Supporting Information

Meroterpenes with Toll-Like Receptor 3 Regulating Activity from Endophytic Fungus *Guignardia mangiferae*

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Correspondence

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Part 1 Experimental section

General experimental procedures
Optical rotations were recorded on a Rudolph Research Analytical Autopol IV automatic polarimeter. IR spectra were recorded in KBr disks on a Nexus 870 FT-IR spectrometer, and UV curves on a Hitachi U-3000 spectrophotometer. Mass spectra were acquired on an Agilent 6210 TOF LC-MS instrument equipped with an electrospray ionization (ESI) probe operating in the positive ion mode with direct infusion. With TMS and solvent signals adopted as internal standards, all NMR experiments were accomplished in CDCl$_3$ (unless stated otherwise) on a Bruker DRX-500 spectrometer with $^1$H and $^{13}$C nuclei observed at 500 and 125 MHz, respectively. Semipreparative RP-HPLC (reverse-phase high-performance liquid chromatography) were accomplished on ODS-2 Hypersil columns (5µ, 250 × 10 mm). Silica gel (SiO$_2$, 200–300 mesh) for CC (column chromatography) and GF254 (10–20 mm) for TLC (thin-layer chromatography) were produced by Qingdao Marine Chemical Company, China. ODS-A GEL (AA12S50) was purchased from YMC Co., Ltd., Japan, and Sephadex LH-20 from Pharmacia Biotech, Sweden. All chemicals used in the study were of analytical grade.

Fungal material
The title strain was isolated from the leaf of *Ilex cornuta* collected in December 2007 from the back hill of Nanjing Forestry University, China, and identified as *Guignardia mangiferae* (Dothideaceae) by Dr. Y. C. Song. The strain of *Guignardia mangiferae* was deposited in the Institute of Functional Biomolecules, Nanjing University, Nanjing, China. The collected plants of *Ilex cornuta* were identified by Dr. Y. C. Song, with a voucher specimen preserved under the number NJ 07-12-15 in the herbarium of Nanjing University.

Spectral data of 4, 5, and 9
Guignarenone C (4)
Colorless crystals, m.p. 98 – 104°C; [α] D 20 = +63 (c 0.28, MeOH); UV/Vis (MeOH): λ max (log ε) = 263 nm (7.11); IR ν max (KBr): 3404, 2970, 2933, 2909, 1666, 1618, 1454, 1364, 1329, 1298, 1246, 1170, 1154, 1041, 1015 cm⁻¹; HRESIMS: m/z: 315.1565 [M + Na]⁺ (calcd. for C17H24O4Na⁺, 315.1567); ¹H and ¹³C NMR data are listed in Tables 2S and 3S.

Guignardone G (5)
Brown oil, [α] D 20 = +45 (c 0.405, MeOH); UV/Vis (MeOH): λ max (log ε) = 264 nm (7.09); IR ν max (KBr): 3407, 2967, 2932, 1620, 1444, 1378, 1326, 1303, 1286, 1237, 1167, 1152, 1099, 1059, 1002. HRESIMS: m/z: 301.1418 [M + Na]⁺ (calcd. for C16H22O4Na⁺, 301.1410); ¹H and ¹³C NMR data are listed in Tables 2S and 3S.

Guignardone F (9)
Colorless crystals, m.p. 133°C; [α] D 20 = −95 (c 0.465, MeOH); UV/Vis (MeOH): λ max (log ε) = 268 nm (7.58); IR ν max (KBr): 3539, 3436, 2976, 2946, 2884, 1644, 1592, 1388, 1337, 1244, 1234, 1166, 1128, 1091, 1014 cm⁻¹; HRESIMS: m/z: 331.1512 [M + Na]⁺ (calcd. for C17H24O5Na⁺, 331.1516); ¹H and ¹³C NMR data are listed in Tables 2S and 3S.

Preparation of 4-O-benzoylguignardone K (2a): Compound 2 (5.0 mg) was treated with 5 equivalent weight benzoyl chloride (13.0 mg) in dry pyridine for 24 h at room temperature. Then the crude product was dried with N₂ and purified by RP-HPLC (MeOH/H₂O = 85/15) to give 2a (3.5 mg). A colorless gum; [α] D 20 = +189.2 (c 0.065, MeOH); UV/Vis (MeOH): λ max (log ε) = 264 nm (4.19), 230 nm (4.16); IR ν max (KBr): 2946, 2833, 1745, 1633, 1450, 1420, 1116, 1035, 669; H RESIMS: m/z: 419.1824 [M + Na]⁺ (calcd. for C24H28O5Na⁺, 419.1829); ¹H NMR data is listed in Table 1S.

Preparation of 2,4-O-dibenzoylguignardone G (5a): Compound 5 (2.5 mg) was treated with 10 equivalent weight benzoyl chloride (65.0 mg) in dry pyridine for 24 h at room temperature. Then the crude product was dried with N₂ and purified by RP-HPLC (MeOH/H₂O = 90/10) to give 5a (2.0 mg). A white amorphous powder; [α] D 20 = +121.9 (c 0.064, MeOH); UV/Vis (MeOH): λ max (log ε) = 268 nm (4.19), 230 nm (4.16); IR ν max (KBr): 2946, 2833, 1745, 1633, 1450, 1420, 1116, 1035, 669; H RESIMS: m/z: 509.1930 [M + Na]⁺ (calcd. for C30H30O6Na⁺, 509.1935); ¹H NMR data is listed in Table 1S.

X-ray crystallographic diffractions
Colorless crystals of 1, 3, 4, and 9 were obtained by crystallization from a solution of MeOH, and the diffraction measurements were performed at 100 K on an Agilent SuperNova diffractometer equipped with Cu–Kα radiation (λ = 1.54184 Å). The structures were solved by direct methods (SHELXS-97) and refined using full-matrix least squares difference Fourier techniques. The absolute configuration was determined by Bijvoet analysis [1]. CCDC numbers 943116–943119 contain the supplementary crystallographic data for this article, which has been deposited in the Cambridge Crystallographic Data Centre [available free of charge at
Guignardone J (1): C_{17}H_{24}O_{5}, M = 308.36, orthorhombic, \( a = 7.4792 \) Å, \( b = 11.5083 \) Å, \( c = 17.7401 \) Å, \( \alpha = \beta = \gamma = 90.00^\circ \), \( V = 1526.94 \) Å\(^3\), \( T = 100 \) K, space group \( P2(1)2(1)2(1) \), \( Z = 4 \), 11302 reflections measured, 3189 independent reflections (\( R_{int} = 0.0288 \)). The final \( R_1 \) value was 0.0320 (\( I > 2\sigma(I) \)). The final \( wR(F^2) \) value was 0.0827 (\( I > 2\sigma(I) \)). The final \( R_1 \) value was 0.0339 (all data). The final \( wR(F^2) \) value was 0.0847 (all data). CCDC number: 943118.

Guignardone L (3): C_{17}H_{24}O_{4}, M = 292.36, monoclinic, \( a = 7.1820 \) Å, \( b = 16.6934 \) Å, \( c = 12.8483 \) Å, \( \alpha = \gamma = 90.00^\circ \), \( \beta = 93.137(3)^\circ \), \( V = 1538.10 \) Å\(^3\), \( T = 100 \) K, space group \( P2(1) \), \( Z = 4 \), 14165 reflections measured, 5364 independent reflections (\( R_{int} = 0.0311 \)). The final \( R_1 \) value was 0.0700 (\( I > 2\sigma(I) \)). The final \( wR(F^2) \) value was 0.1943 (\( I > 2\sigma(I) \)). The final \( R_1 \) value was 0.0716 (all data). The final \( wR(F^2) \) value was 0.1953 (all data). CCDC number: 943117.

Guignarenone C (4): C_{17}H_{24}O_{4}, M = 292.36, orthorhombic, \( a = 7.3990 \) Å, \( b = 7.6684 \) Å, \( c = 27.0059 \) Å, \( \alpha = \beta = \gamma = 90.00^\circ \), \( V = 11352 \) Å\(^3\), \( T = 100 \) K, space group \( P2(1)2(1)2(1) \), \( Z = 4 \), 3197 independent reflections (\( R_{int} = 0.0164 \)). The final \( R_1 \) value was 0.0263 (\( I > 2\sigma(I) \)). The final \( wR(F^2) \) value was 0.0708 (\( I > 2\sigma(I) \)). The final \( R_1 \) value was 0.0267 (all data). The final \( wR(F^2) \) value was 0.0712 (all data). CCDC number: 943116.

Guignardone F (9): C_{17}H_{24}O_{5}, M = 308.36, prism, \( a = 9.7009 \) Å, \( b = 9.7320 \) Å, \( c = 16.7379 \) Å, \( \alpha = \beta = \gamma = 90.00^\circ \), \( V = 1580.21 \) Å\(^3\), \( T = 100 \) K, space group \( P2(1)2(1)2(1) \), \( Z = 4 \), 11700 reflections measured, 3290 independent reflections (\( R_{int} = 0.0168 \)). The final \( R_1 \) value was 0.0288 (\( I > 2\sigma(I) \)). The final \( wR(F^2) \) value was 0.0787 (\( I > 2\sigma(I) \)). The final \( R_1 \) value was 0.0290 (all data). The final \( wR(F^2) \) value was 0.0789 (all data). CCDC number: 943119.

Bioassay

The purity of the tested compounds was determined to be over 95% by using the HPLC-DAD. The positive control, (R)-2-(3-Chloro-6-fluorobenzo[b]thiophene-2-carboxamido)-3-phenylpropanoic acid (TLR3 inhibitor) was purchased from Merck Millipore Co.; its purity was over 99% by HPLC.

Part 2 Tables 1S–4S

Table 1S: \(^1\)H (500 MHz) NMR data of 4-O-benzoylguignardone K (2a) and 2,4-O-dibenzoylguignardone G (5a) (\( J \) in Hz) in CDCl\(_3\).
Table 2S $^1$H (500 MHz) NMR data of guignarenone C (4), guignardone G (5), and F (9) ($J$ in Hz) in CDCl$_3$.

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$^a$Signals observed at $\delta = 8.03$ (2H), $\delta = 7.60$ (1H), $\delta = 7.47$ (2H) attributed to aromatic protons.

$^b$Signals observed at $\delta = 8.06$ (4H), $\delta = 7.59$ (2H), $\delta = 7.46$ (4H) attributed to aromatic protons.
Table 3S $^{13}$C (125 MHz) NMR data of guignarenone C (4), guignardone G (5), and F (9) in CDCl$_3$.

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Part 3 Figs. 1S—27S

Fig. 1S $^1$H NMR spectrum of guignardone J (1) (CDCl$_3$, 500 MHz).

Fig. 2S $^{13}$C NMR spectrum of guignardone J (1) (CDCl$_3$, 125 MHz).

Fig. 3S HSQC spectrum of guignardone J (1) (CDCl$_3$, 500 MHz).

Fig. 4S HMBC spectrum of guignardone J (1) (CDCl$_3$, 500 MHz).

Fig. 5S $^1$H-$^1$H COSY spectrum of guignardone J (1) (CDCl$_3$, 500 MHz).

Fig. 6S $^1$H NMR spectrum of guignardone K (2) (CDCl$_3$, 500 MHz).

Fig. 7S $^{13}$C NMR spectrum of guignardone K (2) (CDCl$_3$, 125 MHz).

Fig. 8S HSQC spectrum of guignardone K (2) (CDCl$_3$, 500 MHz).

Fig. 9S HMBC spectrum of guignardone K (2) (CDCl$_3$, 500 MHz).

Fig. 10S $^1$H-$^1$H COSY spectrum of guignardone K (2) (CDCl$_3$, 500 MHz).
Fig. 11S $^1$H NMR spectrum of 4-O-benzoylguignardone K (2a) (CDCl$_3$, 500 MHz).
Fig. 12S $^1$H NMR spectrum of guignardone L (3) (CDCl$_3$, 500 MHz).
Fig. 13S $^{13}$C NMR spectrum of guignardone L (3) (CDCl$_3$, 125 MHz).
Fig. 14S HSQC spectrum of guignardone L (3) (CDCl$_3$, 500 MHz).
Fig. 15S HMBC spectrum of guignardone L (3) (CDCl$_3$, 500 MHz).
Fig. 16S $^1$H-$^1$H COSY spectrum of guignardone L (3) (CDCl$_3$, 500 MHz).
Fig. 17S $^1$H NMR spectrum of guignardone G (5) (CDCl$_3$, 500 MHz).
Fig. 18S $^{13}$C NMR spectrum of guignardone G (5) (CDCl$_3$, 125 MHz).
Fig. 19S HSQC spectrum of guignardone G (5) (CDCl$_3$, 500 MHz).
Fig. 20S HMBC spectrum of guignardone G (5) (CDCl$_3$, 500 MHz).
Fig. 21S $^1$H-$^1$H COSY spectrum of guignardone G (5) (CDCl$_3$, 500 MHz).
Fig. 22S $^1$H NMR spectrum of 2,4-O-dibenzoylguignardone G (5a) (CDCl$_3$, 500 MHz).
Fig. 23S $^1$H NMR spectrum of guignardone F (9) (CDCl$_3$, 500 MHz).
Fig. 24S $^{13}$C NMR spectrum of guignardone F (9) (CDCl$_3$, 125 MHz).
Fig. 25S HSQC spectrum of guignardone F (9) (CDCl$_3$, 500 MHz).
Fig. 26S HMBC spectrum of guignardone F (9) (CDCl$_3$, 500 MHz).
Fig. 27S $^1$H-$^1$H COSY spectrum of guignardone F (9) (CDCl$_3$, 500 MHz).

References

Fig. 1S $^1$H NMR spectrum of 1.
Fig. 2S 13C NMR spectrum of 1.
Fig. 3S HSQC spectrum of 1.
Fig. 4S HMBC spectrum of 1.
Fig. 5S $^1$H-$^1$H COSY spectrum of 1.
Fig. 6S $^1$H NMR spectrum of 2.
Fig. 7S $^{13}$C NMR spectrum of 2.
Fig. 8S HMQC spectrum of 2.
Fig. 9S HMBC spectrum of 2.
Fig. 10S $^1$H-$^1$H COSY spectrum of 2.
Fig. 11S \(^1\)H NMR spectrum of 2a.
Fig. 12S $^1$H NMR spectrum of 3.
Fig. 13S $^{13}$C NMR spectrum of 3.
Fig. 14S HMQC spectrum of 3.
Fig. 15S HMBC spectrum of 3.
Fig. 16 $^1$H–$^1$H COSY spectrum of 3.
Fig. 17S $^1$H NMR spectrum of $5$. 
Fig. 18S $^{13}$C NMR spectrum of 5.
Fig. 19S HMQC spectrum of 5.
Fig. 20S HMBC spectrum of 5.
Fig. 21S $^1$H-$^1$H COSY spectrum of 5.
Fig. 22S $^1$H NMR spectrum of 5a.
Fig. 23S $^1$H NMR spectrum of 9.
Fig. 24 $^{13}$C NMR spectrum of 9.
Fig. 25S HSQC spectrum of 9.
Fig. 26S HMBC spectrum of 9.
Fig. 27S $^1$H-$^1$H COSY spectrum of 9.