Nine New Farnesylphenols from the Basidiomycete *Albatrellus caeruleoporus*

Liang-Yan Liu · Zheng-Hui Li · Gang-Qiang Wang · Kun Wei · Ze-Jun Dong · Tao Feng · Gen-Tao Li · Yan Li · Ji-Kai Liu

Received: 16 March 2014 / Accepted: 10 April 2014 / Published online: 23 April 2014

© The Author(s) 2014. This article is published with open access at Springerlink.com

**Abstract** Nine previously-unreported farnesylphenols, involving eight neogrifolin derivatives (1–8) and one grifolin analogue (9), together with three known compounds, were isolated from the fruiting bodies of the mushroom *Albatrellus caeruleoporus*. Their structures were elucidated as (S)-17-hydroxy-18,20-ene-neogrifolin (1), (S)-18,19-dihydroxyneogrilin (2), (S)-9-hydroxy-10,22-ene-neogrifolin (3), (9S,10R)-6,10-epoxy-9-hydroxyneogrilin (4), (9S,10R)-6,9-epoxy-10-hydroxyneogrilin (5), (−)-13,14-dihydroxyneogrilin (6), albatrelin G (7), albatrelin H (8), and one grifolin analogue, (S)-10-hydroxygrifolin (9), grifolin (10), neogrilin (11), and albatrellin (12) by extensive spectroscopic analyses and chemical methods. Compounds 7 and 8 showed weak cytotoxic activity to cell lines HL-60, SMMC-7721, A-549, and MCF-7, in vitro.

**Keywords** *Albatrellus caeruleoporus* · Mushroom · Polyporaceae · Farnesylphenols

1 Introduction

Mushrooms of the *Albatrellus* genus are well known for producing farnesylphenols, such as grifolin, neogrilin and their derivatives [1–6]. Farnesylphenols can be divided into two groups: monomers of grifolin and neogrilin derivatives, and dimers of them. The monomers were reported to possess diverse biological activities, such as anti-oxidative activity [3], anti-microbial effect [7, 8], promotion of melanin synthesis [9], activity on human and rat vanilloid receptor 1 [10] inhibition of tumor-cell growth [11], and inhibition of nitric oxide production in RAW 264.7 cells [4]. And the dimers (fungal pigments) are regarded as the chemical base of the conspicuous fruiting bodies of these mushrooms [2, 4].

*Albatrellus caeruleoporus* is a nontoxic and inedible mushroom distributed in central and southwestern China. Its fruiting body is white with a light blue skin on the pileus [12]. Previous investigation on *A. caeruleoporus* led to three grifolin monomers, grifolin, neogrilin, and grifoli
none A, and one dimer, grifolinone B [4]. Their nitor
ite production inhibitory activities were reported [4]. In order to find more farnesylphenols with biological activities a systematic phytochemical investigation on the basidiomycete *A. caeruleoporus* was performed, it led to isolate eight new neogrilin derivatives (1–8), a new grifolin analogue (9), grifolin (10) [10], neogrilin (11) [10], and albatrellin (12) [2]. Their structures were identified by a combination of extensive spectroscopic analyses (NMR, MS, IR, UV, and [\(\alpha\)]$_D$) and chemical methods. Compounds 1–9 were oxygenated farnesylphenols, which...
have not previously been reported in the *Albatrellus* genus, and might be regarded as a chemotaxonomic evidence for identification of this mushroom. All new compounds were tested in a cytotoxicity assay in vitro against five human cancer cell lines.

### 2 Results and Discussion

The chloroform–methanol (1:1) extract of fruiting bodies of *A. caeruleoporus* was subjected to silica gel, RP-18, Sephadex LH-20 column chromatography (CC), and semipreparative HPLC purification steps to give compounds 1–12 (Fig. 1). Compounds 1–8, namely (S)-17-hydroxy-18,20-ene-neogrifolin (1), (S)-18,19-dihydroxy-neogrifolin (2), (S)-9-hydroxy-10,22-ene-neogrifolin (3), (9S,10R)-6,10-epoxy-9-hydroxyneogrigolin (4), (9S,10R)-6,9-epoxy-10-hydroxyneogrigolin (5), (−)-13,14-dihydroxyneogrigolin (6), albatrelin G (7), albatrelin H (8), were neogrigolin derivatives, (S)-10-hydroxygrifolin (9) was a grifolin analogue, and compound 12 was a violet pigment named albatrellin.

Compound 1, colorless oil, displayed a [M]⁺ ion peak at *m/z* 344.2348 in positive HREIMS, corresponding to the molecular formula C_{22}H_{32}O_{3} and seven degrees of unsaturation. The IR spectrum showed absorption at 3421 cm⁻¹ which indicated the presence of OH groups. The ¹H NMR spectrum contained signals for two *m*-coupling aromatic
protons at $\delta_H$ 6.25 (1H, d, $J = 1.8$ Hz) and 6.17 (1H, d, $J = 1.8$ Hz), four olefinic protons, and four singlet methyls. Combined with $^{13}$C NMR (DEPT) experiment, the existence of a 1,2,4,6-tetra-substituted phenyl ring, a terminal double bond, two tri-substituted double bonds, one oxygen-bearing methine, five methylenes, and four methyls were assigned. The $^1$H and $^{13}$C NMR spectroscopic data were close to those of neogrifolin, except that a methylene and a methyl group in neogrifolin were replaced by a hydroxyl methine ($\delta_C 75.3$) and a terminal double bond ($\delta_C$ 110.3 and 149.3), respectively. According to the observed HMBC correlations from $\delta_H$ 6.25 and 6.17 (H-20) to $\delta_C$ 17.8 (C-19) and 75.3 (C-17), the terminal double bond was located at the end of the farnesyl side chain, and the hydroxyl group was at C-17 ($\delta_C$ 75.3). This conclusion was supported by cross peaks from $\delta_H$ 3.73 (OH-17) to $\delta_C$ 34.5 (C-16), 75.3 (C-17) and 149.3 (C-18), and from 3.97 (H-17) to $\delta_C$ 36.4 (C-15) in HMBC spectrum (Fig. 2). The absolute configuration of the only chiral center (C-17) of 1 was deduced to be $S$ by comparing the optical rotation

Fig. 2 Selective 2D NMR correlations for compounds 1–9
value of 1 ([α]D 31 20.9, MeOH) with that of (R)-(−)-3-methyl-3-buten-2-ol ([α]D 20.9, CHCl3) [13]. Therefore, compound 1 was elucidated and named as (S)-17-hydroxy-18,20-ene-neogrofilin.

Compound 2 possessed a molecular formula of C22H32O4 according to HREIMS which showed a molecular ion peak at m/z 362.2452, requiring six degrees of unsaturation. Inspection of the 1H and 13C NMR (DEPT) spectra indicated five methyls, five methylenes, four sp² methines, one oxygen-bearing methine, and seven quaternary carbons. The 1D NMR spectroscopic data were similar to those of 1, except for the terminal double bond being replaced by a methyl and an oxygen-bearing quaternary carbon, which was confirmed by HMBC correlations from δH 3.54 (OH-17) to δC 72.9 (C-18), from δH 3.24 (H-17) to δC 25.8 (C-19 and C-20), from δH 1.11 (H-20) to δC 25.8 (C-19), 72.9 (C-18) and 78.5 (C-17). The absolute configuration of C-17 in 2 was assigned to be S, the same as 1, on a biogenetic point of view. And this assumption was further confirmed by a comparison of the optical rotation values between 2 ([α]D 31 20.9, MeOH) and (R)-2-methylpentane-2,3-diol ([α]D 31 27.3, ether) [14]. Therefore, compound 2 was identified as (S)-18,19-dihydroxyneogrofilin.

Compound 3 was determined to have the molecular formula of C22H34O4 from HREIMS at m/z 344.2364 ([M]⁺). The 13C NMR (DEPT) spectra showed signals of a tetra-substituted phenyl moiety, a terminal double bond, two tri-substituted double bonds, a hydroxyl methane, four methyls, and five methylenes, which resembled those of compound 1. Extensive 2D NMR (COSY, ROESY and HMBC) analyses revealed that the locations of the double bond and the oxygen-bearing methine were different with those of 1. COSY correlations from δH 2.82 and 2.68 (H-8) to δH 4.28 (δC = 77.0) suggested that C-9 was the oxygenated carbon. Moreover, δH 5.11 and 4.84 (δC = 108.9, t) gave HMBC correlations to δC 32.5 (C-11) and 77.0 (C-9), revealing that the Me-22 in compound 1 converted to be a double bond in 3. Therefore, the structure of compound 3 was identified as 9-hydroxy-10,22-ene-neogrofilin. The absolute stereochemistry of the chiral center of C-9 was determined to be S by comparing the optical rotation value of 3 ([α]D 31 -8.8, MeOH) with (S)-3-methyl-1-phenylbutan-2-ol ([α]D 31 -29.5, CHCl3) [15].

The HREIMS of compound 4 showed a [M⁺] ion peak at m/z 344.2348, indicating a molecular formula of C22H34O4 and seven degrees of unsaturation. A comparison of the MS and 1D NMR data of 4 with those of 3 revealed that 4 was another neogrofilin analogue resembled 3 except for the double bond between C-10 and C-22 in 3 being replaced by a methyl (Me-22) and an oxygenated quaternary carbon (C-10) in 4. This structure requires six degrees of unsaturation, and an additional ring was needed to complete the unsaturation. There were two plausible proposals: an epoxy ring between C-6 and C-9 or between C-6 and C-10. HMBC correlations from δH 9 (δC 3.85) to C-1 (δC 111.0), C-10 (δC 78.6), C-11 (δC 38.7), and C-22 (δC 17.9), and from the OH at δH 4.14 to C-8 (δC 29.7), C-9 (δC 68.4) and C-10 (δC 78.6) revealed the location of the free OH at C-9, placing the epoxy ring between C-6 and C-10. Therefore, the planar structure of 4 was elucidated as 6,10-epoxy-9-hydroxyneogrofilin. The ROESY spectrum displayed cross peaks of H-9/H-11 and H-9/H-12, suggesting the same orientation of H-9 and the geranyl group. From a biogenetic point of view, compounds 4 and 3 should share the same absolute configuration on C-9. So the absolute stereochemistry of compound 4 was deduced to be 9S, 10R.

Compound 5 was proposed to be a neogrofilin derivative on basis of HREIMS which displayed the molecular ion peak at m/z 344.2358. A comparison of the 13C NMR (DEPT) spectra of 5 with those of 4 revealed the resemblance of the two structures, for example, the presence of the 1-(2-methyl-4,6-di-hydroxyl)-phenyl group and the geranyl moiety. The structural difference between compounds 4 and 5 was the fragment from C-8 to C-10, according to the different chemical shifts of the corresponding carbons and protons (Tables 1 and 2). In order to establish the structure of 5, extensive 2D NMR experiments were employed. The HMBC correlations of H-9/C-1 and H-9/C-6 indicated the existence of an oxygen bridge between C-6 and C-9. The free hydroxyl group was determined to be located at C-10 by HMBC correlations from OH at 10 to C-9, C-10, C-11 and C-22. Biogenetically speaking, compound 5 would show the same stereochemistry at C-9 and C-10 as compound 4. Therefore, compound 5 was elucidated as (9S,10R)-6,9-epoxy-10-hydroxyneogrofilin.

Compound 6 was obtained as a colorless oil, with a molecular formula of C22H34O4 according to the HREIMS at m/z 362.2432 ([M⁺]). Inspection of the 1H, 13C (DEPT) and HSQC NMR spectra allowed the assignment of five methyls, five methylenes, five methines, seven quaternary carbons, and four active protons. Comparing the 1H and 13C NMR spectroscopic data of 6 with those of neogrofilin indicated that compound 6 shared the “1-(2-methyl-4,6-di-hydroxyl)-phenyl” partial structure with neogrofilin, but had a different side chain, in which one double bond in the farnesyl group was replaced by two oxygen-bearing sp³ carbons. The COSY cross peaks of H-11/H-12/H-13, and HMBC correlations from OH at δH 3.56 to C-12, C-13 and C-14, and from δH 3.20 to C-21, C-13, C-14, and C-15 (Fig. 2) suggested the oxygenated carbons being located at C-13 (δC 77.3, CH) and C-14 (δC 74.2, C). In order to identify the relative configuration of the two chiral centers C-13 and C-14, compound 6 was reacted with 2,2-dimethoxypropane in DMF for 30 min at room temperature to
yield its di-O-isopropylidene derivative \textit{6a}. The observed ROESY correlations of Me-21/H-13 (Fig. 2) indicated that the 13,14-diol existed as the \textit{erythro} form. So, the absolute configuration of \textit{6} should be 13\textit{S},14\textit{S} or 13\textit{R},14\textit{R}.

Compound \textit{7} possessed a molecular formula of C\textsubscript{22}H\textsubscript{32}O\textsubscript{3} from its HREIMS, which displayed a molecular ion peak at \textit{m/z} 344.2348. A comparison of the \textit{1H} and \textit{13C} NMR data of \textit{7} with those of \textit{1} revealed the presence of the 1-(2-methyl-4,6-dihydroxyl)-phenyl group. Combined with MS spectral data, compound \textit{7} was determined to be a neogrifolin derivative unambiguously. Unlike the other neogrifolin analogues (\textit{1–6}) which had straight-chains as “tails”, compound \textit{7} had a cyclohexane moiety—by C-8 connecting to C-13—in its tail. It was supported by COSY correlations of H-9/H-8/H-13/H-12/H-11, and HMBC cross peaks from Me-22 to C-9, C-10 and C-11 (Fig. 2). The remaining part of the “tail” was a 2-(6-methyl)-1,5-heptadiene residue. This residue was connected with C-13, because COSY correlations of H-15/H-16/H-17, and HMBC correlations from H-21 to C-13 and C-15, and from Me-19 and -20 to C-18 and C-17 (Fig. 2) were observed. In order to determine the relative stereochemistry of C-8, C-10 and C-13, a ROESY experiment was performed, combined with further analysis of the coupling constants of several signals in \textit{1H} NMR spectrum. The observed broad singlet (br. s) signal of H-8 (\textit{d}_\text{H} = 3.22) in its \textit{1H} NMR

| Table 1 \textit{1H} NMR spectroscopic data for compounds \textit{1–6} in acetone-\textit{d}_6 (\textit{\delta} in ppm, \textit{J} in Hz) |
|---|---|---|---|---|---|
| No | \textit{1}\textsuperscript{a} | \textit{2}\textsuperscript{a} | \textit{3}\textsuperscript{a} | \textit{4}\textsuperscript{b} | \textit{5}\textsuperscript{a} | \textit{6}\textsuperscript{c} |
| 3 | 6.17, d (1.8) | 6.17, d (2.3) | 6.21, s | 6.25, d (2.1) | 6.12, br. s | 6.17, s |
| 5 | 6.25, d (1.8) | 6.25, d (2.3) | 6.22, s | 6.12, d (2.1) | 6.03, br. s | 6.25, s |
| 7 | 2.15, s | 2.15, s | 2.20, s | 2.11, s | 2.11, s | 2.15, s |
| 8 | 3.26, d (6.7) | 3.26, d (6.7) | 2.82, dd (14.4, 2.2) | 2.79, dd (16.1, 5.9) | 3.06, dd (15.3, 7.9) | 3.27\textsuperscript{a} |
| 9 | 5.09, t (6.7) | 5.09, t (6.7) | 4.28, dd (9.3, 2.2) | 3.85, td (8.5, 5.8) | 4.62, dd (9.5, 7.9) | 5.11\textsuperscript{a} |
| 11 | 1.90–2.00, m | 1.97–1.99, m | 2.22–2.25\textsuperscript{a} | 1.66–1.75, m | 1.50–1.56, m | 2.25, t (9.9) |
| 12 | 2.03–2.11, m | 2.07–2.10, m | 2.22–2.25\textsuperscript{a} | 2.14–2.17, m | 2.09–2.20, m | 1.69–1.74, m |
| 13 | 5.12, t (6.6) | 5.14, t (7.0) | 5.20, br. s | 5.17, t (7.2) | 5.18, t (6.9) | 5.29\textsuperscript{a} |
| 15 | 1.90–2.00, m | 2.21–2.26, m | 1.98, t (7.5) | 1.97, t (7.6) | 1.98, t (7.5) | 1.54–1.58, m |
| 16 | 1.54–1.58, m | 1.62–1.68, m | 2.07–2.09\textsuperscript{a} | 2.06–2.08\textsuperscript{a} | 2.06–2.09\textsuperscript{a} | 2.09–2.13, m |
| 17 | 3.97, m | 3.24, td (5.3, 1.8) | 5.10\textsuperscript{a} | 5.10, t (6.9) | 5.10, t (7.0) | 5.11\textsuperscript{a} |
| 19 | 1.68, s | 1.11, s | 1.59, s | 1.58, s | 1.59, s | 1.58, s |
| 20 | 4.89, br. s | 1.11, s | 1.65, s | 1.65, s | 1.65, s | 1.64, s |
| 21 | 4.74, br. s | 1.58, s | 1.63, s | 1.61, s | 1.63, s | 1.07, s |
| 22 | 1.75, s | 1.75, s | 5.11, br. s | 1.16, s | 1.20, s | 1.76, s |
| 4-OH | 7.86, s | 7.88, s | 8.01, s | 7.89, s | 8.05, s | 7.89, s |
| 6-OH | 8.02, s | 8.06, s | 8.56, s | 8.05, s | 8.05, s |
| 9-OH | 4.88, d (2.8) | 4.14, d (5.6) | 3.54, s |
| 10-OH | 3.54, s | 3.56, d (5.8) |
| 13-OH | 3.20, s |
| 14-OH | 3.73, d (4.2) | 3.54, d (5.3) |
| 17-OH | 3.40, s |

\textsuperscript{a} Measured at 400 MHz  
\textsuperscript{b} Measured at 500 MHz  
\textsuperscript{c} Measured at 600 MHz  
\textsuperscript{a} Signals were overlapped
spectrum indicated that H-8 existed as an equatorial bond in the stable boat conformation of the cyclohexane moiety, as shown in Fig. 2. Likewise, H-13 was proposed to be in an axial position because of the doublet of triplets at $\delta_H$ 3.22 with coupling constants of 12.5 and 2.7 Hz, respectively. Furthermore, The ROESY correlations of H-13/H$_{ax}$-9 and Me-22/H$_{ax}$-9 revealed the same orientation of H-13 and Me-22. Therefore, H-8, H-13 and Me-22 were deduced to be $\alpha$, $\alpha$, $\alpha$-orientated. In compound 7, a ring was formed by new C–C bond connection between C-8 and C-13 in side chain.

Compound 8 exhibited a molecular ion peak at $m/z$ 344.2345 in HREIMS, indicating the molecular formula of C$_{22}$H$_{32}$O$_3$ which required seven degrees of unsaturation. According to the 1H and 13C NMR (DEPT) spectra, 22 carbon signals were recognized as five methyls, four methylenes, five methines, and seven quaternary carbons. Extensive NMR analyses suggested that the structure of 8 resembled that of 7, except for the terminal double bond C-14=C-21 in 7 being saturated to be a methyl and an oxygen-bearing quaternary carbon, which was confirmed by HMBC correlations from $d_H$ 1.13 (Me-21) to $d_C$ 36.8 (C-15), 55.9 (C-13), and 74.0 (C-14), and from $d_H$ 3.30 (OH-14) to $d_C$ 36.8 (C-15), 55.9 (C-13), and 74.0 (C-14). So far, six degrees of unsaturation was assigned, and one more ring should be constructed to complete the structure of 8. The only possible ring to be formed was the oxygen bridge between C-6 and C-10. The stereochemistry of C-13 was identified by analysis of 1H NMR spectrum, in which H-13 showed a doublet-of-triplets peak with the coupling constants of 12.8 and 2.0 Hz, respectively, suggesting the axial bond of H-13. Me-22 had the same orientation as H-13 by the observed ROESY correlations of H-13/H$_{ax}$-11/Me-22, and because of the planar structure of the phenyl group, H-8 and Me-22 should be on the same orientation. Thus, H-8, H-13 and Me-22 were determined to be $\alpha$, $\alpha$, $\alpha$-orientated, the same as for compound 7.

Compound 9 was proposed to possess a molecular formula of C$_{22}$H$_{34}$O$_3$ on basis of HREIMS at $m/z$ 346.2505 ([M]$^+$$)$. Its 13C NMR (DEPT) spectrum showed 20 carbon signals, including two signals at $d_C$ 108.4 (CH) and 156.7 (C) which represented two carbons respectively. The overlapped carbon signals indicated that 9 was a grifolin derivative possessing a symmetric aromatic ring, which

---

Table 2 13C NMR spectroscopic data for compounds 1–6 in acetone-$d_6$ ($\delta$ in ppm)

| No | 1$^a$ | 2$^a$ | 3$^a$ | 4$^b$ | 5$^c$ | 6$^c$ |
|----|------|------|------|------|------|------|
| 1  | 118.3, C | 118.3, C | 117.1, C | 111.0, C | 117.8, C | 118.3, C |
| 2  | 138.9, C | 138.9, C | 139.0, C | 138.6, C | 135.2, C | 138.8, C |
| 3  | 109.4, CH | 109.3, CH | 109.8, CH | 110.1, CH | 108.5, CH | 109.3, CH |
| 4  | 156.5, C | 156.5, C | 157.1, C | 157.1, C | 158.4, C | 156.5, C |
| 5  | 101.0, CH | 101.0, CH | 102.2, CH | 102.0, CH | 95.2, CH | 101.0, CH |
| 6  | 156.4, C | 156.4, C | 157.9, C | 154.7, C | 161.6, C | 156.4, C |
| 7  | 19.9, CH$_{3}$ | 19.9, CH$_{3}$ | 20.5, CH$_{3}$ | 19.3, CH$_{3}$ | 19.0, CH$_{3}$ | 19.9, CH$_{3}$ |
| 8  | 25.1, CH$_{2}$ | 25.1, CH$_{2}$ | 34.4, CH$_{2}$ | 29.7, CH$_{2}$ | 29.1, CH$_{2}$ | 25.1, CH$_{2}$ |
| 9  | 124.8, CH | 124.8, CH | 77.0, CH | 68.4, CH | 89.8, CH | 124.4, CH |
| 10 | 134.1, C | 134.1, C | 153.4, C | 78.6, C | 73.3, C | 134.6, C |
| 11 | 40.4, CH$_{2}$ | 40.4, CH$_{2}$ | 32.5, CH$_{2}$ | 38.7, CH$_{2}$ | 39.3, CH$_{2}$ | 37.8, CH$_{2}$ |
| 12 | 27.2, CH$_{2}$ | 27.2, CH$_{2}$ | 27.3, CH$_{2}$ | 22.1, CH$_{2}$ | 22.5, CH$_{2}$ | 30.2, CH$_{2}$ |
| 13 | 124.8, CH | 124.8, CH | 125.1, CH | 125.4, CH | 125.6, CH | 77.8, CH |
| 14 | 135.5, C | 135.8, C | 135.7, C | 135.4, C | 135.3, C | 74.2, C |
| 15 | 36.4, CH$_{2}$ | 37.6, CH$_{2}$ | 40.4, CH$_{2}$ | 40.4, CH$_{2}$ | 40.4, CH$_{2}$ | 38.5, CH$_{2}$ |
| 16 | 34.5, CH$_{2}$ | 30.7, CH$_{2}$ | 27.3, CH$_{2}$ | 27.4, CH$_{2}$ | 27.3, CH$_{2}$ | 22.6, CH$_{2}$ |
| 17 | 75.3, CH | 78.5, CH | 125.1, CH | 125.1, CH | 125.1, CH | 126.2, CH |
| 18 | 149.3, C | 72.9, C | 131.6, C | 131.6, C | 131.6, C | 131.1, C |
| 19 | 17.8, CH$_{3}$ | 25.8, CH$_{3}$ | 17.7, CH$_{3}$ | 17.7, CH$_{3}$ | 17.7, CH$_{3}$ | 17.6, CH$_{3}$ |
| 20 | 110.3, CH$_{2}$ | 25.8, CH$_{3}$ | 25.8, CH$_{3}$ | 25.8, CH$_{3}$ | 25.8, CH$_{3}$ | 25.8, CH$_{3}$ |
| 21 | 16.1, CH$_{3}$ | 16.1, CH$_{3}$ | 16.1, CH$_{3}$ | 16.0, CH$_{3}$ | 16.0, CH$_{3}$ | 22.8, CH$_{3}$ |
| 22 | 16.1, CH$_{3}$ | 16.1, CH$_{3}$ | 108.9, CH$_{2}$ | 17.9, CH$_{3}$ | 22.3, CH$_{3}$ | 16.3, CH$_{3}$ |

$^a$ Measured at 100 MHz
$^b$ Measured at 125 MHz
$^c$ Measured at 150 MHz
was confirmed by HMBC cross peaks from $\delta_H$ 2.10 (Me-7) to $\delta_C$ 108.4 (C-3 and -5) and 136.5 (C-4), from $\delta_H$ 2.67 (H-8) to $\delta_C$ 113.9 (C-1) and 156.7 (C-2 and -6), and from $\delta_H$ 8.05 (OH-2 and -6) to $\delta_C$ 108.4 (C-3 and -5), 113.9 (C-1) and 156.7 (C-2 and -6). Besides the aromatic ring, the remaining signals represented an oxygenated farnesyl group with four methyls, six methylenes, two pairs of tri-substituted double bonds, and one oxygenated quaternary carbon. The next problem to be resolved was the position of oxygenation, which was addressed by 2D NMR (HMBC and COSY) experiments. The HMBC correlations from $\delta_H$ 2.67 (H-8) to $\delta_C$ 72.6 (C-10), and from $\delta_H$ 1.20 (Me-22) to $\delta_C$ 41.2 (C-9), 72.6 (C-10) and 42.6 (C-11) revealed the hydroxylation of C-10. Hence, the planar structure of 9 was established as 10-hydroxygrifolin. The absolute stereochemistry of C-10 was deduced to be $S$ by a comparison of the optical rotation value of 9 ($[\alpha]_D$ = -8.7, MeOH) with that of (S)-3-methyl-1-phenyl-3-pentanol ($[\alpha]_D$ = -1.6, CHCl$_3$) [16].

All the new compounds were assayed for their cytotoxicity against five human cancer cell lines (HL-60, SMMC-7721, A-549, and MCF-7, with IC$50$ of 12.8, 8.05 (OH-2 and -6) to $\delta_C$ 108.4 (C-3 and -5), 113.9 (C-1) and 156.7 (C-2 and -6). Besides the aromatic ring, the remaining signals represented an oxygenated farnesyl group with four methyls, six methylenes, two pairs of tri-substituted double bonds, and one oxygenated quaternary carbon. The next problem to be resolved was the position of oxygenation, which was addressed by 2D NMR (HMBC and COSY) experiments. The HMBC correlations from $\delta_H$ 2.67 (H-8) to $\delta_C$ 72.6 (C-10), and from $\delta_H$ 1.20 (Me-22) to $\delta_C$ 41.2 (C-9), 72.6 (C-10) and 42.6 (C-11) revealed the hydroxylation of C-10. Hence, the planar structure of 9 was established as 10-hydroxygrifolin. The absolute stereochemistry of C-10 was deduced to be $S$ by a comparison of the optical rotation value of 9 ($[\alpha]_D$ = -8.7, MeOH) with that of (S)-3-methyl-1-phenyl-3-pentanol ($[\alpha]_D$ = -1.6, CHCl$_3$) [16].

All the new compounds were assayed for their cytotoxicity against five human cancer cell lines (HL-60, SMMC-7721, A-549, and SW480) by the MTT method in vitro, with DDP and taxol as positive controls. Compound 7 showed cytotoxic activities to cell lines HL-60, SMMC-7721, A-549, and MCF-7, with IC$50$ of 12.8, 33.8, 33.0, and 33.2 $\mu$M, respectively, and 8 exhibited weak growth inhibition activity to human tumor cell lines HL-60 and A-549, with IC$50$ of 21.8 and 30.3 $\mu$M, respectively.

### 3.2 Fungal Material

The fungus A. caeruleoporus was collected in Anhui province, China, in October, 2011. The voucher specimen (GDGM 29146) has been deposited in the Herbarium of Microbiology Institute of Guangdong.

### 3.3 Extraction and Isolation

The dried fruiting bodies of A. caeruleoporus (about 200 g) were extracted with chloroform/methanol (1:1) for three times ($5 \times 3$). Evaporation of the solvent under reduced pressure gave the crude extract (20 g), which was subjected to silica gel CC using a petroleum ether–acetone gradient (1:0 → 0:1) to afford fractions A–E. Fraction B was purified by CC over silica gel with a petroleum ether–acetone system (20:1 → 10:1) to yield two fractions B$_1$ and B$_2$. Fraction B$_1$ was purified by semi-preparative HPLC (CH$_3$CN/H$_2$O, 6:4) to have 1 (5.2 mg) and 10 (12.0 mg), while B$_2$ was applied on a Sephadex LH-20 (CHCl$_3$/MeOH 1/1) column and then on semi-preparative HPLC eluting with MeCN/H$_2$O (6:4) to give compounds 2 (6.0 mg) and 11 (21.0 mg). Fraction C was subjected to CC with RP-18 silica gel eluting with 90 % methanol, and then purified by semi-preparative HPLC (CH$_3$CN/H$_2$O, 55:45) to get compound 6 (5.1 mg). Fraction D was submitted to silica gel CC eluting with petroleum ether-acetone gradient (15:1) to give two fractions D$_1$ and D$_2$, which were purified first by PR-18 and then by Sephadex LH-20 CC to yield fractions D$_1'$ and D$_2'$, respectively. Fraction D$_1'$ was loaded on a semi-preparative HPLC eluting with MeCN-H$_2$O (60:40 → 65:35) to afford compounds 9 (4.8 mg) and 3 (4.5 mg), while fraction D$_2'$ was passed through a semi-preparative HPLC (MeCN/H$_2$O, 60:40 → 65:35) to yield compounds 8 (6.4 mg) and 5 (4.0 mg). Fraction E was applied on CC over RP-18 to give fractions E$_1$ and E$_2$. Compound 4 (6.5 mg) was obtained from fraction E$_1$ which was passed through silica gel column (petroleum ether-acetone, 10:1), Sephadex LH-20 column (chloroform–methanol, 1:1), and semi-preparative HPLC (MeCN/H$_2$O 7:3), successively. Fraction E$_2$ was passed through Sephadex LH-20, and then loaded on semi-preparative HPLC to yield compound 7 (4.5 mg).
3.4 (S)-17-hydroxy-18,20-ene-neogrifolin (1)

Colorless oil; [α]D 21° = −9.0 (c 0.20, MeOH); UV λmax (MeOH) (log ε) 283 (2.67) nm; IR (KBr) νmax 3421, 3075, 2970, 2922, 2855, 1611, 1447, 1141 cm−1; 1H and 13C NMR spectroscopic data, see Tables 1 and 2; EI-MS m/z: 344 [M]+, 326 [M−H2O]+, 191, 175, 137; HR-EI-MS m/z: 344.2348 [M]+ (calcd for C22H32O4, 344.2351).

3.5 (S)-18,19-dihydroxyneogrifolin (2)

Colorless oil; [α]D 21° = −9.2 (c 0.18, MeOH); UV λmax (MeOH) (log ε) 282 (2.67) nm; IR (KBr) νmax 3423, 2974, 2925, 2855, 1613, 1467, 1141 cm−1; 1H and 13C NMR spectroscopic data, see Tables 1 and 2; EI-MS m/z: 363 [M+H]+, 362 [M]+, 344 [M−H2O]+, 191, 175, 137; HR-EI-MS m/z: 362.2452 [M]+ (calcd for C22H34O4, 362.2457).

3.6 (S)-9-hydroxy-10,22-ene-neogrifolin (3)

Colorless oil; [α]D 21° = −8.8 (c 0.24, MeOH); UV λmax (MeOH) (log ε) 283 (2.90) nm; IR (KBr) νmax 3420, 2966, 2923, 2855, 1614, 1447, 1143 cm−1; 1H and 13C NMR spectroscopic data, see Tables 1 and 2; EI-MS m/z: 344 [M]+, 326 [M−H2O]+, 137; HR-EI-MS m/z: 344.2364 [M]+ (calcd for C22H34O4, 344.2351).

3.7 (9S,10R)-6,10-epoxy-9-hydroxyneogrifolin (4)

Colorless oil; [α]D 22° = −6.7 (c 0.28, MeOH); UV λmax (MeOH) (log ε) 282 (2.74) nm; IR (KBr) νmax 3422, 3038, 2967, 2923, 2854, 1618, 1601, 1460, 1138 cm−1; 1H and 13C NMR spectroscopic data, see Tables 1 and 2; EI-MS m/z: 344 [M]+, 191, 137; HR-EI-MS m/z: 344.2348 [M]+ (calcd for C22H34O4, 344.2351).

3.8 (9S,10R)-6,9-epoxy-10-hydroxyneogrifolin (5)

Colorless oil; [α]D 22° = −8.6 (c 0.19, MeOH); UV λmax (MeOH) (log ε) 282 (2.82) nm; IR (KBr) νmax 3513, 3405, 2969, 2922, 2856, 1629, 1602, 1495, 1449, 1128 cm−1; 1H and 13C NMR spectroscopic data, see Tables 1 and 2; EI-MS m/z: 344 [M]+, 191, 175, 150; HR-EI-MS m/z: 344.2358 [M]+ (calcd for C22H34O4, 344.2351).

3.9 (−)-13,14-Dihydroxyneogrifolin (6)

Colorless oil; [α]D 22° = −9.2 (c 0.20, MeOH); UV λmax (MeOH) (log ε) 282 (2.65) nm; IR (KBr) νmax 3440, 2969, 2924, 2856, 1628, 1452, 1141 cm−1; 1H and 13C NMR spectroscopic data, see Tables 1 and 2; EI-MS m/z: 362 [M]+, 344 [M−H2O]+, 326 [M−2 × H2O]+, 191, 175, 137; HR-EI-MS m/z: 362.2432 [M]+ (calcd for C22H34O4, 362.2457).

3.10 Albatrelin G (7)

Colorless oil; [α]D 22° = −15.2 (c 0.19, MeOH); UV λmax (MeOH) (log ε) 283 (2.93) nm; IR (KBr) νmax 3441, 2969, 2929, 2855, 1640, 1615, 1495, 1452, 1141 cm−1; 1H and 13C NMR spectroscopic data, see Table 3; EI-MS m/z: 344 [M]+, 326 [M−H2O]+, 175; HR-EI-MS m/z: 344.2348 [M]+ (calcd for C22H34O4, 344.2351).

3.11 Albatrelin H (8)

Colorless oil; [α]D 22° = −9.1 (c 0.21, MeOH); UV λmax (MeOH) (log ε) 284 (2.63) nm; IR (KBr) νmax 3441, 2968, 2929, 2872, 2855, 1615, 1595, 1452, 1145 cm−1; 1H and 13C NMR spectroscopic data, see Table 3; EI-MS m/z: 344 [M]+, 175, 137; HR-EI-MS m/z: 344.2345 [M]+ (calcd for C22H34O4, 344.2351).

3.12 (S)-10-hydroxygrifolin (9)

Colorless oil; [α]D 22° = −8.7 (c 0.20, MeOH); UV λmax (MeOH) (log ε) 276 (2.50) nm; IR (KBr) νmax 3441, 2967, 2925, 2856, 1628, 1598, 1452, 1381, 1050 cm−1; 1H and 13C NMR spectroscopic data, see Table 3; EI-MS m/z: 346 [M]+, 328 [M−H2O]+, 175, 137; HR-EI-MS m/z: 346.2505 [M]+ (calcd for C22H34O4, 346.2508).

3.13 Preparation of 6a

To a solution of compound 6 (2.3 mg, 6.35 μmol) in DMF (2 mL) were added 2,2-dimethoxypropane (1.3 mg, 12.7 μmol) and p-toluenesulfonic acid monohydrate (0.6 mg, 3.18 μmol), and the mixture was stirred for 30 min at room temperature. The reaction mixture was added into water, and then extracted by EtOAc for three times. The organic layer was evaporated and the residue was chromatographed on a column of silica gel eluting with petroleum ether-acetone 40:1 to yield 6a (2.1 mg).

3.14 Cytotoxic assay

The following human tumor cell lines were used: HL-60, SMMC-7712, A-549, MCF-7, and SW480. All the cells were cultured in RPMI-1640 or DMEM medium (HyClone, Logan, UT), supplemented with 10 % fetal bovine serum (HyClone) at 37 °C in a humidified atmosphere with 5 % CO2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
briefly, 100 μL of adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1.9 x 10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to the test compounds at various concentrations in triplicate for 48 h, with DDP and toxal as positive controls. After the incubation, MTT (100 μL) was added to each well, and the incubation continued for 4 h at 37 °C. The cells lysed with 200 μL SDS after removal of 100 μL of medium. The optical density of lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_50 value of each compound was calculated by Reed and Muench’s method [17].

Acknowledgment This project was supported by the National Natural Sciences Foundation of China (U1132607).

Conflicts of interest The authors declare no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use,

| No | δC, type | δH (J in Hz) | δC, type | δH (J in Hz) | δC, type | δH (J in Hz) |
|----|---------|--------------|---------|--------------|---------|--------------|
| 1  | 113.7, C | 115.6, C     | 113.9, C| 156.7, C     | 3       | 109.3, CH | 109.6, CH | 6.13, d (2.2)| 108.4, CH | 6.19, s     | 2       | 157.0, C | 157.0, C | 3.52, br. s | 136.5, C | 2.10, s     |
| 5  | 100.6, CH | 100.7, CH | 6.07, d (2.2)| 108.4, CH | 6.19, s     | 6       | 158.6, C | 158.6, C | 4.36, dd (12.8, 3.0)| 41.2, CH | 1.66–1.70, m |
| 7  | 20.0, CH3 | 20.9, CH3 | 2.36, s | 21.2, CH3 | 2.10, s     | 8       | 33.9, CH | 30.3, CH | 5.32, br. s | 18.2, CH2 | 2.67, m     |
| 9  | 38.9, CH2 | 40.4, CH2 | 1.87, dd (12.8, 3.2)| 125.9, CH | 5.15, t (6.9)| 10     | 74.2, C | 74.5, C | 1.62, dd (12.8, 8.0)| 135.0, C | 2.07*        |
| 11 | 40.8, CH2 | 41.5, CH2 | 1.89–1.92, m | 42.6, CH2 | 1.50–1.54, m | 12     | 24.6, CH2 | 21.6, CH2 | 1.53–1.56* | 23.3, CH2 | 2.10–2.15, m |
| 13 | 48.7, CH | 55.9, CH | 1.76, dt (12.8, 2.0) | 125.9, CH | 5.15, t (6.9)| 14     | 148.7, C | 74.0, C | 1.35–1.42, m | 100.0, m | 27.4, CH2 | 2.07* |
| 15 | 37.4, CH2 | 36.8, CH2 | 1.00, m | 125.9, CH | 5.10, t (7.1)| 16     | 27.7, CH2 | 23.0, CH2 | 2.09–2.14, m | 196–2.01, m | 27.4, CH2 | 2.07* |
| 17 | 125.2, CH | 126.1, CH | 4.98, t (7.0) | 125.1, CH | 5.10, t (7.1)| 18     | 131.9, C | 130.9, C | 1.50–1.54, m | 131.6, C | 2.07* |
| 19 | 17.7, CH3 | 17.6, CH3 | 1.56, s | 17.7, CH3 | 1.58, s     | 20     | 25.8, CH3 | 25.8, CH3 | 1.59, s | 25.8, CH3 | 1.65, s     |
| 21 | 109.7, CH2 | 27.3, CH3 | 1.13, s | 15.9, CH3 | 1.62, s     | 22     | 29.1, CH3 | 29.0, CH3 | 1.26, s | 27.5, CH3 | 1.20, s     |

Table 3 1H and 13C NMR spectroscopic data for compounds 7–9 in acetone-d6 (δ in ppm, J in Hz)

| 4-OH | 7.96, s | 8.05, s | 8.05, s | 3.54, s |
| 6-OH | 1.20, s | 8.05, s | 8.05, s | 3.54, s |
| 10-OH | 3.30, s | 3.30, s | 3.30, s | 3.30, s |

a 1H NMR spectra were measured at 400 MHz, and 13C NMR spectra at 100 MHz
b 1H NMR spectra was measured at 500 MHz, and 13C NMR spectra at 125 MHz
c Signals were overlapped

(MTT) (Sigma, St. Louis, MO). Briefly, 100 μL of adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1 x 10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to the test compounds at various concentrations in triplicate for 48 h, with DDP and toxal as positive controls. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells lysed with 200 μL SDS after removal of 100 μL of medium. The optical density of lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_50 value of each compound was calculated by Reed and Muench’s method [17].

Acknowledgment This project was supported by the National Natural Sciences Foundation of China (U1132607).

Conflicts of interest The authors declare no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use,
distribution, and reproduction in any medium, provided the original
author(s) and the source are credited.

References

1. Z.H. Ding, Z.J. Dong, J.K. Liu, Helv. Chim. Acta 84, 259–262
(2001)
2. B. Koch, W. Steglich, Eur. J. Org. Chem. 2007, 1631–1635
(2007)
3. M. Nukata, T. Hashimoto, I. Yamamoto, N. Iwasaki, M. Tanaka,
Y. Asakawa, Phytochemistry 59, 731–737 (2002)
4. D.N. Quang, T. Hashimoto, Y. Arakawa, C. Kohchi, T. Nishizawa,
G.I. Soma, Y. Asakawa, Bioorg. Med. Chem. 14, 164–168
(2006)
5. X.L. Yang, C. Qin, F. Wang, Z.J. Dong, J.K. Liu, Chem. Biodivers.
5, 484–489 (2008)
6. L. Zhang, Z.J. Dong, J.K. Liu, Acta Bot. Yunn. 31, 187–189
(2009)
7. T. Hashimoto, D.N. Quan, M. Nukada, M. Nukada, Y. Asakawa,
Heterocycles 65, 2431–2439 (2005)
8. D.Q. Luo, H.J. Shao, H.J. Zhu, J.K. Liu, Z. Naturforschung C60,
50–56 (2005)
9. H. Kawagishi, A. Tanaka, K. Sugiyama, H. Mori, H. Sakamoto,
Y. Ishoguro, K. Kobayashi, M. Uramoto, Phytochemistry 42,
547–548 (1996)
10. V. Hellwig, R. Nopper, F. Mauler, J. Freitag, J.K. Liu, Z.H. Ding,
M. Stadler, Arch. Pharm. 336, 119–126 (2003)
11. M. Ye, J.K. Liu, Z.X. Lu, Y. Zhao, S.F. Liu, L.L. Li, M. Tan,
X.X. Weng, W. Li, Y. Cao, FEBS Lett. 579, 3437–3443 (2005)
12. H.D. Zheng, P.G. Liu, Microbiol. China 33, 104–107 (2006)
13. S. Jones, D. Valette, Org. Lett. 11, 5358–5361 (2009)
14. M. Nakata, M. Arai, K. Tomooka, N. Ohsawa, M. Kinoshita,
Bull. Chem. Soc. Jpn. 62, 2618–2635 (1989)
15. H. Ziffer, K.I. Kawai, M. Kasai, M. Imuta, C. Froussios, J. Org.
Chem. 48, 3017–3021 (1983)
16. T. Mukaiyama, T. Shintou, K. Fukumoto, J. Am. Chem. Soc. 125,
10538–10539 (2003)
17. L.J. Reed, H. Muench, Am. J. Hyg. 27, 493–497 (1938)