Articles

Novel Potassium Channel Openers: Synthesis and Pharmacological Evaluation of New *N*-(Substituted-3-pyridyl)-*N'*-alkylthioureas and Related Compounds

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This report describes the synthesis and pharmacological evaluation of a series of novel potassium channel openers related to the pinacidil-type compounds. Thioureas, cyanoguanidines, and pyridine *N*-oxides were systematically evaluated for their effects on both the inhibition of spontaneous mechanical activity in rat portal vein (in vitro) and their antihypertensive activity (in vivo), and the structure–activity relationship for this series of compounds was discussed. Good correlation between in vitro and iv antihypertensive activity was observed for these compounds. Among them, cyanoguanidines bearing a conformationally rigid unit such as a norbornyl group generally possessed potent activity in both in vitro and in vivo studies. Especially, *N*-(6-amino-3-pyridyl)-*N'*-cyano-*N''*-(1-methyl-2-norbornyl)guanidine (**23d**) was identified as a more potent potassium channel opener in vitro (EC_{100} = 3 × 10^{-8} M) than pinacidil (EC_{100} = 10^{-7} M).

Introduction

Potassium channel openers have the function of vasorelaxation through hyperpolarization of the cell membrane in vascular smooth muscle.[1-3] Because of their resulting therapeutic potential in the treatment of cardiovascular disorders, these drugs have attracted considerable attention.[4] Several compound types have been identified as potassium channel openers and may be divided into two groups by their specificity toward ATP-sensitive potassium channel. Cromakalim (1),[5] pinacidil (3),[5] RP49356 (6),[6] and minoxidil sulfate (7)[7] possess specific affinity towards the target channel, while diazoxide (2) and nicorandil (5) are less specific[5,7] (Figure 1).

Despite the structural diversity in the members of this class, synthetic elaborations have focused mainly on the modification of cromakalim (1) with few studies based on the structural modification of pinacidil-type compounds (3 and 4).[8,9] The authors' approach to this type of derivative is shown in Figure 2. Thus, appropriate structure modification of 3,4-diaminopyridine, a nonspecific potassium channel blocker (antagonist),[10] could generate a novel class of potassium channel openers (agonist), following the precedent of pinacidil, which was derived from the K^+ channel blocker 4-aminopyridine. This paper reports on a systematic investigation into the synthesis and evaluation of a new series of compounds derived from 3,4-diaminopyridine.

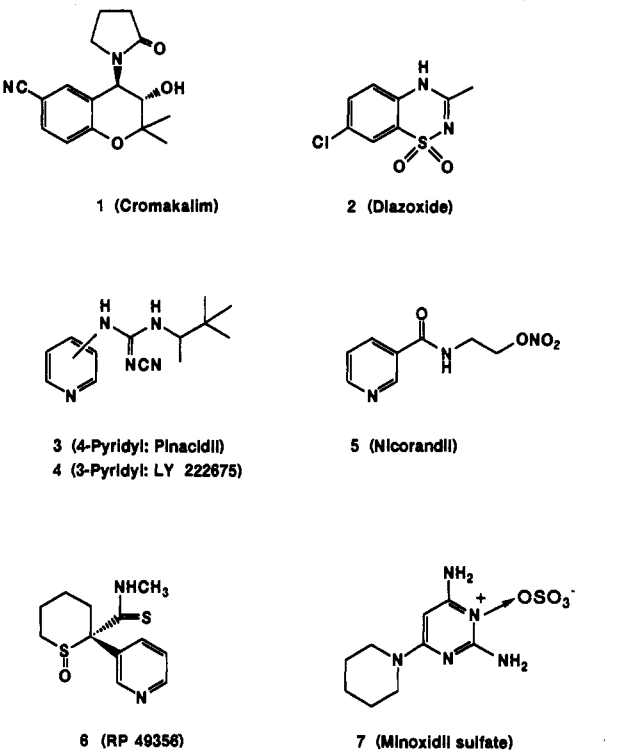

Figure 1.

Chemistry

A general procedure for the synthesis of a novel series of *N*-(3-substituted-pyridyl)-*N'*-alkythioureas was set up as shown in Scheme 1. The target thioureas were synthesized from isothiocyanates (R^1NCS) and from substituted 3-aminopyridines **10**. Required isothiocyanates were prepared from the corresponding amines by treatment with carbon disulfide[11] or thiophosgene.[12] Some cyclic isothiocyanates **9** were derived from amines **8** made by well-known methods (Scheme 2).[13,14] The reactions of amino-substituted 3-aminopyridines with isothiocyanates proceeded regioselectively at C-3 amino group to produce the desired *N*-(amino-substituted-3-pyridyl)-*N'*-alkylthioureas (**11a–i**) in good yields. 2-Chloro or -bromo-5-nitropyridine was a suitable starting material for the introduction of nucleophiles at C-6 on the pyridine ring as shown in Scheme 3 (methods B–D).

Thioureas were converted to the corresponding cyanoguanidines (**23a–f**) via carbodiimide using the reported

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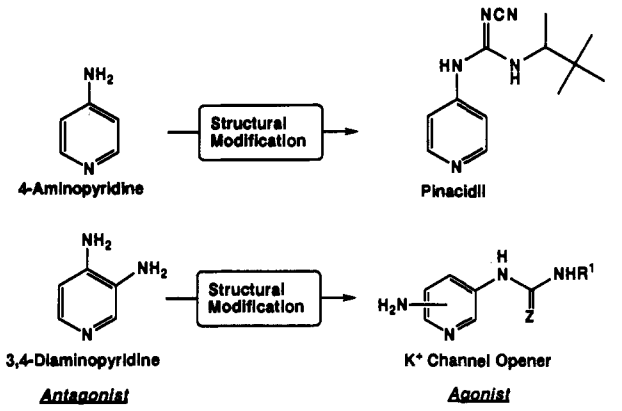
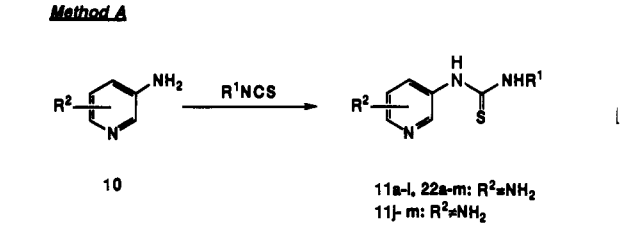
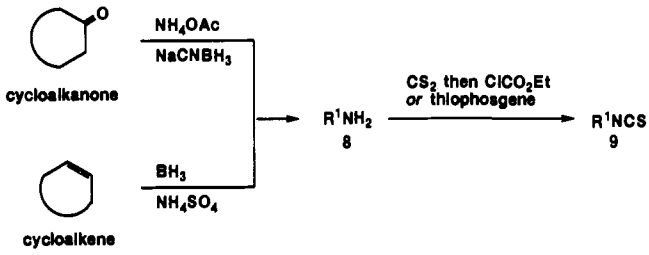


Figure 2.

Scheme 1



Scheme 2. Preparation of Isothiocyanate



procedure,[9] and subsequent treatment with *m*-chloroperbenzoic acid (*m*-CPBA) produced pyridine *N*-oxides (Scheme 4).[15]

Results and Discussion

The compounds were evaluated on the basis of the inhibition of spontaneous mechanical activity in rat portal vein (in vitro)[16-19] and hypotensive activity (in vivo). Hypotensive activity was first evaluated by iv injection in the normotensive rat (NTR). Next, oral antihypertensive activity of the selected compound was determined in spontaneously hypertensive rat (SHR).

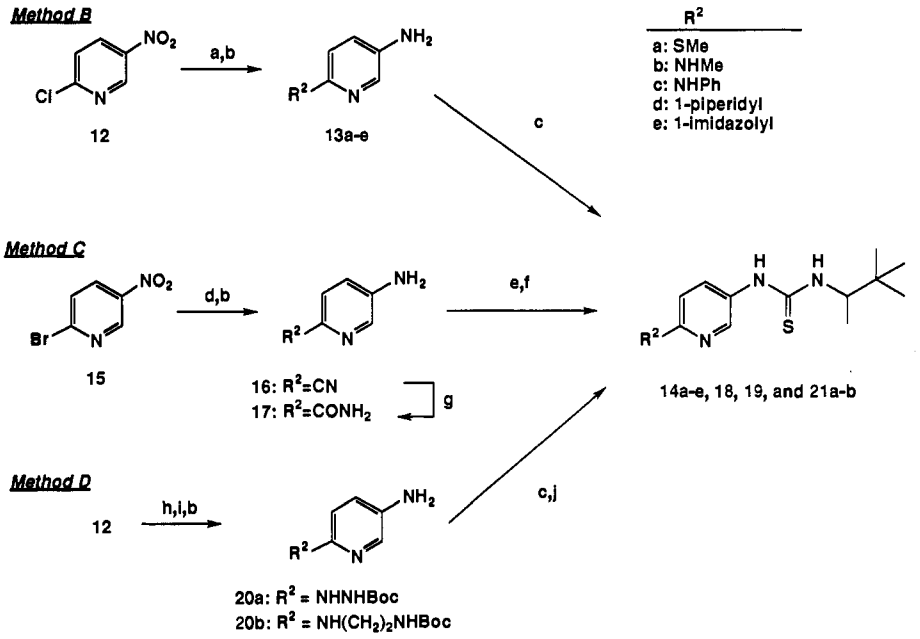
Pharmacological activity of *N*-(3-substituted-pyridyl)-*N'*-alkylthioureas is shown in Table 1. Initially, the effects of various alkyl groups (R^1) placed at the terminal nitrogen in the thiourea group were investigated (**11a–c**). Compound **11a** (R^1 = Me) showed little activity at 10^{-3} M in vitro. By an extension of the methyl group to the *n*-butyl group (**11b**), weak activity was observed, and the change of the alkyl group from linear chain (**11b**) to cyclohexyl ring (**11c**) caused a 100-fold increase of potency. The change of R^1 from cyclohexyl group to 1,2,2-trimethylpropyl group used as a side chain of pinacidil resulted in a marked increase in both in vitro and in vivo activity (**11f** vs **11i**). Altering the position of an amino substituent on the pyridine ring did not appear to significantly affect activity in vitro (**11c–f**). However, the fact that maximal hypotensive response was obtained in the 6-amino-substituted compound **11d** indicated that the amino substituent had a profound influence on the pharmacokinetics rather than on the activity itself. To confirm whether an amino group was optimum as a substituent at the 6-position, compounds **11j–m**, **14a,b**, **18**, **19**, and **21a** were prepared and tested for their activities. In vitro activity increased in the following order: **11l**, **21a** < **11k**, **14b**, **14a**, **19** < **11j**, **11m**, **18** < **11i**. Particularly, the 6-amino compound **11i** was found to have superior activity both in vitro and in vivo. This result promoted an investigation of a series of compounds with a more functionalized 6-amino group such as alkylamino (**14b,d**), arylamino (**14c,e**), and ethylenediamine (**21b**). Unfortunately, both in vitro and in vivo activities were canceled by the functionalizations of the C-6 amino group. This relative inactivity might be due to the additional bulk of the substituents.

The 6-amino substituent having been confirmed as optimal, the effects of the alkyl group on terminal nitrogen in the thiourea portion were extensively studied by introducing substituents into the cyclohexyl ring in **11f**. Results are summarized in Table 2. Although the compounds which have *gem*-dimethyl substituents at C-2 in **22a** and C-3 in **22b** and a *cis-tert*-butyl group at C-2 position in **22c** on the cyclohexyl ring showed the same potency as **11f**, the introduction of a *cis-tert*-butyl group at the C-4 position led to a complete loss of activity. Surprisingly, the change from a cyclohexyl unit to a conformationally more rigid bicycloalkyl unit caused an enhancement of both activities. Norbornyl analogues **22e–g** showed about 10 times the potency of **11i** in both in vitro and in vivo examinations, activities which were not dependent on their exo/endo stereochemistry (**22e,g**) or on the introduction of a double bond(**22f**). Although the compound **22h** methylated at the C-1 bridgehead possessed almost the same activity as **22g**, further alkylation of **22h** diminished the activity shown by **22i** (C-7 *gem*-Me_2) and **22j** (C-3 *gem*-Me_2), which were about 1/3 and 1/30 less potent in vitro than **22h**, respectively. The bridging pattern also affected the biological response, e.g., in vitro activity decreased in the order: bicyclo[2.2.1] (**22e,g**, etc.) = bicyclo[2.2.2] (**22k**) > adamantane (**22l**) > bicyclo[3.1.1] (**22m**).

Isosteric transformation from thiourea to cyanoguanidine is a well-known strategy to obtain improved activity in this area.[24] This approach was applied to the derivatives used (**23a–f**). The biological results are shown in Table 3 together with their *N*-oxide analogues (**24a–e**). No significant change in either in vitro or in vivo activities was observed as a result of this transformation (**23a–c,f**). Exceptionally, **23d** showed more potent in vitro activity than **22h** and was the most potent among the compounds so far tested. On the other hand, the pyridine *N*-oxides **24a–e** were less potent in vitro than the corresponding parent cyanoguanidines in the range from 1/3 to 1/30, and their iv hypotensive activities also decreased in proportion to their magnitude of in vitro activity.

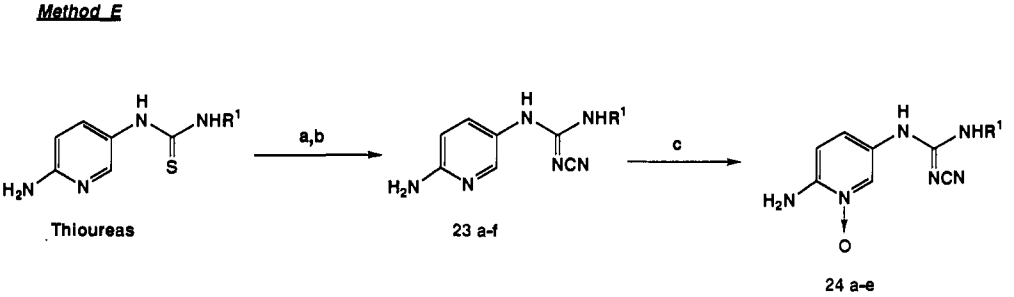
Recently, Manley and Quast[20] postulated a receptor-binding model for the pinacidil-type compounds which consists of three receptor binding elements, i.e., lipophilic site (the alkyl group), hydrogen-bond-donating site (the NH proton in the guanidyl group), and hydrogen-bond-accepting site (the pyridine nitrogen). Since hydrogen-bond-accepting ability would be partly reflected by negative charge density at the site, the value of this indicator on the nitrogen atom in the pyridine ring was calculated for the compounds in Table 1 using MOPAC/

Scheme 3[a]



[a] Reagent: (a) R_2H (nucleophile); (b) hydrogenation or Fe/HCl; (c) ^{t}BuMeCHNCS; (d) CuCN/DMF; (e) $S{=}CCl_2$; (f) ^{t}BuMeCHNH$_2$; (g) $NaOH/H_2O_2$; (h) H_2NNH_2 or $H_2N(CH_2)_2NH_2$; (i) Boc_2O; (j) HCl.

Scheme 4[a]



[a] Reagent: (a) HgO/S; (b) $H_2NCN/^iPr_2EtN$; (c) *m*-CPBA.

AM1, and the order was as follows: **11l > 11k > 11i > 14b > 21a > 14a > 11j > 19 > 11m > 18**.[21] Although the steric factor was not sufficiently taken into account, no apparent relationship was found between activity and charge density. It is noteworthy, in particular, that 18 (R^2 = 6-CN) which seems to be a relatively poor hydrogen-bond-acceptor showed more potent activity than 11i (R^2 = 6-NH_2). Furthermore, the disclosure of arylcyanoguanidines **25**[22] and **26**[23] (Figure 3) as potent antihypertensive agents indicates that the pyridine nitrogen may not necessarily be a prerequisite for the biological activity of pinacidil.

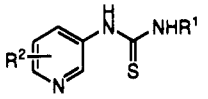
The present results also contain spatial information for the lipophilic site in the binding model proposed. From the observation that compound **23d** showed in vitro activity 30 times stronger than that of the compound **23a**, it seems possible that a 1-methylnorbornyl unit could interact with the lipophilic binding site more favorably than a 1,2,2-trimethylpropyl unit. Further investigation on this subject is currently underway.

Finally, po antihypertensive activity in SHR was determined for the compounds which showed potent iv hypotensive activity in NTR (Figure 4). Although thioureas (**22e,g,h**) bearing a norbornyl unit showed weak activity in oral administration, in contrast to their potency in iv injection, this discrepancy was improved by the minor structural transformation from thiocarbonyl into cyanoimine which gave the derivatives (**23c,d,f**) equipotent to pinacidil. The reason for this phenomenon is still unclear, but it may reflect a difference in drug absorption or metabolic pathway. Interestingly, a marked decrease in po activity was also observed in LY222675 (**4**),[4] which showed the most potent iv activity among the tested compounds. This result suggests that a C-6 amino group on the pyridine ring may be necessary to promote the po antihypertensive activity of 3-pyridylcyanoguanidine derivatives.

An unusual feature was also observed in the series of pyridine *N*-oxides. Although pinacidil *N*-oxide which was identified as a main urinary excretory product in human study[25] did not show iv hypotensive activity in NTR, duration of action for longer than 10 h was observed by po administration in SHR. On the other hand, compounds **24a,c,e** showed sufficient activity and similar duration of action in both iv and po administration. It is most likely that pinacidil *N*-oxide behaves as a prodrug as indicated by Petersen,[15] while the series of pyridine *N*-oxides **24a,c,e** may have different pharmacokinetic or metabolic features.

In conclusion, thioureas, cyanoguanidines, and pyridine *N*-oxides were systematically evaluated for both inhibition of spontaneous mechanical activity in rat portal vein (in vitro) and iv hypotensive activity in NTR. This series of compounds showed good correlation between in vitro and iv hypotensive activities. As to in vitro activity, norbornyl

Table 1. *N*-(Substituted-3-pyridyl)-*N'*-alkylthioureas



no.	R^1	R^2	pEC_{100}[a]	dose (mg/kg)	max. fall in SBP (%)[b]
11a	Me	4-NH_2	no response[c]	3.0	15 ± 1
11b	*n*-Bu	4-NH_2	3.0	3.0	13 ± 4
11c	cyclohexyl	4-NH_2	5.0	1.0	22 ± 4
				3.0	27 ± 4
11d	cyclohexyl	2-NH_2	4.0	1.0	9 ± 2
				3.0	21 ± 4
11e	cyclohexyl	5-NH_2	5.0	1.0	7 ± 4
				3.0	23 ± 4
11f	cyclohexyl	6-NH_2	5.0	1.0	26 ± 3
				3.0	35 ± 2
11g	CH_2CHMe_2	6-NH_2	4.7	0.3	12 ± 2
				1.0	23 ± 4
11h	$CMe_2CH_2CH_3$	6-NH_2	5.0	0.3	15 ± 1
				1.0	31 ± 2
11i	$CHMeCMe_3$	6-NH_2	6.0	0.3	29 ± 2
				1.0	32 ± 3
11j[d]	$CHMeCMe_3$	6-Me	5.7	0.3	18 ± 1
				1.0	30 ± 2
11k[d]	$CHMeCMe_3$	6-OMe	5.0	1.0	17 ± 2
				3.0	30 ± 3
11l[d]	$CHMeCMe_3$	6-OH	4.0	1.0	7 ± 1
11m[d]	$CHMeCMe_3$	6-Cl	5.7	0.3	27 ± 3
				1.0	41 ± 2
14a	$CHMeCMe_3$	6-SMe	5.0	0.3	10 ± 4
14b	$CHMeCMe_3$	6-NHMe	5.0	0.3	8 ± 1
				1.0	25 ± 1
14c	$CHMeCMe_3$	6-NHPh	no response	1.0	5 ± 3
14d	$CHMeCMe_3$	6-(1-piperidyl)	no response	1.0	10 ± 3
14e	$CHMeCMe_3$	6-(1-imidazolyl)	3.7	3.0	11 ± 4
18	$CHMeCMe_3$	6-CN	5.7	0.1	13 ± 1
				0.3	28 ± 3
19	$CHMeCMe_3$	6-$CONH_2$	5.0	1.0	9 ± 1
21a	$CHMeCMe_3$	6-$NHNH_2$	4.0	3.0	26 ± 2
21b	$CHMeCMe_3$	6-$NHCH_2CH_2NH_2$	no response	1.0	3 ± 4

[a] Negative logarithm of the concentration causing a 100% inhibition of spontaneous mechanical activity in rat portal vein. Values represent the means of four independent experiments. [b] Antihypertensive activity in anesthetized normotensive rat (male) by iv injection. Systolic blood pressure (SBP) was measured for 30 min after injection and all values express maximum percent fall in SBP ($n = 4$). [c] Spontaneous mechanical activity was not inhibited by the addition of these compounds. [d] These compounds are disclosed in German Patent Offenleg 2557138 (Leo Pharmaceutical Products).

analogues possessed more potent activity than compounds bearing a conformationally flexible alkyl chain. Cyanoguanidines **23c–f** showed not only potent iv hypotensive activity but also po antihypertensive activity. Thus, compounds **23c–f** were selected for further pharmacological and toxicological studies.

Experimental Section

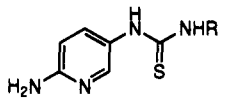
Reagents were purchased from commercial suppliers and used without further purification. Reaction solvents were distilled from an appropriate drying agent before use. Flash column chromatography was carried out at medium pressure using silica gel (Nacalai tesque, 230–400 mesh) with the indicated solvent as eluent. Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. 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 AC-200 spectormeter (DSS or TMS as an internal standard) for NMR. 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). High-resolution mass (HRMS) spectra were obtained on a Hitachi M-2000 instrument at positive SIMS mode.

Method A. *N*-(4-Amino-3-pyridyl)-*N'*-methylthiourea Hydrochloride (11a). To a suspension of 3,4-diaminopyridine **10a** (2.00 g, 18.3 mmol) in 10 mL of pyridine was added methyl isothiocyanate (5.00 g, 68.7 mmol) at room temperature. After being stirred for 14 h, the reaction mixture was concentrated in vacuo. To the residue was added ether (20 mL), and then a precipitated solid was collected by filtration. The filtered cake was washed with Et_2O and dried under reduced pressure at room temperature to give the product (4.7 g, 94%). The obtained product (1.00 g) was dissolved in methanol (10 mL) and treated with 1.75 N HCl–EtOH (3.2 mL) to yield **11a** (816 mg, 66%) as a white powder: mp 278–280 °C; IR (KBr) 3200, 1640, 1580, 1250 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 8.14 (br s, 1 H), 8.04 (dd, J = 1.0, 7.0 Hz, 1 H), 7.03 (d, J = 7.0 Hz, 1 H), 3.04 (s, 3 H); ^{13}C NMR (50.3 MHz, D_2O) δ 184.6, 160.6, 143.1, 141.3, 121.8, 112.8, 34.3; HRMS (SIMS-POSI; M + H) calcd 183.0704, found 183.0678. Anal. ($C_7H_{10}N_4S$·HCl) C, H, N.

Compounds **11b–e** and **22a–m** were similarly prepared by the reaction of the requisite diaminopyridine with the corresponding isothiocyanate. For the preparation of **11f–i**, 2,5-diaminopyridine dihydrochloride was used as starting material and dimethylformamide(DMF) was used as the solvent in the presence of a sufficient amount of pyridine.

Method B. 5-Amino-2-(methylthio)pyridine (13a). To a mixture of 2-chloro-5-nitropyridine (10.0 g, 63.1 mmol), Et_3N (9.60 g, 94.6 mmol), and 15 mL of MeOH was added 20 mL of 30% methyl mercaptan in MeOH (20 mL) at room temperature. After being stirred for 2 h, the resulting suspension was concentrated in vacuo and 10% aqueous K_2CO_3 was added to the residue. The mixture was extracted with $CHCl_3$, and the combined extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The product was purified by the recrystallization from $CHCl_3$–hexane to afford 5-nitro-2-(methylthio)pyridine (8.40 g, 97%) as yellow crystals (mp 109–112 °C). To a suspension of the product (3.80 g, 22.6 mmol) in EtOH/H_2O (4/1, 100 mL) was added iron powder (12.6 g) and concentrated

Table 2. *N*-(6-Amino-3-pyridyl)-*N'*-alkylthioureas



no.	R	pEC_{100}[a]	dose (mg/kg)	max. fall[b] in SBP (%)
	R':			
11f	H	5.0	1.0	25 ± 3
22a	2-*gem*-Me_2	5.5	1.0	26 ± 4
22b	3-*gem*-Me_2	5.0	1.0	25 ± 6
22c	2-*cis*-CMe_3	5.0	1.0	12 ± 2
22d	4-*cis*-CMe_3	no response[c]	1.0	9 ± 1
22e		7.0	0.1	19 ± 1
			0.3	41 ± 1
22f		7.0	0.1	24 ± 3
			0.3	32 ± 5
22g		7.0	0.1	22 ± 2
			0.3	34 ± 1
22h		7.0	0.1	27 ± 1
			0.3	36 ± 2
22i		6.5	0.3	14 ± 1
			1.0	41 ± 4
22j		5.5	1.0	29 ± 4
			3.0	39 ± 5
22k		7.0	0.1	26 ± 2
			0.3	43 ± 2
22l		6.5	1.0	24 ± 4
22m		5.0	1.0	16 ± 5
pinacidil		7.0	0.1	25 ± 4
			0.3	31 ± 3

[a-c] See footnotes to Table 1.

HCl (1 mL) at room temperature. After refluxing for 2 h, the reaction mixture was cooled to room temperature, and insoluble material was filtered off through Celite. The filtrate was concentrated in vacuo and made alkaline with 20% aqueous NaOH solution. The mixture was extracted with $CHCl_3$, and the combined extracts were dried over anhydrous $MgSO_4$, followed by concentration to give **13a** (3.4 g, quantitative) as a brown oil. Compound **13a** was used in the next step without further purification: IR (KBr) 3300, 1620, 1470 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.00 (d, J = 2.9 Hz, 1 H), 7.02 (d, J = 8.5 Hz, 1 H), 6.90 (dd, J = 2.9, 8.5 Hz, 1 H), 3.59 (br s, 2 H), 2.52 (s, 3 H).

***N*-(6-(Methylthio)-3-pyridyl)-*N'*-(1,2,2-trimethylpropyl)-thiourea Hydrochloride (14a).** To a solution of **13a** (1.50 g, 10.7 mmol) in 2 mL of $CHCl_3$ was added a solution of 1,2,2-trimethylpropyl isothiocyanate (1.50 g, 10.7 mmol) in 1 mL of $CHCl_3$ at room temperature. After the mixture was stirred for 16 h, 1,2,2-trimethylpropyl isothiocyanate (1.50 g, 10.7 mmol) was further added, and then the reaction mixture was refluxed for 6 h. The resulting solution was concentrated in vacuo and purified by column chromatography (hexane/EtOAc = 2/1). Subsequent recrystallization ($CHCl_3$/Et_2O) gave the product (1.9 g, 63%) as a white powder (mp 157–158 °C). The product was converted to its HCl salt as described for **11a**: mp 159–164 °C; IR (KBr) 3220, 2950, 1530 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.14 (s, 1 H), 8.63 (s, 1 H), 8.15–7.95 (m, 2 H), 7.33 (d, J = 8.7 Hz, 1 H), 4.40–4.15 (m, 1 H), 2.54 (s, 3 H), 1.05 (d, J = 6.8 Hz, 3 H), 0.93 (s, 9 H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 154.5, 147.6, 136.0, 132.8, 130.1, 52.8, 34.1, 26.3, 16.3, 14.2; HRMS (SIMS-POSI, M + H) calcd 284.1254, found 284.1287.

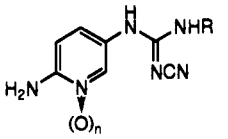
5-Amino-2-(methylamino)pyridine (13b) was prepared by a method similar to that described for **13a** using a 30% EtOH solution of $MeNH_2$ instead of methyl mercaptan and followed by PtO_2-catalyzed hydrogenation: IR (KBr) 3330, 3000, 1600 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.47 (d, J = 2.0 Hz, 1 H), 6.82 (dd, J = 2.0, 8.0 Hz, 1 H), 6.25 (d, J = 8.0 Hz, 1 H), 5.50 (br s, 1 H), 2.66 (br s, 3 H). The compounds **13c–e** were prepared in the same way.

***N*-(6-(Methylamino)-3-pyridyl)-*N'*-(1,2,2-trimethylpropyl)-thiourea (14b).** To a solution of **13b** (0.30 g, 2.4 mmol) in 1.5 mL of pyridine was added 1,2,2-trimethylpropyl isothiocyanate (0.50 g, 3.6 mmol) at room temperature. After the mixture was stirred for 18 h, the resulting precipitate was collected by filtration and washed with Et_2O to yield **14b** (0.33 g, 51%) as a white powder: mp 195–197 °C; IR (KBr) 3300, 2950, 1620 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.99 (d, J = 2.0 Hz, 1 H), 7.43 (s, 1 H), 7.31 (dd, J =2.0, 9.0 Hz, 1 H), 6.44 (d, J = 9.0 Hz, 1 H), 5.58 (d, J = 6.0 Hz, 1 H), 4.90 (s, 1 H), 4.50–4.30 (m, 1 H), 2.96 (d, J = 5.0 Hz, 3 H), 1.08 (d, J = 6.0 Hz, 3 H), 0.85 (s, 9 H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 181.7, 157.1, 144.1, 135.3, 124.9, 106.8, 57.2, 34.4, 28.2, 26.3, 15.4; HRMS (SIMS POSI, M + H) calcd 265.1486 found 265.1457. Thioureas **14c–e** were prepared in the same way.

Method C. 5-Amino-2-cyanopyridine (16). A mixture of 2-bromo-5-nitropyridine (5.60 g, 27.7 mmol), copper(I) cyanide (3.30 g, 35.7 mmol) and 6 mL of DMF was stirred at 100–110 °C for 2 h, and the resulting mixture was cooled to room temperature. After addition of 200 mL of CH_2Cl_2, the insoluble material was removed by filtration through Celite and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (hexane/EtOAc = 3/1) to afford 5-nitro-2-cyanopyridine (2.70 g, 66%): IR (KBr) 3100, 2250, 1600, 1530 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.54 (d, J = 2.0 Hz, 1 H), 8.68 (dd, J = 2.0, 7.5 Hz, 1 H), 7.97 (d, J = 7.5 Hz, 1 H). To a solution of the obtained compound (1.0 g, 6.7 mmol) in 10 mL of dioxane was added 10% Pd on charcoal (0.5 g). After stirring for 3 h under a hydrogen atmosphere, the catalyst was removed by filtration through Celite and the filtrate was concentrated in vacuo to yield **16** (710 mg, 88%) as a brown powder: IR (KBr) 3400, 3300, 3200, 2200, 1660, 1580 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.98 (d, J = 2.8 Hz, 1 H), 7.53 (d, J = 8.6 Hz, 1 H), 6.93 (dd, J = 2.8, 8.6 Hz, 1 H), 6.35 (br s, 2 H).

***N*-(6-Cyano-3-pyridyl)-*N'*-(1,2,2-trimethylpropyl)thiourea (18).** To a suspension of **16** (220 mg, 1.85 mmol) in 10 mL of toluene was added thiophosgene (0.16 mL, 2.10 mmol) at room

Table 3. Cyanoguanidines and Pyridine *N*-Oxides



no.	*n*	R	pEC_{100}[a]	dose (mg/kg)	max. fall[b] in SBP (%)
23a	0	$CHMeCMe_3$	6.0	0.3	24 ± 4
				1.0	30 ± 4
24a	1	$CHMeCMe_3$	5.5	0.3	16 ± 5
				1.0	22 ± 4
23b	0	CMe_3	6.0	0.3	15 ± 1
				1.0	28 ± 3
24b	1	CMe_3	4.5	1.0	19 ± 3
				3.0	20 ± 3
23c	0		7.0	0.1	21 ± 2
				0.3	34 ± 1
24c	1		6.0	1.0	16 ± 3
23d	0		7.5	0.1	26 ± 4
				0.3	33 ± 2
24d	1		6.0	0.3	18 ± 1
				1.0	31 ± 3
23e	0		6.0	0.1	17 ± 2
				0.3	28 ± 4
24e	1		5.0	0.3	12 ± 3
				1.0	20 ± 1
23f	0		6.5	0.1	25 ± 3
				0.3	32 ± 2
pinacicil *N*-oxide			5.0	0.3	13 ± 4
LY 222675			7.0	0.03	26 ± 3
				0.10	37 ± 2

[a,b] See footnotes to Table 1.

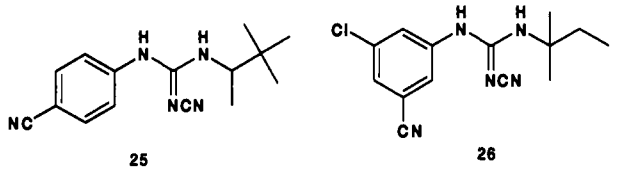


Figure 3.

temperature. After refluxing for 1 h, the reaction mixture was concentrated in vacuo and purified by column chromatography (hexane/EtOAc = 4/1) to yield the corresponding isothiocyanate (80 mg, 27%) as a yellow oil. The isocyanate was treated with 1,2,2-trimethylpropylamine (0.15 mL, 1.12 mmol) in 1 mL of CH_2Cl_2 at room temperature for a few minutes and then directly purified by column chromatography (hexane/EtOAc = 1/1) to afford 18 (130 mg, 99%) as a white powder: mp 146–148 °C; IR (KBr) 3250, 3100, 2950, 2200, 1520 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.97 (s, 1 H), 8.79 (d, *J* = 2.5 Hz, 1 H), 8.56 (dd, *J* = 2.5, 8.6 Hz, 1 H), 7.99 (d, *J* = 9.2 Hz, 1 H), 7.90 (d, *J* = 8.6 Hz, 1 H), 4.40–4.20 (m, 1 H), 1.08 (d, *J* = 6.7 Hz, 3 H), 0.93 (s, 9 H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 179.6, 143.6, 140.2, 128.6, 127.2, 125.2, 117.73, 57.2, 34.2, 26.2, 14.9; HRMS (SIMS POSI, M + H) calcd 263.1330, found 263.1374.

5-Amino-2-pyridinecarboxamide (17). To a mixed solution of 35% H_2O_2/3 N NaOH/MeOH (1/3/2.5 mL) was added **16** (450 mg, 3.78 mmol) at room temperature. After the reaction mixture had been stirred for 30 min, 5 mL of water was added. A precipitate was collected by filtration, and the filtered cake was purified by column chromatography (EtOAc) to give **17** (390 mg, 75%) as a white powder: IR (KBr) 3300, 3150, 1680, 1580 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 7.90 (d, *J* = 2.6 Hz, 1 H), 7.71 (d, *J* = 8.6 Hz, 1H), 6.96 (dd, *J* = 2.6, 8.6 Hz, 1 H), 5.90 (br s, 2 H).

***N*-(6-Carbamoyl-3-pyridyl)-*N'*-(1,2,2-trimethylpropyl)-thiourea (19)** was prepared by method A as a white powder (mp 197–199 °C): IR (KBr) 3200, 3100, 2900, 1690, 1530 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 9.77 (s, 1 H), 8.74 (d, *J* = 2.3 Hz, 1 H), 8.32 (dd, *J* = 2.3, 8.4 Hz, 1 H), 7.96 (d, *J* = 8.4 Hz, 1 H), 7.82 (d, *J* = 9.2 Hz, 1 H), 4.45–4.25 (m, 1 H), 1.08 (d, *J* = 6.7 Hz, 3 H), 0.94 (s, 9 H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 180.1, 165.8, 144.5, 141.5, 139.1, 128.8, 121.7, 57.2, 34.3, 26.3, 15.1; HRMS (SIMS POSI, M + H) calcd 281.1434, found 281.1448.

Method D. 2-(2-(*tert*-Butoxycarbonyl)hydrazino)-5-nitropyridine (20a). To a suspension of 2-chloro-5-nitropyridine (5.00 g, 31.5 mmol) in 60 mL of dioxane was added hydrazine monohydrate (1.70 mL, 34.7 mmol) at room temperature. After the mixture was stirred for 16 h, a precipitate was collected by filtration to give 2-hydrazino-5-nitropyridine as a yellow powder: mp 198–208 °C. To a mixture of the obtained product, (1.00 g, 5.25 mmol), Et_3N (2.50 mL, 18.4 mmol), with DMF (1 mL) and dioxane (20 mL) was added di-*tert*-butyl dicarbonate (2.40 mL, 10.5 mmol) at room temperature. After refluxing for 2 h, the insoluble material was filtered off and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (hexane/EtOAc = 3/2) to give **20a** (1.3 g, 95%) as a yellow powder: mp 132.5–134 °C; IR (KBr) 3300, 3000, 1710, 1600 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.03 (d, *J* = 2.6 Hz, 1 H), 8.32 (dd, *J* = 2.6, 9.2 Hz, 1 H), 7.51 (br s, 1 H), 6.80 (br s, 1 H), 6.76 (d, *J* = 9.2 Hz, 1 H), 1.48 (s, 9 H).

***N*-(6-Hydrazino-3-pyridyl)-*N'*-(1,2,2-trimethylpropyl)-thiourea Hydrochloride (21a).** To a solution of **20a** (570 mg, 2.24 mmol) in 10 mL of EtOH was added PtO_2 (57 mg). After stirring at room temperature under a H_2 atmosphere for 1 h, the catalyst was filtered off and the filtrate was concentrated in vacuo to give the product (500 mg). To a solution of this product (500 mg, 2.23 mmol) in 10 mL of pyridine was added 1,2,2-trimethylpropyl isothiocyanate (350 mg, 2.5 mmol) at room temperature. After stirring at room temperature for 16 h, the reaction mixture was concentrated in vacuo and purified by column chromatography (hexane/EtOAc = 1/3) to give the corresponding thiourea (450 mg, 55%) as a white powder (mp 143–146 °C). To a solution of the thiourea (150 mg, 0.41 mmol) in 2 mL of EtOH was added 8.8 N HCl–EtOH (3.5 mL) at room temperature. After being stirred for 30 min, the reaction mixture was cooled with ice water and a precipitate was isolated by filtration to give **21a** (85 mg, 68%; hygroscopic) as a white powder: IR (KBr) 3200, 1690, 1600 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.07 (s, 1 H), 9.25 (s, 1 H), 8.27 (s, 1 H), 8.07 (d, *J* = 8.6 Hz, 1 H), 7.85 (d, *J* = 9.0 Hz, 1 H), 6.85 (d, *J* = 9 .0 Hz, 1 H), 4.26 (m, 1 H), 4.50–3.0 (br s, 2

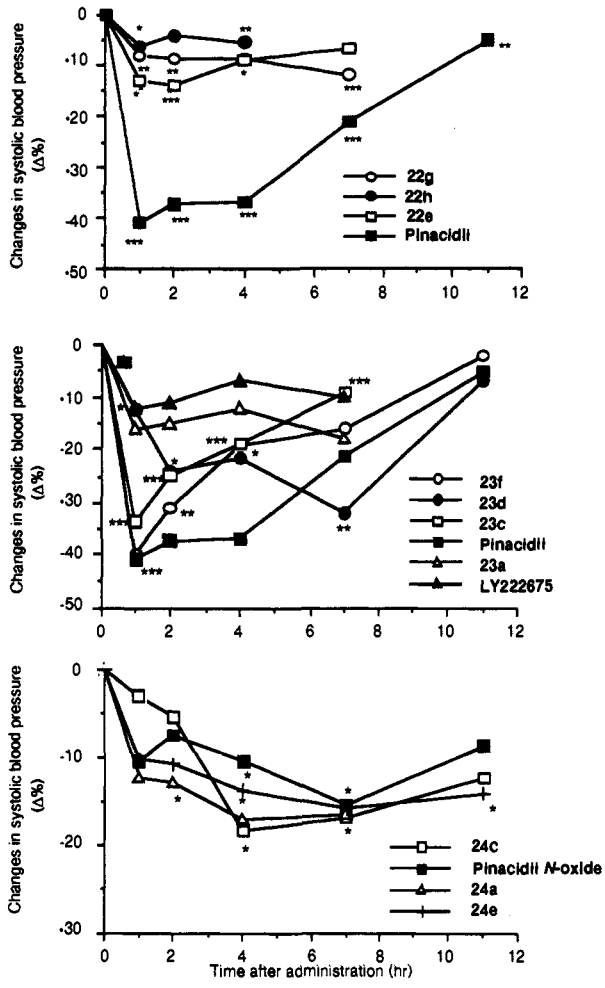


Figure 4. Antihypertensive effects of a single oral administration in conscious male SHR. Dosage of each compound was at 5 mg/kg except for pinacidil *N*-oxide (3 mg/kg). Data points are means ($n = 4$). Asterisk means significantly different from vehicle; $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). Upper: thioureas. Middle: cyanoguanidines. Bottom: pyridine *N*-oxide.

H), 1.04 (d, $J = 6.7$ Hz, 3 H), 0.92 (s, 9 H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 187.9, 156.2, 152.3, 137.6, 133.0, 124.7, 106.6, 79.1, 56.0, 28.1, 18.4; MS (SIMS POSI) 268 (M + H).

***N*-[6-((2-Aminoethyl)amino)-3-pyridyl]-*N'*-(1,2,2-trimethylpropyl)thiourea (21b)** was prepared by the same procedure described for **21a** using ethylenediamine instead of hydrazine in 18% overall yield from 2-chloro-5-nitropyridine as free base (white powder, mp 138–140 °C): IR (KBr) 3300, 2950, 1610, 1530 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.97 (d, J = 4.0 Hz, 1 H), 7.52 (s, 1 H), 7.28 (dd, J = 4.0, 9.0 Hz, 1 H), 6.46 (d, J = 9.0 Hz, 1 H), 5.56 (d, J = 8.0 Hz, 1 H), 5.27 (s, 1 H), 4.60–4.30 (m, 1 H), 3.50–3.30 (m, 2 H), 2.97 (t, J = 6.0 Hz, 2 H), 1.08 (d, J = 6.0 Hz, 3 H), 0.85 (s, 9 H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 181.7, 156.5, 144.0, 135.3, 125.1, 107.4, 57.3, 43.9, 40.8, 34.4, 26.4, 15.4; HRMS (SIMS POSI, M + H) calcd 296.1907, found 296.1874.

***N*-(6-Amino-3-pyridyl)-*N'*-(bicyclo[2.2.2]octan-2-yl)-*N''*-cyanoguanidine Hydrochloride (23e).** A mixture of **22k** (4.70 g, 16.9 mmol), HgO (yellow; 11.0 g, 50.7 mmol), and sulfur (406 mg, 12.7 mol) in EtOH/CH_2Cl_2 (1/1, 100 mL) was stirred at room temperature for 36 h. Insoluble material was removed by filtration through Celite, and the filtrate was concentrated in vacuo to afford the crude product (16.9 g, quantitative), which was used in the next reaction without further purification. To a solution of the crude product (16.9 g) in a mixed solvent of Et_2O/CH_2Cl_2 (1/4, 100 mL) were added cyanamide (1.4 g, 33.8 mmol) and i-Pr_2EtN (1 mL) at room temperature. After stirring at room temperature for 36 h, the resulting precipitate was collected by filtration and the filtered cake was recrystallized from MeOH/Et_2O to give the free base of **23e** (2.3 g, 48%) as a white powder (mp 208–210 °C), which was converted to its hydrochloride **23e** (white powder; mp 220–225 °C) in the same way described for **11a**: IR (KBr) 3250, 2900, 2200 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 13.75 (s, 1 H), 9.35 (s, 1 H), 8.12 (s, 2 H), 7.93 (d, J = 2.2 Hz, 1 H), 7.82 (dd, J = 2.2, 9.4 Hz, 1 H), 7.39 (d, J = 7.2 Hz, 1 H), 7.01 (d, J = 9.4 Hz, 1 H), 4.00 (m, 1 H), 1.96 (t, J = 11.7 Hz, 1 H), 1.90–1.20 (m, 11 H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 157.3, 152.2, 142.1, 130.5, 124.4, 116.6, 113.7, 49.5, 33.2, 28.8, 24.9, 24.2, 23.9, 19.2; HRMS (SIMS POSI, M + H) calcd 285.1827, found 285.1854. Anal. ($C_{15}H_{20}N_6HCl$) C, H, N. Cyanoguanidines **23a–f** were similarly prepared from the corresponding thioureas.

***N*-(6-Amino-1-oxido-3-pyridyl)-*N'*-(bicyclo[2.2.2]octan-2-yl)-*N''*-cyanoguanidine (24e).** To a solution of free base of **23e** (1.00 g, 3.52 mmol) in a mixed solvent of CH_2Cl_2/MeOH (4/1, 25 mL) was added portionwise 3-chloroperbenzoic acid (80% parity; 760 mg, 3.52 mmol) with ice cooling. After being stirred for 3 h with ice cooling, the reaction mixture was diluted with $CHCl_3$ and washed with 10% K_2CO_3 solution (30 mL). After removal of the solvent, the residue was recrystallized from MeOH/Et_2O to afford **24e** (870 mg, 82%) as a light yellow powder: mp 155–161 °C dec; IR (KBr) 3250, 2900, 2150 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.70 (s, 1 H), 7.92 (d, J = 2.1 Hz, 1 H), 6.99 (dd, J = 2.1, 8.9 Hz, 1 H), 6.86 (d, J = 7.0 Hz, 1 H), 6.78 (s, 2 H), 6.75 (d, J = 8.9 Hz, 1 H), 3.82 (m, 1 H), 1.90 (t, J = 12.5 Hz, 1 H), 1.80–1.20 (m, 11 H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 157.8, 148.8, 134.1, 125.4, 123.7, 117.0, 108.5, 49.5, 32.7, 28.6, 24.9, 24.2, 24.0, 23.9, 19.2; HRMS (SIMS POSI, M + H) calcd 301.1776, found 301.1772. Pyridine *N*-oxides **24a–d** were similarly prepared from the corresponding cyanoguanidines.

Biology. Inhibition of Spontaneous Mechanical Activity in Rat Portal Vein (*in Vitro* Activity). Male Wistar rats (11 weeks old, 250–300 g) were sacrificed by cervical dislocation. The portal veins were exposed and attached at either end to a cotton thread. After removal of surrounding connective tissues, 1-cm strips were cut 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 (Nihon Kohden, TB-611T). The strips were equilibrated for 30 min. After the equilibration period, testing compounds were added cumulatively. The portal vein has a pacemaker site which induces automatic mechanical activities.[26] Opening of K^+ channel and K^+ efflux cause a depletion of the mechanical activity by inhibition of 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 channel specificity of the agents, two blockers for potassium ion channels were used: glibenclamide (10^{-6}–10^{-5} M), a selective antagonist for ATP-sensitive potassium channels (K_{ATP}), and 3,4-diaminopyridine (10^{-4}–10^{-3} M), which inhibits mainly voltage-sensitive channels and has a weak effect on K_{ATP}[3].

***In Vivo* Activity: A. iv Antihypertensive Activity in Normotensive Rat.** Male Wistar rats (10–11 weeks old, 250–300 g) were anesthetized with pentobarbital sodium salt (42 mg/kg, ip), and the trachea was cannulated. The right carotid artery was cannulated for arterial pressure and heart rate measurement. BP and HR were measured using a pressure transducer (AP-601G; Nihon Kohden, Osaka, Japan) coupled to a polygraph. The tested compounds were administered in bolus from the tail vein.

B. po Antihypertensive Activity in Spontaneously Hypertensive Rat. The experiments were performed in groups of four male spontaneously hypertensive rats (10–11 weeks old, 250–280 g). Systolic blood pressure (SBP) was measured in a conscious state using the tail cuff plethysmographic method with an electrosphygmomanometer (PS-200Ai Riken, Tokyo, Japan) at 0, 1, 2, 4, 7, and 11 h after administration. The tested 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.

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