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NOTE

Jogyamycin, a new antiprotozoal aminocyclopentitol antibiotic, produced by *Streptomyces* sp. a-WM-JG-16.2

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During our search for new antiprotozoal (antimalarial and antitrypanosomal) agents, we have tested culture extracts of soil microorganisms from a variety of sources, including those isolated by the Kitasato Institute for Life Sciences and the Bioresource Laboratories, MicroBiopharm Japan Corporation. We have previously discovered a variety of microbial metabolites exhibiting antiprotozoal,[1,2] antimalarial[3,4] and antitrypanosomal properties.[5,6] Recently, we have isolated a new derivative of pactamycin, designated as jogyamycin (Figure 1), from a culture broth of *Streptomyces* sp. a-WM-JG-16.2. This compound has proved to have potent antiprotozoal activity (Table 2). Here, we report the fermentation, isolation, structure elucidation and biological activity of this novel aminocyclopentitol antibiotic.

The producing organism, strain a-WM-JG-16.2 was isolated from a soil sample collected in Jogjakarta, Indonesia, using the sucrose density gradient centrifugation method.[7] Using the basic local alignment search tool (BLAST), we compared the 16S rRNA gene sequences available in the EzTaxon server to identify the species of strain a-WM-JG-16.2. *Streptomyces* sp. a-WM-JG-16.2 was classified as a species of the genus *Streptomyces*; the 16S rRNA gene sequence comparison showing a 100% similarity with *S. griseoruber* NBRC12873 (AB184209).

The strain was cultured on a rotary shaker (220 r.p.m.) at 28 °C for 5 days in 500-ml Erlenmeyer flasks (20 flasks) containing 50 ml of a production medium containing 2% rice starch (Rose brand, Cikampek, Indonesia), 2% glucose (Univar, Sydney, NSW, Australia), 2% soybean meal (J-Oil Mills, Tokyo, Japan), 0.5% yeast extract (Himedia, Mumbai, India), 0.25% NaCl (Univar), 0.32% $CaCO_3$ (Univar), 5 p.p.m. $CuSO_4{\cdot}5H_2O$ (Merck, Darmstadt, Germany), 5 p.p.m. $MnCl_2{\cdot}4H_2O$ (Merck) and 5 p.p.m. $ZnSO_4{\cdot}7H_2O$ (Merck), pH 7.4 (before sterilization).

The culture broth (50 ml×20 flasks) was extracted with *n*-butanol (1.5 l). The organic layer was evaporated to dryness *in vacuo*. This *n*-butanol extract (0.87 g) was applied to an ODS column (Pegasil Prep ODS-7515-12A, 2012A, 20 (Senshu Scientific Co., Tokyo, Japan)) pre-equilibrated with 12.5% methanol aq. The column was eluted stepwise with 20, 40 and 60% aqueous acetonitrile (180 ml each) and the active principals were found in the 60% acetonitrile eluate, which was concentrated *in vacuo* to yield a brown material (58.2 mg). The material was washed with 50% aqueous acetonitrile (5.8 ml), and the 50% acetonitrile-soluble portion was purified by HPLC using a L-column2 ODS (20ϕ×250 mm, CERI, Tokyo, Japan) with 20% acetonitrile/0.1% trifluoroacetic acid (TFA) at a flow rate of 7 ml min^{-1} with UV detection at UV 210 nm. The retention time of the active fraction was 9 min. The active fraction was concentrated *in vacuo* to dryness to afford 2.5 mg of jogyamycin (**1**). In the process of purification of **1**, we also identified other known compounds, such as pactamycin (**3**),[8] 7-deoxypactamycin (**2**)[9] and pactamycate.[10]

Compound **1** was obtained as a light yellow powder ($[\alpha]_D^{23}$ −20.4 (*c* 0.1 in MeOH); UV (MeOH) λ_{max} (ε), 239 (5790), 263 (2200) and 350 nm (612)). The IR spectrum showed characteristic absorptions at 3410 and 1679 cm^{-1}, which suggested the presence of hydroxyl and carbonyl groups. The molecular formula of **1** was established by high

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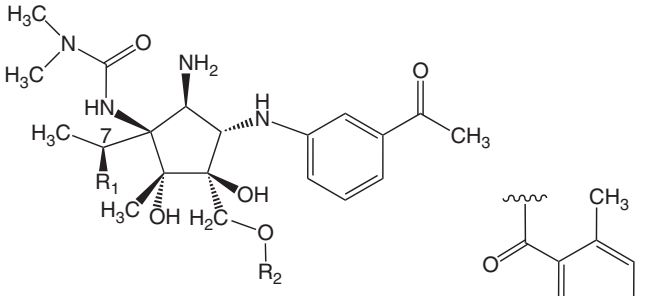
resolution-FAB-MS to be $C_{20}H_{32}O_5N_4$ (*m/z* 409.2437 $[M+H]^+$ calcd. 409.2451), requiring seven double bond equivalents. The ^1H and ^{13}C NMR spectral data of **1**, listed in Table 1, were similar to those of 7-deoxypactamycin.[9] The ^1H NMR, ^{13}C NMR, HSQC and HMBC spectra indicated 20 carbons that were classified into one ketone carbonyl carbon (δ_C 201.5), one urea carbonyl carbon (δ_C 160.9), four sp^2 methine aromatic carbons, two sp^2 quartenary aromatic carbons, two sp^3 methine carbons, three sp^3 quaternary carbons, two sp^3 methylene carbons and five methyl carbons, thus accounting for six double bond equivalents. Therefore, the remaining double bond equivalent was likely to be because of a ring structure. As shown by the bold lines for **1** in Figure 2, two partial structures from H-2 (δ_H 3.65) to H-3 (δ_H 4.11) and from H_2-7 (δ_H 2.13, 2.45) to H_3-8 (δ_H 0.98) were revealed by COSY. The coupling pattern of the aromatic protons and the HMBC correlations from H-2′ (δ_H 7.37) to C-6′ (δ_C 118.8) and C-4′ (δ_C 119.3), from H-4′ (δ_H 7.02) to C-2′ (δ_C 113.1), from H-5′ (δ_H 7.26) to C-1′ (δ_C 139.2) and C-3′ (δ_C 149.9), from H-6′ (δ_H 7.29) to C-2′, and from H_3-8′ (δ_H 2.56) and H-2′ to C-7′ (δ_C 201.5) revealed the presence of a *m*-aminoacetophenone unit. On the basis of ^1H-^{13}C HMBC experiments, the correlations from H-3 to C-4 (δ_C 83.4) and C-5 (δ_C 83.3); from H_2-9 (δ_H 3.48, 4.06) to C-4 and C-5; from H_3-6 (δ_H 1.55) to C-1 (δ_C 69.0), C-4 and C-5; from H_2-7 (δ_H 2.13, 2.45) to C-1 and C-5; from H_3-8 (δ_H 0.98) to C-1; and from H_3-11 and H_3-12 (δ_H 3.00) to C-10 (δ_C 160.9), C-11 and C-12 (δ_C 36.8), indicated the presence of the aminocyclopentitol core attached to an ^1N,^1N-dimethylurea moiety. Finally, by HMBC correlation from H-3 to C-3′, the structure of **1** was elucidated as an analog of 7-deoxypactamycin lacking the 6-methyl salicylic acid (6-MSA) moiety, and it was subsequently designated as jogyamycin (**1**).



	R_1	R_2
Jogyamycin (**1**)	H	H
7-Deoxypactamycin (**2**)	H	6-MSA
Pactamycin (**3**)	OH	6-MSA

Figure 1 Structures of jogyamycin (**1**) and related compounds. 6-MSA, 6-methyl salicylic acid.

In vitro antiprotozoal activities were investigated using the *Plasmodium falciparum* K1 strain (drug resistant), as well as a *Trypanosoma brucei brucei* strain GUTat 3 model.[3,5] As shown in Table 2, jogyamycin (**1**) showed potent antimalarial and antitrypanosomal activities, with IC_{50} values of 1.5 and 12.3 nM, respectively. The effect was 13.5-fold stronger than the antimalarial artemisinin, but 2.6-fold less active than the commonly used antitrypanosomal pentamidine. Compared with our previously reported antiprotozoal activities of pactamycin (**3**) and 7-deoxypactamycin (**2**),[2] the antimalarial activity of **1** was 9.4-fold more potent than that of **3**, whereas its antitrypanosomal activity was similar. However, **1** was 3.8- and 14.7-fold less potent than **2** with regard to antimalarial and antitrypanosomal activity, respectively.

Cytotoxicity against the human diploid embryonic cell line MRC-5 was measured as described previously.[3] Among pactamycin and its two analogs, **1** showed the highest cytotoxicity displaying an IC_{50} value of 5.6 nM. Its cytotoxicity was 5–17-fold more potent than those of **2** and **3**. Jogyamycin showed the lowest selectivity indexes (Selectivity index: cytotoxicity (IC_{50} for the MRC-5 cells)/antitrypanosomal or antimalarial activity (IC_{50} for the GUTat 3.1 strain or the K1 strain)), with ratios of around 0.5–4. These results provide a very interesting insight with regard to structure–activity relationships. The

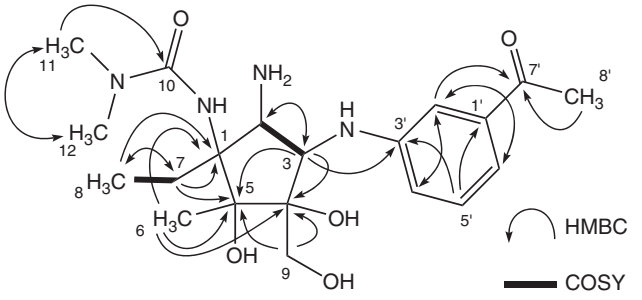


Figure 2 COSY and HMBC correlations of jogyamycin (**1**).

Table 1 ^1H and ^{13}C NMR spectral data of jogyamycin (1) in CD_3OD

	Jogyamycin (**1**)				
Position	δ_C	δ_H *(int., mult., J in Hz)*	*Position*	δ_C	δ_H *(int., mult., J in Hz)*
1	69.0	—	10	160.9	—
2	64.8	3.65 (1H, d, 9.5)	11	36.8	3.00 (3H, s)
3	66.1	4.11 (1H, d, 9.5)	12	36.8	3.00 (3H, s)
4	83.4	—	1′	139.2	—
5	83.3	—	2′	113.1	7.37 (1H, br.s)
6	19.2	1.55 (3H, s)	3′	149.9	—
7	21.5	2.13 (1H, dq, 14.9, 7.5)	4′	119.3	7.02 (1H, d, 8.1)
		2.45 (1H, dq, 14.9, 7.5)	5′	130.3	7.26 (1H, dd, 8.1, 7.5)
8	9.4	0.98 (3H, t, 7.5)	6′	118.8	7.29 (1H, d, 7.5)
9	63.3	3.48 (1H, d, 11.5)	7′	201.5	—
		4.06 (1H, d, 11.5)	8′	26.8	2.56 (3H, s)

Measured in CD_3OD (^1H: 500 MHz, ^{13}C: 125 MHz).

Table 2 ***In vitro*** **antimalarial and antitrypanosomal activity, and cytotoxicity in human MRC-5 cells of jogyamycin (1) and related compounds**

	IC_{50} (nM)				
	Antiprotozoal activity		Cytotoxicity	Selectivity index (SI)	
Compound	*P. f. K1*[a]	*T. b. b. GUTat 3.1*[b]	(MRC-5)	MCR-5/K1	MCR-5/GUTat
Jogyamycin (**1**)	1.5	12.3	5.6	4	0.5
7-Deoxypactamycin (**2**)[c]	0.4	0.9	29.5	74	33
Pactamycin (**3**)[c]	14.2	7.4	95	7	13
Artemisinin[d]	20.2	3333	160 177	7930	48
Chloroquine[d]	575	ND	57 900	101	—
Pentamidine[e]	ND	4.7	16 794	—	3566
Suramin[e]	ND	1106	>69 979	—	>63

Abbreviation: ND, not determined.
[a]*Plasmodium falciparum* K1 (drug resistant).
[b]*Trypanosoma brucei brucei* GUTat 3.1.
[c]The IC_{50} values were reported in ref. 2.
[d]Antimalarial drugs used clinically.
[e]Antitrypanosomal drugs used clinically.

lack of the 6-MSA moiety of 7-deoxypactamycin causes jogyamycin to show 3.8–14.7-fold less antiprotozoal activity and 5-fold more potent cytotoxicity than 7-deoxypactamycin.

Pactamycin and 7-deoxypactamycin, which was originally isolated as cranomycin, are aminocyclopentitol antibiotics, posessing antibacterial and antitumor activity.[8,9] It is known that pactamycin acts by inhibition of the initiation of protein synthesis.[11] Recently, Ito *et al.*[12] isolated de-6-MSA-pactamycin and de-6-MSA-pactamycate from the knockout *ptmQ* (6-MSA synthase gene) mutant of the pactamycin- and pactamycate-producing strain, *Streptomyces pactum*; ATCC 27456. This mutant was not able to produce pactamycin and pactamycate, which accumulated the intermediates of pactamycin biosynthesis. Although de-6-MSA-7-deoxypactamycin (jogyamycin, **1**) was also thought to be a biosynthetic intermediate of pactamycin, it was not isolated from this mutant but from a wild-type organism, *Streptomyces* sp. a-WM-JG-16.2. Ito *et al.*[12] reported that de-6-MSA-pactamycin showed antibacterial and antitumor activities similar to pactamycin, and they suggested that the 6-MSA moiety of pactamycin is not essential for antibacterial and antitumor activities. However, our data indicate that the 6-MSA moiety in 7-deoxypactamycin increases antiprotozoal activity, as well as decreasing cytotoxicity in human cells, suggesting that the 6-MSA moiety has an important role in both antiprotozoal activity and cytotoxicity.

The above results reveal that the analogs of pactamycin are promising lead compounds for the development of new antiprotozoal drugs. Recently, the first total synthesis of pactamycin was achieved, allowing the prospect of synthesis of less cytotoxic analogs that maintain their antibacterial and antiprotozoal properties.[13] Therefore, further investigation of the selective antiprotozoal analogs and their *in vivo* evaluation is in progress. Furthermore, the producing strain of **1**, *Streptomyces* sp. a-WM-JG-16.2, will be useful in analysis of the biosynthesis of pactamycin.

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