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Guaiane sesquiterpenes and isopimarane diterpenes from an endophytic fungus *Xylaria* sp.

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ABSTRACT

Nine oxygenated guaiane-type sesquiterpenes and three isopimarane diterpenes were isolated from the culture broth of an endophytic fungus, *Xylaria* sp. YM 311647, obtained from *Azadirachta indica*. The structures of these compounds were elucidated by interpretation of spectroscopic data. The absolute configurations of two of these were confirmed by X-ray crystallographic analysis. All of the compounds were tested for their antifungal activities against five pathogenic fungal cells. The results showed that nine sesquiterpenes were moderately active against *Candida albicans* and *Hormodendrum compactum* with MIC values ranging from 32 to 256 μg/ml, while the diterpenes were more active; One of these exhibited the most potent inhibitory activity against *C. albicans* and *Pyricularia oryzae* with MIC values of 16 μg/ml.

1. Introduction

Endophytic fungi are often found living in apparently healthy plants and cause no apparent symptoms of disease for the host plant (König et al., 1999). They have proven to be the source of a wide range of novel and bioactive secondary metabolites (Gunatilaka, 2006; Schulz et al., 2002; Zhang et al., 2006). Previous work on the bioactive secondary metabolites from endophytic fungi residing in *Azadirachta indica* led to isolation of ten-membered lactones from *Phomopsis* sp. (Wu et al., 2008) and solanapyrone analogues from *Nigrospora* sp. (Wu et al., 2009). In a continual effort to search for new bioactive compounds from endophytic fungi in *A. indica*, nine new oxygenated guaiane-type sesquiterpenes (**1**–**9**) (Fig. 1) and three new isopimarane diterpenes (**10**–**12**) were identified from the culture broth of an endophytic fungus, *Xylaria* sp. YM 311647, isolated from this plant. Herein described are the isolation, structural elucidation, and *in vitro* antifungal activities of these new compounds.

2. Results and discussion

Compounds **1**–**7** were determined to have the identical molecular formula of $C_{15}H_{28}O_4$ on the basis of their HRESIMS data. The ^1H and ^{13}C NMR spectra indicated that all are tetrahydroxy derivatives of guaiane-type sesquiterpene, with the same distribution of methyl groups and one oxygenated methylene. In addition, compounds **1**–**4** contain five methylenes, three methines, and three oxygenated quaternary carbons, while compounds **5**–**7** contain four methylenes, five methines (one oxygenated), and two oxygenated quaternary carbons.

Compound **1** was obtained as colorless crystals from the $CHCl_3$–MeOH solution. The IR spectrum exhibited a typical absorption band at 3421 cm^{-1} accounting for OH groups. The ^{13}C NMR spectrum (Table 1) indicated the presence of 15 carbons all sp^3-hybridized, and these data, together with two degrees of unsaturation deduced from the molecular formula, suggested a bicyclic structure for **1**. The ^1H NMR spectrum (Table 2) showed that one of the methyl groups at δ 1.04 was a doublet and the other two at δ 1.27 and 1.41, respectively, were single attached to quaternary centers. Detailed analysis of the ^1H–^1H COSY, HMQC, and HMBC spectra led to assignment of a guaiane ring system. The coupling sequence from H-1 to H-4 and from H-6 to H-9 could be established by tracking correlations in the ^1H–^1H COSY spectrum. The ^1H and ^{13}C NMR spectroscopic data of **1** showed similarities to

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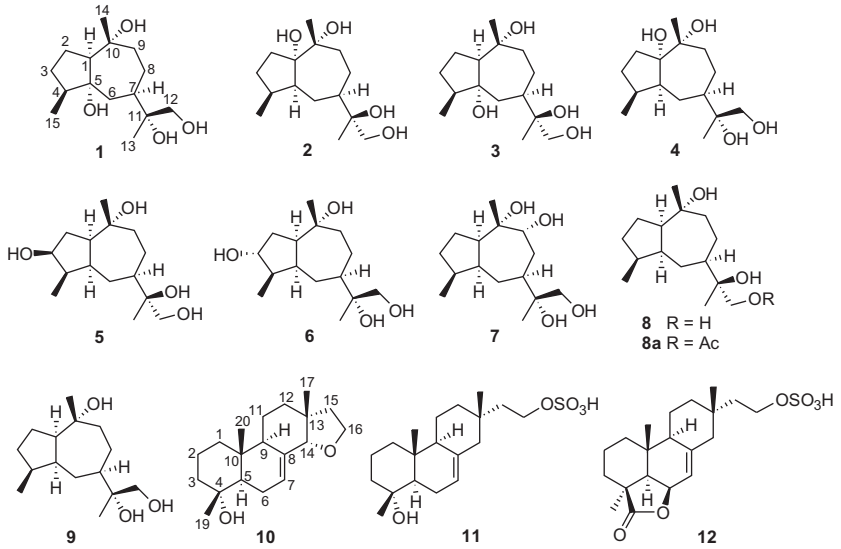

Fig. 1. Structures of compounds **1**–**12**.

Table 1
^{13}C NMR spectroscopic data of compounds **1**–**9** and **8a** (pyridine-d_5, 125 MHz, δ in ppm).

Position	1	2	3	4	5	6	7	8	8a	9
1	61.3	89.4	61.3	89.5	54.2	54.5	51.8	58.0	57.1	57.2
2	24.8	35.3	24.9	35.3	38.2	38.2	27.7	28.6	27.3	27.4
3	29.2	31.7	29.2	31.8	78.6	78.7	33.2	33.3	32.0	32.2
4	47.7	37.9	47.6	37.9	49.5	49.6	40.2	41.5	40.3	40.4
5	82.5	57.1	82.5	57.2	47.2	47.5	46.9	49.3	48.4	48.3
6	28.7	25.6	30.3	24.4	26.6	25.1	26.6	25.7	23.9	23.3
7	37.6	45.9	38.0	45.5	46.9	46.4	42.3	47.0	46.2	45.6
8	25.6	25.4	25.0	26.1	25.9	26.5	36.5	26.3	25.1	26.0
9	33.8	35.5	34.4	35.3	37.9	36.9	75.8	37.1	34.9	35.4
10	75.9	77.0	75.9	76.9	74.3	74.2	77.9	75.6	74.3	74.4
11	76.5	76.4	76.6	76.5	76.4	76.5	76.2	77.7	75.0	76.6
12	70.0	69.3	69.8	69.9	69.4	69.9	69.9	70.4	71.2	69.9
13	21.2	22.8	22.7	20.7	22.7	20.8	21.2	23.8	23.0	20.7
14	33.5	28.7	33.2	28.8	31.4	31.8	27.1	33.4	32.9	32.7
15	14.8	17.2	14.8	17.4	15.5	15.6	17.2	18.5	17.4	17.5
12-OAc									171.8	
									21.7	

Table 2
^{1}H NMR spectroscopic data of compounds **1**–**5** (pyridine-d_5, 500 MHz, δ in ppm, J in Hz).

Position	1	2	3	4	5
1	2.39 br t (10.2)		2.39 br t (10.1)		2.88 dd (17.6, 9.6)
2α	1.46 overlap	1.80 m	1.44 overlap	1.81 m	2.13 m
2β	1.76 m	2.05 overlap	1.75 m	2.04 m	2.26 m
3α	1.80 m	1.97 overlap	1.81 m	2.02 m	4.21 m
3β	1.19 ddd (22.3, 12.0, 5.2)	1.28 m	1.18 ddd (22.6, 11.8, 4.9)	1.29 m	
4	2.28 m	2.79 m	2.25 m	2.85 m	2.30 m
5		2.19 m		2.22 m	2.66 m
6α	2.47 br d (13.9)	1.97 overlap	2.14 br d (14.1)	2.32 br d (13.4)	2.04 br d (13.5)
6β	1.46 overlap	1.13 m	1.46 overlap	1.15 br dd (23.7, 12.9)	1.27 br dd (23.2, 12.9)
7	3.13 dd (17.9, 10.3)	2.52 m	3.12 dd (17.3, 10.3)	2.59 m	2.45 m
8α	2.32 m	2.46 m	2.51 m	2.29 overlap	2.48 m
8β	1.57 m	1.72 m	1.89 m	1.44 m	1.79 m
9α	2.02 m	2.05 overlap	2.04 m	2.29 overlap	2.13 m
9β	1.68 m	1.99 m	1.77 m	1.98 m	1.77 m
12a	3.99 d (10.6)	3.96 d (10.6)	4.06 d (10.8)	3.95 d (10.6)	4.01 d (10.7)
12b	3.90 d (10.6)	3.91 d (10.6)	4.02 d (10.8)	3.86 d (10.6)	3.95 d (10.7)
13	1.41 s	1.45 s	1.52 s	1.36 s	1.48 s
14	1.27 s	1.47 s	1.28 s	1.50 s	1.36 s
15	1.04 d (6.9)	0.94 d (7.1)	1.02 d (7.0)	1.01 d (7.1)	1.14 d (7.0)

those of a known compound (1α-H,4α-H,5α-H,7α-H)-10α,11-dihydroxyguaiane (Bittner et al., 1994). The main differences between them are the presence of an additional oxyquaternary carbon at δ_C 82.5 and an oxygenated methylene (δ_H 3.90 and 3.99, d, J = 10.6 Hz; δ_C 70.0) in **1** instead of one methine and a singlet methyl group, respectively, as in the known compound. The HMBC experiment showed that the oxyquaternary carbon at δ_C 82.5 was correlated with H-1, H_2-3, H-4, H_2-6, H-7, and H_3-15, indicating C-5 to be assigned to this oxyquaternary carbon. The substitution of a hydroxy group at C-12 was confirmed by HMBC correlations from two non-equivalent oxygenated methylene protons at δ_H 3.90 and 3.99 (d, J = 10.6 Hz), as well as the methine proton H-7 (δ_H 3.13, dd, J = 17.9, 10.3 Hz) and the methyl protons H_3-13 (δ_H 1.41, s) to the oxyquaternary carbon of C-11 (δ_C 76.5). To determine the configuration of **1**, a low-temperature single-crystal X-ray diffraction experiment was then performed with Cu Kα, and allowed an unambiguous assignment of the absolute configurations of all the chiral centers as 1*S*, 4*S*, 5*R*, 7*R*, 10*R*, 11*R* (Fig. 2). The X-ray crystallographic analysis of **1** also established that H-1, H-7, and 5-OH were α-oriented, whereas CH_3-14 and CH_3-15 were in β-orientations. These data were further confirmed by the NOE correlations of H-7 with H-6α (δ_H 2.47) and H-8α (δ_H 2.32), as well as the correlations of CH_3-14 (δ_H 1.27) with H-9 β (δ_H 1.68) and CH_3-15 (δ_H 1.04) with H-3β (δ_H 1.19). Thus, compound **1** was conclusively determined to be (1*S*,4*S*,5*R*,7*R*,10*R*,11*R*)-guaiane-5,10,11,12-tetraol.

As for **1**, further analysis of the 2D NMR spectra showed that C-10, C-11 and C-12 in compounds **2**–**7** were also all substituted by hydroxy groups. The only difference among these compounds is the substituted position of the remaining hydroxy group.

The HMBC spectrum of compound **2** showed cross peaks from H-2 (δ_H 1.80 and 2.05), H-4 (δ_H 2.79), H-5 (δ_H 2.19), H-9 (δ_H 1.99 and 2.05), and H-14 (δ_H 1.47) to the oxygenated quaternary carbon at δ_C 89.4, indicating the attachment of a hydroxy group at C-1. A single colorless crystal of **2** was obtained from $CHCl_3$–MeOH mixture. Its absolute configuration was ultimately established by a low-temperature single-crystal X-ray analysis (Fig. 3). It is evident that the second major difference between **1** and **2** is the stereochemistry of C-11 as determined by X-ray analyses. This difference is also apparently reflected in the ^{1}H and ^{13}C NMR spectra, which offers strong evidence to deduce the configuration of other homologues at the same position. Therefore, compound **2** was determined to be (1*S*,4*S*,5*S*,7*R*,10*R*,11*S*)-guaiane-1,10,11,12-tetraol.

Compound **3** had the same substituted position of hydroxy groups as for **1** based on the analysis of its 2D NMR spectra. Significant differences between the ^{1}H NMR spectrum of **3** and **1** were

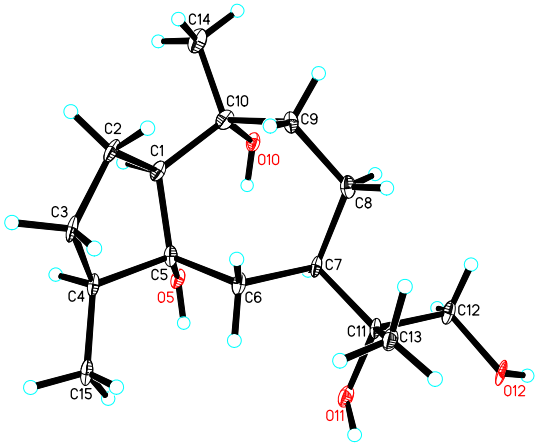

Fig. 2. X-ray crystal structure of compound **1**.

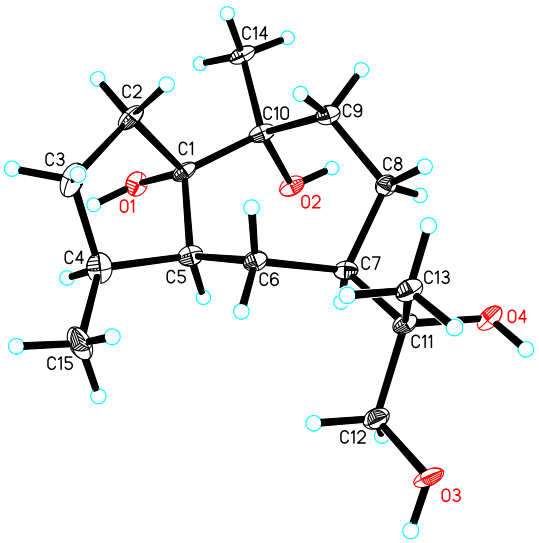

Fig. 3. X-ray crystal structure of compound **2**.

the upfield shift of H-6α (δ_H 2.14, br d, J = 14.1 Hz) and the downfield shift of H-8 (δ_H 1.89 and 2.51, m), H-12 (δ_H 4.02 and 4.06, d, J = 10.8 Hz) and H-13 (δ_H 1.52, s) in **3**, indicating that the latter was an epimer of **1** at C-11. As the absolute configuration at C-11 in **1** was *R*, 11*S* configuration in **3** was accordingly established, which was the same as that in **2**. Therefore, compound **3** was identified to be (1*S*,4*S*,5*R*,7*R*,10*R*,11*S*)-guaiane-5,10,11,12-tetraol.

In a similar manner, compound **4** was an epimer of **2** at C-11. This was confirmed by the upfield shift of H-8 (δ_H 1.44 and 2.29, m), H-12b (δ_H 3.86, d, J = 10.6 Hz), H-13 (δ_H 1.36, s), as well as the downfield shift of H-6α (δ_H 2.32, br d, J = 13.4 Hz) and H-9α (δ_H 2.29) in the ^{1}H NMR spectrum of **4**, when compared with that of **2**. Thus, compound **4** was accordingly determined to be (1*S*,4*S*,5*S*,7*R*,10*R*,11*R*)-guaiane-1,10,11,12-tetraol.

Each of compounds **5**–**7** had two additional methines, one of which is oxygenated, one less methylene, and one less oxyquaternary carbon compared with **1** by analysis of their 1D NMR spectroscopic data. The oxygenated methine (δ_H 4.21, m; δ_C 78.6) in **5** was assigned to be C-3 based on the couplings from this oxygenated proton to H-2 (δ_H 2.13 and 2.26) and H-4 (δ_H 2.30) in the ^{1}H–^{1}H COSY spectrum. The 3-OH group in β-orientation was evidenced by the NOE cross-peaks observed from H-3 to H-1, H-4 and H-5. Compound **6** had the same substitution of hydroxy groups as **5** by detailed analysis of 1D and 2D NMR spectra. The differences between **5** and **6** were the configurations of C-3 and C-11. The absence of a NOE correlation between H-3 and H-1 or between H-3 and H-5 suggested an α-orientation of the 3-OH group in **6**. Careful comparison of H-6α, H-8α, H-8β, H-12, and H-13 in the ^{1}H NMR spectra of **5** and **6** with those signals in **1**–**4** led to the assignment of the 11*S* and 11*R* configurations in **5** and **6**, respectively. Thus, compounds **5** and **6** were identified as (1*R*,3*S*,4*R*,5*S*,7*R*,10*R*,11*S*)-guaiane-3,10,11,12-tetraol and (1*R*,3*R*,4*R*,5*S*,7*R*,10*R*,11*R*)-guaiane-3,10,11,12-tetraol, respectively.

The only difference between **6** and **7** was the position of the oxygenated methine group. The HMBC correlations from H-1 (δ_H 2.81), H-8 (δ_H 1.99 and 2.53), and CH_3-14 (δ_H 1.56) to the oxygenated methine at δ_C 75.8 indicated the assignment of this oxygenated methine to be C-9 in compound **7**. NOE correlations of H-9 (δ_H 4.30) with H-6β (δ_H 1.26), H-8β (δ_H 1.99), and CH_3-14 (δ_H 1.56, s) suggested that the 9-OH group was α-oriented. The configurations at other chiral centers in **7** were identical to those in **6**, as supported by the NOESY experiment and comparison of the ^{1}H

NMR spectroscopic data. Thus, compound **7** was assigned as (1*R*,4*S*,5*S*,7*S*,9*R*,10*S*,11*R*)-guaiane-9,10,11,12-tetraol.

Compound **8** was the most abundant sesquiterpene component from the crude extracts. The molecular formulae of **8** and **9** were determined to be $C_{15}H_{28}O_3$ based on their HRESIMS data, which was 16 mass units less than those of **1**–**7**. The ^{13}C NMR spectra of **8** and **9** were very similar, including three methyl groups, six methylenes (one oxygenated), four methines, and two oxyquaternary carbons. Detailed analysis of the 1D and 2D NMR spectroscopic data established that three hydroxy groups were attached to C-10, C-11 and C-12, respectively. The main differences in the ^1H NMR spectra of these two compounds were the chemical shifts of H-6α, H-8, H-12 and H-13, indicating that **8** and **9** were the pair of C-11 epimers. Direct comparison of the chemical shifts of these protons with those of **1**–**4** assigned the absolute configuration of C-11 in **8** and **9** to be *S* and *R*, respectively. Thus, compounds **8** and **9** were determined to be (1*R*,4*S*,5*S*,7*R*,10*R*,11*S*)-guaiane-10,11,12-triol and (1*R*,4*S*,5*S*,7*R*,10*R*,11*R*)-guaiane-10,11,12-triol, respectively.

Acetylation of **8** gave the monoacetate product **8a**, whose acetoxy group was attached to C-12 on the basis of the HMBC correlations from the methyl signals (δ_H 1.95) and H-12 (δ_H 4.37 and 4.48) to the carbonyl carbon (δ_C 171.8).

When comparing the structures of **8** and **9** with that of the known compound xylaranol B (Li et al., 2010), these three compounds has the same molecular formula of $C_{15}H_{28}O_3$. However, the absolute configuration at C-11 in **8** and **9** were clearly elucidated as *S* and *R*, respectively based on the comparison of ^1H and ^{13}C NMR spectra with those of compounds **1** and **2**, while the stereochemistry of C-11 in xylaranol B remains unsolved.

From a biosynthetic point of view (Dewick, 2009), 11,12-dihydroxy groups are derived from an alkene bond in a fundamental precursor involving peroxidation to form an oxirane ring followed by hydrolysis to the corresponding diol. This hypothesis is supported by the fact that a guaiane intermediate with an 11, 12-double bond had been isolated and reported previously (Amand et al., 2012; Li et al., 2010), so it can be concluded that the epimeric pairs formation at C-11 would result from these reactions.

Compound **10** was assigned the molecular formula of $C_{19}H_{30}O_2$ by HRESIMS at *m*/*z* 313.2147 $[M+Na]^+$ (calcd 313.2143). The IR spectrum showed absorption bands at 3328 cm^{-1} for OH group and 1643 cm^{-1} for olefinic linkage. The ^1H NMR spectrum (Table 4) exhibited signals for an olefinic proton (δ_H 5.83, d, *J* = 5.6 Hz), an oxygenated methylene group (δ_H 3.90 dd, *J* = 16.7, 8.4 Hz; 3.82 dt, *J* = 8.4, 3.5 Hz), and three tertiary methyl singlets (δ_H 1.20, 0.99, and 0.77). Correspondingly, the ^{13}C NMR and DEPT spectra showed two sp^2 hybridized olefinic carbons (δ_C 129.7 and 134.7), three aliphatic quaternary carbons, three methines, eight methylenes, and three methyl groups. These data suggested that **10** was either a pimarane or isopimarane-type norditerpenoid (Lee et al., 1995). The structure of **10** was deduced from the analyses of ^1H–^1H COSY and HMBC correlations. The HMBC correlations from the methyl group at δ_H 0.77 (δ_C 14.6, 20-CH_3) to the quaternary carbon at δ_C 36.4 (C-10), methylene carbon at δ_C 39.4 (C-1), and the two methine carbons at δ_C 48.6 (C-9) and 52.5 (C-5) indicated the connection of C_1–C_{10}–C_9 and C_{20}–C_{10}–C_5. The assessment of the double bond located at C-7 and C-8 was deduced from the HMBC correlations of the olefinic proton at δ_H 5.83 (C-7) to a methylene carbon at δ_C 23.2 (C-6), and two methine carbons at 52.5 (C-5) and 48.6 (C-9). Moreover, based on the HMBC correlations from the proton at δ_H 3.61 to a methyl carbon at δ_C 21.5 (C-17), a methylene carbon at δ_C 32.7 (C-12), a methine carbon at δ_C 48.6 (C-9), and an olefinic methine carbon at δ_C 129.7 (C-7), the oxymethine group (δ_H 3.61(s), δ_C 89.2) was undoubtedly assigned to C-14. According to the HMBC correlations of H-16 (δ_H 3.90 and 3.82) to C-14 and C-15 (δ_C 41.8), an additional five-membered tetrahydrofuran ring formed through the linkage of C_{14}–O–C_{16} was proposed, which was supported by five degrees of unsaturation calculated from the molecular formula of $C_{19}H_{30}O_2$. Notably, the HMBC experiment showed that the proton signals at δ_H 1.49 (H-5), and 1.20 (H-19) were correlated with the oxyquaternary carbon at δ_C 72.7 of C-4 indicated that C-4 was substituted by an OH group instead of a methyl group as usual, thus forming the 18-nor skeleton of **10**. The α-orientation of 4-OH was confirmed by the NOE correlations between H-19 and $H_β$-6 (δ_H 1.91) and H-20, in addition to the absence of the NOE cross peaks for H-19 and H-5. The NOE interactions between H-14 (δ_H 3.61), $H_β$-12 (δ_H 1.58) and H-17 (δ_H 0.99), together with the absence of the NOE correlations between H-9 and H-14 or between H-9 and H-17, indicated the β-orientation of H-14 and 17-CH_3. Thus, the structure of compound **10** was identified as 14α,16-epoxy-18-norisopimar-7-en-4α-ol.

Compound **11** was obtained as a white powder. Its molecular formula was determined as $C_{19}H_{32}O_5S$ by HRESIMS at *m*/*z* 371.1897 $[M-H]^-$ (calcd 371.1892). The ^1H and ^{13}C NMR spectroscopic data of **11** indicated the same 18-norisopimarane skeleton as **10**. The conspicuous difference in ^1H NMR spectrum is the signal of H-14 shifted up-field from δ_H 3.61 (s) in **10** to δ_H 1.93 (s) in **11**,

Table 3
^1H NMR spectroscopic data of compounds **6**–**9** and **8a** (pyridine-d_5, 500 MHz, δ in ppm, *J* in Hz).

Position	6	7	8	8a	9
1	2.85 dd (18.4, 9.9)	2.81 dd (18.1, 9.2)	2.36 dd (17.3, 9.9)	2.35 m	2.34 m
2α	2.11 m	1.87 m	1.62 m	1.57 m	1.64 m
2β	2.24 m	1.87 m	1.76 m	1.69 m	1.70 m
3α		1.62 m	1.66 m	1.70 m	1.62 m
3β	4.22 m	1.29 m	1.26 m	1.24 m	1.29 m
4	2.30 overlap	2.00 m	2.01 m	2.00 m	1.99 m
5	2.60 m	2.32 m	2.24 m	2.20 m	2.24 m
6α	2.36 br d (13.4)	2.30 br d (12.5)	1.90 br d (13.4)	1.88 br d (13.3)	2.21 br d (12.2)
6β	1.25 m	1.26 m	1.17 br dd (22.9, 12.7)	1.13 br dd (23.1, 12.5)	1.16 br dd (23.5, 12.8)
7	2.50 dd (16.4, 9.8)	2.70 m	2.49 m	2.48 m	2.55 dd (17.0, 9.8)
8α	2.30 overlap	2.53 m	2.49 m	2.41 m	2.29 m
8β	1.49 m	1.99 m	1.76 m	1.70 m	1.45 m
9α	2.13 m		2.11 m	2.06 m	2.08 m
9 β	1.75 m	4.30 m	1.74 m	1.69 m	1.69 m
12a	3.95 d (10.7)	4.00 d (10.7)	3.99 d (10.7)	4.48 d (11.0)	3.94 d (10.6)
12b	3.86 d (10.7)	3.92 d (10.7)	3.94 d (10.7)	4.37 d (11.0)	3.85 d (10.6)
13	1.37 s	1.41 s	1.48 s	1.41 s	1.36 s
14	1.38 s	1.56 s	1.36 s	1.33 s	1.34 s
15	1.17 d (7.0)	0.92 d (6.9)	0.96 d (6.8)	0.96 d (6.6)	0.98 d (6.8)
12-OAc				1.95 s	

Table 4
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data for compounds **10**–**12**.

Position	**10**[a]		**11**[b]		**12**[c]	
	δ_C	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)
1α	39.4	1.09 dt (13.2, 3.6)	40.9	1.06 m	34.1	0.94 m
1β		1.83 m		1.86 m		1.24 m
2α	20.8	1.42 m	19.5	1.40 m	19.1	1.28 m
2β		1.77 m		1.78 m		1.48 m
3α	43.8	1.32 m	42.4	1.35 m	29.6	1.31 m
3β		1.73 m		1.68 m		2.16 m
4	72.7		72.5		43.3	
5	52.5	1.49 m	50.8	1.17 m	52.0	1.42 d (4.9)
6α	23.2	2.28 br d (17.7)	23.9	2.11 br d (16.1)	74.8	4.69 br s
6β		1.91 t (13.7)		1.98 t (12.1)		
7	129.7	5.83 d (5.6)	122.7	5.37 br s	118.9	5.53 d (2.2)
8	134.7		137.3		147.0	
9	48.6	2.03 br d (12.3)	53.0	1.66 m	48.7	1.46 m
10	36.4		36.8		34.1	
11α	21.4	1.54 m	21.7	1.55 m	20.8	1.47 m
11β		1.39 m		1.37 m		1.18 m
12α	32.7	1.35 m	38.5	1.28 m	36.9	1.13 m
12β		1.58 m		1.53 m		1.33 m
13	40.8		34.5		34.2	
14	89.2	3.61 s	48.9	1.93 s	47.6	1.93 s
15α	41.8	1.80 m	45.2	1.58 t (7.3)	44.9	1.66 t (7.5)
15β		1.74 m				
16α	65.5	3.82 dt (8.4, 3.5)	66.6	4.11 t (7.3)	65.0	4.51 t (7.5)
16β		3.90 dd (16.7, 8.4)				
17	21.5	0.99 s	22.5	0.83 s	23.2	0.65 s
18					25.3	1.16 s
19	23.5	1.20 s	31.2	1.14 s	183.3	
20	14.6	0.77 s	15.4	1.03 s	19.1	0.86 s

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.
[c] Measured in pyridine-d_5.

which was also reflected in the ^{13}C NMR spectrum, indication of absence of the methine group of C-14 at δ_C 89.2. These data, combined with a less unsaturation degree of **11** indicated that C-14 and C-16 was not connected to each other. This deduction was also supported by the absence of HMBC correlation from H-16 (δ_H 4.11, t, J = 7.3 Hz) to C-14 and the equivalence of two protons at C-16. Furthermore, the ^{1}H and ^{13}C NMR spectroscopic data of C-16 (δ_H 4.11; δ_C 66.6) in **11** were very similar to those in hymatoxins A–D which were substituted by a sulfate group at C-16 (Borgschulte et al., 1991), and they were shifted more downfield than those in hymatoxin E with a hydroxy group at C-16 (δ_H 3.67; δ_C 59.0). Thus, a sulfate group linked to C-16 in **11** was unequivocally deduced. Accordingly, the structure of **11** was determined as 16-*O*-sulfo-18-norisopimar-7-en-4α,16-diol.

Compound **12** was obtained as a white powder, and its molecular formula determined as $C_{20}H_{30}O_6S$ by HRESIMS at *m*/*z* 421.1657 $[M+Na]^+$(calcd 421.1661), with an oxygen atom less than hymatoxin A (Borgschulte et al., 1991). The IR spectrum showed absorption bands at 3455, 1756, and 1640 cm^{-1} for a hydroxy group, a carbonyl group of γ-lactone, and an olefinic linkage, respectively. The ^{13}C NMR spectroscopic data of **12** were similar to those of hymatoxin A except for the absence of an oxyquaternary carbon at δ_C 73.7 and the presence of a methine group at δ_C 48.7 in **12**. This was easily deduced that C-9 was not substituted by hydroxy group in **12**, supported also by the HMBC correlations from C-9 (δ_C 48.7) to H-5 (δ_H 1.42, d, J = 4.9 Hz), H-7 (δ_H 5.53, d, J = 2.2 Hz), and H-20 (δ_H 0.86, s). Thus, compound **12** was named as 9-deoxy-hymatoxin A.

Table 5
Antifungal activities of compounds **1**–**12** against different pathogenic fungi.

Compounds	MIC (μg/ml)				
	C. albicans YM 2005	*A. niger* YM 3029	*P. oryzae* YM 3051	*F. avenaceum* YM 3065	*H. compactum* YM 3077
1	256	128	256	512	128
2	32	64	256	>512	64
3	128	256	128	512	256
4	64	64	256	>512	256
5	64	512	256	>512	128
6	128	512	128	512	128
7	32	128	512	>512	256
8	128	256	512	>512	128
9	128	>512	256	512	256
10	64	64	256	64	128
11	64	128	32	128	64
12	16	32	16	64	64
Nystatin	8	8	8	16	8

Compounds **1–12** were evaluated for their antifungal activities against *Candida albicans*, *Aspergillus niger*, *Pyricularia oryzae*, *Fusarium avenaceum*, and *Hormodendrum compactum*. The results are shown in Table 5 with nystatin as a positive control. As a whole, all nine guaiane-type sesquiterpenes showed a similar inhibitory spectrum against *C. albicans* and *H. compactum* with the minimal inhibitory concentration (MICs) in the range of 32–256 μg/ml. Among them, **2** and **7** were the most potent ones against *C. albicans* with MIC values of 32 μg/ml. In addition, these guaianes demonstrated moderate or weak activities against *A. niger*, *P. oryzae*, and *H. compactum*. Both **2** and **4** with the same substituted position of hydroxy groups exhibited the most potent inhibitory activity against *A. niger* with MIC values of 64 μg/ml. On the other hand, the isopimarane diterpenes (**10**–**12**) showed relatively obvious antifungal activities compared to guaiane sesquiterpenes. Compound **11** exhibited inhibitory activity against *P. oryzae* with MIC value of 32 μg/ml, while **12** with a γ-lactone moiety and a sulfate group showed the most potent activity against *C. albicans* and *P. oryzae* with MIC values of 16 μg/ml, and against *A. niger* with MIC value of 32 μg/ml, respectively.

3. Conclusions

The guaianes are a large group of sesquiterpenes mainly found as constituents of higher plants, and many of them showed various biological activities. However, few guaiane sesquiterpenes have previously been reported from plant endophytes. The only examples are a guaiane mannoside isolated from a *Eutypa*-like fungus in *Murraya paniculata* (Souza et al., 2008) and two guaianes from endophytic fungus S49 in *Cephalotaxus hainanensis* Li. (Mei et al., 2010). Fungi belonging to the genus *Xylaria* are prolific producers of structurally diverse natural products. Several types of sesquiterpenes have been reported from this genus, including eremophilanes (Amaral and Rodrigues-Filho, 2010; Isaka et al., 2010; Li et al., 2010; McDonald et al., 2004; Smith et al., 2002), eudesmanes (Pittayakhajonwut et al., 2009), brasilanes (Hu et al., 2008), and presilphiperfolanes (Silva et al., 2010). To the best of our knowledge, guaiane-type sesquiterpenes are rarely isolated from *Xylaria*, with only one paper including xylaranols A and B in the published literature (Li et al., 2010), while our study is the second time to report this type of compounds isolated from this fungal genus. It is particularly interesting that the positions at C-10, C-11 and C-12 in all isolated guaianes from this strain are oxygenated since this structural feature has rarely been observed from naturally occurring guaiane-type sesquiterpenes, which will lead us into a further investigation from biosynthetic point of view.

Isopimarane diterpenes with a γ-lactone moiety between C-4 and C-6 had been reported from *Xylaria* species (Isaka et al., 2012; Li et al., 2010). The unusual diterpene sulfate was previously isolated from the fungus *Hypoxylon mammatum* (Bodo et al., 1987; Borgschulte et al., 1991). Neither the 18-norisopimarane diterpene nor the diterpene sulfate have been reported in the genus *Xylaria* up to now.

During our screening of endophytic fungi isolated from *A. indica* for antifungal activity, the broth extract of the strain *Xylaria* sp. YM 311647 showed potent activity. In this study, nine guaiane sesquiterpenes and three isopimarane diterpenes were obtained from this strain. All compounds exhibited antifungal activity of various degrees of potency against all pathogenic fungi. Among them, the isopimarane diterpenes (**10**–**12**) were more potent than guaiane sesquiterpenes (**1**–**9**). Comparing the activity of compound **12** with those of compounds **10** and **11**, it seemed that the presence of a γ-lactone moiety was related to the antifungal activity. Compound **12** was the most potent against *C. albicans* and *P. oryzae* (MIC = 16 μg/ml) but showing slightly less potency compared to the standard antibiotic nystatin (MIC = 8 μg/ml), and could possibly provide a starting point for medicinal chemistry efforts and mechanistic studies.

4. Experimental

4.1. General experimental procedures

Melting points were obtained on an XRC-1 apparatus and uncorrected. Optical rotations were measured with a HORIBA SEPA-300 polarimeter. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. NMR spectra were recorded in pyridine-d_5 on a Bruker DRX-500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), using TMS as an internal standard. HRESIMS were acquired using recorded on an Agilent G3250AA LC/MSD TOF spectrometer. X-ray crystallographic data were collected on a Bruker APEX DUO diffractometer. Column chromatography (CC) was performed on silica gel (Qingdao Marine Chemical Factory, China), Sephadex LH-20 (Amersham Pharmacia Biotech) and RP-18 silica gel (40–63 μm, Merck). Precoated silica gel plates (Qingdao Marine Chemical Factory, China) were used for TLC. Detection was done by spraying the plates with 5% H_2SO_4, followed by heating.

4.2. Fungal material

The fungal strain YM 311647 was isolated from the stem of *A. indica* in Yuanjiang County, Yunnan Province, P. R. China., following an isolation protocol described previously (Wu et al., 2008). The strain was deposited in Yunnan Institute of Microbiology, Kunming, P. R. China, and identified as a *Xylaria* sp. on the basis of the sequence data of the ITS genes. Genomic DNA was purified from fungal mycelium using the cetyltrimethylammonium bromide (CTAB) extraction method (Rogers and Bendich, 1994) and then subject to ribosomal internal transcribed spacer (ITS) analysis (White et al., 1990). The nucleotide sequence data of ITS1-5.8S-ITS2 of *Xylaria* sp. YM 311647 is deposited in GenBank with accession number HQ728090. A GenBank search for sequences similar to its ITS region revealed *Xylaria venosula* as the closest match with a 98% sequence identity.

4.3. Fermentation, extraction, and isolation

The fresh mycelium grown on PDA medium at 28 °C for 7 days was inoculated into 500 ml Erlenmeyer flasks (×70) containing PDB medium (120 ml) (200 g potato and 20 g dextrose in 1 l H_2O). After incubation at 28 °C for 4 days on a rotary shaker (200 rpm), each 25 ml portion was transferred into 1 l Erlenmeyer flasks (×300) containing PDB medium (250 ml). The following cultivation was carried out at 28 °C for 9 days on a rotary shaker (200 rpm). The cultures were filtered to remove mycelia. The filtrate was concentrated under reduced pressure to 10 l and then exhaustively extracted with EtOAc (3 × 10 l), and the combined organic phase was concentrated to obtain a brown gum of broth extract (63.5 g). The latter was subjected to silica gel CC, eluted with a gradient of $CHCl_3$/MeOH from 1:0 to 0:1 (v/v) to afford eleven fractions. Fraction 4 (2.3 g) was subjected to RP CC (MeOH/H_2O gradient system, 20:80 → 80:30 v/v) to provide five fractions. Fraction 4–2 (145.2 mg) was further purified by CC on silica gel ($CHCl_3$/MeOH, 95:5) to give **10** (7.2 mg). Fraction 4–3 (39.6 mg) was subjected to CC on Sephadex LH-20 (MeOH) to furnish **8** (28.3 mg) and **9** (12.4 mg). Fraction 5 (1.6 g) was purified by RP CC (MeOH/H_2O, 3:7, 4:6) to yield **2** (16.7 mg) and **4** (8.3 mg), and **7** (9.2 mg). Fraction 6 (2.1 g) was separated by silica gel CC ($CHCl_3$/MeOH, 95:5, 9:1, 85:15) and further purified by RP (MeOH/H_2O, 3:7, 4:6,

1:1) to give **1** (11.2 mg), **3** (7.5 mg), and **12** (5.9 mg). Fraction 8 (2.1 g) was subjected to on silica gel CC ($CHCl_3$/MeOH, 9:1) and then purified by on Sephadex LH-20 CC (MeOH) to afford **5** (10.3 mg), **6** (9.5 mg), and **11** (4.7 mg).

4.4. (1S,4S,5R,7R,10R,11R)-Guaiane-5,10,11,12-tetraol (**1**)

Colorless needles; mp 185–186 °C; $[\alpha]_D^{22}$ −15.8 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3421, 3297, 2944, 1471, 1368, 1039, 921, 762, 599 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z* 295.1889 $[M+Na]^+$(calcd for $C_{15}H_{28}O_4Na$ 295.1885).

4.5. (1S,4S,5S,7R,10R,11S)-Guaiane-1,10,11,12-tetraol (**2**)

Colorless needles; mp 172–173 °C; $[\alpha]_D^{22}$ −15.4 (*c* 0.24, MeOH); IR (KBr) ν_{max} 3396, 2957, 2927, 2869, 1463, 1380, 1367, 1262, 1104, 1052, 795 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z* 295.1887 $[M+Na]^+$(calcd for $C_{15}H_{28}O_4Na$, 295.1885).

4.6. (1S,4S,5R,7R,10R,11S)-Guaiane-5,10,11,12-tetraol (**3**)

Colorless amorphous; $[\alpha]_D^{22}$ −12.6 (*c* 0.33, MeOH); IR (KBr) ν_{max} 3336, 2953, 1465, 1381, 1267, 1096, 1047, 913, 805 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z* 295.1889 $[M+Na]^+$(calcd for $C_{15}H_{28}O_4Na$ 295.1885).

4.7. (1S,4S,5S,7R,10R,11R)-Guaiane-1,10,11,12-tetraol (**4**)

Colorless amorphous; $[\alpha]_D^{22}$ −12.1 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3405, 2958, 1472, 1373, 1279, 1054, 915, 793 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z* 295.1890 $[M+Na]^+$(calcd for $C_{15}H_{28}O_4Na$ 295.1885).

4.8. (1R,3S,4R,5S,7R,10R,11S)-Guaiane-3,10,11,12-tetraol (**5**)

Colorless amorphous; $[\alpha]_D^{22}$ −11.5 (*c* 0.08, MeOH); IR (KBr) ν_{max} 3402, 3291, 2957, 1460, 1379, 1296, 1058, 915 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 295.1889 $[M+Na]^+$(calcd for $C_{15}H_{28}O_4Na$ 295.1885).

4.9. (1R,3R,4R,5S,7R,10R,11R)-Guaiane-3,10,11,12-tetraol (**6**)

Colorless amorphous; $[\alpha]_D^{22}$ −7.7 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3358, 2971, 1477, 1362, 1082, 928, 781 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 295.1889 $[M+Na]^+$(calcd for $C_{15}H_{28}O_4Na$ 295.1885).

4.10. (1R,4S,5S,7S,9R,10S,11R)-Guaiane-9,10,11,12-tetraol (**7**)

Colorless amorphous; $[\alpha]_D^{22}$ −26.8 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3416, 3328, 2956, 2869, 2358, 1451, 1381, 1339, 1270, 1141, 1042, 906 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 295.1888 $[M+Na]^+$(calcd for $C_{15}H_{28}O_4Na$, 295.1885).

4.11. (1R,4S,5S,7R,10R,11S)-Guaiane-10,11,12-triol (**8**)

Colorless oil; $[\alpha]_D^{22}$ −10.0 (*c* 0.69, MeOH); IR (KBr) ν_{max} 3594, 3136, 2973, 2867, 2360, 1455, 1377, 1264, 1045, 908 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z* 279.1939 $[M+Na]^+$(calcd for $C_{15}H_{28}O_3Na$, 279.1936).

4.12. Acetylation of **8**

A sample of **8** (5.0 mg) was reacted with Ac_2O (1.0 ml) and pyridine (1.0 ml). The reaction mixture was stirred for 24 h and then evaporated. The product was purified by silica gel CC (petroleum ether/EtOAc, 9:1) to afford **8a** (4.2 mg) as colorless oil: For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m/z* 321.2047 $[M+Na]^+$(calcd for $C_{17}H_{30}O_4Na$ 321.2042).

4.13. (1R,4S,5S,7R,10R,11R)-Guaiane-10,11,12-triol (**9**)

Colorless amorphous; $[\alpha]_D^{22}$ −10.9 (*c* 0.17, MeOH); IR (KBr) ν_{max} 3524, 2981, 1472, 1381, 1265, 1079, 932, 784 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 279.1932 $[M+Na]^+$(calcd for $C_{15}H_{28}O_3Na$ 279.1936).

4.14. 14α,16-Epoxy-18-norisopimar-7-en-4α-ol (**10**)

Colorless oil; $[\alpha]_D^{22}$ −9.2 (*c* 0.14, MeOH); IR (KBr) ν_{max} 3328, 2934, 1643, 1247, 1092, 948, 796 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Table 4; HRESIMS *m/z* 313.2147 $[M+Na]^+$(calcd for $C_{19}H_{30}O_2Na$, 313.2143).

4.15. 16-O-Sulfo-18-norisopimar-7-en-4α,16-diol (**11**)

White amorphous powder; $[\alpha]_D^{22}$ −36.2 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3454, 2927, 1640, 1430, 1384, 1065, 877 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Table 4; HRESIMS *m/z* 371.1897 $[M-H]^-$ (calcd for $C_{19}H_{31}O_5S$, 371.1892).

4.16. 9-Deoxy-hymatoxin A (**12**)

White amorphous powder; $[\alpha]_D^{22}$ −44.7 (*c* 0.18,); UV (MeOH) λ_{max} (log ε) 216 (3.6), 242 (1.9) nm; IR (KBr) ν_{max} 3455, 2928, 2360, 1756, 1640, 1454, 1383, 1225, 1065, 983, 830, 777 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Table 4; HRESIMS *m/z* 421.1657 $[M+Na]^+$(calcd for $C_{20}H_{30}O_6SNa$, 421.1661).

4.17. X-ray crystallography

The crystal structures of **1** and **2** were determined, using data collected at $T = 100$ (2) K with Cu Kα radiation ($\lambda = 1.54178$ Å) on a Bruker APEX DUO diffractometer, equipped with an Oxford Cryostream 700+ cooler. The structures were solved by the direct method using SHELXS-97, and then refined by full-matrix least-squares on F^2 using SHELXL-97 package software (Sheldrick, 2008). The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in the idealized positions and refined using a riding model. The absolute configurations were determined by computation of the Hooft parameters (Hooft et al., 2008).

Compound ***1****:* Colorless orthorhombic crystals of $C_{15}H_{28}O_4$, $M_r = 272.37$, crystal dimensions 0.56 × 0.17 × 0.13 mm, space group $P2(1)2(1)2$, $a = 9.8830(12)$ Å, $b = 16.2610(13)$ Å, $c = 9.3196(8)$ Å, $\alpha = 90.00°$, $\beta = 90.00°$, $\gamma = 90.00°$, $V = 1497.7(3)$ Å^3, $Z = 4$, $D_{calc} = 1.208$ mg/m^3, $F(000) = 600$, μ(CuKα) = 0.690 mm^{-1}, $T_{min}/T_{max} = 0.70/0.92$, $\theta_{max} = 69.1°$, 6295 reflections measured, 2558 independent reflections ($R_{int} = 0.1182$), final $R_1 = 0.0826$ ($I > 2\sigma(I)$), final $wR(F^2) = 0.2098$ ($I > 2\sigma(I)$), final R_1 (all data) = 0.1185, final $wR(F^2)$ (all data) = 0.2324, goodness of fit on $F^2 = 1.093$. The Hooft parameter is −0.2(3) for 1012 Bijvoet pairs.

Compound ***2****:* Colorless monoclinic crystals of $C_{15}H_{28}O_4$, $M_r = 272.37$, crystal dimensions 0.43 × 0.31 × 0.10 mm, space group $P2(1)$, $a = 9.2942(3)$ Å, $b = 19.3426(7)$ Å, $c = 9.5488(3)$ Å, $\alpha = 90.00°$, $\beta = 118.9610(10)°$, $\gamma = 90.00°$, $V = 1501.96(9)$ Å^3, $Z = 4$, $D_{calc} = 1.205$ mg/m^3, $F(000) = 600$, μ(CuKα) = 0.688 mm^{-1}, $T_{min}/T_{max} = 0.76/0.93$, $\theta_{max} = 69.3°$, 11111 reflections measured,

4127 independent reflections (R_{int} = 0.0476), final R_1 = 0.0660 ($I > 2\sigma(I)$), final $wR(F^2)$ = 0.1793 ($I > 2\sigma(I)$), final R_1 (all data) = 0.0662, final $wR(F^2)$ (all data) = 0.1794, goodness of fit on F^2 = 1.092. The Hooft parameter is 0.33(13) for 1399 Bijvoet pairs.

The above crystallographic data have been deposited with the Cambridge Crystallographic Data Center under CCDC 947539 for compound **1** and CCDC 947540 for compound **2**. These data can be obtained free of charge via www.ccdc.cam.ac.uk/deposit (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; deposit@ccdc.cam.ac.uk).

4.18. Antifungal assays

The minimal inhibitory concentrations (MICs) for fungal strains of compounds **1**–**12** were determined by the broth microdilution method in 96-well culture plates as recommended by the Clinical and Laboratory Standards Institute (NCCLS, 2002a,b). The yeast *C. albicans* (YM 2005) was grown on Sabouraud dextrose agar, and the fungal strains *A. niger* (YM 3029), *P. oryzae* (YM 3051), *F. avenaceum* (YM 3065), and *H. compactum* (YM 3077) were grown on potato dextrose agar. The fungal inocula were prepared from respective broth cultures that were incubated at 28 °C for 48 h. The final suspensions were adjusted to a concentration of 1.0×10^5 spores/ml. A volume of 50 μl of inoculum suspension was loaded into each well of 96-well plates. Each substance was dissolved in a small volume of sterile DMSO and diluted in the appropriate medium giving the final concentrations (prepared from serial 2-fold dilutions) ranging from 512.0 to 1 μg/ml. The plates were then incubated for 48 h at 28 °C. Nystatin was used as positive control. The MIC was defined as the lowest concentration of the test compound at which the microorganism did not demonstrate visible growth. The experiments were repeated three times.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2014.04.016.

References

Amand, S., Langenfeld, A., Blond, A., Dupont, J., Nay, B., Prado, S., 2012. Guaiane sesquiterpenes from *Biscogniauxia nummularia* featuring potent antigerminative activity. J. Nat. Prod. 75, 798–801.

Amaral, L.S., Rodrigues-Filho, E., 2010. Two novel eremophilane sesquiterpenes from an endophytic Xylariaceous fungus isolated from leaves of *Cupressus lusitanica*. J. Braz. Chem. Soc. 21, 1446–1450.

Bittner, M., Silva, M., Rozas, Z., Papastergiou, F., Jakupovic, J., 1994. Sesquiterpenes and other consitituents from *Chilean mutisieae*. Phytochemistry 36, 695–698.

Bodo, B., Davoust, D., Lecommandeur, D., Rebuffat, S., Genetet, I., Pinon, J., 1987. Hymatoxin A, a diterpene sulfate phytotoxin of *Hypoxylon mammatum*, parasite of aspen. Tetrahedron Lett. 28, 2355–2358.

Borgschulte, K., Rebuffat, S., Trowitzsch-Kienast, W., Schomburg, D., Pinon, J., Bodo, B., 1991. Isolation and structure elucidation of hymatoxins B–E and other phytotoxins from *Hypoxylon mammatum* fungal pathogen of leuce poplars. Tetrahedron 47, 8351–8360.

Dewick, P.M., 2009. Medicinal Natural Products: A Biosynthetic Approach, third ed. John Wiley & Sons, West Sussex.

Gunatilaka, A.A.L., 2006. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J. Nat. Prod. 69, 509–526.

Hooft, R.W.W., Straver, L.H., Spek, A.L., 2008. Determination of absolute structure using Bayesian statistics on Bijvoet differences. J. Appl. Crystallogr. 41, 96–103.

Hu, Z.Y., Li, Y.Y., Huang, Y.J., Su, W.J., Shen, Y.M., 2008. Three new sesquiterpenoids from *Xylaria* sp. NCY2. Helv. Chim. Acta 91, 46–52.

Isaka, M., Chinthanom, P., Boonruangprapa, T., Rungjindamai, N., Pinruan, U., 2010. Eremophilane-type sesquiterpenes from the fungus *Xylaria* sp. BCC 21097. J. Nat. Prod. 73, 683–687.

Isaka, M., Srisanoh, U., Sappan, M., Kongthong, S., Srikitikulchai, P., 2012. Eremophilane and eudesmane sesquiterpenoids and a pimarane diterpenoid from the wood-decay fungus *Xylaria* sp. BCC 5484. Phytochem. Lett. 5, 78–82.

König, G.M., Wright, A.D., Aust, H.J., Draeger, S., Schulz, B., 1999. Geniculol, a new biologically active diterpene from the endophytic fungus *Geniculosporium* sp. J. Nat. Prod. 62, 155–157.

Lee, C.K., Fang, J.M., Cheng, Y.S., 1995. Norditerpenes from *Juniperus chinensis*. Phytochemistry 39, 391–394.

Li, Y.Y., Hu, Z.Y., Lu, C.H., Shen, Y.M., 2010. Four new terpenoids from *Xylaria* sp. 101. Helv. Chim. Acta 93, 796–802.

McDonald, L.A., Barbieri, L.R., Bernan, V.S., Janso, J., Lassota, P., Carter, G.T., 2004. 07H239-A, a new cytotoxic eremophilane sesquiterpene from the marine-derived *Xylariaceous* fungus LL-07H239. J. Nat. Prod. 67, 1565–1567.

Mei, W.L., Chen, P., Wang, H., Huang, J.L., Dai, H.F., 2010. Two new sesquiterpenes from endophytic fungus S49 of *Cephalotaxus hainanensis*. J. Asian Nat. Prod. Res. 12, 582–585.

NCCLS, 2002a. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard, second ed., NCCLS Document M27-A2. NCCLS, Wayne.

NCCLS, 2002b. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: Approved Standard, NCCLS Document M38-A. NCCLS, Wayne.

Pittayakhajonwut, P., Usuwan, A., Intaraudom, C., Veeranondha, S., Srikitikulchai, P., 2009. Sesquiterpene lactone 12,8-eudesmanolides from the fungus *Xylaria ianthinovelutina*. Planta Med. 75, 1431–1435.

Rogers, S.O., Bendich, A.J., 1994. Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin, S.B., Schilperoort, R.A. (Eds.), Plant Molecular Biology Manual, second ed. Kluwer Academic Publishers, Dordrecht, pp. 1–8.

Schulz, B., Boyle, C., Draeger, S., Aust, H.J., Römmert, A.K., Krohn, K., 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol. Res. 106, 996–1004.

Sheldrick, G.M., 2008. A short history of *SHELX*. Acta Crystallogr. A64, 112–122.

Silva, G.H., Oliveira, C.M., Teles, H.L., Pauletti, P.M., Castro-Gamboa, I., Silva, D.H.S., Bolzani, V.S., Young, M.C.M., Costa-Neto, C.M., Pfenning, L.H., Berlinck, R.G.S., Araujo, A.R., 2010. Sesquiterpenes from *Xylaria* sp., an endophytic fungus associated with *Piper aduncum* (Piperaceae). Phytochem. Lett. 3, 164–167.

Smith, C.J., Morin, N.R., Bills, G.F., Dombrowski, A.W., Salituro, G.M., Smith, S.K., Zhao, A., Macneil, D.J., 2002. Novel sesquiterpenoids from the fermentation of *Xylaria persicaria* are selective ligands for the NPY Y5 receptor. J. Org. Chem. 67, 5001–5004.

Souza, A.D.L., Rodrigues-Filho, E., Souza, A.Q.L., Henrique-Silva, F., Pereira, J.O., 2008. A new guaiane mannoside from a *Eutypa*-like fungus isolated from *Murraya paniculata* in Brazil. J. Braz. Chem. Soc. 19, 1321–1325.

White, T.J., Bruns, T.D., Lee, S.B., Taylor, J.W., 1990. Isolation of DNA from fungal mycelia and single spores. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.

Wu, S.H., Chen, Y.W., Shao, S.C., Wang, L.D., Li, Z.Y., Yang, L.Y., Li, S.L., Huang, R., 2008. Ten-membered lactones from *Phomopsis* sp., an endophytic fungus of *Azadirachta indica*. J. Nat. Prod. 71, 731–734.

Wu, S.H., Chen, Y.W., Shao, S.C., Wang, L.D., Yu, Y., Li, Z.Y., Yang, L.Y., Li, S.L., Huang, R., 2009. Two new solanapyrone analogues from the endophytic fungus *Nigrospora* sp. YB-141 of *Azadirachta indica*. Chem. Biodiversity 6, 79–85.

Zhang, H.W., Song, Y.C., Tan, R.X., 2006. Biology and chemistry of endophytes. Nat. Prod. Rep. 23, 753–771.