Cytochalasan and Tyrosine-Derived Alkaloids from the Marine Sediment-Derived Fungus *Westerdykella dispersa* and Their Bioactivities

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Six new cytochalasans, designated as 18-oxo-19,20-dihydrophomacin C (1), 18-oxo-19-methoxy-19,20-dihydrophomacin C (2), 18-oxo-19-hydroxyl-19,20-dihydrophomacin C (3), 19,20-dihydrophomacin C (4), 19-methoxy-19,20-dihydrophomacin C (5), 19-hydroxyl-19,20-dihydrophomacin C (6), and one new tyrosine-derived alkaloid named as gymnastatin Z (8), together with two known compounds, phomacin B (7) and triticone D (9), were isolated from a solid-substrate fermentation culture of *Westerdykella dispersa* which was derived from marine sediments. Their structures were established on the basis of spectroscopic analysis using 1D and 2D NMR techniques, and comparison of NMR data to those of known compounds. The anti-bacterial and cytotoxic activities assays of all isolated compounds were evaluated against eight human pathogenic bacteria and five human cancer cell lines, respectively. Compound 8 exhibited moderate activity against *B. subtilis* with MIC values of 12.5 µg/mL, while compounds 5, 7 and 8 displayed moderate inhibitory activities against five human cancer cell lines (MCF-7, HepG2, A549, HT-29 and SGC-7901), with IC₅₀ values ranging from 25.6 to 83.7 µM.

The cytochalasans, a diverse group of fungal polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) hybrid metabolites, have attracted much attention from chemists and pharmacologists in the past nearly 60 years due to their intriguing structures and diverse biological functions. This group of metabolites share a perhydroisoindol-1-one skeleton to which connected is a benzyl group (cytochalasins), a p-methoxybenzyl group (pyrichalasins), a (indol-3-yl)methyl group (chaetoglobosins), or a 2-methylpropyl group (aspochalasins), and which is fused to a 9- to 15-membered carbocyclic (or oxygen containing) ring at positions C-8 and C-9. In 1966, cytochalasin A and B were first discovered from *Phoma strain S 298* and *Helminthosporium dematioideum*, and since then, over 200 related derivatives have been reported from various fungi including ascomycetes as well as basidiomycetes, as exemplified by the genera *Aspergillus*, *Penicillium*, *Chaetomium*, *Zygosporium*, *Phoma*, *Rosellinia*, *Ascochyta*, *Metarhizium*, *Xylaria*, *Phomopsis* or *Hypoxylon*. Various cytochalasans exert a wide range of biological activities, such as interfering with cytokinesis, intracellular motility, monosaccharide transport systems, or intracellular Ca²⁺ regulation, inhibiting thyroid secretion, or displaying cytotoxic, antimicrobial or antiparasitic properties.

As part of our program to discover structurally unique and biologically active secondary metabolites from fungi of unique ecological niches, the chemical investigation on *Westerdykella dispersa* was carried out, resulting...
in the discovery of six new cytochalasans, namely, 18-oxo-19,20-dihydrophomacin C (1), 18-oxo-19-methoxy-19 ,20-dihydrophomacin C (2), 18-oxo-19-hydroxy-19,20-dihydrophomacin C (3), 19,20-dihydrophomacin C (4), 19-methoxy-19,20-dihydrophomacin C (5), 19-hydroxy-19,20- dihydrophomacin C (6), and one new tyrosine-derived alkaloid named as gymnastatin Z (8), together with two known compounds, phomacin B (7) and triticones D (9) (Fig. 1). Herein, we report the fermentation, isolation, structure elucidation, and biological activities of these isolated compounds.

Results and Discussion

Structure Elucidation. Compound 1 was isolated as a colorless block crystal with a molecular formula C_{25}H_{37}NO_{4}, as suggested by the HRESIMS data at m/z 438.26152 [M+Na]^{+} (calcld for 438.26148). Interpretation of its^{1}H, {^{13}}C NMR, DEPT, and HMQC spectra revealed 25 carbon resonances ascribed to five methyls, six sp^{3} methylenes (one of which oxygenated), six sp^{3} methines, two sp^{2} methines, one sp^{3} nonprotonated carbon, two sp^{2} nonprotonated carbons, and three carboxyl groups. The molecular formula requires eight degrees of unsaturation, but only three carboxyl and four olefinic carbons resonating at δ_{C} 175.8 (s, C-1), 207.8 (s, C-18), 208.1 (s, C-21), 139.7 (s, C-6), 125.6 (d, C-7), 124.8 (d, C-13), and 136.7 (s, C-14) were detected, indicating the tricyclic nature of 1. Four spin systems could be detected in the COSY spectrum as depicted in Fig. 2. Detailed analyses of the 1D and 2D NMR spectroscopic data revealed that 1 had a similar structure to phomacin C, a cytochalasin-based alkaloid characterized from Phoma sp.\textsuperscript{2}. The main differences between the two compounds are at positions C-18, C-19 and C-20, with the hydroxyl group (C-18) and the C-19/C-20 trans-olefin in phomacin C being replaced by the two sp^{3} methylenes at positions C-19 and C-20, and ketone substituent at C-18 in 1. This suggested that the C-19/C-20 trans-olefin in phomacin C was reduced, and then oxidative reaction occurred at

![Figure 1. Structures of compounds 1–9.](image1)

![Figure 2. COSY and selected HMBC correlations of compounds 1–6 and 8.](image2)
C-18 to form 1. The observed HMBC and COSY correlations (Fig. 2) supported the above deduction. On the basis of the above data, the gross structure of 1 was established. The relative configurations of 1 were determined to be 3$^{S*}$, 4$^{R*}$, 5$^{S*}$, 8$^{S*}$, 9$^{S*}$, and 16$^{S*}$, by comparing the NMR data with those reported for phomacin C as well as by the NOESY spectroscopic data (Fig. 3), which were in agreement with those of phomacin C. This was confirmed by the X-ray single-crystal diffraction (Fig. 4) using the anomalous scattering of Mo K$\alpha$ radiation. It should be noted that the stereochemistry of the cyclohexene and isoindole moieties in all cytochalasans are the same and have been established as 3$^{S*}$, 4$^{R*}$, 5$^{S*}$, 8$^{S*}$, 9$^{S*}$12. Therefore, compound 1 was characterized as 18-oxo-19,20-dihydrophomacin C.

Compound 2 was found to have the molecular formula $C_{26}H_{39}NO_5$ established by HRESIMS at $m/z$ 468.27164 ([M + Na]$^+$, calcd for 468.27204), suggesting eight degrees of unsaturation. The $^1$H and $^{13}$C NMR data of 2 (Tables 1 and 2) closely resembled those of 1, except for the presence of one additional oxygenated methyl and one oxygenated methine, and the absence of one sp$^3$ methylene in 2. This suggested that the methoxylation occurred at C-19 position in 1 to form 2, evident from HMBC correlations of H-19 with C-21, and H-27 with C-19, combined with correlation of H-19 with H-20 in the COSY spectrum (Fig. 2). The relative configurations of all stereocenters except for C-19 in 2 was characterized the same as in 1 by analysis of NOESY correlations and by comparison of its NMR data with those of 1. While the absolute configuration of C-19 was determined to be S by computational method via calculation of the electronic circular dichroism (ECD) (Fig. 5A), which was also supported by the
confirmed by the detected NOESY correlations (Fig. 3). While the absolute configuration of C-18 was determined to be $R$ and COSY experiments (Fig. 2). The relative stereochemistry of all chiral centers except for C-18 were the same as in phomacin C based upon coupling constants and chemical shift comparisons, which was further confirmed by the HMBC and COSY correlations (Fig. 2). This suggested that compound 3 is the non-methylated derivative of Compound 4 was obtained as an amorphous white powder. The HRESIMS of Compound 4 displayed a pseudomolecular ion peak at m/z 440.27778 [M + Na]$^+$ (calcd for C$_{24}$H$_{39}$NO$_5$Na, 440.27713), corresponding to the formula of C$_{25}$H$_{39}$NO$_5$. The$^1$H and$^{13}$C NMR data were extremely similar to those of 1, except for the absence of a carboxyl group ($\delta_{C}$: 207.8 (s, C-18)) and the appearance of an additional oxygenated methine ($\delta_{C}$: 3.76 (m, H-18); $\delta$$_{H}$: 6.88 (d, C-18)) in 4. This suggested that compound 4 is a reductive derivative of 1, which was confirmed by the HMBC and COSY experiments (Fig. 2). The relative stereochemistry of all chiral centers except for C-18 were the same as in 1-3 and phomacin C based upon coupling constants and chemical shift comparisons, which was further confirmed by the detected NOESY correlations (Fig. 3). While the absolute configuration of C-18 was determined to be $R$ through calculation of the electronic circular dichroism (ECD) (Fig. 5B), which is different from that in 2. Therefore, compound 3 was characterized as 18-oxo-19,20-dihydrophomacin C.

Table 1. $^1$H NMR data for compounds 1–6 in CDCl$_3$ ($\delta$ in ppm, J in Hz). $^a$Spectra were recorded at 400 MHz. $^b$Spectra were recorded at 600 MHz.

| Comp. | $^1$H NMR Data | $^2$H NMR Data | $^3$H NMR Data | $^4$H NMR Data | $^5$H NMR Data | $^6$H NMR Data |
|-------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1     | 6.66, s        | 6.07, s        | 6.17, s        | 6.24, s        | 6.06, s        | 6.20, s        |
| 2     | 5.35, s        | 5.37, brs      | 5.37, brs      | 5.44, brs      | 5.45, brs      | 5.43, brs      |
| 3     | 1.53, m        | 2.91, m        | 2.91, m        | 2.91, m        | 2.92, m        | 2.93, m        |
| 4     | 1.36, s        | 1.35, s        | 1.35, s        | 1.35, s        | 1.35, s        | 1.35, s        |
| 5     | 1.33, d (6.0)  | 1.32, d (6.0)  | 1.32, d (6.0)  | 1.33, d (6.0)  | 1.33, d (6.0)  | 1.33, d (6.0)  |
| 6     | 1.32, d (6.0)  | 1.31, d (6.0)  | 1.31, d (6.0)  | 1.32, d (6.0)  | 1.32, d (6.0)  | 1.32, d (6.0)  |

NOESY correlations of H-25 with H-8 and H-16, and H-16 with H-19 indicating H-19 is $\beta$-oriented (Fig. 3). Therefore, the structure of 2 was characterized as 18-oxo-19-methoxy-19,20-dihydrophomacin C.
suggested that the methylation occurred at C-19 in 4 to form 5, which was further supported by the HMBC correlations of H-19 with C-21, H-27 with C-19, along with the correlations of H-18 with H-19 observed in the COSY spectrum (Fig. 2). The relative stereochemistry of all chiral centers except for C-18 and C-19 were in accord with those of compounds 1-4 based upon coupling constants and chemical shift comparisons, which was further confirmed by the NOESY correlations as depicted in Fig. 3. Furthermore, the α-orientations of hydroxyl group at position C-18 and methoxy group at position C-19 were determined by ROESY correlations of H-13 with H-8, H-16, H-17a and H-20a, H-20a with H-18, and H-19 with H-17a, which allowed us to determine the relative configuration of C-18 and C-19 as S<sup>*</sup> and S<sup>*</sup>, respectively. Therefore, compound 5 was determined to be 19-methoxy-19,20-dihydrophomacin C.

The molecular formula of 6, which was obtained as an amorphous white powder, was determined to be C<sub>25</sub>H<sub>39</sub>NO<sub>5</sub> as deduced by HRESIMS at m/z 456.27169 [M + Na]<sup>+</sup> (calcld for 456.27204), requiring 7 degrees of unsaturation. The molecular weight of 6 was found to be 14 mass units less than that of 5. Its 1H and 13C NMR spectra (Tables 1 and 2) showed resonances for five methyls, five methylenes, ten methines, and five quaternary carbons. Comparison of its NMR spectra with compound 8 based upon coupling constants and chemical shift comparisons, which was different from the COSY and HMBC spectra of 8, as evident from the COSY correlation of H-1 with H-2, and the

| Pos. | 1       | 2       | 3       | 4       | 5       | 6       |
|------|---------|---------|---------|---------|---------|---------|
| 1    | 175.8, C| 174.9, C| 175.4, C| 175.6, C| 175.8, C| 174.8, C|
| 2    | 115.7, CH| 115.7, CH| 115.7, CH| 115.6, CH| 115.8, CH| 115.6, CH|
| 4    | 135.2, CH| 136.7, CH| 135.4, CH| 136.2, CH| 135.6, CH| 135.4, CH|
| 5    | 136.7, CH| 137.2, CH| 135.4, CH| 136.2, CH| 135.6, CH| 135.4, CH|
| 6    | 139.7, CH| 139.8, CH| 139.4, CH| 139.6, CH| 139.8, CH| 139.6, CH|
| 7    | 125.6, CH| 125.6, CH| 125.4, CH| 125.6, CH| 125.4, CH| 125.6, CH|
| 8    | 43.2, CH| 43.9, CH| 43.3, CH| 43.6, CH| 44.1, CH| 44.0, CH|
| 9    | 66.9, C| 65.1, C| 66.4, C| 67.6, C| 67.1, C| 67.5, C|
| 10   | 48.6, CH<sub>2</sub>| 48.1, CH<sub>2</sub>| 48.5, CH<sub>2</sub>| 48.5, CH<sub>2</sub>| 48.7, CH<sub>2</sub>| 48.5, CH<sub>2</sub>|
| 11   | 113.3, CH<sub>3</sub>| 113.3, CH<sub>3</sub>| 113.4, CH<sub>3</sub>| 113.4, CH<sub>3</sub>| 113.4, CH<sub>3</sub>| 113.4, CH<sub>3</sub>|
| 12   | 19.8, CH<sub>4</sub>| 19.8, CH<sub>4</sub>| 19.8, CH<sub>4</sub>| 19.8, CH<sub>4</sub>| 19.8, CH<sub>4</sub>| 19.8, CH<sub>4</sub>|
| 13   | 124.8, CH| 124.6, CH| 124.8, CH| 124.6, CH| 124.8, CH| 124.8, CH|
| 14   | 136.7, C| 137.2, C| 137.4, C| 135.2, C| 134.9, C| 135.2, C|
| 15   | 44.3, CH<sub>2</sub>| 44.1, CH<sub>2</sub>| 43.9, CH<sub>2</sub>| 43.4, CH<sub>2</sub>| 43.7, CH<sub>2</sub>| 43.9, CH<sub>2</sub>|
| 16   | 35.1, CH| 35.2, CH| 33.8, CH| 33.0, CH| 33.9, CH| 33.5, CH|
| 17   | 42.1, CH<sub>3</sub>| 43.2, CH<sub>3</sub>| 38.1, CH<sub>3</sub>| 35.1, CH<sub>3</sub>| 32.6, CH<sub>3</sub>| 32.7, CH<sub>3</sub>|
| 18   | 130.6, CH| 130.6, CH| 130.6, CH| 130.6, CH| 130.6, CH| 130.6, CH|
| 19   | 139.7, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C|
| 20   | 139.7, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C|
| 21   | 139.7, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C|
| 22   | 139.7, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C|
| 23   | 139.7, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C|
| 24   | 139.7, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C|
| 25   | 139.7, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C|
| 26   | 139.7, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C|
| 27   | 139.7, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C| 139.8, C|

Table 2. 13C NMR (100 MHz) data for compounds 1–6 in CDCl<sub>3</sub> (δ in ppm).
HMBC correlation of H-1 with C-3 (Fig. 2). The E-forms of all olefinic double bonds of the side chain as same in gymnastatin were deduced on the basis of their respective coupling constants. Unfortunately, the stereochemistry of C-2 and C-16 could not yet be clarified due to scarcity of material. Thus, compound 8 was identified and designated as gymnastatin Z considering that this compound belonged to gymnastatin derivatives and the gymnastatins A–Y have been already reported26,27.

Two known compounds 7 and 9 were characterized as phomacin B24, and triticone D28, respectively, by comparing of their NMR spectroscopic data with those reported in the literature.

Biological Activity. Cytotoxicity Assay. All isolated compounds were evaluated for cytotoxic activity against human breast cancer cells MCF-7, human hepatocellular carcinoma cells HepG2, human lung cancer cells A549, human colon colorectal adenocarcinoma cells HT-29 and human gastric cancer cells SGC-7901 by the MTT method29. The results (see Supporting Information) indicated that compounds 5, 7 and 8 showed moderate activity against all five cell lines, with IC50 values ranging from 25.6 to 83.7 μM. In addition, compounds 4 and 6 exhibited moderate inhibitory activity against HT-29 cells, with IC50 values 55.5, 49.1 μM, respectively. However, compounds 1–3 and 9 displayed no cytotoxicity.

Antibacterial Activity. All isolated compounds were evaluated for their antibacterial activity against Gram-positive (B. subtilis, M. luteus, B. anthracis and S. enterica) and Gram-negative (P. vulgaris, S. typhimurium, E. coli and E. aerogenes) bacteria30. The results (see Supporting Information) indicated that only compound 8 exhibited moderate activity against B. subtilis with an MIC value of 12.5 μg/mL, and very weak activity against P. vulgaris, S. typhimurium and E. coli with MIC values of 100 μg/mL. None of them are active against M. luteus and S. enterica.

In conclusion, seven cytochalasan alkaloids including six new ones (1–6) and one known derivative (7), one new tyrosine-derived alkaloid (8), and one known 2-pyrrolidinone alkaloid (9) were isolated from Westerdykella dispersa. To the best of our knowledge, so far only several polyenes including gelastatins A–B and dykellic acid have been reported from the genus Westerdykella31,32. Therefore, this is the first report of these types of alkaloids in this genus.

Materials and Methods
General Experimental Procedures. Optical rotations were measured on an Autopol I automatic polarimeter (Rudolph). UV spectra were recorded on an Agilent spectrophotometer (Agilent Cary60). IR spectra were run on a Bruker spectrophotometer (TENSOR 27). HRESIMS spectra were performed on a Bruker instrument (FTICRMS, SolariX). Nuclear magnetic resonance (NMR) spectra were recorded on an Agilent DD2 spectrometer (400 MHz and 600 MHz). Crystal data was collected on a SuperNova area detector diffractometer (Agilent Technologies Inc.) Melting point (m.p.) was obtained on SGW X-4A. Silica gel (200–300 mesh, Anhui liangchen sugar 20.0 g, mannitol 20.0 g, corn steep liquor 1.0 g, yeast extract powder 3.0 g, aginomoto 10.0 g, K2HPO4 0.5 g, magnesium sulfate 0.3 g, CaCO3 2.0 g, distilled water 1000 mL) and incubated at 25 °C for 2 days on a rotating

Fungal Material and Identification. The fungal strain XL602 was isolated from marine sediments, which were collected at South China Sea, Guangzhou, Guangdong province, China, in July 2014. The species was identified to be Westerdykella dispersa based on sequence analysis of the ITS region of 18S rDNA (GenBank Accession No. KY604839), and was deposited at the School of Pharmaceutical Sciences, Chongqing University (Huxi Campus).

Fermentation, Extraction, and Isolation. The strain was cultured on a plate of potato dextrose agar (PDA) at 28 °C for 7 days. Agar plugs were cut into small pieces (approximately 0.5 × 0.5 × 0.5 cm3) under aseptic conditions, and inoculated into four Erlenmeyer flasks (250 mL, 5 pieces per flask) to prepare the seed culture, previously sterilized by autoclaving, each containing 50 mL modified Czapek-Dox medium (glucose 10.0 g, malt sugar 20.0 g, mannitol 20.0 g, corn steep liquor 1.0 g, yeast extract powder 3.0 g, aginomoto 10.0 g, K2HPO4 0.5 g, magnesium sulfate 0.3 g, CaCO3 2.0 g, distilled water 1000 mL) and incubated at 25 °C for 2 days on a rotating
shaker at 180 rpm/min. The scale-up fermentation was carried out in 8 Erlenmeyer flasks (2L) (each containing 300 g of rice, 150 mL modified Czapek-Dox medium, 150 mL H$_2$O, sterilized for 20 minutes at 121 °C). Every flask was inoculated with 5.0 mL of the spore inoculum and incubated at room temperature for 30 days. The fungal cultures of *Westerydikella dispersa* were ultrastructurally extracted four times with MeOH (each time 4L). The solvent was removed to give a crude extract (10.8 g). The organic extracts were combined and concentrated under reduced pressure to yield 10.8 g of brown oil. This extract was chromatographed on column chromatography (CC) over SiO$_2$, using a stepwise gradient of petroleum ether/aceton gradient system (9:1, 8:2, 8:4, and 5:5) to yield nine fractions, Fr. 1–9. Fr. 4 (0.5 g) was purified by CC over Sephadex LH-20 (CH$_3$Cl/MeOH, 1:1), silica gel CC (petroleum ether/aceton, 5:1), and RP-18 (MeOH/H$_2$O, 20:80) to afford compound 9 (4.5 mg). Fr. 6 (0.83 g) was subjected to CC over silica gel (petroleum ether/aceton 6:1, 141:1, 1:1) to yield seven subfractions (6a–6g). Subfraction 6c was separated by repeated CC over Sephadex LH-20 (CH$_3$Cl/MeOH, 1:1) and semi-preparative HPLC using a C18 column (5 μm, 10 × 250 mm, MeOH/H$_2$O, 2 mL/min), yielding compounds 1 (17.4 mg, t$_R$ = 21.6 min, 91% MeOH in H$_2$O) and 2 (7.3 mg, t$_R$ = 21.9 min, 92% MeOH in H$_2$O). Compounds 5 (24.6 mg, t$_R$ = 8.2 min, 78% MeCN in H$_2$O, 3 mL/min) and 8 (3.8 mg, t$_R$ = 22.0 min, 90% MeOH in H$_2$O, 2 mL/min) were obtained from subfraction 6d by silica gel CC (CH$_3$Cl/MeOH, 25:1) and semi-preparative HPLC (5 μm, 10 × 250 mm, MeOH/H$_2$O, 2 mL/min). Subfraction 6e was purified by Sephadex LH-20 (CH$_3$Cl/MeOH, 1:1) and semi-preparative HPLC (5 μm, 10 × 250 mm, MeOH/H$_2$O, 2 mL/min) to afford compound 4 (8.1 mg, t$_R$ = 36.2 min, 75% MeOH in H$_2$O). Subfraction 6f was fractionated on Sephadex LH-20 (CH$_3$Cl/MeOH, 1:1), semi-preparative HPLC (5 μm, 10 × 250 mm, MeOH/H$_2$O, 2 mL/min), and silica gel (petroleum ether/EtOAc, 1:1–5:1) to yield compound 3 (8.1 mg, t$_R$ = 36.0 min, 70% MeOH in H$_2$O) and 7 (2.1 mg). Compound 6 (7.5 mg, t$_R$ = 26.0 min, 75% MeOH in H$_2$O) was obtained from Fr. 7 (0.9 g) by CC over Sephadex LH-20 (MeOH/H$_2$O, 1:1), repeatedly silica gel (CHCl$_3$/MeOH, 50:1–1:1), and semi-preparative HPLC (5 μm, 10 × 250 mm, MeOH/H$_2$O, 2 mL/min).

**Cytotoxicity Assay.** Cytotoxicity activity was evaluated against MCF-7, HepG2, A549, HT-29 and SGC-7901 by the MTT method. All cell lines was grown in RPMI-1640 medium (GIBCO) supplemented with 10% FCS, 100 U/L penicillin, 100 mg/mL streptomycin and 10 mM HEPES, pH 7.4. Cells were kept at 37 °C in a humidified 5% CO$_2$ incubator. An aliquot (180 μL) of these cell suspensions, at a density of 1500 cells mL$^{-1}$ was pipetted into 96-well microtiter plates. Subsequently, 180 μL of sample (in DMSO) at different concentrations was added to each well and incubated for 72 h at the above conditions in a CO$_2$ incubator. MTT solution (20 μL of 5 mg/L in RPMI-1640 medium) was added to each well and further incubated for 4 h at 37 °C. After addition of 100 μL DMSO and incubation for 1 h, the cells were lysed to liberate the formed formazan crystals. The optical density (OD) was read on a Multiscan plate reader at a wavelength of 492 nm. DMSO control well, in which sample was absent, was included in the experiment in order to eliminate the influence of DMSO. The inhibitory rate of cell proliferation was calculated by the following formula:

$$ \text{Inhibition rate} = \frac{1 - (\text{OD}_{\text{treated}} - \text{OD}_{\text{blank}})}{(\text{OD}_{\text{control}} - \text{OD}_{\text{blank}})} \times 100\% $$

**Antibacterial Assay.** All isolated compounds were evaluated for their antibacterial activity against Gram-positive (*B. subtilis, M. luteus, B. anthracis* and *S. enterica*) and Gram-negative (*P. vulgaris, S. typhimurium, E. coli* and *E. aerogenes*) bacteria. They were grown in liquid LB medium (yeast extract 5 g/L, peptone 10 g/L, NaCl 0.5%).
10 g/L, pH = 7.4) overnight at 37 °C, and the diluted bacterial suspension (10^6 CFU per milliliter) was ready for detection. The minimum inhibitory concentrations (MIC) of samples and positive control were determined in sterile 96-well plates by the modified broth dilution test. All of wells were filled with 180 μL of bacterial suspension containing 10^6 CFU per milliliter. Test samples (20 μL) with their different concentrations were added into each well. Medium containing DMSO was used as a negative control, ciprofloxacin was used as the positive control. The final concentrations of ciprofloxacin and test compounds were 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125 μg/mL in medium. After incubation, the minimum inhibitory concentration (MIC) was defined as the lowest test concentration that completely inhibited the growth of the test organisms.

**Table 3.** ^1^H NMR and ^13^C NMR for compound 8 and gymnastatin H in CDCl_3 (δ in ppm, J in Hz). ^a^Spectra were recorded at 400 MHz for ^1^H and at 100 MHz for ^13^C in CDCl_3. ^b^Spectra were recorded at 300 MHz ^1^H and at 75 MHz for ^13^C in CDCl_3.

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Acknowledgements
This work was financially supported by the Fundamental Research Funds for the Central Universities (No. 0903005203401), the Start-up Fund for the “Hundred Young-Talent Scheme” Professorship provided by Chongqing University in China (No. 0236011104424), and the National Natural Science Foundation of China (31301305).

Author Contributions
X.-L. Yang and D. Xu designed the experiments and analyzed the data; M.-H. Luo coordinated the project; D. Xu performed the experiments; M.-Y. Xia, W. Dong, and F.-L. Liu performed the biological activity; X.-L. Yang and D. Xu wrote the paper, while critical revision of the publication was performed by all authors.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-017-12327-1.

Competing Interests: The authors declare that they have no competing interests.

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