Azaphilones and p-Terphenyls from the Mangrove Endophytic Fungus Penicillium chermesinum (ZH4-E2) Isolated from the South China Sea

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · MAY 2011

Impact Factor: 3.8 · DOI: 10.1021/np100889v · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

Azaphilones and *p*-Terphenyls from the Mangrove Endophytic Fungus *Penicillium chermesinum* (ZH4-E2) Isolated from the South China Sea

Hongbo Huang,[†,‡,§] Xiaojun Feng,[†,§] Ze'en Xiao,[†] Lan Liu,[†] Hanxiang Li,[†] Lin Ma,[†] Yongjun Lu,[⊥,∥] Jianhua Ju,[‡] Zhigang She,[*,†,∥] and Yongcheng Lin[*,†,∥]

[†]School of Chemistry and Chemical Engineering, Sun Yat-sen University, 135 West Xingang Road, Guangzhou 510275, People's Republic of China

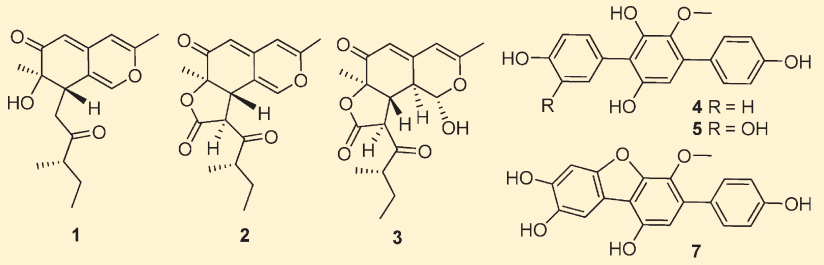
[‡]CAS Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, People's Republic of China

[⊥]School of Life Sciences, Sun Yat-sen University, 135 West Xingang Road, Guangzhou 510275, People's Republic of China

[∥]Key Laboratory of Functional Molecules from Oceanic Microorganisms (Sun Yat-sen University), Department of Education of Guangdong Province, 135 West Xingang Road, Guangzhou 510275, People's Republic of China

Supporting Information

ABSTRACT:



Eight secondary metabolites, including three new azaphilones (chermesinones A−C, **1**−**3**), three new *p*-terphenyls (6′-*O*-desmethylterphenyllin, **4**; 3-hydroxy-6′-*O*-desmethylterphenyllin, **5**; 3″-deoxy-6′-*O*-desmethylcandidusin B, **7**), and two known *p*-terphenyls (**6**, **8**), were isolated from the culture of the mangrove endophytic fungus *Penicillium chermesinum* (ZH4-E2). Their structures were established by spectroscopic analysis. The absolute configuration of **1** was determined by X-ray crystallography. Terphenyls **4**, **5**, and **6** exhibited strong inhibitory effects against α-glucosidase with IC_{50} values of 0.9, 4.9, and 2.5 μM, respectively. Terphenyls **7** and **8** showed inhibitory activity toward acetylcholinesterase with IC_{50} values of 7.8 and 5.2 μM.

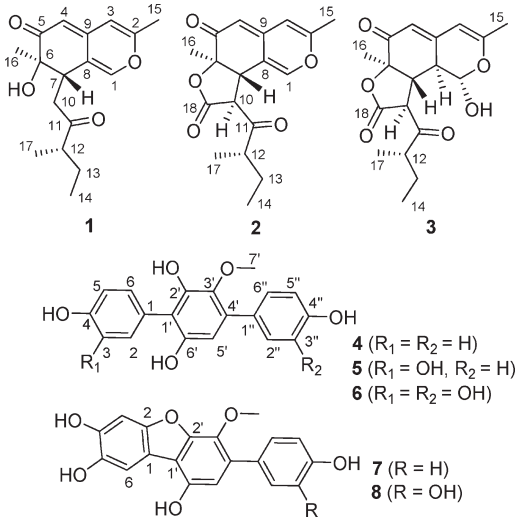
Endophytic fungi are those that inhabit the interior of its host plant for all or part of their life cycle without causing any apparent damage or diseases.[1] Endophytic fungi usually form mutualistic relationships with the host plant and may provide an advantage over other plants or improve resistance to insect and mammalian herbivores by secreting chemical substances.[2,3] Currently, endophytic fungi are receiving renewed attention, not only for their ecological functions but also for their secondary metabolites with agricultural or pharmaceutical potential.[4,5] Accordingly, many structurally unique and biologically active compounds have been obtained from the cultures of endophytic fungi.[6]

Studies have shown that mangrove plants are a rich source of fungal endophytes.[7] Our research group has focused in the past decade on the exploration of bioactive metabolites from mangrove endophytic fungi collected from the South China Sea with the intention of developing potential therapeutics.[8−12] Recently, a chemical investigation of endophyte fungal strain ZH4-E2 was carried out. This fungus was isolated from the stem of the mangrove plant *Kandelia candel* and was identified as a *Penicillium chermesinum* strain. Eight compounds, including three new azaphilones, named chermesinones A−C (**1**−**3**), three new *p*-terphenyls, 6′-*O*-desmethylterphenyllin (**4**), 3-hydroxy-6′-*O*-desmethylterphenyllin (**5**), and 3″-deoxy-6′-*O*-desmethylcandidusin B (**7**), and two known *p*-terphenyls (**6**, **8**), were obtained from the fungal culture. In the enzyme inhibition bioactivity assays, terphenyls **4**, **5**, and **6** exhibited strong inhibitory effect against α-glucosidase (EC 3.2.1.20). Terphenyls **7** and **8** displayed inhibitory activity toward acetylcholinesterase (AChE, EC 3.1.1.7). Herein we report the isolation, structure elucidation, and bioactivities of these compounds.

Received: December 6, 2010
Published: April 21, 2011

RESULTS AND DISCUSSION

Compound **1** was isolated as colorless crystals. Its molecular formula of $C_{17}H_{22}O_4$ was determined by HREIMS (m/z 290.0680 $[M]^+$), which suggested seven degrees of unsaturation. The IR spectrum of **1** displayed characteristic absorptions of hydroxy (3428 cm^{-1}), carbonyl (1711 and 1675 cm^{-1}), and olefinic bond (1596 and 1544 cm^{-1}) functionalilities. The ^1H, ^{13}C, and DEPT NMR spectra (Table 1) indicated the presence of one singlet, two doublet, and one triplet methyl, two methylenes, two aliphatic and three olefinic methines, one oxygen-bearing quaternary carbon, three unsaturated quaternary carbons, and two carbonyls. Six olefinic carbons and two carbonyls accounted for five degrees of unsaturation; the remaining two degrees of unsaturation supported the existence of two rings. The $^1H-^1H$ COSY spectrum of **1** revealed four $^1H-^1H$ spin systems of H-3/H_3-15, H-1/H-7/H_2-10, H-12/H_3-17, and H_2-13/H_3-14, which can assign the fragments of C-15/C-2/C-3, C-1/C-8/C-7/C-10, C-12/C-17, and C-13/C-14 (Figure 1). In the HMBC spectrum, the correlations that originated from H_3-15 and H_3-16, and the three olefinic hydrogens (H-1, H-3, and H-4) established two six-membered rings. The HMBC correlations of H-12/C-10, 11, 13, 14, and 17 indicated the presence of an aliphatic ketone chain from C-10 to C-14, and the HMBC correlations of H-7/C-10, C-11 and of H_2-10/C-6, C-7 located the aliphatic ketone chain at C-7. Accordingly, the planar structure of **1** was established. The absolute configuration of **1** was determined by X-ray crystallography. Fringe crystals of **1** were obtained from EtOAc by a vapor diffusion method. Subsequently, an X-ray crystal structure analysis was conducted using Cu Kα radiation. The diffraction pattern established the absolute configuration of **1** to be 6*R*, 7*S*, and 12*S* (Figure 2) through the refinement of Flack's parameter [x = 0.07(2)] and Hooft's FLEQ parameter [FLEQ = 0.0025(0.0665)].[13,14] This new azaphilone was named chermesinone A after the fungal species *chermesinum*.



Compound **2** was obtained as a white powder. Its HREIMS spectrum exhibited a prominent peak at m/z 316.1305 $[M]^+$, establishing the molecular formula of $C_{18}H_{20}O_5$ and thus suggesting nine degrees of unsatutation. The ^1H and ^{13}C NMR spectroscopic data of **2** closely resembled those of **1**, except that the ^{13}C NMR signal of a methylene (δ_C 37.5) was absent, while a methine [δ_H 4.09 (1H, d, J = 12.9 Hz, H-10), δ_C 51.7, C-10] and an ester carbonyl (δ_C 169.3, C-18) signal were observed. In the HMBC experiment, the correlation of H-10/C-18 indicated the new carbonyl was linked at C-10. In addition, the ^1H NMR signal of the hydroxy proton (δ_H 4.12) was missing and the ^{13}C NMR signal of the oxygen-bearing quaternary carbon (C-6) changed from δ_C 73.0 to δ_C 82.8. The observed changes in the NMR spectra for **2** relative to those of **1**, as well as the degree of unsatutation, suggested that the oxygen atom at C-6 was connected with the carbonyl to form a five-membered lactone ring. Consequently, the planar structure of **2** was established. The relative configurations at C-6, C-7, and C-10 in **2** were determined by a combination of a NOESY experiment and ^1H NMR coupling constant analysis. The NOESY correlation of H-10/H_3-16 placed H-10 and H_3-16 on the same side of the molecule. The ^1H NMR spectrum revealed a large coupling constant between H-7 and H-10 ($J_{H\text{-}7/H\text{-}10}$ = 12.9 Hz), which is consistent with a diaxial-like orientation of H-7/H-10.[15] No NOE correlation was observed between H-7 and H_3-16. The large coupling constant in addition to the lack of an NOE between H-7/H_3-16 was suggestive of the *trans* configurations of H-7/H-10 and H-7/H_3-16. The absolute configuration of C-12 in the side chain was presumed to be *S*, which was identical with that of **1** in light of the similar ^{13}C NMR resonances of CH_3-17 in **2** (δ_C 17.1) and **1** (δ_C 16.4), as well as that **2** and **1** shared a biogenetic pathway. When comparing the NMR data for **2** to those for the known isomeric compound monochaetin,[16,17] the resonance of CH_3-17 exhibited a downfield shift, and the ^{13}C NMR resonance of C-13 showed an upfield shift ($\Delta\delta_{H3\text{-}17}$ = 0.13, $\Delta\delta_{C\text{-}17}$ = 2.7, $\Delta\delta_{C\text{-}13}$ = −1.3, see Table S1). The different chemical shift values between **2** and monochaetin are presumably the result of opposite configurations at C-12. Therefore, **2** was identified to be a new natural product, which is the C-12 epimer of monochaetin, and was named chermesinone B.

Compound **3** had a molecular formula of $C_{18}H_{22}O_6$ from the HREIMS peak at m/z 334.1412 $[M]^+$, indicating eight degrees of unsaturation. The ^1H, ^{13}C, and DEPT NMR spectroscopic data (Table 1) revealed a structure with four methyl, one methylene, seven methine, and six quarternary carbons. Three signals, at δ_C 206.5 (C-11), 193.3 (C-5), and 172.0 (C-18), were ascribed to two ketone carbonyls and one ester carbonyl, respectively. The proton at δ_H 4.82 (1H, d, J = 10.2 Hz, H-1) and the associated carbon at δ_C 97.5 (C-1) supported the presence of a hemiacetal methine group. The COSY associations of H-1/H-8/H-7/H-10 placed this hemiacetal methine in the aliphatic region C-1/C-8/C-7/C-10. The azaphilone skeleton was determined from the HMBC correlations of H-3/C-2, 5, 9; H-4/C-7, 9; H-7/C-1, 5, 6, 8, 9; and H-8/C-1, 9. The COSY correlations of H_3-17/H-12/H_2-13/H_3-14 established a butyl fragment of C-17/C-12/C-13/C-14. This butyl was linked with a carbonyl (C-11) and a methine (C-10) to form a 1,1-disubstituted methyl isobutyl ketone based on the HMBC correlations from H-10, H-12, and H-13 to C-11. Additional HMBC correlations of H-10/C-7 and of H-7/C-10, C-11 placed this aliphatic ketone at C-7. The ester carbonyl (C-18) was located at C-10 on the basis of the HMBC correlation of H-10/C-18. Taking the molecular formula and the degrees of unsaturation into consideration, this carbonyl was connected with an oxygen atom at C-6 (δ_C 84.3) to form a five-membered lactone ring. In addition, two methyls were placed at C-2 and C-6 by the HMBC correlations of H_3-15/C-2 and H_3-16/C-6, respectively. Thus, the planar structure of **3** was inferred to be a hydration product of **2** with H_2O added at the olefinic bond between C-1 and C-8. The *trans* configurations of H-1/H-8, H-8/H-7, and H-7/H-10 were deduced on the basis of the NOESY spectrum, which revealed

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for 1−3 at 400 (^{1}H) and 100 (^{13}C) MHz

	chermesinone A (**1**)[a]		chermesinone B (**2**)[a]		chermesinone C (**3**)[b]	
position	δ_C type	δ_H mult. (J in Hz)	δ_C type	δ_H mult. (J in Hz)	δ_C type	δ_H mult. (J in Hz)
1	144.8, CH	6.81, m	143.5, CH	6.83, s	97.5, CH	4.82, d (10.2)
2	158.4, C		158.8, C		162.8, C	
3	107.1, CH	5.99, s	107.3, CH	6.05, s	101.5, CH	5.64, s
4	103.9, CH	5.39, d (1.1)	106.1, CH	5.34, d (0.8)	115.3, CH	5.62, d (1.7)
5	199.1, C		192.1, C		193.3, C	
6	73.0, C		82.8, C		84.3, C	
7	40.2, CH	3.28, dt (9.8, 2.0)	43.8, CH	3.81, dd (12.9, 1.9)	46.6, CH	2.92, dd (12.3, 11.1)
8	120.3, C		116.5, C		41.8, CH	2.72, ddd (11.1, 10.2, 1.7)
9	147.8, C		145.7, C		155.0, C	
10	37.5, CH_2	3.22, dd (17.6, 2.0)	51.7, CH	4.09, d (12.9)	53.2, CH	4.71, d (12.3)
		2.75, dd (17.6, 9.8)				
11	213.2, C		206.2, C		206.5, C	
12	47.7, CH	2.62, m	47.3, CH	3.15, m	48.5, CH	2.81, m
13	25.9, CH_2	1.74, m; 1.44, m	25.1, CH_2	1.80, m; 1.43, m	24.0, CH_2	1.82, m; 1.32, m
14	11.7, CH_3	0.90, t (7.4)	11.7, CH_3	0.90, t (7.4)	11.9, CH_3	0.85, t (7.4)
15	19.2, CH_3	2.13, d (0.5)	19.8, CH_3	2.17, s	20.7, CH_3	1.93, s
16	21.2, CH_3	1.09, s	19.1, CH_3	1.36, s	18.9, CH_3	1.44, s
17	16.4, CH_3	1.17, d (7.0)	17.1, CH_3	1.24, d (7.1)	14.8, CH_3	1.06, d (6.9)
18			169.3, C		172.0, C	
OH		4.12, br s[c]				7. 78, br s[d]

[a] Recorded in $CDCl_3$. [b] Recorded in DMSO-d_6. [c] Signal of 6-OH. [d] Signal of 1-OH.

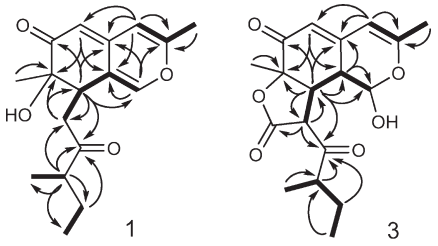


Figure 1. $^{1}H-^{1}H$ COSY (bold) and selected HMBC (arrow) correlations of **1** and **3**.

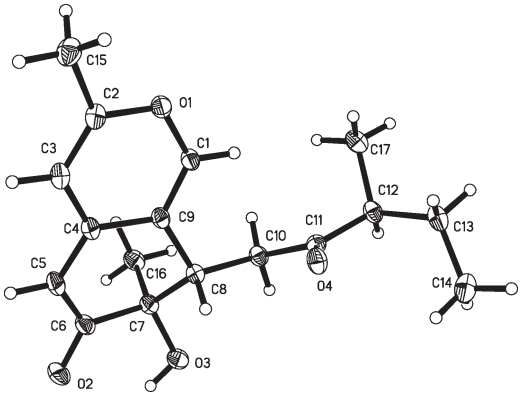


Figure 2. X-ray structure of **1** drawn by ORTEP.

key correlations of H-1/H-7 and H-8/H-10, and the large coupling constants of H-1/H-8 ($J_{H\text{-}1/H\text{-}8}$ = 10.2 Hz), H-8/H-7

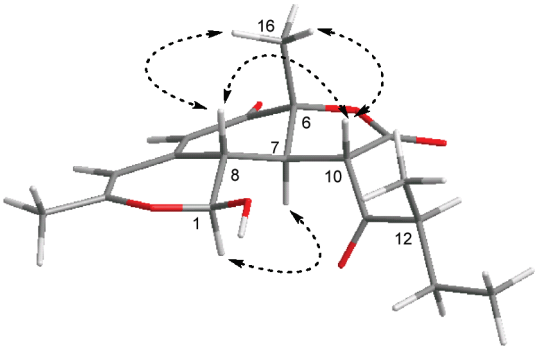


Figure 3. Key NOESY correlations of **3**.

($J_{H\text{-}8/H\text{-}7}$ = 11.1 Hz), and H-7/H-10 ($J_{H\text{-}7/H\text{-}10}$ = 12.3 Hz), which indicated a diaxial-like relationships of H-1/H-8, H-8/H-7, and H-7/H-10.[15] Additional NOESY correlations of H_3-16/H-8, H-10 located H-8, H-10, and H_3-16 on the same face, which led to the *trans* configuration of H_3-16/H-7. The key NOEs and the relative configuration of **3** are shown in Figure 3. Because **3** was insoluble in $CDCl_3$, a comparison of ^{13}C NMR shift of CH_3-17 in DMSO-d_6 was used to support the presumption that **3** shared the 12*S* absolute configuration of **1** and **2** (**3**: δ_C 14.8; **1**: δ_C 15.3; **2**: δ_C 15.8; see Table S2, Figures S2, S4). Furthermore, **3** shared a biosynthetic pathway with **1** and **2**, giving a further evidence for the assignment of 12*S*. This new azaphilone was named chermesinone C.

Compounds **4**, **5**, and **7** had the same terphenyl core structure as deduced from the 18 sp^2 carbons present in the ^{13}C NMR spectra.[18] Compound **4** had the molecular formula $C_{19}H_{16}O_5$, as shown by the HREIMS peak at m/z 324.0990 $[M]^+$. The ^{1}H and

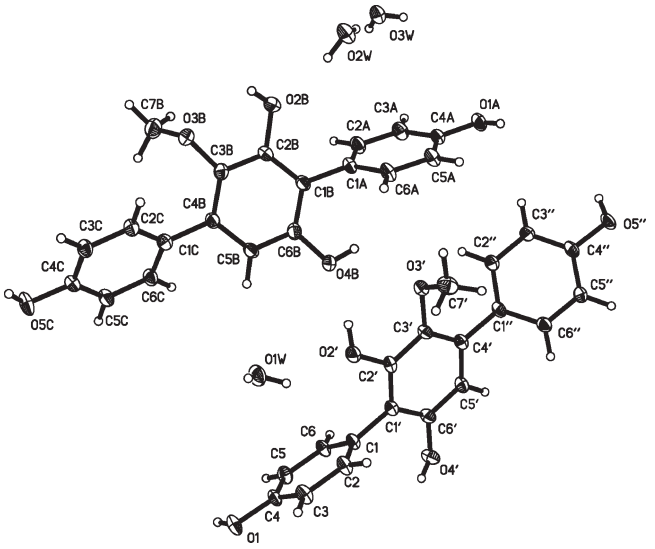


Figure 4. X-ray structure of **4** drawn by ORTEP.

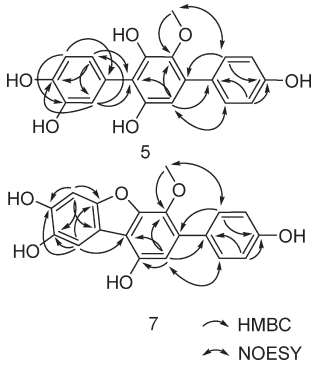


Figure 5. Key HMBC and NOESY correlations of **5** and **7**.

^{13}C NMR spectra showed a methoxy group (δ_H 3.37, δ_C 60.7, 7′-OMe), five aromatic protons, and five oxygen-bearing aromatic carbons. One pentasubstituted benzene ring was identified on the basis of an isolated aromatic proton at δ_H 6.44 (1H, s, H-5′). Two *p*-substituted benzene rings were inferred according to two pairs of *ortho*-coupled aromatic protons. The methoxy was placed at the pentasubstituted aromatic ring by its HMBC correlation with C-3′. The key HMBC correlations of H-2, H-6/C-1′ and of H-2″, H-6″/C-4′, as well as the NOESY correlations of H-5′/H-2″ and of 7′-OMe/H-6″, assigned the connectivity of the three benzene rings. Thus compound **4** was determined to be 6′-*O*-desmethylterphenyllin. This new compound was further confirmed by single-crystal X-ray diffraction analysis (Figure 4).

Compound **5** was obtained as a gray powder. The molecular formula of $C_{19}H_{16}O_6$ for compound **5** was determined by HREIMS (*m*/*z* 340.0944 $[M]^+$), which had one more oxygen atom than **4**. Its ^{1}H and ^{13}C NMR spectroscopic data showed that **5** consists of one pentasubstituted, one *p*-substituted, and one 1,2,4-trisubstituted benzene ring. By detailed analysis of its DEPT, HSQC, HMBC, and NOESY spectroscopic data (Figure 5), compound **5** was identified as 3-hydroxy-6′-*O*-desmethylterphenyllin.

Compound **7** was isolated as a gray powder. The molecular formula of $C_{19}H_{14}O_6$ for **7** was established from the HREIMS peak at *m*/*z* 338.0786 $[M]^+$. Therefore, 13 degrees of unsaturation were obtained, suggesting another ring existed. A fragment containing a *p*-substituted phenyl connected to the pentasubstituted phenyl at C-4′ was deduced by the comparison of the ^{1}H and ^{13}C NMR spectroscopic data with those of **5**. Two isolated aromatic protons at δ_H 7.11 (1H, s, H-3) and δ_H 7.57 (1H, s, H-6) indicated the presence of a 1,2,4,5-tetrasubstitued benzene, which linked at C-2 to the central pentasubstituted ring at C-2′ through an ether bridge to form a dibenzofuran ring. The ^{1}H and ^{13}C NMR spectroscopic data of the substituted dibenzofuran were consistent with those of the related compound **8**, which supported this linkage. The structure of **7** was established to be 3″-deoxy-6′-*O*-desmethylcandidusin B and was further confirmed by the HMBC and NOESY spectra (Figure 5). Furthermore, two known terphenyl derivatives, 3,3″-dihydroxy-6′-*O*-desmethylterphenyllin (**6**) and 6′-*O*-desmethylcandidusin B (**8**), were also isolated and identified by comparing the ^{1}H and ^{13}C NMR and MS data with those reported.[18]

All compounds were tested for their *in vitro* inhibitory activities against α-glucosidase and acetylcholinesterase according to a previously described method.[19,20] In the enzyme assays against α-glucosidase, terphenyls **4**, **5**, and **6** exhibited strong inhibitory effects, with IC_{50} values of 0.9, 4.9, and 2.5 μM, respectively, which were significantly more effective than genistein, the positive control. **7** and **8**, the terphenyls with a furan ring, displayed inhibitory activity against acetylcholinesterase with IC_{50} values of 7.8 and 5.2 μM. Chermesinone A (**1**) showed a mild inhibitory effect on α-glucosidase with an IC_{50} value of 24.5 μM, while chermesinones B and C (**2**, **3**) showed no inhibitory effect on α-glucosidase or acetylcholinesterase (IC_{50} > 100 μM). The results of the two assays are listed in Table 3.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Fisher-Johns hot-stage apparatus and are uncorrected. Optical rotations were determined on a MCP 300 (Anton Paar) polarimeter at 25 °C. UV spectra were measured on a UV-240 spectrophotometer (Shimadzu). IR spectra were obtained on a 5DX-FTIR spectrophotometer (Nicolet). NMR data were recorded on Bruker AVANCE 400 and 500 spectrometers using TMS as an internal standard. HREIMS data were measured on a MAT95XP high-resolution mass spectrometer (Thermo) and EIMS on a DSQ EI-mass spectrometer (Thermo). Column chromatography (CC) was performed using silica gel (200−300 mesh; Qingdao Marine Chemicals) and Sephadex LH-20 (Amersham Pharmacia Biotech AB). Semipreparative HPLC was operated on a 515 pump with a UV 2487 detector (Waters) using an Ultimate XB-C18 column (250 × 10 mm, 5 μm; Welch). Single-crystal data were collected on an Oxford Gemini S Ultra diffractometer using Cu Kα radiation and on a Bruker Smart-1000 CCD diffractometer using Mo Kα radiation.

Fungal Material. The fungal strain ZH4-E2 was isolated from the stem of the mangrove plant *Kandelia candel* collected from the South China Sea in Guangdong Province, China. By classical microscopic analysis, the fungus was identified as a member of the genus *Penicillium*. It was further identified as *P. chermesinum* according to a molecular biological protocol by DNA amplification and sequencing of the ITS region as described below. This fungal strain has been preserved at the China Center for Type Culture Collection (CCTCC) under the patent depository number CCTCC M 2010267.

Molecular Taxonomy of the Fungus. About 500 mg of fresh fungal tissue was collected in a sample tube (15 mL) to extract genomic DNA from the fungus using a fungal DNA midi kit (E.Z.N.A., Omega) according to the manufacturer's protocol. Consequently, PCR was

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for 4, 5, and 7 at 400 (^{1}H) and 100 (^{13}C) MHz

position	(4)[a]		(5)[b]		(7)[a]	
	δ_C type	δ_H mult. (J in Hz)	δ_C type	δ_H mult. (J in Hz)	δ_C type	δ_H mult. (J in Hz)
1	126.0, C		125.4, C		116.1, C	
2	133.1, CH	7.31, d (8.6)	118.6, CH	6.76, d (1.7)	151.1, C	
3	115.5, CH	6.87, d (8.6)	143.7, C		98.9, CH	7.11, s
4	157.1, C		144.1, C		146.0, C	
5	115.5, CH	6.87, d (8.6)	114.7, CH	6.71, d (8.1)	142.8, C	
6	133.1, CH	7.31, d (8.6)	122.0, CH	6.59, dd (8.1, 1.7)	108.2, CH	7.57, s
1′	116.3, C		115.7, C		114.8, C	
2′	149.2, C		148.1, C		150.4, C	
3′	139.2, C		137.8, C		136.6, C	
4′	133.7, C		132.1, C		132.3, C	
5′	107.9, CH	6.44, s	106.5, CH	6.28, s	110.3, CH	6.70, s
6′	152.0, C		151.0, C		148.3, C	
7′-OMe	60.7, CH_3	3.37, s	60.0, CH_3	3.28, s	60.9, CH_3	3.79, s
1″	130.4, C		128.8, C		130.5, C	
2″	130.7, CH	7.47, d (8.5)	129.4, CH	7.34, d (8.5)	131.3, CH	7.42, d (8.7)
3″	116.1, CH	6.91, d (8.5)	115.1, CH	6.82, d (8.5)	115.8, CH	6.91, d (8.7)
4″	157.7, C		156.5, C		157.4, C	
5″	116.1, CH	6.91, d (8.5)	115.1, CH	6.82, d (8.5)	115.8, CH	6.91, d (8.7)
6″	130.7, CH	7.47, d (8.5)	129.4, CH	7.34, d (8.5)	131.3, CH	7.42, d (8.7)

[a] Recorded in acetone-d_6. [b] Recorded in DMSO-d_6.

Table 3. Inhibitory Effects of the Isolates against α-Glucosidase and AChE[a] (n = 3)

compound	IC_{50} (μM)	
	α-glucosidase	AChE
1	24.5	>100
2	>100	>100
3	>100	>100
4	0.9	>100
5	4.9	>100
6	2.9	>100
7	>100	7.8
8	>100	5.2
genistein[b]	9.8	
huperzine A[b]		0.12

[a] Acetylcholinesterase. [b] Positive control.

performed using rTaq polymerase (TaKaRa Taq) and the primer (Invitrogen) pair ITS1F and ITS4 in a SensoQuest LabCycler thermal cycler according to the following protocol: (1) initial denaturation, 94 °C, 5 min; (2) denaturation, 94 °C, 40 s; (3) annealing, 52 °C, 40 s; (4) extension, 72 °C, 1 min; (5) final extension, 72 °C, 10 min. Steps 2−4 were repeated 30 times. Each sample consisted of 5 μL of Taq polymerase master mix, 1 μL of primer mix (20 μmol/mL each), 3 μL of template DNA, and 41 μL of water. From this, 5 μL was loaded onto an agarose gel (2% agarose in 1×TAE, 5 μL of ethidium bromide 1% m/V solution per 100 mL of gel). After electrophoresis at 120 V for 40 min, the band due to the PCR product (approximate size 600 bp) was isolated from the gel slice using a gel extraction kit (E.Z.N.A., Omega) according to the manufacturer's protocol. The PCR product was then submitted for sequencing (Invitrogen) with the primer ITS1F. The sequence data have been deposited at GenBank (accession no. HQ428124). A BLAST search showed that the sequence was most similar (98%) to the sequence of *Penicillium chermesinum* (accession no. AY742693).

Cultivation. Starter cultures were maintained on PDA medium (20 g of glucose, 20 g of agar, and 2 g of sea salt in 1 L of potato infusion). Plugs of agar supporting mycelial growth were cut and transferred aseptically to 250 mL Erlenmeyer flasks containing 100 mL of PDB medium (20 g of glucose and 2 g of sea salt in 1 L of potato infusion). The flasks were incubated at 28 °C on a rotary shaker for seven days, and then the mycelia were aseptically transferred to 500 mL Erlenmeyer flasks containing 300 mL of PDB medium. The flasks were then incubated statically at room temperature (25−30 °C) for one month.

Extraction and Isolation. The fungal culture (70 L) was filtered through cheesecloth. The filtrate was concentrated to 10 L below 50 °C and then extracted with EtOAc (10 L × 3) to give 20.8 g of extract. The extract was subjected to silica gel CC using gradient elution with petroleum ether and an EtOAc mixture from 90:10 to 0:100 (v/v) to give nine fractions (Fr.1−Fr.9). Fr.3 was crystallized from EtOAc to afford **1** (124 mg). Fr.4 was subjected to CC over Sephadex LH-20 eluting with MeOH to give **4** (14 mg). Fr.5 was chromatographed on silica gel eluting with petroleum ether and EtOAc (60:40 → 40:60) to afford **5** (15 mg) and Fr.5-1. Fr.5-1 was isolated by semipreparative HPLC with an ODS column, eluting with MeOH−H_2O (60:40, v/v), to give **2** (18 mg) and **3** (12 mg). Fr.6, Fr.7, and Fr.8 were purified by Sephadex LH-20 CC with $CHCl_3$−MeOH (50:50, v/v) to give **7** (13 mg), **6** (15 mg), and **8** (9 mg), respectively.

Compound ***1****:* colorless needles (EtOAc); mp 117−118 °C; $[\alpha]_D^{25}$ +330 (c 0.045, MeOH); UV (MeOH) λ_{max} (log ε) 244 (3.73), 350 (4.33) nm; IR (KBr) ν_{max} 3428, 2968, 2903, 2877, 1711, 1675, 1596, 1544, 1183 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; EIMS m/z 290 $[M]^+$; HREIMS m/z 290.1513 $[M]^+$ (calcd for $C_{17}H_{22}O_4$, 290.1513).

Compound ***2****:* white powder; $[\alpha]_D^{25}$ +830 (c 0.070, MeOH); UV (MeOH) λ_{max} (log ε) 244 (3.78), 349 (4.43) nm; IR (KBr) ν_{max} 2977, 2935, 2878, 1782, 1706, 1654, 1539, 1383, 1301, 1216, 1183, 1109, 1064 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; EIMS m/z 316 $[M]^+$; HREIMS m/z 316.1305 $[M]^+$ (calcd for $C_{18}H_{20}O_5$, 316.1305).

Compound ***3****:* white powder; $[\alpha]_D^{25}$ +360 (*c* 0.070, MeOH); UV (MeOH) λ_{max} (log ε) 334 (4.14) nm; IR (KBr) ν_{max} 3404, 2969, 2928, 2879, 1778, 1708, 1673, 1619, 1570, 1387, 1305, 1221, 1118, 1095, 1039 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; EIMS *m/z* 334 $[M]^+$; HREIMS *m/z* 334.1412 $[M]^+$ (calcd for $C_{18}H_{22}O_6$, 334.1411).

Compound ***4****:* yellowish powder; UV (MeOH) λ_{max} (log ε) 206 (4.54), 273 (4.31) nm; IR (KBr) ν_{max} 3334, 1609, 1596, 1527, 1492, 1457, 1410, 1246, 1175, 1035 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 2; EIMS *m/z* 324 $[M]^+$; HREIMS *m/z* 324.0990 $[M]^+$ (calcd for $C_{19}H_{16}O_5$, 324.0992).

Compound ***5****:* gray powder; UV (MeOH) λ_{max} (log ε) 209 (4.69), 275 (4.40) nm; IR (KBr) ν_{max} 3327, 1612, 1597, 1523, 1492, 1438, 1359, 1314, 1233, 1176, 1035, 1009 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 2; EIMS *m/z* 340 $[M]^+$; HREIMS *m/z* 340.0944 $[M]^+$ (calcd for $C_{19}H_{16}O_6$, 340.0941).

Compound ***7****:* gray powder; UV (MeOH) λ_{max} (log ε) 213 (4.53), 279(4.29), 335(4.33) nm; IR (KBr) ν_{max} 3359, 1611, 1519, 1475, 1408, 1305, 1285, 1205, 1145, 1037 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 2; EIMS *m/z* 338 $[M]^+$; HREIMS *m/z* 338.0786 $[M]^+$ (calcd for $C_{19}H_{14}O_6$, 338.0785).

X-ray Crystallographic Analysis of 1 and 4. Colorless crystals of **1** and **4** were obtained from EtOAc and MeOH, respectively. The crystal data of **1** were collected at 123 K on an Oxford Gemini S Ultra diffractometer with Cu Kα radiation (λ = 1.54178 Å). Crystal data of **4** were recorded on a Bruker Smart 1000 CCD single-crystal diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The structures were solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Crystallographic data for **1** and **4** have been deposited with the Cambridge Crystallographic Data Centre with the deposition numbers 798113 and 798114, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1233-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Crystal data of ***1****:* monoclinic, $C_{17}H_{22}O_4$, space group $P2_1$, *a* = 5.8825(10) Å, *b* = 8.9553(2) Å, *c* = 14.6363(15) Å, β = 100.671(10)°, *V* = 757.70(2) $Å^3$, *Z* = 2, D_{calcd} = 1.273 g/cm^3, μ = 0.728 mm^{-1}, and *F*(000) = 312. Crystal size: 0.25 × 0.20 × 0.10 mm^3. Independent reflections: 2777 [R_{int} = 0.0311]. The final indices were R_1 = 0.0308, wR_2 = 0.0744 [$I > 2\sigma(I)$].

Crystal data of ***4****:* triclinic, $2(C_{19}H_{16}O_5)\cdot 3H_2O$, space group $P\bar{1}$, *a* = 8.293(12) Å, *b* = 15.048(2) Å, *c* = 15.381(3) Å, α = 109.717(5)°, β = 103.493(4)°, γ = 98.942(4)°, *V* = 1698.6(4) $Å^3$, *Z* = 4, D_{calcd} = 1.374 g/cm^3, μ = 0.104 mm^{-1}, and *F*(000) = 740. Crystal size: 0.19 × 0.14 × 0.10 mm^3. Independent reflections: 6581 [R_{int} = 0.0641]. The final indices were R_1 = 0.0666, wR_2 = 0.1514 [$I > 2\sigma(I)$].

Assays for Enzyme Inhibitory Activities. These two experiments were conducted according to referenced procedures.[19,20]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^1H and ^{13}C NMR spectra of the new compounds and NOESY spectra of **2** and **3**. CIF files and X-ray crystallographic data for **1** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel./Fax: 0086-20-8403 4096. E-mail: cesshzhg@mail.sysu.edu.cn (Z.S.); ceslyc@mail.sysu.edu.cn (Y.L.).

Author Contributions

§These authors contributed equally to this work.

■ ACKNOWLEDGMENT

We are grateful for financial support from the National Natural Science Foundation of China (20772162, 20972197), the 863 Foundation of China (2006AA09Z422, 2007AA09Z448), the Natural Science Foundation of Guangdong Province of China (9151027501000055), the Science & Technology Plan Project of Guangdong Province of China (2010B030600004), and the Fundamental Research Funds for the Central Universities of China (11lgjc01). We also thank Dr. L. Jiang for his helpful instruction in crystallography.

■ REFERENCES

(1) Strobel, G. A. *Crit. Rev. Biotechnol.* **2002**, *22*, 315–333.
(2) Saikkonen, K.; Faeth, S. H.; Helander, M.; Sullivan, T. J. *Annu. Rev. Ecol. Syst.* **1998**, *29*, 319–343.
(3) Lehtonen, P.; Helander., M.; Saikkonen, K. *Oecologia* **2005**, *142*, 38–45.
(4) Zhang, H. W.; Song, Y. C.; Tan, R. X. *Nat. Prod. Rep.* **2006**, *23*, 753–771.
(5) Azevedo, J. L.; Maccheroni, W., Jr.; Pereira, J. O.; Araujo, W. L. *Electron. J. Biotechnol.* **2000**, *3*, 40–65.
(6) Tan, R. X.; Zhou, W. X. *Nat. Prod. Rep.* **2001**, *18*, 448–459.
(7) Ananda, K.; Sridhar, K. R. *Can. J. Microbiol.* **2002**, *48*, 871–878.
(8) Pan, J. H.; Jones, E. B. G.; She, Z. G.; Pang, J. Y.; Lin, Y. C. *Bot. Mar.* **2008**, *51*, 179–190.
(9) Lin, Y.; Wu, X.; Feng, S.; Jiang, G.; Luo, J.; Zhou, S.; Vrijmoed, L. L. P.; Jones, E. B. G.; Krohn, K.; Steingrver, K.; Zsila, F. *J. Org. Chem.* **2001**, *66*, 6252–6256.
(10) Wen, L.; Cai, X.; Xu, F.; She, Z.; Chan, W. L.; Vrijmoed, L. L. P.; Jones, E. B. G.; Lin, Y. *J. Org. Chem.* **2009**, *74*, 1093–1098.
(11) Xu, F.; Zhang, Y.; Wang, J.; Pang, J.; Huang, C.; Wu, X.; She, Z.; Vrijmoed, L. L. P.; Jones, E. B. G.; Lin, Y. *J. Nat. Prod.* **2008**, *71*, 1251–1253.
(12) Huang, H.; She, Z.; Lin, Y.; Vrijmoed, L. L. P.; Lin, W. *J. Nat. Prod.* **2007**, *70*, 1696–1699.
(13) Flack, H. D. *Acta Crystallogr., Sect. A* **1983**, *39*, 876–881.
(14) Hooft, R. W. W.; Straver, L. H.; Spek, A. L. *J. Appl. Crystallogr.* **2008**, *41*, 96–103.
(15) Fotso, S.; Mahmud, T.; Zabriskie, T. M.; Santosa, D. A.; Sulastri; Proteau, P. J. *J. Nat. Prod.* **2008**, *71*, 61–65.
(16) Spencer, H.; Mo, Y. K.; Tertzakian, G.; Slater, G. P.; Haskins, R. H.; Nesbitt, L. R. *Can. J. Chem.* **1970**, *48*, 3654–3661.
(17) Steyn, P. S.; Vleggaar, R. *J. Chem. Soc., Perkin Trans. 1* **1986**, *11*, 1975–1976.
(18) Belofsky, G. N.; Gloer, K. B.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Nat. Prod.* **1998**, *61*, 1115–1119.
(19) Du, Z. Y.; Liu, R. R.; Shao, W. Y.; Mao, X. P.; Ma, L.; Gu, L. Q.; Huang, Z. S.; Chan, A. S. C. *Eur. J. Med. Chem.* **2006**, *41*, 213–218.
(20) Tang, H.; Wei, Y. B.; Zhang, C.; Ning, F. X.; Qiao, W.; Huang, S. L.; Ma, L.; Huang, Z. S.; Gu, L. Q. *Eur. J. Med. Chem.* **2009**, *44*, 2523–2532.