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NMR Analysis of Tautomerisms of Active Pinacidil-Type Potassium Channel Openers and a Less Active One

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Abstract—In order to clarify the structural difference between active pinacidil-type potassium channel openers and a less active one, the tautomerisms of pinacidil derivatives **1**–**3** were investigated by NMR spectrometries. The predominant tautomer of the less active compound **3** was different from those of the active compounds **1** and **2**.

Introduction

The crucial role of potassium channels in controlling the membrane potential and excitability of cells is well established.[1] The opening of potassium channels enhances the efflux of potassium ions, which induces membrane hyperpolarization. This effect leads to a decrease in the opening of voltage-dependent calcium channels, which produces the relaxation of smooth muscle. Hypertension, asthma, angina pectoris, and urinary incontinence are known to be caused by the contraction of smooth muscle. Therefore, compounds with potassium channel opening properties, namely, potassium channel openers are thought to be useful in the treatment of these diseases.[2]

Pinacidil (Fig. 1) is a well-known potassium channel opener,[3] and was proved to be effective for hypertension.[4] However, some drawbacks observed in clinical study, such as short duration,[5] edema,[6] and tachycardia,[6] have limited its utility. To discover more useful compounds and/or to clarify the structure–activity relationships of pinacidil, novel derivatives of pinacidil have been synthesized by some research groups. As a result of the replacement of the pyridine ring with a 3,5-disubstituted benzene ring, we succeeded in the discovery of the phenylcyanoguanidine derivative **1** which is superior to pinacidil in both potency and duration of antihypertensive activity.[7] On the other hand, the replacement of the cyanoguanidine moiety with a nitroethenediamine moiety led to Bayer compound **2** with strong antihypertensive activity.[8] Both compounds **1** and **2** possess a benzene ring, an electron withdrawing group (cyano versus nitro), and a branched alkyl group. Such structural similarities prompted us to synthesize the hybrid compound **3**[9] shown in Figure 1, and to evaluate its antihypertensive activity in dogs (i.v. injection).[10] However, the antihypertensive activity of compound **3** (see relative activity in Fig. 1) was less than one third of that of pinacidil, while the antihypertensive activities of compounds **1** and **2** were twice as strong as that of pinacidil.

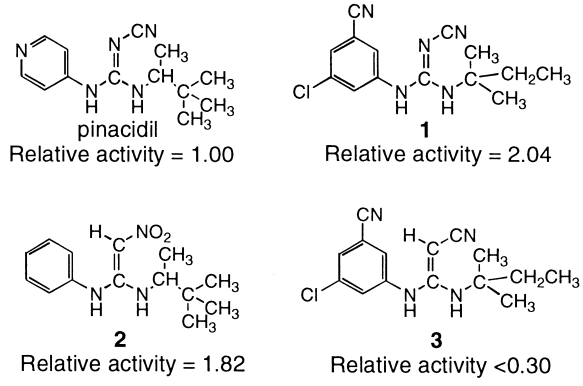

Figure 1. Structures and relative antihypertensive activities of pinacidil and compounds **1**–**3**.

The difference in the activities of compounds **1**–**3** should be explained by some difference in their structures. All of compounds **1**–**3** can exist in three tautomers. We have investigated the predominant tautomers of compounds **1**–**3**, which led to clarification of the structural difference between the active compounds and the less active compound.

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Tautomerism

We can draw three tautomers A, B, and C for compound **1**, as shown in Figure 2, depending upon the position of the double bond in the guanidine moiety, but it has been unclear which tautomer is predominant.

Heteronuclear multiple bond coherence (HMBC) is one of the 2D NMR spectrometries, which can determine long-range ^{1}H–^{13}C connectivities via two or three bonds with high sensitivity.[11] If compound **1** predominantly exists as tautomer A, the HMBC spectrum of compound **1** should give the correlation shown in Figure 3A′. Similarly, if tautomer B or C is predominant, the correlations shown in Figure 3B′ or C′ should be detected, respectively. Based on this hypothesis, at first, the ^{1}H NMR spectrum, the ^{13}C NMR spectrum, and the heteronuclear multiple quantum coherence (HMQC) spectrum of compound **1** were measured.[12] The ^{1}H NMR data and the ^{13}C NMR data are shown in Table 1.

Then, the HMBC spectrum was determined, and correlations were observed between a proton signal at δ_H 7.29 ppm (NH) and a carbon signal at δ_C 33.26 ppm (C-2), δ_C 26.83 ppm (C-3), and δ_C 55.68 ppm (C-4), respectively, and between a proton signal at δ_H 9.43 ppm (NH) and a carbon signal at δ_C 125.70 ppm (C-8). The correlation between NH of δ_H 9.43 ppm and C-6 of δ_C 116.76 ppm was not detected. These results indicate that the predominant tautomer of compound **1** in DMSO-d_6 is B, and the observed correlations can be illustrated in Figure 4.

Dicyanodiamide (Fig. 5) is the simplest compound among cyanoguanidine derivatives. Two tautomers, the cyanoamine-type tautomer D and the cyanoimine-type tautomer E, can be considered for dicyanodiamide, but it is known that dicyanodiamide adopts tautomer E not only in DMSO-d_6[13] but also in water.[14] Among three tautomers of compound **1**, tautomer B corresponds to tautomer E. From the above results, it was predicted that tautomer B could be preferred over tautomers A and C also in water.[15]

Next, the tautomerism of compound **2** that is the other active derivative of pinacidil was investigated. Three tautomers, the nitroethenediamine tautomer F, and the nitroacetamidine tautomers G and H, can be considered for compound **2** (Fig. 6). The ^{1}H NMR spectrum of compound **2** in DMSO-d_6 did not give a signal assigned to methylene protons of the nitroacetamidine tautomer G or H, while an olefin proton signal was observed at δ 6.03 ppm.[16] These data indicate that compound **2** predominantly adopts tautomer F in DMSO-d_6.

Finally, the predominant tautomer of compound **3** that is the less active derivative of pinacidil, was studied. For compound **3**, three tautomers, the cyanoethenediamine tautomer J, and the cyanoacetamidine tautomers K and L, can be represented (Fig. 7). At first, the ^{1}H NMR spectrum of compound **3** was measured, but an olefin proton signal corresponding to tautomer J was not observed in the ^{1}H NMR spectrum. In addition, a broad NH proton signal was detected only at δ 6.91 ppm as a 1H signal. Interestingly, a singlet signal assigned to methylene protons was observed at δ 3.48 ppm as a 2H signal. These findings indicate that compound **3** exists in DMSO-d_6 as cyanoacetamidine-type

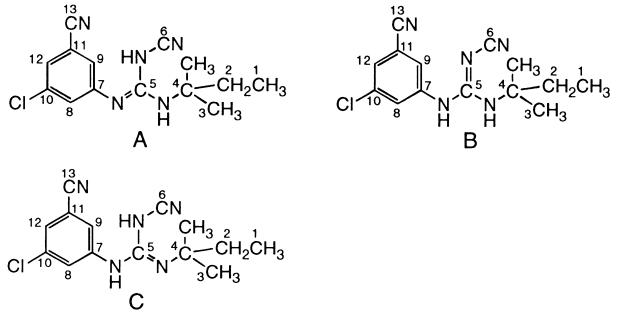

Figure 2. Possible three tautomers of compound **1**.

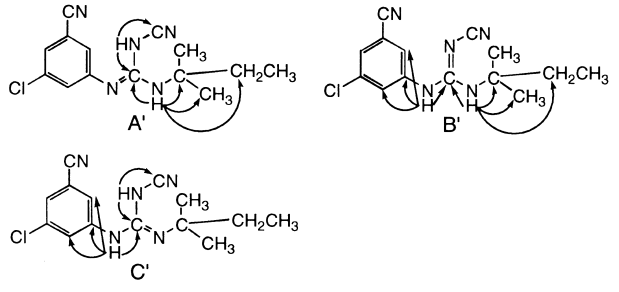

Figure 3. Possible C–H long-range correlations in the HMBC spectra of the tautomers of compound **1**. Correlations which do not relate to tautomerism, such as correlation between H-1 and C-2 are not illustrated.

Table 1. ^{1}H and ^{13}C NMR data of compound **1** in DMSO-d_6[a]

	δ (ppm)
^{1}H NMR	0.83 (t, 3H, J = 7.4 Hz, H-1), 1.29 (s, 6H, H-3), 1.70 (q, 2H, J = 7.4 Hz, H-2), 7.29 (br s, 1H, NH), 7.4–7.5 (m, 2H, H-8 and H-9), 7.6–7.7 (m, 1H, H-12), 9.43 (br s, 1H, NH)
^{13}C NMR	9.13 (C-1), 26.83 (C-3), 33.26 (C-2), 55.68 (C-4), 114.21 (C-11), 116.76 (C-6), 118.18 (C-13), 123.31 (C-9), 125.70 (C-8), 126.57 (C-12), 135.24 (C-10), 142.41 (C-7), 156.99 (C-5)

[a]The assignments were done on the basis of correlations in the HMQC spectrum of compound **1**.

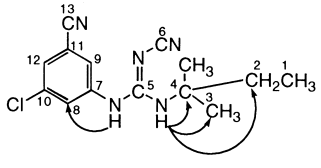

Figure 4. Significant C–H long-range correlations observed in the HMBC spectrum of compound **1**.

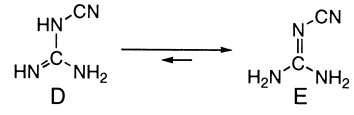

Figure 5. Tautomerism of dicyanodiamide.

tautomer K or L, not as cyanoethenediamine-type tautomer J. The ^{1}H NMR data of compound **3** are listed in Table 2, together with the ^{13}C NMR data.

As a result of the measurement of the HMBC spectrum of compound **3** in DMSO-d_6, correlations were detected between a proton signal at δ_H 6.91 ppm (NH) and a carbon signal at δ_C 26.80 ppm (C-3), δ_C 55.02 ppm (C-4), and δ_C 20.52 ppm (C-6), respectively. If compound **3** adopts tautomer L, the correlation between NH and C-3, and the correlation between NH and C-4, cannot be detected. In addition, neither the correlation between NH and C-9 nor the correlation between NH and C-10 was observed. These results mean that tautomer K predominates over tautomer L, and the observed correlations can be expressed in Figure 8.

Then, our attention was focused on the determination of geometrical isomerism around the imine bond of tautomer K. The NOESY spectrum of compound **3** in DMSO-d_6 gave the correlation between H-6 and H-9 (or H-10), while the correlation between NH and H-9 (or H-10) was not observed. These results indicate that tautomer K exists as geometrical isomer *E*, not *Z* (Fig. 9).

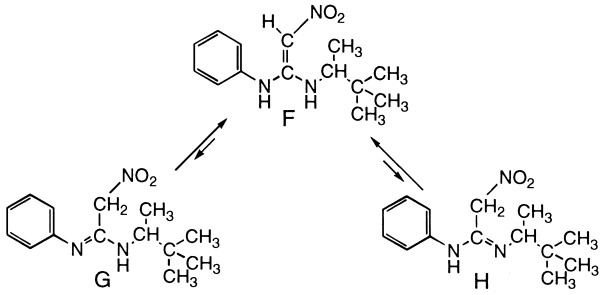

Figure 6. Tautomerism of compound **2** confirmed by ^{1}H NMR spectrum.

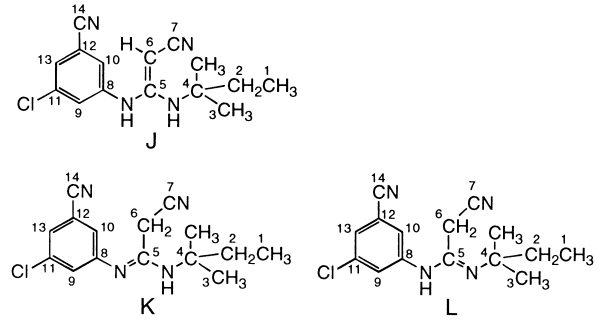

Figure 7. Possible three tautomers of compound **3**.

Table 2. ^{1}H and ^{13}C NMR data of compound **3** in DMSO-d_6[a]

	δ (ppm)
^{1}H NMR	0.82 (t, 3H, $J=7.4$ Hz, H-1), 1.23 (s, 6H, H-3), 1.76 (q, 2H, $J=7.4$ Hz, H-2), 3.48 (s, 2H, H-6), 6.91 (br s, 1H, NH), 7.0–7.1 (m, 2H, H-9 and H-10), 7.5–7.6 (m, 1H, H-13)
^{13}C NMR	9.06 (C-1), 20.52 (C-6), 26.80 (C-3), 31.75 (C-2), 55.02 (C-4), 114.13 (C-12), 117.27 (C-7), 118.57 (C-14), 125.07 (C-10), 125.21 (C-13), 127.70 (C-9), 135.11 (C-11), 148.01 (C-5), 153.21 (C-8)

[a]The assignments were done on the basis of correlations in the HMQC spectrum of compound **3**.

Compounds **1** and **2** of which the predominant tautomers are B and F, respectively, exhibited potent antihypertensive activity. On the other hand, compound **3** which adopts tautomer K, did not show strong antihypertensive activity. Tautomers B and F have an NH group next to the benzene ring, while tautomer K does not (Fig. 10). The poor antihypertensive activity of compound **3** would be due to the lack of the NH group next to the benzene ring.

We have already found that the antihypertensive activity of compound **1** is based on the opening of ATP-sensitive potassium channels.[17] Therefore, it was considered that the ATP-sensitive potassium channel opening activity of compound **3** is weaker than that of compound **1**. In our previous paper,[7] we assumed that the NH group adjacent to the benzene ring is one of the essential factors for binding to potassium channels. Manley et al. also pointed out the importance of the NH group as a

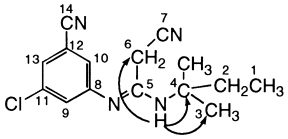

Figure 8. Significant C–H long-range correlations observed in the HMBC spectrum of compound **3**.

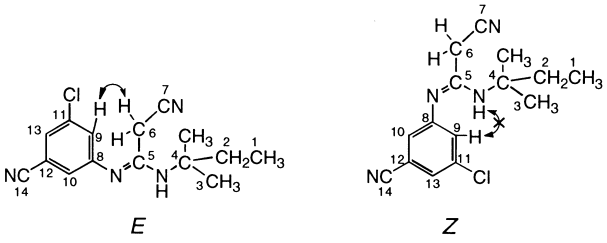

Figure 9. Possible two geometrical isomers of tautomer K and significant correlation observed in the NOESY spectrum of compound **3** in DMSO-d_6.

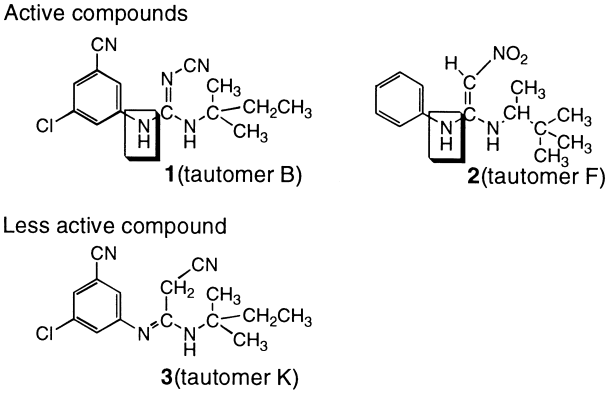

Figure 10. Structural difference between active compounds (**1** and **2**) and the less active compound (**3**).

hydrogen-bond-donor element.[18] The results in the present paper strongly supported that the NH group is essential for binding to potassium channels.

It is of interest to note that compounds **2** and **3** adopt a different tautomer, the nitroethenediamine-type tautomer F and the cyanoacetamidine-type tautomer K, respectively, though both of them have an electron withdrawing group-substituted ethenediamine moiety. Although the reason for the difference in the tautomerisms of compounds **2** and **3** are not clear now, the following possibilities can be considered: (1) the difference in the withdrawing group (nitro versus cyano); (2) the difference in the substituents on the benzene ring (unsubstituted versus 3-chloro-5-cyano); and (3) the difference in the alkyl group (1,2,2-trimethylpropyl versus *tert*-pentyl).

In conclusion, the tautomerisms of compounds **1**–**3** were investigated by NMR spectrometries. The predominant tautomer of the less active compound **3** was different from those of the active compounds **1** and **2** in the absence of an NH group next to the benzene ring. The information obtained through the present study will be useful in the design of novel derivatives of pinacidil-type potassium channel openers.

References and Notes

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9. (a) Compound **3** was prepared by treating *N*-(3-chloro-5-cyanophenyl)-*N*′-*tert*-pentylcarbodiimide with acetonitrile in the presence of *n*-butyllithium. (b) Mp 77.5–79.0. (c) Elemental analysis; calcd for $C_{15}H_{17}ClN_4$: C, 62.39; H, 5.93; N, 19.40; found: C, 62.39; H, 5.99; N, 19.53. (d) TOF-Mass; 289 (M + 1).
10. Antihypertensive activity in anesthetized dogs after intravenous administration. Relative activities were estimated as follows; relative activity = % change of mean blood pressure by tested compound (0.03 mg/kg)/% change of mean blood pressure by pinacidil (0.03 mg/kg).
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15. Because of low solubilities of compounds **1**–**3** in water, their NMR spectra in water could not be taken.
16. ^{1}H NMR (DMSO-d_6) δ (ppm): 0.95 (s, 9H), 1.17 (d, 3H, J = 6.6 Hz), 3.7–3.9 (m, 1H), 6.03 (s, 1H), 7.1–7.5 (m, 5H), 9.20 (br s, 1H), 10.53 (br s, 1H).
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