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Cyclohexadepsipeptides from *Acremonium* sp. BCC 28424

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ABSTRACT

Six new cyclohexadepsipeptides, beauvenniatins A–E (**1**–**5**), and beauvericin J (**6**), together with the known beauvericin (**7**) and enniatin B (**8**), were isolated from the fungus *Acremonium* sp. BCC 28424. The productions of minor derivatives **3**–**6**, possessing an *N*-methyl-L-tyrosine, were enhanced by feeding L-tyrosine in the liquid fermentation medium. Antimalarial, antitubercular, and cytotoxic activities of these cyclodepsipeptides were evaluated.

1. Introduction

Beauvericins and enniatins are cyclohexadepsipeptides composed of three units of *N*-methyl-L-amino acid alternately connected to three units of (*R*)-2-hydroxy acid, most commonly (*R*)-2-hydroxyisovaleric acid (D-Hiv).[1] Beauvericins contain three units of *N*-methyl-L-phenylalanine (*N*-Me-L-Phe), whereas enniatins possess various combinations of three aliphatic amino acids most commonly *N*-methyl-L-valine (*N*-Me-L-Val), *N*-methyl-L-leucine (*N*-Me-L-Leu), and *N*-methyl-L-isoleucine (*N*-Me-L-Ile). Some minor beauvericins[2,3] and enniatins[4–6] possess one or more (2*R*,3*S*)-2-hydroxy-3-methylpentanoic acid residue(s) instead of D-Hiv. These fungal secondary metabolites are known to exhibit a broad range of biological activities, such as insecticidal,[7–9] anthermintic,[10] antibacterial,[11,12] antifungal,[13] antiplasmodial,[3–6,14] antimycobacterial,[3–6,14] and anticancer activities. They are also known inhibitors of acyl-CoA:cholesterol acyltransferase (ACAT)[15] and HIV type 1 integrase.[16] Herein we report the isolation of six new cyclohexadepsipeptides, beauvenniatins A–E (**1**–**5**), and beauvericin J (**6**), along with beauvericin (**7**) and enniatin B (**8**), from the fungus *Acremonium* sp. BCC 28424. Beauvenniatins possess scrambled aromatic/aliphatic *N*-methyl-L-amino acid side chains. Compounds **3**–**6** represent the first examples in the beauvericin/enniatin class cyclohexadepsipeptides containing an *N*-methyl-L-tyrosine (*N*-Me-L-Tyr).

2. Results and discussion

Beauvenniatin A (**1**) was isolated as a colorless solid, and the molecular formula was established by HRESIMS as $C_{41}H_{57}N_3O_9$. The IR spectrum exhibited intense and broad absorption bands at ν_{max} 1742 and 1658 cm^{-1}, indicative of esters and amides, respectively, which are similar to those of known beauvericins and enniatins. The analysis of ^1H and ^{13}C NMR, DEPT135, COSY, HMQC, and HMBC spectroscopic data in $CDCl_3$ revealed that **1** was a hexadepsipeptide composed of three units of Hiv, two units of *N*-Me-Phe and one unit of *N*-Me-Val (Table 1). Three N–CH_3 groups at δ_H 3.03, 2.89, and 3.16 exhibited intense NOESY correlations to H-2 of three Hiv residues at δ_H 4.87, 4.87, and 5.20, respectively, which indicated the presence of three amide linkages. The molecular formula required three ester bonds to form a cyclohexadepsipeptide. The 2*S*-configuration of the *N*-Me-Phe (2 units) and *N*-Me-Val and the 2*R*-configuration of the Hiv (3 units) in **1** were confirmed by HPLC analysis of its acid hydrolyzate using a chiral column. The NMR data assignment of protons and carbons for three nonequivalent D-Hiv and two nonequivalent *N*-Me-L-Phe were addressed by correlation to the data for **4** and **5** (see Table 1), which are discussed below.

The molecular formula of beauvenniatin B (**2**), $C_{37}H_{57}N_3O_9$, was determined by HRESIMS. The analysis of 2D NMR data demonstrated the presence of three D-Hiv, one *N*-Me-L-Phe, and two *N*-Me-L-Val. Intense NOESY correlations from three N–CH_3 groups at δ_H 2.85, 3.02, and 3.04 to H-2 of three Hiv residues at δ_H 4.80, 5.30, and 5.08, respectively, indicated three amide bonds. Taking together the molecular formula (HRESIMS), the only possible structure was the cyclohexadepsipeptide (**2**) with alternate linkages of

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Table 1
^{13}C (125 MHz) and ^{1}H (500 MHz) NMR data for **1**, **4**, and **5** in $CDCl_3$

Position	Beauvenniatin A (**1**)		Beauvenniatin D (**4**)		Beauvenniatin E (**5**)	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
	N-Me-L-Phe-1		*N*-Me-L-Phe		*N*-Me-L-Tyr	
1 (*C*=O)	169.91[a]		169.9[c]		170.1[d]	
2	57.6	5.50, m	57.6	5.51, m	57.4	5.57, m
3	35.2	3.39, dd (14.4, 5.2)	35.2	3.41, dd (14.5, 5.1)	34.4	3.32, dd (14.7, 4.8)
		3.02, m		3.04, dd (14.5, 12.3)		2.94, dd (14.7, 12.4)
4	136.6		136.5		127.5	
5, 9	129.0	7.27–7.22, m	129.0	7.25–7.22, m	130.0	7.08, d (8.4)
6, 8	128.6[b]	7.27–7.22, m	128.5	7.25–7.22, m	115.6	6.79, d (8.4)
7	126.8	7.18, m	126.8	7.17, m	155.2	
N–CH_3	32.6	3.03, s	32.6	3.00, s	32.2	3.08, s
	N-Me-L-Phe-2		*N*-Me-L-Tyr		*N*-Me-L-Phe	
1 (*C*=O)	169.86[a]		169.8[c]		170.0[d]	
2	60.3	4.86, m	60.1	5.07, m	60.5	4.90, m
3	34.8	3.36, dd (14.5, 4.4)	33.9	3.29, dd (14.6, 4.2)	34.7	3.38, dd (14.6, 4.3)
		3.02, m		2.92, dd (14.6, 12.4)		3.03, dd (14.6, 12.0)
4	137.3		128.5		137.4	
5, 9	129.0	7.27–7.22, m	130.0	7.03, d (8.3)	129.0	7.27–7.25, m
6, 8	128.5[b]	7.27–7.22, m	115.6	6.79, d (8.3)	128.6	7.27–7.25, m
7	126.8	7.18, m	155.2		126.7	7.19, m
N–CH_3	34.2	2.89, s	33.8	2.97, s	34.3	2.89, s
	N-Me-L-Val		*N*-Me-L-Val		*N*-Me-L-Val	
1 (*C*=O)	170.4		170.5		170.5	
2	61.2	4.95, d (10.4)	61.4	4.91, d (10.5)	61.2	4.94, d (10.4)
3	27.4	2.22, m	27.5	2.22, m	27.3	2.22, m
4	19.9	1.02, d (6.6)	19.9	1.03, d (6.6)	19.9	1.02, d (6.5)
4′	19.3	0.88, d (6.8)	19.4	0.90, d (7.0)	19.4	0.89, d (6.7)
N–CH_3	31.6	3.16, s	31.7	3.19, s	31.5	3.17, s
	D-Hiv-1		D-Hiv-1		D-Hiv-1	
1 (*C*=O)	169.4		169.7[c]		169.9[d]	
2	76.0	4.87, d (8.7)	76.8	4.82, d (8.9)	76.2	4.89, d (8.9)
3	29.5	1.98, m	29.9	1.92, m	29.5	1.86, m
4	17.5	0.44, d (8.7)	17.5	0.44, d (6.6)	17.4	0.43, d (6.6)
4′	18.4	0.81, d (6.6)	18.3	0.81, d (6.6)	18.2[a]	0.82, d (6.6)
	D-Hiv-2		D-Hiv-2		D-Hiv-2	
1 (*C*=O)	169.3		169.7[a]		169.5	
2	76.0	4.87, d (8.7)	76.0	4.90, d (7.9)	76.0	4.87, d (7.6)
3	29.9	2.01, m	29.5	1.94, m	29.8	1.88, m
4	18.1	0.58, d (6.8)	18.1	0.53, d (6.6)	18.3[e]	0.58, d (6.8)
4′	18.6	0.85, d (6.6)	18.4	0.83, d (6.6)	18.4[e]	0.85, d (6.6)
	D-Hiv-3		D-Hiv-3		D-Hiv-3	
1 (C=O)	169.6		169.6[c]		169.9[d]	
2	75.0	5.20, d (9.1)	75.1	5.08, d (8.7)	75.0	5.18, d (9.0)
3	30.0	2.31, m	30.0	2.27, m	30.1	2.31, m
4	18.3	0.90, d (6.8)	18.2	0.96, d (7.1)	18.2	0.91, d (7.1)
4′	18.2	0.92, d (6.7)	18.6	0.95, d (6.6)	18.6	0.95, d (6.6)

[a–e] The assignment of carbons can be interchanged.

amide and ester. The absolute configuration of each residue was confirmed by the chiral HPLC analysis of the acid hydrolyzate.

Beauvenniatin C (**3**) possessed the molecular formula $C_{37}H_{57}N_3O_{10}$ (HRESIMS), which was one oxygen atom more than **2**. The ^{1}H and ^{13}C NMR spectroscopic data were very similar to those of **2**. The significant difference was found at the aromatic ring moiety. Thus, two pairs of nonequivalent sp^2 methines at δ_H 7.06 (2H, H-5/H-9) and 6.78 (2H, H-6/H-8) vicinally coupled with a *J* value of 8.2 Hz. Inspection of 2D NMR data (COSY, NOESY, HMQC, and HMBC) revealed an *N*-Me-Tyr unit instead of *N*-Me-L-Phe in **2**. Other residues were assigned as three Hiv and two *N*-Me-Val. The close chemical shifts of protons and carbons with those of **2** (Table 2) strongly suggested that **3** was the *N*-Me-L-Tyr variant of **2** and the sequence and the absolute configuration of all residues were also identical to those of **2**.

The ^{1}H NMR spectra and the ESIMS data of the minor analogues **4**–**6** suggested their proposed structures possessing an *N*-Me-L-Tyr, similar to **3**. Due to the poor material quantities, we examined precursor-directed biosynthesis.[3,4,17–19] As expected, feeding L-tyrosine (10 mM) in the liquid medium (potato dextrose broth, PDB) led to increased production of the *N*-Me-L-Tyr-containing analogues **3**–**6** (Fig. 2).

Beauvenniatin D (**4**) had the molecular formula $C_{41}H_{57}N_3O_{10}$ (HRESIMS). The six residues were addressed by 2D NMR spectroscopic analysis to be three Hiv, an *N*-Me-Phe, an *N*-Me-Tyr, and an *N*-Me-Val. NOESY correlations from the N–CH_3 of *N*-Me-Phe to H-2 of Hiv-1, from N–CH_3 of *N*-Me-Tyr to H-2 of Hiv-2, and from N–CH_3 of *N*-Me-Val to H-2 of Hiv-3, and several other key NOESY correlations shown in Fig. 3 indicated the three amide-linked fragments: D-Hiv-1–*N*-Me-L-Phe, D-Hiv-2–*N*-Me-L-Tyr, and D-Hiv-3–*N*-Me-L-Val. Among the six carbonyl carbons (C-1), one of them resonated relatively slightly downfield at δ_C 170.5, while others exhibited very close chemical shifts and overlapped at δ_C 169.9–169.6. HMBC correlations from N–CH_3 of *N*-Me-L-Val (δ_H 3.19, s) and H-2 of D-Hiv-2 (δ_H 4.90, d, *J*=7.9 Hz) to the former resolved carbonyl carbon enabled its assignment as C-1 of *N*-Me-L-Val, and the ester linkage of *N*-Me-L-Val–D-Hiv-2 (Fig. 3). Two remaining ester bonds were not established by HMBC data due to the close chemical shifts of carbonyl carbons. However, on the basis of the molecular formula requirement to form a cyclohexadepsipeptide, the structural formula 4 (Fig. 1) was the only possible sequence for beauvenniatin D.

Beauvenniatin E (**5**) had the same molecular formula as **4**, $C_{41}H_{57}N_3O_{10}$ (HRESIMS). The six residues were the same as those of **4**:

Table 2
^{13}C (125 MHz) and ^{1}H (500 MHz) NMR data for **2** and **3** in $CDCl_3$

Position	Beauvenniatin B (**2**)		Beauvenniatin C (**3**)	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
	N-Me-L-Phe		*N*-Me-L-Tyr	
1 (*C*=O)	169.4[a]		169.5[c]	
2	62.1	4.57, m	61.8	4.68, m
3	35.1	3.41, dd (14.2, 4.4); 3.18, m	34.2	3.35, dd (14.3, 4.3), 3.09, m
4	137.6		129.1	
5, 9	129.2	7.27–7.25, m	130.2	7.09, d (8.2)
6, 8	128.5	7.27–7.25, m	115.5	6.78, d (8.2)
7	126.7	7.19, m	154.8	
N–*CH*$_3$	35.6	2.85, s	35.3	2.91, s
	N-Me-L-Val-1[e]		*N*-Me-L-Val-1[f]	
1 (*C*=O)	170.4		170.4	
2	62.4	4.58, d (10.4)	62.6	4.56, d (10.3)
3	27.4	2.21, m	27.4	2.23, m
4	20.1	1.01, d (6.6)	20.1	1.02, d (6.5)
4′	19.5	0.86, d (6.6)	19.6	0.90, d (6.7)
N–*CH*$_3$	32.1	3.02, s	32.2	3.17, s
	N-Me-L-Val-2[e]		*N*-Me-L-Val-2[f]	
1 (*C*=O)	170.4		170.4	
2	61.1	5.00, d (10.3)	61.2	4.99, d (10.2)
3	27.8	2.23, m	27.8	2.24, m
4	19.8	1.02, d (6.6)	19.8	1.02, d (6.5)
4′	19.1	0.88, m	19.2	0.90, d (6.7)
N–*CH*$_3$	31.5	3.04, s	31.5	3.16, s
	D-Hiv-1		D-Hiv-1	
1 (*C*=O)	169.4[a]		169.5[c]	
2	77.7	4.80, d (9.0)	77.2	4.84, d (8.9)
3	29.5	2.01, m	29.5	2.02, m
4	18.2[a]	0.71, d (6.6)	18.2[a]	0.70, d (6.6)
4′	18.4[a]	0.88, m	18.3[a]	0.89, d (6.5)
	D-Hiv-2[g]		D-Hiv-2[h]	
1 (*C*=O)	169.3[a]		169.4[a]	
2	75.0	5.30, d (8.8)	75.1	5.27, d (8.4)
3	30.3	2.31, m	30.3	2.29, m
4	18.3[a]	0.90, d (6.9)	18.4[a]	0.92, d (6.8)
4′	18.7[a]	0.95, d (6.9)	18.6[a]	0.98, d (6.8)
	D-Hiv-3[g]		D-Hiv-3[h]	
1 (*C*=O)	169.9		170.0[c]	
2	75.3	5.08, d (8.7)	75.2	5.07, d (8.6)
3	29.8	2.24, m	29.9	2.25, m
4	18.4[b]	0.92, d (6.8)	18.5[d]	0.95, d (6.8)
4′	18.9[b]	0.97, d (6.7)	18.9[d]	0.97, d (6.8)

[a–d] The assignment of carbons can be interchanged.
[e,f] Two sets of proton and carbon assignments for *N*-Me-L-Val-1 and *N*-Me-L-Val-1 can be interchanged.
[g,h] Two sets of proton and carbon assignments for D-Hiv-2 and D-Hiv-3 can be interchanged.

three D-Hiv, an *N*-Me-L-Phe, an *N*-Me-L-Tyr, and an *N*-Me-L-Val. NOESY correlations from the N–C*H*$_3$ of *N*-Me-L-Tyr to H-2 of D-Hiv-1, from N–C*H*$_3$ of *N*-Me-L-Phe to H-2 of D-Hiv-2, and from N–C*H*$_3$ of *N*-Me-L-Val to H-2 of D-Hiv-3, revealed three amide-linked fragments: D-Hiv-1–*N*-Me-L-Tyr, D-Hiv-2–*N*-Me-L-Phe, and D-Hiv-3–*N*-Me-L-Val. Similarly to **4**, HMBC correlations from the N–C*H*$_3$ of *N*-Me-L-Val (δ_H 3.17, s) and H-2 of D-Hiv-2 (δ_H 4.87, d, *J*=7.6 Hz) to the relatively downfield shifted carbonyl (δ_C 170.5, C-1 of *N*-Me-L-Val) demonstrated the major fragment of D-Hiv-3–*N*-Me-L-Val–D-Hiv-2–*N*-Me-L-Phe. Other HMBC and NOESY correlations were similar to those observed for **4**. Therefore, beauveniatin E (**5**) was assigned as the isomer of beauveniatin D (**4**), where the location of *N*-Me-L-Phe and *N*-Me-L-Tyr was exchanged. As shown in Table 1, chemical shifts of carbons and protons are very close among **1**, **4**, and **5**, which also suggested that these cyclodepsipeptides adopt identical conformation in $CDCl_3$.

Inspection of the NMR spectroscopic data for beauvericin J (**6**) revealed that it composed of three D-Hiv, an *N*-Me-L-Tyr, and two *N*-Me-L-Phe (Table 3). Intense NOESY correlations from three N–C*H*$_3$ groups (δ_H 3.06, 3.02, and 3.01) to H-2 of D-Hiv strongly suggested the alternate linkage of three amide and three ester bonds. It was therefore assigned as a structural variant of beauvericin (**7**), where one *N*-Me-L-Phe was replaced by *N*-Me-L-Tyr.

Compounds **1**–**8** were evaluated against *Plasmodium falciparum* K1, *Mycobacterium tuberculosis* H37Ra, and three cancer cell lines (KB, MCF-7, and NCI-H187) and nonmalignant Vero cells (Table 4). Beauvenniatins A (**1**) and B (**2**) exhibited similar level of biological activities as the two most commonly occurring analogues beauvericin (**7**) and enniatin B (**8**). Compounds **3**–**6**, possessing an *N*-Me-L-Tyr residue, showed relatively weaker activities. In particular, beauvenniatin D (**4**) was inactive in these assays.

Beauvericin (**7**) was first isolated from the entomopathogenic fungus *Beauveria bassiana*.[7] Later, it was found in other species of *Beauveria* and other entomogenous fungi *Isaria tenuipes* (formerly *Paecilomyces tenuipes*),[14] *Isaria fumosorosea* (formerly *Paecilomyces fumosoroseus*),[20] and *Cordyceps cicadae*,[21] all of these species are members of family *Cordycipitaceae*.[22] Enniatins have been most often isolated from *Fusarium* species. While Cordycipitalean fungi produce only beauvericin (**7**) and its minor analogues, *Fusarium* species often show co-production of both cyclodepsipeptides.[23,24] Beauvericins and enniatins are biosynthesized by the nonribosomal peptide synthetases, respectively, known as beauvericin synthetase and enniatin synthetase. Detailed biosynthesis and biochemistry of these cyclodepsipeptides have been pioneered by Zocher's group.[25] Both synthetases are very similar with the only significant difference found in the adenylation domains with regard to the L-amino acid recognition. The absence of analogues with scrambled aromatic/aliphatic *N*-methyl-L-amino acids in the *Fusarium* species suggests that beauvericins and enniatins were probably produced by independent synthetases existing in the same fungus. The scrambled aromatic/aliphatic amino acid sequence is known only in beauvericin E (possessing two *N*-Me-L-Phe and one *N*-Me-L-Leu), which was isolated as a minor constituent together with beauvericin from *Beauveria* sp. FKI-1366.[8] The compositions of the metabolites from this fungus[8] implies that the structure of the depsipeptide synthetase responsible for the biosynthesis of beauvericin E and beauvericin may be very closely related to usual beauvericin synthetases rather than enniatin synthetases. The present study demonstrates that compounds **1**–**8** are all produced by the same cyclodepsipeptide synthetase, namely beauvenniatin synthetase, of the fungus *Acremonium* sp. BCC 28424. The recognition of the L-amino acid side chain structure by this enzyme should be less specific when compared to those of Cordicipitalean fungi (beauvericin synthetases) and *Fusarium* spp. (beauvericin synthetases and enniatin synthetases). These evidences also suggest that beauvenniatin synthetase should more flexibly accept and incorporate various L-amino acid precursors in directed biosynthesis experiments. In this context, we are currently studying scope and limitation of the strain BCC 28424 in production of various beauvericin–enniatin analogues.

3. Experimental section

3.1. General procedures

Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a GBS Cintra 404 spectrophotometer. FTIR spectra were taken on Bruker VECTOR 22 and ALPHA spectrometers. NMR spectra were recorded on Bruker DRX400 and AV500D spectrometers. ESI-TOF mass spectra were measured with a Bruker micrOTOF mass spectrometer.

3.2. Fungal material

Acremonium sp. was isolated from a soil sample in Khlong Lan National Park, Kampheang Phet Province, Thailand. The living culture was deposited in the BIOTEC Culture Collection (BCC) on November 19, 2007 as BCC 28424.

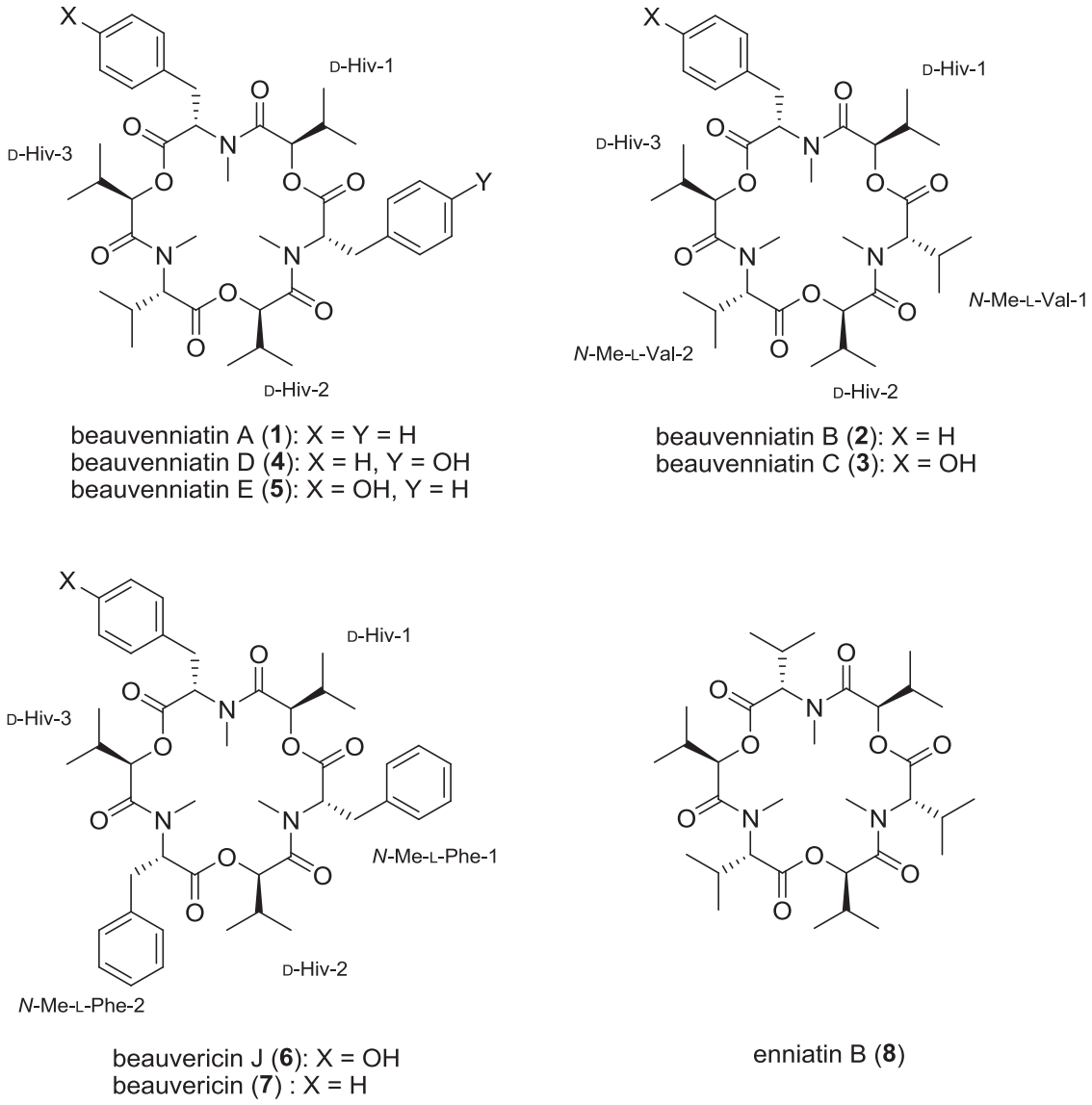

Fig. 1. Structures of cyclohexadepsipeptides isolated from *Acremonium* sp. BCC 28424.

3.3. Fermentation and isolation

The fungus BCC 28424 was maintained on potato dextrose agar at 25 °C. The agar was cut into small plugs and inoculated into 3×250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB; potato starch 4.0 g/L, dextrose 20.0 g/L). After incubation at 25 °C for 4 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (PDB), and incubated at 25 °C for 4 days on a rotary shaker (200 rpm). These secondary cultures were pooled and each 25 mL portion was transferred into 28×1 L Erlenmeyer flasks containing 250 mL of PDB, and final fermentation was carried out at 25 °C for 20 days under static conditions. The cultures were filtered to separate broth (filtrate) and mycelia (residue). The filtrate was extracted with EtOAc (2×6.5 L) to give a brown gum (717 mg, extract A). The wet mycelium was macerated in MeOH (2.8 L, rt, 2 days) and filtered. H_2O (350 mL) and hexanes (2.8 L) were added to the filtrate, and the layers were separated. The aqueous MeOH phase was partially concentrated by evaporation, and the residue was extracted with EtOAc (2×500 mL). The combined EtOAc solution was concentrated under reduced pressure to obtain a brown gum (3.37 g, extract B). The hexanes layer was concentrated under reduced pressure to leave a yellow wax (1.14 g, extract C). Extracts A, B, and C were separately subjected to chromatographic fractionation. Extract B was passed through a column on Sephadex LH-20 (3.8×50 cm) and eluded with MeOH to obtain five pooled fractions, B1 (57 mg), B2 (1.79 g), B3 (187 mg), B4 (721 mg), and B5 (87 mg). Fraction B2 was subjected to column chromatography (CC) on silica gel (3.0×15 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 7:93) to obtain eight fractions: fraction B2-1–B2-8. Fractions B2-1 (264 mg) and B2-2 (949 mg) were purified by preparative HPLC using a reversed-phase column (SunFire Prep C_{18} OBD, 19×250 mm, 10 μm; mobile phase MeCN/H_2O, 65:35, flow rate 15 mL/min) to furnish **8** (256 mg), **2** (375 mg), **1** (141 mg), and **7** (27 mg). Fractions B2-3 (32 mg) and B2-4 (22 mg) were identified as **8**. Fractions B2-5 (27 mg) and B2-6 (12 mg) were also purified by preparative HPLC (SunFire Prep C_{18} OBD, 19×150 mm, 5 μm; mobile phase MeCN/H_2O, 65:35, flow rate 10 mL/min) to afford **3** (3.3 mg), **4** (2.5 mg), **5** (1.7 mg), and **6** (0.8 mg). Fraction B3 was subjected to CC on silica gel (2.0×15 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 8:92) and the fractions were further separated by preparative HPLC (MeCN/H_2O, 65:35) to furnish **8** (9 mg), **2** (27 mg), **1** (19 mg), and **7** (5 mg). Fraction B-4 was fractionated by CC on silica gel (2.8×15 cm,

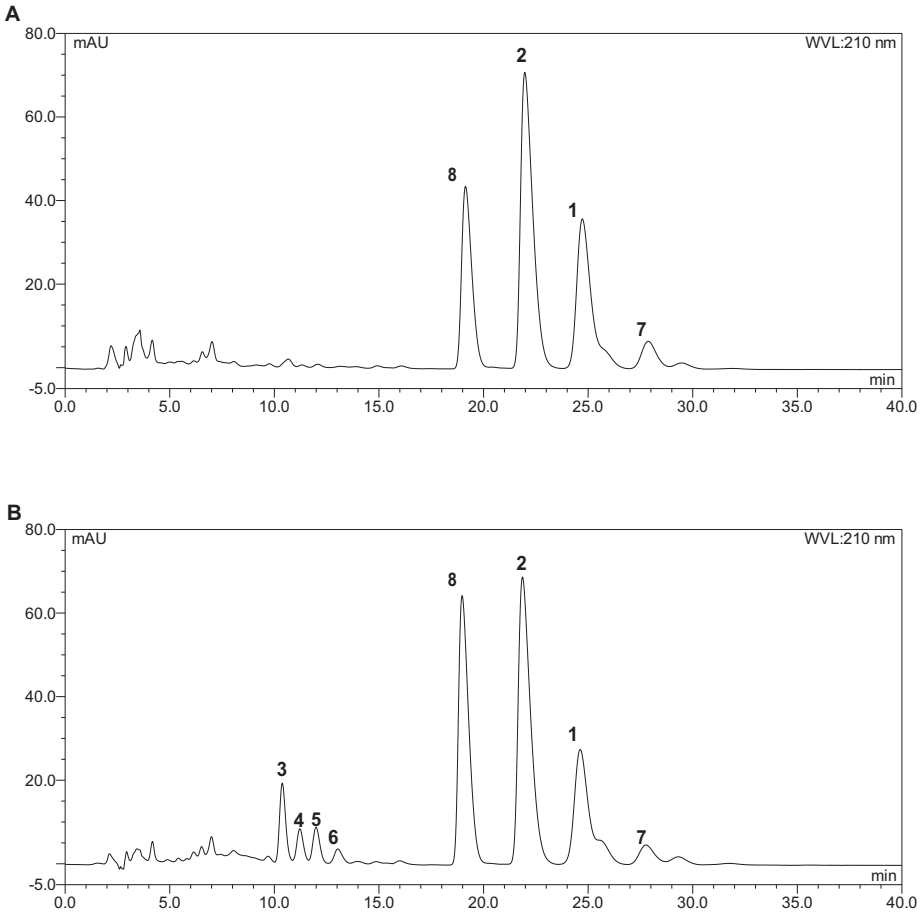

Fig. 2. HPLC chromatogram of the mycelial extracts from *Acremonium* sp. BCC 28424 (detection at 210 nm): (A) culture in PDB (non-precursor); (B) culture in PDB with L-tyrosine (10 mM).

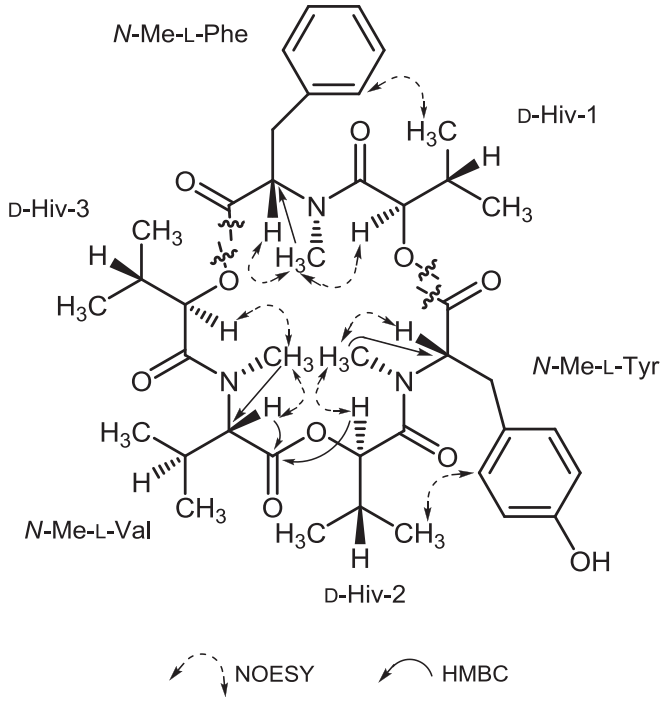

Fig. 3. Selected NOESY and HMBC correlations for beauvenniatin D (**4**).

Table 3
^{13}C (125 MHz) and ^{1}H (500 MHz) NMR data for beauvericin J (**6**) in $CDCl_3$

Position	δ_C	δ_H, mult. (*J* in Hz)
N-Me-L-Tyr		
1 (*C*=O)	170.2[a]	
2	57.3	5.64, m
3	33.9	3.33, dd (14.7, 4.6); 2.85, dd (14.7, 12.7)
4	127.5	7.05, d (8.3)
5, 9	129.7	6.80, d (8.3)
6, 8	115.6	
7	155.5	
N–*CH*$_3$	32.0	3.06, s
N-Me-L-Phe-1, *N*-Me-L-Phe-2		
1 (*C*=O)	170.1,[a] 170.0[a]	
2	57.5, 56.9	5.67, m; 5.61, m
3	34.7, 34.7	3.42, m; 3.39, m; 2.93, m; 2.92, m
4	136.7, 136.5	
5, 9	128.82, 128.77	7.26–7.20, m
6, 8	128.6, 128.6	7.26–7.20, m
7	126.8, 126.8	7.17, m; 7.17, m
N–*CH*$_3$	32.3, 32.0	3.02, s; 3.01, s
D-Hiv-1, D-Hiv-2, D-Hiv-3		
1 (*C*=O)	170.0,[a] 169.9,[a] 169.8[a]	
2	75.8, 75.7, 75.6	4.83, d (9.8); 4.81, d (9.8); 4.79, d (9.1)
3	29.8, 29.7, 29.7	1.95–1.90, m
4	17.5, 17.3, 17.3	0.37, d (6.8); 0.36, d (6.8); 0.33, d (6.8)
4′	18.4, 18.33, 18.26	0.80, d (6.2); 0.79, d (6.4); 0.79, d (6.4)

[a] The assignment of carbons can be interchanged.

Table 4
Biological activities of compounds **1**–**8**

Compound	Cytotoxicity (IC_{50}, μg/mL)[a]				Antimalaria[b] (IC_{50}, μg/mL)	Antituberculosis[c] (MIC, μg/mL)
	NCI-H187	MCF-7	KB	Vero		
Beauvenniatin A (**1**)	1.0	6.2	1.3	2.2	3.0	3.13
Beauvenniatin B (**2**)	0.92	12	1.6	1.3	3.0	3.13
Beauvenniatin C (**3**)	6.6	>50	14	7.0	3.4	>50
Beauvenniatin D (**4**)	>50	>50	>50	>50	>10	>50
Beauvenniatin E (**5**)	7.6	9.6	9.0	8.3	2.9	25
Beauvericin J (**6**)	8.4	>50	10	7.2	3.0	12.5
Beauvericin (**7**)	2.0	8.6	4.5	1.8	3.1	6.25
Enniatin B (**8**)	5.0	>50	4.9	2.7	3.0	25

[a] The IC_{50} values of a standard compound, doxorubicin hydrochloride, against NCI-H187, MCF-7, and KB cells were 0.13, 9.5, and 0.71 μg/mL, respectively. Ellipticine was used as a standard compound for the cytotoxicity assay against Vero cells (IC_{50} 1.6 μg/mL).
[b] Antimalarial activity against *P. falciparum* K1. Standard antimalarial drug, dihidroartemisinin, showed an IC_{50} value of 0.37 ng/mL.
[c] Antituberculosis activity against *M. tuberculosis* H37Ra. Standard anti-TB drug, isoniazid, showed MIC values of 0.0234–0.0468 μg/mL.

MeOH/CH_2Cl_2, step gradient elution from 0:100 to 10:90) to obtain aranorosinol A (156 mg). Extract C was fractionated by CC on silica gel (MeOH/CH_2Cl_2) and preparative HPLC (MeCN/H_2O) to afford **8** (142 mg), **2** (44 mg), **1** (21 mg), **7** (4 mg), and ergosterol (62 mg). Extract A was also purified using similar chromatographic procedures to furnish **8** (195 mg), **2** (15 mg), **1** (2 mg), and **7** (0.7 mg).

*3.3.1. Beauvenniatin A (**1**).* Colorless solid; mp 90–91 °C; $[\alpha]_D^{25}$ −93 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 sh (4.35) nm; IR (KBr disk) ν_{max} 1742, 1658, 1200, 1018 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 758.3981 $[M+Na]^+$ (calcd for $C_{41}H_{57}N_3O_9Na$, 758.3987).

*3.3.2. Beauvenniatin B (**2**).* Colorless solid; mp 84–85 °C; $[\alpha]_D^{26}$ −93 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 sh (4.51) nm; IR (KBr disk) ν_{max} 1739, 1660, 1201, 1018 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 2; HRMS (ESI-TOF) *m*/*z* 710.3989 $[M+Na]^+$ (calcd for $C_{37}H_{57}N_3O_9Na$, 710.3987).

*3.3.3. Beauvenniatin C (**3**).* Colorless solid; mp 127–128 °C; $[\alpha]_D^{26}$ −84 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 sh (4.23), 223 sh (4.11), 278 (3.19) nm; IR (ATR) ν_{max} 1743, 1642, 1177, 1010 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 2; HRMS (ESI-TOF) *m*/*z* 726.3937 $[M+Na]^+$ (calcd for $C_{37}H_{57}N_3O_{10}Na$, 726.3936).

*3.3.4. Beauvericin (**7**).* Colorless solid; $[\alpha]_D^{25}$ +53 (*c* 0.40, MeOH); ESIMS, ^1H NMR, and ^{13}C NMR data consistent with published data.[7,8,14]

*3.3.5. Enniatin B (**8**).* Colorless solid; $[\alpha]_D^{25}$ −100 (*c* 0.60, MeOH); ESIMS, ^1H NMR, and ^{13}C NMR data consistent with published data.[26,27]

3.4. Precursor-directed biosynthesis

The seed cultures were prepared in the same manner as described above. Final fermentation was performed in 8×1 L Erlenmeyer flasks containing 250 mL of PDB with 10 mM of L-tyrosine for 22 days. The cultures were filtered to separate broth (filtrate) and mycelia (residue). The wet mycelia were macerated in MeOH (400 mL, rt, 2 days). H_2O (100 mL) and hexanes (400 mL) were added to the filtrate, and the layers were separated. The aqueous MeOH layer was partially concentrated by evaporation, and the residue was extracted with EtOAc (3×120 mL). The EtOAc solution was concentrated under reduced pressure to obtain a brown gum (1.39 g, mycelial extract). This extract was fractionated by CC on Sephadex LH-20 (3.8×58 cm, MeOH) and the major pooled fraction, which contained cyclodepsipeptides (fraction 2, 1.05 g), was subjected to CC on silica gel (3.5×15 cm, MeOH/CH_2Cl_2, step gradient elution from 0:100 to 9:91) to obtain eight fractions: fraction 2–1 to 2–8. Fractions 2–3 (320 mg) and 2–4 (449 mg) were purified by preparative HPLC (SunFire Prep C_{18} OBD, 19×250 mm, 10 μm; mobile phase MeCN/H_2O, 65:35, flow rate 15 mL/min) to furnish **8** (228 mg), **2** (264 mg), **1** (117 mg), and **7** (22 mg). Fractions 2–6 (21 mg), 2–7 (51 mg), and 2–8 (85 mg) were mixtures of *N*-Me-L-Tyr-containing cyclodepsipeptides, which were further separated by preparative HPLC (SunFire Prep C_{18} OBD, 19×250 mm, 10 μm; mobile phase MeCN/H_2O, 58:42, flow rate 15 mL/min) to obtain **3** (40 mg), **4** (14 mg), **5** (12 mg), and **6** (6.2 mg).

*3.4.1. Beauvenniatin D (**4**).* Colorless solid; mp 123–124 °C; $[\alpha]_D^{26}$ −67 (*c* 0.105, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 sh (4.46), 226 sh (4.17), 278 (3.18) nm; IR (ATR) ν_{max} 1750, 1639, 1186, 1011 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 774.3934 $[M+Na]^+$ (calcd for $C_{41}H_{57}N_3O_{10}Na$, 774.3936).

*3.4.2. Beauvenniatin E (**5**).* Colorless solid; mp 123–124 °C; $[\alpha]_D^{26}$ −63 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 sh (4.45), 226 sh (4.15), 278 (3.20) nm; IR (ATR) ν_{max} 1741, 1647, 1175, 1011 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 774.3938 $[M+Na]^+$ (calcd for $C_{41}H_{57}N_3O_{10}Na$, 774.3936).

*3.4.3. Beauvericin J (**6**).* Colorless solid; mp 117–118 °C; $[\alpha]_D^{26}$ +7 (*c* 0.135, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 sh (4.59), 227 sh (4.18), 278 (3.33) nm; IR (ATR) ν_{max} 1742, 1647, 1175, 1014 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 3; HRMS (ESI-TOF) *m*/*z* 822.3942 $[M+Na]^+$ (calcd for $C_{45}H_{57}N_3O_{10}Na$, 822.3936).

3.5. HPLC analysis of the acid hydrolysates of 1 and 2 using a chiral column

Compound **1** (1.5 mg) was hydrolyzed with 6 M HCl (0.5 mL) at 110 °C for 15 h. After concentration to dryness, the residue was dissolved in MeOH (200 μL) and subjected to HPLC analysis. In the same manner, compound **2** was hydrolyzed. HPLC analysis of the depsipeptide hydrolysates was performed using a ligand-exchange-type chiral column: Phenomenex Chirex 3126 (D)-penicillamine, 4.6×250 mm; mobile phase 2-propanol in 2 mM aqueous $CuSO_4$; flow rate 1 mL/min, UV 235 nm. The 2-hydroxyisovaleric acid standard was purchased from Aldrich. Two mobile phase conditions were employed due to the large retention time differences of the standard samples: (1) 15% 2-propanol in 2 mM aqueous $CuSO_4$, *N*-Me-L-Val and *N*-Me-D-Val (t_R 7.0 min, peaks were superimposed), *N*-Me-L-Phe (t_R 26.6 min), *N*-Me-D-Phe (t_R 30.8 min), L-Hiv (t_R 40.0 min), and D-Hiv (t_R 67.4 min); (2) 5% 2-propanol in 2 mM aqueous $CuSO_4$, *N*-Me-L-Val (t_R 11.0 min), *N*-Me-D-Val (t_R 13.9 min). Hydrolyzates of **1** and **2** contained *N*-Me-L-Val, *N*-Me-L-Phe, and D-Hiv.

3.6. Biological assays

Assay for activity against *P. falciparum* (K1, multi-drug resistant strain) was performed in duplicate using the microculture radioisotope technique.[28] Growth inhibitory activity against *M. tuberculosis* H37Ra and cytotoxicity to Vero cells (African green monkey kidney fibroblasts) were performed in triplicate using the green fluorescent protein microplate assay (GFPMA).[29] Anticancer activities against KB cells (oral human epidermoid carcinoma), MCF-7 cells (human breast cancer), and NCI-H187 cells (human small-cell lung cancer), were evaluated using the resazurin microplate assay (4 replicates).[30]

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Supplementary data

NMR spectra of compounds **1**–**6**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2011.08.041.

References and notes

1. Recent review on fungal cyclooligomer depsipeptides Süssmuth, R.; Müller, J.; von Döhren, H.; Molnár, I. *Nat. Prod. Rep.* **2011**, *28*, 99–124.
2. Gupta, S.; Montllor, C.; Hwang, Y.-S. *J. Nat. Prod.* **1995**, *58*, 733–738.
3. Nilanonta, C.; Isaka, M.; Kittakoop, P.; Trakulnaleamsai, S.; Tanticharoen, M.; Thebtaranonth, Y. *Tetrahedron* **2002**, *58*, 3355–3360.
4. Nilanonta, C.; Isaka, M.; Chanphen, R.; Thong-orn, N.; Tanticharoen, M.; Thebtaranonth, Y. *Tetrahedron* **2003**, *59*, 1015–1020.
5. Supothina, S.; Isaka, M.; Kirtikara, K.; Thebtaranonth, Y. *J. Antibiot.* **2004**, *57*, 732–738.
6. Vongvilai, P.; Isaka, M.; Kittakoop, P.; Srikitikulchai, P.; Kongsaeree, P.; Prabpai, S.; Thebtaranonth, Y. *Helv. Chim. Acta* **2004**, *87*, 2066–2073.
7. Hamill, R. L.; Higgens, C. E.; Boaz, H. E.; Gorman, M. *Tetrahedron Lett.* **1969**, 4225–4228.
8. Gupta, S.; Krasonoff, S. B.; Underwood, N. L.; Renwick, J. A. A.; Roberts, D. W. *Mycopathologia* **1991**, *115*, 185–189.
9. Grove, J. F.; Pople, M. *Mycopathologia* **1980**, *70*, 103–105.
10. Jeschke, P.; Benet-Buchholz, J.; Harder, A.; Etzel, W.; Schindler, M.; Thielking, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3285–3288.
11. Plattner, P. A.; Nager, U.; Boller, A. *Helv. Chim. Acta* **1948**, *31*, 594–602.
12. Gaumann, E.; Roth, S.; Ettlinger, L.; Plattner, P. A.; Nager, U. *Experientia* **1974**, *3*, 202–203.
13. (a) Fukuda, T.; Arai, M.; Yamaguchi, Y.; Masuma, R.; Tomoda, H.; Ōmura, S. *J. Antibiot.* **2004**, *57*, 110–116; (b) Fukuda, T.; Arai, M.; Tomoda, H.; Ōmura, S. *J. Antibiot.* **2004**, *57*, 117–124.
14. Nilanonta, C.; Isaka, M.; Kittakoop, P.; Palittapongarnpim, P.; Kamchonwongpaisan, S.; Pittayakhajonwut, D.; Tanticharoen, M.; Thebtaranonth, Y. *Planta Med.* **2000**, *66*, 756–758.
15. Tomoda, H.; Huang, X.-H.; Cao, J.; Nishida, H.; Nagao, R.; Okuda, S.; Tanaka, H.; Omura, S. *J. Antibiot.* **1992**, *45*, 1626–1632.
16. Shin, C.-G.; An, D.-G.; Song, H.-H.; Lee, C. *J. Antibiot.* **2009**, *62*, 687–690.
17. Klause, M.; Lindemann, A.; Glinski, M.; Hornbogen, T.; Bonse, G.; Jeschke, P.; Thielking, G.; Gau, W.; Kleinkauf, H.; Zocher, R. *J. Antibiot.* **2001**, *54*, 797–804.
18. Feifel, S. C.; Schmiederer, T.; Hornbogen, T.; Berg, H.; Süssmuth, R. D.; Zocher, R. *ChemBioChem* **2007**, *8*, 1767–1770.
19. Xu, Y.; Zhan, J.; Wijeratne, E. M. K.; Burns, A. M.; Gunatilaka, A. A. L.; Molnár, I. *J. Nat. Prod.* **2007**, *70*, 1467–1471.
20. Bernardini, M.; Carilli, A.; Pacioini, G.; Santurbano, B. *Phytochemistry* **1975**, *15*, 1865.
21. Kuo, Y.-C.; Lin, L.-C.; Don, M.-J.; Liao, H.-F.; Tsai, Y.-P.; Lee, G.-H.; Chou, C.-J. *J. Chin. Med.* **2002**, *13*, 209–219.
22. Luangsa-Ard, J. J.; Berkaew, P.; Ridkaew, R.; Hywel-Jones, N. L.; Isaka, M. *Mycol. Res.* **2009**, *113*, 1389–1395.
23. Logrieco, A.; Rizzo, A.; Ferracane, R.; Ritieni, A. *Appl. Environ. Microbiol.* **2002**, *62*, 82–85.
24. (a) Song, H.-H.; Ahn, J.-H.; Lim, Y. H.; Lee, C. *J. Microbiol. Biotechnol.* **2006**, *16*, 1111–1119; (b) Song, H.-H.; Lee, H.-S.; Jeong, J.-H.; Park, H.-S.; Lee, C. *Int. J. Food Microbiol.* **2008**, *122*, 296–301.
25. (a) Zocher, R.; Keller, U.; Kleinkauf, H. *Biochemistry* **1982**, *21*, 43–48; (b) Zocher, R.; Salnikow, J.; Kleinkauf, H. *FEBS Lett.* **1976**, *71*, 13–17; (c) Zocher, R.; Kleinkauf, H. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 1162–1167; (d) Pieper, R.; Kleinkauf, H.; Zocher, R. *J. Antibiot.* **1992**, *45*, 1273–1277; (e) Lee, C.; Gorisch, H.; Kleinkauf, H.; Zocher, R. *J. Biol. Chem.* **1992**, *267*, 11741–11744.
26. Plattner, P. A.; Vogler, K.; Studer, R. O.; Quitt, P.; Keller-Schierlein, W. *Helv. Chim. Acta* **1963**, *46*, 927–935.
27. Shemyakin, M. M.; Ovchinnikov, Y. A.; Kiryushkin, A. A.; Ivanov, V. T. *Tetrahedron Lett.* **1963**, *4*, 885–890.
28. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
29. Changsen, C.; Franzblau, S. G.; Palittapongarnpim, P. *Antimicrob. Agents Chemother.* **2003**, *47*, 3682–3687.
30. O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. *Eur. J. Biochem.* **2000**, *267*, 5421–5426.