Aigialomycins A−E, New Resorcylic Macrolides from the Marine Mangrove Fungus *Aigialus parvus*

Masahiko Isaka,* Chotika Suyarnsestakorn, and Morakot Tanticharoen

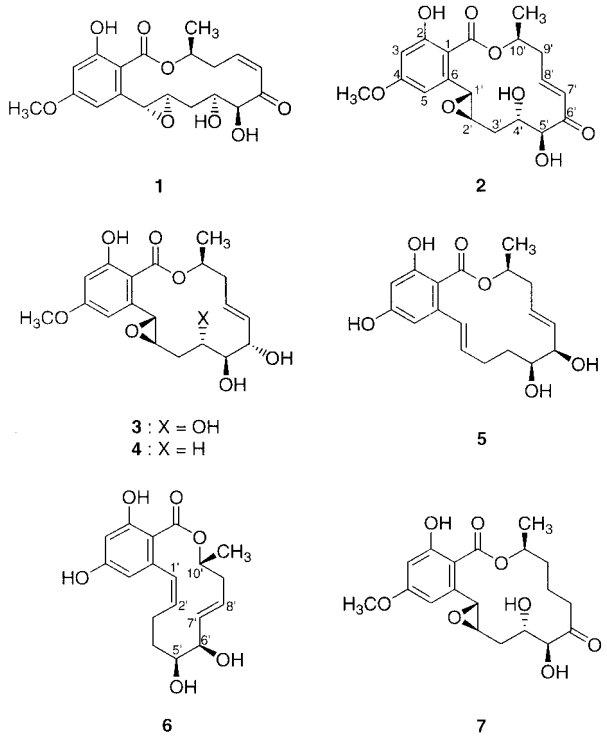
National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Rama 6 Road, Bangkok 10400, Thailand

Palangpon Kongsaeree and Yodhathai Thebtaranonth

Department of Chemistry, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand

Aigialomycins A−E (**2**−**6**), new 14-membered resorcylic macrolides, were isolated together with a known hypothemycin (**1**) from the mangrove fungus, *Aigialus parvus* BCC 5311. Structures of these compounds, including absolute configuration, were elucidated by spectroscopic methods, chemical conversions, and X-ray crystallographic analysis. Hypothemycin and aigialomycin D (**5**) exhibited in vitro antimalarial activity with IC_{50} values of 2.2 and 6.6 μg/mL, respectively, while other analogues were inactive. Cytotoxicities of these compounds were also evaluated.

Marine microorganisms have proved to be rich sources of bioactive secondary metabolites, and numerous compounds with potent biological activities and unique chemical structures have been isolated.[1] In the course of our search for novel bioactive compounds from microbial sources,[2] we came across antimalarial activity (IC_{50} 8 μg/mL) of an extract from a lignicolous mangrove ascomycete, *Aigialus parvus* BCC 5311. Since there has been no report on the secondary metabolites of the genus *Aigialus*, we decided to undertake the investigation of the antimalarial constituents of the strain BCC 5311. Activity-guided fractionation and chromatographic separation led to the isolation of a known resorcylic macrolide, hypothemycin (**1**),[3] as the major secondary metabolite together with its five new analogues, named aigialomycins A−E (**2**−**6**). We report herein the isolation, structural elucidation, and biological activities of these novel macrolides.



Results and Discussion

The major metabolite of *A. parvus* BCC 5311, compound **1**, was obtained as colorless needles. The structure of this compound was elucidated by spectroscopic method (NMR, MS, IR, and UV). Its melting point (170−172 °C), specific rotation ($[\alpha]^{25}_{D}$ −18; *c* 0.50, $CHCl_3$), and other spectral data were identical to those of hypothemycin whose structure has previously been determined by an X-ray crystallographic analysis.[4]

Aigialomycin A (**2**) was isolated as the second major secondary metabolite. HRMS and elemental analysis indicated that this compound possesses the same molecular formula as hypothemycin ($C_{19}H_{22}O_8$). NMR analyses (^{1}H, ^{13}C, DEPTs, COSY, HMQC, and HMBC) revealed that the configuration at C-7′−C-8′ double bond of

* To whom correspondence should be addressed. Tel: +662-6448103. Fax: +662−6448107.

(1) (a) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 1−6. (b) Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1−49.

(2) (a) Isaka, M.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2001**, *66*, 4803−4808. (b) Isaka, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Antibiot.* **2001**, *54*, 36−43. (c) Isaka, M.; Tanticharoen, M.; Thebtaranonth, Y. *Tetrahedron Lett.* **2000**, *41*, 1657−1660. (d) Isaka, M.; Punya, J.; Lertwerawat, Y.; Tanticharoen, M.; Thebtaranonth, Y. *J. Nat. Prod.* **1999**, *62*, 329−331. (e) Ekthawatchai, S.; Isaka, M.; Kittakoop, P.; Kongsaeree, P.; Sirichaiwat, C.; Tanticharoen, M.; Tarnchompoo, B.; Thebtaranonth, Y.; Yuthavong, Y. *J. Heterocycl. Chem.* **1999**, *36*, 1599−1605.

(3) (a) Nair, M. S. R.; Carey, S. T. *Tetrahedron Lett.* **1980**, *21*, 2011−2012. (b) Nair, M. S. R.; Carey, S. T.; James, J. C. *Tetrahedron* **1981**, *37*, 2445−2449.

(4) Revised structure of hypothemycin: Agatsuma, T.; Takahashi, A.; Kabuto, C.; Nozoe, S. *Chem. Pharm. Bull.* **1993**, *41*, 373−375.

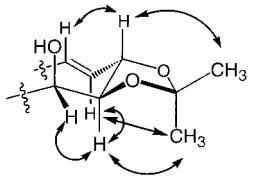
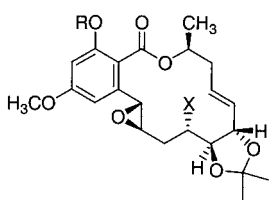


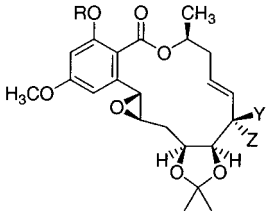
Figure 1. Selected NOESY correlations for **10** (in methanol-d_4).

aigialomycin A (**2**) differed from that of **1**, having the *trans* geometry ($J_{7',8'} = 15.8$ Hz). Catalytic hydrogenation of compounds **1** and **2** (H_2, 10% Pd/C, EtOAc) gave the same 7′, 8′-dihydro analogue **7**; $[\alpha]^{26}_D$ −16 (*c* 0.17, $CHCl_3$) and $[\alpha]^{27}_D$ −14 (*c* 0.17, $CHCl_3$), respectively. Therefore, the 1′*R*,2′*R*,4′*S*,5′*S*,10′*S* configuration of aigialomycin A (**2**), identical to **1**, was established. Physicochemical properties (mp, UV, IR, and ^{1}H NMR) of compound **7** were consistent to those of dihydrohypothemycin which was previously isolated, along with hypothemycin, from *Hypomyces trichothecoides*.[3b] Revised ^{1}H NMR assignments and ^{13}C NMR data of **7** are presented in Experimental Section.

The molecular mass ion $[M + H]^+$ of aigialomycin B (**3**) is 2 mass units more than compound **2**, and the molecular formula of $C_{19}H_{24}O_8$ was established by HRMS and NMR. IR and ^{13}C NMR spectra of **3** indicated the absence of ketone functionality. NMR analyses, particularly COSY, HMQC, and HMBC, clearly revealed that compound **3** was an analogue of **2** but with a different oxidation state at C-6′. The relative configuration of the triol moiety (C-4′ to C-6′) was determined by chemical means. Thus, treatment of **3** under the acetonide-forming conditions (2,2-dimethoxypropane, cat. TsOH, rt, 2 h) resulted in an inseparable mixture of two acetonide isomers (**8** and **9**, analyzed by NMR data) which upon methylation (MeI/K_2CO_3 in 2-butanone, rt, 20 h) followed by preparative reversed-phase HPLC separation provided compounds **10** and **11** (**10**:**11** = 65:35; 95% yield). The $J_{5',6'}$ value of 8.0 Hz (in methanol-d_4) suggested a *cis*-fused acetonide structure for **10**; however, the NOESY spectrum indicated otherwise. Correlation between H-5′ and the δ_H 1.42 acetonide methyl was evident while H-6′ correlated to the δ_H 1.45 acetonide methyl. Also, the intensity of NOESY cross signal between H-5′ and H-6′ was weak, and therefore, H-5′ and H-6′ should be placed on the opposite side of the acetonide ring (*trans*-fused acetonide). The relative configuration from C-4′ to C-8′ in **10** is shown in Figure 1. Compound **11**, $J_{4',5'} = 5.0$ Hz, was assigned as *cis*-fused acetonide by the observation of NOESY correlations (in $CDCl_3$) from both H-4′ and H-5′ to the same acetonide methyl at δ_H 1.37, and not to the other at δ_H 1.49. These experimental results revealed that two hydroxyl groups on C-4′ and C-5′ of aigialomycin B (**3**) are attached to the same side of the macrolide ring and are opposite to that of the hydroxyl group on C-6′. $NaBH_4$ reduction (THF/MeOH, 0 °C, 20 min) of compound **12**, acetonide of aigialomycin A, followed by methylation (MeI, K_2CO_3, 2-butanone) and purification by prep HPLC provided a mixture of two products in a ratio 2:1. Attempted separation of this mixture failed. However, NMR analysis of the mixture revealed that the major product was identical to compound **11** obtained previously from the reaction described above. The minor product, presumably C-6′ epimer of **11**, was not identified. Thus, aigialomycin B (**3**) has a relative configuration identical to **2** and on the basis of these chemical conversions, the 1′*R*,2′*R*,4′*S*,5′*S*,6′*S*,10′*S* configuration, as depicted, was determined for **3**.



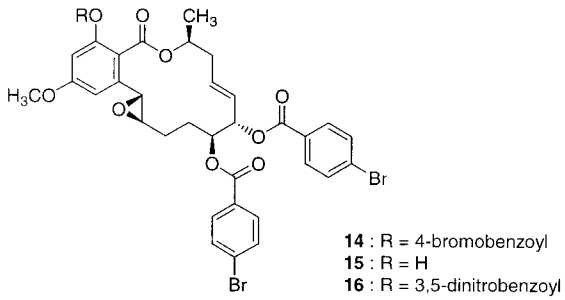
8 : R = H; X = OH
10 : R = CH_3; X = OH
13 : R = CH_3; X = H



9 : R = H; Y = H, Z = OH
11 : R = CH_3; Y = H, Z = OH
12 : R = H; Y = Z = O

Aigialomycin C (**4**), having molecular formula of $C_{19}H_{24}O_7$ (HRMS, NMR), exhibited a structure similar to **3** as elucidated by NMR analyses. The derivative **13**, prepared in two steps from **4**, was found to be a *trans*-fused acetonide by the analysis of NOESY spectra from which the same correlation information as compound **10** was obtained. Thus, aigialomycin C (**4**) was assigned as the 4′-dehydroxy analogue of aigialomycin B (**3**).

There remains an uncertainty on the previously proposed absolute configuration of 1′*R*,2′*R*,4′*S*,5′*S*,10′*S* for **1**, since the elucidation was based on the scattering method using anomalous dispersion of oxygen atoms.[4] We have succeeded, after extensive trials of derivative syntheses, in the X-ray crystallographic analysis of a heavy atom-containing analogue obtained from aigialomycin C (**4**). Thus, acylation of **4** with excess 4-bromobenzoyl chloride in pyridine (rt, 3 days) gave a triacyl derivative **14** as a colorless amorphous solid. Selective hydrolysis of **14** under mild basic conditions (aq K_2CO_3, MeOH, rt) gave **15** as a colorless powder, which was subsequently acylated with 3,5-dinitrobenzoyl chloride (in pyridine, rt, 3 days) to obtain the mixed triester **16**. Crystals of **16** were obtained by recrystallization from EtOAc, one of which was subjected to X-ray diffraction analysis. Absolute configuration of this molecule, containing two bromine atoms, was determined to be 1′*R*,2′*R*,5′*S*,6′*S*,10′*S* by the standard anomalous scattering method. Since the only structural difference between compounds **4** and **3** is the oxidation state at C-4′ and the relative configuration at all five carbon centers is identical, the absolute configuration of **3** was assigned to be 1′*R*,2′*R*,4′*S*,5′*S*, 6′*S*,10′*S*, and not its mirror image. Taken together with the chemical conversions described above, absolute configuration of both hypothemycin (**1**) and aigialomycin A (**2**) was assigned as 1′*R*,2′*R*,4′*S*,5′*S*,10′*S*, identical to the original proposal for **1** by Nozoe et al.[4]



14 : R = 4-bromobenzoyl
15 : R = H
16 : R = 3,5-dinitrobenzoyl

HRMS and NMR analyses of aigialomycin D (**5**), $C_{18}H_{22}O_6$, revealed that this compound bears an ad-

Table 1. ^{1}H NMR Data of Aigialomycins A−E (2−6)

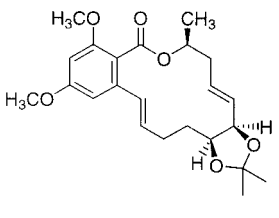
position	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** (acetone-d_6)	**6** (acetone-d_6)
3	6.41 (d, 2.8)	6.37 (d, 2.5)	6.40 (d, 2.6)	6.27 (d, 2.4)	6.28 (d, 2.4)
5	6.43 (d, 2.8)	6.39 (d, 2.4)	6.43 (d, 2.5)	6.52 (d, 2.4)	6.19 (d, 2.2)
1′	4.36 (d, 1.7)	4.42 (d, 1.3)	4.39 (s)	7.14 (d, 15.9)	6.70 (d, 11.6)
2′	2.94 (ddd, 9.1, 2.3, 2.1)	2.90 (brd, 8.7)	2.77 (brd, 7.6)	6.09 (ddd, 15.9, 5.6, 5.4)	5.53 (m)
3′	2.04 (ddd, 14.7, 9.1, 2.7)	2.09 (m)	2.26 (m)	2.31−2.34 (m)	2.19 (m)
	1.08 (ddd, 14.7, 9.1, 2.2))	1.51 (m	1.47 (m)	2.31−2.34 (m)	1.40 (m)
4′	4.15 (m)	3.76 (m)	1.90 (m)	2.14 (m)	2.28 (m)
	−	−	1.55 (m)	1.58 (m)	1.78 (m)
5′	4.64 (dd, 3.8, 3.5)	3.71 (d, 8.5)	3.48 (m)	3.62 (m)	3.65 (m)
6′	−	3.99 (dd, 8.9, 8.7)	3.92 (dd, 8.8, 8.7)	4.35 (brd, 4.3)	4.03 (m)
7′	6.40 (d, 15.8)	5.52 (dd, 15.3, 9.2)	5.43 (dd, 15.5, 8.6)	5.68 (dd, 15.7, 5.0)	5.45 (m)
8′	7.02 (ddd, 15.4, 8.8, 6.0)	5.97 (ddd, 15.3, 9.2, 3.4)	5.89 (ddd, 15.5, 4.9, 4.5)	5.87 (dddd, 15.7, 7.3, 7.3, 1.2)	5.43 (m)
9′	2.85 (m)	2.63 (ddd, 16.1, 9.1, 3.8)	2.68 (ddd, 15.2, 9.5, 3.7)	2.55 (ddd, 14.6, 7.5, 3.2)	2.48 (brd, ca. 14)
	2.61 (m)	2.43 (brd, ca. 16)	2.41 (ddd, 15.2, 3.7, 3.7)	2.42 (m)	2.37 (m)
10′	5.67 (m)	5.60 (m)	5.55 (m)	5.42 (m)	5.22 (m)
10′-CH_3	1.48 (d, 6.6)	1.43 (d, 6.5)	1.44 (d, 6.6)	1.38 (d, 6.4)	1.39 (d, 6.2)
4-OCH_3	3.80 (s)	3.77 (s)	3.80 (s)	−	−
2-O*H*	11.61 (s)	11.67 (s)	11.93 (s)	11.65 (s)	11.7 (br)
4-O*H*	−	−	−	9.5 (br)	9.5 (br)
4′-O*H*	2.48 (brd, 9.7)	3.43 (br)	−	−	−
5′-O*H*	3.81 (br)	4.20 (br)	2.6 (br)	not detected	not detected
6′-O*H*	−	3.58 (br)	2.6 (br)	not detected	not detected

Table 2. ^{13}C NMR Data of Aigialomycins A−E (2−6)

position	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** (acetone-d_6)	**6** (acetone-d_6)
1	104.4 (s)	104.6 (s)	104.2 (s)	104.3 (s)	102.4 (s)
2	165.5 (s)	165.3 (s)	165.7 (s)	165.5 (s)	162.8 (s)
3	101.0 (d)	100.7 (d)	100.8 (d)	102.4 (d)	102.3 (d)
4	165.0 (s)	164.8 (s)	164.8 (s)	163.1 (s)	162.8 (s)
5	104.1 (d)	104.1 (d)	104.5 (d)	107.7 (d)	111.8 (d)
6	141.9 (s)	142.0 (s)	142.3 (s)	144.2 (s)	143.2 (s)
1′	56.2 (d)	56.8 (d)	55.5 (d)	130.6 (d)	131.4 (d)
2′	62.5 (d)	62.3 (d)	63.4 (d)	133.6 (d)	131.7 (d)
3′	34.9 (t)	33.2 (t)	25.8 (t)	27.9 (t)	26.7 (t)
4′	71.1 (d)	69.8 (d)	28.6 (t)	28.5 (t)	31.8 (t)
5′	78.4 (d)	77.2 (d)	72.9 (d)	73.1 (d)[a]	72.3 (d)
6′	197.2 (s)	74.8 (d)	77.1 (d)	76.4 (d)	77.8 (d)
7′	129.3 (d)	131.4 (d)	133.7 (d)	135.6 (d)	135.5 (d)
8′	143.6 (d)	129.6 (d)	128.3 (d)	125.4 (d)	127.7 (d)
9′	37.8 (t)	36.7 (t)	36.4 (t)	37.9 (t)	39.9 (t)
10′	71.0 (d)	71.0 (d)	71.6 (d)	72.9 (d)[a]	72.3 (d)
-*C*OO-	170.8 (s)	170.8 (s)	170.9 (s)	172.1 (s)	175.8 (s)
10′-CH_3	19.0 (q)	19.0 (q)	18.6 (q)	19.1 (q)	20.7 (q)
4-OCH_3	55.5 (q)	55.4 (q)	55.4 (q)	−	−

[a] Assignments can be interchanged.

ditional *trans*-olefin at C-1′−C-2′ ($J_{1',2'}$ = 15.9 Hz) instead of the epoxide functionality, and also free, instead of the methylated, 4-OH group on the aromatic nucleus. The *cis*-fused acetonide structure was assigned by NOESY analyses for the derivative **17** ($J_{5',6'}$ = 5.4 Hz), synthesized from **5**. Hence, structure of aigialomycin D (**5**) was straightforwardly identified.



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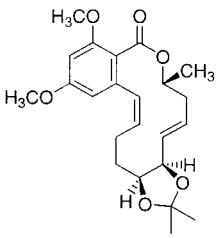

18

Aigialomycin E (**6**) has the same molecular formula to that of **5**. NMR analyses of **6** revealed a similar structural feature to that of **5**, but it differed at the olefinic geometry at C-1′−C-2′, having a *cis* ($J_{1',2'}$ = 11.6 Hz) relationship. The *trans* geometry of the C-7′−C-8′ double bond was not clear in the ^{1}H NMR spectrum of **6** due to signals overlapping. However, the ^{1}H NMR spectrum of acetonide derivative **18**, synthesized from **6**, showed vicinal ^{1}H−^{1}H coupling constants of $J_{1',2'}$ = 11.5 Hz and $J_{7',8'}$ = 15.4 Hz. These data confirmed the *cis* C-1′−C-2′ and *trans* C-7′−C-8′ double bonds' geometries. NOESY spectral analyses indicated the *cis*-acetonide structure of **18** while correlations and vicinal ^{1}H−^{1}H coupling constants revealed the relative configuration between the *cis*-diol moiety and the C-10′ asymmetric center, as well as the probable conformation of **18** (Figure 2). Thus, the large $J_{6',7'}$ value of 9.1 Hz and the intense NOESY correlations between H-6′ and H-8′ as well as the weak intensity of the cross signal between H-6′ and H-7′ were observed. These indicated the antiperiplanar relationship of H-6′ and H-7′ where the *trans*-olefin is vertical to the acetonide ring. H-7′ correlated to one of the H-9′ methylene protons at δ_H 2.33 and correlations to another H-9′ (at δ_H 2.42) from both H-8′ and H-10′ were also evident. Intense NOESY cross signal was also found between H-8′ and H-10′. The δ_H 2.33 proton was coupled to H-8′ with the *J* value of 9.8 Hz, while the NOESY cross signal between these protons was very weak indicating the

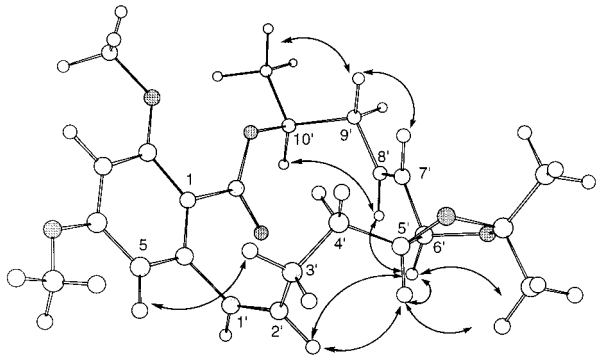

Figure 2. Probable conformation of compound **18**. Selected NOESY correlations are illustrated with solid arrows.

antiperiplanar relationship of these two protons. These NMR data placed H-6′, H-8′, and H-10′ on the same side of the macrolide ring, suggesting an approximate conformation from C-5′ to C-10′ in the molecule. Other important NOESY correlations were that of H-2′ to both H-5′ and H-6′. The structure shown in Figure 2 indicates approximate conformation for the 5′*S*,6′*R*,10′*S* isomer of **18**. Conformational studies using molecular models confirmed the above arguments. Based upon the absolute configuration of aigialomycin C (**4**), the absolute stereochemistry of aigialomycin E (**6**) should be assigned as 5′*S*,6′*R*,10′*S* rather than its mirror image (5′*R*,6′*S*,10′*R*). Aigialomycin D (**5**) should also possess the same sense of absolute configuration as **6**.

Several 14-membered resorcylic macrolides have previously been isolated;[5] e.g., zearalenone,[5a] radicicol,[5b] zeaenol,[5c] LL-Z-1640s,[5d] monocillines,[5e] nordinone,[5f] and nordinonediol.[5f] Hypothemycin and aigialomycins A and B are highly oxygenated analogues in this class. The structure of aigialomycin E, having a *cis*-olefin at C-1′-C-2′, may be of remarkable interest, since all of the previously known compounds possess either a *trans*-olefinic or saturated bond at these two carbons.

Hypothemycin (**1**) and aigialomycins A−E (**2**−**6**) were subjected to antimalarial activity screening against *Plasmodium falciparum* K1. Cytotoxicities of these compounds against two cancer cells (KB, BC-1) and Vero cells (African green monkey kidney fibroblast) were also determined (Table 3). Hypothemycin (**1**) and aigialomycin D (**5**) exhibited moderate antimalarial activity, while other congeners were inactive at a concentration of 20 μg/mL. It should be noted that compounds **1** and **5** also showed cytotoxicity; therefore, the activity of these compounds against *P. falciparum* may well be related to their cytotoxic activity. Hypothemycin is reported to exhibit moderate antibiotic activity against the protozoan, *Tetrahymena furgasoni*, and the plant pathogenic fungi *Ustilago maydis* and *Botrytis allii*.[3] It is also known to be cytotoxic against P388, L1210, Colon 26, A549, and DLD-1 with IC_{50} values of 0.252−1.50 μg/mL.[4]

(5) (a) Urry, W. H.; Wehrmeister, H. L.; Hodge, E. B.; Hidy, P. H. *Tetrahedron Lett*. **1966**, 3109−3114. (b) Mirrington, R. N.; Ritchie, E.; Shoppee, C. W.; Taylor, W. C.; Sternhell, S. *Tetrahedron Lett*. **1964**, 365−370. (c) Sugawara, F.; Kim, K.-W.; Kobayashi, K.; Uzawa, J.; Yoshida, S.; Murofushi, N.; Takahashi, N.; Strobel, G. A. *Phytochemistry* **1992**, *31*, 1987−1990. (d) Ellestad, G. A.; Lovell, F. M.; Perkinson, N. A.; Hargreaves, R. T.; McGahren, W. J. *J. Org. Chem*. **1978**, *43*, 2339−2343. (e) Ayer, W. A.; Lee, S. P.; Tsuneda, A.; Hiratsuka, Y. *Can. J. Microbiol*. **1980**, *26*, 766−773. (f) Ayer, W. A.; Pena-Rodrigues, L. *Phytochemistry* **1987**, *26*, 1353−1355.

Table 3. Antimalarial Activity and Cytotoxicity of Macrolides 1−6

compound	*P. falciparum* K1 (IC_{50}, μg/mL)	cytotoxicity (IC_{50} μg/mL)[b] KB	BC-1	Vero
hypothemycin (**1**)	2.2	17	6.2	6.3
aigialomycin A (**2**)	>20	>20	11	4.3
aigialomycin B (**3**)	>20	>20	>20	>20
aigialomycin C (**4**)	>20	>20	>20	>20
aigialomycin D (**5**)	6.6	3.0	18	1.8
aigialomycin E (**6**)	>20	>20	15	>20
chloroquine diphosphate[a]	0.16	16	>20	>20

[a] Standard antimalarial compound. [b] IC_{50} values of a standard compound ellipticine are 0.46 μg/mL for KB cells, 0.60 μg/mL for BC-1 cells, and 1.0 μg/mL for Vero cells.

Experimental Section

Isolation of Macrolides. *A. parvus* was collected, identified, and isolated from mangrove wood by Prof. E. B. G. Jones. This fungus is deposited at the BIOTEC Culture Collection as BCC 5311. An isolated culture of the strain BCC 5311 was grown on potato dextrose agar (PDA) at 22 °C for 35 days, before inoculation into 56 × 1 L Erlenmeyer flasks each containing 250 mL of potato dextrose broth (PDB). After static incubation at 22 °C for 35 days, the flask cultures were filtered, and the filtrate (14 L) was extracted with equal volume of EtOAc to obtain a light brown solid (1.4 g) after solvent evaporation. The crude extract was passed through a Sephadex LH-20 column chromatography (3.5 × 25 cm) using MeOH/CH_2Cl_2 = 75:25 as an eluent to obtain a mixture of macrolides. This mixture was subjected to column chromatography on silica gel (3.5 × 15 cm; step gradient elution with MeOH/CH_2Cl_2, 2:98 to 30:70). The following fractions were obtained in the order of elution: Fr-A, compounds **2** (major) and **1**, 136 mg; Fr-B, compounds **2**, **1** (major), and **4**, 661 mg; Fr-C, compounds **3**, **5** and **6**, 133 mg. Fr-A was subjected to preparative HPLC using a reversed-phase column (Prep Nova-Pak HR C_{18}, 6 μM, 40 × 100 mm) with MeCN/H_2O = 50:50 as an eluent at a flow rate of 20 mL/min to obtain **2** (61 mg) and **1** (22 mg). Fr-B was triturated twice in MeOH (5 mL, room temp, 24 h) to obtain colorless crystals of **1** (283 mg), and the dried filtrate (354 mg) was subjected to prep HPLC (MeOH/H_2O = 50:50) to yield **2** (40 mg), **1** (73 mg) and **4** (33 mg). Fr-C was chromatographed on silica gel (1.2 × 15 cm, MeOH/EtOAc, step gradient elution, 0:100 to 5:95) to obtain a mixture of **5** and **6** (50 mg), and **3** (40 mg). Compounds **5** and **6** were separated by prep HPLC (MeOH/H_2O = 40:60): **5** (25 mg); **6** (7.5 mg). Compound **3** was further purified by prep HPLC (yield, 28 mg).

Hypothemycin (1). Colorless prisms (MeOH): mp 170−172 °C; $[\alpha]^{25}_D$ −18 (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 220 (4.53), 267 (4.12), 308 (3.80) nm; IR (KBr) ν_{max} 3390, 1685, 1645, 1619, 1576, 1254, 1048 cm^{-1}; MS (ESI-TOF) *m*/*z* 401 $[M + Na]^+$, 379 $[M + H]^+$, 361, 343, 253, 235; ^1H and ^{13}C NMR data were identical to those reported in the literature.[4] Anal. C 60.43%, H 5.79%, calcd for $C_{19}H_{22}O_8$, C 60.31%, H 5.86%.

Aigialomycin A (2). Colorless crystals: mp 166−168 °C (dec); $[\alpha]^{25}_D$ +17 (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 220 (4.50), 266 (4.04), 306 (3.74) nm; IR (KBr) ν_{max} 3509, 1668, 1645, 1623, 1580, 1261, 1044 cm^{-1}; MS (ESI-TOF) *m*/*z* 401 $[M + Na]^+$, 379 $[M + H]^+$, 361, 255, 218; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ 379.1397 (calcd for $C_{19}H_{23}O_8$, 379.1393); ^1H and ^{13}C NMR data, see Tables 1 and 2. Anal. C 60.46%, H 5.87%, calcd for $C_{19}H_{22}O_8$, C 60.31%, H 5.86%.

Aigialomycin B (3). Colorless crystals: mp 82−84 °C; $[\alpha]^{24}_D$ −10 (*c* 0.27, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 220 (4.37), 266 (4.28), 306 (3.74) nm; IR (KBr) ν_{max} 3403, 1651, 1616, 1576, 1256, 1046 cm^{-1}; MS (ESI-TOF) *m*/*z* 381 $[M + H]^+$, 269, 256; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ 381.1548 (calcd for $C_{19}H_{25}O_8$, 381.1549); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Aigialomycin C (4). Colorless amorphous solid: $[\alpha]^{27}_D$ −20 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 219 (4.45), 265 (4.14),

306 (3.82) nm; IR (KBr) ν_{max} 3419, 1646, 1615, 1575, 1318, 1257, 1204, 1160, 1041, 966 cm^{-1}; MS (ESI-TOF) *m/z* 365 [M + H]$^+$, 347, 288; HRMS (ESI-TOF) *m/z* [M + H]$^+$ 365.1617 (calcd for $C_{19}H_{25}O_7$, 365.1600); ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

Aigialomycin D (5). Colorless crystals: mp 83−85 °C; $[\alpha]^{24}_D$ −19 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (4.42), 275 (4.05), 314 (3.71) nm; IR (KBr) ν_{max} 3336, 1640, 1602, 1315, 1262, 1165, 1013, 968 cm^{-1}; MS (EI) *m/z* 334 [M]$^+$, 316, 237, 219, 208, 189, 175, 163; HRMS (ESI-TOF) *m/z* [M + H]$^+$ 335.1499 (calcd for $C_{18}H_{23}O_6$, 335.1494); ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

Aigialomycin E (6). Colorless amorphous solid: mp 91−94 °C; $[\alpha]^{24}_D$ +14 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.31), 268 (4.00), 307 (3.69) nm; IR (KBr) ν_{max} 3401, 1646, 1615, 1318, 1261, 1163, 1021, 969 cm^{-1}; MS (EI) *m/z* 334 [M]$^+$, 316, 237, 219, 189, 175; HRMS (ESI-TOF) *m/z* [M + H]$^+$ 335.1498 (calcd for $C_{18}H_{23}O_6$, 335.1494); ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

Hydrogenation of 1. To a solution of hypothemycin (**1**, 30 mg) in EtOAc (4 mL) was added 10% Pd/C (20 mg), and the mixture was stirred under hydrogen for 26 h. The suspension was filtered by suction, and the filtrate was concentrated and subjected to column chromatography on silica gel (MeOH/CH_2Cl_2) to obtain dihydrohypothemycin **7** (25.4 mg, 84%). Recrystallization from MeOH gave colorless crystals. Hydrogenation of compound **2** (20 mg) under the same reaction conditions gave a compound (13.5 mg, 67%) whose spectral data were identical to **7**.

Dihydrohypothemycin (7). Colorless crystals: mp 175−177 °C; $[\alpha]^{26}_D$ −16 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 (4.44), 265 (4.14), 305 (3.81) nm; IR (KBr) ν_{max} 3404, 1698, 1650, 1618, 1256, 1159, 1051, 1023 cm^{-1}; HRMS (ESI-TOF) *m/z* [M + H]$^+$ 381.1541 (calcd for $C_{19}H_{25}O_8$, 381.1549); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.98 (1H, s, 2-O*H*), 6.50 (1H, d, *J* = 2.6 Hz, H-5), 6.41 (1H, d, *J* = 2.5 Hz, H-3), 5.26 (1H, m, H-10′), 4.52 (1H, brs, H-5′), 4.42 (1H, d, *J* = 1.4 Hz, H-1′), 4.22 (1H, brd, *J* = 10.5 Hz, H-4′), 3.81 (3H, s, 4-OC*H*$_3$), 3.58 (1H, brs, 5′-O*H*), 2.98 (1H, d, *J* = 8.5 Hz, H-2′), 2.82 (1H, m, H-7′a), 2.42 (1H, m, H-7′b), 2.38 (1H, brs, 4′-O*H*), 2.16 (1H, dd, *J* = 15.4, 10.9 Hz, H-3′a), 1.80−1.64 (4H, m, H-8′ and H-9′), 1.39 (3H, d, *J* = 6.3 Hz, 10′-C*H*$_3$), 1.20 (1H, dd, *J* = 15.5, 8.5 Hz, H-3′b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.3 (s, C-6′), 170.9 (s, -*C*OO-), 165.9 (s, C-4), 165.1 (s, C-2), 142.0 (s, C-6), 104.0 (s, C-1), 103.9 (d, C-5), 101.0 (d, C-3), 82.2 (d, C-5′), 73.0 (d, C-10′), 69.7 (d, C-4′), 61.1 (d, C-2′), 58.3 (d, C-1′), 55.5 (q, 4-O*C*H$_3$), 38.5 (t, C-7′), 34.6 (t, C-3′), 34.5 (t, C-9′), 21.2 (t, C-8′), 20.5 (q, 10′-*C*H$_3$).

Preparation of Acetonides 10 and 11. To a solution of aigialomycin B (**3**; 6.0 mg) in 2,2-dimethoxypropane (1 mL) was added a very small amount of *p*-TsOH·H_2O. After stirring at room temperature for 3 h, saturated aq $NaHCO_3$ was added and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to obtain an acetonide mixture (**8** and **9**; 7.3 mg). The crude acetonide mixture was dissolved in 2-butanone (0.3 mL), MeI (50 μL) and K_2CO_3 (s) (50 mg) were added, and the mixture was stirred for 34 h. After standard aqueous workup, the crude product (7.9 mg) was subjected to preparative reversed-phase HPLC (40 × 100 mm; MeCN/H_2O = 35:65; 20 mL/min) to obtain compounds **10** (4.2 mg; t_R 22 min) and **11** (2.3 mg; t_R 16 min).

Acetonide 10. Colorless amorphous solid: IR (KBr) ν_{max} 3486, 1722, 1605, 1460, 1262, 1161, 1045 cm^{-1}; HRMS (ESI-TOF) *m/z* [M + H]$^+$ 435.2032 (calcd for $C_{23}H_{31}O_8$, 435.2019); ^{1}H NMR ($CDCl_3$, 400 MHz) selected signals, δ 4.52 (1H, dd, *J* = 7.7, 7.7 Hz, H-6′), 4.18 (1H, brd, *J* = ca 12 Hz, H-4′), 3.84 (1H, m, H-5′), 1.45 (6H, s, acetonide C*H*$_3$ × 2); ^{1}H NMR (methanol-d_4, 400 MHz) δ 6.58 (1H, d, *J* = 2.2 Hz, H-3), 6.37 (1H, d, *J* = 2.2 Hz, H-5), 6.02 (1H, ddd, *J* = 15.7, 6.4, 5.5 Hz, H-8′), 5.74 (1H, dd, *J* = 15.7, 7.4 Hz, H-7′), 5.61 (1H, m, H-10′), 4.53 (1H, dd, *J* = 7.7, 7.7 Hz, H-6′), 4.19 (1H, ddd, *J* = 11.5, 3.8, 1.5 Hz, H-4′), 3.94 (1H, d, *J* = 1.6 Hz, H-1′), 3.85 (3H, s, 2-OC*H*$_3$ or 4-OC*H*$_3$), 3.84 (3H, s, 4-OC*H*$_3$ or 2-OC*H*$_3$), 3.76 (1H, dd, *J* = 8.0, 1.6 Hz, H-5′), 2.88 (1H, m, H-2′), 2.57 (1H, m, H-9′a), 2.47 (1H, m, H-9′b), 2.28 (1H, ddd, *J* = 14.2, 3.5, 3.3 Hz, H-3′a), 1.57 (1H, ddd, *J* = 14.0, 11.6, 8.8 Hz, H-3′b), 1.45 (3H, s, acetonide), 1.42 (3H, s, acetonide), 1.40 (3H, d, *J* = 6.5 Hz, 10′-C*H*$_3$).

Acetonide 11. Colorless amorphous solid: IR (KBr) ν_{max} 3484, 1723, 1606, 1460, 1261, 1160, 1043 cm^{-1}; HRMS (ESI-TOF) *m/z* [M + H]$^+$ 435.2034 (calcd for $C_{23}H_{31}O_8$, 435.2019); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.39 (2H, brs, H-3 and H-5), 5.97 (1H, ddd, *J* = 16.0, 6.5, 6.1 Hz, H-8′), 5.68 (1H, dd, *J* = 16.0, 7.2 Hz, H-7′), 5.41 (1H, m, H-10′), 4.27 (1H, m, H-4′), 4.13 (1H, dd, *J* = 9.5, 7.2 Hz, H-6′), 4.07 (1H, dd, *J* = 9.6, 5.0 Hz, H-5′), 3.88 (1H, d, *J* = 1.7 Hz, H-1′), 3.81 (3H, s, 2-OC*H*$_3$ or 4-OC*H*$_3$), 3.79 (3H, s, 4-OC*H*$_3$ or 2-OC*H*$_3$), 2.87 (1H, m, H-2′), 2.68 (1H, brs, 6′-O*H*), 2.48 (1H, m, H-9′a), 2.42 (1H, m, H-9′b), 2.02 (1H, m, H-3′a), 1.98 (1H, m, H-3′b), 1.49 (3H, s, acetonide), 1.39 (3H, d, *J* = 6.4 Hz, 10′-C*H*$_3$), 1.37 (3H, s, acetonide).

Acetonide 12. Colorless powder: HRMS (ESI-TOF) *m/z* [M + H]$^+$ 419.1724 (calcd for $C_{22}H_{27}O_8$, 419.1706); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.76 (1H, s, 2-O*H*), 6.96 (1H, ddd, *J* = 16.4, 7.2, 7.1 Hz, H-8′), 6.50 (1H, d, *J* = 2.6 Hz, H-5), 6.42 (1H, d, *J* = 2.6 Hz, H-3), 6.27 (1H, d, *J* = 16.4 Hz, H-7′), 5.55 (1H, m, H-10′), 4.97 (1H, d, *J* = 6.3 Hz, H-5′), 4.54 (1H, ddd, *J* = 6.4, 6.4, 5.4 Hz, H-4′), 4.34 (1H, d, *J* = 1.6 Hz, H-1′), 3.80 (3H, s, 4-OC*H*$_3$), 2.83 (1H, m, H-9′a), 2.66 (1H, m, H-2′), 2.61 (1H, m, H-9′b), 2.14 (1H, m, H-3′a), 1.80 (1H, ddd, *J* = 15.1, 7.0, 6.8 Hz, H-3′b), 1.63 (3H, s, acetonide), 1.46 (3H, d, *J* = 6.5 Hz, 10′-C*H*$_3$), 1.39 (3H, s, acetonide).

Acetonide 13. Colorless powder: HRMS (ESI-TOF) *m/z* [M + H]$^+$ 419.2007 (calcd for $C_{23}H_{31}O_7$, 419.1992); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.41 (1H, d, *J* = 2.3 Hz, H-3), 6.30 (1H, d, *J* = 2.3 Hz, H-5), 6.01 (1H, ddd, *J* = 15.7, 7.3, 4.7 Hz, H-8′), 5.71 (1H, dd, *J* = 15.8, 6.7 Hz, H-7′), 5.61 (1H, m, H-10′), 4.01 (1H, dd, *J* = 7.5, 7.3 Hz, H-6′), 3.85 (1H, d, *J* = 1.7 Hz, H-1′), 3.81 (3H, s, 2-OC*H*$_3$), 3.79 (3H, s, 4-OC*H*$_3$), 3.72 (1H, ddd, *J* = 8.1, 8.1, 4.8 Hz, H-5′), 2.84 (1H, ddd, *J* = 6.9, 2.2, 2.1 Hz, H-2′), 2.48 (1H, m, H-9′a), 2.40 (1H, m, H-9′b), 2.17 (1H, m, H-3′a), 1.94 (1H, m, H-4′a), 1.73 (1H, m, H-4′b), 1.55 (1H, m, H-3′b), 1.43 (3H, s, acetonide), 1.41 (3H, s, acetonide), 1.37 (3H, d, *J* = 6.3 Hz, 10′-C*H*$_3$).

Acetonide 17. Colorless powder: HRMS (ESI-TOF) *m/z* [M + H]$^+$ 403.2126 (calcd for $C_{23}H_{31}O_6$, 403.2120); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.56 (1H, d, *J* = 1.9 Hz, H-5), 6.35 (1H, d, *J* = 1.9 Hz, H-3), 6.26 (1H, d, *J* = 15.4 Hz, H-1′), 6.14 (1H, ddd, *J* = 15.3, 9.9, 4.2 Hz, H-2′), 5.73 (1H, ddd, *J* = 15.2, 9.3, 3.5 Hz, H-8′), 5.59 (1H, ddd, *J* = 15.2, 9.6, 1.6 Hz, H-7′), 5.32 (1H, m, H-10′), 4.57 (1H, dd, *J* = 9.6, 5.4 Hz, H-6′), 4.19 (1H, ddd, *J* = 11.6, 5.3, 3.1 Hz, H-5′), 3.82 (3H, s, 2-OC*H*$_3$), 3.79 (3H, s, 4-OC*H*$_3$), 2.51 (1H, m, H-9′a), 2.47 (1H, m, H-9′b), 2.30 (1H, m, H-3′a), 2.10 (1H, m, H-4′a), 1.82 (1H, m, H-3′a), 1.53 (1H, m, H-4′b), 1.47 (3H, s, acetonide), 1.36 (3H, d, *J* = 6.1 Hz, 10′-C*H*$_3$), 1.35 (3H, s, acetonide).

Acetonide 18. Colorless powder: HRMS (ESI-TOF) *m/z* [M + H]$^+$ 403.2134 (calcd for $C_{23}H_{31}O_6$, 403.2120); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.47 (1H, d, *J* = 11.5 Hz, H-1′), 6.36 (1H, d, *J* = 2.0 Hz, H-3), 6.29 (1H, d, *J* = 1.8 Hz, H-5), 5.74 (1H, ddd, *J* = 11.1, 11.0, 5.5 Hz, H-2′), 5.57 (1H, ddd, *J* = 15.4, 9.8, 3.9 Hz, H-8′), 5.43 (1H, ddd, *J* = 15.3, 9.1, 1.0 Hz, H-7′), 5.20 (1H, m, H-10′), 4.49 (1H, dd, *J* = 9.0, 5.8 Hz, H-6′), 4.15 (1H, ddd, *J* = 8.0, 5.5, 4.3 Hz, H-5′), 3.81 (6H, s, 2-OC*H*$_3$ and 4-OC*H*$_3$), 2.63 (1H, m, H-3′a), 2.42 (1H, m, H-9′a), 2.33 (1H, m, H-9′b), 1.96 (1H, m, H-3′b), 1.64 (1H, m, H-4′a), 1.55 (1H, m, H-4′b), 1.43 (3H, s, acetonide), 1.37 (3H, d, *J* = 6.3 Hz, 10′-C*H*$_3$), 1.35 (3H, s, acetonide).

Synthesis of Compound 16. To a solution of aigialomycin C (**4**; 3.0 mg) in pyridine (0.3 mL) was added excess *p*-bromobenzoyl chloride (10 mg), and the mixture was stirred at room temperature for 2 days. After the usual aqueous workup, the crude mixture was subjected to preparative HPLC (reversed-phase column; MeCN/H_2O = 80:20, then 100:0) to obtain the triester **14** (4.6 mg). This product was treated in aq K_2CO_3 (0.2 mL)/dioxane (0.4 mL)/MeOH (0.2 mL) for 6 h and then concentrated in vacuo. Standard aqueous workup and purification by column chromatography on silica gel (CH_2Cl_2) afforded compound **15** (3.0 mg). A portion of **15** (1.0 mg) was treated with 3,5-dinitrobenzoyl chloride (10 mg) in pyridine (0.15 mL) for 2 days. The usual aqueous workup and subse-

quent CC on silica gel gave the mixed ester **16** (0.5 mg). Recrystallization in EtOAc gave colorless crystals: MS (ESI-TOF) *m/z* 945, 947 and 949 $[M + Na]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.31 (1H, t, $J = 1.9$ Hz), 9.29 (2H, d, $J = 1.8$ Hz), 7.79 (2H, d, $J = 8.4$ Hz), 7.75 (2H, d, $J = 8.5$ Hz), 7.52 (2H, d, $J = 8.4$ Hz), 7.48 (2H, d, $J = 8.5$ Hz), 6.84 (1H, d, $J = 2.4$ Hz), 6.76 (1H, d, $J = 2.2$ Hz), 6.25 (1H, m), 5.78 (1H, dd, $J = 9.3$, 9.1 Hz), 5.65 (1H, dd, $J = 15.8$, 9.3 Hz), 5.48 (1H, m), 5.23 (1H, m), 4.23 (1H, brs), 3.86 (3H, s), 2.81 (1H, brd, $J = 9.2$ Hz), 2.61 (1H, m), 2.46 (1H, m), 2.29 (1H, m), 2.14 (1H, m), 1.72 (1H, m), 1.47 (1H, m), 1.40 (3H, d, $J = 6.3$ Hz).

Single-Crystal X-ray Diffraction Analysis of 16. Crystallographic data: $C_{40}H_{32}N_2O_{14}Br_2$, MW 924.50, monoclinic, $P2_1$, $a = 13.0176(4)$ Å, $b = 9.1772(3)$ Å, $c = 13.7093(6)$ Å, and $\beta = 104.034(2)°$, $V = 2168.4(1)$ Å^3, $Z = 2$, $D_x = 1.465$ g/cm^{-3}. The asymmetric unit contained one molecule of **16** and one disordered solvated methanol. Absolute configuration of **16** was established by using anomalous scattering methods (Flack parameter = +0.069(1) and 0.685(2) for the inverted structure). Of the 7183 unique reflections, the 6768 reflections with $F > 4\sigma(F)$ were used in the least-squares refinement to yield $R = 0.0623$ and $R_w = 0.1661$. The atomic coordinates have been deposited at the Cambridge Crystallographic Data Center (CCDC 168877). Copies of the data can be obtained, on request, from the Director at Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

Biological Assays. The assay for activity against *P. falciparum* K1 was performed using the protocol previously reported[6] which follows the microculture radioisotope technique described by Desjardins.[7] IC_{50} represents the concentration that causes 50% reduction of parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. Cytotoxicity of the purified compounds against human epidermoid carcinoma (KB cells), human breast cancer cells (BC-1 cells), and African green monkey kidney fibroblast (Vero cells) were tested using the colorimetric method.[8]

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Supporting Information Available: Detailed information of the X-ray crystallographic analysis of compound **16** including structure diagram, crystal data, refinement, atomic coordinates, isotropic displacement parameters, bond lengths, and angles. This material is available free of charge via the Internet at http://pubs.acs.org.

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