Appl Microbiol Biotechnol (2013) 97:305–315
DOI 10.1007/s00253-012-4189-7

Cytotoxic metabolites from *Perenniporia tephropora*, an endophytic fungus from *Taxus chinensis* var. *mairei*

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Received: 4 March 2012 / Revised: 3 May 2012 / Accepted: 15 May 2012 / Published online: 3 June 2012

Abstract Based on bioactivity-oriented isolation, the EtOAc extract of a culture broth of the endophytic fungus *Perenniporia tephropora* Z41 from *Taxus chinensis* var. *mairei*, with strong anti-*Pyricularia oryzae* activity, afforded a new sesquiterpenoid, perenniporin A (**1**), together with three known compounds, ergosterol (**2**), *rel*-(+)-(2aR,5R,5aR,8S,8aS,8bR)-decahydro-2,2,5,8-tetramethyl-2H-naphtho[1,8-bc]genfuran-5-ol (**3**), and albicanol (**4**). Their structures were elucidated by means of spectroscopic methods. All the isolated compounds and the EtOAc extract of *P. tephropora* Z41 (EPT) were evaluated for their cytotoxic activity against three human cancer cell lines (HeLa, SMMC-7721, and PANC-1). EPT demonstrated significant cytotoxicity with IC_{50} values ranging from 2 to 15 μg/mL. Compound **2** was the most cytotoxic constituent against the tested cell lines with IC_{50} values of 1.16, 11.63, and 11.80 μg/mL, respectively, while compounds **1**, **3**, and **4** exhibited moderate cytotoxicity with IC_{50} values ranging from 6 to 58 μg/mL. We conclude that the endophytic fungus *P. tephropora* is a promising source of novel and cytotoxic metabolites.

Keywords *Perenniporia tephropora* · *Taxus chinensis* var. *mairei* · Endophytic fungi · Perenniporin A · Cytotoxic activity

Ling-Shang Wu and Chang-Ling Hu contributed equally to this work.

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Introduction

Although the advent of combinatorial chemistry has shifted the research focus away from natural products, fungal endophytes continue to be a new and feasible source for novel drugs (Suryanarayanan et al. 2009). Endophytic fungi, microorganisms occuring within the living tissues of plants without causing visible disease symptoms at a particular time (Rodriguez et al. 2009), have been found in all plant species examined to date (Arnold et al. 2000). They have a capacity to produce an array of metabolites of varied structural groups such as terpenoids, steroids, xanthones, chinones, phenols, isocoumarins, benzopyranones, tetralones, cytochalasines, and enniatines (Schulz et al. 2002) possessing a variety of biological activity include antibacterial, antiviral, antifungal, and anticancer activities (Gunatilaka 2006). However, only few of them have been cultivated and screened for drug use.

Taxus chinensis var. *mairei* (Lemée et Lévl) Cheng et L.K. Fu, one *Taxus* species endemic to China, is ubiquitous to Yangtze River southward in China. Yew trees are a rich source of biological active diterpenoids belonging to the unique structure class of taxanes (Baloglu and Kingston 1999; Parmar et al. 1999) such as taxol (paclitaxel), which is one of the most important anticancer drugs currently on the market for the treatment of ovarian and breast cancers and shows promise for the treatment of neck, lung, gastrointestinal and bladder cancers (Li et al. 2008). In spite of taxol-producing endophytic fungi having generated worldwide interest (Zhang et al. 2009a, b; Raja et al. 2008; Kumaran et al. 2010), there are a few studies on other bioactive compounds produced by endophytes harbored in *Taxus* species (Li et al. 2009).

In the course of our continuous search for novel bioactive secondary metabolites from endophyte cultures (Xu et al. 2009; You et al. 2009; Sun et al. 2011), we isolated a fungus *Perenniporia tephropora* Z41with high anti-*Pyricularia oryzae* activity from the bark of *T. chinensis* var. *mairei* collected in Jingning, Zhejiang Province, People's Republic of China. Through bioassay-oriented fractionation, a new sesquiterpenoid, namely perenniporin A (**1**), together with three known compounds, ergosterol (**2**), *rel*-(+)-(2aR,5R,5aR,8S,8aS,8bR)-decahydro-2,2,5,8-tetramethyl-2H-naphtho[1,8-bc]furan-5-ol (**3**), and albicanol (**4**), were isolated from the culture broth of *P. tephropora* Z41. We report herein the details of the isolation and identification of endophytes and compounds and the evaluation for cytotoxic activity of those isolated compounds.

Materials and methods

Isolation and identification of the endophytic fungus

Healthy bark of *T. chinensis* var. *mairei* was collected in Jingning, Zhejiang Province, PR China. Samples were immediately placed in plastic bags and taken to the laboratory store at 4 °C for isolation of endophytic fungi within 48 h of collection. The samples were cleaned in tap water and then cut into 1×1 cm pieces before surface-sterilization. Bark pieces were consecutive immersion in 75 % ethanol for 1 min, 3 min in 2.5 % sodium hypochlorite, and 30 s in 75 % ethanol. The surface sterilized bark was cut into 0.5× 0.5 cm pieces, and the tissues were placed on potato dextrose agar (PDA) media containing 50 mg/L penicillin to inhibit bacterial contamination and incubated at room temperature for 7–14 days. The hyphal tip was removed and placed on PDA media, incubated at 26 °C, and replated until a pure culture was obtained.

The isolated endophytic fungus Z41 was identified according to its 5.8S gene and ITS (ITS1 and ITS2 regions) sequence by using the universal primers ITS5 and ITS4 following the protocol of Guo et al. (2003). The 5.8S gene and ITS regions sequence was compared by Blast search with reference sequences at the GenBank and aligned with CLUSTAL X software (Thompson et al. 1997) using 1,000 bootstrap replicates. The phylogenetic tree was performed using the neighbor-joining method (Saitou and Nei 1987). Identification at species taxonomic levels was based on ≥97 % ITS similarity (Higgins et al. 2007). The sequence was submitted to GenBank (accession No.JN198488). The fungal strain Z41 was deposited in the China General Microbiological Culture Collection Center (CGMCC) as CGMCC 3.14976.

Fermentation and compounds isolation

The endophytic fungus Z41 initially grown on a PDA medium in Petri dishes was transferred into a shake flask culture by punching out 5 mm of the agar plate culture with a self-designed cutter (Wang et al. 2006). The shake flask culture was carried out (250 mL) containing 100 ml potato dextrose liquid medium at 26 °C with 180 rpm for 7 days and then enriched by transferring into 5 L Erlenmeyer flasks. About 60 L of fermentation liquid was obtained after incubation in the same condition for 10 days. The mycelial pellets were harvested by filtration and then thoroughly crushed in a mortar. The fermentation broths and ground mycelia were subjected to ultrasound-assisted extraction three times with equal volume of ethyl acetate at room temperature for 1 h. All extracts were combined and condensed in a rotating evaporator under reduced pressure. The obtained crude extract (5.62 g) was chromatographed on a silica gel column eluting with a step gradient of petroleum ether- EtOAc (20:1, 10:1, 5:1, 1:1, 1:2, each 1.5 L, *v*/*v*) to give eight fractions (Fr1–Fr8). Fr3 was recrystallized to give compound **2** (18 mg). Fr4 (305 mg) was separated over silica gel with petroleum ether–EtOAc (5:1, 3:1, 1:1, each 0.5 L, *v*/*v*) to give two subfractions (subFr1 and subFr2). SubFr1 (45 mg) was separated using preparative TLC (petroleum ether–EtOAc, 3:1, *v*/*v*) to obtain compound **3** (12 mg). SubFr2 (85 mg) was subjected on Sephadex LH-20 (CH_2Cl_2—MeOH, 1:1; 500 ml, *v*/*v*) to yield compound **4** (8 mg). Fr5 (325 mg) was subjected to silica gel with petroleum ether–EtOAc (3:1, 1:1, each 0.5 L, *v*/*v*) and Sephadex LH-20 (MeOH—H_2O, 1:1; 500 ml, *v*/*v*) to give compound **1** (102 mg).

Chemical analysis

NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer with TMS as an internal standard. ESIMS were measured on an Agilent LC/MSD Trap XCT mass spectrometer, whereas HRESIMS were measured using a Q-TOF micro mass spectrometer (Waters, USA). IR spectra

were recorded on a Bruker Vector 22 spectrometer with KBr pellets. Optical rotations were acquired with a Perkin-Elmer 341 polarimeter. CD data were obtained on a Jasco 810 spectrometer. Materials for column chromatography were silica gel (100–200 mesh; Huiyou Silical Gel Development Co. Ltd. Yantai, China), silica gel H (10–40 μm; Yantai), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-GEL ODS-A (50 μm; YMC, Milford, MA). HSGF254 silica gel TLC plates (Yantai) were used for analytical TLC.

Determination of the absolute configuration of C-10 in compound **1**

According to the published literature (Górecki et al. 2007; Di Bari et al. 2001), a mixture of compound **1** (1.1 mg) and $Mo_2(OAc)_4$ (1.2 mg) was prepared for CD measurement. The mixture was kept for 30 min to form a stable chiral metal complex, the CD spectrum of which was then recorded. The observed sign of the diagnostic ICD (induced CD spectrum) curve at around 305 nm was correlated with the absolute configuration of C-10 in compound **1** (Górecki et al. 2007; Di Bari et al. 2001).

P. oryzae bioassay

The anti-*P. oryzae* activity was evaluated following the method of Xu (Xu et al. 2009). In brief, after the spore suspension of *P. oryzae* P-2b (4×10^4/mL) was added in a 96-well microplate, 50 μL of sample was placed in each well and incubated at 27 °C for 15 h. The spore growth and mycelium shape were observed and compared with Ketoconazole to determine the minimum inhibited concentration (MIC). Each test was done in three replicates and interpretation was based on average value of results.

Cytotoxic assay

The MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazoliumbromide] colorimetric assay, against HeLa, SMMC-7721 and Panc-1 (available in Chinese Academy of Sciences), was performed as described in the literature (Wang et al. 2011). Briefly, the cells were cultured in 10% FBS DMEM medium. Test samples were prepared at six concentrations (0.001, 0.01, 0.1, 1.0, 10.0, and 100.0 μg/mL). After these cell lines were seeded in a 96-well microplate for 12 h, 10 μL of sample was placed in each well and incubated at 37 °C for 48 h, and then 20 μL MTT (5 mg/mL; Sigma, NY) was added for 4 h. After removing the medium and putting 150 μL dimethyl sulfoxide (DMSO) into each well, the plate was shaken for 10 min to dissolve blue formazan crystals and the absorbance was read at a wavelength of 570 nm on a microplate reader (Labsystems, WellscanMR-2).

The cytotoxicity of tested samples against tumor cells was expressed as IC_{50} values, which were calculated by LOGIT method. Results are representative of three individual experiments.

Results

Identification of the endophytic fungus

The phylogenetic tree (Fig. 1) inferred from the 5.8S gene and ITS regions sequences indicated that the endophytic fungus Z41 was classified into the clade including *P. tephropora* AB462323, *Perenniporia corticola* HQ654093, *Perenniporia tenuis* HQ848474, *Perenniporia maackiae* HQ654102. The endophytic fungus Z41 closely related to *P. tephropora* AB462323 with the ITS sequence similarity of 99.3 % and was therefore further identified as *P. tephropora*.

Structural determination of the compounds

Compound **1**, trivially named perenniporin A, was obtained as yellowish powder; $[\alpha]_D^{20}$ −38.9° (*c* 0.175, MeOH); UV (MeOH) λ_{max} 289 nm; IR (KBr) ν_{max} 3,345, 2,940, 1,672, 1,641, 1,431, 1,383, 1,271, and 685. Its molecular formula was determined to be $C_{15}H_{21}O_5$ by positive HRESIMS $[M+Na]^+$ at *m/z* 303.1214 (calcd 303.1216), indicating six degrees of unsaturation, which

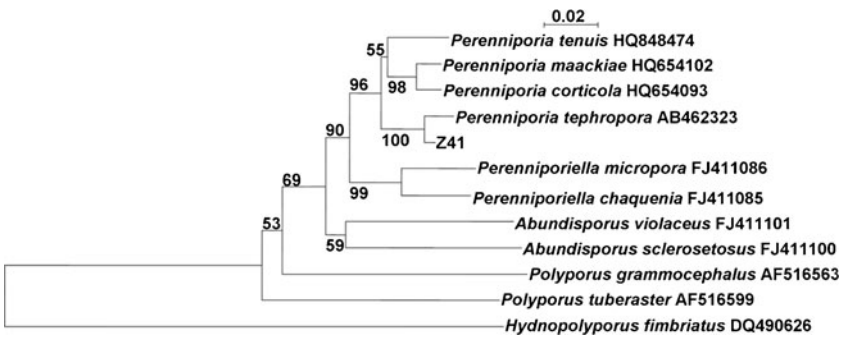


Fig. 1 Phylogenetic tree of the endophytic fungus Z41 based on 5.8S and ITS regions sequences. Bootstrap values above 50 % (1,000 replicates) are shown at branches. *Hydnopolyporus fimbriatus* is used as an outgroup

was supported by its NMR data (Fig. 2). The IR spectrum exhibited an OH absorption band at 3,345 cm^{-1}.

The ^{1}H NMR spectrum of **1** indicated signals due to two tertiary methyl groups (δ_H 1.14 and 1.18), three methylene groups (δ_H 2.53 and 2.23, δ_H 2.39 and 2.26, and δ_H 2.03 and 1.62), one oxygenated methine proton (δ_H 4.39, s), three olefinic protons at δ_H 6.28, 7.24, and 7.02 assigned to H-8, H-14, and H-2, respectively, and an one-proton multiplet at δ_H 2.71 assigned to H-6. The ^{13}C NMR and DEPT (Table 1) analysis revealed 15 carbon signals, including two methyls, three methylenes, five methines, and five quaternary carbons, of which the typical signals were one oxygenated quaternary olefinic carbon (δ_C 155.4, C-9), one oxygenated olefinic methine carbon (δ_C 136.8, C-14), one quaternary

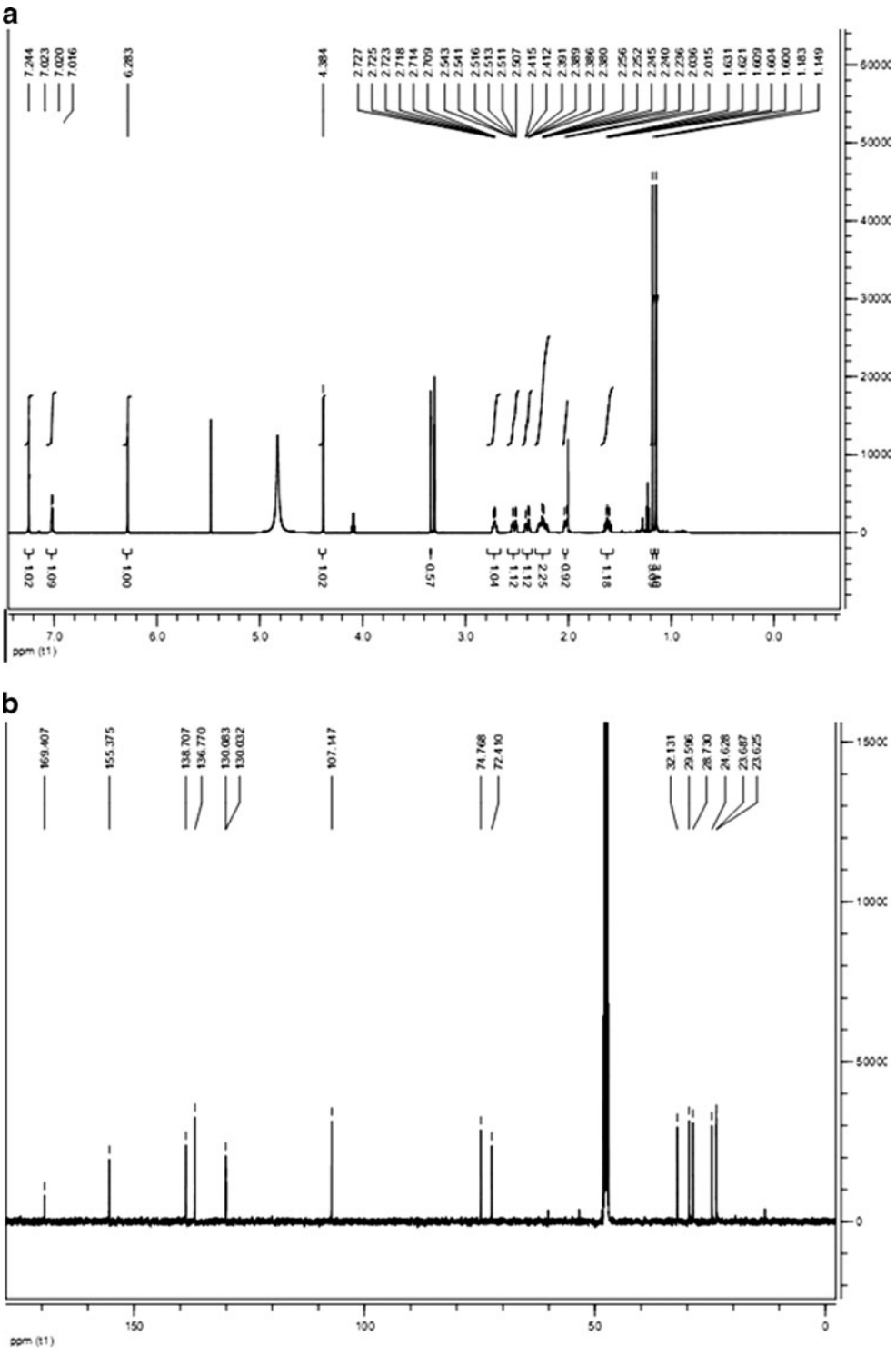
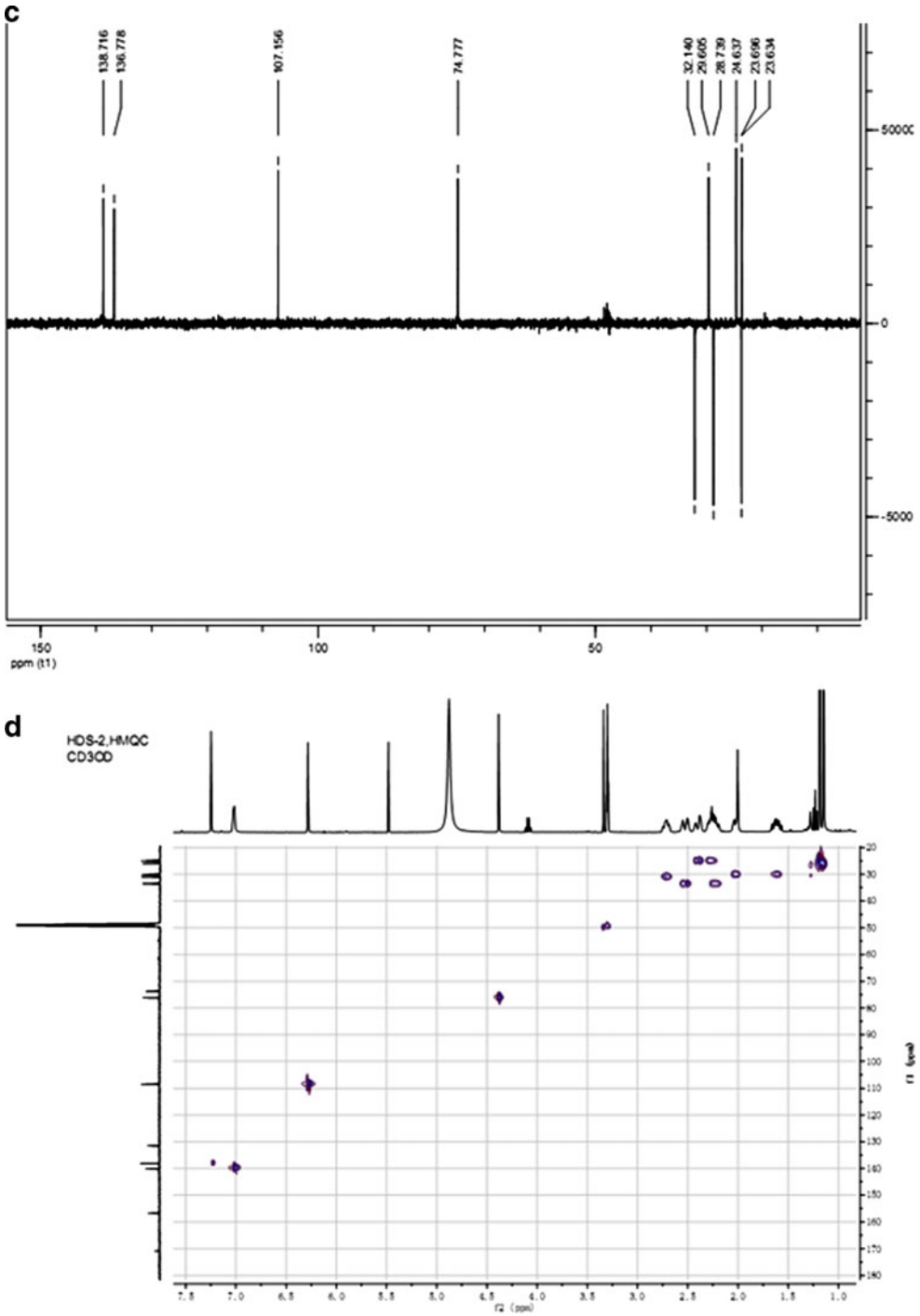


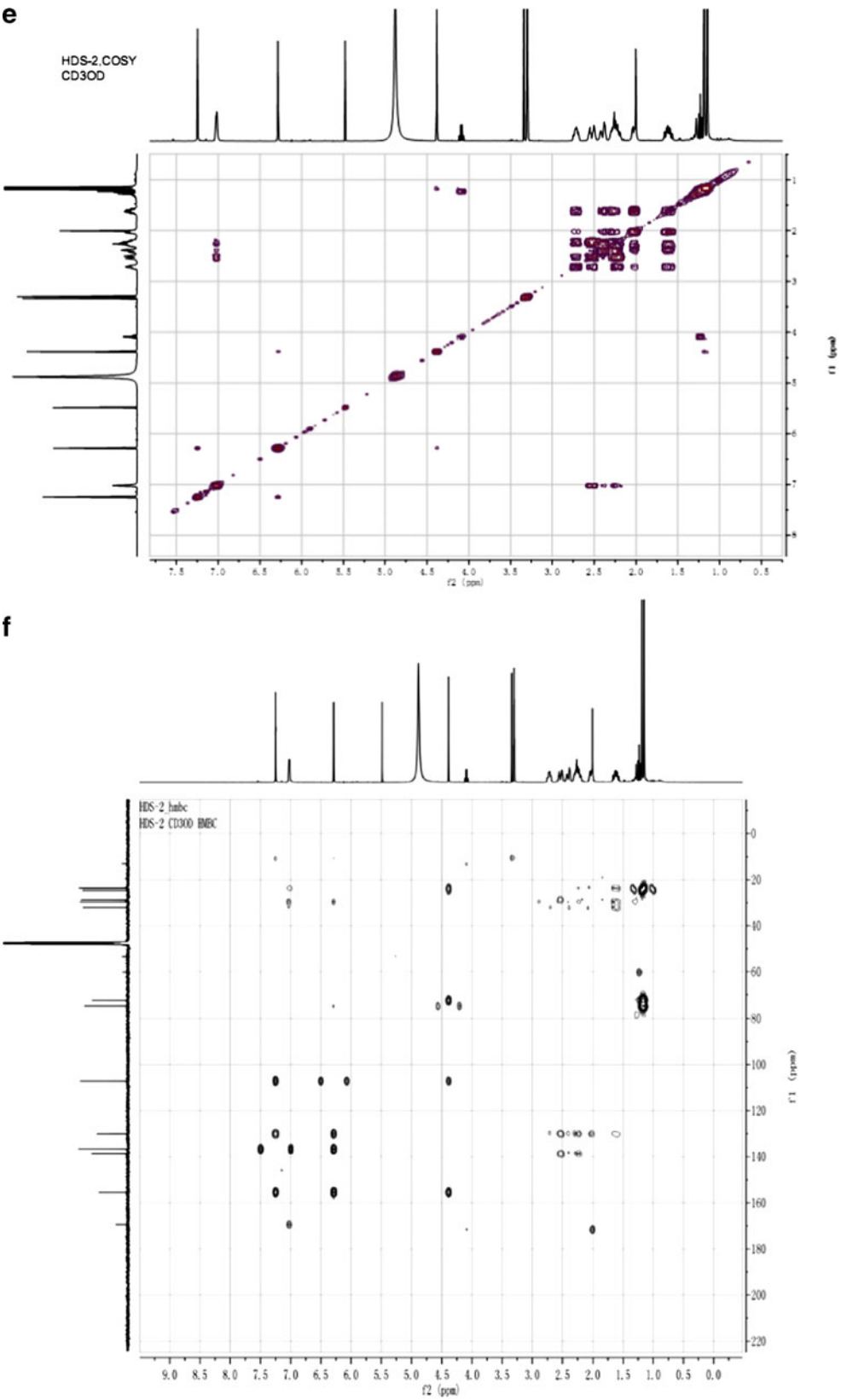
Fig. 2 Supporting Information of compound **1**. **a** ^{1}H-NMR of compound **1**; **b** ^{13}C-NMR of compound **1**; **c** DEPT of compound **1**; **d** HSQC of compound **1**; **e** ^{1}H–$^{1}HCOSY$ of compound **1**; **f** HMBC of compound **1**; **g** ROESY of compound **1**

Fig. 2 (continued)



olefinic carbon (δ_C 130.1, C-7) and one olefinic methine carbon (δ_C 107.1, C-8), which undoubtedly constitute a 2,4-disubstituted furan ring. Furthermore, HMBC correlations observed between H-2/H-4 and C-2/C-4, and between H-1/H-5 and C-1/C-5, strongly supported a 1, 4-disubstituted cyclohexane structure moiety in compound **1**. The ^{1}H–^{1}H COSY spectrum revealed a group of five protons connected in the order 7.02 (δ_H, m, H-2) to 2.53 (δ_H, m, H-1) to 2.71 (δ_H, m, H-6) to 1.62 (δ_H, m, H-5) to 2.26 (δ_H, m, H-4), indicating the existence of fragment –CH–CH_2–CH–CH_2–CH_2–, from C-2 through C-1 to C-6, C-5, and C-4 (Fig. 3). In addition, HMBC cross-peaks from H-4 and H-5 to C-3 (δ_C 130.0) show that the C-2/C-3 double bond is part of the cyclohexene ring, which is conjugated with a carboxy group bond to C-3, evidenced by the HMBC correlation from H-2 (δ_H 7.02, m) to C-15 (δ_C 169.4). A HMBC correlation from H-6 (δ_H 2.73, m) to C-7 (δ_C 130.1), as well as HMBC correlations from H-1 and H-5 to C-7 and from H-8 (δ_H 6.28, s) to C-6 (δ_C 29.6), indicated the suggested linkage of the furan ring and the cyclohexane ring from C-7 to C-6 in

Fig. 2 (continued)



compound **1**. H-14 (δ_H 7.22, s) also gives a HMBC correlation over the furan oxygen to C-9 (δ_C 155.4), to which a 2-methyl-1,2-propylene glycol moiety is attached based on detailed inspection of the HMBC correlations (Fig. 3). Key ROESY correlations were observed between H-6 and H-2, H-8, and H-10, indicating a β- orientation for both H-6 and

Fig. 2 (continued)

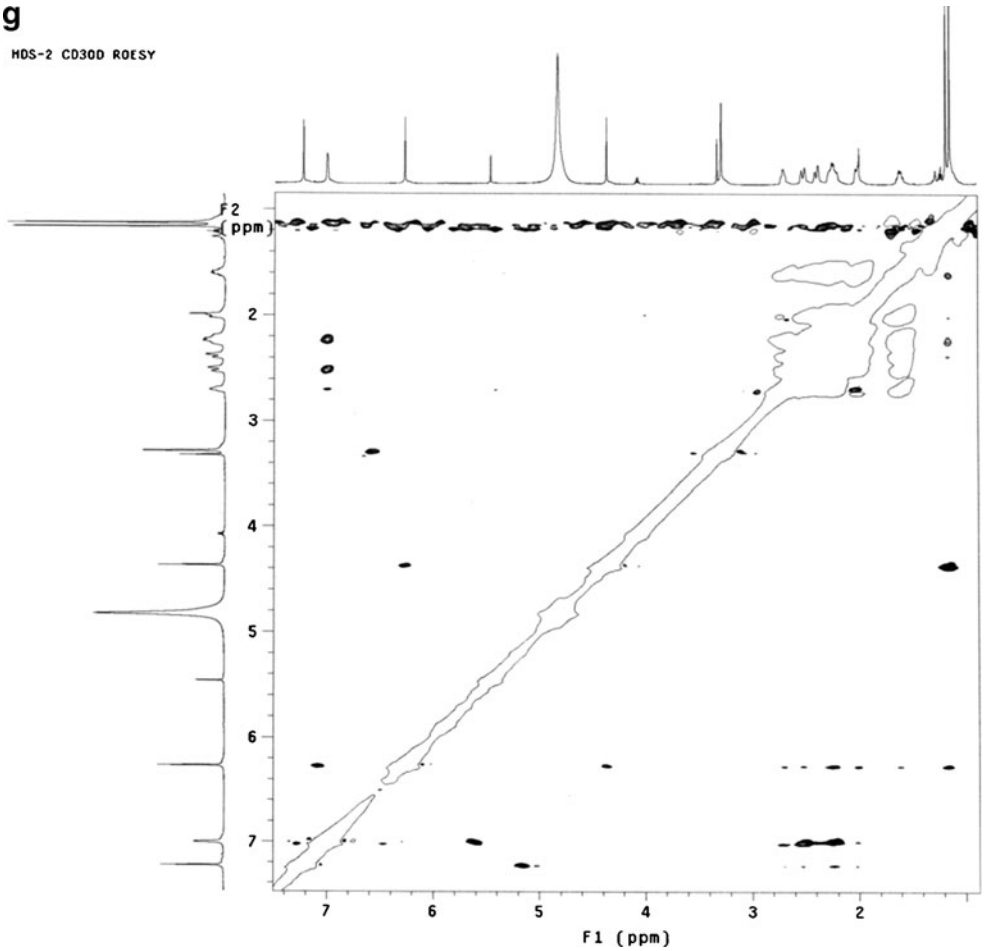


Table 1 ^{1}H-NMR (600 MHz), ^{13}C-NMR (150 MHz), and DEPT spectroscopic data (δ in methanol—d_4) for **1**

Position	δ_C	DEPT	δ_H
1	32.1	CH_2	2.53 (m) and 2.23 (m)
2	138.7	CH	7.02 (m)
3	130	C	
4	23.7	CH_2	2.39 (m) and 2.26 (m)
5	28.7	CH_2	2.03 (m) and 1.62 (m)
6	29.6	CH	2.71 (m)
7	130.1	C	
8	107.1	CH	6.28 (s)
9	155.4	C	
10	74.8	CH	4.39 (s)
11	72.4	C	
12	24.6	CH_3	1.14 (s)
13	23.6	CH_3	1.18 (s)
14	136.8	CH	7.24 (s)
15	169.4	C	

Assignments were confirmed by ^{1}H–^{1}H COSY, ROESY, HMBC, and HSQC

H-10. Accordingly, the structure of compound **1** was therefore deduced to be shown in Fig. 4. And the absolute configuration of C-10 in **1** was further assigned using the Snatzke′s method (Górecki et al. 2007; Di Bari et al. 2001). Metal complex of compound **1** in DMSO gave a significant induced CD spectrum (ICD) (Fig. 5), in which the negative Cotton effect observed at 305 nm permitted the assignment of a 10*R*-configuration for **1**. Compound **1** is a new member of the bisabolane sesquiterpenoid metabolites, and to our knowledge, this is the first reported isolation of bisabolane sesquiterpenoid derivative from the genus *Perenniporia*.

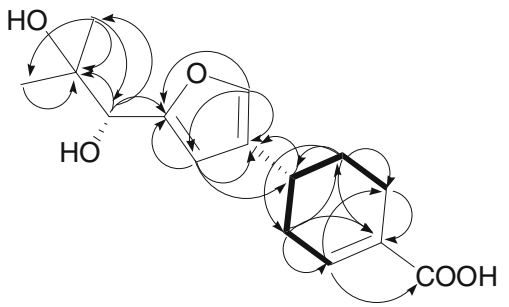
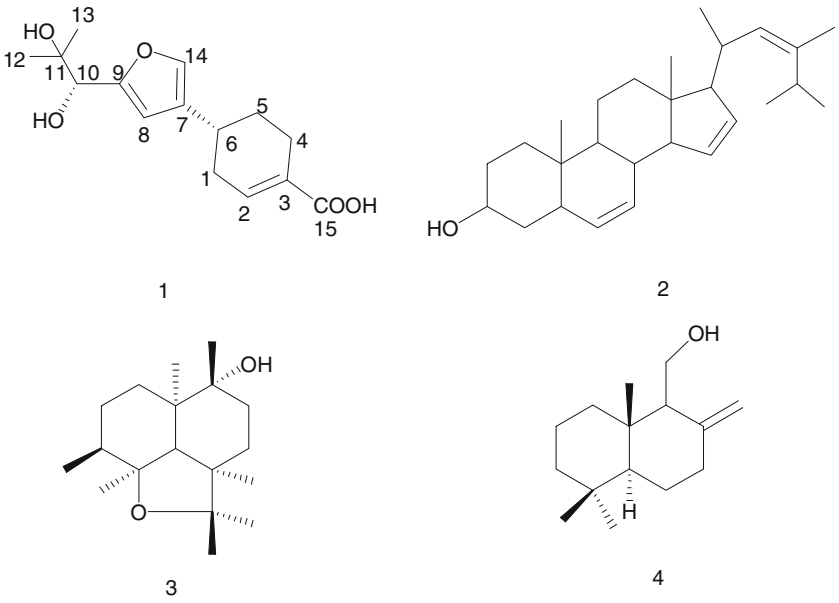


Fig. 3 Key HMBC (*arrows*) correlations and ^{1}H–^{1}H COSY (*solid lines*) of compound **1**

Fig. 4 Structures of compounds **1**–**4**



Based on the MS and NMR data, compounds **2**–**4** were identified as already known compounds: compound **2**, ergosterol (Mooney et al. 2007); compound **3**, *rel*-(+)-(2aR,5R,5aR,8S,8aS,8bR)-decahydro-2,2,5,8-tetramethyl-2H-naphtho[1,8-bc]furan-5-ol (Hashimoto et al. 2003); and compound **4**, albicanol (Ito et al. 2000). Compound **2**, ergosterol, the principal sterol in fungi cell membrane, is abundant in edible mushrooms, yeast lees of wines, and Chinese medicinal mushrooms, such as *Ganoderma lucidum* and *Cordyceps sinensis* (Kuo et al. 2011), and is also known to be an important pharmaceutical intermediate and the precursor of vitamin D_2 and cortisone (Jasinghe et al. 2005). In addition, the antitumor activity of ergosterol has been reported previously (Yasukawa et al. 1994; Kwon et al. 2002; Ding et al. 2009). Compound **3**, a cadinane-type sesquiterpene possessing a cyclic ether linkage, was previously isolated from the liverwort *Ptychanthus striatus* (Hashimoto et al. 2003) and it was isolated from *Perenniporia* species for the first time. Compound **4**, albicanol, a representative drimane sesquiterpene, was reported to be a pungent principle and antifeedant agent against fish and worm, which also exhibited strong inhibitory effects on the EBV-EA activation and significantly suppressed an in vivo two-stage carcinogenesis on mouse skin (Ito et al. 2000). This is the first report of its presence as a metabolite in *Perenniporia* species.

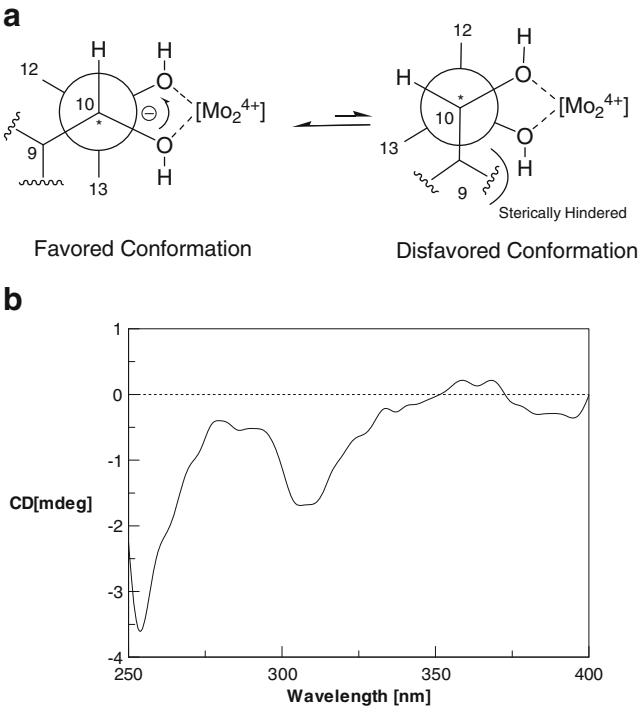


Fig. 5 **a** Conformations of the Mo_2^{4+} complex of **1**. **b** ICD spectrum of Mo_2^{4+} complex of **1** in DMSO

Anti-*P. oryzae* activity

The EtOAc extract of *P. tephropora* Z41 (EPT) displayed strong inhibitiory activity against *P. oryzae* (MIC=7.8 μg/mL). Table 2 showed that compound **2** has the most active anti-*P. oryzae* activity (MIC= 7.8 μg/mL) among the tested pure compounds. The MIC values of compound **1**, **3** and **4** were 15.625, 15.625, and 33.75 μg/mL, respectively.

Cytotoxic activity

The EtOAc extract of *P. tephropora* Z41 (EPT) showed significant cytotoxic activities against HeLa, SMMC-7721 and Panc-1 cell lines with IC_{50} values of 2.83, 15.60, and 12.58 μg/mL. Compound **2** was the most cytotoxic

Table 2 Anti-*Pyricularia oryzae* activity of EPT and compounds **1**–**4**

Compounds	Concentration (μg/mL)							
	1,000	500	250	125	67.5	33.75	15.625	7.8
EPT	×	×	×	×	×/+++	+++	++	++
1	×	×	+++	+++	++	++	++	-
2	×	×	×	×	×	×	+++	++
3	×	×	×	+++	+++	+++	++	–
4	×	×	×/+++	+++	+++	++	–	–
Ketoconazole[a]	×	×	×	×	×	×	×	×

(–) no antagonistic action, (+) presence antagonistic action, (++) moderate antagonistic action, (+++) strong antagonistic action, (×) the growth was inhibited

[a]As positive control

constituent, especially against the HeLa cell line with IC_{50} value of 1.16 μg/mL. In contrast, compounds **1**, **3**, and **4** exhibited moderate cytotoxicity with IC_{50} values ranging from 6 to 58 μg/mL (Table 3).

Discussion

Metabolites product by endophytes could be influenced by the chemistry of their host plants (Huang et al. 2008; Mucciarelli et al. 2007; Aly et al. 2010). During the long period of co-evolution, some endophytes have the ability to produce similar or identical biological active compounds as their host plants, such as paclitaxel, podophyllotoxin, camptothecine, vinblastine, hypericin, and diosgenin (Zhao et al. 2011). We have previously investigated endophytic fungi isolated from bark, branches and leaves of *T. chinensis* var. *mairei* collected from Jiangxi, Zhejiang and Chongqing in China (data not shown). In order to searching for similar antitumor compounds as the yew trees, the EtOAc crude extracts from the fermentation broths and ground mycelia of isolated 145 endophytic fungal taxa were first tested through *P. oryzae* bioassay, which is a good primary model for screening of antifungi and antitumor strains (Gunji et al. 1983). As a result, the crude extract of *P. tephropora* displayed the strongest inhibitiory activity against *P. oryzae* (minimum morphological deformation concentration=7.8 μg/ml), which is therefore selected for the present study on its cytotoxic metabolites.

Table 3 Antitumor activity of EPT and compounds **1**–**4** (IC_{50}, in micrograms per milliliter)

Compounds	Cell line		
	HeLa	SMMC-7721	PANC-1
EPT	2.83	15.60	12.58
1	30.44	45.49	44.22
2	1.16	11.63	11.80
3	6.93	18.19	33.47
4	14.20	34.56	58.57
Doxorubicin	0.38	0.18	1.25

Key to cell lines used: human lung cervical cell lines (HeLa), human hepatocellular carcinoma cell lines (SMMC-7721), and human pancreatic cell lines (PANC-1)

Basidiomyceteous endophytes have been infrequently isolated from plant hosts (Pinruan et al. 2010; Rungjindamai et al. 2008). *P. tephropora*, belonging to Basidiomycetes, has not been isolated from the other *Taxus* species yet. Although members of the genus *Perenniporia* are mostly isolated as wood-inhabiting fungi, some of its species were encountered as endophytes as well, e.g., *Perenniporia* sp. (Evans et al. 2003; Pinruan et al. 2010). Basidiomycetes produce a large number of secondary metabolites which showed antibacterial, antifungal, antiviral, cytotoxic, and hallucinogenic activity (Suay et al. 2000). However, to our knowledge, there have been few bioactive metabolites, except laccase (Ben Younes et al. 2007), reported from *P. tephropora*.

In this study, we found that the endophytic fungus *P. tephropora* Z41 produced different varieties of metabolite classes that were not yet reported from *Perenniporia* species and that showed potent anti-*P. oryzae* and cytotoxic activities. Compound **1** was a new member of the bisabolane sesquiterpenoid metabolites with moderate cytotoxic activity, and it represents the first isolation of bisabolane sesquiterpenoid derivative from the genus *Perenniporia*. Compound **2** was the principal sterol in fungi cell membrane and we found it was the most cytotoxic constituent against the tested three cell lines in our study. Compound **3** was a cadinane-type sesquiterpene and compound **4** was a drimane-type one. Both of them were isolated from *Perenniporia* for the first time. Compound 4 was previously reported to show significant antitumor promoting activity both in vitro and in vivo. In the present work, it also demonstrated moderate cytotoxic activity against the tested cancer cell lines. Our results indicate that ergosterol **2** and sesquiterpenoids **1**, **3**, and **4** could be valuable candidates as potent tumor inhibitors and be beneficial in the therapy of cancer diseases. Our study also underscores that endophyte *P. tephropora* is a promising sources of natural bioactive

and novel sesquiterpenoids metabolites, though without diterpenoids like taxols.

References

Aly AH, Debbab A, Kjer J, Proksch P (2010) Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Divers 41(1):1–16. doi:10.1007/s13225-010-0034-4

Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA (2000) Are tropical fungal endophytes hyperdiverse? Ecol Lett 3:267–274. doi:10.1046/j.1461-0248.2000.00159.x

Baloglu E, Kingston DGI (1999) The taxane diterpenoids. J Nat Prod 62(10):1448–1472

Ben Younes S, Mechichi T, Sayadi S (2007) Purification and characterization of the laccase secreted by the white rot fungus *Perenniporia tephropora* and its role in the decolourization of synthetic dyes. J Appl Microbiol 102(4):1033–1042. doi:10.1111/j.1365-2672.2006.03152.x

Di Bari L, Pescitelli G, Pratelli C, Pini D, Salvadori P (2001) Determination of absolute configuration of acyclic 1, 2-diols with $Mo_2(OAc)_4$. 1. Snatzke's Method Revisited. J Org Chem 66 (14):4819–4825. doi:10.1021/jo010136v

Ding Y, Bao HY, Bau T, Li Y, Kim YH (2009) Antitumor components from *Naematoloma fasciculare*. J Microbiot Biotechnol 19 (10):1135–1138. doi:10.4014/jmb.0901.022

Evans HC, Holmes KA, Thomas SE (2003) Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. Mycol Prog 2(2):149–160. doi:10.1007/s11557-006-0053-4

Górecki M, Jablonska E, Kruszewska A, Suszczynska A, Urbanczyk-Lipkowska Z, Gerards M, Morzycki JW, Szczepek WJ, Frelek J (2007) Practical Method for the Absolute Configuration Assignment of tert/tert 1, 2-Diols Using Their Complexes with $Mo_2(OAc)_4$. J Org Chem 72(8):2906–2916. doi:10.1021/jo062445x

Gunatilaka AAL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69(3):509–526

Gunji S, Arima K, Beppu T (1983) Screening of antifungal antibiotics according to activities inducing morphological abnormalities. Agric Biol Chem 47(9):2061–2069

Guo LD, Huang GR, Wang Y, He WH, Zheng WH, Hyde KD (2003) Molecular identification of white morphotype strains of endophytic fungi from *Pinus tabulaeformis*. Mycol Res 107(Pt 6):680–688. doi:10.1017/S0953756203007834

Hashimoto T, Takaoka S, Tanaka M, Asakawa Y (2003) Structures of two new highly oxygenated labdane-type diterpenoids and a new cadinane-type sesquiterpenoid possessing a cyclic ether linkage from the liverwort *Ptychanthus striatus*. Heterocycles 59(2):645–659

Higgins KL, Arnold AE, Miadlikowska J, Sarvate SD, Lutzoni F (2007) Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. Mol Phylogenet Evol 42(2):543–555. doi:10.1016/j.ympev.2006.07.012

Huang W, Cai Y, Hyde K, Corke H, Sun M (2008) Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. Fungal Divers 33:61–75

Ito H, Muranaka T, Mori K, Jin ZX, Tokuda H, Nishino H, Yoshida T (2000) Ichthyotoxic phloroglucinol derivatives from *Dryopteris fragrans* and their anti-tumor promoting activity. Chem Pharm Bull 48(8):1190–1195

Jasinghe VJ, Perera CO, Barlow PJ (2005) Bioavailability of vitamin D_2 from irradiated mushrooms: an in vivo study. Brit J Nutr 93 (6):951–956. doi:10.1079/BJN20051416

Kumaran RS, Kim HJ, Hur B-K (2010) Taxol promising fungal endophyte, *Pestalotiopsis* species isolated from *Taxus cuspidata*. J Biosci Bioeng 110(5):541–546. doi:10.1016/j.jbiosc.2010.06.007

Kuo CF, Hsieh CH, Lin WY (2011) Proteomic response of LAP-activated RAW 264.7 macrophages to the anti-inflammatory property of fungal ergosterol. Food Chem 126(1):207–212. doi:10.1016/j.foodchem.2010.10.101

Kwon HC, Zee SD, Cho SY, Choi SU, Lee KR (2002) Cytotoxic ergosterols from *Paecilomyces* sp. J300. Arch Phar Res 25 (6):851–855. doi:10.1007/BF02977003

Li C, Huo C, Zhang M, Shi Q (2008) Chemistry of Chinese yew, *Taxus chinensis* var. *mairei*. Biochem Syst Ecol 36(4):266–282. doi:10.1016/j.bse.2007.08.002

Li Y, Lu C, Hu Z, Huang Y, Shen Y (2009) Secondary metabolites of *Tubercularia* sp. TF5, an endophytic fungal strain of *Taxus mairei*. Nat Prod Res 23(1):70–76. doi:10.1080/14786410701852818

Mooney BD, Nichols PD, De Salas MF, Hallegraeff GM (2007) Lipid, fatty acid, and sterol composition of eight species of kareniaceae (dinophyta):chemotaxonomy and putative lipid phycotoxins. J Phycol 43(1):101–111. doi:10.1111/j.1529-8817.2006.00312.x

Mucciarelli M, Camusso W, Maffei M, Panicco P, Bicchi C (2007) Volatile terpenoids of endophyte-free and infected peppermint (*Mentha piperita* L.): chemical partitioning of a symbiosis. Microb Ecol 54(4):685–696. doi:10.1007/s00248-007-9227-0

Parmar VS, Jha A, Bisht KS, Taneja P, Singh SK, Kumar A, Jain R, Olsen CE (1999) Constituents of the yew trees. Phytochemistry 50(8):1267–1304

Pinruan U, Rungjindamai N, Choeyklin R, Lumyong S, Hyde KD, Jones EBG (2010) Occurrence and diversity of basidiomycetous endophytes from the oil palm, *Elaeis guineensis* in Thailand. Fungal Divers 41(1):71–88. doi:10.1007/s13225-010-0029-1

Raja V, Kamalraj S, Muthumary JP (2008) Taxol from *Botryodiplodia theobromae* (BT 115)—AN endophytic fungus of *Taxus baccata*. J Biotechnol 136S:S187–S197. doi:10.1016/j.jbiotec.2008.07.1823, 10.1016/j.jbiotec.2008.07.1820, 10.1016/j.jbiotec.2008.07.1821, 10.1016/j.jbiotec.2008.07.1822, 10.1016/j.jbiotec.2008.07.1824, 10.1016/j.jbiotec.2008.07.1825

Rodriguez RJ, White JF Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182 (2):314–330. doi:10.1111/j.1469-8137.2009.02773.x

Rungjindamai N, Pinruan U, Choeyklin R, Hattori T, Jones E (2008) Molecular characterization of basidiomycetous endophytes isolated from leaves, rachis and petioles of the oil palm, *Elaeis guineensis*. Fungal Divers 33:139–161

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–425

Schulz B, Boyle C, Draeger S, AK Rmmert, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 106(9):996–1004

Suay I, Arenal F, Asensio FJ, Basilio A, Angeles Cabello M, Teresa Díez M, García JB, González del Val A, Gorrochategui J, Hernández P (2000) Screening of basidiomycetes for antimicrobial activities. Antonie Van Leeuwenhoek 78(2):129–140. doi:10.1023/A:1026552024021

Sun PX, Zheng CJ, Li WC, Jin GL, Huang F, Qin LP (2011) Trichodermanin A, a novel diterpenoid from endophytic fungus culture. J Nat Med 1–4. doi:10.1007/s11418-010-0499-1

Suryanarayanan TS, Thirunavukkarasu N, Govindarajulu MB, Sasse F, Jansen R, Murali TS (2009) Fungal endophytes and bioprospecting. Fungal Biology Reviews 23(1–2):9–19. doi:10.1016/j.fbr.2009.07.001

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25(24):4876–4882. doi:10.1093/nar/25.24.4876

Wang J, Wu J, Huang W, Tan R (2006) Laccase production by *Monotospora* sp., an endophytic fungus in *Cynodon dactylon*. Bioresource Technol 97(5):786–789. doi:10.1016/j.biortech.2005.03.025

Wang LW, Xu BG, Wang JY, Su ZZ, Lin FC, Zhang CL, Kubicek CP (2011) Bioactive metabolites from *Phoma* species, an endophytic fungus from the Chinese medicinal plant *Arisaema erubescens*. Appl Microbiol Biotechnol 1–9. doi:10.1007/s00253-011-3472-3

Xu LL, Han T, Wu JZ, Zhang QY, Zhang H, Huang BK, Rahman K, Qin LP (2009) Comparative research of chemical constituents, antifungal and antitumor properties of ether extracts of *Panax ginseng* and its endophytic fungus. Phytomedicine 16(6):609–616. doi:10.1016/j.phymed.2009.03.014

Yasukawa K, Aoki T, Takido M, Ikekawa T, Saito H, Matsuzawa T (1994) Inhibitory effects of ergosterol isolated from the edible mushroom *Hypsizigus marmoreus* on TPA-induced inflammatory ear oedema and tumour promotion in mice. Phytother Res 8 (1):10–13. doi:10.1002/ptr.2650080103

You F, Han T, Wu J, Huang B, Qin L (2009) Antifungal secondary metabolites from endophytic *Verticillium* sp. Biochem Syst Ecol 37(3):162–165. doi:10.1016/j.bse.2009.03.008

Zhang P, Zhou P-P, Yu L-J (2009a) An endophytic taxol-producing fungus from *Taxus media*, *Aspergillus candidus* MD3. FEMS Microbiol Lett 293(2):155–159. doi:10.1111/j.1574-6968.2009.01481.x

Zhang P, Zhou P-P, Yu L-J (2009b) An endophytic taxol-producing fungus from *Taxus media*, *Cladosporium cladosporioides* MD2. Curr Microbiol 59(3):227–232. doi:10.1007/s00284-008-9270-1

Zhao J, Shan T, Mou Y, Zhou L (2011) Plant-derived bioactive compounds produced by endophytic fungi. Mini Rev Med Chem 11 (2):159–168. doi:10.2174/138955711794519492