Cyanoamidines. I. Synthesis and Vasodilatory Activity of *N*-Substituted Heteroaromatic Cyanoamidines

Tatsuo NAKAJIMA,* Toshio IZAWA, Tomoko KASHIWABARA, Shohachi NAKAJIMA, and Yuuji MUNEZUKA

Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., 3, Miyahara-cho, Takasaki-shi, Gunma 370–12, Japan. Received March 10, 1994; accepted July 8, 1994

Various heteroaromatic cyanoamidines were synthesized starting from nitriles *via* cyanoimidates or from amides *via* thioamides. The compounds were tested for inhibitory effect on the 40 mM K^+-induced contraction of rat aorta strips and selected compounds were also evaluated for antagonism of the norepinephrine-induced contraction. Most of the cyanoamidines showed vasodilatory activities. Potent vasoactive compounds were also examined for stimulation of the $^{86}Rb^+$ efflux to determine their potassium channel opening actions. Maximum potency was displayed by *N*-cyano-*N'*-(2-nitroxyethyl)-3-pyridinecarboxamidine (3h). The methanesulfonate of 3h, which was designated as KRN2391, has been selected for further development as an antianginal agent.

Keywords *N*-cyanopyridinecarboxamidine; vasodilator; potassium channel opener; structure–activity relationship; KRN2391; antianginal agent

Potassium channel openers have recently been developed as antihypertensive and antianginal drugs due to their potent smooth muscle relaxant action. The mechanism of action is thought to involve opening of membrane potassium channels, which causes the cell membrane to become hyperpolarized. This hyperpolarization inhibits opening of voltage-operated calcium channels and the intracellular calcium ion level consequently decreases, resulting in smooth muscle relaxation.[1] Potassium channel openers are structurally diverse compounds.[1] Pinacidil (**1**), an alkyl pyridyl cyanoguanidine, is used as an antihypertensive agent.[2] Nicorandil (**2**), a nitrate-containing nicotinamide derivative, induces coronary vasodilation through two different mechanisms of action, *i.e.* potassium channel opening action and soluble guanylate cyclase activation. Nicorandil is used in the treatment of angina pectoris.[3]

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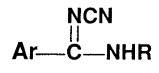
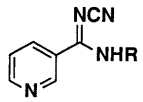


A



3

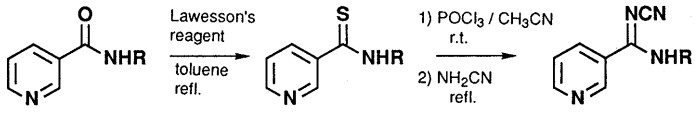
Fig. 1

The objective of this study was to develop a new structural class of potassium channel openers. The heteroaromatic cyanoamidine A was taken as a common structural feature based on the structures of pinacidil and nicorandil. In this paper, we report the synthesis and biological activities of heteroaromatic cyanoamidines.

In order to elucidate the structural requirements for vasodilatory activity, a heteroaromatic moiety was first fixed to 3-pyridyl and synthesis of *N*-cyano-*N'*-substituted-3-pyridinecarboxamidine **3** was studied. Other heteroaromatic cyanoamidines were also synthesized to investigate the relationship between the heteroaromatic moiety and its activity. These compounds were tested for inhibition of the 40 mM K^+-induced contraction of isolated rat aorta, and selected compounds were evaluated for antagonism of the norepinephrine (NE)-induced contraction in isolated rat aorta. Potent vasorelaxant compounds were also examined for stimulation of $^{86}Rb^+$ efflux to clarify their potassium channel opening profile.[4]

Chemistry

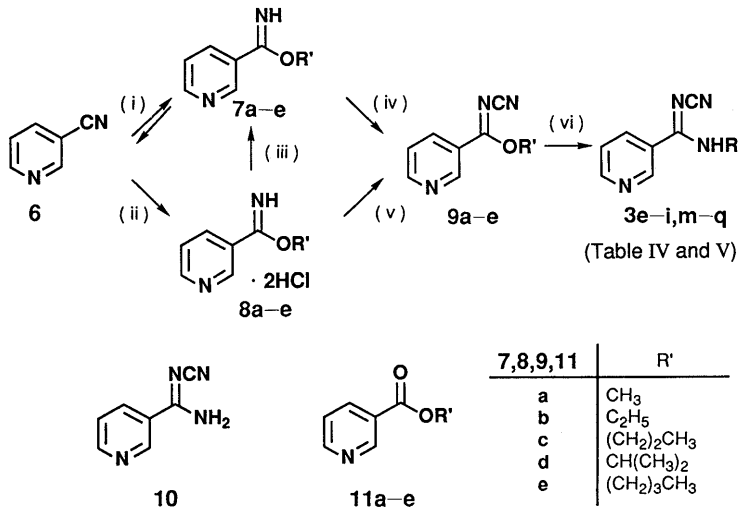
The synthesis of cyanoamidines was first attempted starting from amide *via* thioamide as shown in Chart 1.



4a–e 5a–e 3a–e

3,4,5	R
a	CH_3
b	$CH(CH_3)_2$
c	$C(CH_3)_3$
d	$CH_2CH(CH_3)_2$
e	$CH_2C(CH_3)_3$

Chart 1



(i) cat. NaOMe–R'OH, (ii) excess HCl–R'OH, (iii) excess OH^-,
(iv) NH_2CN, NaH_2PO_4–Na_2HPO_4 (1:4) / H_2O,
(v) NH_2CN, NaH_2PO_4–Na_2HPO_4 (2:3) / H_2O, (vi) NH_2R

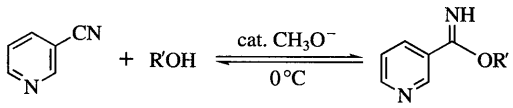
Chart 2

Treatment of *N*-alkylnicotinamides **4a—e** with Lawesson's reagent[5] in toluene yielded thioamides **5a—e**, which were converted to *N*-alkyl-*N'*-cyano-3-pyridinecarboxamidines **3a—e** by reaction with phosphorus oxychloride followed by *in situ* addition of cyanamide.[6] This procedure was not attractive because the yields were generally low in the last step and the corresponding amide must be prepared for each cyanoamidine. Furthermore, the relatively severe synthetic conditions were incompatible with chemically and thermally labile functional groups. In fact, *N*-(2-nitroxyethyl)nicotinamide (nicorandil) could not be transformed to the corresponding thioamide without decomposition.

Therefore an alternative route to cyanoamidines *via* cyanoimidates was investigated starting from 3-cyanopyridine (**6**) as outlined in Chart 2. Imidates **7** were prepared from **6** by either base-catalyzed conversion[7a,b] or acid-promoted reaction (Pinner reaction) followed by alkalization.[7b,c] It is well-known that base-catalyzed reaction of an electron-deficient nitrile with an alcohol is a reversible reaction.[7a] The process was more precisely investigated to elucidate the effect of alcohol on the equilibrium ratio, and the results are summarized in Table I. The equilibrium ratio of imidate to nitrile reached the maximum when 1-propanol and 1-butanol were used.[8] When the more bulky 2-propanol was used, the equilibrium shifted towards nitrile, which in turn decreased the yield of imidate. The effects of the reaction temperature and the molar ratio of both reactants upon the equilibrium were also important. Lower temperature gave a higher equilibrium ratio, and the use of a large quantity of alcohol was favorable for the conversion to imidate as shown in Tables II and III, respectively.

Next, the conversion of imidates **7** to cyanoimidates **9** was studied. When **7a—e** were treated with cyanamide in an organic solvent according to the procedure of Huffman and Schaefer[9] or McCall *et al.*,[10] almost no cyanoimidates **9a—e** were produced. When propyl and isopropyl

Table I. Effect of Alcohol upon Equilibrium[a]

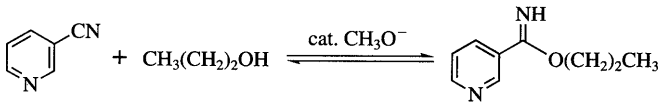


6 **7**

Run	R'	Ratio[b] **6**:**7**
a	CH_3	36:64
b	CH_2CH_3	23:77
c	$(CH_2)_2CH_3$	20:80
d	$CH(CH_3)_2$	42:58
e	$(CH_2)_3CH_3$	20:80

a) 10 eq of alcohol (R'OH) was used. *b*) Determined by HPLC.

Table II. Effect of Temperature upon Equilibrium[a]



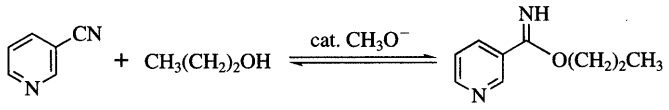
6 **7c**

Run	Temp. (°C)	Ratio[b] **6**:**7c**
1	0	20:80
2	5	18:82
3	15	25:75
4	25	32:68
5	35	39:61

a) 10 eq of 1-propanol was used. *b*) Determined by HPLC.

imidates **7c, d** were treated with cyanamide (2 eq) in aqueous phosphate buffer (NaH_2PO_4 : Na_2HPO_4 = 4 : 1),[11] the corresponding cyanoimidates **9c, d** were obtained in good yields. The pH control was crucial in this reaction.[11a] Under more acidic conditions, the imidates **7c, d** were

TABLE III. Effect of Molar Ratio upon Equilibrium



6 7c

Run	Molar ratio 1-Propanol/6	Equilibrium ratio[a] 6:7c
1	5	28:72
2	7.5	22:78
3	10	20:80
4	15	19:81
5	20	18:82

a) Determined by HPLC.

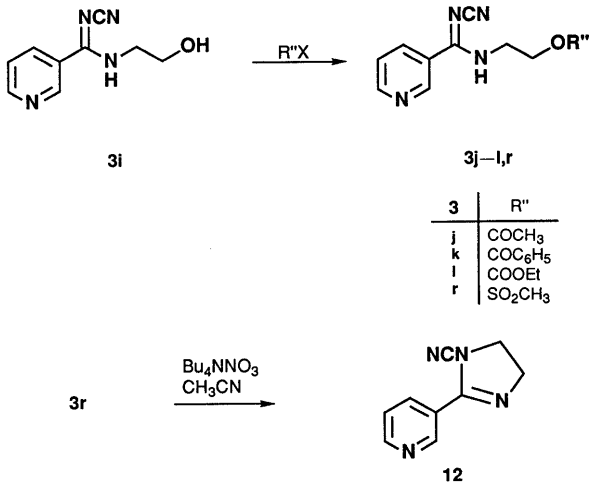


Chart 3

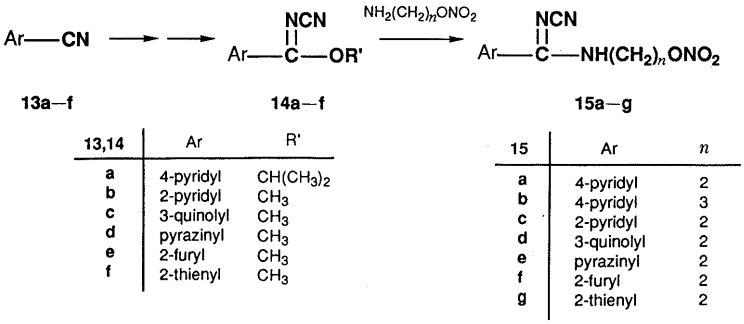


Chart 4

mainly hydrolyzed to nicotinic esters **11c, d**, while under more basic conditions *N*-cyano-3-pyridinecarboxamidine (**10**) was predominantly produced. On the other hand, when methyl and ethyl imidates **7a, b** were used as starting imidates, almost none of the desired cyanoimidates **9a, b** was obtained at any pH, and **10** and **11a, b** were produced as predominant products, the ratio of which depended on the pH of the reaction medium. These results indicated that bulky alkyl imidates such as **7c, d** did not readily undergo hydrolysis to esters **11c, d** and/or cyanoaminolysis to unsubstituted cyanoamidine **10**. Further studies revealed that imidate hydrochlorides **8c, d** could be directly converted to the corresponding cyanoimidates **9c, d** by treatment with cyanamide under a more basic condition ($NaH_2PO_4 : Na_2HPO_4 = 2:3$).[9,11]

Cyanoamidines **3e—i, m—q** were readily obtained in good yields by simply mixing cyanoimidate **9c** or **9d** with the appropriate amines in organic[12] and/or aqueous media. Therefore cyanoimidates **9c** or **9d** are suitable intermediates for the synthesis of various kinds of *N*-cyano-*N'*-substituted-3-pyridinecarboxamidines.

Imidates **7** can be synthesized from the nitrile **6** by two procedures, *i.e.*, both acid-promoted and base-catalized reactions as mentioned before. In terms of the yield of cyanoamidines, the cyanoimidate route was superior to the thioamide route (see **3e** in Table III). The mild conditions of the last step also made it possible to introduce chemically labile groups such as a nitroxyalkyl group without decomposition.

N-(2-Hydroxyethyl) cyanoamidine **3i**, obtained from **9c** and ethanolamine, was subsequently transformed to *N*-(2-acyloxyethyl) cyanoamidines **3j—l** and *N*-(2-methanesulfonylethyl) cyanoamidine **3r** (Chart 3). Preparation of the nitrate **3h** from the alcohol **3i** gave poor results. Nitration of **3i** with fuming nitric oxide gave **3h** in moderate yield with inseparable impurities. Reaction of the mesylate **3r** with tetrabutylammonium nitrate[14] gave a cyclized product, 3-(1-cyanoimidazolin-2-yl)pyridine (**12**), as a sole product instead of the desired **3h** (Chart 3).

Other heteroaromatic cyanoamidines **15a—g** were also synthesized from nitriles **13a—f** *via* imidates (Chart 4). It was found that the size of the alkyl group (R') of imidates had almost no effect on the formation of the corresponding cyanoimidates except for the imidate from 4-cyanopyridine (**13a**). Isopropyl and methyl imidates prepared from nitriles **13a** and **13b—f**, respectively, were converted to the

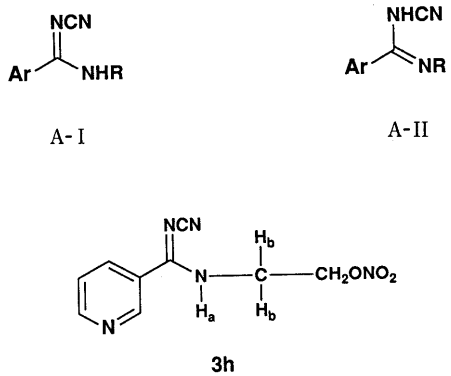
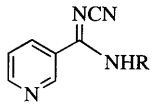


Fig. 2

TABLE IV. Physical and Biological Data for Pyridinecarboxamidines **3**[a]



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Compd. No.	R	Method[b]	Yield[c] (%)	mp (°C)	Formula	Recrystn. solvent	*Anal.*[d]	Vasodilation IC_{50} (M)[e]
3a	CH_3	I	3	181	$C_8H_8N_4$	MeOH/Et_2O	C, H, N	No effect
3b	$CH(CH_3)_2$	I	21	153	$C_{10}H_{12}N_4$	MeOH/Et_2O	C, H, N	1.9×10^{-4}
3c	$C(CH_3)_3$	I	16	128—138	$C_{11}H_{14}N_4$	MeOH/Et_2O	C, H, N	3.0×10^{-3}
3d	$CH_2CH(CH_3)_2$	I	16	83—85	$C_{11}H_{14}N_4$	MeOH/Et_2O	C, H, N	5.7×10^{-4}
3e	$CH_2C(CH_3)_3$	I	14	138—139	$C_{12}H_{16}N_4$	MeOH/Et_2O	C, H, N	5.4×10^{-4}
		II	98					
3f	$CH(CH_3)C(CH_3)_3$	II	63	182—185	$C_{13}H_{18}N_4$	MeOH/Et_2O	C, H, N	1.9×10^{-4}
3g	$(CH_2)_7CH_3$	II	70	99	$C_{15}H_{22}N_4$	MeOH/Et_2O	C, H, N	1.1×10^{-4}
3h	$(CH_2)_2ONO_2$	II	68	99.5—100.2	$C_9H_9N_5O_3$	CH_2Cl_2/Et_2O	C, H, N	5.1×10^{-5}
3i	$(CH_2)_2OH$	II	56	132	$C_9H_{10}N_4O$	MeOH	C, H, N	No effect
3j	$(CH_2)_2OCOCH_3$	III	56	Syrup	$C_{11}H_{12}N_4O_2$	—[f]	—[g]	1.9×10^{-2}
3k	$(CH_2)_2OCOC_6H_5$	III	78	134	$C_{16}H_{14}N_4O_2$	MeOH/Et_2O	C, H, N	No effect
3l[h]	$(CH_2)_2OCOOC_2H_5$	III	72	42	$C_{13}H_{18}N_4O_6S$	MeOH/Et_2O	C, H, N	2.8×10^{-2}
3m[h]	$(CH_2)_2OCH_3$	II	88	114	$C_{11}H_{16}N_4O_4S$	MeOH/Et_2O	C, H, N	No effect
3n	$(CH_2)_2N_3$	II	72	75—76	$C_9H_9N_7$	MeOH/Et_2O	C, H, N	1.5×10^{-2}
3o	$(CH_2)_2CN$	II	70	174	$C_{10}H_9N_5$	MeOH/Et_2O	C, H, N	2.1×10^{-2}
3p	$(CH_2)_2NO_2$	II	77	90	$C_{10}H_{11}N_5O_2$	MeOH/Et_2O	C, H, N	3.8×10^{-4}

a) Structures of all compound were confirmed by IR, NMR, and elemental analysis. *b*) Method I: thioamide route (see Chart 1). Method II: cyanoimidate route (see Chart 2). Compounds **3e**, **3h**, **3i**, **3m**, **3n**, and **3p** were prepared from **9c**. Compounds **3f**, **3g**, and **3o** were prepared from **9d**. Method III: see Chart 3. *c*) The yield was not optimized. Method I: overall yield from amide. Method II: yield from cyanoimidate. Method III: yield from **3i**. *d*) Analysis of indicated elements was within ±0.4% of the theoretical values. *e*) Molar concentration for 50% inhibition of isolated rat aorta precontracted by 40 mM K^+. *f*) Purification by silica-gel column chromatography. *g*) FAB (pos.)-MS *m/z*: Calcd for $(M+H)^+$: 233.250; Found: 233.247. *h*) Methanesulfonate.

corresponding cyanoimidates **14a—f** in good yields. Cyanoimidates **14a—f** gave *N*-nitroxyalkyl cyanoamidines **15a—g** simply by mixing with nitroxyalkylamines.

Due to tautomeric equilibrium about the sp^2 carbon of amidine, cyanoamidine may possess two tautomers (A-I and A-II in Fig. 2). The NMR spectrum of **3h** in dimethylsulfoxide-d_6 (DMSO-d_6) showed that the cyanoamidine proton H_a was coupled to the methylene proton H_b next to the cyanoamidine nitrogen ($J=5.4$ Hz; Fig. 2). This observation confirmed that **3h** exists predominantly as the cyanoimino form (A-I). Similar observations were also made for other cyanoamidines.

Biological Activity Discussion

The vasodilating activity of *N*-cyano-3-pyridinecarboxamidines was first examined by measuring the inhibitory effect on the 40 mM KCl-induced contraction of rat aorta, as shown in Table IV. Compound **3h**, having a *N*-(2-nitroxyethyl) substituent like nicorandil, showed greater activity than *N*-alkyl cyanoamidines **3a—g**. These results showed that the nitroxyethyl group was a more effective *N*-substituent for vasodilation than alkyl groups. When the nitroxyl group of **3h** was displaced by other functional groups, **3i—o** showed a marked reduction in potency,[15] and similar observations were reported for nicorandil.[15,16] However, replacement of the nitroxyl group of **3h** with nitromethyl (**3p**) resulted in retention of the activity.

The vasodilatory activities of various *N*-nitroxyalkyl/heteroaromatic cyanoamidines are listed in Table V. All the cyanoamidines **3q**, **15a—g**, as well as **3h**, showed good activity against both the 40 mM K^+- and NE-induced contractions, and the potencies were comparable to those of nicorandil, pinacidil, and cromakalim. The activity against the NE-induced contraction was $10-10^2$ times more potent than that against the 40 mM K^+-induced contraction. Replacement of the nitroxyethyl (**3h**, **15a**) residue by nitroxypropyl (**3q**, **15b**) decreased the vasodilating activity on the NE-induced contraction, but the activity was still high.

TABLE V. Vasodilatory Activities and Effect on $^{86}Rb^+$ Efflux of *N*-Cyano-*N'*-Nitroxyalkyl Aromatic Cyanoamidines[a]

Compd. No.	Ar	*n*	IC_{50} (M)[b] 40 mK K^+	IC_{50} (M)[b] NE	Increase in $^{86}Rb^+$ efflux over basal rate,[c] %[d]
3h	3-Pyridyl	2	5.0×10^{-5}	2.6×10^{-7}	415
3q	3-Pyridyl	3	2.0×10^{-5}	1.8×10^{-6}	171
15a	4-Pyridyl	2	1.2×10^{-5}	5.5×10^{-7}	35
15b	4-Pyridyl	3	2.0×10^{-5}	2.6×10^{-6}	9
15c	2-Pyridyl	2	4.6×10^{-6}	6.1×10^{-7}	9
15d	3-Pyridyl	2	2.4×10^{-5}	3.4×10^{-7}	304
15e	Pyrazinyl	2	5.3×10^{-5}	3.4×10^{-7}	−2
15f	2-Furyl	2	2.6×10^{-5}	Not tested	0
15g	2-Thienyl	2	1.0×10^{-5}	Not tested	−5
	Nicorandil		2.4×10^{-5}	1.7×10^{-6}	13
	Pinacidil		1.4×10^{-5}	6.4×10^{-7}	163
	Cromakalim		6.6×10^{-5}	4.6×10^{-8}	299

a) Structures of all compounds were confirmed by IR, NMR, and elemental analysis. *b*) Molar concentration for 50% inhibition of isolated rat aorta precontracted by 40 mM K^+ and 10^{-7} M NE, respectively. *c*) At 10^{-5} M of cyanoamidine. *d*) Percentage change of $^{86}Rb^+$ efflux from background control.

TABLE VI. Physical Properties of Cyanoamidines in Table V[a]

Compound No.	Starting material[b]	Yield[c] (%)	mp (°C)	Formula	Recryst. solvents	*Anal.*[d]
3q	**9d**	39	124.9—125.8	$C_{10}H_{11}N_5O_3$	MeOH/Et_2O	C, H, N
15a	**14a**	61	102.5—103.0	$C_9H_9N_5O_3$	CH_2Cl_2/Et_2O	C, H, N
15b	**14a**	41	112.5—112.8	$C_{10}H_{11}N_5O_3$	MeOH/Et_2O	C, H, N
15c	**14b**	63	53.5—54.0	$C_9H_9N_5O_3$	CH_2Cl_2/Et_2O	C, H, N
15d	**14c**	54	126.5—127.0	$C_{13}H_{11}N_5O_3$	CH_2Cl_2/Et_2O	C, H, N
15e	**14d**	26	102.8—103.0	$C_8H_8N_6O_3$	MeOH/Et_2O	C, H, N
15f	**14e**	45	77.0—77.8	$C_8H_8N_4O_4$	CH_2Cl_2/Et_2O	C, H, N
15g	**14f**	40	101.5—102.0	$C_8H_8N_4O_3S$	AcOEt/hexane	C, H, N

a) Physical properties of **3h** are listed in Table III. *b*) Yield from cyanoimidate. The yield was not optimized. *d*) Analysis of indicated elements was within ±0.4% of the theoretical values.

TABLE VII. Physical Properties and Spectral Data for Cyanoimidates

Compd. No.	Yield[a] (%)	mp (°C)	IR[b] ν_{max} (cm^{-1})	^{1}H-NMR: δ (ppm)[c]
9c	60 (65)[d]	Oil	2180, 1610[e]	9.19 (1H, d, J=1.8 Hz), 8.84 (1H, dd, J=4.9, 1.8 Hz), 8.52 (1H, ddd, J=7.9, 1.8, 1.8 Hz), 7.49 (1H, dd, J=7.9, 4.9 Hz), 4.44 (2H, t, J=6.3 Hz), 1.89 (2H, m), 1.07 (3H, t, J=7.6 Hz)
9d	26	Oil	2180, 1610[e]	9.15 (1H, d, J=2.6 Hz), 8.83 (1H, dd, J=4.9, 1.7 Hz), 8.48 (1H, ddd, J=8.1, 2.6, 1.7 Hz), 7.50 (1H, dd, J=8.1, 4.9 Hz), 5.42 (1H, m), 1.48 (6H, d, J=7.2 Hz)[f]
14a	62	Oil	2200, 1620[e]	8.9—8.7 (2H, m), 8.0—7.8 (2H, m), 5.42 (1H, m), 1.50 (6H, d, J=6.1 Hz)[f]
14b	57	81.0—81.5	2200, 1640	8.83 (1H, ddd, J=9.4, 3.4, 2.4 Hz), 7.98 (1H, dd, J=7.3, 2.4 Hz), 7.94 (1H, d, J=3.4 Hz), 7.63 (1H, dd, J=9.4, 7.3 Hz), 4.16 (3H, s)[f,g]
14c	53	113.5—113.8	2190, 1610	9.35 (1H, d, J=2.6 Hz), 9.17 (1H, d, J=2.6 Hz), 8.17 (1H, d, J=8.0 Hz), 8.00 (1H, d, J=8.0 Hz), 7.90 (1H, dd, J=8.0, 8.0 Hz), 7.68 (1H, dd, J=8.0, 8.0 Hz), 4.18 (3H, s)
14d	56	47.5—49.0	2190, 1630	9.33 (1H, s), 8.78 (1H, d, J=2.2 Hz), 8.74 (1H, d, J=2.2 Hz), 4.07 (3H, s)
14e	67	58.5—59.2	2200, 1600	7.78 (1H, d, J=3.8 Hz), 7.69 (1H, d, J=1.8 Hz), 6.64 (1H, dd, J=3.8, 1.8 Hz), 4.05 (3H, s)
14f	54	66.9—67.1	2200, 1580	8.64 (1H, d, J=4.8 Hz), 7.77 (1H, d, J=4.8 Hz), 7.27 (1H, t, dd, J=4.8, 4.8 Hz), 4.10 (3H, s)

a) Overall yield from nitrile *via* imidate prepared by the base-catalyzed reaction except for the numbers in parentheses. *b*) KBr. *c*) Measured in $CDCl_3$; 500 MHz. *d*) Overall yield from nitrile *via* imidate prepared by Pinner's method. *e*) Neat. *f*) 90 MHz. *g*) Measured in $CDCl_3$–CD_3OD.

It is well-known that organic nitrates stimulate soluble guanylate cyclase, causing vasodilation. So, the nitroxyalkyl cyanoamidines might also activate guanylate cyclase.[17] In contrast, **3p** having no nitroxyl moiety retained good activity, as mentioned above. From these results, nitroxyalkyl cyanoamidines might have some other mode of action than guanylate cyclase activation.[18] In earlier pharmacological studies,[4a,19] potassium channel openers seemed to relax the NE-induced contraction more than the contraction induced by a high concentration of K^+. Therefore, nitroxyalkyl cyanoamidines might also possess a potassium channel opening property. This was investigated by studying the increase in the basal efflux rate of $^{86}Rb^+$ as a K^+ marker in rat aorta (Table V).[4] As shown in Table V, vasodilating activities were not correlated with the strength of $^{86}Rb^+$ efflux activity. Compounds **3h, q, 15d** as well as pinacidil and cromakalim produced a marked increase in the efflux. The 3-pyridyl derivatives **3h, q** induced more active efflux than 4-pyridyl (**15a, b**) and 2-pyridyl (**15c**) derivatives. Concerning the chain length of the nitroxyalkyl residue, the nitroxyethyl derivatives **3h, 15a** possessed more potent efflux activity than nitroxypropyl derivatives **3q, 15b**, respectively. The 3-quinolyl analogue **15d** also showed a marked increase in the $^{86}Rb^+$ efflux. In contrast, pyrazinyl (**15e**), 2-furyl (**15f**), and 2-thienyl (**15g**) derivatives did not affect the efflux at the same concentration.[20] Therefore it is considered that the potassium channel opening action contributed to the vasorelaxant effects of **3h, q** and **15d**. In contrast, it could be presumed that the vasodilating activities of **15a—c, e—g** with little or no $^{86}Rb^+$ efflux activities were caused by nitrate action, but not potassium channel opening action.

In summary, aromatic cyanoamidines, especially *N*-nitroxyalkyl cyanoamidines, possess potent vasodilating activity. The $^{86}Rb^+$ efflux study showed that *N*-cyano-*N'*-nitroxyalkylpyridinecarboxamidine possesses potassium channel opening ability. 3-Pyridyl is the optimal aromatic moiety (Ar) and nitroxyethyl was more effective than nitroxypropyl as the *N*-substituent (R) in cyanoamidine A (Fig.1). The nitroxyl group is important not only for its action as a nitrate but also for its potency as a potassium channel opener.[18] A structural comparison of **3h** with nicorandil shows that replacement of the carbonyl oxygen (=O) in nicorandil with a cyanoimino moiety (=NCN), as in **3h**, greatly enhances the stimulation of $^{86}Rb^+$ efflux.[18] The methanesulfonate of the most active *N*-cyano-*N'*-(2-nitroxyethyl)-3-pyridinecarboxamidine (**3h**), designated as KRN2391, has been selected for development as an antianginal agent.

Experimental

Melting points were determined using a Yanagimoto micro melting-point apparatus, without correction. IR spectra were run on a Jasco A-3 spectrophotometer. ^{1}H-NMR spectra were recorded at 500 MHz with a JEOL GX-500 spectrometer and at 90 MHz with a JEOL EX-90 spectrometer using tetramethylsilane as an internal standard, and chemical shifts are given in ppm. Microanalyses were performed on a Perkin-Elmer Model 240c elemental analyzer. Mass

TABLE VIII. Spectral Data for Cyanoamidines Listed in Table IV and V

Compd. No.	IR (KBr) ν_{max} (cm^{-1})	Solvent	^{1}H-NMR: δ (ppm)[a]
3a	3240, 2190, 1600, 1550, 1420, 710	90 MHz DMSO-d_6	9.30 (1H, q, J=4.6 Hz), 8.9—8.7 (2H, m), 8.03 (1H, ddd, J=7.9, 2.2, 1.8 Hz), 7.59 (1H, dd, J=7.9, 4.8 Hz), 2.91 (3H, d, J=4.6 Hz)
3b	3420, 2970, 2180, 1610, 1580, 1550	90 MHz DMSO-d_6	9.17 (1H, d, J=6.3 Hz), 8.9–8.7 (2H, m), 8.01 (1H, ddd, J=7.9, 2.4, 1.8 Hz), 7.58 (1H, ddd, J=7.9, 4.8, 0.9 Hz), 4.18 (1H, m), 1.21 (6H, d, J=6.6 Hz)
3c	2950, 2700, 2200, 1580, 1550, 1480, 1400, 710	90 MHz DMSO-d_6	8.9—8.6 (3H, m), 7.96 (1H, dd, J=7.9, 2.3 Hz), 7.67 (1H, dd, J=7.9, 4.9 Hz), 1.43 (9H, s)
3d	3430, 2960, 2930, 2180, 1610, 1580, 1550, 1390	90 MHz DMSO-d_6	9.35 (1H, br s), 8.9—8.7 (2H, m), 8.03 (1H, ddd, J=8.0, 2.4, 1.8 Hz), 7.59 (1H, ddd, J=8.0, 4.8, 0.9 Hz), 3.19 (2H, br d, J=5.5 Hz), 2.50 (1H, m), 0.94 (6H, d, J=6.6 Hz)
3e	2970, 2200, 1580, 1560	90 MHz DMSO-d_6	9.19 (1H, t, J=6.2 Hz), 8.9—8.7 (2H, m), 8.01 (1H, ddd, J=7.7, 2.2, 2.0 Hz), 7.59 (1H, dd, J=7.7, 4.4 Hz), 3.21 (2H, d, J=6.2 Hz), 0.96 (9H, s)
3f	2960, 2180, 1600, 1580, 1550	90 MHz DMSO-d_6	9.04 (1H, d, J=9.2 Hz), 8.9—8.6 (2H, m), 7.98 (1H, ddd, J=7.9, 2.2, 1.5 Hz), 7.59 (1H, ddd, J=7.9, 4.8, 0.9 Hz), 4.06 (1H, dq, J=9.2, 6.8 Hz), 1.12 (3H, d, J=6.8 Hz), 0.95 (9H, s)
3g	2910, 2150, 1570, 1540, 1440, 1235, 700	90 MHz DMSO-d_6	9.26 (1H, br s), 8.9—8.7 (2H, m), 8.01 (1H, ddd, J=7.9, 2.2, 1.8 Hz), 7.59 (1H, ddd, J=7.9, 4.8, 0.9 Hz), 3.35 (2H, br t, J=6.4 Hz), 1.7—1.1 (12H, m), 0.87 (3H, t, J=6.2 Hz)
3h	2180, 1640, 1590, 1280	500 MHz DMSO-d_6	9.55 (1H, t, J=5.4 Hz), 8.8—8.7 (2H, m), 8.06 (1H, dd, J=8.0, 2.2 Hz), 7.62 (1H, dd, J=8.0, 4.9 Hz), 4.74 (2H, t, J=5.2 Hz), 3.74 (2H, dt, J=5.4, 5.2 Hz)
3i	2200, 1610, 1580, 1550, 1070, 710	90 MHz DMSO-d_6	9.29 (1H, t, J=5.4 Hz), 8.8—8.7 (2H, m), 8.03 (1H, ddd, J=7.9, 2.4, 1.8 Hz), 7.58 (1H, ddd, J=7.9, 4.8, 0.7 Hz), 3.7—3.4 (4H, m)
3j	2180, 1740, 1590, 1230[c]	90 MHz DMSO-d_6	9.44 (1H, br s), 8.9—8.7 (2H, m), 8.03 (1H, ddd, J=7.9, 2.2, 1.5 Hz), 7.60 (1H, ddd, J=7.9, 4.8, 0.9 Hz), 4.23 (2H, t, J=9.9 Hz), 3.61 (2H, t, J=9.9 Hz), 2.03 (3H, s)
3k	2200, 1700, 1610, 1290, 720	90 MHz DMSO-d_6	9.56 (1H, br s), 8.8—8.7 (2H, m), 8.1—7.9 (3H, m), 7.7—7.4 (4H, m), 4.51 (2H, t, J=5.2 Hz), 3.78 (2H, t, J=5.2 Hz)
3l[b]	2170, 1740, 1580, 1260	90 MHz DMSO-d_6	9.57 (1H, t, J=5.3 Hz), 9.0—8.8 (2H, m), 8.19 (1H, ddd, J=7.9, 2.4, 1.8 Hz), 7.71 (1H, ddd, J=7.9, 4.8, 0.7 Hz), 4.5—3.9 (4H, m), 3.68 (2H, dt, J=5.3, 5.3 Hz), 2.40 (3H, s), 1.22 (3H, t, J=7.1 Hz)
3m[b]	2200, 1600, 1210, 1060	90 MHz DMSO-d_6	9.51 (1H, t, J=5.2 Hz), 9.0—8.8 (2H, m), 8.22 (1H, ddd, J=7.9, 2.2, 1.8 Hz), 7.76 (1H, ddd, J=7.9, 4.8, 0.9 Hz), 3.6—3.4 (4H, m), 3.29 (3H, s), 2.41 (3H, s)
3n	2200, 2130, 1600, 1550	500 MHz $CDCl_3$	8.8—8.7 (3H, m), 8.03 (1H, dd, J=7.9, 2.2 Hz), 7.47 (1H, dd, J=7.9, 4.9 Hz), 3.7—3.6 (4H, m)
3o	2200, 1590, 1555, 1440, 1380, 710	90 MHz DMSO-d_6	9.61 (1H, t, J=5.4 Hz), 8.9—8.7 (2H, m), 8.05 (1H, ddd, J=8.0, 2.3, 1.8 Hz), 7.62 (1H, ddd, J=8.0, 4.8, 0.9 Hz), 3.62 (2H, dt, J=5.4, 6.4 Hz), 2.22 (2H, t, J=6.4 Hz)
3p	2160, 1590, 1570, 710	90 MHz DMSO-d_6	9.32 (1H, br s), 8.8—8.7 (2H, m), 8.05 (1H, ddd, J=7.9, 2.4, 1.8 Hz), 7.60 (1H, ddd, J=7.9, 4.8, 0.9 Hz), 4.68 (2H, t, J=6.8 Hz), 3.46 (2H, t, J=6.8 Hz), 2.23 (2H, tt, J=6.8, 6.8 Hz)
3q	2180, 1620, 1600, 1560, 1280	500 MHz $CDCl_3$–CD_3OD	8.8—8.7 (2H, m), 8.10 (1H, ddd, J=7.8, 2.4, 2.4 Hz), 7.54 (1H, dd, J=7.8, 5.2 Hz), 4.59 (2H, t, J=6.0 Hz), 3.61 (2H, t, J=6.0 Hz), 2.14 (2H, m)
15a	2180, 1640, 1580, 1540, 1290, 1280	90 MHz DMSO-d_6	9.58 (1H, t, J=5.4 Hz), 8.82 (2H, dd, J=4.4, 1.5 Hz), 7.56 (2H, dd, J=4.4, 1.5 Hz), 4.74 (2H, t, J=5.2 Hz), 3.73 (2H, dt, J=5.4, 5.2 Hz)
15b	2180, 1600, 1280	500 MHz $CDCl_3$–CD_3OD	8.75 (2H, dd, J=4.4, 1.6 Hz), 7.54 (2H, dd, J=4.4, 1.6 Hz), 4.57 (2H, t, J=6.0 Hz), 3.59 (2H, t, J=6.0 Hz), 2.13 (2H, m)
15c	2180, 1640, 1600, 1580, 1560, 1290	90 MHz CD_3OD	8.73 (1H, m), 8.3—7.9 (2H, m), 7.64 (1H, m), 4.77 (2H, t, J=5.5 Hz), 3.92 (2H, t, J=5.5 Hz)
15d	2190, 1620, 1580, 1560, 1280	500 MHz CD_3OD	9.05 (1H, s), 8.71 (1H, s), 8.13 (1H, d, J=7.9 Hz), 8.10 (1H, d, J=7.9 Hz), 7.93 (1H, dd, J=7.9, 7.9 Hz), 7.74 (1H, dd, J=7.9, 7.9 Hz), 4.80 (2H, t, J=5.7 Hz), 3.92 (2H, t, J=5.7 Hz)
15e	2180, 1630, 1620, 1290	500 MHz $CDCl_3$	9.83 (1H, t, J=5.2 Hz), 8.88 (1H, s), 8.64 (1H, br s), 8.28 (1H, br s), 4.78 (2H, t, J=4.9 Hz), 4.15 (2H, dt, J=5.2, 4.9 Hz)
15f	2180, 1630, 1600, 1570	500 MHz $CDCl_3$	8.04 (1H, d, J=3.7 Hz), 7.57 (1H, d, J=1.2 Hz), 6.79 (1H, t, J=5.3 Hz), 6.66 (1H, dd, J=3.7, 1.2 Hz), 4.69 (2H, t, J=5.5 Hz), 3.87 (2H, dt, J=5.3, 5.5 Hz)
15g	2180, 1630, 1570, 1280	500 MHz $CDCl_3$	7.96 (1H, d, J=3.7 Hz), 7.61 (1H, d, J=3.7 Hz), 7.19 (1H, dd, J=3.7, 3.7 Hz), 4.70 (2H, t, J=4.9 Hz), 3.82 (2H, t, J=4.9 Hz)

a) br s: broad singlet; br d: broad doublet. *b*) Methanesulfonate. *c*) Film.

spectra were determined on a JEOL JMS-SX102A using either fast atom bombardment (FAB) ionization or field desorption (FD) techniques. Analytical liquid chromatograms were obtained with a Hitachi LC (L-6000 solvent delivery system and L-4000 UV detector (at 254 nm)) using a YMC-Pack ODS-AM (S-5 120 A, 150 × 6 mm) with 50% CH_3CN in water, adjusted to pH 2.5 with H_3PO_4, as an eluent and the flow rate was 1.0 ml/min.

Studies of Nitrile-Imidate Equilibrium 3-Cyanopyridine (**6**; 4.8 mmol) was mixed with alcohol (R'OH; 24—96 mmol) and NaOMe (0.09 mmol), and the mixture was stirred at the indicated temperature overnight. A portion of the reaction mixture was diluted with CH_3CN : H_2O = 50 : 50 (v/v) and immediately analyzed by HPLC. The equilibrium ratio was calculated from the area % ratio of the nitrile and imidate peaks. The results are summarized in Tables I, II, and III.

Synthesis The following examples are representative of the experimental procedures for cyanoamidines.

***N*-Cyano-*N'*-(2,2-dimethylpropyl)-3-pyridinecarboxamidine (3e). Method I** A mixture of *N*-(2,2-dimethylpropyl)nicotinamide[21] (**4e**) (1.00 g, 5.2 mmol) and Lawsson's reagent[5] (2.52 g, 6.2 mmol) in toluene (100 ml) was refluxed for 1.5 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was evaporated *in vacuo* and the semi-solid residue was triturated with 2 N HCl (200 ml × 3) and filtered. The aqueous layer was washed with $CHCl_3$ (500 ml) and then neutralized with 2 N NaOH. The solution was extracted with $CHCl_3$ (200 ml × 3) and the combined extracts were dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography (silica gel, $CHCl_3$–MeOH (100 : 1)) to give *N*-(2,2-dimethylpropyl)nicotinethioamide (**5e**) (0.80 g, 74%) as a crystalline solid, mp 134.9—135.5 °C

(AcOEt–hexane).

$POCl_3$ (0.74 g, 4.8 mmol) was added dropwise to a solution of **5e** (0.50 g, 2.2 mmol) in CH_3CN (30 ml) and the solution was stirred overnight at room temperature under an argon atmosphere. This solution was treated with NH_2CN (1.01 g, 24.0 mmol) followed by Et_3N (0.49 g, 4.9 mmol) and heated to reflux for 4 h under an argon atmosphere. After evaporation, the residue was dissolved in $CHCl_3$ (50 ml) and the resultant precipitates were filtered off. The filtrate was evaporated and purified by column chromatography (silica gel, $CHCl_3$–MeOH (50 : 1)) to give **3e** (0.10 g, 14%) as colorless crystals, mp 138.0—139.0 °C (MeOH–Et_2O); *Anal.* Calcd for $C_{12}H_{16}N_4$: C, 66.64; H, 7.46; N, 25.91. Found: C, 66.87; H, 7.48; N, 25.85. Spectral data are shown in Table VII.

Compounds **3a—e** were prepared similarly, and their physical and spectral data are listed in Tables IV and VIII, respectively.

Method II-1 *Via* Base-Catalyzed Conversion: A mixture of 3-cyanopyridine (**6**) (10.0 g, 96.1 mmol) and NaOMe (0.16 g, 2.9 mmol) in PrOH (120 ml) was stirred overnight at 0 °C. AcOH (0.19 g, 3.2 mmol) was then added with stirring and the solution was evaporated *in vacuo*. Hexane (100 ml) was added to the residue and the resultant precipitates were filtered off. The filtrate was evaporated to give crude propyl 3-pyridinecarboximidate (**7c**) (11.3 g) as an oil. Crude **7c** was then added to a mixture of NH_2CN (5.89 g, 0.14 mol), $NaH_2PO_4 \cdot 2H_2O$ (43.7 g, 0.28 mol), and Na_2HPO_4 (9.93 g, 0.07 mol) in water (75 ml). After vigorous stirring for 7 h at room temperature, the reaction mixture was extracted with CH_2Cl_2 (150 ml × 3). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness to yield crude propyl *N*-cyano-3-pyridinecarboximidate (**9c**), which was used directly in the following reaction. Crude **9c** was purified by column chromatography (silica gel, hexane–ether (1 : 2)) to give oily **9c** (10.9 g, 60%). Physical and spectral data are shown in Table VI.

Method II-2 *Via* Pinner Reaction: Dry HCl was passed into a solution of **6** (3.0 g, 28.8 mmol) in PrOH (100 ml) at 0 °C until no further precipitate appeared. After stirring overnight at 0 °C, the suspension was evaporated *in vacuo* and the residual solid was collected and rinsed with ether to yield crude propyl 3-pyridinecarboximidate dihydrochloride (**8c**) (6.45 g, 94%) as a colorless hygroscopic cake. Crude **8c** (1.0 g, 4.22 mmol) was added to a vigorously stirred solution of NH_2CN (355 mg, 8.45 mmol), $NaH_2PO_4 \cdot 2H_2O$ (1.32 g, 8.46 mol) and Na_2HPO_4 (1.79 g, 12.6 mmol) in water (20 ml). After stirring for 2 h at room temperature, the reaction mixture was extracted with CH_2Cl_2 (40 ml × 3). Further work-up as above gave **9c** (0.52 g, 65%).

To a solution of **9c** (0.35 g, 1.85 mmol) in MeOH (6 ml), 2,2-dimethylpropylamine (0.31 ml, 2.65 mmol) was added and stirred overnight at room temperature. After evaporation, the residue was purified by column chromatography (silica gel, $CHCl_3$–MeOH (50 : 1)) to give **3e** (0.39 g, 98%) as a colorless crystalline solid.

Cyanoimidates **9d**, **14a—f** were also prepared either *via* base-catalyzed conversion or *via* the Pinner reaction, and their physical and spectral data are listed in Table V.

Cyanoamidines **3f, g, n—p** were similarly prepared, and their physical and spectral data are listed in Tables IV and VIII, respectively.

***N*-Cyano-*N'*-(2-nitroxyethyl)-3-pyridinecarboxamidine (3h)** To a cooled solution of NaOH (2.4 g, 60.0 mmol) in water (50 ml), 2-nitroxyethylamine · HCl[22] (8.3 g, 58.2 mmol) and **9c** (10.0 g, 52.8 mmol) were added successively. The reaction mixture was stirred at ambient temperature for 1 h and the resulting precipitate was collected by filtration and rinsed several times with water. The precipitates were dried under reduced pressure and recrystallized from CH_2Cl_2–Et_2O to afford **3h** (8.5 g, 68%) as a colorless crystalline solid.

Physical and spectral data are shown in Tables IV and VIII, respectively.

***N*-Cyano-*N'*-(2-nitroxyethyl)-3-pyridinecarboxamidine Methanesulfonate (KRN2391)** Methanesulfonic acid (1.02 g, 10.6 mmol) was added to **3h** (2.50 g, 10.6 mmol) in MeOH (30 ml). Iso-Pr_2O (40 ml) was added to the solution, and the resulting precipitate was collected by filtration and washed with iso-Pr_2O (40 ml). The precipitates were recrystallized from MeOH–iso-Pr_2O to give KRN2391 (3.0 g, 86%) as a colorless crystalline solid, mp 148—150 °C. IR (KBr): 2180, 1620, 1580, 1280, 1220, 540 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO) δ (ppm): 9.66 (1H, t, J=4.9 Hz), 8.95—8.85 (2H, m), 8.26 (1H, ddd, J=7.9, 1.8, 1.8 Hz), 7.80 (1H, dd, J=7.9, 5.5 Hz), 4.74 (2H, t, J=4.9 Hz), 3.75 (2H, dt, J=4.9, 4.9 Hz), 2.43 (3H, s). *Anal.* Calcd for $C_{10}H_{13}N_5O_6S$: C, 36.25; H, 3.88; N, 21.34. Found: C, 36.25; H, 3.95; N, 21.14.

***N*-Cyano-*N'*-(2-hydroxyethyl)-3-pyridinecarboxamidine (3i)** To a solution of 2-aminoethanol (20 g, 0.33 mol) in water (30 ml), **9c** (60 g, 0.32 mol) was added. The mixture was stirred at room temperature for 30 min then kept in a refrigerator overnight. The resulting crystalline precipitate was collected by filtration, rinsed twice with water, and recrystallized from MeOH to give **3i** (34 g, 56%) as colorless crystals. Physical and spectral data are shown in Tables IV and VIII, respectively.

***N*-Cyano-*N'*-(2-methoxyethyl)-3-pyridinecarboxamidine Methanesulfonate (3m)** 2-Methoxyethylamine (0.95 g, 12.6 mmol) was added to a solution of **9c** (2.0 g, 10.6 mmol) in MeOH (20 ml) and the mixture was stirred at room temperature for 1.5 h. After evaporation, the residue was purified by column chromatography (silica gel, $CHCl_3$–MeOH (50 : 1)) to give *N*-cyano-*N'*-(2-methoxyethyl)-3-pyridinecarboxamidine (2.07 g, 10.1 mmol) as a syrup. To this syrup in MeOH (10 ml), methanesulfonic acid (1.0 g, 10.4 mmol) was added. After evaporation, the residue was crystallized from MeOH–Et_2O to give **3m** (2.80 g, 84%). Physical and spectral data are shown in Tables IV and VIII, respectively.

***N*-Cyano-*N'*-(3-nitroxypropyl)-3-pyridinecarboxamidine (3q)** To a solution of isopropyl *N*-cyano-3-pyridinecarboximidate (**9d**) (0.50 g, 2.6 mmol) in MeOH (10 ml), nitroxypropylamine · HNO_3[22] (0.53 g, 2.9 mmol) and NaOMe (0.16 g, 2.9 mmol) were successively added and the reaction mixture was stirred at room temperature for 18 h. After evaporation, the residue was dissolved in $CHCl_3$ (90 ml). The solution was washed with water (100 ml), dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by column chromatography (silica gel, $CHCl_3$–MeOH (60 : 1)) to give **3q** (0.26 g, 39%) as colorless crystals.

Compounds **15a—g** were prepared similarly, and their physical and spectral data are listed in Tables VI and VIII, respectively.

***N*-(2-Acetoxyethyl)-*N'*-cyano-3-pyridinecarboxamidine (3j). Method III** To a solution of **3i** (2.0 g, 10.5 mmol) and pyridine (2.5 ml) in dimethylformamide (DMF) (10 ml), Ac_2O (1.3 g, 12.7 mmol) was added at 0 °C. The mixture was stirred at room temperature for 3 h and then diluted with cold water. After extraction with AcOEt (50 ml × 2), the combined extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (silica gel, $CHCl_3$–MeOH (50 : 1)) to give **3j** (1.96 g, 80%) as a syrup.

Compound **3k** was prepared in a similar manner. Physical and spectral data of **3j, k** are listed in Tables IV and VIII, respectively.

***N*-Cyano-*N'*-(2-ethoxycarbonyloxyethyl)-3-pyridinecarboxamidine Methanesulfonate (3l)** To a solution of **3i** (2.0 g, 10.5 mmol) and pyridine (2.5 ml) in DMF (10 ml), ethyl chloroformate (1.70 g, 15.7 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 5 h and then diluted with cold water. After extraction with AcOEt (50 ml × 2), the combined extracts were washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by column chromatography (silica gel, $CHCl_3$–MeOH (50 : 1)) to give *N*-cyano-*N'*-(2-ethoxycarbonyloxyethyl)-3-pyridinecarboxamidine (2.59 g, 94%) as a syrup. Methanesulfonic acid (1.0 g, 10.4 mmol) was then added to this syrup in MeOH (20 ml). After evaporation, the residue was crystallized from MeOH–Et_2O to give **3l** (2.89 g, 77%). Physical and spectral data are listed in Tables IV and VIII, respectively.

3-(1-Cyanoimidazolin-2-yl)pyridine (12) To a solution of **3i** (0.50 g, 2.63 mmol) and Et_3N (0.7 ml, 5.02 mmol) in DMF (10 ml), methanesulfonyl chloride (0.4 ml, 5.17 mmol) was added at 0 °C and the mixture was stirred at room temperature for 1 h. The resultant solution was poured into water and extracted with AcOEt (20ml). The organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated to give the crude mesylate **3r** (0.54 g) as a syrup. To a solution of **3r** in CH_3CN (20 ml), Bu_4NNO_3[14] (0.80 g, 2.63 mmol) was added and the mixture was refluxed for 1.5 h with stirring. The solvent was removed and the residue was dissolved in AcOEt (50 ml). The solution was washed with water and brine, and then dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (silica gel, $CHCl_3$–MeOH (50 : 1)) to give **12** (1.96 g, 80%) as a syrup. IR (neat): 2230, 1650, 1280 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δ (ppm): 9.10 (1H, d, J=1.5 Hz), 8.76 (1H, dd, J=4.8, 1.5 Hz), 8.16 (1H, ddd, J=7.9, 2.2, 1.8 Hz), 7.41 (1H, ddd, J=7.9, 4.8, 0.9 Hz), 4.2—4.0 (4H). FD-MS m/z: 172 (M^+).

Biological Activities All tissues used in the experiments were obtained from male Wistar rats.

Tissue Bath Studies Thoracic aorta was removed from surrounding connective tissue and cut into ring segments, each about 3 mm long. The endothelium was removed mechanically by rubbing the intimal surface with a wooden stick. Each preparation was mounted in a tissue bath

filled with 10 ml of Krebs–Ringer solution of the following composition (mM): NaCl 112, KCl 4.7, $CaCl_2$ 2.2, $NaHCO_3$ 25, $MgCl_2$ 1.2, KH_2PO_4 1.2 and glucose 14. This was maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. The tension of each segment was measured isometrically with a force-displacement transducer and preparations were loaded at 1 g tension then allowed to equilibrate for 120 min before the acute experiments were started. After the equilibration period, the preparations were contracted by changing the solution in the bath to one containing 40 mM K^+. Contraction of the preparations was also induced by the addition of NE to the bath to give a final concentration of 10^{-7} M. The ability of the compound to relax the established contractions due to high K^+ or NE was determined using a cumulative protocol. The response was expressed as percentage inhibition of each contraction and the mean IC_{50} value (with 95% confidence limits) was calculated.

$^{86}Rb^+$ Efflux The aorta was cut into two rings and each ring was opened into a flat sheet by cutting it longitudinally. Each segment was hooked onto a bent pin then inserted into a tube containing 2 ml Krebs–Ringer solution at 37 °C bubbled with 95% O_2 and 5% CO_2. After a 30 min-equilibration period, the preparation was loaded with $^{86}Rb^+$ (0.74 MBq/ml) for 180 min. $^{86}Rb^+$ was then allowed to leave the tissues using 2 min collection periods and after nine such periods (18 min efflux) the ability of the compound to enhance efflux was tested by exposing the tissue to the compound under test between minutes 18 and 26 of the efflux period. At the end of the efflux period, the radioactivity in the tubes and that remaining in tissues was measured. The rate coefficients were calculated from $^{86}Rb^+$ released during each 2 min period and expressed as a percentage of the mean tissue $^{86}Rb^+$ remaining during that period. The mean rate coefficient over minutes 13—18 of the efflux period was taken as the basal rate. Stimulation of efflux rate by the compound was calculated as the maximum rate observed over minutes 18—26 of the efflux period divided by basal rate and was expressed as a percentage.

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