SYNTHESIS AND ANTIFUNGAL ACTIVITIES OF PRADIMICIN A DERIVATIVES MODIFICATION OF THE ALANINE MOIETY

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Chemical modifications of the carboxyl group in the alanine moiety of pradimicin A were performed and *in vitro* and *in vivo* antifungal activities of the derivatives were examined in comparison with those of pradimicin A. The amide derivatives showed activities comparable to pradimicin A, indicating that the free carboxyl group can be modified without impairing the antifungal activity.

Pradimicin A (**1**), which was isolated from the culture broth of *Actinomadura hibisca* P157-2, is the predecessor of benzo[*a*]naphthacenequinone antifungal antibiotics[1~3]. It exhibited broad-spectrum antifungal activity including activity against clinically important *Candida*, *Aspergillus* and *Cryptococcus* species[4]. Although pradimicin A is relatively non-toxic when compared with amphotericin B, its poor solubility in aqueous media posed some problems in further development. As part of our program aimed at obtaining more water soluble and potent pradimicin derivatives[5], the alanine moiety of pradimicin A was modified chemically.

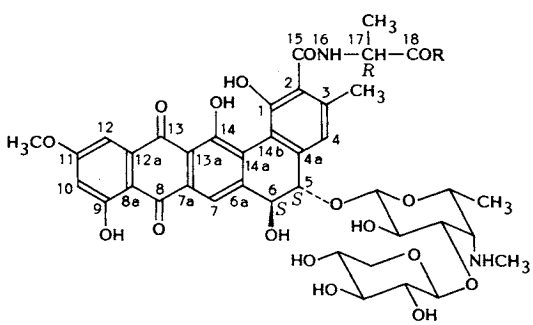
In this paper, we present synthesis and *in vitro* antifungal activities of pradimicin A derivatives, and *in vivo* efficacies of selected compounds in mouse fungal infection models.

Chemistry

Methyl ester (**2a**) and ethyl ester (**2b**) of pradimicin A (**1**) were obtained by condensing **1** with appropriate alcohols. Pivaloyloxymethyl ester (**2c**) was prepared by coupling **1** and chloromethyl pivalate.

Amide derivatives, **3a** (amide), **3b** (*N*-methyl-

Fig. 1. Structures of pradimicin A derivatives.



Compounds	R
1	OH
2a	$\overset{19}{O}CH_3$
2b	$\overset{19}{O}CH_2\overset{20}{C}H_3$
2c	$\overset{19}{O}CH_2\overset{20}{O}C\overset{21}{O}C(CH_3)_3$
3a	$\overset{19}{N}H_2$
3b	$\overset{19}{N}H\overset{20}{C}H_3$
3c	$\overset{19}{N}(CH_3)_2$
3d	$\overset{19}{N}H(CH_2)_3\overset{23}{C}H_3$
4a	$\overset{19}{N}H\overset{20}{C}H(CH_3)\overset{21}{C}OOH(R)$
4b	$\overset{19}{N}H\overset{20}{C}H(CH_3)\overset{21}{C}OOH(S)$
4c	$\overset{19}{N}H\overset{20}{C}H(COOH)\overset{21}{C}H_2\overset{22}{C}OOH(S)$
4d	$\overset{19}{N}H\overset{20}{C}H(COOH)(CH_2)_4\overset{25}{N}H_2(S)$
4e	$\overset{19}{N}H\overset{20}{C}H_2\overset{21}{C}OOH$

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

	MIC (μg/ml) by broth dilution method[a]			MIC (μg/ml) by agar dilution method[b]			
	Candida albicans A9540	*Aspergillus fumigatus* IAM 2034	*Trichophyton mentagrophytes* 4329	*Candida albicans* A9540	*Cryptococcus neoformans* IAM 4514	*Aspergillus fumigatus* IAM 2034	*Trichophyton mentagrophytes* 4329
1	6.3	6.3	12.5	50	0.4	3.1	12.5
2a	>100	>100	>100	>100	12.5	>100	>100
2b	>100	ND	ND	>100	12.5	>100	>100
2c	>100	>100	>100	>100	6.3	12.5	>100
3a	12.5	12.5	3.1	6.3	1.6	6.3	12.5
3b	12.5	12.5	12.5	6.3	1.6	6.3	6.3
3c	100	100	ND	12.5	1.6	6.3	12.5
3d	>100	>100	>100	100	3.1	50	100
4a	>100	ND	ND	25	6.3	>100	>100
4b	>100	>100	>100	>100	>100	>100	>100
4c	>100	>100	>100	>100	>100	>100	>100
4d	>100	>100	>100	>100	>100	>100	>100
4e	>50	ND	ND	>50	12.5	>50	>50

[a] Sabouraud dextrose broth. Inoculum size: 1×10^5 cells/ml. Incubation time: 20 hours for *C. albicans* A9540, 40 hours for *A. fumigatus* IAM 2034 and *T. mentagrophytes* 4329. Temperature: 37°C.
[b] Sabouraud dextrose agar. Inoculum size: 1×10^6 cells/ml. Incubation time: 40 hours.

amide), **3c** (*N*,*N*-dimethylamide) and **3d** (*N*-butylamide), were prepared by reacting methyl ester **2a** with corresponding amines. In the reaction of **2a** and ammonia, we obtained a mixture of **3a** and an unstable product; the latter was readily hydrolyzed to give **3a** under mild alkaline conditions. Although this intermediate was not fully characterized, its UV-visible spectrum was consistent with the 13-imino derivative of **3a**[6,7].

Dipeptidyl derivatives, **4a** (D-alanyl), **4b** (L-alanyl), **4c** (L-aspartyl), **4d** (L-lysyl) and **4e** (glycyl), were prepared by coupling of *N*-benzyloxycarbonyl (*N*-Cbz)-**1** active ester and appropriate amino esters followed by deblocking. All the compounds gave satisfactory spectroscopic data (UV, IR, MS and ^{1}H NMR).

Table 2. *In vivo* antifungal efficacy of pradimicin A derivatives against *Candida albicans* A9540 systemic infection in mice.

	PD_{50}[a] (mg/kg)	
	iv, single[b]	im, bid × 2[c]
1	12	21
2a		32
2b		16
3a	11	
3b	10	
3c	18	
3d	50	
4a	>50	
4b	>50	
4c	>50	
4d	>50	

[a] The PD_{50} was calculated from the survival rate 20 days after the fungal challenge. Inoculum size: 10 LD_{50}.
[b] Single administration immediately after the fungal challenge.
[c] Twice a day for two consecutive days beginning immediately after the fungal challenge.

Biological Activities

The *in vitro* antifungal activities of the pradimicin A derivatives were determined by the broth dilution method in Sabouraud dextrose medium and by the agar dilution method on Sabouraud dextrose agar. MICs are summarized in Table 1. The *in vivo* activities of the selected derivatives were evaluated in mice infected systemically with *Candida albicans* A9540. Groups of 5 mice at each dose level were infected with 10 LD_{50} of *C. albicans* A9540 and then the pradimicin derivatives were given either by im or iv administration. The results are shown in Table 2.

The amide derivatives, **3a**, **3b** and **3c**, were effective in protecting mice from lethal infection as predicted from their MIC values. The longer alkyl amide **3d** and dipeptidyl derivatives, **4a**, **4b**, **4c** and **4d**, on the other hand, were almost inactive *in vitro* and *in vivo*. Esters **2a** and **2b** were inactive *in vitro* but showed efficacy *in vivo* against *C. albicans* infection. Detection of **1** (3 μg/ml) in plasma 18 hours after im administration of **2a** at 50 mg/kg dose indicated that these esters might serve as prodrugs and release **1** *in vivo*.

These pradimicin A derivatives did not show oral activity in mice even at an oral dose of 50 mg/kg

Discussion

Pradimicin A is an amphoteric compound having basic amino and acidic carboxyl functionalities. The activity of benanomicin A and BMS-181184 suggest that the amino group in the sugar moiety is not essential for the antifungal activity[8,9]. The purpose of the present study was to examine the role of the carboxyl group in the alanine side chain of pradimicin A. The results show that the carboxyl group can be modified without impairing antifungal activity. The amide **3a** and the small alkyl amide derivatives **3b** and **3c** are as effective as the parent compound, giving PD_{50} values comparable to that of **1**. But the fact that butyl amide derivative **3d** and dipeptidyl derivatives **4a**～**4d** showed much reduced activity indicates that only small (alkyl) amide functionalities can act as bioisoteres of the carboxyl group in **1**. Unfortunately, however, those bioactive amide derivatives appear not suitable for iv formulations owing to their limited solubility in saline at physiological pH values.

Experimental

General

Mp's were determined with a Yanaco micro melting point apparatus and are uncorrected. UV and IR spectra were recorded on a JASCO UVIDEC-610C spectrometer and a JASCO IR-810 spectrometer, respectively. ^{1}H NMR spectra were recorded on a JEOL JNM-GX400 spectrometer. Mass spectra were obtained with a JEOL JMS-AX505H spectrometer or HITACHI M80B spectrometer.

Pradimicin A Methyl Ester (2a)

1 HCl salt (105 mg) was refluxed in 20.5 ml of 0.04 N methanolic HCl for 1.5 hours. The reaction mixture was cooled and concentrated to dryness to yield **2a** HCl salt (108 mg). An aqueous solution of the HCl salt (15 mg) was adjusted to pH 5.0 to deposit **2a** (12 mg). MP: 236～239°C (dec.). IR ν_{max} (KBr) cm^{-1} 3380, 1740, 1625, 1440, 1255. UV λ_{max} (0.02 N NaOH-MeOH (1:1)) nm (ε) 241 (27,400), 320 (12,700), 497 (11,200). SI-MS *m/z* 857 $(M+3H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (3H, d, J=7.3 Hz, 17-CH_3), 2.25 (3H, s, 3-CH_3), 3.67 (3H, s, 19-CH_3), 3.90 (3H, s, 11-OCH_3), 4.38～4.48 (3H, m, 17-, 5-, 6-H), 6.72 (1H, d, J=2.4 Hz, 10-H), 6.83 (1H, s, 4-H), 7.13 (1H, d, J=2.4 Hz, 12-H), 7.70 (1H, s, 7-H), 8.92 (1H, d, J=6.4 Hz, 16-NH).

Pradimicin A Ethyl Ester (2b)

To a stirred solution of **1** HCl salt (290 mg) in 50 ml of EtOH was added dropwise 3 ml of thionyl chloride at 0°C. The stirring was continued for one hour at 0°C, and two hours at room temperature. The reaction mixture was concentrated to dryness and the residue was chromatographed on a reversed phase silica gel column (YMC ODS A60, 20 mm i.d. × 320 mm). Elution was carried out with a mixture of acetonitrile-0.15% KH_2PO_4, pH 3.5 (50:50). The fractions containing pure **2b** were pooled, concentrated and desalted by Diaion HP-20 chromatography (100 ml, eluent: acetone-1 N HCl, pH 3.0) to yield **2b** HCl salt (263 mg). An aqueous solution of the HCl salt (60 mg) was adjusted to pH 6.0 to deposit **2b** (50 mg). MP: 218～221°C (dec.). IR ν_{max} (KBr) cm^{-1} 3400, 1740, 1630, 1440, 1255. UV λ_{max} (0.02 N NaOH-MeOH (1:1)) nm (ε) 242 (24,800), 320 (11,100), 498 (10,100). SI-MS *m/z* 871 $(M+3H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 (3H, t, J=7.3 Hz, 19-CH_3), 1.34 (3H, d, J=7.3 Hz, 17-CH_3), 2.29 (3H, s, 3-CH_3), 3.91 (3H, s, 11-OCH_3), 4.42 (1H, quint, J=7.3 Hz, 17-H), 4.49 (2H, d-like, 5-, 6-H), 6.73 (1H, d, J=2.4 Hz, 10-H), 6.85 (1H, s, 4-H), 7.14 (1H, d, J=2.4 Hz, 12-H), 7.67 (1H, s, 7-H), 8.89 (1H, d, J=6.8 Hz, 16-NH).

Pradimicin A Pivaloyloxymethyl Ester (**2c**)

Chloromethyl pivalate (46 μl), NaI (48 mg) and 1,8-diazabicyclo[5,4,0]-7-undecene (48 μl) were added to a stirred solution of **1** Na salt (250 mg) and K_2CO_3 (22 mg) in 20 ml of DMSO at 5°C. The stirring was continued for one hour at 5°C, and 55 hours at room temperature. The reaction mixture was diluted with water (400 ml, pH 3.5 by 6 N HCl), and then applied to a column of Diaion HP-20 (250 ml, eluent: acetone - 1 N HCl, pH 3.0) to yield semi-pure **2c** HCl salt (221 mg). This material was applied to a column of reversed phase silica gel (LiChroprep RP-18, 20 mm i.d. × 450 mm). Elution was carried out with a mixture of acetonitrile - 0.15% KH_2PO_4, pH 3.5 (45 : 55). The fractions containing pure **2c** were pooled, concentrated and desalted by Diaion HP-20 chromatography (100 ml) to yield **2c** HCl salt (77 mg). An aqueous solution of the HCl salt (10 mg) was adjusted to pH 5.5 to deposit **2c** (7 mg). MP: 205~208°C (dec.), IR ν_{max} (KBr) cm^{-1} 3380, 1755, 1625, 1440, 1255. UV λ_{max} (0.02 N NaOH - MeOH (1 : 1)) nm (ε) 241 (33,700), 319 (14,700), 497 (13,900). SI-MS *m/z* 957 $(M+3H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 1.27 (9H, s, 21-$(CH_3)_3$), 1.27 (3H, d, J=6.3 Hz, 17-CH_3), 2.28 (3H, s, 3-CH_3), 3.91 (3H, s, 11-OCH_3), 4.4~4.6 (3H, m, 17-, 5-, 6-H), 5.72, 5.80 (2H, d, J=5.9 Hz, 19-H_2), 6.72 (1H, d, J=2.4 Hz, 10-H), 6.85 (1H, s, 4-H), 7.12 (1H, d, J=2.4 Hz, 12-H), 7.68 (1H, s, 7-H), 9.04 (1H, d, J=5.9 Hz, 16-NH).

Pradimicin A Amide (**3a**)

A solution of **2a** HCl salt (95 mg) in 2 ml MeOH was added dropwise to a stirred solution of 28% ammonia (25 ml) and stirring was continued for 4 hours at room temperature. The solvent was evaporated and the solid residue was treated with 0.25 N NaOH in aqueous MeOH (30 ml) at room temperature for 4 hours. The solution was then acidified to pH 3.5 with 1 N HCl, concentrated and desalted by Diaion HP-20 column chromatography (150 ml, eluent: acetone - 1 N HCl, pH 3.0) to yield semi-pure **3a** HCl salt (105 mg). The solid (100 mg) was applied to a column of reversed phase silica gel (YMC ODS A60, 20 mm i.d. × 450 mm). Elution was carried out with a mixture of acetonitrile - 0.15% KH_2PO_4, pH 3.5 (21 : 79). The fractions containing pure **3a** were pooled, concentrated and desalted by Diaion HP-20 chromatography (100 ml) to yield **3a** HCl salt (59 mg). An aqueous solution of the HCl salt was adjusted to pH 6.0 to deposit **3a** (50 mg). MP: 205~208°C (dec.). IR ν_{max} (KBr) cm^{-1} 3400, 1620, 1440, 1250. UV λ_{max} (0.02 N NaOH - MeOH (1 : 1)) nm (ε) 245 (30,100), 320 (13,000), 496 (11,800). SI-MS *m/z* 840 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 1.29 (3H, d, J=7.3 Hz, 17-CH_3), 2.19 (3H, s, 3-CH_3), 3.91 (3H, s, 11-OCH_3), 4.30 (1H, quint, J=7.8 Hz, 17-H), 4.45 (1H, d, J=8.3 Hz, 5-H), 4.52 (1H, d, J=8.3 Hz, 6-H), 6.72 (1H, d, J=2.4 Hz, 10-H), 6.82 (1H, s, 4-H), 7.11 (1H, d, J=2.4 Hz, 12-H), 7.64 (1H, s, 7-H), 8.03 (2H, s, 19-NH_2), 8.44 (1H, d, J=8.3 Hz, 16-NH).

Pradimicin A Methylamide (**3b**)

3b (117 mg) was prepared by the procedure described for **3a** except that starting **2a** HCl salt (160 mg) was reacted with 40% aqueous methylamine (15 ml). MP: 202~205°C (dec.). IR ν_{max} (KBr) cm^{-1} 3400, 1620, 1440, 1290. UV λ_{max} (0.02 N NaOH - MeOH (1 : 1)) nm (ε) 245 (30,500), 320 (13,500), 496 (12,300). SI-MS *m/z* 854 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 1.29 (3H, d, J=7.3 Hz, 17-CH_3), 2.20 (3H, s, 3-CH_3), 2.63 (3H, d, J=4.4 Hz, 20-CH_3), 3.91 (3H, s, 11-OCH_3), 4.36 (1H, quint, J=7.8 Hz, 17-H), 4.48 (1H, d, J=8.3 Hz, 5-H), 4.55 (1H, d, J=8.3 Hz, 6-H), 6.72 (1H, d, J=2.7 Hz, 10-H), 6.80 (1H, s, 4-H), 7.13 (1H, d, J=2.7 Hz, 12-H), 7.64 (1H, s, 7-H), 8.62 (1H, q, J=4.4 Hz, 19-NH), 8.48 (1H, d, J=7.8 Hz, 16-NH).

Pradimicin A Dimethylamide (**3c**)

A mixture of *N*-Cbz-**1** (224 mg)[10], *N*-hydroxysuccinimide (32 mg) and 1,3-dicyclohexylcarbodiimide (DCC, 58 mg) in THF (5 ml) was stirred for 1 hour at room temperature and the resulting precipitate was filtered off. The filtrate was added to a 50% aqueous solution of dimethylamine (0.04 ml) and the mixture was stirred at room temperature overnight and then concentrated *in vacuo*. The residue was chromatographed on a silica gel column to yield 215 mg of *N*-Cbz-**3c**. A mixture of *N*-Cbz-**3c** (100 mg), MeOH (6 ml), water (1.5 ml) and acetic acid (1.5 ml) was stirred with 10% palladium on carbon (30 mg) under hydrogen atmosphere overnight. The solid was filtered off and the filtrate was concentrated and purified on a reversed phase silica gel column (25 mm i.d. × 150 mm), eluting with 40% aqueous acetonitrile. The desired fractions were combined, concentrated to small volume and lyophilized to yield

53 mg of **3c**. MP: >230°C (grad. dec.). IR ν_{max} (KBr) cm^{-1} 3400, 1630, 1600, 1440, 1255. UV λ_{max} (0.01 N NaOH) nm (ε) 245 (30,500), 319 (13,600), 496 (12,400). ^1H NMR (400 MHz, DMSO-d_6) δ 1.25 (3H, d, J=6.9 Hz, 17-CH_3), 2.25 (3H, s, 3-CH_3), 2.86 and 3.11 (3H each, s, 19-CH_3), 3.91 (3H, s, 11-OCH_3), 4.91 (1H, quint, J=6.9 Hz, 17-H), 6.71 (1H, d, J=2.6 Hz, 10-H), 6.84 (1H, s, 4-H), 7.12 (1H, d, J=2.6 Hz, 12-H), 7.62 (1H, s, 7-H).

Anal Calcd for $C_{42}H_{49}N_3O_{17}\cdot 3H_2O$: C 54.72, H 6.01, N 4.56.
Found: C 54.54, H 5.63, N 4.72.

Pradimicin A Butylamide (**3d**)

3d (62 mg) was prepared by the procedure described for **3a** except that starting **2a** HCl salt (135 mg) was reacted with 1-butylamine (10 ml). MP: 200~205°C (dec.). IR ν_{max} (KBr) cm^{-1} 3400, 1625, 1600, 1440, 1255. UV λ_{max} (0.02 N NaOH-MeOH (1:1)) nm (ε) 245 (31,600), 320 (13,300), 496 (12,400). SI-MS m/z 898 $(M+3H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 0.85 (3H, t, J=7.3 Hz, 23-CH_3), 1.28 (3H, d, J=7.3 Hz, 17-CH_3), 1.31 (2H, m, 21-H_2), 1.45 (2H, quint, J=7.3 Hz, 22-H_2), 2.19 (3H, s, 3-CH_3), 3.4 (2H, m, 20-CH_2), 3.91 (3H, s, 11-OCH_3), 4.35 (1H, quint, J=7.6 Hz, 17-H), 4.47 (1H, d, J=8.3 Hz, 5-H), 4.55 (1H, d, J=8.3 Hz, 6-H), 6.71 (1H, d, J=2.4 Hz, 10-H), 6.79 (1H, s, 4-H), 7.12 (1H, d, J=2.4 Hz, 12-H), 7.62 (1H, s, 7-H), 8.54 (1H, t, J=4.4 Hz, 19-NH), 8.43 (1H, d, J=7.8 Hz, 16-NH).

D-Alanyl Pradimicin A (**4a**)

A suspension of D-alanine benzyl ester tosylate (116 mg) and triethylamine (46 μl) in THF (2 ml) was mixed with a solution of *N*-Cbz-**1** (300 mg), *N*-hydroxybenzotriazole (49 mg) and DCC (68 mg) in THF (20 ml). After the mixture was stirred for 2 hours at 0°C and then 13 hours at room temperature, it was diluted with water (100 ml) and extracted with EtOAc (100 ml). The organic solvent was evaporated to give a solid residue which was dissolved in a mixture of MeOH (30 ml), EtOH (10 ml) and water (20 ml), and hydrogenated in the presence of 5% palladium on carbon for 15 hours. The catalyst was filtered off and the filtrate was concentrated and applied to a reversed phase silica gel column (LiChroprep PR-18, E. Merck, 22 mm i.d. × 450 mm). Elution was carried out with acetonitrile - 0.15% KH_2PO_4, pH 3.5 (21:79) and fractions containing the homogeneous product were pooled, concentrated and desalted by Diaion HP-20 column chromatography (acetone - 1 N HCl, pH 3.0) to yield **4a** HCl salt (27 mg). The HCl salt was dissolved in water and the solution was adjusted to pH 5.5 to provide **4a** (20 mg). MP: 213~221°C (dec.). IR ν_{max} (KBr) cm^{-1} 3400, 1620, 1445, 1265. UV λ_{max} (0.02 N NaOH-MeOH (1:1)) nm (ε) 242 (26,200), 318 (11,700), 502 (11,700). SI-MS m/z 912 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 1.20 (3H, d, J=6.4 Hz, 21-CH_3), 1.27 (3H, d, J=7.3 Hz, 17-CH_3), 2.19 (3H, s, 3-CH_3), 3.90 (3H, s, 11-OCH_3), 4.3~4.4 (2H, m, 17-, 20-H), 4.43 (1H, d, J=9.8 Hz, 5-H), 4.48 (1H, d, J=9.8 Hz, 6-H), 6.73 (1H, d, J=2.4 Hz, 10-H), 6.94 (1H, s, 4-H), 7.20 (1H, d, J=2.4 Hz, 12-H), 7.70 (1H, s, 7-H), 7.90 (1H, d-like, 19-NH), 8.56 (1H, d, J=9.0 Hz, 16-NH).

L-Alanyl Pradimicin A (**4b**)

The reaction of a L-alanine ethyl ester HCl salt (54 mg) with *N*-Cbz-**1** (300 mg) as described above yielded **4b** ethyl ester HCl salt (111 mg). A part of the solid (105 mg) was stirred in 0.3 N NaOH aqueous MeOH solution (70%, 23 ml) for 30 minutes at 5°C and then 1 hour at room temperature. After being adjusted to pH 3.5, the reaction solution was concentrated and desalted by Diaion HP-20 chromatography (acetone - 1 N HCl, pH 3.0). The HCl salt thus obtained was dissolved in water and adjusted to pH 5.5 to give **4b** (46 mg). MP: 212~216°C (dec.). IR ν_{max} (KBr) cm^{-1} 3400~3300, 1620, 1445, 1295, 1255. UV λ_{max} (0.02 N NaOH-MeOH (1:1)) nm (ε) 240 (28,400), 320 (11,600), 499 (10,700). SI-MS m/z 912 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 1.23 (3H, d, J=7.3 Hz, 21-CH_3), 1.30 (3H, d, J=7.3 Hz, 17-CH_3), 2.18 (3H, s, 3-CH_3), 3.88 (3H, s, 11-OCH_3), 4.3~4.4 (4H, m, 5-, 6-, 17-, 20-H), 6.70 (1H, d, J=2.4 Hz, 10-H), 6.97 (1H, s, 4-H), 7.16 (1H, d, J=2.4 Hz, 12-H), 7.72 (1H, s, 7-H), 8.32 (1H, d-like, 19-NH), 8.43 (1H, d-like, 16-NH).

L-Aspartyl Pradimicin A (**4c**)

The reaction of L-aspartic acid α,β-dimethyl ester HCl salt (69 mg) with *N*-Cbz-**1** (300 mg) as described for **4a** yielded **4c** (109 mg). MP: 190~197°C (dec.). IR ν_{max} (KBr) cm^{-1} 3400~3300, 1620, 1600, 1440,

1255. UV λ_{max} (0.02 N NaOH - MeOH (1 : 1)) nm (ε) 240 (35,800), 320 (16,100), 500 (15,400). SI-MS *m/z* 956 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 1.30 (3H, d, J=7.3 Hz, 17-CH_3), 2.20 (3H, s, 3-CH_3), 2.35, 2.62 (2H, t-like, 21-H_2), 3.92 (3H, s, 11-OCH_3), 4.19 (1H, m, 20-H), 4.3~4.4 (3H, m, 17-, 5-, 6-H), 6.74 (1H, d, J=2.4 Hz, 10-H), 6.99 (1H, s, 4-H), 7.17 (1H, d, J=2.4 Hz, 12-H), 7.76 (1H, s, 7-H), 8.17 (1H, d, J=7.3 Hz, 19-NH), 8.49 (1H, d, J=6.4 Hz, 16-NH).

L-Lysyl Pradimicin A (**4d**)

4d (120 mg) was prepared from *N*-Cbz-lysine methyl ester HCl salt (116 mg) and *N*-Cbz-**1** (300 mg) as described for **4a**. MP: 170~175°C (dec.). IR ν_{max} (KBr) cm^{-1} 3400, 1620, 1600, 1440, 1255. UV λ_{max} (0.02 N NaOH - MeOH (1 : 1)) nm (ε) 244 (32,700), 319 (13,900), 500 (13,900). SI-MS *m/z* 969 $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 1.29 (3H, d, J=7.3 Hz, 17-CH_3), 1.44, 1.56, 1.69 (8H, m, 21-~24-H_2), 2.20 (3H, s, 3-CH_3), 3.90 (3H, s, 11-OCH_3), 4.33 (1H, m, 20-H), 4.3~4.6 (3H, m, 17-, 5-, 6-H), 6.74 (1H, d, J=2.2 Hz, 10-H), 6.80 (1H, s, 4-H), 7.16 (1H, d, J=2.2 Hz, 12-H), 7.57 (1H, s, 7-H), 8.03 (1H, d, J=7.3 Hz, 19-NH), 8.69 (1H, d, J=6.9 Hz, 16-NH).

Glycyl Pradimicin A (**4e**)

The reaction of glycine ethyl ester HCl salt (21 mg) with *N*-Cbz-**1** (107 mg) as described for **4a** yielded **4e** (9.5 mg). MP: >195°C (grad. dec.). IR ν_{max} (KBr) cm^{-1} 3400~3300, 1730, 1610, 1560. ^1H NMR (400 MHz, DMSO-d_6) δ 1.31 (3H, d, J=7.3 Hz, 17-CH_3), 2.29 (3H, s, 3-CH_3), 3.83 (2H, dd, J=5.6 and 17 Hz, 20-H_2), 3.97 (3H, s, 11-OCH_3), 6.97 (1H, d, J=2.6 Hz, 10-H), 7.16 (1H, s, 4-H), 7.32 (1H, d, J=2.6 Hz, 12-H), 8.05 (1H, s, 7-H), 8.20 (1H, t, J=5.6 Hz, 19-NH).

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References

1) OKI, T.; M. KONISHI, K. TOMATSU, K. TOMITA, K. SAITOH, M. TSUNAKAWA, M. NISHIO, T. MIYAKI & H. KAWAGUCHI: Pradimicin, a novel class of potent antifungal antibiotics. J. Antibiotics 41: 1701~1704, 1988
2) TSUNAKAWA, M.; M. NISHIO, H. OHKUMA, T. TSUNO, M. KONISHI, T. NAITO, T. OKI & H. KAWAGUCHI: The structures of pradimicins A, B and C: A novel family of antifungal antibiotics. J. Org. Chem. 54: 2532~2536, 1989
3) TOMITA, K.; M. NISHIO, K. SAITOH, H. YAMAMOTO, Y. HOSHINO, H. OHKUMA, M. KONISHI, T. MIYAKI & T. OKI: Pradimicins A, B and C: New antifungal antibiotics. I. Taxonomy, production, isolation and physico-chemical properties. J. Antibiotics 43: 755~762, 1990
4) OKI, T.; O. TENMYO, M. HIRANO, K. TOMATSU & H. KAMEI: Pradimicins A, B and C: New antifungal antibiotics. II. *In vitro* and *in vivo* biological activities. J. Antibiotics 43: 763~770, 1990
5) KAMACHI, H.; S. IIMURA, S. OKUYAMA, H. HOSHI, S. TAMURA, M. SHINODA, K. SAITOH, M. KONISHI & T. OKI: Synthesis and antifungal activities of pradimicin derivatives, modification at C4'-position. J. Antibiotics 45: 1518~1525, 1992
6) ACTON, E. M. & G. L. TONG: Synthesis and preliminary antitumor evaluation of 5-iminodoxorubicin. J. Med. Chem. 24: 669~673, 1981
7) OKI, T. & N. YAMAMOTO (Bristol-Myers Squibb Co.): Antiviral method for human immunodeficiency virus with BU-3608. Jpn. Kokai 48530 ('90), Feb. 19, 1990
8) TAKEUCHI, T.; T. HARA, H. NAGANAWA, M. OKADA, M. HAMADA, H. UMEZAWA, S. GOMI, M. SEZAKI & S. KONDO: New antifungal antibiotics, benanomicins A and B from an *Actinomycete*. J. Antibiotics 41: 807~811, 1988
9) OKI, T.; M. KAKUSHIMA, M. HIRANO, A. TAKAHASHI, A. OHTA, S. MASUYOSHI, M. HATORI & H. KAMEI: *In vitro* and *in vivo* antifungal activities of BMS-181184. J. Antibiotics 45: 1512~1517, 1992
10) KAKUSHIMA, M.; M. NISHIO, K. NUMATA, M. KONISHI & T. OKI: Effect of stereochemistry at the C-17 position on the antifungal activity of pradimicin A. J. Antibiotics 43: 1028~1030, 1990