JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

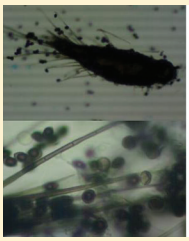
Cytotoxic and Antimalarial Azaphilones from *Chaetomium longirostre*

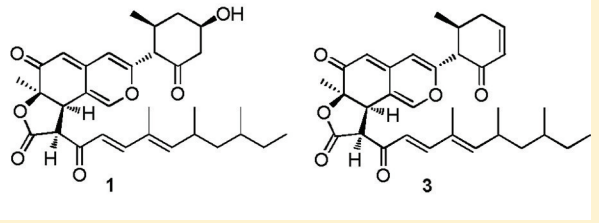
Natcha Panthama,[†] Somdej Kanokmedhakul,*[,†] Kwanjai Kanokmedhakul,[†] and Kasem Soytong[‡]

[†]Natural Products Research Unit, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

[‡]Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok 10520, Thailand

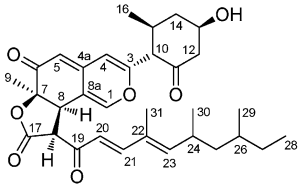
Supporting Information



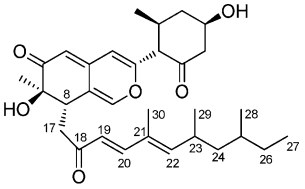


ABSTRACT: Four new azaphilones named longirostrerones A–D (**1**–**4**) and three known sterols, ergosteryl palmitate, ergosterol, and ergosterol peroxide, have been isolated from ethyl acetate extract of the fungus *Chaetomium longirostre*. These structures were determined by 1D and 2D NMR, IR, UV, MS, and CD spectroscopy. Compounds **1**–**4** exhibited strong cytotoxicity against KB cancer cell lines (IC_{50} 0.23–6.38 μM), while only **1** showed potent cytotoxicity against MCF7 and NCI-H187 cell lines (IC_{50} 0.24 and 3.08 μM, respectively). In addition, **1**–**3** showed antimalarial activity against *Plasmodium falciparum* (IC_{50} 0.62–3.73 μM).

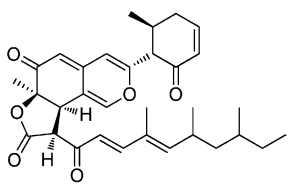
Chaetomium belongs to the family Chaetomiaceae, which is the largest genus of saprophytic ascomycetes. The family includes more than 300 species worldwide, and 24 species have been found in Thailand.[1−3] Previous investigation of secondary metabolites from *Chaetomium* species resulted in the isolation of compounds such as benzoquinone derivatives,[4] tetra-*S*-methyl derivatives,[5] azaphilones,[6−8] bis-azaphilones,[8] indo-3-yl-[13]cytochalasans and chaetogobosin analogues,[9−16] anthraquinone-chromanone,[14] orsellinic acid, globosumones,[17] cheatochalasin A,[18] and depsidones.[18−20] As part of our search for bioactive compounds from fungi isolated from Thai soil, an EtOAc extract of *C. longirostre* showed cytotoxicity against KB, MCF7, and NCIH-187 cell lines with 60.2%, 31.9%, and 22.5% inhibition, respectively, at a concentration of 50 μg/mL. This work will describe the isolation, structural determination, and bioactivities of four new azaphilones, i.e., the longirostrerones A–D (**1**–**4**), and three known sterols from *C. longirostre*.



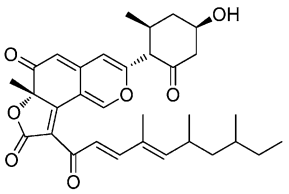
1



2



3



4

RESULTS AND DISCUSSION

The EtOAc extract of dried fungal biomass of *C. longirostre* was fractionated by flash column chromatography on silica gel and preparative TLC to yield four new azaphilones, longirostrerones A–D (**1**–**4**), and three known sterols. The structures of these known compounds were identified by physical and spectroscopic data measurements (IR, ^{1}H and ^{13}C NMR, 2D NMR, and MS) and by comparing the data obtained with published values, as ergosteryl palmitate,[21] ergosterol,[22] and ergosterol epoxide.[23] Azaphilones have previously been reported from *Chaetomium* species such as cheatoviridins A–D, an angular type of azaphilone from *C. globosum* var. Flavo-viridae.[6] The linear type of azaphilones and their biological activity were first reported from *C. cupreum* CC3003.[7] In addition, bis-azaphilones have been reported from *C. cochliodes* VTh01 and *C. cochliodes* CTh05.[8]

Compound **1** was assigned the molecular formula $C_{32}H_{40}O_7$, as deduced from the HRESITOFMS, indicating 13 degrees of unsaturation. The UV spectrum displayed an absorption maximum due to a $\alpha,\beta,\gamma,\delta$-conjugated ketone at 295 and 350 nm.[24] The IR spectrum of **1** showed characteristics of hydroxy (3445 cm^{-1}), lactone (1787 cm^{-1}), ketone (1717 cm^{-1}), and $\alpha,\beta,\gamma,\delta$-conjugated ketone (1636 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** showed the typical pattern of an azaphilone

Received: June 13, 2011
Published: October 17, 2011

dx.doi.org/10.1021/np2004903 | *J. Nat. Prod.* 2011, 74, 2395–2399

skeleton, joined in a nonlinear five-membered ring lactone with three olefinic protons at δ 7.00 (s, H-1), 6.04 (s, H-4), and 5.37 (s, H-5), together with a singlet signal of a methyl group at C-9 (δ 1.42), and two methine protons at δ 3.85 (dd, J = 1.7, 12.9 Hz, H-8) and 4.14 (d, J = 12.9 Hz, H-18), as reported for sassafrin A.[25] Analysis of the 2D NMR spectral data (COSY, HSQC, and HMBC) indicated the presence of two partial structures of **1** connecting to the main azaphilone at C-3 and C-18. The ^{1}H NMR spectrum of the first partial structure of **1** showed signals at δ 2.92 (1H, d, J = 12.0 Hz, H-10), 2.84 (1H, dd, J = 4.6, 13.4 Hz, H-12a), 2.42 (1H, dd, J = 11.7, 13.1 Hz, H-12b), 4.0 (1H, m, H-13), 2.30 (1H, brd, J = 12.3, H-14a), 1.57 (1H, m, H-14b), 2.06 (1H, m, H-15), and 1.04 (3H, d, J = 6.4 Hz, H_3-16), consistent with the 4-hydroxy-6-methyl-2-oxocyclohexyl reported for multiformin D.[26] The COSY spectrum showed correlations of H-10/H-15/H-16 and H-14/H-13/H-12, together with the HMBC correlations of H-10 to C-3, C-11, C-12, C-14, C-15, and C-16, H-16 to C-10, C-14, and C-15, H-15 to C-10, C-11, C-13, C-14, and C-16, and H-12 to C-10, C-13, and C-14, indicating that the cyclohexanone moiety was connected to the azaphilone unit at C-3 (Figure 1). The second

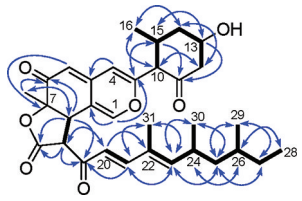


Figure 1. COSY (bold lines) and selected HMBC correlations (H→C) of **1**.

partial structure was identified as 4,6,8-trimethyldecyl-2,4-dienone from the ^{1}H, ^{13}C NMR and 2D NMR spectra (COSY and HMBC). The ^{1}H NMR spectrum showed the presence of *trans* alkene protons at δ 6.46 (H-20) and 7.39 (H-21), 5.84 (H-23), 2.69 (H-24), 1.34 and 1.14 (H_2-25), 1.22 (H-26), 1.27 and 1.14 (H_2-27), 0.83 (H_3-28), 0.85 (H_3-29), 0.98 (H_3-30), and 1.85 (H_3-31). The COSY displayed correlations of H-20/H-21, H-31(allylic)/H-23/H-24/H-25/H-26/H-27/H-28, H-24/H-30, and H-26/H-29 (Figure 1). The HMBC spectrum exhibited the correlations of H-8 to C-17, C-18, and C-19, H-18 to C-8, C-8a, C-17, and C-19, H-20 to C-18, C-19, and C-22, H-21 to C-19, C-20, C-22, C-23, and C-31, H-23 to C-24, C-25, C-30, and C-31, H-24 to C-30, H-25 to C-23, C-26, and C-30, H-26 to C-24, C-28, and C-29, H-27 to C-28, and C-29, and H-28 to C-26 and C-27, indicating the side chain is connected to the core structure azaphalone at C-18. Selected HMBC correlations of **1** are summarized in Figure 1.

The NOESY spectrum of **1** (Figure 2) showed correlations between H-8 and methyl protons (H-9), H-18 and H-8, and H-9, indicating that those protons were located on the same side. In addition, H-10, H-15, and H-13 were all axial, resulting from the NOESY correlation between H-10 and methyl protons (H-16); H-16 and H-14$_{ax}$ (δ 2.30); and H-13 and H-12$_{eq}$ as well as the coupling constant of H-10 (δ 2.92). An attempt to prepare the (*S*)- and (*R*)-MTPA esters of **1** for determining the absolute configuration of C-13 in the cyclohexanone ring by the modified Mosher's method was not successful. The reaction yielded an α,β-unsaturated compound **3**. The assignment of the absolute stereochemistry at C-7 was then concluded to be *R* by CD spectrum. The CD spectrum of **1** showed a negative Cotton effect at 209 nm ($\Delta\varepsilon$ −19.6) and 240 ($\Delta\varepsilon$ −3.0) and a positive one at 350 nm ($\Delta\varepsilon$ 31.8), which are similar patterns to those reported for sassafrins A and B[25] and cohaerin C.[27] From the above evidence, **1** was determined to be 3-(4-hydroxy-6-methyl-2-oxocyclohexyl)-18-(4,6,8-trimethyldeca-2,4-dienoyl)-(7*R*)-7-methyl-8,18-dihydro-7*H*-furo[2,3-*h*]isochromene-6,17-dione, which we named longirostrerone A.

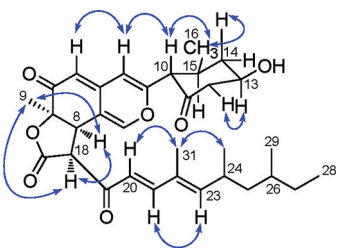


Figure 2. NOESY correlations of **1**.

Compound **2** was shown to have the molecular formula $C_{31}H_{42}O_6$ by means of HRESITOFMS, indicating 11 degrees of unsaturation. The IR spectrum showed characteristics of hydroxyl (3420 cm^{-1}), ketones (1716 cm^{-1}), and conjugated ketone (1617 cm^{-1}) groups. The ^{1}H NMR spectrum of **2** was similar to that of **1**, except for a missing lactone ring and the appearance of signals of a methylene group at $\delta_{H/C}$ 3.40 (H-17a) and 2.83 (H-17b)/36.6. These chemical shifts were comparable to those of the analogue cohaerin F[27] [$\delta_{H/C}$ 3.22 and 2.79/40.2 for H/C-18, and 3.32/40.2 for H/C-8]. However, slight differences in chemical shift between **1** and cohaerin F in the ^{13}C NMR spectra were noted at δ_C 41.0 and 40.2 (for C-8) and at 36.6 and 40.2 (for C-17). This suggested an opposite configuration at C-8 for the two compounds.

The COSY spectrum exhibited correlations between H-17 and H-8, and the HMBC showed correlations of H-17 to C-7, C-8, C-8a, and C-18, which supported the connection of a side chain at C-8. The NOESY spectral data showed correlations between H-10 and H-16; H-16 and H-14$_{ax}$; and H-13 and H-14$_{eq}$, together with the coupling constants of H-10 (d, J = 12.0 Hz), which indicated that H-10, H-15, and H-13 are axial. The absolute stereochemistry at C-7 was deduced to be *R* from the CD spectrum, which revealed the presence of positive (370 and 318 nm) and negative (269 and 209 nm) Cotton effects, as reported for cohaerin F.[27] Thus, **2** was judged to be 7-hydroxy-3-(4-hydroxy-6-methyl-2-oxocyclohexyl)-8-(5,7,9-trimethylundeca-3,5-dienoyl)-(7*R*)-7-methyl-8-hydro-7*H*-furo-[2,3-*h*]isochromene-6,18-dione, which we named longirostrerone B.

Compound **3** was obtained as a yellow solid, and its molecular formula $C_{32}H_{38}O_6$ was assigned from HRESITOFMS, indicating 14 degrees of unsaturation. The UV spectrum displayed an absorption maximum due to an $\alpha,\beta,\gamma,\delta$-conjugated ketone at 296 and 348 nm. The IR spectrum showed absorption bands of hydroxyl (3461 cm^{-1}), lactone (1785 cm^{-1}), and α,β-unsaturated ketone (1646 cm^{-1}) groups. The ^{1}H NMR spectrum of **3** was similar to that of **1**, except for the absence of the hydroxyl group at C-13, which was replaced by the α,β-unsaturated ketone, $\delta_{H/C}$ 6.09 (H-12)/129.1 and 7.03 (H-13)/150.1. The COSY spectrum confirmed the double bond of the cyclohexenone by the correlations of H-10/H-15/H-14/H-13/H-12. The stereochemistry at C-10 and C-15 was assigned to be the same as **1** on the basis of the coupling constant of H-10 (J = 12.4 Hz) and the NOESY correlation between H-10 and the

dx.doi.org/10.1021/np2004903 | *J. Nat. Prod.* 2011, 74, 2395–2399

methyl protons (H-16). The absolute stereochemistry of C-7 was assigned as *R* from the CD spectrum, which showed positive (332 and 365 nm) and negative (240 and 276 nm) Cotton effects.[25,27] On the basis of the above data, the structure of **3** was determined as 3-(6-methyl-2-oxocyclohex-3-enyl)-18-(4,6,8-trimethyldeca-2,4-dienoyl)-(7*R*)-7-methyl-8,18-dihydro-7*H*-furo[2,3-*h*]isochromene-6,17-dione and has been named longirostrerone C.

Compound **4** was obtained as a red solid, and its molecular formula $C_{32}H_{38}O_7$ was deduced from HRESITOFMS, indicating 14 degrees of unsaturation. The IR spectrum showed the presence of hydroxyl (3411 cm^{-1}), lactone (1762 cm^{-1}), and conjugated ketone (1632 cm^{-1}) groups. Compound **4** was similar to **1** but differed in the following ways; a signal of H-1 (δ_H 8.83), which appeared downfield in **4**, and the signals of H-8 and H-18 disappeared from **1** and were replaced by a double bond in **4**. The ^{13}C NMR signals of **4** at C-8 (δ_C, 165.0) and C-18 (δ_C, 124.6) appeared downfield shifted compared to **1**, which confirmed an α,β-unsaturated lactone. The sterochemistry of the cyclohexanone system was similar to that of **1** by comparing the chemical shifts in the NMR spectrum and NOESY correlations of the protons. The absolute configuration of C-7 was concluded as *S* on the basis of the CD spectrum, which revealed the negative (417 and 264 nm) and positive (347 nm) Cotton effects, which were opposite of those of sassafrin C [positive (436 and 277 nm) and negative (386 nm) Cotton effects].[25] Thus, the structure of **4** was determined as 3-(4-hydroxy-6-methyl-2-oxocyclohexyl)-18-(4,6,8-trimethyldeca-2,4-dienoyl)-(7*S*)-7-methyl-7*H*-furo[2,3-*h*]isochromene-6,17-dione, which has been named longirostrerone D.

The new isolated compounds **1**−**4** were tested for their bioactivities, and the results are given in Table 2. Compounds **1**−**4** exhibited cytotoxicity against KB cell lines with IC_{50} values of 1.04, 1.52, 0.23, and 6.38 μM, respectively. Among these, **3** showed significant cytotoxicity against KB (IC_{50} 0.23 μM), which was close to the control drugs, doxorubicine and ellipticine, while compound **1** showed strong cytotoxicity against MCF7 and NCIH-187 cell lines with respective IC_{50} values of 0.24 and 3.08 μM. In addition, compounds **1**, **2**, and **3** showed potent antimalarial activity against *Plasmodium falciparum* with IC_{50} values of 0.63, 3.73, and 0.62 μM, respectively, compared to the control, dihydroartemisinine, which showed an IC_{50} of 0.004 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained using a JASCO DIP-1000 digital polarimeter, and CD spectra were obtained using a JASCO J-810 apparatus. UV spectra were measured on an Agilent 8453 UV−visible spectrophotometer. IR spectra were taken on a Perkin-Elmer Spectrum One spectrophotometer. NMR spectra were recorded in $CDCl_3$ on a Varian Mercury Plus 400 spectrometer, using residual $CHCl_3$ as internal standard. HRESITOFMS were recorded on a Micromass Q-TOF-2 spectrometer. Column chromatography was carried out on Merck silica gel 60 (230−400 mesh). TLC was performed with precoated Merck silica gel 60 PF_{254} aluminum sheets; the spots were visualized under UV light (254 and 366 nm) and further by spraying with anisaldehyde and heating until charred.

Fungal Materials. The fungus was isolated from a pineapple plantation at Prachupkirikhan Province, Thailand, in 2008 and was identified by one of the authors (K.S.). A voucher specimen (Chl01) was deposited at the Department of Plant Production Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The fungus was cultured in conical flasks (1 L, 80 flasks) with potato dextrose broth (200 mL/flask) and incubated stationary at 28−30 °C for 30 days. The culture broth was filtered to give fungal biomass and then air-dried at room temperature.

Extraction and Isolation. Dried fungal biomass of *C. longirostre* (273 g) was ground into powder and extracted successively with EtOAc (3 × 400 mL) and MeOH (3 × 400 mL). Removal of solvents under reduced pressure gave crude EtOAc (11.49 g, 4.2%) and MeOH (31.2 g, 11.4%) extracts. The EtOAc extract (11.0 g) was applied to silica gel flash column chromatography (FCC), eluted with a gradient solvent system of CH_2Cl_2−EtOAc and EtOAc−MeOH, to give eight fractions, F_1−F_8. Fraction F_1 was dissolved with hexane to give ergosteryl palmitate (1.5360 g). Fraction F_2 was further dissolved with hexane to yield ergosterol (53.4 mg). Fraction F_4 was purified by silica gel FCC, eluted with 60% EtOAc−hexane, affording three subfractions, $F_{4.1}$−$F_{4.3}$. Subfraction $F_{4.2}$ was purified by preparative TLC and developed with 60% EtOAc−hexane (×3) to yield compound **2** (10 mg). Subfraction $F_{4.3}$ was purified by preparative TLC, using 60% EtOAc−hexane as eluent (×4), to give ergosterol (23.2 mg). Fraction F_5 was separated by silica gel FCC, eluted with 1% MeOH−CH_2Cl_2, to give two subfractions, $F_{5.1}$ and $F_{5.2}$. Subfraction $F_{5.1}$ was dissolved with hexane to yield ergosterol peroxide (47.8 mg). Fraction F_7 was purified by silica gel FCC, eluted with a gradient system of CH_2Cl_2−MeOH, to obtain three subfractions, $F_{7.1}$−$F_{7.3}$. Subfraction $F_{7.1}$ was purified by preparative TLC and developed three times with 1% MeOH−CH_2Cl_2 to give compound **3** (6.1 mg). Fraction F_8 was applied on silica gel FCC, eluted with 2% MeOH−CH_2Cl_2, to give three fractions, $F_{8.1}$−$F_{8.3}$. Fraction $F_{8.1}$ was separated by PLC and developed with 3% MeOH−CH_2Cl_2 to yield compound **4** (5.0 mg). Fraction $F_{8.2}$ was purified by silica gel FCC, eluted with 1% MeOH−CH_2Cl_2, to give **1** (7.0 mg) and an additional amount of **4** (82.7 mg). Subraction $F_{8.3}$ was separated by silica gel FCC, eluted with 1% MeOH−CH_2Cl_2, to give an additional amount of **1** (110.8 mg) and **4** (53.6 mg).

*Longirostrerone A (**1**):* colorless crystals; $[\alpha]^{22.7}_D$ +387.4 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.8), 295 (4.0), 350 (3.9); CD (MeOH) nm 350 (+31.8), 209 (−19.6); IR (KBr) ν_{max} 3445, 2961, 2926, 1787, 1717, 1636, 1558, 1175, 1109, 1048 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HRESITOFMS *m*/*z* 537.2855 $[M + H]^+$ (calcd for $C_{32}H_{40}O_7$ + H, 537.2852).

*Longirostrerone B (**2**):* red, amorphous solid; $[\alpha]^{25.9}_D$ +341.2 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.4), 285 (4.4), 350 (4.3); CD (MeOH) nm 370 (+7.0), 318 (+8.1), 231 (+0.3), 247 (−2.4), 209 (−5.6); IR (KBr) ν_{max} 3420, 2961, 2927, 1716, 1617, 1170, 1032 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HRESITOFMS *m*/*z* 511.3065 $[M + H]^+$ (calc for $C_{31}H_{42}O_6$ + H, 511.3060).

*Longirostrerone C (**3**):* yellow solid; $[\alpha]^{22.9}_D$ +158.6 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 201 (3.4), 225 (3.3), 296 (3.5), 348 (3.5); CD (MeOH) nm: 365 (+23.1), 332 (+25.1), 276 (−2.7), 240 (−14.2); IR (KBr) ν_{max} 3461, 2961, 2917, 1785, 1646, 1175, 1110, 1049 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HRESITOFMS *m*/*z* 519.2748 $[M + H]^+$ (calcd for $C_{32}H_{38}O_6$ + H, 519.2747).

*Longirostrerone D (**4**):* red solid; $[\alpha]^{22.7}_D$ −846.4 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 (4.2), 270 (4.3), 341 (4.4); CD (MeOH) nm: 417 (−34.6), 347 (+49.7), 264 (−43.9); IR (KBr) ν_{max} 3411, 2960, 2925, 1762, 1632, 1173, 1106, 1033 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HRESITOFMS *m*/*z* 557.2510 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_3$ + Na, 557.2503).

Antimalarial Assay. Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multi-drug-resistant strain), using the method of Trager and Jensen.[28] Quantitative assessment of activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[29] The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of $[^3H]$-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin (Table 2).

Cytotoxicity Assay. Cytotoxic assays against human epidermoid carcinoma (KB), human breast adenocarcinoma (MCF7), and human small cell lung cancer (NCI-H187) were performed employing the

Table 1. NMR Spectroscopic Data (400 MHz, $CDCl_3$) for 1−4

	longirostrerone A (1)		longirostrerone B (2)		longirostrerone C (3)		longirostrerone D (4)	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	143.2, CH	7.00, s	145.5, CH	7.40, s	143.2, CH	6.96, s	153.4, CH	8.83, s
3	156.6, C		156.5, C		157.2, C		157.1, C	
4	110.5, CH	6.04, s	110.5, CH	5.97, s	110.7, CH	6.10, s	111.9, CH	6.11, s
5	106.7, CH	5.37, s	104.7, CH	5.40, s	106.7, CH	5.39, s	105.6, CH	5.29, s
6	192.0, C		199.4, C		192.0, C		190.8, C	
7	82.7, C		73.3, C		82.8, C		87.8, C	
8	44.2, CH	3.85, dd (1.7, 12.9)	41.0, CH	3.34, brd (10.2)	44.2, CH	3.89, dd (1.7, 12.9)	165.0, C	
9	19.0, CH_3	1.42, s	21.3, CH_3	1.14, s	19.0, CH_3	1.42, s	26.2, CH_3	1.70, s
10	61.8, CH	2.92, d (12.0)	61.6, CH	2.88, d (12.0)	59.9, CH	3.02, d (12.4)	61.5, CH	3.00, d (12.7)
11	203.1, C		203.3, C		194.8, C		203.2, C	
12	50.4, CH_2	2.84, dd (4.6, 13.4), 2.42, dd (11.7, 13.1)	50.5, CH_2	2.85, dt (3.9, 13.2), 2.40, dd (11.8, 13.2)	129.1, CH	6.09, d (7.6)	50.4, CH_2	2.87, brdd (2.0, 13.1), 2.46, t (13.1)
13	67.9, CH	4.0, m	67.9, CH	3.99, m	150.1, CH	7.03, m	67.6, CH	4.20, m
14	42.5, CH_2	2.30, brd (12.3), 1.57, m	42.6, CH_2	2.28, brd (13.1), 1.54, q (12.5)	33.4, CH_2	2.54, m, 2.20, dd (10.6, 18.6)	42.3, CH_2	2.30, brd (12.5), 1.59, quin (12.5)
15	30.9, CH	2.06, m	30.9, CH	2.07, m	33.1, CH	2.49, m	30.7, CH	2.11, m
16	20.5, CH_3	1.04, d (6.4)	20.5, CH_3	1.02, d (6.5)	19.9, CH_3	1.03, d (6.4)	20.5, CH_3	1.05, d (6.4)
17	169.7, C		36.6, CH_2	3.40, dd (1.8, 16.3), 2.83, brd (16.3)	169.7, C		168.3, C	
18	51.7, CH	4.14, d (12.9)	199.4, C		51.7, CH	4.14, d (12.9)	124.6, C	
19	190.8, C		123.4, CH	6.13, d (15.9)	190.8, C		184.8, C	
20	121.4, CH	6.46, d (15.5)	149.8, CH	7.36, d (15.9)	121.4, CH	6.46, d (15.5 Hz)	121.4, CH	6.93, d (15.4)
21	152.0, CH	7.39, d (15.5)	131.5, C		152.0, CH	7.40, d (15.5 Hz)	151.5, CH	7.38, d (15.4)
22	131.8, C		151.1, CH	5.73, d (9.9)	131.8, C		132.4, C	
23	153.6, CH	5.84, d (9.9)	31.1, CH	2.66, m	153.6, CH	5.83, d (9.9)	153.2, CH	5.79, d (9.9)
24	31.3, CH	2.69, m	44.4, CH_2	1.33, 1.11, m	31.3, CH	2.69, m	31.3, CH	2.66, m
25	44.3, CH_2	1.34, 1.14, m	32.3, CH	1.23, m	44.3, CH_2	1.34, 1.44 m	44.3, CH_2	1.90, 1.10, m
26	32.4, CH	1.22, m	30.1, CH_2	1.30, 1.11, m	32.4, CH	1.25, m	32.4, CH	1.24, m
27	30.1, CH_2	1.27, 1.14, m	11.2, CH_3	0.83, t (7.0)	30.1, CH_2	1.28, 1.44, m	30.0, CH_2	1.23, 1.10, m
28	18.9, CH_3	0.83, t (6.2)	19.0, CH_3	0.85, d (7.0)	11.2, CH_3	0.83, t (6.0)	11.2, CH_3	0.78, t (6.0)
29	11.2, CH_3	0.85, d (7.1)	21.0, CH_3	0.98, d (6.6)	19.0, CH_3	0.84, d (7.0)	19.0 CH_3	0.80, d (7.2)
30	20.8, CH_3	0.98, d (6.6)	12.3, CH_3	1.80, s	20.9, CH_3	0.98, d (6.6)	20.9, CH_3	0.95, d (6.5)
31	12.3, CH_3	1.85, s			12.3, CH_3	1.85, s	12.4, CH_3	1.83, s
4a	144.6, C		146.9, C		144.6, C		143.5, C	
8a	116.3, C		120.7, C		116.3, C		111.5, C	

Table 2. Biological Activity of Compounds 1−4

	cytotoxicity ($IC_{50}\mu M$)			
compound	KB[a]	MCF7[b]	NCIH-187[c]	antimalarial ($IC_{50}\mu M$)
1	1.04	0.24	3.08	0.63
2	1.52	inactive	inactive	3.73
3	0.23	inactive	inactive	0.62
4	6.38	38.22	18.39	inactive
dihydroartemisinine				0.004
doxorubicine	0.33	2.29	0.11	
ellipticine	1.25		1.82	

[a]Human epidermoid carcinoma of the mouth. [b]Human breast adenocarcinoma (MCF7). [c]Human small-cell lung cancer.

colorimetric method as described by Skehan and co-worker.[30] The reference substances were doxorubicine and ellipticine (Table 2).

■ ASSOCIATED CONTENT

Supporting Information

^1H NMR and ^{13}C NMR spectra for compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +66-43-202222-41, ext. 12243. Fax: +66-43-202373. E-mail: somdej@kku.ac.th.

■ ACKNOWLEDGMENTS

Financial support from the Thailand Research Fund (grant no. DBG5380047) for S.K. is gratefully appreciated. We acknowledge the Office of the Higher Education Commission, Thailand, for supporting by grant fund under the program Strategic Scholarships for Frontier Research Network for the Joint Ph.D. Program Thai Doctoral degree for N.P. We thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC) and Rajamangala University of Technology Isan for partial support. We are indebted to the National Center for Genetic Engineering and Biotechnology via the Bioresources Research Network (BRN) for bioactivity tests.

■ REFERENCES

(1) Pornsuriya, C.; Lin, F. C; Kanokmedhakul, S.; Soytong, K. *J. Agric. Tech.* **2008**, *4*, 91−103.

(2) Soytong, K. *Thai Phytopathong.* **1991**, *11*, 86−111.
(3) Pornsuriya1, C.; Soytong, K.; Poeaim, S.; Kanokmedhakul, S.; Khumkomkhet, P.; Lin, F.-C.; Wang, H. K.; Hyde, K. D. *Mycotaxol* **2011**, *115*, 19−27.
(4) Brewer, D.; Jerram, W. A.; Taylor, A. *Can. J. Microbiol.* **1968**, *14*, 861−866.
(5) Safe, S.; Taylor, A. *J. Chem. Soc., Perkin Trans. 1* **1972**, 472−479.
(6) Takahashi, M.; Koyama, K.; Natori, S. *Chem. Pharm. Bull.* **1990**, *38*, 625−628.
(7) Kanokmedhakul, S.; Kanokmedhakul, K.; Nasomjai, P.; Soytong, K.; Isobe, M.; Kongsaeree, P.; Prabpai, S.; Suksamrarn, A. *J. Nat. Prod.* **2006**, *69*, 891−895.
(8) Phonkerd, N.; Kanokmedhakul, S.; Kanokmedhakul, K.; Soytong, K.; Prabpai, S.; Kongsaeree, P. *Tetrahedron* **2008**, *68*, 9636−9645.
(9) Sekita, S.; Yoshihira, K.; Natori, S. *Tetrahedron Lett.* **1976**, *17*, 1351−1354.
(10) Udagawa, S.; Muroi, T.; Kurata, H.; Sekita, S.; Yoshihira, K.; Natori, S.; Umeda, M. *Can. J. Microbiol.* **1979**, *25*, 170−177.
(11) Probst, A.; Tamm, C. *Helv. Chim. Acta* **1981**, *64*, 2056−2064.
(12) Sekita, S.; Yoshihira, K.; Natori, S.; Kuwano, H. *Chem. Pharm. Bull.* **1982**, *30*, 1629−1638.
(13) Sekita, S.; Yoshihira, K.; Natori, S. *Chem. Pharm. Bull.* **1983**, *31*, 490−498.
(14) Kanokmedhakul, S.; Kanokmedhakul, K.; Phonkerd, N.; Soytong, K.; Kongsaeree, P.; Suksamrarn, A. *Planta Med.* **2002**, *68*, 834−836.
(15) Ding, G.; Song, Y. C.; Chen, J. R.; Xu, C.; Ge, Wang, X. T.; H., M.; Tan, R. X. *J. Nat. Prod.* **2006**, *69*, 302−304.
(16) Thohinung, S.; Kanokmedhakul, S.; Kanokmedhakul, K.; Kukongviriyapan, V.; Tusskorn, O.; Soytong, K. *Arch. Pharm. Res.* **2010**, *33*, 1135−1141.
(17) Bashyal, B. P.; Wijeratne, E. M. K.; Faeth, S. H.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2005**, *68*, 724−728.
(18) Oh, H.; Swenson, D. C.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Tetrahedron Lett.* **1998**, *39*, 7633−7636.
(19) Li, G. Y.; Li, B. G.; Yang, T.; Liu, G. Y.; Zhang, G. L. *Helv. Chim. Acta* **2008**, *91*, 124−129.
(20) Khumkomkhet, P.; Kanokmedhakul, S.; Kanokmedhakul, K.; Hahnvajanawong, C.; Soytong, K. *J. Nat. Prod.* **2009**, *72*, 1487−1491.
(21) Makin, H. L. J.; Kirk, D. N.; Murphy, G. M. Chemical data, structures and bibliographies. Index: Hill, R. A., Ed.; *Dictionary of Steroids*; University Press: Cambridge: Chapman & Hall Ltd, 1991; p 409.
(22) Bok, J. W.; Lermer, L.; Chilton, J.; Klingeman, H. G.; Towers, G. H. *Phytochemistry* **1999**, *51*, 891−898.
(23) Rösecke, J.; König, W. A. *Phytochemistry* **2000**, *54*, 757−762.
(24) Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Vyvyan, J. R. *Introduction to Spectroscopy*, 4th ed.; Books/Cole, Cengage Learning: Belmont, CA, USA, 2008; pp 241−246.
(25) Quang, N. D.; Hashimoto, T.; Fournier, J.; Stadler, M.; Radulovic, N.; Asakawa, Y. *Tetrahetron* **2005**, *61*, 1743−1748.
(26) Quang, N. D.; Hashimoto, T.; Stadler, M.; Radulovic, N.; Asakawa, Y. *Planta Med.* **2005**, *71*, 1058−1062.
(27) Quang, N. D.; Stadler, M.; Fournier, J.; Tomita, A.; Hashimoto, T. *Tetrahetron* **2006**, *62*, 6349−6354.
(28) Trager, W.; Jensen, J. B. *Science* **1967**, *193*, 673−675.
(29) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.
(30) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, T. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer. Inst.* **1990**, *82*, 1107−1112.