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Sesquiterpenes from cultures of the fungus *Phellinus igniarius* and their Cytotoxicities

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ABSTRACT

Four new sesquiterpenoids, phellinignins A–D (**1**–**4**), together with four known ones (**5**–**8**), were isolated from cultures of the fungus *Phellinus igniarius*. The structures were established by extensive spectroscopic methods including MS, NMR, and the single crystal X-ray diffraction. Compounds **1**–**3** and **5**–**8** are tremulane sesquiterpenoids, while compound **4** possesses a new carbon skeleton that might derive from an illudane framework. Compounds **1**, **2**, **4**, and **5** showed certain cytotoxicities to three human cancer cell lines.

1. Introduction

Natural products play an important in drug discovery [1], while fungal products are one of the main components due to their structural and bioactive diversities [2]. The fungus *Phellinus igniarius*, a well-known mushroom belonging to the Polyporaceae family, is distributed in many East Asian countries like China, Korea, and Japan [3,4], where it is used as a physiologically functional food and exemplary source of natural medicines. In China, the extract of *P. igniarius* is used as a traditional Chinese medicine for the treatment of fester, abdominalgia, bloody gonorrhea, and antidiarrheal [5]. Previous chemical investigations on both fruiting bodies and their cultures have demonstrated various secondary metabolites including polysaccharides, flavonoids, polyphenols, steroids, terpenoids, and organic acids [6–12]. Some recent studies suggested that these constituents have an application prospect in the prevention and treatment of cancer [13,14]. In order to search for more bioactive compounds from this fungus, a chemical investigation on the cultures of *P. igniarius* was carried out. As a result, eight sesquiterpenoids including four new ones, phellinignins A–D (**1**–**4**), were obtained (Fig. 1). Their structures were elucidated by means of spectroscopic methods including MS, NMR, and the single crystal X-ray diffraction. Compounds **1**–**3** and **5**–**8** are tremulane type sesquiterpenoids. Compound **4** possesses a new framework that might derive from an illudane skeleton. All compounds were tested for their cytotoxicities against three human cancer cell lines. Herein, the isolation, structural elucidation, and cytotoxicities of the isolates are described.

2. Results and discussion

Compound **1** was isolated as colorless crystals. Its molecular formula $C_{15}H_{22}O_3$ was established on the basis of the HRESIMS data at *m/z* 251.16408 $[M + H]^+$ (calcd for $C_{15}H_{23}O_3^+$: 251.16417), implying for three degrees of unsaturation. The IR absorption bands at 3340 and 1702 cm^{-1} indicated the presence of hydroxy and carbonyl groups, respectively. In the ^1H NMR spectrum (Table 1), signals for three methyls were readily identified as one doublet at δ_H 0.92 (3H, d, J = 7.2 Hz, Me-13) and two singlets at δ_H 1.12 (3H, s, Me-14) and 0.99 (3H, s, Me-15). In addition, several signals for protons of oxygenated carbons were observed at δ_H 3.60 (1H, d, J = 4.9 Hz, H-10), 4.65 (1H, dd, J = 16.3, 2.7 Hz, H-11a) and 4.54 (1H, dd, J = 16.3, 2.7 Hz, H-11b). In the ^{13}C NMR and DEPT spectra (Table 1), a total of 15 carbon resonances were revealed, which were classified as three CH_3, four CH_2, four CH, and four C. In association with HSQC spectrum, two oxygenated carbons at δ_C 82.3 (d, C-10) and 72.5 (t, C-11) were in agreement with those ^1H NMR data as mentioned before. In addition, an ester carbonyl carbon at δ_C 175.8 (s, C-12) and two olefinic carbons at 157.3 (s, C-2) and 128.4 (s, C-3) were also identified, while the UV data at

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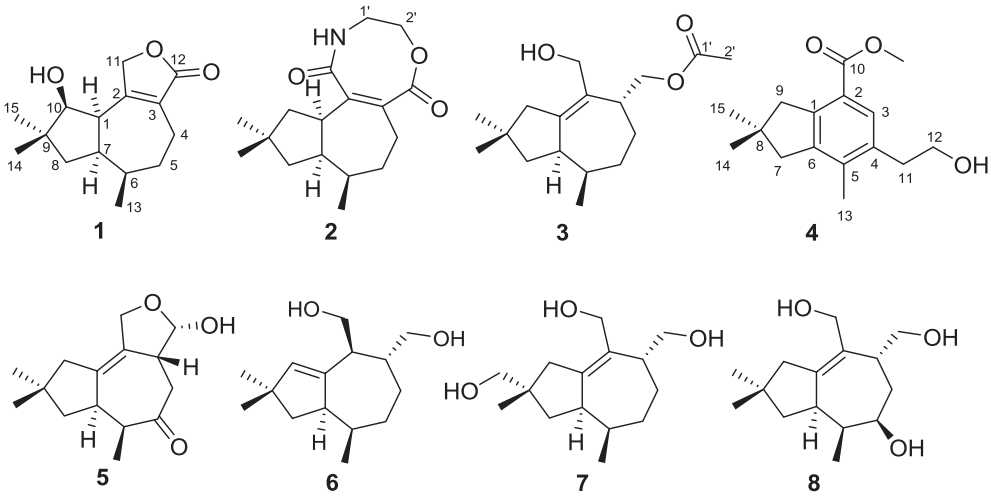


Fig. 1. Structures of compounds **1–8**.

Table 1
[1] (600 MHz) and ^{13}C (150 MHz) NMR data for **1–4** in $CDCl_3$ (δ in ppm, J in Hz).

No	1		2		3		4	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.19, dd (11.3, 4.7)	45.5, d	2.74, m	37.1, d		146.1, s		144.0, s
2		157.3, s		144.0, s		131.4, s		123.8, s
3		128.4, s		142.6, s	2.74, m	40.6, d	7.67, s	129.5, d
4a	2.10, m	25.7, t	2.62, m	19.9, t	1.81, m	21.5, t		134.3, s
4b	1.36, m		2.47, m		1.66, m			
5a	2.45, m	26.2, t	1.82, m	33.4, t	1.88, m	31.8, t		137.7, s
5b	2.35, m		1.64, m		1.62, m			
6	1.84, m	33.8, d	2.10, m	31.8, d	1.77, m	31.4, d		144.3, s
7	2.71, m	45.5, d	2.17, m	48.7, d	3.04, m	46.1, d	2.68, s	46.7, t
8a	1.73, t (11.7)	38.0, t	1.47, dd (12.7, 8.0)	44.3, t	1.51, m	45.2, t		38.9, t
8b	1.47, dd (12.6, 8.0)		1.39, dd (12.7, 11.3)		1.38, t (11.5)			
9		43.0, s		35.9, s		37.0, s	3.09, s	48.9, t
10a	3.60, d (4.9)	82.3, d	2.38, dd (13.0, 7.6)	45.6, t	2.25, dd (15.0, 2.4)	47.7, t		167.7, s
10b			1.53, dd (13.0, 11.5)		1.91, m			
11a	4.64, dd (16.3, 2.7)	72.5, t		172.1, s	4.11, d (11.5)	66.2, t	2.92, t (6.9)	36.1, t
11b	4.54, dd (16.3, 2.7)				4.01, d (11.5)			
12		175.8, s		172.3, s	4.29, dd (10.7, 7.4)	63.6, t	3.84, m	62.8, t
					4.24, dd (10.7, 8.5)			
13	0.92, d (7.2)	21.9, q	0.92, d (7.0)	12.6, q	0.82, d (7.0)	11.6, q	2.24, s	16.1, q
14a	1.12, s	22.9, q	1.05, s	31.1, q	1.06, s	28.4, q	1.15, s	29.3, q
14b								
15	0.99, s	26.1, q	1.05, s	31.4, q	0.83, s	26.7, q	1.15, s	29.3, q
1’			3.67, m	40.8, t		171.1, s		
2’			3.76, m	61.5, t	2.06, s	21.0, q		
OMe							3.86, s	51.6, q

248 nm indicated that an α,β-unsaturated keto moiety might be built by these three carbons (C-2, C-3, C-12). The $^1H-^1H$ COSY spectrum afford fragments as shown in Fig. 2. Based on this, the HMBC correlations from two methyl singlets of H-14 and H-15 to δ_C 43.0 (s, C-9), 38.0 (t, C-8), and C-10 indicated a five-membered ring, while the HMBC correlations from H-11 to C-2 and δ_C 45.5 (d, C-1), and from δ_H 2.10 (1H, m, H-4a) and 1.36 (1H, m, H-4b) to C-3 and C-12 indicated a seven-membered ring (Fig. 2). In addition, the HMBC correlation from H-11 to C-12 indicated a γ-lactone ring, and these data also confirmed an 2,3-unsaturated-12-keto moiety as mentioned. One additional hydroxy group at C-10 was also confirmed by NMR and MS data. Therefore, the planar structure of **1** was identified as a 5/7/5-tricyclic compound which should be a tremulane sesquiterpenoid with respect to those reported in the literature [10,15,16]. In the ROESY spectrum (Fig. 2), correlations of H-1/H-7 and H-1/H-6 were observed, which indicated that H-1, H-7, and H-6 should array in the same side. However, the stereoconfiguration of C-10 could not be well established by the ROESY data. Fortunately, the results of the single crystal X-ray diffraction (Flack parameter = 0.06(8); CCDC: 1959030) not only established the structure of **1** as elucidated, but also revealed the absolute configuration (Fig. 3). Compound **1** was, therefore, identified and named phellinignin A.

Compound **2** was isolated as a colorless oil. The molecular formula $C_{17}H_{25}O_4N$ was determined on the basis of its HRESIMS data at m/z 308.18558 $[M + H]^+$ (calcd for $C_{17}H_{26}O_4N^+$: 308.18563). The NMR spectroscopic data (Tables 1 and 2) showed some related patterns to those of **1** including signals for three methyls, one sp^3 quaternary carbon at δ_C 35.9 (s, C-9), one carbonyl carbon at δ_C 172.3 (s, C-12) and two olefinic carbons at δ_C 144.0 (s, C-2) and 142.6 (s, C-3). The significant differences were that one more carbonyl carbon at δ_C 172.1 (s, C-11) in **2** instead of the oxygenated methylene in **1**, and two additional carbons were observed at δ_C 40.8 (t, C-1′) and 61.5 (t, C-2′). The HMBC correlations from δ_H 3.67 (2H, m, H-1′) to C-11 and from δ_H 3.76 (2H, m, H-2′) to C-12, as well as the $^1H-^1H$ COSY cross peak between H-1′

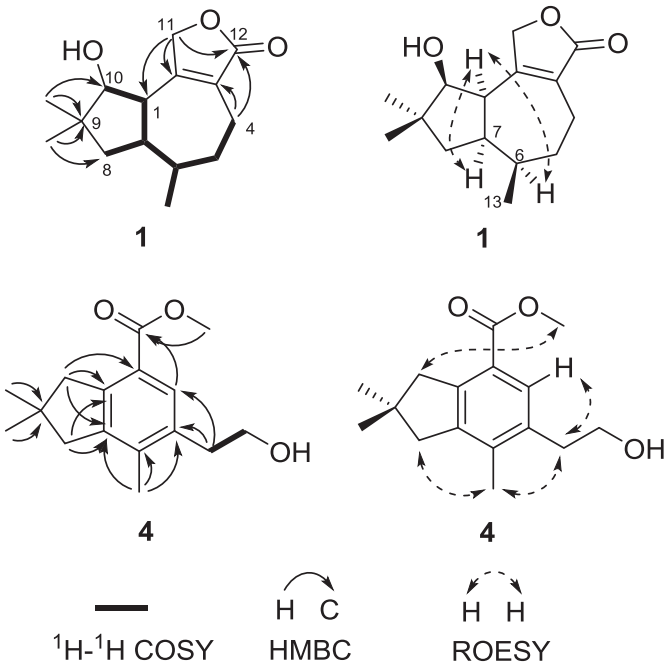


Fig. 2. Key 2D NMR correlations for compounds **1** and **4**.

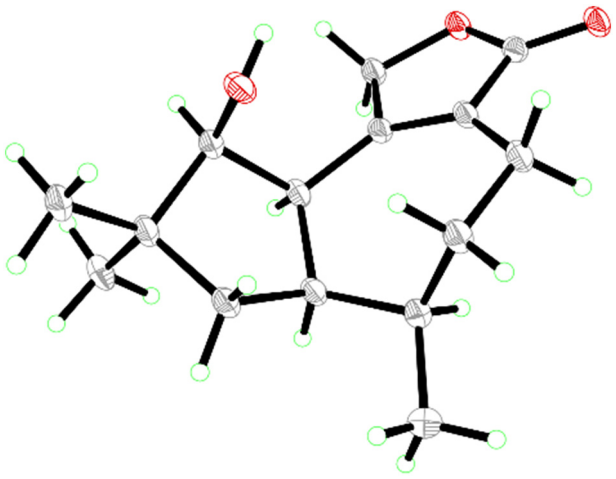


Fig. 3. ORTEP drawing of **1** showing the absolute configuration.

Table 2
Cytotoxicities for **1–8** against three human cancer cell lines (IC_{50}, μM).

No.	HL-60	SMMC-7721	SW480
1	3.8	12.1	0.7
2	> 40	17.4	7.9
3	> 40	> 40	> 40
4	21.1	12.3	13.9
5	7.6	2.6	1.1
6	> 40	> 40	> 40
7	29.8	> 40	> 40
8	> 40	> 40	16.7
Taxol	< 0.008	< 0.008	< 0.008

and H-2′, suggested a 2-aminoethoxyl group which might origin from choline during the biosynthesis. Detailed analysis of 2D NMR data elucidated the structure of **2** as shown. The ROESY correlations of H-1/H-7 and H-1/H-6 suggested that stereochemistry of **2** would be the same to that of **1**.

Compound **3** was isolated as a colorless oil. The molecular formula $C_{17}H_{28}O_4$ was identifed by the HRESIMS data at *m/z* 303.19275 $[M + Na]^+$ (calcd for $C_{17}H_{28}O_4Na^+$: 303.19307). The ^{1}H and ^{13}C NMR data also implied that compound **3** might be a tremulane sesquiterpenoid with a related structure to that of tremulenediol A [17,18]. Two additional carbons at δ_C 171.1 (s, C-1′) and 21.0 (q, C-2′) suggested one *O*-acetyl group in **3**, which was substituted at C-12 as supported by the HMBC correlation from δ_H 4.29 (1H, dd, J = 10.7, 7.4 Hz, H-12a) and 4.24 (1H, dd, J = 10.7, 8.5 Hz, H-12b) to C-1′. Detailed analysis of 2D NMR data suggested that the other parts of **3** were the same to those of tremulenediol A. The structure of compound **3** was, therefore, elucidated and named phellinignin C.

Compound **4** was isolated as a colorless oil. Its molecular formula $C_{16}H_{22}O_3$ was determined by the HRESIMS at *m/z* 263.16418 $[M + H]^+$ (calcd for $C_{16}H_{23}O_3^+$: 263.16417). The ^{13}C NMR and DEPT spectra revealed 16 carbon resonances (Table 1). Except one methoxy group at δ_C 51.6 (q, OMe), the other 15 carbon resonances were likely come from a sesquiterpenoid skeleton including three methyl carbons at δ_C 16.1 (q, C-13) and 29.3 × 2 (q, for C-14 and C-15, respectively). However, six olefinic carbon at δ_C 144.0 (s, C-1), 128.3 (s, C-2), 129.5 (d, C-3), 134.3 (s, C-4), 137.7 (s, C-5), and 144.3 (s, C-6), as well as the UV data at 282 and 256 nm, suggested a penta-substituted phenyl moiety. Analysis of HMBC data, a cyclopentane with a gem-dimethyl substituent was identified as shown in Fig. 2. A methyl ester group was also identified by the HMBC correlation from δ_H 3.86 to δ_C 167.7 (s, C-10). Therefore, a 5/6 bicyclic system for **4** was established. Detailed analysis of 2D NMR data established the substituents on the phenyl including a methyl group, a hydroxylethyl, and the methyl ester group (Fig. 2). Meanwhile, the ROESY cross peaks of H-13/H-7, H-7/H-11, H-11/H-3, and H-9/H-OMe also helped with the location of the substituents (Fig. 2). Finally, the structure of **4** was identified, which was named phellinignin D. Compound **4** represents a new carbon skeleton, which might derive from an illudane precursor [19] by methyl shift and aromatization, as shown in Scheme 1.

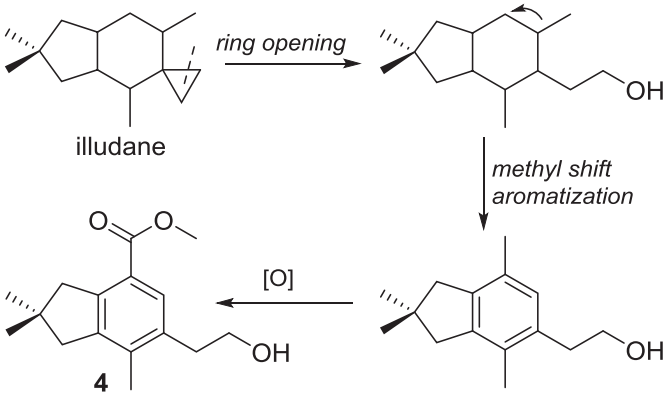
Besides the four new compounds as described, four known ones were identified by comparing their spectroscopic data with those in literature as 11,12-epoxy-12β-hydroxy-1-tremulen-5-one (**6**) [15], tremulenediol B (**7**) [17], conocenols A (**7**) and B (**8**) [18].

Compounds **1–8** were tested for their cytotoxicites against three human cancer cell lines using the modified MTT method. The results (Table 2) showed that compounds **1** and **5** showed better cytotoxicities than those of others, while compound **3** and **6–8** were almost inactive. These data suggested that the γ-lactone in **1** and furan moiety in **5** may contribute much to their cytotoxicities.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Jasco-P-1020 polarimeter



Scheme 1. Possible generation of the skeleton of **4** from an illudane precursor.

(Horiba, Japan). IR spectra were obtained by using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets (Bruker, Germany). NMR spectra were acquired with instrument of a Bruker Avance III with deuterated solvent signals used as internal standards at room temperature (Bruker, Germany). HRESIMS were measured on a Waters Auto Premier P776 spectrometer (Waters, USA). Silica gel (200–300 mesh and 80–100 mesh, Qingdao Marine Chemical Inc., China) and RP-18 gel (20–45 μm, Fuji Silysia Chemical Ltd., Japan) were used for column chromatography (CC). Preparative HPLC was performed on an Agilent 1100 series with a Zorbax SB-C18 (5 μm, 9.4 × 150 mm) column (Agilent Technologies, USA). Fractions were monitored by TLC (thin layer chromatography) (Qingdao Marine Chemical Inc., China) and spots were visualized by heating silica gel plates immersed in H_2SO_4 in EtOH, in combination with the Agilent 1200 series HPLC system (Eclipse XDB-C18 column, 5 μm, 4.6 × 150 mm) (Agilent Technologies, USA).

3.2. Fungal material and cultivation conditions

The fresh fruiting bodies of *Phellinus igniarius* were collected in Guizhou province, China in 2016. A specimen (No. FAMP20160811–2) was deposited at State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University. The culture medium was composed of glucose (5%), pork peptone (0.15%), yeast powder (0.5%), KH_2PO_4 (0.05%) and $MgSO_4$ (0.05%). The initial pH was adjusted to 6.0 and the fermentation was carried out on a shaker at 150 rpm for 23 days.

3.3. Extraction and isolation

The cultural broth (22*L*) was extracted three times with EtOAc. The organic layer was concentrated by evaporator to yield a crude extract (25 g). The extract was subjected to silica gel CC eluted with $CHCl_3$/MeOH (from 1:0 to 0:1) to give seven fractions (A–G). Fraction B (1.3 g) was further isolated by the silica gel CC eluted with petroleum ether/acetone (from 10:1 to 1:1) to give five subfractions B1–B5. Fraction B2 (120 mg) was purified by the HPLC (MeCN/H_2O from 20/80 to 60/40 in 25 min) to give compounds **2** (2.3 mg, retention time (t_R) = 12.4 min), **3** (4.1 mg, t_R = 14.7 min), and **5** (7 mg, t_R = 16.1 min). Compound **1** (3 mg), colorless needles, deposited from fraction B4 after methanol evaporated. Fraction C (2.3 g) was isolated by the RP-18 gel CC (MeOH/H_2O from 30:70 to 90:10) to give four subfractions C1-C3. Fraction C1 (210 mg) was purified by HPLC (MeCN/H_2O from 20/80 to 40/60 in 20 min) to give compounds **8** (9 mg, t_R = 11.3 min) and **7** (12 mg, t_R = 14.4 min). Fraction C3 (78 mg) was purified by HPLC (MeCN/H_2O from 20/80 to 50/50 in 20 min) to give compounds **4** (0.9 mg, t_R = 10.6 min) and **6** (4.1 mg, t_R = 12.7 min).

Phellinignin A (1): colorless crystal (MeOH), mp: 168 °C; $[\alpha]_D^{27}$ + 142.9 (c 0.15, MeOH); UV λ_{max} (log ε): 248 (3.68), 221 (3.88) nm; IR (KBr) ν_{max}: 3340, 2943, 2831, 1702, 1450, 1114, 1031 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 1; HRESIMS: *m/z* 251.16408 $[M + H]^+$ (calcd for $C_{15}H_{23}O_3^+$: 251.16417).

X-Ray crystallographic data for Cu_phellinignin A (1)_0m: a light colorless needle-like of $C_{15}H_{22}O_3$, *M* = 250.32, approximate dimensions 0.071 mm × 0.095 mm × 0.291 mm, was used for the X-ray crystallographic analysis on the BRUKER D8 QUEST. The integration of the data using a orthorhombic unit cell yielded a total of 30,950 reflections to a maximum θ angle of 79.44° (0.78 Å resolution), of which 2874 were independent (average redundancy 10.769, completeness = 99.6%, R_{int} = 7.05%, R_{sig} = 3.40%) and 2706 (94.15%) were greater than 2σ(F^2). The final cell constants of a = 6.3601(6) Å, b = 13.3041(12) Å, c = 15.7450(13) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 1332.3(2) Å3, *T* = 150.(2) K. Data were corrected for absorption effects using the Multi-Scan method (SADABS). The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group P 21 21 21, with Z = 4. The final anisotropic full-matrix least-squares refinement on F^2 with 168 variables converged at R1 = 3.47%, for the observed data and wR2 = 8.90% for all data. The goodness-of-fit was 1.048. The absolute configuration was determined by the Flack parameter = 0.06(8), which was determined using 1096 quotients [(I+)-(I-)]/[(I+) + (I-)]. Crystallographic data for **1** have been deposited in the Cambridge Crystallographic Data Centre at https://www.ccdc.cam.ac.uk (deposition number: CCDC 1959030).

Phellinignin B (2): colorless oil; $[\alpha]_D^{27}$ + 42.7 (c 0.25, MeOH); UV λ_{max} (log ε): 253 (3.67), 224 (3.71) nm; IR (KBr) ν_{max}: 3338, 2943, 2831, 1682, 1452, 1114, 1031 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 1.10; HRESIMS: *m/z* 308.18558 $[M + H]^+$ (calcd for $C_{17}H_{26}O_4N^+$: 308.18563).

Phellinignin C (3): colorless oil; $[\alpha]_D^{27}$ + 32.2 (c 0.1, MeOH); IR (KBr) ν_{max}: 3365, 2947, 2833, 1651, 1450, 1114, 1031 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 1.5; HRESIMS: *m/z* 303.19275 $[M + Na]^+$ (calcd for $C_{17}H_{28}O_4Na^+$: 303.19307).

Phellinignin D (4): colorless oil; $[\alpha]_D^{27}$ – 22.2 (c 0.1, MeOH); UV λ_{max} (log ε): 282 (3.43), 256 (3.61), 221 (3.72) nm; IR (KBr) ν_{max}: 3338, 2943, 2831, 1450, 1114, 1031 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 1.11; HRESIMS: *m/z* 263.16418 $[M + H]^+$ (calcd for $C_{16}H_{23}O_3^+$: 263.16417).

3.4. Cytotoxicity assay

Three human cancer cell lines including human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, and colon cancer SW480 were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The assays were performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates [20]. Briefly, 100 μL of adherent cells was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of 1 × 10^5 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with taxol as a positive control. After each compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method [21].

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Declaration of Competing Interest

The authors declare no Conflict of Interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.104415.

References

[1] D.J. Newman, G.M. Cragg, Natural products as sources of new drugs from 1981 to 2014, J. Nat. Prod. 79 (2016) 629–661.
[2] H.P. Chen, J.K. Liu, Secondary metabolites from higher fungi, Prog. Chem. Org. Nat. Prod. 106 (2017) 1–201.

[3] X.L. Mao, The macrofungi in China, Henan Science and Technology Publishing House: Zhengzhou. 477 (2000).
[4] Y. Dong, P. Qiu, R. Zhu, L.S. Zhao, P.H. Zhang, Y.Q. Wang, C.Y. Li, K.Q. Chai, D. Shou, H.J. Zhao, A combined phytochemistry and network pharmacology approach to reveal the potential antitumor effective substances and mechanism of *Phellinus igniarius*, Front. Pharmacol. 19 (2019), https://doi.org/10.3389/fphar.2019.00266.
[5] S.Y. Mo, Y.C. Yang, J.G. Shi, Studies on chemical constituents of *Phellinus igniarius*, Zhongguo Zhongyao Zazhi 28 (2003) 339–341.
[6] S.Y. Mo, S. Wang, G. Zhou, Y. Yang, Y. Li, X. Chen, J,G. Shi, Phelligridins C–F: cytotoxic pyrano[4,3-c][2]benzopyran-1,6-dione and furo[3,2-c]pyran-4-one derivatives from the fungus *Phellinus igniarius*, J. Nat. Prod. 67 (2004) 823–828.
[7] Y. Wang, S.Y. Mo, S.J. Wang, S. Li, Y.C. Yang, J.G. Shi, A unique highly oxygenated pyrano[4,3-c][2]benzopyran-1,6-dione derivative with antioxidant and cycotoxic activities from the fungus *Phellinus igniarius*, Org. Lett. 7 (2005) 1675–1678.
[8] Y. Wang, S.J. Wang, S.Y. Mo, S. Li, Y.C. Yang, J.G. Shi, A. Phelligridimer, a highly oxygenated and unsaturated 26-membered macrocyclic metabolite with antioxidant activity from the fungus *Phellinus igniarius*, Org. Lett. 7 (2005) 4733–4736.
[9] Y. Wang, X.Y. Shang, S.J. Wang, S.Y. Mo, S. Li, Y.C. Yang, L. He, J.G. Shi, Structures, biogenesis, and biological activities of pyrano[4,3-c]isochromen-4-one derivatives from the fungus *Phellinus igniarius*, J. Nat. Prod. 70 (2007) 296–299.
[10] R.H. Yin, Z.Z. Zhao, H.P. Chen, X. Yin, X. Ji, Z.J. Dong, Z.H. Li, T. Feng, J.K. Liu, Tremulane sesquiterpenes from cultures of the fungus *Phellinus igniarius* and their vascular-relaxing activities, Phytochem. Lett. 10 (2014) 300–303.
[11] Y. Dong, H. Wang, Y. Zhang, N. An, Y. Zhang, D. Shou, Ultra high performance liquid chromatography with synapt high-definitionmass spectrometry and a pattern recognition approach to characterize chemical constituents and rat metabolites after the oral administration of *Phellinus igniarius*, J. Sep. Sci. 38 (2015) 1137–1148.
[12] D. Shou, Y. Dong, N.N. Wang, H.Y. Li, Y. Zhang, Y. Zhu, Simultaneous quantification of antioxidant compounds in *Phellinus igniarius* using ultra performance liquid chromatography-photodiode array detection electrospray ionization tandem mass spectrometry, PLoS One 11 (2016) e0163797, , https://doi.org/10.1371/journal.pone.0163797.
[13] C. Zhou, S.S. Jiang, C.Y. Wang, R. Li, H.L. Che, Different immunology mechanisms of *Phellinus igniarius* in inhibiting growth of liver cancer and melanoma cells, Asian Pac. J. Cancer Prev. 15 (2014) 3659–3665.
[14] K. Sangdee, B. Buranrat, K. Naksuwankul, P. Jaihan, A. Sangdee, Antibacterial and anti-breast cancer cell line activities of Sanghuang Porus sp. 1 extracts, Trop. J. Pharm. Res. 16 (2017) 613–620.
[15] Z.Y. Zhou, J.G. Tang, F. Wang, Z.J. Dong, J.K. Liu, Sesquiterpenes and aliphatic diketones from cultures of the basidiomycete *Conocybe siliginea*, J. Nat. Prod. 71 (2008) 1423–1426.
[16] L.Y. Liu, Z.H. Li, J. Si, Z.J. Dong, J.K. Liu, Two new sesquiterpenoids from the fungus *Ceriporia alachuana*, J. Asian Nat. Prod. Res. 15 (2013) 300–304.
[17] W.A. Ayer, E.R. Cruz, The tremulanes, a new group of sesquiterpenes from the aspen rotting fungus *Phellinus tremulae*, J. Org. Chem. 58 (1993) 7529–7534.
[18] D.Z. Liu, F. Wang, J.K. Liu, Sesquiterpenes from cultures of the basidiomycete *Conocybe siliginea*, J. Nat. Prod. 70 (2007) 1503–1506.
[19] B.M. Fraga, Natural sesquiterpenoids, Nat. Prod. Rep. 28 (2011) 1580–1610.
[20] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Meth. 65 (1983) 55–63.
[21] L.J. Reed, H. Muench, A simple method of estimating fifty per cent endpoints, Am. J. Hyg. 27 (1938) 493–497.