Absolute Configurations of 14,15-Hydroxylated Prenylxanthones from a Marine-Derived Aspergillus sp. Fungus by Chiroptical Methods

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Determination of the absolute configurations for natural products is one of the most important and challenging tasks, especially when the molecules display high conformational flexibility. In this paper, eight new prenylxanthones, aspergixanthones A–H (1–8), and one known analogue (9), were isolated from the marine-derived fungus Aspergillus sp. ZA-01. The absolute configurations of C-14 and C-15 in 1–8 were difficult to be assigned due to the high conformational flexibility of the chains. To solve this problem, the experimental ECD, ORD, and VCD spectra of 1 were combined for analysis with the corresponding theoretical predictions for its different diastereomers. This study suggested that a concerted application of more than one chiroptical methods could be used as a preferable approach for the stereochemical characterizations of flexible molecules. Compounds 1–9 were evaluated for their cytotoxic and antibacterial activities. Among them, 6 showed cytotoxicity against the A-549 cell line with the IC₅₀ value of 1.1 μM, and 7 exhibited antibacterial activity against Micrococcus lysodeikticus with the MIC value of 0.78 μg/mL.

Prenylated derivatives, including prenylated polyketides¹⁻⁷, prenylated alkaloids⁸⁻⁹, prenylated flavones¹⁰, and so on, which contained conformationally flexible terpenoid-derived carbon chains, made such a task extremely challenging to assign their absolute configurations. Specially, when the terpenoid-derived carbons in prenylated derivatives were hydroxylated, it was more difficult to solve this problem by common means as the high free rotation of the stereogenic centers in chains³⁻⁷. Fortunately, the concerted application of more than one chiroptical methods has recently emerged as a hopeful approach for the stereochemical characterizations of prenylated derivatives¹¹⁻¹³. In the course of our ongoing works to discover bioactive secondary metabolites from fungal sources¹⁴⁻¹⁶, the marine-derived fungus Aspergillus sp. ZA-01 was chosen for research as its TLC and HPLC-UV profiles of the secondary metabolites. As a result, eight new prenylated xanthones (prenylxanthones), aspergixanthones A–H (1–8) and one known shamixanthone (9)⁵, were obtained from the fungus Aspergillus sp., which was grown on rice in solid culture. It was hard to assign the absolute configurations of C-14 and C-15 in chains of 1–8 as the free rotation of the stereogenic centers. To address this problem, a combined analysis of ECD, ORD, and VCD was performed for 1, its conclusion was further confirmed by using the Snatzke’s method. The cytotoxic and antibacterial activities of 1–9 were also evaluated. Herein, we report the isolation, absolute configurations, and biological activities of 1–9 (Fig. 1).

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Results and Discussion
Aspergixanthone A (1) was isolated as a yellow powder, with the molecular formula of C_{28}H_{32}O_9 (12 degrees of unsaturation) established by positive HRESIMS (m/z 535.1935 [M + Na]^+, calcd. for 535.1939). In the NMR data of 1 (Tables 1 and 2), one keto carbonyl [δ_C 183.0 (C-13)], three aromatic signals [δ_H 7.66 (1 H, d, J = 8.4 Hz, H-3), 7.24 (1 H, s, H-5), and 6.82 (1 H, d, J = 8.4 Hz, H-2); δ_C 135.3 (C-3), 120.2 (C-5), and 110.4 (C-2)], one disubstituted double bond [δ_H 4.80 (1 H, s, H-22a), 4.75 (1 H, s, H-22b); δ_C 141.5 (C-21) and 112.7 (C-22)], and four methyls [δ_H 2.35 (3 H, s, H-24), 1.88 (3 H, s, H-23), 1.40 (3 H, s, H-18), and 1.25 (3 H, s, H-17); δ_C 26.5 (C-18), 26.4 (C-17), 22.3 (C-23) and 17.2 (C-24)] were present. Along with the IR absorptions of 1, it was found that 1 contains the presence of hydroxy (3445 cm⁻¹), aromatic ring (1635 cm⁻¹), and aromatic ketone (1591 cm⁻¹) groups. The above characteristic NMR and IR data indicated that 1 belongs to the family of prenylxanthones skeleton. Careful comparison of the 1D and 2D NMR spectra of 1 with those of the known compound tajixanthone hydrate, previously isolated from the fungus *Emericella variecolor*, indicated that 1 and tajixanthone hydrate share the same prenylxanthone nucleus structure. The detailed comparison of 1D NMR data between 1 and tajixanthone hydrate suggested the presence of an additional acetoxy [δ_H 2.07 (3 H, s, 25-OCOCH_3); δ_C 170.0 (25-OCOCH_3) and 21.2 (25-OCOCH_3)] and an additional methoxyl [δ_H 3.28 (3 H, s, 14-OCH_3); δ_C 56.6 (14-OCH_3)] in 1. The observed key HMBC correlations (Fig. 2) from H-14 to C-3, C-4 and C-15, and from 14-OCH_3 to C-14 revealed the methoxyl connected to position C-14 in 1, while the key HMBC correlations from H-25 to 25-OCOCH_3 implied that the additional acetoxy was attached to C-25 in 1. Therefore, 1 was identified as the 14-methoxyl-25-acetyl derivative of tajixanthone hydrate.

Figure 1. Chemical structures of 1–9.
revealed their similar prenylxanthone nucleus to those of 3 and 4, which was verified by the each HMBC correlations from H-14 to C-3, C-4 and C-15 in 1 and 3. The methoxy at C-14 in 1 and 3 was absent in 2 and MS data of 2 was thus identified as the 25-deacetylation derivative of 1.

Aspergixanthones C and D (3 and 4) were also isolated as yellow powder with the molecular formulas of C27H30O9 and C25H28O8 by positive HRESIMS, respectively. The 1D and 2D NMR data of 3 and 4 (Tables 1 and 2) revealed their similar prenylxanthone nucleus to those of 1 and 2. Particularly, the NMR data showed the presence of a C-25 acetoxy group in 3 and a C-25 hydroxy group in 4. Detailed analysis and comparison of the NMR and MS data of 3 and 4 with those of 1 and 2 revealed that the methoxy at C-14 in 1 and 2 was absent in 3 and 4, which was verified by the each HMBC correlations from H-14 to C-3, C-4 and C-15 in 3 and 4.

Aspergixanthones E and F (5 and 6) displayed quasi-molecular ions at m/z 577.2048 and 535.1539 [M + Na]+ in the positive HRESIMS, corresponding to the molecular formulas of C26H29O10 and C24H27O9, respectively. Compounds 5 and 6 were also prenylxanthone analogues by the comparison of the strikingly similar NMR data of 5 and 6 (Tables 2 and 3) with those of 1 and 2, with the appearance of the additional acetoxy groups in 5 and 6. The additional acetoxy groups laid within their respective side chains at C-15, demonstrated by the detailed analysis of their 1H NMR data with those of reported prenylxanthone derivatives 3–6. In the NOESY spectra of 5 and 6, the correlation between H-25 and CH2-22/CH3-23 was observed, suggesting these protons should be on the same face of the molecule. And, the 1H NMR data about 25-OH/OAc in 5 and 6 had the molecular formulas of C27H30O9 and C25H28O8, respectively. The molecular structures of 25-OH/OAc in 1 and 2 were assigned by NOESY experiments and comparison with those of compound epitajixanthone hydrate [4].

| No. | 1H NMR Data (δ) of 1–4 (600 MHz, δ in ppm, J in Hz). | 1H NMR Data (δ) of 1–4 (600 MHz, δ in ppm, J in Hz). |
|-----|--------------------------------------------------|--------------------------------------------------|
| 2   | 6.82, d (8.4) 6.86, d (8.4) 6.77, d (8.4) 6.78, d (8.4) 6.78, d (8.4) | 6.78, d (8.4) |
| 3   | 7.66, d (8.4) 7.72, d (8.4) 7.82, d (8.4) 7.82, d (8.4) 7.83, d (8.4) | 7.83, d (8.4) |
| 5   | 7.24, s 7.21, s 7.50, s 7.40, s 7.40, s | 7.40, s |
| 14  | 5.10, brs 5.13, d (2.4) 5.47, d (2.8) 5.48, brs | 5.48, brs |
| 15  | 3.45, brs 3.46, brs 3.27, brd (7.2) 3.27, brd (6.6) | 3.27, brd (6.6) |
| 17  | 1.25, s 1.25, s 1.21, s 1.22, s 1.22, s | 1.22, s |
| 18  | 1.40, s 1.42, s 1.28, s 1.29, s 1.29, s | 1.29, s |
| 19  | 4.54, brd (10.8) 4.43, brd (10.8) 4.56, brd (11.4) 4.46, brd (11.4) | 4.46, brd (11.4) |
| 20  | 4.31, dd (10.8, 2.4) 4.35, dd (10.8, 2.4) 4.20, dd (11.4, 2.4) 4.34, dd (11.4, 2.4) | 4.34, dd (11.4, 2.4) |
| 21  | 2.71, brs 2.72, brs 2.68, brs 2.51, brs | 2.51, brs |
| 22  | 4.80, s 4.78, s 4.79, s 4.74, s | 4.74, s |
| 23  | 4.75, s 4.56, s 4.61, s 4.55, s | 4.55, s |
| 24  | 1.88, s 1.84, s 1.81, s 1.78, s | 1.78, s |
| 25  | 2.35, s 2.35, s 2.31, s 2.29, s | 2.29, s |
| 26  | 6.89, brs 5.40, brs 6.81, brs 5.81, brs | 5.81, brs |
| 1-OH | 12.97, brs 12.71, brs 12.78, brs 12.84, brs | 12.84, brs |
| 14-OH/OCH3 | 3.28, s 3.29, s 5.19, d (5.4) 5.17, d (3.6) | 5.17, d (3.6) |
| 15-OH | 2.73, brs 2.95, brs 4.44, brd (7.2) 4.46, brd (6.6) | 4.46, brd (6.6) |
| 16-OH | — — 4.55, brs 4.54, brs | 4.54, brs |
| 25-OH/OAc | 2.07, s 4.94, d (2.4) 1.99, s 5.26, d (3.6) | 5.26, d (3.6) |

Table 1. 1H NMR Data (δ) of 1–4 (600 MHz, δ in ppm, J in Hz). aRecorded in CDCl3. bRecorded in DMSO-d6.
different structures. ECD had one or two orders of magnitude higher sensitivity, but the stereogenic centers should be close to UV-Vis chromophores. ORD has been popularly used in recent research, yet, it was hard to explain the spectra. VCD, which requires no chromophores in the UV-Vis region and has a larger scope than ECD, was limited by the sample quantity. Thus, the use of more than one chiroptical technique could provide more reliable results for complex products, especially for some flexible compounds. In this paper, a combined analysis of ECD, ORD, and VCD properties was applied to elucidate the absolute configuration of conformationally flexible 1.

Table 2. 13C NMR Data (δ) of 1–8 (150 MHz, δ in ppm). aRecorded in CDCl3. bRecorded in DMSO-d6.

| No. | 1a | 2a | 3a | 4a | 5a | 6a | 7a | 8a |
|-----|----|----|----|----|----|----|----|----|
| 1   | 161.7, C | 161.5, C | 159.6, C | 159.6, C | 162.0, C | 161.8, C | 161.7, C | 161.4, C |
| 2   | 110.4, CH | 110.5, CH | 109.2, CH | 109.0, CH | 110.0, CH | 110.1, CH | 110.3, CH | 110.3, CH |
| 3   | 135.3, CH | 135.9, CH | 135.8, CH | 135.6, CH | 134.1, CH | 134.6, CH | 134.5, CH | 135.0, CH |
| 4   | 115.0, C | 115.5, C | 122.3, C | 122.2, C | 113.4, C | 113.8, C | 117.7, C | 118.2, C |
| 5   | 120.2, CH | 119.0, C | 120.7, CH | 119.1, CH | 120.3, CH | 119.1, CH | 120.2, CH | 118.9, CH |
| 6   | 137.6, C | 138.6, C | 137.6, C | 137.2, C | 137.6, C | 138.6, C | 137.7, C | 138.7, C |
| 7   | 150.2, C | 149.6, C | 149.9, C | 148.7, C | 150.3, C | 149.7, C | 150.3, C | 149.8, C |
| 8   | 115.2, C | 121.2, C | 114.7, C | 121.0, C | 115.1, C | 121.2, C | 115.0, C | 121.4, C |
| 9   | 109.0, C | 108.9, C | 108.1, C | 108.1, C | 109.1, C | 109.0, C | 108.2, C | 108.7, C |
| 10  | 152.2, C | 152.4, C | 151.4, C | 151.1, C | 152.2, C | 152.4, C | 152.0, C | 152.1, C |
| 11  | 151.5, C | 151.7, C | 150.5, C | 150.5, C | 151.5, C | 151.7, C | 151.6, C | 151.8, C |
| 12  | 116.3, C | 116.9, C | 115.5, C | 115.8, C | 116.5, C | 117.0, C | 116.3, C | 116.8, C |
| 13  | 183.0, C | 184.2, C | 183.1, C | 183.4, C | 183.0, C | 184.2, C | 183.1, C | 184.3, C |
| 14  | 76.2, CH | 76.2, CH | 65.3, CH | 65.2, CH | 76.4, CH | 76.5, CH | 68.8, CH | 68.8, CH |
| 15  | 78.3, CH | 78.2, CH | 78.0, CH | 78.0, CH | 77.0, CH | 77.0, CH | 79.1, CH | 79.1, CH |
| 16  | 72.9, C | 72.9, C | 72.7, C | 72.7, C | 72.7, C | 72.7, C | 143.4, C | 143.4, C |
| 17  | 26.4, CH3 | 26.5, CH3 | 26.2, CH3 | 26.2, CH3 | 26.9, CH3 | 26.9, CH3 | 113.7, CH3 | 113.7, CH3 |
| 18  | 26.5, CH3 | 26.5, CH3 | 27.5, CH3 | 27.5, CH3 | 27.9, CH3 | 27.9, CH3 | 18.6, CH3 | 18.6, CH3 |
| 19  | 63.8, CH2 | 64.5, CH2 | 63.5, CH2 | 63.5, CH2 | 63.8, CH2 | 64.4, CH2 | 63.8, CH2 | 64.8, CH2 |
| 20  | 42.5, CH2 | 44.8, CH2 | 41.7, CH2 | 44.4, CH2 | 42.5, CH2 | 44.8, CH2 | 42.5, CH2 | 45.0, CH2 |
| 21  | 141.5, C | 142.5, C | 141.8, C | 142.8, C | 142.6, C | 141.6, C | 142.5, C | 141.4, C |
| 22  | 112.7, CH3 | 112.2, CH3 | 112.6, CH3 | 111.9, CH3 | 112.7, CH3 | 112.2, CH3 | 112.8, CH3 | 112.3, CH3 |
| 23  | 22.3, CH3 | 22.5, CH3 | 22.1, CH3 | 22.4, CH3 | 22.4, CH3 | 22.5, CH3 | 22.4, CH3 | 22.5, CH3 |
| 24  | 17.2, CH3 | 17.3, CH3 | 16.9, CH3 | 16.9, CH3 | 17.3, CH3 | 17.4, CH3 | 17.3, CH3 | 17.4, CH3 |
| 25  | 65.4, CH3 | 63.1, CH3 | 64.8, CH3 | 60.9, CH3 | 65.4, CH3 | 63.0, CH3 | 65.5, CH3 | 63.3, CH3 |
| 14-OCH3 | 56.6, CH3 | 56.6, CH3 | — | — | 57.1, CH3 | 57.1, CH3 | — | — |
| 15-OAc | — | — | — | — | 170.2, C | 170.1, C | — | — |
| 25-OAc | 170.0, C | — | 169.3, C | — | 170.1, C | — | 170.0, C | — |
| 21.2, CH3 | — | 21.0, CH3 | — | 21.3, CH3 | — | 21.2, CH3 | — |

Figure 2. COSY and key HMBC correlations of 1.
were assigned as 20R, 25S-25 in C-25, suggesting that the absolute configurations of C-20 and
1 were calculated using the gas-phase B3LYP/6-311+G(d) level to compare the experimental IR and VCD
data of 1. All most of the calculated VCD signals of (14R, 15R, 20S, 25R)-1 and (14S, 15S, 20S, 25R)-1 had agreements with the experimental VCD signals of 1, while the signals of 4, 8 and 9 in the calculated VCD spectrum of (14R, 15S, 20S, 25R)-1 had disagreements with the corresponding signals in the experimental VCD spectrum of 1 (Fig. 5), suggesting that the structure of (14R, 15R,20S,25R)-1 was closer to the real structure of 1. In order to further verify the absolute configuration of 1, the dimolybdenum tetraacetate [Mo6(SO4)4] induced circular dichroism (ICD) procedure (Snatzke’s method) was used. The negative ICD Cotton effects around 300 and 400 nm of 1 (Fig. 6) gave the new-
man form of Mo-complexes of 1. It was found that a counterclockwise rotation, suggesting the R configuration for C-15 in 1. Therefore, on the basis of the above ECD, ORD, VCD, and Snatzke’s results, the absolute configuration of 1 could be defined as 14R, 15R, 20S, 25R, unambiguously.

It was showed that the ECD spectra of 2-8 closely matched that of 1 (Figures S1 and S2), suggesting the 20S, 25R absolute configuration for 2-3 and 5-8, and 20R, 25R for 4. Due to the sample quantity limitation of 2-8, it was difficult to elucidate the absolute configurations of C-14 and C-15 in 2-8 by VCD method, directly. Their absolute configurations could be tentatively assigned on the basis of a shared biogenesis with the co-isolated 1, whose absolute configuration had been unambiguously established firstly. The absolute configurations could be proposed as 14R, 15S, 20S, 25R for 2, 3, 5 and 6, 14R, 15S, 20R, 25R for 4, and 14R, 15R, 20S, 25R for 7 and 8.

Xanthones, which were isolated from many different species within fungi, bacteria, and higher plants are widespread classes of typically polysubstituted dibenzo-γ-pyryrole derivatives. Among them, prenyl xanthones

| No. | 5    | 6    | 7    | 8    |
|-----|------|------|------|------|
| 2   | 6.77, d (8.4) | 6.62, d (8.4) | 6.79, d (8.4) | 6.79, d (8.4) |
| 3   | 7.59, d (8.4) | 7.65, d (8.4) | 7.73, d (8.4) | 7.76, d (8.4) |
| 5   | 7.24, s     | 7.22, s     | 7.24, s     | 7.20, s     |
| 14  | 5.41, d (1.8) | 5.42, d (1.2) | 5.28, brs  | 5.28, brs  |
| 15  | 5.00, d (2.4) | 5.02, d (2.4) | 4.27, d (6.0) | 4.26, d (5.4) |
| 17  | 1.22, s     | 1.22, s     | 4.82, s     | 4.82, s     |
| 18  | 1.56, s     | 1.56, s     | 1.82, s     | 1.83, s     |
| 19  | 4.54, brd (10.8) | 4.43, brd (10.8) | 4.55, brd (10.8) | 4.41, brd (10.8) |
|     | 4.32, dd (10.8, 3.0) | 4.36, dd (10.8, 3.0) | 4.27, dd (10.8, 2.4) | 4.33, dd (10.8, 2.4) |
| 20  | 2.72, brs   | 2.73, brs   | 2.72, brs   | 2.73, d (2.4) |
| 22  | 4.80, s     | 4.87, s     | 4.85, s     | 4.83, s     |
| 24  | 1.88, s     | 1.84, s     | 1.89, s     | 1.86, s     |
| 25  | 2.36, s     | 2.37, s     | 2.35, s     | 2.35, s     |
|     | 6.88, brs   | 5.40, brs   | 6.90, brs   | 5.41, brs   |
| 1-OH | 12.97, brs | 12.74, brs | 13.00, brs | 12.70, brs |
| 14-OH/OCH3 | 3.34, s | 3.34, s | 2.76, brs | 2.94, brs |
| 15-OH/OAc | 1.92, s | 1.91, s | 2.52, brs | 2.59, brs |
| 25-OH/OAc | 2.10, s | 4.86, d (3.0) | 2.08, s | 4.99, d (4.2) |

Table 3. 1H NMR Data (δ) of 5–8 (600 MHz, δ in ppm, CDCl3, J in Hz).
(1–9) represent the family of naturally occurring xanthones with C-4 terpenoid-derived side chain\(^\text{25}\). In the previous literature, twenty prenylxanthones have been mainly obtained from the genus *Aspergillus*/*Emericella* fungi, such as 14-hydroxyltajixanthone hydrate\(^\text{1}\), emerixanthones A–D\(^\text{4}\), ruguloxanthones A–C\(^\text{27}\), and so on. The configurations of C-14 and C-15 in prenylxanthone derivatives were challenging to be assigned due to the high conformational flexibility of the terpenoid-derived side chains. It was un-accommodated to determine the relative configuration of C-14 and C-15 by comparison of the coupling constants (\(J_{14,15}\)) as the free rotation of the flexible side chain\(^\text{5}\). Also, it was problem to define the absolute configuration of C-15 by comparison of the optical rotatory\(^\text{27}\). This work demonstrated that using multiple chiroptical methods in combination with DFT calculations allowed one to determine absolute configurations with high confidence for chiral natural products, which possessed rotatable bonds.

Compounds 1–9 were subjected to test their cytotoxic activities by MTT method against human breast cancer (MDA-MB-231 and MCF-7), human gastric cancer (MGC-803), cervical cancer (HeLa), and human lung epithelial carcinoma (A-549) cell lines. Compound 1 displayed selective cytotoxicity against the A-549 cell line with the IC\(_{50}\) value of 1.8 \(\mu\)M. Compounds 3 and 6 showed broad-spectrum cytotoxicities against five tumor cell lines with the IC\(_{50}\) values ranging from 1.1 to 9.8 \(\mu\)M (Table 4). However, the other compounds (2, 4, 5, 7, 8, 9) exhibited very low cytotoxicity to any of the above cell lines (IC\(_{50}\) > 10.0 \(\mu\)M).

Compounds 1–9 were further tested their antibacterial activities against a panel of pathogenic bacteria, including *Micrococcus lysodeikticus*, *Bacillus anthraci*, *Salmonella typhi*, and *Enterobacter aerogenes*. Only 7 and 8 showed antibacterial activity against *M. lysodeikticus*, *B. anthraci*, *S. typhi*, and *E. aerogenes*, with MIC values of 0.78, 12.5, 6.13 and 6.13 \(\mu\)g/mL for 7, and 6.13, 12.5, 6.13 and 6.13 \(\mu\)g/mL for 8, respectively. These data indicated that the antibacterial activities may be due to the double bonds between C-16 and C-17 in 7 and 8. In addition, ciprofloxacin showed antibacterial activity against *M. lysodeikticus*, *B. anthraci*, *S. typhi*, and *E. aerogenes*, with MIC values of 0.19, 1.56, 3.13, 1.56 \(\mu\)g/mL, respectively.
In summary, three chiroptical methods, including ECD, ORD and VCD, combined with quantum theory calculation were carried out to elucidate the absolute configuration of the prenylxanthone 1, which was difficult to be determined by single method due to high flexibility of the molecule. The interesting chemical structures and
potent biological activities of these prenylxyanthones (1–9) may encourage further investigations on this cluster of metabolites for drug discovery.

**Methods**

**General Experimental Procedures.** Optical rotatory dispersions were acquired using a JASCO P-2000 spectrometer. Optical rotations were obtained on an Optical Activity AA-55 series polarimeter. UV data were performed using a Perkin-Elmer model 241 spectrophotometer in MeOH. Electronic circular dichroism spectra were measured using a JASCO J-715 circular dichroism spectrometer. IR spectra were determined using KBr pellets with a Nicolet NEXUS 470 spectrophotometer. Vibrational circular dichroism spectra were taken on a BioTools ChiralIR 2X spectrophotometer. 1D and 2D NMR data (600 MHz for 1H and 150 MHz for 13C) were acquired on Bruker Avance-III 600 MHz NMR spectrometer with TMS as an internal standard. High-resolution mass data were obtained from a Thermo Scientific LTQ Orbitrap XL spectrometer. HPLC analysis and semi-preparation was performed on a Shimadzu LC-20AT system with a SPD-M20A photodiode array detector, using a Waters RP-18 column at a flow rate of 2.0 mL/min (MeOH/ H2O, 85:15) at a wavelength of 200–300 nm (Qingdao Marine Chemical Factory), and Sephadex LH-20 (18–110 μm, Pharmacia Fine Chemical Co., Ltd., Sweden).

**Fungal Material.** The fungus *Aspergillus* sp. ZA-01 was obtained from sediment, collected from the Bohai Sea of Huanghuagang, Hebei Province of China, in June 2016. The strain was identified according to its 16S rRNA amplification and sequencing of the ITS region. The strain was deposited in College of Pharmaceutical Sciences, Key Laboratory of Medicinal Chemistry and Molecular Diagnostics of Education Ministry of China, Hebei University, Baoding, China.

**Extraction and Purification.** The fermentation of the fungus *Aspergillus* sp. ZA-01 was carried out using solid culture in eighty erlenmeyers (each erlenmeyer flask containing rice 100 g, water 100 mL, NaNO3 0.3 g, KH2PO4 0.1 g, MgSO4·7H2O 0.05 g, NaCl 0.05 g, FeSO4 0.01 g, sucrose 3.0 g, pH adjusted to 7.3) at room temperature. After 45 days, the fermented solid medium was repeatedly extracted with a CH2Cl2/MeOH (1:1) mixture to give the EtOAc extract (60.0 g). The EtOAc extract was then subjected to silica gel column chromatography (CC) [10 × 20 cm, stepwise gradient, petroleum ether (PE)-EtOAc to offer six fractions: Fr.1-Fr.6. Fr.2 (7.5 g) was applied to silica gel CC (PE:EtOAc 85:15) to give the EtOAc extract (60.0 g). The EtOAc extract was then subjected to silica gel column chromatography (CC) [10 × 20 cm, stepwise gradient, petroleum ether (PE)-EtOAc to offer six fractions: Fr.1-Fr.6. Fr.2 (7.5 g) was applied to silica gel CC (PE:EtOAc = 3:1), followed by Sephadex LH-20 (CH2Cl2/MeOH = 1:1) to obtain three subfractions: Fr.3-1-2, Fr.3-3. Fr.3-3 was further purified by preparative HPLC by a Waters RP-18 column at a flow rate of 2.0 mL/min (MeOH/ H2O, 85:15) to give 1 (15.0 mg), 2 (4.5 mg), 3 (5.0 mg), 4 (5.2 mg), 5 (5.0 mg), 6 (4.5 mg), 7 (5.0 mg), 8 (4.7 mg) and 9 (2.5 mg).

Aspergixanthone A (1): yellow, amorphous powder; [α]20 D = −97 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 231 (4.7), 241 (4.3), 265 (4.9), 286 (2.0), 383 (1.7) nm; CD (MeOH) (Δε) 219 (16.3), 241 (−9.4), 271 (−8.1), 289 (1.4), 319 (1.0), 332 (−3.2) nm; IR (KBr) νmax 3445, 2929, 2355, 1635, 1591, 1475, 1246, 1065, 895 cm−1; NMR data, see Tables 1 and 2; HRESIMS m/z 535.1935 [M + Na]+, (calcd. for C30H34O10Na, 535.1939).

Aspergixanthone B (2): yellow, amorphous powder; [α]20 D = −120 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 232 (4.6), 245 (4.3), 267 (4.8), 284 (2.0), 385 (1.8) nm; CD (MeOH) (Δε) 219 (3.3), 242 (−15.1), 267 (−10.5), 285 (0.2), 318 (−1.8), 330 (−6.6) nm; IR (KBr) νmax 3442, 2929, 2358, 1639, 1593, 1471, 1242, 1072, 894 cm−1; NMR data, see Tables 1 and 2; HRESIMS m/z 493.1839 [M + Na]+, (calcd. for C28H32O9Na, 493.1833).

Aspergixanthone C (3): yellow, amorphous powder; [α]20 D = −107 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 235 (4.7), 242 (4.5), 269 (4.9), 286 (1.9), 384 (1.7) nm; CD (MeOH) (Δε) 220 (16.9), 238 (−12.7), 271 (−12.7), 289 (0.8), 318 (0.6), 332 (−4.6) nm; IR (KBr) νmax 3419, 2966, 2585, 1737, 1733, 1473, 1238, 1020, 829 cm−1; NMR data, see Tables 1 and 2; HRESIMS m/z 521.1784 [M + Na]+, (calcd. for C26H29O9Na, 521.1782).

Aspergixanthone D (4): yellow, amorphous powder; [α]20 D = +39.0 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 231 (4.3), 249 (4.1), 262 (4.3), 288 (1.8), 389 (1.6) nm; CD (MeOH) (Δε) 217 (−3.7), 243 (−19.2), 270 (−9.2), 286 (0.1), 316 (−3.6), 332 (−7.9) nm; IR (KBr) νmax 3421, 2964, 2566, 1716, 1718, 1475, 1236, 1016, 835 cm−1; NMR data, see Tables 1 and 2; HRESIMS m/z 457.1857 [M + H]+, (calcd. for C25H28O8Na, 457.1857).

Aspergixanthone E (5): yellow, amorphous powder; [α]20 D = −92 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 230 (4.7), 245 (4.5), 266 (4.7), 285 (1.9), 384 (1.8) nm; CD (MeOH) (Δε) 221 (7.1), 240 (−18.5), 270 (−13.5), 286 (−1.0), 314 (−1.1), 331 (−1.8) nm; IR (KBr) νmax 3432, 2926, 2357, 1662, 1587, 1465, 1246, 1064, 847 cm−1; NMR data, see Tables 2 and 3; HRESIMS m/z 577.2048 [M + Na]+, (calcd. for C32H33O11Na, 577.2044).

Table 4. Cytotoxicity of compounds 1–9.
Aspergixanthone F (6): yellow, amorphous powder; [α]_D^20 = −73 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.7), 246 (4.5), 269 (4.7), 285 (1.8), 386 (1.8) nm; CD (MeOH) λ_{max} (Δε) 223 (−10.5), 240 (−33.1), 267 (−19.1), 280 (−2.1), 315 (−2.0), 331 (−4.1) nm; IR (KBr) ν_{max} 2344, 2929, 2354, 1665, 1587, 1466, 1232, 1069, 852 cm⁻¹; NMR data, see Tables 2 and 3; HRESIMS m/z 535.1939 [M + Na]^⁺, (calcd. for C_{25}H_{26}O_{7}Na, 535.1939).

Aspergixanthone G (7): yellow, amorphous powder; [α]_D^20 = −56 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 237 (4.6), 242 (4.4), 271 (4.6), 285 (1.8), 385 (1.8) nm; CD (MeOH) λ_{max} (Δε) 222 (13.1), 235 (−11.7), 271 (−6.2), 283 (2.9), 314 (0.1), 332 (−4.0) nm; IR (KBr) ν_{max} 3446, 2972, 2368, 1733, 1645, 1471, 1249, 1029, 831 cm⁻¹; NMR data, see Tables 2 and 3; HRESIMS m/z 503.1667 [M + Na]^⁺, (calcd. for C_{25}H_{26}O_{7}Na, 503.1676).

Aspergixanthone H (8): yellow, amorphous powder; [α]_D^20 = −113 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 234 (4.8), 246 (4.5), 271 (4.7), 286 (1.8), 389 (1.8) nm; CD (MeOH) λ_{max} (Δε) 222 (−3.1), 240 (−10.7), 275 (1.2), 283 (1.5), 316 (−2.6), 331 (−4.7) nm; IR (KBr) ν_{max} 3431, 2972, 2352, 1733, 1637, 1471, 1249, 1054, 817 cm⁻¹; NMR data, see Tables 2 and 3; HRESIMS m/z 461.1569 [M + Na]^⁺, (calcd. for C_{24}H_{25}O_{7}Na, 461.1571).

Computational section. The four possibly diastereomers of 1 [(14R, 15R, 20S, 25R)-1, (14S, 15S, 20S, 25R)-1 and (14S, 15S, 20S, 25R)-1] were constructed and used for conformational searchings using M06-2X/6-311+G(d,p) force field by the BARISTA software (CONPLEX Corporation). Totally 63 stable conformers for (14R, 15R, 20S, 25R)-1 with relative energy within a 10.0 kcal/mol energy window, 56 conformers for (14S, 15S, 20S, 25R)-1, 70 conformers for (14S, 15R, 20S, 25R)-1 and 75 conformers for (14S, 15S, 20S, 25R)-1 were recorded, respectively. These corresponding minimum geometries were optimized at the gas-phase B3LYP/6-31 G(d) level using Gaussian 09 package. The re-optimizations were performed at the gas-phase B3LYP/6-311 + G(d) level for four possibly diastereomers of 1 with relative energy lower than 4.6 kcal/mol (ten conformers for (14R, 15S, 20S, 25R)-1, two conformers for (14S, 15S, 20S, 25R)-1, three conformers for (14S, 15R, 20S, 25R)-1, and eight conformers for (14S, 15S, 20S, 25R)-1), respectively. Time-dependent density functional theory (TD-DFT) at the set of gas-phase B3LYP/6-311 + G(2d,p)//B3LYP/6-311 + G(d) level was used for ECD calculations with total of 60 excited states for the four diastereomers. ORD calculations were performed at the B3LYP/6-311 + G(2d,p)// B3LYP/6-311 + G(d) level. VCD predictions were carried out at the gas-phase B3LYP/6-311 + G(d)//B3LYP/6-311 + G(d) level. Boltzmann statistics were used for all simulations of ECD, ORD and VCD.

Snatzke’s method. The LCD spectrum of 1 by adding MoO₄(OAc)₂ was measured according to the referenced procedure.

Cytotoxicity Assays. The cytotoxicities against human breast cancer (MDA-MB-231 and MCF-7), human gastric cancer (MGC-803), cervical cancer (HeLa), and human lung epithelial carcinoma (A-549) cell lines were evaluated using the MTT method. Cisplatin was used as a positive control.

Antibacterial Assays. Antibacterial activity was evaluated by the conventional broth dilution assay. Four pathogenic bacterial strains, Micrococcus lysodeikticus, Bacillus anthraci, Salmonella typhi, and Enterobacter aerogenes were used, and ciprofloxacin was used as a positive control.

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Author Contributions
A.Z. and M.Y.Y. contributed to extraction, isolation, identification, and manuscript preparation. Y.H.Z. and L.D.H. contributed to bioactivities test. C.L.S. and C.Y.W. contributed to NMR and MS analysis. H.J.Z. contributed to VCD analysis. F.C. conceived of and proposed the idea.

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