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ORIGINAL ARTICLE

Two new polyketides from the ascomycete fungus *Leptosphaeria* sp.

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Leptosphaerins H and I (1 and 2), two new xanthone derivatives, and six known compounds, leptosphaerin F (3), monodictysin B (4), norlichexanthone (5), leptosphaerin D (6), moniliphenone (7) and emodinbianthrone (8) have been isolated from a scale-up fermentation of the ascomycete fungus *Leptosphaeria* sp. Their structures were primarily elucidated by interpretation of NMR spectroscopic data. The absolute configuration of 1 was assigned using the modified Mosher method, whereas that of C-8a in 2 was determined via the CD data. Compound 6 showed modest cytotoxicity against a panel of three human tumor cell lines.
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INTRODUCTION

Fungi inhabiting special and competitive environments are more likely to produce bioactive secondary metabolites with diverse structural features, presumably due to their highly evolved metabolic systems adapted during the natural selection process.[1–5] In recent years, more and more bioactive compounds from *Cordyceps*-colonizing fungi, the species that colonize the fruiting body of *Cordyceps sinensis*, and the fungi isolated from the soil samples in the *C. sinensis*, have been reported.[6–9] In the course of investigating new bioactive compounds from the fungus, a strain of *Leptosphaeria* sp. (XZC04-CS-304), isolated from the soil sample on the surface of the fruiting body of *C. sinensis* collected in Linzhi, Tibet, China was investigated, leading to the discovery of antifungal polyketides leptosphaerins A–G.[10] Subsequent chemical investigations of the extract from a larger-scale fermentation of this fungus led to the isolation of two new xanthone derivatives, leptosphaerins H and I (**1** and **2**), together with the known compounds, monodictysin B (**4**),[11] norlichexanthone (**5**),[12] moniliphenone (**7**)[13] and emodinbianthrone (**8**)[14] (Figure 1). The previously identified polyketides, leptosphaerins F (**3**) and D (**6**) were also re-isolated in the current work. Details of the isolation, structure elucidation and biological activities of these compounds are reported herein.

RESULTS AND DISCUSSION

Leptosphaerin H (**1**) was obtained as light yellow powder. The molecular formula was determined to be $C_{15}H_{16}O_4$ (eight degrees of unsaturation) on the basis of its high-resolution electrospray ionisation mass spectrometry (HRESIMS) (*m/z* 283.0941 $[M+Na]^+$; Δ+0.2 m.m.u.). The ^1H and ^{13}C NMR spectra of **1** (Table 1) revealed the presence of two methyl groups, one methylene unit, three methines (one oxygenated), eight aromatic/olefinic carbons (three oxygenated) and one α, β-unsaturated ketone carbon (δ_C 183.8). These data together with two exchangeable protons (δ_H 3.91, 12.9) accounted for all ^1H and ^{13}C resonances and required the presence of three rings for **1**. Interpretation of the ^1H–^1H COSY NMR (Figure 2) data of **1** identified two isolated proton spin systems of C-2–C-4 and C-5–C-8 (including 7-OH, C-10 and C-11). Analysis of ^1H NMR spectrum for **1** (Table 1) displayed the presence of one 1, 2, 3-trisubstituted benzene ring, which was also supported by HMBC correlations (Figure 2) from H-2 to C-1 and C-9a, H-3 to C-1 and C-4a, and from H-4 to C-4a. HMBC correlations from H-5 and H-8 to C-8a and C-10a, and from H_3-11 to C-7, C-8 and C-8a indicated that the C-8a/C-10a olefin were attached to C-8 and C-5, respectively, establishing the cyclohexene ring. The cross peaks from OH-1 (δ_H 12.9) to C-1 and C-2 indicated that the hydroxyl group was attached to C-1. Further correlation from H-8 to C-9, together with the four-bond HMBC correlation from H-4 to C-9 implied that the C-9 was located between C-9a and C-8a. Considering the chemical shift values of C-4a (δ_C 157.1) and C-10a (δ_C 166.3), and the unsaturation requirement for 1, the two carbons were attached to the same oxygen to form tetrahydro-9*H*-xanthen-9-one skeleton. Collectively, these data permitted assignment of the planar structure of **1**.

The relative configuration of **1** was assigned by analysis of the ^1H–^1H coupling constants and NOESY experimental data. The small coupling constant of between H-7 and H-8 ($J = 2.0$ Hz) indicated that these two protons had a *cis* relationship with respect to the corresponding cyclohexene ring. NOESY correlations of H-6 with H_3-11 placed H-6 and H_3-11 on the same face of the ring system. Therefore, the relative configuration was proposed as shown.

The absolute configuration of **1** was determined using the modified Mosher method.[15,16] Treatment of **1** with (*S*)- and (*R*)-MTPA Cl alpha-Methoxy-alpha-(trifluoromethyl)phenylacetyl chloride afforded the major products, the *R*- (**1a**) and *S*-MTPA (**1b**) mono-esters, respectively. The difference in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for

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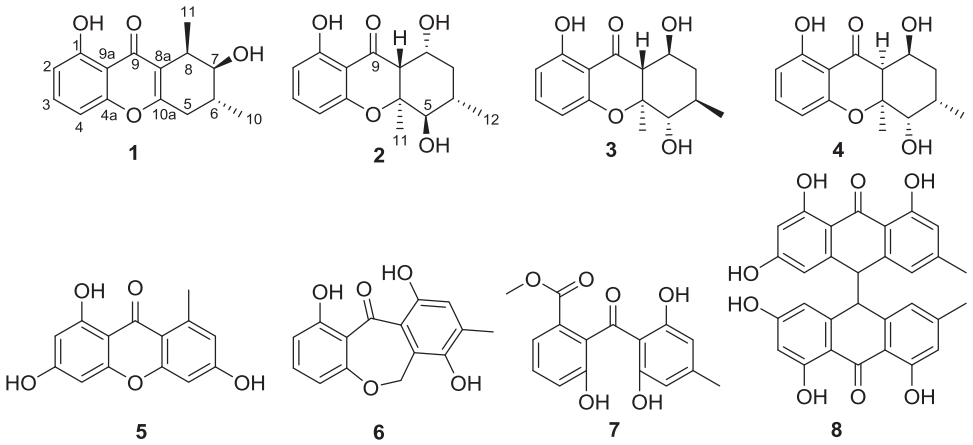

Figure 1 Chemical structures of compounds **1**–**8**.

Table 1 NMR data (500 MHz, acetone-d_6) for compound 1

	1			
Position	δ_C, *mult*	δ_H (*J in Hz*)	*HMBC*[a]	*NOESY*
1	161.7, qC			
2	111.0, CH	6.70, d (8.0)	1, 4, 9a	
3	136.0, CH	7.57, t (8.0)	1, 2, 4, 4a	
4	111.0, CH	6.90, d (8.0)	2, 4a, 9	
4a	157.1, qC			
5	31.8, CH_2	2.59, d (8.5)	7, 8a, 10, 10a	
6	28.7, CH	2.22, dq (1.0, 7.0)	5, 10	7, 10, 11
7	73.7, CH	3.70, s	5, 6, 8a, 10	6, 8, 10, 11
8	36.1, CH	3.07, dq (2.0, 7.0)	6, 7, 8a, 9, 10,10a	7, 11
8a	119.6, qC			
9	183.8, qC			
9a	107.5, qC			
10	18.0, CH_3	1.16, d (7.0)	5, 6, 7, 10a	6, 7
10a	166.3, qC			
11	18.2, CH_3	1.16, d (7.0)	7, 8, 8a	6, 7, 8
OH-1		12.9, s	1, 2	
OH-7		3.91, d (3.0)		

[a]HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

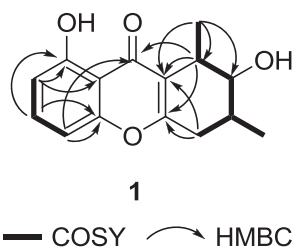

Figure 2 Key ^1H–^1H COSY, HMBC correlations for **1**.

the diastereomeric esters **1b** and **1a** was calculated to assign the 7*S* absolute configuration. Therefore, the 6*R*, 7*S*, 8*R* configuration was proposed for **1** on the basis of the $\Delta\delta$ results summarized in Figure 3.

The molecular formula of leptosphaerin I (**2**) was deduced as $C_{15}H_{18}O_5$ (seven degrees of unsaturation) by HRESIMS (*m/z* 301.1050 $[M+Na]^+$; Δ − 0.4 m.m.u.). A molecular formula search identified a tetrahydroxanthone, leptosphaerin F (**3**)[10] and monodictysin B (**4**),[11] which possess the same elemental composition as **2**. Analysis of the NMR data of **2** (Table 2) revealed the same gross structure as **3** and **4**, indicating their isomeric relationship. The relative configuration of **2** was assigned by analysis of its ^1H-^1H coupling constants and NOESY data. A large coupling constant of 10.1 Hz between H-5 and H-6 in **2** revealed their *trans* relationship. The small coupling constant 4.1 Hz between H-8a and H-8 in **2**, which is similar with that in compound **4** (4.3 Hz),[11] indicated that H-8a and H-8 were located on the same side of the cyclohexane-1,4-diol moiety. These assignments were confirmed by NOESY correlations (Figure 4) of H-6 with H-8 and H-8a. NOESY correlations of H_3-11 with H_3-12 revealed their proximity in space. Therefore, the relative configuration of **2** was proposed as shown.

The absolute configuration of C-8a in **2** was assigned by application of the CD exciton chirality method. The CD spectrum of **2** (Figure 5) showed a negative Cotton effect at 293 ($\Delta\varepsilon$–0.3) and a positive Cotton effect at 368 ($\Delta\varepsilon$+0.4) nm, respectively, similar to that of (−)-rotoic acid,[17] suggesting 8a*R* absolute configuration. Considering the relative configuration determined by ^1H-^1H coupling constants and NOESY data, **2** was assigned 5*R*, 6*S*, 8*R*, 8a*R* and 10a*S* absolute configuration.

The known compounds **3**–**8**, isolated from the crude extract were identified as leptosphaerin F,[10] monodictysin B,[11] norlichexanthone,[12] leptosphaerin D,[10] moniliphenone[13] and emodinbianthrone,[14] respectively, by comparison of their NMR and MS data with those reported.

Compounds **1**–**8** were tested for cytotoxicity against three human tumor cell lines, HeLa (human cervical cancer), MCF-7 (human breast cancer) and HepG2 (human hepatocellular carcinoma). Compound **6** was cytotoxic to the three cell lines, showing IC_{50} values of 11.0, 14.7 and 11.0 μM, respectively, whereas the positive control 5-fluorouracil showed IC_{50} values of 10.0, 15.0 and 23.1 μM, respectively. Compounds **1**–**5**, **7** and **8** did not show detectable inhibitory effects on the cell lines tested at 20 μg ml^{-1}.

Chemical constituents of the fungus *Leptosphaeria* sp. have been studied to afford five new polyketides leptosphaerins A–G[10] in our previous study. In the present study, chemical reinvestigation of *Leptosphaeria* sp. has led to the isolation of two additional new xanthone derivatives, leptosphaerins H and I (**1** and **2**). Compound **1** differs from the known 3, 4-dihydroglobosuxanthone A by having one more methyl group at C-6, and only a methyl group at C-8 instead of a hydroxyl group and acetyl in the latter.[18] Compound **2** is a C-8 and C-10 a stereoisomer of leptosphaerin F.[10] Biogenetically, compounds

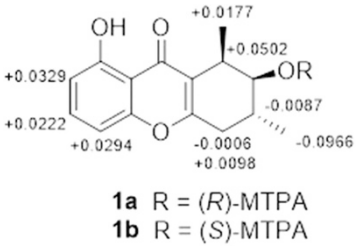

Figure 3 $\Delta\delta$ Values (in p.p.m.) $=\delta_S-\delta_R$ obtained for (*S*)- and (*R*)-MTPA esters **1b** and **1a**.

Table 2 NMR data (500 MHz, acetone-d_6) for compounds 2 and 3

	2			*3*	
Position	δ_C[a], *mult*	δ_H (*J in Hz*)	*NOESY*	δ_C[a], *mult*	δ_H (*J in Hz*)
1	162.5, qC			161.6, qC	
2	108.3, CH	6.36, d (8)		108.0, CH	6.41, d (8.5)
3	138.4, CH	7.32, t (8)		138.9, CH	7.41, t (8.5)
4	108.7, CH	6.34, d (8)		108.0, CH	6.39, d (8.5)
4a	161.6, qC			159.8, qC	
5	80.3, CH	3.07, t (10.1)	11, 12	79.6, CH	3.57, d (11.0)
6	28.8, CH	2.38, m	8, 8a	32.6, CH	1.62, m
7a	41.2, CH_2	1.87, dt (14, 3)		40.0, CH_2	1.92, dt (12.9, 4.5)
7b		1.49, td (14, 2.4)			1.23, dd (12.9, 13)
8	68.6, CH	4.18, m	6	66.0, CH	4.07, m
8a	56.3, CH	2.54, d (4.1)	6, 7a, 7b	57.2, CH	2.94, d (9.6)
9	201.8, qC			202.1, qC	
9a	110.6, qC			108.4, qC	
10a	81.4, qC			84.1, qC	
11	21.8, CH_3	1.42, s	5, 12	18.3, CH_3	1.25, s
12	18.7, CH_3	1.06, d (6.5)	5, 11	12.0, CH_3	1.06, d (6.5)
OH-1		11.84			
OH-5		3.67, d (10.1)			
OH-8		3.81, d (5)			4.64, br s

[a]HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

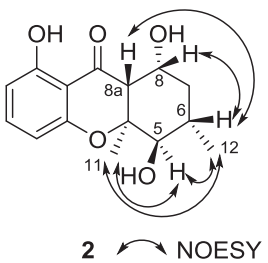

Figure 4 Key NOESY correlations for **2**.

1–8 could be derived from the cleavage of an anthraquinone/anthrone precursor.[19,20]

METHODS

General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter (Perkin-Elmer Waltham, MA, USA), and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer (Shimadzu, Columbia, MD, USA). CD spectra were recorded on a JASCO J-815 spectropolarimeter (Jasco, Tokyo, Japan) using MeOH as solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^1H and ^{13}C NMR data were acquired with Varian Mercury -500 and -600 spectrometers (Varian, Inc., Palo Alto, CA, USA) using solvent signals (acetone-d_6; δ_H 2.05/δ_C 29.8, 206.1) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer (Bruker, Bremen, Germany), and HRESIMS data were obtained using Bruker APEX III 7.0 T and APEXII FT-ICR spectrometers (Bruker Daltonics, Billerica, MA, USA), respectively.

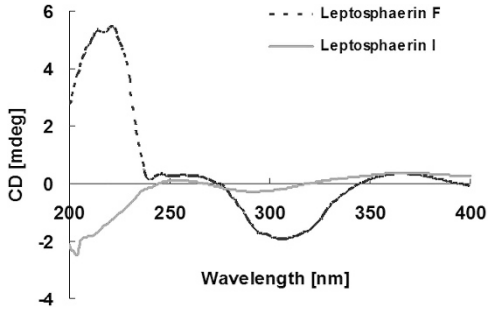

Figure 5 CD spectrum of **2** and **3.** A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Fungal material

The ascomycete fungus *Leptosphaeria* sp. (XZC04-CS-304) was isolated by Dr Mu Wang from the soil sample on the surface of the fruiting body of *C. sinensis* collected in Linzhi, Tibet, China. A fresh sample of *C. sinensis* was collected, together with the soil partially covered on the surface of its fruiting body, and sealed in a plastic bag. The strain was isolated from the soil suspension in distilled water by the spread-plate technique on a potato dextrose agar plate with streptomycin. The isolate was identified by one of the authors and assigned the accession number XZC04-CS–304 in X.L.'s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar at 25 °C for 10 days. Agar plugs were used to inoculate in 250 ml Erlenmeyer flasks, each containing 50 ml of media (0.4% glucose, 1% malt extract and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 r.p.m. for 5 days. Fermentation was carried out in 12 Fernbach flasks (500 ml), each containing 80 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6 ml^{-1}. Distilled H_2O (100 ml) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 ml of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and isolation

The fermented material was extracted repeatedly with ethyl acetate (4×500 ml), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (8.2 g), which was fractionated by silica gel VLC using petroleum ether–EtOAc gradient elution. The fraction (286 mg) eluted with 45% EtOAc was separated by Sephadex LH-20 column chromatography eluting with 1:1 CH_2Cl_2–MeOH. The resulting subfractions were combined and further purified by reverse phase HPLC (Agilent Zorbax SB-C_{18} column (Agilent Technologies, Wilmington, DE, USA); 5 μm; 9.4×250 mm; 20% MeOH in H_2O for 2 min, from 20 to 65% in 30 min; 2 ml min^{-1}) to afford **5** (10.7 mg, t_R 21.9 min) and **7** (25.0 mg, t_R 27.5 min). The fraction (90 mg) eluted with 25% EtOAc was purified by RP HPLC (25% MeOH in H_2O for 5 min, from 25 to 80% in 40 min) to afford **1** (6.9 mg, t_R 16.6 min), **2** (8.2 mg, t_R 25.1 min), **3** (7.4 mg, t_R 25.2 min) and **4** (10.0 mg, t_R 25.4 min). The fraction (90 mg) eluted with 50% EtOAc was also separated by RP HPLC (60% MeOH in H_2O for 5 min, from 80 to 90% in 25 min) to afford **6** (6.9 mg, t_R 22.5 min) and **8** (28.1 mg, t_R 24.2 min).

Leptosphaerin H (1). Light yellow powder; $[\alpha]^{25}_{D}$ −33.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 237 (3.79), 330 (3.03) nm; IR (neat) ν_{max} 3482, 3451, 2969, 2933, 1648, 1616, 1587, 1487, 1362, 1270, 1234, 986, 775 cm^{-1}; ^{1}H NMR, ^{13}C NMR, HMBC and NOESY data see Table 1; HRESIMS *m/z* 283.0941 (calcd for $C_{15}H_{16}O_4Na$, 283.0943).

Preparation of (R)-MTPA ester (1a) and (S)-MTPA ester (1b). A sample of **1** (1.0 mg, 0.004 mmol), (*S*)-MTPA Cl (2.0 µl, 0.011 mmol) and pyridine-d_5 (0.5 ml) was allowed to react in an NMR tube at ambient temperature for 24 h. The ^{1}H NMR data of the *R*-MTPA ester derivative (**1a**) were obtained directly on the reaction mixture: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.50 (1H, t, 8.0 Hz, H-3), 6.80 (1H, d, 8.0 Hz, H-2), 6.78 (1H, d, 8.0 Hz, H-4), 3.31 (1H, dq, 2.0, 7.1 Hz, H-8), 2.65 (1H, dd, 4.3, 11.4 Hz, H-5a), 2.44 (1H, dd, 11.4, 16.6 Hz, H-5b), 2.40 (1H, m, H-6), 1.30 (3H, d, 7.1 Hz, H_3-11), 1.18 (3H, d, 7, H_3-10).

Another sample of **1** (1.0 mg, 0.004 mmol), (*R*)-MTPA Cl (2.0 µl, 0.011 mmol), and pyridine-d_5 (0.5 ml) was processed as described above for **1a** to afford **1b**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.52 (1H, t, 8.0 Hz, H-3), 6.84 (1H, d, 8.0 Hz, H-2), 6.82 (1H, d, 8.0 Hz, H-4), 3.37 (1H, dq, 2.0, 7.1 Hz, H-8), 2.66 (1H, dd, 4.3, 11.4 Hz, H-5a), 2.43 (1H, dd, 11.4, 16.6 Hz, H-5b), 2.39 (1H, m, H-6), 1.32 (3H, d, 7.1 Hz, H_3-11), 1.09 (3H, d, 7, H_3-10).

Leptosphaerin I (2). White solid; $[\alpha]^{25}_{D}$ −81.5 (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} (log ε) 218 (3.93), 277 (3.78), 353 (3.28) nm; CD (*c* 1.0×10^{-4} M, CH_3OH) λ_{max} ($\Delta\varepsilon$) 293 (−0.3), 368 (+0.4) nm; IR (neat) ν_{max} 3460, 2975, 2920, 1614, 1460, 1358, 1229, 1044, 797, 768, 731 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 301.1050 (calcd for $C_{15}H_{18}O_5Na$, 301.1046).

Leptosphaerin F (3). ^{1}H, ^{13}C NMR and the MS data were consistent with literature values.[10]

Monodictysin B (4). ^{1}H, ^{13}C NMR and the MS data were consistent with literature values.[11]

Norlichexanthone (5). ^{1}H, ^{13}C NMR and the MS data were consistent with literature values.[12]

Leptosphaerin D (6). ^{1}H, ^{13}C NMR and the MS data were consistent with literature values.[10]

Moniliphenone (7). ^{1}H, ^{13}C NMR and the MS data were consistent with literature values.[13]

Emodinbianthrone (8). ^{1}H, ^{13}C NMR and the MS data were consistent with literature values.[14]

MTT assay

The assay was run in triplicate.[21] In 96-well plates, each well was plated with 10^4 cells. After cell attachment overnight, the medium was removed, and each well was treated with 50 µl of medium containing 0.2% DMSO, or appropriate concentrations of the test compounds and positive control 5-fluorouracil (10 mg ml^{-1} as stock solution of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). Cells were treated at 37 °C for 4 h in a humidified incubator at 5% CO_2 first, and were allowed to grow for another 48 h after the medium was changed to fresh Dulbecco's Modified Eagle Medium (DMEM). MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (Sigma, St Louis, MO, USA) was dissolved in serum-free medium or phosphate-buffered saline at 0.5 mg ml^{-1} and sonicated briefly. In the dark, 50 µl of MTT/medium was added into each well after the medium was removed from wells, and incubated at 37 °C for 3 h. Upon removal of MTT/medium, 100 µl of DMSO was added to each well, and agitated at 60 r.p.m. for 5 min to dissolve the precipitate. The assay plate was read at 540 nm using a microplate reader.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Gloer, J. B. Antiinsectan natural products from fungal sclerotia. *Acc. Chem. Res.* **28**, 343–350 (2002).
2 Macíasrubalcava, M. L., Sobrino, R. V., Meléndezgonzález, C. & Hernándezortega, S. Naphthoquinone spiroketals and organic extracts from the endophytic fungus edenia gomezpompae as potential herbicides. *J. Agric. Food Chem.* **62**, 3553–3562 (2014).
3 Keller, N. P. & Wiemann, P. Strategies for mining fungal natural products. *J. Ind. Microbiol. Biotechnol.* **41**, 301–313 (2014).
4 Tan, R. X. & Zou, W. X. Endophytes: a rich source of functional metabolites. *Nat. Prod. Rep.* **18**, 448–459 (2001).
5 Dreyfuss, M. M. & Chapela, I. H. CHAPTER 3–Potential of fungi in the discovery of novel, low-molecular weight pharmaceuticals. *Biotechnology* **26**, 49–80 (1994).
6 Ma, C. *et al*. N-hydroxypyridones, phenylhydrazones, and a quinazolinone from *Isaria farinosa*. *J. Nat. Prod.* **74**, 32–37 (2011).
7 Zhang, Y., Liu, S., Che, Y. & Liu, X. Epicoccins A-D, Epipolythiodioxopiperazines from a *Cordyceps*-colonizing isolate of *Epicoccum nigrum*. *J. Nat. Prod.* **70**, 1522–1525 (2007).
8 Guo, H. *et al*. Bioactive p-terphenyl derivatives from a *Cordyceps*-colonizing isolate of *Gliocladium* sp. *J. Nat. Prod.* **70**, 1519–1521 (2007).
9 Lin, J. *et al*. Isolation and characterization of aphidicolin and chlamydosporol derivatives from *tolypocladium inflatum*. *J. Nat. Prod.* **74**, 1798–1804 (2011).
10 Lin, J. *et al*. Polyketides from the ascomycete fungus *Leptosphaeria* sp. *J. Nat. Prod.* **73**, 905–910 (2010).
11 Krick, A. *et al*. Potential cancer chemopreventive *in vitro* activities of monomeric xanthone derivatives from the marine algicolous fungus *Monodictys putredinis*. *J. Nat. Prod.* **70**, 353–360 (2007).
12 Mutanyatta, J., Matapa, B. G., Shushu, D. D. & Abegaz, B. M. Homoisoflavonoids and xanthones from the tubers of wild and *in vitro* regenerated *Ledebouria graminifolia* and cytotoxic activities of some of the homoisoflavonoids. *Phytochemistry* **62**, 797–804 (2003).
13 Kachi, H., Sassa, T., Kachi, H. & Sassa, T. Isolation of monilphenone, a key intermediate in xanthone biosynthesis from *Monilinia fructicola*. *Agric. Biol. Chem.* **50**, 1669–1671 (1986).
14 Abegaz, B. M. & Peter, M. G. Emodin and emodinanthrone rhamnoside acetates from fruits of *Rhamnus prinoides*. *Phytochemistry* **39**, 1411–1414 (1995).
15 Dale, J. A. & Mosher, H. S. Nuclear magnetic resonance enantiomer regents. Configurational correlations via nuclear magnetic resonance chemical shifts of diastereomeric mandelate, O-methylmandelate, and. alpha.-methoxy-.alpha.-trifluoromethylphenylacetate (MTPA) esters. *J. Am. Chem. Soc.* **95**, 512–519 (1973).
16 Ohtani, I., Kusumi, T., Kashman, Y. & Kakisawa, H. A new aspect of the high-field NMR application of Mosher's method. The absolute configuration of marine triterpene sipholenol A. *J.Org.Chem.* **56**, 1296–1298 (1991).
17 Lawson, M. A., Kaouadji, M., Allais, D. P., Champavier, Y. & Chulia, A. J. Substituted tubaic acids, new oxidative rotenoid metabolites from *Lonchocarpus nicou*. *Tetrahedron Lett.* **47**, 451–454 (2006).
18 Karsten, K. *et al*. Xanthones and oxepino[2, 3-]chromones from three endophytic fungi. *Chemistry* **15**, 12121–12132 (2009).
19 Turner, W. B. & Aldridge, D. C. *Fungal Metabolites II* 156–167 (Academic Press, New York, USA, 1983).
20 Chexal, K. K., Holker, J. S. E. & Simpson, T. J. ChemInform abstract: the biosynthesis of fungal metabolites. Part V. Structure of variecoxanthones A, B, and C, metabolites of *Aspergillus variecolor*. *J. Chem. Soc. Perkin 1* **6**, 549–554 (1975).
21 Koparal, A. T., Tüylü, B. A. & Türk, H. *In vitro* cytotoxic activities of (+)-usnic acid and (-)-usnic acid on V79, A549, and human lymphocyte cells and their non-genotoxicity on human lymphocytes. *Nat. Prod. Res.* **20**, 1300–1307 (2006).

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