Secondary Metabolites from the Endophytic Fungus *Xylaria* sp. hg1009

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Abstract

A detailed chemical investigation of the secondary metabolites produced by the endophytic fungus *Xylaria* sp. isolated from the stems of *Isodon sculponeatus* afforded six new compounds, xylariahgin A–F (**1–6**), two new natural products (**7** and **8**), along with two known compounds (**9** and **10**) (Fig. 1). The structures of all compounds were unambiguously established by analyzing their spectroscopic data or referring to pertinent literature. Compounds **1–8** were tested for their cytotoxic activity against five human tumor cell lines.

Keywords Endophytic fungus · *Xylaria* sp. · Secondary metabolites · Cytotoxicity

1 Introduction

Endophytic fungi are microorganisms, parasitizing plants without causing any obvious disease at any specific moment of their colonizing period [1]. Although interactions between endophytes with their hosts are not fully understood in most cases, many endophytes produce bioactive secondary metabolites that may protect hosts from herbivores, plant pathogens, and abiotic stressors [2, 3]. The bioactive secondary metabolites produced by endophytes may be useful in medicine, industry and agriculture [4–8]. In addition, plants which possess significant biological activity have the potential of harbouring endophytes with great biodiversity [9]. The genus *Isodon* is an abundant source of diterpenoids with diverse chemical structures, of which some ones exhibit antitumor and anti-

inflammatory activities [10–12]. Therefore, we assumed that the secondary metabolites produced by endophytic fungi in *Isodon* species could be functional in biology and structural diversity. Our previous investigation had already testified that some secondary metabolites secreted by endophytic fungus from *I. eriocalyx* var. *laxiflora* possessed interesting structure and diverse bioactivities, such as penicillifuranone A isolated from *Penicillium* sp. sh18 [13], phomopchalsins A and B isolated from *Phomopsis* sp. shj2 [14] and phomopsiketones A–C isolated from *Phomopsis* sp. sh917 [15]. Accordingly, we have extended our attention to endophytic fungus in *Isodon* species. In this research, we have conducted our research with the fungus *Xylaria* sp., isolated from the stems of *I. sculponeatus*. Fungi in the genus *Xylaria* have been a rich source of compounds with intriguing structure, including pestalotin 4'-*O*-methyl- β -mannopyranoside and 3*S*,4*R*-(+)-4-hydroxymellein from *Xylaria feejeensis* [16], nigriterpenes A–F from *X. nigripes* YMJ653 [17], cytochalasin Z₂₇ and cytochalasin Z₂₈ from *Xylaria* sp. XC-16 [18].

Through OSMAC strategy analysis [19], Rice and MEA media were selected as the larger fermentation media for subsequent study. As a result, ten compounds, including six new ones, named xylariahgin A–F (**1–6**), two new natural products, 3-(2,3-dihydroxypropyl)-6,8-dimethoxyisocoumarin and methyl 4-(hexanoylamino) benzoate (**7** and **8**), and two known ones, (–)-5-carboxymellein and 4-methoxyisoquinolin-1(2*H*)-one (**9** and **10**), were discovered from two culture extract. The isolation, structure

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Fig. 1 Structures of compounds **1–10** and orthosporin dimethyl ether

elucidation and bioactivity of these compounds, are herein described (Figs. 1, 2).

2 Results and Discussion

On the basis of HRESIMS ($[M+Na]^+$ 231.0624, calcd for 231.0628) and ^{13}C NMR data, xylariahgin A (**1**) has the molecular formula $C_{11}H_{12}O_4$, indicating six degrees of unsaturation. Its IR absorptions at 1781 and 1663 cm^{-1} implied the existence of lactone and $C=C$ groups. The 1H , ^{13}C NMR and DEPT spectra revealed the presence of one methyl group, two methylenes, five methines including three olefinic methines, three quaternary carbons (two carbonyls, one olefinic group) (Table 1). The 1H – 1H COSY spectrum of **1** exhibited the correlations of H_3 – H_2 /H–9/H–9/H–8/H–7. While its HMBC spectrum showed the

correlations from H_b –10 (δ_H 1.53, m) to C–8 and C–12, H_b –8 (δ_H 2.06, m) to C–10 and C–12, H –7 (δ_H 5.28, dd, 9.9, 6.1) to C–12. These results indicated that **1** has a furanone moiety. Similarly, the structure of pyrone is supported by the HMBC correlations from H –5 (δ_H 6.26, dd, 5.9, 2.5) to C–6 and C–3, from H –3 (δ_H 6.37, d, 2.5) to C–4, C–7 and C–2, from H –6 (δ_H 8.05, d, 5.9) to C–4, C–2 and C–5 (Fig. 2). Furthermore, the HMBC correlations from H_b –8 to C–4, from H –3 to C–7 determined the direct connection of the furanone moiety with the pyrone moiety. Additionally, the key ROESY correlation between H –7 and H –9 supported that the H –7 and H –9 were cofacial and were arbitrarily assigned as β -oriented (Fig. 3). Therefore, the structure and relative configuration of compound **1** was determined.

Xylariahgin B (**2**) was obtained as colorless oil. Its IR absorptions at 3422, 2932, 1657 and 1595 cm^{-1} implied

Fig. 2 Key HMBC (blue arrows H $\rightarrow$ C), ^1H - ^1H COSY (red lines) correlations of **1**–**8**

the presence of OH, COOH, lactone and C=C groups. Its HRESIMS displayed a molecular ion peak $[\text{M}+\text{Na}]^+$ at m/z 249.0730 (calcd for 249.0733), corresponding to the molecular formula $\text{C}_{11}\text{H}_{14}\text{O}_5$. Comparison of its ^1H and ^{13}C NMR spectra (Table 1) with those of **1** indicated that these two compounds were closely related. The only difference was that **2** had less one degree of unsaturation than **1**. Furthermore, the HMBC correlations from H_3 -11 (δ_{H} 0.96, t, 7.4) to C-10 and C-9, H_2 -10 (δ_{H} 1.70, m; 1.58, m) to C-9, C-8 and C-12, H_2 -8 (δ_{H} 2.13, m; 1.96, m) to C-10, C-9, C-12 and C-4 suggested that the furanone ring of **1** was opened in **2** (Fig. 2). Above data together with ^1H - ^1H COSY and other key HMBC correlations from H-5 (δ_{H} 6.34, dd, 5.8, 2.4) to C-6 and C-3, from H-3 (δ_{H} 6.59, d, 2.4) to C-4, C-7 and C-2, from H-6 (δ_{H} 7.77, d, 5.8) to C-4, C-2 and C-5 elucidated the planar structure of **2**. The relative configuration of **2** was determined by a hydrolysis reaction of **1**. **1** was exposed to $\text{K}_2\text{CO}_3/\text{MeOH}$ at room temperature for 4 h, with HPLC–DAD analysis detecting conversion to **2** (Fig. S31).

Xylariahgin C (**3**) was found by HRESIMS ($[\text{M}+\text{Na}]^+$ m/z 263.0884, calcd for 263.0890) to possess the molecular formula $\text{C}_{12}\text{H}_{16}\text{O}_6$ as determined. Its IR absorptions at 3395, 1732 and 1660 cm^{-1} implied the presence of OH, lactone and C=C groups. The ^1H and ^{13}C NMR spectra

(Table 1) of **3** were similar to those of **2** except that there was one more methoxy group in compound **3**. The HMBC correlation from CH_3O -12 to C-12 verified the presence of methoxy signal. The ^1H - ^1H COSY correlations of H_3 -11/ H_2 -10/H-9/H-8/H-7 and H-5/H-6, coupled with the key HMBC correlations from H-8 (δ_{H} 2.15, m; 1.93, m) to C-4 and C-12, and from H-10 (δ_{H} 1.62, m) to C-12 determined the structure of **3** (Fig. 2). Similarly, the relative configuration of **3** was also supported by the hydrolysis reaction of **1**. Similar treatment of **1** with $\text{K}_2\text{CO}_3/\text{MeOH}$ resulted in conversion to **2** and **3**, simultaneously. Therefore, the relative configuration of **3** was confirmed (Fig. S31).

Xylariahgin D (**4**) was found to have the molecular formula $\text{C}_{11}\text{H}_{14}\text{O}_4$ established by HRESIMS at m/z 233.0784 $[\text{M}+\text{Na}]^+$ (calcd for 233.0784), indicating five degrees of unsaturation. Its IR absorptions at 1779 and 1671 cm^{-1} implied the presence of lactone and C=C groups. The ^1H NMR spectrum (Table 1) showed typical signals assignable to one methyl at δ_{H} (1.01, t, 7.5), one olefinic methine at δ_{H} (5.61, s). The ^{13}C NMR and DEPT spectra of **4** displayed resonances for 11 carbons, ascribed to one methyls, four methylenes (including one oxygenated methylene), three methines (one olefinic, one oxygenated methine), and three quaternary carbons (two carbonyls). These data accounted for all ^1H and ^{13}C NMR resonances

Table 1 NMR data of **1–4** (δ in ppm, J in Hz)

No	1 ^a		2 ^b		3 ^c		4 ^b	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1								
2		178.3, s		180.4, s		178.6, s		171.6, s
3	6.37, 1H (d, 2.5)	115.1, d	6.59, 1H (d, 2.4)	112.7, d	6.30, 1H (d, 2.5)	113.5, d	5.61, 1H (s)	103.7, d
4		165.2, s		171.3, s		171.2, s		191.6, s
5	6.26, 1H (dd, 5.9, 2.5)	117.7, d	6.34, 1H (dd, 5.8, 2.4)	116.7, d	6.17, 1H (dd, 5.8, 2.5)	117.4, d	2.60, 2H (m)	36.1, t
6	8.05, 1H (d, 5.9)	156.9, d	7.77, 1H (d, 5.8)	155.6, d	7.97, 1H (d, 5.8)	156.3, d	4.56, 2H (m)	68.7, t
7	5.28, 1H (dd, 9.9, 6.1)	75.4, d	4.56, 1H (dd, 8.6, 4.2)	69.7, d	4.53, 1H (m)	69.1, d	4.80, 1H (dd, 9.5, 6.5)	75.0, d
8	2.82, 1H (m)	32.8, t	2.13, 1H (m)	36.8, t	2.15, 1H (m)	37.7, t	2.62, 1H (m)	32.3, t
	2.06, 1H (m)		1.96, 1H (m)		1.93, 1H (m)		1.96, 1H (m)	
9	2.83, 1H (m)	41.9, d	2.49, 1H (m)	44.3, d	2.55, 1H (m)	44.3, d	2.61, 1H (m)	41.3, d
10	1.86, 1H (m)	23.9, t	1.70, 1H (m)	26.1, t	1.62, 2H (m)	26.1, t	1.95, 1H (m)	23.5, t
	1.53, 1H (m)		1.58, 1H (m)				1.55, 1H (m)	
11	0.99, 3H (t, 7.5)	11.7, q	0.96, 3H (t, 7.4)	11.5, q	0.88, 3H (t, 7.4)	11.7, q	1.01, 3H (t, 7.5)	11.6, q
12		177.8, s		179.9, s		176.3, s		177.2, s
12-OMe					3.60, 3H (s)	51.7, q		

The assignments were based on HSQC, ^1H - ^1H COSY, and HMBC experiments

^a ^1H and ^{13}C NMR data were recorded at 500 MHz and 125 MHz in Acetone- d_6 , respectively

^b ^1H and ^{13}C NMR data were recorded at 500 MHz and 125 MHz in CDCl_3 , respectively

^c ^1H and ^{13}C NMR data were recorded at 600 MHz and 150 MHz in Acetone- d_6 , respectively

Fig. 3 ROESY correlation (blue dashed double-headed arrow) of **1**

and indicated **4** is a polyketide. The ^1H - ^1H COSY correlations of $\text{H}_2\text{-5}/\text{H}_2\text{-6}$, suggested the connection from C-5 to C-6. The detailed planar structure of **4** was further constructed by the HMBC correlations. The HMBC correlations from H-3 (δ_{H} 5.61, s) to C-2, C-4 and C-5, $\text{H}_2\text{-5}$ (δ_{H} 2.60, m) to C-4, and $\text{H}_2\text{-6}$ (δ_{H} 4.56, m) to C-4, C-2, indicated the presence of pyrone. In the HMBC spectrum of **4**, correlations from $\text{H}_2\text{-8}$ (δ_{H} 2.62, m; 1.96, m) to C-7, C-12 and C-10, coupled with the ^1H - ^1H COSY correlations of H-7/ $\text{H}_2\text{-8}$ / $\text{H}_2\text{-9}$ / $\text{H}_2\text{-10}$ / $\text{H}_3\text{-11}$ proved the presence of furanone moiety (Fig. 2).

In **4**, the pyrone moiety with the connection to furanone moiety was confirmed from key HMBC correlations from H-7 to C-2, C-3, and H-8 to C-2. Herein, the structure of pyranone derivative was elucidated. In addition, there was no obvious ROESY correlation of H-7/H-9, indicative of their opposite oriented, and H-7 were arbitrarily assigned as β -oriented, H-9 were arbitrarily assigned as α -oriented.

Xylariahgin E (**5**) was isolated as yellowish oil, and its molecular formula was established to be $\text{C}_{13}\text{H}_{18}\text{O}_5$ by HRESIMS at m/z 253.1081 [$\text{M}-\text{H}$] $^-$ (calcd for 253.1087). Its IR absorptions at 2928, 1773 and 1745 cm^{-1} implied the presence of lactone and C=C groups. Comparison of the ^1H and ^{13}C NMR data (Table 2) of compound **5** with that of 2-carboxymethyl-3-hexylmaleic anhydride indicated that both compounds had identical skeleton and substitution patterns, differing only in that compound **5** was esterified in C-6 [20]. This conclusion was verified by the key HMBC correlations from $\text{H}_2\text{-5}$ (δ_{H} 3.52, s) to C-1 and C-6, from $\text{H}_2\text{-7}$ (δ_{H} 2.48, m) to C-4, and from 6-OMe (δ_{H} 3.75, s) to C-6 (Fig. 2). Herein, **5** was assigned as a new dialkylmaleic anhydride derivative.

Xylariahgin F (**6**) was obtained as a yellowish amorphous powder and gave a HRESIMS ion peak at m/z 320.1108 [$\text{M}+\text{Na}$] $^+$, calcd for 320.1105), which

Table 2 NMR data of **5-8** (δ in ppm, J in Hz)

No	5^b		No	6^b		No	7^a		No	8^c	
	δ_{H}	δ_{C}		δ_{H}	δ_{C}		δ_{H}	δ_{C}		δ_{H}	δ_{C}
1		165.2, s	1		170.2, s	1		167.8, s	1		125.3, s
2		136.2, s	2	3.49, 2H (s)	28.8, t	2		103.5, s	2	8.92, 1H (d, 8.7)	131.1, d
3		147.7, s	3		132.8, s	3		164.8, s	3	7.77, 1H (d, 8.7)	119.1, d
4		165.2, s	4	2.42, 2H (t, 7.5)	24.2, t	4	6.58, 1H (d, 1.9)	99.7, d	4		144.7, s
5	3.52, 2H (s)	29.2, t	5	1.52, 2H (m)	28.0, t	5		162.2, s	5	7.77, 1H (d, 8.7)	119.1, d
6		167.7, s	6	1.30, 2H (overlap)	29.2, t	6	6.55, 1H (d, 1.9)	101.5, d	6	8.92, 1H (d, 8.7)	131.1, d
7	2.48, 2H (m)	24.9, t	7		145.3, s	7		144.1, s	7		166.8, s
8	1.58, 2H (m)	27.5, t	8	1.28, 2H (overlap)	31.3, t	8	6.41, 1H (s)	106.8, d	7-OMe	3.82, 3H (s)	52.0, q
9	1.33, 2H (overlap)	29.1, t	9	1.29, 2H (overlap)	22.4, t	9		157.2, s	1'		172.5, s
10	1.30, 2H (overlap)	31.3, t	10	0.85, 3H (t, 6.4)	14.0, q	10	2.53, 1H (dd, 4.7, 8.7)	38.8, t	2'	2.38, 2H (t, 7.5)	37.7, t
11	1.31, 2H (overlap)	22.4, t	1'		174.2, s		2.74, 1H (dd, 4.7, 4.2)		3'	1.67, 2H (m)	25.7, t
12	0.88, 3H (t, 6.9)	14.0, q	2'		172.8, s	11	4.02, 1H (m)	70.8, d	4'	1.33, 2H (m)	32.1, t
6-OMe	3.75, 3H (s)	52.9, q	3'	4.27, 2H (s)	38.7, t	12	3.55, 2H (d, 5.2)	67.0, t	5'	1.31, 2H (m)	23.0, t
			4'		170.3, s	3-OMe	3.90, 3H (s)	56.5, q	6'	0.87, 3H (t, 7.0)	14.2, q
						5-OMe	3.91, 3H (s)	56.7, q			

The assignments were based on HSQC, ^1H - ^1H COSY, and HMBC experiments

^a ^1H and ^{13}C NMR data were recorded at 600 MHz and 150 MHz in CD_3OD , respectively

^b ^1H and ^{13}C NMR data were recorded at 500 MHz and 125 MHz in CDCl_3 , respectively

^c ^1H and ^{13}C NMR data were recorded at 600 MHz and 150 MHz in Acetone- d_6 , respectively

corresponded to a molecular formula of $C_{14}H_{19}NO_6$ with six degrees of unsaturation. Its IR absorptions at 2928, 1717 and 1429 cm^{-1} implied the presence of COOH, C=O and C=C groups. The ^1H and ^{13}C NMR spectra (Table 2) exhibited 14 carbon resonances including one methyl (δ_{C} 14.0), seven methylenes (δ_{C} 22.4, 24.2, 28.0, 28.8, 29.2, 31.3, and 38.7), two olefinic quaternary carbons (δ_{C} 132.8, 145.3), four carbonyl carbons (δ_{C} 170.2, 170.3, 172.8, and 174.2). The ^1H - ^1H COSY correlations of H_2 -4/ H_2 -5/ H_2 -6, and H_2 -9/ H_3 -10 were observed, HMBC correlations from H_3 -10 (δ_{H} 0.85, t, 6.4) to C-9 and C-8, from H_2 -4 (δ_{H} 2.42, t, 7.5) to C-3, C-5 and C-7, from H-5 (δ_{H} 1.52, m) to C-7, from H-2 (δ_{H} 3.49, s) to C-1, C-3 and C-7 suggested compound **6** was a 2-propylcyclopenteneacetic acid derivative. The HMBC correlations of H_2 -3' (δ_{H} 4.27, s) with C-2' and C-4', revealed compound **6** was a propane-dioic acid derivative (Fig. 2). Herein, the structure of two units was elucidated, and suggested compound **6** was a monocyclic polyketide derivative. The rest of units and the connections of the all the units in **6** was supported by EI^+ mass spectrum. In EI^+ mass spectrum, it seemed certain that m/z 297 is the molecular ion. The spectrum displayed the fragment ion m/z 252 due to the loss of COOH on the base of the molecular ion. And the ion at m/z 209 was strong in spectrum, probably was a daughter of m/z 251 via loss of $\text{C}_2\text{H}_2\text{O}$. The major fragment ions at m/z 123, 169, 195 which were due to the processes ($\text{M}^+ - \text{COOCONHCOCH}_2\text{COOH}$), ($\text{M}^+ - \text{CONHCOCH}_2\text{COOH} + 2\text{H}^+$), ($\text{M}^+ - \text{NHCOCH}_2\text{COOH}$), respectively. Then the peak at m/z 181 was a daughter of m/z 195 by means of losing CH_2 . Similarly, the ion at m/z 137 was a daughter of m/z 181 via loss CO_2 . Herein, the ion m/z at 123, 137, 181, 252, 251, 169, and 209 in EI^+ mass spectrum proved the presence of the fragment ion I-VII (Fig. S61). Therefore, the structure of compound **6** was elucidated.

Compound **7** had a molecular formula of $C_{14}H_{16}O_6$ based on HRESIMS ($[\text{M}+\text{Na}]^+$ m/z 303.0838, calcd for 303.0839). Its IR absorptions at 3416, 1711, 1602, 1570, 1457 and 1429 cm^{-1} implied the presence of OH, lactone and phenyl groups. Comparison of the ^1H and ^{13}C NMR data (Table 2) of compound **7** with that of orthosporin dimethyl ether indicated that both compounds had identical skeletons and substitution patterns, differing only in that compound **7** was hydroxylated at C-12 rather than a methyl group in orthosporin dimethyl ether [21, 22]. This conclusion was verified by the HMBC correlation from H_2 -12 (δ_{H} 3.55, d, 5.2) to C-11, and the ^1H - ^1H COSY correlations of H_a -10/ H -11/ H_2 -12 (Fig. 2). Accordingly, the planar structure of compound **7** was elucidated. For its absolute configuration, it was hardly to identify the configuration of **7** ($[\alpha] - 6.7$, c 0.14, MeOH) by comparison with the values of specific rotation of orthosporin dimethyl ether ($[\alpha]$

+18.9, c 0.03, MeOH) on account of the values of specific rotation was too small to discriminate whether it was experimental error or not [15]. Moreover, **7** was further subjected to the chiral chromatographic column (DAICEL AD-RH) ($4.6\text{ mm} \times 250\text{ mm}$) to exclude the possibility of racemate. Unfortunately, studies about the absolute configuration of **7** was restricted due to the sample shortage (1.1 mg), therefore, the configuration of C-11 couldn't be determined under the current situation. Consequently, **7** was named 3-(2,3-dihydroxypropyl)-6,8-dimethoxyisocoumarin, and defined as a new natural product, previously reported as a reactant for the total synthesis of desmethyldiaportinol [23].

Compound **8** was obtained as a yellowish solid. Its IR absorptions at 1720, 1664, 1601, 1534 and 1407 cm^{-1} implied the presence of C=O and phenyl groups. It had the molecular formula of $C_{14}H_{19}NO_3$, established by HRESIMS ($[\text{M}+\text{H}]^+$ 250.1439, calcd for $C_{14}H_{20}NO_3$, 250.1438) with six degrees of unsaturation. The ^1H and ^{13}C NMR data (Table 2) of **8** revealed the presence of one methyl, one methoxy group, four methylenes, four methines, and four quaternary carbons. All the above data suggested that **8** is a *p*-substituted phenyl derivative containing one nitrogen. The HMBC correlations from H_2 -2' (δ_{H} 2.38, t, 7.5) to C-4' and C-1', from H_2 -3' (δ_{H} 1.67, m) to C-5' and C-1', from H-6' (δ_{H} 0.87, t, 7.0) to C-5' and C-4', coupled with the ^1H - ^1H COSY correlation of H_2 -2'/ H_2 -3' proved the presence of the hexanamide group. Furthermore, the HMBC spectrum showed the correlations from H-3 (δ_{H} 7.77, d, 8.7) to C-1, from H-2 (δ_{H} 8.92, d, 8.7) to C-4 and C-7, from CH_3O -7 (δ_{H} 3.82, s) to C-7 and C-1, corresponding with the ^1H - ^1H COSY correlation of H-2/H-3 established the *p*-substituted phenyl with methoxycarbonyl group (Fig. 2). Herein, the structure of **8** was elucidated, and named as methyl 4-(hexanoylamino) benzoate. **8** was defined as a new natural product, previously reported as a reactant for the preparation of oxindole hydrazide modulators of protein tyrosine phosphatases [24].

The structures of known compounds (**9** and **10**) were identified by comparison of their spectroscopic data with the literature as (–)-5-carboxymellein (**9**) [25], 4-methoxyisoquinolin-1(2*H*)-one (**10**) [26], respectively.

Compounds **1–8** were tested for their cytotoxicity against HL-60, A-549, SMMC-7721, MCF-7, and SW-480 human tumor cell lines by MTS methods using cisplatin and paclitaxel as positive controls. Neither of them showed cytotoxic activity against all cell lines.

3 Experimental

3.1 General

Optical rotations were measured in MeOH with JASCO P-1020 polarimeters. 1D and 2D NMR spectra were recorded on Advance III 500 or 600 spectrometers using pyridine-*d*₅ as the internal standard. Chemical shifts (δ) are expressed in ppm relative to the pyridine-*d*₅ signals. HRESIMS was performed on an API QSTAR spectrometer. UV spectra were obtained on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained on a Bruker Tensor-27 FT-IR spectrometer using KBr pellets. Column chromatography (CC) was performed with silica gel (100–200 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), MCI gel (CHP20P, 75–150 μ m, Mitsubishi Chemical Corporation, Tokyo, Japan). Semi-preparative HPLC was performed on an Agilent 1200 liquid chromatograph with a Zorbax SB-C18 (9.4 mm $\times$ 250 mm) column. Fractions were monitored by thin layer chromatography, spots were visualized by UV light (254 nm and 365 nm) and by heating silica gel plates sprayed with 10% H₂SO₄ in EtOH. All solvents used in column chromatography were distilled including petroleum ether.

3.2 Fungal Material and Identification

The fungal strain of *Xylaria* sp. hg1009 was isolated from fresh stems of *I. sculponeatus* collected from Weishan, DaLi, People's Republic of China, in December 2012. Fungal identification was based on sequence analysis (GenBank Accession No. MG739623) of internal transcribed spacer (ITS) regions of the rDNA.

3.3 Fermentation, Extraction and Isolation

The fungus was cultivated in solid rice medium in 135 Fernbach flasks (500 mL, 60 mL distilled water was added to 80 g rice and kept overnight before autoclaving) for 40 days at 28 °C in a static incubator. The fungus was cultivated in 200 mL MEA liquid medium in 58 Erlenmeyer flasks (500 mL) for two weeks at 28 °C on a rotary shaker at 180 rpm.

The rice medium was overlaid and extracted with 70% aqueous acetone. The solvent was then evaporated in vacuo to afford a crude extract (80 g). The extraction was subjected to column chromatography on silica gel with a CHCl₃/Me₂CO gradient system (1:0, 9:1, 8:2, 7:3, 6:4, 1:1, 0:1) to yield seven fractions, A–G. Fraction A (CHCl₃/Me₂CO 1:0, 12 g) was subjected to column chromatography on silica gel with a petroleum ether/CH₃CH₂OAc

gradient system (from 10:1 to 0:1) to afford fractions A1–A9, fraction A7 was purified by semipreparative HPLC to yield **1** (25 mg), fraction A8 was purified by semipreparative HPLC to yield **9** (2.3 mg) and **10** (1.9 mg).

Fraction B (CHCl₃/Me₂CO 9:1, 19 g) was chromatographed on RP-18 with MeOH/H₂O (30:70, 40:60, 50:50, 60:40, 70:30, 100:0) to obtain B1–B11, B3 (65 mg) and B4 (80 mg) was purified by semipreparative HPLC to afford **2** (5.9 mg), **3** (30 mg), and **7** (1.1 mg), respectively. B8 (1.2 g) chromatographed on a silica gel column with a petroleum ether/Me₂CO gradient system (from 20:1 to 1:1) to afford fractions B8-1 to B8-7. Fraction B8-5 was purified by preparative HPLC to yield **4** (3.5 mg). Fraction C (CHCl₃/Me₂CO 8:2, 15 g) was purified by silica gel column with a petroleum ether/CH₃CH₂OAc gradient system (from 10:1 to 1:2) to afford fractions C1–C9. Fraction C6 was purified by preparative HPLC yield subfractions C6-1 to C6-5, then subfraction C6-2 were purified by semipreparative HPLC to yield **6** (60 mg).

The MEA culture broth was extracted with EtOAc 3 times to yield a crude extract (3.2 g). The extraction was subjected to RP-18 with MeOH/H₂O (20:80, 30:70, 50:50, 70:30, and 100:0) to yield five fractions, A–E. Fraction B (0.6 g) was chromatographed on a silica gel column with a petroleum ether/Me₂CO gradient system (from 10:1 to 0:1) to afford fractions B1–B3. Fraction B1 was subsequently purified by semipreparative HPLC to yield **5** (1.4 mg) and **8** (2.1 mg).

3.4 The Cytotoxicity Assay

The human tumor cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW-480 were used in the cytotoxic assay. These cell lines were obtained from ATCC (Manassas, VA, USA). Cells were cultured in RMPI-1640 or DMEM medium (Biological Industries, Kibbutz Beit-Haemek, Israel) supplemented with 10% fetal bovine serum (Biological Industries) at 37 °C in a humidified atmosphere with 5% CO₂. The cytotoxicity assay was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) (Promega, Madison, WI, USA) assay [27]. Briefly, cells were seeded into each well of a 96-well cell culture plate. After 12 h of incubation at 37 °C, the test compound (40 μ M) was added. After incubated for 48 h, cells were subjected to the MTS assay [28, 29]. Compounds with a growth inhibition rate of 50% were further evaluated at concentrations of 0.064, 0.32, 1.6, 8, and 40 μ M in triplicate, with cisplatin and paclitaxel (Sigma, St. Louis, MO, USA) as positive controls. The IC₅₀ value of each compound was calculated with Reed and Muench's method [30].

Xylariahgin A (**1**) yellow oil. $[\alpha]_D^{23}$ – 56.9 (*c* 0.18, MeOH), UV (MeOH) $\lambda_{\max}$ (log ϵ) 414 (1.70), 327 (2.48),

248 (3.97), 207 (3.86) nm, IR (KBr) $\nu_{\max}$ 2967, 2878, 1781, 1663, 1623, 1414, 1386, 1342, 1153, 1029, 956, 931, 871 cm^{-1} . ESIMS m/z 231 $[\text{M}+\text{Na}]^+$, HRESIMS m/z 231.0624 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{11}\text{H}_{12}\text{O}_4\text{Na}$ 231.0628). ^1H NMR (Acetone- d_6 , 500 MHz) and ^{13}C NMR (Acetone- d_6 , 125 MHz), see Table 1.

Xylariahgin B (**2**) colorless oil. $[\alpha]_{\text{D}}^{20}$ – 20.0 (c 0.18, MeOH), UV (MeOH) $\lambda_{\max}$ (log ϵ) 248 (3.97), 208 (3.83) nm, IR (KBr) $\nu_{\max}$ 3422, 2932, 1657, 1595, 1422, 1384, 1328, 1236, 1103, 935, 874 cm^{-1} . ESIMS m/z 249 $[\text{M}+\text{Na}]^+$, HRESIMS m/z 249.0730 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{11}\text{H}_{14}\text{O}_5\text{Na}$ 249.0733). ^1H NMR (CDCl_3 , 500 MHz) and ^{13}C NMR (CDCl_3 , 125 MHz), see Table 1.

Xylariahgin C (**3**) yellow oil. $[\alpha]_{\text{D}}^{24}$ – 23.1 (c 0.14, MeOH), UV (MeOH) $\lambda_{\max}$ (log ϵ) 248 (4.15), 207 (3.99) nm, IR (KBr) $\nu_{\max}$ 3395, 2964, 2933, 1783, 1732, 1660, 1422, 1384, 1157, 1103, 933, 873 cm^{-1} . ESIMS m/z 263 $[\text{M}+\text{Na}]^+$, HRESIMS m/z 263.0884 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{12}\text{H}_{16}\text{O}_6\text{Na}$ 263.0884). ^1H NMR (Acetone- d_6 , 600 MHz) and ^{13}C NMR (Acetone- d_6 , 150 MHz), see Table 1.

Xylariahgin D (**4**) yellow oil. $[\alpha]_{\text{D}}^{23}$ – 44.9 (c 0.10, MeOH), UV (MeOH) $\lambda_{\max}$ (log ϵ) 295 (3.07), 260 (3.98) nm, IR (KBr) $\nu_{\max}$ 2966, 2934, 2878, 1779, 1671, 1618, 1464, 1384, 1291, 1229, 1186, 1163, 1076, 984 cm^{-1} . ESIMS m/z 233 $[\text{M}+\text{Na}]^+$, HRESIMS m/z 233.0784 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{11}\text{H}_{14}\text{O}_4\text{Na}$ 233.0784). ^1H NMR (CDCl_3 , 500 MHz) and ^{13}C NMR (CDCl_3 , 125 MHz), see Table 1.

Xylariahgin E (**5**) yellowish oil. UV (MeOH) $\lambda_{\max}$ (log ϵ) 206 (3.94) nm, IR (KBr) $\nu_{\max}$ 2956, 2928, 2856, 1773, 1745, 1582, 1384, 1275, 1174, 924, 765 cm^{-1} . ESIMS m/z 253 $[\text{M}-\text{H}]^-$, HRESIMS m/z 253.1081 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{13}\text{H}_{17}\text{O}_5$ 253.1087). ^1H NMR (CDCl_3 , 500 MHz) and ^{13}C NMR (CDCl_3 , 125 MHz), see Table 2.

Xylariahgin F (**6**) yellowish amorphous power. UV (MeOH) $\lambda_{\max}$ (log ϵ) 225 (4.15) nm, IR (KBr) $\nu_{\max}$ 2928, 2860, 1717, 1429, 1313, 1194, 1124, 980, 944, 765, 616 cm^{-1} . ESIMS m/z 320 $[\text{M}+\text{Na}]^+$, HRESIMS m/z 320.1108 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{14}\text{H}_{19}\text{NO}_6\text{Na}$ 320.1105). ^1H NMR (CDCl_3 , 500 MHz) and ^{13}C NMR (CDCl_3 , 125 MHz), see Table 2.

3-(2,3-Dihydroxypropyl)-6,8-dimethoxyisocoumarin (**7**): yellowish solid. $[\alpha]_{\text{D}}^{24}$ – 6.7 (c 0.14, MeOH). UV (MeOH) $\lambda_{\max}$ (log ϵ) 323 (3.74), 287 (3.72), 278 (3.77), 242 (4.55) nm. IR (KBr) $\nu_{\max}$ 3416, 2928, 2854, 1711, 1602, 1570, 1457, 1429, 1382, 1246, 1217, 1205, 1165, 1061 cm^{-1} . ESIMS m/z 303 $[\text{M}+\text{Na}]^+$, HRESIMS m/z 303.0838 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{14}\text{H}_{16}\text{O}_6\text{Na}$ 303.0839). ^1H NMR (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz), see Table 2.

Methyl 4-(hexanoylamino) benzoate (**8**): yellowish solid. UV (MeOH) $\lambda_{\max}$ (log ϵ) 272 (4.44) nm. IR (KBr) $\nu_{\max}$ 2955, 2930, 1720, 1664, 1601, 1534, 1407, 1281,

1175, 1111, 1019, 771 cm^{-1} . ESIMS m/z 250 $[\text{M}+\text{H}]^+$, HRESIMS m/z 250.1439 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{14}\text{H}_{20}\text{NO}_3$ 250.1438). ^1H NMR (Acetone- d_6 , 600 MHz) and ^{13}C NMR (Acetone- d_6 , 150 MHz), see Table 2.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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