ANTIFUNGAL ACTIVITIES OF PRADIMICIN DERIVATIVES MODIFIED AT C4′-AMINO GROUP

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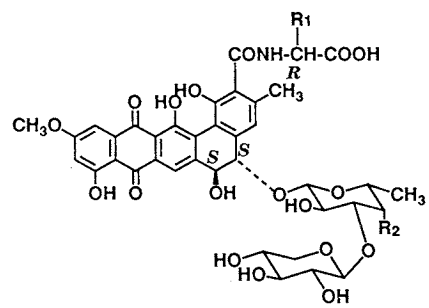
In order to explore potent derivatives of pradimicins (PRMs), modification of their C4′-amino group was carried out. 4′-*N*-Cyano (**1**, **2**), 4′-deamino-4′-nitroguanidino (**4**), 4′-deamino-4′-ureido (**7**～**9**) and 4′-deamino-4′-thioureido (**10**) derivatives were synthesized by trimethylsilylation of PRMs A and C, followed by condensation with appropriate reagents. 4′-Deamino-4′-guanidino (**5**) and 4′-deamino-4′-amidino (**6**) derivatives were synthesized by catalytic hydrogenation of **4** and **2**, respectively. 4′-*N*-Nitroso derivative **3** was prepared by treatment of PRM A with nitrous acid. Among these compounds, the 4′-*N*-cyano derivative of PRM C (**2**) exhibited *in vitro* and *in vivo* antifungal activities comparable to the parent compounds together with good water-solubility.

The pradimicins (PRMs) are a new family of antibiotics (Fig. 1)[1～5] that exhibited broad-spectrum antifungal activity both *in vitro* and *in vivo* studies. Although they are relatively nontoxic, their limited water-solubility due to amphoteric nature hampered further development studies. Thus, we initiated chemical modification of PRMs focused on the C4′-position[6～8] in order to improve water-solubility. In the previous paper[8], we reported water-soluble 4′-*N*-alkyl and -acyl and 4′-deamino-4′-hydroxy PRM derivatives. This report describes the syntheses and antifungal activities of the other 4′-amino-modified PRM derivatives; 4′-*N*-cyano, 4′-*N*-nitroso, 4′-guanidino, 4′-amidino, 4′-ureido and 4′-thioureido derivatives.

Synthesis

PRMs A and C have many functional groups that may interfere with substitution of the 4′-amino group. In the previous paper[3], we reported 4′-*N*-alkylation and -acylation of PRMs, with alkyl halides and activated carboxylic acids respectively, proceed smoothly in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (BSA). *N*-Substitution of PRMs with the other reagents also went smoothly. Thus, the BSA-pretreated PRMs A and C were reacted with cyanogen bromide in dichloromethane at room temperature, followed by detrimethylsilylation with HCl-MeOH afforded the 4′-*N*-cyano derivatives, **1** and **2**, respectively, in good yields. The

Fig. 1. Structures of natural pradimicins.



	R_1	R_2
Pradimicin A	CH_3	$NHCH_3$
Pradimicin C	CH_3	NH_2
Pradimicin FA-1	CH_2OH	$NHCH_3$
Pradimicin FA-2	CH_2OH	NH_2

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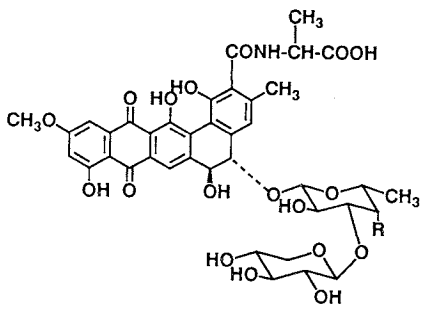
Fig. 2. 4′-*N*-modified pradimicins (**1**～**10**).



No.	R
1	NCH_3CN
2	NHCN
3	NCH_3NO
4	$NHC(=NH)NHNO_2$
5	$NHC(=NH)NH_2$
6	NHCH=NH
7	$NHCONH_2$
8	NCH_3CONH_2
9	$NCH_3CONHCH_3$
10	$NCH_3CSNHCH_3$

Table 1. *In vitro* activity of pradimicin derivatives.

Compound	MIC (μg/ml)[a]			
	Candida albicans A9540	*Candida tropicalis* IFO 10241	*Cryptococcus neoformans* IAM 4514	*Aspergillus fumigatus* IAM 2034
1	3.1	12.5	3.1	12.5
2	3.1	12.5	1.6	3.1
3	6.3	12.5	1.6	3.1
4	3.1	12.5	1.6	3.1
5	12.5	>100	6.3	3.1
6	12.5	>100	3.1	1.6
7	6.3	12.5	6.3	50
8	12.5	25	25	>100
9	6.3	25	25	>100
10	12.5	25	50	>100
PRM A	12.5	>100	1.6	0.8
PRM C	25	>100	0.8	3.1

[a] MIC's were determined by the 2-fold agar dilution method on yeast morphology agar buffered at pH 7.0 (Incubation, 28°C, 48 hours).

N-unsubstituted cyanoamino derivative **2** was rather unstable in acidic media to give the 4′-ureido derivative **7**, while the *N*-methylcyanoamino derivative **1** was stable in the same conditions. In a similar way to **1** and **2**, BSA-pretreated PRM A was reacted with methyl isocyanate and methyl isothiocyanate to afford the 4′-dimethylureido derivative (**9**) and 4′-dimethylthioureido derivative (**10**), respectively. PRMs A and C were reacted with trichloroacetyl isocyanate in a similar way, followed by alkaline hydrolysis, to give the 4′-ureido (**7**) and the 4′-monomethylureido (**8**) derivatives, respectively. The reaction of BSA-treated PRM C with *N*-nitro-*S*-methylisothiourea required more severe conditions. It proceeded in DMF at high temperature (80°C for 2 hours) to give the 4′-nitroguanidino derivative **4**, which was subjected to catalytic hydrogenation to afford the 4′-guanidino derivative **5**. Catalytic hydrogenation of the 4′-*N*-cyano derivative **2** in aqueous acetic acid afforded the 4′-amidino derivative **6**, together with the 4′-ureido derivative **7**, which was considered to be derived from **2** by action of acetic acid. PRM A was treated with sodium nitrite in aqueous acetic acid to give the 4′-nitrosoamino derivative **3**.

In Vitro Activity

The antifungal activity was determined by the 2-fold agar dilution method on yeast morphology agar buffered with 0.067 M phosphate, pH 7.0 and the results are summarized in Table 1. The 4′-*N*-cyano derivative **2** showed improved antifungal activity against *Candida albicans* and *Candida tropicalis* and retained antifungal activity of the parent PRM C against *Cryptococcus neoformans* and *Aspergillus fumigatus*, while the 4′-*N*-methylcyano derivative **1** was moderately active against *A. fumigatus* although the activity against the yeasts was very similar to **2**. The 4′-*N*-nitroso (**3**) and 4′-nitroguanidino (**4**) derivatives also showed improved antifungal activity against *C. tropicalis* with retention of activity against all the other strains as compared with PRMs A and C. On the contrary, the 4′-guanidino (**5**) and 4′-amidino (**6**) derivatives showed no activity against *C. tropicalis* at 100 μg/ml with retention of the activity against the other strains. The

4′-ureido derivative **7** was active against only yeasts and the other *N*-substituted ureido or thioureido derivatives (**8**～**10**) showed no activity against *A. fumigatus*. Among the C4′-amino-modified derivatives, the derivatives **1** through **4** retain *in vitro* activity of PRMs, but the 4′-ureido and 4′-thioureido derivatives, **7** through **10**, lose activity against *A. fumigatus*.

Table 2. *In vitro* activity of pradimicin derivatives against *Candida* and *Aspergillus* systemic infections in mice.

Compound	PD_{50} (mg/kg, iv)	
	Candida albicans A9540	*Aspergillus fumigatus* IAM 2034
1	13	>50
2	13	27
3	27	>50
4	12	>50
PRM A	10	23
PRM C	13	NT[a]

[a] Not tested.

In Vivo Activity and Water-solubility of the 4′-*N*-Cyano Derivative **2**

The *in vivo* activity of **1**, **2**, **3** and **4**, which showed broader *in vitro* antifungal spectrum than PRMs A and C was determined in mice infected with *C. albicans* A9540 and *A. fumigatus* IAM 2034 after intravenous administration, and the results are summarized in Table 2. All of the compounds were as effective as PRMs A and C against *C. albicans* A9540. However, against *A. fumigatus* IAM 2034, only compound **2** was as effective as PRM A among these compounds.

The water solubility of **2** was more than 20 mg/ml in phosphate-buffered saline containing Ca^{2+} and Mg^{2+} at pH 7.2[7]. Due to the electron withdrawing property of the cyano group, the basicity of 4′-amino group of **2** is thought to be much lower than the parent antibiotic resulting in the improvement of its water-solubility at a neutral pH.

In summary, among the 4′-amino-modified derivatives of PRMs A and C synthesized in this study, the 4′-*N*-cyano PRM C (**2**) shows antifungal activity comparable to the PRMs both *in vitro* and *in vivo* together with high water-solubility.

Experimental

MPs were determined using a Yanagimoto micro hot-stage apparatus and are uncorrected. NMR spectra were recorded on a JEOL GX-400 (400 MHz). Mass spectra were recorded on a JEOL JMS-AX505H (FAB) mass spectrometer.

Synthesis

4′-*N*-Cyano Derivatives (**1** and **2**)

Cyanogen bromide (180 mg, 1.7 mmol) was added to a mixture of PRM C (67 mg, 0.081 mmol) and BSA (0.5 ml, 2.02 mmol) in dichloromethane (2 ml) and the mixture was stirred overnight at room temperature. After removal of the solvent, MeOH (2 ml) and 1N HCl (1 ml) was added and the mixture was chromatographed on a column of Cosmosil 75C_{18}-OPN (Nacalai Tesque, Inc., 100 g). The column was eluted with water and 10～40% acetonitrile successively. The eluate was collected in fractions, which were monitored by HPLC. The fractions containing **2** were combined, concentrated *in vacuo* and freeze-dried to give 48 mg (79%) of a light red amorphous powder. MP 220～230°C (dec); IR ν_{max} (KBr) cm^{-1} 2210, 1720, 1620, 1290, 1060; Mass (FAB) *m/z* 852 $(M+H)^+$; UV λ_{max} (1/100N NaOH) nm (ε) 319 (14,800), 496 (15,400); ^1H NMR (DMSO-d_6) δ 1.15 (3H, d, J=7 Hz, 5′-CH_3), 1.33 (3H, d, J=7 Hz, 17-CH_3), 2.29 (3H, s, 3-CH_3), 3.93 (3H, s, OCH_3), 4.39 (1H, q, J=7 Hz, 17-H), 4.48 (1H, d, J=10 Hz, 5-H), 4.52 (1H, br d, J=10 Hz, 6-H), 4.64 (1H, d, J=8 Hz, 1′-H), 6.73 (1H, d, J=12 Hz, NHCN), 6.87 (1H, d, J=2 Hz, 10-H), 7.11 (1H, s, 4-H), 7.25 (1H, d, J=2 Hz, 12-H), 7.93 (1H, s, 7-H), 8.62 (1H, d, J=7 Hz, 16-NH).

Compound **1** was synthesized from PRM A by a similar procedure to the above. Yield 81%; mp >250°C (dec); IR ν_{max} (KBr) cm^{-1} 2200, 1720, 1600, 1290, 1165; Mass (FAB) *m/z* 866 $(M+H)^+$; UV

λ_{max} (1/100 N NaOH) nm (ε) 319 (14,800), 496 (13,900); ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, d, J=7 Hz, 5'-CH_3), 1.33 (3H, d, J=7 Hz, 17-CH_3), 2.28 (3H, s, 3-CH_3), 3.00 (3H, s, 4'-NCH_3), 3.74 (1H, dd, J=5 and 11 Hz, 5''-H), 3.95 (3H, s, OCH_3), 4.39 (1H, q, J=7 Hz, 17-H), 4.41 (1H, d, J=10 Hz, 5-H), 4.50 (1H, br d, J=10 Hz, 6-H), 4.64 (1H, d, J=8 Hz, 1'-H), 6.89 (1H, d, J=2 Hz, 10-H), 7.19 (1H, s, 4-H), 7.26 (1H, s, 12-H), 8.00 (1H, s, 7-H), 8.59 (1H, s, 16-NH).

4'-*N*-Nitroso Derivative (**3**)

Sodium nitrite (1 M aqueous solution, 0.5 ml) was added dropwise to a stirred solution of PRM A (100 mg, 1.19 mmol) in 0.25 M aqueous acetic acid (10 ml). The mixture was stirred for 2 hours at room temperature. By a similar purification to **2**, 75 mg (72%) of **3** was obtained. MP 230~240°C (dec); IR ν_{max} (KBr) cm^{-1} 1720, 1600, 1450, 1295, 1160, 1060; Mass (FAB) m/z 870 $(M+H)^+$; UV λ_{max} (1/100 N NaOH) nm (ε) 320 (14,100), 498 (14,000); ^{1}H NMR (DMSO-d_6) δ 0.98 (3H, d, J=7 Hz, 5'-CH_3), 1.33 (3H, d, J=7 Hz, 17-CH_3), 2.29 (3H, s, 3-CH_3), 3.15 (3H, s, 4'-NCH_3), 3.67 (1H, dd, J=5 and 11 Hz, 5''-H), 3.95 (3H, s, OCH_3), 4.40 (1H, q, J=7 Hz, 17-H), 4.47 (1H, d, J=7 Hz, 1''-H), 4.54 (2H, br s, 5-H and 6-H), 4.81 (1H, d, J=8 Hz, 1'-H), 6.92 (1H, d, J=2 Hz, 10-H), 7.04 (1H, s, 4-H), 7.28 (1H, d, J=2 Hz, 12-H), 8.20 (1H, s, 7-H), 8.59 (1H, d, J=7 Hz, 16-NH).

4'-Nitroguanidino Derivative (**4**)

N-Nitro-*S*-methylisothiourea (150 mg, 1.14 mmol) was added to a mixture of PRM A (100 mg, 1.21 mmol) and BSA (0.5 ml, 2.02 mmol) in DMF (2 ml) and the mixture was heated at 80°C for 2 hours. After removal of the solvent, MeOH (2 ml) and 1 N HCl (1 ml) was added and the mixture was purified by a similar way to that of compound **2** to give 69 mg (63%) of **4**. MP 220~230°C (dec); IR ν_{max} (KBr) cm^{-1} 1720, 1600, 1290, 1160, 1050; Mass (FAB) m/z 914 $(M+H)^+$; UV λ_{max} (1/100 N NaOH) nm (ε) 316 (16,000), 497 (14,700); ^{1}H NMR (DMSO-d_6) δ 1.06 (3H, d, J=7 Hz, 5'-CH_3), 1.34 (3H, d, J=7 Hz, 17-CH_3), 2.32 (3H, s, 3-CH_3), 3.95 (3H, s, OCH_3), 4.40 (1H, q, J=7 Hz, 17-H), 4.44 (1H, d, J=7 Hz, 1''-H), 4.50 (1H, d, J=10 Hz, 5-H), 4.60 (1H, br d, J=10 Hz, 6-H), 4.76 (1H, d, J=8 Hz, 1'-H), 6.91 (1H, s, 10-H), 7.11 (1H, s, 4-H), 7.28 (1H, s, 12-H), 8.02 (1H, s, 7-H), 8.59 (1H, d, J=7 Hz, 16-NH).

4'-Guanidino PRM C (**5**)

A mixture of **4** (50 mg, 0.055 mmol) and 10% palladium on charcoal (20 mg) in 1 N HCl - MeOH (1 : 10, 5 ml) was hydrogenated overnight under atmospheric pressure. The mixture was chromatographed on a column of Cosmosil 75C_{18}-OPN (Nacalai Tesque, Inc., 100 g) eluting with water and then with 1/1,000 N hydrochloric acid - acetonitrile (80 : 20~60 : 40) successively. Concentration of the appropriate fractions gave 18 mg (38%) of **5**. MP 220~230°C (dec); IR ν_{max}(KBr) cm^{-1} 1720, 1605, 1290, 1150; Mass (FAB) m/z 869 $(M+H)^+$; UV λ_{max} (1/100 N NaOH) nm (ε) 318 (14,700), 498 (14,100); ^{1}H NMR (DMSO-d_6) δ 1.08 (3H, d, J=7 Hz, 5'-CH_3), 1.34 (3H, d, J=7 Hz, 17-CH_3), 2.30 (3H, s, 3-CH_3), 3.95 (3H, s, OCH_3), 4.40 (1H, q, J=7 Hz, 17-H), 4.43 (1H, d, J=7 Hz, 1''-H), 4.55 (2H, br s, 5-H and 6-H), 4.76 (1H, d, J=8 Hz, 1'-H), 6.85 (1H, s, 10-H), 7.04 (1H, s, 4-H), 7.24 (1H, s, 12-H), 7.88 (1H, s, 7-H), 8.65 (1H, d, J=7 Hz, 16-NH).

4'-Amidino PRM C (**6**)

A mixture of **2** (35 mg, 0.041 mmol) and 10% palladium on charcoal (10 mg) in 25% aqueous acetic acid was hydrogenated overnight under atmospheric pressure. HPLC showed the presence of two products. Chromatographic separation by Cosmosil 75C_{18}-OPN column (100 g) eluting with 10~40% acetonitrile afforded 10 mg (27%) of **7**. Further elution with 1/1,000 N HCl - acetonitrile (80 : 20~60 : 40) afforded 5 mg (14%) of **6**. MP 220~230°C (dec); IR ν_{max} (KBr) cm^{-1} 1700, 1605, 1290, 1160; Mass (FAB) m/z 854 $(M+H)^+$; UV λ_{max} 1/100 N NaOH nm (ε) 319 (14,100), 496 (14,900); ^{1}H NMR (DMSO-d_6) δ 1.09 (3H, d, J=7 Hz, 5'-CH_3), 1.34 (3H, d, J=7 Hz, 17-CH_3), 2.29 (3H, s, 3-CH_3), 3.94 (3H, s, OCH_3), 4.39 (1H, q, J=7 Hz, 17-H), 4.55 (2H, br, 5-H and 6-H), 6.83 (1H, s, 10-H), 7.02 (1H, s, 4-H), 7.22 (1H, s, 12-H), 7.82 (1H, s, 7-H), 7.90 (1H, s, NHC*H*=NH), 8.68 (1H, br, 16-NH).

4'-Ureido PRMs (**7**~**9**) and 4'-Thioureido PRM (**10**)

Trichloroacetyl isocyanate (316 mg, 1.7 mmol) was added to a mixture of PRM C (85 mg, 0.102 mmol) and BSA (0.5 ml, 2.02 mmol) in dichloromethane (2 ml) and the mixture was stirred overnight at room

temperature. After removal of the solvent, MeOH (2 ml) and 1 N HCl (1 ml) was added. Separation of the mixture by Cosmosil 75C_{18}-OPN column with 40% acetonitrile elution afforded *N*-trichloroacetyl ureido derivative (80 mg). This was dissolved in 1 N NaOH (1 ml) and stirred for 1 hour at room temperature. The solution was acidified and chromatographed on a column of Cosmosil 75C_{18}-OPN. Elution with 20% acetonitrile afforded 47 mg (53%) of **7**. MP 220~230°C (dec); IR ν_{max} (KBr) cm^{-1} 1600, 1300, 1050; Mass (FAB) *m/z* 870 $(M+H)^+$; UV λ_{max} (1/100 N NaOH) nm (ε) 319 (14,900), 498 (14,900); ^1H NMR (DMSO-d_6) δ 1.00 (3H, d, J=7 Hz, 5'-CH_3), 1.34 (3H, d, J=7 Hz, 17-CH_3), 2.31 (3H, s, 3-CH_3), 3.95 (3H, s, OCH_3), 4.39 (1H, q, J=7 Hz, 17-H), 4.42 (1H, d, J=7 Hz, 1"-H), 4.53 (2H, s, 5-H and 6-H), 4.66 (1H, d, J=8 Hz, 1'-H), 6.91 (1H, d, J=2 Hz, 10-H), 7.12 (1H, s, 4-H), 7.27 (1H, d, J=2 Hz, 12-H), 7.98 (1H, s, 7-H), 8.61 (1H, d, J=7 Hz, 16-NH).

Compounds **8** was synthesized from PRM A by a similar procedure to above. Yield 50%; MP 215~235°C (dec); IR ν_{max} (KBr) cm^{-1} 1600, 1300, 1060; Mass (FAB) *m/z* 884 $(M+H)^+$; UV ν_{max} (1/100 N NaOH) nm (ε) 317 (13,900), 498 (13,300); ^1H NMR (DMSO-d_6) δ 1.02 (3H, d, J=7 Hz, 5'-CH_3), 1.33 (3H, d, J=7 Hz, 17-CH_3), 2.29 (3H, s, 3-CH_3), 3.02 (3H, s, N-CH_3), 3.95 (3H, s, OCH_3), 4.39 (1H, q, J=7 Hz, 17-H), 4.45 (1H, d, J=11 Hz, 5-H), 4.51 (2H, m, 6-H and 1"-H), 4.67 (1H, d, J=8 Hz, 1'-H), 6.87 (1H, d, J=2 Hz, 10-H), 7.00 (1H, s, 4-H), 7.24 (1H, d, J=2 Hz, 12-H), 7.95 (1H, s, 7-H), 8.65 (1H, d, J=7 Hz, 16-NH).

Compound **9** and **10** were synthesized by coupling of PRM A with methyl isocyanate and methyl isothiocyanate, respectively, in a similar reaction conditions to above. **9**; yield 52%; MP 210~230°C (dec); IR ν_{max} (KBr) cm^{-1} 1600, 1300, 1060; Mass (FAB) *m/z* 897 $(M+H)^-$; UV λ_{max} (1/100 N NaOH) nm (ε) 319 (14,700), 498 (14,400); ^1H NMR (DMSO-d_6) δ 1.01 (3H, d, J=7 Hz, 5'-CH_3), 1.33 (3H, d, J=7 Hz, 17-CH_3), 2.28 (3H, s, 3-CH_3), 2.55 (3H, d, J=5 Hz, $NHCH_3$), 3.02 (3H, s, N-CH_3), 3.95 (3H, s, OCH_3), 4.39 (1H, q, J=7 Hz, 17-H), 4.45 (1H, d, J=11 Hz, 5-H), 4.51 (2H, m, 6-H and 1"-H), 4.68 (1H, d, J=8 Hz, 1'-H), 6.84 (1H, d, J=2 Hz, 10-H), 6.99 (1H, s, 4-H), 7.22 (1H, d, J=2 Hz, 12-H), 7.91 (1H, s, 7-H), 8.70 (1H, d, J=7 Hz, 16-NH). **10**; yield 76%; MP 230~235°C (dec); IR ν_{max} (KBr) cm^{-1} 1600, 1300, 1060; Mass (FAB) *m/z* 914 $(M+H)^-$; UV λ_{max} (1/100 N NaOH) nm (ε) 319 (16,600), 498 (15,900); ^1H NMR (DMSO-d_6) δ 1.05 (3H, d, J=7 Hz, 5'-CH_3), 1.37 (3H, d, J=7 Hz, 17-CH_3), 2.29 (3H, s, 3-CH_3), 2.90 (3H, d, J=4 Hz, $NHCH_3$), 3.94 (3H, s, OCH_3), 4.39 (1H, q, J=7 Hz, 17-H), 4.47 (1H, d, J=11 Hz, 5-H), 4.52 (2H, m, 6-H and 1"-H), 4.70 (1H, d, J=8 Hz, 1'-H), 6.91 (1H, d, J=2 Hz, 10-H), 7.03 (1H, s, 4-H), 7.27 (1H, d, J=2 Hz, 12-H), 8.00 (1H, s, 7-H), 8.62 (1H, d, J=7 Hz, 16-NH).

Susceptibility Testing

MICs were determined on yeasts morphology agar (YMA, Difco Laboratories, Detroit, Mich., U.S.A.) buffered with 0.067 M phosphate, pH 7.0. Nine parts of molten agar were combined with one part of antibiotic dilution petri dishes. A 5-μl suspension containing 2×10^6 cells per ml was spotted on the surface of the agar plates. The plates were incubated at 28°C for 60 hours. MICs were recorded after 40 hours of incubation and defined as the lowest antibiotic concentrations showing no growth or less than five discrete colonies per spot.

Experimental Infection in Mice

Groups of 5 male ICR mice weighing 20~24 g at each dose level received 10-LD_{50} of *C. albicans* A9540 or *A. fumigatus* IAM 2034 intravenously and test compounds given intravenously once immediately after the infection. The 50% protective dose (PD_{50}) was calculated by the method of LITCHFIELD and WILCOXON[9] from the survival rate 20 days after the fungal infection.

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