Tetrahedron 64 (2008) 9636–9645

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Bis-spiro-azaphilones and azaphilones from the fungi *Chaetomium cochliodes* VTh01 and *C. cochliodes* CTh05

Nutchanat Phonkerd [a], Somdej Kanokmedhakul [a,*], Kwanjai Kanokmedhakul [a], Kasem Soytong [b], Samran Prabpai [c], Palangpon Kongsearee [c,d]

[a] *Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*
[b] *Department of Plant Pest Management, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok 10520, Thailand*
[c] *Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*
[d] *Center for Excellence in Protein Structure and Function, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

ARTICLE INFO

Article history:
Received 18 June 2008
Accepted 10 July 2008
Available online 16 July 2008

Dedicated to Professor Dr. Yodhathai Thebtaranonth in honor of his 65th birthday

Keywords:
Cochliodone
Bis-spiro-azaphilones
Atropisomer
Antimalaria
Antimycobacteria
Anticancer
Chaetomium
Chaetomium cochliodes

ABSTRACT

Four new dimeric spiro-azaplilones, cochliodones A–D (**1**–**4**), two new azaphliones, chaetoviridines E and F (**5** and **6**), a new *epi*-chaetoviridin A (**7**), together with five known compounds, chaetoviridin A (**8**), ergosterol (**9**), chaetochalasin A (**10**), 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol (**11**), and ergosterol-β-D-glucoside (**12**) were isolated from the fungi *Chaetomium cochliodes* VTh01 and *C. cochliodes* CTh05. Structures and stereochemistry of the atropisomers **1**–**3** were determined by single-crystal X-ray diffraction analysis. Compounds **5**, **10**, and **11** exhibited antimalarial activity against *Plasmodium falciparum*, while **3**, **5**, **6**, **10**, and **11** showed antimycobacterial activity against *Mycobacterium tuberculosis*. In addition, **5** and **6** also showed cytotoxicity against the KB, BC1, and NCI-H187 cell lines.

1. Introduction

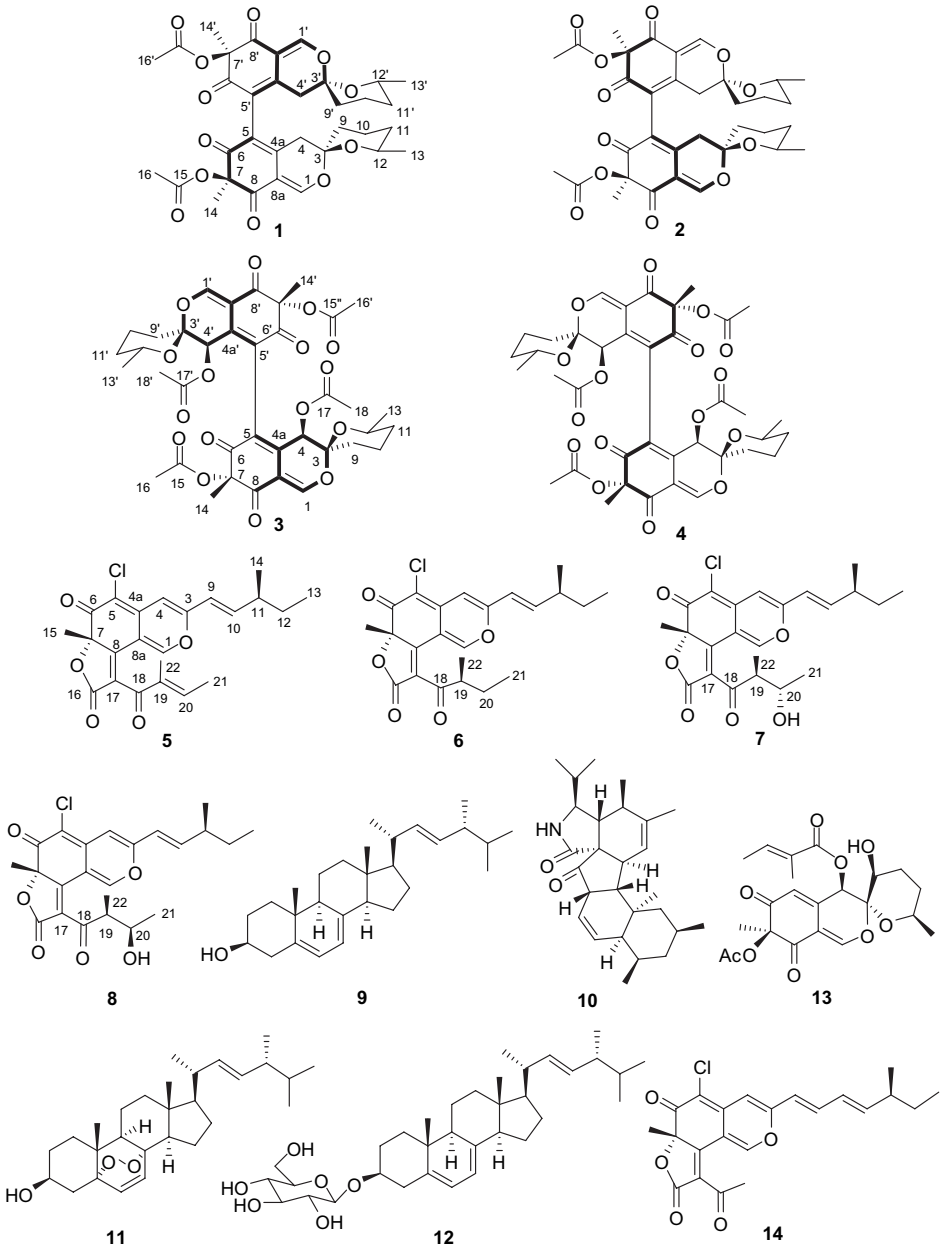
Chaetomium is the largest genera of saprophytic ascomycetes. It belongs to the Chaetomiaceae family and comprises ca. 92 species[1] of which about 20 species have been found in Thailand.[2,3] Previous investigation on secondary metabolites from the *Chaetomium* species resulted in the isolation of numerous types of compounds such as benzoquinone derivatives,[4] tetra-*S*-methyl derivatives,[5] azaphilones,[6,7] indol-3-yl-[13]cytochalasans, and chaetoglobosin analogs. Many of these are mycotoxins.[8–14] In addition, anthraquinone-chromanone,[13] orsellinic acid, and globosumones[15] have also been reported. Apart from the above mentioned compounds, investigation of other strains of *Chaetomium cochliodes* have led to the isolation of other types of chemical structure.[4,16–18] As part of our work on bioactive constituents from microorganisms, we noted that hexane and EtOAc extracts of the fungus *C. cochliodes* VTh01 showed antimalarial activity against *Plasmodium falciparum* (IC_{50} 5.1 and 4.2 μg/mL, respectively) and antimycobacterial activity against *Mycobacterium tuberculosis* (MIC 100 μg/mL of both). In addition, the hexane, EtOAc, and MeOH extracts of the fungus *C. cochliodes* CTh05 showed anti-TB activity against *M. tuberculosis* (MIC 50, 0.39, and 1.56 μg/mL, respectively). The EtOAc extract showed antimalarial activity against *P. falciparum* (IC_{50} 3.8 μg/mL). Besides, EtOAc and MeOH extracts exhibited cytotoxicity against cancer cell lines, KB and NCI-H187 (IC_{50} range 0.44–10.5 μg/mL). We report herein the isolation, characterization, and bioactivity of four new dimeric spiro-azaplilones, **1**–**4**, two new azaphliones, **5** and **6**, a new *epi*-chaetoviridin A (**7**), together with five known compounds, **8**–**12**.

2. Results and discussion

C. cochliodes strains VTh01 and CTh05 were isolated from soils of the northeastern and northern parts of Thailand, respectively. Both of them showed activity against *Fusarium oxysporum* f. sp. *lycopersici* causing tomato wilt, while the *C. cochliodes* CTh05 also

* Corresponding author. Tel.: +66 43 202222–41x12243, 12370–4; fax: +66 43 202373.
E-mail address: somdej@kku.ac.th (S. Kanokmedhakul).

0040-4020/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.07.040



showed activity against *Phytophthora parasitica* causing citrus root rot. Colonies of both strains showed a daily growth rate of 5 mm on potato dextrose agar with pale aerial mycelia. The mycelia turned to yellow and then to grayish green. The fruiting bodies become mature within 30 days, having ovate shape with ostiolate of an average size 120×84 μm (for *C. cochliodes* VTh01) and 138–378×229–293 μm (for *C. cochliodes* CTh05). The walls of the fruiting bodies are brown, surrounded with numerous unbranched hairs, undulate, flexuous, broad at the base, tapering at the end, and 300 μm long. Asci develop inside fruiting bodies with clavate shape of an average size 25×10 μm. There are eight spores per ascus with evanescent walls. Spores are lemon shape, biapiculate, thick walled with an apical germ pore, averaging 4×3 μm (for *C. cochliodes* VTh01) and 10×7 μm (for *C. cochliodes* CTh05) and brown when mature. These characteristics are similar to those reported by Skolko and Grove.[19]

Cochliodone A (**1**) was assigned the molecular formula $C_{34}H_{38}O_{12}$, as deduced from the HRESITOFMS (observed *m*/*z* 639.2437 $[M+H]^+$). The UV spectrum displayed an absorption maximum due to α,β,γ,δ-conjugated ketone at 312 nm. The IR spectrum showed characteristic ester (1738 cm^{-1}), α,β-unsaturated ketone (1708 cm^{-1}), and α,β,γ,δ-conjugated ketone (1665 cm^{-1}) bands. The ^{13}C NMR and DEPT spectra contained only 17 signals attributable to 3 carbonyl (δ 192.2, 191.8, 170.0), 3 sp^2 quaternary (δ 145.0, 122.6, 111.2), 1 sp^2 methine (δ 157.6), 2 sp^3 quaternary (δ 102.9, 84.7), 1 sp^3 methine (δ 68.7), 4 sp^3 methylene, and 3 methyl carbons. Since the ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed only half the number of resonance signals expected for 38 protons and 34 carbons, the structure must be a symmetrical dimer. The monomeric unit exhibited the presence of an olefinic proton at δ 7.78 (s, H-1/H-1′), nonequivalent methylene protons at δ 2.70 (d, *J*=16.2 Hz, H-4β/H-4β′) and 2.42 (d, *J*=16.2 Hz, H-4α/H-4α′), C-7 methyl protons at δ 1.58 (s, H_3-14/H_3-14′), and a methyl of acetoxy groups at δ 2.16 (s, H_3-16/H_3-16′) in the azaphilone unit. The tetrahydropyran unit was indicated by three sets of methylene groups, at δ 1.88 (m, H-9_{eq}/H-$9'_{eq}$) and 1.46 (dt, *J*=4.6, 14.4 Hz, H-9_{ax}/H-$9'_{ax}$), 1.70 (m, H-10/H-10′), 1.62 (m, H-11_{eq}/H-$11'_{eq}$), and 1.25 (dq, *J*=3.3, 12.3 Hz, H-11_{ax}/H-$11'_{ax}$) together with an oxymethine proton at δ 3.85 (m, H-12/H-12′) and C-12 methyl protons at δ 1.08 (d, *J*=6.4 Hz, H_3-13/H_3-13′). The structure of **1** was then elucidated to be a bis-spiro-azaphilone by analysis of

Table 1
^{1}H NMR data for compounds **1**–**4** ($CDCl_3$)[a]

Position	1	2	3	4
1,1′	7.78 (s)	7.75 (s)	8.04 (s)	7.84 (s)
4β,4β′	2.70 (d, 16.2)	2.71 (d, 16.2)	5.41 (s)	5.87 (s)
4α,4α′	2.42 (d, 16.2)	2.52 (d, 16.2)		
9_{eq},$9'_{eq}$	1.88 (m)	1.92 (m)	2.10 (m)	2.36 (m),
9_{ax},$9'_{ax}$	1.46 (dt, 4.6, 14.4)	1.45 (dt, 4.6, 14.4)	1.40 (m)	1.39 (m)
10,10′	1.70 (m)	1.71 (m)	1.75 (m)	1.78 (m)
11_{eq},$11'_{eq}$	1.62 (m)	1.90 (m)	1.80 (m)	1.74 (m)
11_{ax},$11'_{ax}$	1.25 (dq, 3.3, 12.3)	1.23 (dq, 3.2, 12.2)	1.35 (m)	1.25 (m)
12_{ax},$12'_{ax}$	3.85 (m)	3.82 (m)	4.22 (m)	4.19 (m)
13,13′	1.08 (d, 6.4)	1.08 (d, 6.4)	1.25 (d, 6.4)	1.16 (d, 6.24)
14,14′	1.58 (s)	1.53 (s)	1.90 (s)	1.60 (s)
16,16′	2.16 (s)	2.11 (s)	2.22 (s)	2.13 (s)
18,18′			2.15 (s)	1.95 (s)

[a] Figures in parentheses are coupling constants in hertz.

Table 2
^{13}C NMR data for compounds **1**–**4** ($CDCl_3$)

Position	1	2	3	4
1,1′	157.6 (d)[a]	157.2 (d)	159.8 (d)	158.2 (d)
3,3′	102.9 (s)	102.1 (s)	104.7 (s)	103.2 (s)
4,4′	37.6 (t)	37.2 (t)	68.2 (d)	66.5 (d)
4a,4a′	145.0 (s)	143.9 (s)	109.0 (s)	109.3 (s)
5,5′	122.6 (s)	122.6 (s)	123.0 (s)	124.5 (s)
6,6′	192.2 (s)	192.2 (s)	194.0 (s)	193.4 (s)
7,7′	84.7 (s)	83.8 (s)	84.5 (s)	84.7 (s)
8,8′	191.8 (s)	191.1 (s)	191.1 (s)	190.6 (s)
8a,8a′	111.2 (s)	111.3 (s)	143.8 (s)	140.0 (s)
9,9′	32.0 (t)	32.4 (t)	27.4 (t)	28.6 (t)
10,10′	18.2 (t)	18.1 (t)	18.1 (t)	18.0 (t)
11,11′	31.4 (t)	31.7 (t)	31.4 (t)	31.7 (t)
12,12′	68.7 (d)	68.5 (d)	69.5 (d)	69.0 (d)
13,13′	21.6 (q)	21.8 (q)	21.5 (q)	21.2 (q)
14,14′	22.4 (q)	22.9 (q)	22.1 (q)	22.1 (q)
15,15′	170.0 (s)	169.1 (s)	169.8 (s)	168.8 (s)
16,16′	20.2 (q)	19.9 (q)	19.9 (q)	19.6 (q)
17,17′			169.3 (s)	168.2 (s)
18,18′			20.8 (q)	19.7 (q)

[a] Multiplicities were determined by analysis of the DEPT and HSQC spectra.

the 2D NMR spectra including COSY, HSQC, HMBC, NOESY, and by comparison with the spectral data for spiro-azaphlilone, daldinin C (**13**).[20] In addition, the absence of proton signals at C-5 and C-5′ indicated that the bridge in **1** was connected via C-5 and C-5′. The HMBC spectrum confirmed the above observations by showing correlations of H-1 to C-3, C-4a, and C-8; H-4 to C-5, C-8a, and C-9; H-9 to C-3 and C-11; H-10 to C-3 and C-11; H-11 to C-9 and C-13; H-12 to C-10 and C-13; H-13 to C-11 and C-12; H-14 to C-6, C-7, and C-8; and H-16 to C-15. The COSY spectrum also supported the tetrahydropyran ring with methyl attached to carbon adjacent to the oxygen atom from the correlations between H-9 and H-10, H-10 and H-11, H-11 and H-12, and H-12 and H-13. Thus, X-ray crystallographic analysis was performed to confirm the structure and configuration of **1** (Fig. 1). Finally, the structure of **1** was defined as a new dimeric bis-spiro-azaphilone, which we named cochliodone A.

Cochliodone B (**2**) possessed the same molecular formula ($C_{34}H_{38}O_{12}$) as cochliodone A (**1**) as determined by HRESITOFMS and ^{1}H and ^{13}C NMR spectra. The IR, UV, ^{1}H, and ^{13}C NMR spectra (Tables 1 and 2) of **2** were similar to those of **1**, except for the difference of the proton chemical shifts of H-4α (**1**, δ 2.42; **2**, δ 2.52) and H-11_{eq} (**1**, δ 1.62; **2**, δ 1.90). Determination of the ^{1}H and ^{13}C NMR spectra including COSY, HSQC, and HMBC experiments led to the elucidation of the structure analogous to that of **1**. However, the different physiochemical and specific rotations [**1**, −226 (*c* 0.14, $CHCl_3$); **2**, −42 (*c* 0.14, $CHCl_3$)] between the two suggested an

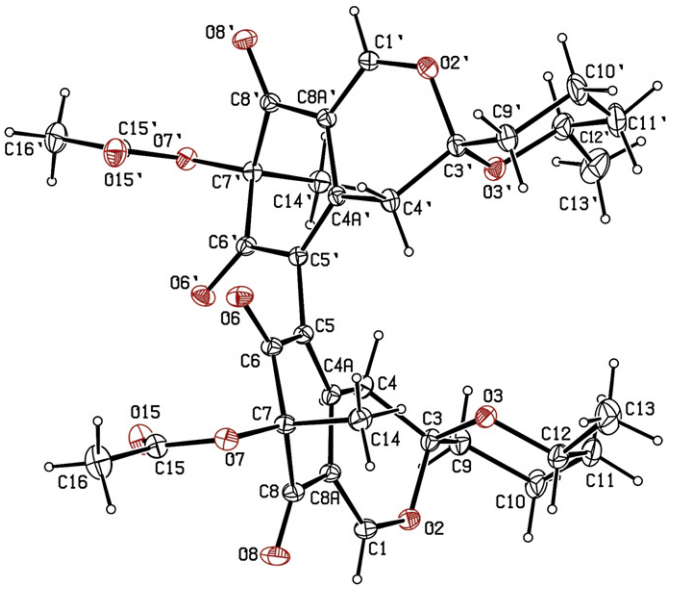


Figure 1. ORTEP drawing of the crystal structure of cochliodone A (**1**).

atropisomeric relationship to each other. The structure and configuration of **2** were finally confirmed by X-ray crystallographic analysis (Fig. 2). Crystals of compounds **1** and **2** were obtained as tetragonal and orthorhombic, respectively, by crystallization from EtOAc/hexane.

Cochliodone C (**3**) was obtained as pale yellow crystals and was assigned the molecular formula $C_{38}H_{42}O_{16}$ as deduced from the HRESITOFMS (observed *m*/*z* 777.2369 $[M+Na]^+$). The UV spectrum displayed an absorption maximum due to α,β,γ,δ-conjugated ketone at 230 and 345 nm. The IR spectrum showed the presence of esters (1756, 1738 cm^{-1}), α,β-unsaturated ketone (1706 cm^{-1}), and α,β,γ,δ-conjugated ketone (1672 cm^{-1}). The ^{13}C NMR and DEPT spectra contained only 19 signals attributable to 4 carbonyl (δ 194.0, 191.1, 169.8, 169.3), 3 sp^2 quaternary (δ 143.8, 123.0, 109.0), 1 sp^2 methine (δ 159.8), 2 sp^3 quaternary (an acetal, δ 104.7 and 84.5), 2 sp^3 methine (oxymethine, δ 69.5, 68.2), 3 sp^3 methylene, and 4 methyl carbons. Since the ^{1}H and ^{13}C NMR spectrum (Tables 1 and

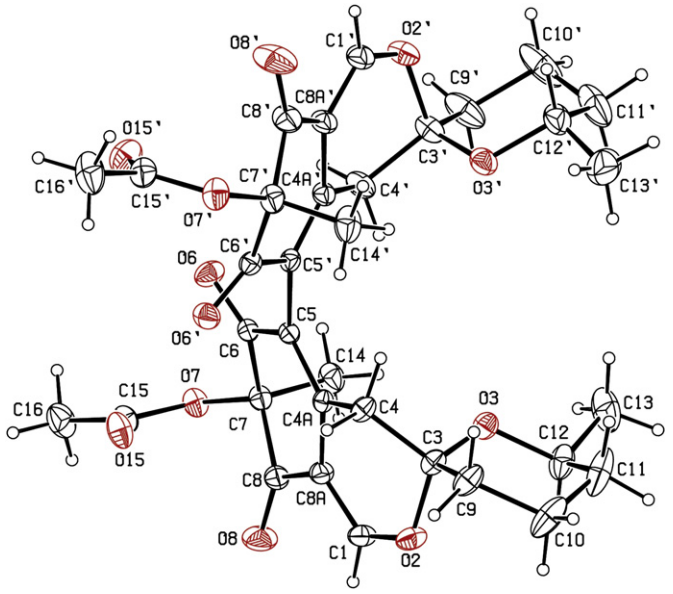


Figure 2. ORTEP drawing of the crystal structure of cochliodone B (**2**).

2) showed only half the number of resonance signals expected for 42 protons and 38 carbons, the structure must be a symmetrical dimer. The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of bis-spiro-azaphilone, cochliodone A (**1**), except for the presence of two more acetoxy groups (δ_H 2.15, δ_C 20.8, 169.3) in **3** than in **1**. The singlet signal at δ_H 5.41 (H-4/H-4′) correlated to the acetate carbonyl C-17 (δ_C 169.3) in the HMBC experiment revealing that the acetoxy group was located at C-4. The structure of **3** was then elucidated to be a bis-spiro-azaphilone by analysis of the 2D NMR spectra including COSY, HSQC, HMBC, and NOESY. Thus, X-ray crystallographic analysis was performed to confirm the structure and relative configuration of **3** (Fig. 3). Finally, the structure of **3** was defined as a new dimeric bis-spiro-azaphilone, which we named cochliodone C.

Cochliodone D (**4**) possessed the same molecular formula ($C_{38}H_{42}O_{16}$) as cochliodone C (**3**) as determined by HRESITOFMS and ^{1}H and ^{13}C NMR spectra. The IR, UV, ^{1}H, and ^{13}C NMR spectra of **4** were similar to those of **3**, except for the difference of their proton chemical shifts of H-1 (**3**, δ 8.04; **4**, 7.84), H-4 (**3**, δ 5.41; **4**, 5.87), H-9 (**3**, δ 2.10 and 1.40; **4**, 2.36 and 1.39), H-14 (**3**, δ 1.90; **4**, 1.60), and H-18 (**3**, δ 2.15; **4**, δ 1.95). Determination of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) including 2D NMR techniques (COSY, HSQC, and HMBC) supported the structure being analogous to that of **3**. However, the different physiochemical and specific rotations [**3**, −42 (*c* 0.22, $CHCl_3$); **4**, −276 (*c* 0.22, $CHCl_3$)] suggested an atropisomeric relationship between these two compounds.

Slow rotation about C–C single bonds resulting in the structural conversion of compounds **1** and **2**, and **3** and **4** were also performed to support the proposed architectural features of these two pairs of atropisomers. Heating a solution of each compound in MeCN at 65 °C for varying periods of time resulted in the interconversion of **1**⇆**2** and **3**⇆**4** (determined by HPLC). For the first pair, compound **1** gave a mixture ratios of **1**/**2**=100:0 at 0 h, 59:41 at 10 h, 40:60 at 24 h, 37:63 at 48 h, and 36:64 at 72 h, whereas compound **2** provided a mixture ratios of **1**/**2**=0:100 at 0 h, 21:79 at 10 h, 33:67 at 24 h, 37:63 at 48 h, and 36:64 at 72 h (Fig. 4). For the second pair, compound **3** gave a mixture ratios of **3**/**4**=100:0 at 0 day, 79:21 at 8 days, 77:23 at 10 days, 74:26 at 12 days, 72:28 at 14 days, and 71:29 at 16 days, while compound **4** provided a mixture ratios of **3**/**4**=0:100 at 0 day, 45:54 at 8 days, 52:48 at 10 days, 57:43 at 12 days, 60:40 at 14 days, and 63:37 at 16 days (Fig. 5).

During the purity check by HPLC analysis of the stock solutions of both **1** and **2** in MeCN at room temperature, slow interconversion was observed with the ratios of **1**/**2**=64:36 (for **1**) and 14:86 (for **2**) at 30 days; 53:47 (for **1**) and 19:81 (for **2**) at 40 days; and 49:51 (for **1**) and 22:78 (for **2**) at 50 days. In order to clarify that **1** and **2** were naturally occurring compounds and not artifacts produced during the isolation process, controlled fermentation–extraction of the fungus *C. cochliodes* VTh01 was performed. After quick extraction of dried mycelial mat with hexane at room temperature for 1 h, the crude residue was then dissolved in MeCN and subjected to HPLC determination. The chromatogram showed the presence of **1** and a trace of **2** at a ratio of 93:7 (Fig. 6). Thermal conversions of this crude residue were also observed. Heating the solution at 65 °C for 72 h resulted in a mixture of **1**/**2** with a ratio of 36:64 (Fig. 6). On the basis of the above experimental evidence, it was concluded that compounds **1** and **2** were atropisomers, which could be converted in solution, both at room and high temperatures. It is most likely, though not absolutely certain, that compound **2** isolated in our experiment was not a naturally occurring product but rather the artifact of compound **1**.

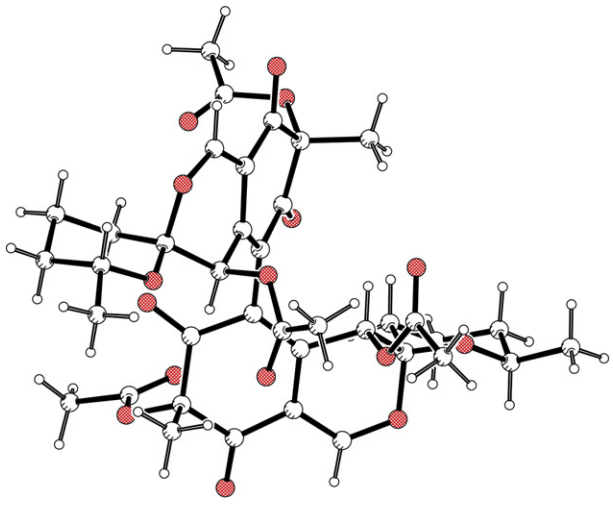


Figure 3. A perspective drawing of the crystal structure of cochliodone C (**3**).

In comparison with the cochliodones A and B (**1** and **2**), cochliodones C and D (**3** and **4**) were stored in MeCN at room temperature for 60 days, and no interconversion occurred. This suggests the steric interference of the acetoxy groups at C-4 and C-4′. Clarification that compounds **3** and **4** are naturally occurring substances from *C. cochliodes* CTh05 by using the method described above for **1** and **2** provided a mixture ratio of **3**/**4**=7:93. In addition, heating a solution of **3** or **4** in DMSO at 110 °C for 48 h provided the same mixture ratio of **3**/**4**=70:30 on HPLC analysis. Unfortunately, both compounds started to decompose after heating for 3 h. On the basis of this evidence, it was concluded that compounds **3** and **4** were atropisomers, which could be converted in solution at high temperatures. It is not unreasonable to conclude that **3** and **4** are naturally occurring compounds.

Chaetoviridin E (**5**) was assigned the molecular formula $C_{23}H_{23}ClO_5$, as deduced from the HRESITOFMS (observed *m*/*z* 415.1327 $[M+H]^+$) indicating the presence of 12 degrees of unsaturation in the molecule. The IR spectrum showed γ-lactone (1760 cm^{-1}) and α,β-unsaturated ketone (1645, 1626 cm^{-1}) bands. The ^{1}H and ^{13}C NMR spectra of **5** (Tables 3 and 4) showed five olefinic protons, five methyls, two conjugated carbonyls (δ 183.7, 190.3), and one conjugated ester (δ 167.4). The structure could then be elucidated as being of azaphilone type by analysis of the data obtained by 2D NMR (COSY, HSQC, HMBC, and ROESY) and by comparison with that of the closely related compound, chaetoviridin A (**8**).[6] The spectral data of **5** were similar to **8**, except that **5** showed an olefinic proton at δ 6.55 (q, *J*=7.6 Hz), which was connected to sp^2 carbon at δ_C 146.7 (C-20) in the HSQC experiment, instead of two methine protons (H-19 and H-20). The specific rotation of **5** [−385 (*c* 0.02, $CHCl_3$)] showed the same sign as that of rubrorotiorin (**14**) [−368 (*c* 0.019, $CHCl_3$)],[21,22] which has *S* absolute configuration at the C-7 position, implying that **5** possessed the same stereochemistry at C-7 as that of **14**. However, the specific rotation of **5** showed the opposite sign to that of chaetoviridin A (**8**) [+98 (*c* 0.05, $CHCl_3$)],[6] suggesting the loss of two stereogenic centers at C-19 and C-20 in **5**.

Treating compound **8** with H_3PO_4 in acetone and heating for 24 h yielded a product (25%) that was identical (mp, IR, NMR, specific rotation, and behavior on TLC) to the isolated compound **5**. Compound **5** and the dehydration product of **8** displayed negative specific rotation values of −385 and −390, respectively, which supported the *S* configuration at C-7 of **5** and **8** as in the reference compound **14**. Also, the stereochemistry of C-11 in the side chain at C-3 was assigned to be *S* similar to those reported for azaphilones.[6] On the basis of the above data, the structure of **5** was deduced as a dehydrated derivative of chaetoviridin A (**8**), which we named chaetoviridin E.

Chaetoviridin F (**6**) was assigned the molecular formula $C_{23}H_{25}ClO_5$, as deduced from the HRESITOFMS (observed *m*/*z* 417.1467 $[M+H]^+$) indicating the presence of 11 degrees of

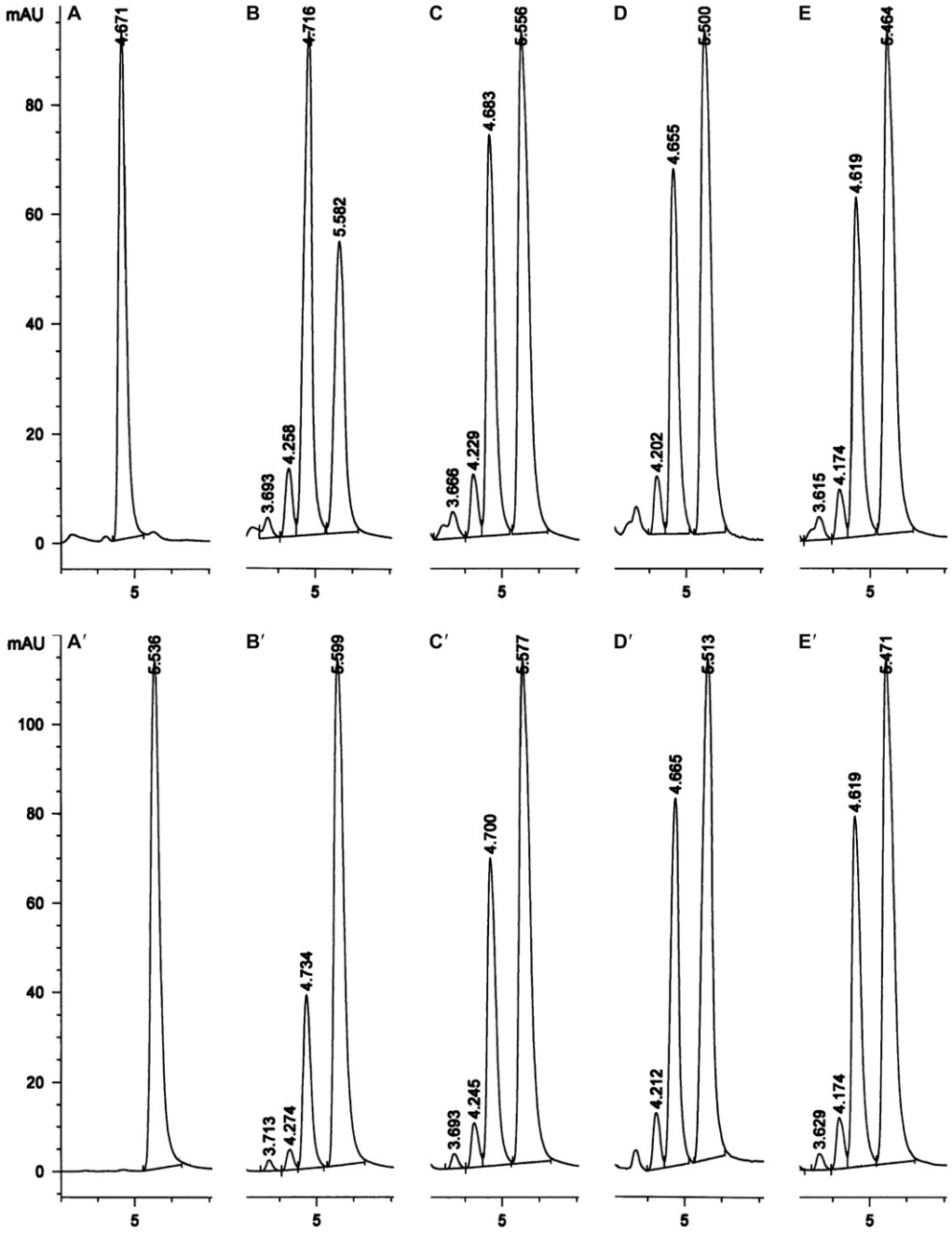


Figure 4. HPLC chromatogram of thermal conversions of **1** and **2** in MeCN solution at 65 °C. Compound **1**: A at 0 h, B at 10 h, C at 24 h, D at 48 h, and E at 72 h. Compound **2**: A′ at 0 h, B′ at 10 h, C′ at 24 h, D′ at 48 h, and E′ 72 h.

unsaturation in the molecule. The IR spectrum showed γ-lactone (1769 cm^{-1}) and α,β-unsaturated ketone (1684 and 1620 cm^{-1}) bands. Comparison of the ^{1}H and ^{13}C NMR spectral data (Tables 3 and 4) indicated that **6** was similar to **8**, except for the absence of an oxymethine proton at C-20, which was displaced by methylene protons [δ_H 1.51 (m), 1.25 (m); δ_C 26.5]. The specific rotation of **6** [+40 (*c* 0.02, $CHCl_3$) showed the same sign as that of chaetoviridin A (**8**) [+98 (*c* 0.05, $CHCl_3$)][6] suggesting the loss of a stereogenic center at C-20 in **6**. On the basis of the above data, the structure of **6** was assigned as a desoxy form of chaetoviridin A (**8**), which we named chaetoviridin F.

epi-Chaetoviridin A (**7**) was obtained as a red solid, and had molecular formula $C_{23}H_{25}ClO_6$, as deduced from the HRESITOFMS (observed *m*/*z* 455.1237 $[M+Na]^+$) indicating the presence of 11 degrees of unsaturation in the molecule. The IR spectrum of **7** indicated hydroxyl (3436 cm^{-1}), γ-lactone (1769 cm^{-1}), and α,β-unsaturated ketone (1682 and 1619 cm^{-1}) groups. The ^{1}H and ^{13}C NMR data (Tables 3 and 4) and 2D NMR techniques (COSY, HSQC, HMBC, and NOESY) of **7** indicated a structure similar to chaetoviridin A (**8**). However, there were some differences in chemical shifts between compounds **7** and **8**. Differences in the ^{1}H and ^{13}C NMR spectra were noted at δ_H 4.29 and 3.89 (for H-20), 1.23 and 1.18 (for H_3-21), and 0.98 and 1.18 (for H_3-22), while the carbon signals differed at δ_C 48.7 and 50.9 (for C-19), 67.2 and 70.9 (for C-20), 19.5 and 13.5 (for C-21), and 9.8 and 21.4 (for C-22) of **7** and **8**, respectively. This suggested an opposite configuration at C-20 between the two compounds. The absolute configurations of C-7, C-19, and C-20 in chaetoviridin A (**8**) have been reported as *S*, *S*, and

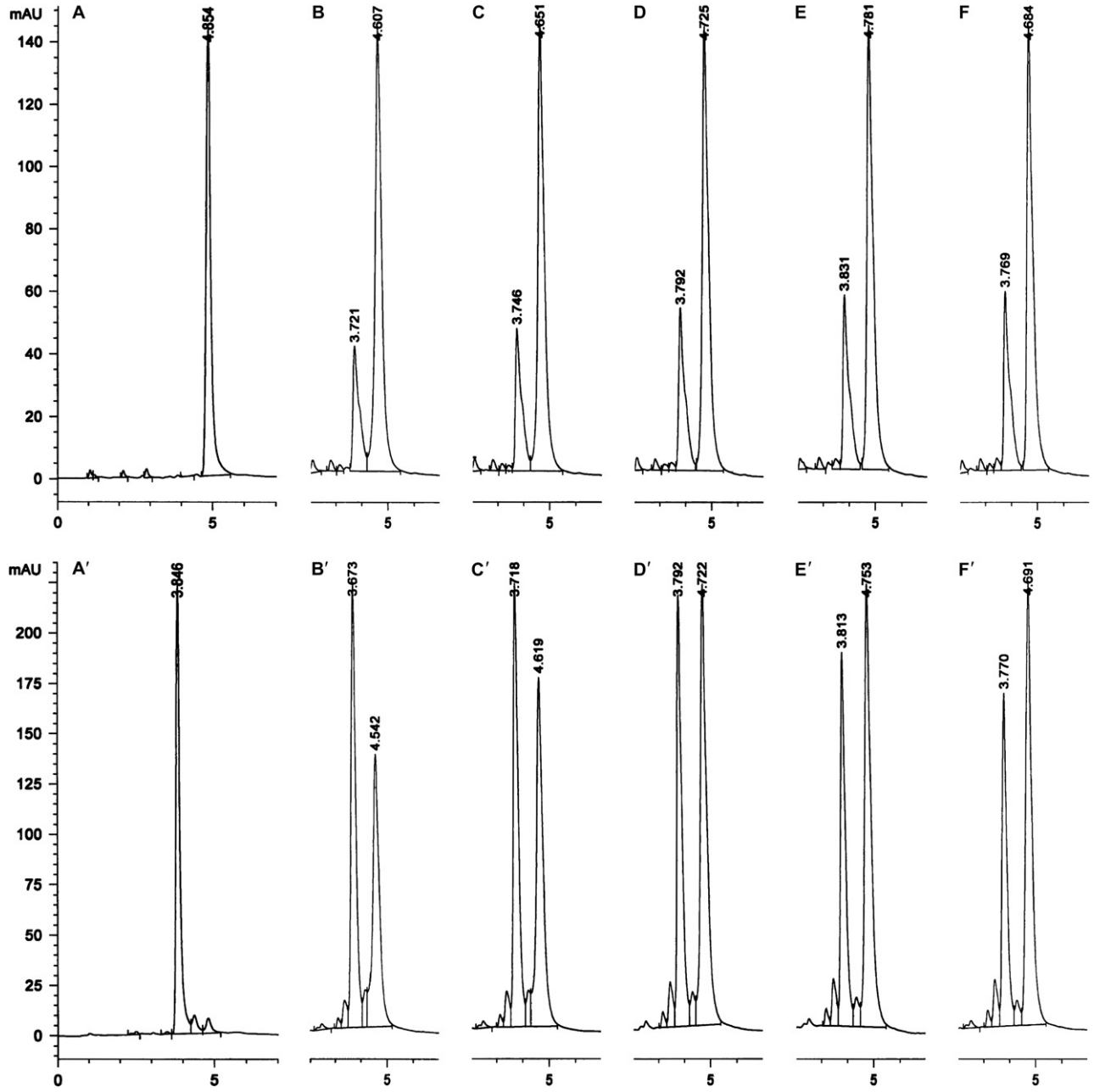


Figure 5. HPLC chromatogram of thermal conversions of **3** and **4** in MeCN solution at 65 °C. Compound **3**: A at 0 day, B at 8 days, C at 10 days, D at 12 days, E at 14 days, and F at 16 days. Compound **4**; A′ at 0 day, B′ at 8 days, C′ at 10 days, D′ at 12 days, E′ at 14 days, and F′ at 16 days.

R, respectively.[6] The stereochemistry at C-7 of **7** was assigned to *S* configuration by comparison of the chemical shift in the NMR data with that of **8**. The NOESY spectrum of **7** showed no correlation between H-19 and H-20, which has been observed in **8**, indicating *S* configuration at C-20. Besides, compound **7** showed an opposite sign of specific optical rotation [−44 (*c* 0.03, $CHCl_3$)] to the analogue **8** [+98 (*c* 0.05, $CHCl_3$)][6] suggesting a difference in stereogenic center at C-20. Thus, **7** was deduced to be *epi*-chaetoviridin A.

The five known compounds were identified by spectroscopic methods as well as by comparison with those of literature data or authentic samples, as chaetoviridin A (**8**),[6] ergosterol (**9**),[23] chaetochalasin A (**10**),[24] 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol (**11**),[23,25] and ergosterol-β-D-glucoside (**12**).[23]

Compounds **1**–**6**, **8**, **10**, and **11** were evaluated for their bioactivities (Table 5). Compounds **5**, **10**, and **11** showed antimalarial activity against *P. falciparum* with IC_{50} values of 2.9, 3.7, and 3.1 μg/mL, respectively. Compounds **3**, **5**, **6**, **10**, and **11** showed antimycobacterial activity against *M. tuberculosis* with MIC values of 200, 50, 100, 100, and 200 μg/mL, respectively. In addition, **5** and **6** had respective IC_{50} values against KB of 10.7 and 12.3 μg/mL, BC1 of 5.6 and 13.4 μg/mL, and NCI-H187 of 3.5 and 4.5 μg/mL.

3. Experimental

3.1. General

Melting points were determined using a Gallenkamp melting point apparatus and were uncorrected. Optical rotations were obtained using a JASCO DIP-1000 digital polarimeter. UV spectra were measured on an Agilent 8453 UV–visible spectrophotometer.

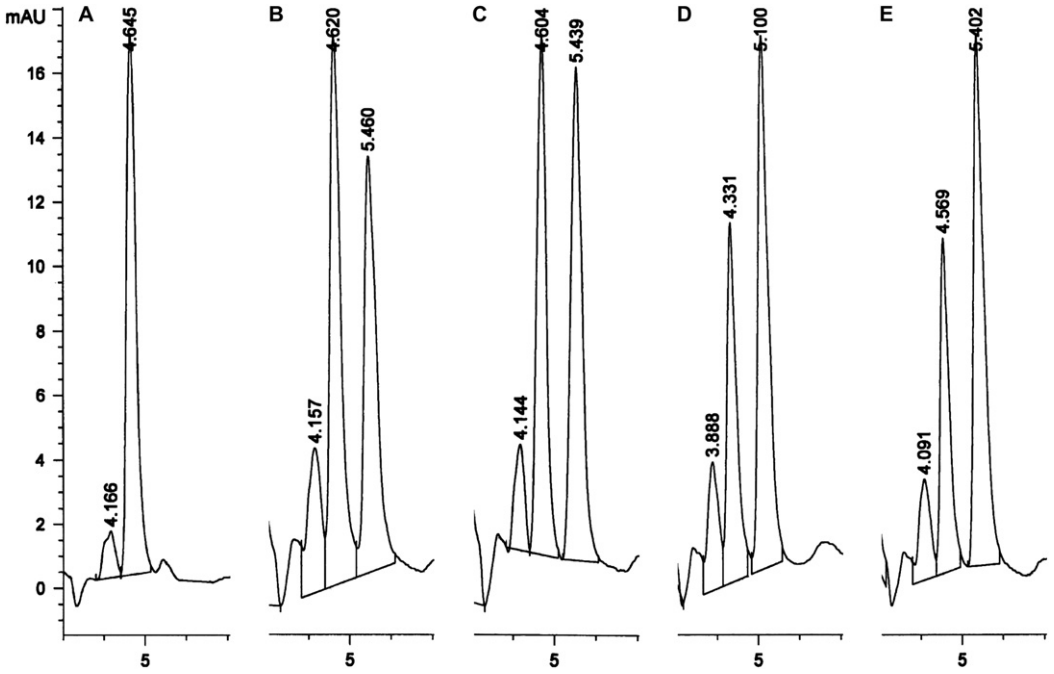


Figure 6. HPLC chromatogram of thermal conversions of hexane extract of *C. cochliodes* VTh01 in MeCN solution at 65 °C. A at 0 h, B at 10 h, C at 24 h, D at 48 h, and E at 72 h. Co-injections with standard samples were performed to confirm compound identities.

IR spectra were taken on a Perkin–Elmer Spectrum One spectrophotometer. NMR spectra were recorded in $CDCl_3$ on a Varian Mercury Plus 400 spectrometer, using residual $CHCl_3$ as an internal standard. HRESITOFMS spectra were obtained using a Micromass LCT mass spectrometer and the lock mass calibration was applied for the determination of accurate masses. Column chromatography and preparative TLC were carried out on silica gel 60 (230–400 mesh) and PF_{254}, respectively. HPLC was carried out on an Agilent 1100 series instrument with an analytical C18 column (Agilent, 5 μm, 3.2×150 mm).

3.2. Fungi material

The fungi *C. cochliodes* VTh01 and *C. cochliodes* CTh05 were collected from Ubon Rajathanee and Chiangrai provinces, respectively, and were identified by Assoc. Prof. K. Soytong. Voucher specimens CcoVTh01 and CcoCTh05 were deposited at the Department of Plant Pest Management, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The fungi were cultivated in Potato Dextrose Broth (PDB) at 25–28 °C for 4 weeks.

3.3. Extraction and isolation

3.3.1. *C. cochliodes VTh01*

Air-dried mycelial mat (200 g) was ground and extracted successively with hexane (500 mL×3), EtOAc (500 mL×3), and MeOH (500 mL×3) to yield the crude hexane (7.1 g), EtOAc (10.3 g), and MeOH extracts (31 g), respectively. EtOAc (50 mL) and hexane (300 mL) were added to the hexane extract to give a solid (1.4 g), which was further recrystallized from EtOAc/hexane to yield yellowish crystals of **1** (37 mg). The filtrate was evaporated to yield a residue, which was recrystallized from EtOAc/hexane to give **2** (50 mg). The filtrate was further separated by preparative TLC using 50% EtOAc/hexane as eluent to give an additional amount of **1** (R_f=0.52, 51 mg) and **2** (R_f=0.22, 17 mg). The residue was then separated over a silica gel column, eluted with a gradient system of EtOAc/hexane followed by MeOH/EtOAc. Each fraction (100 mL) was monitored by TLC; fractions with similar TLC patterns were combined to yield fractions F_1–F_5. Fraction F_3 was chromatographed on silica gel and eluted with a gradient system of EtOAc/hexane to give **9** (36.1 mg), **10** (39.3 mg), and **11** (33.6 mg). Fraction F_4 was

Table 3
^{1}H NMR data (δ, ppm) for compounds **5**–**8** in $CDCl_3$[a]

Position	5	6	7	8
1	8.04 (s)	8.74 (s)	8.70 (s)	8.79 (s)
4	6.55 (s)	6.50 (s)	6.57 (s)	6.58 (s)
9	6.07 (d, 15.6)	6.04 (d, 15.6)	6.09 (d, 15.6)	6.10 (d, 15.7)
10	6.56 (dd, 15.6, 8.0)	6.57 (dd, 15.6, 7.8)	6.62 (dd, 15.6, 7.5)	6.62 (dd, 15.7, 8.0)
11	2.28 (septet, 6.9)	2.23 (septet, 7.0)	2.29 (septet, 7.0)	2.30 (septet, 6.9)
12	1.44 (quint, 7.3)	1.39 (quint, 7.2)	1.45 (quint, 7.2)	1.42 (quint, 7.4)
13	0.91 (t, 7.4)	0.85 (t, 7.5)	0.83 (t, 7.4)	0.92 (t, 7.4)
14	1.08 (d, 6.7)	1.03 (d, 6.8)	1.09 (d, 7.0)	1.10 (d, 6.7)
15	1.72 (s)	1.64 (s)	1.71 (s)	1.70 (s)
19		3.46 (sextet, 6.2)	3.69 (dq, 7.4, 3.0)	3.62 (quint, 7.4)
20	6.55 (q, 7.6)	1.51 (m), 1.25 (m)	4.29 (dq, 6.2, 3.0)	3.89 (quint, 6.6)
21	1.90 (d, 7.1)	0.72 (t, 7.6)	1.23 (d, 6.6)	1.18 (d, 6.5)
22	1.87 (s)	1.06 (d, 6.4)	0.98 (d, 7.0)	1.18 (d, 6.5)

[a] Figures in parentheses are multiplicity and coupling constants in hertz.

Table 4
^{13}C NMR data (δ, ppm) for compounds **5**–**8** in $CDCl_3$

Position	5	6	7	8
1	148.3 (d)[a]	151.8 (d)	151.9	151.5
3	157.1 (s)	157.5 (s)	157.1	157.1
4	105.1 (d)	105.5 (d)	105.3	105.4
4a	139.3 (s)	140.0 (s)	139.7	139.7
5	109.5 (s)	109.0 (s)	109.0	108.9
6	183.7 (s)	183.9 (s)	183.1	183.4
7	87.6 (s)	87.8 (s)	87.8	87.6
8	159.1 (s)	164.0 (s)	165.1	162.7
8a	110.8 (s)	110.5 (s)	110.2	110.4
9	119.9 (d)	119.9 (d)	119.7	119.7
10	147.9 (d)	148.3 (d)	148.3	148.1
11	38.9 (d)	39.1 (d)	38.9	38.9
12	29.1 (t)	29.3 (t)	29.1	29.1
13	11.6 (q)	11.9 (q)	11.7	11.7
14	19.2 (q)	19.4 (q)	19.2	19.2
15	25.6 (q)	26.3 (q)	26.2	26.2
16	167.4 (s)	167.9 (s)	167.8	167.9
17	126.2 (s)	124.5 (s)	123.0	125.1
18	190.3 (s)	201.2 (s)	201.0	201.2
19	137.7 (s)	45.1 (d)	48.7	50.9
20	146.7 (d)	26.5 (t)	67.2	70.9
21	15.4 (q)	11.7 (q)	19.5	13.5
22	10.8 (q)	14.3 (q)	9.8	21.4

[a] Multiplicities were determined by analysis of the DEPT and HSQC spectra.

Table 5
Biological activities of the isolated compounds

Compound	Antimalarial (IC_{50}, μg/mL)	Antimycobacterial (MIC, μg/mL)	Cytotoxicity (IC_{50}, μg/mL)		
			KB[a]	BC1[b]	NCI-H187[c]
3		200			
5	2.9	50	10.7	5.6	3.5
6		100	12.3	13.4	4.5
10	3.7	100			
11	3.1	200			
Artemisinin	0.001				
Isoniazid		0.05			
Kanamycin sulfate		2.5			
Ellipticine			0.36	0.26	0.32

[a] Human epidermoid carcinoma in the mouth.
[b] Human breast cancer cells.
[c] Human lung cancer cells.

separated on silica gel CC eluted with a gradient system of hexane/EtOAc to yield additional **11** (16.4 mg). The EtOAc extract (10.0 g) was separated on a silica gel column and eluted with increasing EtOAc in hexane followed by MeOH in EtOAc to yield five fractions, F_1'–F_5'. Fraction F_3' gave additional **9** (69.7 mg). Fractions F_5' afforded additional **11** (35.6 mg). Fraction F_4' was further purified by CC using MeOH/CH_2Cl_2 (10:90) as eluent to yield **8** (100.6 mg). Fraction F_9' was recrystallized from MeOH/CH_2Cl_2 to give colorless needles of **12** (5.3 mg). The MeOH extract was partitioned between EtOAc and water. The EtOAc residue was subjected to silica gel CC, gradually eluted with MeOH/CH_2Cl_2 to give four fractions, F_1''–F_4''. Fraction F_2'' gave additional **11** (11.2 mg) and **12** (24.4 mg).

3.3.2. *C. cochliodes CTh05*

Air-dried mycelial mat (735 g) was ground into powder and extracted successively with hexane (3 L×3), EtOAc (3 L×3), and MeOH (3 L×3) at room temperature. Filtrates were combined and the solvents were removed in vacuo to yield crude hexane (7.8 g), EtOAc (17.4 g), and MeOH (23.4 g) extracts. CH_2Cl_2 (100 mL) was added to the hexane extract (7.8 g) to afford a pale yellow solid of **3** (34.4 mg). The filtrate was evaporated in vacuo and the residue was subjected to silica gel column chromatography, eluted with a gradient system of EtOAc in hexane followed by MeOH in CH_2Cl_2. Each fraction (75 ml) was monitored by TLC; fractions with similar TLC patterns were combined to yield fractions, F_1–F_8. Fraction F_2 was further separated by silica gel column eluted with a gradient solvent system of EtOAc in hexane to obtain five subfractions designated as $F_{2/1}$–$F_{2/5}$. Subfraction $F_{2/3}$ gave a red solid of **8** (92.5 mg). Fraction F_3 was purified by preparative TLC using 5% EtOAc/CH_2Cl_2 as eluent to yield a yellow solid of **5** (66.4 mg). Fraction F_4 was separated on silica gel column eluted with a gradient solvent system of EtOAc in hexane to yield seven subfractions designated as $F_{4/1}$–$F_{4/7}$. Subfraction $F_{4/2}$ afforded **8** (48.7 mg). Fraction F_7 was further separated by silica gel column eluted with a gradient system of EtOAc in hexane to yield seven subfractions, $F_{7/1}$–$F_{7/7}$. Subfraction $F_{7/3}$ was further purified by preparative TLC using 50% EtOAc/hexane as eluent to give an additional amount of **3** (R_f=0.49, 4 mg) and a pale yellow solid of **4** (R_f=0.41, 242.9 mg). Fraction F_8 was rechromatographed on a silica gel column, gradually eluted with EtOAc in CH_2Cl_2 to obtain five subfractions, $F_{8/1}$–$F_{8/5}$. Subfraction $F_{8/3}$ gave an additional amount of **3** (7.3 mg).

The crude EtOAc extract (17.4 g) was redissolved with EtOAc and filtered. The filtrate was evaporated in vacuo, and the residue was chromatographed on a silica gel column eluted with the same gradient system as the hexane extract above to give 10 fractions, F_1'-F_{10}'. Fraction F_5' was separated by silica gel column eluted with EtOAc and hexane by increasing polarity of solvents to give five subfractions designated as $F_{5/1}'$–$F_{5/5}'$. Subfraction $F_{5/2}'$ was purified by PLC using 20% EtOAc/CH_2Cl_2 as eluent to give an additional amount of **5** (91.3 mg). Fraction F_6' was recrystallized with MeOH to yield an additional amount of **8** (1.5 g). Fraction F_7' was chromatographed on silica gel column eluted with a gradient solvent system of EtOAc in hexane to obtain 10 subfractions designated as $F_{7/1}'$–$F_{7/10}'$. Subfraction $F_{7/3}'$ gave an additional amount of **8** (41.9 mg). Subfraction $F_{7/5}'$ was further purified by preparative TLC using 40% EtOAc/hexane as eluent to yield a red solid of **7** (R_f=0.38, 13 mg).

3.3.3. *Cochliodone A* (***1***)

Yellow crystals; mp 225–226 °C (decomp.); $[\alpha]_D^{26}$ −226 (*c* 0.14, $CHCl_3$); UV (CH_2Cl_2) λ_{max} nm (log ε): 217 (3.97), 312 (4.34); IR (KBr) ν_{max}: 3080, 2982, 2941, 1738, 1708, 1665, 1598, 1577, 1448, 1407, 1347, 1251, 1088 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS *m*/*z* $[M+H]^+$ 639.2437 (calcd for $C_{34}H_{38}O_{12}+H^+$, 639.2441).

3.3.4. *Cochliodone B* (***2***)

Yellow crystals; mp 223–225 °C (decomp.); $[\alpha]_D^{26}$ −42 (*c* 0.14, $CHCl_3$); UV (CH_2Cl_2) λ_{max} nm (log ε): 218 (4.0), 311 (4.30); IR (KBr) ν_{max}: 3080, 2982, 2941, 1738, 1708, 1665, 1598, 1577, 1448, 1407, 1347, 1251, 1088 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS *m*/*z* $[M+H]^+$ 639.2439 (calcd for $C_{34}H_{38}O_{12}+H^+$, 639.2441).

3.3.5. *Cochliodone C* (***3***)

Pale yellow crystals; mp 273–274 °C (decomp.); $[\alpha]_D^{31}$ −42 (*c* 0.22, $CHCl_3$); UV (CH_2Cl_2) λ_{max} nm (log ε): 230 (4.06), 345 (4.42); IR (KBr) ν_{max}: 3057, 2936, 2871, 1756, 1738,1706, 1672, 1597, 1559, 1455, 1371, 1328, 1240, 1083 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS *m*/*z* 777.2369 $[M+Na]^+$ (calcd for $C_{38}H_{42}O_{16}+Na$, 777.2365).

3.3.6. *Cochliodone D* (***4***)

Pale yellow crystals; mp 158–160 °C; UV (CH_2Cl_2) λ_{max} nm (log ε): 230 (3.81), 334 (4.26); $[\alpha]_D^{29}$ −276 (*c* 0.22, $CHCl_3$); IR (KBr) ν_{max}: 3059, 2975, 2940, 2877, 1764, 1746, 1713, 1676, 1604, 1582, 1446, 1411, 1372, 1345, 1243, 1219, 1084 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS *m*/*z* 777.2371 $[M+Na]^+$ (calcd for $C_{38}H_{42}O_{16}+Na$, 777.2365).

*3.3.7. Chaetoviridin E (**5**)*

A yellow solid; mp 108–109 °C; $[\alpha]_D^{25}$ −385 (*c* 0.02, $CHCl_3$); UV (CH_2Cl_2) λ_{max} nm (log ε): 230 (4.03), 302 (4.11), 365 (3.97); IR (KBr) ν_{max}: 3083, 2960, 2925, 2872, 1760, 1645, 1626, 1565, 1514, 1456, 1407, 1349, 1160, 1017 cm^{-1}; ^1H and ^{13}C NMR, see Tables 3 and 4; HRESITOFMS *m*/*z* 415.1327 $[M+H]^+$ (calcd for $C_{23}H_{23}ClO_5$+H, 415.1307).

*3.3.8. Chaetoviridin F (**6**)*

A red solid; mp 84–86 °C; $[\alpha]_D^{27}$ +40 (*c* 0.02, $CHCl_3$); UV (CH_2Cl_2) λ_{max} nm (log ε): 229 (3.97), 305 (4.27), 365 (4.12); IR (KBr) ν_{max}: 3107, 2962, 2927, 2873, 1769, 1684, 1620, 1509, 1458, 1402, 1348, 1325, 1163, 1014 cm^{-1}; ^1H and ^{13}C NMR, see Tables 3 and 4; HRESITOFMS *m*/*z* 417.1467 $[M+H]^+$ (calcd for $C_{23}H_{25}ClO_5$+H, 417.1463).

*3.3.9. epi-Chaetoviridin A (**7**)*

A red solid; $[\alpha]_D^{30}$ -44 (*c* 0.03, $CHCl_3$); UV (CH_2Cl_2) λ_{max} nm (log ε): 229 (3.99), 306 (4.16), 365 (4.04); IR (KBr) ν_{max}: 3436, 3079, 2962, 2925, 2873, 2836, 1769, 1682, 1619, 1511, 1452, 1406, 1376, 1350, 1259; ^1H and ^{13}C NMR, see Tables 3 and 4; HRESITOFMS *m*/*z* 455.1237 $[M+Na]^+$ (calcd for $C_{23}H_{25}ClO_6$+Na, 455.132).

*3.3.10. Chaetoviridin A (**8**)*

Red crystals; mp 157–158 °C (lit.[6] 121–124 °C); UV (CH_2Cl_2) λ_{max} nm (log ε): 229 (3.95), 306 (4.32), 366 (4.15); $[\alpha]_D^{27}$ +97 (*c* 0.05, $CHCl_3$) {lit.[6] $[\alpha]_D^{30}$ +98 (*c* 0.05, $CHCl_3$)}; IR (KBr) ν_{max}: 3436, 3108, 2968, 2930, 2875, 1774, 1678, 1619, 1516, 1453, 1402, 1375, 1358, 1248, 1016 cm^{-1}; ^1H and ^{13}C NMR, see Tables 3 and 4; ESITOFMS *m*/*z* 455 $[M+Na]^+$ (calcd for $C_{23}H_{25}ClO_6$+Na, 455).

3.4. X-ray crystallographic analyses of 1–3

Yellowish crystals of **1**–**3** were obtained in the mixture of EtOAc/hexane by slow evaporation. X-ray diffraction data were measured on a Bruker–Nonius kappaCCD diffractometer with graphite monochromated Mo Kα radiation (λ=0.71073 Å) at 298(2) K. The structure was solved by direct methods by SIR97,[26] and refined with full-matrix least-squares calculations on F^2 using SHELXL-97.[27] All non-hydrogen atoms were refined anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. Crystallographic data of cochliodone A (**1**), cochliodone B (**2**), and cochliodone C (**3**) have been deposited at the Cambridge Crystallographic Data Centre under the reference numbers CCDC 672198, CCDC 672199, and CCDC685491, respectively.

*3.4.1. X-ray data of **1***

$C_{34}H_{38}O_{12}$, *M*=638.37, tetragonal, dimensions: 0.10×0.15×0.20 mm, *D*=1.130 g/cm^3, space group $P4_12_12$, *Z*=8, *a*=*b*=13.264(1), *c*=42.672(7) Å, *V*=7508(2) $Å^3$, reflections collected/unique: 11,634/5853 (R_{int}=0.026), number of observation [$>2\sigma(I)$] 4457, final *R* indices [$I>2\sigma(I)$]: R_1=0.0690, wR_2=0.1948.

*3.4.2. X-ray data of **2***

$C_{34}H_{38}O_{12}$, *M*=638.37, orthorhombic, dimensions: 0.15×0.15×0.25 mm, *D*=1.246 g/cm^3, space group $P2_12_12_1$, *Z*=4, *a*=12.3511(2), *b*=12.8552(4), *c*=21.4484(4) Å, *V*=3405.5(1) $Å^3$, reflections collected/unique: 18,077/3594 (R_{int}=0.046) number of observation [$>2\sigma(I)$]: 3262, final *R* indices [$I>2\sigma(I)$]: R_1=0.0433, wR_2=0.1166.

*3.4.3. X-ray data of **3***

$C_{38}H_{42}O_{16}$, M_r=754.74, orthorhombic, dimensions: 0.30×0.10×0.10 mm, *D*=1.153 g/cm^3, space group $P2_12_12_1$, *Z*=4, *a*=9.2189(2), *b*=21.3464(9), *c*=22.0889(10) Å, *V*=4346.9(3) $Å^3$, reflections collected/unique: 18,742/3328 (R_{int}=0.051), number of observation [$>2\sigma(I)$]: 2833, final *R* indices [$I>2\sigma(I)$]: R_1=0.0605, wR_2=0.1624.

3.5. Bioassays

3.5.1. Antimalarial assay

Antimalarial activity was evaluated against the parasite *P. falciparum* (K1, multidrug resistant strain), using the method of Trager and Jensen.[28] Quantitative assessment of malarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[29] The inhibitory concentration (IC_{50}) represents the concentration, which causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^3H]-hypoxanthine by *P. falciparum*. The standard compound, artemisinin, exhibited an IC_{50} value of 1.0 ng/mL.

3.5.2. Antimycobacterial assay

Antimycobacterial activity was assessed against *M. tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).[30] The standard drugs, isoniazid and kanamycin sulfate, showed respective MIC values of 0.04–0.09 and 2.0–5.0 μg/mL.

3.5.3. Cytotoxicity assay

Cytotoxicity assays against human epidermoid carcinoma (KB), human breast cancer (BC1), and human small cell lung cancer (NCI-H187) cell lines were performed employing the colorimetric method as described by Skehan et al.[31] The reference substance was ellipticine, which showed IC_{50} values of 0.36, 0.32, and 0.26 μg/mL, respectively.

Acknowledgements

Financial support from the Thailand Research Fund (Grant no. RMU4980029) for S.K. and the Thailand Research Fund via the Royal Golden Jubilee Ph.D. program (PHD/0058/2548) and the Center for Innovation in Chemistry Postgraduate Education and Research in Chemistry (PERCH-CIC), Ministry of Education, Thailand for N.P. are acknowledged. P.K. thanks for BRG50080008. S.K. is grateful to Khon Kaen University and the Bioresources Research Network (BRN) of the National Center for Genetic Engineering Biotechnology (BIOTEC) for a research grant.

References and notes

1. Von Arx, J. A.; Guarro, J.; Figueras, M. J. *Nova Hedwigia* **1986**, *84*, 1–162.
2. Soytong, K. *Thai Phytopathol.* **1991**, *11*, 86–94.
3. Petcharat, V.; Soytong, K. *Songklanakarin J. Sci. Technol.* **1991**, *13*, 129–132.
4. Brewer, D.; Jerram, W. A.; Taylor, A. *Can. J. Microbiol.* **1968**, *14*, 861–866.
5. Safe, S.; Taylor, A. *J. Chem. Soc., Perkin Trans. 1* **1972**, 472–479.
6. Takahashi, M.; Koyama, K.; Natori, S. *Chem. Pharm. Bull.* **1990**, *38*, 625–628.
7. Kanokmedhakul, S.; Kanokmedhakul, K.; Nasomjai, P.; Soytong, K.; Isobe, M.; Kongsaeree, P.; Prabpai, S.; Suksamrarn, A. *J. Nat. Prod.* **2006**, *69*, 891–895.
8. Sekita, S.; Yoshihira, K.; Natori, S. *Tetrahedron Lett.* **1976**, *17*, 1351–1354.
9. Udagawa, S.; Muroi, T.; Kurata, H.; Sekita, S.; Yoshihira, K.; Natori, S.; Umeda, M. *Can. J. Microbiol.* **1979**, *25*, 170–177.
10. Probst, A.; Tamm, C. *Helv. Chim. Acta* **1981**, *64*, 2056–2064.
11. Sekita, S.; Yoshihira, K.; Natori, S.; Kuwano, H. *Chem. Pharm. Bull.* **1982**, *30*, 1629–1638.
12. Sekita, S.; Yoshihira, K.; Natori, S. *Chem. Pharm. Bull.* **1983**, *31*, 490–498.
13. Kanokmedhakul, S.; Kanokmedhakul, K.; Phonkerd, N.; Soytong, K.; Kongsaeree, P.; Suksamrarn, A. *Planta Med.* **2002**, *68*, 834–836.
14. Ding, G.; Song, Y. C.; Chen, J. R.; Xu, C.; Ge, H. M.; Wang, X. T.; Tan, R. X. *J. Nat. Prod.* **2006**, *69*, 302–304.
15. Bashyal, B. P.; Wijeratne, E. M. K.; Faeth, S. H.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2005**, *68*, 724–728.
16. Brewer, D.; Jerram, W. A.; Leach, C. K.; Safe, S.; Taylor, A.; Vining, L. C.; Archibald, R.; McGStevenson, R. G. *Can. J. Microbiol.* **1972**, *18*, 1129–1137.
17. Abraham, W. R.; Arfmann, H. A. *Phytochemistry* **1992**, *31*, 2405–2408.
18. Kang, J. G.; Kim, K. K.; Kang, K. Y. *Agric. Chem. Biotechnol.* **1999**, *42*, 146–150.
19. Skolko, A. J.; Grove, J. W. *Can. J. Bot.* **1953**, *31*, 779–809.

20. Hashimoto, T.; Tahara, S.; Takaoka, S.; Tori, M.; Asakawa, Y. *Chem. Pharm. Bull.* **1994**, *42*, 2397–2399.
21. Gray, R. W.; Whalley, W. B. *J. Chem. Soc.* **1971**, 3575–3577.
22. Ahbab, M.; Borthwick, A. D.; Hooper, J. W.; Millership, J. S.; Whalley, B.; Ferguson, G.; Marsh, F. C. *J. Chem. Soc.* **1976**, 1366–1369.
23. Bok, J. W.; Lermer, L.; Chilton, J.; Klingeman, H. G.; Towers, G. H. *Phytochemistry* **1999**, *51*, 891–898.
24. Oh, H.; Swenson, D. C.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Tetrahedron Lett.* **1998**, *39*, 7633–7636.
25. Rösecke, J.; Köning, W. A. *Phytochemistry* **2000**, *54*, 757–762.
26. Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115–119.
27. Sheldrick, G. M. *SHELXL-97: Program for X-ray Crystal Structure Solution and Refinement*; University of Göttingen: Göttingen, Germany, 1997.
28. Trager, W.; Jensen, J. B. *Science* **1967**, *193*, 673–675.
29. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
30. Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *4*, 1004–1009.
31. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.