Thieme

Cytotoxic Secondary Metabolites from the Endolichenic Fungus *Hypoxylon fuscum*

Authors
Buddha Bahadur Basnet[1,2*], Baosong Chen[1,3*], Yerlan Melsuly Suleimen[1,4], Ke Ma[1,3], Shouyu Guo[1], Li Bao[1,3], Ying Huang[5], Hongwei Liu[1,3]

Affiliations
1 State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No. 1 Beichenxi Road, Chaoyang District, Beijing, China
2 International College, University of Chinese Academy of Sciences, Beijing, China
3 Savaid Medical School, University of Chinese Academy of Sciences, Beijing, China
4 Institute of Applied Chemistry, Chemistry Department of L. N. Gumilyov Eurasian National University, Astana, the Republic of Kazakhstan
5 State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences. No. 1 Beichenxi Road, Chaoyang District, Beijing, P. R. China

Key words
Hypoxylon fuscum, Hypoxylaceae, endolichenic fungi, polyacetylene, sesquiterpene, cytotoxicity, antimicrobial activity

received March 17, 2019
revised May 28, 2019
accepted June 10, 2019

Bibliography
DOI https://doi.org/10.1055/a-0957-3567
Published online | Planta Med © Georg Thieme Verlag KG Stuttgart · New York | ISSN 0032-0943

Correspondence
Prof. Dr. Hongwei Liu
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences
No. 1 Beichenxi Road, Chaoyang District, 100101 Beijing, P. R. China
Phone: + 86 10 64 80 60 74, Fax: + 86 10 64 80 75 15
liuhw@im.ac.cn

Correspondence
Dr. Li Bao
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences
No. 1 Beichenxi Road, Chaoyang District, 100101 Beijing, P. R. China
Phone: + 86 10 64 80 60 74, Fax: + 86 10 64 80 75 15
baol@im.ac.cn

Supporting information available online at http://www.thieme-connect.de/products

ABSTRACT

As part of our search for new cytotoxic and antimicrobial natural products from endolichenic fungi, 19 compounds including 1 new 10-member lactone (**2**), 1 new polyacetylene glycoside (**3**), 1 new brasilane-type sesquiterpenoid glycoside (**4**), and 2 isobenzofuran-1(3H)-one derivatives (**5** and **6**) were isolated from the solid culture of the endolichenic fungus *Hypoxylon fuscum*. Their structures were unambiguously elucidated by NMR spectroscopic data, MS, ECD (electronic circular dichroism) calculation, and chemical methods. The cytotoxic effects on K562, SW480, and HEPG2 cell lines and the antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, and *Candida albicans* were assessed. Compounds **1**, **2**, and **5** exhibited moderate cytotoxicity against K562, SW480, and HEPG2 cell lines while compounds **1**, **9**, and **11** displayed weak antibacterial activity against *S. aureus*.

* These authors contributed equally to this work

Downloaded by: University of Iowa Libraries. Copyrighted material.

ABBREVIATIONS

BLAST	basic local alignment search tool
DFT	density-functional theory
ECD	electronic circular dichroism
LB medium	lysogeny broth medium
MMFF	Merck Molecular Force Field
ODS	octadecylsilyl silica
SDB medium	Sabouraud Dextrose Broth medium
TDDTF	time-dependent density-functional theory
TFA	trifluoroacetic acid

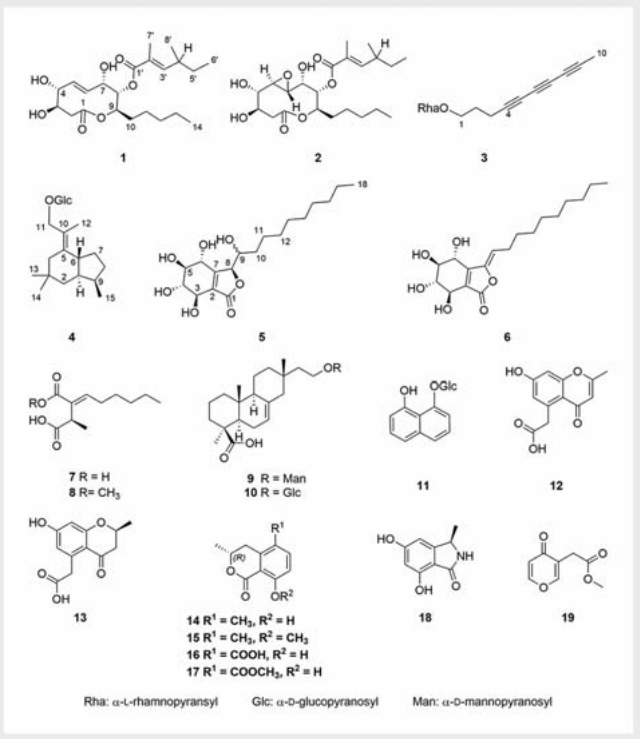
Introduction

Lichens represent a unique symbiotic system composed of mycobiont and photobiont [1]. Recently, endolichenic fungi that inhabit the inner part of lichen without causing disease symptoms have been shown to be an attractive and valuable biological resource for the discovery of new bioactive natural products [2]. A number of secondary metabolites with diverse biological activities including antimicrobial, anti-inflammatory, and antiproliferative properties have been reported from endolichenic fungi [2–4]. However, in comparison with their tremendous species diversity, the number chemically investigated endolichenic fungi are limited.

Hypoxylon fuscum (Pers.) Fr. (Hypoxylaceae) is an ascomycete fungus belonging to the order Xylariales. It has a cosmopolitan distribution in temperate to tropical regions of the world and grows on the bark or decorticated parts of Alnus, Betula, and Corylus tree species [5, 6]. Previous chemical studies on this fungus resulted in the isolation of daldinins A, C, and E–F [7, 8] and 4,5,40,50-tetrahydroxy-1:10-binaphthyl [8]. In addition, cytotoxic compounds, such as fendleryl A and C [9], hypoxyvermelhotins A–C [10], and daldinones C and D [11], antimicrobial compounds including atromentin [9], multiformins A–D [12], and carneic acids A and B [13], were reported from other *Hypoxylon* species. In our preliminary screening, the EtOAc extract of solid culture of *H. fuscum* that was isolated from the lichen *Usnea* sp. by one of the coauthors, Prof. Shouyu Guo, was found to exhibit cytotoxicity against K562 cells (IC_{50} = 250 µg/mL). Chemical investigation on the culture extract of *H. fuscum* fermented on rice led to the isolation of 19 secondary metabolites including 1 new 10-membered lactone (**2**), 1 new polyacetylene glycoside (**3**), 1 new brasilane-type sesquiterpenoid glycoside (**4**), and 2 new isobenzofuran-1(3H)-one derivatives (**5** and **6**). Herein, we report the isolation and structure elucidation of the new compounds, as well as the cytotoxicity and antimicrobial evaluation of all isolates.

Results and Discussion

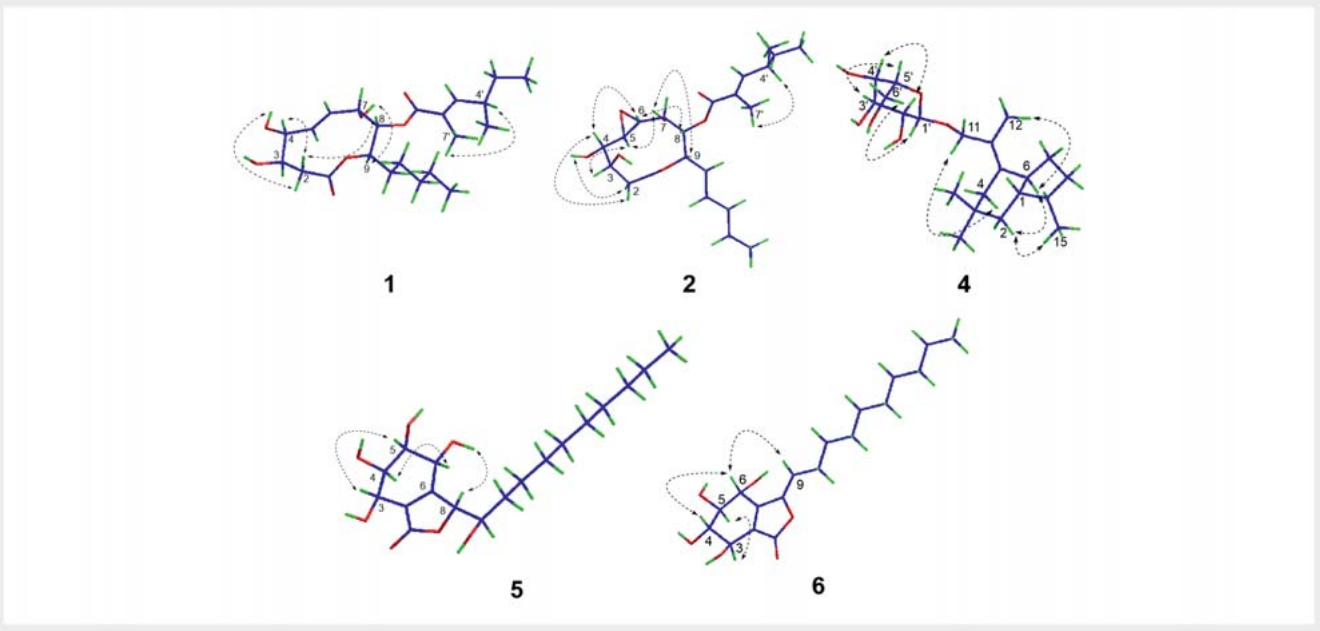
The known compounds **7**–**19** (▶ **Fig. 1**) 2-hexylidene-3-methylsuccinic acid (**7**) [14], 2-hexylidene-3-methylsuccinic acid 4-methyl ester (**8**) [15], 16-α-D-mannopyranosyloxyisopimar-7-en-19-oic acid (**9**) [16], 16-α-D-glucopyranosyloxyisopimar-7-en-19-oic acid (**10**) [16], 8-methoxy-1-naphthyl-β-glucopyranoside (**11**) [17], 2-(7-hydroxy-2-methyl-4-oxo-4H-chromen-5-yl) acetic acid (**12**) [18], (*S*)-2-(7-hydroxy-2-methyl-4-oxochroman-5-yl) acetic acid (**13**) [18], (3*R*)-5-methylmellein (**14**) [19], 3,5-dimethyl-8-methoxy-3,4-dihydroisocoumarin (**15**) [20], (3*R*)-5-carboxymellein (**16**) [19], (3*R*)-5-carbomethoxymellein (**17**) [19], (3*R*)-5,7-dihydroxy-3-methylisoindolin-1-one (**18**) [21], and methyl 4-oxo-4H-pyran-3-acetate (**19**) [22] were identified by comparison of their spectroscopic data with literature values. The elucidation of the structures of the new compounds **2**–**6** (▶ **Fig. 1**) as well as of the relative configuration of the known compound **1** (▶ **Fig. 2**) are reported herein.



▶ **Fig. 1** Structure of compounds **1**–**19**.

The structure of **1** was determined to be the same as that of phomol that was isolated from *Phomopsis* sp. [23], by detailed analysis of its 1D and 2D NMR data (▶ **Table 1**). The relative configuration of phomol was left unassigned in literature. With the help of coupling constants and ROE correlations in the ROESY spectrum, the relative configuration of phomol was assigned herein. When recorded in DMSO-d_6, 3 active hydrogens at δ_H 4.99 (d, *J* = 4.6 Hz, HO-3), 4.86 (d, *J* = 4.1 Hz, HO-4), and 5.30 (d, *J* = 4.5 Hz, HO-7) were observed in the ^{1}H spectrum of **1** (▶ **Table 1**). The ROE correlations (▶ **Fig. 2**) of H-9 with HO-7 and Hβ-2 (δ_H 2.14 [dd, J = 13.3, 11.3]) with H-8 and H-4, the large *trans*-diaxial-type coupling constant between Hβ-2 and H-3 (*J* = 11.3 Hz), H-3 and H-4 (*J* = 8.9 Hz), and H-8 and H-9 (*J* = 9.9 Hz), and the equatorial-axial-type coupling constant between Hα-2 and H-3 (*J* = 3.1 Hz) collectively assigned the α-orientation of H-3 and H-9 and the β-orientation of H-4, H-7, and H-8. The large coupling constant (*J* = 15.6 Hz) between H-5 and H-6 proved the *E*-configuration for the double bond at C-5 and C-6. Moreover, the ROE correlation of H_3-7′ with H-4′ indicated the *E*-configuration for the double bond at C-2′ and C-3′. The configuration at C-4′ was not

Downloaded by: University of Iowa Libraries. Copyrighted material.



▶ **Fig. 2** ROESY correlations of compounds **1**, **2**, and **4**–**6**.

assigned. From the above analysis, the relative configuration was established, as shown in ▶ **Fig. 1**.

5,6-Epoxy-phomol (**2**) was obtained as an oil. Its molecular formula was established as $C_{22}H_{36}O_8$ (5 degrees of unsaturation) by the protonated ion peak at *m/z* 429.2484 $[M + H]^+$ in the HRESI-MS. By careful analysis of ^1H and ^{13}C NMR spectral data (▶ **Table 1**), compound **2** was deduced to have a similar planar structure with that of **1** except for the absence of the double bond between C-5 and C-6. The extra NMR signals at δ_C/δ_H 54.2/2.78 and 58.3/3.20 observed in the ^1H and ^{13}C NMR spectra of **2** and the requirement of the molecular formula suggested the presence of an epoxide moiety. The ^1H-^1H COSY correlations of H-3/H-4/H-5/H-6/H-7 and the HMBC correlations from H-5/H-6 to H-4 and H-7 (▶ **Fig. 3**) confirmed the epoxide moiety at C-5 and C-6. The ROE correlations of H-5 with H-3, OH-4, and OH-7, H-6 with H-4 and H-8, and H-9 with HO-7, the smaller coupling constant (J = 1.9 Hz) between H-5 and H-6 that is consistent with the data reported for 1,2-disubstituted *trans*-oxirane [24], and the larger vicinal coupling constants of 9.2 Hz between H-4 and H-5 and 10.1 Hz between H-8 and H-9 supported α-orientation for H-3, H-5, and H-9 and β-orientation for H-4, H-6, H-7, and H-8. The configuration at C-4′ was left unassigned. Based on the above evidences, the structure of **2** was assigned as 5,6-epoxy-phomol.

Fuscuyne (**3**) was obtained as an amorphous powder. Its molecular formula, $C_{16}H_{20}O_5$, accounting for 7 degree of unsaturation, was established on the basis of HRESI-MS quasi-molecular ion at *m/z* 315.1211 $[M + Na]^+$. The ^1H and ^{13}C NMR spectra (▶ **Table 2**) showed the presence of a rhamnose unit (δ_C/δ_H 99.9/4.53 [d, J = 1.7 Hz], 71.9/3.17 [td, J = 9.4, 5.7 Hz], 70.5/3.58 [m], 70.7/3.40 [m], 68.5/3.38 [m], 18.0/1.13 [d, J = 6.2 Hz]). Apart from the rhamnose moiety, 3 methylene groups (δ_H 3.61 [m]/3.35 [m]; 1.72 [m] and 2.43 [t, J = 6.9 Hz]) and 1 methyl group (δ_H 2.01 [s]) were observed in the ^1H NMR spectrum of **3**. The ^{13}C NMR data in conjunction with the HSQC spectrum suggested the presence of 16 carbons including 6 quaternary carbons (δ_C 79.8, 77.3, 65.2, 64.1, 60.3, 59.2), 1 oxymethylene carbon (δ_C 64.4), 2 methylene carbons (δ_C 27.4, 15.5), 1 methyl carbon (δ_C 3.9), and 6 carbon signals from rhamnose unit (▶ **Table 2**). The structure of **3** was elucidated based on the interpretation of ^1H-^1H COSY and HMBC correlations (▶ **Fig. 3**). The HMBC spectra of **3** showed correlations from H-2 (δ_H 1.72 m) to C-4, from H-3 to C-4, C-5, and C-6, from H_3-10 to C-9, C-8, and C-7, and from the rhamnose anomeric proton (δ_H 4.53) to C-1 (δ_H 3.61), which in combination with NMR data comparison between **3** and deca-4,6,8-triyn-1-ol [25] indicated a deca-4,6,8-triyane moiety linked to a rhamnose unit at C-1 via an ether bond. The α-anomeric configuration of the rhamnose unit was deduced from the C-3′ (δ_C 70.7) and C-5′ (δ_C 68.5) resonances, which appeared at higher fields than those of the corresponding β-anomers [26–27]. To determine the absolute configuration of the rhamnose, compound **3** was hydrolyzed in 2 M HCl and further reacted with L-cysteine methyl ester. The L-configuration of the rhamnose unit was established by HPLC comparison with the derivatives of monosaccharide standards (**Fig. 1S**, Supporting Information). Thus, the structure of **3** was determined to be 1-*O*-α-L-rhamnoside-deca-4, 6, 8-triyne.

Hypoxyside A (**4**) was obtained as a white solid and its molecular formula $C_{21}H_{36}O_6$ (4 degrees of unsaturation) was determined based on the quasi-molecular ion peak at *m/z* 407.2417 $[M + Na]^+$ in the positive HRESI-MS spectrum. Inspection of its ^1H and ^{13}C spectra (▶ **Table 3**) revealed the presence of glucopyranosyl moiety (δ_C/δ_H 97.2/4.57 [d, J = 3.7 Hz], 73.3/3.38 [m], 71.9/3.19 [dd, J = 9.6, 3.8 Hz], 70.4/3.05 [dd, J = 9.6, 8.7 Hz], 72.8/3.38 [m], 61.0/3.62 [dd, J = 11.5, 2.0 Hz]/3.45 [dd, J = 11.5, 5.7 Hz]). Apart from glucopyranosyl moiety, the remaining resonances in ^1H

Downloaded by: University of Iowa Libraries. Copyrighted material.

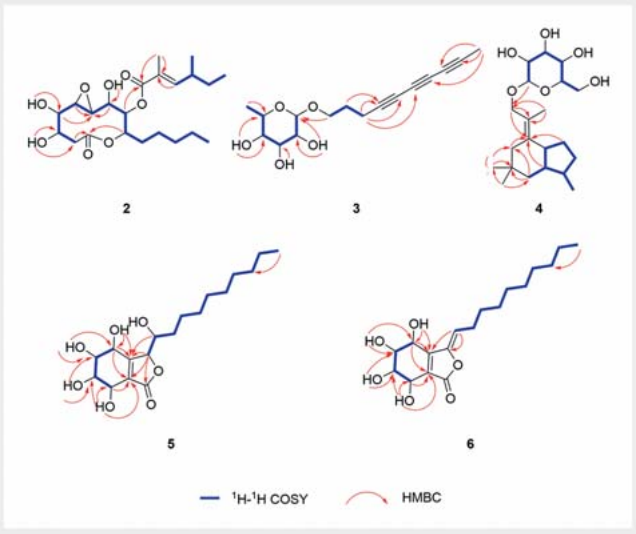
▶ **Table 1** ^{1}H and ^{13}C NMR data of compounds **1** and **2**.

No.	1 (in DMSO-d_6)		1 (in $CDCl_3$)		2 (in DMSO-d_6)	
	δ_C[a]	δ_H[b], mult (*J* in Hz)	δ_C[a]	δ_H[b], mult (*J* in Hz)	δ_C[a]	δ_H[b], mult (*J* in Hz)
1	170.7		171.4		169.6	
2	41.4	2.14, dd (13.3, 11.3)	39.9	2.29, dd (11.0, 12.8)	38.9	2.59, dd (13.4, 3.1)
		2.36, dd (13.3, 3.1)		2.62, d (12.8)		2.31, dd (13.4, 11.3)
3	72.1	3.53, m	72.6	3.95, m	70.9	3.73, m
3-OH		4.99, d (4.6)				5.07, d (4.5)
4	78.8	3.70, td (9.2, 4.1)	79.4	3.96, m	77.6	2.83, m
4-OH		4.86, d (4.1)				5.17, d (4.5)
5	125.9	5.38, ddd (15.6, 9.2, 2.3)	124.6	5.62, dd, (15.7, 7.6)	54.2	2.78, dd (9.2, 1.9)
6	131.8	5.82, dd (15.6, 2.3)	132.6	5.93, dd, (15.7,1.4)	58.3	3.20, d (1.9)
7	68.1	4.35, dq (4.5, 2.3)	70.2	4.58, m	66.5	4.02, dd (5.6, 1.9)
7-OH		5.30, d (4.5)				5.33, d (5.6)
8	74.0	4.69, dd (9.9, 2.3)	74.2	4.90, dd, (10.3,1.7)	73.8	4.87, dd (10.1, 1.9)
9	67.3	5.20, td (9.9, 8.6, 3.1)	68.1	5.34, ddd, (10.3, 8.6, 2.8)	67.4	5.34, ddd (10.1, 8.5, 3.0)
10	30.4	1.33, m	31.0	1.45, m	30.4	1.40, m
		1.44, m		1.55, m		1.49, m
11	23.2	1.19, m	23.3	1.26, m	23.3	1.24, m
12	21.7	1.17, m	22.3	1.26, m	21.7	1.21, m
13	30.8	1.19, m	31.4	1.26, m	30.8	1.20, m
14	13.7	0.79, t (7.1)	13.9	0.85, t (7.0)	13.8	0.83, ov[c]
1′	166.4		167.0		166.4	
2′	125.9		125.8		125.9	
3′	148.4	6.53, dd (10.1, 1.5)	150.0	6.60, dd (10.0, 1.2)	148.6	6.54, dd (10.2, 1.5)
4′	34.2	2.44, m	35.0	2.43, m	34.3	2.45, m
5′	28.9	1.33, m		1.36, m	29.0	1.40, m
		1.42, m		1.44, m		1.29, m
6′	11.7	0.81, t (7.4)		0.87, t (7.3)	11.7	0.83, ov[c]
7′	12.4	1.81, d (1.5)		1.86, s	12.5	1.80, d (1.5)
8′	19.4	0.96, d (6.7)		1.01, d (6.6)	19.4	0.97, d (6.6)

[a] Recorded at 125 MHz with internal reference TMS; [b] Recorded at 500 MHz with internal reference TMS; [c] ov: overlapped signal

NMR accounted for 4 methyl protons (δ_H 0.78 [d, *J* = 6.4 Hz], 0.79 [s], 0.94 [s] and 1.79 [s]) and 1 oxygenated methylene proton (δ_H 4.02 [d, *J* = 10.9 Hz]/3.87 [d, *J* = 10.9 Hz]). The ^{13}C NMR data in conjunction with the HSQC spectrum showed the presence of 21 carbons including 3 quaternary carbons including 2 olefinic ones (δ_C 137.1, 123.7), 5 methylene carbons (δ_C 68.1, 43.7, 40.6, 33.1, 30.8), 4 methyl carbons (δ_C 18.0, 32.1, 25.7, 17.3), 3 methine carbons (δ_C 47.6, 45.1, 31.2), and 6 carbons from the glucose moiety. The 2D NMR data, including the ^{1}H-^{1}H COSY, HSQC, and HMBC (▶ **Fig. 3**), suggested that the planar structure of **4** was a brasilane-type sesquiterpenoid glucoside [28]. In brief, ^{1}H-^{1}H COSY correlations disclosed the spin system from C-2 to C-15 through C-1, C-6, C-7, C-8, and C-9, which together with the HMBC correlations from H-6 to C-4, C-5, and C-10, from H_3-12 and H_2-11 to C-10 and C-5, and from H_3-13 and H_3-14 to C-2, C-3, and C-4 elucidated the structure of the sesquiterpenoid moiety (▶ **Fig. 3**). Furthermore, the HMBC correlation from H-1′ to C-11 established the linkage between the sesquiterpenoid moiety and the glucosyl residue through C-11. Accordingly, the planar structure of **4** was unambiguously established as depicted in ▶ **Fig. 1**. The relative configuration was determined by analysis of coupling constants and ROESY and 1D selective NOE experiments (**Fig. 27S**, Supporting Information). The NOE or ROE correlations Hβ-2 (δ_H 1.14) with H-6 and H_3-15 in combination with the large coupling constant (*J* = 12.8 Hz) between Hβ-2 and H-1 (δ_H 1.61) indicated the α-orientation of H-1 and the β-orientation of H-6 and CH_3-15. The *E*-configuration of the double bond was confirmed by the ROE correlations of H_2-11 with H_2-4 and H_3-12 with H_2-7 (▶ **Fig. 2**). The α-configuration of glucopyranoside unit was determined from the small coupling constant of the anomeric proton ($^{3}J_{H1', H2'}$ 3.7 Hz) (an equatorial-axial coupling) and in comparison with the literature data [29]. The D-configuration was confirmed by chemical hydrolysis followed by derivatization and HPLC analysis as described for compound **3** (**Fig. 1S**, Supporting Informa-

Downloaded by: University of Iowa Libraries. Copyrighted material.



▶ **Fig. 3** ^{1}H-^{1}H COSY and HMBC correlations of compounds **2**–**6**.

▶ **Table 2** ^{1}H and ^{13}C NMR data of compound **3**.

No.	δ_C[a]	δ_H[b], mult (*J* in Hz)
1	64.4	3.61, m
		3.35, m
2	27.4	1.72, m
3	15.5	2.43, t (6.9)
4	79.8	
5	65.2	
6	59.2	
7	64.1	
8	60.3	
9	77.3	
10	3.9	2.01, s
1′	99.9	4.53, d (1.7)
2′	70.5	3.58, m
2′-OH		4.72, d (4.5)
3′	70.7	3.40, m
3′-OH		4.52, d (5.9)
4′	71.9	3.17, td (9.4, 5.7)
4′-OH		4.70, d (5.9)
5′	68.5	3.38, m
6′	18.0	1.13, d (6.2)

[a] Recorded at 125 MHz in DMSO-d_6 with internal reference TMS;
[b] Recorded at 500 MHz in DMSO-d_6 with internal reference TMS

▶ **Table 3** ^{1}H and ^{13}C NMR data of compound **4**.

No.	δ_C[a]	δ_H[b], mult (*J* in Hz)
1	45.1	1.61, m
2	40.6	1.14, t (12.8)
		1.34, dd (12.8, 3.0)
3	33.2	
4	43.7	2.41, dd (13.8, 1.7)
		1.53, d (13.8)
5	137.1	
6	47.6	1.94, m
7	30.8	2.07, m
		1.59, m
8	33.1	2.02, m
		1.04, m
9	31.2	1.96, m
10	123.7	
11	68.1	4.02, d (10.9)
		3.87, d (10.9)
12	17.3	1.79, s
13	32.1	0.94, s
14	25.8	0.79, s
15	18.0	0.78, d (6.4)
1′	97.2	4.57, d (3.7)
2′	73.3	3.38, m
3′	71.9	3.19, dd (9.6, 3.8)
4′	70.4	3.05, dd (9.6, 8.7)
5′	72.8	3.38, m
6′	61.0	3.62, dd (11.5, 2.0)
		3.45, dd (11.5, 5.7)

[a] Recorded at 125 MHz in DMSO-d_6 with internal reference TMS;
[b] Recorded at 500 MHz in DMSO-d_6 with internal reference TMS

tion). Therefore, the structure of compound **4** was determined and named hypoxyside A.

Hypoxyolide A (**5**) was obtained as a white powder and its molecular formula of $C_{18}H_{30}O_7$ (4 degrees of unsaturation) was assigned by a positive HRESI-MS ion peak at *m*/*z* [M + H]$^+$ 359.2060. The ^{1}H NMR spectroscopic data (▶ **Table 4**) exhibited the characteristic resonances of 1 methyl (δ_H 0.86 [t, *J* = 6.7 Hz]), 5 overlapped methylenes at δ_H 1.29, 6 oxygenate methine groups (δ_H 4.85, 4.26, 4.09, 3.31, 3.80, 3.28), and 5 hydroxyl protons at δ_H 5.57, 5.29, 5.10, 5.17, and 4.71. The ^{13}C NMR spectrum of **5** displayed 18 resonances due to 1 methyl carbon (δ_C 14.0), 8 methylene carbons (δ_C 33.9, 31.3, 29.0, 29.0, 28.9, 28.7, 25.6, 22.1), 6 methine carbons (δ_C 82.1, 76.8, 75.9, 68.9, 67.6, 67.4), 2 tetra-substituted olefinic carbons (δ_C 163.4, 126.6), and a lactone carbonyl carbon (δ_C 170.7). The ^{1}H-^{1}H COSY correlations of **5** suggested a contiguous fragment C3–C6 consisting of 4 oxymethines and an alkyl chain fragment C8-C18 that contained 2 oxymethines (C-8 and C-9). Moreover, the HMBC spectrum showed correlations from H-3 and H-6 to C-2 and C-7, from H-8 to C-1, C-2, and C-7, from H-9 to C-7 and C-2, and from HO-6 to C-7 (▶ **Fig. 3**). Thus, the planar structure of **5** was assigned. To determine the relative configuration, the NMR data of **5** were re-measured in pyridine-d_5 (▶ **Table 4**). The large coupling constants

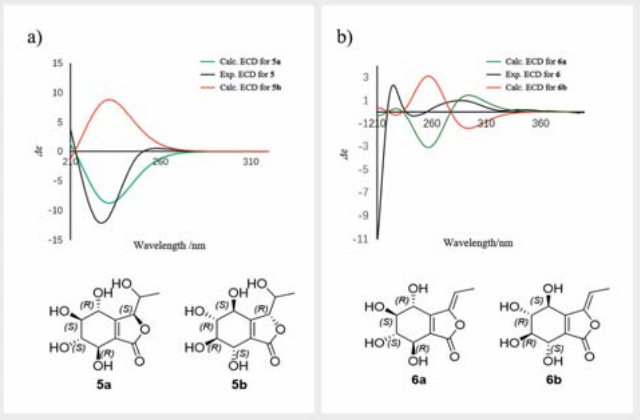
Downloaded by: University of Iowa Libraries. Copyrighted material.

▶ **Table 4** ^{1}H and ^{13}C NMR data of compounds **5** and **6**.

No.	5 (in DMSO-d_6)		5 (in pyridine-d_5)		6 (in DMSO-d_6)	
	δ_C[a]	δ_H[b], mult (*J* in Hz)	δ_C[a]	δ_H[b], mult (*J* in Hz)	δ_C[a]	δ_H[b], mult (*J* in Hz)
1	170.7		172.0		166.1	
2	126.6		128.4		126.2	
3	67.5	4.09, td (2.8, 6.5)	69.5	5.12, dd (7.2, 2.8)	67.4	4.17, td (6.9, 2.6)
3-OH		5.29, d (6.5)				5.34, d (6.9)
4	76.8	3.31, m	78.4	4.46, dd (10.1, 7.2)	75.7	3.35, ddd (10.2, 6.9, 4.0)
4-OH		5.10, d (4.4)				5.23, d (4.0)
5	75.9	3.28, ddd (10.2, 7.1, 4.0)	77.6	4.38, dd (10.1, 7.9)	76.5	3.33, m
5-OH		5.17, d (4.0)				5.19, d (4.0)
6	68.9	4.26, td (7.1, 2.8)	70.6	5.33, dd (7.9, 2.8)	68.5	4.35, td (7.2, 2.6)
6-OH		5.57, d (6.9)				5.68, d (7.2)
7	163.4		164.2		150.9	
8	82.1	4.85, br s	83.6	5.64, br s	145.8	
9	67.4	3.80, m	69.4	4.61, m	115.7	5.81, t (8.0)
9-OH		4.71, d (6.5)				
10	33.9	1.53, m	34.8	2.07, m	25.7	2.28, q (7.5)
				1.93, m		
11	25.6	1.42, m	26.4	1.73, m	28.5	1.41, m
				1.53, m		
12–15	28.7–29.0	1.29, m	29.3–29.7	1.24, m	28.7–28.9	1.26, m
16	31.3	1.25, m	31.8		31.3	1.26, m
17	22.1	1.26, m	22.6	1.21, m	22.1	1.26, m
18	14.0	0.86, t (6.7)	14.0	0.84, t (7.0)	14.0	0.85, t (6.8)

[a] Recorded at 125 MHz with internal reference TMS; [b] Recorded at 500 MHz with internal reference TMS

of $^{3}J_{3H,\,4H}$ (7.2 Hz), $^{3}J_{4H,\,5H}$ (10.1 Hz), and $^{3}J_{5H,\,6H}$ (7.9 Hz) placed these protons in an anti-orientation each other, as shown in ▶ **Fig. 2**. The ROE correlation of H-8 with HO-6 supported that H-8 and H-6 were orientated on the different sides of the ring system. Thus, the α-orientation of H-3, H-5, and H-8 and the β-orientation of H-4 and H-6 were assigned. To determine the absolute configuration of the tetrahydroisobenzofuran-1(3H)-one moiety of **5**, TDDFT-ECD calculations were performed. ECD curves for the 2 simplified stereo isomers (**5a** and **5b**) were calculated using the TD-DFT theory method. As shown in ▶ **Fig. 4**, the calculated curve of isomer **5a** was similar with the experimental CD spectrum. Thus, the absolute configurations of the chiral carbons in **5** were established as 3*R*, 4*S*, 5*S*, 6*R*, and 8*S*. The configuration at C-9 was left unsolved in this study.



▶ **Fig. 4** Comparison of calculated and experimental ECD spectrum (in MeOH) for compounds **5** and **6**.

Hypoxyolide B (**6**) was obtained as a white powder and assigned a molecular formula of $C_{18}H_{28}O_6$ (5 degrees of unsaturation) from the positive HRESI-MS spectrum (*m/z* [M + Na]$^{+}$ 363.1788). The comparison of the ^{1}H and ^{13}C NMR spectra between **5** and **6** (▶ **Table 4**) revealed high structural similarity including the presence of a 4,5,6,7-tetrahydroxy-4,5,6,7-tetrahydroisobenzofuran-1(3H)-one moiety linked to an aliphatic chain. The HMBC cross-peaks of olefinic protons at δ_H 5.81 (H-9) with the C-10 methylene carbon (δ_C 25.7), C-7 (δ_C 150.9), and C-8 olefinic carbon (δ_C 145.8) confirmed the presence of a double bond between C-8 and C-9 (▶ **Fig. 3**). The length of side chain at C-9 was deduced to be nonane due to the requirement of the molecular formula. The similar ^{3}J value observed in the 4,5,6,7-tetrahydroxy-4,5,6,7-tetrahydroisobenzofuran-1(3H)-one moiety between **5** and **6** supported the same relative configuration (▶ **Table**

Downloaded by: University of Iowa Libraries. Copyrighted material.

▶ **Table 5** Cytotoxicity of compounds **1**, **2**, **4**, and **5**.

Compound	IC_{50} (μM)		
	K562	SW480	HepG2
1	19.4 (14.9–25.4)	15.9 (13.1–19.3)	32.7 (20.1–53.2)
2	15.9 (13.1–19.3)	12.0 (8.8–16.4)	28.3 (23.2–34.6)
4	18.7 (15.6–22.3)	> 100	> 100
5	20.6 (14.0–30.3)	20.3 (16.8–24.4)	20.4 (16.4–25.4)
Cisplatin	3.8 (3.1–4.5)	5.5 (3.8–7.9)	6.8 (5.0–9.2)

All data are presented as the mean of IC_{50} values with lower and upper 95 % CI from triplicate measurement (n = 3).

4). In addition, the ROE correlation of H-9 with H-6 indicated the *Z*-configuration for the double bond between C-8 and C-9 (▶ **Fig. 2**). As described for **5**, the absolute configuration of **6** was determined by ECD calculation. The calculated curve of isomer **6a** was similar with the experimental CD spectrum of **6** (▶ **Fig. 4**). Thus, the absolute configurations in **6** were assigned as 3*R*, 4*S*, 5*S*, and 6*R*.

Compounds **1**–**19** were evaluated for their cytotoxicity against a human leukemia cell line (K562), a colon adenocarcinoma cell line (SW480), and a human liver carcinoma cell line (HepG2) (▶ **Table 5**). Compounds **1**, **2**, and **5** showed moderate cytotoxic activity against all the tested cell lines with IC_{50} ranging from 12.0 to 32.7 μM. In addition, compound **4** showed moderate cytotoxicity towards K562 cells with an IC_{50} value of 18.7 μM. Cisplatin was used as the positive control with IC_{50} values of 3.8, 5.5, and 6.8 μM toward K562, SW480, and HepG2 cells, respectively. Compounds **1**–**19** were evaluated for antimicrobial activity against Gram-positive and Gram-negative bacteria and the yeast *Candida albicans*. Compounds 1, 9, and 11 displayed weak antibacterial activity against the Gram-positive bacterium *Staphylococcus* aureus with MICs of 51.2, 96.5, and 89.4 μM, respectively, compared to the positive control vancomycin hydrochloride (MIC, 2.1 μM). None of the tested compounds showed inhibition against *Bacillus subtilis*, *Escherichia coli*, and *C. albicans*.

1D and 2D NMR spectra of compounds **1**–**6**, structures of conformers of **5a** and **6a**, ITS sequence of the isolated fungus, and cytotoxicity activity at various concentrations are available as Supporting Information.

Method and Materials

General experimental procedures

The NMR (^1H, ^{13}C NMR and 2D NMR) were acquired on a Bruker Avance-500 spectrometer. The mass spectra were recorded using an Agilent Accurate-Mass-Q-TOF LC/MS 6520 spectrometer. Analytical HPLC was performed on a Shimadzu (DGU-20A5R) HPLC system using an RP-YMC Pack column (250 × 10 mm, Kromasil, 2.5 μm) and UV detection with a flow rate of 1 mL/min. Preparative HPLC was performed on a Waters 1489 HPLC system using a RP-18 column (250 × 10 mm, Kromasil, 5 μm) and UV detection with a flow rate of 2 mL/min. IR and UV data were recorded on a Nicolet IS5FTIR spectrophotometer and a Thermo Genesys-10S UV-vis spectrophotometer, respectively. CD spectra and optical rotations were obtained on a JASCO J-815 spectral polarimeter and a PerkinElmer 241 polarimeter, respectively. Solvents including MeOH, dichloromethane, and EtOAc used for extraction and chromatographic separation were of analytical grade, and TLC was carried out on silica gel $HSGF_{254}$ plates. The spots were visualized under UV at 254 nm or spraying with 10 % H_2SO_4 followed by heating. Silica gel (150–250 μm, Qingdao Haiyang Chemical Co., Ltd.), octadecylsilyl silica gel (ODS, 50 μm, YMC Co., Ltd.), and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography (CC).

Fungus material

The endolichenic fungus was isolated from the lichen *Usnea* sp. collected from spruce bark at 3400 m in the Lilong Snow Mountain in Lijiang, Yunnan, China, in August 2015. The fungus was identified as *H. fuscum* using morphological techniques and confirmed by amplification, sequencing, and BLAST analysis of internal transcribed spacer (ITS) sequences (Supporting Information) by Dr. Shouyu Guo, one of the co-authors. A lichen voucher specimen was deposited in the Mycological Herbarium of Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS 143163). The fungus was deposited in China General Microbiological Culture Collection (CGMCC 3.19500).

Fermentation and extraction

The fresh mycelia of *H. fuscum* grown on potato dextrose agar medium (28 °C, 10 d) were inoculated in potato dextrose broth and cultured at 17 °C (3 bottles, 100 mL media/250 mL-Erlenmeyer flasks). After 10 d, 5–10 mL of inoculum was inoculated in rice media (30 bottles, 80 g of rice in 100 mL ddH_2O/bottle) and cultured (17 °C, 35 d) in darkness. After 35 d of fermentation, the rice cultures were extracted with EtOAc (2.5 L × 3) under sonication and the extract was dried by evaporation under reduced pressure to yield the crude extract (30.3 g).

Isolation procedure

The EtOAc crude extract was subjected to silica CC (455.7 g) eluted with petroleum ether/EtOAc (100 : 0, 100 : 1, 50 : 1, 30 : 1, 20 : 1, 10 : 1, 5 : 1, 3 : 1, and 2 : 1, v/v) followed by CH_2Cl_2/MeOH (100 : 0, 100 : 1, 50 : 1, 30 : 1, 20 : 1, 10 : 1, 5 : 1, 3 : 1, and 2 : 1, v/v), with 3 L solvent for each step. The collected fractions were combined by TLC analysis to yield 14 fractions (F-1 to F-14).

Downloaded by: University of Iowa Libraries. Copyrighted material.

Fraction 7 (F-7, 1.64 g) eluted with ether/EtOAc 20 : 1 was purified by Sephadex LH-20 CC using MeOH as eluent to give 2 subfractions (F-7-1 and F-7-2). Compound **17** (119.2 mg; t_R = 21.8 min) was obtained by RP-HPLC (60% MeOH in H_2O; 2 mL/min) from F-7-1. Compound **14** (73.2 mg, t_R = 21.2 min) was purified by RP-HPLC (57% MeOH in H_2O; 2 mL/min) from F-7-2. Fraction 8 (F-8, 1.87 g) eluted with CH_2Cl_2/MeOH 100 : 0 was subjected to ODS CC with a gradient elution of MeOH-H_2O (20, 30, 50, 70, and 100%, v/v, 1 L each) to yield 8 subtractions (F-8-1 to F-8-8). Compound **16** (64.9 mg, t_R = 17.2 min) was purified from the subfraction F-8-3 by RP-HPLC (45% MeCN in H_2O [0.01% TFA]; 2 mL/min). Ten subfractions (F-9-1 to F-9–10) were obtained from fraction 9 (F-9, 1.56 g) by ODS CC with a gradient elution of MeOH–H_2O (20, 30, 50, 70, and 100%, v/v, 1 L each). F-9-7 (67 mg) was further purified with RP-HPLC (30% MeCN in H_2O [0.01% TFA]; 2 mL/min) to yield compound **15** (17.3 mg; t_R = 16.7 min). Fraction 10 (F-10, 1.861 g) eluted with CH_2Cl_2/MeOH 20 : 1 was separated by Sephadex LH-20 CC to give 9 subfractions F-10–1 to F-10–9. Compound **19** (7.4 mg) was obtained from F-10–4 (51.3 mg) by preparative TLC using CH_2Cl_2-MeOH (10 : 1, v/v). F-10–5 (43.4 mg) was purified by RP-HPLC (28% MeCN in H_2O [0.01% TFA]; 2 mL/min) to give compounds **3** (7.2 mg; t_R = 16.1 min), **7** (21.3 mg; t_R = 22.6 min), and **8** (35.2 mg; t_R = 32.2 min). F-10–6 (61.4 mg) was separated by RP-HPLC (38% MeOH in H_2O [0.01% TFA]; 2 mL/min) to afford compounds **12** (2.1 mg; t_R = 14.2 min) and **13** (2.0 mg; t_R = 13.9 min). Compound **18** (4.2 mg; t_R = 9.5 min) was obtained from the fraction F-10–7 (33.4 mg) by RP-HPLC (38% MeOH in H_2O [0.01% TFA]; 2 mL/min). Compounds **2** (2.8 mg; t_R = 25.2 min) and **1** (122.3 mg; t_R = 27.5 min) were isolated from the fraction F-10–8 (189.9 mg) by RP-HPLC (70% MeOH in H_2O [0.01% TFA]; 2 mL/min), respectively. Fraction 11 (F-11, 804.7 mg) eluted with CH_2Cl_2/MeOH 10 : 1 was separated into 9 subfractions F-11-1 to F-11–9 by ODS CC (MeOH–H_2O, 20, 30, 50, 70, and 100%, v/v, 1 L each). Compounds **9** (13.1 mg; t_R = 17.5 min) and **5** (6.7 mg; t_R = 21.7 min) were obtained from subfraction F-11–6 (99.3 mg) by RP-HPLC (42% MeCN in H_2O [0.01% TFA]; 2 mL/min). F-11–7 (163.11 mg) was separated with MeOH on Sephadex LH-20 CC followed by purification with RP-HPLC (32% MeCN in H_2O [0.01% TFA]; 2 mL/min) to afford compounds **10** (78.3 mg; t_R = 18.1 min) and **6** (6.5 mg; t_R = 24 min). F-11–8 (123.63 mg) was purified by RP-HPLC (33% MeCN in H_2O [0.01% TFA]; 2 mL/min) to give compounds **4** (9.8 mg; t_R = 21.5 min) and **11** (10.2 mg; t_R = 24.7 min).

*5,6-Epoxyphomol (**2**):* colorless oil; $[\alpha]_D^{25}$ + 62 (*c* 0.2 MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.5); CD (*c* 1.2 × 10^{-6} M, MeOH) λ_{max} ($\Delta\varepsilon$) 223 (− 1.13), 246 (0.32); IR (neat) ν_{max}: 3417, 2958, 2929, 2872, 1682, 1651, 1556, 1538, 1455, 1270, 1235, 1151, 1039, 748, 457 cm^{-1}; HRESI-MS (positive ion mode) *m/z* 429.2484 $[M + H]^+$ (calcd for $C_{22}H_{37}O_8$, 429.2488)·^{1}H and ^{13}C NMR data, see ▶ **Table 1**.

*Fuscuyne (**3**):* white powder; $[\alpha]_D^{25}$ − 131 (*c* 0.2 MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.0); IR (neat) ν_{max}: 3415, 2934, 2892, 2871, 2223, 1669, 1439, 1425, 1127, 1098, 1052, 979, 908, 838, 804 cm^{-1}; HRESI-MS (positive ion mode) *m/z* 315.1211 $[M + Na]^+$, (calcd for $C_{16}H_{20}$ NaO_5, 315.1208). ^{1}H and ^{13}C NMR data, see ▶ **Table 2**.

*Hypoxyside (**4**):* colorless oil; $[\alpha]_D^{25}$ + 77 (*c* 0.2 MeOH); UV (MeOH) λ_{max} (log ε) 220 (2.7); IR (neat) ν_{max}: 3365, 2951, 2869, 1715, 1669, 1556, 1456, 1362, 1146, 1031, 631, 601, 594, 585 cm^{-1}; HRESI-MS (positive ion mode) *m/z* 407.2417 $[M + Na]^+$, (calcd for $C_{21}H_{36}$ NaO_6, 407.2410). ^{1}H and ^{13}C NMR data, see ▶ **Table 3**.

*Hypoxyolide A (**5**):* white powder; $[\alpha]_D^{25}$ + 16 (*c* 0.1 MeOH); UV (MeOH) λ_{max} (log ε) 216 (3.5); CD (*c* 1.5 × 10^{-6} M, MeOH) λ_{max} ($\Delta\varepsilon$) 229 (− 11.7), 252 (0.45); IR (neat) ν_{max}: 3364, 2924, 1752, 1713, 1678, 1346, 1204, 1138, 988, 722, 606, 568, 456 cm^{-1}; HRESI-MS (positive ion mode) *m/z* 359.2060 $[M + H]^+$, (calcd for $C_{18}H_{31}O_7$, 359.2070). ^{1}H and ^{13}C NMR data, see ▶ **Table 4**.

*Hypoxyolide B (**6**):* white powder; $[\alpha]_D^{25}$ + 3 (*c* 0.1 MeOH); UV (MeOH) λ_{max} (log ε) 279 (2.6); CD (*c* 1.5 × 10^{-6} M, MeOH) λ_{max} ($\Delta\varepsilon$) 226 (1.57), 247 (− 0.38),292 (0.25); IR (neat) ν_{max}: 3390, 2955, 2926, 2871, 1746, 1702, 1455, 1287, 1105, 1030, 983, 834, 716 cm^{-1}; HRESI-MS (positive ion mode) *m/z* 363.1788 $[M + Na]^+$ (calcd for $C_{18}H_{28}Na$ O_6, 363.1784). ^{1}H and ^{13}C NMR data, see ▶ **Table 4**.

ECD calculations

Systematic conformation analysis of **5a** and **6a** were conducted with CONFLEX (version 7 Rev. A; CONFLEX Corporation) using the MMFF94 molecular mechanics force field. Optimization with DFT calculation at the B3LYP/6-31G(d) level in MeOH by the Gaussian09 program (Revision C.01, Gaussian Inc.) [30] afforded the MMFF minima. At the B3LYP/6-31G(d) level, the exciting states were calculated using time-dependent density-functional theory (TDDFT) methodology for **5a** and **6a**. The overall ECD spectra were then produced on the basis of Boltzmann weighting of each conformer as described in literature [31].

Acid hydrolysis and determination of absolute configurations of monosaccharides by HPLC

The procedure reported in [32] was followed. Each glycoside (1.0 mg) was hydrolyzed with 2 M HCl (2 mL) for 3 h at 90 °C. After extraction 3 times with $CHCl_3$ (3 × 5 mL), the aqueous layer was evaporated to dryness under vacuum. The dried residue was dissolved in pyridine (0.5 mL, Sigma) containing L-cysteine methyl ester (1.0 mg, Sigma) and heated at 60 °C for 1 h. A solution of *O*-tolylisothiocyanate (5.0 µL, Sigma) was added to the mixture and heated at 60 °C for another 1 h. The reaction mixture was analyzed by HPLC (C_{18} column [Phenomenex Gemini, 5 µm, 4.6 × 250 mm]; mobile phase: MeCN–H_2O–1‰ formic acid [25 : 75, v/v]; detection wavelength: 254 nm; flow rate: 0.8 mL/min; column temperature: 35 °C). The monosaccharides were identified by comparison of their retention time with those of authentic samples treated in the same way (D-glucose: t_R = 19.5 min; L-glucose: t_R = 17.9 min; L-rhamnose: t_R = 33.6 min).

Cytotoxicity assay

The cytotoxicities of compounds **1–19** were investigated with the Cell Counting Kit 8 (CCK8) cell viability assay [4, 33]. K562, SW480, HEPG2 cells were purchased from National Infrastructure of Cell Line Resource. Cells were maintained in RPMI 1640 medium (Sigma) with 10% FBS (Gemini Bio-Products) and grown with 5% CO_2 in an incubator at 37 °C. Cells (1 × 10^4 cells/well) were

Downloaded by: University of Iowa Libraries. Copyrighted material.

cultured in 96-well tissue culture plates at regular culture conditions with appropriate cell number in 100 µL media per well and then treated with cisplatin (Sigma, purity ≥ 98 %) or compounds **1**–**19** for 24 h. All test compounds were dissolved in DMSO as a stock solution, and the final concentration of DMSO in each well was no more than 1 %. Subsequently, viable cells were detected using CCK8. The absorbance of each sample at 450 nm was measured using a microplate reader (Molecular Devices Inc.). The inhibition rate (Inhibitory rate [%] = [1 – ($OD_{treated}/OD_{control}$)] × 100 %) was calculated and plotted versus test concentrations to afford the IC_{50}. All data were calculated by GraphPad Prism 5 and WebApp MDRA [34–35] and presented as geometric mean with confidence intervals of 3 independent experiments.

Antimicrobial assay

Antimicrobial activities of compounds **1**–**19** were evaluated in triplicate as per National Center for Clinical Laboratory Standards recommendations using broth micro dilution method to determine the MIC values [36] with some modifications [37]. The respective MIC values were determined using 2 Gram-positive bacteria, *S. aureus* (CGMCC 1.2465) and *B. subtilis* (ATCC 6633), 1 Gram-negative bacterium, *E. coli* (CGMCC 1.2340), and 1 yeast, *C. albicans* (ATCC 18804). *S. aureus* and *E. coli* were purchased from the CGMCC. *B. subtilis* and *C. albicans* were obtained from the ATCC. In brief, the bacteria were grown in a LB medium (0.5 % yeast extract, 1 % peptone, 0.5 % NaCl in deionized H_2O) while *C. albicans* was grown in SDB medium (Liquid Sabouraud Medium, 0.4 % glucose, 1 % peptone in deionized H_2O). The assay was carried out in flat bottom 96-well microtiter plates. Microorganisms were pre-incubated at 37 °C for 24 h in medium. Compounds **1**–**19** were dissolved in DMSO at an initial concentration at 20 mM, and then 1 µL was added to 99-µL medium in 96-well microtiter plates. After serial 2-fold dilution with the medium, each well contained 50 µL of the test compounds at different concentrations (final DMSO concentration less than 5 %). Then the microorganism solution was added into the 96-well plate (50 µL per well). The densities of the cells were approximately 1.0×10^6 CFU/mL. Positive control drugs were vancomycin hydrochloride (Sigma, purity > 900 µg/mg) for *S. aureus* and *B. subtilis*, ampicillin (Sigma, purity ≥ 99 %) for *E. coli*, and amphotericin B (Sigma, approximately 80 %) for *C. albicans*. After 24 h incubation, the absorbance was determined at 600 nm by a microplate reader. The MIC value was determined as the lowest concentration inhibiting microbial growth.

Supporting Information

1D and 2D NMR spectra of compounds **1**–**6**, structures of conformers of **5a** and **6a**, ITS sequence of the isolated fungus, and cytotoxicity activity at various concentrations are available as Supporting Information.

Acknowledgements

This work was supported by National Natural Science Foundation of China (Grant 81673334). Dr. Jinwei Ren and Dr. Wenzhao Wang (State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences) are appreciated for their help in measuring the NMR and MS data.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Honegger R. The lichen symbiosis-what is so spectacular about it? Lichenologist 1998; 30: 193–212

[2] Kellogg JJ, Raja HA. Endolichenic fungi: a new source of rich bioactive secondary metabolites on the horizon. Phytochem Rev 2017; 16: 271–293

[3] Suryanarayanan TS, Thirunavukkarasu N. Endolichenic fungi: the lesser known fungal associates of lichens. Mycology 2017; 8: 189–196

[4] Basnet BB, Liu L, Chen B, Suleimen YM, Yu H, Guo S, Li B, Liu H. Four new cytotoxic arborinane-type triterpenes from the endolichenic fungus *Myrothecium inundatum*. Planta Med 2019. doi:10.1055/a-0855-4051

[5] Bodo B, Tih RG, Davoust D, Jacquemin H. Hypoxylone, a naphthyl-naphthoquinone pigment from the fungus *Hypoxylon sclerophaeum*. Phytochemistry 1983; 22: 2579–2581

[6] Fournier J, Köpcke B, Stadler M. New species of *Hypoxylon* from Western Europe and Ethiopia. Mycotaxon 2010; 113: 209–235

[7] Mühlbauer A, Triebel D, Persoh D, Wollweber H, Seip S, Stadler M. Macrocarpones, novel metabolites from stromata of *Hypoxylon macrocarpum*, and new evidence on the chemotaxonomy of *Hypoxylon* species. Mycol Prog 2002; 1: 235–248

[8] Quang DN, Hashimoto T, Tanaka M, Stadler M, Asakawa Y. Cyclic azaphilones daldinins E and F from the ascomycete fungus *Hypoxylon fuscum* (Xylariaceae). Phytochemistry 2004; 65: 469–473

[9] Intaraudom C, Bunbamrung N, Dramae A, Boonyuen N, Kongsaeree P, Srichomthong K, Supothina S, Pittayakhajonwut P. Terphenyl derivatives and drimane – Phathalide/isoindolinones from *Hypoxylon fendleri* BCC32408. Phytochemistry 2017; 139: 8–17

[10] Kuhnert E, Heitkämper S, Fournier J, Surup F, Stadler M. Hypoxyvermelhotins A–C, new pigments from *Hypoxylon lechatii* sp. nov. Fungal Biol 2014; 118: 242–252

[11] Gu W, Ge HM, Song YC, Ding H, Zhu HL, Zhao XA, Tan RX. Cytotoxic benzo[j]fluoranthene metabolites from *Hypoxylon truncatum* IFB-18, an endophyte of *Artemisia annua*. J Nat Prod 2007; 70: 114–117

[12] Quang DN, Hashimoto T, Stadler M, Radulović N, Asakawa Y. Antimicrobial azaphilones from the fungus *Hypoxylon multiforme*. Planta Med 2005; 71: 1058–1062

[13] Quang DN, Stadler M, Fournier J, Asakawa Y. Carneic Acids A and B, chemotaxonomically significant antimicrobial agents from the Xylariaceous ascomycete *Hypoxylon carneum*. J Nat Prod 2006; 69: 1198–1202

[14] Chinworrungsee M, Kittakoop P, Isaka M, Rungrod A, Tanticharoen M, Thebtaranonth Y. Antimalarial halorosellinic acid from the marine fungus *Halorosellinia oceanica*. Bioorg Med Chem Lett 2001; 11: 1965–1969

[15] Sabitha G, Yadagiri K, Swapna R, Yadav JS. The first total synthesis of putaminoxin and determination of its absolute configuration. Tetrahedron Lett 2009; 50: 5417–5419

[16] Shiono Y, Motoki S, Koseki T, Murayama T, Tojima M, Kimura K. Isopimarane diterpene glycosides, apoptosis inducers, obtained from fruiting bodies of the ascomycete *Xylaria polymorpha*. Phytochemistry 2009; 70: 935–939

Downloaded by: University of Iowa Libraries. Copyrighted material.

[17] Cameron DW, Craik JCA. Colouring matters of the aphididae. Part XXXVI. The configuration of the glucoside linkage in protoaphins. J Chem Soc C Org 1968; 0: 3068–3072

[18] Kashiwada Y, Nonaka G, Nishioka I. Studies on rhubarb (rhei rhizoma). V. Isolation and characterization of chromone and chromanone derivatives. Chem Pharm Bull (Tokyo) 1984; 32: 3493–3500

[19] Sumarah MW, Puniani E, Blackwell BA, Miller JD. Characterization of polyketide metabolites from foliar endophytes of *Picea glauca*. J Nat Prod 2008; 71: 1393–1398

[20] Kokubun T, Shiu W, Gibbons S. Inhibitory activities of lichen-derived compounds against methicillin- and multidrug-resistant *Staphylococcus aureus*. Planta Med 2007; 73: 176–179

[21] El Amrani M, Debbab A, Aly AH, Wray V, Dobretsov S, Müller WEG, Lin W, Lai D, Proksch P. Farinomalein derivatives from an unidentified endophytic fungus isolated from the mangrove plant *Avicennia marina*. Tetrahedron Lett 2012; 53: 6721–6724

[22] Edwards RL, Maitland DJ, Pittayakhajonwut P, Whalley AJS. Metabolites of the higher fungi. Part 33. Grammicin, a novel bicyclic $C_7H_6O_4$ furanopyranol from the fungus *Xylaria grammica* (Mont.) Fr. J Chem Soc Perkin 1 2001; 11: 1296–1299

[23] Weber D, Sterner O, Anke T, Gorzalczancy S, Martino V, Acevedo C. Phomol, a new anti-inflammatory metabolite from an endophyte of the medicinal plant *Erythrina crista*-galli. J Antibiot (Tokyo) 2004; 57: 559–563

[24] Evidente A, Capasso R, Abouzeid MA, Lanzetta R, Vurro M, Bottalico A. Three new toxic pinolidoxins from *Ascochyta pinodes*. J Nat Prod 1993; 56: 1937–1943

[25] Machado VR, Biavatti MW, Danheiser RL. A short and efficient synthesis of the polyacetylene natural product deca-4,6,8-triyn-1-ol. Tetrahedron Lett 2018; 59: 3405–3408

[26] Lee MH, Son YK, Han YN. Tissue factor inhibitory sesquiterpene glycoside from *Eriobotrya japonica*. Arch Pharm Res 2004; 27: 619

[27] Kasai R, Okihara M, Asakawa J, Mizutani K, Tanaka O. ^{13}C NMR study of α- and β-anomeric pairs of D-mannopyranosides and L-rhamnopyranosides. Tetrahedron 1979; 35: 1427–1432

[28] Hu ZY, Li YY, Huang YJ, Su WJ, Shen YM. Three new sesquiterpenoids from *Xylaria* sp. NCY2. Helv Chim Acta 2008; 91: 46–52

[29] Roslund MU, Tähtinen P, Niemitz M, Sjöholm R. Complete assignments of the ^1H and ^{13}C chemical shifts and $J_{H,H}$ coupling constants in NMR spectra of D-glucopyranose and all D-glucopyranosyl-D-glucopyranosides. Carbohydr Res 2008; 343: 101–112

[30] Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery JA jr., Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Keith T, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ. Gaussian 09, Revision C.01. Gaussian Inc.: Wallingford; 2010

[31] Xue GM, Zhu DR, Han C, Wang XB, Luo JG, Kong LY. Artemisianins A–D, new stereoisomers of seco-guaianolide involved heterodimeric [4 + 2] adducts from *Artemisia argyi* induce apoptosis via enhancement of endoplasmic reticulum stress. Bioorg Chem 2019; 84: 295–301

[32] Tanaka T, Nakashima T, Ueda T, Tomii K, Kouno I. Facile discrimination of aldose enantiomers by reversed-phase HPLC. Chem Pharm Bull (Tokyo) 2007; 55: 899–901

[33] Liu L, Wang L, Bao L, Ren J, Bahadur Basnet B, Liu R, He L, Han J, Yin WB, Liu H. Versicoamides F–H, prenylated indole alkaloids from *Aspergillus tennesseensis*. Org Lett 2017; 19: 942–945

[34] Jiang X, Kopp-Schneider A. Statistical strategies for averaging EC50 from multiple dose-response experiments. Arch Toxicol 2015; 89: 2119–2127

[35] Jiang X. Dose-Response Analysis of Multiple Experiments. Available at http://biostatistics.dkfz.de/mdra_v1.3/. Accessed May 16, 2019

[36] Eloff J. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 1998; 64: 711–713

[37] Nzogong R, Nganou B, Tedonkeu A, Awouafack M, Tene M, Ito T, Tan P, Morita H. Three new abietane-type diterpenoids from *Plectranthus africanus* and their antibacterial activities. Planta Med 2018; 84: 59–64

Downloaded by: University of Iowa Libraries. Copyrighted material.