ORIGINAL ARTICLE

Bipolamides A and B, triene amides isolated from the endophytic fungus *Bipolaris* sp. MU34

Ratklao Siriwach¹, Hiroshi Kinoshita¹, Shigeru Kitani¹, Yasuhiro Igarashi², Kanokthip Pansuksan³, Watanalai Panbangred³ and Takuya Nihira^{1,4}

As a result of the continued screening for new metabolites produced by endophytic fungi from Thai medicinal plants, two new triene fatty acid amides, bipolamides A (1) and B (2), were discovered from the endophytic fungus *Bipolaris* sp. MU34. The structures of all of the isolated compounds were elucidated on the basis of the spectroscopic data of NMR and MS. An antimicrobial assay revealed that bipolamide B (2) had moderate antifungal activity against *Cladosporium cladosporioides* FERMS-9, *Cladosporium cucumerinum* NBRC 6370, *Saccharomyces cerevisiae* ATCC 9804, *Aspergillus niger* ATCC 6275 and *Rhizopus oryzae* ATCC 10404, with Minimum inhibitory concentration (MIC) values of 16, 32, 32, 64 and 64 $\mu\text{g ml}^{-1}$, respectively. *The Journal of Antibiotics* (2014) 67, 167–170; doi:10.1038/ja.2013.103; published online 6 November 2013

Keywords: *Bipolaris*; endophytic fungi; triene amide

INTRODUCTION

Endophytic fungi from plants have been recognized as a promising source of a variety of secondary metabolites with novel chemical structures and diverse biological activities.^{1–4} Thailand is located in a tropical zone with high biodiversity and abundant bioresources, especially in plants, and thus is a highly promising area for obtaining useful endophytes. Particular attention has been paid to plants used for medicinal purposes in Thailand,^{5–8} because their medicinal activity may derive from secondary metabolites of the endophytes.

During our preliminary screening of our in-house HPLC-UV/visible database to find structurally novel secondary metabolites produced by endophytic fungi that inhabit medicinal plants in Thailand,^{9,10} *Bipolaris* sp. MU34 from *Gynura hispida* was revealed to produce unknown metabolites that had a maximum UV/visible spectrum around 300 nm. Although *Bipolaris* sp. is well known as a plant-associated fungus, only a few studies have been reported regarding the discovery of new compounds, such as cochlioquinones,¹¹ bipolaramide¹² and 11-epiterpestacin,¹³ but some of them showed interesting activity, such as antitumor activity¹⁴ and antagonist activity toward HIV-1 in binding human chemokine receptor CCR5.¹⁵ Hence, we isolated unknown metabolites from *Bipolaris* sp. MU34, identified their chemical structures and determined their biological activities. Herein, we describe the isolation of new triene amides, bipolamides A (1) and B (2).

RESULTS AND DISCUSSION

Bipolaris sp. MU34 was cultivated under static conditions in liquid medium containing soluble starch 5%, Pharmamedia (ADM Traders

Protein, Lubbock, TX, USA) 2%, oatmeal (Quaker Oats Company, Chicago, IL, USA) 0.5%, KH_2PO_4 0.35%, Na_2HPO_4 0.25% and $(\text{NH}_4)_2\text{SO}_4$ 0.6% for 21 days at 28 °C. The whole culture was extracted with EtOAc. Compounds 1 and 2 were purified by a series of steps with C_{18} column chromatography and preparative reversed-phase HPLCs.

Compound 1 was obtained as a pale yellow powder. The molecular formula was identified as $\text{C}_{18}\text{H}_{29}\text{NO}_4$ based on high-resolution fast atom bombardment mass spectrometry (HRFABMS) (obs. m/z 324.2166 $[\text{M} + \text{H}]^+$, calcd 324.2140 for $\text{C}_{18}\text{H}_{30}\text{NO}_4$). The IR spectrum showed absorptions at 3340, 1715, 1646 and 1603 cm^{-1} , characteristic of hydroxyl groups, carbonyl groups, amide carbonyl groups and double bonds, respectively. Because of the instability of 1 during NMR measurements, 1 was acetylated, yielding compound 3 as a monoacetate. The structure of 1 was elucidated based on the monoacetate form.

The ^1H -NMR spectrum of 3 indicated six methyl signals at δ 0.83 (t, $J = 7.5$ Hz), 0.96 (d, $J = 6.6$ Hz), 1.41 (s, 3H), 1.77 (s, 3H), 2.03 (s, 3H) and 2.28 (s, 3H); two methylene protons at δ 1.29 (m, 1H), 1.38 (m, 1H) and 4.26 (dd, $J = 8.4, 11.4$ Hz, 1H), 4.43 (dd, $J = 4.2, 11.4$ Hz, 1H); two methine protons at δ 2.40 (m, 1H) and 4.77 (ddd, $J = 4.2, 8.4, 9.6$ Hz); five olefinic protons at δ 5.45 (d, $J = 10.2$ Hz, 1H), 5.74 (d, $J = 15.0$ Hz, 1H), 6.17 (dd, $J = 11.4, 15.6$ Hz, 1H), 6.53 (d, $J = 15.6$ Hz, 1H) and 7.22 (dd, $J = 11.4, 15.0$ Hz, 1H); a NH proton at 5.70 (d, $J = 9.6$ Hz, 1H); and an OH proton at 4.31 (s, br). The ^{13}C -NMR and distortionless enhancement by polarization transfer (DEPT) data of 3 revealed the presence of 20 carbon signals, including three carbonyl carbons at δ 210.4, 171.1 and 166.2; two

¹International Center for Biotechnology, Osaka University, 2-1 Yamadaoka, Suita, Osaka, Japan; ²Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama, Japan; ³Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand and ⁴MU-OU Collaborative Research Center for Bioscience and Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand
Correspondence: Professor T Nihira, International Center for Biotechnology, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan.
E-mail: nihira@icb.osaka-u.ac.jp

Received 22 May 2013; revised 6 September 2013; accepted 19 September 2013; published online 6 November 2013

quaternary carbons at δ 79.4 and 132.4; six methyl carbons at δ 11.9, 12.5, 20.3, 20.8, 22.6 and 23.5; two sp^3 methylene carbons at δ 30.1 and 62.7; two sp^3 methine carbons at δ 34.7 and 52.5; and five sp^2 methine carbons at δ 121.0, 123.4, 142.9, 144.6 and 145.9.

Comprehensive interpretation of its UV, IR, ^1H -NMR, ^{13}C -NMR, HH-COSY, HSQC and HMBC spectra indicated that **3** contains a decatriene fatty acid amide moiety. The partial structure was identical to that of the reported triene fatty acid moiety of dictyopanine C,¹⁶ judging from the matched ^1H -NMR and ^{13}C -NMR spectra, except for the signal from a secondary amide. The presence of NH was confirmed by the data of MS and HMBC correlation between proton on the nitrogen (δ 5.70) and C1 (δ 166.2). The remaining part of **3** was deduced to contain one carbonyl group, one acetoxy group, one hydroxyl group, two methyls, one methylene, one methine and one quaternary carbon. The fatty acid amide part was linked to the methine C2' by the HH-COSY correlation of NH (δ 5.70) to H-2' (δ 4.77). The remaining units and functional groups were connected on the basis of HH-COSY and HMBC correlations. HH-COSY and HMBC demonstrated the connection between C1' and C2'; HMBC of H6' revealed the correlation with methine C2', quaternary C3' and carbonyl C4'; and HMBC of H5' displayed the correlation with carbonyl C4' and quaternary C3'. Together, these results indicated the partial structure as a short-branched five-carbon unit. Consequently, the complete structure of **3** was elucidated, as depicted in Figure 1. According to the structure of compound **3**, which was estimated as a monoacetylated compound of **1**, the complete structure of compound **1** was also identified (Figure 2).

The stereochemistry of the triene in the fatty acid amide moiety was deduced from the vicinal coupling constants between olefinic protons, and from the detection of peaks in NOESY (nuclear Overhauser effect spectroscopy). The constants of $J_{2,3}$ and $J_{4,5}$ were

Figure 1 HH COSY and HMBC correlation of monoacetate bipolamide A (**3**).

Figure 2 Bipolamide A (**1**), monoacetate bipolamide A (**3**) and bipolamide B (**2**).

15.0 and 15.6 Hz, respectively. NOESY cross-peaks were detected for H4/H6-CH₃, H3/H5 and H5/H7, whereas H4 and H6-CH₃ did not show any correlations to H3, H5 and H7. All these data supported that the geometry of all three olefins was (*E*)-configuration. The relative configuration for C2' and C3' were also clarified by NOESY analysis. NOEs observed for H1'/H6', H2'/H5' and H2'/H6' indicated the relative relationship between the two chiral centers as depicted in Figure 3 (see also Supplementary S1). From these data, the relative configuration of compound **1** was determined as shown in Figure 2.

Compound **2** was obtained as a colorless powder. The molecular formula was determined to be C₁₂H₁₉NO based on high-resolution electron ionization mass spectrometry (HREIMS) (obs. m/z 193.1470 [M]⁺, calcd 193.1490 for C₁₂H₁₉NO). The UV and IR spectra suggested that it was a conjugated primary amide. The ^1H and ^{13}C -NMR spectra of **2** closely resembled those of the fatty acid amide moiety of compound **3** (Table 1). The structure was finally elucidated as a linear triene primary amide, supported by HH-COSY, HSQC and HMBC correlations. Judging from the same vicinal coupling constants of olefinic protons in compound **1** together with probably the same biosynthetic pathway, **2** was deduced to possess the same (*E*)-configurations.

To the best of our knowledge, Bipolamides A (**1**) and B (**2**) are novel compounds that have not previously been identified in natural resources or derived from chemical synthesis. Especially, bipolamide A (**1**) was regarded as a rare natural compound because it was composed of an acyloin moiety and a triene fatty acid secondary amide, bipolamide B (**2**) moiety, neither of which has been reported in nature. Thus, the biosynthetic pathway of bipolamide A, particularly the biosynthetic mechanism underlying the acyloin moiety, needs to be clarified.

The antimicrobial activities of compounds **1** and **2** were evaluated by the procedures of the CLSI (Clinical and Laboratory Standards Institute). No activities were detected against bacterial strains (minimum inhibitory concentration (MIC) > 512 $\mu\text{g ml}^{-1}$) for either compound. However, compound **2** showed mild antifungal activities against *Cladosporium cladosporioides* FERMS-9, *Cladosporium cucumerinum* NBRC 6370, *Saccharomyces cerevisiae* ATCC 6275, *Aspergillus niger* ATCC 6275 and *Rhizopus oryzae* ATCC 10404, with MIC values of 16, 32, 32, 64 and 64 $\mu\text{g ml}^{-1}$, respectively, whereas no activities were observed in compound **1** (MIC > 128 $\mu\text{g ml}^{-1}$) (Supplementary S2).

Although the result of general antimicrobial activity assay might not reveal the role of these two compounds to the fungus itself and/or host plant, some fatty acid amide including *N*-acylethanolamines are proved to have an important role as chemical signaling in plant and animal for physiological processes.¹⁷ The two newly identified compounds containing linear fatty acid amide moiety might be used as precursors or intermediates to synthesize signaling molecule, or function as signaling molecules themselves to regulate the

Figure 3 Partial NOE correlation of bipolamide A (**1**).

Table 1 NMR spectroscopic data of monoacetate bipolamide A (**3**) and bipolamide B (**2**) in CDCl₃

Position	3				2			
	δ_C	δ_H	HH COSY	HMBC	δ_C	δ_H	HH COSY	HMBC
1	166.2 (qC)	—	—	—	168.3 (qC)	—	—	—
2	121.0 (CH)	5.74 (d, $J=15.0$ Hz)	H3	C1, C4	120.8 (CH)	5.89 (d, $J=15.0$ Hz)	H3	C1, C4
3	142.9 (CH)	7.22 (dd, $J=11.4, 15.0$ Hz)	H2, H4	C1, C5	143.0 (CH)	7.29 (dd, $J=11.1, 15.0$ Hz)	H2, H4	—
4	123.4 (CH)	6.17 (dd, $J=11.4, 15.6$ Hz)	H3, H5	C2	123.4 (CH)	6.21 (dd, $J=11.1, 15.0$ Hz)	H3, H5	—
5	145.9 (CH)	6.53 (d, $J=15.6$ Hz)	H4	C3, C7, C12	145.9 (CH)	6.55 (d, $J=15.0$ Hz)	H4	C3, C7, C12
6	132.4 (qC)	—	—	—	132.4 (qC)	—	—	—
7	144.6 (CH)	5.45 (d, $J=10.2$ Hz)	H8	C5, C9, C11, C12	144.6 (CH)	5.46 (d, $J=9.7$ Hz)	H8	C12
8	34.7 (CH)	2.40 (m)	H7, H11	—	34.7 (CH ₂)	2.40 (m)	H7, H9, H11	—
9	30.1 (CH ₂)	1.29, 1.38 (m)	H10	C7, C8, C10, C11	30.1 (CH)	1.27, 1.37 (m)	H8, H10	C7, C8, C10, C11
10	11.9 (CH ₃)	0.83 (t, $J=7.5$ Hz)	H9	C8, C9	11.9 (CH ₃)	0.82 (t, $J=7.6$ Hz)	H9	C8, C9
11	20.3 (CH ₃)	0.96 (d, $J=6.6$ Hz)	H8	C7, C8, C9	20.4 (CH ₃)	0.96 (d, $J=6.5$ Hz)	H8	C7, C8, C9
12	12.5 (CH ₃)	1.77 (s)	—	C5, C7	12.5 (CH ₃)	1.76 (s)	—	C5, C6, C7
NH	—	5.70 (d, $J=9.6$ Hz)	H2'	C1	—	—	—	—
NH ₂	—	—	—	—	—	5.60 (s, br)	H2'	—
1'	62.7 (CH ₂)	4.26, 4.43 (dd, $J=8.4, 11.4$; dd, $J=4.2, 11.4$ Hz)	H2'	C2', C3', 1'-OCOCH ₃	—	—	H2', H4'	—
2'	52.5 (CH)	4.77 (ddd, $J=4.2, 8.4, 9.6$ Hz)	H1', NH	—	—	—	H1', NH ₂	—
3'	79.4 (qC)	—	—	—	—	—	—	—
4'	210.4 (qC)	—	—	—	—	—	—	—
5'	23.5 (CH ₃)	2.28 (s)	—	C3', C4'	—	—	—	—
6'	22.6 (CH ₃)	1.41 (s)	—	C2', C3', C4'	—	—	—	—
3'-OH	—	4.31 (s, br)	—	—	—	—	—	—
4'-OCH ₃	—	—	—	—	—	—	—	—
1'-OCOCH ₃	171.1 (qC)	—	—	—	—	—	—	—
1'-OCOCH ₃	20.8 (CH ₃)	2.03 (s)	—	1'-OCOCH ₃	—	—	—	—

¹H, ¹³C-NMR and 2D NMR spectra were obtained on Varian UNITY INOVA 600 spectrometers, in CDCl₃ at room temperature and the solvent peak was used as an internal standard (δ_H 7.26 and δ_C 77.0 in CDCl₃).

metabolism in fungus and/or host plant. In addition, judging from the result that only compound **2** exhibited the broad spectrum to various fungal cells, the short-branched five-carbon unit chain, only existing on compound **1**, might function to control their toxicity. Further study will clarify the function of these two compounds in the host plant in the future.

EXPERIMENTAL PROCEDURE

General experimental procedures

The UV spectrum was recorded on a Hitachi U-3200 spectrophotometer. NMR spectra were recorded on a UNITY INOVA 600 (Varian, Palo Alto, CA, USA) for ¹H (600 MHz) and ¹³C (150 MHz). The ¹H and ¹³C chemical shifts were referenced to the solvent signals (δ_H 7.26 and δ_C 77.0 in CDCl₃). HREIMS, HRFABMS and HRCIMS were recorded on a JMS-700 spectrometer (JEOL, Tokyo, Japan). Optical rotation was measured on a P-1020 polarimeter (Jasco, Tokyo, Japan). IR spectra were recorded on a Jasco FTIR-4100. HPLC was performed on a Jasco PU-1570 with a UVDEC-100-V detector using a CAPCELL PAK C₁₈ column (250 × 10 mm, UG80S5; Shiseido, Tokyo, Japan) for preparative purification, and on a 1200 Series instrument with a binary pump equipped with a photodiode array detector (1200 Series; Agilent, Böblingen, Germany) using a Cadenza CD-C18 (75 × 4.6 mm, Imtakt, Kyoto, Japan) for acetate bipolamide A (**3**) purification.

Fungal material

The endophytic fungus MU34 was isolated from leaves of *G. hispida* Thwaites obtained at the botanical garden in Mahidol University, Bangkok, Thailand. The leaves were sterilized with 1% NaOCl solution followed by washing with 70% ethanol. They were then incubated on water agar plates containing streptomycin (20 µg ml⁻¹) and chloramphenicol (30 µg ml⁻¹) at 28 °C. After 2 weeks, the developed mycelia on the plate were picked and transferred to

potato dextrose agar plates as stock culture. The fungus was identified as a *Bipolaris* sp. based on the homology of the ITS rRNA region (DDBJ accession number AB813872); the highest identity (98%) was with *Bipolaris* sp. in GenBank. The fungus was deposited as *Bipolaris* sp. MU34 at the culture collection of the International Center for Biotechnology (ICBiotech; Osaka University, Osaka, Japan).

Fermentation, extraction and isolation

All chemicals, media and reagents were purchased from Wako (Osaka, Japan) unless stated otherwise. For the preparation of seed culture, the mycelia of *Bipolaris* sp. MU34 grown on a potato dextrose agar slant were inoculated into 5 ml of medium 2 (soluble starch 5%, Pharmamedia (ADM Traders Protein, Lubbock, TX, USA) 2%, oatmeal (Quaker Oats Company, Chicago, IL, USA) 0.5%, KH₂PO₄ 0.35%, Na₂HPO₄ 0.25% and (NH₄)₂SO₄ 0.6%) in test tubes (Ø12.5 mm × 10.5 cm), and incubated for 3 days at 28 °C on a reciprocal shaker at 120 rpm. Two milliliters of seed culture was inoculated into 100 ml of medium 2 in 500-ml baffled flasks and cultivated for 21 days at 28 °C under static conditions to obtain **1** and **2**.

After static cultivation, the whole cultures (100 ml × 10 flasks) were mixed with an equal amount of EtOAc and left to be stirred for 2 h at room temperature. Mycelia were removed by filtration with Miracloth (Calbiochem, La Jolla, CA, USA), and the EtOAc layer was recovered with a separation funnel, dried over anhydrous Na₂SO₄, and evaporated to afford a crude extract (brown gum, 2 g).

The crude extract (2 g) was separated on C₁₈ column chromatography by stepwise elution with increasing MeOH concentrations (MeOH/H₂O = 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and 10:0 v/v). The mixture of compounds **1** and **2** was obtained by evaporation of the fractions eluted with 90% MeOH (140 mg). Compounds **1** and **2** were further purified by preparative reversed-phase HPLC with a shallow MeCN gradient in 0.1% trifluoroacetic acid (TFA) (40–70% MeCN gradient over a period of 30 min, flow rate 5 ml min⁻¹),

yielding pure **1** (17.3 mg) and a mixture of **2** (19.0 mg). Compound **2** was further purified with the second-round HPLC (32% MeCN isocratic elution over a period of 55 min, flow rate 5 ml min⁻¹), yielding pure **2** (8.1 mg).

Compound (**1**): a pale yellow powder; $[\alpha]_{\text{D}}^{28}$ -28° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 301 (4.69) nm; HRFABMS *m/z* 324.2166 $[\text{M} + \text{H}]^+$ (calcd for C₁₈H₃₀NO₄, 324.2140). IR ν_{max} (film) 3340 (OH), 2961, 2926, 1715 (C=O), 1646 (C=O), 1603, 1539, 1455, 1362, 1258, 1145, 1120, 997 cm⁻¹. For ¹H, ¹³C, see Supplementary S3 and S4.

Compound (**2**): a colorless powder; $[\alpha]_{\text{D}}^{28}$ -27° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 300 (4.41) nm; HREIMS *m/z* 193.1470 $[\text{M}]^+$ (calcd for C₁₂H₁₉NO, 193.1490). IR ν_{max} (film) 3332 (NH), 3166 (NH), 2959, 2924, 1670 (C=O), 1595, 1455, 1398, 1290, 991 cm⁻¹. For ¹H, ¹³C, H-H COSY and HMBC, see Table 1 (see also Supplementary S5 and S6).

Acetylation of compound 1

Six milligrams of compound (**1**) in dry pyridine (0.2 ml) was mixed with 0.2 ml of acetic anhydride and left for 2 h with stirring at room temperature. The reaction mixture was evaporated to dryness under reduced pressure to afford a white powder. The residue was purified by reversed-phase HPLC under the following conditions: MeCN gradient in 0.1% TFA (15–85% MeCN gradient over a period of 32 min, flow rate 1.2 ml min⁻¹), yielding a pure monoacetate compound (**3**) (3.5 mg).

Compound (**3**): a white powder; $[\alpha]_{\text{D}}^{27}$ -46° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 302 (4.28) nm; HRCIMS *m/z* 366.2274 $[\text{M} + \text{H}]^+$ (calcd for C₂₀H₃₂NO₅, 366.2256). IR ν_{max} (film) 3290 (OH), 2961, 2925, 1742 (C=O), 1716 (C=O), 16516 (C=O), 1606, 1540, 1520, 1507, 1456, 1362, 1228, 1151, 1041, 997 cm⁻¹. For ¹H, ¹³C, H-H COSY and HMBC (see Table 1 and Supplementary S7 and S8).

Antibacterial, anti-yeast and antifungal activity

The MICs of bipolamides (**1** and **2**) were determined by the twofold broth microdilution method according to the procedures of the CLSI for antimicrobial, anti-yeast and antifungal activities.¹⁸ The MIC is defined as the lowest concentration of the compound at which there is no visible growth of the indicator strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29923, *Enterococcus faecalis* ATCC 29212, *S. cerevisiae* ATCC 9804, *Candida albicans* OUT 6266, *A. niger* ATCC 6275, *R. oryzae* ATCC10404, *Geotrichum candidum* IFO 4598, *C. cladosporioides* FERMS-9, *Alternaria mali* NBRC 8984, *C. cucumerinum* NBRC 6370 and *Fusarium oxysporum* subsp. *Cucumerinum* NBRC 31224 (Supplementary S2).

ACKNOWLEDGEMENTS

This study was supported in part by a scholarship from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan to R.S., and by funds from the joint program in the field of biotechnology under the

Japan Society for the Promotion of Science (JSPS) and the National Research Council of Thailand (NRCT) to T.N., H.K. and S.K.

- Schulz, B., Boyle, C., Draeger, S., Römmert, A.-K. & Krohn, K. Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycol. Res.* **106**, 996–1004 (2002).
- Strobel, G., Daisy, B., Castillo, U. & Harper, J. Natural products from endophytic microorganisms. *J. Nat. Prod.* **67**, 257–268 (2004).
- Guo, B., Wang, Y., Sun, X. & Tang, K. Bioactive natural products from endophytes: a review. *Appl. Biochem. Microbiol.* **44**, 136–142 (2008).
- Suryanarayanan, T. S. *et al.* Fungal endophytes and bioprospecting. *Fungal Biol. Rev.* **23**, 9–19 (2009).
- Tansuwan, S. *et al.* Antimalarial benzoquinones from an endophytic fungus, *Xylaria* sp. *J. Nat. Prod.* **70**, 1620–1623 (2007).
- Pongcharoen, W., Rukachaisirikul, V., Phongpaichit, S., Rungjindamai, N. & Sakayaroj, J. Pimarane diterpene and cytochalasin derivatives from the endophytic fungus *Eutypella scoparia* PSU-D44. *J. Nat. Prod.* **69**, 856–858 (2006).
- Sappapan, R. *et al.* 11-Hydroxymonocerin from the plant endophytic fungus *Exserohilum rostratum*. *J. Nat. Prod.* **71**, 1657–1659 (2008).
- Rukachaisirikul, V., Sommart, U., Phongpaichit, S., Sakayaroj, J. & Kirtikara, K. Metabolites from the endophytic fungus *Phomopsis* sp. PSU-D15. *Phytochemistry* **69**, 783–787 (2008).
- Siriwach, R. *et al.* Xylaropyrone, a new γ -pyrone from the endophytic fungus *Xylaria feejeensis* MU18. *J. Antibiot.* **64**, 217–219 (2011).
- Siriwach, R. *et al.* Mycoleptione, a new chromone derivative isolated from the endophytic fungus *Mycoleptodiscus* sp. MU41. *J. Antibiot.* **65**, 627–629 (2012).
- Jung, H. J., Burm Lee, H., Lim, C.-H., Kim, C.-J. & Kwon, H. J. Cochlioquinone A1, a new anti-angiogenic agent from *Bipolaris zeicola*. *Bioorg. Med. Chem.* **11**, 4743–4747 (2003).
- Maes, C. M. *et al.* Structure and biosynthesis of bipolaramide, a novel dioxopiperazine from *bipolaris sorokiniana*. *J. Chem. Soc., Perkin Trans. 1*, 2489–2492 (1985).
- Nihashi, Y., Lim, C.-H., Tanaka, C., Miyagawa, H. & Ueno, T. Phytotoxic sesterterpene, 11-epiterpestacin, from *Bipolaris sorokiniana* NSDR-011. *Biosci. Biotechnol. Biochem.* **66**, 685–688 (2002).
- Phuwapraisrisan, P., Sawang, K., Siripong, P. & Tip-pyang, S. Anhydrocochlioquinone A, a new antitumor compound from *Bipolaris oryzae*. *Tetrahedron Lett.* **48**, 5193–5195 (2007).
- Yoganathan, K. *et al.* Cochlioquinones and epi-cochlioquinones: antagonists of the human chemokine receptor CCR5 from *Bipolaris brizae* and *Stachybotrys chartarum*. *J. Antibiot.* **57**, 59–63 (2004).
- Dörfelt, H., Schlegel, B. & Gräfe, U. Dictyopanines A, B and C, new bicyclic sesquiterpene esters from *Dictyopanus* sp. HKI 0181. *J. Antibiot.* **53**, 839–843 (2000).
- Wang, Y. S. *et al.* Manipulation of Arabidopsis fatty acid amide hydrolase expression modifies plant growth and sensitivity to *N*-acylethanolamines. *Proc. Natl Acad. Sci. USA* **103**, 12197–12202 (2006).
- Clinical and Laboratory Standards Institute (CLSI), 2007. (a) *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*—Seventh Edition; Approved Standard M07-07, (b) *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*—Third Edition; Approved Standard M27-A3 (c) *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*—Second Edition; Approved Standard M38-A2 (CLSI, Wayne, PA).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (<http://www.nature.com/ja>)