

# Antibacterial Oligomeric Polyphenols from the Green Alga *Cladophora socialis*

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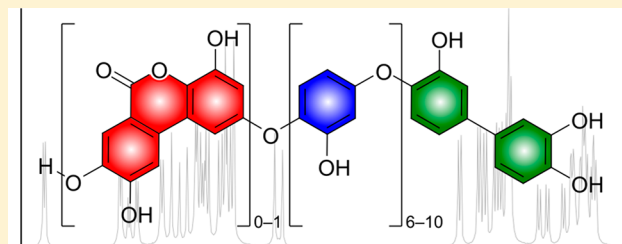
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## Supporting Information

**ABSTRACT:** A series of oligomeric phenols including the known natural product 3,4,3',4'-tetrahydroxy-1,1'-biphenyl (3), the previously synthesized 2,3,8,9-tetrahydroxybenzo[*c*]chromen-6-one (4), and eight new related natural products, cladophorols B–I (5–12), were isolated from the Fijian green alga *Cladophora socialis* and identified by a combination of NMR spectroscopy, mass spectrometric analysis, and computational modeling using DFT calculations. *J*-resolved spectroscopy and line width reduction by picric acid addition aided in resolving the heavily overlapped aromatic signals. A panel of Gram-positive and Gram-negative pathogens used to evaluate pharmacological potential led to the determination that cladophorol C (6) exhibits potent antibiotic activity selective toward methicillin-resistant *Staphylococcus aureus* (MRSA) with an MIC of 1.4  $\mu\text{g}/\text{mL}$ . Cladophorols B (5) and D–H (7–11) had more modest but also selective antibiotic potency. Activities of cladophorols A–I (4–12) were also assessed against the asexual blood stages of *Plasmodium falciparum* and revealed cladophorols A (4) and B (5) to have modest activity with EC<sub>50</sub> values of 0.7 and 1.9  $\mu\text{g}/\text{mL}$ , respectively.



## INTRODUCTION

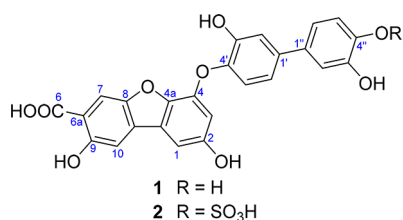
Aromatic systems are ubiquitous within natural products, reflecting the availability of their biosynthetic precursors in plants, algae, and microorganisms via the shikimate and polyketide pathways and the varied biological effects of such functional groups.<sup>1,2</sup> When incorporated as building blocks in vascular plants, aromatic polymers<sup>3–6</sup> such as lignin provide structural support that ultimately enabled plants to occupy terrestrial ecosystems leading to the evolution of trees.<sup>7</sup> Aromatic natural products conferring structural rigidity were unknown in the marine realm until lignin was reported in the coralline red alga *Calliarthron cheilosporioides* (phylum Rhodophyta).<sup>8</sup> This startling discovery suggested that an ancestor of green and red algae acquired the capacity to polymerize phenolic monomers but that this feature was silenced in most evolving lineages. Other red algae produce a variety of structurally simple, often halogenated phenolics of mostly unknown biological function,<sup>9</sup> although more complex, aromatic natural products of mixed shikimate and isoprenoid

biosynthetic origin have also been observed.<sup>10</sup> Many members of the brown algae (phylum Ochrophyta), which evolutionarily diverged just before land plants, produce polymeric, aceto-genic, phenolic compounds (phlorotannins) constituting 1–20% of dry mass.<sup>11</sup> These oligomers of phloroglucinol protect tissues against UV radiation and herbivory<sup>11,12</sup> and may contribute to structural support.<sup>12</sup> In contrast, significantly fewer phenolic natural products have been reported in green algae (phylum Chlorophyta). Brominated polyphenolic compounds including the feeding deterrent avrainvilleol and the condensation product of two molecules of avrainvilleol, rawsonol, are produced by green algae belonging to the genus *Avrainvillea*.<sup>13–15</sup> Another example of phenolic compounds from green algae are two vanillic acid analogues (1–2) from an Australian *Cladophora socialis* that have been found to disrupt insulin cell signaling.<sup>16</sup>

Received: December 19, 2018

Published: March 25, 2019

The present study describes a series of related oligomeric phenols, cladophorols A–I (4–12), from the green alga *C. socialis* that attracted our attention for its antibiotic activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Analysis of mass spectrometric and NMR spectroscopic data enabled structure elucidation despite the high molecular weights and repeating structural patterns of members of this natural product family. We used *J*-resolved NMR spectroscopy to reduce signal overlap in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra and employed picric acid line width-narrowing to reveal the exact linkage pattern of the subunits.<sup>17</sup> In addition, we propose a structural revision of known vanillic acid analogues (1–2) based on a combination of spectroscopic and computational approaches.



## RESULTS AND DISCUSSION

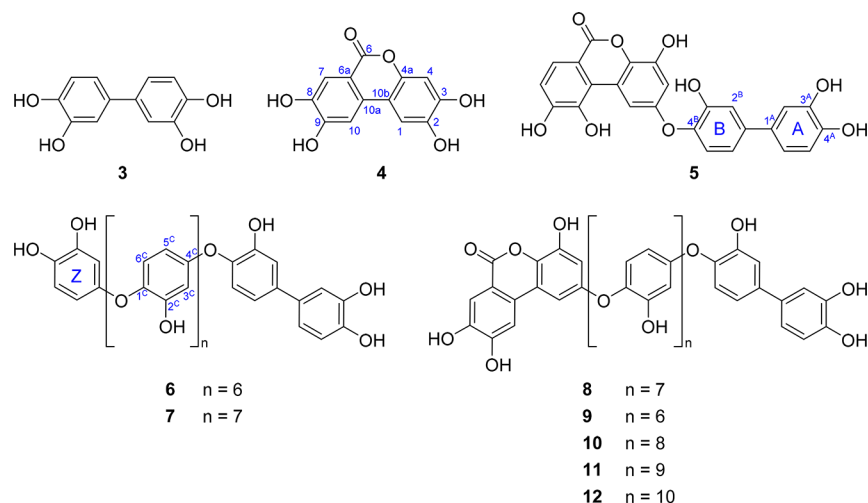
**Collection and Isolation.** Offshore of Vanua Levu Island in Fiji, *C. socialis*, identified by morphological features and nuclear small-subunit 18S rRNA analyses (Figure S1), was collected floating at the ocean surface. The aqueous methanol extract was partitioned using a sequence of biphasic organic solvents and water. Following evaluation of MRSA activity, the ethyl acetate-soluble fraction was chromatographed on silica gel followed by repeated reversed-phase semipreparative HPLC, yielding the known compound 3,4,3',4'-tetrahydroxy-1,1'-biphenyl (3), previously synthesized 2,3,8,9-tetrahydroxybenzo[*c*]chromen-6-one (4),<sup>18</sup> and eight new phenolic natural products (5–12) (Figure 1).

**Characterization of 4.** HRESIMS was used to assign the molecular formula of 4 as C<sub>13</sub>H<sub>8</sub>O<sub>6</sub>. The  $^1\text{H}$  NMR spectrum exhibited only four singlets at  $\delta_{\text{H}}$  7.58, 7.33, 7.32, and 6.73 (Table 1), attributed to two aromatic rings based on HSQC correlations with  $\delta_{\text{C}}$  115.4, 108.3, 107.4, and 104.4, respectively, and the absence of coupling between the protons.

**Table 1.**  $^1\text{H}$  (800 MHz) and  $^{13}\text{C}$  (200 MHz) NMR Spectroscopic Data of Cladophorols A and B (4–5) in CD<sub>3</sub>OD

position	4		5	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$
Benzo[ <i>c</i> ]chromenone				
1	107.4 (d)	7.32 s	108.2 (d)	8.37 d (2.8)
2	144.4 (s)		155.6 (s)	
3	148.6 (s)		106.9 (d)	6.69 d (2.8)
4	104.4 (d)	6.73 s	146.6 (s)	
4a	146.0 (s)		136.3 (s)	
6	164.0 (s)		163.4 (s)	
6a	112.9 (s)		114.5 (s)	
7	115.4 (d)	7.58 s	123.9 (d)	7.84 d (8.5)
8	147.3 (s)		116.4 (d)	7.08 d (8.5)
9	154.8 (s)		152.7 (s)	
10	108.3 (d)	7.33 s	144.3 (s)	
10a	131.7 (s)		123.1 (s)	
10b	111.4 (s)		120.7 (s)	
Biphenyl				
1 <sup>A</sup>			139.6 (s)	
2 <sup>A</sup>			116.0 (d)	7.14 d (2.2)
3 <sup>A</sup>			150.1 (s)	
4 <sup>A</sup>			144.0 (s)	
5 <sup>A</sup>			122.1 (d)	6.98 d (8.3)
6 <sup>A</sup>			119.1 (d)	7.02 dd (8.3, 2.2)
1 <sup>B</sup>			133.9 (s)	
2 <sup>B</sup>			114.9 (d)	7.04 d (2.2)
3 <sup>B</sup>			146.5 (s)	
4 <sup>B</sup>			145.9 (s)	
5 <sup>B</sup>			116.6 (d)	6.82 d (8.2)
6 <sup>B</sup>			119.2 (d)	6.93 dd (8.2, 2.2)

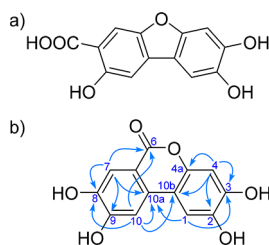
Correlations observed in HMBC from the signal at  $\delta_{\text{H}}$  6.73 (H-4) to  $\delta_{\text{C}}$  111.4 (C-10b), 144.4 (C-2), 146.0 (C-4a), and 148.6 (C-3) established the first ring. The second ring was determined from HMBC correlations from  $\delta_{\text{H}}$  7.58 (H-7) to  $\delta_{\text{C}}$  131.7 (C-10a), 147.3 (C-8), 154.8 (C-9), and 164.0 (C-6). Direct linkage between the two aromatic rings was established by HMBC correlations from  $\delta_{\text{H}}$  7.32 (H-1) to C-10a and from  $\delta_{\text{H}}$  7.33 (H-10) to C-10b. With connectivity of the carbonyl functionality and the two aromatic rings established, a third



**Figure 1.** Structures of compounds 3–12 from *C. socialis*.

ring was necessary to account for the 10 degrees of unsaturation calculated for this compound.

Since the  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts of **4** were similar to those reported for **1**, the structure depicted in Figure 2a was



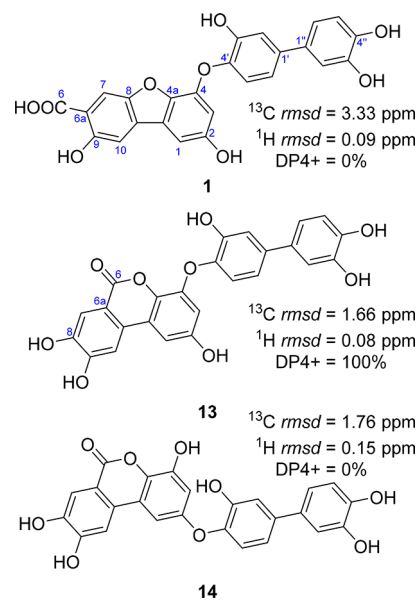
**Figure 2.** Possible structures (a and b) for cladophorol A (**4**) based on selected observed HMBC correlations indicated by arrows.

considered. Although it did not conflict with any of the observed NMR spectroscopic features, full consideration of all plausible structures and evaluation of these data to determine the best fit led to a more plausible alternative (Figure 2b). An HMBC spectrum recorded in DMSO- $d_6$  with picric acid,<sup>17</sup> which reduced the line widths of the phenolic hydroxy region, revealed four exchangeable protons at  $\delta_{\text{H}}$  10.41, 9.89, 9.74, and 9.23, each correlating with three carbons (Figure S13). In the originally considered structure (Figure 2a), only three hydroxyl groups are within this range; the carboxylic acid group is expected to shift the fourth labile proton downfield. Additionally, HMBC signals from  $\delta_{\text{H}}$  9.89 to C-7, C-8, and C-9 indicated an alcohol group at C-8. Since none of the labile protons correlated with carbonyl C-6, we concluded that it was part of the third ring. Compound **4** was thus identified as 2,3,8,9-tetrahydroxybenzo[*c*]chromen-6-one and named cladophorol A. This compound was previously reported as a synthetic analogue of ellagic acid created to improve potency for DNA gyrase inhibition, but the spectroscopic data were not disclosed at that time.<sup>18</sup>

**Characterization of 5.** Compound **5** was obtained as a pale red amorphous solid with a molecular formula of  $\text{C}_{25}\text{H}_{16}\text{O}_9$  deduced by HRESIMS. The  $^1\text{H}$  and COSY NMR spectra exhibited resonances accounting for four aromatic rings.  $^1\text{H}$ – $^1\text{H}$  coupling constants were used to establish substitution patterns including one 1,2,3,5-tetrasubstituted ring, one 1,2,3,4-tetrasubstituted ring, and two 1,2,4-trisubstituted rings. HMBC correlations from H-1 to  $\delta_{\text{C}}$  123.1 (C-10a), 136.3 (C-4a), and 155.6 (C-2), from H-7 to C-10a,  $\delta_{\text{C}}$  152.7 (C-9), and 163.4 (C-6), and from H-8 to 114.5 (C-6a) and 144.3 (C-10) indicated the first two rings were connected in a benzo[*c*]chromen-6-one moiety similar to **4** (Figure S2), although the location of hydroxy groups differed with substitution at positions C-2, C-4, C-9, and C-10. The two other rings formed a biphenyl group based on HMBC correlations from H-2<sup>A</sup> and H-6<sup>A</sup> to  $\delta_{\text{C}}$  133.9 (C-1<sup>B</sup>), from H-5<sup>A</sup> to  $\delta_{\text{C}}$  139.6 (C-1<sup>A</sup>), from H-2<sup>B</sup> and H-6<sup>B</sup> to C-1<sup>A</sup>, and from H-5<sup>B</sup> to C-1<sup>B</sup>. Despite careful examination of the HMBC spectra, a connecting point between the biphenyl and benzo[*c*]chromen-6-one could not be identified. Whereas a  $^4J$  correlation was reported between H-5' and C-4 for **1**,<sup>16</sup> a similar correlation was not found in **5**, despite recording HMBC spectra in DMSO- $d_6$  or acetone- $d_6$  and optimizing for 2 Hz long-range coupling. Adding picric acid to DMSO- $d_6$  for the NMR acquisition led to six labile proton signals (Figure S23) which showed correlations with all carbons bearing an

alcohol group and their neighbor (Figure S25). The four singlets at  $\delta_{\text{H}}$  11.04, 9.99, 9.68, and 9.57 exhibited HMBC correlations with  $\delta_{\text{C}}$  106.9 (C-3), 146.6 (C-4), C-4a, 116.4 (C-8), C-9, C-10, C-10a, 116.0 (C-2<sup>B</sup>), 150.1 (C-3<sup>B</sup>), and 144.0 (C-4<sup>B</sup>), clarifying that the benzo[*c*]chromen-6-one and the biphenyl were linked via an oxygen attached to C-2 and C-4<sup>B</sup>. For this reason, **5** was identified as 4,9,10-trihydroxy-2-[(3,3',4'-trihydroxy-(1,1'-biphenyl)-4-yl)oxy]benzo[*c*]chromen-6-one and named cladophorol B.

**Structure Revision of 1.** Since **4** and **5** were well characterized, the validity of the structure previously reported for **1** was questioned.<sup>16</sup> When recorded in DMSO- $d_6$ , the carboxyl carbons of **4** and **5**, which are part of a lactone ring, produced NMR signals at  $\delta_{\text{C}}$  164.5 and 160.3, respectively. The carboxyl carbon of **1** was reported at  $\delta_{\text{C}}$  161.1, a value upfield of expectations for a free carboxylic acid.<sup>16</sup> DFT calculations of the  $^1\text{H}$  and  $^{13}\text{C}$  chemical shift values were performed for the published structure **1** and for two other hypothetical structures (**13** and **14**) having a benzo[*c*]chromen-6-one unit (Figure 3).<sup>19,20</sup> Indeed, the



**Figure 3.** Original and hypothetical structures proposed for **1**, supporting reassignment of previously published **1** as **13**.

mPW1PW91/6-311+G(d,p)  $^{13}\text{C}$  chemical shift values did not match the experimental values for the originally published structure **1** with an rmsd of 3.3 ppm (Table 2). The largest discrepancies were due to C-4a and C-6 which are involved in the lactone ring. The hypothetical structures **13** and **14** showed smaller deviations for  $\delta_{\text{C}}$  with rmsd of 1.7 and 1.8 ppm, respectively, but only **13** exhibited a small  $\delta_{\text{H}}$  rmsd (0.08 ppm). When DP4+ probabilities were calculated, structure **13** scored 100% when considering chemical shift data for both  $^1\text{H}$  and  $^{13}\text{C}$ . For this reason, **1** should be revised to **13** and named 2,8,9-trihydroxy-4-((3,3',4'-trihydroxy[1,1'-biphenyl]-4-yl)oxy)benzo[*c*]chromen-6-one.

**Characterization of 6.** Compound **6** was isolated as a pale red solid with a molecular formula of  $\text{C}_{54}\text{H}_{38}\text{O}_{18}$  based on an  $[\text{M} - \text{H}]^-$  pseudomolecular ion in the HRESIMS at  $m/z$  973.1967. The  $^1\text{H}$  NMR spectrum was difficult to interpret because of severe signal overlap in the aromatic region. *J*-resolved 2D NMR spectroscopy aided the interpretation and

Table 2. Experimental and Calculated NMR Chemical Shifts for 1, 13, and 14

position	exp. <sup>16</sup>	1		13		14	
		calc.	$\Delta$	calc.	$\Delta$	calc.	$\Delta$
$\delta_H^a$							
H-1	6.92	7.00	-0.08	7.01	-0.09	6.71	+0.21
H-3	6.55	6.61	-0.06	6.47	+0.08	6.83	-0.28
H-7	7.55	7.63	-0.08	7.47	+0.08	7.59	-0.04
H-10	7.38	7.22	+0.16	7.35	+0.03	7.10	+0.28
H-2 <sup>A</sup>	6.96	6.92	+0.04	6.92	+0.04	7.02	-0.06
H-5 <sup>A</sup>	6.80	6.88	-0.08	6.89	-0.09	6.84	-0.04
H-6 <sup>A</sup>	6.87	6.99	-0.12	7.04	-0.17	7.01	-0.14
H-2 <sup>B</sup>	7.14	7.11	+0.03	7.15	-0.01	7.12	+0.02
H-5 <sup>B</sup>	7.00	6.94	+0.06	6.97	+0.03	6.99	+0.01
H-6 <sup>B</sup>	7.04	6.89	+0.15	6.96	+0.08	6.99	+0.05
	<i>rmsd</i> <sup>b</sup>	0.09		0.08		0.15	
	MAE <sup>c</sup>	0.16		0.17		0.28	
	DP4+ <sup>1</sup> H data <sup>d</sup>	3.7 %		96.3 %		0.0 %	
$\delta_C$							
C-1	98.7	102.1	-3.4	100.2	-1.5	97.7	+1.0
C-2	154.2	150.3	+3.9	153.5	+0.7	155.6	-1.4
C-3	104.2	106.4	-2.2	103.6	+0.6	104.8	-0.6
C-4	145.9	140.5	+5.4	146.3	-0.4	145.5	+0.4
C-4a	134.0	140.1	-6.1	135.9	-1.9	134.3	-0.3
C-6	161.1	166.7	-5.6	160.7	+0.4	159.5	+1.6
C-6a	112.8	108.3	+4.5	112.2	+0.6	113.0	-0.2
C-7	114.0	113.2	+0.8	113.3	+0.7	114.6	-0.6
C-8	146.9	145.4	+1.5	144.2	+2.7	144.4	+2.5
C-9	152.6	155.8	-3.2	151.6	+1.0	150.8	+1.8
C-10	107.9	108.3	-0.4	107.1	+0.8	107.0	+0.9
C-10a	128.1	130.7	-2.6	129.5	-1.4	129.6	-1.5
C-10b	118.6	124.4	-5.8	119.8	-1.2	118.4	+0.2
C-1 <sup>A</sup>	130.6	131.5	-0.9	133.8	-3.2	133.7	-3.1
C-2 <sup>A</sup>	113.7	112.4	+1.3	112.3	+1.4	112.1	+1.6
C-3 <sup>A</sup>	145.5	140.5	+5.0	143.8	+1.7	143.7	+1.8
C-4 <sup>A</sup>	144.6	141.7	+2.9	144.0	+0.6	143.6	+1.0
C-5 <sup>A</sup>	115.5	114.4	+1.1	113.6	+1.9	114.0	+1.5
C-6 <sup>A</sup>	116.9	119.7	-2.8	119.7	-2.8	119.2	-2.3
C-1 <sup>B</sup>	137.8	137.9	-0.1	139.9	-2.1	140.6	-2.8
C-2 <sup>B</sup>	114.6	114.1	+0.5	113.4	+1.2	113.8	+0.8
C-3 <sup>B</sup>	148.7	145.7	+3.0	149.4	-0.7	150.1	-1.4
C-4 <sup>B</sup>	141.1	138.9	+2.2	141.2	-0.1	140.6	+0.5
C-5 <sup>B</sup>	116.8	118.9	-2.1	119.1	-2.3	120.9	-4.1
C-6 <sup>B</sup>	121.3	118.1	+3.2	118.1	+3.2	118.6	+2.7
	<i>rmsd</i> <sup>b</sup>	3.3		1.7		1.8	
	MAE <sup>c</sup>	6.1		3.2		4.1	
	DP4+ <sup>13</sup> C data <sup>d</sup>	0.0 %		96.8 %		3.2 %	
	DP4+ all data <sup>d</sup>	0.0 %		100.0 %		0.0 %	

<sup>a</sup> $\delta$  values calculated at the mPW1PW91/6-311+G(d,p)//B3LYP/6-31G(d) level of theory performed with the polarizable continuum model (PCM) using DMSO. Red color highlights large differences in calculated values:  $|\Delta\delta_H| > 0.2$ ,  $|\Delta\delta_C| > 3.0$ . <sup>b</sup>Root-mean-square deviation. <sup>c</sup>Maximum absolute error. <sup>d</sup>DP4+ probability.<sup>21</sup>

allowed assignment of all chemical shifts and coupling constants (Table 3). Using DQF-COSY and *J*-resolved spectroscopy, seven AMX aromatic spin systems resulting from 1,2,4-trihydroxyphenyl groups were observed as doublets at  $\delta_H$  6.67–6.91 and 6.42–6.57 and doublet of doublets at  $\delta_H$  6.27–6.47. Interestingly, within each of these ranges, a gradient of proton chemical shifts was noted whereby each set of signals from a given ring appeared in a predictable order. This feature was highlighted by simulating each set of protons using the extracted  $\delta_C$  and *J* from NMR spectra (Figure 4). When comparing proton signals for H-3, H-5, and H-6, the two signals corresponding to rings Y and Z appeared to be outliers.

Most of the carbon signals were resolved in the <sup>13</sup>C NMR spectrum, although HSQC and HMBC spectra showed large clusters of correlations between protons of rings D–G and carbons averaged at 157.5 (C-4<sup>D–G</sup>),  $\delta_C$  151.2 (C-2<sup>D–G</sup>), 139.6 (C-1<sup>D–G</sup>), 123.1 (C-6<sup>D–G</sup>), 108.8 (C-5<sup>D–G</sup>), and 106.4 (C-3<sup>D–G</sup>). This prevented a precise assignment of signals but also indicated these rings were similar. Two other spin systems unrelated to the gradient of chemical shifts showed HMBC correlations from H-2<sup>A</sup> to  $\delta_C$  139.9 (C-1<sup>B</sup>) and from H-2<sup>B</sup> and H-6<sup>B</sup> to  $\delta_C$  133.9 (C-1<sup>A</sup>) allowing assignment of these two spin systems to a biphenyl unit (Figure S2). Given all these data,

Table 3.  $^1\text{H}$  (800 MHz) and  $^{13}\text{C}$  (200 MHz) NMR Spectroscopic Data of Cladophorols C and D (6–7) in  $\text{CD}_3\text{OD}$ 

position	6		7	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$
Rings A–B (Biphenyl)				
1 <sup>A</sup>	134.0		134.0	
2 <sup>A</sup>	114.9	7.01 (d, 2.2)	114.9	7.01 (d, 2.2)
3 <sup>A</sup>	146.5		146.5	
4 <sup>A</sup>	146.0		146.0	
5 <sup>A</sup>	116.6	6.81 (d, 8.2)	116.6	6.81 (d, 8.2)
6 <sup>A</sup>	119.3	6.91 <sub>2</sub> (dd, 8.2, 2.2)	119.3	6.91 <sub>2</sub> (dd, 8.2, 2.3)
1 <sup>B</sup>	139.9		139.9	
2 <sup>B</sup>	116.1	7.11 (d, 2.2)	116.1	7.11 (d, 2.2)
3 <sup>B</sup>	150.2		150.2	
4 <sup>B</sup>	143.9		143.9	
5 <sup>B</sup>	122.3	6.94 (d, 8.3)	122.2	6.94 (d, 8.3)
6 <sup>B</sup>	119.2	7.00 (dd, 8.3, 2.2)	119.2	7.00 (dd, 8.3, 2.2)
Ring C				
1	139.8		139.8 <sub>7</sub>	
2	151.3		151.3	
3	107.0	6.57 (d, 2.9)	107.0	6.58 (d, 2.9)
4	157.0		157.0	
5	109.4	6.47 (dd, 8.8, 2.9)	109.4	6.47 (dd, 8.8, 2.9)
6	123.2 <sup>b</sup>	6.90 <sub>5</sub> (dd, 8.8)	123.2 <sup>b</sup>	6.90 <sub>5</sub> (dd, 8.8)
Middle Rings				
1	139.7, 139.5 <sub>4</sub> , 139.5 <sub>3</sub> , 139.5 <sub>0</sub>		139.7 <sub>6</sub> , 139.5 <sub>7</sub> , 139.5 <sub>5</sub> , 139.5 <sub>2</sub> (2×)	
2	151.2 <sub>5</sub> , 151.2 <sub>3</sub> , 151.2 <sub>1</sub> , 151.1		151.2 <sub>6</sub> , 151.2 <sub>4</sub> , 151.2 <sub>3</sub> , 151.2 <sub>1</sub> , 151.1	
3	106.5, 106.3 <sub>6</sub> , 106.3 <sub>4</sub> , 106.3 <sub>1</sub>	6.53, 6.51, 6.50 <sub>3</sub> , 6.49 <sub>6</sub> (all d, 2.9)	106.5 (2×), 106.3 <sub>7</sub> , 106.3 <sub>6</sub> , 106.3 <sub>3</sub>	6.53, 6.51 <sub>2</sub> , 6.51, 6.50 <sub>3</sub> , 6.49 <sub>7</sub> (all d, 2.9)
4	157.6, 157.5 <sub>4</sub> , 157.5 <sub>1</sub> , 157.4		157.6 (2×), 157.5 <sub>4</sub> , 157.5 <sub>1</sub> , 157.4	
5	108.8, 108.7 <sub>7</sub> , 108.7 <sub>5</sub> , 108.7 <sub>4</sub>	6.42, 6.41, 6.40, 6.39 (all dd, 8.8, 2.9)	108.8, 108.7 <sub>8</sub> , 108.7 <sub>7</sub> (2×), 108.7 <sub>5</sub>	6.42 <sub>3</sub> , 6.41, 6.40, 6.39 <sub>9</sub> , 6.38 <sub>8</sub> (all dd, 8.8, 2.9)
6	123.1 <sub>9</sub> (2×), 123.1 <sub>4</sub> , 122.9	6.88, 6.87, 6.86, 6.84 (all d, 8.8)	123.1 <sub>9</sub> (2×), 123.1 <sub>7</sub> , 123.1 <sub>3</sub> , 122.9	6.88, 6.87, 6.86 <sub>8</sub> , 6.86 <sub>3</sub> , 6.84 (all d, 8.8)
Ring Y <sup>a</sup>				
1	141.0		141.0	
2	150.6		150.6	
3	106.3 <sub>4</sub> <sup>b</sup>	6.48 (d, 2.9)	106.3 <sub>6</sub> <sup>b</sup>	6.48 (d, 2.9)
4	156.7		156.7	
5	108.6 <sub>6</sub> <sup>b</sup>	6.34 (dd, 8.8, 2.9)	108.6 <sub>8</sub> <sup>b</sup>	6.34 (dd, 8.8, 2.9)
6	121.7	6.74 (d, 8.8)	121.7	6.74 (d, 8.8)
Ring Z <sup>a</sup>				
1	141.7		141.7	
2	147.0		147.0	
3	106.4 <sup>b</sup>	6.42 (d, 2.9)	106.4 <sub>5</sub> <sup>b</sup>	6.42 <sub>0</sub> (d, 2.9)
4	152.9		152.9	
5	109.1	6.27 (dd, 8.6, 2.9)	109.1	6.27 (d, 8.6, 2.9)
6	116.4	6.67 (d, 8.6)	116.4	6.67 (d, 8.6)

<sup>a</sup>Rings Y and Z are the last two rings of the oligomer. <sup>b</sup> $^{13}\text{C}$  signal can be interchanged with one of the corresponding signals of rings D–G.

two structures were hypothesized, differing by the position of the free alcohol groups on each hydroxyphenyl (Figure 5).

Two model structures, **15a** and **15b**, containing a biphenyl unit and three 1,2,4-trihydroxyphenyl rings (Figure 5), were computationally tested to determine which arrangement was more probable. Their  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts were predicted (Tables S26 and S27) following DFT computation at the mPW1PW91/6-311+G(d,p) level of theory and compared to the corresponding position of **6**. The rmsd and MAE values calculated for both  $^1\text{H}$  and  $^{13}\text{C}$  were lower when **15a** was considered, indicating that **6a** is the correct structure. Based on these results, **6** was identified as  $\alpha$ -hydro- $\omega$ -[3,4-dihydroxyphenyl]octa[oxy(2-hydroxyphen-4-yl)] and named cladophorol C.

**Characterization of 7.** The molecular formula for **7** was established as  $\text{C}_{60}\text{H}_{42}\text{O}_{20}$  based on HRESIMS, accounting for one additional dihydroxyphenyl moiety. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra almost perfectly superimposed those of **6**, the only differences being three additional proton signals at  $\delta_{\text{H}}$  6.86 (d,  $J = 8.8$  Hz), 6.50 (d,  $J = 2.9$  Hz), and 6.40 (dd,  $J = 8.8$  Hz) and six additional carbon signals at  $\delta_{\text{C}}$  157.6, 151.2<sub>3</sub>, 139.5<sub>7</sub>, 123.1<sub>7</sub>, 108.7<sub>7</sub>, and 106.5. As a result, **7** was identified as  $\alpha$ -hydro- $\omega$ -[3,4-dihydroxyphenyl]nona[oxy(2-hydroxyphen-4-yl)] and named cladophorol D.

**Characterization of 8.** Compound **8** was isolated as a reddish solid with the molecular formula  $\text{C}_{67}\text{H}_{44}\text{O}_{23}$  based on an  $[\text{M} - \text{H}]^-$  HRESIMS peak at  $m/z$  1215.2188. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra strongly resembled those of **6** and **7**,



Table 4.  $^{13}\text{C}$  (200 MHz) NMR Spectroscopic Data of Cladophorols E–I (8–12) in  $\text{CD}_3\text{OD}$ 

position	8	9	10	11	12
Rings A–B (Biphenyl)					
1 <sup>A</sup>	134.0	134.0	134.0	134.0	134.0
2 <sup>A</sup>	114.9	114.9	114.9	114.9	114.9
3 <sup>A</sup>	146.5	146.5	146.5	146.5	146.5
4 <sup>A</sup>	146.0	146.0	146.0	146.0	146.0
5 <sup>A</sup>	116.6	116.7	116.6	116.6	116.6
6 <sup>A</sup>	119.3	119.3	119.3	119.3	119.3
1 <sup>B</sup>	139.9 <sub>3</sub>	139.9 <sub>1</sub>	139.9 <sub>2</sub>	139.9 <sub>2</sub>	139.9 <sub>3</sub>
2 <sup>B</sup>	116.1	116.1	116.1	116.1	116.1
3 <sup>B</sup>	150.2 <sub>1</sub>	150.2	150.2 <sub>0</sub>	150.2	150.2 <sub>3</sub>
4 <sup>B</sup>	143.9	143.9	143.9	143.9	143.9
5 <sup>B</sup>	122.2	122.2	122.2	122.2	122.2
6 <sup>B</sup>	119.2	119.2	119.2	119.2	119.2
Middle Rings					
1	139.8 <sub>7</sub> , 139.6, 139.5 <sub>2</sub> , 139.5 <sub>0</sub> , 139.4 <sub>9</sub> , 139.3 <sub>8</sub> , 139.2	139.8 <sub>7</sub> , 139.6, 139.5, 139.4 <sub>8</sub> , 139.3 <sub>8</sub> , 139.2	139.8 <sub>6</sub> , 139.6, 139.5, 139.4 <sub>9</sub> (2×), 139.4 <sub>8</sub> , 139.3 <sub>7</sub> , 139.2	139.8 <sub>6</sub> , 139.6, 139.4 <sub>9</sub> (4×), 139.4 <sub>7</sub> , 139.3 <sub>6</sub> , 139.2	139.85, 139.5, 139.48 (5×), 139.46, 139.36, 139.2
2	151.3 <sub>2</sub> , 151.3 <sub>0</sub> , 151.2 <sub>7</sub> , 151.2 <sub>5</sub> , 151.2 <sub>3</sub> (3×)	151.3, 151.2 <sub>8</sub> , 151.2 <sub>6</sub> , 151.2 <sub>3</sub> , 151.2 <sub>2</sub> (2×)	151.3, 151.2 <sub>9</sub> , 151.2 <sub>7</sub> , 151.2 <sub>4</sub> , 151.22 (4×)	151.3 <sub>2</sub> , 151.3 <sub>0</sub> , 151.2 <sub>7</sub> , 151.2 <sub>4</sub> , 151.23 (3×), 151.2 <sub>2</sub> (2×)	151.33, 151.31, 151.28, 151.25, 151.24 (6×)
3	107.0, 106.5, 106.3 <sub>8</sub> (3×), 106.3 <sub>7</sub> (2×)	107.1, 106.5, 106.3 <sub>9</sub> , 106.3 <sub>8</sub> , 106.3 <sub>7</sub> (2×)	107.0, 106.4, 106.3 <sub>7</sub> (3×), 106.3 <sub>6</sub> (3×)	107.0, 106.4, 106.3 <sub>7</sub> (2×), 106.3 <sub>6</sub> (5×)	107.0, 106.4, 106.3 (8×)
4	157.8, 157.7, 157.5 <sub>9</sub> , 157.5 <sub>7</sub> , 157.5 <sub>6</sub> , 157.5 <sub>0</sub> , 157.0	157.8, 157.6, 157.5 <sub>7</sub> , 157.5 <sub>5</sub> , 157.4 <sub>9</sub> , 157.0	157.8, 157.6 <sub>5</sub> , 157.5 <sub>8</sub> , 157.5 <sub>7</sub> , 157.5 <sub>6</sub> (2×), 157.5 <sub>0</sub> , 157.0	157.8, 157.6, 157.5 <sub>8</sub> , 157.5 <sub>6</sub> (4×), 157.5 <sub>0</sub> , 157.0	157.8, 157.7, 157.58 (3), 157.57 (3×), 157.52, 157.0
5	109.4, 108.8 <sub>5</sub> , 108.8 <sub>1</sub> , 108.7 <sub>9</sub> (2×), 108.7 <sub>8</sub> (2×)	109.4, 108.8 <sub>4</sub> , 108.8 <sub>0</sub> , 108.7 <sub>9</sub> , 108.7 <sub>8</sub> , 108.7 <sub>7</sub>	109.4, 108.8 <sub>4</sub> , 108.8 <sub>0</sub> , 108.7 <sub>8</sub> , 108.77 (4×)	109.4, 108.8 <sub>4</sub> , 108.8 <sub>0</sub> , 108.7 <sub>8</sub> , 108.7 <sub>7</sub> (5×)	109.4, 108.8, 108.79, 108.76 (7×)
6	123.4, 123.3, 123.2 <sub>0</sub> (3×), 123.1 <sub>8</sub> (2×)	123.4, 123.3, 123.2 <sub>0</sub> (3×), 123.1 <sub>9</sub>	123.4, 123.3, 123.2 <sub>0</sub> (3×), 123.1 <sub>9</sub> (3×)	123.4, 123.3, 123.2 <sub>0</sub> , 123.1 <sub>8</sub> (6×)	123.41, 123.35, 123.22 (2), 123.20 (6×)
Benzo[c]chromenone					
1	100.4	100.4	100.4	100.4	100.4
2	156.9	156.9	156.9	156.9	156.9
3	105.8	105.8	105.8	105.8	105.8
4	147.5	147.5	147.5	147.5	136.2
4a	136.2	136.2	136.2	136.2	147.5
6	162.7	162.8	162.8	162.7	162.7
6a	114.5	114.4	114.5	114.5	114.5
7	115.6	115.5	115.6	115.6	115.6
8	148.6	148.7	148.6	148.6	148.7
9	154.6	155.3	154.6	154.6	154.6
10	108.6	108.6	108.6	108.6	108.6
10a	130.7	130.7	130.7	130.7	130.7
10b	120.7	120.7	120.7	120.7	120.7

(Figure S2). Altogether, these data supported the assignment of these natural products as  $\alpha$ -[(4,8,9-trihydroxy-2-benzo[c]chromen-6-on-2-yl)oxy]- $\omega$ -[3,4-dihydroxyphenyl]hepta[oxy(2-hydroxyphen-4-yl)] (9),  $\alpha$ -[(4,8,9-trihydroxy-2-benzo[c]chromen-6-on-2-yl)oxy]- $\omega$ -[3,4-dihydroxyphenyl]nona[oxy(2-hydroxyphen-4-yl)] (10),  $\alpha$ -[(4,8,9-trihydroxy-2-benzo[c]chromen-6-on-2-yl)oxy]- $\omega$ -[3,4-dihydroxyphenyl]deca[oxy(2-hydroxyphen-4-yl)] (11), and  $\alpha$ -[(4,8,9-trihydroxy-2-benzo[c]chromen-6-on-2-yl)oxy]- $\omega$ -[3,4-dihydroxyphenyl]undeca[oxy(2-hydroxyphen-4-yl)] (12) and named cladophorols F–I.

**Pharmacological Assays.** The antimicrobial activities of all isolated natural products (3–12) were assessed against multiple drug-resistant (MDR) Gram-positive and Gram-negative human pathogens as well as amphotericin-resistant *Candida albicans* (Table 6, Figure S3). Cladophorol C (6) was found to be very potent only against MRSA with a minimal inhibitory concentration (MIC) value of 1.4  $\mu\text{g}/\text{mL}$ . The bioactivity is negatively correlated by the presence of a benzo[c]chromen-6-one unit, as shown by the weaker MIC values for 5 and 8–12 (13–49  $\mu\text{g}/\text{mL}$ ) relative to 6 and 7

(1.4–9  $\mu\text{g}/\text{mL}$ ). The total number of phenol rings is also an important factor since the most potent compounds in both series (6–7 vs 8–12) have 9–10 rings and the activity weakens as this number increases. Surprisingly, all of the other cell lines, including the other Gram-positive bacterium, VREF, were much less sensitive to this family of natural products. The bioactivities of 4–12 were also evaluated in an assay using intraerythrocytic *Plasmodium falciparum*.<sup>22</sup> Only 4 and 5 inhibited the parasite with half-maximal effective concentration ( $\text{EC}_{50}$ ) values of 0.7 and 1.9  $\mu\text{g}/\text{mL}$ , respectively. In addition, the cytotoxicities of 3–12 were evaluated using immortalized human keratinocytes (HaCaT) and human kidney cells (HEK293T). Although compounds 5–11 exhibited weak toxicity (<25%) at their respective maximum concentrations against HaCaT (Figure 7) and 4 and 5 had weak toxicity toward HEK293T (half maximal cytotoxicity concentration ( $\text{CC}_{50}$ ) of 9.4 and 4.5  $\mu\text{g}/\text{mL}$ , respectively), no toxicity was observed at the MRSA MICs for 5–11 or the *P. falciparum*  $\text{EC}_{50}$  values for 4 and 5.

Table 5. <sup>1</sup>H (800 MHz) NMR Spectroscopic Data of Cladophorols E–I (8–12) in CD<sub>3</sub>OD

position	8	9	10	11	12
Rings A–B (Biphenyl)					
2 <sup>A</sup>	7.01 (d, 2.2)	7.02 (d, 2.2)	7.02 (d, 2.2)	7.01 (d, 2.2)	7.01 (d, 2.2)
5 <sup>A</sup>	6.81 (d, 8.2)	6.81 (d, 8.2)	6.81 (d, 8.2)	6.81 (d, 8.2)	6.81 (d, 8.2)
6 <sup>A</sup>	6.91 (dd, 8.2, 2.4)	6.91 (dd, 8.2, 2.2)	6.91 <sub>3</sub> (dd, 8.2, 2.2)	6.91 <sub>6</sub> (dd, 8.2, 2.2)	6.91 (dd, 8.2, 2.3)
2 <sup>B</sup>	7.11 (d, 2.2)	7.11 (d, 2.2)	7.11 (d, 2.2)	7.11 (d, 2.2)	7.11 (d, 2.3)
5 <sup>B</sup>	6.94 (d, 8.3)	6.94 (d, 8.3)	6.94 (d, 8.3)	6.94 (d, 8.3)	6.94 (d, 8.3)
6 <sup>B</sup>	7.00 (dd, 8.4, 2.2)	7.00 (dd, 8.3, 2.2)	7.00 (dd, 8.3, 2.2)	7.00 (dd, 8.3, 2.2)	7.00 (dd, 8.3, 2.2)
Middle Rings					
3	6.58, 6.54, 6.53, 6.52, 6.51 <sub>3</sub> , 6.51 <sub>1</sub> , 6.51 <sub>6</sub> (all d, 2.9)	6.58, 6.54, 6.53, 6.52, 6.51 <sub>7</sub> , 6.51 <sub>4</sub> (all d, 2.9)	6.58, 6.54 <sub>5</sub> , 6.53, 6.52 <sub>5</sub> , 6.51 <sub>6</sub> , 6.51 <sub>4</sub> , 6.51 <sub>1</sub> (2×) (all d, 2.9)	6.57, 6.54, 6.53, 6.52, 6.51 <sub>1</sub> , 6.51 <sub>6</sub> , 6.50 <sub>6</sub> , 6.50 <sub>4</sub> (2×) (all d, 2.9)	6.57, 6.54, 6.53, 6.52, 6.51, 6.50 <sub>8</sub> , 6.50 <sub>6</sub> (2×), 6.50 <sub>4</sub> (2×) (all d, 2.9)
5	6.47, 6.44, 6.42 <sub>3</sub> , 6.42 <sub>2</sub> , 6.40 <sub>6</sub> , 6.40 <sub>7</sub> , 6.40 <sub>5</sub> (all dd, 8.8, 2.9)	6.47, 6.43, 6.42 <sub>3</sub> , 6.42 <sub>2</sub> , 6.41 <sub>6</sub> , 6.40 <sub>7</sub> (all dd, 8.8, 2.9)	6.47, 6.44, 6.43, 6.42, 6.41, 6.40 <sub>6</sub> , 6.40 <sub>8</sub> , 6.40 <sub>6</sub> (all dd, 8.8, 2.9)	6.47, 6.43, 6.42 <sub>3</sub> , 6.42 <sub>1</sub> , 6.40 <sub>7</sub> , 6.40 <sub>5</sub> , 6.40 <sub>3</sub> , 6.40 <sub>2</sub> (2×) (all dd, 8.8, 2.9)	6.47, 6.43, 6.42 <sub>2</sub> , 6.42 <sub>1</sub> , 6.40 <sub>7</sub> , 6.40 <sub>5</sub> , 6.40 <sub>3</sub> , 6.40 <sub>1</sub> (3×) (all dd, 8.8, 2.9)
6	6.92, 6.91, 6.90, 6.88, 6.87 <sub>6</sub> , 6.87 <sub>2</sub> (2×) (all d, 8.8)	6.92, 6.90 <sub>8</sub> , 6.90 <sub>5</sub> , 6.88, 6.87 <sub>7</sub> , 6.87 <sub>5</sub> (all d, 8.8)	6.92, 6.91 <sub>1</sub> , 6.90 <sub>7</sub> , 6.88, 6.87 <sub>9</sub> , 6.87 <sub>5</sub> (2×), 6.87 <sub>2</sub> (all d, 8.8)	6.92, 6.91, 6.90, 6.88, 6.87 <sub>6</sub> , 6.87 <sub>1</sub> (2×), 6.86 <sub>8</sub> (2×) (all d, 8.8)	6.92, 6.90 <sub>8</sub> , 6.90 <sub>5</sub> , 6.88, 6.87 <sub>6</sub> , 6.87 <sub>1</sub> (2×), 6.86 <sub>6</sub> (3×) (all d, 8.8)
Benzo[c]chromenone					
1	6.96 (d, 2.7)	6.96 (d, 2.7)	6.96 (d, 2.7)	6.96 (d, 2.7)	6.96 (d, 2.8)
3	6.55 (d, 2.7)	6.55 (d, 2.7)	6.55 <sub>2</sub> (d, 2.7)	6.55 <sub>6</sub> (d, 2.7)	6.55 (d, 2.7)
7	7.63 (s)	7.63 (s)	7.64 (s)	7.63 (s)	7.63 (s)
10	7.32 (s)	7.32 (s)	7.33 (s)	7.32 (s)	7.33 (s)

Table 6. Pharmacological Activities<sup>a</sup> of 3,4,3',4'-Tetrahydroxy-1,1'-biphenyl (3) and Cladophorols A–I (4–12)

compd	no. of rings <sup>b</sup>	microbial strains <sup>c</sup>					<i>P. falciparum</i> <sup>d</sup>
		MRSA	VREF	Gram–	WTCA	ARCA	
3	2	>50	>50	>50	>50	>50	NT
4	2	>50	>50	>50	>50	>50	0.7 ± 0.1
5	4	13 ± 2	>50	>50	>50	>50	1.9 ± 0.2
6	9	1.4 ± 0.3	>50	>50	46 ± 13	46 ± 9	>2
7	10	9 ± 2	>50	>50	>50	>50	>2
8 <sup>e</sup>	11	25 ± 3	>50	>50	>50	>50	>2
9	10	14 ± 3	>50	>50	>50	>50	>2
10	12	31 ± 3	>50	>50	>50	>50	>2
11	13	49 ± 8	>50	>50	>50	>50	>2
12 <sup>e</sup>	14	>50	>50	>50 <sup>e</sup>	>50	>50	>2

<sup>a</sup>The values are expressed as a 95% confidence interval. <sup>b</sup>Total number of phenyl rings. <sup>c</sup>Minimal inhibitory concentration (μg/mL). Cell lines are MRSA = methicillin-resistant *Staphylococcus aureus*; VREF = vancomycin-resistant *Enterococcus faecium*; Gram–: [*Escherichia coli*; multiple drug-resistant (MDR) *E. coli*; MDR *Klebsiella pneumoniae*; MDR *K. pneumoniae*; MDR *Acinetobacter baumannii*; *A. baumannii*; MDR *Enterobacter aerogenes*; MDR *Enterobacter cloacae*; wild type *Pseudomonas aeruginosa*]; WTCA = wild type *Candida albicans*; ARCA = amphotericin B-resistant *C. albicans*. Details for the positive controls are provided in the [Experimental Section](#). <sup>d</sup>Half-maximal effective concentration (μg/mL). <sup>e</sup>Tested only against *E. coli*.

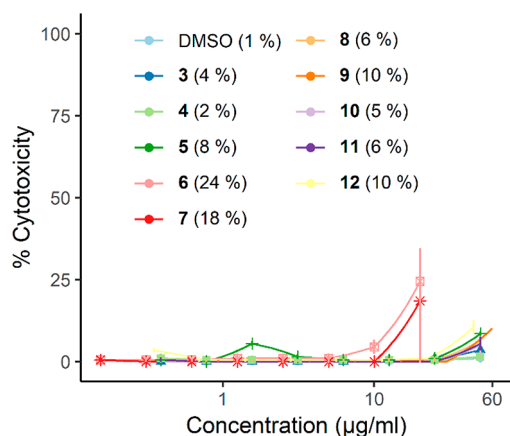
## EXPERIMENTAL DETAILS

**General Experimental Procedures.** NMR spectra (<sup>1</sup>H, HMBC, HSQC, DQF-COSY, and *J*-resolved) were recorded on an 18.8 T (800 MHz for <sup>1</sup>H and 200 MHz for <sup>13</sup>C) Bruker Avance IIIHD 800 instrument equipped with a 5 mm triple resonance broadband cryoprobe. All spectra were acquired in CD<sub>3</sub>OD or DMSO-*d*<sub>6</sub>, and chemical shifts were reported in ppm (δ) relative to the residual solvent peaks (δ<sub>H</sub> 3.31 and δ<sub>C</sub> 49.00 for CD<sub>3</sub>OD, δ<sub>H</sub> 2.50 and δ<sub>C</sub> 39.52 for DMSO-*d*<sub>6</sub>). All spectra were processed using MestReNova 11.0. HPLC separations were performed with a Waters 1525 binary pump and a Waters 2487 dual wavelength absorbance detector set at 260 nm, using two different columns: a 9.4 × 250 mm, C<sub>18</sub> silica reversed-phase (Zorbax stable-bond, 5 μm particle size), and a 4.6 × 250 mm phenylhexyl phase (Phenomenex Luna, 5 μm particle size). High-resolution mass spectrometry was conducted on an Orbitrap spectrometer in negative ion mode. The masses of isolated compounds were estimated by qNMR using a capillary filled with benzene-*d*<sub>6</sub> and calibrated against caffeine.<sup>23</sup>

**Specimen Collection.** Green alga (collection G-1240) was harvested as floating clumps on Dec 3, 2015, at the ocean surface near Titi Island, Viti Levu, Fiji (16°16'26" S, 179°26'02" E). This collection was identified as *Cladophora socialis* Kützinger by morphological and 18S rRNA phylogenetic analyses. Voucher specimens were preserved in aqueous formaldehyde and stored at the University of South Pacific. The collection was stored at –80 °C until extraction.

**Species Identification.** The collected green alga (G-1240) was identified by comparing its morphological traits with that of previously described *Cladophora* species<sup>24</sup> and by sequence analysis of nuclear, small subunit (SSU) rRNA (18S rRNA). Genomic DNA from an ethanol-preserved algal specimen was extracted using the innuPREP plant DNA kit (Analytik Jena, Germany) according to the manufacturer's protocol. The three overlapping 18S rRNA gene fragments from genomic DNA were amplified via the polymerase chain reaction (PCR) in three separate reactions using universal primer pairs, NS3/NS4, NSS/NS6, and NS7/NS8.<sup>25</sup> Each PCR amplification was performed in a 25 μL reaction volume consisting of





**Figure 7.** Cytotoxicity of 3–12 against human keratinocytes (HaCaT). The parenthetical values provide the relative cytotoxicities (%) at the maximum concentration tested (60 µg/mL for 9, 50 µg/mL for 3–5, 8, 10, 11, 45 µg/mL for 12, and 20 µg/mL for 6 and 7).

5–50 ng of purified genomic DNA; 200 µM of each of the dNTPs; 1 µM of each of the oligonucleotide primer; 1.0 U Taq DNA polymerase, and 1× standard PCR reaction buffer (NEB, Ipswich, MA). All PCR amplifications were performed in a GeneAmp PCR system 2700 (Applied Biosystems, Foster City, CA) thermocycler using the following temperature cycling parameters: initial denaturation at 94 °C for 5 min followed by a total of 40 cycles of amplification in which each cycle consisted of denaturation at 94 °C for 40 s, primer annealing at 50 °C for 40 s and primer extension at 72 °C for 1 min. After amplification, final extension of the incompletely synthesized DNA was carried out at 72 °C for 7 min. The PCR fragments were analyzed by agarose gel electrophoresis (1% wt/vol). The gel was stained with ethidium bromide and visualized under a UV transilluminator. All of the PCR fragments were either sequenced with forward or reverse primers, and sequences were manually edited and assembled using CAP3 Sequence Assembly Program.<sup>26</sup> The assembled G-1240 18S rRNA sequence (1334 bp) was submitted to GenBank (accession no. MK127549). The sequence similarity of the assembled contig of G-1240 18S rRNA to other known *Cladophora* spp. was determined by comparing it with the nonredundant nucleotide database (NCBI) using the blastn program.<sup>27</sup> The top ranked matches according to *E* values and maximum scores revealed high similarity to *C. socialis* with 96% identity.

Further, the 18S rRNA sequence of G-1240 was compared with those of other known species of the genus *Cladophora* and two outgroup taxa *Ulva curvata* and *Ulothrix zonata* from the same class but different orders, obtained from GenBank. Phylogenetic analysis was conducted in MEGA X<sup>28</sup> using the Maximum Likelihood method based on the Tamura–Nei model<sup>29</sup> with 1000 bootstrap iterations. The phylogenetic analysis revealed that G-1240 is closely related to *C. socialis* (Figure S1). Overall, morphological and phylogenetic analyses are consistent with identification of the green alga G-1240 as *C. socialis*.

**Isolation of 3–12.** Frozen material (659 g) was thawed and broken into small pieces (~1 cm) before being agitated in 60% aqueous MeOH (1.3 L) for 3 days. The mixture was filtered with a metal wire mesh, and the residues were extracted four more times with pure MeOH (2 × 1 L) and CHCl<sub>3</sub>/MeOH 1:1 (2 × 1 L). The solutions were pooled, filtered, and evaporated under reduced pressure to yield a brown gummy extract (33.4 g). The residue was dissolved in MeOH/H<sub>2</sub>O 9:1 (500 mL) and partitioned with hexanes (4 × 250 mL, 1.56 g). The amount of water in the MeOH/H<sub>2</sub>O phase was increased to a 3:2 ratio, and the resulting solution was partitioned with CHCl<sub>3</sub> (3 × 500 mL, 0.75 g). The methanol was removed from the MeOH/H<sub>2</sub>O phase by vacuum evaporation, and the aqueous phase was extracted with EtOAc (3 × 250 mL, 2.05 g). The EtOAc fraction was subjected to silica gel column chromatography eluting

with a gradient of CH<sub>2</sub>Cl<sub>2</sub>/MeOH from 49:1 to 1:2 to yield nine fractions (3.1–3.9). Fraction 3.5 (97.4 mg) was separated by reversed-phase semipreparative HPLC eluting with H<sub>2</sub>O + 0.05% TFA and MeCN (25 to 75% gradient, in 25 min). In addition to pure 3 (*t*<sub>R</sub> = 3.8 min, 0.50 mg), 4 (*t*<sub>R</sub> = 4.9 min, 0.68 mg), and 5 (*t*<sub>R</sub> = 8.7 min, 3.4 mg), eight other fractions were collected (3.5.1–3.5.8). Fraction 3.5.1 was subjected to HPLC using a phenylhexyl column and eluting with H<sub>2</sub>O + 0.05% TFA and MeOH (75 to 85% gradient, in 15 min) to yield 6 (*t*<sub>R</sub> = 7.9 min, 0.057 mg) and 11 (*t*<sub>R</sub> = 12.8 min, 0.62 mg). Fraction 3.5.2 was separated by HPLC with a phenylhexyl column eluting with H<sub>2</sub>O + 0.05% TFA and MeOH (75 to 85% gradient, in 15 min) to obtain 9 (*t*<sub>R</sub> = 9.5 min, 0.10 mg) and 12 (*t*<sub>R</sub> = 12.8 min, 0.027 mg). The same conditions were used to purify fraction 3.5.3 yielding 7 (*t*<sub>R</sub> = 9.0 min, 0.32 mg). Fraction 3.5.4 was purified by HPLC using a phenylhexyl column and eluting with H<sub>2</sub>O + 0.05% TFA and MeOH (80 to 82.5% in 7.5 min) to obtain 8 (*t*<sub>R</sub> = 6.5 min, 0.60 mg). Fraction 3.5.6 was subjected to HPLC using a phenylhexyl column and eluting with H<sub>2</sub>O + 0.05% TFA and MeOH (20 to 82.5% gradient, in 7.5 min) to yield 10 (*t*<sub>R</sub> = 6.4 min, 2.3 mg).

**Cladophorol A (4):** pale red amorphous solid; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; HRMS (ESI) *m/z* [M – H]<sup>–</sup> calcd for C<sub>13</sub>H<sub>7</sub>O<sub>6</sub><sup>–</sup> 259.0248, found 259.0245.

**Cladophorol B (5):** pale red amorphous solid; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; HRMS (ESI) *m/z* [M – H]<sup>–</sup> calcd for C<sub>25</sub>H<sub>15</sub>O<sub>9</sub><sup>–</sup> 459.0722, found 459.0718.

**Cladophorol C (6):** pale red amorphous solid; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 3; HRMS (ESI) *m/z* [M – H]<sup>–</sup> calcd for C<sub>54</sub>H<sub>37</sub>O<sub>18</sub><sup>–</sup> 973.1985, found 973.1967.

**Cladophorol D (7):** pale red amorphous solid; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 3; HRMS (ESI) *m/z* [M – H]<sup>–</sup> calcd for C<sub>60</sub>H<sub>41</sub>O<sub>20</sub><sup>–</sup> 1081.2197, found 1081.2177.

**Cladophorol E (8):** pale red amorphous solid; <sup>1</sup>H NMR data, see Table 5; <sup>13</sup>C NMR data, see Table 4; HRMS (ESI) *m/z* [M – H]<sup>–</sup> calcd for C<sub>67</sub>H<sub>43</sub>O<sub>23</sub><sup>–</sup> 1215.2201, found 1215.2188.

**Cladophorol F (9):** pale red amorphous solid; <sup>1</sup>H NMR data, see Table 5; <sup>13</sup>C NMR data, see Table 4; HRMS (ESI) *m/z* [M – H]<sup>–</sup> calcd for C<sub>61</sub>H<sub>39</sub>O<sub>21</sub><sup>–</sup> 1107.1989, found 1107.1973.

**Cladophorol G (10):** pale red amorphous solid; <sup>1</sup>H NMR data, see Table 5; <sup>13</sup>C NMR data, see Table 4; HRMS (ESI) *m/z* [M – H]<sup>–</sup> calcd for C<sub>73</sub>H<sub>47</sub>O<sub>25</sub><sup>–</sup> 1323.2412, found 1323.2394.

**Cladophorol H (11):** pale red amorphous solid; <sup>1</sup>H NMR data, see Table 5; <sup>13</sup>C NMR data, see Table 4; HRMS (ESI) *m/z* [M – H]<sup>–</sup> calcd for C<sub>79</sub>H<sub>51</sub>O<sub>27</sub><sup>–</sup> 1431.2623, found 1431.2617.

**Cladophorol I (12):** pale red amorphous solid; <sup>1</sup>H NMR data, see Table 5; <sup>13</sup>C NMR data, see Table 4; HRMS (ESI) *m/z* [M – H]<sup>–</sup> calcd for C<sub>85</sub>H<sub>55</sub>O<sub>29</sub><sup>–</sup> 1539.2834, found 1539.2797.

**Computational Methods.** Model compounds (1, 13, 14, 15a, and 15b) were subjected to a Monte Carlo conformational search using the MMFF94 force field in Spartan '16.<sup>30</sup> The geometry of all conformers within 21 kJ/mol were optimized using density functional theory (DFT) in the Gaussian D09 package,<sup>31</sup> using the B3LYP/6-31G(d) level of theory. Solvent interactions, either DMSO (1, 13, 14) or MeOH (15a, 15b), were considered with PCM. Frequencies computation was performed to ascertain true minima were obtained (zero imaginary frequency), and the resulting free energies were extracted to calculate the Boltzmann distribution. All conformers representing more than 2% of the population were considered for the computation of the shielding tensors which was performed at the mPW1PW91/6-311+G(d,p) level of theory. The shielding tensors (GIAO) were averaged using their respective Boltzmann weight, and the chemical shifts were finally derived by scaling the shielding tensors against the experimental values.

**Pharmacological Assays.** Antibacterial and antifungal assays were performed as previously described<sup>32</sup> using methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 33591), vancomycin-resistant *Enterococcus faecium* (VREF, ATCC 700221), *Escherichia coli* (EC, ATCC 25922), MDR *Escherichia coli* (MDREC, ATCC BAA-1743), wild type *Candida albicans* (WTCA, ATCC 32354), and amphotericin B-resistant *C. albicans* (ARCA, ATCC 90873). The positive controls used were vancomycin for MRSA (MIC < 4 µg/mL), chloramphenicol for VREF (MIC < 4 µg/mL), and amphotericin B for WTCA (MIC < 4 µg/mL).

nicol for VREF (MIC < 8  $\mu\text{g}/\text{mL}$ ) and EC (MIC < 8  $\mu\text{g}/\text{mL}$ ), nitrofurantoin for MDREC (MIC =  $14 \pm 1$   $\mu\text{g}/\text{mL}$ ), amphotericin B for WTCA (MIC =  $16 \pm 1$   $\mu\text{g}/\text{mL}$ ), and nystatin/cycloheximide for ARCA (MIC > 500  $\mu\text{g}/\text{mL}$ ). Activities were expressed as a 95% confidence interval of concentration required for at least 90% inhibition of growth (MIC<sub>90</sub>).

Additional antibacterial assays were performed using MDR *Klebsiella pneumonia* (MDRKP1, CDC0087), MDR *K. pneumonia* (MDRKP2, CDC0003), MDR *Acinobacter baumannii* (MDRAB1, CDC0280), MDR *A. baumannii* (MDRAB2, CDC0033), MDR *Enterobacter aerogenes* (MDREA, CDC0018), MDR *Enterobacter cloacae* (MDRECL, CDC0050), and wild type *Pseudomonas aeruginosa* (WTPA, PAO1). The bacterial cultures were grown in tryptic soy broth (TSB). Minimum inhibitory concentrations were evaluated with cation-adjusted Mueller Hinton broth (CAMHB) following Clinical and Laboratory Standards Institute (CLSI) methods.<sup>33</sup> Overnight cultures were standardized by optical density (OD) to  $5 \times 10^5$  CFU/mL, and this was confirmed by plate counts of colonies. MIC values was assigned as described;<sup>34</sup> this was determined by reading at an OD600 nm in a Cytation 3 multimode plate reader after 18–22 h incubation. Gentamicin was used as a positive control.

The human keratinocyte cell line (HaCaT) was maintained, and cytotoxicity of isolated compounds were assessed using LDH cytotoxicity assay as previously described.<sup>34</sup>

Antimalarial activity was assessed using a standard parasite proliferation assay with asexual blood-stage *P. falciparum* and SYBR Green detection.<sup>22</sup> Screening media (complete media supplemented with 0.05% Albumax II but without human serum) and fresh O<sup>+</sup> erythrocytes (TSRI Normal Blood Donation) were used for cultures of *P. falciparum* strain Dd2L (gift from Dr. David Fidock, Colombia University). A Labcyte ECHO acoustic liquid handler transferred compounds into assay plates. The plates were inoculated (Multi-Flo; BioTek, VT) with parasitized erythrocytes and fresh erythrocytes prepared in screening media, resulting in a final parasitemia of 0.3% and hematocrit of 2.5%. A chamber with a low oxygen gas mixture at 37 °C was used to culture the assay plates for 72 h with daily gas exchanges. SYBR Green lysis buffer was added to the wells after incubation using a Multi-Flo liquid dispenser, and plates were incubated at room temperature for an additional 24 h to achieve optimal development of the fluorescence signal which was read by an Envision Multimode Reader (PerkinElmer, MA). The positive controls used were atovaquone (EC<sub>50</sub> = 0.0006  $\mu\text{M}$ ) and artemisinin (EC<sub>50</sub> = 0.03  $\mu\text{M}$ ).

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.8b03218.

Phylogenetic relationship of G-1240 with other species of the genus *Cladophora*; key HMBC correlations for compounds **5**, **6**, **8**, and **10**; dose–response curves of **3**–**12** against multiple cell lines; spectroscopic data (NMR, MS, UV) for compounds **3**–**12**; DFT energies and geometries for compounds **1**, **13**, **14**, **15a**, and **15b**; NMR spectroscopic data comparison between **6** and **15a/15b** (PDF)

NMR FIDs for all isolated compounds (ZIP)

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## Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work was supported by ICBG Grant U19-TW007401 and by NIAID Grant R21 AI136563 from the U.S. National Institutes of Health and the Bill & Melinda Gates Foundation, Seattle, WA (Grant No. OPP1107194). The content is solely the responsibility of the authors and does not necessarily reflect the official views of ICBG, NIAID, or NIH. The funding agencies had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. This research was supported in part through research cyberinfrastructure resources and services provided by the Partnership for an Advanced Computing Environment (PACE) at the Georgia Institute of Technology, Atlanta, GA. We thank the Government of Fiji and the customary fishing right owners for permission to perform research in their territorial waters. We thank M. E. Hay, T. Waqa, R. X. Poulin, R. Brooker, and K. Feussner for collection of algal samples; D.E Bostwick and C. Sullards for mass spectrometric analyses; L. T. Gelbaum for NMR assistance; A. Burns, J. Mathew-Valayil, and G. O. Longo for antimicrobial assays, and B. K. Chhetri for manuscript revision. We finally thank Dr. Ronald Quinn for the useful discussions related to the manuscript.

## ■ DEDICATION

This paper is dedicated to William Aalbersberg, in memoriam, for his active mentoring of junior scientists, for his engaged international collaborations, and for his advocacy on behalf of Fijian science and society.

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**NOTE ADDED AFTER ASAP PUBLICATION**

A Supporting Information file containing NMR FIDs was added on April 9, 2019.