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Novel cyclopropyl diketones and 14-membered macrolides from the soil fungus *Hamigera avellanea* BCC 17816

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ABSTRACT

Two novel cyclopropyl diketones, hamavellone A (**1**) and B (**2**), and two new 14-membered nonaketide macrolactones, hamigeromycin A (**3**) and B (**4**), together with six known compounds, 89-250904-F1 (radicicol analogue A, **5**), pseurotin A (**6**), emodin (**7**), ω-hydroxyemodin (**8**), and emodin bianthrones (**9** and **10**) were isolated from the soil fungus *Hamigera avellanea* BCC 17816. The structures of the new compounds were defined by analysis of NMR and MS data. The absolute stereochemistry of **3** was addressed by chemical correlation to **5**. Hamavellone B (**2**) exhibited antimalarial activity with an IC_{50} of 5.2 μg/mL, whereas it also showed comparable cytotoxicity.

1. Introduction

During a search for novel bioactive fungal metabolites we investigated the soil fungus *Hamigera avellanea* BCC 17816 as an extract from fermentation broth of this strain displayed cytotoxicity to NCI-H187 cells (human small-cell lung cancer) with an IC_{50} value of 10 μg/mL and exhibited a unique ^{1}H NMR profile. Scale-up fermentation and chemical studies led to the isolation of two novel cyclopropyl diketones, hamavellone A (**1**) and B (**2**), and two new 14-membered macrolactones, hamigeromycin A (**3**) and B (**4**), together with six known compounds, 89-250904-F1 (radicicol analogue A, **5**),[1,2] pseurotin A (**6**),[3,4] emodin (**7**), ω-hydroxyemodin (**8**), and emodin bianthrones (**9** and **10**).[5,6] We report herein the detailed isolation, structure elucidation, and biological activities of these compounds.

2. Results and discussion

Hamavellone A (**1**) was a pale yellow viscous oil whose molecular formula was established as $C_{11}H_{16}O_2$ by HRMS (ESI-TOF). The UV spectrum showed λ_{max} at 252 nm. The IR spectrum exhibited a broad carbonyl absorption band (1714–1633 cm^{-1}) with a maximum at 1667 cm^{-1}. Inspection of ^{1}H and ^{13}C NMR, DEPT135, and HMQC data revealed that **1** was consisted of two ketones (δ_C 208.7 and 196.9), a *trans*-olefin (J=15.6 Hz) resonated at δ_H 6.35 (δ_C 133.2) and 6.58 (δ_C 142.3), a quaternary carbon (δ_C 38.2), two methines at δ_H 2.29 (δ_C 34.0) and 1.95 (δ_C 28.9), and four methyl groups at δ_H 2.25 (3H, s; δ_C 27.9), 2.23 (3H, s; δ_C 27.3), 1.42 (3H, s; δ_C 10.5), and 1.19 (3H, d, J=6.6 Hz; δ_C 8.9). The *trans*-olefin (C-3/C-4) was attached to an acetyl group (C-1/C-2) to form an enone, which was evident from the HMBC correlations from H_3-1, H-3, and H-4 to the upfield ketone carbon at δ_C 196.9 (C-2). The COSY correlations revealed that the other side of the *trans*-olefin (C-4) was connected to δ_H 2.29 methine (H-5), which was further attached to the other methine at δ_H 1.95 (H-6). This methine (C-6) was flanked by a methyl group (δ_H 1.19, H_3-10). The HMBC correlations demonstrated that the two methines (C-5 and C-6) together with a quaternary carbon (δ_C 38.2, C-7) form a cyclopropane, wherein the quaternary carbon (C-7) was substituted with a methyl group (C-11) and an acetyl group (C-8, C-9). Thus, H_3-9 showed correlations to C-5, C-6, and C-7, and H_3-10 also correlated to these cyclopropyl carbons. The connection of an acetyl group (C-8/C-9) to C-7 was confirmed by the HMBC correlations from H-5, H-6, H_3-11, and H_3-9 to the δ_C 208.7 ketone carbonyl carbon (C-8). On the basis of these data, the planar structure of hamavellone A was established as shown in **1**.

Hamavellone B (**2**) had the same molecular formula as **1**, $C_{11}H_{16}O_2$ (HRMS). The UV and IR spectra of **2** were very similar to those of **1**. Analysis of the 1D and 2D NMR spectra of **2** resulted in the establishment of the same gross structure as **1**; therefore, these compounds should be diastereomers (Table 1).

The relative configurations of **1** and **2** were deduced from the ^{1}H NMR J-values and NOESY data (Fig. 1). The ^{1}H NMR spectrum of **1** showed that the cyclopropyl protons H-5 and H-6 were vicinally

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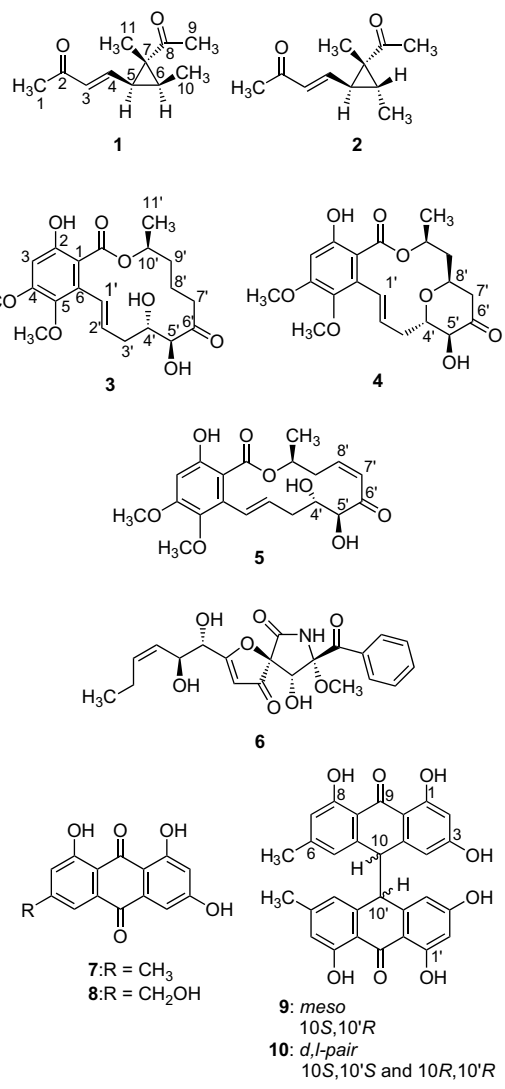


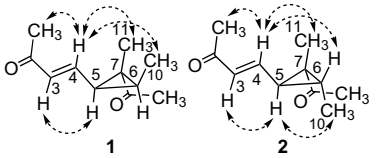


Figure 1. Key NOESY correlations for **1** and **2**.

coupled with a large *J*-value of 9.4 Hz, which indicated a cis relation,[7] although their NOESY correlation was not clear because of the close proton chemical shifts. The NOESY spectrum of **1** exhibited intense correlation between H-3 and H-5, while the cross-peak between H-4 and H-5 was relatively much weaker. The large vicinal coupling constant of *J*=9.4 Hz for H-4 and H-5 strongly suggested an antiperiplanar relation of these protons in $CDCl_3$. Correlations from H-4 to two methyl groups (H_3-9 and H_3-10) attached to the cyclopropane and the lack of the cross-peak between H-4 and H-5 established the relative configuration of the tetrasubstituted cyclopropane. The downfield shift of H-6 (δ_H 1.95) could be explained by the deshielding by the C-8 ketone carbonyl. As for compound **2**, relatively smaller vicinal *J*-value of 6.5 Hz for the cyclopropyl protons, H-5 and H-6, suggested a trans relation.[7] The antiperiplanar relation of H-4 and H-5 was evident from the large *J*-value of 9.8 Hz and the intense NOESY correlation between H-3 and H-5. NOESY correlations from H-4 to H-6 and H_3-10, and from H-5 to H_3-9 clearly indicated the relative configuration of **2** as shown in Figure 1. The absolute configurations for **1** and **2** remain undefined. The optical rotation values for **1** and **2**, $[\alpha]_D^{25}$ +144 (*c* 0.115, MeOH) and $[\alpha]_D^{25}$ +169 (*c* 0.11, MeOH), respectively, suggested that these compounds possess the same sense of absolute configuration at C-5 and C-7, and opposite at C-6.

Hamigeromycin A (**3**), the most abundant macrolide constituent, was isolated as a colorless solid, and its molecular formula of $C_{20}H_{26}O_8$ was determined on the basis of the HRESIMS and ^{13}C NMR data. Analysis of the ^1H and ^{13}C NMR, DEPT135, and HMQC data revealed that the molecule possessed a ketone (δ_C 209.7), an ester (δ_C 171.2), a *trans*-olefin at δ_C 126.9 (δ_H 6.64, dd, *J*=15.9, 1.9 Hz) and 130.4 (δ_H 6.03, ddd, *J*=15.9, 10.7, 3.1 Hz), an sp^2 methine at δ_C 99.8 (δ_H 6.44, s), five sp^2 quaternary carbons resonated at δ_C 161.5, 158.9, 140.0, 133.5, and 103.6, three oxymethines at δ_C 73.0 (δ_H 5.16), 79.8(δ_H 4.42), and 73.3(δ_H 4.11), four methylenes at δ_C 39.5, 38.0, 34.9, and 20.9, a methyl group at δ_C 20.1 (δ_H 1.40, d, *J*=6.2 Hz), and two methoxy groups at δ_C 55.9 (δ_H 3.89) and 60.4(δ_H 3.60). In addition, a chelated OH proton was observed at δ_H 11.94. The structure of the pentasubstituted benzene ring was addressed from the HMBC correlations: from a chelated OH proton (δ_H 11.94, 2-O*H*) to C-1, C-2, and C-3, from H-3 (δ_H 6.44, s) to C-1, C-2, C-4, and C-5, and from methoxy protons at δ_H 3.89 (3H, s) and 3.60 (3H, s), respectively, to C-4 and C-5. The aromatic methine proton (H-3) showed weak four-bond correlation to the δ_C 171.2 carbonyl, which confirmed the attachment of the ester carbonyl to C-1. The benzene ring was attached to a *trans*-olefin at C-6 position as indicated by the HMBC correlations from the olefinic proton H-1′ (δ_H 6.64) to C-1, C-5, and C-6, and from H-2′ (δ_H 6.03) to C-6. The connection from C-1′ to C-5′ as well as the local structure from C-7′ to C-11′ was

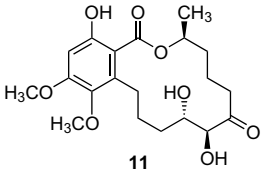
Table 1
NMR data for hamavellone A (**1**) and B (**2**) in $CDCl_3$ (500 MHz for ^1H, and 125 MHz for ^{13}C)

Position	**1**			**2**		
	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC
1	27.9, CH_3	2.25, s	2, 3	27.7, CH_3	2.23, s	2,3
2	196.9, qC			197.2, qC		
3	133.2, CH	6.35, d (15.6)	2, 5	131.6, CH	6.28, d (15.6)	2,5
4	142.3, CH	6.58, dd (15.6, 9.4)	2	146.3, CH	6.53, dd (15.6, 9.8)	2
5	34.0, CH	2.29, t (9.4)	3, 8	34.3,[a] CH	2.39, dd (9.8, 6.5)	3,8,10
6	28.9, CH	1.95, dq (9.4, 6.6)	8	34.2,[a] CH	1.37, m	
7	38.2, qC			40.3, qC		
8	208.7, qC			206.4, qC		
9	27.3, CH_3	2.23, s	7, 8	29.2, CH_3	2.28, s	7,8
10	8.9, CH_3	1.19, d (6.6)	5, 6, 7	11.7, CH_3	1.09, d (6.2)	5,6,7
11	10.5, CH_3	1.42, s	5, 6, 7, 8	17.4, CH_3	1.49, s	5,6,7,8

[a] Assignment of carbons can be interchanged.

addressed on the basis of the COSY correlations. The δ_C 209.7 ketone was placed in the C-6′ position, since the HMBC spectrum exhibited correlations from H-4′, H-5′, and H_2-7′ (δ_H 2.81, m; δ_H 2.42, m) to this carbon. Finally, the macrolactone ring was required by downfield shift of H-10′ (δ_H 5.16) and from the molecular formula (HRMS), thus, the gross structure of hamigeromycin A was established as depicted.

The absolute stereochemistry of **3** was determined by correlation to the co-metabolite 89-250904-F1 (**5**). The ^{1}H NMR and ^{13}C NMR data and optical rotation of **5**, isolated from BCC 17816 ($[\alpha]_D^{27}$ −44 (*c* 0.09, MeOH)), were consistent with the literature values ($[\alpha]_D^{25}$ −43.6 (*c* 0.76, MeOH)).[2,8] Hydrogenation of **3** (H_2, Pd/C, THF; rt, 2 h) gave a sole product whose ESIMS and ^{1}H and ^{13}C NMR spectra were identical to those of the known compound **11**, which was previously obtained by double bond isomerization of **5** to the *trans*-enone (pyridine, 50 °C) and subsequent hydrogenation (H_2, Pd/C, THF).[9] For clarity, compound **5**, isolated from BCC 17816, was also subjected to hydrogenation under the same conditions, giving a sole product spectroscopically (ESIMS, ^{1}H and ^{13}C NMR) identical to **11** from **3**. These results indicated that hamigeromycin A (**3**) is the 7′,8′-dihydro derivative of **5**, possessing 4′*S*,5′*S*,10′*S* configuration.



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Hamigeromycin B (**4**) was obtained as a minor constituent. The molecular formula, $C_{20}H_{24}O_8$ (HRMS), was the same as that of **5**. The ^{1}H and ^{13}C NMR spectra were similar to those of hamigeromycin A (**3**). The significant difference with **3** was the replacement of one of the methylenes in **3** by an oxymethine in **4**, which was assigned to the C-8′ position on the basis of the COSY correlations. The structure of the aromatic portion was proved to be the same as **3** and **5** on the basis of the HMBC correlations. The trans configuration of the C-1′/C-2′ double bond (1′*E*) was evident from the ^{1}H–^{1}H *J*-value of 16.1 Hz. The linkage from C-1′ to C-11′, through the C-6′ ketone (δ_C 206.5), was established by analysis of the COSY and HMBC data, including the HMBC correlations from H-4′ (δ_H 3.79, dt, *J*=2.9, 9.7 Hz), H_2-7′ (δ_H 2.99, ddd, *J*=14.4, 7.4, 1.0 Hz; δ_H 2.38, dd, *J*=14.4, 2.6 Hz), and H-8′ (δ_H 4.61, m) to C-6′. The ether linkage of C-4′ and C-8′, to construct a tetrahydro-γ-pyrone, was unambiguously indicated by the HMBC correlations from H-4′ to C-8′ and from H-8′ to C-4′. The relative configuration was assigned by analysis of relevant ^{1}H NMR *J*-values and NOESY correlations (Fig. 2). The large vicinal coupling (*J*=9.7 Hz) between H-4′ and H-5′, and the lack of NOESY correlation indicated the antiperiplanar relationship of these protons. H-8′ was coupled with non-equivalent H_2-7′ methylenes, resonated at δ_H 2.99 and 2.38, with respective *J*-values of 7.4 and 2.5 Hz. Also, H-8′ showed NOESY correlations to both of these methylene protons. These data suggested the half-chair conformation of the tetrahydro-γ-pyrone, wherein H-4′ and H-5′ were placed in pseudoaxial positions. Intense NOESY correlation was observed for H-4′ and one of the H_2-9′ methylene protons at δ_H 2.25 (H_β-9′). On the other hand, the NOESY cross-peak for H-8′ and H_α-9′ (δ_H 1.60) was much stronger than that for H-8′ and H_β-9′. The large *J*-value (11.8 Hz) for H-8′ and H_β-9′ indicated antiperiplanar relation of these protons. These data are consistent with the *anti*-facial assignment of H-4′ and H-8′ to the six-membered ring. H_β-9′ showed NOESY correlation to H_3-11′, while H_α-9′ showed correlation to H-10′. The *J*-value of 8.8 Hz for H_β-9′ and H-10′ demonstrated the antiperiplanar relation. Local conformation of the other part of the macrolactone, C-1′ through C-5′, was also suggested from the NMR data. H_α-3′ showed strong

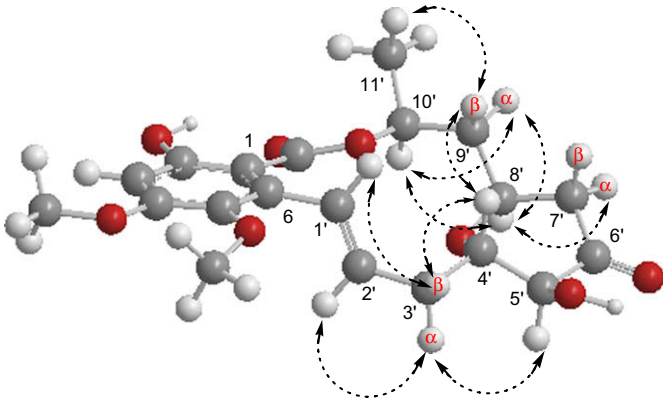

Figure 2. Probable conformation and selected NOESY correlations for hamigeromycin B (**4**).

NOESY correlations to H-2′ and H-5′, whereas H_β-3′ exhibited intense cross-peaks with H-1′ and H-4′. The antiperiplanar relation of H_α-3′ and H-4′ was evident from their vicinal *J*-value of 9.7 Hz. Consequently, the relative configuration and preferred conformation of **4** (in acetone-d_6) were consistent with those shown in Figure 2. Since hamigeromycin B (**4**) was co-produced with the major macrolides **3** and **5**, its absolute configuration can be proposed as 4′*S*,5′*S*,8′*S*,10′*S*. Compound **4** was probably derived from **5** or its *trans*-enone (7′*E*) isomer through intramolecular hetero Michael addition of 4′-OH to the enone.

In addition to the close relationship to the co-metabolite **5**, hamigeromycin A (**3**) also structurally resembles to queenslandon (isolated from *Chrysosporium queenslandicum* IFM 51121), which is reported to possess translocated ketone and secondary alcohol functionalities at C-5′ and C-6′, and proposed to have 4′*R**,6′*S**,10′*S** relative configuration.[10] Very recently, structurally related nematicidal macrolides caryospomycins A–C were isolated from *Caryospora callicarpa* YMF1.01026.[11] Caryospomycin C is a *trans*-enone (7′*E*) isomer of **5**, with the 4′*R**,5′*S**,10′*S** relative configuration.

Pseurotin A (**6**) was first described from *Pseudeurotium ovalis* S2269/F,[3a] and it has been isolated from various fungi such as *Aspergillus* species[4,12] and *Pochonia chlamydosporia* var. *catenulata*.[13] Emodin (**7**) and its hydroxy analogue **8** are commonly occurring as constituents of plants and fungi. Emodin bianthrones (**9** and **10**) and structurally related bianthrones have been known as constituents of plants.[5,6] To the best of our knowledge, this is the first report of the isolation of **9** and **10** as fungal secondary metabolites.

There have been only a few other reports on the new metabolites of *Hamigera* species, such as the cyclopentapeptides, avellanins A and B from *H. avellanea*[14] and their analogues PF1171A–PF1171E from *H. avellanea* PF1171,[15] and polyketide metabolites, hamigerone and dihydrohamigerone from *H. avellanea* NN005492.[16] In the present study four different chemical classes of polyketide metabolites, comprising 10 compounds, have been isolated from *H. avellanea* BCC 17816, demonstrating that fungi in this genus are potent sources of bioactive compounds.

All isolated compounds were subjected to our in vitro biological protocols, inclusive of antimalarial (*Plasmodium falciparum* K1), antitubercular (*Mycobacterium tuberculosis* H37Ra), and antifungal (*Candida albicans*) activities, and cytotoxicity against three human cancer cell-lines (KB, MCF7, and NCI-H187 cells) and noncancerous Vero cells (Table 3). Hamavellone B (**2**) exhibited moderate antimalarial activity, while it also showed cytotoxic activities. Among 14-membered macrolides, only compound **5** exhibited moderate cytotoxicity, and no other biological activities were shown in this class of compounds. It should be noted that emodin bianthrones (**9** and **10**) strongly inhibited the proliferation of the malarial parasite, although they showed comparable cytotoxicity to Vero cells.

3. Experimental

3.1. General procedures

Melting points were measured with an Electrothermal IA9100 digital melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on an analytikjena SPEKOL 1200 UV–visible spectrophotometer. FTIR spectra were recorded on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on a Bruker AV500D spectrometer. ESI-TOF mass spectra were measured with Bruker micrOTOF and Micromass LCT mass spectrometers.

3.2. Fungal material

H. avellanea was isolated from a soil sample collected in the Queen Sirikit Botanic Garden, Chiang Mai province, Thailand. The identification of the fungus is based on morphology and sequence data of the ITS rDNA. This fungus was deposited at the BIOTEC Culture Collection as BCC 17816 on June 22, 2005.

3.3. Fermentation and isolation

The fungus BCC 17816 was maintained on potato dextrose agar at 25 °C, which was then cut into plugs and inoculated into 3×250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB; potato starch 4.0 g, dextrose 20.0 g, per liter). After incubation at 25 °C for 6 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 6 days on a rotary shaker (200 rpm). These secondary cultures (700 mL) were transferred into a 10 L bioreactor containing 6.3 L of a liquid medium (composition, sucrose 30.0 g, malt extract 20.0 g, bacto-peptone 2.0 g, yeast extract 1.0 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KH_2PO_4 0.5 g, per liter), and final fermentation was carried out at 25 °C for 7 days. The culture was filtered to separate the residue (mycelium) and the filtrate (broth). The broth was extracted with EtOAc (3×6 L) and concentrated to a brown gum (2.19 g; extract A). The mycelium was macerated in MeOH (1.2 L, rt, 2 days) and filtered. This extraction was repeated one more time. The filtrate was defatted with equal volume of hexane, and the MeOH phase was evaporated. The residue was diluted with EtOAc (2.5 L), washed with H_2O (200 mL), concentrated under reduced pressure to leave a pale brown gum (1.42 g, extract B). Extract A was passed through a Sephadex LH-20 column (3.5×50 cm) using MeOH as eluent to obtain six fractions: A-1 (249 mg), A-2 (1180 mg), A-3 (610 mg), A-4 (55 mg), A-5 (25 mg), and A-6 (9 mg). Fraction A-2 was subjected to column chromatography (CC) on Si gel (2.5×20 cm, step gradient elution with CH_2Cl_2/MeOH) to obtain seven subfractions A-2-1 to A-2-7. Subfractions A-2-2 (31 mg), A-2-3 (128 mg), and A-2-4 (12 mg) were combined and further purified by HPLC using a reverse phase column (phenomenex® Luna 10u C18(2) 100A, 21.2×250 mm, 10 μm; mobile phase MeCN/H_2O=25:75, flow rate 15 mL/min) to furnish **1** (7.0 mg, t_R 38 min) and **2** (46 mg, t_R 45 min). Fraction A-3 was subjected to CC on Si gel (3.5×17 cm, step gradient elution with CH_2Cl_2/MeOH) to obtain nine subfractions A-3-1 to A-3-9. Subfraction A-3-1 (42 mg) was further purified by reverse phase HPLC (MeCN/H_2O=25:75) to obtain **4** (12 mg, t_R 34 min). Subfractions A-3-2 to A-3-6 were combined (354 mg) and purified by HPLC (MeCN/H_2O=25:75) to afford **3** (113 mg, t_R 50 min) and **5** (53 mg, t_R 60 min). The mycelium extract (extract B, 1.42 g) was passed through a column on Sephadex LH-20 (3.5×54 cm) and eluted with MeOH to obtain seven fractions B-1 to B-7. Fraction B-3 (174 mg) was subjected to CC on Si gel (2.5×20 cm, step gradient elution with CH_2Cl_2/MeOH) to provide nine subfractions B-3-1 to B-3-9. Subfractions B-3-5 (28 mg) and B-3-6 (10 mg) were combined and further fractionated by HPLC (MeCN/H_2O=25:75) to furnish **3** (24 mg) and **4** (2.8 mg). Fraction B-6 (224 mg) was fractionated by CC on Si gel (2.5×17 cm, step gradient elution with CH_2Cl_2/EtOAc) to six subfractions B-6-1 to B-6-6. Subfraction B-6-4 (92 mg) was purified by HPLC (MeCN/H_2O=55:45) to furnish emodin bianthrones **10** (40 mg, t_R 27 min) and **9** (12 mg, t_R 34 min). Compounds **6** (17 mg), **7** (20 mg), and **8** (26 mg) were isolated from another batch of fermentation (the same scale and conditions), together with **3** (133 mg) and **4** (5 mg).

Table 2
NMR data for hamigeromycin A (**3**) and B (**4**) (500 MHz for ^1H, and 125 MHz for ^{13}C)

position	**3** (in $CDCl_3$)			**4** (in acetone-d_6)		
	δ_C, mult.	δ_H (J in Hz)	HMBC	δ_C, mult.	δ_H (J in Hz)	HMBC
1-*C*OO–	171.2, qC			171.4, qC		
1	103.6, qC			105.6, qC		
2	161.5, qC			159.3, qC		
2-O*H*		11.94, s	1,2,3		10.76, s	1,2,3
3	99.8, CH	6.44, s	1,2,4,5,*C*OO	99.5, CH	6.49, s	1,2,4,5,*C*OO
4	158.9, qC			157.7, qC		
4-O*C*H_3	55.9, CH_3	3.89, s	4	55.4, CH_3	3.89, s	4
5	140.0, qC			139.9, qC		
5-O*C*H_3	60.4, CH_3	3.60, s	5	59.5, CH_3	3.62, s	5
6	133.5, qC			134.0, qC		
1′	126.9, CH	6.64, dd (15.9, 1.9)	1,5,6,2′,3′	127.1, CH	6.74, d (16.1)	1,5,6,2′,3′
2′	130.4, CH	6.03, ddd (15.9, 10.7, 3.1)	6	131.2, CH	5.82, ddd (16.1, 8.1, 6.1)	6,3′
3′	38.0, CH_2	2.70, m		33.8, CH_2	2.70, dddd (13.4, 8.1, 2.8, 1.3)	1′,2′
		2.18, ddd (15.8, 10.7, 5.0)			2.33, dddd (13.4, 9.7, 6.1, 0.6)	1′,2′,4′
4′	73.3, CH	4.11, m	2′,3′,6′	78.9, CH	3.79, dt (2.8, 9.7)	2′,6′,8′
5′	79.8, CH	4.42, d (1.7)	3′,4′,6′	76.4, CH	4.08, dd (9.7, 4.9)	
5′-O*H*		Not detected			4.25, d (4.9)	5′
6′	209.7, qC			206.5, qC		
7′	39.5, CH_2	2.81, m	6′,8′,9′	45.5, CH_2	2.99, ddd (14.4, 7.4, 1.0)	5′,6′,8′,9′
		2.42, m	6′,8′,9′		2.38, dd (14.4, 2.5)	6′,9′
8′	20.9, CH_2	1.88, m		74.3, CH	4.61, ddt (11.8, 7.4, 2.2)	4′,6′
		1.76, m				
9′	34.9, CH_2	1.75, m	8′	35.9, CH_2	2.25, ddd (15.7, 11.8, 8.8)	8′,10′
		1.73, m			1.60, dt (15.7, 2.0)	
10′	73.0, CH	5.16, m		73.2, CH	5.31, m	
11′	20.1, CH_3	1.40, d (6.2)	9′,10′	21.0, CH_3	1.28, d (6.3)	9′,10′

*3.3.1. Hamavellone A (**1**)*

Pale yellow oil; $[\alpha]_D^{25}$ +144 (*c* 0.115, MeOH); UV (MeOH) λ_{max} (log ε) 252 (4.00), 322 sh (3.32) nm; IR (KBr) ν_{max} 1691 sh, 1667, 1615, 1360, 1261, 1104, 975 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* 203.1052 $[M+Na]^+$ (calcd for $C_{11}H_{16}O_2Na$, 203.1048).

*3.3.2. Hamavellone B (**2**)*

Pale yellow oil; $[\alpha]_D^{25}$ +169 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 250 (4.15), 320 sh (3.16) nm; IR (KBr) ν_{max} 1689, 1666, 1613, 1358, 1261, 1151, 966 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* 203.1052 $[M+Na]^+$ (calcd for $C_{11}H_{16}O_2Na$, 203.1048).

*3.3.3. Hamigeromycin A (**3**)*

Colorless solid; mp 182–184 °C; $[\alpha]_D^{27}$ −1 (*c* 0.105, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.53), 267 (4.07), 321 (3.89) nm; IR (KBr) ν_{max} 3423, 1708, 1648, 1601, 1252, 1226, 1064 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* 417.1535 $[M+Na]^+$ (calcd for $C_{20}H_{26}O_8Na$, 417.1520).

*3.3.4. Hamigeromycin B (**4**)*

Colorless solid; mp 168–170 °C; $[\alpha]_D^{27}$ +56 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.50), 263 (3.93), 316 (3.85) nm; IR (KBr) ν_{max} 3454, 1727, 1632, 1595, 1246, 1222, 1116, 1016 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Table 2; HRMS (ESI-TOF) *m*/*z* 415.1370 $[M+Na]^+$ (calcd for $C_{20}H_{24}O_8Na$, 415.1369).

*3.3.5. 87-250904-F1 (Radicicol analogue A) (**5**)*

Colorless solid; mp 156–158 °C; $[\alpha]_D^{27}$ −44 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.72), 269 (4.15), 324 (4.02) nm; IR (KBr) ν_{max} 3407, 1693, 1644, 1596, 1359, 1312, 1247, 1226, 1013 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 12.11 (1H, s, 2-O*H*), 6.47 (1H, dd, *J*=15.7, 1.4 Hz, H-1′), 6.42 (1H, s, H-3), 6.35 (1H, dd, *J*=11.5, 2.8 Hz, H-7′), 6.21 (1H, ddd, *J*=11.5, 11.1, 2.6 Hz, H-8′), 6.13 (1H, ddd, *J*=15.7, 10.5, 3.7 Hz, H-2′), 5.34 (1H, m, H-10′), 4.56 (1H, d, *J*=2.1 Hz, H-5′), 3.99 (1H, m, H-4′), 3.89 (3H, s, 4-OC*H*$_3$), 3.58 (3H, s, 5-OC*H*$_3$), 3.42 (1H, m, Ha-9′), 2.54 (1H, m, Hb-9′), 2.34 (1H, m, Ha-3′), 2.08 (1H, ddd, *J*=15.9, 10.5, 1.8 Hz, Hb-3′), 1.44 (3H, d, *J*=6.1 Hz, H-11′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.5 (s, C-6′), 171.6 (s, –*C*OO–), 161.9 (s, C-2), 159.1 (s, C-4), 146.5 (d, C-8′), 140.3 (s, C-5), 133.6 (s, C-6), 132.9 (d, C-2′), 125.7 (d, C-7′), 125.2 (d, C-1′), 103.2 (s, C-1), 99.6 (d, C-3), 80.9 (d, C-5′), 73.6 (d) and 73.5 (d) (C-4′ and C-10′), 60.3 (q, 5-OCH$_3$), 55.9 (q, 4-OCH$_3$), 37.8 (t, C-3′), 37.1 (t, C-9′), 20.8 (q, C-11′); HRMS (ESI-TOF) *m*/*z* 415.1379 $[M+Na]^+$ (calcd for $C_{20}H_{24}O_8Na$, 415.1369).

3.4. Hydrogenation of 3

To a solution of **3** (11 mg) in THF (0.8 mL) was added 10% Pd/C (6 mg), and the mixture was vigorously stirred under hydrogen for 2 h. The suspension was filtered, and the filtrate was concentrated in vacuo to leave a colorless solid (11 mg). The ^1H and ^{13}C NMR spectra in DMSO-d_6, and MS data (ESI-TOF) were identical to those of **11** reported in the literature.[9]

*3.4.1. Compound **11***

Colorless solid; mp 190–192 °C; $[\alpha]_D^{26}$ +63 (*c* 0.105, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.32), 263 (3.98), 314 (3.72) nm; IR (KBr) ν_{max} 3531, 3547, 1688, 1646, 1600, 1312, 1242, 1223, 1117, 1053 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 9.79 (1H, s, 2-O*H*), 6.38 (1H, s, C-3), 5.11 (1H, m, H-10′), 5.10 (1H, d, *J*=4.7 Hz, 5′-O*H*), 4.63 (1H, d, *J*=6.0 Hz, 4′-O*H*), 4.03 (1H, dd, *J*=4.7, 2.9 Hz, H-5′), 3.76 (3H, s, 4-OC*H*$_3$), 3.69 (1H, m, H-4′), 3.61 (3H, s, 5-OC*H*$_3$), 2.72 (1H, m, Ha-7′), 2.64 (1H, m, Ha-1′), 2.47 (1H, m, Hb-1′), 2.32 (1H, m, Hb-7′), 1.62 (1H, m, Ha-9′), 1.60 (1H, m, Ha-8′), 1.52 (2H, m, Hb-8′ and Hb-9′), 1.50 (1H, m. Ha-2′), 1.24 (1H, m, Ha-3′), 1.23 (1H, m, Hb-2′), 1.23 (3H, d, *J*=6.2 Hz, H-11′), 1.13 (1H, m, Hb-3′); ^{13}C NMR (125 MHz, DMSO-d_6) δ 213.3 (s, C-6′), 168.5 (s, –*C*OO–), 154.5 (s, C-4), 153.1 (s, C-2), 139.9 (s, C-5), 134.2 (s, C-6), 113.2 (s, C-1), 99.3 (d, C-3), 81.3 (d, C-5′), 72.6 (d, C-4′), 71.6 (d, C-10′), 60.6 (q, 5-OCH$_3$), 55.9 (q, 4-OCH$_3$), 39.0 (t, C-7′), 33.2 (t, C-9′), 31.4 (t, C-3′), 27.4 (t) and 27.3(t) (C-1′ and C-2′), 20.1 (q, C-11′), 19.0 (t, C-8′); HRMS (ESI-TOF) *m*/*z* 419.1685 $[M+Na]^+$ (calcd for $C_{20}H_{28}O_8Na$, 419.1682).

3.5. Biological assays

Assay for activity against *P. falciparum* (K1, multi-drug resistant strain) was performed using the microculture radioisotope technique.[17] Growth inhibitory activity against *M. tuberculosis* H37Ra and cytotoxicity to Vero cells (African green monkey kidney fibroblasts) were performed using the green fluorescent protein microplate assay (GFPMA).[18] Antifungal activity against *C. albicans* and anticancer activities against KB cells (oral human epidermoid carcinoma), MCF7 cells (human breast cancer), and NCI-H187 cells (human small-cell lung cancer) were evaluated using the resazurin microplate assay (Table 3).[19]

Table 3
Biological activities of the compounds isolated from *H. avellanea* BCC 17816

Compound	*P. falciparum*[a] (IC_{50}, μg/mL)	*M. tuberculosis*[b] (MIC, μg/mL)	*C. albicans*[c] (IC_{50}, μg/mL)	Cytotoxicity (IC_{50}, μg/mL)			
				KB[d]	MCF7[d]	NCI-H187[d]	Vero[e]
Hamavellone A (**1**)	>10	>50	39	28	12	18	47
Hamavellone B (**2**)	5.2	>50	>50	13	6.7	11	24
Hamigeromycin A (**3**)	>10	>50	—[f]	>50	>50	>50	42
Hamigeromycin B (**4**)	>10	>50	—[f]	>50	>50	>50	>50
87-250904-F1 (**5**)	>10	>50	—[f]	24	24	13	3.6
11	>10	>50	—[f]	>50	>50	>50	>50
Pseurotin A (**6**)	>10	>50	>50	2.3	>50	12	>50
Emodin (**7**)	>10	12.5	11	0.88	2.8	11	9.0
ω-Hydroxyemodin (**8**)	>10	12.5	>50	4.5	22	39	>50
9	0.71	>50	30	—[f]	—[f]	—[f]	1.6
10	0.27	50	24	—[f]	—[f]	—[f]	1.4

[a] Antimalarial activity against *P. falciparm* K1. Standard antimalarial drug, dihydroartemisinin, showed an IC_{50} value of 0.0011 μg/mL.
[b] Antitubercular activity against *M. tuberculosis* H37Ra. Standard anti-TB drug, isoniazid, showed MIC values of 0.025–0.050 μg/mL.
[c] Antifungal activity against *C. albicans*. Standard compound, amphotericin B, showed an IC_{50} value of 0.037 μg/mL.
[d] The IC_{50} values of a standard compound, doxorubicin, against KB, MCF7, and NCI-H187 cells were 0.185, 1.754, and 0.072 μg/mL, respectively.
[e] The IC_{50} value of a standard cytotoxic compound, ellipticine, against Vero cells was 0.495 μg/mL.
[f] Not tested.

References and notes

1. Kastelic, T.; Schnyder, J.; Leutwiler, A.; Traber, R.; Streit, B.; Niggli, H.; MacKenzie, A.; Cheneval, D. *Cytokines* **1996**, *8*, 751–761.
2. Dreyfuss, M. M.; Leutwiler, A.; MacKenzie, A. R.; Schnyder, J.; Traber, R.; Mattes, H. Eur. Pat. Appl. 606044, 1994; *Chem Abstr.* **2004**, *122*, 81004s.
3. (a) Bloch, P.; Tamm, C.; Bollinger, P.; Petcher, T. J.; Weber, H. P. *Helv. Chim. Acta* **1976**, *59*, 133–137; (b) Bloch, P.; Tamm, C. *Helv. Chim. Acta* **1981**, *64*, 304–315.
4. Boot, C. M.; Gassner, L. C.; Compton, J. E.; Tenny, K.; Tamble, C. M.; Lokey, R. S.; Holman, T. R.; Crews, P. *J. Nat. Prod.* **2007**, *70*, 1672–1675.
5. Mai, L. P.; Guéritte, F.; Dumontet, V.; Tri, M. V.; Hill, B.; Thoison, O.; Guénard, D.; Sévenet, T. *J. Nat. Prod.* **2001**, *64*, 1162–1168.
6. (a) Kinget, R. *Planta Med.* **1967**, *5*, 233–239; (b) Rideout, J. A.; Sutherland, M. D. *Aust. J. Chem.* **1985**, *38*, 793–808; (c) Abegaz, B. M.; Dagne, E. *Bull. Chem. Soc. Ethiop.* **1988**, *2*, 15–20.
7. Jackman, L. M.; Sternhell, S. *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon: Oxford, 1969; p 286.
8. Dakas, P.-Y.; Barluenga, S.; Totzke, F.; Zirrgiebel, U.; Winssinger, N. *Angew. Chem., Int. Ed.* **2007**, *46*, 6899–6902.
9. Grosche, P.; Akyel, K. G.; Marzinzik, A. L. *Synthesis* **2005**, 2015–2021.
10. Hoshino, Y.; Ivanova, V. B.; Yazawa, K.; Ando, A.; Mikami, Y.; Zaki, S. M.; Karam, A.-Z. A.; Youssef, Y. A.; Gräfe, U. *J. Antibiot.* **2002**, *55*, 516–519.
11. Dong, J.; Zhu, Y.; Song, H.; Li, R.; He, H.; Liu, H.; Huang, R.; Zhou, Y.; Wang, L.; Cao, Y.; Zhang, K. *J. Chem. Ecol.* **2007**, *33*, 1115–1126.
12. Hayashi, A.; Fujioka, S.; Nukina, M.; Kawano, T.; Shimada, A.; Kimura, Y. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 1697–1702.
13. Hellwig, V.; Mayer-Bardschmid, A.; Müller, H.; Greif, G.; Kleymann, G.; Zitzmann, W.; Tichy, H.-V.; Stadler, M. *J. Nat. Prod.* **2003**, *66*, 829–837.
14. Yamazaki, M.; Horie, Y.; Bae, K.; Maebayashi, Y.; Jisai, Y.; Hujimoto, H. *Chem. Pharm. Bull.* **1987**, *35*, 2122–2124.
15. Umagome, K.; Nagase, K.; Harimaya, K.; Nakamura, F.; Yamaguchi, T.; Satou, E.; Hoshiko, S.; Kamitoo, N.; Soneda, T.; Hachisu, M. Jpn Kokai Tokkyo Koho, JP 1102–1297, 1999.
16. (a) Breinholt, J.; Kjoer, A.; Olsen, C. E.; Rassing, E. R.; Rosendahl, C. N. *Acta Chem. Scand.* **1997**, *51*, 1241–1244; (b) Breinholt, J. WO9626199, 1996.
17. Desjardins, R. E.; Canfield, C. J.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
18. Changsen, C.; Franzblau, S. G.; Palittapongarnpim, P. *Antimicrob. Agents Chemother.* **2003**, *47*, 3682–3687.
19. O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. *Eur. J. Biochem.* **2000**, *267*, 5421–5426.