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Polyketides with α-Glucosidase Inhibitory Activity from a Mangrove Endophytic Fungus, *Penicillium* sp. HN29-3B1

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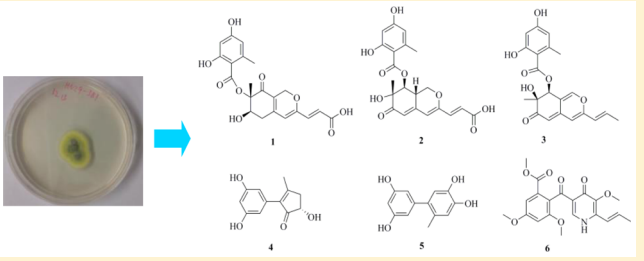
Supporting Information

ABSTRACT: Five new compounds, pinazaphilones A and B (**1**, **2**), two phenolic compounds (**4**, **5**), and penicidone D (**6**), together with the known Sch 1385568 (**3**), (±)-penifupyrone (**7**), 3-*O*-methylfunicone (**8**), 5-methylbenzene-1,3-diol (**9**), and 2,4-dihydroxy-6-methylbenzoic acid (**10**) were obtained from the culture of the endophytic fungus *Penicillium* sp. HN29-3B1, which was isolated from a fresh branch of the mangrove plant *Cerbera manghas* collected from the South China Sea. Their structures were determined by analysis of 1D and 2D NMR and mass spectroscopic data. Structures of compounds **4** and **7** were further confirmed by a single-crystal X-ray diffraction experiment using Cu Kα radiation. The absolute configurations of compounds **1**−**3** were assigned by quantum chemical calculations of the electronic circular dichroic spectra. Compounds **2**, **3**, **5**, and **7** inhibited α-glucosidase with IC_{50} values of 28.0, 16.6, 2.2, and 14.4 μM, respectively, and are thus more potent than the positive control, acarbose.



Diabetes mellitus is a chronic, polygenic complex metabolic disorder characterized by elevated blood glucose. It is considered to be one of the main threats to human health in the 21st century.[1] In 2014, an estimated 387 million people had diabetes worldwide, with type II diabetes making up about 90% of the cases.[2,3] α-Glucosidase is a key enzyme for breaking down carbohydrates for absorption, and α-glucosidase inhibitors such as acarbose, miglitol, and voglibose, all originating from natural products, are widely used to treat type II diabetes.[4−6]

Endophytic fungi have been demonstrated to be a reservoir of natural compounds possessing significant pharmacological activity that could be used for the development of new medicinal agents.[7] In the past decade our research group had focused on the exploration of bioactive metabolites from mangrove endophytic fungi collected from the South China Sea,[8−13] including some α-glucosidase inhibitors.[12,13] A chemical investigation of the mangrove-derived fungus *Penicillium* sp. HN29-3B1, from the branches of *Cerbera manghas*, had led to the isolation and characterization of five new polyketides (**1**, **2**, **4**, **5**, **6**) as well as five previously reported compounds (**3**, **7**, **8**, **9**, **10**). Compounds **2**, **3**, **5**, and **7** exhibited significant inhibitory activities toward α-glucosidase. Herein, details of the isolation, structure elucidation, and activity against α-glucosidase of these compounds are reported.

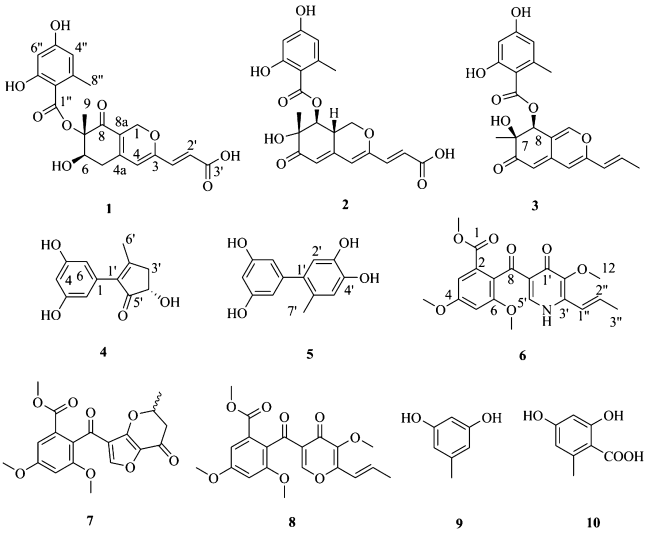


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Table 1. ^{1}H and ^{13}C NMR Data of Compounds 1 and 2[a]

	1			2		
position	δ_C, type	δ_H, mult (J in Hz)	HMBC (H→C)	δ_C, type	δ_H, mult (J in Hz)	HMBC (H→C)
1a	64.7, CH_2	4.88, dt (13.1, 2.0)	3, 4a, 8a	69.1, CH_2	3.94, dd (10.9, 13.5)	4a, 8, 8a
1b		5.02, dd (13.1, 2.0)	3, 4a, 8a		4.61, dd (10.9, 5.2)	3, 4a, 8a
3	157.3, C			157.8, C		
4	111.9, CH	6.03, brs	3, 5, 8, 8a, 2′	110.9, CH	6.23, s	3, 4a, 5, 8, 8a, 2′
4a	147.4, C			149.5, C		
5a	35.3, CH_2	2.66, ddt (17.8, 10.7, 2.0)	4a, 6, 8a	120.4, CH	5.91, d (1.9)	4, 7, 8a
5b		2.81, dd (17.8, 6.0)	4, 4a, 6, 7, 8a			
6	68.2, CH	5.10, dd (10.7, 6.0)	5, 7, 9	195.4, C		
7	88.1, C			74.8, C		
8	192.2, C			76.3, CH	5.35, d (9.8)	1, 4a, 7, 8a, 9, 1″
8a	119.3, C			35.8, CH	3.54, m	1, 4a, 8
9	16.5, CH_3	1.55, s	6, 7, 8, 1″	19.8, CH_3	1.33, s	6, 7, 8a
1′	123.3, CH	6.35, d (15.5)	3, 4, 2′, 3′	123.6, CH	6.32, d (15.5)	3, 4, 2′, 3′
2′	137.3, CH	7.13, d (15.5)	3, 4, 1′, 3′	137.8, CH	7.10, d (15.5)	3, 4, 1′, 3′
3′	166.9, C			167.4, C		
1″	171.0, C			171.6, C		
2″	106.1, C			104.9, C		
3″	144.8, C			145.2, C		
4″	112.3, CH	6.31, d (2.5)	2″, 5″, 6″, 8″	112.8, CH	6.35, brs	2″, 5″, 6″, 8″
5″	163.2, C			163.9, C		
6″	101.5, CH	6.22, d (2.5)	1″, 2″, 4″, 5″, 7″	101.8, CH	6.29, d (2.4)	1″, 2″, 4″, 5″, 7″
7″	166.1, C			166.3, C		
8″	24.3, CH_3	2.54, s	1″, 2″, 3″, 4″, 5″	24.9, CH_3	2.64, s	1″, 2″, 3″, 4″, 5″

[a]Data were recorded in acetone-d_6 at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR.

RESULTS AND DISCUSSION

The fungus *Penicillium* sp. HN29-3B1 was grown on a rice-based medium and then extracted with MeOH. The extract was subjected to silica gel column chromatography (CC) using gradient elution to afford seven fractions (Fr. 1−Fr. 7). Fr. 3, Fr. 4, Fr. 5, and Fr. 6 were further separated by silica gel CC and Sephadex LH-20 CC to give compounds **1**−**10**. The structures of Sch 1385568 (**3**),[14] (±)-penifupyrone (**7**),[15] 3-*O*-methylfunicone (**8**),[16] 5-methylbenzene-1,3-diol (**9**),[17] and 2,4-dihydroxy-6-methylbenzoic acid (**10**)[18] were established by comparison of their NMR, MS, and optical rotation data with literature values.

Compound **1** was obtained as a yellow, amorphous powder. Its molecular formula was assigned as $C_{21}H_{20}O_9$ on the basis of HRESIMS analysis and was determined to possess 12 degrees of unsaturation. The presence of hydroxy and carbonyl groups was revealed by the IR absorption bands at ν_{max} 3428 and 1639 cm^{-1}. In the ^{1}H NMR spectrum, the signals for two *meta*-coupled aromatic protons at δ_H 6.31 (d, J = 2.5 Hz, H-4″) and 6.22 (d, J = 2.5 Hz, H-6″), two *E*-configured olefinic protons at δ_H 6.35 (J = 15.5 Hz, H-1′) and 7.13 (J = 15.5 Hz, H-2′), one singlet olefinic proton (δ_H 6.03, H-4), one oxymethine (δ_H 5.10, H-6), two methylenes (δ_H 4.88 and 5.02, H_2-1; δ_H 2.66 and 2.81, H_2-5), and two methyls (δ_H 1.55, H_3-9; 2.54, H_3-8″) were observed (Table 1). The $^{1}H-^{1}H$ COSY data indicated the presence of two spin fragments, −OCH−CH_2− and −CH═CH− (Figure 1). The HMBC correlations from H_3-9 to C-6, C-7, and C-8; from H-6 to C-5, C-7, and C-9; from H-5a to C-4a, C-6, and C-8a, and from H-5b to C-4, C-4a, C-6, C-7, and C-8a constructed a cyclohexenone fragment. The HMBC correlations from H-1 to C-3, C-4a, C-8, and C-8a and from the singlet olefinic proton H-4 to C-3, C-8a, and C-5 assembled a 2*H*-pyran ring, which connected with the cyclohexenone to form an azaphilone skeleton.[19] From the following HMBC correlations of H-4″ to C-2″, C-5″, C-6″, and C-8″; of H-6″ to C-2″, C-4″, C-5″, and C-7″; and of H_3-8″ to C-1″, C-2″, C-3″, and C-4″, the moiety of orsellinic acid was deduced, and this was connected to C-7 (δ_C 88.1) on the basis of an HMBC correlation from H_3-9 to C-1″. The HMBC correlations from H-1′ to C-3, C-4, and C-3′ determined that the fragment −CH═CH−COOH was connected to the azaphilone skeleton at C-3. Thus, the planar structure of **1** was deduced.

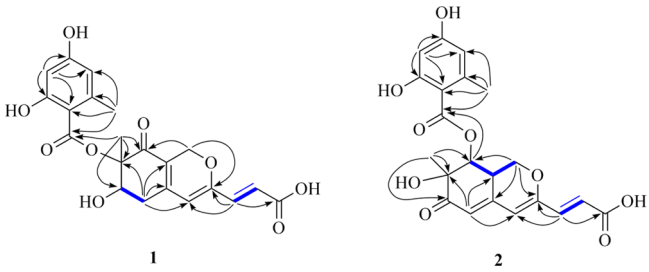

Figure 1. Selected $^{1}H-^{1}H$ COSY (bold line) and HMBC (arrow) correlations of compounds **1** and **2**.

The relative configuration of **1** was established by interpretation of ^{1}H NMR and NOESY data. In the ^{1}H NMR spectrum, H-6 showed ax/eq coupling (J = 6.0 Hz) to H-5b and ax/ax coupling (J = 10.7 Hz) to H-5a. In the NOESY spectrum, the NOE correlations between H-5a and H_3-9 as shown in Figure 2 supported a *syn* relationship of 6-OH and H_3-9. On the basis of the above analysis, the structure of **1** was established as shown. To assign the absolute configuration of **1**, the CD spectrum was measured in methanol and compared with its calculated electronic circular dichroic (ECD) of 6*S*,7*R*-**1** using the quantum chemical method. After conformational analysis and geometry optimizations at the B3LYP/6-31G(d)

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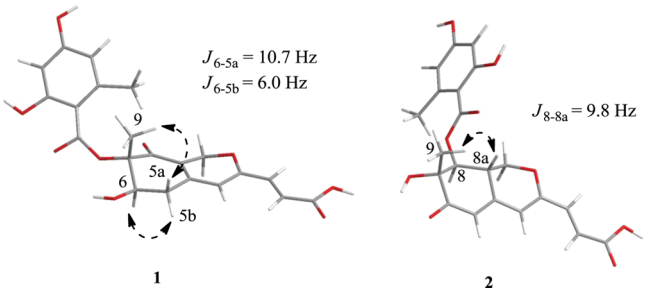

Figure 2. Key NOESY (dashed lines) correlations of compounds **1** and **2**.

level, 10 low-energy conformers with energies of 0−2.5 kcal/mol were obtained (see Supporting Information S35). The ECD were calculated at the B3LYP/6-311+G(d) level using the reported effective methods that Zhu has summarized.[20] As illustrated in Figure 3a, the theoretical ECD curve for 6*S*,7*R*-**1** showed an excellent fit with the experimental one. Therefore, the absolute configuration of **1** was determined to be 6*S*, 7*R*. **1** was named pinazaphilone A.

Compound **2** was obtained as a pale yellow powder. The molecular formula was determined as $C_{21}H_{20}O_9$ on the basis of HRESIMS data. The ^{1}H and ^{13}C NMR spectroscopic data are listed in Table 1. A literature survey suggested that the structure of **2** was similar to the known pinophilin B.[21] Detailed analysis of 2D NMR data for **2** showed that an oxymethylene group in pinophilin B was replaced by a carboxyl group (δ_C 167.4) in compound **2** (Figure 2). The relative configuration of **2** was established on the basis of ^{1}H−^{1}H coupling constants and NOESY correlations. The coupling constant of J_{8-8a} = 9.8 Hz indicated the *trans*-diaxial relationship of H-8/H-8a. In the NOESY spectrum, the correlation of H_3-9 and H-8a was observed, which indicated the *syn* relationship between H-8a and H_3-9 (Figure 2). Furthermore, the predicted ECD curves of **2** were calculated by a quantum chemical method at the B3LYP/6-311+G(d)//B3LYP/6-31G(d) level, and the predicted ECD curve of 7*R*,8*S*,8a*S*-**2** was similar to the experimental one (Figure 3b). Therefore, the absolute configuration of **2** was identified as 7*R*, 8*S*, 8a*S*, and the compound named pinazaphilone B. To the best of our knowledge, pinazaphilone B (**2**) is the fifth example of a C-8 benzoyl-substituted azaphilone.[14,21−23]

A molecular formula of $C_{12}H_{12}O_4$ (seven degrees of unsaturation) was determined for compound **4** on the basis of HREIMS. The ^{13}C NMR data showed nine sp^2-hybridized carbon atoms suggesting the presence of four double bonds and one ketone group. A bicyclic system accommodated two remaining degrees of unsaturation. The ^{1}H NMR spectrum of compound **4** showed one methyl signal at δ_H 2.17 (s), two methylene signals at δ_H 3.01 (dd, *J* = 6.9, 18.4 Hz) and 2.46 (ddd, *J* = 1.3, 3.1, 18.4 Hz), and three aromatic protons at δ_H 6.33 (3H, overlap). The ^{1}H−^{1}H COSY correlation between H-3′ and H-4′ indicated the presence of −CH_2−CH−. Additionally, the HMBC correlations from H-3′a to C-1′, C-2′, C-4′, C-5′, and C-6′, from H-3′b to C-1′, C-2′, and C-4′, and from H-6′ to C-1′, C-2′, C-3′, and C-5′ suggested the presence of the fragment methylcyclopentenone. The ^{1}H and ^{13}C NMR spectra revealed that compound **4** had a resorcinol fragment (δ_H 6.33, 3H; δ_C 159.1, 159.1, 134.8, 108.5, 108.5, 102.5). Furthermore, HMBC correlations of H-2 to C-1′ and of H-6 to C-1′ and C-1 revealed that the two fragments were linked via C-1−C-1′. The absolute configuration of compound **4** was established as 4′*S* by a single-crystal X-ray diffraction experiment using Cu Kα radiation (Flack parameter = −0.02(15)) (Figure 4).[24,25]

Compound **5** was isolated as a white, amorphous powder. Its molecular formula $C_{13}H_{12}O_4$ (eight degrees of unsaturation) was established on the basis of HREIMS analysis. Analysis of ^{1}H and ^{13}C NMR spectroscopic data (Table 2) revealed that compound **5** was very similar to 5′-methoxy-6-methylbiphenyl-3,4,3′-triol,[26] except for the absence of the methoxy signal at C-3. Combined with HMQC and HMBC, compound **5** was determined to be 6′-methyl-[1,1′-biphenyl]-3,3′,4′,5-tetraol.

Compound **6** was obtained as a white, amorphous powder. Its molecular formula was deduced to be $C_{20}H_{21}O_7N$ on the basis of HREIMS analysis. The IR spectrum showed absorptions due to −NH (3429 cm^{-1}) and carbonyl (1662 cm^{-1}) groups. Overall inspections of the ^{1}H and ^{13}C NMR

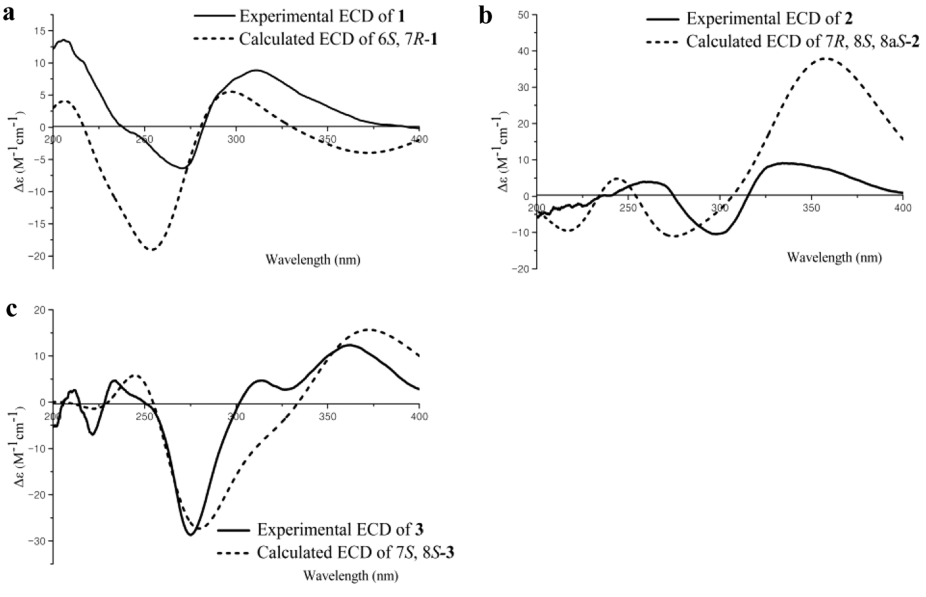

Figure 3. Experimental CD and calculated ECD spectra of **1**−**3** in methanol.

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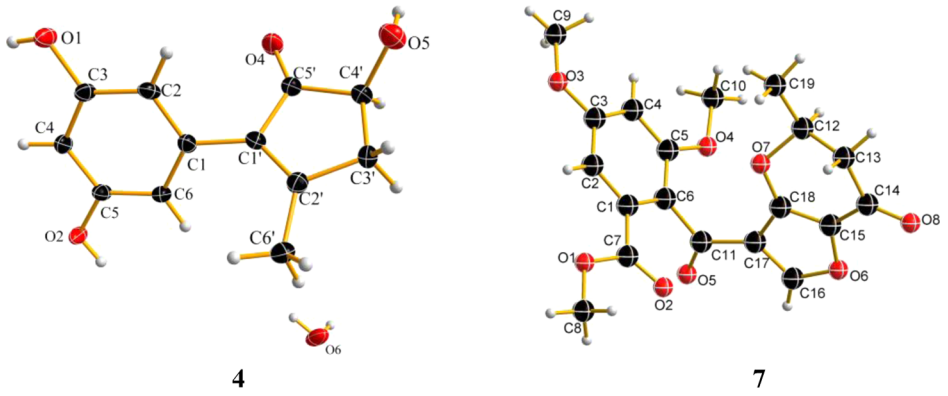

4 7

Figure 4. Perspective ORTEP drawing for compounds **4** and **7**.

Table 2. ^{1}H and ^{13}C NMR Data of Compounds 4 and 5[a]

	4		5	
position	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)
1	134.8, C		144.5, C	
2	108.5, CH	6.33, overlap	108.3, CH	6.26, d (2.2)
3	159.1, C		158.4, C	
4	102.8, CH	6.33, overlap	101.1, CH	6.31, t (2.2)
5	159.1, C		158.4, C	
6	108.5, CH	6.33, overlap	108.3, CH	6.26, d (2.2)
1′	138.1, C		134.0, C	
2′	168.9, C		116.7, CH	6.68, s
3′a				
3′b	41.4, CH_2	3.01, dd (6.9, 18.5)	144.4, C	
		2.46, ddd (1.3, 3.1, 18.5)		
4′	72.3, CH	4.23, dd (3.1, 6.9)	142.9, C	
5′	206.7, C		117.4, CH	6.71, s
6′	18.5, CH_3	2.17, s	126.3, C	
7′			19.3, CH_3	2.11, s
5′-OH		4.54		
3-OH		8.22		
5-OH		8.22		

[a]Data were recorded in acetone-d_6 at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR).

Table 3. ^{1}H and ^{13}C NMR Data of Compound 6[a]

position	δ_C, type	δ_H, mult (J in Hz)
1	166.3, C	
2	131.4, C	
3	107.5, CH	7.20, brs
4	162.9, C	
5	103.7, CH	7.01, brs
6	159.0, C	
7	122.0, C	
8	190.7, C	
9	52.8, CH_3	3.73, s
10	56.3, CH_3	3.96, s
11	56.8, CH_3	3.83, s
12	60.1, CH_3	3.93, s
1′	172.6, C	
2′	141.6, C	
3′	153.7, C	
5′	149.1, CH	7.98, s
6′	119.5, C	
1″	125.4, CH	6.85, d (15.6)
2″	135.8, CH	7.05, dd (15.6, 6.4)
3″	18.8, CH_3	1.94, d (6.4)
	NH	12.52, brs

[a]Data were recorded in acetone-d_6 at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR).

spectroscopic data (Table 3) indicated compound **6** and penicidone C shared the same skeleton,[27] except that compound **6** possessed an additional methoxy group at δ_H 3.93 and δ_C 60.1 (OCH_3-12). Further HMBC correlation of H_3-12/C-2′ confirmed that the additional methoxy group was located at C-2′. The double bond Δ1″, 2″ was determined as *trans*, supported by the typical large coupling constant (J = 15.6 Hz). Compound **6** was named penicidone D. The γ-pyridone nucleus found in **6** is rare in natural products, with only penicidones A−C having similar structures.[27]

In addition, the absolute configuration of Sch 1385568 (**3**)[14] was determined as 7*S*, 8*S* by comparing its experimental CD spectrum (negative, 274 nm, and positive, 312 and 360 nm) and theoretical ECD spectrum (Figure 3c). (±)-Penifupyrone (**7**)[15] was obtained as a racemic mixture, and the structure was subsequently confirmed by a single-crystal X-ray diffraction experiment using Cu Kα radiation (Figure 4).[24,25] Due to the limited amount available (less than 2 mg), we were unable to separate the isomers.

On the basis of literature reports, biosynthetic routes leading to compounds **1**−**3** and **6**−**8** were proposed based on the polyketide pathway (see Supporting Information Scheme 1).[23,28−30]

Compounds were tested for their *in vitro* inhibitory activities against α-glucosidase.[31] The results are given in Table 4. Compounds **2**, **3**, **5**, and **7** were more potent than acarbose (used as a positive control), with IC_{50} values ranging from 2.2 to 28.0 μM. According to reports,[12,32] triphenyl polyphenols showed good α-glucosidase inhibitory activities. It is worth noting that compound **5**, a diphenyl polyphenol, exhibited a strong inhibitory effect against α-glucosidase, with an IC_{50} value of 2.2 μM, while polyhydroxybenzenes (**9** and **10**) were inactive (IC_{50} > 100 μM) in this test.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Fisher-Johns hot-stage apparatus and were uncorrected. Optical rotations were measured on an Anton Paar MCP 300 polarimeter at 25 °C. UV data were recorded on a Shimadzu UV-240 spectrophotometer. IR spectra were recorded on a Nicolet 5DX-FTIR, in KBr discs. CD spectra were recorded on a JASCO J-815 spectropolarimeter using a Nicolet Magna-IR 750 spectrophotometer.

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Table 4. Inhibitory Effects of the Isolates against α-Glucosidase

compound	IC_{50} (μM)
1	81.7 ± 0.5
2	28.0 ± 0.2
3	16.6 ± 0.5
4	>100
5	2.2 ± 0.1
6	>100
7	14.4 ± 0.5
8	>100
9	>100
10	>100
acarbose[a]	446.7 ± 1.6

[a]Positive control.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 (400 and 100 MHz) or Varian Mercury-Plus 300 (300 and 75 MHz) NMR spectrometer in $CDCl_3$ or acetone-d_6. Chemical shifts were reported in δ (ppm), using TMS as an internal standard, and coupling constants (*J*) are reported in hertz (Hz). EIMS spectra were measured on a Thermo DSQ EIMS spectrometer, and HREIMS on a Thermo MAT95XP high-resolution mass spectrometer. ESIMS spectra were obtained using a Micro mass Q-TOF spectrometer, and HRESIMS from a Thermofisher LTQ Orbitrp Elite LC-MS spectrometer. Single-crystal data were measured on an Agilent Gemini Ultra diffractometer (Cu Kα radiation). Column chromatography was performed using silica gel (200−300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Pharmacia Biotech AB) as stationery phases. The chiral HPLC separation of compound **7** was accomplished over a Chiralpak AY-H (column size: 4.6 × 250 mm 5 μm; Daicel Chemical Industries, Ltd.; flow rate: 1.0 mL/min; solvent: *n*-hexane (0.1% DEA)−EtOH (0.1% DEA) = 50:50. α-Glucosidase from *Saccharomyces cerevisiae* was purchased from Sigma-Aldrich Co. (CAS number: 9001-42-7, E.C 3.2.1.20).

Fungal Material. The fungal strain HN29-3B1 used in this study was isolated from a fresh branch of *Cerbera manghas*, which was collected from Dongzhaigang Mangrove National Nature Reserve in Hainan Island, China, in April 2009. The strain was identified as *Penicillium* sp., Trichocomaceae, according to morphologic traits and molecular identification. Its 444 base pair ITS sequence had 99% sequence identity to that of *Penicillium pinophilum* (HQ671193.1). The sequence data had been submitted to GenBank with accession number KF572480. A voucher specimen (registration number HN29-3B1) has been deposited at Sun Yat-Sen University, China.

Molecular Taxonomy of the Fungus. The DNA amplification and sequencing of the fungus HN29-3B1 were carried out as described below. About 120 mg of fresh fungal mycelium was collected in a microcentrifuge tube (1.5 mL) to extract genomic DNA from the fungus using a fungal DNA kit (50) (E.Z.N.A., Omega) according to the manufacturer's protocol. The PCR reactions were performed in a final volume of 50 μL, which was composed of template DNA (2 μL), 5 μL of 10× buffer, 1 μL of dNTP, 0.5 μL of ITS1F, 0.5 μL of ITS4 (20 μmol/mL each), 0.25 μL of Taq polymerase, and appropriate ultrapure water under the following conditions: (1) initial denaturation at 94.0 °C for 5 min; (2) desmolysis at 94.0 °C for 50 s; (3) annealing at 52.5 °C for 50 s; (4) extension at 72.0 °C for 1 min; (5) final extension at 72.0 °C for 10 min. Steps 2−4 were repeated 32 times. Then, 5 μL of the amplification products was loaded on an agarose gel (1.2% agarose in 0.5× TAE, 5 μL of ethidium bromide 1% m/v solution per 100 mL of gel). After electrophoresis at 100 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, Shanghai, China) with the primer ITS1F.

Fermentation, Extraction, and Isolation. The fungus *Penicillium* sp. HN29-3B1 was grown on a solid autoclaved rice substrate medium (60 500 mL Erlenmeyer flasks, each containing 50 g of rice and 50 mL of 3‰ of saline water) for 28 days at 25 °C. The mycelia and solid rice medium were extracted with MeOH three times. The organic solvents were evaporated to dryness under reduced pressure to yield 12.6 g, which was subjected to a silica gel column (80 × 10 cm), eluting with a gradient of petroleum ether to ethyl acetate to afford seven fractions (Fr. 1−Fr. 7), containing 1.8, 2.0, 2.1, 1.3, 2.2, 1.8, and 1.4 g of material, respectively. Fr. 3 (2.1 g) was subjected to a silica gel column (30 × 3 cm) using gradient mixtures of petroleum ether and ethyl acetate to yield seven subfractions (Fr. 3-1−Fr. 3-7). Fr. 3-1 (550 mg) was rechromatographed on a silica gel column (20 × 4 cm) with a gradient of petroleum ether and ethyl acetate from 70:30 to 30:70 v/v to give **4** (5.2 mg). Fr. 4 (1.3 g) was subjected to CC on silica gel (40 × 6 cm column) eluting with a gradient of petroleum ether and ethyl acetate from 80:20 to 20:80 v/v, affording 7 (1.8 mg) and **5** (7.2 mg). Fr. 5 (2.2 g) was further fractioned on Sephadex LH-20 CC (110 × 3 cm), eluting with $CHCl_3$−MeOH (1:1, v/v), to obtain five subfractions (Fr. 5-1−Fr. 5-5). Fr. 5-1 (910 mg) was further fractioned by silica gel (30 × 6 cm column) eluting with a gradient of petroleum ether and ethyl acetate from 80:20 to 20:80 v/v to give **3** (5.1 mg) and **6** (4.0 mg). Fr. 5-2 (310 mg) was chromatographed on silica gel (10 × 4 cm column) eluting with CH_2Cl_2−MeOH from 1:0 to 10:1 v/v to obtain **8** (4.0 mg) and **9** (5.0 mg). Fr. 6 (1.8 g) was chromatographed on silica gel (60 × 4 cm column) eluting with petroleum ether and ethyl acetate adding 0.5‰ acetic acid (60:40 to 40:60 v/v) to afford **1** (4.0 mg) and **2** (6.2 mg). Fr. 6-1 (70 mg) was purified by recrystallization with MeOH−H_2O (10:1, v/v) to yield **10** (5.8 mg).

*Pinazaphilone A (**1**):* yellow, amorphous powder; $[\alpha]_D^{25}$ −203 (*c* 0.002 MeOH); UV (MeOH) λ_{max} (log ε) 306 (3.01), 265 (3.34), 217 (3.63) nm; CD (CH_3OH) λ_{max} (Δε) 310 (+8.7), 270 (−6.4), 205 (+13.5) nm; IR (KBr) ν_{max} 3428, 2919, 1639, 1564, 1444, 1318, 1262, 1165, 1061, 802 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz), see Table 1; ESIMS *m*/*z* 415 $[M - H]^-$; HRESIMS *m*/*z* 415.10318 $[M - H]^-$ (calcd for $C_{21}H_{19}O_9$, 415.10346).

*Pinazaphilone B (**2**):* pale yellow powder; $[\alpha]_D^{25}$ −50 (*c* 0.001 MeOH); UV (MeOH) λ_{max} (log ε) 339 (3.73), 267 (3.72), 215 (4.05) nm; CD (CH_3OH) λ_{max} (Δε) 330 (+8.8), 298 (−10.6), 264 (+3.7) nm; IR (KBr) ν_{max} 3433, 2920, 1633, 1458, 1261, 1163, 1061, 802 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz), see Table 1; ESIMS *m*/*z* 415 $[M - H]^-$; HRESIMS *m*/*z* 415.10321 $[M - H]^-$ (calcd for $C_{21}H_{19}O_9$, 415.10346).

*Sch 1385568 (**3**):* yellow, amorphous powder; $[\alpha]_D^{25}$ +65 (*c* 0.002 MeOH); CD (CH_3OH) λ_{max} (Δε) 360 (+12.1), 312 (+4.9), 274 (−28.5), 232 (+4.7), 221 (−7.1), 211 (+2.4) nm.

*4′-(S)-(3,5-Dihydroxyphenyl)-4′-hydroxy-6′-methylcyclopent-1′-en-5′-one (**4**):* colorless crystals; mp 179−180 °C; $[\alpha]_D^{25}$ +170 (*c* 0.001 MeOH); UV (MeOH) λ_{max} (log ε) 266 (3.22), 226 (3.41), 205 (3.50) nm; IR (KBr) ν_{max} 3408, 2916, 1684, 1611, 1445, 1264, 1101, 1079 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz), see Table 2; EIMS *m*/*z* 220 $[M]^+$; HREIMS *m*/*z* 220.0733 $[M]^+$ (calcd for $C_{12}H_{12}O_4$, 220.0730).

*6′-Methyl-[1,1′-biphenyl]-3,3′,4′,5-tetraol (**5**):* white, amorphous powder; $[\alpha]_D^{25}$ −95 (*c* 0.001 MeOH); UV(MeOH) λ_{max} (log ε) 264 (2.63), 224 (2.91), 212 (3.26) nm; IR (KBr) ν_{max} 3409, 2963, 1722, 1597, 1444, 1262, 1098, 801 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz), see Table 2; EIMS *m*/*z* 232 $[M]^+$; HREIMS *m*/*z* 232.0736 $[M]^+$ (calcd for $C_{13}H_{12}O_4$, 232.0730).

*Penicidone D (**6**):* white, amorphous powder; $[\alpha]_D^{25}$ +30 (*c* 0.001 MeOH); UV (MeOH) λ_{max} (log ε) 258 (3.67), 207 (3.79) nm; IR (KBr) ν_{max} 3429, 2921, 1623, 1578, 1438, 1373, 1244, 1159, 1070 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz), see Table 3; EIMS *m*/*z* 387 $[M]^+$; HREIMS *m*/*z* 387.1311 $[M]^+$ (calcd for $C_{20}H_{21}O_7N$, 387.1313).

X-ray Crystallographic Analysis of 4 and 7. Colorless crystals of **4** were obtained from MeOH−H_2O. Colorless crystals of **7** were obtained from acetone; all single-crystal X-ray diffraction data were collected at 123 K on an Oxford Gemini S Ultra diffractometer with

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Cu Kα radiation (λ = 1.541 78 Å). The structures were solved by direct methods (SHELXL-2013) 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. The crystallographic data of **4** and **7** have been deposited at the Cambridge Crystallographic Data Centre with the deposition numbers CCDC 979698 and CCDC 970259, respectively (submitted on November 6, 2013). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: +44(0)-1233-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Crystal data of ***4****:* orthorhombic, $C_{12}H_{14}O_5$, space group $P2(1)2(1)2(1)$, a = 7.8143(4) Å, b = 7.9133(3) Å, c = 18.3147(9) Å, α = 90°, β = 90°, γ = 90°, Z = 4, D_{calcd} = 1.397 g/cm^3, μ(Cu Kα) = 0.921 mm^{-1}, and $F(000)$ = 504, Flack = −0.02(15). Crystal size: 0.40 × 0.39 × 0.08 mm^3. Independent reflections: 5807 [R_{int} = 0.0306]. The final indices were R_1 = 0.0447, wR_2 = 0.1167 [$I > 2\sigma(I)$].

Crystal data of ***7****:* monoclinic, $C_{19}H_{18}O_8$, space group $P2(1)/c$, a = 13.6047(5) Å, b = 7.4884(2) Å, c = 17.5684(5) Å, α = 90.00°, β = 101.622(3)°, γ = 1753.13(9)°, Z = 4, D_{calcd} = 1.418 g/cm^3, μ(Cu Kα) = 0.947 mm^{-1}, and $F(000)$ = 784. Crystal size: 0.40 × 0.34 × 0.14 mm^3. Independent reflections: 3098 [R_{int} = 0.0478]. The final indices were R_1 = 0.0474, wR_2 = 0.1236 [$I > 2\sigma(I)$].

***In Vitro* Inhibition Studies on α-Glucosidase.** All the assays were performed using 0.01 M KH_2PO_4–K_2HPO_4 buffers, pH 7.0, and a Shimadzu 2450 spectrophotometer. Enzyme solution was prepared to give 2.0 units/mL in 2 mL aliquots. The assay medium contained phosphate buffer, pH 7.0 (950 μL), 10 μL of enzyme solution, 20 μL of DMSO or inhibitor (dissolved in DMSO), and 20 μL of 0.01 M substrate (*p*-nitrophenyl glycoside, 3 mg/mL). The substrate was added to the assay medium containing enzyme and buffer with inhibitor added after 20 min of incubation time. The activity was determined by measuring the increase in absorbance at 400 nm for a 1 min interval at 37 °C. Calculations were performed according to the equation η (%) = [($B - S$)/B] × 100% (B stands for the assay medium with DMSO; S stands for the assay medium with inhibitor). All measurements were done in triplicate from two independent experiments. The reported IC_{50} was the average value of two independent experiments.

Quantum Mechanical Calculation. Molecular mechanics calculations were performed using BARISTA version 1.4.2 (CONFLEX Corporation, Tokyo, Japan) with standard parameters and convergence criteria. Low-energy conformations within 0–3.5 kcal/mol were further computed at the B3LYP/6-31G(d) level in the gas phase using Gaussian 09 (Gaussian, Wallingford, CT, USA) with default grids and convergence criteria. All low-energy conformers with relative energy of 0–2.5 kcal/mol were further used in TD-DFT calculations at the B3LYP/6-311+G(d) level in the gas phase and included 35 single excited states for each case. ECD simulations were generated using Boltzmann statistics, and ECD spectra were generated by applying Gaussian band shapes with a 0.40 eV exponential half-width from dipole-length rotational strengths. ECD predictions were performed by Supercomputing Center of Chinese Academy of Sciences (SCCAS).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Proposed biosynthetic pathways for compounds **1**–**3** and **6**–**8**, 1D and 2D NMR spectra, HREIMS and HRESIMS spectra for new compounds, CIF files for compounds **4** and **7**, and the quantum mechanical calculation data for **1**–**3**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/np500885f.

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Notes

The authors declare no competing financial interest.

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