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Dihydronaphthalenones from endophytic fungus *Fusarium* sp. BCC14842

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ABSTRACT

Eleven new dihydronaphthalenones **1**–**11**, together with five known compounds; 5-hydroxydihydrofusarubin C (**12**), javanicin, bostrycoidin, anhydrofusarubin, and 3-*O*-methylfusarubin, were isolated from the endophytic fungus *Fusarium* sp. BCC14842. Structures were elucidated by analyses of the NMR spectroscopic and mass spectrometry data. Javanicin, 3-*O*-methylfusarubin, compounds **3** and **7** exhibited antimycobacterial and cytotoxic activities. Javanicin also displayed antifungal activity with IC_{50} of 6.16 μg/mL, while compounds **2**, **4**, **5**, **8**, and **12** showed only cytotoxic activity.

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1. Introduction

Endophytic fungi in the genus *Fusarium* are reported to produce a diversity of bioactive secondary metabolites including naphthoquinones, e.g., javanicin, fusarubin, solaniol, marticin, and nectraiafurone.[1–3] This class of compounds is of interest due to the broad spectrum of their biological activities, such as antibacterial,[4–6] antifungal,[6,7] phytotoxic,[1] insecticidal,[8] and cytotoxic[9] properties. As part of our research program on bioactive compounds from fungi, we investigated the constituents of endophytic fungus *Fusarium* sp. BCC14842, of which the crude extract exhibited strong cytotoxic activity against human breast cancer (MCF-7, IC_{50} value of 8.14 μg/mL). The study led to the isolation of eleven new compounds, 4-hydroxydihydronorjavanicin (**1**), dihydronaphthalenone **2** and its diastereomer **3**, 5-hydroxydihydrofusarubins A, B, and D (**4**–**6**), and the methyl ether derivatives **7**–**11**, together with five known compounds, 5-hydroxydihydrofusarubin C (**12**),[10] javanicin,[4] bostrycoidin,[11] anhydrofusarubin,[12] and 3-*O*-methylfusarubin.[9] Biological activities of these compounds were also evaluated.

2. Results and discussion

Javanicin, 3-*O*-methylfusarubin, 5-hydroxydihydrofusarubin C (**12**), and compounds **2**–**4** were obtained from both culture broth and mycelia extract of BCC14842. Anhydrofusarubin, compounds **1**, and **5**–**11** were obtained from the culture broth extract, while bostrycoidin was solely isolated from an *n*-hexane extract of the mycelium.

Compound **1** was obtained as a red solid. The molecular formula was established by HRMS (ESITOF), in combination with ^{13}C NMR spectroscopy, as $C_{14}H_{16}O_6$. The IR spectrum showed major absorption bands at 3415, 1712, and 1625 cm^{-1} corresponding, respectively, to a hydroxyl, a carbonyl and a conjugated carbonyl group. The unusually low IR absorption frequency of the conjugated carbonyl is brought about by the H-bonding between the unsaturated ketone and β-OH group. The ^1H NMR spectrum showed signals for one chelated hydroxy proton, one aromatic proton, two hydroxy protons, two methine protons, one methoxy, one methyl, and two methylene groups. The cross peak between H-2 and H-3, H-3, and H-4 in COSY spectrum and the correlations from H-2 to C-1/C-4/C-8a, from H-3 to C-1/C-4a, from H-4 to C-2/C-4a/C-5/C-8a, and from H-7 to C-1, C-8a in HMBC spectrum established a dihydronaphthalenone nucleus. The location of OH-5, OH-8 and methoxy group (H_3-12) at C-5, C-8 and C-6 of the benzene ring, respectively, was indicated by HMBC correlations between these protons and their corresponding carbons. The acetonyl side chain at C-3 was determined by the COSY correlation between H-3 and H-9 and the HMBC correlations from H_3-11 to C-9/C-10 and from H-9 to C-2/C-3/C-4/C-10. The remaining OH-group was indicated at C-4 on the basis of its chemical shift, together with HMBC correlation from this proton (OH-4) to the

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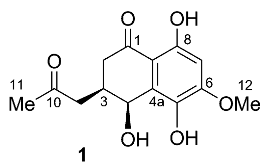
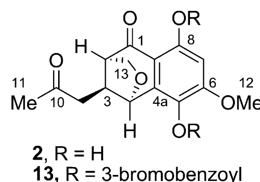
corresponding carbon. The relative configuration of compound **1** was deduced on the basis of the coupling constants and NOESY data. The cross peak from axial Ha-2 to H-4 in NOESY spectrum established the axial orientation of H-4 and the small coupling constant between H-3 and H-4 (4.7 Hz) indicated the cis relationship between H-3 and H-4. Compound **1** was therefore assigned as 4-hydroxydihydronorjavanicin.

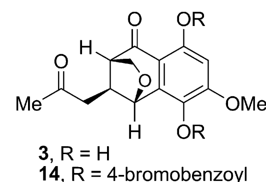
1624 cm^{-1} for an unsaturated carbonyl group of which its IR absorption frequency is unusually low due to the H-bonding between the unsaturated ketone and β-OH group. The ^{1}H and ^{13}C NMR data as well as the optical rotation of compound **12** were identical to those of 5-hydroxydihydrofusarubin,[10] however, the configurational assignment of the stereocenters has not clearly been reported. The relative configuration of compound **12** has now been



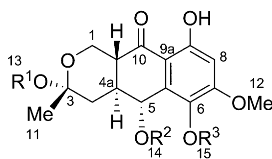
1



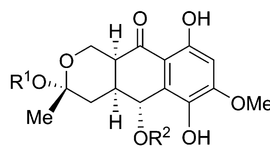
2, R = H
13, R = 3-bromobenzoyl



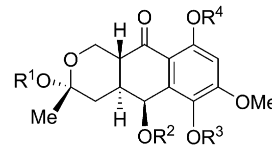
3, R = H
14, R = 4-bromobenzoyl



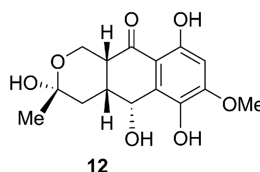
4, $R^1 = R^2 = R^3 = H$
7, R^1 = Me, $R^2 = R^3 = H$
15a, $R^1 = R^3$ = Me, R^2 = *(S)*-THENA
15b, $R^1 = R^3$ = Me, R^2 = *(R)*-THENA



5, $R^1 = R^2 = H$
8, R^1 = H, R^2 = Me
9, $R^1 = R^2$ = Me



6, $R^1 = R^2 = R^3 = R^4 = H$
10, R^1 = Me, $R^2 = R^3 = R^4 = H$
11, $R^1 = R^2$ = Me, $R^3 = R^4$ = H,
16a, $R^1 = R^3$ = Me, $R^2 = R^4$ = *(S)*-MTPA
16b, $R^1 = R^3$ = Me, $R^2 = R^4$ = *(R)*-MTPA



12

Compound **2** was obtained as a pale orange solid, possessing the molecular formula $C_{15}H_{16}O_6$ as deduced from HRMS (ESITOF), in combination with ^{13}C NMR spectroscopy. The ^{1}H NMR data of compound **2** were closely related to those of compound **1**. Analysis of ^{13}C and 2D NMR spectra revealed the similar structures of these two compounds except for the presence of an additional oxymethylene group at C-2 and the replacement of hydroxyl group at C-4 with alkoxy group. The COSY correlation between H-2 and H-13 and the HMBC correlations from H-13 to C-1/C-2/C-3/C-4 established an oxa-bicyclic ring system. The relative configuration of compound **2** was assigned on the basis of NOESY spectroscopic data, which the cross peak from H-3 to Hb-13 suggested the trans relationship between H-2 and H-3, and between H-3 and H-4. The absolute configurations at C-2, C-3, and C-4 were addressed by the X-ray diffraction analysis of 6, 8-bis-*O*-(3-bromobenzoyl) derivative **13** (Fig. 1), which were assigned as *R*, *S*, and *R*, respectively.

Compound **3** with the molecular formula $C_{15}H_{16}O_6$ from HRMS (ESITOF) was obtained as a pale orange solid. The UV and IR spectra of compound **3** were almost identical to those of compound **2**. Their ^{1}H NMR spectra were also similar. Analysis of 2D NMR data revealed the same structure for these two compounds except for the stereogenic centers at C-2 and C-4. The NOESY correlation between H-9 and Hb-13 indicated cis relationship between H-2, H-3 and H-4. The X-ray diffraction analysis of 6, 8- bis-*O*-(4-bromobenzoyl) derivative **14** (Fig. 2) established the *S*, *S*, and *S* configurations at C-2, C-3, and C-4, respectively.[13]

Compound **12** was obtained as an orange solid, possessing the molecular formula $C_{15}H_{18}O_7$ as deduced from HRMS (ESITOF), in combination with ^{13}C NMR spectroscopy. The IR spectrum showed absorption bands at 3274 cm^{-1} for a hydroxyl group and at

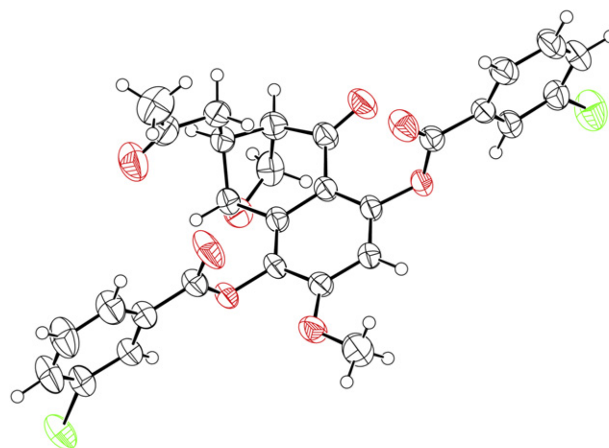


Fig. 1. X-ray crystal structure of compound **13**.

deduced on the basis of the coupling constants and NOESY data. The large coupling constant between axial Ha-1 and H-10a (9.5 Hz) and the small coupling constant between H-10a and H-4a (3.8 Hz) established the axial and equatorial orientation of H-10a and H-4a, respectively, which indicated the cis ring junction through H-10a and H-4a. The cross peak between H-10a and axial Ha-4 in NOESY spectrum also supported the axial position of H-10a. The cis relationship between H-4a and H-5 was deduced from the cross peak

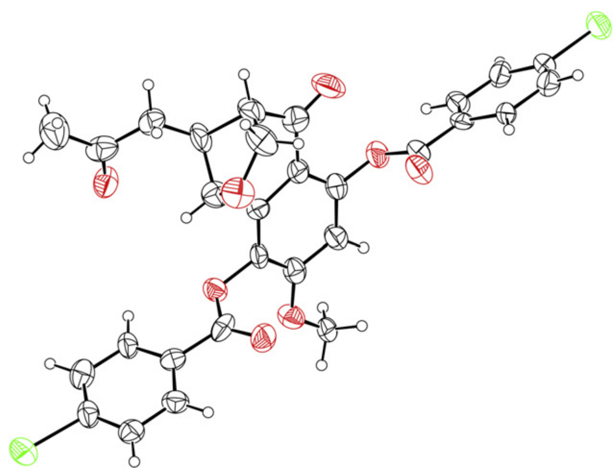


Fig. 2. X-ray crystal structure of compound **14**.

between H-4a and H-5 in NOESY spectrum. The NOESY correlation between axial Ha-4 and CH_3-3 indicated the equatorial orientation of CH_3 group at C-3, which established cis relationship between CH_3 group and H-10a (Fig. 3). Compound **12** was therefore determined as 5-hydroxydihydrofusarubin C.

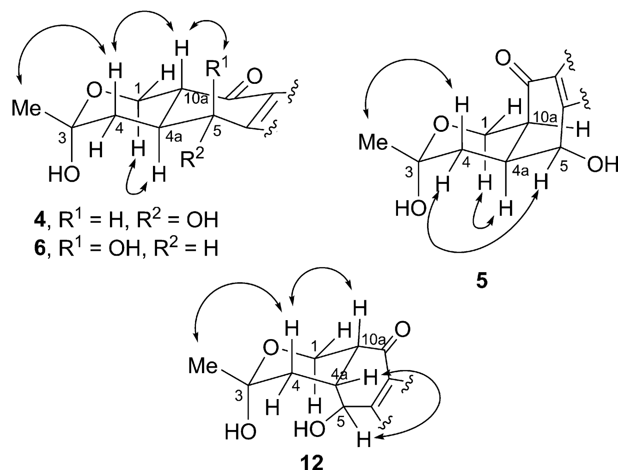


Fig. 3. Selected NOESY correlations of compounds **4**–**6** and **12**.

Compound **4** was obtained as a pale orange solid. The HRMS (ESITOF) spectrum indicated the molecular formula as $C_{15}H_{18}O_7$. The UV and IR spectra were identical to those of compound **12**. Their ^{1}H NMR spectrum was also closely related. Analysis of 2D NMR data as well as coupling constants revealed the same structural features for both compounds with the difference being in the relative configuration at C-4a. The trans diaxial arrangement between H-4a and H-10a and between H-4a and H-5 were assigned on the basis of large coupling constant of 13.1 Hz and 9.8 Hz, respectively. The cross peak between H-10a and H-5 in NOESY spectrum also supported the cis relationship of these two protons. The absolute configuration at C-5 was addressed by application of THENA (Tetrahydro-1,4-epoxynaphthalene-1-carboxylic acid) method.[14] Compound **4** was treated with MeI (2.5 equiv) in K_2CO_3/acetone to afford dimethylated derivative, which was further converted into (*S*)- and (*R*)-THENA esters **15a** and **15b**, respectively. The differences in chemical shift values ($\Delta\delta_{S-R}$, Fig. 4) were consistent with the 5*R* configuration.[15] Therefore, compound **4** was determined as (3*R*, 4a*S*, 5*R*, 10a*R*)-5-hydroxydihydrofusarubin A.

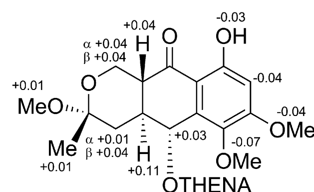


Fig. 4. $\Delta\delta$-Values ($\delta_S-\delta_R$) of (*S*)- and (*R*)-THENA esters **15a** and **15b**.

Compound **7** with the molecular formula $C_{16}H_{20}O_7$ from HRMS (ESITOF) was obtained as a red solid. The ^{1}H NMR spectrum was similar to that of compound **4** except for the presence of an additional methoxy signal at δ_H 3.18. The HMBC correlation from the additional methoxy protons (H_3-13) to C-3 revealed the replacement of hydroxyl group in compound **4** with methoxy group in compound **7** at C-3. Analysis of NOESY spectrum and the coupling constants suggested that the relative configurations at C-3, C-4a, C-5, and C-10a are the same as those in compound **4**. The almost identical optical rotation and CD spectrum of compound **4** and compound **7** indicated the same absolute configuration of these two compounds. Therefore, the absolute configurations at C-3, C-4a, C-5, and C-10a of compound **7** were assigned as *R*, *S*, *R*, and *R*, respectively, and compound **7** was determined as 5-hydroxy-3-methoxydihydrofusarubin A.

Compound **5** with the molecular formula $C_{15}H_{18}O_7$ from HRMS (ESITOF) was obtained as a pale orange solid. The UV and IR spectra were identical to those of compound **4**. Their ^{1}H NMR spectra were also similar. The coupling constants and 2D NMR data analysis revealed that the structures of these two compounds were the same except for the relative configuration at C-10a. The large coupling constant (13.2 Hz) between axial Ha-4 and H-4a and the small coupling constant (3.2 Hz) between axial Ha-1 and H-10a established the axial and equatorial orientation of H-4a and H-10a, respectively, which indicated the cis ring junction through H-4a and H-10a. The cross peak between equatorial Hb-4 and H-5 in NOESY spectrum deduced the trans relationships between H-4a and H-5 (Fig. 3). Therefore, compound **5** was the (10a*S*)-epimer of 5-hydroxydihydrofusarubin A and was assigned as 5-hydroxydihydrofusarubin B.

Compound **8** with the molecular formula $C_{16}H_{20}O_7$ from HRMS (ESITOF) was obtained as a yellow solid. The ^{1}H NMR spectrum indicated that compound **8** was closely related to compound **5** except for the presence of an additional methoxy group at δ_H 3.36. The position of methoxy group at C-5 was determined by the HMBC correlation from these protons to C-5. The relative configurations at C-3, C-4a, C-5, and C-10a of compound **8** were assigned to be the same as those of compound **5** by the analysis of NMR data of these two compounds. Compound **8** was therefore determined as 5-methoxydihydrofusarubin B.

Compound **9** was obtained as a red solid. The molecular formula was deduced from HRMS (ESITOF), in combination with ^{13}C NMR data as $C_{17}H_{22}O_7$. The ^{1}H NMR spectrum was similar to that of compound **8** except for the presence of an additional methoxy signal at δ_H 3.19. The HMBC correlation from the additional methoxy protons to C-3 revealed the replacement of hydroxyl group in compound **8** with methoxy group in compound **9** at C-3. The analysis of NOESY spectrum and the coupling constants suggested that the relative configurations at C-3, C-4a, C-5, and C-10a were the same as those in compound **8**. Therefore, compound **9** was determined as 3,5-dimethoxydihydrofusarubin B.

Compound **6** was obtained as an orange solid. The HRMS (ESITOF) spectrum indicated the molecular formula as $C_{15}H_{18}O_7$. The UV and IR spectra were identical to those of compound **4**. Their ^{1}H

NMR spectrum was also closely related. The analysis of 2D NMR data as well as coupling constants revealed the same structure for both compounds with the difference in relative configuration at C-5. The trans diaxial arrangement between H-4a and H-10a and cis relationship between H-4a and H-5 were assigned on the basis of large coupling constant of 12.0 Hz and small coupling constant of 3.1 Hz, respectively. The cross peak between equatorial Ha-4 and H-5 in NOESY spectrum also supported cis relationship of H-4a and H-5. Application of modified Mosher method[16] with compound **6**, by methylation and conversion into bis-(*S*)- and bis-(*R*)-MTPA esters **16a** and **16b**, established (5*S*) configuration (Fig. 5). Compound **6** was (5*S*)-epimer of compound **4** and was therefore assigned as (3*R*, 4a*S*, 5*S*, 10a*R*)-5-hydroxydihydrofusarubin D.

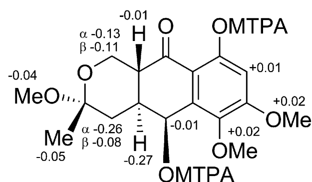


Fig. 5. Δδ-Values ($\delta_S-\delta_R$) of bis-(*S*)- and bis (*R*)-MTPA esters **16a** and **16b**.

Compound **10** with the molecular formula $C_{16}H_{20}O_7$ from HRMS (ESITOF) was obtained as an orange solid. The ^{1}H NMR spectrum was similar to that of compound **6** except for the presence of an additional methoxy signal at δ_H 3.17. The HMBC correlation from the additional methoxy protons (H_3-13) to C-3 revealed the replacement of hydroxyl group in compound **6** with methoxy group in compound **10** at C-3. The analysis of NOESY spectrum and the coupling constants suggested that the relative configurations at C-3, C-4a, C-5, and C-10a are the same as those in compound **6**. The almost identical optical rotation and CD spectrum of compound **6** and compound **10** indicated the same absolute configuration of these two compounds. Therefore, the absolute configurations at C-3, C-4a, C-5, and C-10a of compound **10** were assigned as *R*, *S*, *S*, and *R*, respectively, and compound **10** was determined as 5-hydroxy-3-methoxydihydrofusarubin D.

Compound **11** was obtained as a red solid. The molecular formula was deduced from HRMS (ESITOF), in combination with ^{13}C NMR data as $C_{17}H_{22}O_7$. The ^{1}H NMR spectrum indicated that compound **11** was closely related to compound **10** except for the presence of an additional methoxy group at δ_H 3.36. The position of methoxy group at C-5 was determined by the HMBC correlation from these protons to C-5. The configurations at C-3, C-4a, C-5, and C-10a of compound **11** were assigned to be the same as those of compound **6** by the analysis of NMR data as well as the almost identical of both optical rotations and CD spectra of these two compounds. Compound **11** was therefore determined as 3,5-dimethoxydihydrofusarubin D.

Due to the shortage of the sample, the absolute configuration of compounds **1**, **5**, **8**, **9**, and **12** were not determined.

The structure of five known compounds were elucidated on the basis of HRMS and NMR spectroscopic data, which were identical to those of 5-hydroxydihydrofusarubin C (**12**),[10] javanicin,[4] bostrycoidin,[11] anhydrofusarubin,[12] and 3-*O*-methylfusarubin.[9]

Six new compounds, **2**–**5**, **7**, and **8**, 5-hydroxydihydrofusarubin A (**12**), javanicin, and 3-*O*-methylfusarubin were tested for antifungal, antimycobacterial, and cytotoxic activities (Table 1). Javanicin, 3-*O*-methylfusarubin, compounds **3** and **7** showed weak to moderate antimycobacterial activity against *Mycobacterium tuberculosis*. Only javanicin was strongly active against *Candida albicans* with IC_{50} of 6.16 μg/mL. All of these compounds exhibited weak to moderate cytotoxic activity against KB and NCI-H187 cell lines (IC_{50} 1.62–31.69 μg/mL). Compounds **2** and **5** were inactive against both MCF-7 and vero cells, while compounds **4** and **7** were inactive against only vero cells.

Table 1
Biological activities of javanicin, 3-*O*-methylfusarubin, 5-hydroxydihydrofusarubin C (**12**), compounds **2**–**5**, **7**, and **8**

Compound	Anti-TB, MIC (μg/mL)	Antifungal, IC_{50} (μg/mL)	Cytotoxicity, IC_{50} (μg/mL)			
			KB cells	MCF-7 cells	NCI–H187 cells	Vero cells
Javanicin[a]	25.0	6.16	1.62	3.40	1.91	6.98
3-*O*-Methyl fusarubin[a]	50.0	>50	9.28	8.50	5.38	12.77
Compound**12**[a]	>200	>50	9.34	16.58	9.99	26.76
Compound **2**	>200	>50	31.69	>50	23.10	>50
Compound **3**	25.0	>50	31.29	>50	26.88	12.94
Compound **4**	>200	>50	21.25	10.99	12.14	>50
Compound **5**	>200	>50	31.50	>50	11.89	>50
Compound **7**	50.0	>50	25.48	14.50	26.03	>50
Compound **8**	>200	>50	23.59	23.43	13.47	28.26
Isoniazid[b]	0.03	—	—	—	—	—
Amphotericin B[c]	—	0.06	—	—	—	—
Doxorubicin[d]	—	—	0.28	0.90	0.07	—
Ellipticine[d]	—	—	0.32	—	1.21	1.72

[a] Antimicrobial activities of javanicin, 3-*O*-methylfurarubin, and 5-hydroxydihydrofusarubin A against other organisms using a different method were previously reported.[6,9,10]
[b] Anti-TB control.
[c] Antifungal control.
[d] Cytotoxicity controls.

3. Conclusion

In conclusion, 11 new compounds, 4-hydroxydihydronorjavanicin (**1**), dihydronaphthalenone **2** and its diastereomer **3**, 5-hydroxydihydrofusarubins A, B, and D (**4**–**6**), and the methyl ether derivatives **7**–**11**, together with five known compounds; 5-hydroxydihydrofusarubin C (**12**), javanicin, bostrycoidin, anhydrofusarubin, and 3-*O*-methylfusarubin, were isolated from the endophytic fungus *Fusarium* sp. BCC14842. Javanicin, 3-*O*-methylfusarubin, compounds **3** and **7** exhibited antimycobacterial and cytotoxic activities. Only javanicin displayed antifungal activity

with IC_{50} of 6.16 μg/mL, while compounds **2**, **4**, **5**, **8**, and **12** showed only cytotoxic activity.

4. Experimental

4.1. General procedures

Melting points were measured using an electrothermali IA9100 digital melting point apparatus and are uncorrected. Optical rotation measurements were obtained using a JASCO P-1030 digital polarimeter. UV and FT-IR spectra were recorded on a Varian Cary 1E UV–vis spectrophotometer and a Bruker VECTOR 22 spectrometer. The CD spectra were recorded on a JASCO J-180 spectropolarimeter. NMR spectra were recorded on Bruker DRX400 and Bruker AV500D spectrometers. ESITOF MS data were obtained on Micromass LCT and Bruker micrOTOF mass spectrometers.

4.2. Fungal material

The fungus used in this study was isolated from Bamboo leaf by Mr. Prasert Srikitikulchai, collected in the Bamboo forest at Nam Nao National Park, Phetchabun Province, Thailand. This fungus was deposited as BCC14842 at the BIOTEC Culture Collection Laboratory (BCC) on January 5, 2004. It was identified as *Fusarium* sp. on the basis of the sequence data of 18S rDNA, 28S rDNA, and internal transcribed spacer (ITS) genes by Mr. Nattawut Boonyuen.

4.3. Fermentation and isolation

Fusarium sp. BCC14842 was maintained on potato dextrose agar at 25 °C, and the agar was cut into pieces (1×1 cm) and inoculated into 4×250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB, potato starch 4.0 g, dextrose 20.0 g/L). After incubation at 25 °C for 7 days on a rotary shaker (200 rpm), each primary culture was transferred into 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (PDB) and incubated under the same conditions for 4 days. Each 25 mL portion of the secondary culture was transferred into 40×1 L Erlenmeyer flasks containing 250 mL of a liquid medium (PDB). The fungus was cultivated under shaking conditions at 250 rpm, 25 °C for 20 days.

After filtration of the mycelium, the culture broth was extracted with (3×10 L) EtOAc and evaporated to dryness leaving a dark brown solid (2.45 g). The crude extract was fractionated using a silica gel column (4×20 cm), eluted with 2% MeOH/CH_2Cl_2, to provide five fractions. Fraction 1 was subjected to silica gel column chromatography (3×15 cm), using 30% EtOAc/*n*-hexane as eluent, to give seven fractions (1–1–1–7). Javanicin (24.6 mg) and compound **3** (100.2 mg) were obtained from fractions 1–3 and 1–7, respectively. Fraction 1–2 was subjected to preparative HPLC using a reverse phase column (SunFire C_{18} OBD, 5 μm, 19,150 mm, step gradient elution with 20–60% $MeCN/H_2O$, flow rate 10 mL/min) to furnish compounds **8** (4.7 mg), **9** (3.2 mg), and javanicin (1.4 mg). After further purification by silica gel chromatography, anhydrofusarubin (5.5 mg), compounds **2** (43.9 mg), 5-hydroxydihydrofusarubin C (**12**, 9.1 mg), and an additional amount of javanicin (14.7 mg) were obtained from fraction 1–1, 1–4, 1–5, and 1–6, respectively. Trituration of fraction 2 with MeOH followed by filtration provided the insoluble compound **7** (330 mg) as a red solid. The filtrate was subjected to preparative HPLC (step gradient elution with 15–60% $MeCN/H_2O$, flow rate 10 mL/min) to afford 3-*O*-methylfusarubin (11.6 mg), compounds **4** (67.9 mg), **11** (5.0 mg), **2** (14.4 mg), **3** (14.1 mg), and **7** (123.1 mg). Further purification of fraction 3 by preparative HPLC (step gradient elution with 10–50% $MeCN/H_2O$) yielded compounds **1** (4.2 mg), **5** (9.1 mg), **6** (3.2 mg), **10** (5.0 mg) and an additional amount of compounds **2** (2.6 mg), **4** (35.6 mg), and **8** (6.7 mg). More of compound **4** (29.7 mg) was obtained from fractions 4 and 5 after further purification by preparative HPLC (step gradient elution with 20–60% $MeCN/H_2O$) and silica gel column chromatography, respectively.

The cells were macerated in MeOH for 3 days and then in CH_2Cl_2 for 3 days. The MeOH and CH_2Cl_2 extracts were combined and evaporated under reduced pressure. Water (200 mL) was added, and the mixture was extracted with *n*-hexane (3×200 mL), followed by EtOAc (3×200 mL). The dark brown solid (0.31 g), obtained from *n*-hexane extraction of the mycelium, was subjected to preparative HPLC (step gradient elution with 15–60% MeCN/H_2O) to furnish bostrycoidin (3.2 mg), javanicin (5.7 mg), 3-*O*-methylfusarubin (1.1 mg), and compound **3** (1.4 mg). Purification of the crude EtOAc extract (0.57 g) by preparative HPLC (step gradient elution with 15–50% $MeCN/H_2O$) provided an additional amount of javanicin (2.5 mg), compounds **2** (11.4 mg), **3** (19.9 mg), **4** (10.1 mg), and 5-hydroxydihydrofusarubin C (**12**, 2.1 mg).

*4.3.1. Compound **1**.* Red solid; $[\alpha]_D^{25}$ −57.7 (*c* 0.02, acetone); UV (MeOH) λ_{max} (log ε) 214 (4.00), 242 (4.07), 282 (3.95), 357 (3.85) nm; IR (KBr) ν_{max} 3415, 2925, 2853, 1712, 1625, 1493, 1440, 1356, 1297, 1277, 1226, 1205, 1163, 1095, 1079, 1036 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 2.09 (3H, s, 11-CH_3), 2.36 (1H, dd, *J*=5.7, 17.2 Hz, Ha-2), 2.45 (1H, dd, *J*=8.1, 17.7 Hz, Ha-9), 2.65 (1H, dd, *J*=5.5, 17.7 Hz, Hb-9), 2.78 (1H, m, H-3), 3.03 (1H, dd, *J*=4.7, 17.2 Hz, Hb-2), 3.93 (3H, s, 12-OCH_3), 4.74 (1H, d, *J*=4.3 Hz, OH-4), 5.04 (1H, dd, *J*=4.3, 4.7 Hz, H-4), 6.45 (1H, s, ArH-7), 7.92 (1H, s, OH-5), 12.63 (1H, s, OH-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 29.2 (C-11), 36.3 (C-3), 38.4 (C-2), 44.6 (C-9), 55.7 (C-12), 66.1 (C-4), 98.9 (C-7), 108.1 (C-8a), 126.6 (C-4a), 137.4 (C-5), 155.6 (C-6), 158.6 (C-8), 202.0 (C-1), 206.0 (C-10); HRMS (ESITOF) *m*/*z* 303.0837 $[M+Na]^+$ (calcd for: $C_{14}H_{16}O_6Na$, 303.0839).

*4.3.2. Compound **2**.* Pale orange solid; $[\alpha]_D^{24}$ −78.22 (*c* 0.12, acetone); UV (MeOH) λ_{max} (log ε) 215 (4.10), 244 (4.12), 284 (4.03), 355 (3.94) nm; IR (KBr) ν_{max} 3443, 2923, 1713, 1632, 1486, 1441, 1386, 1292, 1201, 1162, 1106 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.04 (3H, s, 11-CH_3), 2.41 (2H, d, *J*=6.9 Hz, H-9), 2.98 (1H, m, H-3), 3.20 (1H, t, *J*=4.9, H-2), 3.80 (1H, d, *J*=9.0 Hz, Ha-13), 3.81 (3H, s, 12-OCH_3), 4.26 (1H, dd, *J*=4.9, 9.0 Hz, Hb-13), 5.62 (1H, d, *J*=5.1 Hz, H-4), 5.94 (1H, s, OH-5), 6.29 (1H, s, ArH-7), 11.74 (1H, s, OH-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.2 (C-11), 40.4 (C-9), 43.1 (C-3), 52.9 (C-2), 56.3 (C-12), 68.3 (C-13), 72.4 (C-4), 99.0 (C-7), 108.1 (C-8a), 125.0 (C-4a), 135.7 (C-5), 155.0 (C-6), 158.5 (C-8), 202.3 (C-1), 205.8 (C-10); HRMS (ESITOF) *m*/*z* 315.0843 $[M+Na]^+$ (calcd for: $C_{15}H_{16}O_6Na$, 315.0839).

*4.3.3. Compound **3**.* Pale orange solid; $[\alpha]_D^{26}$ −39.9 (*c* 0.12, acetone); UV (MeOH) λ_{max} (log ε) 214 (3.68), 243 (3.73), 282 (3.61), 352 (3.54) nm; IR ($CHCl_3$) ν_{max} 3453, 2922, 1713, 1633, 1487, 1441, 1389, 1362, 1286, 1225, 1201, 1166, 1108 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.19 (3H, s, 11-CH_3), 2.49 (1H, dd, *J*=5.8, 18.0 Hz, Ha-9), 2.76 (1H, dd, *J*=7.9, 18.0 Hz, Hb-9), 2.87 (1H, dd, *J*=5.8, 7.9 Hz, H-3), 2.99 (1H, d, *J*=5.6, H-2), 3.77 (1H, d, *J*=9.3 Hz, Ha-13), 3.91 (3H, s, 12-OCH_3), 4.19 (1H, dd, *J*=5.6, 9.3 Hz, Hb-13), 5.39 (1H, s, H-4), 5.41 (1H, s, OH-5), 6.38 (1H, s, ArH-7), 11.66 (1H, s, OH-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.3 (C-11), 44.0 (C-3, C-9), 54.1 (C-2), 56.4 (C-12), 66.4 (C-13), 74.6 (C-4), 99.0 (C-7), 107.3 (C-8a), 127.6 (C-4a), 134.5 (C-5), 154.2 (C-6), 158.8 (C-8), 203.5 (C-1), 205.7 (C-10); HRMS (ESITOF) *m*/*z* 315.0844 $[M+Na]^+$ (calcd for: $C_{15}H_{16}O_6Na$, 315.0839).

*4.3.4. Compound **4**.* Pale orange solid; $[\alpha]_D^{26}$ −123.2 (*c* 0.03, acetone); UV (MeOH) λ_{max} (log ε) 217 (3.94), 243 (4.06), 282 (3.97), 354 (3.87) nm; CD (MeOH) Δε (nm) +16.36 (243), −1.56 (264), +12.85 (286), −9.96 (310), +1.05 (335), −2.47 (360); IR ($CHCl_3$) ν_{max} 3442, 2927, 2360, 1626, 1492, 1441, 1387, 1268, 1246, 1203, 1162, 1091 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 1.40 (3H, s, 11-CH_3),

1.45 (1H, ddd, J=2.0, 12.0, 13.2 Hz, Ha-4), 2.36 (1H, dd, J=3.8, 12.0 Hz, Hb-4), 2.49 (1H, m, H-4a), 2.59 (1H, ddd, J=5.1, 10.1, 13.1 Hz, H-10a), 3.90 (3H, s, 12-OCH$_3$), 3.96 (1H, dd, J=10.1, 11.2 Hz, Ha-1), 4.02 (1H, dd, J=5.1, 11.2 Hz, Hb-1), 4.63 (1H, d, J=2.0 Hz, OH-3), 4.87 (1H, dd, J=4.5, 9.8 Hz, H-5), 5.51 (1H, d, J=4.5 Hz, OH-5), 6.45 (1H, s, ArH-8), 8.89 (1H, br s, OH-6), 12.48 (1H, s, OH-9); ^{13}C NMR (125 MHz, acetone-d_6) δ 29.3 (C-11), 38.3 (C-4), 39.9 (C-4a), 45.0 (C-10a), 55.6 (C-12), 58.6 (C-1), 72.1 (C-5), 94.3 (C-3), 99.1 (C-8), 107.9 (C-9a), 127.3 (C-5a), 137.8 (C-6), 155.9 (C-7), 158.8 (C-9), 201.3 (C-10); HRMS (ESITOF) m/z 333.0949 [M+Na]$^+$ (calcd for: $C_{15}H_{18}O_7Na$, 333.0945).

*4.3.5. Compound **5**.* Pale orange solid; $[\alpha]_D^{26}$ −51.9 (c 0.06, acetone); UV (MeOH) λ_{max} (log ε) 214 (4.01), 243 (4.07), 282 (3.97), 357 (3.86) nm; CD (MeOH) $\Delta\varepsilon$ (nm) +11.15 (219), −3.55 (245), +4.30 (273), +1.12 (291), +9.03 (315), −7.51 (350); IR (CHCl$_3$) ν_{max} 3443, 2925, 1627, 1494, 1442, 1387, 1293, 1204, 1164, 1105, 1032 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.08 (1H, dt, J=1.7, 13.2 Hz, Ha-4), 1.21 (3H, s, 11-CH$_3$), 1.63 (1H, dd, J=3.2, 13.2 Hz, Hb-4), 2.94 (1H, m, H-10a), 2.98 (1H, m, H-4a), 3.94 (3H, s, 12-OCH$_3$), 4.01 (1H, dd, J=3.2, 11.2 Hz, Ha-1), 4.28 (1H, br s, OH-5), 4.47 (1H, dd, J=1.4, 11.2 Hz, Hb-1), 4.59 (1H, d, J=1.7 Hz, OH-3), 4.99 (1H, s, H-5), 6.46 (1H, s, ArH-8), 7.52 (1H, br s, OH-6), 12.86 (1H, s, OH-9); ^{13}C NMR (125 MHz, acetone-d_6) δ 29.3 (C-11), 36.2 (C-4a), 36.3 (C-4), 40.4 (C-10a), 55.7 (C-12), 58.1 (C-1), 63.92 (C-5), 94.0 (C-3), 98.9 (C-8), 108.2 (C-9a), 126.5 (C-5a), 137.2 (C-6), 155.6 (C-7), 158.7 (C-9), 202.3 (C-10); HRMS (ESITOF) m/z 333.0946 [M+Na]$^+$ (calcd for: $C_{15}H_{18}O_7Na$, 333.0945).

*4.3.6. Compound **6**.* Orange solid; $[\alpha]_D^{25}$ −64.0 (c 0.13, acetone); UV (MeOH) λ_{max} (log ε) 214 (3.95), 243 (4.01), 282 (3.90), 357 (3.81) nm; CD (MeOH) $\Delta\varepsilon$ (nm) +20.35 (211), −11.35 (229), +6.25 (247), −3.49 (270), +1.75 (292), −3.16 (312), +0.52 (341); IR (KBr) ν_{max} 3403, 2923, 2852, 1715, 1629, 1492, 1441, 1391, 1294, 1212, 1611, 1061, 1026 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.40 (3H, s, 11-CH$_3$), 1.76 (1H, dd, J=3.8, 12.8 Hz, Ha-4), 1.96 (1H, ddd, J=2.0, 12.4, 12.8 Hz, Hb-4), 2.43 (1H, ddd, J=3.1, 12.4, 12.0 Hz, H-4a), 3.00 (1H, ddd, J=5.1, 11.7, 12.0 Hz, H-10a), 3.92 (3H, s, 12-OCH$_3$), 3.94 (1H, m, Ha-1), 4.08 (1H, dd, J=5.1, 11.2 Hz, Hb-1), 4.19 (1H, d, J=4.5 Hz, OH-5), 4.55 (1H, d, J=2.0 Hz, OH-3), 4.98 (1H, m, H-5), 6.45 (1H, s, ArH-8), 7.45 (1H, s, OH-6), 12.52 (1H, s, OH-9); ^{13}C NMR (125 MHz, acetone-d_6) δ 29.3 (C-11), 37.3 (C-4a), 37.4 (C-4), 41.0 (C-10a), 55.7 (C-12), 59.0 (C-1), 62.3 (C-5), 94.6 (C-3), 98.9 (C-8), 108.0 (C-9a), 128.7 (C-5a), 136.3 (C-6), 155.3 (C-7), 158.8 (C-9), 203.0 (C-10); HRMS (ESITOF) m/z 333.0947 [M+Na]$^+$ (calcd for: $C_{15}H_{18}O_7Na$, 333.0945).

*4.3.7. Compound **7**.* Red solid; $[\alpha]_D^{26}$ −148.8 (c 0.09, acetone); UV (MeOH) λ_{max} (log ε) 216 (4.09), 243 (4.18), 282 (4.09), 353 (3.97) nm; CD (MeOH) $\Delta\varepsilon$ (nm) +23.21 (243), −2.89 (264), +18.0 (284), −13.45 (310), +1.14 (335), −3.98 (360); IR (CHCl$_3$) ν_{max} 3443, 2923, 2360, 1626, 1493, 1441, 1388, 1289, 1245, 1203, 1161, 1045 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.31 (3H, s, 11-CH$_3$), 1.49 (1H, dd, J=11.7, 13.0 Hz, Ha-4), 2.35 (1H, dd, J=3.9, 13.0 Hz, Hb-4), 2.44 (1H, m, H-4a), 2.61 (1H, ddd, J=5.0, 10.7, 13.1 Hz, H-10a), 3.18 (3H, s, 13-OCH$_3$), 3.59 (1H, dd, J=10.7, 11.5 Hz, Ha-1), 3.90 (3H, s, 12-OCH$_3$), 4.04 (1H, dd, J=5.0, 11.5 Hz, Hb-1), 4.85 (1H, dd, J=4.9, 10.1 Hz, H-5), 5.49 (1H, d, J=4.9 Hz, OH-5), 6.45 (1H, s, ArH-8), 8.86 (1H, br s, OH-6), 12.44 (1H, s, OH-9); ^{13}C NMR (125 MHz, acetone-d_6) δ 22.8 (C-11), 38.5 (C-4), 39.5 (C-4a), 44.6 (C-10a), 47.0 (C-13), 55.6 (C-12), 59.1 (C-1), 71.9 (C-5), 97.3 (C-3), 99.1 (C-8), 107.9 (C-9a), 127.2 (C-5a), 137.8 (C-6), 160.0 (C-7), 158.8 (C-9), 201.1 (C-10); HRMS (ESITOF) m/z 347.1103 [M+Na]$^+$ (calcd for: $C_{16}H_{20}O_7Na$, 347.1101).

*4.3.8. Compound **8**.* Yellow solid; $[\alpha]_D^{27}$ −84.0 (c 0.06, acetone); UV (MeOH) λ_{max} (log ε) 216 (4.06), 243 (4.10), 282 (4.01), 357 (3.92) nm; CD (MeOH) $\Delta\varepsilon$ (nm) +5.06 (217), −4.62 (240), +5.03 (271), +1.03 (290), +11.29 (315), −9.34 (351); IR (KBr) ν_{max} 3415, 2928, 2854, 1710, 1627, 1491, 1441, 1395, 1260, 1202, 1163, 1085, 1018 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.07 (1H, ddd, J=1.7, 13.2, 13.3 Hz, Ha-4), 1.20 (3H, s, 11-CH$_3$), 1.61 (1H, dd, J=3.2, 13.2 Hz, Hb-4), 2.80 (1H, t, J=3.8 Hz, H-10a), 3.10 (1H, m, H-4a), 3.37 (3H, s, 14-OCH$_3$), 3.96 (3H, s, 12-OCH$_3$), 4.02 (1H, dd, J=3.8, 11.2 Hz, Ha-1), 4.41 (1H, d, J=11.2 Hz, Hb-1), 4.63 (1H, s, OH-3), 4.64 (1H, s, H-5), 6.50 (1H, s, ArH-8), 7.64 (1H, br s, OH-6), 12.89 (1H, s, OH-9); ^{13}C NMR (125 MHz, acetone-d_6) δ 29.3 (C-11), 33.6 (C-4a), 35.9 (C-4), 40.6 (C-10a), 55.7 (C-12), 56.0 (C-13), 58.1 (C-1), 72.6 (C-5), 94.0 (C-3), 99.2 (C-8), 108.3 (C-9a), 123.6 (C-5a), 137.9 (C-6), 155.5 (C-7), 159.1 (C-9), 202.2 (C-10); HRMS (ESITOF) m/z 347.1108 [M+Na]$^+$ (calcd for: $C_{16}H_{20}O_7Na$, 347.1101).

*4.3.9. Compound **9**.* Red solid; $[\alpha]_D^{24}$ −46.8 (c 0.16, acetone); UV (MeOH) λ_{max} (log ε) 214 (3.91), 243 (3.93), 283 (3.85), 357 (3.74), 423 (2.59) nm; CD (MeOH) $\Delta\varepsilon$ (nm) +0.88 (215), −4.72 (238), +4.05 (271), +1.18 (295), +8.19 (315), −6.64 (351); IR (KBr) ν_{max} 3378, 2925, 2853, 1707, 1627, 1490, 1441, 1396, 1364, 1258, 1163, 1091, 1034, 1019 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.09 (1H, t, J=13.2 Hz, Ha-4), 1.10 (3H, s, 11-CH$_3$), 1.62 (1H, ddd, J=1.0, 4.1, 13.2 Hz, Hb-4), 2.81 (1H, m, H-10a), 3.04 (1H, m, H-4a), 3.19 (3H, s, 13-OCH$_3$), 3.36 (3H, s, 14-OCH$_3$), 3.64 (1H, dd, J=3.5, 11.2 Hz, Ha-1), 3.96 (3H, s, 12-OCH$_3$), 4.44 (1H, d, J=11.2 Hz, Hb-1), 4.63 (1H, d, J=3.0 Hz, H-5), 6.49 (1H, s, ArH-8), 7.66 (1H, s, OH-6), 12.86 (1H, s, OH-9); ^{13}C NMR (125 MHz, acetone-d_6) δ 22.8 (C-11), 33.2 (C-4a), 35.9 (C-4), 40.3 (C-10a), 47.2 (C-13), 55.7 (C-12), 56.0 (C-14), 58.6 (C-1), 72.5 (C-5), 96.9 (C-3), 99.2 (C-8), 108.2 (C-9a), 123.5 (C-5a), 137.9 (C-6), 155.6 (C-7), 159.1 (C-9), 201.9 (C-10); HRMS (ESITOF) m/z 361.1261 [M+Na]$^+$ (calcd for: $C_{17}H_{22}O_7Na$, 361.1258).

*4.3.10. Compound **10**.* Orange solid; $[\alpha]_D^{25}$ −106.8 (c 0.23, acetone); UV (MeOH) λ_{max} (log ε) 214 (3.68), 243 (3.72), 282 (3.61), 357 (3.52) nm; CD (MeOH) $\Delta\varepsilon$ (nm) +6.67 (211), −3.69 (229), +3.65 (247), −1.86 (270), +1.18 (292), −1.94 (312), +0.56 (341); IR (KBr) ν_{max} 3470, 2929, 1745, 1630, 1554, 1492, 1441, 1371, 1226, 1161, 1054 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.31 (3H, s, 11-CH$_3$), 1.76 (1H, dd, J=3.5, 12.8 Hz, Ha-4), 1.98 (1H, dd, J=12.3, 12.8 Hz, Hb-4), 2.37 (1H, ddd, J=3.5, 11.8, 12.3 Hz, H-4a), 3.01 (1H, ddd, J=5.1, 11.4, 11.8 Hz, H-10a), 3.17 (3H, s, 13-OCH$_3$), 3.56 (1H, dd, J=11.2, 11.4 Hz, Ha-1), 3.93 (3H, s, 12-OCH$_3$), 4.10 (1H, dd, J=5.1, 11.2 Hz, Hb-1), 4.21 (1H, br s, OH-5), 4.96 (1H, s, H-5), 6.45 (1H, s, ArH-8), 7.55 (1H, br s, OH-6), 12.49 (1H, s, OH-9); ^{13}C NMR (125 MHz, acetone-d_6) δ 22.9 (C-11), 37.0 (C-4a), 37.6 (C-4), 40.6 (C-10a), 47.0 (C-13), 55.7 (C-12), 59.5 (C-1), 62.1 (C-5), 97.5 (C-3), 98.9 (C-8), 107.9 (C-9a), 128.5 (C-5a), 136.3 (C-6), 155.4 (C-7), 158.8 (C-9), 202.7 (C-10); HRMS (ESITOF) m/z 347.1107 [M+Na]$^+$ (calcd for: $C_{16}H_{20}O_7Na$, 347.1101).

*4.3.11. Compound **11**.* Red solid; $[\alpha]_D^{24}$ −35.5 (c 0.25, acetone); UV (MeOH) λ_{max} (log ε) 214 (3.90), 243 (3.88), 282 (3.80), 357 (3.65), 424 (2.91) nm; CD (MeOH) $\Delta\varepsilon$ (nm) +1771 (211), −5.26 (229), +4.83 (244), −2.09 (266), +1.64 (290), −2.68 (312), +0.95 (339); IR (KBr) ν_{max} 3416, 2922, 2851, 1715, 1628, 1490, 1460, 1441, 1390, 1291, 1261, 1243, 1203, 1161, 1093 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.29 (3H, s, 11-CH$_3$), 1.81 (1H, dd, J=3.9, 13.0 Hz, Hb-4), 1.92 (1H, dd, J=12.6, 13.0 Hz, Ha-4), 2.41 (1H, m, H-4a), 2.90 (1H, m, H-10a), 3.15 (3H, s, 13-OCH$_3$), 3.36 (3H, s, 14-OCH$_3$), 3.50 (1H, t, J=11.1 Hz, Ha-1), 3.95 (3H, s, 12-OCH$_3$), 4.09 (1H, dd, J=5.0, 11.1 Hz, Hb-1), 4.63 (1H, d, J=2.0 Hz, H-5), 6.48 (1H, s, ArH-8), 7.75 (1H, s, OH-6), 12.63 (1H, s, OH-9); ^{13}C NMR (125 MHz, acetone-d_6) δ 22.8 (C-11), 37.5 (C-4a), 37.7 (C-4), 41.4 (C-10a), 47.0 (C-13), 55.8 (C-12), 56.9 (C-14), 59.7 (C-1), 70.9 (C-5), 97.5 (C-3), 99.3 (C-8), 107.9 (C-9a), 125.5 (C-5a), 136.9 (C-6), 155.3 (C-7), 159.6 (C-9), 202.4 (C-10); HRMS (ESITOF) m/z 361.1264 [M+Na]$^+$ (calcd for: $C_{17}H_{22}O_7Na$, 361.1258).

*4.3.12. Compound **12**.* Orange solid; $[\alpha]_D^{24}$ −172.5 (c 0.03, acetone); UV (MeOH) λ_{max} (log ε) 217 (3.94), 243 (4.06), 282 (3.97), 354 (3.87) nm; CD (MeOH) $\Delta\varepsilon$ (nm) −68.04 (233), +31.25 (254), −17.35 (274),

+10.31 (293), −8.90 (318), +4.54 (338), +8.78 (362); IR (KBr) ν_{max} 3274, 2935, 2852, 1624, 1492, 1459, 1442, 1410, 1382, 1369, 1258, 1224, 1204, 1155, 1125, 1097 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.58 (3H, s, 11-CH$_3$), 1.99 (1H, dd, *J*=4.0, 11.6 Hz, Ha-4), 2.03 (1H, d, *J*=11.6 Hz, Hb-4), 2.94 (1H, ddd, *J*=3.4, 3.8, 4.0 Hz, H-4a), 3.00 (1H, ddd, *J*=3.8, 8.7, 9.5 Hz, H-10a), 3.80 (1H, dd, *J*=9.5, 11.3 Hz, Ha-1), 3.93 (1H, s, OH-5), 3.94 (3H, s, 12-OCH$_3$), 4.00 (1H, dd, *J*=8.7, 11.3 Hz, Hb-1), 5.34 (1H, d, *J*=3.4 Hz, H-5), 5.65 (1H, br s, OH-6), 6.48 (1H, s, ArH-8), 12.00 (1H, s, OH-9); ^{13}C NMR (125 MHz, CDCl$_3$) δ 23.9 (C-11), 38.9 (C-4a), 40.1 (C-4), 45.0 (C-10a), 56.4 (C-12), 62.8 (C-1), 72.0 (C-5), 100.1 (C-8), 106.2 (C-3), 107.2 (C-9a), 121.2 (C-5a), 137.8 (C-6), 154.5 (C-7), 158.7 (C-9), 203.1 (C-10); HRMS (ESITOF) *m*/*z* 309.0961 [M−H]$^-$ (calcd for: C$_{15}$H$_{17}$O$_7$, 309.0969).

4.4. Preparation of bis-*O*-(bromobenzoyl) derivatives 13 and 14

To the suspension of **2** (10 mg) and NaH (2.9 mg) in THF (1 mL) was added the solution of 3-bromobenzoyl chloride (10.0 μL) in THF (0.5 mL), at 0 °C. The mixture was left stirring at room temperature for 1 h, diluted with satd NaHCO$_3$ (2 mL) and then extracted with EtOAc (2 mL). The organic layer was concentrated under reduced pressure, then purified by preparative HPLC (step gradient elution with 50–100% MeCN/H$_2$O) to yield a white solid of **13** (10.1 mg). Compound **14** was prepared from **3** (10 mg) and 4-bromobenzoylchloride (2 equiv) and was obtained in 11.1 mg as a white solid after purification by preparative HPLC. Recrystallization of both **13** (10.1 mg) and **14** (11.1 mg) in 1:1 CH$_2$Cl$_2$/MeOH gave colorless needles.

*4.4.1. Bis-O-(3-bromobenzoyl) derivative **13**.* Colorless needles; mp 171.6–173.5 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 2.08 (3H, s, 11-CH$_3$), 2.47 (1H, dd, *J*=7.9, 18.9 Hz, Ha-9), 2.58 (1H, dd, *J*=8.0, 18.9 Hz, Hb-9), 2.98 (1H, m, H-3), 3.18 (1H, t, *J*=4.8, H-2), 3.80 (1H, d, *J*=9.0 Hz, Ha-13), 3.88 (3H, s, 12-OCH$_3$), 4.19 (1H, dd, *J*=4.8, 9.0 Hz, Hb-13), 5.30 (1H, m, H-4), 6.81 (1H, s, ArH-7), 7.42 (2H, dt, *J*=2.0, 7.8 Hz, 3-bromobenzoyl), 7.79 (2H, t, *J*=8.8 Hz, 3-bromobenzoyl), 8.15 (2H, d, *J*=7.4 Hz, 3-bromobenzoyl), 8.36 (2H, d, *J*=2.0 Hz, 3-bromobenzoyl); HRMS (ESITOF) *m*/*z* 656.9824 and 660.9752 [M+H]$^+$ (calcd for: C$_{29}$H$_{23}$O$_8^{79}$Br$_2$, 656.9754; calcd for: C$_{29}$H$_{23}$O$_8^{81}$Br$_2$, 660.9723).

*4.4.2. Bis-O-(4-bromobenzoyl) derivative **14**.* Colorless needles; mp 110.0–112.0 °C; ^{1}H NMR (400 MHz, acetone-*d*$_6$) δ 2.08 (3H, s, 11-CH$_3$), 2.61 (1H, dd, *J*=6.6, 18.2 Hz, Ha-9), 2.71 (1H, dd, *J*=6.8, 18.2 Hz, Hb-9), 2.82 (1H, m, H-3), 3.09 (1H, d, *J*=5.1 Hz, H-2), 3.61 (1H, d, *J*=9.1 Hz, Ha-13), 3.94 (3H, s, 12-OCH$_3$), 4.16 (1H, dd, *J*=5.5, 9.1 Hz, Hb-13), 5.20 (1H, m, H-4), 7.14 (1H, s, ArH-7), 7.79 (2H, d, *J*=8.3 Hz, 3-bromobenzoyl), 7.81 (2H, t, *J*=8.4 Hz, 3-bromobenzoyl), 8.09 (2H, d, *J*=8.3 Hz, 3-bromobenzoyl), 8.14 (2H, d, *J*=8.4 Hz, 3-bromobenzoyl); HRMS (ESITOF) *m*/*z* 656.9843 and 660.9760 [M+H]$^+$ (calcd for: C$_{29}$H$_{23}$O$_8^{79}$Br$_2$, 656.9754; calcd for: C$_{29}$H$_{23}$O$_8^{81}$Br$_2$, 660.9723).

4.5. X-ray crystallographic analysis of 13 and 14

X-ray diffraction data of **13** and **14** were measured on a Bruker–Nonius kappaCCD diffractometer with graphite monochromated Mo Kα radiation (λ=0.71073 Å) at 298 (2) K for **13** and at 150 (2) K for **14**. The structures were solved by direct methods by SIR97,[17] and refined with full-matrix least-squares calculations on F^2 using SHELXL-97.[18]

Crystal data for compound **13**: 2(C$_{29}$H$_{22}$Br$_2$O$_8$)·CH$_2$Cl$_2$, *MW*=1401.523, monoclinic, dimensions: 0.15×0.15×0.10 mm^3, *D*=1.663 g/cm^3, space group *C*2, *Z*=2, *a*=13.8367 (3), *b*=9.64360 (10), *c*=20.9872 (4) Å, β=91.496 (1)°, *V*=2799.5 (1) Å^3, reflections collected/unique: 24,140/5404 (R_{int}=0.0239), number of observation [>2σ(*I*)] 4,978, final *R* indices [*I*>2σ(*I*)]: R_1=0.0391, *wR*$_2$=0.0926. Flack parameter=0.048 (7).

Crystal data for compound **14**: C$_{29}$H$_{22}$Br$_2$O$_8$, MW=658.31, triclinic, dimensions: 0.15×0.10×0.10 mm^3, *D*=1.441 g/cm^3, space group *P*1, *Z*=2, *a*=9.3789 (8), *b*=12.2707 (11), *c*=14.6566 (13) Å, *a*=100.09 (1)°, *b*=102.66(1)°, *g*=107.43(1)°, *V*=1516.8(2) Å^3, reflections collected/unique: 14,278/8742 (R_{int}=0.0433), number of observation [>2σ(*I*)] 7,308, final *R* indices [*I*>2σ(*I*)]: R_1=0.0751, w*R*$_2$=0.2267. Flack parameter=0.11 (2).

Crystallographic data (excluding structure factors) for the structures **13** and **14** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 818559 and 818560, respectively. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0) 1223 336033 or e-mail: deposit@cdcc.cam.ac.uk).

4.6. Preparation of the THENA esters 15a and 15b

Compound **4** (3.8 mg) was methylated with MeI (2.5 equiv) in K$_2$CO$_3$ (2.5 equiv) and acetone (0.5 mL) at room temperature for 18 h. The reaction mixture was evaporated then diluted with H$_2$O and extracted with EtOAc. The organic layer was concentrated under reduced pressure to leave dark brown solid, which was purified by column chromatography on silica gel (using 40% EtOAc/*n*-hexane as eluent) to furnish the dimethylated product (4.0 mg) as a pale orange solid. The replacement of hydroxyl groups at C-3 and C-6 with methoxy groups (δ_H 3.17 and 3.78) was revealed in the 3,6-*O*-dimethyl ether derivative.

The dimethylated product (1.8 mg) was treated with (*S*)-THENACl (20 μL) in CH$_2$Cl$_2$ (0.2 mL) and pyridine (0.2 mL) at room temperature for 16 h. The mixture was diluted with EtOAc (2 mL) and washed with H$_2$O (2 mL), and the organic layer was evaporated under reduced pressure. The residue was purified by preparative HPLC (step gradient elution with 50–100% MeCN/H$_2$O) to obtained the (*S*)-THENA ester **15a** (1.4 mg). Similarly, (*R*)-THENA ester **15b** was prepared from the dimethylated product (1.8 mg) and (*R*)-THENACl (20 μL) and was obtained in 1.5 mg after purification by preparative HPLC. The assignment of protons for **15a** and **15b** was achieved by analysis of COSY and NOESY data.

*4.6.1. (S)-THENA ester **15a**.* White solid; ^{1}H NMR (500 MHz, acetone-*d*$_6$) δ 1.17 (3H, s, 11-CH$_3$), 1.24 (1H, t, *J*=13.3 Hz, Ha-4), 1.37 and 1.63 (2H, m, CH$_2$ of THENA), 1.87 (1H, dd, *J*=3.5, 13.3 Hz, Hb-4), 2.16 (2H, m, CH$_2$ of THENA), 2.97 (1H, m, H-4a), 3.19 (3H, s, 13-OCH$_3$), 3.23 (1H, m, H-10a), 3.70 (1H, dd, *J*=3.2, 11.3 Hz, Ha-1), 3.71 (3H, s, 15-OCH$_3$), 3.96 (3H, s, 12-OCH$_3$), 4.49 (1H, d, *J*=11.3 Hz, Hb-1), 5.41 (1H, d, *J*=4.6 Hz, CH of THENA), 6.31 (1H, d, *J*=2.4 Hz, H-5), 6.62 (1H, s, ArH-8), 7.20 (2H, m, ArH of THENA), 7.30 (1H, dd, *J*=2.1, 6.8 Hz, ArH of THENA), 7.46 (1H, dd, *J*=2.1, 6.9 Hz, ArH of THENA); HRMS (ESITOF) *m*/*z* 533.1785 [M+Na]$^+$ (calcd for: C$_{28}$H$_{30}$O$_9$Na, 533.1782).

*4.6.2. (R)-THENA ester **15b**.* White solid; ^{1}H NMR (500 MHz, acetone-*d*$_6$) δ 1.16 (3H, s, 11-CH$_3$), 1.23 (1H, t, *J*=13.2 Hz, Ha-4), 1.36 and 1.64 (2H, m, CH$_2$ of THENA), 1.86 (1H, dd, *J*=3.8, 13.2 Hz, Hb-4), 2.14 (2H, m, CH$_2$ of THENA), 2.86 (1H, m, H-4a), 3.18 (3H, s, 13-OCH$_3$), 3.19 (1H, m, H-10a), 3.66 (1H, dd, *J*=3.2, 11.4 Hz, Ha-1), 3.78 (3H, s, 15-OCH$_3$), 4.00 (3H, s, 12-OCH$_3$), 4.45 (1H, d, *J*=11.4 Hz, Hb-1), 5.42 (1H, d, *J*=4.6 Hz, CH of THENA), 6.34 (1H, d, *J*=2.5 Hz, H-5), 6.66 (1H, s, ArH-8), 7.12–7.21 (2H, m, ArH of THENA), 7.31 (2H, d, *J*=7.5 Hz, ArH of THENA); HRMS (ESITOF) *m*/*z* 533.1787 [M+Na]$^+$ (calcd for: C$_{28}$H$_{30}$O$_9$Na, 533.1782).

4.7. Preparation of the bis-MTPA esters 16a and 16b

The bis-MTPA esters, **16a** and **16b**, were prepared from compound **6** with (−)-(*R*)-MTPACl and (+)-(*S*)-MTPACl, respectively, by

the same method as that used for **15a** and **15b** and were obtained as a white solid in 1.5 mg and 1.7 mg, respectively.

*4.7.1. Bis-(S)-MTPA ester **16a***. White solid; ^{1}H NMR (400 MHz, acetone-d_6) δ 1.19 (3H, s, 11-CH_3), 1.23 (1H, t, J=12.7 Hz, Ha-4), 1.85 (1H, dd, J=3.5, 12.7 Hz, Hb-4), 2.50 (1H, m, H-4a), 2.77 (1H, m, H-10a), 3.14 (3H, s, 13-OCH_3), 3.46 (1H, m, Ha-1), 3.47 (3H, s, OCH_3 of MTPA), 3.76 (3H, s, OCH_3 of MTPA), 3.88 (1H, dd, J=5.1, 11.6 Hz, Hb-1), 3.96 (3H, s, 15-OCH_3), 4.01 (3H, s, 12-OCH_3), 6.69 (1H, d, J=2.3 Hz, H-5), 6.80 (1H, s, ArH-8), 7.44 (3H, m, ArH of MTPA), 7.53 (3H, m, ArH of MTPA), 7.60 (2H, m, ArH of MTPA), 7.75 (2H, m, ArH of MTPA); HRMS (ESITOF) m/z 793.2059 $[M+Na]^+$ (calcd for: $C_{37}H_{36}O_{11}F_6Na$, 793.2054).

*4.7.2. Bis-(R)-MTPA ester **16b***. White solid; ^{1}H NMR (400 MHz, acetone-d_6) δ 1.27 (3H, s, 11-CH_3), 1.49 (1H, t, J=12.7 Hz, Ha-4), 1.93 (1H, dd, J=3.4, 12.7 Hz, Hb-4), 2.77 (1H, m, H-4a), 2.78 (1H, m, H-10a), 3.18 (3H, s, 13-OCH_3), 3.57 (1H, t, J=11.0 Hz, Ha-1), 3.46 (3H, s, OCH_3 of MTPA), 3.75 (3H, s, OCH_3 of MTPA), 3.94 (3H, s, 14-OCH_3), 3.99 (3H, s, 12-OCH_3), 4.01 (1H, dd, J=4.0, 11.0 Hz, Hb-1), 6.70 (1H, d, J=2.1 Hz, H-5), 6.79 (1H, s, ArH-8), 7.47 (3H, m, ArH of MTPA), 7.53 (3H, m, ArH of MTPA), 7.57 (2H, m, ArH of MTPA), 7.77 (2H, m, ArH of MTPA); HRMS (ESITOF) m/z 793.2053 $[M+Na]^+$ (calcd for: $C_{37}H_{36}O_{11}F_6Na$, 793.2054).

4.8. Biological assays

Growth inhibitory activity against *M. tuberculosis* $H_{37}Ra$ and cytotoxicity to Vero cells (African green monkey kidney fibroblasts) were performed using the green fluorescent protein (GFP)-based method.[19] Antifungal activity against *C. albicans* and anticancer activities against KB cells (oral human epidermoid carcinoma), MCF-7 cells (human breast cancer), and NCI-H187 cells (human small-cell lung cancer) were evaluated using the resazurin microplate assay.[20]

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2011.07.078. These data include NMR spectra of the most important compounds described in this article.

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