Synthesis and Pharmacological Evaluation of *N*-(6-Functionalized-amino-3-pyridyl)-*N'*-bicycloalkyl-*N''*-cyanoguanidines as Antihypertensive Agents

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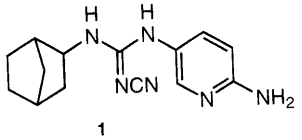
A series of amino acid conjugates of *N*-(6-amino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (4) were prepared and evaluated as antihypertensive agents. The parent compound 4 showed potent potassium channel-opening and antihypertensive activities, but with undesirable changes of the urinary balance of electrolytes. However, alanine and histidine congeners (9, 19) reduced this undesirable side effect of 4 through improved pharmacokinetics without loss of antihypertensive activity. They also provided additional information on the structural requirements for pinacidil-type potassium channel openers.

Key words potassium channel opener; functionalized congener approach; antihypertensive agent

Potassium channel (K^+ channel) openers induce vasorelaxation through hyperpolarization of the cell membrane in vascular smooth muscle.[1-3] A number of compounds have shown effectiveness in animal models of hypertension by opening ATP-sensitive K^+ channels (K^+_{ATP}). However, their clinical trials as antihypertensive agents have often proved disappointing because of undesirable side effects, such as reflex tachycardia, increase in plasma renin activity, and sodium and water retention.[4] These may have a variety of causes, such as the opening of K^+_{ATP} in a number of different tissues or a rapid fall in blood pressure due to rapid absorption. Our approach to these problems is to change the pharmacokinetics by structural modification of *N*-(6-amino-3-pyridyl)-*N'*-bicycloalkyl-*N''*-cyanoguanidine derivatives (**1**), which have recently been reported as pinacidil (**2**) type K^+ channel openers (Fig. 1).[5] The fact that little attention has been given to pinacidil-type compounds,[6,7] in contrast to the considerable progress of structure–activity relationship (SAR) studies on cromakalim (**3**) type compounds,[8-12] also stimulated us to expand our work in this area.

It may be possible to improve pharmacokinetics without loss of K^+ channel opening activity through incorporation of a functionalized chain into the primary structure at a point where it does not disturb the biological activity. This approach is described as a "congener approach" by Goodman and co-workers[13] and has been established successfully in the field of adenosine receptors[14] and catecholamines.[15] Their approach to isoproterenol in particular has resulted in dramatic modulation of both potency and duration of action *in vivo* through attachment of long-chain amines or oligopeptides to the pharmacophore. Our findings suggest that a functionalized congener approach could provide the desired pharmacological profile for new K^+ channel openers.



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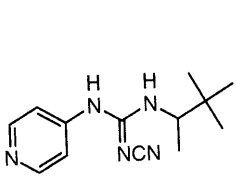
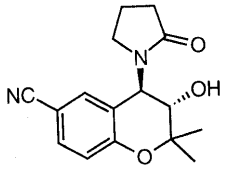


2 Pinacidil



3 Cromakalim

Fig. 1. Chemical Structures of K^+ Channel Openers

Chemistry

A general procedure for the synthesis of a novel series of *N*-(6-functionalized-amino-3-pyridyl)-*N'*-bicycloalkyl-*N''*-cyanoguanidine was set up as shown in Chart 1. Racemic *exo*-norbornyl amine was used to prepare the parent compound **4** in all cases. Since L- or D-amino acid was used, the products were mixtures of diastereomers. No attempt was made to separate these mixtures.

Introduction of an amino acid moiety at C-6 on the pyridine ring in **4** was carried out by condensation with an *N*-protected amino acid using dicyclohexylcarbodiimide (DCC) in pyridine, followed by deprotection, using an *N*-*tert*-butyloxycarbonyl (Boc) moiety under acidic conditions, or an *N*-benzyloxycarbonyl (Z) moiety under catalytic hydrogenation with 10% palladium on activated carbon, to give the desired amide.

Results and Discussion

The model compound **4** chosen for functionalization has shown potent K^+ channel opening activity and antihypertensive activity in earlier studies.[5] From structure–activity relationships in analogues of **4**, the amino group at C-6 on the pyridine ring of **4** was selected as the attachment point of the functionalized chain, since the norbornyl or cyanoguanidyl moiety had proved very sensitive to structural change.

The compounds were first evaluated by measuring inhibition of spontaneous mechanical activity in rat portal vein (*in vitro* activity)[16-19] and i.v. hypotensive activity in normotensive rats (NTRs) (*in vivo* activity). The results are summarized in Table 1. As was previously observed,[5] simple substituents on the amine functional group merely abrogated the activity (**5**—**7**). Interestingly,

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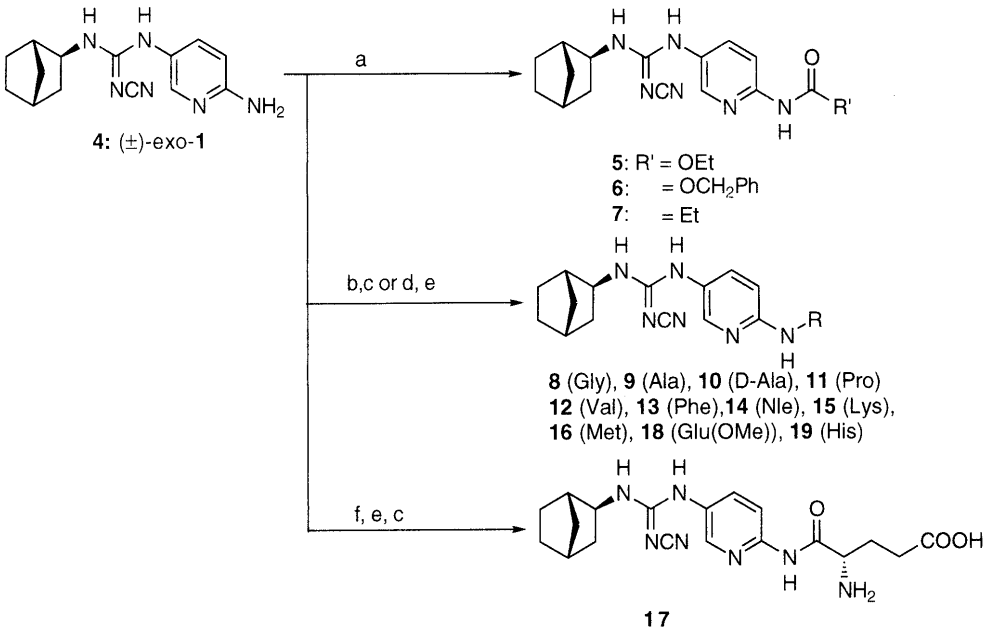


[a] Reagents: (a) R'COCl, pyridine; (b) Boc-amino acid, DCC, pyridine; (c) HCl/dioxane; (d) Z-amino acid, DCC, pyridine; (e) H_2, Pd/C; (f) Boc-Glu (OBzl), DCC, pyridine

Chart 1

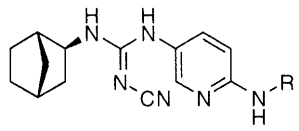
the attachment of glycine to the C-6 amino group gave a compound (**8**) with hypotensive activity, although the potency of *in vitro* activity was 30-fold less than that of **4**. Further syntheses were based on the expectation that the attachment of an amino acid to the amine function on the pyridine ring would not abrogate the hypotensive activity. The alanine derivative **9** showed a marked increase in both *in vitro* and *in vivo* activity, and was practically equipotent to the parent compound (**4**), while the reduced potency of the proline derivative **11** suggests that *N*-alkylation of the α-amino group exceeds the steric limitation for efficient binding. The low potency of **7** indicated that the α-amino group in the amino acid moiety may significantly influence the retention of biological activity. A comparison of **9**, the valine derivative **12**, and the phenylalanine derivative **13** showed that a branched chain or a sterically bulky substituent is not well tolerated near the primary pharmacophore. While potency was reduced by extension of the alkyl chain of alanine to norleucine (**14**), it was maintained upon introduction of an amine moiety at the terminal of an extended chain (lysine derivative, **15**). The methionine derivative **16** showed the most potent *in vitro* activity among this series of congeners, although *in vivo* it was not particularly potent compared to **9**. In view of the relatively low *in vivo* activity of the carboxylic acid congener (glutamic acid, **17**) and ester congener (glutamic acid γ-methyl ester, **18**), a basic group provided a higher hypotensive activity than an acidic or a neutral group as a terminal substituent. These *in vitro* activities were antagonized by glibenclamide and 3,4-diaminopyridine, known to be K^+ channel blockers.

We previously reported that no significant difference was found between the potency of (+)-**4** and (−)-**4**.[20] Pfeiffer's rule states that highly potent chiral compounds display a large difference in potency between their enantiomers.[21,22] For pinacidil, the relatively small eudismic ratio ((*R*)-(−)-pinacidil being **12** times more potent than (*S*)-(+)-pinacidil)[23] seems unusual in that it is counter to Pfeiffer's rule; cromakalim enantiomers possess a eudismic ratio of 100 to 200.[24] The L-alanine derivative **9** was found to be 100 times more potent than the D-alanine derivative **10** *in vitro*. This suggests that there must be an important region on the channel to the pharmacophore-binding site that recognizes the chirality of the amino acid moiety in the congeners. This may provide helpful information for constructing an SAR model using overlapping pinacidil- and cromakalim-type K^+ channel openers that have apparently unrelated structures.

Figure 2 shows the effects of **4**, **9**, **15**, **16** (0.3 mg/kg, respectively), and **19** (1.0 mg/kg) on systolic blood pressure (SBP) (% change) at various time intervals (min). Compounds **9**, **15**, and **16** produced rapid falls in blood pressure, which were similar to that of the parent compound **4**. It is noteworthy in the case of the histidine derivative **19** that, even though the maximum hypotensive activity was several-fold less potent than that of **4**, its duration of action was prolonged. Thus, the i.v. hypotensive activity of **19** (1 mg/kg) was characterized by slow onset and prolonged duration of action compared with that of **4** (0.3 mg/kg). This result suggests the possibility of achieving prolonged duration of action by using the functionalized congener approach to K^+ channel openers.

Next, *p.o.* antihypertensive activity in spontaneously hypertensive rats (SHRs) was determined for selected compounds that showed potent (**4**, **9**, **12**, **16**, **19**) and poor (**6**) *in vivo* activity. As indicated in Fig. 3, considerable antihypertensive activity was observed in the alanine derivative **9**, and the order of potency (**4**>**9**>**12**, **16**>**6**) was nearly identical to that for *in vitro* activity. The

Table 1. Inhibition of *in Vitro* and *in Vivo* Activities by Test Compounds



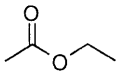
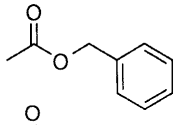
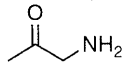
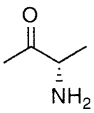
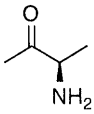
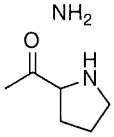
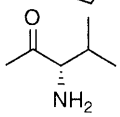
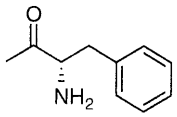
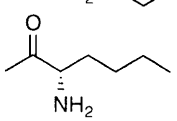
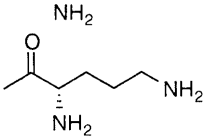
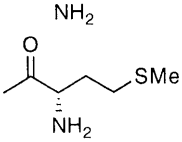
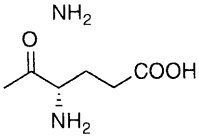
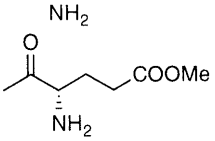
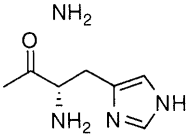
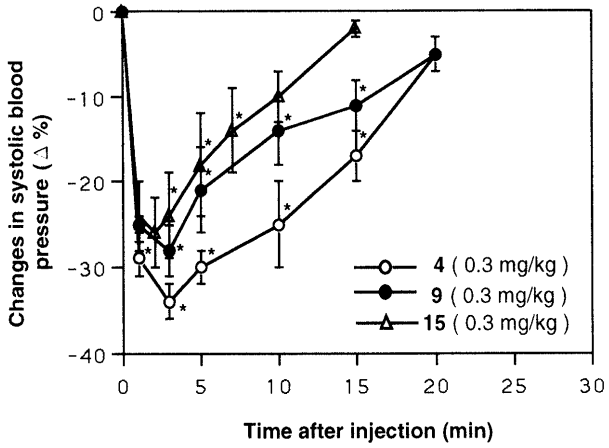
No.	R[a]	pEC_{100}[b]	Dose (mg/kg)	Max. fall in SBP (%)[c]
4	H	7.0	0.1	21±2
			0.3	34±1
5		No response		
6		No response		
7		No response	0.3	1±4
			1.0	4±3
8 Gly		5.5	0.3	16±3
			1.0	28±6
			3.0	32±2
9 Ala		6.5	0.1	22±4
			0.3	30±1
			1.0	34±3
10 D-Ala		4.5	0.3	8±5
			1.0	12±9
11 Pro		4.5	1.0	11±1
			3.0	21±4
12 Val		6.0	0.1	12±1
			0.3	16±3
			1.0	24±5
13 Phe		6.0	0.3	16±2
			1.0	25±1
14 Nle		5.0	0.1	10±1
			0.3	16±3
			1.0	23±5
15 Lys		6.1	0.1	12±3
			0.3	27±4
			1.0	33±7
16 Met		6.9	0.1	17±3
			0.3	23±3
			1.0	30±4
17 Glu		6.0	0.1	9±2
			0.3	17±4
			1.0	25±3
18 Glu(OMe)		5.9	0.1	10±3
			0.3	19±5
			1.0	24±4
19 His		5.8	0.1	7±3
			0.3	17±4
			1.0	22±4

Table 1. (continued)

No.	R[a]	pEC_{100}[b]	Dose (mg/kg)	Max. fall in SBP (%)[c]
Pinacidil		7.0	0.1	25±4
			0.3	31±3

a) Absolute configurations of the amino acid moiety are (L)-form except for **10** ((D)-form). *b*) Negative logarithm of concentration causing 100% inhibition of spontaneous mechanical activity in rat portal vein. Values represent means of 4 independent experiments. *c*) Hypotensive activity in anesthetized normotensive rat (male) by *i.v.* injection. SBP was measured for 30 min after injection; all values express maximum % fall in SBP ($n=4$).



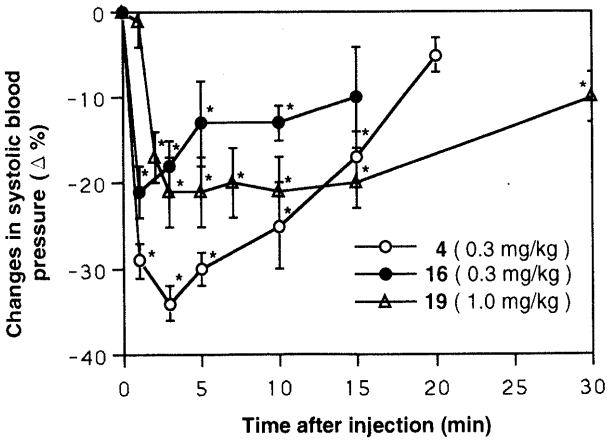


Fig. 2. Hypotensive Activity at Various Time Intervals (min) after i.v. Injection in Anesthetized Normotensive Rats

Dosage of each compound was 0.3 mg/kg except for **19**. Values are mean ± S.E.M. ($n=4$—5). Asterisks indicate significant differences from before injection; $*p<0.05$ (Dunnett's method).

histidine derivative **19** at an equal dosage to **4** (5 mg/kg) was found to have an equal duration of action (>7 h), although its *in vitro* activity was 100 times less than that of **4**.

In the *in vitro* assay, the drug was added directly to the medium and, therefore, absorption and metabolism of the drug played no significant part in the drug effect. On the other hand, the effects observed in *in vivo* hypotensive activity and antihypertensive activity are a composite of drug-channel interaction plus absorption, biodisposition and metabolism of the drug. The good correlation between *in vitro* activity and i.v. hypotensive activity suggest that the congener is not merely a prodrug which acts after

hydrolysis from the pharmacophore of the attached amino acid moiety, but rather a directed active analogue, for the activation of which no metabolic cleavage step is necessary. Moreover **9** showed equipotent antihypertensive activity to **4** in oral administration, indicating that it was readily absorbed in the gut and that its antihypertensive effect arose mainly from its action not as a pro-drug, but as a drug in its own right. The reason for the lasting antihypertensive effect of **19** is still unclear; however, its similar duration of action in i.v. and *p.o.* administration suggests that it may follow a different metabolic pathway from other congeners after ready absorption.

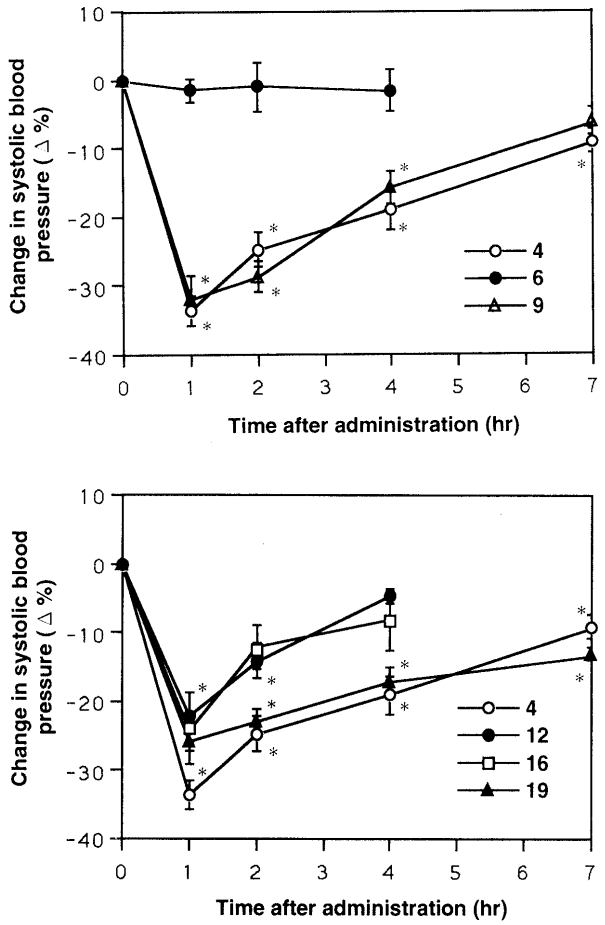


Fig 3. Antihypertensive Effects of a Single Oral Administration in Conscious SHR

Dosage of each compound was 5 mg/kg. Values are mean ± S.E.M. ($n=4$—5). Asterisks indicate significant differences from before administration; $*p<0.05$ (Dunnett's method).

In line with the previous finding[20] of oral diuretic activity in SHR at a dose of 5 mg/kg of **4**, urinary volume did not decrease significantly. However, the urinary Na^+/K^+ ratio did decrease significantly. This profile is regarded as a disadvantage of **4** in the treatment of hypertension. Attention was therefore focused on evaluation of oral diuretic activity of **9** and **19**, which have potent antihypertensive activity. As shown in Table 2, compound **4** increased urinary volume, which differed from the previous result.[20] The urinary Na^+/K^+ ratio, however, decreased significantly in a similar manner. At a dose which was sufficient to produce antihypertensive activity, compound **9** had the effect of increasing urinary volume but without changing excretion of Na^+ and K^+ or the urinary ratio of Na^+/K^+, whereas pinacidil significantly decreased both. Compound **19** did not change any of these. The results obtained in this study indicate that **9** possesses a diuretic activity which does not affect the urinary balance of electrolytes. This is a more desirable profile for an antihypertensive agent than that of the parent compound **4**.

In conclusion, the alanine and histidine congeners (**9**, **19**) display improved pharmacokinetics without loss of the antihypertensive activity of the corresponding parent compound **4**. They also provided valuable information on the structural requirements for pinacidil-type K^+ channel openers. We have thus demonstrated that the functionalized congener approach is an effective method of overcoming the disadvantages of K^+ channel openers.

Experimental

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 micro melting point apparatus and are uncorrected. IR and NMR spectra, which were consistent with the proposed structures, were recorded on a Shimadzu IR-420 instrument for IR and, unless otherwise indicated, a Bruker AC-200 spectrometer (200 MHz for ^{1}H-NMR and 50 MHz for ^{13}C-NMR) for NMR using dimethyl sul-

Table 2. Diuretic Effects of Compounds **4**, **9**, **19**, and Pinacidil[a]

No.	Dose (mg/kg)	Urinary volume (ml/kg/5 h)	Na^+ excretion (meq/kg/5 h)	K^+ excretion (meq/kg/5 h)	Na/K ratio
4	Vehicle	4.2±0.9	1.24±0.12	0.40±0.08	3.4 ±0.3
	1	9.4±3.2	1.59±0.27	0.54±0.08	3.0 ±0.3
	5	15.5±4.2*	1.85±0.40	0.77±0.07**	2.3 ±0.3*
9	Vehicle	10.5±1.2	1.77±0.24	1.05±0.07	1.7 ±0.2
	1	9.3±2.3	1.35±0.17	0.96±0.12	1.4 ±0.1
	5	26.1±3.3***	1.82±0.46	1.06±0.17	1.6 ±0.2
19	Vehicle	10.5±1.5	1.38±0.34	1.42±0.21	0.92±0.18
	1	11.6±1.1	1.50±0.20	1.80±0.06	0.83±0.10
	5	14.2±3.2	1.41±0.35	1.87±0.36	0.77±0.20
Pinacidil	Vehicle	7.8±0.6	1.88±0.13	1.73±0.16	1.0 ±0.2
	1	7.3±1.8	1.52±0.17	1.63±0.23	1.0 ±0.2
	5	2.8±0.6***	0.19±0.05***	1.31±0.26	0.1 ±0.0***

a) Compounds were administered orally to spontaneously hypertensive rats ($n=5$ or 6). Values are means ± S.E.M. Asterisks (*, **, or ***) represent significant differences from control values at $p<0.05$, 0.01, and 0.001, respectively.

foxide-d_6 as a solvent and tetramethylsilane (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.

***N*-(6-Benzyloxycarbonylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine Hydrochloride (6)** Benzyl chloroformate (0.55 ml, 3.85 mmol) was added to a solution of **4** (1.00 g, 3.70 mmol) in pyridine (5 ml) and tetrahydrofuran (3 ml) at 0 °C. After having been stirred for 15 h at room temperature, the mixture was evaporated *in vacuo*, and the residue was washed with saturated aqueous $NaHCO_3$. Further purification was accomplished by column chromatography (silica gel, $CHCl_3$: MeOH = 50 : 1) to give the product (1.00 g, 67%) as a white powder. The product (1.00 g) was dissolved in MeOH (5 ml) and treated with 1.58 N HCl–MeOH (1.56 ml) to give **6** (0.99 g, 91%) as a white powder: mp 152—153 °C. ^{1}H-NMR δ: 1.72—1.08 (m, 8H), 2.23 (m, 2H), 3.70 (m, 1H), 5.21 (s, 2H), 7.27 (d, 1H, J=6.7 Hz), 7.43 (m, 5H), 7.82—7.71 (m, 2H), 8.21 (d, 1H, J=1.5 Hz), 9.29 (s, 1H), 10.76 (s, 1H). ^{13}C-NMR δ: 25.8 (t), 27.9 (t), 34.8 (t), 35.2 (d), 38.7 (t), 42.0 (d), 54.8 (d), 66.3 (t), 113.2 (d), 116.6 (s), 128.0 (d), 128.1 (d), 128.4 (d), 130.4 (s), 135.2 (d), 136.2 (s), 139.7 (d), 147.4 (s), 153.3 (s), 156.8 (s). IR (KBr) cm^{-1}: 3200, 2950, 2200, 1720. HR-SIMS m/z: 405.1995 $(M+H)^+$. Calcd for $C_{22}H_{24}N_6O_2$: 405.2028. *Anal.* Calcd for $C_{22}H_{24}N_6O_2 \cdot HCl$: C, 59.93; H, 5.71; N, 19.06. Found: C, 59.63; H, 5.57; N, 18.78.

Compounds **5** and **7** were prepared in the same way.

***N*-(6-Ethoxycarbonylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine Hydrochloride (5)** Yield 55%. mp 144—145 °C. ^{1}H-NMR δ: 1.26 (t, 3H, J=7.1 Hz), 1.07—1.72 (m, 8H), 2.24 (m, 2H), 3.68 (m, 1H), 4.18 (q, 2H, J=7.1 Hz), 7.18 (d, 1H, J=6.0 Hz), 7.74 (m, 2H), 8.17 (d, 1H, J=1.4 Hz), 9.16 (s, 1H), 10.50 (s, 1H). ^{13}C-NMR δ: 14.4 (q), 25.8 (q), 27.9 (t), 34.8 (t), 35.2 (d), 41.9 (d), 54.9 (d), 60.9 (t), 113.0 (d), 116.6 (s), 130.1 (s), 135.1 (d), 140.1 (d), 147.7 (s), 153.5 (s), 156.9 (s). IR (KBr) cm^{-1}: 3200, 2950, 2200, 1740. HR-SIMS m/z: 343.1862 $(M+H)^+$. Calcd for $C_{17}H_{22}N_6O_2$: 343.1872. *Anal.* Calcd for $C_{17}H_{22}N_6O_2 \cdot 0.6HCl$: C, 56.05; H, 6.25; N, 23.07. Found: C, 55.90; H, 6.24; N, 23.05.

***N*-(6-Propionylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine Hydrochloride (7)** Yield 31%. mp 228—229 °C. ^{1}H-NMR δ: 1.07 (t, 3H, J=7.5 Hz), 1.1—1.3 (m, 3H), 1.42 (br, 4H), 1.64 (m, 1H), 2.23 (br, 2H), 2.38 (q, 2H, J=7.5 Hz), 3.61 (br, 1H), 6.95 (d, 1H, J=6.6 Hz), 7.58 (dd, 1H, J=8.9, 2.5 Hz), 8.05 (d, 1H, J=8.9 Hz), 8.13 (d, 1H, J=2.5 Hz), 8.85 (s, 1H), 10.41 (s, 1H). ^{13}C-NMR δ: 9.4 (q), 25.8 (t), 27.8 (t), 29.2 (t), 34.7 (t), 35.1 (d), 38.6 (t), 41.7 (d), 54.8 (d), 113.0 (d), 116.8 (s), 130.0 (s), 133.5 (d), 143.1 (d), 148.8 (s), 157.2 (s), 172.6 (s). IR (KBr) cm^{-1}: 3250, 2950, 2150, 1700. HR-SIMS m/z: 327.1902 $(M+H)^+$. Calcd for $C_{17}H_{22}N_6O$: 327.1923. *Anal.* Calcd for $C_{17}H_{22}N_6O \cdot 0.3H_2O$: C, 61.54; H, 6.87; N, 25.33. Found: C, 61.39; H, 6.61; N, 25.30.

***N*-(6-Alanylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (9)** DCC (20.3 g, 98.5 mmol) was added to a solution of **4** (24.2 g, 89.5 mmol) and *tert*-butyloxycarbonyl-L-alanine (18.6 g, 98.5 mmol) in pyridine (300 ml) at room temperature. The mixture was stirred for 21 h at room temperature, then insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by recrystallization from MeOH–$CHCl_3$–Et_2O to give the product (30.6 g, 77%). This product (30.0 g) was dissolved in $CHCl_3$ (200 ml), and 5.93 N HCl ethylene glycol dimethyl ether solution (229 ml) was added slowly over a 30 min period. Stirring was continued for 30 min at 0 °C and then for 1 h at room temperature after the addition was completed. Potassium carbonate was added to adjust the pH to pH 8—9, and the mixture was extracted with $CHCl_3$ (1 l × 3). The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated to give **9** (17.5 g, 75%) as a white powder: mp 179—180 °C. ^{1}H-NMR δ: 1.23 (d, 3H, J=6.0 Hz), 1.00—1.55 (m, 7H), 1.55—1.75 (m, 1H), 2.20 (m, 2H), 3.49 (q, 1H, J=7.0 Hz), 3.68 (m, 1H), 7.92 (d, 1H, J=6.0 Hz), 7.63 (dd, 1H, J=10.0, 2.0 Hz), 8.08 (d, 1H, J=10.0 Hz), 8.15 (d, 1H, J=2.0 Hz). ^{13}C-NMR δ: 21.1 (q), 25.9 (d), 27.9 (d), 34.8 (t), 35.2 (d), 38.5 (t), 41.9 (d), 50.6 (d), 54.9 (d), 112.6 (d), 116.9 (s), 130.4 (s), 133.5 (d), 143.2 (d), 148.1 (s), 157.1 (s), 175.1 (s). IR (KBr) cm^{-1}: 3300, 2900, 2150, 1680. HR-SIMS m/z: 342.1021 $(M+H)^+$. Calcd for $C_{17}H_{23}N_7O$: 342.2031. *Anal.* Calcd for $C_{17}H_{23}N_7O$: C, 59.81; H, 6.79; N, 28.72. Found: C, 59.81; H, 6.81; N, 28.53.

Compounds **10** and **13** were prepared in the same way.

***N*-(6-D-Alanylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (10)** Yield 29%. mp 133—138 °C. ^{1}H-NMR δ: 1.0—1.1 (m, 3H), 1.19 (d, 3H, J=6.9 Hz), 1.41 (br, 4H), 1.62 (m, 1H), 2.49 (m, 2H), 3.47 (q, 1H, J=6.9 Hz), 3.60 (br, 1H), 6.95 (d, 1H, J=6.6 Hz), 7.60 (dd, 1H, J=2.5, 8.7 Hz), 8.06 (d, 1H, J=8.7 Hz), 8.13 (d, 1H, J=2.5 Hz). ^{13}C-NMR δ: 21.0 (q), 25.8 (t), 27.8 (t), 34.7 (t), 35.1 (d), 39.0 (t), 41.7 (d), 50.6 (d), 54.8 (d), 112.5 (d), 116.7 (s), 130.4 (s), 133.6 (d), 143.2 (d), 148.1 (s), 157.1 (s), 175.0 (s). IR (KBr) cm^{-1}: 3250, 2950, 2150, 1560. HR-SIMS m/z: 342.2018 $(M+H)^+$. Calcd for $C_{17}H_{23}N_7O$: 342.2031. *Anal.* Calcd for $C_{17}H_{23}N_7O \cdot 0.3H_2O$: C, 58.87; H, 6.86; N, 28.27. Found: C, 58.92; H, 6.88; N, 28.07.

***N*-(6-Phenylalanylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (13)** Yield 62%. A white amorphous solid. ^{1}H-NMR δ: 1.0—1.35 (m, 3H), 1.35—1.6 (m, 4H), 1.65 (dd, 1H, J=8.0, 13.2 Hz), 2.23 (br, 2H), 2.73 (dd, 1H, J=8.4, 13.4 Hz), 3.06 (dd, 1H, J=5.0, 13.4 Hz), 3.64 (br, 1H), 3.72 (dd, 1H, J=5.0, 8.4 Hz), 7.08 (d, 1H, J=6.6 Hz), 7.1—7.4 (m, 5H), 7.63 (dd, 1H, J=2.6, 8.8 Hz), 8.08 (d, 1H, J=8.8 Hz), 8.15 (d, 1H, J=2.6 Hz), 8.99 (br, 1H). ^{13}C-NMR δ: 25.9 (t), 27.9 (t), 34.8 (t), 35.2 (d), 38.7 (t), 40.5 (t), 41.8 (d), 54.7 (d), 54.9 (d), 56.5 (d), 112.7 (d), 116.8 (s), 126.1 (d), 128.1 (d), 129.2 (d), 130.4 (s), 133.3 (d), 138.4 (s), 143.0 (d), 148.0 (s), 157.0 (s), 173.6 (s). IR (KBr) cm^{-1}: 3200, 2900, 2020, 1670. HR-SIMS m/z: 418.2385 $(M+H)^+$. Calcd for $C_{17}H_{23}N_7O$: 418.2354. *Anal.* Calcd for $C_{23}H_{27}N_7O \cdot 0.5H_2O$: C, 64.77; H, 6.62; N, 22.99. Found: C, 64.76; H, 6.47; N, 23.23.

***N*-(6-Methionylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (16)** Compound **16** was prepared using a method similar to that described for **9**, except for the deprotection of the Boc group, which was carried out by treatment with 25% HBr in acetic acid in a mixed solvent (CH_2Cl_2 : MeOH = 3 : 1) for 1 h at room temperature. Yield 48%. mp 78—82 °C. ^{1}H-NMR δ: 1.0—2.0 (m, 10H), 2.05 (s, 3H), 2.15—2.3 (m, 2H), 2.45—2.7 (m, 2H), 3.4—3.55 (m, 1H), 3.55—3.7 (m, 1H), 6.99 (d, 1H, J=6.0 Hz), 7.62 (dd, 1H, J=8.0, 2.0 Hz), 8.09 (d, 1H, J=8.0 Hz), 8.15 (d, 1H, J=2.0 Hz), 8.9 (br s, 1H). IR (KBr) cm^{-1}: 3600—3000, 2950, 2850, 2160, 1670. HR-SIMS m/z: 402.2087 $(M+H)^+$. Calcd for $C_{19}H_{27}N_7OS$: 402.2074. *Anal.* Calcd for $C_{19}H_{27}N_7OS \cdot 0.5H_2O$: C, 55.58; H, 6.87; N, 23.88. Found: C, 55.78; H, 6.94; N, 23.78.

***N*-(6-Norleucylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (14)** A solution of **4** (0.50 g, 1.9 mmol) and carbobenzyloxy-L-norleucine (0.59 g, 2.2 mmol) was prepared in pyridine (10 ml), and DCC (0.46 g, 2.2 mmol) was added at room temperature. The mixture was stirred for 24 h at room temperature, then insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, $CHCl_3$: MeOH = 30 : 1) to give the product (0.76 g, 80%). This product (0.40 g) was dissolved in MeOH (2 ml), 10% Pd on charcoal (0.05 g) was added, and the mixture was stirred for 3 h under a hydrogen atmosphere. The catalyst was removed by filtration through Celite and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, $CHCl_3$: MeOH = 10 : 1) to give **14** (168 mg, 49%) as a white amorphous solid. ^{1}H-NMR (Bruker AM-500 spectrometer, 500 MHz) δ: 0.86 (t, 3H, J=7.1 Hz), 1.09 (br d, 2H, J=9.7 Hz), 1.18 (br t, 1H, J=10.6 Hz), 1.27—1.35 (m, 4H), 1.39—1.47 (m, 5H), 1.63—1.69 (m, 2H), 2.20 (br, 1H), 2.24 (br d, 1H, J=6.7 Hz), 3.35 (dd, 1H, J=7.7, 5.2 Hz), 3.62 (br, 1H), 6.95 (d, 1H, J=6.5 Hz), 7.61 (dd, 1H, J=8.9, 2.7 Hz), 8.08 (d, 2H, J=8.9 Hz), 8.14 (d, 1H, J=2.7 Hz), 8.5—9.1 (br, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 13.8 (q), 22.0 (d), 25.8 (d), 27.4 (d), 27.8 (d), 34.3 (t), 34.7 (t), 35.1 (d), 38.4 (t), 41.7 (d), 54.8 (d), 55.0 (d), 112.6 (d), 116.8 (s), 130.3 (s), 133.6 (d), 143.2 (d), 148.1 (s), 157.1 (s), 174.7 (s). IR (KBr) cm^{-1}: 3200, 2920, 2150, 1560, 1500. HR-SIMS m/z: 384.2451 $(M+H)^+$. Calcd for $C_{20}H_{29}N_7O$: 384.2499. *Anal.* Calcd for $C_{20}H_{29}N_7O \cdot 0.3H_2O$: C, 61.77; H, 7.67; N, 25.21. Found: C, 61.64; H, 7.70; N, 24.90.

Compounds **8**, **11**, **12**, and **18** were prepared in the same way.

***N*-(6-Glycylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (8)** Yield 21%. mp 210—211 °C. ^{1}H-NMR δ: 1.01—1.30 (m, 3H), 1.35—1.60 (m, 4H), 1.61—1.72 (m, 1H), 2.18—2.30 (m, 2H), 3.31 (s, 2H), 3.64 (br, 1H), 7.00 (d, 1H, J=6.4 Hz), 7.62 (dd, 1H, J=8.9, 2.5 Hz), 8.09 (d, 1H, J=8.9 Hz), 8.15 (d, 1H, J=2.5 Hz). ^{13}C-NMR δ: 25.9 (t), 27.9 (t), 34.9 (t), 35.2 (d), 38.7 (t), 41.8 (d), 45.0 (t), 54.7 (d), 112.6 (d), 116.8 (s), 130.4 (s), 133.4 (d), 143.0 (d), 147.9 (s), 157.1 (s), 172.1 (s). IR (KBr) cm^{-1}: 3400—3000, 2950, 2850, 2150. HR-SIMS m/z: 328.1836 $(M+H)^+$. Calcd for $C_{16}H_{21}N_7O$: 328.1884.

Anal. Calcd for $C_{16}H_{21}N_7O \cdot 0.5H_2O$: C, 58.06; H, 6.52; N, 29.62. Found: C, 57.95; H, 6.43; N, 29.25.

***N*-(6-Prolylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (11)** Yield 20%. mp 123—126 °C. ^{1}H-NMR δ: 1.0—1.9 (m, 11H), 1.9—2.15 (m, 1H), 2.15—2.35 (m, 2H), 2.7—3.0 (m, 2H), 3.55—3.7 (m, 1H), 3.75 (dd, 1H, J=9.0, 5.0 Hz), 7.01 (d, 1H, J=6.0 Hz), 7.63 (dd, 1H, J=8.0, 2.0 Hz), 8.08 (d, 1H, J=8.0 Hz), 8.14 (d, 1H, J=2.0 Hz), 8.95 (br s, 1H), 10.29 (br s, 1H). ^{13}C-NMR δ: 25.9 (t), 26.0 (t), 27.9 (t), 30.3 (t), 34.8 (t), 35.2 (d), 38.4 (t), 41.6 (d), 46.7 (t), 54.9 (d), 60.5 (d), 112.4 (d), 116.6 (s), 130.6 (s), 133.6 (d), 143.3 (d), 147.6 (s), 157.2 (s), 173.6 (s). IR (KBr) cm^{-1}: 3600—3000, 2900, 2850, 2150, 1670. HR-SIMS *m/z*: 368.2201 $(M+H)^+$. Calcd for $C_{19}H_{26}N_7O$: 368.2197. *Anal.* Calcd for $C_{19}H_{26}N_7O \cdot 0.7H_2O$: C, 59.89; H, 7.25; N, 25.73. Found: C, 59.79; H, 6.61; N, 25.38.

***N*-(6-Valylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (12)** Yield 11%. mp 114—116 °C. ^{1}H-NMR δ: 0.83 (d, 3H, J=6.0 Hz), 0.93 (d, 3H, J=6.0 Hz), 1.0—1.5 (m, 7H), 1.55—1.75 (m, 1H), 1.9—2.15 (m, 1H), 2.15—2.3 (m, 2H), 3.23 (d, 1H, J=5.0 Hz), 3.55—3.7 (m, 1H), 7.01 (d, 1H, J=6.0 Hz), 7.61 (dd, 1H, J=6.0, 3.0 Hz), 8.13 (d, J=3.0 Hz). ^{13}C-NMR δ: 16.9 (q), 19.5 (q), 25.9 (t), 27.9 (t), 31.4 (d), 34.8 (t), 35.2 (d), 38.4 (t), 41.8 (d), 54.9 (d), 79.1 (d), 112.8 (d), 117.2 (s), 130.4 (s), 133.6 (d), 143.2 (d), 148.1 (s), 157.5 (s), 174.1 (s). IR (KBr) cm^{-1}: 3600—3000, 2950, 2850, 2200, 1680. HR-SIMS *m/z*: 370.2376 $(M+H)^+$. Calcd for $C_{19}H_{28}N_7O$: 370.2354. *Anal.* Calcd for $C_{19}H_{28}N_7O \cdot 0.7H_2O$: C, 59.57; H, 7.74; N, 25.59. Found: C, 59.35; H, 7.29; N, 25.26.

***N*-[6-*O*^{5}-Methyl-α-glutamylamino-3-pyridyl]-*N'*-[*exo*-bicyclo[2.2.1]-hept-2-yl]-*N''*-cyanoguanidine (18)** Yield 39%. A colorless amorphous solid. ^{1}H-NMR δ: 1.09 (d, 2H, J=9.5 Hz), 1.18 (m, 1H), 1.4—1.5 (m, 4H), 1.6—1.75 (m, 2H), 1.95 (m, 1H), 2.20 (br, 1H), 2.24 (d, 1H, J=3.8 Hz), 2.42 (m, 2H), 3.38 (dd, 1H, J=8.1, 5.1 Hz), 3.58 (s, 3H), 3.63 (br, 1H), 6.95 (d, 1H, J=6.5Hz), 7.62 (dd, 1H, J=8.9, 2.6 Hz), 8.07 (d, 1H, J=8.9 Hz), 8.15 (d, 1H, J=2.6 Hz), 8.83 (br, 1H). ^{13}C-NMR δ: 25.8 (t), 27.8 (t), 29.6 (t), 30.0 (t), 34.7 (t), 35.1 (d), 39.3 (t), 41.7 (d), 51.2 (q), 54.4 (d), 54.8 (d), 112.7 (d), 116.7 (s), 130.4 (s), 133.5 (d), 143.1 (d), 148.0 (s), 157.1 (s), 173.1 (s), 174.1 (s). IR (KBr) cm^{-1}: 3200, 2920, 2150, 1720, 1680, 1570. HR-SIMS *m/z*: 414.2290 $(M+H)^+$. Calcd for $C_{20}H_{27}N_7O_3$: 414.2241. *Anal.* Calcd for $C_{20}H_{27}N_7O_3 \cdot 0.6H_2O$: C, 56.62; H, 6.70; N, 23.11. Found: C, 56.62; H, 6.38; N, 23.33.

***N*-(6-Lysylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (15)** A solution of **4** (0.50 g, 1.9 mmol) and N^{α},N^{ε}-dicarbobenzyloxy-L-lysine (0.92 g, 2.2 mmol) was prepared in pyridine (10 ml), and DCC (0.46 g, 2.2 mmol) was added at room temperature. The mixture was stirred for 24 h at room temperature, then insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, $CHCl_3$: MeOH = 20 : 1) to give the product (0.77 g, 52%). This product (0.26 g) was dissolved in MeOH (2 ml), 10% Pd on charcoal (0.03 g) was added, and stirred for 24 h under a hydrogen atmosphere. The catalyst was removed by filtration through Celite and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (Chromatorex NH-DM 1020, Fuji Silsia Chemical Ltd., $CHCl_3$: MeOH = 20 : 1) to give **15** (100 mg, 64%) as a white powder: mp 93—99 °C. ^{1}H-NMR δ: 1.10 (m, 2H), 1.10 (br t, 1H), 1.3—1.5 (m, 9H), 1.65 (m, 2H), 2.20 (br s, 1H), 2.24 (br s, 1H), 3.35 (m, 2H), 3.63 (d, 1H, J=5.1 Hz), 6.92 (br, 1H), 7.60 (dd, 1H, J=8.9, 2.5 Hz), 8.07 (d, J=8.9 Hz), 8.13 (d, 1H, J=2.5 Hz). ^{13}C-NMR δ: 22.6 (t), 25.8 (t), 27.8 (t), 32.9 (t), 34.5 (t), 34.7 (t), 35.1 (d), 38.4 (t), 41.4 (t), 41.8 (d), 54.8 (d), 55.1 (d), 112.6 (d), 116.9 (s), 130.9 (s), 133.4 (d), 143.1 (d), 147.9 (s), 157.1 (s), 174.7 (s). IR (KBr) cm^{-1}: 3250, 2920, 2150, 1670, 1570. HR-SIMS *m/z*: 399.2651 $(M+H)^+$. Calcd for $C_{20}H_{30}N_8O$: 399.2607. *Anal.* Calcd for $C_{20}H_{30}N_8O \cdot H_2O$: C, 57.67; H, 7.74; N, 26.90. Found: C, 57.50; H, 7.37; N, 26.66.

***N*-(6-α-L-Glutamylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine Hydrochloride (17)** A solution of **4** (1.00 g, 3.70 mmol) and *tert*-butyloxycarbonyl-L-glutamic acid γ-benzyl ester (1.37 g, 4.06 mmol) was prepared in pyridine (20 ml), and DCC (0.85 g, 4.11 mmol) was added at room temperature. The mixture was stirred for 24 h at room temperature, then insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (300 ml) and the solution was washed with saturated aqueous $NaHCO_3$, water, and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, $CHCl_3$: MeOH = 20 : 1) to give the product (1.30 g, 60%). This product (0.30 g) was dissolved in MeOH (2 ml), Pd–black (0.03 g) was added, and the mixture was stirred for 3 h under a hydrogen atmosphere. The catalyst was removed by filtration through Celite and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, $CHCl_3$: MeOH = 5 : 1) to give the product (230 mg, 90%) as a white amorphous solid. To this product (0.17 g), 5.3 N HCl dioxane solution (1.3 ml) was added slowly at 0 °C. Stirring was continued at room temperature for 4 h after the addition was completed. Then Et_2O was added, and the precipitate was collected by filtration to give **17** (85 mg, 57%) as a white powder: mp 189—210 °C. ^{1}H-NMR δ: 1.0—1.3 (m, 3H), 1.50 (m, 4H), 1.65 (br, 1H), 2.08 (m, 2H), 2.22 (br, 2H), 2.39 (br, 2H), 3.72 (br, 1H), 4.08 (br, 1H), 7.42 (d, 1H, J=6.8 Hz), 7.70 (dd, 1H, J=8.9, 2.5 Hz), 7.99 (d, 1H, J=8.9 Hz), 8.22 (d, 1H, J=2.4 Hz), 8.50 (br, 3H), 9.41 (br, 1H), 11.11 (br, 1H). ^{13}C-NMR δ: 25.7 (t), 26.3 (t), 27.9 (t), 29.2 (t), 34.8 (t), 35.2 (d), 38.7 (t), 42.0 (d), 52.1 (d), 54.8 (d), 66.3 (t), 113.8 (d), 116.5 (s), 131.5 (s), 132.9 (d), 142.1 (d), 147.1 (s), 156.7 (s), 167.5 (s), 173.0 (s). IR (KBr) cm^{-1}: 2950, 2170, 1700, 1580. HR-SIMS *m/z*: 400.2097 $(M+H)^+$. Calcd for $C_{19}H_{25}N_7O_3$: 400.2085. *Anal.* Calcd for $C_{19}H_{25}N_7O_3 \cdot HCl \cdot 1.8H_2O$: C, 48.73; H, 6.37; N, 20.93. Found: C, 48.38; H, 5.99; N, 20.89.

***N*-(6-Histidylamino-3-pyridyl)-*N'*-[*exo*-bicyclo[2.2.1]hept-2-yl]-*N''*-cyanoguanidine (19)** A solution of **4** (1.00 g, 3.70 mmol) and N^{α},N^{π}-di-*tert*-butyloxycarbonyl-L-histidine (1.46 g, 4.11 mmol) was prepared in pyridine (20 ml), and DCC (0.85 g, 4.12 mmol) was added at room temperature. The mixture was stirred for 24 h at room temperature, then insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography ($CHCl_3$: MeOH = 20 : 1) to give the product (0.96 g, 43%). To this product (0.40 g), 5.3 N HCl dioxane solution (2.5 ml) was added slowly over a 30 min period at 0 °C. Stirring was continued for 4 h at room temperature after the addition was completed. Then Et_2O was added and the precipitate was collected by filtration, and purified by column chromatography (Chromatorex NH-DM 1020, Fuji Silsia Chemical Ltd., $CHCl_3$: MeOH = 20 : 1) to give **19** (0.14 g, 54%) as a colorless amorphous solid. ^{1}H-NMR δ: 1.0—1.3 (m, 3H), 1.43 (m, 4H), 1.64 (m, 1H), 2.23 (br, 2H), 2.97 (dd, 1H, J=8.0, 14.2 Hz), 3.59 (m, 2H), 6.84 (br, 1H), 6.97 (d, 1H, J=6.5 Hz), 7.53 (s, 1H), 7.62 (dd, 1H, J=2.7, 8.9 Hz), 8.09 (d, 2 H, J= 8.9 Hz), 8.13 (d, 1H, J=2.7 Hz), 8.7—9.1 (br, 1H), 11.5—12.5 (br, 1H). ^{13}C-NMR δ: 25.9 (d), 27.9 (d), 32.0 (d), 34.8 (t), 35.2 (t), 38.5 (t), 41.8 (d), 54.9 (d), 55.5 (d), 112.6 (d), 116.9 (s), 130.5 (s), 133.6 (d), 134.8 (d), 143.3 (d), 148.0 (s), 157.2 (s), 173.7 (s). IR (KBr) cm^{-1}: 3250, 2950, 2150, 1580, 1500. HR-SIMS *m/z*: 408.2211 $(M+H)^+$. Calcd for $C_{19}H_{25}N_7O_3$: 408.2247. *Anal.* Calcd for $C_{20}H_{25}N_9O \cdot HCl$: C, 54.11; H, 5.90; N, 28.40. Found: C, 54.12; H, 6.07; N, 28.25.

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

Inhibition of Spontaneous Mechanical Activity in Rat Portal Vein (*in Vitro* Activity) Male Wistar rats (11 weeks old, 250—300 g) were killed by cervical dislocation. The portal veins were excised and, after removal of surrounding connective tissues, cut into 1 cm strips along the longitudinal axis. The strips were suspended in an organ bath containing Locke's solution (composition mM; NaCl 154, KCl 5.6, $CaCl_2$ 1.63, $NaHCO_3$ 6.0, glucose 5.6; pH 7.2) warmed to 37 °C and gassed with 5% CO_2 in O_2. A tension of 0.5 g was applied, and the developed tension was measured isometrically with a force-displacement transducer (TB-611T; Nihon Kohden, Osaka, Japan). The strips were equilibrated for 30 min. After the equilibration period, test compounds were added cumulatively. The portal vein has a pacemaker site which induces automatic mechanical activities.[25] Opening of K^+ channels and K^+ efflux cause a depletion of the mechanical activity by inhibition of the action potential. Cumulative addition of drugs decreased the automatic activity of the portal vein. The concentration which depleted the activity completely was defined as pEC_{100}. In order to evaluate the channel specificity of the agents, two blockers of potassium ion channels were used: glibenclamide (10^{-6} M—10^{-5} M), a selective antagonist for K^+_{ATP}, and 3,4-diaminopyridine (10^{-4} M—10^{-3} M), which inhibits mainly voltage-sensitive channels and has a weak effect on K^+_{ATP}.[3]

i.v. Hypotensive Activity in Normotensive Rats (*in Vivo* Activity) Male Wistar rats (10 to 11 weeks old, 250—300 g) were anesthetized with pentobarbital sodium salt (42 mg/kg, i.p.) and the trachea was cannulated. The right carotid artery was cannulated for arterial pressure measurement. BP and HR were measured using a pressure transducer (AP-601G; Nihon Kohden, Osaka, Japan) coupled to a polygraph.

The test compounds were administered as a bolus into the tail vein.

***p.o.* Antihypertensive Activity in Spontaneously Hypertensive Rats** The experiments were performed in groups of 4 or 5 male spontaneously hypertensive rats (10 to 11 weeks old, 250—280 g). SBP was measured in the conscious state using the tail cuff plethysmographic method with an electrosphygmomanometer (PS-200A; Riken, Tokyo, Japan) at 0, 1, 2, 4, and 7 h after administration. The test compounds were orally administered in a solution or a suspension in 1% Tween 80 solution at the dosage described. Hypotensive activities are expressed as reduction in SBP (%) from the 0 h value.

***p.o.* Diuretic Study in Rats** SHR (250—280 g) were starved overnight, orally given a test drug at a dose of 5 mg/kg as drug solution or the vehicle, and immediately confined to metabolic cages. After drug administration, individual urine was collected for 5 h. Urinary volume, as well as Na^+, K^+, and Cl^- excretion, was measured using an electrolyte autoanalyzer (NAKL-2, Olympus Optical, Japan).

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