# Synthesis and Structure–Activity Relationships of Novel Phenylcyanoguanidine Derivatives as Potassium Channel Openers

Kazuya YOSHIIZUMI,* Shoji IKEDA, Katsumi GOTO, Tominori MORITA, Noriyasu NISHIMURA, Takayuki SUKAMOTO, and Kohichiro YOSHINO

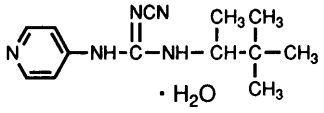
*New Drug Discovery Research Laboratory, Kanebo Ltd., 1–5–90 Tomobuchi-cho, Miyakojima-ku, Osaka 534, Japan.*
Received April 26, 1996; accepted August 9, 1996

**3,5-Di-substituted phenylcyanoguanidine derivatives with halogen, cyano, and/or nitro groups at the 3- and 5-positions of the benzene ring exhibited very strong smooth muscle relaxation activity *in vitro*, as compared to pinacidil. Among them, *N*-(3-chloro-5-cyanophenyl)-*N'*-cyano-*N''*-*tert*-pentylguanidine (5s) showed 27-fold more potent activity than pinacidil, and exhibited a stronger and more lasting antihypertensive effect than pinacidil by oral administration to spontaneously hypertensive rats. We propose a new pharmacophore model in which the essential factors for binding to the potassium channel are an NH and a bulky alkyl group.**

**Key words** potassium channel opener; phenylcyanoguanidine; antihypertensive effect; smooth muscle relaxation activity; pinacidil

Potassium channels are an important class of ionic channels, and the opening of potassium channels in the membranes of cells such as smooth muscle cells enhances the efflux of potassium ions, which induces membrane hyperpolarization. This effect causes a decrease in the opening of voltage-dependent calcium channels and a reduction in the release of calcium ions from intracellular stores, and produces smooth muscle relaxation. Therefore, potassium channel openers are thought to be useful in the treatment of diseases caused by smooth muscle contraction, such as hypertension, asthma, angina pectoris, and urinary incontinence.[1,2]

Pinacidil[3] is a well-known potassium channel opener, but its use is often accompanied by some problems, including edema,[4,5] tachycardia,[4,5] and a short duration of the effect.[6] Moreover, the potassium channel opening activity of pinacidil[7] is not as strong as that of cromakalim,[8] an another class of potassium channel opener. For the purpose of overcoming such drawbacks in pinacidil and/or clarifying its structure–activity relationship, a few studies on its derivatives have been carried out.[9–11] Nitrobenzene is known to be similar to pyridine in electronic distribution,[12] so we were interested in the activities and properties of pinacidil analogues in which the pyridine ring was replaced by a benzene ring with an electron-withdrawing group, such as a nitro, cyano, or halogen substituent. We synthesized a variety of phenylcyanoguanidine derivatives possessing various substituents on the benzene ring, and evaluated them for smooth muscle relaxation activity in order to uncover out novel potassium channel openers with excellent activity.



pinacidil

Fig. 1

## Chemistry

The substituted phenylthioureas **3a—u** listed in Table 1 were synthesized from the corresponding alkylamine and phenylisothiocyanate **2**, which was obtained by treating aniline **1** with thiophosgene (Chart 2). The substituted phenylcyanoguanidines **5a—u** listed in Table 2 were prepared by either method A or B, according to the reported procedures.[3] In method A, thiourea **3** was converted to carbodiimide **4** by treatment with triphenylphosphine and carbon tetrachloride, or dicyclohexylcarbodiimide. The reaction of carbodiimide **4** with cyanamide gave phenylcyanoguanidine **5**. Phenylcyanoguanidines **5c**, **5d**, **5g**, **5j**, and **5q** were directly prepared from thiourea **3** by treatment with cyanamide and dicyclohexylcarbodiimide (method B).

## Results and Discussion

All of the phenylcyanoguanidine derivatives were tested for smooth muscle relaxation activity *in vitro* to monitor their potassium channel opening activity. We used the taenia caecum of guinea pig as smooth muscle owing to the ease of preparation of test specimens.[13] The obtained results are given in Table 3.

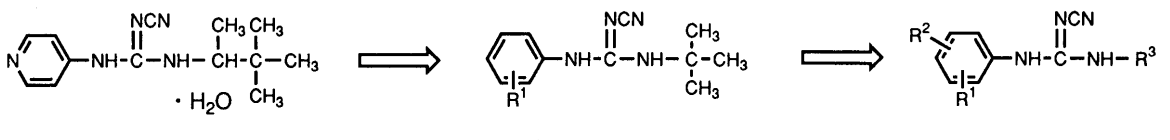
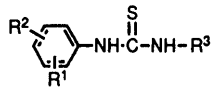



Chart 1

* To whom correspondence should be addressed.

Table 1. Physical Properties of Substituted Phenylthioureas **3**



| Compd. | $R^1$ | $R^2$ | $R^3$ | Yield (%)[a] | Recryst. solvent[b] | mp (°C) | Formula | Elemental analysis (%) Calcd (Found) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | C | H | N |
| **3a** | 2-$NO_2$ | H | $C(CH_3)_3$ | 58 | A–C | 155.5—157.0[c] | $C_{11}H_{15}N_3O_2S$ | 52.16 | 5.97 | 16.59 |
| | | | | | | | | (52.16 | 5.98 | 16.63) |
| **3b** | 3-$NO_2$ | H | $C(CH_3)_3$ | 61 | A–C | 145.5—146.5 | $C_{11}H_{15}N_3O_2S$ | 52.16 | 5.97 | 16.59 |
| | | | | | | | | (52.06 | 6.23 | 16.47) |
| **3c** | 4-$NO_2$ | H | $C(CH_3)_3$ | 45 | F | 171.0—172.0 | $C_{11}H_{15}N_3O_2S$ | 52.16 | 5.97 | 16.59 |
| | | | | | | | | (52.07 | 5.91 | 16.62) |
| **3d** | 3-CN | H | $C(CH_3)_3$ | 49 | B | 140.5—141.5 | $C_{12}H_{15}N_3S$ | 61.77 | 6.48 | 18.01 |
| | | | | | | | | (61.83 | 6.46 | 18.03) |
| **3e** | 3-F | H | $C(CH_3)_3$ | 99 | C | 117.5—119.5 | $C_{11}H_{15}FN_2S$ | 58.38 | 6.68 | 12.38 |
| | | | | | | | | (58.35 | 6.70 | 12.37) |
| **3f** | 3-Cl | H | $C(CH_3)_3$ | 84 | A–I | 140.0—141.0 | $C_{11}H_{15}ClN_2S$ | 54.42 | 6.23 | 11.54 |
| | | | | | | | | (54.44 | 6.23 | 11.55) |
| **3g** | 3-Br | H | $C(CH_3)_3$ | 74 | A–C | 132.0—134.0 | $C_{11}H_{15}BrN_2S$ | 46.00 | 5.26 | 9.75 |
| | | | | | | | | (46.01 | 5.12 | 9.76) |
| **3h** | 3-I | H | $C(CH_3)_3$ | 85 | A–C | 134.5—133.5 | $C_{11}H_{15}IN_2S$ | 39.53 | 4.52 | 8.38 |
| | | | | | | | | (39.42 | 4.58 | 8.29) |
| **3i** | 3-$CH_3$ | H | $C(CH_3)_3$ | 72 | A–C | 130.0—131.5 | $C_{12}H_{18}N_2S$ | 64.82 | 8.16 | 12.60 |
| | | | | | | | | (64.84 | 7.99 | 12.61) |
| **3j** | 3-$N(CH_3)_2$ | H | $C(CH_3)_3$ | 30 | A–C | 133.0—135.0 | $C_{13}H_{21}N_3S$ | 62.11 | 8.42 | 16.72 |
| | | | | | | | | (62.18 | 8.38 | 16.75) |
| **3k** | H | H | $C(CH_3)_3$ | 85 | C | 120.5—121.5[d] | $C_{11}H_{16}N_2S$ | 63.42 | 7.74 | 13.45 |
| | | | | | | | | (63.43 | 7.74 | 13.56) |
| **3l** | 3-Cl | 4-Cl | $C(CH_3)_3$ | 80 | A–C | 146.0—147.5 | $C_{11}H_{14}Cl_2N_2S$ | 47.66 | 5.09 | 10.11 |
| | | | | | | | | (47.77 | 5.15 | 10.08) |
| **3m**[e] | 3-Cl | 5-Cl | $C(CH_3)_3$ | 81 | B | 164.0—165.0 | $C_{11}H_{14}Cl_2N_2S$ | 47.66 | 5.09 | 10.11 |
| | | | | | | | | (47.67 | 5.07 | 10.13) |
| **3n** | 2-Cl | 6-Cl | $C(CH_3)_3$ | 32 | A–D | 136.0—138.0 | $C_{11}H_{14}Cl_2N_2S$ | 47.66 | 5.09 | 10.11 |
| | | | | | | | | (47.76 | 5.29 | 9.77) |
| **3o** | 3-Cl | 5-CN | $C(CH_3)_3$ | 73 | B–C | 135.0—137.0 | $C_{12}H_{14}ClN_3S$ | 53.82 | 5.27 | 15.69 |
| | | | | | | | | (53.99 | 5.28 | 15.72) |
| **3p** | 3-Cl | 5-$NO_2$ | $C(CH_3)_3$ | 95 | B–C | 138.0—140.0 | $C_{11}H_{14}ClN_3O_2S$ | 45.91 | 4.90 | 14.60 |
| | | | | | | | | (46.08 | 4.88 | 14.60) |
| **3q** | 3-CN | 5-CN | $C(CH_3)_3$ | 80 | B–C | 160.0—162.0 | $C_{13}H_{14}N_4S$ | 60.44 | 5.46 | 21.69 |
| | | | | | | | | (60.65 | 5.49 | 21.58) |
| **3r** | 3-Cl | 5-Cl | $C(CH_3)_2CH_2CH_3$ | 77 | A–C | 134.0—136.0 | $C_{11}H_{16}Cl_2N_2S$ | 49.49 | 5.54 | 9.62 |
| | | | | | | | | (49.58 | 5.47 | 9.59) |
| **3s** | 3-Cl | 5-CN | $C(CH_3)_2CH_2CH_3$ | 39 | B–C | 109.0—111.0 | $C_{13}H_{16}ClN_3S$ | 55.41 | 5.72 | 14.91 |
| | | | | | | | | (55.60 | 5.65 | 14.84) |
| **3t** | 3-Cl | 5-Cl | $CH(CH_3)C(CH_3)_3$ | 61 | A–C | 152.0—154.0 | $C_{13}H_{18}Cl_2N_2S$ | 51.15 | 5.94 | 9.18 |
| | | | | | | | | (51.26 | 5.97 | 9.18) |
| **3u** | 3-Cl | 5-Cl | $CH_2CH_2CH_2CH_3$ | 71 | A–D | 78.0—80.0 | $C_{11}H_{14}Cl_2N_2S$ | 47.66 | 5.09 | 10.11 |
| | | | | | | | | (47.63 | 5.06 | 9.90) |

*a*) The yield from thiophosgene. *b*) The symbols are as follows: A, AcOEt; B, benzene; C, cyclohexane; D, *n*-hexane; E, EtOH; F, iso-PrOH; G, $H_2O$; H, MeOH; I, iso-$Pr_2O$. *c*) Ref. 22, mp 155—165 °C. *d*) Ref. 23, mp 125 °C. *e*) The smooth muscle relaxation activity of **3m**, $ED_{50}=6.9\,\mu M$ ($n=5$).

Initially, we synthesized 2-, 3-, and 4-nitrophenylcyanoguanidines in order to investigate the effect of the substituted position. The alkyl moiety of the phenylcyanoguanidine derivatives was tentatively fixed to a *tert*-butyl group, because it had been reported to be preferred over $CH(CH_3)C(CH_3)_3$ in pyridylcyanoguanidine derivatives.[3] The 3-nitro compound (**5b**) showed the most potent activity among the three compounds (**5a—c**). In the next step, the substitution position was fixed at the 3-position, and other substituents were investigated (**5d—k**). Among them, the 3-chloro compound (**5f**) exhibited the best activity. In the course of our study,[11,14] Atwal *et al.* reported the smooth muscle relaxing and antihypertensive activities of several monosubstituted phenylcyanoguanidine derivatives, such as cyano or nitrophenylcyanoguanidines. They described that the 4-cyano compound (**6**) relaxed rat aorta with higher potency than pinacidil. We found out that the 3-chloro compound (**5f**) is more potent than **6**.

Manley and Quast reported that the potassium channel opening activity of arylcyanoguanidine derivatives is proportional to the acidity of the NH proton adjacent to the aromatic ring.[9] If the role of the substituent on the benzene ring is only to increase the acidity of the NH proton, the 4-nitro compound (**5c**) should have shown the most potent activity. However, the most active compound

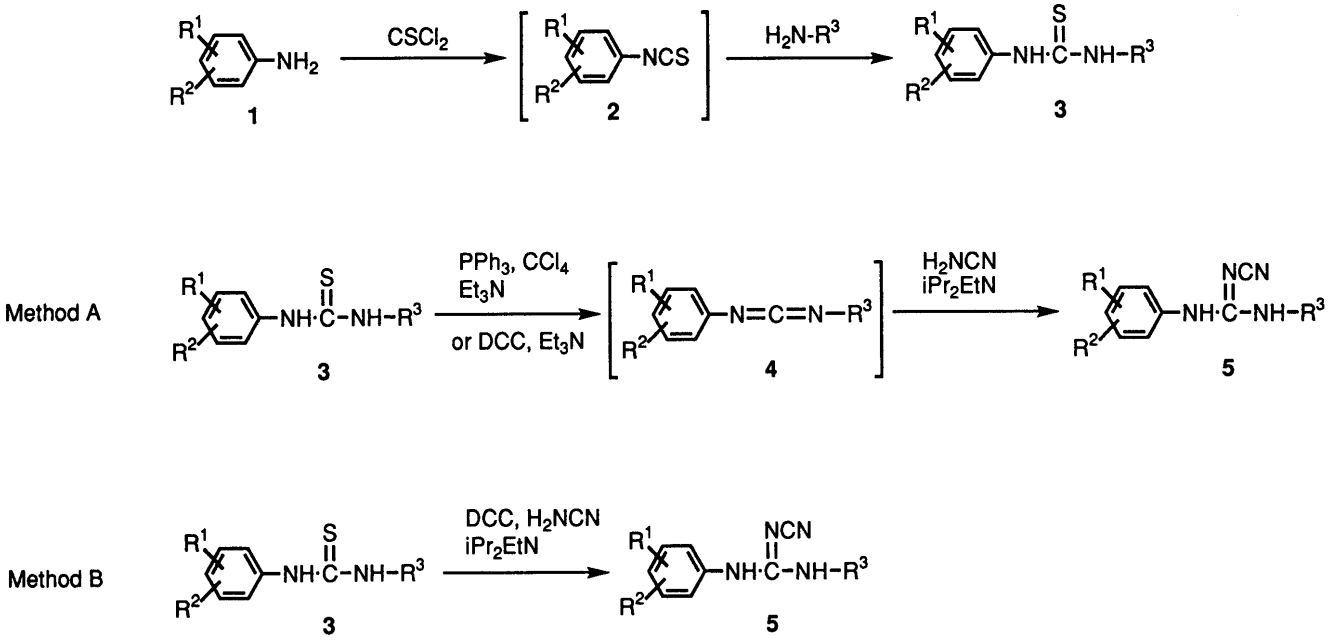



Chart 2

was actually the 3-chloro compound (**5f**). These results suggest the possibility that other properties, such as lipophilicity, may also be involved in the activity. So, a quantitative structure–activity relationship (QSAR) analysis was carried out with the aim of obtaining a basis for the design of superior potassium channel openers.

At first we analyzed only 3-monosubstituted phenylcyanoguanidine derivatives (**5b**, **5d**—**k**) using a typical electronic parameter, $\sigma_m$,[15] but the obtained correlation was not high (Eq. 1).

$$\log 1/ED_{50} = 1.971(\pm 1.644)\sigma_m + 4.976(\pm 0.642) \quad (1)$$
$$n=9,\ r=0.731,\ s=0.572,\ F=8.036$$

In Eq. 1, the numbers in parentheses, $n$, $r$, $s$, and $F$ mean, respectively, the 95% confidence interval, the number of compounds, the correlation coefficient, the standard deviation, and the $F$-ratio between the variances of the calculated and observed activities. We also analyzed the derivatives by use of a hydrophobic parameter, $\pi$,[15] or a steric parameter, $MR$,[15] instead of $\sigma_m$, but each correlation was quite low (when by use of $\pi$, $r=0.337$, and when by use of $MR$, $r=0.022$). Next, both $\sigma_m$ and $\pi$ together were employed for the analysis, and excellent correlation was obtained, as shown in Eq. 2.

$$\log 1/ED_{50} = 2.312(\pm 1.214)\sigma_m + 0.764(\pm 0.639)\pi + 4.651(\pm 0.535) \quad (2)$$
$$n=9,\ r=0.899,\ s=0.397,\ F=12.626$$

In this analysis, the correlation coefficient between $\sigma_m$ and $\pi$ was 0.235. An analysis using both $\sigma_m$ and $MR$ led to a lower correlation ($r=0.733$). These results reveal that both the electron withdrawing property and the lipophilicity of the substituent affect the activities of the 3-monosubstituted compounds. When 11 compounds, including the 2-nitro derivative (**5a**) and the 4-nitro derivative (**5c**), were analyzed, only a poor correlation was obtained ($r=0.626$).

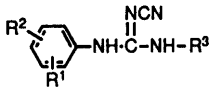
We suppose from Eq. 2 that an increase in the electron-withdrawing property and the lipophilicity would enhance the activity. So, phenylcyanoguanidine derivatives with two substituents were synthesized and evaluated for their smooth muscle relaxation activity. At first, both of two substituents were fixed to chlorine atoms, and the substitution pattern was examined (Table 3, **5l**—**5n**). The 3,5-dichloro compound (**5m**) showed the most potent activity, and exhibited more potent activity than the 3-monochloro derivative (**5f**). The 3,4-dichloro derivative (**5l**) showed moderate activity, but its activity was less potent than that of **5f**. This finding means that the further introduction of chlorine atom to the 4-position causes a drop in the activity.

In the next step, the substitution positions were fixed at the 3- and 5-positions, and the effects of other substituents were examined. The 3,5-dicyano compound (**5q**) also exhibited higher activity than the 3-monocyano compound (**5d**). The 3-chloro-5-cyano compound (**5o**) and the 3-chloro-5-nitro compound (**5p**) also showed excellent activity (**5o** *vs.* **5d**, **5p** *vs.* **5b**). The tendency for the chlorine atom to be more favorable than the cyano group or nitro group was also observed in the 3,5-di-substituted compounds (**5m** > **5o** > **5q**, **5m** > **5p**).

Both the 3-substituted and the 3,5-di-substituted compounds (**5b**, **5d**—**5k**, **5m**, **5o**—**5q**) were subjected to QSAR analysis. Initially, we analyzed the compounds using $\Sigma\sigma_m$, which means the sum of the $\sigma_m$ values of the substituents, but the correlation was not high ($r=0.733$), just as in Eq. 1. Next, when both $\Sigma\sigma_m$ and $\Sigma\pi$, which means the sum of the $\pi$ values of the substituents, were employed, an excellent correlation was obtained (in Eq. 3, the correlation coefficient between $\Sigma\sigma_m$ and $\Sigma\pi$ was 0.267).

$$\log 1/ED_{50} = 1.718(\pm 0.622)\Sigma\sigma_m + 0.637(\pm 0.372)\Sigma\pi + 4.804(\pm 0.421) \quad (3)$$
$$n=13,\ r=0.901,\ s=0.388,\ F=21.504$$

Table 2. Physical Properties of Substituted Phenylcyanoguanidines **5**



| Compd. | $R^1$ | $R^2$ | $R^3$ | Method[a] | Recryst. solvent[b] | Yield (%)[c] | mp (°C) | Formula | Elemental analysis (%) Calcd (Found) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | C | H | N |
| **5a** | 2-$NO_2$ | H | $C(CH_3)_3$ | A | H | 33 | 166.0—167.0 | $C_{12}H_{15}N_5O_2$ | 55.16 | 5.79 | 26.81 |
| | | | | | | | | | (55.24 | 5.81 | 27.00) |
| **5b** | 3-$NO_2$ | H | $C(CH_3)_3$ | A | B | 26 | 196.0—197.5 | $C_{12}H_{15}N_5O_2$ | 55.16 | 5.79 | 26.81 |
| | | | | | | | | | (55.31 | 5.66 | 26.62) |
| **5c** | 4-$NO_2$ | H | $C(CH_3)_3$ | B | A | 24 | 215.0—216.0 | $C_{12}H_{15}N_5O_2$ | 55.16 | 5.79 | 26.81 |
| | | | | | | | | | (55.31 | 5.60 | 26.66) |
| **5d** | 3-CN | H | $C(CH_3)_3$ | B | H | 18 | 187.0—189.0 | $C_{13}H_{15}N_5$ | 64.71 | 6.27 | 29.03 |
| | | | | | | | | | (64.72 | 6.28 | 28.92) |
| **5e** | 3-F | H | $C(CH_3)_3$ | A | B–C | 31 | 157.0—159.0 | $C_{12}H_{15}FN_4$ | 61.52 | 6.45 | 23.91 |
| | | | | | | | | | (61.73 | 6.47 | 23.80) |
| **5f** | 3-Cl | H | $C(CH_3)_3$ | A | A–I | 62 | 145.0—146.0 | $C_{12}H_{15}ClN_4$ | 57.48 | 6.03 | 22.35 |
| | | | | | | | | | (57.53 | 6.05 | 22.46) |
| **5g** | 3-Br | H | $C(CH_3)_3$ | B | B | 39 | 153.0—156.0 | $C_{12}H_{15}BrN_4$ | 48.83 | 5.12 | 18.98 |
| | | | | | | | | | (48.90 | 5.16 | 19.02) |
| **5h** | 3-I | H | $C(CH_3)_3$ | A | G–H | 33 | 184.5—186.5 | $C_{12}H_{15}IN_4$ | 42.12 | 4.42 | 16.37 |
| | | | | | | | | | (42.26 | 4.35 | 16.41) |
| **5i** | 3-$CH_3$ | H | $C(CH_3)_3$ | A | C | 21 | 140.5—142.5 | $C_{13}H_{18}N_4$ | 67.80 | 7.88 | 24.33 |
| | | | | | | | | | (68.03 | 7.89 | 24.34) |
| **5j** | 3-$N(CH_3)_2$ | H | $C(CH_3)_3$ | B | B–C | 14 | 172.0—174.0 | $C_{14}H_{21}N_5$ | 64.84 | 8.16 | 27.00 |
| | | | | | | | | | (65.05 | 8.21 | 26.74) |
| **5k** | H | H | $C(CH_3)_3$ | A | B–C | 50 | 151.0—152.0 | $C_{12}H_{16}N_4$ | 66.64 | 7.46 | 25.90 |
| | | | | | | | | | (66.87 | 7.43 | 25.70) |
| **5l** | 3-Cl | 4-Cl | $C(CH_3)_3$ | A | B | 30 | 174.0—175.0 | $C_{12}H_{14}Cl_2N_4$ | 50.54 | 4.95 | 19.65 |
| | | | | | | | | | (50.46 | 4.83 | 19.55) |
| **5m** | 3-Cl | 5-Cl | $C(CH_3)_3$ | A | F | 65 | 193.0—195.0 | $C_{12}H_{14}Cl_2N_4$ | 50.54 | 4.95 | 19.65 |
| | | | | | | | | | (50.53 | 4.89 | 19.68) |
| **5n** | 2-Cl | 6-Cl | $C(CH_3)_3$ | A | F | 36 | 211.0—212.5 | $C_{12}H_{14}Cl_2N_4$ | 50.54 | 4.95 | 19.65 |
| | | | | | | | | | (50.57 | 4.97 | 19.64) |
| **5o** | 3-Cl | 5-CN | $C(CH_3)_3$ | A | E | 37 | 185.0—187.0 | $C_{13}H_{14}ClN_5$ | 56.62 | 5.12 | 25.40 |
| | | | | | | | | | (56.54 | 5.04 | 25.09) |
| **5p** | 3-Cl | 5-$NO_2$ | $C(CH_3)_3$ | A | A–B | 35 | 181.0—183.0 | $C_{12}H_{14}ClN_5O_2$ | 48.74 | 4.77 | 23.68 |
| | | | | | | | | | (48.79 | 4.75 | 23.70) |
| **5q** | 3-CN | 5-CN | $C(CH_3)_3$ | B | A–B | 30 | 213.0—215.0 | $C_{14}H_{14}N_6$ | 63.14 | 5.30 | 31.56 |
| | | | | | | | | | (63.19 | 5.30 | 31.52) |
| **5r** | 3-Cl | 5-Cl | $C(CH_3)_2CH_2CH_3$ | A | A–I | 62 | 151.0—153.0 | $C_{13}H_{16}Cl_2N_4$ | 52.18 | 5.39 | 18.73 |
| | | | | | | | | | (52.26 | 5.41 | 18.79) |
| **5s** | 3-Cl | 5-CN | $C(CH_3)_2CH_2CH_3$ | A | B | 64 | 181.0—183.0 | $C_{14}H_{16}ClN_5$ | 58.03 | 5.57 | 24.17 |
| | | | | | | | | | (58.06 | 5.55 | 24.19) |
| **5t** | 3-Cl | 5-Cl | $CH(CH_3)C(CH_3)_3$ | A | B | 47 | 154.0—156.0 | $C_{14}H_{18}Cl_2N_4$ | 53.68 | 5.79 | 17.89 |
| | | | | | | | | | (53.76 | 5.83 | 17.92) |
| **5u** | 3-Cl | 5-Cl | $CH_2CH_2CH_2CH_3$ | A | B–C | 39 | 129.5—131.0 | $C_{12}H_{14}Cl_2N_4$ | 50.54 | 4.95 | 19.65 |
| | | | | | | | | | (50.64 | 4.91 | 19.91) |

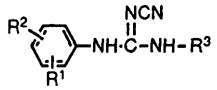
*a*) See Chemistry and Experimental for details. *b*) See footnotes *b* in Table 1. *c*) The yield from the corresponding thiourea **3** after recrystallization.

This equation is essentially the same as Eq. 2. From Eq. 3, it is confirmed that both the electron-withdrawing property and the lipophilicity of the substituent contribute to the activity in both the 3-mono-substituted and the 3,5-di-substituted series.

Next, the effect of an alkyl group on the activity was examined. Replacement of the *tert*-butyl group with a *tert*-pentyl group enhanced the activity (**5r** *vs.* **5m**, **5s** *vs.* **5o**). Among the novel phenylcyanoguanidine derivatives synthesized here, the 3,5-dichloro compound with a *tert*-pentyl group (**5r**) showed the most potent activity, which was 77-fold stronger than that of pinacidil. On the other hand, **5t**, with a $CH(CH_3)C(CH_3)_3$ group which was used in pinacidil and **6**, exhibited less potent activity as compared to **5m**. The substitution of the *tert*-butyl group in **5m** with an *n*-butyl group led to a 400-fold less active compound (**5u**). These results indicate that a bulky alkyl group is essential for good activity.

Manley and Quast postulated a pharmacophore model for the binding of pinacidil-related compounds to the potassium channel.[9] Their model consisted of a hydrogen-bond acceptor moiety, a hydrogen-bond donor moiety, and a lipophilic moiety (Fig. 2A). They proposed that the nitrogen atom in the pyridine ring can act as a hydrogen-bond acceptor. However, the 3,5-dichloro derivative (**5r**), which does not have a hydrogen-bond acceptor moiety on the benzene ring, showed excellent activity. A hydrogen-bond between the channel and the

Table 3. Smooth Muscle Relaxation Activities of Substituted Phenylcyanoguanidines **5** and Related Compounds



| Compd. | $R^1$ | $R^2$ | $R^3$ | Smooth muscle relaxation activity[a] $n$ | $ED_{50}$ ($\mu$M) | Compd. | $R^1$ | $R^2$ | $R^3$ | Smooth muscle relaxation activity[a] $n$ | $ED_{50}$ ($\mu$M) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **5a** | 2-$NO_2$ | H | $C(CH_3)_3$ | 5 | 49 | **5l** | 3-Cl | 4-Cl | $C(CH_3)_3$ | 5 | 0.89 |
| **5b** | 3-$NO_2$ | H | $C(CH_3)_3$ | 5 | 1.2 | **5m** | 3-Cl | 5-Cl | $C(CH_3)_3$ | 5 | 0.087 |
| **5c** | 4-$NO_2$ | H | $C(CH_3)_3$ | 5 | 38 | **5n** | 2-Cl | 6-Cl | $C(CH_3)_3$ | 5 | [c] |
| **5d** | 3-CN | H | $C(CH_3)_3$ | 5 | 2.7 | **5o** | 3-Cl | 5-CN | $C(CH_3)_3$ | 5 | 0.45 |
| **5e** | 3-F | H | $C(CH_3)_3$ | 5 | 6.4 | **5p** | 3-Cl | 5-$NO_2$ | $C(CH_3)_3$ | 5 | 0.38 |
| **5f** | 3-Cl | H | $C(CH_3)_3$ | 5 | 0.21 | **5q** | 3-CN | 5-CN | $C(CH_3)_3$ | 5 | 0.75 |
| **5g** | 3-Br | H | $C(CH_3)_3$ | 5 | 0.82 | **5r** | 2-Cl | 5-Cl | $C(CH_3)_2CH_2CH_3$ | 5 | 0.026 |
| **5h** | 3-I | H | $C(CH_3)_3$ | 5 | 0.61 | **5s** | 3-Cl | 5-CN | $C(CH_3)_2CH_2CH_3$ | 5 | 0.075 |
| **5i** | 3-$CH_3$ | H | $C(CH_3)_3$ | 5 | 23 | **5t** | 5-Cl | 5-Cl | $CHCH_3C(CH_3)_3$ | 3 | 0.18 |
| **5j** | 3-$N(CH_3)_2$ | H | $C(CH_3)_3$ | 5 | 56 | **5u** | 5-Cl | 5-Cl | $CH_2CH_2CH_2CH_3$ | 5 | 38 |
| **5k** | H | H | $C(CH_3)_3$ | 5 | 6.9 | | | | | | |
| **6**[b] | 4-CN | H | $CHCH_3C(CH_3)_3$ | 5 | 0.32 | | | | | | |
| Pinacidil | | | | 20 | 2.0 | | | | | | |
| Cromakalim | | | | 5 | 0.47 | | | | | | |

*a*) Drug concentration required to relax a spontaneous contraction in guinea pig taenia caecum by 50%. See Experimental for details. *b*) Prepared by Atwal *et al.*, see reference 11. *c*) No effect at a concentration of 100 $\mu$M.

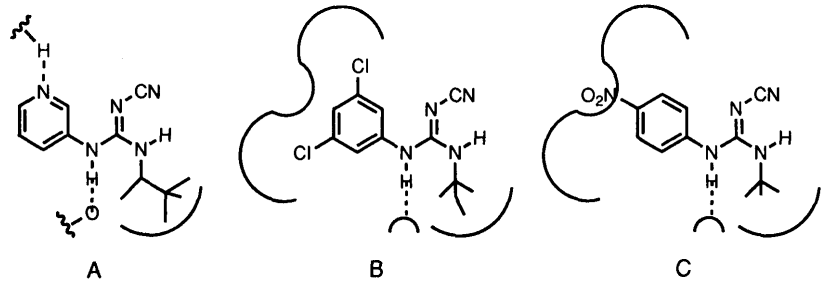



Fig. 2. Pharmacophore Model for Potassium Channel Opener

A, the pharmacophore model proposed by Manley and Quast for a pinacidil analogue; B, our novel pharmacophore model for **5r**; C, for **5c**.

pyridine ring is not essential for binding to the potassium channel. From the above results, we propose a new pharmacophore model, as shown in Fig. 2B. In our model, 1) the essential factors for binding to the channel are an NH and a bulky alkyl group, and 2) the potassium channel has two spaces which the two substituents at the 3- and 5-positions can occupy. The reason the 3,5-dichloro *tert*-pentyl compound (**5r**) showed excellent activity must be due to that: 1) the acidity of the NH proton was enhanced by the substitutions of the two chlorine atoms,[16] so the hydrogen-bond between NH and the channel was strengthened, 2) the hydrophobic interaction between substituents and the channel was enhanced by the two chlorine atoms, and 3) the *tert*-pentyl group also enhanced the affinity to the channel.

The 4-nitro compound (**5c**) did not show strong activity, in spite of the powerful electron withdrawing property of the 4-nitro group. The 3,4-dichloro compound (**5l**) was less potent than the 3-chloro derivative (**5f**). These results seem to mean that the activity decreases when a phenylcyanoguanidine derivative possesses a substituent at the 4-position. We speculate that a compound, such as **5c**, can not bind to the potassium channel with high affinity because of the steric hindrance between the 4-substituent and the channel, as shown in Fig. 2C. If the presence of a substituent at the 4-position weakens the activity, the 4-cyano derivative (**6**) should also show weak activity. However, **6** was actually more potent than pinacidil. **5c** and **6** differ from each other in terms of an alkyl group (*tert*-butyl *vs.* $CH(CH_3)C(CH_3)_3$). The reason the 4-cyano compound (**6**) exhibited the strong activity may be associated with its alkyl group.[17] We assume that the narrow cyano group of **6** could be located in one of two hydrophobic spaces because of its alkyl group, $CH(CH_3)C(CH_3)_3$, and **6** could bind to the channel with good affinity.

It is known that calcium channel antagonists also relax the guinea pig caecum.[18] In order to confirm that the smooth muscle relaxation effect of the 3-chloro-5-cyano *tert*-pentyl compound (**5s**) is based on a potassium channel opening effect, the following experiment was carried out. After a section of taenia caecum was pretreated with glibenclamide, a known potassium channel blocker, **5s** was cumulatively added to the solution in which the specimen was suspended. As shown in Fig. 3, the dose-response curve was shifted to the right by pretreatment with glibenclamide. In addition, higher concentrations of glibenclamide caused the curve to shift

more to the right. These results means that the smooth muscle relaxation effect of **5s** is based on its potassium channel opening activity.

Those phenylcyanoguanidine derivatives which showed strong activities *in vitro* ($ED_{50}$ values less than 0.2 $\mu$M) were evaluated for antihypertensive activity using dogs (i.v.) and spontaneously hypertensive rats (SHR, i.v. and *p.o.*). The 3-chloro-5-cyano-*tert*-pentyl compound (**5s**) was selected as the most preferable compound from several candidates. When administered to SHRs by i.v. injection, **5s** exhibited ten times more potent antihypertensive activity than pinacidil (Table 4). The activity of **5s** by the oral administration in SHRs was slightly more potent, as compared to that of pinacidil. **5s** evoked reflex tachycardia in the same manner as pinacidil, but **5s** was superior to pinacidil in the duration of the effect (Fig. 4). On the other hand, the antihypertensive activity of the 4-cyano compound (**6**) was weaker than that of both **5s** and pinacidil. **5s** did not show as strong activity by oral administration as expected from the result of the i.v. injection, which may be due to its low absorption from the intestinal tract.

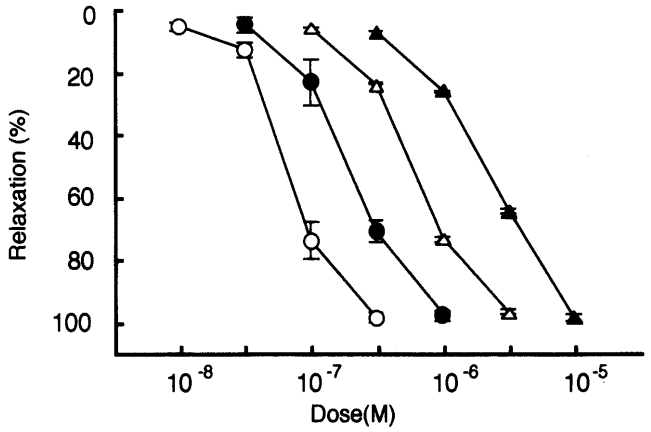



Fig. 3. Effect of Glibenclamide on Smooth Muscle Relaxation Activity of **5s**

**5s** was cumulatively added to the solution, in which the taenia caecum was suspended, without pretreatment by glibenclamide (—○—), or with pretreatment by glibenclamide (—●— $10^{-7}$ M, —△— $3\times10^{-7}$ M, —▲— $10^{-6}$ M). Each point represents the mean±S.E. of five experiments.

## Conclusion

In order to discover novel, highly active potassium channel openers, we synthesized the 21 substituted phenylcyanoguanidine derivatives. Among the mono-substituted series, the 3-chloro compound (**5f**) showed more potent smooth muscle relaxation activity than either pinacidil or the 4-cyano compound (**6**) reported by Atwal *et al.* The results of the QSAR-analysis prompted us to synthesize di-substituted phenylcyanoguanidine derivatives which had not been investigated. In the di-substituted series, the 3,5-di-substituted compounds having halogen, cyano, and/or nitro groups showed 2 to 77-fold better activities than pinacidil. Among them, the 3-chloro-5-cyano *tert*-pentyl compound (**5s**) exhibited a stronger and more lasting antihypertensive effect than pinacidil by

Table 4. Antihypertensive Activities of **5s**, **6** and Pinacidil in SHRs

| Compd. | Antihypertensive activity (*p.o.*) | | Antihypertensive activity (i.v.) | |
|---|---|---|---|---|
| | $\Delta$ mmHg[a] | $ED_{30}$ (mg/kg)[b] | Dose ($\mu$g/kg) | $\Delta$ mmHg[c] |
| **5s** | −32.6±4.0 | 0.72 | 10 | −31.0±3.6 |
| **6** | −17.2±3.7 | 3.3 | NT[d] | |
| Pinacidil | −21.2±8.1 | 0.95 | 100 | −27.0±2.7 |

*a*) Antihypertensive activities in conscious SHRs (male) by oral administration at a dose 1 mg/kg. Arterial pressure was measured for 24 h after administration. Each value represents the mean±S.E. of a maximum decrease in mean blood pressure ($n=7$ for **5s**, $n=5$ for **6**, and $n=6$ for pinacidil). *b*) The dose required to cause a 30 mmHg decrease in mean blood pressure ($n=5$—7 for **5s**, $n=5$—6 for **6**, and $n=5$—6 for pinacidil). *c*) Antihypertensive activities in anesthetized SHRs by intravenous administration, and each value represents the mean±S.E. of a maximum decrease in mean blood pressure ($n=11$ for **5s**, and $n=8$ for pinacidil). *d*) Not tested.

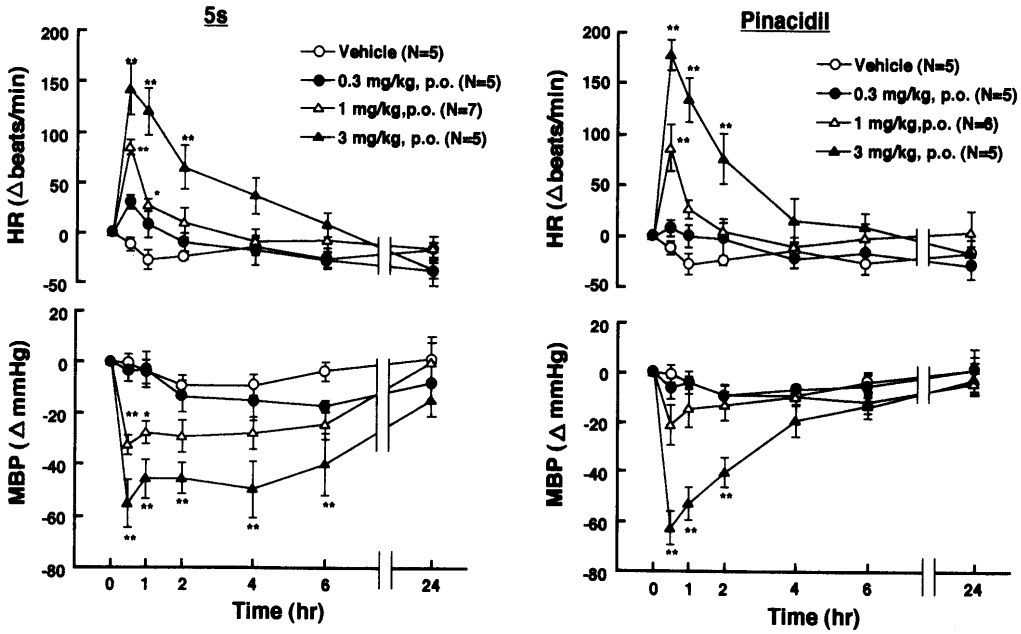



Fig. 4. Effects of Oral Administration of **5s** (Left) and Pinacidil (Right) on Heart Rate (HR, Top) and Mean Blood Pressure (MBP, Bottom) in Conscious SHRs

Each point represents the mean±S.E., $*p<0.05$ and $**p<0.01$, significantly different from the vehicle group (Dunnett's test).

oral administration to SHRs. It was confirmed by QSAR analysis that both the electron-withdrawing and lipophilic properties of the substituents were associated with the activities of the 3-mono-substituted series and the 3,5-di-substituted series.

## Experimental

Melting points were determined on a capillary melting point apparatus (Yamato MR-21) and are uncorrected. The structures of all compounds were supported by their 60- or 300-MHz $^1$H-NMR spectra (Hitachi R-24A and Bruker AM300) using tetramethylsilane as an internal standard. All compounds were analyzed for C, H, and N, and the results were within $\pm 0.4\%$ 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 anilines were prepared according to the literature: 3-chloro-5-cyanoaniline,[19] 3-chloro-5-nitroaniline,[20] 3,5-dicyanoaniline.[21]

***N*-(3-Chloro-5-cyanophenyl)-*N'*-*tert*-pentylthiourea (3s)** To a solution of 3-chloro-5-cyanoaniline (52.0 g, 341 mmol) in ethyl acetate (500 ml) was added dropwise thiophosgene (13.0 g, 113 mmol), and then the mixture was stirred at 75 °C for 1.5 h. After being cooled to room temperature, insoluble matter was removed by filtration. *tert*-Pentylamine (19.8 g, 227 mmol) was added to the filtrate at room temperature, and the mixture was stirred at the same temperature for 1 h. The reaction mixture was concentrated under reduced pressure, then the precipitated crystals were collected by filtration and washed with benzene to give **3s**

Table 5. $^1$H-NMR Data for Substituted Phenylthioureas **3** and Substituted Phenylcyanoguanidines **5**

| Compd. | Solvent[a] | $\delta$ ppm |
|---|---|---|
| **3a** | A | 1.57 (9H, s), 6.47 (1H, br s), 7.1—7.3 (1H, m), 7.5—7.7 (1H, m), 8.0—8.2 (1H, m), 8.48 (1H, d, $J$=8.3 Hz), 9.68 (1H, br s) |
| **3b** | A | 1.55 (9H, s), 6.14 (1H, br s), 7.5—7.7 (3H, m), 8.0—8.2 (2H, m) |
| **3c** | B | 1.55 (9H, s), 7.6—8.2 (5H, m), 9.80 (1H, br s) |
| **3d** | A | 1.54 (9H, s), 6.09 (1H, br s), 7.4—7.6 (4H, m), 7.75 (1H, br s) |
| **3e** | A | 1.53 (9H, s), 6.09 (1H, br s), 6.9—7.0 (3H, m), 7.3—7.5 (1H, m), 7.59 (1H, br s) |
| **3f** | A | 1.52 (9H, s), 6.03 (1H, br s), 7.0—7.4 (4H, m), H, 7.53 (1H, br s) |
| **3g** | B | 1.48 (9H, s), 7.2—7.4 (3H, m), 7.58 (1H, br s), 7.9—8.0 (1H, m), 9.40 (1H, br s) |
| **3h** | A | 1.52 (9H, s), 6.00 (1H, br s), 7.2—7.2 (2H, m), 7.29 (1H, br s), 7.5—7.7 (2H, m) |
| **3i** | A | 1.51 (9H, s), 2.36 (3H, s), 6.05 (1H, br s), 6.9—7.1 (3H, m), 7.2—7.4 (1H, m), 7.46 (1H, br s) |
| **3j** | A | 1.52 (9H, s), 2.95 (6H, s), 6.26 (1H, br s), 6.4—6.7 (3H, m), 7.23 (1H, t, $J$=8.0 Hz), 7.32 (1H, br s) |
| **3k** | A | 1.51 (9H, s), 6.02 (1H, br s), 7.1—7.5 (6H, m) |
| **3l** | A | 1.52 (9H, s), 6.00 (1H, br s), 7.09 (1H, dd, $J$=2.5, 8.6 Hz), 7.34 (1H, d, $J$=2.5 Hz), 7.46 (1H, d, $J$=8.6 Hz), 7.52 (1H, br s) |
| **3m** | B | 1.48 (9H, s), 7.22 (1H, t, $J$=1.8 Hz), 7.61 (2H, d, $J$=1.8 Hz), 7.75 (1H, br s), 9.52 (1H, br s) |
| **3n** | A | 1.52 (9H, s), 5.73 (1H, br s), 6.97 (1H, br s), 7.2—7.3 (1H, m), 7.43 (2H, d, $J$=7.8 Hz) |
| **3o** | A | 1.55 (9H, s), 6.23 (1H, br s), 7.45 (1H, t, $J$=1.6 Hz), 7.5—7.7 (2H, m), 7.84 (1H, br s) |
| **3p** | A | 1.56 (9H, s), 6.25 (1H, br s), 7.65 (1H, br s), 7.7—7.8 (1H, m), 8.03 (1H, t, $J$=1.9 Hz), 8.08 (1H, t, $J$=1.8 Hz) |
| **3q** | B | 1.49 (9H, s), 7.96 (1H, br s), 8.09 (1H, t, $J$=1.4 Hz), 8.26 (2H, d, $J$=1.4 Hz), 9.69 (1H, br s) |
| **3r** | B | 0.82 (3H, t, $J$=7.4 Hz), 1.42 (6H, s), 1.93 (2H, q, $J$=7.4 Hz), 7.23 (1H, t, $J$=1.9 Hz), 7.5—7.7 (3H, m), 9.55 (1H, br s) |
| **3s** | A | 0.93 (3H, t, $J$=7.5 Hz), 1.49 (6H, s), 1.91 (2H, q, $J$=7.5 Hz), 6.11 (1H, br s), 7.45 (1H, t, $J$=1.6 Hz), 7.5—7.7 (2H, m), 7.86 (1H, br s) |
| **3t** | B | 0.92 (9H, s), 1.05 (3H, d, $J$=6.7 Hz), 4.2—4.4 (1H, m), 7.23 (1H, t, $J$=1.8 Hz), 7.73 (2H, d, $J$=1.5 Hz), 7.89 (1H, d, $J$=9.2 Hz), 9.86 (1H, br s) |
| **3u** | A | 0.95 (3H, t, $J$=7.2 Hz), 1.3—1.7 (4H, m), 3.5—3.7 (2H, m), 6.02 (1H, br s), 7.13 (2H, d, $J$=1.7 Hz), 7.27 (1H, t, $J$=1.8 Hz), 7.52 (1H, br s) |
| **5a** | A | 1.54 (9H, s), 5.60 (1H, br s), 7.2—7.3 (1H, m), 7.6—7.7 (1H, m), 8.1—8.2 (2H, m), 9.53 (1H, br s) |
| **5b** | B | 1.36 (9H, s), 7.37 (1H, br s), 7.5—7.7 (2H, m), 7.8—8.0 (2H, m), 9.45 (1H, br s) |
| **5c** | B | 1.36 (9H, s), 7.1—7.3 (2H, m), 7.77 (1H, br s), 8.1—8.3 (2H, m), 9.75 (1H, br s) |
| **5d** | B | 1.32 (9H, s), 7.20 (1H, br s), 7.3—7.6 (4H, m), 9.24 (1H, br s) |
| **5e** | B | 1.34 (9H, s), 6.8—7.0 (3H, m), 7.08 (1H, br s), 7.3—7.4 (1H, m), 9.12 (1H, br s) |
| **5f** | B | 1.34 (9H, s), 6.82 (1H, br s), 6.9—7.4 (4H, m), 8.92 (1H, br s) |
| **5g** | B | 1.33 (9H, s), 7.0—7.4 (5H, m), 9.08 (1H, br s) |
| **5h** | B | 1.33 (9H, s), 7.04 (1H, br s), 7.1—7.2 (2H, m), 7.3—7.5 (2H, m), 9.04 (1H, br s) |
| **5i** | B | 1.32 (9H, s), 2.28 (3H, s), 6.58 (1H, br s), 6.8—7.0 (3H, m), 7.1—7.2 (1H, m), 8.87 (1H, br s) |
| **5j** | B | 1.32 (9H, s), 2.88 (6H, s), 6.3—6.6 (4H, m), 7.12 (1H, t, $J$=8.0 Hz), 8.81 (1H, br s) |
| **5k** | A | 1.35 (9H, s), 4.70 (1H, br s), 7.1—7.4 (3H, m), 7.4—7.5 (2H, m), 7.57 (1H, br s) |
| **5l** | B | 1.33 (9H, s), 7.10 (1H, dd, $J$=2.6, 8.7 Hz), 7.18 (1H, br s), 7.31 (1H, d, $J$=2.5 Hz), 7.55 (1H, d, $J$=8.7 Hz), 9.20 (1H, br s) |
| **5m** | B | 1.34 (9H, s), 7.09 (2H, d, $J$=1.8 Hz), 7.23 (1H, t, $J$=1.8 Hz), 7.37 (1H, br s), 9.27 (1H, br s) |
| **5n** | B | 1.34 (9H, s), 6.40 (1H, br s), 7.3—7.4 (1H, m), 7.51 (2H, d, $J$=8.0 Hz), 8.65 (1H, br s) |
| **5o** | B | 1.34 (9H, s), 7.4—7.5 (3H, m), 7.6—7.7 (1H, m), 9.42 (1H, br s) |
| **5p** | B | 1.36 (9H, s), 7.54 (1H, t, $J$=1.9 Hz), 7.58 (1H, br s), 7.86 (1H, t, $J$=2.0 Hz), 7.91 (1H, t, $J$=1.9 Hz), 9.62 (1H, br s) |
| **5q** | B | 1.35 (9H, s), 7.44 (1H, br s), 7.81 (2H, d, $J$=1.4 Hz), 8.11 (1H, t, $J$=1.3 Hz), 9.54 (1H, br s) |
| **5r** | B | 0.82 (3H, t, $J$=7.4 Hz), 1.29 (6H, s), 1.70 (2H, q, $J$=7.4 Hz), 7.08 (2H, d, $J$=1.7 Hz), 7.2—7.3 (2H, m), 9.30 (1H, br s) |
| **5s** | B | 0.83 (3H, t, $J$=7.4 Hz), 1.29 (6H, s), 1.70 (2H, q, $J$=7.4 Hz), 7.29 (1H, br s), 7.4—7.5 (2H, m), 7.6—7.7 (1H, m), 9.43 (1H, br s) |
| **5t** | B | 0.90 (9H, s), 1.07 (3H, d, $J$=6.7 Hz), 3.7—3.9 (1H, m), 7.2—7.4 (4H, m), 9.27 (1H, br s) |
| **5u** | B | 0.89 (3H, t, $J$=7.3 Hz), 1.2—1.4 (2H, m), 1.4—1.6 (2H, m), 3.23 (2H, q, $J$=6.6 Hz), 7.32 (3H, s), 7.63 (1H, t, $J$=5.6 Hz), 9.17 (1H, br s) |

a) A, $CDCl_3$. B, DMSO-$d_6$.

(12.4 g, 39%). Recrystallization from a mixture of benzene and cyclohexane provided a more pure sample for analysis. The physical properties and spectral data of **3s** are listed in Tables 1 and 5, respectively.

Compounds **3a—i**, **3k—m**, **3o—r**, and **3t—u** were prepared by the same method as described above. 3-*N*,*N*-Dimethylaminophenylisothiocyanate, which was required to synthesize **3j**, was prepared by stirring a mixture of *N*,*N*-dimethyl-1,3-phenylenediamine dihydrochloride (0.50 g, 2.39 mmol), thiophosgene (0.30 g, 2.61 mmol), diisopropylethylamine (0.62 g, 4.80 mmol), and toluene (5 ml) at 75 °C. The 2,6-dichlorophenylisothiocyanate needed to prepare **3n** was synthesized by stirring a mixture of 2,6-dichloroaniline, (0.50 g, 3.09 mmol), thiophosgene (0.13 g, 1.13 mmol), toluene (5 ml), and one drop of pyridine at 75 °C.

**Method A. *N*-*tert*-Butyl-*N'*-(3-chlorophenyl)-*N''*-cyanoguanidine (5f)** A mixture of *N*-*tert*-butyl-*N'*-(3-chlorophenyl)thiourea (**3f**, 6.3 g, 26.0 mmol), dicyclohexylcarbodiimide (8.0 g, 38.8 mmol), triethylamine (0.5 ml), and tetrahydrofuran (50 ml) was stirred at 50—60 °C overnight. After being cooled to room temperature, the reaction mixture was evaporated *in vacuo*. The obtained residue was stirred with petroleum ether, then the insoluble solid was removed by filtration. The filtrate was evaporated *in vacuo* to give *N*-*tert*-butyl-*N'*-(3-chlorophenyl)carbodiimide as an oil. To a solution of this oil in tetrahydrofuran (40 ml) were added cyanamide (3.0 g, 71.4 mmol) and diisopropylethylamine (0.5 ml), and then the mixture was stirred at 80—90 °C overnight. The mixture was evaporated *in vacuo*, and the obtained residue was purified by column chromatography using a 100 : 1 mixture of chloroform and methanol as an eluent. The obtained crude **5f** was recrystallized from a mixture of ethyl acetate and diisopropylether to afford **5f** as colorless needles (4.0 g, 62%). Physical properties and spectral data of **5f** are listed in Tables 2 and 5, respectively.

Compounds **5a**, **5l—5n**, **5r**, and **5t** were synthesized by a method similar to that used for the preparation of **5f**.

**Method A. *N*-(3-Chloro-5-cyanophenyl)-*N'*-cyano-*N''*-*tert*-pentylguanidine (5s)** A mixture of *N*-(3-chloro-5-cyanophenyl)-*N'*-*tert*-pentylthiourea (**3s**, 19.0 g, 67.4 mmol), triphenylphosphine (35.5 g, 135 mmol), carbon tetrachloride (21.5 g, 135 mmol), triethylamine (13.7 g, 135 mmol), and dichloromethane (350 ml) was refluxed for 5 h, and then evaporated *in vacuo*. The obtained residue was stirred with ligroin, and the resulting solid was removed by filtration. The filtrate was evaporated *in vacuo* to give *N*-(3-chloro-5-cyanophenyl)-*N'*-*tert*-pentylcarbodiimide as an oil. To a solution of this oil in DMF (100 ml) were added cyanamide (16.0 g, 381 mmol) and diisopropylethylamine (0.1 ml). The mixture was stirred at 100 °C for 15 h, cooled to room temperature, and poured into water (1500 ml). The resulting crystals were collected by filtration and washed with cyclohexane. Recrystallization from benzene gave **5s** as colorless needles (12.6 g, 64%).

Compounds **5b**, **5e**, **5h**, **5i**, **5k**, **5o**, **5p**, and **5u** were synthesized by a method similar to that used for the preparation of **5s**.

**Method B. *N*-*tert*-Butyl-*N'*-cyano-*N''*-(3,5-dicyanophenyl)guanidine (5q)** A mixture of *N*-*tert*-butyl-*N'*-(3,5-dicyanophenyl)thiourea (**3q**, (4.3 g, 16.6 mmol), cyanamide (1.4 g, 33.3 mmol), dicyclohexylcarbodiimide (6.9 g, 33.4 mmol), diisopropylethylamine (0.4 ml), and tetrahydrofuran (100 ml) was refluxed for 12 h. After being cooled to room temperature, the reaction mixture was evaporated *in vacuo*. The obtained residue was purified by column chromatography using a 9 : 1 mixture of benzene and ethyl acetate as an eluent to give crude **5q** (1.2 g). Recrystallization from a mixture of benzene and ethyl acetate afforded **5q** as colorless needles (1.0 g, 23%).

Compounds **5c**, **5d**, **5g**, and **5j** were synthesized by a method similar to that used for the preparation of **5q**.

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

In the experiment investigating the effect of glibenclamide on the smooth muscle relaxation activity of **5s**, each specimen was pretreated with glibenclamide for 15 min before **5s** was cumulatively added.

**Antihypertensive Activity in SHRs** Male SHRs, 13—24 weeks old (Charles River Japan Inc., Atsugi, Japan), were anesthetized with diethyl ether and instrumented with an abdominal aorta catheter *via* the left femoral artery. The catheters were passed subcutaneously to the dorsal side of the neck and exteriorized. At least 15 h after the surgery, the carotid catheter was connected to a pressure transducer (Nihon Kohden, TP400T, Tokyo, Japan) coupled to a polygraph (Nihon Kohden, RM-6000) for monitoring arterial pressure. Heart rate was recorded by a tachograph (Nihon Kohden, AT-601G). Compounds were suspended in 5% Arabic gum. After a 30-min stabilization period, conscious SHRs were treated orally with the vehicle and **5s**, **6**, or pinacidil at 0.3, 1 or 3 mg/kg, respectively, and the arterial pressure and heart rate were monitored for 24 h.

The antihypertensive activity by intravenous injection was carried out as follows. Male SHRs were anesthetized with pentobarbital sodium salt (50 mg/kg, i.p.), and both the left femoral artery and vein were cannulated. Compounds were dissolved in a mixture of 10% dimethylacetamide, 10% ethanol and 80% saline. After a 30-min stabilization period, single intravenous doses of **5s** at 1, 3, 10, or 30 μg/kg, or pinacidil at 10, 30, 100, 300 μg/kg were given to the anesthetized SHRs, and the arterial pressure and heart rate were monitored for 5 min.

## References and Notes


1) Robertson D. W., Steinberg M. I., *J. Med. Chem.*, **33**, 1529—1541 (1990).
2) Longman S. D., Hamilton T. C., *Med. Res. Rev.*, **12**, 73—148 (1992).
3) Petersen H. J., Nielsen C. K., Arrigoni-Martelli E., *J. Med. Chem.*, **21**, 773—781 (1978).
4) Goldberg M. R., Sushak C. S., Rockhold F. W., Thompson W. L., *Clin. Pharmacol. Ther.*, **44**, 78—92 (1988).
5) Sterndorff B., Johansen P., *Acta Med. Scand.*, **224**, 329—336 (1988).
6) Ramsey L. E., Freestone S., *Br. J. Clin. Pharmacol.*, **16**, 366—338 (1983).
7) Cook N. S., Quast U., Hof R. P., Baumlin Y., Pally C., *J. Cardiovasc. Pharmacol.*, **11**, 90—99 (1988).
8) Ashwood V. A., Buckingham R. E., Cassidy F., Evans J. M., Faruk E. A., Hamilton T. C., Nash D. J., Stemp G., Willcocks K. J., *J. Med. Chem.*, **29**, 2194—2201 (1986).
9) Manley P. W., Quast U., *J. Med. Chem.*, **35**, 2327—2340 (1992).
10) 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).
11) 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).
12) Barton S. D., Ollis W. D., "Comprehensive Organic Chemisty: The Synthesis and Reactions of Organic Compounds," Vol. 4 Heterocyclic Compounds, ed. by Sammes P. G., Pergamon Press Ltd., Oxford, 1979, pp. 3—84 (Chapter 16.1).
13) Weir S. W., Weston A. H., *Br. J. Pharmacol.*, **88**, 113—120 (1986).
14) *a*) Atwal K. S., McCullough J. R., Grover G. J. (E. R. Squibb & Sons Inc.), U. S. Patent 5011837 (1991), priority date: 1988. 8. 9, [*Chem. Abstr.*, **113**, 114834*g* (1990)]; *b*) Morita T., Yoshiizumi K., Nishimura N., Goto K., Sukamoto T., Yoshino K. (Kanebo Ltd.), Japan. Patent 3-31250 (1991), priority date: 1989. 6. 28, [*Chem. Abstr.*, **115**, 8333*z* (1991)].
15) Hansch C., Leo A., Unger S. H., Kim K. H., Nikaitani D., Lien E. J., *J. Med. Chem.*, **16**, 1207—1217 (1973).
16) *a*) We did not measure the $pK_a$ values of substituted phenylcyanoguanidines, including **5r**, but it is known that the $pK_a$ value of 3,5-dichloroaniline was smaller than that of 3-chloroaniline or aniline ($pK_a$ value of 3,5-dichloroaniline at 25 °C, 2.371; 3-chloaniline, 3.521; aniline, 4.596), as described in reference 16*b*. Therefore, we assume that the acidity of the NH proton in **5r** would be enhanced; *b*) Perrin D. D., "Dissociation Constants of Organic Bases in Aqueous Solution: Supplement 1972," Butterworth's Scientific Publications, London, 1972.
17) Atwal *et al.* also reported a similar result in reference 11. Thus, a

4-cyano compound with a *tert*-butyl group was 10-fold less potent than **6** with $CHCH_3C(CH_3)_3$.
18) Matsui K., Ogawa Y., Imai S., *Arch. Int. Pharmacodyn.*, **283**, 124—133 (1986).
19) Grivsky E. M., Hitchings G. H., *Ind. Chim. Belg.*, **39**, 490—500 (1974).
20) White W. N., Klink J. R., *J. Org. Chem.*, **42**, 166—169 (1977).
21) Teach E. G. (Stauffer Chemical Co.), U.S. Patent 3903130 (1975) [*Chem. Abstr.*, **83**, 205985*k* (1975)].
22) Houghton P. G., Pipe D. F., Rees C. W., *J. Chem. Soc., Perkin Trans. 1*, **1985**, 1471—1479.
23) Nair G. V., *Indian J. Chem.*, **4**, 516—520 (1966) [*Chem. Abstr.*, **67**, 11290*d* (1967)].