ORGANIC LETTERS

2013
Vol. 15, No. 7
1674–1677

Periconiasins A–C, New Cytotoxic Cytochalasans with an Unprecedented 9/6/5 Tricyclic Ring System from Endophytic Fungus *Periconia* sp.

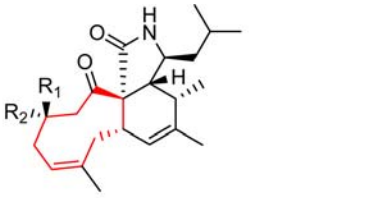
Dewu Zhang, Hanlin Ge, Dan Xie, Ridao Chen, Jian-hua Zou, Xiaoyu Tao, and Jungui Dai*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Peking Union Medical College & Chinese Academy of Medical Sciences, 1 Xian Nong Tan Street, Beijing 100050, P. R. China

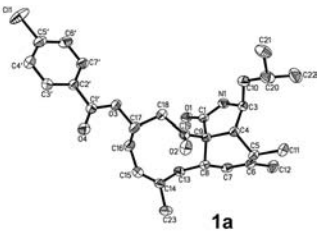
jgdai@imm.ac.cn

Received February 19, 2013

ABSTRACT



1 R_1 = H, R_2 = OH **1a** R_1 = H, R_2 = 4-ClPhCO$_2$
2 R_1 = OH, R_2 = H **3** R_1 = R_2 = O



1a

Periconiasins A–C (1–3), new cytochalasans with an unprecedented 9/6/5 tricyclic ring system, were isolated from the endophytic fungus *Periconia* sp. F-31. Their structures and absolute configurations were elucidated by extensive spectroscopic and X-ray crystallographic analyses. Their biosynthesis is proposed to occur from an unusual seven acetate/malonate polyketide backbone attached to one leucine moiety by a PKS-NRPS followed by Diels–Alder and other reactions. 1 and 2 showed significant cytotoxicity against human HCT-8 cancer cells.

Cytochalasans are a group of polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) hybrid metabolites from a number of fungal genera (including *Phomopsis*, *Aspergillus*, and *Penicillium*) that display a wide range of biological properties such as cytotoxic,[1] antimicrobial,[2] antiviral,[3] and phytotoxic[4] activities. In general, cytochalasans are structurally characterized by their tricyclic core, which consists of a macrocyclic ring fused to an isoindolone moiety contributed by a highly reduced polyketide backbone and an amino acid (for example, leucine or phenylalanine). Over 80 different cytochalasans have been isolated, and their macrocyclic rings all featured 11- to 13-membered carbocyclic or 12- to 14-membered lactone rings, which were assembled by the condensation of 8/9 acetate/malonate units. The lactone rings in some cytochalasan structures are thought to be generated through Baeyer–Villiger insertion of oxygen atom(s) into the carbocyclic ring.[5]

As part of our ongoing work searching novel bioactive natural compounds from endophytic fungus of medicinal plants,[6] more than 40 fungal strains were isolated from the medicinal plant *Annona muricata* distributed in Hainan

(1) (a) Jiao, W.; Feng, Y.; Blunt, J. W.; Cole, A. L. J.; Munro, M. H. G. *J. Nat. Prod.* **2004**, *67*, 1722–1725. (b) Alvi, K. A.; Nair, B.; Pu, H.; Ursino, R.; Gallo, C.; Mocek, U. *J. Org. Chem.* **1997**, *62*, 2148–2151.

(2) (a) Betina, B. V.; Micekova, D.; Nemec, P. *J. Gen. Microbiol.* **1972**, *71*, 343–349. (b) Horn, W. S.; Simmonds, M. S. J.; Schwartz, R. E.; Blaney, W. M. *Tetrahedron* **1995**, *51*, 3969–3978. (c) Pongcharoen, W.; Rukachaisirikul, V.; Phongpaichit, S.; Rungjindamai, N.; Sakayaroj, J. *J. Nat. Prod.* **2006**, *69*, 856–858.

(3) (a) Lingham, R. B.; Hsu, A.; Silverman, K. C.; Bills, G. F.; Dombrowski, A.; Goldman, M. E.; Darke, P. L.; Huang, L.; Koch, G.; Ondeyka, J. G.; Goetz, M. A. *J. Antibiol.* **1992**, *45*, 686–691. (b) Zhang, Y.; Tian, R.; Liu, S.; Chen, X.; Liu, X.; Che, Y. *Bioorg. Med. Chem.* **2008**, *16*, 2627–2634.

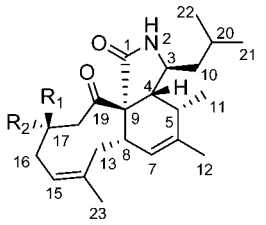
(4) (a) Cimmino, A.; Andolfi, A.; Berestetskiy, A.; Evidente, A. *J. Agric. Food Chem.* **2008**, *56*, 6304–6309. (b) Berestetskiy, A.; Dmitriev, A.; Mitina, G.; Lisker, I.; Andolfi, A.; Evidente, A. *Phytochemistry* **2008**, *69*, 953–960. (c) Evidente, A.; Andolfi, A.; Vurro, M.; Zonno, M. C.; Motta, A. *J. Nat. Prod.* **2003**, *66*, 1540–1544.

(5) (a) Scherlach, K.; Boettger, D.; Remme, N.; Hertweck, C. *Nat. Prod. Rep.* **2010**, *27*, 869–886. (b) Schümann, J.; Hertweck, C. *J. Am. Chem. Soc.* **2007**, *129*, 9564–9565. (c) Qiao, K.; Chooi, Y. H.; Tang, Y. *Metab. Eng.* **2011**, *13*, 723–732.

10.1021/ol400458n
Published on Web 03/18/2013

Province, China. Bioassay results revealed that the EtOAc extract of one strain identified as *Periconia* sp. F-31 displayed potent cytotoxicity against several human cancer cell lines (see Table S3). Bioassay-guided fractionation of the EtOAc extract of the fermentation broth of this strain led to the isolation of three novel cytochalasans, periconiasins A−C (**1**−**3**), which featured an unusual 9/6/5 tricyclic ring system. Their molecular structures together with the relative configuration were unambiguously established on the basis of extensive spectroscopic data analysis. The absolute configurations were determined by analyzing the single-crystal X-ray diffraction data of one of the esterified derivatives of **1** (**1a**, Figure 2) and circular dichroism (CD) spectroscopic data. The biosynthetic pathway of **1**−**3** was proposed to be typical of fungal PKS-NRPS hybrids formed from one acetyl-CoA starter and six malonyl-CoA extenders coupled with one leucine-CoA. The formation of a polyketide backbone and 9-membered macrocyclic ring is the first such example among the structures of cytochalasans and constitutes a new type of skeleton in the cytochalasan family. Herein, we report the isolation, structural elucidation, plausible biogenetic pathway, and in vitro cytotoxicity of **1**−**3**.

The filtrate of 140 L of fermentation cultures was applied through an Amberlite XAD-16 macroporous adsorbent resin column followed by eluting with H_2O and 95% EtOH (see pp S5−S6). The 95% EtOH fraction was extracted with EtOAc to afford 25 g of residue, which was subjected to silica gel and Sephadex LH-20 column chromatography and further purified by semipreparative normal-phase and reversed-phase HPLC to yield **1** (185 mg), **2** (40 mg), and **3** (5 mg).



1 R_1 = H, R_2 = OH **1a** R_1 = H, R_2 = 4-ClPhCO$_2$
2 R_1 = OH, R_2 = H **3** R_1 = R_2 = O

Periconiasin A (**1**)[7] was obtained as a colorless gum and gave an HR-ESI-MS ion peak at m/z 360.2528 $[M + H]^+$, which corresponded to a molecular formula of $C_{22}H_{33}NO_3$ with seven degrees of unsaturation. The IR absorption bands at 3351, 3270, 3217, and 1690 cm^{-1} indicated the presence of hydroxy, amidogen, and carbonyl moieties. The ^{13}C NMR and DEPT spectra exhibited 22 carbon resonances including two carbonyl carbons (δ_C 212.2 and 175.7), four olefinic carbons (δ_C 138.7, 136.6, 128.9, and 122.7), one quaternary carbon (δ_C 65.9), six methines (δ_C 69.3, 55.3, 49.5, 43.6, 34.8, and 23.9), four methylenes (δ_C 48.8, 46.7, 37.3, and 30.8), and five methyls (δ_C 23.6, 23.5, 21.6, 19.4, and 12.9). The proton and protonated carbon resonances in the NMR spectra of **1** were unambiguously assigned by interpretation of ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, and HSQC spectroscopic data. The HMBC correlations of H-2/C-3, C-4, and C-9; H-3/C-1, C-4, and C-5; H-4/C-3, C-5, C-6, C-9, C-10, and C-11; and H-12/C-5, C-6, and C-7, together with the ^{1}H−^{1}H COSY correlations of H-8/H-7 and H-13 (Figure 1), indicated the presence of a pentabasic lactam ring and cyclohexene substructures (the isoindolone moiety). Furthermore, the HMBC correlations of H-13/C-7, C-8, C-9, C-14, and C-15; H-16/C-14, C-15, C-17, and C-18; H-17/C-15 and C-19; and H-4/C-19, along with the spin system from H-15 to H-18 on the basis of the ^{1}H−^{1}H COSY correlations (Figure 1), established another 9-membered ring. Accordingly, the molecular structure of **1** was determined to be a typical tricyclic core structure seen in cytochalasan structures in which a macrocyclic ring is fused to an isoindolone moiety. However, **1** represents a new type of cytochalasan bearing a unique 9-membered carbocyclic ring.

The relative configuration of **1** was determined by NOESY spectral data analysis (Figure 1). The NOEs of H-3/H_3-11 suggested their α-orientations, while the NOEs of H-4/H-5 and H-8 indicated their β-orientations. The NOEs of H-13α/H-16α and H-18α; H-17/H-16β and H-18β; and H-13β/H-8 established the α-orientation of 17-OH.

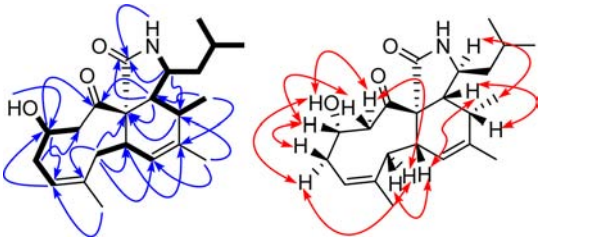


Figure 1. ^{1}H−^{1}H COSY (bold —), key HMBC (→), and NOESY (↔) correlations of **1**.

For the purpose of determining the absolute configuration of **1**, a number of experiments were deliberately developed to culture available single crystals for the X-ray diffraction analysis, but the desired single crystals were not obtained. However, the existence of a functional group, 17-OH, in the molecule, gave an opportunity for its further chemical derivatization. Accordingly, several esterified derivatives were designed and chemically synthesized. A single crystal X-ray diffraction pattern of one of the derivatives (the 4-Cl-benzoxylated one, **1a**; see pp S7−13) was fortunately obtained by anomalous scattering of Cu

(6) (a) Wang, J. M.; Ding, G. Z.; Fang, L.; Dai, J. G.; Yu, S. S.; Wang, Y. H.; Chen, X. G.; Ma, S. G.; Qu, J.; Du, D. *J. Nat. Prod.* **2010**, *73*, 1240–1249. (b) Wang, J. M.; Jiang, N.; Ma, J.; Yu, S. S.; Tan, R. X.; Dai, J. G.; Si, Y. K.; Ding, G. Z.; Ma, S. G.; Qu, J.; Fang, L.; Du, D. *Tetrahedron* **2013**, *69*, 1199–1201. (c) Ge, H. L.; Zhang, D. W.; Li, L.; Xie, D.; Zou, J. H.; Si, Y. K.; Dai, J. *Chem. Pharm. Bull.* **2011**, *59*, 1541–1544. (d) Shen, Y.; Zou, J.; Xie, D.; Ge, H.; Cao, X.; Dai, J. *Chem. Pharm. Bull.* **2012**, *60*, 1437–1441.

(7) Periconiasin A (**1**): colorless gum; $[\alpha]^{20}_D$ −21.7 (*c* 0.12, MeOH); IR (ν_{max}): 3351, 3270, 3217, 2961, 2927, 1690, 1462, 1383, and 1050 cm^{-1}; UV (MeOH) λ_{max} (log ε): 205 (0.87), 223 (0.15) nm; CD (MeOH) $\Delta\varepsilon$ (nm): +1.75 (218), +3.55 (237), −1.91 (301.5); ESI-MS m/z 360.3 $[M + H]^+$; HR-ESI-MS m/z 360.2528 $[M + H]^+$ (calcd for $C_{22}H_{34}NO_3$, 360.2533); ^{1}H and ^{13}C NMR data, see Table 1.

Table 1. NMR Data of Periconiasins A–C (**1**–**3**)[a]

	1[b]		**2**[b]		**3**[c]	
no.	δ_C	δ_H[d]	δ_C	δ_H[d]	δ_C	δ_H[d]
1	175.7		173.8		176.3	
2		8.19 (1H, s)		7.86 (1H, s)		7.38 (1H, s)
3	49.5	3.02 (1H, br s)	50.2	3.03 (1H, m)	51.4	3.17 (1H, m)
4	55.3	2.11 (1H, dd, 5.6, 1.6)	50.3	2.07 (1H, overlapped)	57.8	2.20 (1H, dd, 5.4, 3.0)
5	34.8	2.39 (1H, overlapped)	34.9	2.40 (1H, overlapped)	36.2	2.58 (1H, m)
6	138.7		138.3		140.6	
7	128.9	5.40 (1H, s)	129.7	5.51 (1H, s)	128.8	5.48 (1H, d, 1.8)
8	43.6	2.41 (1H, overlapped)	40.3	2.42 (1H, overlapped)	44.6	2.79 (1H, m)
9	65.9		67.6		66.9	
10	48.8	1.02 (2H, m)	48.1	1.08 (1H, m), 0.98 (1H, m)	50.1	1.28 (1H, m), 1.16 (1H, m)
11	12.9	1.11 (3H, d, 7.2)	12.9	1.13 (3H, d, 7.2)	13.7	1.22 (3H, d, 7.2)
12	19.4	1.71 (3H, s)	19.6	1.71 (1H, s)	19.9	1.78 (3H, d, 1.8)
13	30.8	4.08 (1H, m, Hα)	32.4	3.16 (1H, dd, 14.8, 4.4, Hα)	33.2	4.00 (1H, dd, 13.8, 11.4, Hα)
		1.58 (1H, m, Hβ)		1.77 (1H, dd, 14.8, 2.8, Hβ)		1.85 (1H, dd, 13.8, 1.8, Hβ)
14	136.6		140.3		142.8	
15	122.7	5.08 (1H, dd, 10.4, 6.8)	118.5	5.16 (1H, t, 8.0)	120.1	5.08 (1H, dd, 10.2, 6.0)
16	37.3	2.69 (1H, dd, 12.4, 10.4, Hα)	32.9	2.05 (2H, overlapped)	44.8	4.32 (1H, dd, 13.2, 11.4, Hα)
		1.99 (1H, dd, 12.4, 6.8, Hβ)				2.71 (1H, dd, 13.2, 6.0, Hβ)
17	69.3	3.75 (1H, m)	68.4	4.11 (1H, m)	201.0	
18	46.7	3.30 (1H, m, Hα)	42.4	2.38 (1H, dd, 13.6, 5.4, Hα)	57.4	3.83 (1H, d, 13.8, Hα)
		2.35 (1H, overlapped, Hβ)		2.83 (1H, dd, 13.6, 10.8, Hβ)		3.20 (1H, d, 13.8, Hβ)
19	212.2		210.7		206.6	
20	23.9	1.60 (1H, m)	23.9	1.60 (1H, m)	25.4	1.71 (1H, m)
21	21.6	0.83 (3H, d, 6.4)	21.5	0.81 (3H, d, 6.4)	21.6	0.89 (3H, d, 6.6)
22	23.5	0.83 (3H, d, 6.4)	23.5	0.82 (3H, d, 6.4)	23.9	0.88 (3H, d, 7.2)
23	23.6	1.54 (3H, s)	24.2	1.71 (3H, s)	24.0	1.64 (3H, s)
–OH		4.82 (1H, d, 4.0)		4.76 (1H, d, 4.0)		

[a] Peaks were assigned by analyses of the 1D and 2D NMR spectra. [b] ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), DMSO-d_6. [c] ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), acetone-d_6. [d] Multiplicities and coupling constants (J) in Hz are in parentheses.

Kα radiation. An ORTEP drawing including the atom-numbering scheme is shown in Figure 2, and it demonstrates the absolute configuration of 3*S*, 4*R*, 5*S*, 8*S*, 9*S*, and 17*S* for **1a**. Thus, the structure of **1** was unambiguously assigned as (3*S*, 4*R*, 5*S*, 8*S*, 9*S*, 17*S*)-periconiasin A.

In addition, the presence of the carbonyl group (C-19) suggests that the CD octant rule is applicable to confirm the absolute configuration of the chiral center (C-9) near the carbonyl moiety.[8] A negative Cotton effect at 301.5 nm for the n–π* transition (see Figure S17) is indicative of the 9*S*, which is in accord with that analyzed by X-ray diffraction.

Periconiasin B (**2**)[9] was isolated as a colorless gum and was assigned the same molecular formula $C_{22}H_{33}NO_3$ as **1** based on HR-ESI-MS results (m/z 360.2525 $[M + H]^+$). Its ^{1}H and ^{13}C NMR spectra showed resonances nearly identical to those of **1**. Interpretation of its 1D and 2D NMR data established the same molecular structure as **1**.

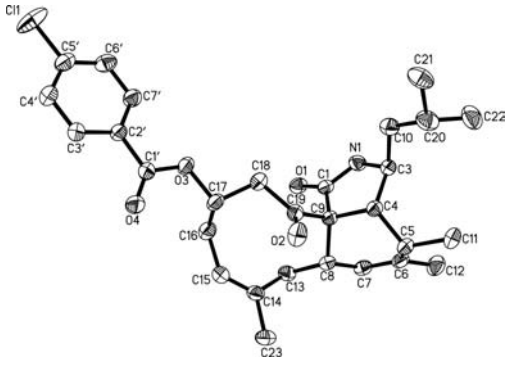


Figure 2. ORTEP diagram of **1a**.

Compared with those of **1**, the chemical shifts for H-16α, H-17, H-18α, and H-18β of **2** were altered by $\Delta\delta$ values of −0.64, +0.36, −0.92, and +0.48 ppm, respectively, and the resonances of C-16, C-17, and C-18 were upfield shifted by $\Delta\delta$ values of 4.4, 0.9, and 4.3 ppm, respectively, which indicates a β-orientation of the 17-OH in **2** rather than the α-orientation found in **1**. This conclusion was further confirmed by the NOEs of H-8/H-18β and H-17/H-18α and the presence of $^3J_{H\text{-}17,H\text{-}18\alpha}$ (5.4 Hz) and $^3J_{H\text{-}17,H\text{-}18\beta}$

(8) Ye, X. L. *Stereochemistry*; Beijing University Press: Beijing, 1999; pp 241–257.

(9) Periconiasin B (**2**): colorless gum; $[\alpha]^{20}_D$ −4.6 (*c* 0.35, MeOH); IR (ν_{max}): 3337, 3255, 2961, 2927, 1703, 1682, 1461, 1384, and 1049 cm^{-1}; UV (MeOH) λ_{max} (log ε): 204 (0.82), 222 (0.13) nm; CD (MeOH) $\Delta\varepsilon$ (nm): −1.57 (296); ESI-MS m/z 360.3 $[M + H]^+$; HR-ESI-MS m/z 360.2525 $[M + H]^+$ (calcd for $C_{22}H_{34}NO_3$, 360.2533); ^{1}H and ^{13}C NMR data, see Table 1.

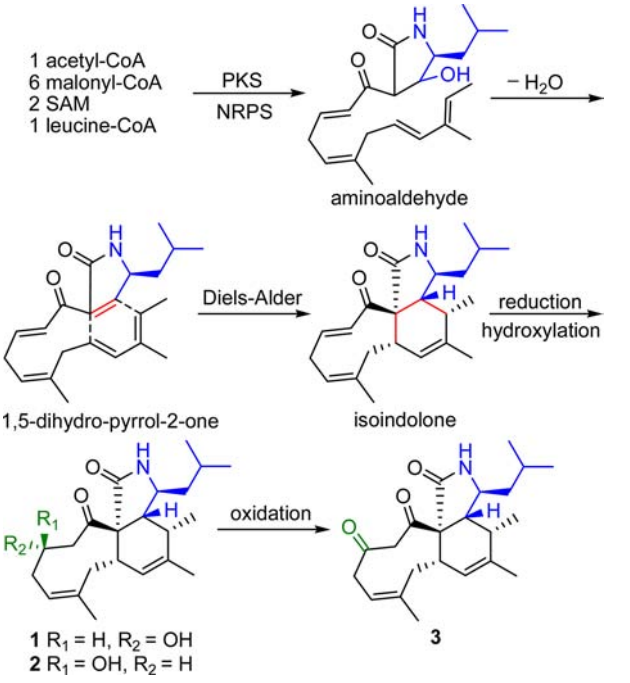
(10.8 Hz) coupling constants (see Figure S1 and Table 1). In the CD spectrum, a negative Cotton effect at 296 nm was observed for **2**, which is similar to that of **1** (see Figure S39) and indicates that **2** has the same configuration of C-9 as **1**. Therefore, the structure of **2** was identified as (3*S*, 4*R*, 5*S*, 8*S*, 9*S*, 17*R*)-periconiasin B.

Periconiasin C (**3**)[10] was obtained as a colorless gum. Its molecular formula was determined to be $C_{22}H_{31}NO_3$ with 8 degrees of unsaturation using HR-ESI-MS, in which an ion peak $[M + H]^+$ at *m*/*z* 358.2384 (calcd for $C_{22}H_{32}NO_3$, 358.2377) was observed. The ^1H and ^{13}C NMR spectra of **3** were similar to those of **1**, except for the presence of a carbonyl moiety (δ_C 201.0) instead of the hydroxy group (δ_C 69.3) in **1**. The location of the carbonyl moiety at C-17 was assigned from the HMBC correlations of C-17/H-16 and H-18 (see Figure S2). The relative configuration of **3** was determined by its NOESY spectrum, in which the NOEs of H-3/H_3-11, and H-5/H-4 and H-8 were observed; the results suggest that **3** has the same relative configuration as **1**. The negative Cotton effect at 294 nm was observed for **3** in the CD spectrum (see Figure S51), which is similar to those of **1** and **2** and indicates that **1**−**3** all have the same absolute configurations. Accordingly, the structure of **3** was elucidated as (3*S*, 4*R*, 5*S*, 8*S*, 9*S*)-periconiasin C.

To the best of our knowledge, periconiasins A−C are the first examples of cytochalansans bearing a 9/6/5 tricyclic ring system. The biosynthesis of **1**−**3** is proposed to be through a hybrid PKS-NRPS biosynthetic pathway (Scheme 1).[5] The incorporation of a polyketide backbone originated from one acetyl-CoA starter and six malonyl-CoA extenders and one leucine moiety by a PKS-NRPS would lead to an aminoaldehyde intermediate, which can readily undergo an intramolecular Knoevenagel condensation to produce the 1,5-dihydro-pyrrol-2-one. A subsequent intramolecular Diels−Alder reaction gives rise to the interesting 9/6/5 tricyclic ring system, isoindolone, after which **1**−**3** can be generated via reduction and oxidation(s). Interestingly, the *Z* configuration of the double bonds at C-14(15) in **1**−**3** is observed while the *E* configuration of the double bonds at C-13(14) is observed in all previously reported cytochalasans, which might be attributable to the unusual 9/6/5 tricyclic ring system.

Compounds **1**−**3** were biologically evaluated for in vitro cytotoxicity against five human cancer cell lines (A2780, HCT-8, Bel-7402, BGC-823, and A549) by using an MTT method with camptothecin as the positive control (see Table S4).[11] Compound **1** showed selective and significant cytotoxicity against the HCT-8 and BGC-823 cell lines with IC_{50} values of 0.9 and 2.1 μM, respectively. Compound **2** showed selective cytotoxic activity against the HCT-8, Bel-7402, and BGC-823 cell lines with IC_{50} values of 0.8, 5.1, and 9.4 μM, respectively. These results imply that **1** and **2** might be candidates as lead compounds against HCT-8 cancer cells.

Scheme 1. Proposed Biosynthesis of **1**−**3**



Acknowledgment. This work was supported by the Science & Technology Project of Guangdong Province (2011A080403020), the Fundamental Research Funds for the Central Universities (Grant No. 2012N06), and the National Science & Technology Major Project 'Key New Drug Creation and Manufacturing', China (No. 2012ZX09301002-001-005).

Supporting Information Available. The experimental procedures, selected HR-ESI-MS, IR, UV, CD, and 1D and 2D NMR spectra of compounds **1**−**3**; HR-ESI-MS, NMR data and X-ray crystallographic data for **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(10) Periconiasin C (**3**): colorless gum; $[\alpha]^{20}_D$ −306.0 (*c* 0.05, MeOH); IR (ν_{max}): 3196, 2962, 2923, 1715, 1687, 1459, and 1383 cm^{-1}; UV (MeOH) λ_{max} (log ε): 204 (0.89), 223 (0.36) nm; CD (MeOH) $\Delta\varepsilon$ (nm): +12.55 (218), −11.42 (294); ESI-MS *m*/*z* 358.3 $[M + H]^+$; HR-ESI-MS *m*/*z* 358.2384 $[M + H]^+$ (calcd for $C_{22}H_{32}NO_3$, 358.2377). ^1H and ^{13}C NMR data, see Table 1.

(11) (a) Mosumann, T. *J. Immunol. Methods* **1983**, *65*, 55–63. (b) Carmichael, J.; Degraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936–943.