ORIGINAL ARTICLE

New angucycline C-glycosides from *Streptomyces* sp. RI33

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In the course of our screening program for anticancer agents from natural sources, five new angucyclines, JBIR-90 (1), -116 (2), -91 (3), -92 (4) and -93 (5), were isolated from the fermentation broth of *Streptomyces* sp. RI33. The structures of 1–5 were elucidated on the basis of 1D and 2D NMR spectroscopy and MS analyses. Compounds 2–4 showed cytotoxic activities against malignant pleural mesothelioma cells at IC₅₀ values of 20–50 μ M.

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INTRODUCTION

Malignant pleural mesothelioma (MPM), which is associated with exposure to asbestos fibers, is an aggressive neoplasm that develops from the pleura and is highly invasive to surrounding tissues.^{1,2} MPM has been shown to be resistant to all conventional therapies, including chemotherapy, radiotherapy and surgery, and the prognosis of patients remains very poor. Consequently, it is a tumor that continues to be a difficult clinical problem.^{3–5} Therefore, developing novel therapeutic agents against MPM cells are strongly desired. We have already reported that a novel compound, JBIR-23, which was isolated from *Streptomyces* sp. AK-AB27, showed antitumor activity against MPM cells, with tubulin polymerization-promoting activity.^{6,7} We have also discovered novel anti-MPM compounds, namely, the teleocidin analog JBIR-31,⁸ the aminocaprophenone alkaloid ficuseptamine B⁹ and the 1,1-dichlorocyclopropane-skeleton-containing angucycline JBIR-88.¹⁰ In the course of our screening program for cytotoxic compounds against MPM cells from microbial metabolites, we succeeded in isolating new compounds termed JBIR-90 (1), -116 (2), -91 (3), -92 (4) and -93 (5) from the culture of *Streptomyces* sp. RI33 (Figure 1). This paper describes the fermentation, isolation, structure elucidation and, in brief, the biological activity of 1–5.

RESULTS AND DISCUSSION

Structure elucidation of 1

Compound 1 was obtained as a yellow powder. The IR spectrum of 1 revealed the characteristic absorptions of a ketone ($\nu_{\max}$ 1705 cm⁻¹) and a quinone ($\nu_{\max}$ 1620 cm⁻¹) group. The molecular formula of 1 was determined to be C₂₅H₂₄O₇ on the basis of HR-ESI-MS data. The

direct connectivity between each proton and carbon was established by the heteronuclear single-quantum coherence spectrum, and the ¹³C and ¹H NMR spectral data for 1 are shown in Table 1. The analyses of double-quantum-filtered (DQF)-COSY and constant time heteronuclear multiple bond correlation (HMBC)¹¹ spectra revealed the structure of 1 as follows.

The sequence from methylene protons 2-H (δ_{H} 3.02, 2.56) to methylene protons 4-H (δ_{H} 2.98, 2.67) through a methine proton 3-H (δ_{H} 2.46), which was in turn coupled to a methyl proton 3-CH₃ (δ_{H} 1.20), was observed in the DQF-COSY (Figure 2). The HMBC correlations from 2-H to an α,β -unsaturated ketone carbonyl carbon C-1 (δ_{C} 199.3) and an aromatic carbon C-12b (δ_{C} 136.6), and from 4-H to two aromatic carbons, C-4a (δ_{C} 150.4) and C-12b, established a 3-methylhex-5-en-1-one substructure. The spin coupling between two aromatic protons, 5-H (δ_{H} 7.53) and 6-H (δ_{H} 8.28), together with ¹H–¹³C long-range couplings from 5-H to two aromatic carbons, C-6a (δ_{C} 133.5) and C-12b, and from 6-H to two aromatic carbons, C-4a and C-12a (δ_{C} 135.9), indicated a 3-methyl-1-oxo-1,2,3,4-tetrahydronaphthalene structure. An aromatic proton 11-H (δ_{H} 7.71), which was spin-coupled with another aromatic proton 10-H (δ_{H} 7.90), was strongly *meta*-coupled to two aromatic carbons, C-7a (δ_{C} 114.8) and C-9 (δ_{C} 137.1), and long-range coupled to an aromatic carbon C-11a (δ_{C} 133.7), a quinone carbonyl carbon C-12 (δ_{C} 182.8) located at the *peri*-position, and another quinone carbonyl carbon C-7 (δ_{C} 188.0). The aromatic proton 10-H was *meta*-coupled to two aromatic carbons, C-8 (δ_{C} 158.2) and C-11a, and W-letter long-range-coupled to a quinone carbonyl carbon C-12. The long-range coupling from a hydrogen-bonded phenolic hydroxyl proton 8-OH (δ_{H} 12.72) to three

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Figure 1 Structures of 1–5.

aromatic carbons, C-7a, C-8 and C-9, established a naphthoquinone substructure. The connectivity between these two substructures was established by the long-range couplings from 5-H to the quinone carbonyl carbon C-7, which suggested a 1,2,3,4-tetrahydrotetraphene skeleton. This chromophore moiety was also supported by the UV absorptions (λ_{max} 268 and 405 nm), which corresponded to those of urdamycin B^{12,13} and urdamycinone B.¹⁴

A remaining pseudo-glycoside unit was established by the sequence from an oxymethine proton 1'-H (δ_{H} 5.19) to a methyl proton 6'-H (δ_{H} 1.37) through two methylene protons, 2'-H (δ_{H} 2.44, 1.40), and three oxymethine protons, 3'-H (δ_{H} 4.03), 4'-H (δ_{H} 3.76) and 5'-H (δ_{H} 4.44), observed in the DQF-COSY, and a long-range coupling between 1'-H and C-5' (δ_{C} 72.6). The large coupling constants between 1'-H/2'-H_{ax} ($J_{1'-\text{H}-2'-\text{H}_{\text{ax}}}$ ~12.0 Hz), 2'-H_{ax}/3'-H ($J_{2'-\text{H}_{\text{ax}}-3'-\text{H}}$ ~11.5 Hz), 3'-H/4'-H ($J_{3'-\text{H}-4'-\text{H}}$ ~9.0 Hz) and 4'-H/5'-H ($J_{4'-\text{H}-5'-\text{H}}$ ~6.0 Hz) deduced a β -2,6-dideoxy-*arabino*-hexopyranose (olivopyranose) structure. The high-field shift at the anomeric carbon

C-1' (δ_{C} 64.3), compared with those of usual anomeric carbon shifts, and the HMBC correlations from 1'-H to three aromatic carbons, C-8, C-9 and C-10 (δ_{C} 133.7), indicated that the saccharide unit directly attached at C-9. Thus, the structure of 1 was determined as an angucycline C-olivopyranoside, 3-deoxyurdamycinone B, as shown in Figure 1.

Structure elucidation of 2

Compound 2 was obtained as a dark yellow powder. The IR spectrum of 2 revealed the characteristic absorptions of a ketone (ν_{max} 1720 cm⁻¹) and a quinone (ν_{max} 1640 cm⁻¹) group. Its molecular formula was determined as C₃₇H₄₆O₁₅ by analysis of the HR-ESI-MS spectrum. The ¹³C and ¹H NMR spectral data for 2 are summarized in Table 1.

The sequence from methylene protons 2-H (δ_{H} 2.60, 2.25) to methylene protons 4-H (δ_{H} 1.98, 1.56) through a methine proton 3-H (δ_{H} 2.24), which was in turn coupled to a methyl proton 3-CH₃

Table 1 ^{13}C and ^1H NMR data for 1–5

Position	1^{a,d}		2^{b,e}		3^{b,d}		4^{b,e}		5^{c,e}	
	δ_{H} (multiplicity, J in Hz)		δ_{H} (multiplicity, J in Hz)		δ_{H} (multiplicity, J in Hz)		δ_{H} (multiplicity, J in Hz)		δ_{H} (multiplicity, J in Hz)	
	δ_{C}		δ_{C}		δ_{C}		δ_{C}		δ_{C}	
1	199.3		208.2		208.5		209.1		208.0	
2	47.5	3.02 (ddd, 16.0, 5.5, 1.5)	47.8	2.60 (dd, 8.0, 2.0)	48.1	2.58 (dd, 8.5, 2.0)	47.5	2.72 (dt, 12.5, 3.5)	46.7	2.66 (dt, 12.5, 3.0)
		2.56 (dd, 16.0, 11.0)		2.25 (m)		2.29 (m)		2.52 (t, 12.5)		2.58 (t, 12.5)
3	30.7	2.46 (br sept-like, ~5.5)	32.3	2.24 (m)	32.3	2.30 (m)	31.1	2.32 (m)	30.7	2.31 (m)
3-CH ₃	21.5	1.20 (d, 6.5)	21.8	1.01 (d, 5.0)	21.8	1.02 (d, 6.0)	21.9	0.99 (d, 6.5)	21.7	0.92 (d, 6.5)
4	38.4	2.98 (dd, 16.5, 1.5)	44.3	1.98 (dd, 13.5, 2.0)	44.3	1.99 (m)	44.8	2.01 (dt, 14.0, 2.0)	44.3	2.00 (dd, 14.0, 2.0)
		2.67 (dd, 16.5, 11.0)		1.56 (dd, 13.5, 11.5)		1.63 (dd, 14.0, 11.5)		1.56 (dd, 14.0, 12.5)		1.57 (dd, 14.0, 12.5)
4a	150.4		87.6		87.7		86.5		85.7	
5	133.0	7.53 (d, 8.0)	142.7	6.95 (d, 10.0)	142.1	7.00 (d, 10.0)	134.6	6.76 (d, 10.0)	134.1	6.80 (d, 10.0)
6	128.9	8.28 (d, 8.0)	119.7	7.10 (d, 10.0)	120.3	7.06 (d, 10.0)	121.3	7.20 (d, 10.0)	120.5	7.12 (d, 10.0)
6a	133.5		139.9		139.2		126.1		124.3	
7	188.0		189.8		189.9		161.0		156.7	
7a	114.8		115.2		115.4		112.9		111.3	
8	158.2		158.9		159.1		187.6		187.9	
8-OH		12.72 (s)				8.52 (br s)				
9	137.1		139.3		139.7		145.7		144.5	
10	133.7	7.90 (d, 8.0)	134.4	7.87 (d, 8.0)	134.4	7.93 (d, 8.0)	134.6	7.80 (s)	135.8	8.13 (s)
11	119.7	7.71 (dd, 8.0, 1.5)	120.1	7.55 (d, 8.0)	120.1	7.64 (d, 8.0)	134.6		127.7	
11a	133.7		132.0		132.1		117.5		117.3	
12	182.8		183.5		183.6		141.6		141.1	
12a	135.9		139.5		140.4		129.9		129.4	
12b	136.6		78.9		79.3		80.4		79.7	
9-C-olivose										
1'	64.3	5.19 (dd, 11.5, 2.0)	65.5	5.19 (dd, 11.0, 1.5)	65.5	5.24 (dd, 7.5, 1.5)	66.0	5.03 (m)	65.4	4.99 (d, 11.0)
2'	39.5	2.44 (ddd, 12.5, 5.0, 2.0)	41.0	2.41 (ddd, 11.0, 4.5, 2.0)	41.1	2.44 (ddd, 12.5, 5.0, 2.0)	40.7	2.46 (ddd, 12.5, 4.5, 2.5)	41.0	2.42 (m)
		1.40 (q-like, ~12.0)		1.34 (m)		1.37 (m)		1.30 (m)		1.30 (m)
3'	68.4	4.03 (ddd, 11.0, 9.5, 5.0)	68.8	3.96 (ddd, 11.0, 9.0, 5.5)	68.8	3.97 (ddd, 11.0, 9.0, 5.0)	68.7	3.94 (m)	68.4	3.91 (ddd, 11.0, 9.0, 4.5)
4'	74.2	3.76 (dd, 9.0, 6.0)	74.9	3.66 (dd, 9.0, 5.5)	75.0	3.66 (dd, 9.0, 6.0)	74.7	3.55 (dd, 9.0, 6.0)	74.7	3.39 (dd, 9.0, 4.5)
5'	72.6	4.44 (qui, 6.5)	74.6	4.37 (qui, 6.5)	74.6	4.38 (qui, 6.5)	74.4	4.27 (qui, 6.5)	73.6	4.05 (m)
6'	12.0	1.37 (d, 7.0)	12.5	1.37 (d, 7.0)	12.5	1.37 (d, 7.0)	12.6	1.30 (d, 7.5)	12.4	1.10 (d, 6.5)
12-O-p-hydroxyphenylacetyl/12-O-indoleacetyl										
2''							161.1		159.6	
3''							133.0		131.5	
4''							123.5		109.2	
5''							134.6	7.36 (d, 8.5)	132.7	7.89 (s)
6''							116.7	6.93 (d, 8.5)		
6a''									137.5	
7''							162.5		113.1	7.55 (d, 8.0)
8''							116.7	6.93 (d, 8.5)	121.0	7.57 (d, 8.0)
9''							134.6	7.36 (d, 8.5)	121.6	7.14 (d, 8.0)
10''									123.6	7.23 (d, 8.0)
10a''									128.3	
4a-O-olivose/4a-O-rhodamine										
r1			98.7	5.43 (br s)	97.1	5.52 (br s)	97.7	5.56 (br s)	97.2	5.50 (br d, 2.0)
r2			34.8	1.90 (br d, 12.5)	29.0	2.41 (br d, 12.0)	30.5	2.14 (dd, 12.0, 2.5)	30.3	1.98 (dd, 12.5, 2.5)
				1.84 (m)		2.00 (m)		1.84 (td, 12.5, 3.5)		1.79 (td, 12.5, 3.5)
r3			66.7	3.98 (m)	64.0	3.40 (m)	63.4	2.35 (m)	63.0	2.27 (m)
r3-N-dimethyl					42.1	3.03 (br s)	43.9	2.36 (s)	43.8	2.27 (s)
r4			81.4	3.65 (br s)	74.5	4.18 (br s)	75.5	3.87 (br s)	75.0	3.75 (br s)
r5			68.3	4.13 (qui, 6.5)	68.6	4.15 (qui, 6.5)	69.4	4.09 (q, 7.0)	68.6	4.03 (m)
r6			17.7	1.27 (d, 6.5)	18.3	1.35 (d, 6.5)	18.7	1.29 (d, 5.5)	18.4	1.22 (d, 6.5)
r4-O-olivose										
o1			101.4	4.99 (br s)	101.1	5.23 (br s)	100.4	5.10 (t-like)	100.0	4.93 (t-like)
o2			33.2	1.95 (td, 11.7, 4.0)	33.1	2.06 (td, 12.5, 4.0)	33.3	1.95 (dd, 9.0, 3.0)	33.6	1.83 (m)
						1.98 (m)				
o3			66.6	4.07 (ddd, 11.7, 4.0, 2.5)	66.3	4.05 (ddd, 11.5, 4.0, 2.5)	66.8	4.08 (td, 7.5, 3.0)	66.2	3.98 (m)
o4			72.3	3.61 (m)	71.9	3.64 (m)	72.5	3.58 (br d, 2.0)	72.1	3.50 (br d, 2.0)
o5			68.5	4.30 (qui, 6.5)	68.9	4.13 (qui, 6.0)	67.9	4.42 (q, 6.5)	67.2	4.46 (q, 6.5)
o6			17.2	1.23 (d, 6.5)	17.3	1.27 (d, 6.5)	17.4	1.23 (d, 6.5)	17.3	1.14 (d, 6.5)

^{a-c}Measured in CDCl₃, CD₃OD and CD₃COCD₃, respectively.^d ^{13}C and ^1H NMR were observed at 150 and 600 MHz, respectively.^e ^{13}C and ^1H NMR were observed at 125 and 500 MHz, respectively.

Figure 2 Key correlations of ^1H - ^1H double-quantum-filtered COSY (bold lines) and constant time heteronuclear multiple bond correlation (solid arrows) of **1**–**5**.

(δ_{H} 1.01), and a coupling between two olefinic protons, 5-H (δ_{H} 6.95) and 6-H (δ_{H} 7.10), were observed in the DQF-COSY for **2** (Figure 2). The HMBC from 2-H to a ketone carbonyl carbon C-1 (δ_{C} 208.2) and an oxygenated quaternary carbon C-12b (δ_{C} 78.9), from 4-H to two oxygenated quaternary carbons, C-4a (δ_{C} 87.6) and C-12b, and an olefinic carbon C-5 (δ_{C} 142.7), from 5-H to C-4a, an aromatic carbon C-6a (δ_{C} 139.9), and C-12b, and from 6-H to C-4a and an aromatic carbon C-12a (δ_{C} 139.5) indicated the presence of a 3,4,4a,8a-tetrahydro-4a,8a-dihydroxy-3-methyl-1(2*H*)-naphthalenone substructure. Other COSY and HMBC correlations were the same as those of **1**, establishing an angucycline 9-*C*-olivoside structure, as shown in Figure 2. This 4a,12b-dihydroxyl form of angucycline was also supported by its UV absorption (λ_{max} 318 and 425 nm).^{12,13} The presence of two additional sugar moieties was determined as follows. The sequences from the anomeric proton r1-H (δ_{H} 5.43) through the two methylene protons r2-H (δ_{H} 1.90, 1.84) to an oxymethine proton r3-H (δ_{H} 3.98), and from an oxymethine proton r4-H (δ_{H} 3.65) through an oxymethine proton r5-H (δ_{H} 4.13) to a methyl proton r6-H (δ_{H} 1.27), were observed in the DQF-COSY. Long-range couplings from r1-H to C-r5 (δ_{C} 68.3) and r4-H to C-r3 (δ_{C} 66.7) indicated a 2,6-dideoxyhexopyranoside structure. Their relative stereochemistry was determined by the coupling constants shown in Table 1, establishing an α -2,6-dideoxy-*lyxo*-hexopyranoside (olipyranside) structure.

Another *O*-glycoside unit, an α -olipyranside, was deduced in the same manner, as shown in Figure 2. The ^1H - ^{13}C long-range correlations from the two anomeric protons r1-H and o1-H (δ_{H} 4.99) to C-4a and C-r4 (δ_{C} 81.4), respectively, concluded the substituted position of these glycosides, as shown in Figure 2. Thus, the structure of **2** was determined, as shown in Figure 1.

Structure elucidation of **3**

Compound **3** was obtained as a dark yellow powder. The IR spectrum of **3** revealed the characteristic absorptions of a ketone (ν_{max} 1720 cm^{-1}) and a quinone (ν_{max} 1630 cm^{-1}) group. Its molecular formula was determined as $\text{C}_{39}\text{H}_{51}\text{NO}_{14}$ by the HR-ESI-MS analysis. The ^{13}C and ^1H NMR spectral data for **3** (Table 1) revealed similar signals to those of **2**, except for the additional signal of an *N*-dimethyl group. The long-range couplings from r1-H (δ_{H} 5.52) to C-r5 (δ_{C} 68.6), and from r4-H (δ_{H} 4.18) and an aminodimethyl proton r3-*N*-CH₃ (δ_{H} 3.03) to C-r3 (δ_{C} 64.0) indicated a 3-(dimethylamino)-2,3,6-trideoxyhexopyranoside moiety. Their relative stereochemistry was determined by the coupling constants to establish an α -3-(dimethylamino)-2,3,6-trideoxy-*lyxo*-hexopyranoside (rhodosaminopyranoside) structure. Another *O*-glycoside unit, an α -olipyranside, was deduced in the same manner, as shown in Figure 2. Thus, the structure of **3** was determined, as shown in Figure 1.

Structure elucidation of 4

Compound **4** was obtained as a red powder and showed a protonated ion at m/z 890.3604 (calcd. for $C_{47}H_{56}NO_{16}$, 890.3599) in HR-ESI-MS, corresponding to the molecular formula $C_{47}H_{55}NO_{16}$. The IR spectrum (KBr) of **4** revealed the characteristic absorptions of an ester and/or a ketone group ($\nu_{\max}$ 1720 cm^{-1}) and an unsaturated ketone functional group ($\nu_{\max}$ 1655, 1640 cm^{-1}). The ^{13}C and ^1H NMR spectral data for **4** (Table 1) revealed similar signals to those of **3** and an additional six signals, including AB-type aromatic signals (C-5'', 9'', δ_{C} 134.6, δ_{H} 7.36; C-6'', 8'', δ_{C} 116.7, δ_{H} 6.93) and an ester carbonyl signal (C-2'', δ_{C} 161.1).

The DQF-COSY and HMBC spectral analyses of **4** revealed that a 1,2,3,4,4a,12b-hexahydrotetraphene, an α -olivopyranoside, an α -rhodosaminopyranoside and an α -oliopyranoside moiety were conserved, as in **3** (Figure 2). The HMBC correlations from the AB-type aromatic proton 5'', 9''-H to a phenolic carbon C-7'' (δ_{C} 162.5) and an aromatic carbon C-3'' (δ_{C} 133.0) at the *peri*-position, and from another AB-type aromatic proton 6'', 8''-H to an aromatic quaternary carbon C-4'' (δ_{C} 123.5) revealed the phenol moiety. Long-range couplings from 10-H (δ_{H} 7.80) to C-3'' suggested that the phenol moiety and the tetraphene unit are attached through C-3''. A remaining unit, an ester carbonyl carbon C-2'' (δ_{C} 161.1), was found to form a six-membered ring system consisting of C-2'', C-3'', C-11, C-11a, C-12 and an oxygen atom, as shown in Figure 2. This characteristic chromophore was supported by UV and visible absorption data ($\lambda_{\max}$ 310 and 504 nm) in comparison with those of urdamycin C.^{12,14} Thus, the structure of **4** was determined as a *p*-hydroxyphenylacetic acid complex of **3**, as shown in Figure 1.

Structure elucidation of 5

Compound **5** was obtained as a violet powder, and its HR-ESI-MS (m/z 911.3621; calcd. for $C_{49}H_{55}N_2O_{15}$, 911.3602) indicated the molecular formula $C_{49}H_{56}N_2O_{15}$. The IR spectrum (KBr) of **5** revealed the characteristic absorptions of an ester and/or a ketone group ($\nu_{\max}$ 1720 cm^{-1}) and an unsaturated ketone functional group ($\nu_{\max}$ 1630, 1600 cm^{-1}). The ^{13}C and ^1H NMR spectral data for **5** (Table 1) revealed that the signals in the condensed ring unit and the sugar units were super-imposable with those of **4**. The structural differences between **4** and **5** are as follows.

The sequence from an aromatic proton 7''-H (δ_{H} 7.55) through two aromatic protons, 8''-H (δ_{H} 7.57) and 9''-H (δ_{H} 7.14), to an aromatic proton 10''-H (δ_{H} 7.23) was observed in the DQF-COSY (Figure 2). ^1H - ^{13}C long-range couplings from an aromatic proton 5''-H (δ_{H} 7.89) to three aromatic carbons, C-4'' (δ_{C} 109.2), C-6a'' (δ_{C} 137.5) and C-10a'' (δ_{C} 128.3), and a low-field shifted ^{13}C chemical shift of C-5'' (δ_{C} 132.7) and C-6a'' indicated the presence of an indole structure. The UV and visible absorption spectra of **5** ($\lambda_{\max}$ 328 and 578 nm) were the same as those of the known indoleacetic acid-conjugated angucycline urdamycin D.^{12,14} Thus, the structure of **5** was determined as a 3''-(3-indolyl)-substituted congener of **4** instead of the hydroxyphenyl group, as shown in Figure 1.

Cytotoxicity

The cytotoxic effects of **1**–**5** on MPM and HeLa cells were determined by the WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) colorimetric assay. Compounds **2**, **3** and **4** exhibited cytotoxic activities against ACC-MESO-1 cells, with IC_{50} values of 45, 23 and 46 μM , respectively (Table 2). However, **1** and **5** did not show cytotoxicity against ACC-MESO-1 cells at a concentration of 100 μM . In addition, **1**–**5** exhibited cytotoxic activity against HeLa cells with IC_{50} values of 18–75 μM (Table 2).

Table 2 Cytotoxic effects of **1**–**5** on MPM and HeLa cells

Cancer cell lines	IC_{50} (μM)				
	1	2	3	4	5
ACC-MESO-1	> 100	45	23	46	> 100
HeLa	47	23	18	18	75

METHODS

General experimental procedures

Optical rotations were obtained on an SEPA-300 polarimeter (Horiba, Kyoto, Japan). UV and IR spectra were measured on a DU730 UV/Vis spectrophotometer (Beckman Coulter, Brea, CA, USA) and an FT-720 spectrophotometer (Horiba), respectively. NMR spectra were recorded on a Varian NMR System 500 or 600 NB CL (Varian, Palo Alto, CA, USA) in CDCl_3 (7.25 p.p.m. for ^1H , 77.0 p.p.m. for ^{13}C) or CD_3OD (3.35 p.p.m. for ^1H , 49.0 p.p.m. for ^{13}C) or acetone- d_6 (2.04 p.p.m. for ^1H , 29.8 p.p.m. for ^{13}C), with the residual solvent peak as the internal standard. HR-ESI-MS data were recorded on an LCT Premier XE mass spectrometer (Waters, Milford, MA, USA). Normal-phase medium-pressure liquid chromatography was performed using a Purif-Pack SI 30 column (30 μm ; Shoko Scientific, Yokohama, Japan). Analytical reversed-phase HPLC was carried out using a Capcell Pak C18 MGII column (5.0 μm , 4.6 mm i.d. $\times$ 150 mm; Shiseido, Tokyo, Japan) equipped with a 2996 photodiode array detector (Waters) and a 3100 Mass Detector (Waters). Preparative reversed-phase HPLC was carried out using a Capcell Pak C18 MGII column (5.0 μm , 20 mm i.d. $\times$ 150 mm; Shiseido).

Microorganisms

The producing *Streptomyces* designated as RI33 was isolated from a lichen obtained from a soil sample collected at Shuri (Okinawa Prefecture, Japan) by the SDS-yeast extract method.¹⁵ The genus of the strain RI33 was identified with the partial 16S rRNA gene sequences (660 bp) by using EzTaxon.¹⁶ The strain belonged to the genus *Streptomyces*, as the 16S rRNA gene sequence comparison exhibited high sequence similarity of 99.24% to that of *Streptomyces albiacialis* strain NRRL B-24327^T (accession no. AY999901).

Fermentation

Streptomyces sp. RI33 was cultivated in 50-ml test tubes each containing 15 ml of a seed medium consisting of starch 1% (Kosokagaku, Tokyo, Japan), Polypepton 1% (Nihon Pharmaceutical, Tokyo, Japan), molasses 1% (Dai Nippon Meiji Sugar, Tokyo, Japan) and meat extract 1% (Extract Ehrlich; Wako Pure Chemical Industries, Osaka, Japan), pH 7.2 (before sterilization). The test tubes were shaken on a reciprocal shaker (355 r.p.m.) at 27 °C for 2 days. Aliquots (2.5 ml) of the broths were transferred to 500-ml baffled Erlenmeyer flasks (20 flasks) containing 100 ml of a production medium consisting of starch 2.5% (Kosokagaku), soybean meal 1.5% (Nisshin Oillio Group, Tokyo, Japan), dry yeast 0.2% (Mitsubishi Tanabe Pharma, Osaka, Japan), CaCO_3 0.4% (Kozakai Pharmaceutical, Tokyo, Japan) and Diaion HP-20 1% (Mitsubishi Chemical, Tokyo, Japan), pH 7.2 (before sterilization), and cultured on a rotary shaker (180 r.p.m.) at 27 °C for 5 days.

Isolation

The mycelial cake obtained from the fermentation broth (21) was extracted with Me_2CO (500 ml). The extract was concentrated *in vacuo*, and the aqueous concentrate was extracted with EtOAc (100 ml $\times$ 3). After drying over anhydrous Na_2SO_4 , the organic layer was evaporated to dryness. The residue (845 mg) was subjected to normal-phase medium-pressure liquid chromatography and successively eluted with a stepwise solvent system of *n*-hexane– EtOAc (0, 10, 20 and 25% EtOAc) and CHCl_3 – MeOH (0, 2, 5, 10, 20 and 50% MeOH). The 5% MeOH fraction (54.7 mg) was purified by preparative reversed-phase HPLC with 65% MeOH – H_2O (flow rate 10 ml min^{-1}) to yield **1** (6.3 mg, Rt 24.8 min). The 10% MeOH fraction (31.4 mg) was purified by preparative reversed-phase HPLC with 55% MeOH – H_2O (flow rate

10 ml min⁻¹) to yield **2** (4.0 mg, Rt 18.8 min). The 20% MeOH fraction (31.4 mg) was purified by preparative reversed-phase HPLC with a 35–70% MeOH–H₂O linear gradient system (30 min, flow rate 10 ml min⁻¹) to yield **3** (12.6 mg, Rt 23.4 min). The 50% MeOH fraction (27.1 mg) was subjected to preparative reversed-phase HPLC with a 50–90% MeOH–H₂O linear gradient system (30 min) followed by a 90% MeOH (10 min) isocratic system to yield two fractions (4.1 mg, Rt 26.8 min and 5.9 mg, Rt 30.9 min) containing **4** and **5**, respectively. From the former fraction (Rt 26.8 min), **4** (0.9 mg) was isolated by a Toyopearl HW-40F gel filtration column (15 × 500 mm²; Tosoh, Tokyo, Japan) developed with 100% MeOH. From the latter fraction (Rt 30.9 min), **5** (1.3 mg, Rt 13.8 min) was obtained by using preparative reversed-phase HPLC with a linear gradient system (35–90% MeOH–H₂O, 20 min, flow rate 10 ml min⁻¹). The physicochemical properties of compounds thus obtained are described below.

JBIR-90 (1). Yellow powder; [α]_D²⁵ +86.3 (c 0.15, MeOH); UV (MeOH) $\lambda_{\max}$ (ϵ) 268 (22 200) and 405 (4100) nm; IR (KBr) $\nu_{\max}$ 1705 and 1620 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃), see Table 1; HR-ESI-MS m/z 437.1592 [M+H]⁺ (calcd. for C₂₅H₂₅O₇, 437.1600).

JBIR-116 (2). Dark yellow powder; optical rotation could be not measured by the yellow sodium D line near 589 nm; UV (MeOH) $\lambda_{\max}$ (ϵ) 318 (5400) and 425 (6500) nm; IR (KBr) $\nu_{\max}$ 1720 and 1640 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) and ¹³C NMR (125 MHz, CD₃OD), see Table 1; HR-ESI-MS m/z 729.2750 [M–H][–] (calcd. for C₃₇H₄₅O₁₅, 729.2758).

JBIR-91 (3). Dark yellow powder; optical rotation could be not measured; UV (MeOH) $\lambda_{\max}$ (ϵ) 319 (5600) and 423 (6800) nm; IR (KBr) $\nu_{\max}$ 1720 and 1630 cm⁻¹; ¹H NMR (600 MHz, CD₃OD) and ¹³C NMR (150 MHz, CD₃OD), see Table 1; HR-ESI-MS m/z 758.3375 [M+H]⁺ (calcd. for C₃₉H₅₂NO₁₄, 758.3388).

JBIR-92 (4). Red powder; optical rotation could be not measured; UV (MeOH) $\lambda_{\max}$ (ϵ) 310 (15 900) and 504 (11 100) nm; IR (KBr) $\nu_{\max}$ 1720, 1655 and 1640 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) and ¹³C NMR (125 MHz, CD₃OD), see Table 1; HR-ESI-MS m/z 890.3604 [M+H]⁺ (calcd. for C₄₇H₅₆NO₁₆, 890.3599).

JBIR-93 (5). Violet powder; optical rotation could be not measured; UV (MeOH) $\lambda_{\max}$ (ϵ) 328 (11 900) and 578 (10 400) nm; IR (KBr) $\nu_{\max}$ 1720, 1630 and 1600 cm⁻¹; ¹H NMR (500 MHz, CD₃COCD₃) and ¹³C NMR (125 MHz, CD₃COCD₃), see Table 1; HR-ESI-MS m/z 911.3621 [M–H][–] (calcd. for C₄₉H₅₅N₂O₁₅, 911.3602).

Cytotoxic assay

Cytotoxic activities against human MPM ACC-MESO-1 cells¹⁷ and human cervical carcinoma HeLa cells were determined by a colorimetric assay, using WST-8. ACC-MESO-1 and HeLa cells were cultured in RPMI 1640 (Nacalai Tesque, Kyoto, Japan) and Dulbecco's modified Eagle's medium (Sigma, St Louis, MO, USA), respectively, supplemented with 10% (v/v⁻¹) fetal bovine serum (Life Technologies, Carlsbad, CA, USA), penicillin (100 U ml⁻¹) and streptomycin (100 µg ml⁻¹) at 37 °C in a humidified incubator under a 5% CO₂ atmosphere. The 384-well plates were seeded with aliquots of a 20-µl medium containing 1 × 10³ cells per well and were incubated overnight before

being treated with compounds at various concentrations for 48 h. Plates were incubated for 1 h at 37 °C after the addition of 2 µl of WST-8 reagent solution (Cell Counting Kit; Dojindo, Kumamoto, Japan) per well. The absorption of the formed formazan dye was measured at 450 nm. The vehicle solvent (DMSO) was used as a negative control. We screened all the samples at a final concentration of 1% and selected a sample that showed ≥70% inhibition of the growth of ACC-MESO-1 and HeLa cells.

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