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Bioactive polyketides from the fungus *Astrocystis* sp. BCC 22166

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ABSTRACT

Five new compounds, phthalide **1**, dihydroisocoumarin **2**, and **3**, pyrone **4**, and benzophenone **5**, together with nine known compounds, 3,4-dihydro-4,5,8-trihydroxy-3-methylisocoumarin, sclerotinin A, methyl-8-hydroxy-6-methylxanthone-1-carboxylate, sydowinin A, conioxanthone A, 1,3,8-trihydroxy-6-methylxanthone, 1,8-dihydroxy-3-methoxy-6-methylxanthone, coniochaetone B, and xylaranol B, were isolated from the fungus *Astrocystis* sp. BCC 22166. Structures of these compounds were elucidated using NMR spectroscopic and MS spectrometric analyses. Compound **1** exhibited antibacterial activity against *Bacillus cereus* (IC_{50}=12.5 μg/mL) while compound **2** showed cytotoxicity to KB and Vero cells (IC_{50}=22.6 and 48.2 μg/mL).

1. Introduction

Astrocystis is a genus of fungi in the family *Xylariaceae*, which is known to be a potential source for bioactive compounds.[1,2] However, to our knowledge, the study of secondary metabolites of the genus *Astrocystis* has never been reported. As part of our continuing research into bioactive compounds from Thai microorganisms, we investigated the constituents of the fungus *Astrocystis* sp. BCC 22166, of which the crude extract exhibited antibacterial activity against *Bacillus cereus* (IC_{50}=4.79 μg/mL) and cytotoxic activity to oral human epidermoid carcinoma (KB, IC_{50}=48.09 μg/mL) cells. The study led to the isolation and structure elucidation of five new compounds, phthalide **1**, dihydroisocoumarin **2**, and **3**, pyrone **4**, and benzophenone **5**, together with nine known compounds, 3,4-dihydro-4,5,8-trihydroxy-3-methylisocoumarin (**6**),[3] sclerotinin A (**7**),[4] methyl-8-hydroxy-6-methylxanthone-1-carboxylate (**8**),[5] sydowinin A (**9**),[6] conioxanthone A (**10**),[7] 1,3,8-trihydroxy-6-methylxanthone (**11**),[8] 1,8-dihydroxy-3-methoxy-6-methylxanthone (**12**),[9] coniochaetone B,[10] and xylaranol B.[11] Biological activities of these compounds were also evaluated.

2. Results and discussion

Compound **2**, methyl-8-hydroxy-6-methylxanthone-1-carboxylate, conioxanthone A, sydowinin A, and coniochaetone B were obtained from both culture broth and mycelia extracts of BCC 22166.

Compounds **1**, **3**, **4**, **5**, 1,8-dihydroxy-3-methoxy-6-methyl xanthone, 3,4-dihydro-4,5,8-trihydroxy-3-methylisocoumarin, sclerotinin A, and xylaranol B were obtained from the culture broth extract while 1,3,8-trihydroxy-6-methylxanthone was isolated solely from an *n*-hexane extract of the mycelium.

Compound **1** was obtained as a white solid, possessing the molecular formula of $C_{12}H_{12}O_6$ as deduced from ESIMS, in combination with ^{13}C NMR spectroscopy. The IR spectrum showed major absorption bands at 3372, 1752, and 1680 cm^{-1} corresponding, respectively, to a hydroxyl, a carbonyl, and H-bonding carbonyl group. The ^1H and ^{13}C NMR spectra revealed the presence of one methyl group, one methoxy group, two oxymethine protons, 1,2,3,4-tetrasubstitued benzene, two hydroxyl groups (D_2O exchangeable), and two carboxyl carbons (Table 1). The HMBC correlations from H-3 to C-1/C-3a/C-4, H-5 to C-3a/C-7, and H-6 to C-4/C-7a established the isobenzofuranone ring. The hydroxyl group was assigned at C-7 on the basis of its chemical shift. The HMBC correlations from the methoxy protons to C-10 and from H-5 to C-10 established the methylcarboxylate group at C-4. The cross peaks from H-3 to H-8 and from H-8 to the methyl protons (H_3-9) and OH-8 in the COSY spectrum and the correlations from methyl protons to C-3/C-8, OH-8 to C-8, and H-3 to C-8/C-9 in the HMBC spectrum indicated the 1-hydroxyethyl side chain at C-3. This assignment was also supported by the NOESY correlations from the methoxy protons to H-8 and OH-8. Therefore, compound **1** was named as astrophthalide (Fig. 1).

The cross peak between H-3 and H-8 in the NOESY spectrum and the small coupling constant between these two protons (1.9 Hz)

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Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for **1** in acetone-d_6

Position	**1**		
	δ_C	δ_H mult. (*J* in Hz)	HMBC
1	169.1	—	—
3	85.8	5.88 d (1.9)	1, 3a, 4, 8, 9
3a	150.7	—	—
4	119.0	—	—
5	131.7	7.93 d (8.5)	3a, 7, 10
6	120.1	7.20 d (8.5)	4, 7, 7a
7	167.0	—	—
7a	108.6	—	—
8	68.7	4.39 m	—
9	15.5	0.76 d (6.0)	3, 8
10	169.6	—	—
7-OH	—	11.5 s	—
8-OH	—	4.39 m	8
11-OMe	52.7	4.08 s	10

showed signals for two non-chelated phenolic protons, one aromatic proton, one oxymethine proton, one methoxy, one methylene, and one methyl groups (Table 2). Analysis of ^{13}C and 2D NMR spectroscopic data revealed the structural feature of dihydroisocoumarin derivative. The cross peak between the methyl protons and H-3 in the COSY spectrum together with the correlations from the methyl protons to C-3/C-4 in the HMBC spectrum established the methyl group at C-3. The positions of the substituents on the aromatic ring were established on the basis of their chemical shifts together with HMBC correlations from H-4 to C-5, methoxy protons to C-8, and H-6 to C-4a/C-5/C-7/C-8. Cross peaks between H-6 and the two hydroxyl protons in the NOESY spectrum also supported this assignment. Compound **2** was, therefore, assigned as 5,7-dihydroxy-*O*-methylmellein. The almost identical CD spectrum and optical rotations of compound **2** and those of (3*R*)-5,6-dihydroxymellein[15] indicated the *R* configuration at C-3 as depicted in Fig 1.

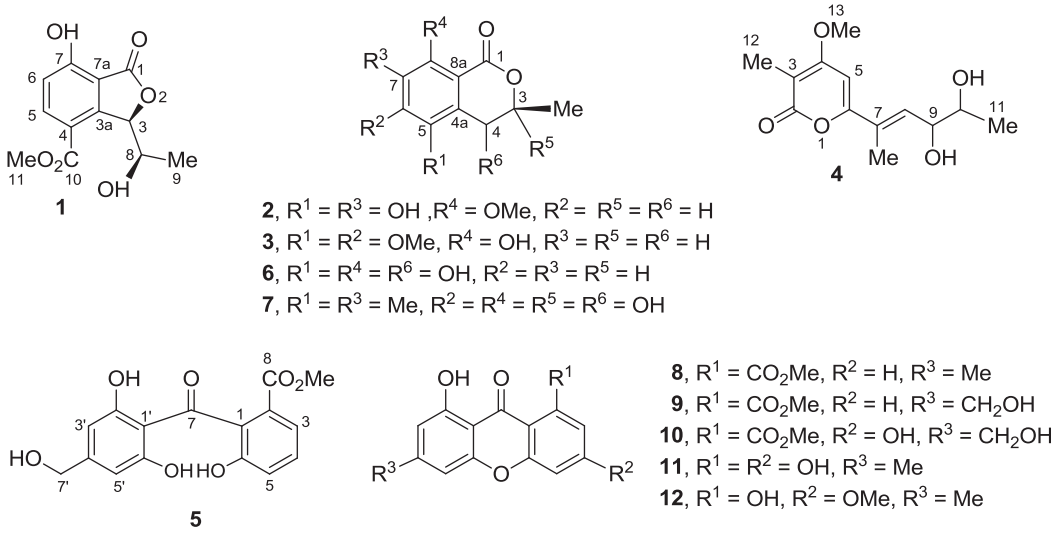
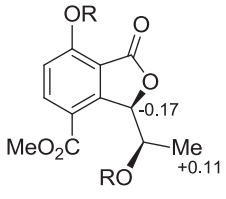


Fig. 1. Structures of compounds **1**–**12**.

suggested the *cis* relationship between H-3 and H-8. The absolute configuration at C-8 was addressed by application of the modified Mosher method.[12] Compound **1** was treated with (*R*)-MTPACl and (*S*)-MTPACl to afford (*S*)- and (*R*)-MTPA esters **1a** and **b**, respectively. The differences in chemical shift values ($\Delta\delta_{S-R}$, Fig. 2) were consistent with the 8*R* configuration. Considering the relative configuration deduced by NOESY and coupling constant data, the absolute configuration at C-3 was assigned as *R*. The CD spectra of compound **1**, which showed the same chirality as those of spirolaxine[13] and pestaphthalide B,[14] also supported the *R* configuration at C-3.



1a, R = (*S*)-MTPA
1b, R = (*R*)-MTPA

Fig. 2. $\Delta\delta$-Values (δ_S–δ_R) of bis-(*S*)- and (*R*)-MTPA esters **1a** and **b**.

Compound **2** with the molecular formula $C_{11}H_{12}O_5$ from ESIMS was obtained as a pale yellow solid. The ^{1}H NMR spectrum

Table 2
^{1}H and ^{13}C data for **2** and **3**

Position	**2**[a] (In acetone-d_6)		**3**[b] (In $CDCl_3$)	
	δ_C	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)
1	161.9	—	169.9	—
3	74.5	4.50 m	75.6	4.63 m
4	28.6	2.47 dd (11.4, 16.5)	28.8	2.68 dd (11.5, 16.7)
		3.05 dd (2.8, 16.5)		3.08 dd (3.2, 16.7)
4a	117.9	—	131.4	—
5	149.5	—	137.8	—
6	108.1	6.73 s	159.6	—
7	150.2	—	99.0	6.42 s
8	142.4	—	160.8	—
8a	119.0	—	100.2	—
9	20.3	1.42 d (6.3)	20.8	1.53 d (6.3)
5-OMe/OH	—	8.08 s	60.9	3.74 s
6-OMe	—	—	55.9	3.90 s
7-OH	—	8.54 s	—	—
8-OMe/OH	61.1	3.77 s	—	11.14 s

[a] Recorded at 400 (^{1}H) and 100 (^{13}C) MHz.
[b] Recorded at 500 (^{1}H) and 125 (^{13}C) MHz.

Compound **3** was obtained as a pale yellow solid, and the molecular formula was deduced as $C_{12}H_{14}O_5$ by ESIMS in combination with ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum was similar to that of 5,6-dihydroxymellein[15] except for the presence of two additional methoxy groups (Table 2). The hydroxyl group at C-8 was

assigned on the basis of its chemical shift and the correlations from the hydroxyl proton to C-7/C-8/C-8a in the HMBC spectrum. The attachment of two additional methoxy groups at C-5 and C-6 was established by HMBC correlations from these protons to their corresponding carbons, from H-4 to C-5, and from H-7 to C-5/C-8a. Thus, the structure of compound **3** was determined as 5,6-dimethoxymellein, which was known as a synthetic product[16] but is now isolated for the first time as a natural product. The same absolute configuration of compound **3** with (3*R*)-5,6-dihydroxymellein[15] was indicated by the almost identical optical rotations and CD spectra of these two compounds.

Compound **4** was assigned the molecular formula $C_{13}H_{18}O_5$ on the basis of ESIMS, in combination with ^{13}C NMR spectroscopy. The ^1H NMR spectrum indicated the presence of three methyl groups, one methoxy group, two oxymethine protons, two vinyl protons, and two hydroxyl protons. The HMBC correlations from H_3-12 to C-2/C-3/C-4, from the methoxy protons (H_3-13) to C-4, and from H-5 to C-3/C-4/C-6 established the α-pyrone substituted by the methyl and methoxy group at C-3 and C-4, respectively. The side chain at C-6 was deduced by the COSY correlations from H_3-14 to H-8, H-8 to H-9, H-9 to H-10, and H-10 to H_3-11 together with HMBC correlations from H-5 to C-7, H-8 to C-6/C-14, and H_3-14 to C-6/C-7/C-8. The attachment of the hydroxyl groups at C-9 and C-10 were indicated by their chemical shifts. The cross peak between H-9 and H_3-14 in the NOESY spectrum led to the proposal of *E*-configuration at the 7,8-double bond. The magnitude of the allylic coupling between H-8 and H_3-14 (J^4=1.1 Hz), which was the same value as that reported for pestalopyrone,[17] also supported this assignment. Compound **4** was, thus, assigned to be astropyrone (Fig. 1). However, the absolute configurations at C-9 and C-10 were not determined due to the shortage of the sample.

Compound **5** was obtained as a pale yellow solid. The ESIMS, in combination with ^{13}C NMR spectroscopy, gave the molecular formula as $C_{16}H_{14}O_7$. ^1H NMR spectrum was similar to that of moniliphenone[3] except the methyl signal at δ_H 2.19 of moniliphenone was replaced by oxymethylene signal at δ_H 4.52. ^1H and ^{13}C NMR assignments were secured by the analyses of 2D NMR spectroscopic data. The HMBC spectrum showed correlations from H-3 to C-1/C-5/C-8, H-4 to C-2/C-6, H-5 to C-1/C-3, methoxy protons to C-8, H-3′ to C-1′/C-5′/C-7′, H-5′ to C-1′/C-3′/C-7′ and H-7′ to C-3′/C-5′. The attachment of hydroxyl groups at C-6, C-2′, and C-6′ were indicated on the basis of their chemical shifts. Therefore, compound **5**, named as astrophenone, was depicted as shown (Fig. 1). The benzophenone derivatives are proposed to be the intermediates in the biosynthesis of fungal xanthones.[18,19] Astrophenone, thus, was identified as a precursor in the biosynthesis of the isolated sydowinin A (**9**). Although compound **5** is commercially available,[20] it is first reported to be isolated from natural source.

The structure of nine known compounds were elucidated on the basis of HRMS and NMR spectroscopic data, which were identical in all respects to those of 3,4-dihydro-4,5,8-trihydroxy-3-methylisocoumarin (**6**),[3] sclerotinin A (**7**),[4] methyl-8-hydroxy-6-methylxanthone-1-carboxylate (**8**),[5] sydowinin A (**9**),[6] conioxanthone A (**10**),[7] 1,3,8-trihydroxy-6-methylxanthone (**11**),[8] 1,8-dihydroxy-3-methoxy-6-methylxanthone (**12**),[9] coniochaetone B,[10] and xylaranol B.[11]

All isolated compounds were subjected to biological assays for antibacterial activity against *B. cereus* and cytotoxicity to three cancer cell lines, KB, MCF-7, and NCI-H187 and non-cancer Vero cells. Compound **1** was weakly active to *B. cereus* (IC_{50} 12.5 μg/mL). Compound **3** exhibited cytotoxic activity against KB and Vero cells with IC_{50} 22.6 and 48.2 μg/mL, respectively. 1,3,8-Trihydroxy-6-methylxanthone displayed both antibacterial, against *B. cereus*, and cytotoxic activities, against MCF-7, BC, and Vero cells, with IC_{50} 12.5, 44.76, 14.026 and 88.3 μg/mL, respectively. It would be noted that the bioactivity of the crude extract against *B. cereus* was more potent than that of any of the compounds isolated. This observation could result from the amount of the active compounds being too small to be detected during the separation process.

3. Conclusion

In conclusion, five new compounds, phthalide **1**, dihydroisocoumarin **2**, naturally new dihydroisocoumarin **3**, pyrone **4**, and synthetically known benzophenone **5**, together with nine known compounds, 3,4-dihydro-4,5,8-trihydroxy-3-methylisocoumarin, sclerotinin A, methyl-8-hydroxy-6-methylxanthone-1-carboxylate, sydowinin A, conioxanthone A, 1,3,8-trihydroxy-6-methylxanthone, 1,8-dihydroxy-3-methoxy-6-methylxanthone, coniochaetone B, and xylaranol B, were isolated from the fungus *Astrocystis* sp. BCC 22166. Compounds **1** and **3** showed weak antibacterial and cytotoxic activities, respectively.

4. Experimental

4.1. General procedures

Melting points were measured using a BUCHI M565 melting point apparatus and are uncorrected. Optical rotation measurements were obtained using a JASCO P-1030 polarimeter. The CD spectrum was recorded on a JASCO J-180 spectropolarimeter. UV and FT-IR spectra were recorded on a Spekol 1200, Analytic Jena, spectrophotometer and a Bruker Alpha spectrometer. NMR spectra were recorded on Bruker AV400 and Bruker AV500D spectrometers. ESITOF MS data were obtained on a Bruker MicrOTOF mass spectrometer. Preparative thin layer chromatography was performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel 60 (70–230 mesh ASTM, Merck). HPLC were performed using Dionex-Ultimate 3000 series equipped with a binary pump, an autosampler, and a diode array detector.

4.2. Fungal material

The fungus *Astrocystis* sp. was isolated from a mangrove palm, Nypa, at Hat Khanom-Mu Ko Thale Tai National Park, Nakhon Si Thammarat Province, Thailand. The specimen was identified by Mr. Jones E.B.G., BIOTEC. This fungus was deposited at the BIOTEC Culture Collection Laboratory (BCC) as BCC 22166.

4.3. Fermentation and isolation

Astrocystis sp. BCC 22166 was maintained on potato dextrose agar at 25 °C, 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 PDB liquid medium and fermentation was carried out under shaking conditions at 200 rpm, 25 °C for 20 days.

After filtration of the mycelium, the culture broth was extracted with EtOAc (3×10 L) and evaporated to dryness leaving a dark brown gum (2.464 g). The crude extract was fractionated using Sephadex LH 20 (3.5×50 cm), eluted with 100% MeOH, to provide four fractions (1–4). Fraction 2 was subjected to silica gel column chromatography (5.5×18 cm), step gradient elution with 1–20% MeOH/CH_2Cl_2, to give eight fractions (2-1–2-8). Compound **3** (3.0 mg), methyl-8-hydroxy-6-methylxanthone-1-carboxylate

(0.5 mg), and 3,4-dihydro-4,5,8-trihydroxy-3-methylisocoumarin (12.4 mg) were obtained from fractions 2-2, 2-3, and 2-8, respectively. Purification of fraction 2-4 by silica gel column chromatography, using 30% EtOAc/*n*-hexane as eluent, followed by preparative HPLC using reverse phase column (SunFire C_{18} OBD, 5 μm, 19×150 mm, step gradient elution with 45–60% MeCN/H_2O) furnished compound **1** (1.6 mg), sclerotinin A (4.0 mg), and coniochaetone B (40.1 mg). Fraction 2-5 was subjected to silica gel column chromatography (4.0×18 cm), using 45% EtOAc/*n*-hexane as eluent, then was further purified by preparative HPLC (step gradient elution with 20–60% MeCN/H_2O) to yielded compounds **1** (7.5 mg) and **2** (14.8 mg). Compound **4** (4.6 mg), 1,8-dihydroxy-3-methoxy-6-methylxanthone (2.0 mg), and sydowinin A (4.2 mg) were provided from fraction 2-6 after consecutive purification by silica gel column chromatography (using 60% EtOAc/*n*-hexane as eluent) and preparative HPLC (step gradient elution with 10–40% MeCN/H_2O). Fractions 2-7 was further purified by preparative HPLC (step gradient elution with 20–50% MeCN/H_2O) to afford compound **5** (4.2 mg) and xylaranol B (34.5 mg). Fraction 3 was subjected to silica gel column chromatography, using 5% MeOH/CH_2Cl_2 as an eluent, to give six fractions (3-1–3-6). After further purification by preparative HPLC (step gradient elution with 20–40% MeCN/H_2O), coniochaetone B (6.5 mg) and conioxanthone A (7.6 mg) were obtained from fractions 3-2 and 3-4, respectively.

The cells were macerated in MeOH (1 L) for 3 days and then in CH_2Cl_2 (1 L) for 3 days. The MeOH and the 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). Purification of the crude EtOAc extracted (0.585 g) by silica gel column chromatography (4.0×18 cm), step gradient elution with 65% EtOAc/*n*-hexane–10% MeOH/EtOAc, followed by preparative thin layer chromatography provided compound **2** (2.6 mg), methyl-8-hydroxy-6-methylxanthone-1-carboxylate (2.5 mg), sydowinin A (2.4 mg), and conioxanthone A (8.6 mg). The dark brown oil (0.489 g), obtained from *n*-hexane extraction of the mycelium, was subjected to silica gel column chromatography step gradient elution with 10% EtOAc/*n*-hexane–100% EtOAc, then was further purified by preparative thin layer chromatography to yield methyl-8-hydroxy-6-methylxanthone-1-carboxylate (20.9 mg), 1,3,8-trihydroxy-6-methylxanthone (2.0 mg), and coniochaetone B (4.3 mg).

*4.3.1. Compound **1**.* White solid; mp 149.5–151.5 °C; $[\alpha]_D^{26}$ +127.6 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.10), 250 (3.79), 305 (3.40) nm; CD (MeOH) $\Delta\varepsilon$ (nm) −3.79 (228), +5.05 (242), −3.36 (260), +1.31 (273), −0.51 (299); IR (ATR) ν_{max} 3372, 2921, 2851, 1752, 1680, 1616, 1593, 1446, 1353, 1307, 1250, 1220, 1133, 1090 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data in acetone-d_6, Table 1; HRMS (ESITOF) *m*/*z* 275.0529 $[M+Na]^+$ (calcd for: $C_{12}H_{12}O_6Na$, 275.0526).

*4.3.2. Compound **2**.* Pale yellow solid; mp 110.0–112.0 °C; $[\alpha]_D^{26}$ −132.9 (*c* 0.19, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 223 (3.77), 257 (3.64), 335 (3.50) nm; CD (EtOH) $\Delta\varepsilon$ (nm) −10.77 (212), +1.02 (244), −4.10 (267), +0.27 (292); IR (ATR) ν_{max} 3431, 3222, 2924, 2851, 1681, 1597, 1498, 1459, 1342, 1274, 1241, 1193, 1050 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data in acetone-d_6, Table 2; HRMS (ESITOF) *m*/*z* 247.0579 $[M+Na]^+$ (calcd for: $C_{11}H_{12}O_5Na$, 247.0577).

*4.3.3. Compound **3**.* Pale yellow solid; mp 131.5–132.5 °C; $[\alpha]_D^{26}$ −35.2 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (3.65), 264 (3.61), 312 (3.38) nm; CD (EtOH) $\Delta\varepsilon$ (nm) −4.39 (238), +3.13 (254), −2.15 (270), +1.25 (289); IR (ATR) ν_{max} 2926, 2853, 1667, 1625, 1494, 1438, 1376, 1338, 1311, 1283, 1249, 1204, 1168, 1098 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data in $CDCl_3$, Table 2; HRMS (ESITOF) *m*/*z* 239.0919 $[M+H]^+$ (calcd for: $C_{12}H_{15}O_5$, 239.0914).

*4.3.4. Compound **4**.* White solid; $[\alpha]_D^{25}$ +6.6 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 227 (3.69), 324 (3.43) nm; IR (ATR) ν_{max} 3377, 2930, 2872, 1681, 1617, 1554, 1459, 1384, 1359, 1246, 1169, 1071 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6) δ 1.10 (3H, d, *J*=6.0 Hz, CH_3-11), 1.84 (3H, s, CH_3-12), 2.0 (3H, d, *J*=1.1 Hz, CH_3-14), 3.78–3.81 (2H, m, H-10, OH-10), 3.96 (1H, s, OH-9), 4.0 (3H, s, OCH_3-13), 4.34 (1H, dd, *J*=4.9, 8.7 Hz, H-9), 6.51 (1H, dd, *J*=0.9, 8.7 Hz, H-8), 6.53 (1H, s, H-5); ^{13}C NMR (100 MHz, acetone-d_6) δ 8.2 (C-12), 12.5 (C-14), 18.3 (C-11), 56.3 (OCH_3-13), 70.4 (C-10), 72.5 (C-9), 93.4 (C-5), 101.6 (C-3), 128.6 (C-7), 133.9 (C-8), 159.5 (C-6), 163.7 (C-2), 166.1 (C-4); HRMS (ESITOF) *m*/*z* 277.1045 $[M+Na]^+$ (calcd for: $C_{13}H_{18}O_5Na$, 277.1046).

*4.3.5. Compound **5**.* Pale yellow solid; UV (MeOH) λ_{max} (log ε) 218 (4.08), 284 (3.93), 355 (3.39) nm; IR (ATR) ν_{max} 3241, 2930, 2875, 1714, 1633, 1599, 1492, 1433, 1375, 1298, 1021 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6) δ 3.69 (3H, s, OCH_3-8), 4.52 (2H, s, H-7′), 6.38 (2H, s, H-3′, H-5′), 7.12 (1H, d, *J*=7.9 Hz, H-5), 7.28 (1H, t, *J*=7.9 Hz, H-4), 7.47 (1H, d, *J*=7.9 Hz, H-3); ^{13}C NMR (100 MHz, acetone-d_6) δ 51.5 (OCH_3-8), 63.4 (C-7′), 104.9 (C-3′, C-5′), 110.4 (C-1′), 120.1 (C-5), 120.8 (C-3), 128.5 (C-2), 128.8 (C-4), 133.7 (C-1), 152.2 (C-4′), 153.7 (C-6), 162.3 (C-2′, C-6′), 166.2 (C-8), 200.4 (C-7); HRMS (ESITOF) *m*/*z* 341.0631 $[M+Na]^+$ (calcd for: $C_{16}H_{14}O_7Na$, 341.0632).

4.4. Preparation of the bis-MTPA ester 1a and 1b

Compound **1** (2.8 mg) was treated with (*R*)-MTPACl (20 μL) in CH_2Cl_2 (0.2 mL) and pyridine (0.2 mL) at room temperature for 16 h. The mixture was diluted with EtOAc and washed with H_2O, and the organic layer was evaporated under reduced pressure. The residue was purified by preparative thin layer chromatography (45% EtOAc/*n*-hexane) to obtained bis-(*S*)-MTPA ester **1a** (4.2 mg). Similarly, bis-(*R*)-MTPA ester **1b** was prepared from compound **1** (2.1 mg) and (*S*)-MTPACl (20 μL) and was obtained in 3.2 mg after purification by preparative thin layer chromatography.

*4.4.1. Bis-(S)-MTPA ester **1a**.* ^1H NMR (400 MHz, acetone-d_6) δ 1.04 (3H, d, *J*=6.6 Hz, H-9), 3.60 (3H, s, OCH_3 of MTPA), 3.76 (3H, s, OCH_3 of MTPA), 3.93 (3H, s, OCH_3-11), 5.91 (1H, dq, *J*=2.0, 6.6 Hz, H-8), 6.17 (1H, d, *J*=2.0 Hz, H-3), 7.50 (3H, m, ArH of MTPA), 7.60 (5H, m, ArH of MTPA), 7.64 (1H, d, *J*=8.3 Hz, H-6), 7.75 (2H, m, ArH of MTPA), 8.19 (1H, d, *J*=8.3 Hz, H-5); HRMS (ESITOF) *m*/*z* 707.1324 $[M+Na]^+$ (calcd for: $C_{32}H_{26}O_{10}F_6Na$, 707.1322).

*4.4.2. Bis-(S)-MTPA ester **1b**.* ^1H NMR (400 MHz, acetone-d_6) δ 0.93 (3H, d, *J*=6.6 Hz, H-9), 3.66 (3H, s, OCH_3 of MTPA), 3.76 (3H, s, OCH_3 of MTPA), 3.92 (3H, s, OCH_3-11), 5.91 (1H, dq, *J*=2.0, 6.6 Hz, H-8), 6.34 (1H, d, *J*=2.0 Hz, H-3), 7.50 (3H, m, ArH of MTPA), 7.59 (5H, m, ArH of MTPA), 7.61 (1H, d, *J*=8.3 Hz, H-6), 7.75 (2H, m, ArH of MTPA), 8.21 (1H, d, *J*=8.3 Hz, H-5); HRMS (ESITOF) *m*/*z* 707.1320 $[M+Na]^+$ (calcd for: $C_{32}H_{26}O_{10}F_6Na$, 707.1322).

4.5. Biological assays

Cytotoxicity to Vero cells (African green monkey kidney fibroblasts) were performed using the green fluorescent protein (GFP)-based method[21] and the standard compound, ellipticine, showed IC_{50} value of 0.73 μg/mL. Anticancer activities against KB cells (oral human epidermoid carcinoma), MCF-7 cells (human breast cancer), and NCI-H187 cells (human small-cell lung cancer) and antibacterial activity to *B. cereus* were evaluated using the resazurin microplate assay.[22] The IC_{50} values of standard compound, doxorubicin, against KB, MCF-7, and NCI-H187 cells were 0.50, 8.09, and 0.16 μg/

mL, respectively. Vancomycin, was used as a standard compound for antibacterial against *B. cereus* (IC_{50} value 2.0 μg/mL).

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Supplementary data

Supplementary data associated with this article can be found. These data include NMR spectra of the most important compounds described in the article. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.02.004.

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