cm^{-1}.

Compounds **18a,b,d** were prepared in the same way.

(−)-(*1R,2S,4S*)-Bicyclo[2.2.1]heptane-2-carboxylic acid (18d) was prepared quantitatively as a colorless oil: $[\alpha]_D$ = −30.6° (c = 1.1) (lit.[15] $[\alpha]_D$ = −30.6° (26 °C, c = 1.2, 95% EtOH)). The spectral data were identical to those of **18c**.

(+)-(*1S,2S,4R*)-Bicyclo[2.2.1]heptane-2-carboxylic acid (18a) was prepared quantitatively as a crystal: mp 43.5–45 °C; $[\alpha]_D$ = +25.7° (c = 3.5); ^1H NMR (200 MHz, $CDCl_3$) δ 9.0–6.0 (br, 1 H), 2.55 (br s, 1 H), 2.4–2.1 (m, 2 H), 1.9–1.7 (m, 1H), 1.7–1.3 (m, 4 H), 1.3–1.0 (m, 3 H); IR ($CHCl_3$) 3000, 2920, 1700 cm^{-1}.

(−)-(*1R,2R,4S*)-Bicyclo[2.2.1]heptane-2-carboxylic acid (18b) was prepared quantitatively as a crystal: mp 42–45 °C; $[\alpha]_D$ = −25.5° (c = 3.7, $CHCl_3$). The spectral data were identical to those of **18a**.

(−)-(*1S,2R,4R*)-2-Aminobicyclo[2.2.1]heptane (19c). To a solution of ethyl chloroformate (52.5 mL) in hexane (500 mL) were added dropwise for 1 h a solution of **18c** (85.5 g) and triethylamine (85 mL) in hexane (500 mL) with temperature maintained below 10 °C. After being stirred for 30 min at 0 °C, the reaction mixture was filtered and concentrated in vacuo. The residual oil was dissolved in acetone (400 mL) and added dropwise for 45 min to a solution of sodium azide (59.5 g) in water (500 mL) with temperature maintained below 10 °C. After being stirred for 2 h at 0 °C, the reaction mixture was poured into ice water and extracted with hexane. The organic layer was dried over anhydrous $MgSO_4$ and filtered and the filtrate added dropwise to hexane (500 mL) under reflux. After being refluxed for 5 h, the reaction mixture was cooled to room temperature and concentrated in vacuo. To the residue redissolved in xylene (350 mL) was added slowly concentrated HCl (150 mL) at room temperature. After being stirred for 1 h at room temperature, the mixture was heated at reflux for 2 h. Xylene (130 mL) was then distilled off and the residue cooled to 0 °C to give the HCl salt of the title compound as a white solid (66.3 g, 74%): mp >250 °C; $[\alpha]_D$ = +4.6° (c = 0.5).

To the solution of 6 N NaOH (1 L) was added the HCl salt (145 g) at 0 °C. After being stirred for 1 h at 0 °C, the mixture was extracted with CH_2Cl_2. The extracts were washed with brine. Most of the solvent was distilled off under atmospheric pressure, and a small portion of the residue was distilled (bp 156 °C) to give the title compound as a waxy solid (94.3 g, 87%): $[\alpha]_D$ = −25.5° (c = 2.2); ^1H NMR (200 MHz, $CDCl_3$) δ 3.17 (m, 1 H), 2.06 (m, 1 H), 1.99 (m, 1 H), 1.88–1.13 (m, 9 H), 0.60 (m, 1H); IR (KBr) 3250, 2950, 1560, 1490, 1460 cm^{-1}; MS (EI) M^+ 111.

Compounds **19a,b,d** were prepared in the same way.

***endo*-2-Amino-1-methylbicyclo[2.2.1]heptane (22).** To 1-methylnorbornanone (**21**)[12] (24.2 g) were added formic acid (45 mL) and formamide (45 mL), and the mixture was azeotropically refluxed at 160 °C. After 3 h, an additional 100 mL of formic acid was added and the reaction mixture kept at 150 °C for 23 h, after which it was cooled to room temperature and extracted with CH_2Cl_2. The extracts were washed successively with water, saturated aqueous $NaHCO_3$, and water. The organic layer was dried over anhydrous MgSO4, filtered, and concentrated in vacuo to give the crude (±)-2-(formylamino)-1-methylbicyclo[2.2.1]-heptane (29.0 g). This material was added to 6 N HCl (300 mL) and refluxed for 4 h. The reaction mixture was cooled and washed with CH_2Cl_2 and the aqueous layer made alkaline (pH >10) with 28% aqueous ammonia and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and filtered to give the mixture of exo and endo amine (18.2 g, 75%) in a ratio of 13:87 as determined by 500-MHz NMR. This mixture (18.2 g) was converted to the salt of fumalic acid and recrystallized from MeOH. The crystalline was dissolved in water and the solution made alkaline and extracted with Et_2O to give the pure endo amine (9.4 g, >99% purity as determined by 500-MHz NMR): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.87 (ddd, J = 1.8, 5.1, 10.6 Hz, 1 H), 2.10–2.04 (m, 2 H), 1.65–1.59 (m, 2 H), 1.37–1.12 (m, 6 H), 1.05 (s, 3 H), 0.75 (m, 1 H); MS (EI) M^+ 125.

Resolution of *endo*-2-Amino-1-methylbicyclo[2.2.1]heptane. To a solution of **22** (1.2 g), carbobenzoxy-L-phenylalanine (Z-Phe, 0.50 g), and 1-hydroxybenzotriazole (0.54 g) in THF (20 mL) was added 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (0.77 g) at room temperature. After being stirred for 16 h, the reaction mixture was poured into water and extracted with AcOEt. The organic layer was successively washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine and dried over anhydrous MgSO4. The extract was filtered and concentrated in vacuo. The residue was purified by recrystallization from Et_2O/hexane to give the diastereomer **23** (1.43 g, 88%). This diastereomer was resolved by preparative HPLC to give the (−)-amide of (*1S,2R,4R*)-2-amino-1-methylbicyclo[2.2.1]-heptane ((−)-**23**) (first peak) as a white powder ($[\alpha]_D$ = −3.0° (25 °C, c = 1.9, 99% EtOH); 99.8% de; mp 105–106 °C) and the (+)-amide of (*1R,2S,4S*)-2-amino-1-methylbicyclo[2.2.1]heptane ((+)-**23**) (second peak) as a colorless crystal ($[\alpha]_D$ = +7.2° (c = 1.6); 99.2% de; mp 132.5–133.5 °C).

Separation conditions for preparative HPLC: column, YMC-Pack R&D D-SIL-5 (250 mm × 20 mm o.d.) (Yamamura Chemical Lab. Co., Ltd.); eluent, THF/hexane, 1:7 (v/v); flow rate, 10 mL/min; detection, 235 nm; sample size, 100 mg/mL; injection volume, 100 μL.

Analytical conditions: YMC-Pack R&D R-SIL-5 (250 mm × 4.6 mm o.d.); eluent, THF/hexane, 1:7 (v/v); flow rate, 1 mL/min; detection, 235 nm; sample size, 1 mg/mL; injection volume, 10 μL.

Spectral data for (−)-**23**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.28 (m, 7 H), 7.25–7.21 (m, 3 H), 5.51 (br, 1 H), 5.38 (br, 1 H), 5.13 (d, J = 12.3 Hz, 1 H), 5.09 (d, J = 12.3 Hz, 1 H), 4.34 (q, J = 6.0 Hz, 1 H), 3.88–3.83 (m, 1 H), 3.18 (dd, J = 6.0, 13.5 Hz, 1 H), 2.93 (dd, J = 8.8, 13.5 Hz, 1 H), 2.08–2.01 (m, 2 H), 1.60–1.53 (m, 1 H), 1.29 (d, J = 9.9 Hz, 1 H), 1.20 (d, J = 9.9 Hz, 1 H), 1.11–1.01 (m, 3 H), 0.99 (s, 3 H), 0.48 (d, J = 11.0, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6 (s), 136.8 (s), 136.2 (s), 129.3 (d), 128.8 (d), 128.5 (d), 128.2 (d), 128.0 (d), 127.1 (d), 67.0 (t), 56.6 (d), 54.7 (d), 47.3 (s), 45.1 (t), 39.1 (t), 38.9 (t), 36.6 (d), 31.0 (t), 28.6 (t), 18.6 (q); IR (KBr) 3300, 2950, 1700, 1650, 1540 cm^{-1}; MS (SI) M + H^+ 407.

Spectral data for (+)-**23**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.26 (m, 7 H), 7.24–7.21 (m, 3 H), 5.60 (br, 1 H), 5.42 (br, 1 H), 5.11 (d, J = 12.3 Hz, 1 H), 5.07 (d, J = 12.3 Hz, 1 H), 4.36 (q, J = 6.6 Hz, 1 H), 3.11 (dd, J = 6.0, 13.7 Hz, 1 H), 3.09 (m, 1 H), 3.00 (dd, J = 8.2, 13.7 Hz, 1 H), 2.16–2.09 (m, 1 H), 2.07 (t, J = 4.4 Hz, 1 H), 1.61–1.55 (m, 1 H), 1.30 (d, J = 9.9 Hz, 1 H), 1.21 (d, J = 9.9 Hz, 1 H), 1.17–1.14 (m, 1 H), 1.04–1.01 (m, 2 H), 0.94 (s, 3 H), 0.72 (d, J = 12.6, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (s), 136.6 (s), 136.2 (s), 129.2 (d), 128.8 (d), 128.5 (d), 128.2 (d), 128.0 (d), 127.1 (d), 67.0 (t), 56.7 (d), 54.9 (d), 47.3 (s), 45.2 (t), 39.2 (t), 39.0 (t), 36.6 (d), 31.0 (t), 28.5 (t), 18.5 (q); IR (KBr) 3300, 2950, 1700, 1650, 1540 cm^{-1}; MS (SI) M + H^+ 407.

Each of amide (+)-**23** and (−)-**23** was heated with concentrated HCl at 110 °C for 72 h in a sealed tube and then cooled to room temperature, diluted with water, made alkaline with KOH, and extracted with Et_2O. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under atmospheric pressure to give respectively the crude enantiomers **24a** and **24b** containing Et_2O. These were used in the next step without further purification.

***N*-(*R*)-MTPA Amide of 24b.** To a solution of (*1S,2R,4R*)-2-amino-1-methylbicyclo[2.2.1]heptane (**24b**) (23 mg) and triethylamine (50 mg) in CH_2Cl_2 (1 mL) was added (+)-MTPA chloride at room temperature. After being stirred for 1 h, the reaction mixture was poured into water and extracted with Et_2O. The organic layer was washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by HPLC (t_R = 12.5–15 min) to give the title compound (36 mg, 57%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55–7.50 (m, 2 H), 7.45–7.35 (m, 3 H), 6.72 (br d, 1 H), 4.09 (m, 1 H), 3.45 (s, 3 H), 2.21 (m, 1 H), 2.17 (m, 1 H), 1.71 (m, 1 H), 1.49 (m, 1 H), 1.41 (dd, J = 1.3, 9.9 Hz, 1 H), 1.31 (d, J = 9.9 Hz, 1 H), 1.30 (m, 1 H), 1.28 (ddd, J = 2.1, 2.6, 6.0 Hz), 1.13 (s, 3 H), 0.84 (ddd, J = 1.5, 3.2, 13.0 Hz, 1 H).

Conditions for HPLC: column, YMC-Pack R&D D-SIL-5 (250 mm × 20 mm o.d.); eluent, THF/hexane 1:20 (v/v); flow rate, 10 mL/min; detection, 235 nm; sample size, 360 mg/mL; injection volume, 100 μL.

***N*-(*S*)-MTPA amide of 24b** was prepared in the way described above: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58–7.53 (m, 2 H), 7.42–7.38 (m, 3 H), 6.69 (br d, 1 H), 4.08 (m, 1 H), 3.43 (s, 3 H), 2.26 (m, 1 H), 2.18 (t, J = 4.4 Hz, 1 H), 1.71 (m, 1 H), 1.40 (dd, J = 2.0, 10.0 Hz, 1 H), 1.36 (m, 1 H), 1.32 (m, 1 H), 1.27 (m, 1 H), 1.22 (dt, J = 2.0, 12.2 Hz), 1.06 (s, 3 H), 0.92 (ddd, J = 3.3, 4.4, 13.0 Hz, 1 H).

***N*-(*R*)-MTPA amide of 19c** was prepared in the way described above: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54–7.52 (m, 2 H), 7.41–7.38 (m, 3 H), 6.80 (br d, J = 4.9 Hz, 1 H), 4.15 (m, 1 H), 3.42 (q, J = 1.4 Hz, 3 H), 2.52 (t, J = 4.0 Hz, 1 H), 2.24 (t, J = 4.4 Hz, 1 H), 2.09 (ddd, J = 3.1, 4.7, 12.1 Hz, 1 H), 1.60 (ddd, J = 4.6, 7.8, 12.0 Hz, 1 H), 1.49 (m, 2 H), 1.45 (ddd, J = 1.7, 2.7, 10.0 Hz, 1 H), 1.35 (ddd, J = 1.8, 2.4, 10.4 Hz, 1 H), 1.23 (ddd, J = 2.0, 4.5, 11.8 Hz, 1 H), 0.75 (ddd, J = 3.1, 4.6, 13.0 Hz, 1 H).

***N*-(*S*)-MTPA amide of 19c** was prepared in the way described above: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54–7.52 (m, 2 H), 7.41–7.38 (m, 3 H), 6.73 (br d, J = 4.9 Hz, 1 H), 4.16 (m, 1 H), 3.44 (q, J = 1.4 Hz, 3 H), 2.47 (t, J = 4.0 Hz, 1 H), 2.24 (t, J = 4.4 Hz, 1 H), 2.13 (ddd, J = 3.1, 4.7, 12.1 Hz, 1 H), 1.57 (ddd, J = 4.6, 7.8, 12.0 Hz, 1 H), 1.45 (ddd, J = 1.7, 2.7, 10.0 Hz, 1 H), 1.40 (ddd, J = 1.6, 4.6, 12.1 Hz, 1 H), 1.33 (ddd, J = 1.8, 2.4, 10.4 Hz, 1 H), 1.31 (m, 1 H), 1.21 (ddd, J = 2.0, 4.5, 11.8 Hz, 1 H), 0.80 (ddd, J = 3.1, 4.6, 13.0 Hz, 1 H).

***N*-(6-Amino-3-pyridyl)-*N'*-cyano-*O*-phenylisourea (20).** To a solution of 2,5-diaminopyridine (1.66 g) in *i*-PrOH (40 mL) was added *N*-cyanodiphenoxyimidocarbonate[4] (3.62 g) at room temperature. After the mixture had been stirred for 2 h, *i*-Pr_2O (20 mL) was added and the mixture cooled to 0 °C. After further stirring for 1 h at 0 °C, the resulting precipitate was collected by filtration to give the title compound as a purple powder (3.38 g), the color of which was removed by refluxing in MeOH with charcoal for 5 min to give a white powder (2.37 g, 70%): mp >172 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.41 (br s, 1 H), 7.92 (d, J = 2.6 Hz, 1 H), 7.5–7.4 (m, 3 H), 7.3–7.2 (m, 3 H), 6.43 (d, J = 8.8 Hz, 1 H), 6.04 (br s, 2 H); IR (KBr) 3450, 3350, 2200, 1620 cm^{-1}; MS (SI) M + H^+ 254.

(+)-*N*-(6-Amino-3-pyridyl)-*N'*-[(*1S,2R,4R*)-bicyclo[2.2.1]-hept-2-yl]-*N''*-cyanoguanidine ((+)-10). To a solution of isourea (**20**) (76.0 g) in AcOEt (500 mL) was added amine **19c** (36.7 g) at room temperature. After being refluxed for 16 h, the reaction mixture was cooled to room temperature and the resulting precipitate collected by filtration. The powder was recrystallized successively from acetonitrile and water/MeOH to give the title compound (59.6 g, 68%) as a white powder: mp 184–185 °C; $[\alpha]_D$ = +12.0° (c = 0.6); 99.9% ee based on chiral HPLC (t_R = 37.0 min); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.45 (s, 1 H), 7.74 (d, J = 2.6 Hz, 1 H), 7.21 (dd, J = 2.6, 8.7 Hz, 1 H), 6.58 (d, J = 5.3 Hz, 1 H), 6.43 (d, J = 8.7 Hz, 1 H), 5.91 (s, 2 H), 3.94 (q, J = 5.3 Hz, 1 H), 2.41 (br s, 1 H), 2.13 (br s, 1 H), 1.87

(m, 1 H), 1.45 (m, 2 H), 1.34 (m, 2 H), 1.26 (m, 2 H), 1.02 (d, J = 12.4 Hz, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.7 (s), 157.8 (s), 144.7 (d), 135.2 (d), 122.9 (s), 117.1 (s), 107.7 (d), 52.9 (d), 40.0 (d), 37.5 (t), 36.0 (d), 35.0 (t), 29.1 (t), 21.0 (t); IR (KBr) 3300, 2900, 2300, 1590 cm^{-1}. Anal. ($C_{14}H_{18}N_6$) C, H, N.

(−)-*N*-(6-Amino-3-pyridyl)-*N'*-[(*1R,2S,4S*)-bicyclo[2.2.1]-hept-2-yl]-*N''*-cyanoguanidine ((−)-10) was prepared as a white powder using a method similar to that described above (68%): mp 184–185 °C; $[\alpha]_D$ = −12.0° (c = 0.6); 99.9% ee based on chiral HPLC (t_R = 30.6 min). The spectral data were identical to those of (+)-10. Anal. ($C_{14}H_{18}N_6 \cdot {}^1/_2H_2O$) C, H, N.

(+)-*N*-(6-Amino-3-pyridyl)-*N'*-[(*1S,2S,4R*)-bicyclo[2.2.1]-hept-2-yl]-*N''*-cyanoguanidine ((+)-9) was prepared as a white powder using a method similar to that described above (27%): mp 174–175 °C; $[\alpha]_D$ = +16.6° (c = 0.5); 99% ee based on chiral HPLC (t_R = 32.4 min). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.43 (s, 1 H), 7.71 (d, J = 2.6 Hz, 1 H), 7.19 (dd, J = 2.6, 8.8 Hz, 1 H), 6.44 (br, 1 H), 6.41 (d, J = 8.8 Hz, 1 H), 5.90 (br, 2 H), 3.56 (br, 1 H), 2.19 (br, 2 H), 1.61 (br, 1 H), 1.5–1.3 (m, 4 H), 1.16 (br t, J = 10.3 Hz, 1 H), 1.07 (br d, J = 8.9 Hz, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.9 (s), 157.8 (s), 144.8 (d), 135.2 (d), 122.9 (s), 117.1 (s), 107.7 (d), 54.7 (d), 41.7 (d), 38.4 (t), 35.1 (d), 34.7 (t), 27.8 (t), 25.8 (t); IR (KBr) 3300, 2950, 2150, 1580 cm^{-1}. Anal. ($C_{14}H_{18}N_6 \cdot {}^3/_5H_2O$) C, H, N.

(−)-*N*-(6-Amino-3-pyridyl)-*N'*-[(*1R,2R,4S*)-bicyclo[2.2.1]-hept-2-yl]-*N''*-cyanoguanidine ((−)-9) was prepared as a white powder using a method similar to that described above (50%): mp 173.5–175 °C; $[\alpha]_D$ = −15.8° (c = 0.5); 99% ee based on chiral HPLC (t_R = 49.8 min). The spectral data were identical to those of (+)-9. Anal. ($C_{14}H_{18}N_6$) C, H, N.

(+)-*N*-(6-Amino-3-pyridyl)-*N'*-cyano-*N''*-[(*1R,2S,4S*)-1-methylbicyclo[2.2.1]hept-2-yl]guanidine ((+)-11) was prepared as a white powder using a method similar to that described above (25%): $[\alpha]_D$ = +84.3° (c = 0.5); 99% ee based on chiral HPLC (t_R = 33.2 min); mp 225.5–227 °C;^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55 (s, 1 H), 7.73 (d, J = 2.6 Hz, 1 H), 7.19 (dd, J = 2.6, 8.7 Hz, 1 H), 6.42 (d, J = 8.7, 1 H), 6.38 (d, J = 7.1 Hz, 1 H), 5.93 (br s, 2 H), 4.02–3.90 (m, 1 H), 2.18–1.97 (m, 2 H), 1.58–1.45 (m, 2 H), 1.40–1.20 (m, 4 H), 1.08 (s, 3 H), 1.02–0.92 (m, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.0 (s), 157.7 (s), 144.4 (d), 135.0 (d), 122.9 (s), 117.1 (s), 107.8 (d), 56.5 (d), 47.5 (s), 44.7 (t), 37.0 (t), 36.0 (d), 30.5 (t), 28.1 (t), 18.7 (q); IR (KBr) 3450, 3320, 3280, 2920, 2850 cm^{-1}; MS (SI) M + H$^+$ 285. Anal. ($C_{15}H_{20}N_6$) C, H, N.

(−)-*N*-(6-Amino-3-pyridyl)-N'-cyano-*N''*-[(*1S,2R,4R*)-1-methylbicyclo[2.2.1]hept-2-yl]guanidine ((−)-11) was prepared as a white powder using a method similar to that described above (75%): mp 225.5–227 °C; $[\alpha]_D$ = −79.8° (c = 0.5); 99% ee based on chiral HPLC (t_R = 58.9 min). The spectral data were identical to those of (+)-11. Anal. ($C_{15}H_{20}N_6$) C, H, N.

Biology. All compounds were dissolved in DMSO and diluted with experimental vehicle (described in the following procedure) to the appropriate concentration.

Vasorelaxant Studies in Aorta. Male Wister rats (200–300 g) were sacrificed and the thoracic aorta removed, cleaned of extraneous fat, and cut into spiral strips. The strips were suspended in an organ bath containing physiological salt solution (PSS) maintained at 37 °C and aerated with 5% CO_2 in O_2. A resting tension of 1.0 g was applied, and developed tension was measured isometrically with a force-displacement transducer (TB612T; Nihon Kohden, Osaka, Japan). The strips were equilibrated for 30 min in the bathing solution with replacement of fresh solution every 20 min. After equilibration, the tissues were contracted by the addition of 20 mM KCl and the contractile responses allowed to reach a plateau. The ability of test compounds to relax the tissues was then examined using a cumulative dose–response protocol. At the end of the experiments, 10^{-4} M papaverine was added to obtain maximum relaxation. Drug activity is expressed as the IC_{50} value, that is, the concentration of a drug producing 50% of the maximum relaxation as induced by papaverine. The PSS had the following composition (mM): NaCl 117.0, KCl 5.0, $CaCl_2$ 1.5, NaH_2PO_4 1.0, $NaHCO_3$ 25.0, $MgSO_4$ 1.2, glucose 11.5.

iv Hypotensive Activity in Normotensive Rats. Male Wistar rats (250–300 g) were anesthetized with pentobarbital sodium salt (42 mg/kg, ip) and the tracheas cannulated. The right carotid artery was cannulated for arterial pressure and heart rate measurement. SBP were measured using a pressure transducer (Statham P - 50; Gould) coupled to a polygraph (AP-601G; Nihon Kohden, Osaka, Japan). The tested compounds were administered in bolus from the tail vein. An estimate of the dose level of a compound inducing a 30% fall in SBP (ED_{30}) was made using linear regression of change in SBP versus log dose.

Diuretic Study in Rats. SHR (250–280 g) were fasted overnight, gavaged with the drug dose in a total volume of 5 mg/kg of drug solution and vehicle, and immediately confined to metabolic cages. Individual urine was collected for 5 h after drug administration. Urinary volume as well as Na^+, K^+, and Cl^- excretion was measured using an electrolyte autoanalyzer (NAKL-2; Olympus Optical, Japan).

X-ray Structure Determinations. Intensity data were collected with a Rigaku AFC5R diffractometer and TeXsan software. Linear and empirical absorption corrections[38] as well as secondary extinction corrections were applied to the intensity data. The crystal structure was solved using a direct method[39] and expanded using Fourier techniques[40] and then refined with a full-matrix least-squares procedure against a set of unique reflections. Anomalous dispersion effects were taken into account in structure factor calculations. Absolute configuration of the structure was determined by comparing the observed Bijvoet differences with those calculated from the refined model; see also Table 4.

Table 4. Single-Crystal X-ray Crystallographic Analysis of the Hydrochloric Acid Salt of AL0670

formula	$C_{14}H_{25}N_6ClO_3$
formula weight	360.84
crystal size (mm)	0.3 × 0.25 × 0.20
crystal system	monoclinic
space group	$P2_1$
molecules/unit cell	4
unit cell dimensions: a, Å	11.510(1)
b, Å	14.880(1)
c, Å	11.612(1)
β, degrees	115.406(7)
density (calcd), g/cm^3	1.334
absorption coefficient, cm^{-1}	21.09
system used	Rigaku AFC5R
solution	direct methods
refinement method	full-matrix least-squares
number of reflections	2373
observed reflections	2093
wavelength, λ, Å	1.54178
R index	0.044

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Supplementary Material Available: Methods of data collection and crystal data for (+)-10 and tables listing atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, and torsion angles (21 pages). Ordering information is given on any current masthead page.

References

(1) Hamilton, T. C.; Weir, S. W.; Weston, A. H. Comparison of the Effects of BRL 34915 and Verapamil on Electrical and Mechanical Activity in Rat Portal Vein. *Br. J. Pharmacol.* **1986**, *88*, 103–111.
(2) Gillespie, J. S.; Sheng, H. The Lack of Involvement of Cyclic Nucleotides in the Smooth Muscle Relaxant Action of BRL 34915. *Br. J. Pharmacol.* **1988**, *94*, 1189–1197.
(3) Foster, C. D.; Fujii, K.; Kingdon, J.; Brading, A. F. The Effect of Cromakalim on the Smooth Muscle of the Guinea-pig Urinary Bladder. *Br. J. Pharmacol.* **1989**, *97*, 281–291.
(4) Farraway, L.; Huizinga, J. D. Potassium Channel Activation by Cromakalim Affects the Slow Wave Type Action Potential of Colonic Smooth Muscle. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 35–41.

(5) Edwards, G.; Henshaw, M.; Miller, M.; Weston, A. H. Comparison of the Effects of Several Potassium-Channel Openers on Rat Bladder and Rat Portal Veins *in vitro*. *Br. J. Pharmacol.* **1991**, *102*, 679–686.
(6) Robertson, D. W.; Steinberg, M. I. Potassium Channel Modulators: Scientific Applications and Therapeutic Promise. *J. Med. Chem.* **1990**, *33*, 1529–1541.
(7) Andersson, K.-E. Clinical Pharmacology of Potassium Channel Openers. *Pharmacol. Toxicol.* **1992**, *70*, 244–254.
(8) Takemoto, T.; Eda, M.; Okada, T.; Sakashita, H.; Matzno, S.; et al. Novel Potassium Channel Openers: Synthesis and Pharmacological Evaluation of New *N*-(Substituted-3-pyridyl)-*N'*-alkylthioureas and Related Compounds. *J. Med. Chem.*, **1994**, *37*, 18–25.
(9) Poll, T.; Sobczak, A.; Hartmann, H.; Helmchen, G. Diastereoface-Discriminative Metal Coordination in Asymmetric Synthesis: D-Pantolactone as Practical Chiral Auxiliary for Lewis Acid Catalyzed Diels-Alder Reactions. *Tetrahedron Lett.* **1985**, *26*, 3095–3098.
(10) Poll, T.; Abdel Hady, A. F.; Karge, R.; Linz, G.; Weetman, J.; Helmchen, G. *N*-Substituted Hydroxysuccinimides From (S)-Malic Acid as New Reagents for Asymmetric Diels-Alder Additions to Enoates. *Tetrahedron Lett.* **1989**, *30*, 5595–5598.
(11) Berson, J. A.; Ben-Efraim, D. A. Resolution, Stereochemical Correlation and Maximum Rotations of the Norbornane- and 5-Norbornene-2-carboxylic Acids. Isotope Dilution as an Aid in the Determination of Optical Purity. *J. Am. Chem. Soc.* **1959**, *81*, 4083–4087.
(12) Weinstock, J. A Modified Curtuis Reaction. *J. Org. Chem.* **1961**, *26*, 3511.
(13) Webb, R. L.; Labaw, C. S. Diphenyl Cyanocarbonimidate. A Versatile Synthon for the Construction of Heterocyclic Systems. *J. Heterocycl. Chem.* **1982**, *19*, 1205–1206.
(14) Ingersoll, A. W.; DeWitt, H. D. The Preparation and Resolution of DL-α-Fenchylamine. *J. Am. Chem. Soc.* **1951**, *73*, 3360–3362.
(15) Brown, H. C.; Ravindranathan, M.; Gundu Rao, C.; Chloupek, F. J.; Min-Hon, Rei. Structural Effects in Solvolytic Reactions. 27. Solvolysis of the *exo*- and *endo*-1,2-Diphenyl-2-norbornyl and -1,2-Dimethyl-2-norbornyl p-Nitrobenzoates and Chlorides. Definitive Evidence for the Classical Nature of the 1,2-Disubstituted Tertiary 2-Norbornyl Cations and Implications for the Structure of the Parent 2-Norbornyl Cation. *J. Org. Chem.* **1978**, *43*, 3667–3678.
(16) Kusumi, T.; Fukushima, T.; Ohtani, I.; Kakisawa, H. Elucidation of the Absolute Configurations of Amino Acids and Amines by the Modified Mosher's Method. *Tetrahedron Lett.* **1991**, *32*, 2939–2942.
(17) Manley, P. W.; Quast, U. Structure-Activity Studies for Potassium-Channel Opening in Pinacidil-Type Cyanoguanidine. *Chimia* **1991**, *45*, 88–89.
(18) Hof, R. P.; Quast, U.; Cook, N. S.; Blarer, S. Mechanism of Action and Systemic and Regional Hemodynamics of the Potassium Channel Activator BRL 34915 and its Enantiomers. *Circ. Res.* **1988**, *62*, 679–686.
(19) Pfeiffer, C. C. Optical Isomerism and Pharmacological Action, a Generation. *Science (Washington, D.C.)* **1956**, *124*, 29–31.
(20) Lehmann, F. P. A. A Quantitative Stereo-structure Activity Relationship Analysis of the Binding of Promiscuous Chiral Ligands to Different Receptors. *Quant. Struct. Act. Relat.* **1987**, *6*, 57–65.
(21) Ahnfelt-Rønne, I. Pinacidil. Preclinical Investigations. *Drugs* **1988**, *36*, 4–9.
(22) Goldberg, M. R. Clinical Pharmacology of Pinacidil, A Prototype for Drugs That Affect Potassium Channels. *J. Cardiovasc. Pharmacol.* **1988**, *12* (Suppl. 2), S41–S47.
(23) Atwal, K. S. Modulation of Potassium Channels by Organic Molecules. *Med. Res. Rev.* **1992**, *12*, 569–591.
(24) Hayashi, K.; Uchida, T.; Kido, Y.; Watanabe, M. Effects of the Novel Potassium Channel Opener AL0671 on Renal Hemodynamics and Function in Anesthetized Dogs. *Jpn. J. Pharmacol.* **1993**, *61* (Suppl. 1), 172P.
(25) Dunne, M. J.; Bullett, M. J.; Li, G.; Wollheim, C. B.; Petersen, O. H. Galanin Activates Nucleotide-dependent K+ Channels in Insulin-secreting Cells via a Pertussis Toxin-sensitive G Protein. *EMBO J.* **1989**, *8*, 413–420.
(26) Garrino, M. G.; Plant, T. D.; Henquin, J. C. Effects of Putative Activators of K^+ Channels in Mouse Pancreatic β-Cells. *Br. J. Pharmacol.* **1989**, *98*, 957–965.
(27) Newgreen, D. T.; Bray, K. M.; McHarg, A. D.; Weston, A. H.; Duty, S.; et al. The Action of Diazoxide and Minoxidil Sulfate on Rat Blood Vessels: a Comparison With Cromakalim. *Br. J. Pharmacol.* **1990**, *100*, 605–613.
(28) Hamilton, T. C. Cromakalim, Nicorandil and Pinacidil: Novel Drugs Which Open Potassium Channels in Smooth Muscle. *Gen. Pharmacol.* **1989**, *20*, 1–9.
(29) Pirotte, B.; De Tullio, P.; Lebrun, P.; Antoine, M.-H.; Fontaine, J.; et al. 3-(Alkylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-Dioxides as Powerful Inhibitors of Insulin Release from Rat Pancreatic B-Cells: A New Class of Potassium Channel Openers? *J. Med. Chem.* **1993**, *36*, 3211–3213.
(30) Pirotte, B.; Dupont, L.; De Tullio, P.; Masereel, B.; Schynts, M.; Delarge, J. Structural Study of Pinacidil, a Potassium-Channel Opener Belonging to the Chemical Class of *N*-Alkyl-*N''*-cyano-*N'*-pyridylguanidines. *Helv. Chim. Acta* **1993**, *76*, 1311–1318.
(31) Manley, P. W.; Quast, U. Structure-Activity Studies of Potassium Channel Opening in Pinacidil-Type Cyanoguanidines, Nitroethenediamines, Thioureas, and Ureas. *J. Med. Chem.* **1992**, *35*, 2327–2340.
(32) Nishikawa, M.; Gohda, M.; Matzno, S.; Uchida, T.; Nakamura, N.; et al. Vasorelaxant Properties of AL0671, A Novel Potassium Channel Opener, in Comparison With Other Potassium Channel Openers. *Jpn. J. Pharmacol.* **1993**, *61* (Suppl. 1), 116P.
(33) Gohda, M.; Ebisu, H.; Matzno, S.; Okita, M.; Kido, H.; et al. Antihypertensive and Serum Lipids Lowering Effects of AL0671, A Novel Potassium Channel Opener. *Jpn. J. Pharmacol.* **1993**, *61* (Suppl. 1), 209P.
(34) Matzno, M.; Sato, H.; Takai, S.; Aida, Y.; Karasaki, S.; et al. Active Mechanism of AL0671, A Novel Potassium Channel Opener, on Potassium Current in Rat Aortic Smooth Muscle Cells. *Jpn. J. Pharmacol.* **1993**, *61* (Suppl. 1), 128P.
(35) Kroz, A.; Helmchen, G. Total Synthesis of Sandalwood Fragrances: (Z)- and (E)-β-santalol and Their Enantiomers, ent-β-Santalene. *Tetrahedron: Asymmetry* **1990**, *1*, 537–540.
(36) Poll, T.; Helmchen, G.; Bauer, B. Influence of Polar Groups in Thermal and Lewis Acid Promoted Asymmetric Diels-Alder Additions: Lactic acid Derivatives as Practical Highly Selective and Configurationally Dichotomic Reagents. *Tetrahedron Lett.* **1984**, *21*, 2191–2194.
(37) Evans, D. A.; Chapman, K. T.; Bisaha, J. Asymmetric Diels-Alder Cycloaddition Reactions with Chiral α,β-Unsaturated *N*-Acyloxazolinones. *J. Am. Chem. Soc.* **1988**, *110*, 1238–1256.
(38) Walker, N.; Stuart, D. An Empirical Method for Correcting Diffractometer Data for Absorption Effects. *Acta Crystallogr.* **1983**, *A39*, 158–166.
(39) Burla, M. C.; Camalli, M.; Cascarano, G.; Giacovazzo, C.; Polidori, G.; et al. SIR88 - A Direct Methods Program for the Automatic Solution of Crystal Structures. *J. Appl. Crystallogr.* **1989**, *22*, 389–393.
(40) Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; Garcia-Granda, S.; et al. The DIRDIF program system. Technical Report of Crystallography Laboratory, University of Nijmegen: The Netherlands, 1992.