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Bioactive Metabolites from Cultures of Basidiomycete *Favolaschia tonkinensis*

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Two strobilurins, 9-methoxystrobilurin B (**1**) and 9-methoxystrobilurin G (**2**), two monochlorinated 2,3-dihydro-1-benzoxepin derivatives, **3** and **4a**, and butenolide **5**, together with four known compounds, strobilurin B, 9-methoxystrobilurin A, and oudemansins A and B, were isolated from culture BCC 18689 of the fungus *Favolaschia tonkinensis*. 9-Methoxystrobilurins A, B (**1**), and G (**2**) and oudemansins A and B exhibited antimalarial, antifungal, and cytotoxic activities, while compounds **3**, **4a**, and **5** displayed only cytotoxic activity.

Fungi in the Basidiomycetes class are regarded as an important source of biologically active secondary metabolites including antifungal, antiviral, antimicrobial, and antitumor compounds. For example, pterulone and pterulinic acid (from *Pterula* species),[1] drosophilin A (from *Psathyrella* species),[2] chlorinated orcinols (from *Hericium erinaceum*),[3] laschiatrion (from *Favolaschia* species),[4] and strobilurins and oudemansins (from the genera *Strobilurus*, *Xerula*, *Oudemansiella*, and *Favolaschia*)[5−8] exhibit potent antifungal activity. As a part of our research program on novel bioactive secondary metabolites,[9] we investigated the basidiomycete fungus *Favolaschia tonkinensis* BCC 18689, of which the crude extract exhibited strong antifungal activity against *Candida albicans* (IC_{50} value of 0.03 μg/mL). Chemical investigation of BCC 18689 subsequently led to the isolation and structural elucidation of five new compounds, 9-methoxystrobilurin B (**1**), 9-methoxystrobilurin G (**2**), monochlorinated 2,3-dihydro-1-benzoxepin derivatives **3** and **4a**, and butenolide **5**, together with four known compounds, strobilurin B,[5,10,11] 9-methoxystrobilurin A,[12] and (−)-oudemansins A[7] and B.[6] Biological activities of these compounds were also investigated.

Compounds **3**, **5**, 9-methoxystrobilurin A, and oudemansins A and B were obtained from both culture broth and mycelia extracts of BCC 18689. Compound **4a** was obtained from the culture broth extract, while compounds **1**, **2**, and strobilurin B were isolated solely from an *n*-hexane extract of the mycelium.

Compound **1** was obtained as a yellow oil. The molecular formula was established by HRMS (ESITOF), in combination with ^{13}C NMR spectroscopy, as $C_{18}H_{21}O_5Cl$. The IR spectrum showed an absorption band at 1708 cm^{-1} for a carbonyl group. The ^1H NMR spectrum showed signals for a methyl and four methoxy groups, one methine singlet, the three protons of a 1,2,4-trisubstituted benzene ring and an isolated *trans* double bond. The NMR data indicated that compound **1** was closely related to strobilurin B[5,10] except for the presence of an additional methoxy signal at δ_H 3.68. The HMBC correlations from H-16 to C-13, H-15 to C-12, H-12 to C-10/C-11/C-13, and H-14 to C-11 indicated the presence of a methyl β-methoxyacrylate moiety connected to C-10 at the α-position of the carbonyl carbon. Locations of the methoxy group at C-2 and the *trans* double bond at C-6 of the aromatic ring were established by the HMBC correlations from H-18 to C-2 and from H-7 to C-1/C-5/C-6, respectively. A chlorine group was assigned to C-3 on the basis of its chemical shift. The presence of methoxy and methyl groups at C-9 and C-10, respectively, was indicated by the HMBC correlations between H-17 and H-14 and their corresponding carbons (C-9 and C-10). The linkage of the isolated *trans* double bond to C-9 was established on the basis of an HMBC correlation from H-8 to C-9. The *E*-configurations of the C-9−C-10 and C-11−C-12 olefinic bonds were established by the presence of a cross-peak between the methoxy (H-17) and the methyl protons (H-14) in the NOESY spectrum and also based on the chemical shift of H-12 as compared to the data previously reported for other strobilurins.[11,12] Therefore, compound **1** was determined as the new 9-methoxystrobilurin B (**1**).

Compound **2** was obtained as a yellow oil. Its molecular formula was determined as $C_{27}H_{36}O_7$ on the basis of HRMS (ESITOF), in combination with ^{13}C NMR data. Analysis of the NMR data revealed the structural fragments of a 9-methoxystrobilurin side chain, 1,2,4-trisubstituted benzene ring, and two oxygen-linked isoprene units. Comparison of the NMR data of the 9-methoxystrobilurin substructure with those of the relevant fragment in compound **1** showed close agreement, thus confirming the presence of the indicated side chain in compound **2**. The first isoprene unit was assigned by correlation between the vinylic H-24 and methylene protons H-23 in the COSY spectrum and the correlations from Hb-23 to C-25 along with the correlations from methyl protons H-26 and H-27 to C-24 and C-25 in the HMBC spectrum. Similarly, the second isoprene unit was determined by the COSY correlation between H-19 and H-18 as well as the correlations from H-18 and methyl protons H-21 and H-22 to C-20 in the HMBC spectrum. The presence of the cross-peaks between H-21 and Ha-18 and between H-22 and H-19 in the NOESY spectrum also supported the assignment of this isoprene unit. The oxygen atoms were suggested to attach to the isoprene units according to the chemical shifts of C-18, C-20, and C-23. The linkage of two isoprene units was established by the HMBC correlations from H-19 to C-23 and Ha-23 to C-19. The HMBC correlation from Ha-18 to C-3 and the NOESY correlation between H-22 and H-1 indicated the attachment of the isoprene unit to C-2 and C-3 of the aromatic ring. The NMR data of the 1,5-benzodioxepin moiety were consistent with those reported for the relevant fragment of strobilurin G.[11] Therefore, compound **2** was assigned as the new 9-methoxystrobilurin G (**2**).[13]

Compound **3** was obtained as a white solid, and the molecular formula was deduced as $C_{12}H_{10}O_2NCl$ by HRMS (ESITOF). The 3:1 ratio of $[M]^+$ and $[M + 2]^+$ observed in the ESI mass spectrum indicated the presence of a chlorine atom in the molecule. The ^1H NMR data of compound **3** were similar to those of pterulone.[1] The IR spectrum showed characteristic absorption bands of an amide at 1680, 3197, and 3424 cm^{-1}. The location of the amide group at C-7 was determined by the HMBC correlations from H-6 and H-8 to C-11 of the amide group. The presence of a dihydrobenzoxepin

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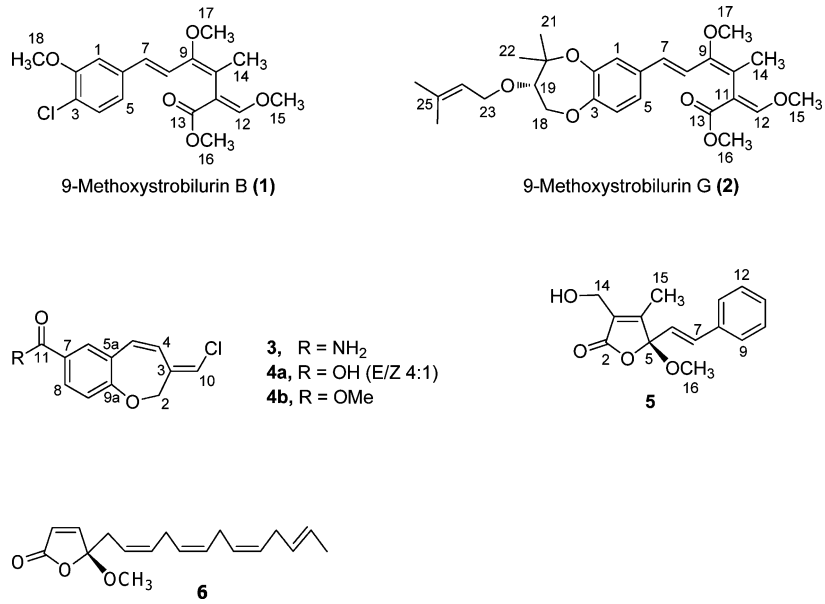

Figure 1. Structures of compounds **1**−**6**.

ring was established on the basis of the chemical shift and the COSY correlation between H-4 and H-5 as well as the HMBC correlations from H-5 to C-3/C-4/C-6/C-9a, from H-2 to C-3/C-4/C-9a, and from H-6 to C-5. The chemical shift of C-10 indicated the presence of the chlorine atom. The connection of alkene C-10 to C-3 was assigned by the HMBC correlations from H-4 to C-10 and H-10 to C-3. The NOESY correlation between H-10 and H-2 established the *E*-configuration of the olefinic bond.

Compound **4a** was obtained as a white solid. The HRMS spectrum of compound **4a** indicated its molecular formula of $C_{12}H_9O_3Cl$. The ^{1}H NMR data of compound **4a** were closely related to those of compound **3**. Analysis of ^{13}C and 2D NMR spectra also revealed similar structures for these two compounds. Compound **4a** was obtained as a 4:1 mixture of two inseparable *E*/*Z* isomers, according to the integration in the ^{1}H NMR spectrum. However, the NMR signals of these two isomers were clearly resolved, and thus their structures could be determined independently. The presence of a carboxylic acid group was established on the basis of the molecular formula and the IR absorptions at 3450 and 1686 cm^{-1}. The correlations from H-6 and H-8 to the carbonyl carbon C-11 in the HMBC spectrum indicated the position of the carboxylic acid moiety at C-7. In order to confirm the presence of the carboxylic group, transformation of **4a** to its methyl ester **4b** was performed. The ^{1}H NMR spectrum of **4b** was similar to that of **4a** except for the presence of an additional methyl ester signal at δ_H 3.87 (and 3.89 for the *Z* isomer). Both compounds **4a** and **4b** are synthetically known,[14,15] but the former is now isolated and reported for the first time from natural sources.

Compound **5** was obtained as a yellow oil. The molecular formula was deduced from HRMS (ESITOF) in combination with ^{13}C NMR data as $C_{15}H_{16}O_4$. The ^{1}H NMR spectrum showed the pattern of a monosubstituted benzene ring, one isolated *trans* double bond, one methylene, one methyl, and one methoxy group. The ^{13}C NMR chemical shift and the absorption band at 1759 cm^{-1} in the IR spectrum indicated the presence of a γ-lactone ring. The HMBC correlations from the allylic oxymethylene protons H-14 to C-2/C-3/C-4, the methyl proton H-15 to C-3/C-4/C-5, and the methoxy proton H-16 to C-5 established the location of these substituents, respectively, at C-3, C-4, and C-5 of the lactone moiety. The connection of the isolated *trans* double bond to the benzene and γ-lactone rings at C-8 and C-5, respectively, was established on the basis of the correlations from H-13 and H-9 to C-7, H-7 to C-13/C-5, and H-6 to C-5/C-8 in the HMBC spectrum. The remaining hydroxy group was placed at C-14 according to the chemical shift. The absolute configuration of **5** could be deduced by the comparison of both the optical rotation and the CD spectrum with those of the known γ-methoxy-γ-alkyl-disubstituted butenolide **6**.[16] The positive specific rotation of compound **5** ($[\alpha]_D$ +3.7) was related to the positive specific rotation of butenolide **6** ($[\alpha]_D$ +14). Furthermore, the CD spectrum of **5** showed a strong positive Cotton effect at 214 nm (a slight red shift when compared to **6** possibly due to 2,3-dialkyl substitution),[17,18] corresponding to the $\pi \rightarrow \pi^*$ transition of the 2(5*H*)-furanone moiety. This Cotton effect was similar to that found in compound **6** and other 5,5-disubstitued-2(5*H*)-furanones possessing an *S*-configuration.[16,18,19] Accordingly, a 5*S*-configuration could be assigned and the structure of compound **5** shown in Figure 1 defined.

The structures of the four known compounds were elucidated on the basis of HRMS and NMR spectroscopic data, which were identical to those of strobilurin B,[5,10,11] 9-methoxystrobilurin A,[12] and (−)-oudemansins A[7] and B.[6]

Compounds **1**−**5**, 9-methoxystrobilurin A, and oudemansins A and B were subjected to biological assays for antifungal activity (*Candida albicans*), antiplasmodial activity (*Plasmodium falciparum* K1), and cytotoxicity against KB, MCF-7, NCI-H187, and Vero cells. Strobilurin B was not tested due to paucity of the sample. 9-Methoxystrobilurins A, B, and G and oudemansins A and B exhibited moderate to strong antimalarial (IC_{50} 0.03−3.0 μg/mL) and antifungal (IC_{50} 0.06−0.89 μg/mL) activities. The strong bioactivity of the strobilurins and oudemansins was also observed in other naturally occurring (*E*)-β-methoxyacrylates.[20,21] Plausibly, the presence of an (*E*)-β-methoxyacrylate moiety, which could inhibit mitochondrial respiration, might be responsible for its activity. Accordingly, a number of strobilurins served as lead compounds for the development of effective agriculture fungicides.[20−22] Compounds **3**, **4a**, and **5** were inactive against both *P. falciparum* and *C. albicans*.

Most of these compounds showed weak to moderate cytotoxicity against all tested cell lines (IC_{50} 0.40−48.71 μg/mL). Oudemansin A was inactive against MCF-7 cells. Compound **3** displayed cytotoxic activity against KB cells with an IC_{50} value of 12.15 μg/mL, while compound **4a** exhibited cytotoxicity against both KB and NCI-H187 cells with IC_{50} values of 0.78 and 11.66 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were measured using an Electrothermal IA9100 digital melting point apparatus and

Table 1. Biological Activities of Compounds **1**−**5**, 9-Methoxystrobilurin A, and Oudemansins A and B

compound	antimalarial, IC_{50} (μg/mL)	antifungal, IC_{50} (μg/mL)	cytotoxicity, IC_{50} (μg/mL) KB cells	MCF-7 cells	NCI-H187 cells	Vero cells
9-methoxystrobilurin B (**1**)	0.30	0.22	5.45	4.86	0.40	7.91
9-methoxystrobilurin G (**2**)	0.03	0.50	14.32	15.41	0.36	23.98
3	>10	>50	12.15	>50	>50	>50
4a	>10	>50	0.78	>50	11.66	>50
5	>10	>50	23.89	48.71	27.30	48.44
9-methoxystrobilurin A	0.39	0.06	1.39	12.42	1.57	21.28
(−)-oudemansin A[a]	3.00	0.76	5.48	>50	15.10	39.19
(−)-oudemansin B[a]	0.40	0.89	0.73	27.84	0.02	14.27
dihydroartemisinin[b]	0.0011					
amphotericin B[c]		0.07				
doxorubicin[d]			0.25	0.57	0.04	
ellipticine[d]			0.45		0.68	1.18

[a] Antimicrobial activities of oudemansins A and B against other organisms using a different method were previously reported.[6,7] [b] Antimalarial control. [c] Antifungal control. [d] Cytotoxicity controls.

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 spectrum was recorded on a JASCO J-180 spectropolarimeter. NMR spectra were recorded on a Bruker AV500D spectrometer. ESITOF MS data were obtained on a Micromass LCT and a Bruker micrOTOF mass spectrometer.

Fungal Material. The fungus *Favolaschia tonkinensis* (Pat.) Singer was collected from a bamboo stem, planted at the Bamboo Plantation, Ban Noen Sung Penitentiary, Prachin Buri Province, Thailand. The specimen was identified by Rattaket Choeyklin, BIOTEC. This fungus was deposited in the BIOTEC Culture Collection (BCC) as BCC 18689 on November 3, 2005.

Fermentation and Isolation. *F. tonkinensis* BCC 18689 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 a 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 21 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 (1.02 g). The crude extract was fractionated using a silica gel column (3 × 20 cm), gradient elution with 0 to 40% EtOAc/*n*-hexane, to provide seven fractions (A-1−A-7). Fraction A-2 was further purified by preparative thin-layer chromatography (60% EtOAc/*n*-hexane) to give 9-methoxystrobilurin A (1.8 mg), oudemansin A (8.3 mg), and oudemansin B (4.7 mg). The combined A-4 and A-5 fractions was subjected to preparative TLC to give pure compound **5** (6.0 mg). Fraction A-6 contained compounds **3** and **4a**, in which compound **3** was precipitated from MeOH as a white solid (6.3 mg). The residue was passed through a Sephadex LH-20 column with MeOH as eluent to yield compound **4a** (1.2 mg).

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 crude *n*-hexane extract (0.36 g) was fractionated using a silica gel column (1.5 × 15 cm), eluted with 20% EtOAc/*n*-hexane, to provide seven fractions (B-1−B-7). After further purification by silica gel column chromatography, additional amounts of oudemansin A (14.7 mg), 9-methoxystrobilurin A (12.9 mg), oudemansin B (18.9 mg), and compound **3** (2.4 mg) were obtained from fractions B-1, B-3, B-4, and B-7, respectively. Fraction B-2 was subjected to preparative TLC (100% CH_2Cl_2) to afford 9-methoxystrobilurin G, **2** (2.3 mg). Fraction B-5 was further purified by preparative TLC (80% CH_2Cl_2/*n*-hexane) to yield 9-methoxystrobilurin B, **1** (1.2 mg), and strobilurin B (1.6 mg).

The dark brown solid (0.27 g), obtained from EtOAc extraction of the mycelium, was purified over silica gel column chromatography using 30% EtOAc/*n*-hexane as eluent to provide eight fractions (C-1−C-8). Additional amounts of oudemansin A (1.7 mg), oudemansin B (1.6 mg), and compounds **5** (2.5 mg) and **3** (6.7 mg) were obtained from fractions C-1, C-2, C-6, and C-8, respectively.

9-Methoxystrobilurin B (1): yellow oil; UV (MeOH) λ_{max} (log ε) 228 (5.15), 306 (5.07) nm; IR (KBr) ν_{max} 2936, 2851, 1708, 1627, 1492, 1463, 1408, 1381, 1253, 1195, 1127, 1066, 1029 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.92 (3H, s, 14-CH_3), 3.68 (3H, s, 17-OMe), 3.73 (3H, s, 16-OMe), 3.83 (3H, s, 15-OMe), 3.92 (3H, s, 18-OMe), 6.48 (1H, d, J = 15.9 Hz, H-8), 6.66 (1H, d, J = 15.9 Hz, H-7), 6.88 (1H, d, J = 1.8 Hz, ArH-1), 6.94 (1H, dd, J = 1.8, 8.2 Hz, ArH-5), 7.27 (1H, d, J = 8.2 Hz, ArH-4), 7.41 (1H, s, H-12); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.4 (C-14), 51.5 (C-16), 56.2 (C-18), 59.5 (C-17), 61.8 (C-15), 110.6 (C-1, 11), 119.2 (C-5), 119.6 (C-10), 121.3 (C-3), 122.3 (C-8), 126.7 (C-7), 130.1 (C-4), 137.6 (C-6), 152.5 (C-9), 155.0 (C-2), 159.3 (C-12), 168.0 (C-13); HRMS (ESITOF) *m*/*z* 375.0968 $[M + Na]^+$ (calcd for $C_{18}H_{21}O_5ClNa$, 375.0970).

9-Methoxystrobilurin G (2): yellow oil; $[\alpha]^{24}_D$ +11.40 (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (4.33), 303 (4.27) nm; IR (KBr) ν_{max} 2935, 1708, 1626, 1569, 1502, 1435, 1264, 1195, 1125, 1070, 1023 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.22 (3H, s, 21-CH_3), 1.46 (3H, s, 22-CH_3), 1.68 (3H, s 26-CH_3), 1.75 (3H, s, 27-CH_3), 1.89 (3H, s, 14-CH_3), 3.50 (1H, dd, J = 3.2, 7.8 Hz, H-19), 3.65 (3H, s, 17-CH_3), 3.71 (3H, s, 16-CH_3), 3.81 (3H, s, 15-CH_3), 3.95 (1H, dd, J = 7.8, 12.5 Hz, Ha-18), 4.06 (1H, dd, J = 6.9, 11.6 Hz, Ha-23), 4.15 (1H, dd, J = 6.9, 11.6 Hz, Hb-23), 4.23 (1H, dd, J = 3.2, 12.5 Hz, Hb-18), 5.34 (1H, brt, J = 6.9 Hz, H-24), 6.38 (1H, d, J = 15.9 Hz, H-8), 6.58 (1H, d, J = 15.9 Hz, H-7), 6.86 (1H, d, J = 8.6 Hz, ArH-4), 6.95 (1H, dd, J = 2.1, 8.6 Hz, ArH-5), 6.97 (1H, d, J = 2.1 Hz, ArH-1), 7.39 (1H, s, H-12); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.3 (C-14), 18.1 (C-26), 20.8 (C-21), 25.8 (C-27), 27.7 (C-22), 51.9 (C-16), 59.4 (C-17), 61.8 (C-15), 68.8 (C-18), 67.4 (C-23), 80.7 (C-20), 82.0 (C-19), 110.7 (C-11), 118.2 (C-10), 120.6 (C-4, 8), 120.9 (C-24), 121.9 (C-1), 122.7 (C-5), 126.8 (C-7), 133.3 (C-6), 137.4 (C-25), 146.9 (C-2), 150.9 (C-3), 152.7 (C-9), 159.3 (C-12), 168.1 (C-13); HRMS (ESITOF) *m*/*z* 473.2541 $[M + H]^+$ (calcd for $C_{27}H_{36}O_7$+H, 473.2534).

Compound 3: white solid; UV (MeOH) λ_{max} (log ε) 251 (4.01), 297 (3.65) nm; IR (KBr) ν_{max} 3496, 3424, 3197, 3071, 2924, 1680, 1604, 1493, 1453, 1434, 1405, 1381, 1362, 1332, 1267, 1139, 1111, 1004, 779 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 4.68 (2H, s, H-2), 6.48 (1H, s, H-10), 6.62 (1H, br s, NH), 6.70 (1H, d, J = 12 Hz, H-5), 6.85 (1H, d, J = 12 Hz, H-4), 7.03 (1H, d, J = 8.0 Hz, H-9), 7.47 (1H, br s, NH), 7.80 (1H, dd, J = 1.5, 8.4 Hz, H-8), 7.97 (1H, s, H-6); ^{13}C NMR (125 MHz, acetone-d_6) δ 72.0 (C-2), 119.6 (C-10), 119.7 (C-9), 124.3 (C-4), 126.5 (C-5a), 128.7 (C-8), 129.3 (C-7), 131.0 (C-5), 133.3 (C-6), 136.5 (C-3), 161.7 (C-9a), 167.5 (C-11); HRMS (ESITOF) *m*/*z* 236.0474 $[M + H]^+$ (calcd for $C_{12}H_{10}O_2NCl$+H, 236.0478).

Compound 4a: white solid; UV (MeOH) λ_{max} (log ε) 248 (4.09), 298 (3.80) nm; IR (KBr) ν_{max} 3450, 2922, 1686, 1605, 1421, 1333, 1300, 1251, 1110, 1070, 1023 cm^{-1}; (***E***)-**7a**: ^1H NMR (500 MHz, $CDCl_3$) δ 4.61 (1H, s, H-2), 6.19 (1H, s, H-10), 6.60 (1H, d, J = 11.6 Hz, H-5), 6.88 (1H, d, J = 11.6 Hz, H-4), 7.06 (1H, d, J = 8.5 Hz, H-9), 7.92 (1H, dd, J = 2.1, 8.5 Hz, H-8), 8.06 (1H, d, J = 2.1 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 72.5 (C-2), 120.4 (C-10), 120.5 (C-9), 123.9 (C-7), 124.9 (C-4), 126.7 (C-5a), 130.5 (C-5), 131.1 (C-8), 135.7 (C-3), 136.1 (C-6), 163.5 (C-9a), 169.2 (C-11); (***Z***)-**7a**: ^1H

NMR (500 MHz, $CDCl_3$) δ 4.91 (1H, s, H-2), 6.39 (1H, s, H-10), 6.41 (2H, s, H-4, 5), 7.09 (1H, d, $J = 8.4$ Hz, H-9), 7.90 (1H, dd, $J = 2.1$, 8.4 Hz, H-8), 8.01 (1H, d, $J = 2.1$ Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 68.1 (C-2), 120.6 (C-9), 121.4 (C-10), 122.9 (C-7), 124.1 (C-5a), 127.6 (C-5), 128.8 (C-4), 130.8 (C-8), 135.2 (C-6), 137.7 (C-3), 163.3 (C-9a), 169.2 (C-11); HRMS (ESITOF) *m/z* 259.0130 $[M + Na]^+$ (calcd for $C_{12}H_9O_3ClNa$, 259.0132).

Compound 5: yellow oil; $[\alpha]^{24}_D$ +3.7 (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.52), 258 (4.30) nm; CD (MeOH) $\Delta\varepsilon$ (nm) +5.47 (214), −1.93 (240), +0.47 (265); IR (KBr) ν_{max} 3420, 2938, 1759, 1686, 1649, 1449, 1234, 1190, 1074, 1027, 957, cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.02 (3H, s, 15-CH_3), 3.35 (3H, s, 16-OMe), 4.42 (2H, d, $J = 2.7$ Hz, H-14), 6.07 (1H, d, $J = 16.0$ Hz, H-6), 6.94 (1H, d, $J = 16.0$ Hz, H-7), 7.34 (3H, m, ArH), 7.40 (2H, d, $J = 7.0$ Hz, ArH-9,13); ^{13}C NMR (125 MHz, $CDCl_3$) δ 10.8 (C-15), 51.4 (C-16), 55.0 (C-14), 108.11 (C-5), 122.5 (C-6), 127.0 (C-9, 13), 127.8 (C-3), 128.8 (C-10, 12), 128.9 (C-11), 134.8 (C-7), 135.2 (C-8), 159.4 (C-4), 171.2 (C-2); HRMS (ESITOF) *m/z* 283.0958 $[M + Na]^+$ (calcd for $C_{15}H_{16}O_4Na$, 283.0941).

Methylation of 4a. Compound **4a** (1.0 mg) was methylated with CH_2N_2 in dioxane (0.2 mL) at room temperature for 12 h. The reaction mixture was evaporated to dryness under vacuum to yield **4b** (1.2 mg).

Biological Assays. Antimalarial activity against *P. falciparum* K1 was evaluated by using the microculture radioisotope technique.[23] 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.[24] Cytotoxicity to Vero cells (African green monkey kidney fibroblasts) was evaluated using the green fluorescent protein (GFP)-based method.[25]

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Supporting Information Available: NMR, mass spectra of compounds **1**, **2**, **3**, **4a**, and **5**, and CD spectrum of compound **5** are available free of charge via the Internet at http://pubs.acs.org.

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(13) Due to the limited availability of the material, the absolute configuration of compound **2** could not be determined. However, the specific rotation as well as UV absorption of compound **2** ($[\alpha]^{24}_D$ +21.4 (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} 227, 303 nm) and those of strobilurin G ($[\alpha]^{24}_D$ +26.8 (*c* 0.8, EtOH); UV (EtOH) λ_{max} 229, 301 nm)[26,27] were closely related and might suggest similar *S*-configurations for both compounds.
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