SUPPLEMENTARY MATERIAL

Xanthones from a lignicolous freshwater fungus (BCC 28210)

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ABSTRACT
Four xanthones (1–4) and a known compound, mansonone D (5), were isolated from the lignicolous freshwater fungus BCC 28210 (family, Chaetosphaeriaceae). The structures of these compounds were elucidated by extensive spectroscopic analysis. Among the isolated metabolites, compound 2 and the known mansonone D (5) displayed antimalarial activity against Plasmodium falciparum K1 with IC$_{50}$ values of 7.75 and 0.55 μg/mL, respectively. Compound 4 displayed antibacterial activity against Bacillus cereus with an MIC value of 6.25 μg/mL.

KEYWORDS
Xanthone; Freshwater fungus; Chaetosphaeriaceae
Content

Experimental Section

Table S1. NMR spectroscopic data [\(^1\)H (500 MHz) and \(^{13}\)C (125 MHz)] of 1, 4 in acetone-\(d_6\), and 2, 3 in methanol-\(d_4\).

Figure S1. \(^1\)H NMR spectrum of 1 in acetone-\(d_6\).
Figure S2. \(^{13}\)C NMR spectrum of 1 in acetone-\(d_6\).
Figure S3. COSY spectrum of 1 in acetone-\(d_6\).
Figure S4. NOESY spectrum of 1 in acetone-\(d_6\).
Figure S5. HMBC spectrum of 1 in acetone-\(d_6\).

Figure S6. \(^1\)H NMR spectrum of 2 in methanol-\(d_4\).
Figure S7. \(^{13}\)C NMR spectrum of 2 in methanol-\(d_4\).
Figure S8. COSY spectrum of 2 in methanol-\(d_4\).
Figure S9. NOESY spectrum of 2 in methanol-\(d_4\).
Figure S10. Expanded NOESY spectrum of 2 in methanol-\(d_4\).
Figure S11. HMBC spectrum of 2 in methanol-\(d_4\).

Figure S12. \(^1\)H NMR spectrum of 3 in methanol-\(d_4\).
Figure S13. \(^{13}\)C NMR spectrum of 3 in methanol-\(d_4\).
Figure S14. COSY spectrum of 3 in methanol-\(d_4\).
Figure S15. NOESY spectrum of 3 in methanol-\(d_4\).
Figure S16. Expanded NOESY spectrum of 3 in methanol-\(d_4\).
Figure S17. HMBC spectrum of 3 in methanol-\(d_4\).

Figure S18. \(^1\)H NMR spectrum of 4 in acetone-\(d_6\).
Figure S19. \(^{13}\)C NMR spectrum of 4 in acetone-\(d_6\).
Figure S20. COSY spectrum of 4 in acetone-\(d_6\).
Figure S21. NOESY spectrum of 4 in acetone-\(d_6\).
Figure S22. HMBC spectrum of 4 in acetone-\(d_6\).
Experimental

General experimental procedures

Melting points were measured on a MP90 Mettler Toledo or an Electrothermal IA9100 digital melting point apparatus. Optical rotations were determined using a JASCO P-1030 digital polarimeter. UV and IR spectra were taken on Analytik Jena SPEKOL 1200 spectrophotometer and a Bruker Alpha-E FTIR spectrometer, respectively. NMR spectra were taken on a Bruker AVANCE III 400 MHz and a Bruker AVANCE III HD 500 MHz NMR spectrometers. ESI-TOF mass spectra were recorded on a Bruker micrOTOF mass spectrometer.

Fungal material

The fungus was isolated by Dr. Somsak Sivichai from a submerged wood, collected from Tai Rom Yen National Park, Surat Thani province, Thailand. It was deposited at the BIOTEC Culture Collection as BCC 28210 on November 7, 2007. Total genomic DNA was extracted from actively growing mycelia on PDA. The partial large subunit (LSU) and ITS1-5.8S-ITS2 rDNA regions were PCR amplified, sequenced by Macrogen Inc, Korea, and subsequently analyzed using BLAST (Altschul et al. 1990). The LSU data showed 96-97% gene similarity with their closest relatives in Genbank, belonging to family Chaetosphaeriaceae (order Chaetosphaeriales, class Sordariomycetes). The ITS sequence showed 91% similarity with Pyrigemmula aurantiaca CBS 126743 and P. aurantiaca CBS 126744 of the family Chaetosphaeriaceae (Magyar et al. 2011). However, due to limitation of existing database in the genus Pyrigemmula (which contained only two isolates as described), and taxonomic uncertainty in the family Chaetosphaeriaceae, the fungus BCC 28210 was therefore unable to identified into genus level, and classified only as a member of this family. The fungus sequences were submitted to GenBank with accession numbers as MH250050 (LSU) and MH251907 (ITS).

Fermentation, extraction, and isolation

The fungal strain was fermented in liquid medium composing of malt extract 6 g, maltose 1.8 g, dextrose 6 g, and yeast extract 1.2 g in 1 L of distilled water. After incubation at 25 °C for 26 days on a rotary shaker (200 rpm), the whole culture (10 L) was filtered to separate broth and mycelia. The culture broth was extracted three times with an equal volume of EtOAc.
The organic layer was concentrated under reduced pressure to obtain a brown gum (3.3 g). The crude extract was fractionated by Sephadex LH-20 column chromatography (elution with 100% MeOH) to provide 10 fractions (1–10). Fraction 4 (1.1 g) was subjected to silica gel column chromatography (elution with 0–10% MeOH in CH₂Cl₂) to afford 14 fractions (4-1–4-14). Fraction 4-4 (50.6 mg) was purified by reversed-phase HPLC (SunFire C18 column, 19 × 150 mm, 5 μm; flow rate 10 mL/min) using a linear gradient of MeCN:H₂O (50:50→60:40) over 25 min to yield 5 (21.9 mg, t_R 12.0 min).

The mycelia cakes were macerated in MeOH (1 L, 2 days) and filtered. To the filtrate was defatted with hexane (3 × 800 mL). The MeOH layer was concentrated under reduced pressure, and the residue was dissolved in EtOAc (700 mL), then washed with H₂O (400 mL). The EtOAc layer was concentrated under reduced pressure to provide a dark brown gum (1.6 g). The extract was subsequently fractionated by silica gel column chromatography (elution with 0–30% acetone in CH₂Cl₂) to afford 20 fractions (1–20). Fraction 13 (70.4 mg) was purified by reversed-phase HPLC (SunFire C18 column, 19 × 250 mm, 10 µm; flow rate 15 mL/min) using a linear gradient of MeCN:H₂O (50:50→60:40) over 25 min to provide 3 (6.7 mg, t_R 5.0 min), 2 (7.5 mg, t_R 6.3 min), 4 (4.2 mg, t_R 7.4 min), and 1 (6.7 mg, t_R 7.9 min).

3-(1,4-dihydroxy-4-methylpentyl)-1,2,8-trihydroxy-9H-xanthen-9-one (1)

Yellow powder; melting point: 174–176 °C; [α]_D^27 +31.7 (c 0.10, MeOH); UV (MeOH) λ_max (log ε) 240 (4.38), 263 (4.42), 301 (4.08), 335 (4.00), 407 (3.75) nm; IR (ATR, film, acetone) ν_max 3218, 1630, 1599, 1484, 1446, 1280, 1234, 1033, 697 cm⁻¹; ^1H and ^13C NMR data, see Table S1; HRMS (ESI-TOF) m/z 361.1248 [M + H]⁺ (Calcd for C₁₉H₂₁O₇, 361.1282).

1,2,8-trihydroxy-3-(1R*,3S*,4-trihydroxy-4-methylpentyl)-9H-xanthen-9-one (2)

Yellow powder; melting point: 161–163 °C; [α]_D^27 +29.7 (c 0.10, MeOH); UV (MeOH) λ_max (log ε) 240 (4.44), 263 (4.48), 302 (4.12), 335 (4.04), 407 (3.75) nm; IR (ATR, film, acetone) ν_max 3232, 1655, 1631, 1601, 1488, 1447, 1289, 1230, 1035, 1085, 811 cm⁻¹; ^1H and ^13C NMR data, see Table S1; HRMS (ESI-TOF) m/z 399.1082 [M + Na]⁺ (Calcd for C₁₉H₂₀NaO₈, 399.1050).

1,2,8-trihydroxy-3-(1R*,3R*,4-trihydroxy-4-methylpentyl)-9H-xanthen-9-one (3)
Yellow powder; melting point: 162–164 °C; \([\alpha]_{D}^{24} +75.4 \ (c \ 0.05, \text{MeOH}), \) UV (MeOH) \(\lambda_{\text{max}}\) (log \(\varepsilon\)) 239 (3.79), 263 (3.81), 302 (3.53), 334 (3.45), 400 (3.23) nm; IR (ATR, neat) \(\nu_{\text{max}}\) 3283, 1657, 1637, 1604, 1491, 1292, 1054, 708 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR data, see Table S1; HRMS (ESI-TOF) \(m/z\) 399.1051 [M + Na]\(^+\) (Calcd for C\(_{19}\)H\(_{20}\)NaO\(_8\), 399.1050).

\((E)-3-(1,4\text{-dihydroxy-4-methylpent-2-enyl})-1,2,8\text{-trihydroxy-9H-xanthen-9-one (4)}\)

Yellow powder; melting point: 172–174 °C; \([\alpha]_{D}^{28} +26.3 \ (c \ 0.025, \text{MeOH}), \) UV (MeOH) \(\lambda_{\text{max}}\) (log \(\varepsilon\)) 241 (4.38), 263 (4.41), 302 (4.09), 334 (4.01), 408 (3.75) nm; IR (ATR, film, acetone) \(\nu_{\text{max}}\) 3247, 1654, 1631, 1600, 1486, 1449, 1268, 1229, 707 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR data, see Table S1; HRMS (ESI-TOF) \(m/z\) 357.1003 [M – H]\(^-\) (Calcd for C\(_{19}\)H\(_{17}\)O\(_7\), 357.0980).

**Biological assays**

Growth inhibition of *Plasmodium falciparum* (K1, multi-drug resistant strain) was assessed using the microculture radioisotope technique (Desjardins et al. 1979). Antibacterial activity against *Bacillus cereus* (Sarker et al. 2007), and antifungal activity against *Candida albicans* and *Curvularia lunata* (O’Brien et al. 2000) were evaluated using resazurin microplate assay. Minimum concentration required to inhibit 90% of growth of *B. cereus* (MIC\(_{90}\)) was applied. Green fluorescent protein-based assay was used to measure cytotoxicity against African green monkey kidney fibroblast (Vero) cells (Hunt et al. 1999).

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Table S1. NMR spectroscopic data $[^1]H$ (500 MHz) and $^{13}C$ (125 MHz) of 1, 4 in acetone-$d_6$, and 2, 3 in methanol-$d_4$.

| position | 1 | 2 | 3 | 4 |
|----------|---|---|---|---|
|          | $\Delta H$, mult. ($J$ in Hz) | $\Delta C$ | $\Delta H$, mult. ($J$ in Hz) | $\Delta C$ | $\Delta H$, mult. ($J$ in Hz) | $\Delta C$ | $\Delta H$, mult. ($J$ in Hz) | $\Delta C$ |
| 1        | 146.3 | 146.4 | 147.7 | 146.5 |
| 2        | 136.9 | 136.8 | 138.0 | 137.2 |
| 3        | 144.1 | 142.5 | 145.0 | 142.1 |
| 4        | 7.15, s | 7.09, s | 7.12, s | 7.13, s |
| 4a       | 148.9 | 149.0 | 150.3 | 148.9 |
| 5        | 6.99, dd (8.4, 0.7) | 6.91, d (8.4) | 6.94, d (8.4) | 7.00, d (8.4) |
| 6        | 7.72, dd (8.4, 8.4) | 7.62, dd (8.4, 8.2) | 7.64, dd (8.4, 8.2) | 7.73, dd (8.4, 8.2) |
| 7        | 6.77, dd (8.4, 0.7) | 6.72, d (8.2) | 6.74, d (8.2) | 6.78, d (8.2) |
| 8        | 161.3 | 161.0 | 162.4 | 161.3 |
| 8a       | 107.3 | 107.1 | 108.5 | 107.3 |
| 9        | 186.2 | 186.0 | 187.4 | 186.2 |
| 9a       | 106.3 | 106.4 | 107.7 | 106.5 |
| 10a      | 156.7 | 157.0 | 158.1 | 156.8 |
| 1'       | 5.16, br m | 5.34, dd (8.0, 5.0) | 5.40, dd (9.0, 2.7) | 5.69, br m |
| 2'       | 1.98, m; 1.81, m | 2.18, dddd (14.0, 5.0, 2.1); 1.71, dddd (14.0, 10.0, 8.0) | 1.92, dddd (14.1, 9.0, 1.5); 1.84, dddd (14.1, 10.4, 2.7) | 5.90, dd (15.5, 5.5) |
| 3'       | 1.72, dddd (13.5, 10.8, 5.2); 1.60, dddd (13.5, 10.6, 5.3) | 3.56, dd (10.0, 2.1) | 3.70, dd (10.4, 1.5) | 6.01, dd (15.5, 1.2) |
| 4'       | 69.2 | 72.1 | 73.6 | 69.2 |
| 5', 6'   | 1.17, s | 1.16, s | 1.17, s | 1.23, s |
| 5'       | 1.17, s | 1.14, s | 1.15, s | 2 × 29.5 |
| 1'-OH    | 11.55, br s | 11.59, br s |
| 2'-OH    | 8.30, br s | 8.26, br s |
| 8'-OH    | 11.82, s | 11.82, s |
| 1'-OH    | 4.95, br m | 4.89, br m |
| 4'-OH    | 3.57, br s | 3.60, br s |
Figure S1. $^1$H NMR spectrum of 1 in acetone-$d_6$. 
Figure S2. $^{13}$C NMR spectrum of 1 in acetone-$d_6$. 
Figure S3. COSY spectrum of 1 in acetone-$d_6$. 
Figure S4. NOESY spectrum of 1 in acetone-$d_6$. 
Figure S5. HMBC spectrum of 1 in acetone-$d_6$. 
Figure S6. $^1$H NMR spectrum of 2 in methanol-$d_4$. 
Figure S7. $^{13}$C NMR spectrum of 2 in methanol-$d_4$. 
Figure S8. COSY spectrum of 2 in methanol-$d_4$. 
Figure S9. NOESY spectrum of 2 in methanol-\textit{d}_4.
Figure S10. Expanded NOESY spectrum of 2 in methanol-$d_4$. 
Figure S11. HMBC spectrum of 2 in methanol-$d_4$. 
Figure S12. $^1$H NMR spectrum of 3 in methanol-$d_4$. 
Figure S13. $^{13}$C NMR spectrum of 3 in methanol-$d_4$. 
Figure S14. COSY spectrum of 3 in methanol-$d_4$. 
Figure S15. NOESY spectrum of 3 in methanol-$d_4$. 
Figure S16. Expanded NOESY spectrum of 3 in methanol-$d_4$. 
Figure S17. HMBC spectrum of 3 in methanol-$d_4$. 
Figure S18. $^1$H NMR spectrum of 4 in acetone-$d_6$. 
Figure S19. $^{13}$C NMR spectrum of 4 in acetone-$d_6$. 
Figure S20. COSY spectrum of 4 in acetone-$d_6$. 
Figure S21. NOESY spectrum of 4 in acetone-$d_6$. 
Figure S22. HMBC spectrum of 4 in acetone-$d_6$. 

(Figure and text)