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Aigialomycins and related polyketide metabolites from the mangrove fungus *Aigialus parvus* BCC 5311

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ABSTRACT

Reinvestigation of the secondary metabolites from the marine mangrove fungus *Aigialus parvus* BCC 5311 led to the isolation of six new nonaketide metabolites, aigialomycins F (**4**) and G (**5a**/**5b**), 7′,8′-dihydroaigialospirol (**7**), 4′-deoxy-7′,8′-dihydroaigialospirol (**8**), and rearranged macrolides **9** and **10**, along with six previously described compounds, hypothemycin (**1**), aigialomycins A (**2**) and B (**3**), aigialospirol (**6**), 4-*O*-demethylhypothemycin (**11**), and aigialone (**12**). The structures of the new compounds were elucidated by analyses of the NMR spectroscopic and mass spectrometry data in combination with chemical means.

1. Introduction

Hypothemycin (**1**)[1,2] is one of the highly oxygenated analogues in the group of 14-membered resorcylic acid lactones.[3] Although only marginal antifungal and cytotoxic activities of **1** were initially reported,[1,2] the recent discovery that hypothemycin (**1**) and several closely related compounds are potent protein kinase inhibitors has stimulated a renewed interest in this class of natural products.[4–7] We previously isolated hypothemycin along with five resorcylic acid lactones aigialomycins A–E from the mangrove fungus *Aigialus parvus* BCC 5311.[8] In the subsequent studies, extension of the fungus incubation period in a liquid medium from 35 to 80 days resulted in the production of a hypothemycin-derived spiroacetal compound, aigialospirol (**6**), along with a biogenetically different class of metabolite, aigialone (**12**).[9] Because of the increasing attention of hypothemycin (**1**) and aigialomycins,[3,10] we have reinvestigated the fungus BCC 5311. We report herein the isolation and structure elucidation of six new derivatives, aigialomycins F (**4**) and G (**5a**/**5b**), 7′,8′-dihydroaigialospirol (**7**), 4′-deoxy-7′,8′-dihydroaigialospirol (**8**), and rearranged 13-membered macrolides **9** and **10**, together with previously described compounds, hypothemycin (**1**), aigialomycins A (**2**) and B (**3**), aigialospirol (**6**), 4-*O*-demethylhypothemycin (**11**),[11] and aigialone (**12**).

2. Results and discussion

A. parvus BCC 5311 was fermented under static conditions for two incubation periods of 35 and 80 days in PDB (20 L for each, 80×250 mL). Although no new hypothemycin/aigialomycin analogue was found in the 35-day culture broth, investigation of the EtOAc extract of the 80-day culture broth resulted in the isolation of six new compounds **4**, **5a**/**5b**, and **7**–**10** as minor constituents along with **1** (most abundant constituent), aigialomycins A (**2**) and B (**3**), and aigialospirol (**6**). The same extract also provided 4-*O*-demethylhypothemycin (**11**) and aigialone (**12**). Compound **11** was recently isolated from *Hypomyces subiculosus* DSM 11931 and DSM 11932 as one of the minor co-metabolites with **1**.[11]

Aigialomycin F (**4**) was the most polar constituent whose molecular formula was established as $C_{19}H_{26}O_9$ by HRMS (ESI-TOF). The ^{1}H NMR spectrum in DMSO-d_6 showed a chelated phenolic proton at δ_H 11.16 (s, 2-O*H*) and five hydroxy protons (D_2O exchangeable) of aliphatic secondary alcohols, all appearing as doublets. The structure of the resorcylic acid region was addressed by HMBC correlations: from H-3 to C-1 and C-2, from H-5 to C-1 and C-3, from 2-O*H* to C-1, C-2, and C-3, and from the methoxy protons (δ_H

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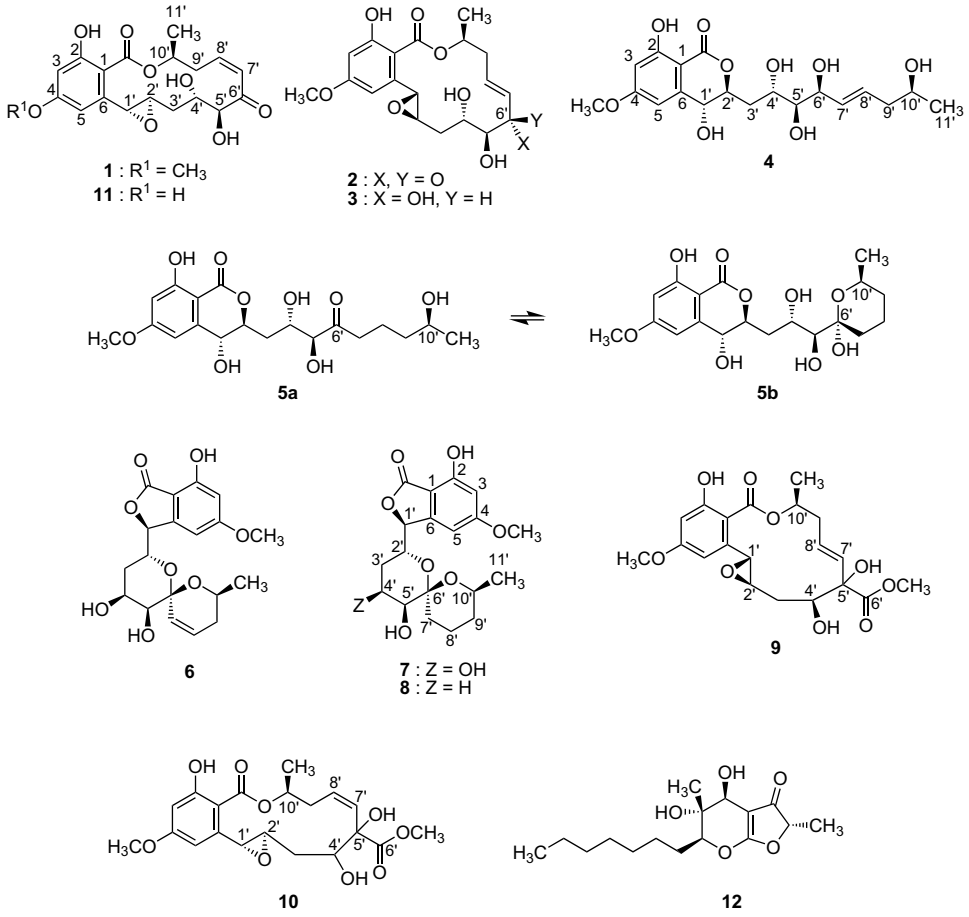


Table 1
NMR data for **4** and **5a**/**5b** (500 MHz for ^{1}H and 125 MHz for ^{13}C)

Position	**4** (in DMSO-d_6)			**5a**/**5b** (in acetone-d_6)			
	δ_C, mult.	δ_H, mult (J in Hz)	HMBC	δ_C, mult.	δ_H, mult (J in Hz)	δ_C	δ_H, mult (J in Hz)
1-COO–	168.5, C			168.5, C		168.6	
1	100.6, C			100.0, C		100.1	
2	163.6, C			164.1, C		164.1	
2-O*H*		11.16, s			11.22, s		11.26, s
3	100.5, CH	6.48, br s	1,2	99.9, CH	6.43, d (2.4)	99.8	6.43
4	166.2, C			166.5, C		166.5	
4-O*C*H_3	56.3, CH_3	3.83, s	4	55.3, CH_3	3.88, s	55.3	3.88, s
5	105.3, CH	6.59, br s	1,3	104.3, CH	6.69, dd (2.4, 0.7)	104.4	6.69
6	145.6, C			145.3, C		145.3	
1′	67.2, CH	4.51, dd (6.6, 6.1)	1,6,3′	67.4, CH	4.68, br dd (8.3, 6.1)	67.6	4.68
1′-O*H*		6.07, d (6.1)			5.28, br d (6.1)		5.29, m
2′	80.8, CH	4.59, m		80.0, CH	4.64, ddd (10.0, 8.3, 2.4)	80.0	4.68
3′	36.0, CH_2	1.84, m	2′	35.0, CH_2	2.13, ddd (14.3, 10.8, 2.4)	36.9	2.24, m
		1.70, m			1.76, ddd (14.3, 10.0, 1.9)		1.99, m
4′	66.4, CH	3.72, m		68.3, CH	4.16, m	67.2	4.17, m
4′-O*H*		4.82, d (6.3)	4′,5′		4.37, br d (5.7)		4.80, br d (4.7)
5′	77.6, CH	3.07, dt (3.1, 6.8)		80.2, CH	4.11, m	77.7	3.20, t (7.9)
5′-O*H*		4.40, d (6.8)	4′,5′,6′		4.48, br d (5.1)		3.72, m
6′	71.5, CH	4.08, m		211.6, C		98.0	
6′-O*H*		4.48, d (6.0)	5′,6′				5.28, m
7′	133.9, CH	5.52, dd (15.5, 5.4)		39.3, CH_2	2.72, ddd (17.9, 8.3, 6.5)	29.7	1.73, m
					2.63, ddd (17.9, 8.2, 6.3)		1.59, m
8′	127.9, CH	5.56, dt (15.5, 6.3)		19.4, CH_2	1.67, m	18.4	1.87, m
					1.58, m		1.59, m
9′	42.7, CH_2	2.10, m	7′,8′,10′,11′	38.6, CH_2	1.40, m	32.9	1.57, m
		2.00, m	7′,8′,10′,11′		1.38, m		1.15, m
10′	66.4, CH	3.61, m		66.5, CH	3.71, m	65.9	4.08, m
10′-O*H*		4.43, d (4.4)	10′,11′		3.49, br d (3.8)		
11′	23.5, CH_3	1.02, d (6.1)	9′,10′	23.1, CH_3	1.10, d (6.2)	21.4	1.07, d (6.3)

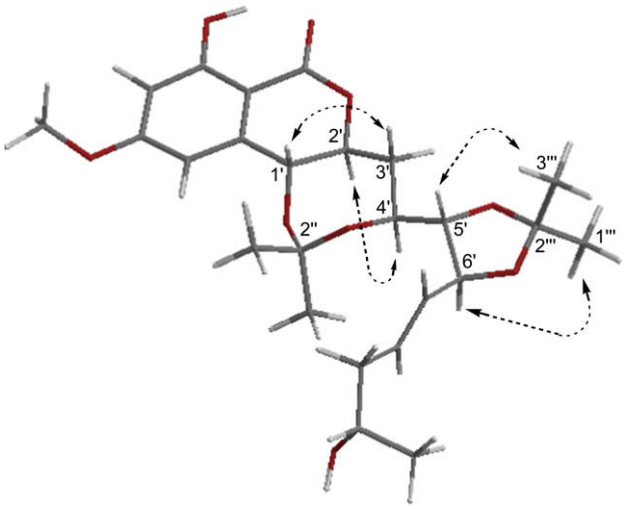


Figure 1. Selected NOESY correlations for **13**.

3.83, 3H, s) to C-4. The side-chain structure from C-1′ to C-11′, through a *trans*-olefin (C-7′/C-8′, J=15.5 Hz), was elucidated primarily by COSY correlations, which also revealed that five hydroxy groups were attached to C-1′, C-4′, C-5′, C-6′, and C-10′. HMBC correlations from H-1′ to C-1 (δ_C 100.6) and C-6 (δ_C 145.6) indicated the connection of C-1′ to C-6. The downfield H-2′ (δ_H 4.59, m) and C-2′ (δ_C 80.8) strongly suggested the δ-lactone form, which was also required from the molecular formula (HRMS). The ^1H–^1H $J_{1',2'}$ values of 6.6 Hz in DMSO-d_6 and 7.7 Hz in acetone-d_6–D_2O suggested a trans relation of these protons. The remaining stereochemistry was addressed by derivatization and correlation to aigialomycin G (**5a**/**5b**) as described below.

The molecular formula of aigialomycin G was determined by HRMS as $C_{19}H_{26}O_9$. The ^1H and ^{13}C NMR spectra indicated that it exists as a linear form (**5a**) and a hemiacetal form (**5b**), which interconvert each other. The **5a**/**5b** ratio in acetone-d_6 was probably sensitive to concentrations of the compound and trace H_2O, as it varied in each sample from 3:1 to 1.3:1 (^1H NMR spectroscopy). The ^1H and ^{13}C resonances of **5a** and **5b** were superimposed for the resorcylic acid moiety, whereas the resonances were clearly resolved for the C-1′–C-11′ portion. The connectivity of the ketone **5a** from C-1′ to C-5′, and from C-7′ to C-11′ was addressed by analysis of COSY spectrum. The ketone (δ_C 211.6) was placed at the C-6′ position on the basis of the HMBC correlations from four methylene protons, H-7′ (δ_H 2.72 and 2.63) and H-8′ (δ_H 1.67 and 1.58) to this

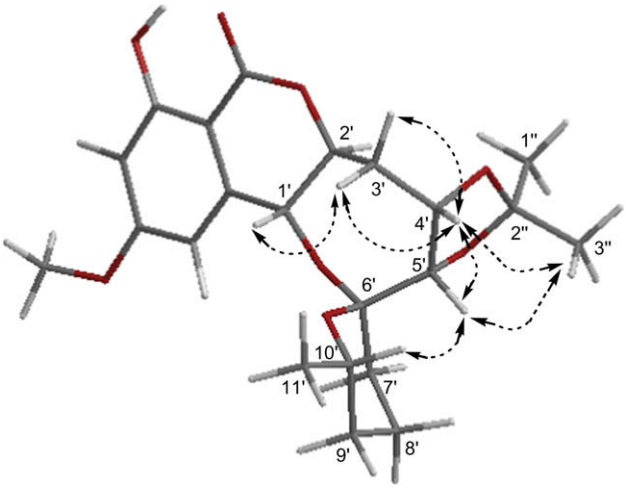
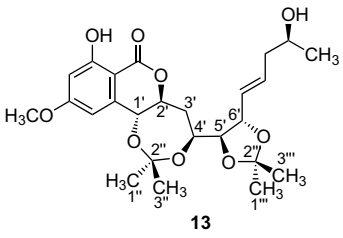


Figure 2. Selected NOESY correlations for **14**.



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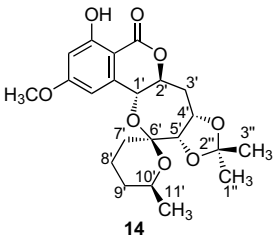

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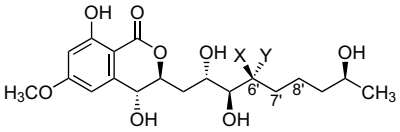


15 : X = OH, Y = H
16 : X = H, Y = OH

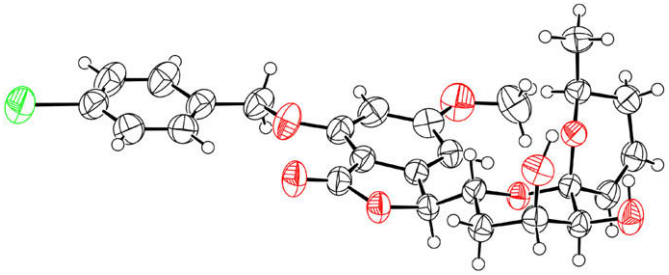


Figure 3. Crystallographic structure of 2-*O*-(4-bromobenzyl)aigialospirol (**17**). The ORTEP diagram is drawn at 50% probability level.

carbon. The $J_{1',2'}$ value of 8.3 Hz indicated the pseudoaxial orientation of these protons. The hemiacetal form **5b** was apparent from the presence of a quaternary carbon, which resonated at δ_C 98.0 (C-6′) and showed HMBC correlations from H-5′ and δ_H 5.28 hydroxy proton (6′-O*H*). This hemiacetal hydroxy proton also showed HMBC correlation to C-7′. Although the key HMBC correlation from H-10′

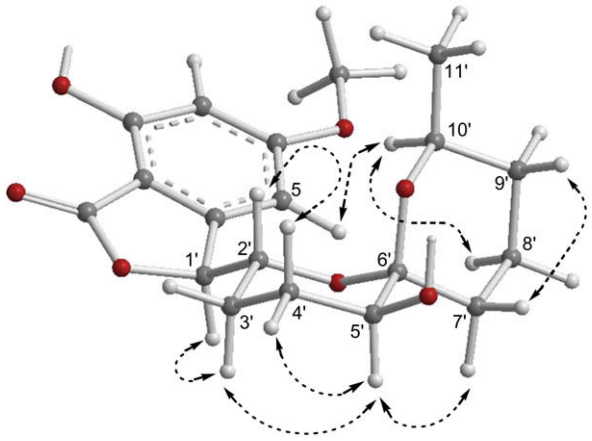


Figure 4. Selected NOESY correlations for **8**.

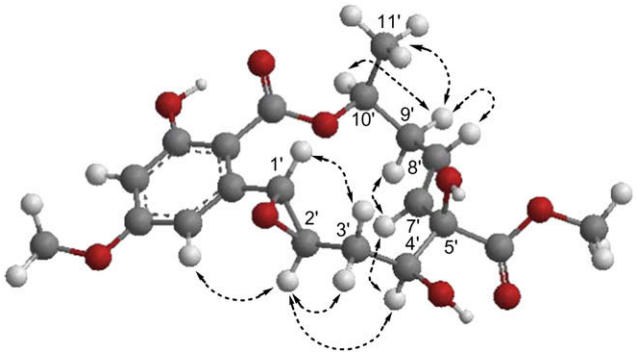


Figure 5. Probable stereo structure and key NOESY correlations of **9**. The absolute configuration of the tertiary alcohol (C-5′) is uncertain, therefore, the methoxycarbonyl group and the hydroxyl group attached to this carbon could be interchanged.

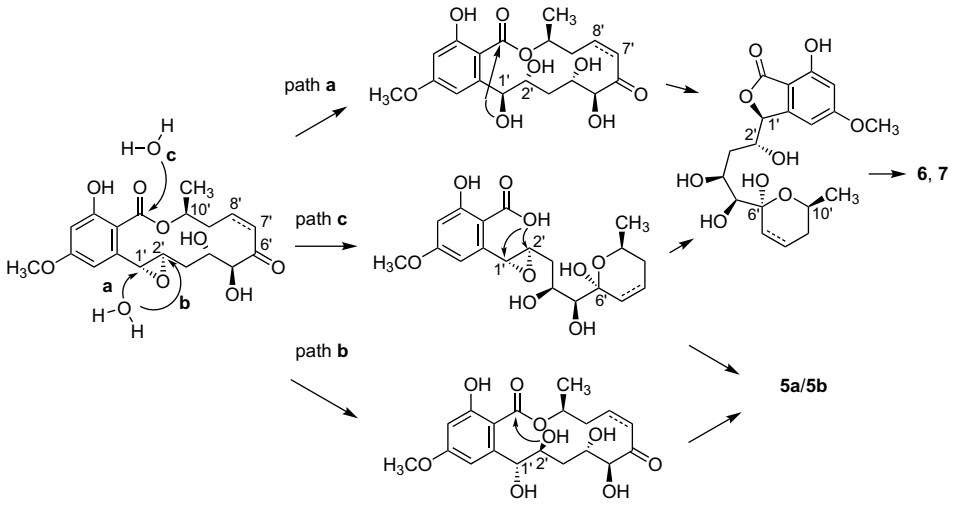
(δ_H 4.08, m) to C-6′ was not observed at long range J_{CH} of 15.4 and 10 Hz, this proton (H-10′) was downfield shifted when compared to the linear form **5a** (δ_H 3.71).

The stereochemistry of aigialomycins F (**4**) and G (**5a**/**5b**) was addressed by conversion to acetonide derivatives. We first examined preparation of two five-membered acetonide derivatives of aigialomycin F (**4**), due to the involvement of 4′,5′-diol and 5′,6′-diol. Unexpectedly, treatment of **4** with *p*-TsOH in 2,2-dimethoxypropane (rt, 4 h) gave a bis-acetonide derivative **13** as the sole product. The structure of the seven-membered acetonide moiety was confirmed by the HMBC correlations from H-1′ and H-4′ to the acetal carbon (δ_C 102.2, C-2″). The large *J* value of 10.6 Hz for H-1′ and H-2′ indicated the rigid δ-lactone conformation, placing both these protons as pseudoaxial (Fig. 1). One of the H-3′ methylene protons that resonated at δ_H 1.90 was coupled to H-2′ and H-4′, both with *J*=11.2 Hz and lacked NOESY correlation to these protons, hence there was an antiperiplanar relation. This proton ($H_β$-3′) showed an intense NOESY correlation to H-1′, which was consistent with their synfacial relation. On the other hand, an intense NOESY cross-peak was observed between H-2′ and H-4′. These data indicated the relative configuration of the seven-membered ring moiety as depicted. Proton H-5′ showed a NOESY correlation to one of the five-membered acetonide methyl (δ_H 1.46, H-1‴), whereas H-6′ showed a NOESY cross-peak with the other acetonide methyl at δ_H 1.43 (H-3‴). Under the acetonide-forming reaction conditions (*p*-TsOH, 2,2-dimethoxypropane, rt, 4 h), aigialomycin G (**5a**/**5b**) was converted into a spiroacetal–acetonide derivative **14**. The five-membered acetonide due to the 4′,5′-diol had a cis-form, since both H-4′ and H-5′ showed NOESY correlations to the same acetonide methyl at δ_H 1.32 (H-3″), but not to the other (δ_H 1.40, H-1″). Intense NOESY cross-peaks between H-5′ and H-10′ revealed the configuration of the spiroacetal carbon (C-6′) (Fig. 2). Finally, **4** and **5a**/**5b** were chemically correlated. Hydrogenation of **4** (H_2, Pd/C, THF, rt, 3 h) gave the 7′,8′-dihydro derivative **15**. $NaBH_4$ reduction of **5a**/**5b** (THF, rt, 30 min) gave a 1:5 mixture of two epimeric C-6′ alcohols (**15** and **16**), wherein ^{1}H NMR (CD_3OD) spectroscopic data for the minor isomer were identical to **15**. These results revealed that aigialomycins F (**4**) and G (**5a**/**5b**) possess the same relative stereochemistry. On the basis of these experimental data and by biogenetic correlation to aigialomycin B (**3**), as discussed below, the absolute configurations of **4** and **5a** were deduced to be 1′*R*,2′*S*,4′*S*,5′*S*,6′*S*,10′*S* and 1′*R*,2′*S*,4′*S*,5′*S*,10′*S*, respectively.

Compound **7** was isolated as a colorless solid, which has the molecular formula $C_{19}H_{24}O_8$ (HRESIMS). The ^{1}H and ^{13}C NMR spectra were very similar to those of aigialospirol (**6**). The only differences were the absence of the olefin (C-7′/C-8′) in **7**, instead of the presence of two additional methylenes. A dihydroisobenzofuranone structure was confirmed by the downfield shift of H-1′ (δ_H 5.33) and HMBC correlations from this proton to the lactone carbonyl (δ_C 171.2), C-1, C-5, and C-6. The linkage from C-1′ to C-11′, through a quaternary acetal carbon (C-6′, δ_C 101.39), was established by analysis of the 2D NMR spectroscopic data, including the HMBC correlations from H-2′ (δ_H 3.94, ddd, *J*=12.0, 6.5, 2.2 Hz) and H_{ax}-7′ (δ_H 2.08, dt, *J*=4.9, 13.6 Hz) to C-6′. Analysis of the ^{1}H–^{1}H *J* values and NOESY correlations revealed the relative configuration of the spirocyclic region and its rigid conformation, demonstrating that both tetrahydropyran rings adopt a chair conformation. Thus, an intense NOESY cross-peak between one of the C-3′ methylene protons at δ_H 1.88 (H_{ax}-3′) and H-5′ indicated the 1,3-diaxial relationship of these protons. The axial

Table 2
NMR data for **7** and **8** in $CDCl_3$ (500 MHz for ^{1}H, and 125 MHz for ^{13}C)

Position	**7**			**8**		
	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC
1-*C*OO-	171.2, C			171.5, C		
1	104.5, C			104.5, C		
2	157.8, C			157.7, C		
3	101.43, CH	6.48, d (1.5)	*C*OO,1,2,4,5	101.4, CH	6.48, s	*C*OO,1,2,4,5
4	167.3, C			167.2, C		
4-O*C*H_3	56.1, CH_3	3.88, s	4	56.0, CH_3	3.88, s	4
5	101.9, CH	6.67, br s	1,3,4,1′	101.8, CH	6.71, s	1,3,4,1′
6	149.2, C			150.0, C		
1′	82.6, CH	5.33, d (6.5)	*C*OO,1,5,6,2′,3′	83.1, CH	5.20, d (7.3)	*C*OO,5,6,2′
2′	65.5, CH	3.94, ddd (12.0, 6.5, 2.2)	6,1′,6′	70.2, CH	3.62, m	6,1′
3′	34.4, CH_2	2.16, ddd (14.0, 5.2, 2.2)	4′,5′	27.2, CH_2	1.96, m	
		1.88, m	1′,2′		1.64, m	
4′	68.5, CH	4.07, m	2′	27.6, CH_2	1.93, m	5′
					1.73, m	5′
5′	71.1, CH	3.29, d (3.4)	7′	71.6, CH	3.32, dd (11.2, 4.8)	
6′	101.39, C			98.0, C		
7′	29.2, CH_2	2.08, m	5′,6′,8′,9′	29.7, CH_2	1.99, dt (4.7, 13.4)	6′,8′
		1.51, m	6′,9′		1.54, m	6′,9′
8′	18.2, CH_2	1.85, m		18.8, CH_2	1.88, tq (3.8, 13.3)	7′
		1.68, m			1.69, ddd (12.0, 3.2, 2.6)	
9′	31.8, CH_2	1.59, m	8′	32.4, CH_2	1.57, m	11′
		1.27, m			1.19, dq (3.8, 12.2)	10′
10′	67.5, CH	3.68, m	8′,11′	66.2, CH	3.57, m	
11′	21.9, CH_3	1.14, d (6.3)	9′	21.8, CH_3	1.07, d (6.2)	9′,10′

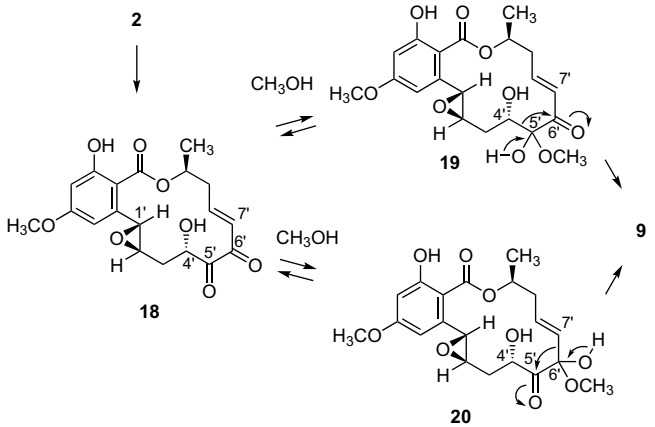


Scheme 1. Possible biosynthetic pathways from hypothemycin/dihydrohypothemycin to dihydroisobenzofuranone–spiroacetal and dihydroisicoumarin derivatives.

orientation of H-2′ was indicated by its large ^1H–^1H coupling (J=12.0 Hz) to H_{ax}-3′. The small J value (3.4 Hz) for H-4′ and H-5′, and NOESY cross-peaks from H-4′ to H_{ax}-3′, H_{eq}-3′, and H-5′ with close intensity placed H-4′ in an equatorial position. A chair conformation of the outer tetrahydropyran ring in $CDCl_3$ was suggested by NOESY correlations between H_{ax}-7′ and H_{ax}-9′, and between H_{ax}-8′ and H-10′, therefore, the C-11′ methyl group occupied an equatorial position. To confirm the identical stereochemistry of **7** with aigialospirol (**6**), these compounds were chemically correlated. Hydrogenation of **6** (H_2, Pd/C, EtOAc, rt, 15 h) gave a single product whose ESIMS and ^1H NMR spectroscopic data were identical to those of **7**, thus, compound **7** is designated as 7′,8′-dihydroaigialospirol. In the previous report,[9] the C-1′ configuration of aigialospirol (**6**) was proposed solely based on the observation of a weak NOESY cross-peak of H-5 and H-10′, and the absolute configuration of this molecule was proposed by analogy to hypothemycin. Recently, Hsung and co-workers reported total synthesis of **6**.[12] Since the synthetic route involves a 1′-OH secondary alcohol intermediate, which quickly epimerized under the reaction conditions, the chirality of the starting material cannot be correlated to the C-1′ absolute configuration of the natural product. Fortunately, the 2-*O*-(4-bromobenzyl) derivative **17**, prepared from **6**, upon crystallization in MeOH, provided colorless needles suitable for X-ray diffraction analysis (Fig. 3). This experiment also unambiguously confirmed the absolute configuration of aigialospirol (**6**) as depicted (1′*S*,2′*R*,4′*S*,5′*S*,6′*R*,10′*S*).

The HRESIMS and ^{13}C NMR spectroscopic data of compound **8** indicated its molecular formula to be $C_{19}H_{24}O_7$, which is lacking one oxygen atom from **7**. The ^1H and ^{13}C NMR spectra were closely related to those of **7**, except for the replacement of an oxymethine (C-4′) to a methylene at δ_C 27.6 (δ_H 1.93 and 1.73). The gross structure of **8** was deduced by detailed analysis of COSY, HMQC, and HMBC data in similar fashion as described for **7**. The relative configuration of the spirocyclic region was determined on the basis of the NOESY correlations: H-2′ to H_{ax}-4′, H_{ax}-3′ to H-5′, H-5′ to H_{eq}-7′, and H_{ax}-8′ to H-10′ (Fig. 4). Therefore, both tetrahydropyran rings adopt chair conformation wherein H-2′, H-5′, and H-10′ are placed in axial position. A weak NOESY cross-peak of H-5 and H-10′, as also found for **6** and **7**, indicated the 1′*S*-configuration. Compound **8** is, hence, designated as 4′-deoxy-7′,8′-dihydroaigialospirol. The absolute configuration of **8** should be identical to that of the co-metabolites **6** and **7**.

The molecular formula of compound **9** was determined to be $C_{20}H_{24}O_9$ by HRMS. The ^1H and ^{13}C NMR data and HMBC correlations for the resorcylic acid moiety were consistent with those of **1** and **2**. A *trans*-epoxide (C-1′/C-2′), attached to C-6, was connected at the other side with a methylene (C-3′), which was further bonded to an oxymethine (C-4′). The substructure from C-7′ to C-11′, including a *trans*-olefin (C-7′/C-8′, J=15.5 Hz), was addressed from COSY correlations. The remaining fragments were a tertiary alcohol (δ_C 81.0) and a methoxycarbonyl group (δ_C 174.2; δ_C 53.6, δ_H 3.83). Therefore, the quaternary carbon (δ_C 81.0, C-5′) should link C-3′ and C-7′, and also substituted with the methoxycarbonyl group. The absolute configuration of the epoxide carbons (C-1′ and C-2′) and the lactone-linking oxymethine (C-10′) should be identical to all other co-metabolites (1′*R*,2′*R*,10′*S*-configuration). The 4′*S*-isomer with a conformation shown in Figure 5 is consistent with the observed NOESY correlations;



Scheme 2. Proposed mechanisms for the production of **9** from aigialomycin A (**2**).

Table 3
Antimalarial and cytotoxic activities of aigialomycin-related compounds

Compound	Antimalarial (IC_{50}, μg/mL)[a] *P. falciparum* K1	Cytotoxicity (IC_{50}, μg/mL)[b]			
		KB	BC	NCI-H187	Vero
1	2.8	>20	—[c]	2.0	2.1
4	>10	>20	>20	>20	>50
5a/5b	>10	>20	>20	>20	>50
6	>10	>20	>20	>20	>50
7	>10	>20	>20	>20	>50
8	>10	>20	>20	>20	>50
11	3.0	>20	2.6	3.6	0.77

[a] Inhibition of the proliferation of *P. falciparum* K1. Standard antimalarial drug, dihidroartemisinin, showed an IC_{50} value of 0.0012 μg/mL.

[b] The IC_{50} values of a standard compound, doxorubicin, against KB, BC, and NCI-H187 were 0.18, 0.13, and 0.40 μg/mL, respectively. Ellipticine was used as a standard compound for the cytotoxicity assay against Vero cells (IC_{50} 0.40 μg/mL).

[c] Not tested.

however, the configuration of the tertiary alcohol (C-5′) could not be proposed from available NMR spectroscopic data.

Compound **10** possessed the same molecular formula as **9**, $C_{20}H_{24}O_9$ (HRMS). The UV, IR, and IR data were similar to those of **9**. The only difference was that compound **10** possessed *cis*-olefinic geometry (C-7′/C-8′, J=12.4 Hz in acetone-d_6, and J=11.3 Hz in $CDCl_3$). Significant peak broadening was observed for the macrolactone moiety in the ^1H and ^{13}C NMR spectra acquired in acetone-d_6 and $CDCl_3$, in particular, the resonances of the olefinic carbons (C-7′ and C-8′) were not detected and the HMQC spectra lacked corresponding H–C correlations. Due to sample limitation, further NMR studies at variable temperature or chemical correlation to **9** (by hydrogenation) were not examined, therefore, the configurations at C-4′ and C-5′ of **10** remain unassigned.

We previously proposed that aigialospirol (**6**) should be biogenetically derived from hypothemycin (**1**).[9] Similarly, likely precursors for dihydroisobenzofuranone–spiroacetal type compounds **7** and **8** are dihydrohypothemycin (known as a co-metabolite of **1** from *Hypomyces trichothecoides*)[1] and 4′-deoxy-dihydrohypothemycin, respectively, although we have not isolated these compounds in extracts of *A. parvus* BCC 5311 (Scheme 1). One of the possible mechanisms accounting for the production of the dihydroisobenzofuranone–spiroacetal derivatives is the hydrative epoxide cleavage by attack of a hydroxyl oxygen to the benzylic carbon (C-1′) with stereo inversion (S_N2) (path a), giving a 1′*S*,2′*R*-diol, which should subsequently undergo intramolecular trans-lactonization and spiroacetal formation. An alternate pathway involving hydrolysis of the ester linkage (path c) and subsequent attack at the epoxide (C-1′), giving the same final precursor as path a, may also be possible. In the present study dihydroisocoumarin derivatives **4** and **5a**/**5b**, possessing a 1′*R*,2′*S*-configuration, have been additionally isolated. The stereochemistry of **4** and **5a**/**5b** can be correlated to aigialomycin B (**3**) and dihydrohypothemycin, respectively, with only difference was the inversion of C-2′ configuration. The dihydroisocoumarin formation can be explained by two routes: (1) hydrative epoxide cleavage at C-2′ (path b) and subsequent intramolecular trans-lactonization, and (2) initial macrolactone hydrolysis (path c) and attack of the resulting carboxyl oxygen to the epoxide at C-2′. Path c accounts for the appropriate stereochemistry of both dihydroisobenzofuranone–spiroacetal (C-1′ inversion) and dihydroisocoumarin (C-2′ inversion) derivatives; however, this route is not consistent with the composition of isolated compounds. Thus, 7′*Z*-olefinic derivatives are absent in the dihydroisocoumarin series, whereas no 7′*E*-olefinic derivative was isolated in the dihydroisobenzofuranone series. The selectivity of dihydroisobenzofuranone and dihydroisocoumarin production could be better explained by considering the competition of path a and path b, which should be significantly influenced by the macrolide conformation. Further experimental evidences would be necessary for conclusive proposal of the mechanisms of the transformations.

HPLC/UV analysis of an EtOAc extract from culture broth of BCC 5311 revealed the presence of aigialospirol (**6**) and aigialomycin F (**4**) along with **1**, **2**, and **3**, which was confirmed by co-injections of the standard compounds. These results strongly suggested that both dihydroisobenzofuranone–spiroacetal and dihydroisocoumarin derivatives are not isolation artifacts. On the other hand, 13-membered macrolides **9** and **10** were possibly produced, respectively, from aigialomycin A (**2**) and hypothemycin (**1**), during extensive silica gel (eluent, MeOH/CH_2Cl_2) and Sephadex LH-20 (eluent, MeOH) column chromatography. Possible mechanisms for the production of **9** from aigialomycin A (**2**) are proposed in Scheme 2. Air oxidation of **2** will give a diketone **18**, which should form hemiacetal **19** and/or **20** in the presence of MeOH. Rearrangement from a 14-membered to 13-membered macrolide could be explained by carbon migration of hemiacetal **19** or **20**.

Four new compounds **4**, **5a**/**5b**, **7**, and **8**, and 4-*O*-demethylhypothemycin (**11**) were tested for antimalarial and cytotoxic activities. For comparison, hypothemycin (**1**) and aigialospirol (**6**) were also subjected to the same assays (Table 3). Non-macrolide derivatives **4**, **5a**/**5b**, **6**, **7**, and **8** were inactive in these assays. Biological activities of 4-*O*-demethylhypothemycin (**11**) were similar to those of hypothemycin (**1**).

3. Conclusion

In conclusion, reinvestigation of the mangrove fungus *A. parvus* BCC 5311 resulted in the isolation of six new aigialomycin-related compounds, including two dihydroisocoumarin-type derivatives, aigialomycins F (**4**) and G (**5**), and their post-PKS biogenetic relations were proposed. The compositions of the metabolites are significantly influenced by the duration of incubation and also differ in each fermentation batch. Taking together with our previous studies,[8,9] the rarely investigated fungal species has been proved to be a potent source of novel bioactive compounds.

4. Experimental

4.1. General procedures

Melting points were measured with an Electrothermal IA9100 digital melting point apparatus. Optical rotations were measured with a JASCO D-1030 digital polarimeter. UV spectra were recorded on an analytikjena SPEKOL 1200 UV–visible spectrophotometer. FTIR spectra were taken on a Perkin–Elmer 2000 spectrometer. NMR spectra were recorded on Bruker DRX400 and AV500D spectrometers. ESI-TOF mass spectra were measured with Micromass LCT and Bruker micrOTOF mass spectrometers.

4.2. Fungal material

A. parvus was collected, identified, and isolated from mangrove wood by one of the authors (E. B. G. Jones). This fungus was deposited at the Thailand BIOTEC Culture Collection as BCC 5311 in June 1999.[9]

4.3. Fermentation and isolation

A. parvus BCC 5311 was fermented in 80×1 L Erlenmeyer flasks each containing 250 mL of potato dextrose broth (PDB) under static conditions at 25 °C for 80 days. The cultures were filtered, and the filtrate (20 L) was extracted with an equal volume of EtOAc and concentrated to a brown solid (5.41 g). This crude extract was passed through a Sephadex LH-20 column (4.0×60 cm) using MeOH as eluent to obtain a major fraction containing a complex mixture of compounds (5.01 g) and a late-eluting fraction (314 mg) mostly composed of hypothemycin. The former was subjected to column chromatography (CC) on Si gel (5.0×20 cm, step gradient elution with 0–40% MeOH/CH_2Cl_2) to furnish 12 fractions. Fraction 3 (192 mg, major components were hypothemycin and aigialomycin A) was purified by combination of preparative HPLC using a reverse phase column (NovaPak HR C_{18}, 10 μm, 4.0×10.0 cm; MeCN/H_2O=40:60; flow rate, 20 mL/min) and CC on Si gel (5–30% EtOAc/CH_2Cl_2) to furnish compound **8** (4.2 mg). Fraction 4 (1.16 g) was triturated in MeOH and filtered. The filtrate was subjected to preparative HPLC (MeCN/H_2O=25:75, then MeOH/H_2O=35:65) to afford **7** (35.4 mg). The residual solid (877 mg) was mainly composed of hypothemycin (**1**) and aigialomycin A (**2**) (ca. 2:1, ^1H NMR). Further fractionation of this material by repeated CC on Si gel (CH_2Cl_2/MeOH and CH_2Cl_2/EtOAc) and preparative HPLC (MeOH/H_2O) provided **1** (522 mg), **2** (22 mg), **9** (2.5 mg), and **10** (1.2 mg). It was observed that aigialomycin A (**2**) was unstable under these Si gel column chromatographic conditions, although we did not experience such phenomenon in the previous studies.

Fraction 5 (321 mg) was subjected to preparative HPLC (MeCN/H_2O=50:50) and CC on Si gel (5–30% acetone in CH_2Cl_2) to obtain **6** (98 mg). Fraction 6 (272 mg) was fractionated by CC on Si gel (3.0×15 cm, 1–25% MeOH in CH_2Cl_2) to furnish **12** (11.6 mg) and **5a**/**5b** (20.5 mg). Fraction 7 (253 mg) was repeatedly fractionated by CC on Si gel (2–10% MeOH in CH_2Cl_2) and preparative HPLC (MeCN/H_2O and MeOH/H_2O) to furnish **7** (21.9 mg) and **12** (44.8 mg). Fraction 8 (1.18 g) was repeatedly fractionated by CC on Si gel (MeOH/CH_2Cl_2) to afford **11** (98.9 mg), **3** (203 mg), and **4** (23.6 mg). Fraction 10 (427 mg) also provided **4** (57.4 mg).

4.3.1. *Aigialomycin F (**4**)*

Colorless solid; mp 186–187 °C; $[\alpha]_D^{27}$ −39 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.42), 266 (4.15), 301 (3.85) nm; IR (KBr) ν_{max} 3363, 1637, 1379, 1202, 1060, 1034, 712 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 421.1464 $[M+Na]^+$ (calcd for $C_{19}H_{26}O_9Na$ 421.1469).

4.3.2. *Aigialomycin G (**5a**/**5b**)*

Colorless solid; mp 94–95 °C; $[\alpha]_D^{27}$ −35 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.46), 267 (4.18), 303 (3.89) nm; IR (KBr) ν_{max} 3424, 1713, 1677, 1650, 1629, 1584, 1372, 1254, 1206, 1162, 1032 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 421.1461 $[M+Na]^+$ (calcd for $C_{19}H_{26}O_9Na$, 421.1469).

4.3.3. *7′,8′-Dihydroaigialospirol (**7**)*

Colorless solid; mp 80–83 °C; $[\alpha]_D^{28}$ +14 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 217 (4.47), 257 (4.20), 290 (3.81) nm; IR (KBr) ν_{max} 3459 (br), 1749, 1614, 1216, 1158, 1073 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 2; HRMS (ESI-TOF) *m*/*z* 403.1372 $[M+Na]^+$ (calcd for $C_{19}H_{24}O_8Na$ 403.1369).

4.3.4. *4′-Deoxy-7′,8′-dihydroaigialospirol (**8**)*

Colorless solid; mp 70–71 °C; $[\alpha]_D^{28}$ +12 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 217 (4.44), 257 (4.05), 292 (3.64) nm; IR (KBr) ν_{max} 3488, 3385, 1733, 1614, 1221, 1168, 1085, 1075, 976 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 2; HRMS (ESI-TOF) *m*/*z* 387.1412 $[M+Na]^+$ (calcd for $C_{19}H_{24}O_7Na$ 387.1420).

4.3.5. *Compound **9***

Colorless solid; mp 128–129 °C; $[\alpha]_D^{25}$ +8 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.17), 265 (3.83), 305 (3.52) nm; IR (KBr) ν_{max} 3444, 1734, 1645, 1615, 1257, 1159 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 11.75 (1H, s, 2-O*H*), 6.51 (1H, d, *J*=2.7 Hz, H-5), 6.42 (1H, d, *J*=2.7 Hz, H-3), 6.21 (1H, ddd, *J*=15.5, 8.2, 5.6 Hz, H-8′), 5.52 (1H, m, H-10′), 5.51 (1H, br d, *J*=15.5 Hz, H-7′), 4.55 (1H, d, *J*=1.7 Hz, H-1′), 4.05 (1H, m, H-4′), 3.83 (3H, s, –COOC*H*$_3$), 3.82 (3H, s, 4-OC*H*$_3$), 2.72 (1H, dt, *J*=8.9, 2.1 Hz, H-2′), 2.57 (1H, m, Ha-9′), 2.44 (1H, ddt, *J*=15.7, 1.6, 5.6 Hz, Hb-9′), 2.37 (1H, br d, *J*=10.7 Hz, 4′-O*H*), 2.07 (1H, dt, *J*=15.5, 2.7 Hz, Ha-3′), 2.03 (1H, ddd, *J*=15.5, 8.9, 5.6 Hz, Hb-3′), 1.45 (3H, d, *J*=6.5 Hz, H-11′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.2 (C, C-6′), 170.7 (C, –*C*OO–), 165.4 (C, C-2), 165.1 (C, C-4), 142.3 (C, C-6), 129.8 (CH, C-7′), 127.3 (CH, C-8′), 104.4 (C, C-1), 104.0 (CH, C-5), 100.8 (CH, C-3), 81.0 (C, C-5′), 72.8 (CH, C-4′), 70.7 (CH, C-10′), 63.4 (CH, C-2′), 58.2 (CH, C-1′), 55.5 (CH$_3$, 4-OC*H*$_3$), 53.6 (CH$_3$, –COOC*H*$_3$), 36.6×2 (CH$_2$, C-3′; and CH$_2$, C-9′), 19.0 (CH$_3$, C-11′); HRMS (ESI-TOF) *m*/*z* 431.1315 $[M+Na]^+$ (calcd for $C_{20}H_{24}O_9Na$ 431.1318).

4.3.6. *Compound **10***

Colorless solid; mp 156–159 °C; $[\alpha]_D^{25}$ +21 (*c* 0.065, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.17), 265 (3.87), 305 (3.56) nm; IR (KBr) ν_{max} 3460, 1747, 1644, 1618, 1260 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 12.15 (1H, s, 2-O*H*), 6.52 (1H, d, *J*=2.7 Hz, H-5), 6.41 (1H, d, *J*=2.7 Hz, H-3), 5.72 (1H, br, H-8′), 5.57 (1H, m, H-10′), 5.52 (1H, br d, *J*=12.4 Hz, H-7′), 4.44 (1H, br s, 5′-OH), 4.39 (1H, m, H-4′), 4.29 (1H, br d, *J*=7.3 Hz, 4′-O*H*), 4.17 (1H, br s, H-1′), 3.83 (3H, s, 4-OC*H*$_3$), 3.74 (3H, s, –COOC*H*$_3$), 2.84 (1H, m, H-2′), 2.52–2.48 (2H, m, H-9′), 2.09–2.05 (2H, m, H-3′), 1.49 (3H, d, *J*=6.4 Hz, H-11′); ^{13}C NMR (125 MHz, acetone-d_6) δ 173.7 (C, C-6′), 171.3 (C, –*C*OO–), 166.1 (C, C-2), 165.1 (C, C-4), 143.1 (C, C-6), 103.8 (CH, C-5), 103.6 (C, C-1), 100.2 (CH, C-3), 81.6 (C, C-5′), 73.6 (CH, C-4′), 72.5 (CH, C-10′), 62.1 (CH, C-2′), 58.1 (CH, C-1′), 55.0 (CH$_3$, 4-OC*H*$_3$), 51.9 (CH$_3$, –COOC*H*$_3$), 36.4 (CH$_2$, C-3′), 34.1 (CH$_2$, C-9′), 17.8 (CH$_3$, C-11′), resonances for C-7′ and C-8′ were not clearly detected because of the peak broadening; HRMS (ESI-TOF) *m*/*z* 431.1322 $[M+Na]^+$ (calcd for $C_{20}H_{24}O_9Na$ 431.1318).

4.4. Synthesis of the acetonide derivatives 13 and 14

To a suspension of aigialomycin F (**4**, 2.0 mg) in 2,2-dimethoxypropane (0.5 mL) was added *p*-TsOH·H_2O (ca. 0.5 mg), and the mixture was stirred at room temperature for 4 h. The mixture was diluted with EtOAc and washed with satd $NaHCO_3$. The organic layer was concentrated under reduced pressure to leave a colorless solid, which was purified by preparative HPLC using a reverse phase column (MeCN/H_2O=45:55) to furnish compound **13** (1.2 mg) as a colorless solid. Similarly, compound **14** (0.3 mg) was synthesized from aigialomycin G (**5a**/**5b**, 1.0 mg).

4.4.1. *Compound **13***

Colorless solid; ^1H NMR (500 MHz, $CDCl_3$) δ 11.19 (1H, s, 2-O*H*), 6.52 (1H, dd, *J*=2.4, 1.2 Hz, H-5), 6.44 (1H, d, *J*=2.4 Hz, H-3), 5.88 (1H, dt, *J*=15.4, 7.3 Hz, H-8′), 5.63 (1H, dd, *J*=15.4, 7.5 Hz, H-7′), 4.89 (1H, d, *J*=10.6 Hz, H-1′), 4.35 (1H, dt, *J*=5.6, 10.6 Hz, H-2′), 4.42 (1H, t, *J*=7.7 Hz, H-6′), 4.03 (1H, dd, *J*=11.2, 5.2 Hz, H-4′), 3.89 (1H, m, H-10′), 3.88 (3H, s, 4-OC*H*$_3$), 3.75 (1H, dd, *J*=7.9, 5.2 Hz, H-5′), 2.38 (1H, dd, *J*=13.5, 5.6 Hz, Ha-3′), 2.28–2.26 (2H, m, H-9′), 1.90 (1H, dt, *J*=13.5, 11.2 Hz, Hb-3′), 1.49 (3H, s, H-1″), 1.48 (3H, s, H-3″), 1.46 (3H, s, H-1‴), 1.43 (3H, s, H-3‴), 1.24 (3H, d, *J*=6.3 Hz, H-11′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.4 (C, –*C*OO–), 165.1 (C, C-4), 164.7 (C, C-2), 143.1 (C, C-6), 131.5 (CH, C-8′), 131.3 (CH, C-7′), 109.3 (C, C-2‴), 104.4 (CH, C-5), 102.2 (C, C-2″), 99.8 (C, C-1), 99.8 (CH, C-3), 82.5 (CH, C-5′), 79.1 (CH, C-6′), 79.0 (CH, C-2′), 67.5 (CH, C-1′), 67.5 (CH, C-4′), 67.2 (CH, C-10′), 55.6 (CH$_3$, 4-OC*H*$_3$), 42.1 (CH$_2$, C-9′), 37.0 (CH$_2$, C-3′), 27.1 (CH$_3$, C-1‴), 26.8 (CH$_3$, C-3‴), 24.9 (CH$_3$, C-1″), 24.9 (CH$_3$, C-3″), 23.0 (CH$_3$, C-11′); HRMS (ESI-TOF) *m*/*z* 501.2095 $[M+Na]^+$ (calcd for $C_{25}H_{34}O_9Na$ 501.2101).

4.4.2. *Compound **14***

Colorless solid; ^1H NMR (500 MHz, $CDCl_3$) δ 11.14 (1H, s, 2-O*H*), 6.68 (1H, dd, *J*=2.4, 1.3 Hz, H-5), 6.40 (1H, d, *J*=2.2 Hz, H-3), 5.10 (1H, d, *J*=10.9 Hz, H-1′), 4.52 (1H, dt, *J*=2.5, 10.9 Hz, H-2′), 4.51 (1H, m, H-4′), 4.24 (1H, d, *J*=7.9 Hz, H-5′), 3.94 (1H, m, H-10′), 3.85 (3H, s, 4-OC*H*$_3$), 2.54 (1H, ddd, *J*=14.2, 11.3, 2.3 Hz, Ha-3′), 2.46 (1H, ddd, *J*=14.2, 4.5, 2.5 Hz, Hb-3′), 2.03 (1H, m, Ha-7′), 1.96 (1H, m, Ha-8′), 1.93 (1H, m, Hb-7′), 1.72 (1H, m, Ha-9′), 1.70 (1H, m, Hb-8′), 1.40 (3H, s, H-1″), 1.39 (1H, m, Hb-9′), 1.32 (3H, s, H-3‴), 1.20 (3H, d, *J*=6.2 Hz, H-11′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4 (C, –*C*OO–), 166.5 (C, C-4), 164.4 (C, C-2), 144.3 (C, C-6), 107.7 (C, C-2″), 103.4 (CH, C-5), 100.5 (C, C-1), 100.0 (C, C-6′), 99.5 (CH, C-3), 78.8 (CH, C-5′), 77.2 (CH, C-2′), 69.8 (CH, C-4′), 69.8 (CH, C-10′), 67.5 (CH, C-1′), 55.5 (CH$_3$, 4-OC*H*$_3$), 35.2 (CH$_2$, C-3′), 30.2 (CH$_2$, C-7′), 30.0 (CH$_2$, C-9′), 26.2 (CH$_3$, C-1″), 23.9 (CH$_3$, C-3″), 21.8 (CH$_3$, C-11′), 16.6 (CH$_2$, C-8′); HRMS (ESI-TOF) *m*/*z* 443.1686 $[M+Na]^+$ (calcd for $C_{22}H_{28}O_8Na$ 443.1682).

4.5. Hydrogenation of aigialomycin F (4)

To a solution of aigialomycin F (**7**, 3.0 mg) in THF (0.5 mL) was added 10% Pd/C (5 mg), and the mixture was vigorously stirred under hydrogen for 3 h. The suspension was filtered, and the filtrate was concentrated in vacuo to leave a colorless solid, which was

purified by preparative HPLC using a reverse phase column (MeCN/H_2O=25:75) to furnish **15** (1.4 mg) as a colorless gum.

*4.5.1. 7′,8′-Dihydroaigialomycin F (**15**)*

Colorless gum; ^{1}H NMR (500 MHz, CD_3OD) δ 6.66 (1H, d, J=2.3 Hz, H-5), 6.46 (1H, d, J=2.3 Hz, H-3), 4.68 (1H, ddd, J=10.6, 7.4, 2.4 Hz, H-2′), 4.58 (1H, d, J=7.4 Hz, H-1′), 3.98 (1H, ddd, J=10.9, 7.5, 1.8 Hz, H-4′), 3.86 (3H, s, 4-OCH_3), 3.80 (1H, m, H-6′), 3.73 (1H, m, H-10′), 3.21 (1H, dd, J=7.5, 2.2 Hz, H-5′), 2.06 (1H, d, J=14.6, 10.6, 1.8 Hz, Ha-3′), 1.90 (1H, ddd, J=14.6, 10.9, 2.4 Hz, Hb-3′), 1.58–1.55 (2H, m, H-7′), 1.54 (1H, m, Ha-8′), 1.48–1.46 (2H, m, H-9′), 1.39 (1H, m, Hb-8′), 1.15 (3H, d, J=6.2 Hz, H-11′); ^{13}C NMR (125 MHz, CD_3OD) δ 168.5 (C, –*C*OO–), 166.7 (C, C-4), 164.0 (C, C-2), 144.7 (C, C-6), 104.5 (CH, C-5), 100.3 (C, C-1), 99.9 (CH, C-3), 80.3 (CH, C-2′), 76.1 (CH, C-5′), 70.0 (CH, C-6′), 67.5 (CH, C-1′), 67.1 (CH, C-10′), 66.8 (CH, C-4′), 54.9 (CH_3, 4-O*C*H$_3$), 38.8 (CH_2, C-9′), 35.8 (CH_2, C-3′), 33.3 (CH_2, C-7′), 22.1 (CH_3, C-11′), 21.8 (CH_2, C-8′); HRMS (ESI-TOF) m/z 401.1813 [M+H$^+$] (calcd for $C_{19}H_{29}O_9$ 401.1811).

4.6. $NaBH_4$ reduction of aigialomycin G (5a/5b)

To a mixture of $NaBH_4$ (2 mg) in THF (0.2 mL) was added aigialomycin G (1.0 mg) in THF (0.2 mL), and the mixture was stirred for 30 min. The reaction was terminated by addition of H_2O, and the resulting mixture was partially concentrated by evaporation. The residue was extracted twice with EtOAc, and the combined organic layer was concentrated in vacuo to obtain a colorless gum (0.9 mg). The ^{1}H NMR (500 MHz, CD_3OD) spectrum of the crude product suggested the presence of a 1:5 mixture of the diastereomeric C-6′ alcohols. The resonances of the minor isomer were identical to those of **15**. ^{1}H NMR spectral data (500 MHz, CD_3OD) for the major isomer **16** were assigned by ^{1}H–^{1}H COSY; δ 6.66 (1H, d, J=2.3 Hz, H-5), 6.46 (1H, d, J=2.3 Hz, H-3), 4.68 (1H, m, H-2′), 4.58 (1H, d, J=7.4 Hz, H-1′), 4.03 (1H, m, H-4′), 3.86 (3H, s, 4-OCH_3), 3.74 (1H, m, H-10′), 3.58 (1H, m, H-6′), 3.39 (1H, t, J=6.3 Hz, H-5′), 1.99–1.98 (2H, m, H-3′), 1.70–1.42 (6H, m, H-7′, H-8′, and H-9′), 1.16 (3H, d, J=6.2 Hz, H-11′).

4.7. Hydrogenation of 6

To a solution of aigialospirol (**6**, 3.0 mg) in EtOAc (0.5 mL) was added 10% Pd/C (5 mg), and the mixture was vigorously stirred under hydrogen for 15 h. The suspension was filtered, and the filtrate was concentrated in vacuo to leave a colorless solid (2.8 mg). The ^{1}H NMR (400 MHz, $CDCl_3$) spectra and ESIMS data were identical to those of **7**.

4.8. Synthesis of compound 17

To a solution of aigialospirol (**6**, 5.0 mg) and 4-bromobenzyl bromide (8.0 mg) in 2-butanone (0.3 mL) was added K_2CO_3 (10 mg), and the mixture was stirred at room temperature for 3 days. The mixture was diluted with EtOAc and washed with H_2O. The organic layer was concentrated under reduced pressure to leave a pale brown solid (11.7 mg), which was purified by CC on Si gel (MeOH/CH_2Cl_2) to furnish compound **17** (5.9 mg) as a colorless solid. Recrystallization in MeOH gave colorless needles.

*4.8.1. 2-O-(4-Bromobenzyl)aigialospirol (**17**)*

Colorless needles (MeOH); mp 173–175 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.51 (2H, d, J=8.4 Hz, 4-bromobenzyl), 7.37 (2H, d, J=8.4 Hz, 4-bromobenzyl), 6.62 (1H, br s, H-5), 6.41 (1H, d, J=1.6 Hz, H-3), 6.15 (1H, m, H-8′), 5.66 (1H, m, H-7′), 5.24 (1H, d, J=6.2 Hz, H-1′), 5.20 (2H, s, 4-bromobenzyl), 4.09–4.03 (2H, m, H-2′ and H-4′), 3.97 (1H, m, H-10′), 3.80 (3H, s, 4-OCH_3), 3.51 (1H, m, H-5′), 3.41 (1H, d, J=10.6 Hz, 4′-O*H* or 5′-O*H*), 2.56 (1H, d, J=10.4 Hz, 5′-O*H* or 4′-O*H*), 2.03–1.89 (4H, m, H-3′ and H-9′), 1.23 (3H, d, J=6.3 Hz, H-11′); HRMS (ESI-TOF) m/z 569.0785 and 571.0771 [M+Na]$^+$ (calcd for $C_{26}H_{27}O_8{}^{79}$BrNa 569.0782; calcd for $C_{26}H_{27}O_8{}^{81}$BrNa 571.0765).

4.9. X-ray crystallographic analysis of 17

Crystal data for compound **17** at 298(2) K: $C_{26}H_{27}O_8Br$, M_r=547.40, monoclinic, space group $P2_1$ (No. 4) with a=6.2147(1) Å, b=14.5504(7) Å, c=13.5813(6) Å, β=90.669(3)°, V=1228.02(8) Å^3, Z=2, D_{calcd}=1.480 Mg/m^3. F_{000}=564, λ(Mo Kα)=0.71073 Å, μ=1.72 mm^{-1}. Data collection and reduction: crystal size 0.10×0.15×0.20 mm, θ range 1.00–26.02°, 9204 reflection collected, 4250 independent reflections (R_{int}=0.048), final R indices $I>2\sigma(I)$: 0.0434, wR_2=0.1234 for 316 parameters, GOF=1.021. Flack parameter=0.019(11). Intensity data were measured on a Bruker–Nonius kappaCCD diffractometer. Crystallographic data for the structure **17** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-711607. Copies of the data can be obtained, free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

4.10. Biological assays

Assay for activity against *Plasmodium falciparum* (K1, multi-drug resistant strain) was performed using the microculture radioisotope technique described by Desjardins et al.[13] Cytotoxicity against KB cells (oral human epidermoid carcinoma), BC cells (human breast cancer), NCI-H187 cells (human small-cell lung cancer), and Vero cells (African green monkey kidney fibroblasts) were evaluated using the resazurin microplate assay.[14]

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