Synthesis and Structure–Activity Relationships of Thienylcyanoguanidine Derivatives as Potassium Channel Openers[1)]

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In our series of studies on potassium channel openers, several thienylcyanoguanidine derivatives were synthesized and evaluated for smooth muscle relaxation activity *in vitro*. Among the newly synthesized compounds, *N*-cyano-*N'*-(5-cyano-3-thienyl)-*N''*-*tert*-pentylguanidine (4b) and *N*-(5-bromo-3-thienyl)-*N'*-cyano-*N''*-*tert*-pentylguanidine (4f) exhibited excellent activity which was proved to be based on potassium channel-opening action. Bioisosterism between benzene and thiophene ring was observed in the arylcyanoguanidines. After intravenous administration to dogs, 4b lowered the blood pressure more strongly than pinacidil.

Key words potassium channel opener; thienylcyanoguanidine; pinacidil; smooth muscle relaxation activity

Hypertension, asthma, angina pectoris, and urinary incontinence are known to be caused by contraction of smooth muscles. Potassium channel openers have attracted considerable attention over the past ten years, because potassium channels play a crucial role in controlling cellular membrane potential and the opening of potassium channels leads to relaxation of smooth muscles. Thus, potassium channel openers are thought to be useful in the treatment of these diseases.[2,3)] Pinacidil,[4)] a well-known potassium channel opener, was proved to be effective in the therapy of hypertension.[5)]

Some studies on its derivatives, including replacement of the pyridine ring with another aromatic ring, have been carried out[6–8)] in order to clarify the structure–activity relationships and/or to overcome drawbacks[9–11)] of pinacidil. In our previous paper, we also reported that phenylcyanoguanidines substituted with electron-withdrawing groups showed more potent activity than pinacidil.[1)]

Thiophene, a five-membered heterocycle with six-π electron aromaticity, is electronically similar to benzene, though it is different from benzene in shape and in the presence of the hetero atom. Such features of thiophene have prompted medicinal chemists to convert a benzene ring in pharmacologically active compounds into a thiophene ring.[12–14)] In some cases, bioisosterism has been observed between the benzene and thiophene rings,[12,14)] but, in other cases, replacement of the benzene ring with a thiophene ring resulted in a marked drop of the activity.[13,14)] Among potassium channel openers, thiophene analogues of cromakalim, show comparable antihypertensive activity to that of cromakalim.[15)] These reports encouraged us to synthesize thienylcyanoguanidines with the intention of investigating their biological activities, and elucidating whether bioisosterism between benzene and thiophene rings exists in this case.

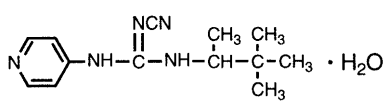
Chemistry

Compounds listed in Table 1 were synthesized by method A, B, or C, as shown in Chart 1. Thienylcyanoguanidines **4b**, **4d**, **4e**, and **4g** were prepared by replacing the two phenoxy groups of diphenyl cyanocarbonimidate **1** with alkylamines and aminothiophenes **3** successively (method A).[16)] When method A was applied for the synthesis of **4a** and **4c**, the thieno[2,3-*d*]pyrimidine derivative **5a** or **5c** was formed in the second replacement reaction (Table 2). Thus, another method (method B) was employed for the preparation of **4a**, **4c**, and **4f**. An aminothiophene was treated with *tert*-pentyl isocyanide in the presence of silver oxide and palladium chloride, followed by heating of the obtained carbodiimide **6** with cyanamide.[17)] The thiourea **7** was prepared from 4-amino-2-cyanothiophene (**3b**) *via* isothiocyanate (method C).

Results and Discussion

All of the compounds were evaluated for smooth muscle relaxation activity *in vitro* for the sake of monitoring their potassium channel opening activity. Taenia cecum of guinea pig was used as smooth muscle owing to the ease of the preparation of test specimens.[18)] The results are given in Table 3.

In our previous study on substituted phenylcyanoguanidine derivatives, we discovered that; 1) compounds having an electron-withdrawing group on the benzene ring could show potent activity, 2) substitution position has a great influence on the activity; for example, the 3-nitro derivative **9** (Fig. 1) was much more potent than the corresponding 2-nitro, or 4-nitro derivative. We tentatively chose a cyano group as the substituent on the thiophene ring because it is easy to prepare the aminocyanothiophenes required to synthesize thienylcyanoguanidines, and we chose a *tert*-pentyl group as the alkyl group because it was the most favorable one in our previous work. Then, three *N*-cyano-*N'*-cyanothienyl-*N''*-*tert*-pentylguanidines **4a**—**c** were pre-



pinacidil

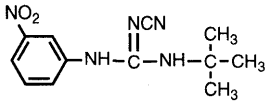


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Fig. 1

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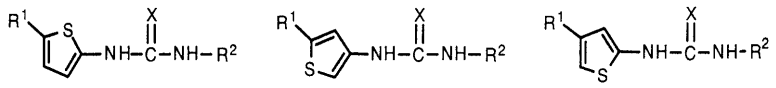
Table 1. Physical Properties of Thienylcyanoguanidines **4** and Thienylthiourea **7**



A B C

Compd.	Type of Structure	X	R^1	R^2	Method[a]	Recryst. solvent[b]	Yield (%)	mp (°C)	Formula	Elemental analysis (%) Calcd (Found) C	H	N
4a	A	NCN	CN	$C(CH_3)_2CH_2CH_3$	B	B–C	40	155.5—157.0	$C_{12}H_{15}N_5S$	55.15 (55.30)	5.78 5.69	26.80 26.72)
4b	B	NCN	CN	$C(CH_3)_2CH_2CH_3$	A	B	61	162.5—165.0	$C_{12}H_{15}N_5S$	55.15 (55.28)	5.78 5.81	26.80 26.82)
4c	C	NCN	CN	$C(CH_3)_2CH_2CH_3$	B	B	64	172.5—175.0	$C_{12}H_{15}N_5S$	55.15 (55.32)	5.78 5.71	26.80 26.94)
4d	B	NCN	CN	$C(CH_3)_3$	A	B	36	176.0—178.5	$C_{11}H_{13}N_5S$	53.42 (53.67)	5.30 5.40	28.32 28.37)
4e	B	NCN	CN	$CH(CH_3)C(CH_3)_3$	A	B	48	165.5—167.5	$C_{13}H_{17}N_5S$	56.70 (56.59)	6.22 6.27	25.43 25.37)
4f	B	NCN	Br	$C(CH_3)_2CH_2CH_3$	B	C	41	122.5—124.0	$C_{11}H_{15}BrN_4S$	41.91 (41.80)	4.80 4.68	17.77 17.90)
4g	B	NCN	CO_2CH_3	$C(CH_3)_2CH_2CH_3$	A	B–C	34	152.5—154.5	$C_{13}H_{18}N_4O_2S$	53.04 (53.08)	6.16 6.17	19.03 19.01)
7	B	S	CN	$C(CH_3)_2CH_2CH_3$	C	B–C	67	101.5—103.0	$C_{11}H_{15}N_3S_2$	52.14 (52.13)	5.97 5.86	16.58 16.70)

a) See Chemistry and Experimental. *b*) The symbols are as follows: B, benzene; C, cyclohexane.

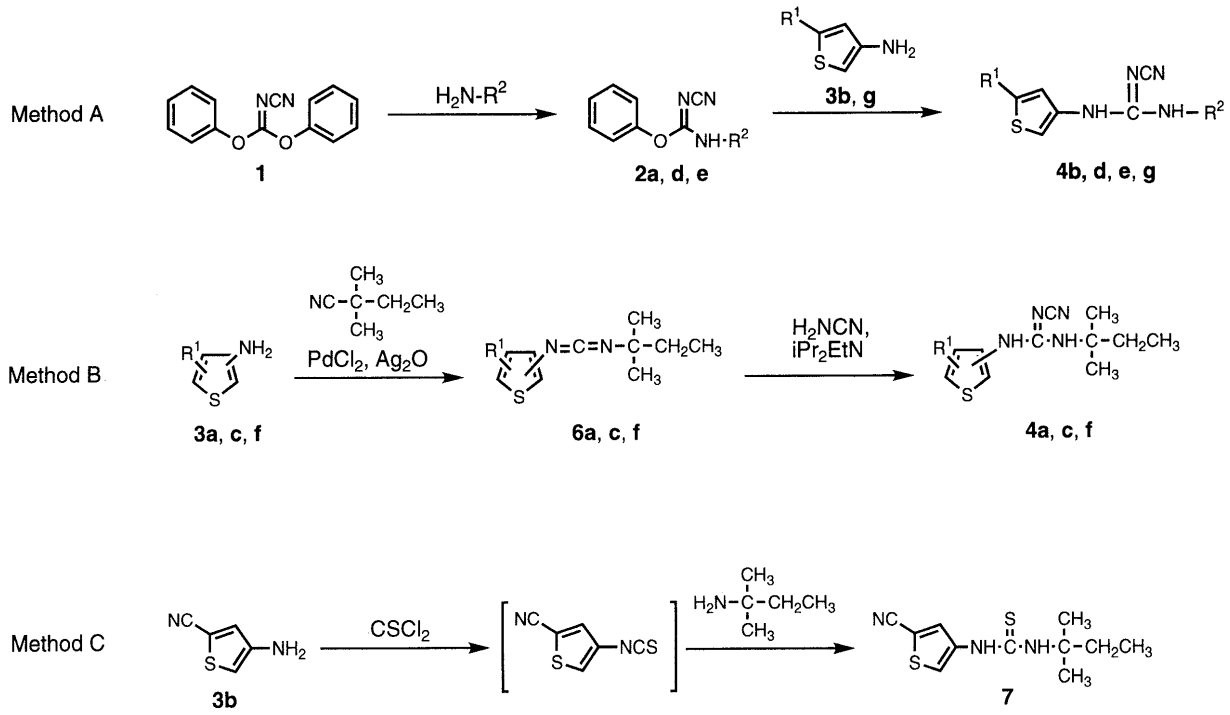
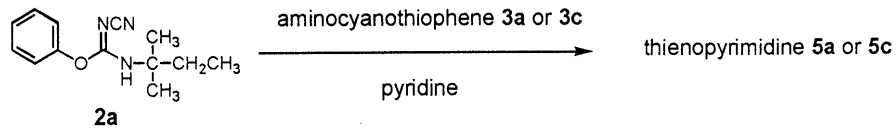


Chart 1

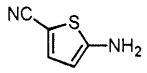
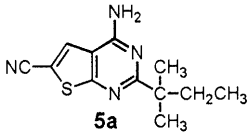
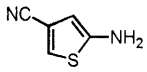
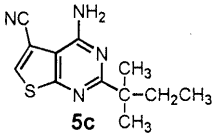
pared in order to investigate the optimum substitution pattern on the thiophene ring (Chart 2, these compounds correspond to thiophene analogues of cyanophenylcyanoguanidine **8**). Among them, the 2-cyano-4-guanidine derivative **4b** showed the most potent activity, and the order of activity was as follows: **4b** > **4a** > **4c**. In the three isomers, the orientation of the cyano group on the thiophene ring is thought to be similar.[19] Therefore, the weaker activities of the 2-cyano-5-guanidine derivative **4a** and the 3-cyano-5-guanidine derivative **4c** should not be due to a difference in the orientation of the cyano group. In **4a** and **4c**, the cyanoguanidine group is located at the 5-position on the thiophene ring. The activities of 2-pyridylcyanoguanidines have been reported to be much less potent than those of 3-pyridylcyanoguanidines, or 4-pyridylcyanoguanidines.[4] The reason why **4a** and **4c** were less active than **4b**, may be attributed to the existence of a hetero atom adjacent to the cyanoguanidine group-binding carbon.

Then, the substitution pattern was fixed to 2-cyano-4-guanidine type, and we focused on the effect of the alkyl

Table 2. Physical Properties of Thienopyrimidines **5a**, **c** Obtained by Reaction of *O*-Phenylurea **2a** and Aminocyanothiophenes **3a**, **c**



2a

Aminothiophene	Thienopyrimidine	Yield (%)	mp (°C)	Recryst. solvent[a]	Elemental analysis (%) Calcd (Found) C	H	N
 **3a**	 **5a**	24	165.0—167.0	B–C	55.15 (55.31	5.78 5.74	26.80 26.85)
 **3c**	 **5c**	47	82.0— 84.0	C–N	55.15 (55.22	5.78 5.72	26.80 26.90)

a) B, C, see footnote in Table 1; N, *n*-hexane.

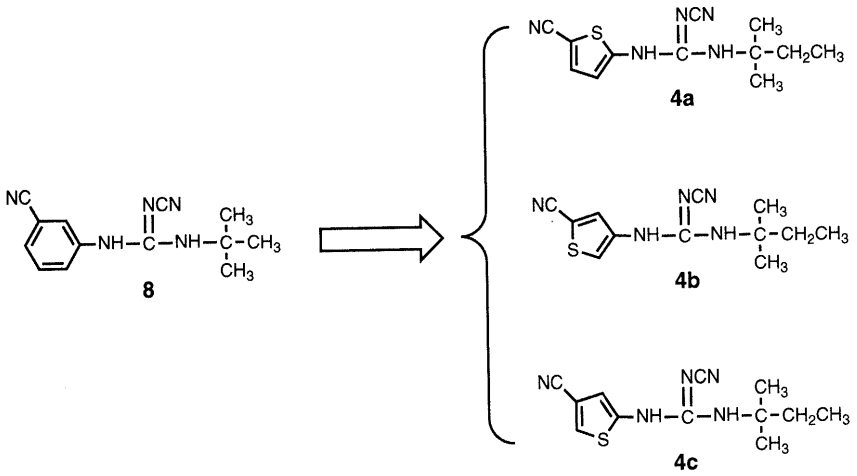


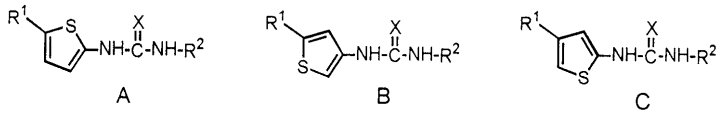
Chart 2

group on the activity. Among **4b**, **4d**, and **4e**, the *tert*-pentyl compound **4b** exhibited the most potent activity, which was 15-fold stronger than that of **4e** with $CH(CH_3)C(CH_3)_3$. This was consistent with the result in our previous work that conversion of the *tert*-pentyl group in the 3,5-dicholorophenylcyanoguanidine derivative **10** to a $CH(CH_3)C(CH_3)_3$ group (derivative **11**) results in a remarkable decrease in the activity. We had proposed a novel pharmacophore model in which the essential factors for binding to the potassium channel consist of an NH and a bulky alkyl group, and in which the potassium channel possesses two hydrophobic spaces that substituents on the benzene ring can occupy.[1] Based on this model, the difference of the activity between **10** and **11** could be explained in terms of the effect of the alkyl group on the orientation of substituents (Fig. 2, top). It can be presumed that the cyano group of **4b** can be located in one of the two spaces, but that of **4e** can not, due to its alkyl group ($CH(CH_3)C(CH_3)_3$), and consequently **4e** can not bind to the channel with high affinity.

The activity of the *tert*-butyl compound **4d** was only slightly weaker than that of the 3-cyano phenylcyanoguanidine **8** with a *tert*-butyl group. Therefore, we conclude that bioisosterism between benzene and thiophene rings does hold in the arylcyanoguanidine derivatives. The replacement of the cyanoguanidine moiety in **4b** with a thiourea moiety led to a less potent compound (**7**). This finding is in agreement with the results which have been reported so far,[4,7] and this indicates the importance of the cyanoguanidine moiety for the activity.

The effect of a substituent of the thiophene ring on the activity was investigated next (**4f**, **4g**). The bromo derivative **4f**[20] was more active than the cyano derivative **4b**, whereas the methoxycarbonyl derivative **4g** was less active. An attempt to synthesize an unsubstituted thienylcyanoguanidine derivative resulted in failure because of the instability of 3-aminothiophene. Among the compounds newly synthesized, the bromo derivative **4f** showed

Table 3. Smooth Muscle Relaxation Activities of Thienylcyanoguanidines **4** and Related Compounds



Compd.	Type of structure	X	R^1	R^2	Smooth muscle relaxation activity		
					n	ED_{50} (μM)[a]	(95% Confidence limit)
4a	A	NCN	CN	$C(CH_3)_2CH_2CH_3$	5	2.1	(1.61—2.74)
4b	B	NCN	CN	$C(CH_3)_2CH_2CH_3$	5	1.2	(0.96—1.62)
4c	C	NCN	CN	$C(CH_3)_2CH_2CH_3$	5	3.8	(3.05—4.62)
4d	B	NCN	CN	$C(CH_3)_3$	5	4.7	(3.53—6.73)
4e	B	NCN	CN	$CHCH_3C(CH_3)_3$	5	18	(13.6—24.6)
4f	B	NCN	Br	$C(CH_3)_2CH_2CH_3$	5	0.43	(0.36—0.51)
4g	B	NCN	CO_2CH_3	$C(CH_3)_2CH_2CH_3$	4	4.3	(3.39—5.39)
7	B	S	CN	$C(CH_3)_2CH_2CH_3$	5	5.3	(4.07—6.87)
8					5	2.7	(2.23—3.21)
Pinacidil					20	2.0	(1.68—2.33)

a) Drug concentration required to relax a spontaneous contraction in guinea pig taenia cecum by 50%. See Experimental for details.

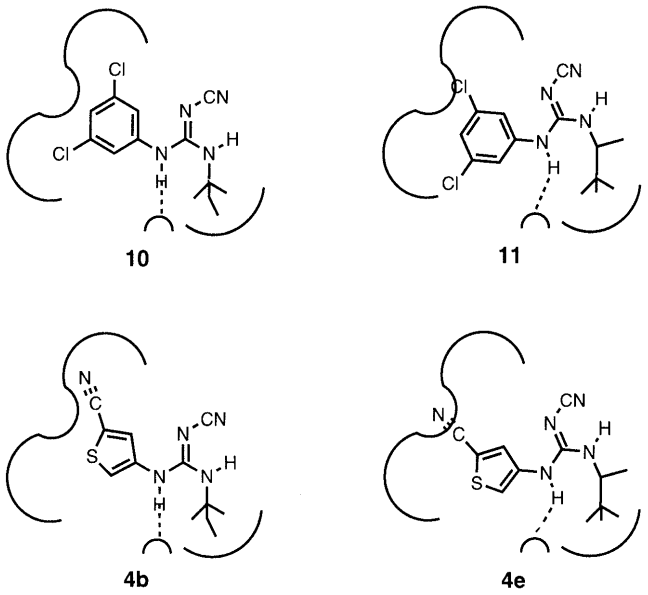


Fig. 2. Putative Interaction between Arylcyanoguanidine Derivative and Potassium Channel

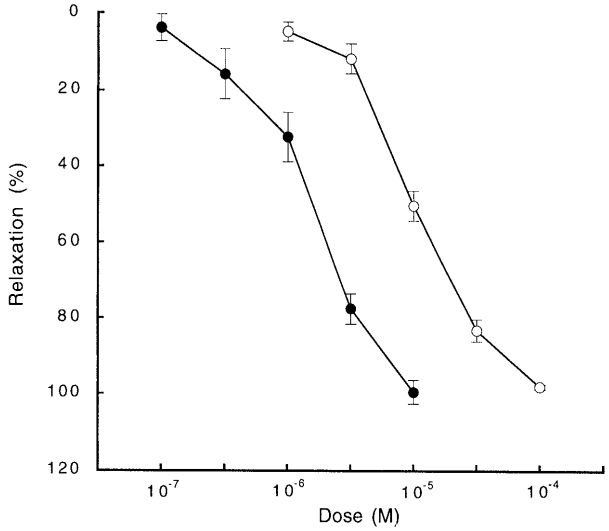


Fig. 3. Effect of Glibenclamide on Smooth Muscle Relaxation Activity of **4b**

4b was cumulatively added to the solution in which the taenia cecum was suspended, without glibenclamide pretreatment (—●—), or with glibenclamide pretreatment (—○—). Each point represents the mean ± S.E. of five experiments.

the best activity, which was 5-fold stronger than that of pinacidil. The tendency that a halogen-substituted arylcyanoguanidine derivative exhibits more potent activity than a cyano-substituted one had also been observed in the phenylcyanoguanidine derivatives. In the case of 3-monosubstituted phenylcyanoguanidine derivatives, it had been concluded that both the electron-withdrawing property and the lipophilicity of the substituent are associated with the activity. Both properties also contribute to the activities of the thienylcyanoguanidine derivatives.

The effect of a potassium channel blocker on the smooth muscle relaxation was examined in order to confirm that the activities of thienylcyanoguanidine derivatives are based on potassium channel opening.[21] After a section of taenia cecum was pretreated with glibenclamide,[22] a well-known potassium channel blocker, **4b** was cumulatively added to the bathing solution in which the specimen was suspended. As shown in Fig. 3, the pretreatment with glibenclamide caused a shift of the dose-response curve to the right. Glibenclamide had a similar effect in the case of **4f** (data not shown). These findings mean that the smooth muscle relaxation activities of **4b** and **4f** are based on their potassium channel opening action.

Table 4. Antihypertensive Activities of **4b**, **4f**, and Pinacidil in Dogs

Compound	Antihypertensive activity (i.v.)[a]	
	Dose (μg/kg)	Δ mmHg[b]
4b	30	−17.4±2.4
4f	60	−15.6±2.0
Pinacidil	30	−11.3±1.1
	60	−17.3±2.2

a) Antihypertensive activities in anesthetized dogs after intravenous administration. *b*) Each value represents the mean ±S.E. of maximum decrease in mean blood pressure ($n=7$ for **4b**, $n=5$ for **4f**, and $n=6$ for pinacidil).

Table 5. Spectral Data for Thienylcyanoguanidines **4** and Related Compounds

Compd.	IR[a] ν_{CN} (cm^{-1})	^{1}H-NMR	
		Solvent[b]	δ ppm
4a	2228 2180	A	0.83 (3H, t, J=7.4 Hz), 1.32 (6H, s), 1.74 (2H, q, J=7.4 Hz), 6.79 (1H, dd, J=2.3, 4.2 Hz), 7.27 (1H, br s), 7.72 (1H, d, J=4.2 Hz), 10.20 (1H, br s)
4b	2220 2176	A	0.80 (3H, t, J=7.4 Hz), 1.27 (6H, s), 1.70 (2H, q, J=7.4 Hz), 6.69 (1H, br s), 7.53 (1H, d, J=1.6 Hz), 7.76 (1H, d, J=1.7 Hz), 9.24 (1H, br s)
4c	2224 2184	A	0.80 (3H, t, J=7.4 Hz), 1.27 (6H, s), 1.71 (2H, q, J=7.4 Hz), 6.76 (1H, br s), 7.09 (1H, d, J=1.5 Hz), 8.20 (1H, d, J=1.5 Hz), 9.6 (1H, br s)
4d	2220 2176	A	1.33 (9H, s), 6.83 (1H, br s), 7.54 (1H, d, J=1.6 Hz), 7.79 (1H, d, J=1.6 Hz), 9.26 (1H, br s)
4e	2216 2176	A	0.88 (9H, s), 1.09 (3H, d, J=6.7 Hz), 3.8—4.0 (1H, m), 6.84 (1H, d, J=9.8 Hz), 7.70 (1H, d, J=1.4 Hz), 7.81 (1H, d, J=1.5 Hz), 9.29 (1H, br s)
4f	2184	A	0.79 (3H, t, J=7.4 Hz), 1.26 (6H, s), 1.69 (2H, q, J=7.5 Hz), 6.47 (1H, br s), 7.06 (1H, d, J=1.8 Hz), 7.08 (1H, d, J=1.8 Hz), 9.06 (1H, br s)
4g	2180	A	0.80 (3H, t, J=7.4 Hz), 1.27 (6H, s), 1.70 (2H, q, J=7.4 Hz), 3.82 (3H, s), 6.63 (1H, br s), 7.4—7.5 (1H, m), 7.5—7.7 (1H, m), 9.14 (1H, br s)
5a	2204	A	0.76 (3H, t, J=7.4 Hz), 1.31 (6H, s), 1.81 (2H, q, J=7.4 Hz), 6.56 (1H, br s), 7.39 (2H, br s), 8.12 (1H, s)
5c	2220	A	0.77 (3H, t, J=7.4 Hz), 1.31 (6H, s), 1.81 (2H, q, J=7.4 Hz), 6.36 (1H, br s), 6.53 (2H, br s), 8.10 (1H, s)
7	2220	B	0.89 (3H, t, J=7.4 Hz), 1.47 (6H, s), 1.91 (2H, q, J=7.5 Hz), 5.97 (1H, br s), 7.55 (2H, s), 7.84 (1H, br s)

a) KBr. *b*) A, DMSO-d_6; B, $CDCl_3$.

Two thienylcyanoguanidine derivatives **4b** and **4f**, which showed excellent activity *in vitro*, were evaluated for antihypertensive activity using dogs. The administration of **4b** by intravenous injection to anesthetized dogs caused a large fall of blood pressure compared to that of pinacidil (Table 4). On the other hand, **4f** did not exhibit as powerful an antihypertensive activity as expected from the *in vitro* result. One possible reason is that **4f** binds to serum proteins with higher affinity than **4b** or pinacidil, because **4f** has a large lipophilicity[23] compared to **4b**.

In conclusion, arylcyanoguanidines of a novel type with good potassium channel opening activity, namely, the thienylcyanoguanidine derivatives **4b** and **4f**, were found. It was confirmed that bioisosterism between benzene and thiophene rings exists in the arylcyanoguanidines. The information on structure–activity relationships obtained from the present work provides further support for our pharmacophore model on the potassium channel, proposed in our previous paper.

Experimental

Melting points were determined on a capillary melting apparatus (Yamato MR-21) and are uncorrected. The structures of all compounds were supported by their IR spectra (Shimadzu IR-440) and 60- or 300-MHz ^{1}H-NMR spectra (Hitachi R-24A or Bruker AM300; tetramethylsilane as an internal standard). All of the new compounds were analyzed for C, H, and N, and the results were within ±0.4% of the calculated theoretical values. Column chromatography was performed using silica gel (YMC-GEL, SIL-60) under medium pressure. No attempt was made to maximize the yields. The following known compounds were prepared according to the literature: 2-amino-5-cyanothiophene (**3a**),[24] 4-amino-2-cyanothiophene (**3b**),[24] 4-amino-2-bromothiophene (**3f**),[25] 2-methoxycarbonyl-4-nitrothiophene,[25] *tert*-pentyl isocyanide.[17c]

2-Amino-4-cyanothiophene (3c) A mixture of 4-cyano-2-nitrothiophene[26] (3.00 g, 19.5 mmol), 5% palladium–carbon (2.07 g, 0.973 mmol as palladium), ethyl acetate (60 ml), and dioxane (3 ml) was stirred at room temperature for 17 h under hydrogen atmosphere (4.0 kgf/cm^2). After removal of palladium–carbon by filtration, the filtrate was evaporated *in vacuo*. The obtained residue was purified by column chromatography using chloroform to give 2-amino-4-cyanothiophene (**3c**) as a brown solid (1.45 g, 60%). ^{1}H-NMR ($CDCl_3$) δ: 3.89 (2H, br s), 6.29 (1H, d, J=1.5 Hz), 7.11(1H, d, J=1.6 Hz).

Compound **3g** was synthesized from 2-methoxycarbonyl-4-nitrothiophene by a similar method to that used for the preparation of **3c**.

4-Amino-2-methoxycarbonylthiophene (**3g**): ^{1}H-NMR ($CDCl_3$) δ: 3.67 (2H, br s), 3.86 (3H, s), 6.41 (1H, d, J=1.8 Hz), 7.31 (1H, d, J=1.8 Hz).

Method A. a) *N*-Cyano-*N'*-*tert*-pentyl-*O*-phenylisourea (2a) A mixture of diphenyl cyanocarbonimidate (**1**) (5.00 g, 21.0 mmol), *tert*-pentylamine (2.00 g, 22.9 mmol), and isopropyl alcohol (50 ml) was stirred at room temperature for 15 h. The resulting precipitate was collected by filtration to give *N*-cyano-*N'*-*tert*-pentyl-*O*-phenylisourea as a colorless powder (3.50 g, 72%). Recrystallization from cyclohexane provided a better sample for analysis. mp 134.5—135.5 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, t, J=7.4 Hz), 1.32 (6H, s), 1.73 (2H, q, J=7.5 Hz), 7.1—7.5 (5H, m), 8.45 (1H, br s). *Anal.* Calcd for $C_{13}H_{17}N_3O$: C, 67.51; H, 7.41; N, 18.17. Found: C, 67.50; H, 7.28; N, 18.19.

The following compounds were prepared in the same manner as described above.

N-*tert*-Butyl-*N'*-cyano-*O*-phenylisourea (**2d**): Yield 73%. mp 150.5—152.5 °C.[27] ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 5.82 (1H, br s), 7.0—7.5 (5H, m). *Anal.* Calcd for $C_{12}H_{15}N_3O$: C, 66.34; H, 6.96; N, 19.34. Found: C, 66.53; H, 6.90; N, 19.45.

N-Cyano-*O*-phenyl-*N'*-1,2,2-trimethylpropylisourea (**2e**): Yield 84%. mp 152.0—153.0 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.91 (9H, s), 1.12 (3H, s), 3.7—3.9 (1H, m), 7.0—7.5 (5H, m), 8.41 (br s), 8.66 (br s). *Anal.* Calcd for $C_{14}H_{19}N_3O$: C, 68.54; H, 7.81; N, 17.13. Found: C, 68.66; H, 7.70; N, 17.23.

b) *N*-Cyano-*N'*-(5-cyano-3-thienyl)-*N''*-*tert*-pentylguanidine (4b) A mixture of 4-amino-2-cyanothiophene (**3b**) (6.30 g, 27.2 mmol), *N*-cyano-*N'*-*tert*-pentyl-*O*-phenylisourea (**2a**) (3.40 g, 27.4 mmol), and pyridine (34 ml) was refluxed for 3 h. It was cooled to room temperature, then 2 N HCl (200 ml) was added. The whole was extracted with chloroform (150 ml × 3). The extract was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The obtained oil was purified by column chromatography using a 2 : 1 mixture solution of cyclohexane and ethyl acetate to give a pale brown powder. Finally, the powder was recrystallized from benzene to give **4b** as colorless plates (4.35 g, 61%). Physical properties and spectral data of **4b** are listed in Tables 1 and 5.

Compounds **4d** (from **3b** and **2d**), **4e** (from **3b** and **2e**), and **4g** (from **3g** and **2a**) were synthesized by a similar method to that used for the preparation of **4b**.

Method B. *N*-Cyano-*N'*-(5-cyano-2-thienyl)-*N''*-*tert*-pentylguanidine (4a) A mixture of 2-amino-5-cyanothiophene (**3a**) (1.00 g, 8.05 mmol), *tert*-pentyl isocyanide (1.20 g, 12.4 mmol), palladium chloride (0.12 g, 0.68 mmol), silver oxide (1.90 g, 8.20 mmol), molecular sieves (2.00 g),

and freshly distilled benzene (15 ml) was refluxed for 1 h. It was cooled to room temperature, and insoluble matter was removed by filtration. The filtrate was evaporated *in vacuo*, and then the residue was purified by column chromatography using a 10 : 1 mixture of cyclohexane and ethyl acetate to give *N*-(5-cyano-2-thienyl)-*N'*-*tert*-pentylcarbodiimide as an oil (0.56 g, 22%).

Cyanamide (0.54 g, 12.9 mmol) and diisopropylethylamine (0.1 ml) were added to a solution of the above oil (0.56 g, 2.56 mmol) in *N*,*N*-dimethylformamide (DMF) (5.6 ml). The mixture was stirred at 70 °C for 30 min, cooled to room temperature, and poured into water (100 ml). The whole was extracted with ethyl acetate (50 ml × 3), and then the extract was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The resulting residue was purified by using preparative TLC (developing solvent: a 1 : 1 mixture of cyclohexane and ethyl acetate) to give a pale brown powder (400 mg, 61%). Recrystallization of this powder from a mixture of benzene and cyclohexane gave **4a** as a colorless powder (270 mg, 40% from carbodiimide).

Compounds **4c** (from **3c**) and **4f** (from **3f**) were synthesized by a similar method to that used for the preparation of **4a**.

Method C. *N*-(5-Cyano-3-thienyl)-*N'*-*tert*-pentylthiourea (7) Thiophosgene (0.74 g, 6.44 mmol) was added dropwise to a solution of 4-amino-2-cyanothiophene (**3b**) (2.00 g, 16.1 mmol) in benzene (30 ml) and then the mixture was stirred at 50—60 °C for 1 h. After cooling to room temperature, and insoluble matter was removed by filtration. *tert*-Pentylamine (5.60 g, 64.2 mmol) was added to the filtrate at room temperature, and the mixture was stirred at the same temperature for 30 min. After addition of concentrated HCl (20 ml), the organic layer was separated, washed with water (20 ml × 2), and dried over $MgSO_4$. The solution was evaporated *in vacuo* to afford a brown oil, which was purified by column chromatography using chloroform. The obtained crude material was recrystallized from a mixture of benzene and cyclohexane to provide **7** as pale brown needles (1.10 g, yield based on thiophosgene: 67%).

Smooth Muscle Relaxation Activity Male guinea pigs weighing 300—600 g were stunned by a blow on the head. Taeniae were isolated from the cecum and cut into lengths of about 1 cm to prepare test specimens. These specimens were each suspended in an organ bath filled with 3-(*N*-morpholino)propanesulfonic acid-physiological salt solution (MOPS-PSS) containing 129.7 mM NaCl, 5.9 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM $MgCl_2$, 10.0 mM MOPS and 11.1 mM glucose, pH 7.4. The bathing solution was continuously bubbled with 100% O_2 gas and maintained at 37 ± 1 °C. Resting tension of each specimen was adjusted to 1 g and the spontaneous response was recorded isotonically. The specimens were allowed to stabilize before the start of the test. A solution of test compound in dimethyl sulfoxide was cumulatively added to the bathing solution. Relaxation induced with papaverine (10^{-4} M) was taken as 100% relaxation. The relaxing effect of each test compound was expressed in terms of the dose giving 50% relaxation (ED_{50}) as determined by linear regression analysis.

In the experiment investigating the effect of glibenclamide on the smooth muscle relaxation activities of **4b** and **4f**, each specimen was pretreated with glibenclamide for 15 min before **4b** or **4f** was cumulatively added.

Antihypertensive Activity in Dogs Mongrel dogs of both sexes, weighing 11—22 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Mean blood pressure was measured with a carrier amplifier (Nihon Koden, AP-621G) *via* a pressure transducer (Nihon Koden, TP-200T) connected to the cannulated left femoral artery. Each test compound was dissolved in 0.9% saline containing 50% (v/v) ethanol, and injected into the femoral vein in volumes of 0.1 ml/kg. The antihypertensive activity of each test compound was expressed in terms of the maximum decrease in the mean blood pressure (ΔmmHg) at a dose of 30 or 60 μg/kg.

References and Notes

1) Previous paper : Yoshiizumi K., Ikeda S., Goto K., Morita T., Nishimura N., Sukamoto T., Yoshino K., *Chem. Pharm. Bull.*, **44**, 2042—2050 (1996).
2) Robertson D. W., Steinberg M. I., *J. Med. Chem.*, **33**, 1529—1541 (1990).
3) Longman S. D., Hamilton T. C., *Med. Res. Rev.*, **12**, 73—148 (1992).
4) Petersen H. J., Nielsen C. K., Arrigoni-Martelli E., *J. Med. Chem.*, **21**, 773—781 (1978).
5) Goldberg M. R., Rockhold F. W., Offen W. W., Dornseif B. E., *Clin. Pharmacol. Ther.*, **46**, 208—218 (1989).
6) Atwal K. S., Moreland S., McCullough J. R., O'Reilly B. C., Ahmed S. Z., Normandin D. E., *Bioorg. Med. Chem. Lett.*, **2**, 83—86 (1992).
7) Manley P. W., Quast U., *J. Med. Chem.*, **35**, 2327—2340 (1992).
8) Takemoto T., Eda M., Okada T., Sakashita H., Matzno S., Gohda M., Ebisu H., Nakamura N., Fukaya C., Hihara M., Eiraku M., Yamanouchi K., Yokoyama K., *J. Med. Chem.*, **37**, 18—25 (1994).
9) Goldberg M. R., Sushak C. S., Rockhold F. W., Thompson W. L., *Clin. Pharmacol. Ther.*, **44**, 78—92 (1988).
10) Sterndorff B., Johansen P., *Acta Med. Scand.*, **224**, 329—336 (1988).
11) Ramsey L. E., Freestone S., *Br. J. Clin. Pharmcol.*, **16**, 336—338 (1983).
12) Reports describing bioisosterism, see; *a*) Russell R. K., Press J. B., Rampulla R. A., McNally J. J., Falotico R. F., Keiser J. A., Bright D. A., Tobia A., *J. Med. Chem.*, **31**, 1786—1793 (1988); *b*) Press J. B., Russell R. K., McNally J. J., Rampulla R. A., Falotico R., Scott C., Moore J. B., Offord S. J., Tobia J., *Eur. J. Med. Chem.*, **26**, 807—813 (1991); *c*) Wong G., Skolnick P., *Eur. J. Pharm. Mol. Pharm. Sec.*, **225**, 63—68 (1992).
13) Reports describing absence of bioisosterism, see; *a*) Estenne G., Dodey P., Renaut P., Leclerc G., *Bioorg. Med. Chem. Lett.*, **5**, 15—18 (1995); *b*) Binder D., Koch A., Rocvenszky F., Stroissnig H., *Arch. Pharm. Weinheim.*, **325**, 797—801 (1992); *c*) Schove L. T., Chen S.-W., Beatty M. B., Maguire P. A., Davies M. F., Loew G. H., *Bioorg. Med. Chem.*, **3**, 1547—1561 (1995).
14) Pharmacologically active compounds containing a thiophene ring prior to 1985, see: Press J. B., "The Chemistry of Heterocyclic Compounds," Vol. 44, Thiophene and Its Derivatives Part 1 ed. by Weissberger A., Taylor E. C., John Wiley and Sons, Inc., New York, 1985, pp. 353—456 (Chapter V).
15) Sanfilippo P. J., McNally J. J., Press J. B., Fitzpatrick L. J., Urbanski M. J., Katz L. B., Giardino E., Falotico R., Salata J., Moore J. B., Miller W., *J. Med. Chem.*, **35**, 4425—4433 (1992).
16) Buschauer A., Schunack W., *J. Heterocycl. Chem.*, **21**, 753—757 (1984).
17) *a*) Ito Y., Hirao T., Saegusa T., *J. Org. Chem.*, **40**, 2981—2982 (1975); *b*) Kiyoi T., Seko N., Yoshino K., Ito Y., *ibid.*, **58**, 5118—5120 (1993); *c*) Baumagarten H. E. (ed.), "Organic Synthesis," Coll. Vol. 5, John Wiley and Sons, Inc., New York, 1973, p. 300.
18) Weir S. W., Weston A. H., *Br. J. Pharmacol.*, **88**, 113—120 (1986).
19) *a*) Thiophene is not a perfect pentagon as reported in reference 19*b*. Nevertheless, the orientation of the cyano group in **4b** is thought to be almost the same as that of the cyano group in **4c**, because **4b** and **4c** are isomers in which the cyano group and the cyanoguanidine moiety are exchanged on the thiophene ring; *b*) Bak B., Christensen D., Hansen-Nygaard L., Rasrup-Andersen J., *J. Mol. Spectrosc.*, **7**, 58—63 (1961).
20) In the monosubstituted phenylcyanoguanidine derivatives, the most favorable substituent was chloro, but the synthesis of 4-amino-2-chlorothiophene is known to be more difficult than that of 4-amino-2-bromothiophene. So, we chose the bromo atom as the halogen substituent.
21) Calcium antagonists also relax taeniae cecum of guinea pig. See; Matsui K., Ogawa Y., Imai S., *Arch. Int. Pharmacodyn.*, **283**, 124—133 (1986).
22) *a*) Schmid-Antomarch H., Weille J. D., Fosset M., Lazdunski M., *J. Biol. Chem.*, **262**, 15840—15844 (1987); *b*) Cavero I., Mondot S., Mestre M., Escande D., *Br. J. Pharmacol.*, **95**, 643P (1988).
23) Hansch C., Kiehs K., Lawrence G. L., *J. Am. Chem. Soc.*, **87**, 5770—5773 (1965).
24) Hammond M. L., Zambias R. A., Chang M. N., Jensen N. P., McDonald J., Thompson K., Baulton D. A., Kopka I. E., Hand K. M., Opas E. E., Luell S., Bach T., Davies P., MacIntyre D. E., Bonney R. J., Humes J. L., *J. Med. Chem.*, **33**, 908—918 (1990).
25) Motoyama Y., Nishimura S., Imoto E., *Nihon Kagaku Zasshi*, **78**, 788—792 (1957).
26) Maybridge Chemical Co., Ltd.
27) *a*) Reference 27*b*, mp 150—151 °C; *b*) Tsuzuki R., Matsumoto Y., Kondo Y., Fujikura T., Uchida W., Asano M. (Yamanouchi Pharmaceutical Co., Ltd.), Japan. Patent 2-172984 (1990).