Nine New and Five Known Polyketides Derived from a Deep Sea-Sourced Aspergillus sp. 16-02-1

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Received: 24 March 2014; in revised form: 12 April 2014 / Accepted: 16 April 2014 / Published: 27 May 2014

Abstract: Nine new C₉ polyketides, named aspiketolactonol (1), aspilactonols A–F (2–7), aspyronol (9) and epiaspinonediol (11), were isolated together with five known polyketides, (S)-2-(2′-hydroxyethyl)-4-methyl-γ-butyrolactone (8), dihydroaspyrone (10), aspinotriol A (12), aspinotriol B (13) and chaetoquadrin F (14), from the secondary metabolites of an Aspergillus sp. 16-02-1 that was isolated from a deep-sea sediment sample. Structures of the new compounds, including their absolute configurations, were determined by spectroscopic methods, especially the 2D NMR, circular dichroism (CD), Mo₂-induced CD and Mosher’s ¹H NMR analyses. Compound 8 was isolated from natural sources for the first time, and the possible biosynthetic pathways for 1–14 were also proposed and discussed. Compounds 1–14 inhibited human cancer cell lines, K562, HL-60, HeLa and BGC-823, to varying extents.
Keywords: Aspergillus sp. 16-02-1; fungal strain from deep sea sediment; aspiketolactonol; aspilactonol; aspyronol; lactone; epiaspinonediol; polyketide; structure; cytotoxicity

1. Introduction

In recent five years from 2008 to 2012, more than 5500 new compounds have been discovered from marine natural products [1–5]. Marine microorganisms, especially the marine fungi, are increasingly a major focus of marine natural product researches, providing a lot of structurally novel and bioactive compounds [1–10]. Fungi from marine environments have great potential to be a rich source of drug leads [6–10] and over 30 compounds derived from marine microbes are currently in preclinical studies or clinical trials [10,11]. Recently, microorganisms from deep-sea habitats, including the hydrothermal vents, have become an interesting and newly emerging source of novel bioactive compounds [3,8,9,12]. Although a handful of reports described new compounds from fungi derived from these habitats [9], relevant researches have attracted considerable, growing research interest [13–16].

During the ongoing search for new bioactive natural products from marine-sourced fungi, we have evaluated cytotoxicity and antifungal activities for 16 fungal strains from deep-sea habitats, and found that an Aspergillus sp. 16-02-1 produced metabolites with both cytotoxic and antifungal activities. The strain Aspergillus sp. 16-02-1 was isolated from a deep-sea sediment sample that was collected at a Lau Basin hydrothermal vent (depth 2255 m, temperature 114 °C) in southwest Pacific. We previously reported 8 known metabolites from this strain by a liquid fermentation [17]. In a continuation, we re-fermented this strain using solid-substrate fermentation medium and obtained nine new (1–7, 9 and 11) and five known (8, 10, and 12–14) polyketides shown in Figure 1. We report herein the isolation, structure elucidation, and cytotoxicity evaluation of these compounds in detail.

Figure 1. Structures of 1–14.
2. Results and Discussion

2.1. Fermentation, Isolation, and Identification of Known Compounds

The fermentation and extraction of *Aspergillus* sp. 16-02-1 gave an ethyl acetate (EtOAc) extract that inhibited K562 cells with an inhibition rate (IR%) of 76.8% at 100 μg/mL. The repeated column chromatography of the EtOAc extract coupled with HPLC purification afforded 14 metabolites 1–14 (Figure 1). Among them, structures of the nine new compounds 1–7, 9 and 11 were elucidated by modern spectroscopic methods, and named aspiketolactonol (1), aspilactonols A–F (2–7), aspyronol (9) and epiaspinonediol (11). The five known compounds 8, 10, and 12–14 were identified as (S)-2-(2′-hydroxyethyl)-4-methyl-γ-butyrolactone (8) [18], dihydroaspyrone (10) [19], aspinotriol A (12) [19], aspinotriol B (13) [19] and chaetoquadrin F (14) [20] by the physicochemical and spectroscopic data.

2.2. Structure Determination of New Compounds

Aspiketolactonol (1), colorless oil (from MeOH), [α]_D^25 +10.5 (c 0.12, MeOH), was assigned the molecular formula C_6H_{12}O_4 by HRESIMS (measured 185.0811 [M + H]^+; calculated for C_6H_{13}O_4 [M + H]^+ 185.0814). It showed end UV absorption, and the IR spectrum showed the absorptions due to OH (3417 cm\(^{-1}\)), CH_3/CH_2 (2980, 2938, 2906, 1422 and 1360 cm\(^{-1}\)), α,β-unsaturated γ-lactone (1752 and 1656 cm\(^{-1}\)) [21,22] and keto carbonyl (1722 cm\(^{-1}\)) groups. The olefinic proton and carbon signals at the lower field (δ_\(H\) 7.43 and δ_\(C\) 149.4) [22] of \(^{1}\)H and \(^{13}\)C NMR spectra (Tables 1 and 2) and the ester carbonyl carbon signal at δ_\(C\) 173.5 in the \(^{13}\)C NMR spectrum (Table 2) supported the presence of the α,β-unsaturated γ-lactone moiety. The carbonyl carbon signal at δ_\(C\) 203.6 in the \(^{13}\)C NMR spectrum further supported the presence of one keto carbonyl in 1. Interpretation of the \(^{1}\)H–\(^{1}\)H COSY, HMQC and HMBC data (Table S1 in the Supplementary Information) established the planar structure. The structural part related to the \(^{1}\)H spin system, C-7–C-4 (via quaternary sp\(^2\) C-3)–C-8, was deduced from the \(^{1}\)H–\(^{1}\)H COSY and HMQC data, including the allylic coupling between H-4/H_2-8, which was confirmed by the HMBC correlations of H-4, H-5 and H_2-8 with C-3. The acetyl group that consisted of the C-9 keto carbonyl and the C-10 methyl was linked to C-8 by the HMBCs on H_2-8/C-9, H_3-10/C-8 and H_3-10/C-9. The ester carbonyl carbon (C-2, δ_\(C\) 173.5) was linked to C-3 by the HMBC correlations of H-4 and H_2-8 with C-2. Then, C-2 was further linked to C-5 by an ester linkage to form the α,β-unsaturated γ-lactone ring according to the IR absorption at 1752 cm\(^{-1}\).

The stereochemistry of 1 was determined as follows. The coupling of H-5/H-6 (4.7 Hz) indicated the erythro relative configuration of 5,6-diol in 1. The couplings of the same protons are larger than 4 Hz in erythro isomers but smaller than 2 Hz in threo isomers in the α,β-unsaturated γ-lactones [23–25]. The CD of the α,β-unsaturated γ-lactone rings with a chiral γ-carbon shows Cotton effects associated with the π→π* transition in the region 200–235 nm [26] and the n→π* transition in the region 235–270 nm [26–29]. Generally the π→π* Cotton effect is decisive to assign the absolute configuration of the α,β-unsaturated γ-lactone ring because of the easy influence of the n→π* Cotton effect by external asymmetry [26,27]. Compound 1 showed positive π→π* Cotton effect at 233 nm (Figure 2), indicating the R absolute configuration at C-5 [26,27]. The absolute configuration at C-6 was thus assigned to be S according to the erythro relative configuration of 5,6-diol in 1.
Figure 2. Circular dichroism (CD) spectra of compounds 1–10 in MeOH.

Aspilactonol A (2), colorless oil (MeOH), \([\alpha]^\text{25}_\text{D} +24.6\) (c 0.23, MeOH), was assigned the molecular composition \(\text{C}_9\text{H}_{14}\text{O}_3\) by HRESIMS (measured 171.1015 \([\text{M} + \text{H}]^+\), calculated for \(\text{C}_9\text{H}_{15}\text{O}_3\) \([\text{M} + \text{H}]^+\) 171.1021). The IR spectrum showed absorptions ascribable to the OH, CH\(_3\)/CH\(_2\) and \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone groups (Experimental Section). Similar to 1, the olefinic proton and carbon signals at the lower field (\(\delta_H 7.04, H-4\) and \(\delta_C 149.8, C-4\)) \([22]\) of the \(^1\text{H}\) and \(^13\text{C}\) NMR spectra (Tables 1 and 2) together with the ester carbonyl (\(\delta_C 174.3, C-2\)) and the \(\text{sp}^2\) carbon (\(\delta_C 134.0, C-3\)) signals in the \(^13\text{C}\) NMR spectrum (Table 2) supported the presence of the \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone moiety in 2. Then, the planar structure was established by interpretation of the \(^1\text{H}–^1\text{H}\) COSY, HMQC and HMBC data (Table S2 in the Supplementary Information). The carbon backbone chain related to the \(^1\text{H}\) spin system, C-6-C-4 (via quaternary \(\text{sp}^3\) C-3)–C-7–C-10, could be derived from the \(^1\text{H}–^1\text{H}\) COSY and HMQC data (Table S2 in the Supplementary Information), including the allylic coupling between H-4/H\(_2\)-7, which was confirmed by the HMBC correlations of H-4, H-5, H\(_2\)-7 and H\(_2\)-8 with C-3. The carbonyl carbon C-2 (\(\delta_C 174.3\)) was linked to C-5 and C-3 by the HMBC correlations between H-5/C-2, H-4/C-2 and H\(_2\)-7/C-2 to form the \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone ring with the ester linkage between C-5 and C-2.
The absolute configuration at C-5 was assigned to be S by the positive \(\pi \rightarrow \pi^*\) Cotton effect at 225 nm and the negative \(\pi \rightarrow \pi^*\) Cotton effect around 239 nm (Figure 2) [26,27]. The absolute configuration at C-9 was assigned by the modified Mosher’s method [30,31]. Treatment of 2 with (S)-\(\alpha\)-methoxy-\(\alpha\)-trifluoromethyl phenylacetyl chloride [(S)-MTPA-Cl] and (R)-MTPA-Cl gave (S)-MTPA ester (2a) and (R)-MTPA ester (2b), respectively. The \(\Delta \delta\) (\(\delta_S - \delta_R\)) values of relevant proton signals from 2a and 2b established the S absolute configuration of C-9 in 2 (Figure 3).
Aspilactanol B (3), [α]_D^{25} +27.2 (c 0.32, MeOH), and aspilactanol C (4), [α]_D^{25} +34.5 (c 0.41, MeOH), colorless oils (MeOH), were assigned the molecular composition C_{10}H_{16}O_3 by HRESIMS (measured 203.0919 [M + H]^+ for 3 and 203.0910 [M + H]^+ for 4, calculated for C_{10}H_{16}O_3 [M + H]^+ 203.0919). Both they showed end UV absorptions, and similar to 1 and 2, their IR spectra revealed the presence of α,β-unsaturated γ-lactone moieties (around 1740 and 1652 cm\(^{-1}\)) [21,22]. This was supported by the lower field olefinic proton and carbon signals (around δ_H 7.55, H-4; around δ_C 150.4, C-4) [22] in the \(^1\)H and \(^{13}\)C NMR spectra (Tables 1 and 2) and the ester carbonyl (δ_C 174.5, C-2) and the sp\(^2\) carbon (around δ_C 137, C-3) signals in the \(^{13}\)C NMR spectra (Table 2). Their IR spectra also indicated the presence of OH and CH\(_2/\_CH\_2\) groups, and the strong OH signals around 3384 cm\(^{-1}\) further revealed more OH groups in 3 and 4 than in 1 and 2 (the IR spectra in the Supplementary Information). Interpretation of the \(^1\)H–\(^1\)H COSY and HMBC data (Tables S3 and S4 in the Supplementary Information) established the carbon backbone chain related to the proton spin system, C-7–C-4 (via quaternary sp\(^2\) C-3)–C-8–C-10. The allylic couplings between H-4 and H-8 in 3 and 4 indicated the connection of C-4 and C-8 via the quaternary sp\(^2\) carbon C-3, and the C-3 carbon was assigned by the HMBC correlations of H-4, H-5, H-8 and H-9 with C-3 (Tables S3 and S4 in the Supplementary Information). The C-2 carbonyl carbons in 3 and 4 were linked to C-5 and C-3 by the HMBC correlations between H-5/C-2, H-4/C-2 and H-8/C-2 to form the α,β-unsaturated γ-lactone rings with the ester linkage between C-5 and C-2.

The absolute configuration at C-5 in 3 and 4 was assigned both to be R by the positive π→π* Cotton effect around 226 nm in the CD of 3 and 4 (Figure 2) [26,27]. Because the coupling of H-5 and H-6 (4.6 Hz for 3 and 5.0 Hz for 4) indicated the erythro relative configuration of 5,6-diols in 3 and 4 [23–25], the absolute configuration at C-6 in 3 and 4 was also assigned both to be S. Thus, 3 and 4 should be stereoisomers at the vicinal diol methine carbons C-8/C-9. There are many reports recorded that the coupling of vicinal diol methine protons is generally larger than 6 Hz in threo isomers but smaller than 5 Hz in erythro isomers of the relevant compounds with a vicinal diol unit similar to the 8,9-diols in 3 and 4 [32–36]. The coupling of H-8 and H-9 (4.6 Hz for 3 and 4.9 Hz for 4) indicated the erythro relative configuration of the 8,9-diols in 3 and 4 [32–36]. The absolute configuration of the erythro-8,9-diols in 3 and 4 was determined by the dimolybdenum induced CD (ICD) analysis. In the ICDs by the Snatzke’s method using dimolybdenum tetraacetate (Mo\(_2\)(OAc)\(_4\)) in DMSO [37,38], the Mo\(_2\)-complex of 3 gave negative CD bands II (near 400 nm) and IV (around 329 nm), while the Mo\(_2\)-comlex of 4 gave the positive bands II and IV (Figure 4). By the Snatzke’s helicity rule, the sign of O–C–C–O torsional angle in the favored conformation of the chiral Mo\(_2\)-complexes determines the
sign of the bands II and IV [37,38]. We have demonstrated that in the erythro-diols closely resembled 3 and 4, the conformation with an antiperiplanar orientation of the OH and methyl groups, O–C–C–CH3, is favored conformation of the Mo2-complexes [39], as shown for 3 and 4 in Figure 4. Therefore, the absolute configuration at C-8 and C-9 in 3 and 4 could be assigned to be 8S,9R for 3 and 8R,9S for 4 on the basis of their band II and IV signs (Figure 4), respectively.

Figure 4. Induced CD (ICD) spectra from the Mo2-complexes of 3 and 4 in DMSO.

Aspilactonol D (5), colorless oil (MeOH), [α]D25° = -1.7 (c 0.38, MeOH), was assigned the molecular formula C10H16O5 by HRESIMS (measured 217.1071 [M + H]+, calculated for C10H17O5 [M + H]+ 217.1076), which had a CH2 composition more than in 3 and 4. It showed UV and IR absorptions similar to 3 and 4, and the IR absorptions revealed the presence of OH, CH3/CH2 and α,β-unsaturated γ-lactone groups (Experimental Section). The 1H and 13C NMR spectra of 5 in CD3OD showed signals similar to 3 and 4 except additional signals ascribable to an O-methyl group were detected (Tables 1 and 2). These NMR data indicated that 5 was an O-methylated. Analyses of the 1H–1H COSY, HMQC and HMBC spectra (Table S5 in the Supplementary Information) established its planar structure. The α,β-unsaturated γ-lactone ring was confirmed by the HMBC correlations between H-5/C-2, H-4/C-2 and H-8/C-2. The O-methyl group was located at C-8 by the HMBC correlations of the O-methyl protons with C-8. The absolute configuration at C-5 in 5 was assigned to be R by the positive π→π* Cotton effect at 231.5 nm in the CD spectrum (Figure 2) [26,27]. The coupling of H-5/H-6 (4.5 Hz) indicated the erythro relative configuration of 5,6-diol in 5 [23–25], and thus the absolute configuration at C-6 was assigned to be S. The R absolute configuration of C-9 was determined by the modified Mosher’s method [30,31] on the basis of the Δδ (δS–δR) values from the (S)- and (R)-MTRA esters (Figure 3). Since the coupling of H-8/H-9 (4.7 Hz) indicated the erythro relative configuration of 8,9-diol in 5 [32–36], the absolute configuration at C-8 was assigned to be S.

Aspilactonols E/F (6/7) were obtained as a mixture of epimers as a colorless oil from MeOH, [α]D25° +78.1 (c 1.00, MeOH), and the molecular formula C9H14O4 was determined by HRESIMS (measured 187.0967 [M + H]+, calculated for C9H15O5 [M + H]+ 187.0970). In the UV spectrum, 6/7 showed the end absorption, and the IR spectrum showed absorptions due to the OH, CH3/CH2 and α,β-unsaturated γ-lactone groups (Experimental Section). The 1H and 13C NMR spectra of 6/7 in CD3OD showed signals similar to 3–5, but they were characterized by the appearance of methylene signals instead of the signals from an oxygenated methine in 3–5 and an O-methyl group in 5 (Tables 1
and 2). These NMR data indicated the same skeletal structures in 3–7. The appearance of the proton H-4 and the carbon signals except for the C-10 signal as pairs in an approximate 1:1 ratio indicated that 6/7 was a 1:1 mixture of stereoisomers. The planar structure of 6/7 was deduced by the $^1$H–$^1$H COSY, HMQC and HMBC data (Table S6 in the Supplementary Information), coupled with the IR absorptions at 1748 and 1651 cm$^{-1}$ from an α,β-unsaturated γ-lactone ring [21,22]. The absolute configuration at C-5 in 6/7 was assigned both to be R by the strong positive π→π$^*$ Cotton effect at 228.5 nm in the CD spectrum (Figure 2) [26,27]. Since the coupling of H-5/H-6 (4.4 Hz) indicated the erythro relative configuration of 5,6-diols in 6/7 [23–25], the absolute configuration at C-6 was assigned to be S for both 6/7. Thus, 6/7 was a 1:1 mixture of epimers at C-9. Although a Doctor’s Thesis has recorded the same planar structure of 6/7, its stereochemistry was not elucidated [40]. We therefore named 6/7 as asplactonols E/F as new compounds.

Aspyronol (9), colorless oil from MeOH, [α]$^D_{25}$ =–41.6 (c 0.22, MeOH), was assigned the molecular formula C$_{10}$H$_{16}$O$_5$ by HRESIMS (measured 217.1073 [M + H]$^+$, calculated for C$_{10}$H$_{17}$O$_5$ [M + H]$^+$ 217.1076). It showed end UV absorption and the IR absorptions due to OH (3398 cm$^{-1}$), CH$_3$/CH$_2$ (2982, 2938, 2905, 2835, 1451 and 1384 cm$^{-1}$), C=O (1714 cm$^{-1}$) and C=C (1649 cm$^{-1}$) groups. The $^1$H and $^{13}$C NMR spectra (Table 3) resembled those of dihydroaspyrone (10) except additional signals from a methoxy and an oxygenated methine groups were detected instead of the methylene signals in 10. There were also slight changes in several $^1$H and $^{13}$C signals. These NMR data suggested that 9 was a methoxylated derivative of 10, and this was confirmed by analysis of the $^1$H–$^1$H COSY, HMQC and HMBC spectra (Table S7 in the Supplementary Information) to complete the planar structure. The carbon chain related to the proton spin system, C-7–C-4(via quaternary sp$^2$ C-3)–C-8–C-10, was deduced by interpretation of the $^1$H–$^1$H COSY and HMQC data (Table S7 in the Supplementary Information). The allylic coupling between H-4 and H-8 suggested the connectivity of C-4 and C-8 via a quaternary sp$^2$ carbon C-3, and C-3 was assigned by the HMBC correlations between H-8/C-3 and H-9/C-3. The OCH$_3$ group was located at C-8 by the HMBCs of the methoxy protons with the carbon C-8. The carbonyl carbon C-2 was linked to C-3 by the HMBCs on H-4/C-2 and H-8/C-2. The ester linkage of the C-2 carbonyl was then linked to C-6 to form a δ-lactone ring by the coupling of H-5/H-6 (9.4 Hz), which requires C-5/C-6 fixed in a six-membered ring with trans orientated H-5/H-6. This was supported by the chemical shift of C-6, δC 80.0 in 9 and δC 79.6 in 10. The R absolute configuration at C-9 was determined by the modified Mosher’s method [30,31] on the basis of the Δδ (δ$_S$–δ$_R$) values from the (S)- and (R)-MTRA esters of 9 (Figure 3), and the absolute configuration at C-8 was assigned to be S because the coupling of H-8/H-9 (4.6 Hz) indicated the erythro relative configuration of 8,9-diol in 9 [32–36]. The absolute configuration at C-5 and C-6 in 9 was determined to be S,S,6R, the same as 10, according to the negative signs of the Cotton effects around 260 nm both from the chiral α,β-unsaturated δ-lactone units in 9 and 10 (Figure 2) [28]. This was also supported by the co-generation of 9 and 10 by the same Aspergillus sp. 16-02-1 strain from a biogenetic consideration.
Epiospinonediol (11), yellow oil (MeOH), $[\alpha]_{D}^{25} = -5.4$ (c 0.14, MeOH), was assigned the molecular formula $C_{9}H_{14}O_{3}$ by HRESIMS (measured 193.0842 [M + Na]$^{+}$, calculated for $C_{9}H_{14}O_{3}Na$ [M + Na]$^{+}$ 193.0841). The $^1$H and $^{13}$C NMR data of 11 in CD$_3$OD were identical with those of spinonediol in CD$_3$OD [19], indicating the same planar structures of both compounds. Aspinonediol had the absolute configuration $7'S$ and showed $[\alpha]_{D}^{25} +2.4$ (c 0.63, MeOH) [19]. In contrast, 11 showed the opposite optical rotation. Thus, the epiospinonediol (11) was determined to be the epimer of spinonediol at C-7 with the 7R absolute configuration.

2.3. Inhibitory Effects of 1–14 on Several Human Cancer Cell Lines

Antitumor activities of 1–14 were tested by the MTT method using the human cancer K562, HL-60, HeLa, and BGC-823 cell lines. Compounds 1–14 inhibited some of the tested four cell lines and the IR% values at 100 μg/mL are given below. 1: 11.7% (HL-60); 2: 17.7% (HL-60); 3: 22.2% (HL-60); 4: 20.0% (K562), 16.7% (HL-60); 5: 20.0% (HL-60), 13.2% (BGC-823); 6/7: 14.1% (K562), 13.4% (HeLa); 8: 14.2% (HL-60), 15.7% (HeLa); 9: 27.9% (K562), 67.2% (HL-60), 14.0% (HeLa); 10: 19.9% (HL-60), 10.1% (HeLa); 11: 79.7% (K562); 72.5% (HL-60), 14.9% (HeLa); 21.8 (BGC-823); 12: 17.0% (K562); 14.1% (HeLa); 13: 20.3% (K562), 39.4% (HL-60), 12.3% (HeLa), 15.7% (BGC-823); 14: 13.5% (HeLa). The half-inhibitory concentration (IC$_{50}$) of 9 on HL-60 cells was determined to be 52.1 μg/mL (241.2 μM), and the IC$_{50}$ for 11 on K562 and HL-60 cells to be 44.3 μg/mL (260.6 μM) and 32.8 μg/mL (192.9 μM), respectively. The positive control docetaxol inhibited these cell lines with the IR% values of 55.6% (K562), 49.9% (HL-60), 45.1% (HeLa), and 61.5% (BGC-823) at 100 μg/mL.

2.4. Discussions

Chemical investigation of a deep sea-sourced Aspergillus sp. 16-02-1 has resulted in the elucidation of 14 secondary metabolites 1–14, including nine new (1–7, 9 and 11) and five known (8, 10, and 12–14) compounds, shown in Figure 1. Although compound 8 has been chemically prepared [18], it is the first time to report 8 from natural sources in present study. Structures of the new compounds, including

| NO. | in CD$_3$OD $\delta_{c}$ (J in Hz) | in CDCl$_3$ $\delta_{c}$ (J in Hz) | NOE |
|-----|----------------------------------|----------------------------------|-----|
| 2   | 165.7 s                          | -                                | -   |
| 3   | 129.9 s                          | -                                | -   |
| 4   | 147.8 d                          | 6.78 t (1.4)                     | 6.79 dd (2.6, 0.9) | H-7 |
| 5   | 68.7 d                           | 4.21 dd (9.4,1.4)                | 4.25–4.20 m (masked by H-8) |
| 6   | 80.0 d                           | 4.24 dq (9.4, 5.8)               | 4.35 dq (8.5, 6.4) |
| 7   | 18.20 q                          | 1.44 3H d (5.8)                  | 1.48 3H d (6.4) | H-5 |
| 8   | 83.5 d                           | 4.08 dd (4.9, 1.4)               | 4.22 br d (4.6) |
| 9   | 70.1 d                           | 3.82 qd (6.4,4.9)                | 3.94 qd (6.5,4.6) |
| 10  | 18.17 q                          | 1.12 3H d (6.4)                  | 1.10 3H d (6.5) |
| OCH$_3$ | 58.0 q                         | 3.32 3H s                        | 3.31 3H s |

* Chemical shifts were recorded in δ values using the solvent signals (CDCl$_3$: $\delta_{H}$ 7.26; CD$_3$OD: $\delta_{C}$ 3.31/$\delta_{C}$ 49.00) as references, respectively. Signals were assigned on the basis of $^1$H-$^1$H COSY, HMQC and HMBC experiments.

Table 3. 400 MHz $^1$H and 100 MHz $^{13}$C NMR data of 9.
their absolute configurations, were determined by extensive spectroscopic methods, especially the 2D NMR, CD, ICD and Mosher’s $^1$H NMR analyses. The determination of the absolute configuration of α,β-unsaturated γ-lactone ring in 1–7 mainly relied on the CD data. In most cases, a chiral α,β-unsaturated γ-lactone ring gave both π→π* and n→π* Cotton effects with the opposite sign [26,27] in 200–235 nm and 235–270 nm regions, respectively [26–29]. However, usually the n→π* Cotton effect is weak and sometimes could not be observed [26,27] or even appeared with the same sign of the π→π* Cotton effect [26]. The same is true of the case of 1–8. As shown in Figure 2, 2 and 8 gave both opposite n→π* and π→π* Cotton effects in similar magnitude and 4 also showed a weak n→π* Cotton effect opposite to the π→π* transition. However, the others did not give opposite n→π* Cotton effect and rather they showed a weak CD curve with the same sign of the π→π* Cotton effect in the n→π* transition region (Figure 2). Since the easy influence of the n→π* Cotton effect by the external asymmetry [26] and the decisive role of the π→π* Cotton effect in absolute configuration assignment has been known [26,27], the absolute configurations of the α,β-unsaturated γ-lactone rings in 1–7 could be assigned by the sign of their π→π* Cotton effects shown in Figure 2.

Compounds 1–14 are all polyketides derived from a branched C$_9$ (1–7 and 9–14) or C$_7$ (8) carbon skeleton, and are derivatives of α,β-unsaturated γ-lactone (1–8), α,β-unsaturated δ-lactone (9–10 and 14) or branched, acyclic linear C$_8$ carbon backbone chain with a conjugated diene group (11–13). The biosynthesis of aspyrone [41–46], asperlactone and isoasperlactone [44–46], and aspinonene [47], their structures were closely related to the compounds, 9–10/14, 1–8, and 11–13, respectively, have been exhaustively studied [41–47], and as indicated in Scheme 1 [46,47], it has been demonstrated that these metabolites derive from a common biosynthetic precursor, 1–4, which originates from the intermediate 1, the ultimate product of polyketide synthesis (PKS), by post-PKS modifications (see in Scheme 1). Reduction of the aldehyde in 1–4 into the primary alcohol give 5–5, which undergo further modification to produce aspinonene [47], while oxidation of the aldehyde in 1–4 into the carboxyl afford 1–7, which switches the formation of aspyrone and asperlactone by nucleophilic attack of the carboxyl group on either site of the carbons in one of the two epoxide groups [44–47].

Relating to the above mentioned metabolites, plausible biosynthetic pathways for 1–7 and 9–13 are proposed in Scheme 1. Reduction of either one of the two epoxides in 1–5, the precursor of aspinonene [47], coupled with hydration at either site of the other epoxide ring followed by dehydration, would give 1–6 and compounds 12 and 13, which further underwent oxidation at C-2 to produce compound 11 and aspinonediol, the epimer of 11 at C-7 (Scheme 1). Aspyrone and asperlactone are proposed to be intermediates for 9–10 and 1/3–7. Reduction of the epoxide in aspyrone would give 10, and hydration at C-8 of the epoxide, followed by methylation, would afford 9 (Scheme 1). Reduction or hydration of the epoxide in asperlactone and further modification of the products by methylation, reduction or dehydration followed by keto-enol tautomerization, and oxidation/reduction reactions would produce 1 and 3–7, as shown in Scheme 1. The γ-lactone 2 seems likely to be produced from the intermediate 1–7. Reduction of the epoxides in 1–7 coupled with double bond rearrangement, followed by lactonization of the product 1–8, would give 2 (Scheme 1).
In view of the structural features of 1, 3–4 and 6–7, one of these compounds may probably form from some or other during extraction with EtOAc at the slightly acidic conditions by the acid-catalyzed chemical reactions artificially. In order to confirm whether this occurred in truth, each 0.3 mg of the crude compound samples was dissolved in 0.2 mL water-saturated EtOAc in a 0.5 mL Eppendorf tube, capped the tube and kept at room temperature for 6 days, and then further treated at 50 °C for 16 h. These treatment conditions simulated the extraction conditions (whole extraction was achieved within 4 days with a total of 12 h evaporating times at the temperature lower than 40 °C). Then, the aqueous EtOAc was removed by blowing inside of the tube with nitrogen gas to dryness. The residue was dissolved in MeOH and then subjected to HPLC analysis. No any one of them was detected in other compound samples by the HPLC analysis (Figure S1 in the Supplementary Information), confirming that none of these compounds are artificial product formed from the others in the extraction conditions.

Differing from 1–7/9–13, they are all pentaketide derivatives as shown in Scheme 1, compound 8 seems likely to be a tetraketide derivative. A plausible pathway for the 8 biosynthesis is proposed in Scheme 2. A first intermediate in this pathway was proposed to be II, the ultimate tetraketide product of PKS, like the intermediate I in Scheme 1. Similar to the 1–7/9–13 biosynthesis from I as shown in Scheme 1, post-PKS modifications of II would give a key intermediate II-4, which underwent further modification would produce 8.
Compound 14 is also a pentaketide derivative that seems likely to be derived from the intermediate I shown for 1–7 and 9–13 in Scheme 1. A plausible biosynthetic pathway for 14 is proposed in Scheme 3. Similar to the biosynthesis of 1–7/9–13 (Scheme 1), epoxidation of the double bond adjacent to the methyl group in I, followed by rearrangement, dehydration and decarboxylation reactions, would give a key intermediate III-2, which underwent further modification would produce 14 (Scheme 3).

In present MTT assay, 9 and 11 showed stronger inhibitory effect than the others on human cancer K562 and/or HL-60 cell lines. Compound 9 inhibited the HL-60 cells with the IR% value of 67.2% at 100 μg/mL, while 11 inhibited the K562 and HL-60 cells with the IR% values of 79.7% and 72.5% at 100 μg/mL, respectively. Both compounds also inhibited to some extents the K562 (9: an IR% of 27.9% at 100 μg/mL) and the BGC-823 (11: an IR% of 21.8% at 100 μg/mL) cells. These data suggested that the new compounds 9 and 11 showed somewhat selective inhibitory effect on the HL-60 cells and the K562 and HL-60 cells, respectively, although the inhibitory effect of both compounds by the IC₅₀ of 9 (52.1 μg/mL or 241.2 μM on HL-60) and 11 (44.3 μg/mL or 260.6 μM on K562 and 32.8 μg/mL or 192.9 μM on HL-60) is not so exciting. On the other hand, except three other new compounds 3–4 weakly inhibited the K562 and HL-60 cells with the IR% values larger than 20% at 100 μg/mL, all the others showed very weak effect on some of the tested four cancer cell lines with the IR% values lower than 20% at 100 μg/mL, as shown in Section 2.3. Aspyrone and asperlactone have been identified for some biological activities, including the remarkable insect growth regulator activity of asperlactone against Tribolium castaneum and Nezara viridula [48], the nematicidal activity of aspyrone on Pratylenchus penetrans [49], and the antifungal and antibacterial activities of both.
compounds on several fungal and bacterial strains [50]. Antibacterial activities were also reported for several chlorine containing derivatives from aspyrone and asperlactone by opening of the epoxy ring therein [22,51]. Although aspinonene and mono (S)- and (R)-MTRA esters of dihydroaspyrone (10) at C-9 have been reported to show a low cytotoxicity on mouse lymphocytic leukemia cells [19], there are few of reports recorded the antitumor activities for the compounds structurally closely related to 1–14. The present bioassay results, as well as the report in the literature [19], seem to suggest that the branched C9 polyketides structurally related to 1–14 were likely worthy for further extended studies to obtain antitumor agents with more strong activity and higher selectivity.

3. Experimental Section

3.1. General Experimental

Melting point was measured on a Beijing Tiandiyu X-4 exact micro melting point apparatus (Tiandiyu science and technology Co., Ltd., Beijing, China) and the temperatures were not corrected. Optical rotations were measured on an Optical Activity Limited poLAar 3005 spectropolarimeter (Optical Activity Limited, Ramsey, UK). ESIMS was recorded on an Applied Biosystems API 3000 LC-MS spectrometer (AB SCIX, Framingham, MA, USA) and HRESIMS was measured on an Agilent 6520 Q-TOF LC-MS spectrometer (Agilent Technologies, Santa Clara, CA, USA). IR spectra were taken on a Bruker Tensor-27 infrared spectrophotometer (Bruker, Karlsruhe, Germany). CD and ICD data were recorded on a Biologic Science MOS450 CD spectropolarimeter (Bio-Logic, Pont-de-Clai, France). 1D and 2D NMR spectra were obtained on a JEOL JNM-GX 400 (400 MHz 1H and 100 MHz 13C NMR) NMR spectrometer (JEOL Ltd., Tokyo, Japan). The chemical shifts of 1H and 13C NMR signals were recorded in δ values using the solvent signals (CDCl3: δH 7.26/δC 77.1; CD3OD: δH 3.31/δC 49.0) as references, respectively.

Precoated silica gel GF254 plates (10 cm × 20 cm, 0.25 mm thickness, Yantai Chemical Industrial Institute, Yantai, China) were used in TLC, and spots were detected under UV lights (254 and 365 nm) or by using the 10% sulfuric acid reagent or Vaughan’s reagent [39,52]. Silica gel H (100–200 mesh, Yantai Chemical Industrial Institute, Yantai, China), YMC*GEL® ODS-A-HG (12 nm S-50 μm, YMC Co., Ltd., Kyoto, Japan), and Sephadex™ LH-20 (GE Healthcare, Uppsala, Sweden) were used for column chromatography. HPLC was performed on a Waters HPLC systems equipped with Waters 600 controller, Waters 600 pump, Waters 2414 refractive index detector, Waters 2996 (for analytical HPLC) or 2998 (for preparative HPLC) photodiode array detector, and Waters Empower™ software. The Capcell Pak C18 columns (UG120 Å, 4.6 × 250 mm and 20 × 250 mm; Shiseido Co., Ltd., Tokyo, Japan) were used in analytical and preparative HPLC, respectively.

Human chronic myelogenous leukemia K562 cell line was provided by Prof. Dr. Lili Wang (Beijing Institute of Pharmacology and Toxicology, Beijing, China). Human acute promyelocytic leukemia HL-60, human cervical cancer HeLa, and Human gastric adenocarcinoma BGC-823 cell lines were provided by Prof. Dr. Wenzia Zhou (Beijing Institute of Pharmacology and Toxicology). Fetal bovine serum was purchased from Tianjin Hao Yang Biological manufacture Co., Ltd. (Tianjin, China). The RPMI-1640 medium was purchased from Gibco (lot No. 1403238) and MTT from Amresco (lot No. 0793). Streptomycin (lot No. 071104) and penicillin (lot No. X1103302) were purchased from
North China Pharmaceutical Group Corporation, China. Docetaxol (DOC, lot No. 20110326) was purchased from Beijing Chimivo Technology Co., Ltd. (Beijing, China).

3.2. Producing Strain, Fermentation and Extraction

The producing fungal strain 16-02-1 was isolated from a deep-sea sediment, DY19-4-TVCG11, which was collected at a Lau Basin hydrothermal vent (depth 2255 m, temperature 114 °C) in the southwest Pacific (south latitude 20.9280°, west longitude 176.2401°) during round-the-world ocean research of Dayangyihao in May 2007. This strain was identified as a species of the genus Aspergillus by sequence analysis of the ITS region of the rDNA and by morphological characteristics.

For fermentation of the 16-02-1 strain, a spore suspension was prepared using fresh spores by the method that we have previously reported [39, 52] at first. Then, the fermentation was carried out in sixteen of 500 mL Erlenmeyer flasks, each containing 80 g of rice. Distilled water (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 121 °C for 30 min. After cooling to room temperature, each flask was inoculated with 200 μL of the 16-02-1 spore suspension and incubated at 28 °C for 36 days. The fermented material was extracted repeatedly with EtOAc (3 × 6 L), and the organic solvent was evaporated under reduced pressure to obtain an EtOAc extract (21.9 g).

3.3. Isolation of 1–14

The EtOAc extract (21.9 g) was subjected to silica gel column (silica gel 140 g, bed 6.0 × 30 cm) chromatography by stepwise elution with b.p. 60–90 °C petroleum ether (P)–dichloromethane (D)–MeOH (M) to obtain three fractions: Fr-4 (5.7 g, eluted by DM 30:1, IR% 55.9% at 100 μg/mL on K562 cells: the following IR% value in each bracket all indicate the IR% of the fraction at 100 μg/mL on K562 cells), Fr-5 (5.4 g, eluted by DM 20:1, IR% 80.2%) and Fr-6 (2.5 g, eluted by DM 9:1, IR% 78.6%).

Fr-4 (5.7 g) was separated by Sephadex LH-20 column (bed 1.5 × 135 cm) chromatography using DM 1:1 as eluting solvent to obtain three fractions Fr-4-1–Fr-4-3. Fr-4-1 (2.2 g, IR% 75.4%) was subjected to ODS column (bed 1.2 × 10 cm) and eluted with MeOH–H₂O (20:80→90:10) to afford three fractions Fr-4-1-1–Fr-4-1-3. Fr-4-1-2 was then subjected to preparative HPLC (Capcell Pak C18, 20 mm × 250 mm, room temperature; 20% MeOH at initial time 0 min→100% MeOH at 20 min→100% MeOH at 50 min; flow rate, 10 mL/min; detecting wave length 210 nm) to obtain 2 (6.5 mg, tᵣ = 14.1 min) and 8 (13 mg, tᵣ = 9.9 min). Fr-4-2 (2.7 g, IR% 42.3%) was subjected to ODS column (bed 1.2 × 10 cm) chromatography and stepwise elution by MeOH–H₂O (20:80→90:10) afforded three fractions Fr-4-2-1–Fr-4-2-3. A preparative HPLC separation (Capcell Pak C18 column, 20 mm × 250 mm, at the room temperature; 20% MeOH at initial time 0 min→100% MeOH at 30 min; flow rate, 10 mL/min; detecting wave length 210 nm) of Fr-4-2-3 (1.8 g, IR% 40.1%) gave 1 (9 mg, tᵣ = 9.2 min) and five fractions Fr-4-2-3-1–Fr-4-2-3-5. Separation of Fr-4-2-3-3 (28 mg) by preparative HPLC at the same condition except for the mobile phase MeOH–H₂O 95:5 gave 5 (6.5 mg, tᵣ = 35.5 min), and separation of Fr-4-2-3-4 (28 mg) by preparative HPLC at the same condition using the mobile phase MeOH–H₂O 94:6 afforded 9 (7 mg, tᵣ = 33.3 min).
Fr-5 (5.4 g) was subjected Sephadex LH-20 column (bed 1.5 x 135 cm) chromatography eluted with DM 1:1 to separate into three fractions Fr-5-1–Fr-5-3. Fr-5-2 (1.2 g, IR% 40.1%) was subjected to preparative HPLC (Capcell Pak C18 column, 20 mm x 250 mm, room temperature; 20% MeOH at initial time 0 min→100% MeOH at 50 min; flow rate, 10 mL/min; detecting wave length 210 nm) to obtain 11 (12 mg, *t*<sub>r</sub> = 13.2 min) and 10 (141 mg, *t*<sub>r</sub> = 11.8 min).

Fr-6 (2.5 g) was subjected to ODS column (bed 1.2 x 10 cm) chromatography eluted with MeOH–H₂O (20:80→90:10) to give three fractions Fr-6-1–Fr-6-3. Fr-6-2 (1.7 g, IR% 62.5%) was separated by preparative HPLC (Capcell Pak C18 column, 20 mm x 250 mm, room temperature; 10% MeOH at initial time 0 min→100% MeOH at 50 min; flow rate, 10 mL/min; detecting wave length 210 nm) to obtain 6/7 (19 mg, *t*<sub>r</sub> = 13.6 min), 13 (19 mg, *t*<sub>r</sub> = 15.8 min) and a fraction (29 mg, *t*<sub>r</sub> = 6 min) containing 3 and 4. This fraction (29 mg) was further separated by preparative HPLC at the same condition except for the mobile phase MeOH–H₂O 90:10 to obtain 3 (9 mg, *t*<sub>r</sub> = 13.6 min) and 4 (7 mg, *t*<sub>r</sub> = 16.0 min). Fr-6-3 (872 mg, IR% 30.1%) was separated by preparative HPLC at the same conditions as described for Fr-6-2 to obtain 12 (21 mg, *t*<sub>r</sub> = 18.8 min) and 14 (31 mg, *t*<sub>r</sub> = 23.7 min).

### 3.4. Physicochemical and Spectroscopic Data of 1–14

Aspiketolactonol (1): Colorless oil (MeOH), [α]<sub>D</sub>+10.5 (c 1.2, MeOH). Positive ion ESIMS *m/z*: 185 [M + H]<sup>+</sup>, 207 [M + Na]<sup>+</sup>; Negative ion ESIMS *m/z*: 183 [M – H]<sup>−</sup>. Positive ion HRESIMS *m/z*: measured 185.0811 [M + H]<sup>+</sup>, calculated for C₉H₁₃O₄ [M + H]<sup>+</sup> 185.0814; measured 207.0631 [M + Na]<sup>+</sup>, calculated for C₉H₁₃O₄Na [M + Na]<sup>+</sup> 207.0633. UV *λ*<sub>max</sub> nm in MeOH: 213 nm. IR ν<sub>max</sub> cm<sup>−1</sup> (Diamond ATR crystal): 3417, 3090, 2980, 2938, 2906, 1752, 1722, 1656, 1422, 1360, 1323, 1207, 1166, 1138, 1081, 1024, 982, 935, 896. CD Δε (nm): 0 (192.5), −0.44 (198.0), −0.30 (207.5), −0.32 (209.5), −0.21 (215.0), −0.23 (216.5), 0 (226.0), +0.34 (233.0), +0.07 (244.5), 0 (282). <sup>1</sup>H and <sup>13</sup>C NMR data: Tables 1 and 2.

Aspilactonol A (2): Colorless oil (MeOH), [α]<sub>D</sub>+24.6 (c 0.23, MeOH). Positive ion ESIMS *m/z*: 171 [M + H]<sup>+</sup>, 193 [M + Na]<sup>+</sup>. Positive ion HRESIMS *m/z*: measured 171.1015 [M + H]<sup>+</sup>, calculated for C₉H₁₃O₃ [M + H]<sup>+</sup> 171.1021; measured 193.0836 [M + Na]<sup>+</sup>, calculated for C₉H₁₄O₃Na [M + Na]<sup>+</sup> 193.0841; measured 209.0573 [M + K]<sup>+</sup>, calculated for C₉H₁₄O₃K [M + K]<sup>+</sup> 209.0580. UV *λ*<sub>max</sub> nm in MeOH: 210 nm. IR ν<sub>max</sub> cm<sup>−1</sup> (Diamond ATR crystal): 3436, 3080, 2971, 2932, 1746, 1653, 1449, 1406, 1374, 1320, 1201, 1030, 960, 870, 786. CD Δε (nm): 0 (191.0), −0.49 (198.0), −0.30 (206.0), −0.33 (208.5), −0.25 (214.0), −0.26 (215.5), 0 (219.5), +0.70 (225.0), 0 (229.5), −0.60 (237.5), −0.59 (240.5), 0 (279.5). <sup>1</sup>H and <sup>13</sup>C NMR data: Tables 1 and 2.

Aspilactonol B (3): Colorless oil (MeOH), [α]<sub>D</sub>+34.5 (c 0.41, MeOH). Positive ion ESIMS *m/z*: 203 [M + H]<sup>+</sup>, 225 [M + Na]<sup>+</sup>; Negative ion ESIMS *m/z*: 201 [M – H]<sup>−</sup>. Positive ion HRESIMS *m/z*: measured 203.0919 [M + H]<sup>+</sup>, calculated for C₉H₁₅O₃ [M + H]<sup>+</sup> 203.0919; measured 225.0736 [M + Na]<sup>+</sup>, calculated for C₉H₁₄O₃Na [M + Na]<sup>+</sup> 225.0739. UV *λ*<sub>max</sub> nm in MeOH: 214 nm. IR ν<sub>max</sub> cm<sup>−1</sup> (Diamond ATR crystal): 3388, 3095, 2978, 2936, 2900, 2831, 1742, 1653, 1451, 1406, 1378, 1314, 1200, 1136, 1069, 996, 934, 894, 870, 830, 803, 770, 721, 698. CD Δε (nm): 0 (192.0), −0.78 (197.0), 0 (206.5), +2.02 (226.5), 0 (282.5). <sup>1</sup>H and <sup>13</sup>C NMR data: Tables 1 and 2.
Aspilactonol C (4): Colorless oil (MeOH), [α]_D^20 +27.2 (c 0.32, MeOH). Positive ion ESIMS m/z: 203 [M + H]^+, 225 [M + Na]^+. Negative ion ESIMS m/z: 201 [M − H]−. Positive ion HRESIMS m/z: measured 203.0910 [M + H]^+, calculated for C9H15O3 [M + H]^+ 203.0919; measured 225.0732 [M + Na]^+, calculated for C9H14O3Na [M + Na]^+ 225.0739; measured 241.0476 [M + K]^+, calculated for C9H14O3K [M + K]^+ 241.0478. UV λ_{max} nm in MeOH: 214 nm. IR ν_{max} cm⁻¹ (Diamond ATR crystal): 3379, 3092, 2979, 2935, 1737, 1651, 1450, 1402, 1377, 1300, 1200, 1133, 1063, 994, 933, 894, 868, 826, 763, 693. CD Δε (nm): 0 (192.0), −0.43 (196.5), −0.39 (197.0), −0.41 (197.5), 0 (201.5), +0.37 (210.5), +1.64 (225.5), +1.65 (226.0), 0 (242.0), −0.19 (251.5), 0 (275.0). ¹H and ¹³C NMR data: Tables 1 and 2.

Aspilactonol D (5): Colorless oil (MeOH), [α]_D^25 −1.7 (c 0.38, MeOH). Positive ion ESIMS m/z: 217 [M + H]^+, 239 [M + Na]^+. Positive ion HRESIMS m/z: measured 217.1071 [M + H]^+, calculated for C10H17O3 [M + H]^+ 217.1076; measured 239.0890 [M + Na]^+, calculated for C10H16O3Na [M + Na]^+ 239.0895; measured 255.0635 [M + K]^+, calculated for C10H16O3K [M + K]^+ 255.0635. UV λ_{max} nm in MeOH: 213 nm. IR ν_{max} cm⁻¹ (Diamond ATR crystal): 3401, 3088, 2978, 2937, 2902, 2832, 1750, 1655, 1451, 1378, 1298, 1256, 1200, 1136, 1108, 1066, 1022, 977, 950, 936, 894, 853, 833, 803, 780, 717. CD Δε (nm): 0 (193.5), −0.29 (196.5), 0 (203.5), +1.41 (231.5), 0 (271.0). ¹H and ¹³C NMR data: Tables 1 and 2.

Aspilactonols E/F (6/7): Colorless oil (MeOH), [α]_D^25 +78.1 (c 1.00, MeOH). Positive ion ESIMS m/z: 187 [M + H]^+, 209 [M + Na]^+. Positive ion HRESIMS m/z: measured 187.0967 [M + H]^+, calculated for C9H15O3 [M + H]^+ 187.0970; measured 209.0789 [M + Na]^+, calculated for C9H16O3Na [M + Na]^+ 209.0790. UV λ_{max} nm in MeOH: 213 nm. IR ν_{max} cm⁻¹ (Diamond ATR crystal): 3396, 3093, 2975, 2934, 1748, 1651, 1456, 1417, 1377, 1205, 1127, 1070, 990, 942, 882, 841, 677. CD Δε (nm): 0 (185.0), −0.55 (196.5), −0.09 (201.5), −0.05 (202.5), −0.07 (214.0), 0 (216.5), +2.78 (228.5), 0 (280.5). ¹H and ¹³C NMR data: Tables 1 and 2.

Aspyronol (9): Colorless oil (MeOH), [α]_D^25 −41.6 (c 0.22, MeOH). Positive ion ESIMS m/z: 217 [M + H]^+, 239 [M + Na]^+. Positive ion HRESIMS m/z: measured 217.1073 [M + H]^+, calculated for C10H17O3 [M + H]^+ 217.1076; measured 239.0896 [M + Na]^+, calculated for C10H16O3Na [M + Na]^+ 239.0895; measured 255.0634 [M + K]^+, calculated for C10H16O3K [M + K]^+ 255.0635. UV λ_{max} nm in MeOH: 208 nm. IR ν_{max} cm⁻¹ (Diamond ATR crystal): 3398, 2982, 2938, 2905, 2835, 1714, 1649, 1451, 1384, 1309, 1216, 1044, 1018, 783, 721, 669. CD Δε (nm): 0 (192.0), −0.53 (196.5), −0.72 (209.5), −2.81 (223.5), 0 (242.0), −1.22 (263.0), 0 (301.0). ¹H and ¹³C NMR data: Table 3.

Epiaspinodonediol (11): Colorless oil (MeOH), [α]_D^25 −5.4 (c 0.14, MeOH). Positive ion ESIMS m/z: 193 [M + Na]^+, 209 [M + K]^+. Positive ion HRESIMS m/z: measured 171.1003 [M + H]^+, calculated for C9H13O3 [M + H]^+ 171.1021; measured 193.0842 [M + Na]^+, calculated for C9H14O3Na [M + Na]^+ 193.0841. ¹H NMR (400 MHz, CD3OD) δ: 7.20 (1H, d, J = 16.2 Hz, H-4), 6.41 (1H, d, J = 16.2 Hz, H-3), 6.05 (1H, d, J = 8.4 Hz, H-6), 4.77 (1H, dq, J = 8.4, 6.4 Hz, H-7), 4.33 (2H, s, H-9), 2.31 (3H, s, H-1), 1.29 (3H, d, J = 6.4 Hz, H-8). ¹³C NMR (100 MHz, CD3OD) δ: 201.6 (C-2), 148.3 (C-6), 147.5 (C-4), 137.0 (C-5), 128.4 (C-3), 64.8 (C-7), 56.9 (C-9), 27.3 (C), 23.6 (C-8).

The data for five known compounds, 8, 10 and 12–14, and the spectra of the new compounds, 1–7, 9 and 11, are all given in the Supplementary Information.
3.5. Preparation of (S)- and (R)-MTPA Esters of 1, 5 and 9

Each of duplicate 2 (0.3 mg, 1.8 μmol), 5 (0.5 mg, 2.3 μmol) and 9 (0.3 mg, 1.4 μmol) in 0.2 mL unhydrous pyridine-\textsubscript{d\textsubscript{6}} in NMR tubes was reacted with (S)- and (R)-MPTA-Cl (1.65 μL, 8.8 μmol for 2; 4.40 μL, 23.1 μmol for 5; 2.63 μL, 13.9 μmol for 9), respectively. The reaction was performed at room temperature for 72 h. Then, the \textsuperscript{1}H NMR data of the (S)- and (R)-MTPA esters were obtained without purification.

Compound 2a (pyridine-\textsubscript{d\textsubscript{5}}, 400 MHz) \(\delta\): 7.10 (1H, br s, H-4), 5.30 (1H, m, H-9), 4.99 (1H, m, H-5), 2.41 (2H, m, H-7), 1.87 (2H, m, H-8), 1.24 (3H, d, \(J = 6.5\) Hz, H-6), 1.23 (3H, d, \(J = 6.2\) Hz, H-10).

Compound 2b (pyridine-\textsubscript{d\textsubscript{5}}, 400 MHz) \(\delta\): 7.02 (1H, br s, H-4), 5.31 (1H, m, H-9), 4.97 (1H, m, H-5), 2.28 (2H, m, H-7), 1.84 (2H, m, H-8), 1.31 (3H, d, \(J = 6.3\) Hz, H-10), 1.22 (3H, d, \(J = 6.9\) Hz, H-6).

Compound 5a (pyridine-\textsubscript{d\textsubscript{5}}, 400 MHz) \(\delta\): 5.87 (1H, m, H-9), 5.25 (1H, m, H-5), 4.59 (1H, m, H-6), 4.42 (1H, br d, \(J = 4.0\) Hz, H-8), 3.24 (3H, s, OCH\textsubscript{3}), 1.79 (3H, d, \(J = 7.4\) Hz, H-7), 1.37 (3H, d, \(J = 6.5\) Hz, H-10).

Compound 5b (pyridine-\textsubscript{d\textsubscript{5}}, 400 MHz) \(\delta\): 5.83 (1H, m, H-9), 5.33 (1H, m, H-5), 4.60 (1H, m, 6-H), 4.54 (1H, br d, \(J = 3.9\) Hz, H-8), 3.34 (3H, s, OCH\textsubscript{3}), 1.80 (3H, d, \(J = 7.4\) Hz, H-7), 1.31 (3H, d, \(J = 6.5\) Hz, H-10).

Compound 9a (pyridine-\textsubscript{d\textsubscript{5}}, 400 MHz) \(\delta\): 7.18 (1H, m, H-4), 5.93 (1H, m, H-5), 5.7860 (1H, m, H-9), 4.58 (1H, m, H-8), 4.57 (1H, m, H-6), 3.19 (3H, s, OCH\textsubscript{3}), 1.39 (3H, d, \(J = 6.3\) Hz, H-7), 1.28 (3H, d, \(J = 6.4\) Hz, H-10).

Compound 9b (pyridine-\textsubscript{d\textsubscript{5}}, 400 MHz) \(\delta\): 7.19 (1H, m, H-4), 6.03 (1H, m, H-5), 5.7827 (1H, m, H-9), 4.88 (1H, m, H-6), 4.72 (1H, m, H-8), 3.29 (3H, s, OCH\textsubscript{3}), 1.45 (3H, d, \(J = 6.5\) Hz, H-7), 1.22 (3H, d, \(J = 6.6\) Hz, H-10).

3.6. Measurement of ICD Spectra of 3 and 4 Using Mo\textsubscript{2}(OAc)\textsubscript{4}

ICD was measured according to our previous procedure [39] using spectroscopy grade unhydrous DMSO. A mixture of the ligand (3 or 4) and Mo\textsubscript{2}(OAc)\textsubscript{4} in DMSO in an approximate 1:2 molar ratio was subjected to the ICD measurement. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary ICD was reached about 10 min after mixing. After the inherent CD data of the compound were subtracted, the ICD spectrum was normalized to the molar concentration of 3 or 4 and is presented as the Δε' values. The observed signs of the bands II and IV in the ICD were correlated to the absolute configuration of the 1,2-diol moiety.

3.7. MTT Assay

All samples and DOC were dissolved in MeOH to prepare the MeOH solution at 10.0 mg/mL, and serial dilutions were made for compounds 9 and 11. These solutions were subjected to MTT assay. DOC was used as positive control and MeOH was used as blank control. The assay was run in triplicate on human cancer K562, HL-60, HeLa and BGC-823 cell lines by the method that we have previously reported [52]. The OD was read at 570 nm, and the IR% value was calculated using the OD
mean values by the formula, IR% = (OD_{control} − OD_{sample})/OD_{control} × 100%. The IC_{50} for 9 and 11 was obtained from their IR% values at different concentrations.

4. Conclusions

Chemical investigation on a deep sea-sourced *Aspergillus* sp. 16-02-1 has resulted in the elucidation of 14 secondary metabolites, including nine new branched C_9 polyketides, named aspiketolactonol (1), asplactonols A–F (2–7), aspyronol (9) and epiaspinonediol (11), and five known branched polyketides identified as (S)-2-(2′-hydroxyethyl)-4-methyl-γ-butyrolactone (8), dihydroaspyrone (10), aspinotriol A (12), aspinotriol B (13) and chaetoquadrin F (14). Structures of the new compounds, including their absolute configurations, were determined by extensive spectroscopic methods, especially the 2D NMR, CD, ICD and Mosher’s 1H NMR analyses. Compound 8 was isolated from the natural sources for the first time, and the possible biosynthetic pathways for 1–14 were also proposed and discussed. Compounds 1–14 inhibited human cancer K562, HL-60, HeLa and BGC-823 cells to varying extents.

Acknowledgments

This work was supported by the grants from the NHTRDP (2013AA092901, 2007AA09Z411), NSFC (81172976), NSTMP (2012ZX09301-003, 2009ZX09103-019, 2009ZX09301-002), CAS (KSCX2-EW-G-6), and COMRA (DYXM-115-02-2-09), China. Dedicated to Xin-Sheng Yao on the occasion of his 80th birthday.

Author Contributions

Xiu-Wen Chen, Chang-Wei Li and Wei Hua performed the experiments for biological and chemical investigations on polyketides. Chang-Wei Li especially contributed to the structure elucidation of new compounds and also contributed to the preparation of the manuscript. Tian-Jiao Zhu and Qian-Qun Gu isolated and identified the producing strain *Aspergillus* sp. 16-02-1. Cheng-Bin Cui conceived and designed the experiments, accomplished the CD and ICD data analyses and absolute configuration assignments, and wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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