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NOTE

Isolation of two new terpeptin analogs—JBIR-81 and JBIR-82—from a seaweed-derived fungus, *Aspergillus* sp. SpD081030G1f1

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The marine environment has recently been described as a source of novel chemical diversity for drug discovery, as many bioactive substances are isolated from marine organisms, including phytoplankton, algae, sponges, tunicates and mollusks.[1] Our group has reported the isolation of azaspiracid-22[2] and JBIR-44[3] from marine sponges. Microorganisms from marine habitats, especially fungi,[4] also constitute a promising untapped resource for discovering novel bioactive substances such as diketopiperazine alkaloids,[5] trichodermatides[6] and carbonarones.[7] We also have reported new sesquiterpenes—JBIR-27 and JBIR-28—from a tunicate-derived fungus *Penicillium* sp. SS080624SCf1,[8] a new aspochracin derivative—JBIR-15—from a marine sponge-derived fungus *Aspergillus sclerotiorum* Huber Sp080903f04,[9] and new glycosyl benzenediols—JBIR-37 and JBIR-38—from a marine sponge-derived fungus, *Acremonium* sp. SpF080624G1f01.[10] Further investigation resulted in the discovery of two new terpeptin analogs, designated as JBIR-81 (**1**) and JBIR-82 (**2**), together with terpeptin[11] (**3**), from the culture of a marine-derived *Aspergillus* sp. SpD081030G1f1 (Figure 1a). This paper describes the fermentation, isolation, structure elucidation, and in brief, the biological activity of **1** and **2**.

The fungus, *Aspergillus* sp. SpD081030G1f1, was isolated from a seaweed, *Sargassum* sp., collected at the sea shore of Ishigaki Island, Okinawa Prefecture, Japan. The strain was cultivated in 50-ml test tubes containing 15 ml of the seed medium (24 g l^{-1} potato dextrose broth, BD Biosciences, San Jose, CA, USA). The test tubes were shaken on a reciprocal shaker (355 r.p.m.) at 27 °C for 3 days. Aliquots (5 ml) of the seed culture were inoculated into 500-ml Erlenmeyer flasks containing 9 g oatmeal (Quaker, Chicago, IL, USA), 30 ml vegetable juice (KAGOME, Nagoya, Japan) and incubated at 27 °C for 14 days in static culture.

The solid culture (20 flasks) was extracted with 80% aqueous acetone (400 ml). The extract was evaporated *in vacuo*, and the residual aqueous concentrate was partitioned with EtOAc (100 ml×3). After drying over Na_2SO_4, the EtOAc layer was evaporated. The dried residue (845 mg) was subjected to normal-phase medium-pressure liquid chromatography (Purif-Pack SI-60, Moritex, Tokyo, Japan) using a $CHCl_3$–MeOH stepwise solvent system (0, 5 and 10% MeOH). The fractions containing **1**–**3** were collected by HPLC-MS monitoring. The target eluate (5% MeOH, 28 mg) was further purified by preparative reverse-phase HPLC using an L-column 2 ODS column (20 i.d.×150 mm; Chemical Evaluation and Research Institute, Tokyo, Japan) with a 2996 photodiode array detector (Waters, Milford, MA, USA) and a 3100 mass detector (Waters) developed using 25% aqueous MeOH containing 0.1% formic acid (flow rate, 10 ml min^{-1}) to yield **1** (1.1 mg, retention time (Rt) 9.7 min), **2** (1.4 mg, Rt 21.4 min) and **3** (2.0 mg, Rt 19.2 min).

Compounds **1** and **2** were obtained as colorless amorphous solids ($[\alpha]_D^{25}$ –177.7 for **1** and –145.3 for **2**, *c* 0.1, in MeOH). The molecular formulas of **1** and **2** were determined by HR-electrospray ionization-MS to be $C_{24}H_{34}N_4O_3$ (found: 427.2712 $[M+H]^+$, calcd: 427.2709) for **1** and $C_{29}H_{42}N_4O_3$ (found: 495.3340 $[M+H]^+$, calcd: 495.3335) for **2**. The presence of an amide group was deduced from their IR spectra (ν_{max} (KBr) 3315, 1631 cm^{-1} for **1**; and 3313, 1631 cm^{-1} for **2**). The UV spectra revealed the presence of an indole chromophore (λ_{max} (MeOH) 230 (ε=13 880), 280 (ε=6780) nm for **1** and 230 (ε=15 030), 285 (ε=6770) nm for **2**] similar to those of **3**.[11] The structures of **1** and **2** were determined by NMR spectral analyses. The direct connectivity of protons and carbons were established by heteronuclear single quantum coherence spectra, and the tabulated ^{13}C and ^{1}H NMR spectral data for **1** and **2** are shown in Table 1. The structures of **1** and **2** were elucidated in a series of double-quantum-filtered (DQF)-COSY and constant time heteronuclear multiple bond correlation (HMBC)[12] as follows.

The ^{1}H–^{1}H correlations observed in the DQF-COSY spectrum, together with the spin coupling constants among aromatic protons

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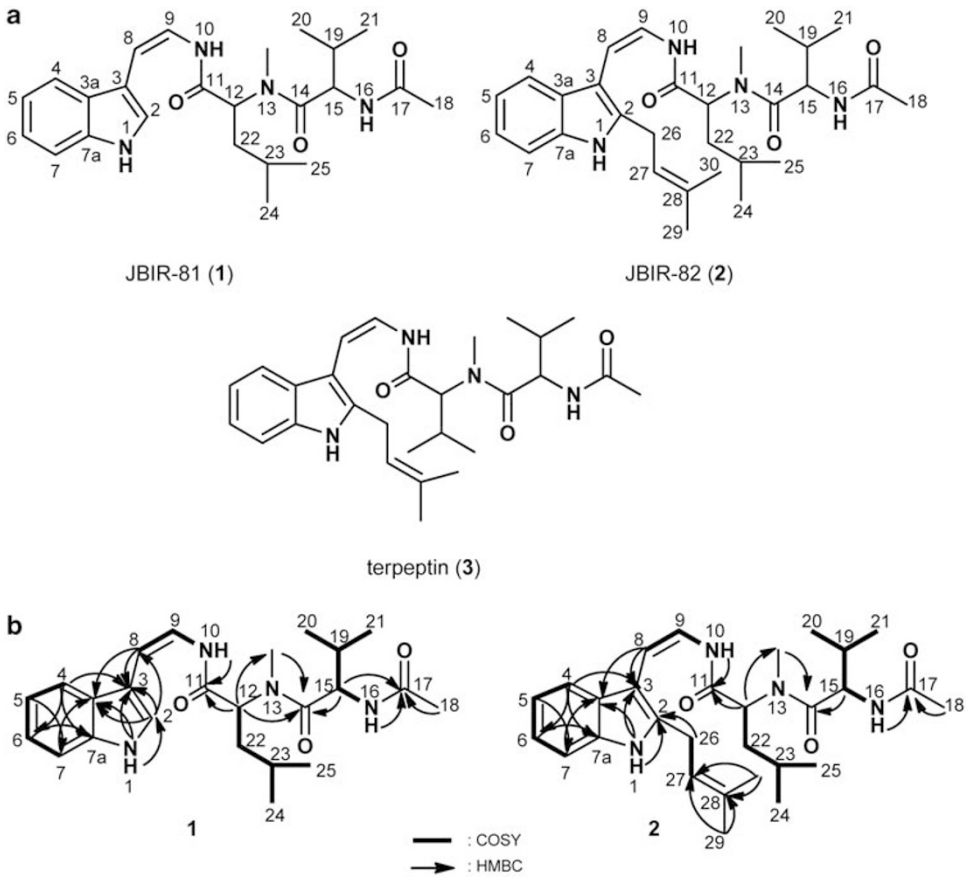

Figure 1 (**a**) Structures of JBIR-81 (**1**), JBIR-82 (**2**) and terpeptin (**3**). (**b**) Correlations in DQF-COSY (bold lines) and CT-HMBC (arrows) spectra of **1** and **2**.

4-H (δ_H 7.57), 5-H (δ_H 7.03), 6-H (δ_H 7.12) and 7-H (δ_H 7.38), revealed a 1,2-disubstituted benzene ring. In the HMBC spectrum, the aromatic proton 4-H was *peri* coupled to an aromatic quaternary carbon C-3 (δ_C 109.8), an olefinic proton 2-H (δ_H 7.56) was coupled to C-3 and an aromatic quaternary carbon C-3a (δ_C 126.8), and an amino proton 1-NH (δ_H 11.32) was coupled to an olefinic carbon C-2 (δ_C 123.7), C-3 and C-3a. Taking into consideration these correlations, the UV absorption and ^{13}C chemical shifts, a 3-substituted indole chromophore was established. The sequence from an olefinic proton 8-H (δ_H 5.93) to an amide proton 10-NH (δ_H 8.95) through an olefinic proton 9-H (δ_H 6.62) in the DQF-COSY spectrum determined an ethenamine moiety. The configuration at C-8 was deduced to be *cis* by the coupling constants between 8-H and 9-H (J=9.4 Hz). The ethenamine moiety was connected to C-3 of the indole by the ^1H-^{13}C long-range couplings from 8-H to C-3 and C-3a, and from 2-H to C-8 (δ_C 103.3). Therefore, these results indicated (*Z*)-2-(1*H*-indol-3-yl)ethenamine.

The remaining peptide sequence, *N*-methyl leucine-*N*-acetyl valine, was elucidated as follows. The sequence from an α-methine proton 12-H (δ_H 5.25) to a methyl proton 24-H (δ_H 0.88) through methylene protons 22-H (δ_H 1.68, 1.59) and a methine proton 23-H (δ_H 1.40), which in turn spin coupled to a methyl proton 25-H (δ_H 0.79), was observed in the DQF-COSY spectrum. In addition, ^1H-^{13}C long-range couplings from the α-methine proton 12-H to an amide carbonyl carbon C-11 (δ_c 169.3) and an *N*-methyl carbon 13-CH_3 (δ_c 31.2) established an *N*-methylleucine residue. Furthermore, the sequence from an amide proton 16-NH (δ_H 8.11) to a methyl proton 20-H (δ_H 0.73) through an α-methine proton 15-H (δ_H 4.47) and a methine proton 19-H (δ_H 1.91), which was coupled to a methyl proton 21-H (δ_H 0.81), together with the ^1H-^{13}C long-range couplings between the α-methine proton 15-H and an amide carbonyl carbon C-14 (δ_c 173.3) revealed a valine residue. Finally, ^1H-^{13}C long-range couplings from the amide proton 10-NH to the amide carbonyl carbon C-11, from the *N*-methyl proton 13-CH_3 (δ_H 3.02) to the amide carbonyl carbon C-14, and from the amide proton 16-NH and an acyl methyl proton 18-H (δ_H 1.81) to an amide carbonyl carbon C-17 (δ_c 169.2), established the connectivity of these amino-acid derived partial residues. Thus, the planar structure of **1** was determined as shown in Figure 1a.

Compound **2** is structurally related to **1,** in which the olefinic proton 2-H is replaced by a dimethylallyl moiety. Correlations interpreted by DQF-COSY and HMBC spectra proved common partial structures in **2** (Figure 1b). Both singlet methyl protons 29-H (δ_H 1.68) and 30-H (δ_H 1.67) were ^1H-^{13}C long-range coupled to each other, and commonly coupled to an olefinic quaternary carbon C-28 (δ_C 132.6) and an olefinic methine carbon C-27 (δ_C 121.1), of which proton was ^1H spin-coupled to methylene proton 26-H (δ_H 3.35). Thus, a dimethylallyl moiety was determined as shown in Figure 1b. ^1H-^{13}C long-range couplings from the methylene proton 26-H and the amino proton 1-NH (δ_H 10.99) to an olefinic quaternary carbon C-2 (δ_C 136.9) confirmed the structure of **2** as shown in Figure 1a. To our knowledge, this is the first report of these terpeptin analogs.

To evaluate the free radical scavenging activities of **1–3**, we tested the protective activity against L-glutamate toxicity in N18-RE-105 cells,[13–15] which can be used to assess free radical scavenging activity. Compounds **1–3** showed stronger protective activity against

Table 1 ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR data for 1 and 2

	1		*2*	
Number	δ_C	δ_H *(multiplicity, J=Hz)*	δ_C	δ_H *(multiplicity, J=Hz)*
1		11.32 (d, 8.2)		10.99 (s)
2	123.7	7.56 (d, 8.2)	136.9	
3	109.8		105.4	
3a	126.8		127.1	
4	118.6	7.57 (d, 7.3)	118.7	7.25 (d, 7.3)
5	119.3	7.03 (t, 7.3)	119.0	6.95 (t, 7.3)
6	121.9	7.12 (dd, 8.2, 7.3)	120.7	7.03 (t, 7.3)
7	111.7	7.38 (d, 8.2)	111.1	7.30 (d, 7.3)
7a	135.8		135.9	
8	103.3	5.93 (d, 9.4)	103.8	5.77 (d, 9.1)
9	118.4	6.62 (dd, 10.3, 9.4)	121.1	6.76 (dd, 10.0, 9.1)
10		8.95 (d, 10.3)		8.78 (d, 10.0)
11	169.3		169.1	
12	54.2	5.25 (dd, 10.1, 5.6)	54.0	5.14 (dd, 10.9, 5.0)
13-Me	31.2	3.02 (s)	31.2	2.97 (s)
14	173.3		172.9	
15	54.3	4.47 (dd, 8.8, 8.5)	53.9	4.40 (dd, 8.8, 8.5)
16		8.11 (d, 8.5)		8.05 (d, 8.5)
17	169.2		169.2	
18	22.3	1.81 (s)	22.3	1.77 (s)
19	30.1	1.91 (dqq, 8.8, 7.8, 7.0)	30.0	1.71 (dqq, 8.8, 6.8, 6.5)
20	19.0	0.73 (d, 7.8)	19.0	0.64 (d, 6.5)
21	18.6	0.81 (d, 7.0)	18.3	0.74 (d, 6.8)
22	36.5	1.68 (ddd, 14.0, 5.6, 4.7)	37.0	1.65 (ddd, 14.8, 5.0, 4.2)
		1.59 (ddd, 14.0, 10.1, 5.8)		1.54 (ddd, 14.8, 10.9, 5.4)
23	24.7	1.40 (ddqq, 6.5, 6.5, 5.8, 4.7)	24.6	1.33 (ddqq, 6.5, 6.5, 5.4, 4.2)
24	23.4	0.88 (d, 6.5)	23.4	0.83 (d, 6.5)
25	21.6	0.79 (d, 6.5)	21.4	0.71 (d, 6.5)
26			25.9	3.35 (d, 6.0)
27			121.1	5.31 (t, 6.0)
28			132.6	
29			25.7	1.68 (s)
30			17.9	1.67 (s)

NMR spectra were obtained with the Varian NMR system 600 NB CL (Palo Alto, CA, USA) in DMSO-d_6, and the solvent peak was used as an internal standard (δ_H 2.49 and δ_C 37.9 p.p.m.).

L-glutamate toxicity in cells with EC_{50} values of 0.7, 1.5 and 0.9 μM, respectively, compared to that of a representative antioxidant, α-tocopherol (EC_{50}=8.8 μM). Details of the biological activities of **1–3** are now under examination.

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