Cytotoxic and Antiplasmodial Compounds from the Roots of *Strophioblachia fimbricalyx*

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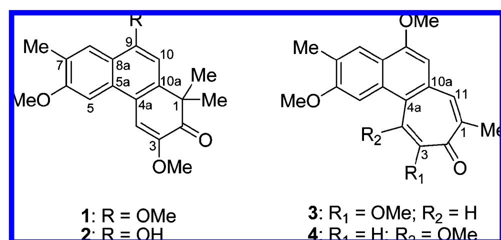
The known phenanthrenone trigonostemone (**1**), along with a new phenanthrenone, 9-*O*-demethyltrigonostemone (**2**), and two new phenanthropolones, 3,6,9-trimethoxyphenanthropolone (**3**) and 4,6,9-trimethoxyphenanthropolone (**4**), were isolated from the roots of *Strophioblachia fimbricalyx*. Compound **2** showed cytotoxicity against NCI-H187, KB, and MCF7 cancer cells with IC_{50} values of 0.8, 0.8, and 2.9 μg/mL, respectively, while **3** and **4** showed reduced cytotoxicity. Compounds **2** and **3** displayed antiplasmodial activity in vitro (IC_{50} values of 2.7 and 3.2 μg/mL, respectively) against *Plasmodium falciparum* (K1, resistant strain). In addition, the antioxidant activity of **1**−**4** toward DPPH radicals was determined, but only compound **2** showed any discernible activity.

Plants of the genus of *Strophioblachia* (family Euphorbiaceae) are very rare in Thailand. Only one species, *Strophioblachia fimbricalyx* Boerl. (Thai name "Dok Khrui" or "Ba Sad"), a small shrub up to 2.5 m high, is classified as a native species and is distributed widely in northeast Thailand.[1] Information from traditional healers and local people has indicated that a decoction of roots of *B. fimbricalyx* with *Capparis micracantha*, *Embelia ribes*, and *Xylopia vielana* has been used in a traditional recipe to treat fevers.[2] This plant is also used in Thai traditional medicine for migraine alleviation,[3] and the fermented liquor of its roots, together with other medicinal plants, has been used to treat cancer. There is only one report of the chemical constituents of *S. fimbricalyx*, from which megastigmane glucosides and flavone glycosides were isolated from the aerial parts of this plant species.[3] As part of the present study, the crude EtOAc extract from the roots of *S. fimbricalyx* was investigated chemically. We report herein the isolation and structure elucidation of the new compounds 9-*O*-demethyltrigonostemone (**2**), 3,6,9-trimethoxyphenanthropolone (**3**), and 4,6,9-trimethoxyphenanthropolone (**4**), together with the ^{1}H and ^{13}C NMR spectroscopic data reassignments of the previously known compound trigonostemone (**1**). The cytotoxicity of **1**−**4** is also reported against four cancer cell lines along with their antiplasmodial and radical-scavenging activities.



Compound **1** was isolated as a yellow powder and was identified as the known compound trigonostemone from analysis of its NMR spectroscopic and MS data. This compound was first isolated from the roots of *Trigonostemon reidioides* (Euphorbiaceae), and its chemical structure was confirmed by X-ray crystallography.[4] The melting point, IR, and UV data of the isolated **1** were identical to those reported in the literature.[4] However, comparison of the NMR

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **1** and **2**

	1[a]		**2**[b]	
position	δ_H (*J* in Hz)	δ_c, mult.	δ_H (*J* in Hz)	δ_c, mult.
1		49.6, qC		48.8, qC
2		199.4, qC		197.8, qC
3		147.8, qC		147.7, qC
4	7.34 (s)	112.1, CH	7.57 (s)	112.8, CH
5	7.31 (s)	99.4, CH	7.59 (s)	100.2, CH
6		158.2, qC		158.1, qC
7		127.6, qC		126.6, qC
8	8.05 (d, 0.9)	123.7, CH	8.02 (s)	123.5, CH
9		155.4, qC		153.1, qC
10	6.77 (s)	100.9, CH	6.95 (s)	105.2, CH
4a		115.1, qC		114.6, qC
5a		131.1, qC		131.8, qC
8a		119.5, qC		118.9, qC
10a		143.0, qC		143.2, qC
Me-1 × 2	1.57 (s)	28.4, CH_3	1.43 (s)	27.6, CH_3
Me-7	2.40 (d, 0.5)	16.8, CH_3	2.36 (s)	16.0, CH_3
OMe-3	3.96 (s)	55.3[c], CH_3	3.85 (s)	55.1[d], CH_3
OMe-6	4.03 (s)	55.6[c], CH_3	4.03 (s)	55.0[d], CH_3
OMe-9	4.04 (s)	55.7[c], CH_3		
OH-9			9.13 (brs.)	

[a] Recorded in $CDCl_3$. [b] Recorded in acetone-d_6. [c,d] Assignments are interchangeable.

chemical shifts of isolated **1** with those reported in the literature indicated that the ^{1}H NMR assignments for H-4 and H-10 and the ^{13}C NMR assignments for C-4, C-5, C-4a, C-10a, Me-1, and Me-7 required correction (Supporting Information). The corrected ^{1}H and ^{13}C NMR data and assignments for compound **1** are shown in Table 1.

Compound **2** gave similar ^{1}H and ^{13}C NMR spectroscopic data to those of **1** (Table 1), except for the absence of a signal due to the methoxy group on position C-9 and the presence of a hydroxy proton signal at δ 9.13 (br s). Analysis of the ^{1}H and ^{13}C NMR spectroscopic data of compound **2** revealed signals of 20 protons and 19 carbon atoms (one carbon signal at δ 27.6 was overlapped). The molecular formula of compound **2** was determined as $C_{19}H_{20}O_4$ by HRESIMS, showing a protonated molecular ion peak. This suggested that for compound **2** a hydroxy group replaced the methoxy group in the molecule of **1**. NOESY correlations between H-4 and H-5 and OMe-3; H-5 and OMe-6; H-8 and Me-7; and H-10 and Me-1 confirmed the position of the hydroxy group in **2**. The IR and UV spectra of **2** were also similar to those of **1**. On the basis of the above spectroscopic data, compound **2** was assigned as 9-*O*-demethyltrigonostemone, a new derivative of phenanthrenone.

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Table 2. ^1H and ^{13}C NMR Spectroscopic Data of Compounds **3** and **4** In $CDCl_3$

	3		**4**	
position	δ_H (J in Hz)	δ_c, mult.	δ_H (J in Hz)	δ_c, mult.
1		141.5, qC		141.1, qC
2		180.7, qC		180.3, qC
3		156.0, qC	7.64 (s)	112.0, CH
4	7.98 (s)	112.7, CH		155.6, qC
5	7.63 (s)	101.6, CH	7.32 (s)	101.3, CH
6		158.4, qC		158.1, qC
7		129.4, qC		129.2, qC
8	8.13 (d, 0.7)	123.9, CH	8.02 (s)	123.7, CH
9		154.9, qC		154.5, qC
10	6.80 (s)	105.6, CH	6.57 (s)	105.4, CH
11	7.84 (s)	140.4, CH	7.62 (s)	140.3, CH
4a		121.4, qC		121.1, qC
5a		132.8, qC		132.4, qC
8a		132.7, qC		132.5, qC
10a		123.4, qC		123.1, qC
Me-1	2.45 (s)	23.4, CH_3	2.41 (s)	23.3, CH_3
Me-7	2.48 (d, 0.5)	16.7, CH_3	2.41 (s)	16.7, CH_3
OMe-3	4.06 (s)	55.6[a], CH_3		
OMe-4			3.93 (s)	55.0[b], CH_3
OMe-6	4.05 (s)	55.2[a], CH_3	3.94 (s)	56.1[b], CH_3
OMe-9	4.09 (s)	56.5[a], CH_3	4.01 (s)	55.4[b], CH_3

[a,b] Assignments are interchangeable.

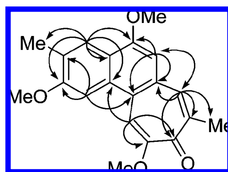

Figure 1. Selected HMBC correlations for compound **3**.

Compound **3** was assigned the molecular formula $C_{20}H_{20}O_4$ from the HRESIMS of the protonated molecular ion at *m/z* 325.1447 $[M + H]^+$ (calcd for $C_{20}H_{21}O_4$ 325.1440). The ^1H and ^{13}C NMR spectroscopic data for **3** were similar to those of **2**, except for an additional singlet aromatic proton resonance at δ 7.84 and one less methyl group resonance (Table 2). The ^1H NMR spectrum of **3** showed four aromatic proton singlets at δ 7.98 (H-4), 7.84 (H-11), 7.63 (H-5), and 6.80 (H-10), one aromatic proton doublet at δ 8.13 (d, $J = 0.7$ Hz; H-8), two methyl groups at δ 2.48 (d, $J = 0.5$ Hz; Me-7) and 2.45 (s, Me-1), and three methoxy groups at δ 4.09 (OMe-9), 4.06 (OMe-3), and 4.05 (OMe-6). From the HMQC correlations, the five aromatic protons were assigned as being attached to the aromatic methine carbons at δ 140.4 (C-11), 123.9 (C-8), 112.7 (C-4), 105.6 (C-10), and 101.6 (C-5). Further connectivity of the structure was determined using HMBC correlations between aromatic protons and nearby quaternary carbons, as shown in Figure 1. The core structure of compound **3** was assigned as a phenanthropolone unit with the aromatic C ring of the phenanthrene modified to a seven-membered tropolone ring. The HMBC correlations of the H-11 and H-4 protons to the carbonyl carbon C-2 (δ 180.7) supported the protons of a tropolone ring moiety in compound **3**. HMBC correlations of protons H-11 to Me-1 (δ 23.4), H-4 to C-3 (δ 156.0), H-5 to C-6 (δ 158.4), H-8 to C-6, C-9 (δ 154.9) and Me-7 (δ 16.7), and H-10 to C-9 were used to identify the positions of the methyl and methoxy groups. NOESY correlations between the aromatic protons and the methyl and methoxy group protons also confirmed the assigned structure of **3**. NOESY correlations between protons OMe-9 and H-10, H-10 and H-11, and H-11 and Me-1 were observed, while H-8 displayed a correlation to Me-7. The NOESY correlations from H-4 to OMe-3 and H-5 and from H-5 to OMe-6 strongly supported the position of OMe-3 on a tropolone ring (Figure 2). An IR absorption band for a conjugated carbonyl group (1616 cm^{-1}) and UV absorption bands at 328 and 279 nm were in good agreement with the tropolone

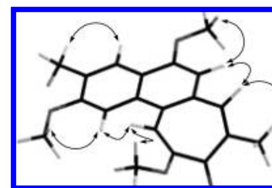

Figure 2. Molecular structure (Chem 3D model) and selected NOESY correlations of compound **3**.

incorporated in the skeleton of compound **3**.[5] On the basis of these spectroscopic data, the structure of new compound **3** was deduced as 3,6,9-trimethoxyphenanthropolone.

Compound **4** showed signals for five aromatic proton singlets, two methyl groups, and two methoxy groups in the ^1H NMR spectrum and exhibited 20 carbon signals, including that for a carbonyl group in the ^{13}C NMR spectrum (Table 2). The molecular formula of **4** was determined as $C_{20}H_{20}O_4$ from the HRESIMS $[M + H]^+$ peak at *m/z* 325.1445 (calcd for $C_{20}H_{21}O_4$ 325.1440). These data indicated that **4** is an isomer of compound **3**. HMBC correlations from H-8 (δ 8.02) to C-8a and C-5a, H-10 (δ 6.57) to C-9, C-10a, C-4a, and C-11, Me-7 (δ 2.41, d, $J = 0.5$ Hz) to C-6, C-7, and C-8, and Me-1 (δ 2.41) to C-1, C-2, and C-11 demonstrated that this compound has the same core structure as **3**. Assignments of the H-3 and OMe-4 (δ 3.93) positions on the tropolone ring were carried out using NOESY correlations between H-3 and OMe-4 and between H-5 and OMe-6 (δ 3.94). On the basis of these spectroscopic data, compound **4** was elucidated as 4,6,9-trimethoxyphenanthropolone.

The biological activities determined for compounds **1**−**4** are shown in Table 3. Compounds **3** and **4** displayed cytotoxic activity against the KB and NCI-H187 cell lines but were found to be noncytotoxic to MCF7 and Vero cells. Interestingly, the phenolic compound **2** showed cytotoxicity against NCI-H187, KB, MCF7, and Vero cell lines with IC_{50} values of 0.8, 0.8, 2.9, and 3.1 μg/mL, respectively, while **1**, its nonphenolic analogue, was found to be inactive for all these cell lines. Compounds **2** and **3** also showed antiplasmodial activity in vitro against *Plasmodium falciparum* (K1, multidrug-resistant strain), with IC_{50} values of 2.7 and 3.2 μg/mL, respectively. Radical-scavenging activity of **2** was shown with an IC_{50} value of 29.1 $\pm$ 0.4 μg/mL toward the DPPH radical, using BHT (IC_{50} value of 18.8 $\pm$ 0.27 μg/mL) as a positive control.

Experimental Section

General Experimental Procedures. Melting points were measured on an Electrothermal 9100 melting point apparatus. UV−visible spectra were recorded on a Varian CARY 1E spectrometer. FT-IR spectra were recorded on a Bruker vector 22 spectrometer. NMR spectra were recorded in $CDCl_3$ and acetone-d_6 solvents on a Bruker AV500D spectrometer at 500 MHz (^1H NMR) and 125 MHz (^{13}C NMR). ESITOF mass spectra were obtained on a Bruker microTOF mass spectrometer.

Plant Material. *Strophioblachia fimbricalyx* was collected from indigenous forest near Pang Village, Kosum Phisai District, Maha Sarakham Province, Northeast Thailand, in September 2005. A voucher specimen [number P. Bunchalee 011005 (MSUT)] was deposited at Mahasarakham University.

Extraction and Isolation. Air-dried roots of *S. fimbricalyx* were roughly ground, and 1 kg was taken for extraction with MeOH (3 L, 5 days, three times) at room temperature. The MeOH layer (ca. 9 L) was evaporated under reduced pressure to give a brownish-black crude extract, which was suspended in H_2O (200 mL) and then partitioned successively with hexane (200 mL × 2), CH_2Cl_2 (200 mL × 5), and EtOAc (200 mL × 10). The hexane (ca. 400 mL), CH_2Cl_2 (ca. 1 L), and EtOAc (ca. 2 L) layers were concentrated under reduced pressure to obtain crude extracts from hexane (2.52 g), CH_2Cl_2 (1.57 g), and EtOAc (6.42 g), respectively. The crude EtOAc extract was subjected to silica gel column chromatography, eluting with a stepwise gradient from CH_2Cl_2−hexane (70:30 to 100:0) to MeOH−CH_2Cl_2 (5:95 to 30:70), and three main fractions, fractions A−C, were collected based on

Table 3. Biological Activities of Compounds **1**−**4**

compound	cytotoxicity (IC_{50} values; μg/mL)				antiplasmodial assay[a] (μg/mL)	radical scavenging assay[b] (μg/mL)
	NCI-H187	KB	MCF7	Vero		
1	>5	>5	>5	>20	>20	>100
2	0.8	0.8	2.9	3.1	2.7	29.1 ± 0.4
3	>5	4.0	>5	>20	3.2	>100
4	>5	3.9	>5	>20	>20	>100
ellipticine[c]	0.44	0.48		0.48		
doxorubicin[c]	0.04	0.13	2.2			
dihydroartemisinin[c]					0.004	
BHT[c]						18.8 ± 0.27

[a] Tested against *Plasmodium falciparum* (K1, multidrug-resistant strain). [b] Using the DPPH method. [c] Positive control substance.

similar TLC profiles. Fraction A (466 mg) was further chromatographed on a silica gel column, using CH_2Cl_2−hexane (70:30 to 100:0) and then MeOH−CH_2Cl_2 (5:95 to 20:80), as eluting solvents, to obtain compound **1** (118 mg). Fraction B (205 mg) was purified using silica gel column chromatography, eluting with EtOAc−CH_2Cl_2 (20:80 to 80:20) in a stepwise manner, and gave compounds **3** (33 mg) and **4** (52 mg). Fraction C (330 mg) was passed through a silica gel column and eluted with a stepwise gradient of EtOAc−CH_2Cl_2 (20:80 to 80:20), and then the collected fraction was subjected to passage over a Sephadex LH-20 column, using MeOH−CH_2Cl_2 (80:20) as eluent, to yield compound **1** (35 mg) and compound **2** (252 mg).

Trigonostemone (1): yellow powder; mp 185−186 °C (lit. 184−185 °C);[4] UV−vis (MeOH) λ_{max} (log ε) 405 (4.17), 255 (4.69), 235 (sh) (4.59), 214 (4.59) nm; IR (KBr) ν_{max} 1666, 1613, 1595, 1463, 1377, 1280, 1241, 1170, 1067 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 349.1413 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_4Na$, 349.1416).

9-*O*-Demethyltrigonostemone (2): yellow powder; mp 222−223 °C; UV−vis (MeOH) λ_{max} (log ε) 408 (3.95), 257 (4.47), 213 (4.58) nm; IR (KBr) ν_{max} 1630, 1592, 1464, 1385, 1364, 1263, 1237, 1162 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 313.1449 $[M + H]^+$ (calcd for $C_{19}H_{21}O_4$, 313.1440).

3,6,9-Trimethoxyphenanthropolone (3): yellow powder; mp 209−210 °C; UV (MeOH) λ_{max} (log ε) 328 (4.47), 279 (4.45), 260 (sh) (4.46), 231 (4.32) nm; IR (KBr) ν_{max} 1616, 1584, 1465, 1432, 1235, 1181, 1149 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m/z* 325.1447 $[M + H]^+$ (calcd for $C_{20}H_{21}O_4$, 325.1440).

4,6,9-Trimethoxyphenanthropolone (4): yellow powder; mp 211−212 °C; UV (MeOH) λ_{max} (log ε) 329 (4.47), 279 (4.51), 261 (sh) (4.46), 232 (4.32) nm; IR (KBr) ν_{max} 1616, 1581, 1464, 1432, 1236, 1181, 1146 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m/z* 325.1445 $[M + H]^+$ (calcd for $C_{20}H_{21}O_4$, 325.1440).

Biological Assays. An assay for cytotoxicity against NCI-H187 (human small-cell lung cancer), KB (oral human epidermoid carcinoma), MCF-7 (human breast cancer), and Vero (African green monkey kidney fibroblasts) cell lines was evaluated using a colorimetric method.[6] The antiplasmodial activity against *Plasmodium falciparum* (K1, multidrug-resistant strain) was performed using the microculture radioisotope technique described by Desjardins and associates.[7] Radical-scavenging activity (DPPH assay) was measured according to the method of Chu et al.[8]

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Supporting Information Available: ^1H and ^{13}C NMR and HRMS spectra of compounds **1**−**4** and ^1H and ^{13}C NMR spectroscopic data of compound **1** compared with the literature. This information is available free of charge via the Internet at http://pubs.acs.org.

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