

ORIGINAL ARTICLE

Salinisporamycin, a novel metabolite from *Salinispora arenicora*

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A new rifamycin antibiotic, salinisporamycin (**1**), has been isolated from a culture of a marine actinomycete. The producing organism was identified as *Salinispora arenicora* on the basis of the 16S rRNA sequence. High-resolution FAB-MS established the molecular formula of **1** as C₃₃H₄₃NO₉. The planar structure of **1** was elucidated by NMR spectral analysis including COSY, heteronuclear single quantum coherence and heteronuclear multiple bond correlation. The relative stereochemistry of **1** was determined on the basis of rotating frame nuclear Overhauser effect spectroscopy. In addition, the solvatochromic behavior of **1** was investigated by measuring the UV spectra. This compound inhibited the growth of A549 cells, the human lung adenocarcinoma cell line, with an IC₅₀ value of 3 μg ml⁻¹, and also showed antimicrobial activity.

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Keywords: antimicrobial; cytotoxic; rifamycin antibiotic; salinisporamycin; *Salinispora arenicora*; YM23-082

INTRODUCTION

It is thought that the exploration of novel natural products in the marine environment is valuable in view of the diversity of marine microbial and metabolic products.¹ Novel marine natural products have been reported on a continuous basis.² Therefore, it can be expected that the LC-MS (HPLC/PDA-ESI-MS) system with simulated data of many antibiotics and others may have an important role for identification of many classes of novel marine natural products.^{3,4}

Salinispora sp. was identified as the first seawater-requiring marine actinomycete.^{1,5} This bacterium includes the potent proteasome inhibitor salinosporamide A, which is under investigation in a phase 1 clinical trial for the treatment of cancer.^{1,6,7} The analysis of the *Salinispora tropica* genome revealed the presence of a polyketide synthase system and nonribosomal peptide synthases, with a large percentage of its genome (~10%) devoted to secondary metabolite biosynthesis, which is greater than the *Streptomyces* genome sequence.^{1,7}

In this study, we investigated bioactive products from *Salinispora* sp. Results of this screening showed that YM23-082 extracts had strong antitumor and antimicrobial activities among 17 species of *Salinispora*. Therefore, bioactive products were isolated from YM23-082 extracts using LC-MS methods. The results showed that saliniketals A (**2**)^{8,9} and rifamycin S (**3**)¹⁰ were isolated from YM23-082 extracts. In salinisporamycin (**1**), it was expected that the ansa chain partial structure could be assigned as saliniketals^{8,9} and connected to the naphthoquinone ring system. **1** and **2** show structural resemblance to the construct of rifamycin antibiotics. Therefore, **1** and **2** would be biosynthetically related products of

rifamycin antibiotics. Also, **1** showed moderate cytotoxic activity against A549 and antimicrobial activities. Their structures are shown in Figure 1.

In our study, we elucidated the taxonomy, physicochemical properties, structure and biological activity of salinisporamycin (**1**) from *Salinispora arenicora* YM23-082.

RESULTS AND DISCUSSION

Identification of taxonomy

The strain YM23-082 was isolated from marine sediment collected in the Yap State in the Federated States of Micronesia, N: 9°31'11.0", E: 138°10'26.7" and grew at room temperature on a marine agar 2206 (BD Difco, Tokyo, Japan) plate. The culture YM23-082 showed colonies and an orange pigment was produced frequently.⁵ The 16S rRNA sequencing of this strain revealed high sequence identity with *S. arenicola* CNH643 (AY040619.2) (100.0%).⁵ This strain included **2**, **3**, staurosporine and K-252C⁶ (data not shown). On account of this characteristic, the strain YM23-082 was tentatively identified as a member of the *S. arenicola* CNH643.

Physicochemical properties

The physicochemical properties of **1** are summarized in Table 1. **1** was isolated as an amorphous compound ([α]_D²⁴ +36.0, *c* 0.7, MeOH). It was soluble in DMSO, MeOH, Me₂CO and CHCl₃, poorly soluble in water, and insoluble in n-Hex. **1** was identified as having an R_F value at 0.84 (CHCl₃:MeOH=4:1). Analysis of LC-MS spectral data on positive and negative ions revealed the molecular mass to be 597. The molecular formula of **1** was established as C₃₃H₄₃NO₉

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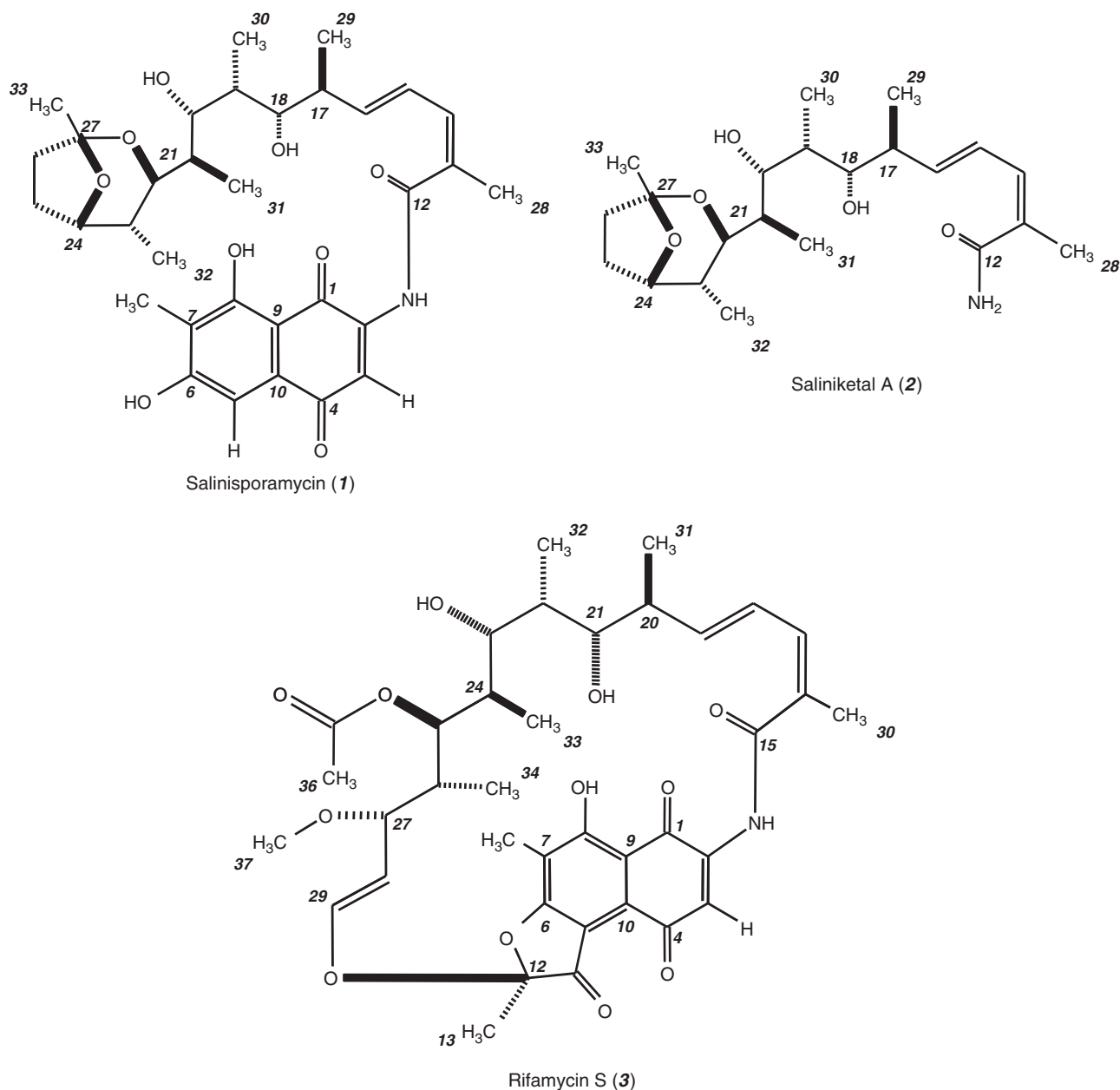


Figure 1 Structures of salinisporamycin (1), saliniketol A (2) and rifamycin S (3).

by high-resolution FAB-MS data ($[M+H]^+$: found, m/z 598.3027, calcd for $C_{33}H_{44}NO_9$, m/z 598.3011). In addition, the exchangeable protons of **1** were established by hydrogen–deuterium (H–D) exchange mass spectrometry (ESI-IT-MS, profile mode). The H–D exchange signals were found at m/z 625.47 $[M+Na]^+$ (calcd for $C_{33}H_{38}D_5NNaO_9$, m/z 625.32) with a dimer ion at m/z 1227.13 $[2M+Na]^+$, indicating the presence of the intermolecular exchange of 5 hydrogen atoms (data not shown). The UV spectrum of **1** was nearly identical with that of **3** in solvent MeOH. Also, the UV spectrum of **1** showed a short wavelength at 595 nm in the solvent DMSO. The absorption at 595 nm was possibly due to the presence of conjugated double bonds between the naphthoquinone chromophore and the α -, β -, γ - and δ -unsaturated system. However, the imide-amide tautomeric form of **1** could not be distinguished from the NMR spectral data.

Structural elucidation

1H and ^{13}C NMR spectral data for **1** are summarized in Table 2. All one-bond 1H - ^{13}C connections were confirmed by heteronuclear single quantum coherence (HSQC) correlations. Two methylene signals at δ_c 24.1 (C-25) and 35.4 (C-26) were observed in the DEPT spectrum and HSQC correlations. Also, other carbon signal types were assigned by the DEPT spectrum and HSQC correlations.

The ansa chain partial structure of **1** was confirmed by COSY and heteronuclear multiple bond correlations (HMBCs) in solvent CD_3OD . These results showed a structural resemblance to the ansa chain of rifamycin antibiotics.¹⁰ These assignments are summarized in Figure 2.

The α -, β -, γ - and δ -unsaturated system signals at δ_H 6.46 (1H, br d, 11.3, H-14), 6.79 (1H, dd, 15.0, 10.9, H-15), 6.03 (1H, dd, 15.0, 8.3, H-16), 2.07 (3H, d, 1.1, CH_3 -28) and a quaternary sp^2 carbon

Table 1 Physicochemical properties of **1**

Appearance	Amorphous
Molecular formula	C ₃₃ H ₄₃ NO ₉
LC-ESI-MS (<i>m/z</i>)	598 [M+H] ⁺ 596 [M-H] ⁻
<i>High-resolution FAB-MS (m/z)</i>	
Found	598.3027 [M+H] ⁺
Calcd	598.3011 [M+H] ⁺
[α] _D	+36.0 (<i>c</i> 0.7, MeOH)
TLC (<i>R_f</i> value)	CHCl ₃ :MeOH (4:1)
(SiO ₂ , Merck F ₂₅₄)	<i>R_f</i> 0.84
UV λ_{\max} nm (log ϵ) in MeOH	225 (4.66), 272 (4.52), 319 (4.26), 408 (3.83)
UV λ_{\max} nm (log ϵ) in DMSO	281 (4.58), 307 (4.49), 320 (4.49), 358 (4.27)
IR ν_{\max} (KBr) cm ⁻¹	3399, 2926, 1600, 1496, 1458, 1387, 1327, 1098, 974

signal at δ_C 129.7 (C-13) were mainly confirmed by $^3J_{HH}$ coupling constants of olefinic protons and HMBC correlations. The $^4J_{HH}$ coupling constant of proton signals at CH₃-28 and H-14 was determined by COSY correlation. In addition, the configuration of C-15/C-16 was assigned as *E* on the basis of the $^3J_{HH}$ coupling constant of H-15/H-16 at 15.0 Hz. This result was supported by the fact that H-14 and CH₃-28 were determined by rotating frame nuclear Overhauser effect spectroscopy (ROESY) correlations (data not shown). Also, the amide bond signals at δ_H 9.14 (1H, br s, 2-NH) and δ_C 167.1 (C-12) were determined by HMBC correlations in solvent DMSO-*d*₆ (Table 2). This result was supported by assignment of the amide bond as a primary amide on the basis of the molecular formula.

In contrast, for determination of the terminal ansa chain partial structure of **1**, the tetrahydrofuran ring signals at δ_H 4.20 (1H, br dd, 6.8, 3.8, H-24) and the ketal functional group at δ_C 106.6 (C-27) were confirmed by HMBC correlations. Those results were supported by the finding that the IR spectrum showed characteristic absorption bands at 1098 cm⁻¹, indicating the presence of ether bonds in the molecule.

The two exchangeable proton signals at δ_H 4.57 (1H, m, 18-OH) and 4.64 (1H, m, 20-OH) were mainly determined by COSY correlations in solvent DMSO-*d*₆. In support of these results, exchangeable proton signals were observed in the pre-saturation spectrum in solvent DMSO-*d*₆, decreasing the integral intensity of the peak area. Also, the IR spectrum showed characteristic absorption bands at 3399 cm⁻¹ (3431 cm⁻¹ of **3**), indicating the presence of a hydroxyl group in the molecule. Consequently, these results showed a structural resemblance to the saliniketals.^{8,9}

In the detailed analysis, the chemical shifts in the ansa chain of **1** were compared with values in the literature reported for saliniketal A⁸ (Table 2). The chemical shifts revealed that **1** was nearly identical with the spectra of saliniketal A. In addition, the chiral centers of C-17/C-18, C-18/C-19, C-19/C-20, C-20/C-21, C-21/C-22 and C-22/C-23 were mainly determined by the $^3J_{HH}$ coupling constants, COSY and ROESY correlations (Figure 3, Table 2). These results revealed that $^3J_{HH}$ coupling constants of **1** were almost identical with the literature values reported for saliniketal A⁸ and the recorded NMR spectra of **2**. The COSY correlation in the ansa chain partial structure signals at δ_H 3.62 (1H, m, 7.5, H-18) and 1.71 (1H, m, H-19) were not observed in solvent DMSO-*d*₆, because the torsion angle between H-18/H-19 is close to 90°¹¹ (Figure 3).

The bicyclic ring structure signals at δ_H 1.93 (1H, m, H-25a), 2.02 (1H, m, H-26a) and 3.94 (1H, br d, 10.5, 1.5, H-22) were determined by ROESY correlations (Figure 4). This result was supported by stereochemical assignments in a bicyclic ring structure that a diaxial arrangement signals at δ_H 3.82 (1H, br d, 10.5, 0.9, H-22) and 1.84 (1H, br dq, 10.5, 6.8, 3.8, H-23), and was confirmed by a large coupling constant at 10.5 Hz (Figures 3 and 4). Consequently, the ansa chain of **1** was established to be the same as saliniketal.^{8,9}

The naphthoquinone ring system of **1** was mainly confirmed by the ¹³C NMR spectral data and HMBC correlations (Figure 2, Table 3). With the help of a detailed analysis, the quaternary carbon signal at δ_C 164.5 (C-6) and other carbon signals were nearly identical with values reported in the literature for 31-homorifamycin W.¹² The two quaternary carbon signals at δ_C 181.3 (br, C-1) and 172.0 (br, C-8) gave broad peak signals possibly due to tautomerization (Table 3). These results were supported by the finding that the IR and UV spectra showed characteristic absorption bands at 1496 cm⁻¹ (1465 cm⁻¹ of **3**) and wavelengths at 319 and 408 nm (315 and 410 nm of **3**), indicating the presence of a chromophoric system in the naphthoquinone form.^{10,12} In addition, the IR spectrum showed a characteristic absorption band at 1600 cm⁻¹ (1606 cm⁻¹ of **3**), indicating the presence of a naphthoquinone carbonyl group linked in the intramolecular hydrogen bond.¹²

The naphthoquinone ring system of **1** was established by the ¹³C NMR spectral data and HMBC correlations in solvent DMSO-*d*₆ (Table 3, Figure 5). With the help of a detailed analysis, the naphthoquinone ring system of **1** had chemical shifts comparable to the literature values reported for rifamycin Z.¹³ This result revealed that the ¹³C NMR signals at δ_C 170.3 (C-1, diff. (-11.5 p.p.m.)) and δ_C 180.4 (br, C-6, diff. (+20.4 p.p.m.)) could not be distinguished from the spectra of rifamycin Z. Therefore, the quaternary carbon signal at C-6 was assigned as a carbonyl carbon on the basis of its chemical shift. Intriguingly, the two quaternary carbon signals at δ_C 180.4 (br, C-6) and 162.1 (br, C-8) gave broad peak signals possibly due to tautomerization. The enolic proton signal at δ_H 12.55 (1H, br s, 8-OH) was confirmed by HMBC correlations in solvent DMSO-*d*₆. These results were supported from the assignment of ¹H-¹³C connections made by HSQC correlations in solvent DMSO-*d*₆. Consequently, these results showed the isomerization of the naphthoquinone ring system of **1** by different solvents. However, the enolic proton signal (1-OH) was not determined by the ¹H NMR spectral data (Table 3). Finally, the amide bond between the ansa chain and the naphthoquinone ring system signals at δ_H 9.14 (1H, br s, 2-NH) and δ_C 111.7 (C-3) were determined by HMBC correlations in solvent DMSO-*d*₆. These results were supported by the finding by H-D exchange mass spectrometry that showed the characteristic intermolecular exchange of five hydrogen atoms.

Biological activity

Compound **1** showed moderate cytotoxic activity against A549 cells with an IC₅₀ value of 3 μ g ml⁻¹. Compound **3** did not show cytotoxic activity against A549 cells at 200 μ g ml⁻¹.^{10,12,14} Among all rifamycin antibiotics, **1** showed cytotoxic activity.¹⁴

The antimicrobial activity of **1** was tested against six microorganisms by paper disk methods. This compound showed moderate activity against two microorganisms (Table 4). The antimicrobial activity of **1** was shown to be weaker than that of **3**. The sensitivity of bacteria to **1** seems to be inhibited by the presence of hydroxyl groups (1, 8, 18, 20-OH) (Figures 1 and 5). An earlier study found that the high sensitivity of bacteria to **3** was promoted by four hydrogen bond interactions between four free hydroxyl groups (1, 8,

Table 2 750 MHz ¹H and 125 MHz ¹³C NMR data on 1, 2 and 3

		DMSO-d ₆				CD ₃ OD						
		1		2		3		1		Saliniketal a ^b		
Position	δ_C	δ_H	Multi, J (Hz)	HMBC	δ_H	Multi, J (Hz)	Position	δ_H	Multi, J (Hz)	δ_C	δ_H	Multi, J (Hz)
1	170.3						1	181.3 (br)				
2	143.1						2	143.2				
2-NH		9.14 (br s)		C-1, C-3, C-12	7.21 (br s)		15-NH	9.41 (br s)				
3	111.7	7.18 (s)		C-1, C-2, C-10	7.03 (br s)		3	116.4	7.55 (s)			
4	186.7						4	187.9				
5	117.7	6.32 (s)		C-4, C-7, C-9			5	112.7	6.96 (s)			
6	180.4 (br) ^b						6	164.5				
7	112.2						7	117.8				
8	162.1 (br)						8	172.0				
8-OH		12.55 (br s)		C-7, C-8, C-9			9	106.6				
9	100.6						10	132.5				
10	131.1						11	8.2	2.06 (s)			
11	7.9	1.75 (s)		C-6, C-7, C-8			12	170.1		175.1		
12	167.1						13	129.7		131.4		
13	127.1						14	138.6	6.46 (br d, 11.3)	134.1	6.17 (br d, 11.1, 1.2)	
14	137.7	6.43 (br d, 11.3)		C-12, C-16, C-28	6.02 (br d, 11.3)		15	127.6	6.79 (dd, 15.0, 10.9)	128.3	6.60 (dd, 15.3, 11.1)	
15	125.3	6.78 (dd, 15.0, 12.0)		C-17	6.57 (dd, 15.0, 11.3)		16	146.0	6.03 (dd, 15.0, 8.3)	142.0	5.78 (dd, 15.3, 8.4)	
16	145.7	6.07 (dd, 15.0, 7.5)		C-14, C-29	5.76 (dd, 15.0, 8.3)		17	42.4	2.43 (m, 8.3, 7.5)	42.3	2.35 (m, 9.3, 8.4, 6.8)	
17	40.4	2.31 (m, 8.3, 7.5, 6.8)		C-15, C-16, C-18, C-29	2.22 (m, 7.5)		18	75.8	3.78 (dd, 9.8, 1.9)	75.8	3.71 (dd, 9.3, 1.8)	
18	73.3	3.62 (m, 7.5)			3.57 (m, 9.0)		19	36.4	1.83 (m, 6.8, 4.5, 1.9)	35.7	1.88 (m, 7.4, 4.9, 1.8)	
18-OH		4.57 (m)			4.52 (br s)		20	78.4	3.50 (dd, 8.3, 4.5)	78.2	3.52 (dd, 8.3, 4.9)	
19	34.3	1.71 (m)		C-20	1.73 (m)		21	37.1	1.82 (br dq, 8.3, 6.8, 1.5)	37.1	1.84 (br dq, 8.3, 7.2, 1.4)	
20	75.6	3.33 ^c (m, 8.3, 3.8, 3.0)			3.33 ^c (m)		22	75.2	3.94 (br d, 10.5, 1.5)	74.9	3.97 (br d, 10.8, 1.4)	
20-OH		4.64 (m)			4.67 (br s)		23	35.3	1.97 (br dq, 10.5, 6.8, 3.8)	35.2	2.00 (br dq, 10.8, 7.3, 3.4)	
21	35.7	1.69 (m, 0.9)		C-31	1.69 (m, 6.8)		24	81.8	4.20 (br dd, 6.8, 3.8)	81.6	4.23 (br dd, 6.3, 3.4)	
22	72.8	3.82 (br d, 10.5, 0.9)		C-20, C-21, C-31	3.83 (br d, 10.5)		25a	24.1	1.93 (m)	24.9	1.90 (m)	
23	33.4	1.84 (br dq, 10.5, 6.8, 3.8)			1.85 (m, 3.8)		25b	35.4	1.88 (m)	35.1	2.05 (m)	
24	79.1	4.14 (br dd, 6.8, 3.8)			4.15 (br dd, 6.8, 3.8)		26a	106.4	1.80 (m)	106.4	1.80 (m)	
25a	23.6	1.81 (m)			1.83 (m)		26b	20.7	2.07 (d, 1.1)	20.9	1.94 (d, 1.2)	
25b	1.77 (m)				1.76 (m)		27	17.1	0.99 (d, 6.8)	17.1	0.96 (d, 6.8)	
26a	33.9	1.92 (m)			1.92 (m)		28	106.6		106.6		
26b	1.69 (m)				1.71 (m)		29	17.1	0.99 (d, 6.8)	17.1	0.96 (d, 6.8)	
27	104.2						30	2.01 (br s)		2.01 (br s)		
28	20.1	2.03 (br s)		C-12, C-13, C-14	1.86 (br s)		31	0.84 (d, 7.5)		0.84 (d, 7.5)		
29	16.2	0.90 (d, 6.8)		C-16, C-17, C-18	0.87 (d, 7.5)							

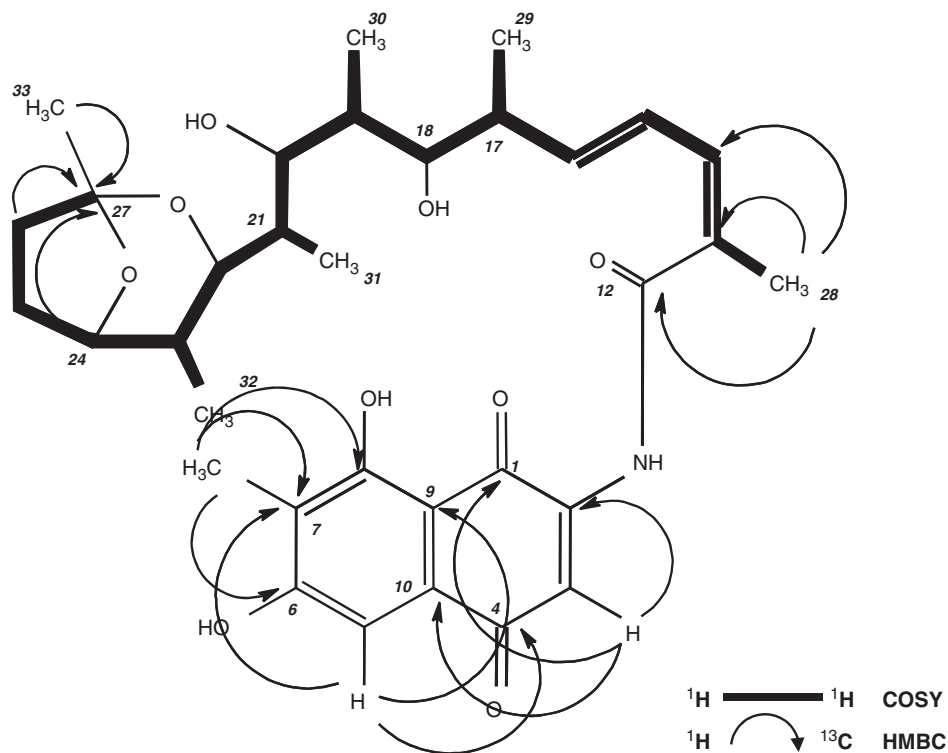


Figure 2 2D NMR spectral data in solvent CD₃OD.

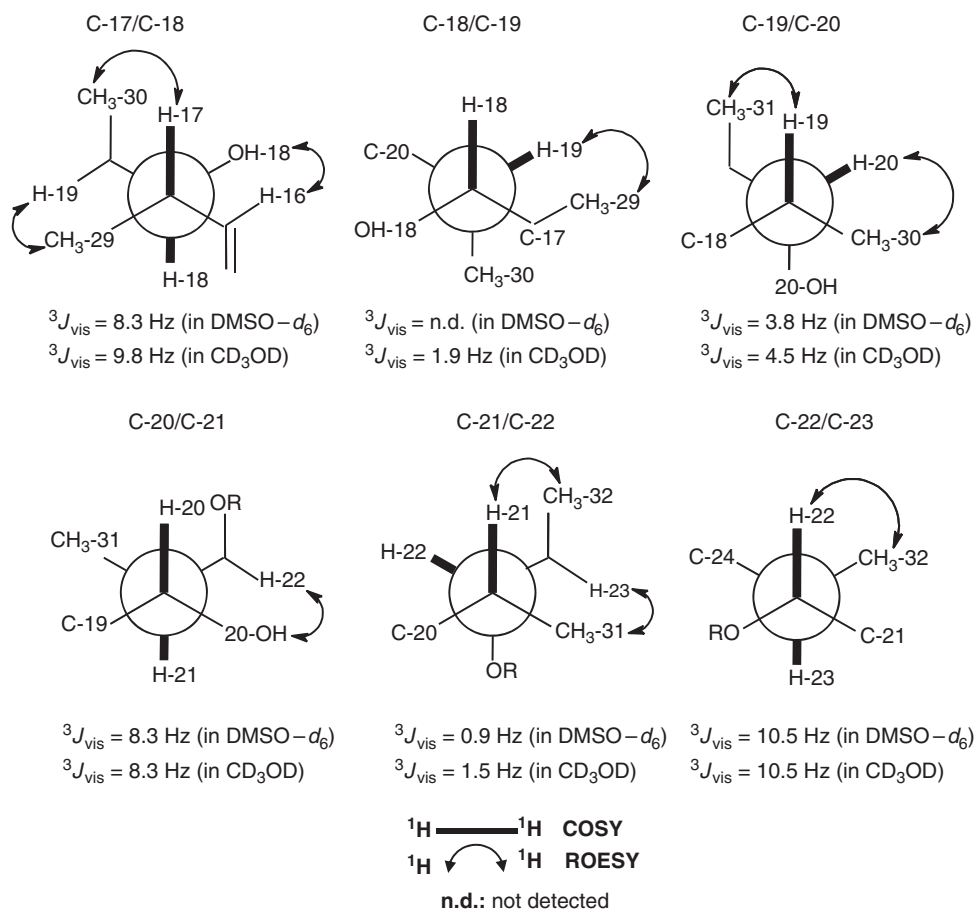


Figure 3 Data on ROESY correlations, and coupling constants and partial structures of **1**.

Salinisporamycin (1)

The UV spectrum pattern of **1** was nearly identical with the spectrum of rifamycin antibiotics.¹⁰ However, the MW was not the same in this experiment as in the databases mentioned above. Therefore, the silica gel chromatographed eluates were separated by HPLC (Inertsil ODS-2 column, 4.6 mm i.d. ×250 mm) with a linear gradient from 10 to 100% CH₃CN to afford **1** (0.6 mg). Compound **1** LC-MS: RT, 38.4 min; PDA, 240, 270, 326, 409 nm; MS, *m/z* 598 [M+H]⁺, *m/z* 596 [M-H]⁻.

Saliniketol A (2)

The silica gel chromatographed eluates were separated by HPLC (Inertsil ODS-2 column, 4.6 mm i.d. ×250 mm) with isocratic elution of 30% CH₃CN to afford **2**^{8,9} (2.6 mg). Compound **2** LC-MS: RT, 25.1 min; PDA, 252 nm; MS, *m/z* 396 [M+H]⁺, n.d. [M-H]⁻; 750 MHz, DMSO-*d*₆; Table 2. The

chemical shifts revealed that **2** was identical with the values reported in the literature for saliniketol A.⁸

Rifamycin S (3)

The silica gel chromatographed eluates were separated by HPLC (Inertsil ODS-2 column, 4.6 mm i.d. ×250 mm) with a linear gradient from 20 to 100% CH₃CN to afford **3**¹⁰ (4.6 mg). Compound **3** LC-MS: RT; 37.5 min, PDA; 239, 276, 334, 409 nm, MS; *m/z* 696 [M+H]⁺, *m/z* 694 [M-H]⁻; TLC: *R_f* value at 0.87 (CHCl₃:MeOH=4:1); UV λ_{max} nm (log ε) in MeOH: 228 (4.86), 271 (4.71), 315 (4.43), 410 (3.93); IR ν_{max} (KBr) cm⁻¹: 3431, 2926, 2854, 1710, 1606, 1465, 1415, 1384, 1354, 1259, 1164, 1074, 976; 750 MHz, DMSO-*d*₆;

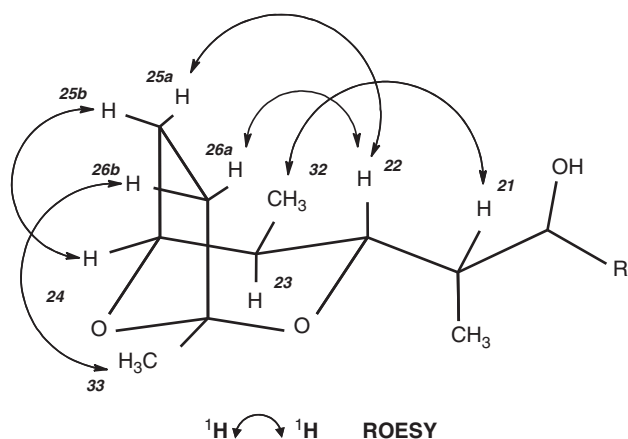


Figure 4 ROESY correlations and partial structure of **1**.

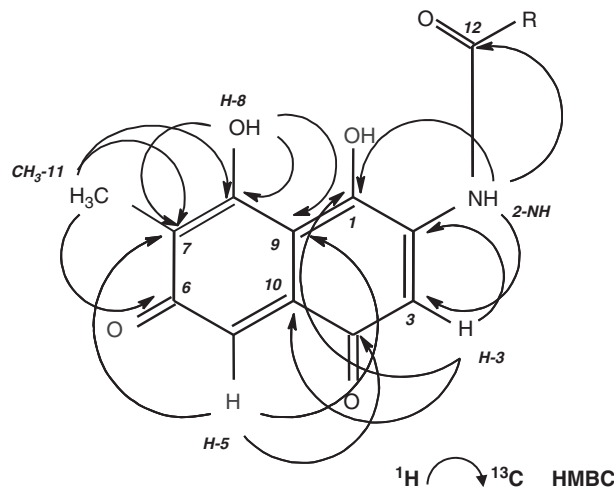


Figure 5 Heteronuclear multiple bond correlations and naphthoquinone ring system of **1** in solvent DMSO-*d*₆.

Table 3 750 MHz ¹H and 125 MHz ¹³C NMR data of naphthoquinone ring system of **1**, 31-homo rifamycin W and rifamycin Z

Position	CD ₃ OD				Diff. (p.p.m.) ^f	DMSO- <i>d</i> ₆				Diff. (p.p.m.)
	1		31-Homo rifamycin W ^a			1		Rifamycin Z ^b		
	δ _C	δ _H	δ _C	δ _H		δ _C	δ _H	δ _C	δ _H ^d	
1	181.3 (br) ^e	—	182.3	—	-1.0	170.3	—	181.8	—	-11.5
1-OH	—	—	—	—	—	—	ND	—	—	—
2	143.2	—	142.7	—	0.5	143.1	—	140.4	—	2.7
2-NH	—	—	—	—	—	—	9.14	—	8.90 (ND)	—
3	116.4	7.55	117.6	7.52	-1.2	111.7	7.18	114.2	7.30 (7.70)	-2.5
4	187.9	—	187.3	—	0.6	186.7	—	184.0	—	2.7
5 ^f	112.7	6.96	127.1	—	—	117.7	6.32	123.5	—	—
6	164.5	—	164.2	—	0.3	180.4 (br)	—	160.0	—	20.4
6-OH	—	—	—	—	—	—	—	—	10.50 (7.70)	—
7	117.8	—	118.7	—	-0.9	112.2	—	117.6	—	-5.4
8	172.0 (br)	—	166.5	—	5.5	162.1 (br)	—	162.1	—	0.0
8-OH	—	—	—	—	—	—	12.55	—	12.70 (9.40)	—
9	106.6	—	106.7	—	-0.1	100.6	—	106.5	—	-5.9
10	132.5	—	129.7	—	2.8	131.1	—	127.7	—	3.4
11	8.2	2.06	8.5	2.12	-0.3	7.9	1.75	8.7	2.15 (1.62)	-0.8
12	170.1	—	172.0	—	-1.9	167.1	—	171.6	—	-4.5

Abbreviation: ND, not detected.

^aData adapted from Wang *et al.*¹²

^bData adapted from Cricchio *et al.*¹³

^cDiff. (p.p.m.)=obs. [δ_C]-ref. [δ_C].

^dIn solvent DMSO-*d*₆/acetone-*d*₆ (7:3) (Pyridine-*d*₅).

^eBroad peak signal.

^fC-6 of rifamycin Z and 31-homo rifamycin W were assigned as diagnostic of quaternary carbon signal.

Table 4 Antimicrobial activity properties of **1**, **2** and **3**

Taxin	Strain	1	2	3
Firmicutes	<i>Staphylococcus aureus</i> IFO 12732	0.46	37	0.0056
Firmicutes	<i>Bacillus subtilis</i> IFO 3134	4.1	111	1.4
Bacteroidetes	<i>Cytophaga marinoflava</i> IFO 14170	>200	>200	12.3
Gammaproteobacteria	<i>Escherichia coli</i> IFO 3301	>200	>200	>200
Gammaproteobacteria	<i>Pseudomonas aeruginosa</i> IFO3446	>200	>200	>200
Yeast	<i>Candida albicans</i> IFO 1060	>200	>200	>200

Antimicrobial activity (MIC, $\mu\text{g ml}^{-1}$).

Table 2. **3** was established by 2D NMR, and the chemical shifts showed that **3** was nearly identical with the values reported in the literature for proansamycin B,¹⁰ 8-deoxy-rifamycin¹⁸ and 31-homorifamycin W.¹²

Cytotoxic and antibacterial activity

A549 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. The cells were seeded in a flat-bottomed 96-well microplate (4000 cells per 200 μl per well), and then cultured for 14 h at 37 °C in a CO₂ incubator (5% CO₂-air). Serially diluted **1** was added to each well, and the cells were further cultured for 48 h. The number of cells was counted by Alamar Blue assay.¹⁹ The MIC of **1**, **2** and **3** were analyzed by paper disk methods.²⁰

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