Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 69 (2008) 1273–1278
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Polyketides from *Penicillium* sp. JP-1, an endophytic fungus associated with the mangrove plant *Aegiceras corniculatum*

Zhenjian Lin, Tianjiao Zhu, Yuchun Fang, Qianqun Gu *, Weiming Zhu *

Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, PR China

Received 22 February 2007; received in revised form 6 October 2007
Available online 11 December 2007

Abstract

Four polyketides, leptosphaerone C (**1**), penicillenone (**2**), arugosin I (**3**) and 9-demethyl FR-901235 (**4**), as well as five known compounds, bacillosporin A (**5**), bacillosporin C (**6**), sequoiamonascin D (**7**), sequoiatone A (**8**), and sequoiatone B (**9**) were isolated from the *Penicillium* sp. JP-1, an endophytic fungus isolated from *Aegiceras corniculatum*. Their structures were determined by spectroscopic methods, mainly by 2D NMR spectroscopic analyses. Compound **1** showed cytotoxicity against A-549 cells with an IC_{50} value of 1.45 μM, while compound **2** showed cytotoxicity against P388 cells with an IC_{50} value of 1.38 μM.

Keywords: *Penicillium*; Endophytic fungi; *Aegiceras corniculatum*; *Myrsinaceae*; Polyketides; Cytotoxicity

1. Introduction

Recent research has shown that plant endosymbionts may produce secondary metabolites that enable them to survive in the competitive world of plant interstitial space. These bioactive compounds might prove suitable for specific medicinal or agrochemical applications (Stierle et al., 1993). Mangrove plants are found in the tidal mudflats, where streams and rivers join the sea. Due to the special living environment of tidal mudflats, mangrove plants have been considered as a source of compounds possessing physiological activities. Investigation of the secondary metabolites of microorganisms isolated from unusual or mangrove plants residing in niche environments may increase the chance of finding novel active compounds. In our search for new antitumor compounds from endophytic fungi from mangrove plants, a fungus, authenticated as *Penicillium* sp., was isolated from the inner bark of a mangrove tree. Its extract exhibited cytotoxicity against the P388 cell line. Studies on the active constituents of this fungus led to isolation of four new polyketides along with five known compounds. All new compounds were evaluated for their cytotoxicities against the A-549 and P388 cell lines by the MTT method.

2. Results and discussion

2.1. Production and identification

The isolated fungus *Penicillium* sp. JP-1 was grown in a static liquid medium. The culture broth was extracted with EtOAc and the organic extract was separated using silica gel column, Sephadex LH-20 with $CHCl_3$–MeOH (50:50) and semipreparative HPLC to yield nine compounds (**1**–**9**) (Fig. 1).

Compound **1** was obtained as a colorless oil. HREIMS *m*/*z* 156.0788 $[M]^+$ (calcd. for $C_8H_{12}O_3$, 156.0786) indicated that **1** had a formula of $C_8H_{12}O_3$. The ^1H and ^{13}C NMR spectroscopic data (Table 1) showed that the molecule possessed one oxygenated quaternary carbon (δ_C

* Corresponding authors. Tel.: +86 532 82032065; fax: +86 532 82033054.
E-mail addresses: guqianq@ouc.edu.cn (Q. Gu), weimingzhu@ouc.edu.cn (W. Zhu).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.10.030

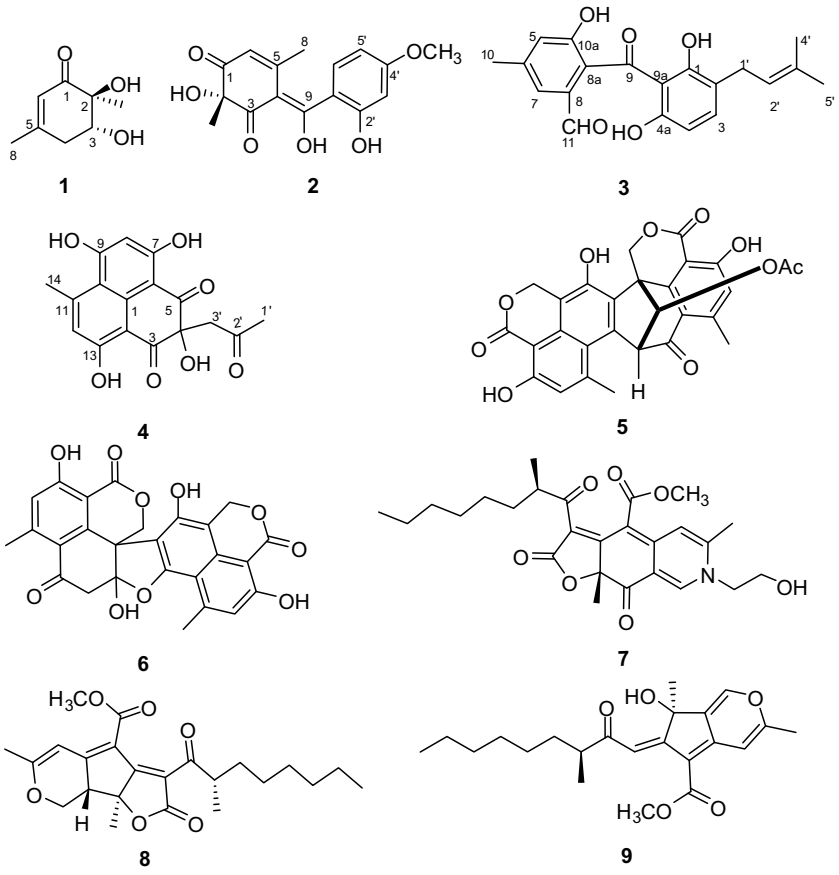

Fig. 1. Structures of compounds **1**–**9**.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data for **1** in DMSO-d_6[a]

Position	^{1}H (*J*, Hz)	^{13}C	HMBC
1		201.0 *s*	
2		75.6 *s*	
3	3.73 *dd* (5.2, 8.3)	71.9 *d*	C-1, C-5, C-2, C-4
4a	2.53 *dd* (5.2, 18.4)	37.8 *t*	C-2, C-3, C-5
4b	2.22 *dd* (8.3, 18.4)		C-2, C-3, C-5
5		159.5 *s*	
6	5.73 *s*	123.5 *d*	C-2, C-4
7	1.08 *s*	18.2 *q*	C-1, C-2, C-3
8	1.91 *s*	23.8 *q*	C-4, C-6, C-5
OH	4.97 *brs*		

[a] Chemical shifts (relative to TMS) are in (δ) ppm, coupling constants in Hz in parentheses. Assignments were aided by ^{1}H–^{1}H COSY and HMQC.

75.6), one oxygenated methine (δ_H 3.73, δ_C 71.9), one methylene (δ_H 2.22, 2.53, δ_C 37.8), two methyls (δ_H 1.08, δ_C 18.2 and δ_H 1.91, δ_C 23.8, respectively), and one α,β-unsaturated ketone moiety (δ_H 5.73, δ_C 201.0 *s*, 123.5 *d*, 159.5 *s*). All these data indicated **1** had the same gross structure as leptosphaerone A (Ayer et al., 1993). Compound **1** showed a CD spectra [320 nm ($\Delta\varepsilon$ −0.15), 238 nm ($\Delta\varepsilon$ +2.48)], which was opposite to that of leptosphaerone A [325 nm ($\Delta\varepsilon$ +0.4), 240 nm ($\Delta\varepsilon$ −1.5)] (Ayer et al., 1993). This implied that the chirality of the asymmetric carbons in **1** is different from that in leptosphaerone A. The absolute configuration of **1** was determined by its CD spectrum. On the basis of the octant rule for the cyclohexanone (Ye, 1999), the negative Cotton effect at 320 nm ($\Delta\varepsilon_{max}$ −0.15) for n → π* and the positive Cotton effect at 233 nm ($\Delta\varepsilon_{max}$ +2.48) for π → π* indicated that the conformation of **1** was as depicted in Fig. 2. Therefore, the structure of **1** was determined as (2*S*,3*R*)-2,3-dihydroxy-2,5-dimethylcyclohex-5-enone, and was given the name leptosphaerone C.

Compound **2** was obtained as a red amorphous solid. High resolution mass measurement on the $[M+Na]^+$ peak (*m*/*z* 327.0840, calcd for $C_{16}H_{16}O_6Na$, 327.0845) in ESIMS, in combination with ^{1}H and ^{13}C NMR spectroscopic data (Table 2), supported the molecular formula $C_{16}H_{16}O_6$. Two unsaturated carbonyl groups were identified based on the two vibrations in the IR spectrum at 1669 and 1612 cm^{-1}. The ^{1}H NMR data showed two aliphatic methyls (δ_H 1.35, 2.20), one methoxy group (δ_H

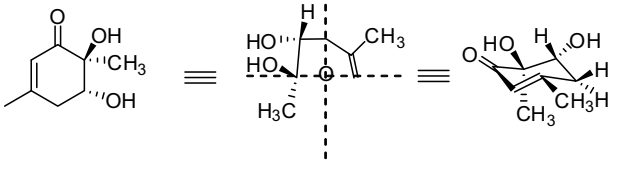

Fig. 2. Configuration of compound **1**.

Table 2
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data for **2** in DMSO-d_6[a]

Position	^{1}H (*J*, Hz)	^{13}C	COSY	HMBC	NOIESY
1		196.0 *s*			
2		81.2 *s*			
2-OH	5.68 *brs*			C-1, C-3, C-2, C-7	
3		197.3 *s*			
4		127.7 *s*			
5		154.4 *s*			
6	5.98 *s*	119.9 *d*	H-8	C-4, C-2	
7	1.35 *s*	27.3 *q*		C-1, C-3, C-2	
8	2.20 *s*	18.7 *q*		C-6, C-5, C-4	H-6′
9		156.4 *s*			
9-OH	14.32 *brs*			C-4, C-1′	
1′		122.7 *s*			
2′		156.4 *s*			
2′-OH	9.74 *brs*				
3′	6.55 *d* (2.3)	99.6 *d*	H-5′	C-1′, C-2′, C-4′, C-5′	
4′		148.9 *s*			
4′-OCH_3	3.89 *s*	56.1 *q*		C-4′	
5′	6.48 *dd* (2.3, 8.7)	108.2 *d*	H-6′	C-1′, C-3′	
6′	7.48 *d* (8.7)	115.4 *d*	H-5′	C-9, C-2′, C-4′	H-8

[a] Chemical shifts (relative to TMS) are in (δ) ppm, coupling constants in Hz in parentheses. Assignments were aided by ^{1}H–^{1}H COSY and HMQC.

3.89) and four aromatic protons, three of which exhibited *ortho* and *meta* couplings (δ_H 6.48 *dd*, $J = 8.7$, 2.4 Hz, δ_H 7.48 *d*, $J = 8.7$ Hz and δ_H 6.55 *d*, $J = 2.4$ Hz, respectively), and three exchangeable protons (δ_H 5.68, 9.74 and 14.32), including one hydrogen-bonded proton. The ^{13}C NMR spectrum indicated two unsaturated carbonyl carbons (δ_C 197.3, 196.04), one sp^3-hybridized oxygenated quaternary carbon (δ_C 81.2), ten sp^2-hybridized carbons [four of which were in an α, β, γ, δ-unsaturated ketone (δ_C 165.4, 127.9, 154.4, 119.9)] (Stierle et al., 1999). Further analysis and comparison of the 1D NMR spectroscopic data of **2** with **1** established two key moieties, leptosphaeronyl and 1,2,4-trisubstituted benzene ring. The C-3 hydroxyl and the C-4 methylene in **1** were replaced by a carbonyl and a double bond carbon in **2**.

HMBC correlations from the 9-OH to C-4 and C-1′ (Table 2) established the connectivity of the two moieties through C-9. The location of the methoxy group (δ 3.89) at C-4′ was supported by the HMBC correlation between the CH_3O protons and C-4′. This completed the structure of **2**.

The observation of only one correlation (Table 2) between the H-8 and H-6′ in NOESY spectrum suggested a common $\Delta^{4,9}$ *Z* stereochemistry. In a previous report, leptosphaerones A and B, with the same biosynthesis pathway, had the same configuration at C-2 (Ayer et al., 1993). Analogously, compounds **1** and **2** may also have a similar biosynthesis pathway and the same stereochemical configuration at C-2. Furthermore, the CD spectrum of **2** showing Cotton effects at 302 nm ($\Delta\varepsilon$ −4.58) for n → π, and 240 nm ($\Delta\varepsilon$ +16.43) for π → π*, which were similar to that for **1**, supported this assignment. Therefore, compound **2** may have an *R* configuration at C-2, and was given the name penicillenone.

Compound **3** was obtained as a yellow amorphous solid. The high resolution-electron ionization mass spectra HRESIMS ($[M+Na]^+$, *m/z* 363.1219, calcd. for $C_{20}H_{20}O_5Na$, 363.1208) indicated that **3** had the formula $C_{20}H_{20}O_5$. In the ^{1}HNMR spectrum, two *ortho* coupling aromatic protons signals (δ_H 7.10, 6.12, $J = 8.3$ Hz) and two *meta* coupling aromatic protons signals (δ_H 7.23 *brs*, 6.97 *brs*) indicated one 1,2,3,4-substituted and one 1,2,3,5-substituted benzene ring, respectively, which were also confirmed by 12 aromatic signals (Table 3) in ^{13}C NMR spectrum of **3**. The presence of the one 3-methyl-2-butenyl group was indicated from the resonances at (δ_H 3.18 *d*, 5.27 *t*, 1.70 *s*, 1.69 *s*, δ_C 26.9, 122.7, 25.6, 17.6). All of these data are very similar to those of arugosin H (Kralj et al., 2006), except for a proton (δ 7.23 *brs*) on C-7 in **3** instead of a hydroxyl in arugosin H, which was consistent with the molecular formula. That was supported by the HMBC correlations of H-7 with C-8 and C-5. Therefore the structure of **3** was elucidated to be a 7-dehydroxylated derivative of arugosin H, and was given the name arugosin I.

Compound **4** was obtained as a yellow amorphous solid. The high resolution-electron ionization mass spectra HRESIMS ($[M+Na]^+$, *m/z* 353.0647, calcd. for $C_{17}H_{14}O_7Na$, 353. 0637) indicated **4** to have the formula $C_{17}H_{14}O_7$. An acetyl group (δ_H 2.12, δ_C 29.4, C-1′; 208.0, C-2′), two unsaturated ketone carbons (δ_C 200.7, 198.1), ten aromatic carbons and two aromatic protons (δ_H 6.42 *s*, 6.87 *s*) were established from analysis of the ^{1}H and ^{13}C NMR spectroscopic data (Table 4). In addition, one oxygenated quaternary carbon (δ_C 73.3), one methyl group (δ_H 2.84 *s*, δ_C 25.6), one methylene group (δ_H 3.58 *d*, 3.63 *d*, δ_C 49.3), and four D_2O exchangeable protons (δ_H 13.74, 13.24, 12.13 and 6.98) were identified. The 1D NMR spectra of **4** were very close to those of FR-901235 except for the observation that a methoxy group in FR-901235 was replaced by a hydroxyl group in **4**, which was 14 amu smaller than FR-901235 (Shibata et al., 1989), with a difference

Table 3
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data for **3** in DMSO-d_6[a]

Position	^{1}H (*J*, Hz)	^{13}C	COSY	HMBC
9a		110.8 *s*		
1	13.19 *s*	160.6 *s*		
1-OH				C-9a, C-1, C-2
2		118.6 *s*		
3	7.10 *d* (8.3)	136.4 *d*	H-4	C-1, C-1′, C-4a
4	6.12 *d* (8.3)	105.8 *d*	H-3	C-9a, C-2
4a		158.7 *s*		
4a-OH	9.92 *s*			C-9a
1′	3.18 *d* (7.3)	26.9 *t*	H-2′	C-1, C-2, C-2′, C-3′, C-3
2′	5.27 *t* (7.3)	122.7 *d*	H-1′	C-1′, C-5′
3′		131.5 *s*		
4′	1.70 *s*	25.6 *q*		C-5′, C-2′, C-3′
5′	1.69 *s*	17.6 *q*		C-4′, C-2′, C-3′
9		201.4 *s*		
8a		128.6 *s*		
10a		153.4 *s*		
10a-OH	9.88 *s*			C-8a
5	6.97 *s*	122.0 *d*		C-9, C-8a, C-7, C-10
6		139.0 *s*		
7	7.23 *s*	123.8 *d*		C-8a, C-5, C-11, C-10
8		134.1 *s*		
11	9.80 *s*	192.2 *d*		C-8a, C-8, C-7
10	2.34 *s*	20.6 *q*		C-7, C-6, C-5

[a] Chemical shifts (relative to TMS) are in (δ) ppm, coupling constants in Hz in parentheses. Assignments were aided by ^{1}H–^{1}H COSY and HMQC.

Table 4
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data for **4** in DMSO-d_6[a]

Position	^{1}H (*J*, Hz)	^{13}C	HMBC
1		137.7 *s*	
2		105.4 *s*	
3		200.7 *s*	
4		73.3 *s*	
4-OH	6.98 *s*		
5		198.1 *s*	
6		101.2 *s*	
7		168.1 *s*	
7-OH	13.74 *s*		C-6, C-7, C-8
8	6.42 *s*	99.2 *d*	C-6, C-7, C-9, C-10
9		166.6 *s*	
9-OH	12.13 *brs*		
10		112.5 *s*	
11		149.7 *s*	
12	6.87 *s*	117.9 *d*	C-2, C-13, C-14, C-10, C-3
13		165.2 *s*	
13-OH	13.24 *s*		C-2, C-12, C-13, C-11, C-3
14	2.84 *s*	25.6 *q*	
1′	2.12 *s*	29.3 *q*	C-2′, C-3′
2′		208.0 *s*	
3′	3.58 *d* (17.8), 3.63 *d* (17.8)	49.3 *t*	C-2′, C-4, C-3, C-5

[a] Chemical shifts (relative to TMS) are in (δ) ppm, coupling constants in Hz in parentheses. Assignments were aided by ^{1}H–^{1}H COSY and HMQC.

of a H_2C in the molecular formula. COSY and HMBC correlations also supported this structure. Therefore, the structure of **4** is the 9-demethyl derivative of FR-901235.

The remaining compounds were identified as bacillosporin A (**5**) (Yamazaki and Okuyama, 1980), bacillosporin C (**6**) (Yamazaki and Okuyama, 1980), sequoiamonascin D (**7**) (Stierle et al., 2003), and sequoiatones A (**8**) and B (**9**) (Stierle et al., 1999) by comparison of their spectroscopic data with the literature values.

Compounds **1**–**4** were evaluated for their cytotoxicities against the A-549 and P388 cell lines by the MTT method (Mosmann, 1983). Compound **1** showed cytotoxicity against the A-549 cell line, with an IC_{50} value of 1.45 μM, and compound **2** showed cytotoxicity against the P388 cell line with an IC_{50} value of 1.38 μM.

2.2. Identification of the producing strain

Strain JP-1 was isolated from the inner bark of an *Aegiceras corniculatum* tree collected in Fujian, China. On potato dextrose agar it showed floccose colonies, the surface at first white then dark green, and the reverse appeared red. A GenBank search with the 18S ribosomal DNA gene of JP-1 (1718 nucleotides; GenBank accession number EF654656) was carried out. The results indicate several species of *Penicillium* as the closest matches, with sequence identities of the 21 closest hits 99%. On the basis of its 18S ribosomal DNA gene sequence, JP-1 is a *Penicillium* species.

2.3. Concluding remarks

In summary, we have isolated and determined the structures of four new polyketides (**1**–**4**) together with five known compounds (**5**–**9**). To the best of our knowledge, the leptosphaerone skeleton with a benzoyl substituent at C-4 has not been previously reported, and this paper represents the first report of the cytotoxicities of leptosphaerones.

3. Experimental

3.1. General experimental procedure

Optical rotations were obtained on a JASCO P-1020 digital polarimeter. CD spectra were obtained on a JASCO J-810 spectropolarimeter. UV spectra were recorded on a Beckmen DU 640 spectrophotometer. IR spectra were taken on a Nicolet NEXUS 470 spectrophotometer in KBr disks. ^{1}H, ^{13}C NMR and DEPT spectra and 2D NMR were recorded on a JEOL JNM-ECP-600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. NOESY experiments were carried out using a mixing time of 0.5 s. 1D NOE spectra were obtained on a Varian INOVA-400 spectrometer. ESIMS was measured on a Micromass Q-TOF Ultima Global GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [YMC-pack ODS (A), 10 × 250 mm, 5 μm, 4 mL/min].

3.1.1. Fungal collection

A sample of *Penicillium* sp. JP-1 was isolated from the inner bark of an *A. corniculatum* tree collected in Fujian,

China. The outer bark was removed from a branch of this mangrove plant. The surface was sterilized by sequential washes in 0.53% NaOCl (2 min) and EtOH–H_2O (3:1, v/v) (2 min), rinsed with sterile distilled H_2O, and allowed to surface dry under aseptic conditions. The surface-sterilized branch was cut into several segments, which were placed on 2% malt extract agar in Petri dishes. All plates were incubated at room temperature for a maximum of 3 weeks. Fungi growing out from the plant tissues were transferred to potato dextrose agar slants and stored at 4 °C.

3.1.2. Determination of rDNA sequences

The methods and reagents for DNA extraction and amplification were essentially as described by Köpcke et al. (2002a). For the amplification of a 5′ segment of the 18S rDNA gene, primers NS1 (5′-GTAGTCATATGCTTGTCTC) and SN8 (5′-TCCGCAGGTTCACCTACGGA) were used (White et al., 1990; http://www.biology.duke.edu/fungi/mycolab). The PCR mixture (50 μL) contained 1 μL of a 50 μM solution of each primer (TaKaRa, Japan), 1 μL DNA (200 ng/mL), 0.15 μL Ex Taq (5 U/μL; TaKaRa, Japan), 5 μL 10× Ex Taq Buffer (Mg^+ Plus), 5 μL dNTP (2.5 mM) and 36.85 μL H_2O. After an initial pre-heat at 94 °C for 3 min, 35 amplification cycles were performed (30 s at 94 °C, 45 s at 56 °C, 2 min at 72 °C), followed by a final extension period of 15 min at 72 °C. Independent sequencing of the PCR product was performed at Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. Sequences were assigned and proof-read by eye using Chromas 2.23 (Technelysium, Helensvale, Australia). Searches for matching sequences were performed using BLAST in GenBank.

3.2. Fermentation, extraction and isolation

The fungus was grown under static conditions at 24 °C for 30 days in 120 1000-mL conical flasks containing the liquid medium (300 mL/flask) composed of glucose (10 g/L), maltose (20 g/L), mannitol (20 g/L), monosodium glutamate (10 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.3 g/L), corn steep liquor (1 g/L) and yeast extract (3 g/L) and seawater after adjusting its pH to 7.0. The fermented whole broth (36 L) was filtered through cheesecloth to separate into supernatant and mycelia. The former was concentrated under reduced pressure to about a quarter of the original volume and then extracted with EtOAc (3 × 35 L) to give an ethyl acetate solution, while the latter was extracted with acetone–H_2O (4:1, v/v) (3 × 15 L). The acetone–H_2O solution was concentrated under reduced pressure to afford an H_2O solution (11 L). The H_2O solution was extracted with EtOAc (3 × 11 L) to give another EtOAc solution. Both EtOAc solutions were combined and concentrated under reduced pressure to give a crude extract (35.0 g), which was separated into 14 fractions on a silica gel column using a step gradient elution of $CHCl_3$–MeOH (1:0 → 1:1). Fraction 1 was further separated by reversed-phase CC (MeOH–H_2O = 3:2 → 4:1 → MeOH) to afford Fractions 1–5 (40 mg), which was then further separated by extensive PHPLC (MeOH–H_2O = 85:15, 4.0 mL/min) to yield compound **9** (15 mg) and compound **8** (26 mg). Fraction 2 was further subjected to silica gel CC with a gradient of $CHCl_3$–MeOH (20:1 → 10:1), and Sephadex LH-20 with $CHCl_3$–MeOH (1:1) repeatedly. The obtained subfraction was further purified by semipreparative HPLC employing an isocratic elution with MeOH–H_2O (75:25, 4.0 mL/min) to give compound **2** (30 mg), and with MeOH–H_2O (50:50, 4.0 mL/min) to give compound **1** (10 mg). Fraction 3 was further subjected to silica gel CC with a gradient elution of $CHCl_3$–MeOH (30:1 → 10:1) to obtain three subfractions Fr. 3-1, Fr. 3-2 and Fr. 3-3. Fr. 3-1 was further purified by Sephadex LH-20 with $CHCl_3$–MeOH (1:1) and semipreparative HPLC employing isocratic elution with MeOH–H_2O (70:30, 4.0 mL/min) to give compound **3** (41.1 mg) and compound **4** (13.0 mg). Fr. 3-2 was separated by extensive PHPLC (MeOH–H_2O = 75:25, 4.0 mL/min) to yield compound **7** (30 mg). Fr. 3-3 was subjected to Sephadex LH-20 CC with $CHCl_3$–MeOH (1:1) and then purified by semipreparative HPLC employing isocratic elution with MeOH–H_2O (80:20, 4.0 mL/min) to give compound **5** (15 mg) and compound **6** (9 mg).

3.2.1. Leptosphaerone C (*1*)

Colorless oil; $[\alpha]_D^{25}$ − 0.8 ($CHCl_3$; *c* 0.30); UV μ_{max}^{MeOH} : 232 nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 320 (−0.15), 238 (+2.48) nm; IR ν_{max} (KBr) 3405, 2970, 2920, 2858, 1669, 1390, 1172, 1114, 1056, 1029, 998, 450 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HREIMS *m/z* 156.0788 $[M]^+$ (calcd. for $C_8H_{12}O_3$, 156.0786).

3.2.2. Penicillenone (*2*)

Red amorphous solid; $[\alpha]_D^{25}$ + 24 (MeOH; *c* 0.10); UV μ_{max}^{MeOH} : 454 nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 192 (−17.85), 194 (+10.90), 210 (−9.40), 240 (+16.43), 301 (−4.58), 322 (+1.63) nm; IR ν_{max} (KBr) 3401, 2920, 1669, 1611, 1584, 1518, 1405, 1293, 1211, 1079, 1021, 834 cm^{-1}; ^1H and ^{13}C NMR (see Table 2); HRESIMS *m/z* 327.0840 $[M+Na]^+$ (calcd. for $C_{16}H_{16}O_6Na$, 327.0845).

3.2.3. Arugosin I (*3*)

Yellow amorphous solid; UV μ_{max}^{MeOH} : 214, 279, 351 nm; IR ν_{max} (KBr) 3436, 2950, 2915, 1626, 1584, 1468, 1418, 1344, 1225, 1174, 1047, 947, 827 cm^{-1}; ^1H and ^{13}C NMR (see Table 3); HRESIMS *m/z* 363.1219 $[M+Na]^+$ (calcd. for $C_{20}H_{20}O_5Na$, 363.1208).

3.2.4. 9-Demethyl FR-901235 (*4*)

Yellow amorphous solid; $[\alpha]_D^{25}$ + 8 (MeOH; *c* 0.035); UV μ_{max}^{MeOH} : 214, 254, 330 nm; IR ν_{max} (KBr) 3436, 3393, 1703, 1599, 1452, 1414, 1388, 1256, 1200, 1178, 840 cm^{-1}; ^1H and

^{13}C NMR (see Table 4); HRESIMS *m/z* 353.0647 $[M+Na]^+$ (calcd. for $C_{17}H_{14}O_7Na$, 353.0637).

4. Biological assays

Cytotoxic activity was evaluated by the MTT method using A-549 and P388 cell lines. The cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. These cell suspensions (200 μL) at a density of 5×10^4 cell mL^{-1} were plated in 96-well microtiter plates and incubated for 24 h in the above conditions. The test compound solution (2 μL in DMSO) at different concentrations was added to each well and further incubated for 72 h in the same condition. Then 20 μL of the MTT solution (5 mg/mL in RPMI-1640 medium) was added to each well and incubated for 4 h. The old medium containing MTT (150 μL) was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a Spectra Max Plus plate reader at 540 nm.

Acknowledgements

This work was financially supported by the Chinese National Natural Science Fund (No. 30472136) and the International Cooperation Project of Australia and China (2005 DFA 30030-4). The anti-tumor assay was performed at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

References

Ayer, W.A., Gökdemir, T., Miao, S.-C., Trifonov, L.S., 1993. Leptosphaerones A and B, new cyclohexenones from *Leptosphaeria herpotrichoides*. J. Nat. Prod. 56, 1647–1650.

Köpcke, B., Weber, R.W.S., Anke, H., 2002a. Galiellalactone and its biogenetic precursors as chemotaxonomic markers of the Sarcosomataceae (Ascomycota). Phytochemistry 60, 709–714.

Kralj, A., Kehraus, S., Krick, A., Eguereva, E., Kelter, G., Maurer, M., Wortmann, A., Fiebig, H.-H., König, G.M., 2006. Arugosins G and H: prenylated polyketides from the marine-derived fungus *Emericella nidulans* var. *acristata*. J. Nat. Prod. 69, 955–1000.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays. J. Immunol. Meth. 65, 55–63.

Shibata, T., Nishikawa, M., Tsurumi, Y., Takase, S., Terano, H., Kohsaka, M., 1989. A new immunomodulator, FR-901235. J. Antibiot. 42, 1356–1361.

Stierle, A.A., Strobel, G.A., Stierle, D.B., 1993. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific Yew. Science 260, 214–216.

Stierle, A.A., Stierle, D.B., Bugni, T., 1999. Sequoiatones A and B: novel antitumor metabolites isolated from a redwood endophyte. J. Org. Chem. 64, 5479–5484.

Stierle, D.B., Stierle, A.A., Bugni, T., 2003. Sequoiamonascins A-D: novel anticancer metabolites isolated from a redwood endophyte. J. Org. Chem. 68, 4966–4969.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.

Yamazaki, M., Okuyama, E., 1980. Isolation and structures of oxaphenalenone dimers from *Talaromyces bacillosporus*. Chem. Pharm. Bull. 28, 3649–3655.

Ye, X.L., 1999. Stereochemistry. Beijing University Express, Beijing, pp. 257–259.