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Halogenated Anthraquinones from the Marine-Derived Fungus *Aspergillus* sp. SCSIO F063

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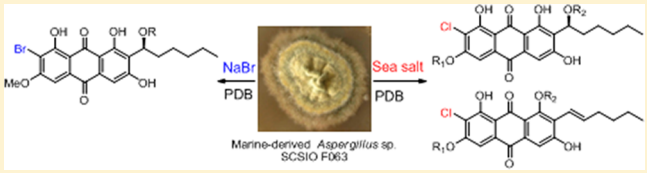
Supporting Information

ABSTRACT: Metabolomic investigations focusing on the marine-derived fungus *Aspergillus* sp. SCSIO F063 have unveiled seven new chlorinated anthraquinones (**1**–**7**) related to averantin, together with five known analogues (**11**–**15**) when the fungus was fermented using sea salt-containing potato dextrose broth. Through the addition of sodium bromide to the broth, two new brominated anthraquinones (**8**, **9**) and one new nonhalogenated anthraquinone (**10**) were obtained from the fungal mycelia. Their structures were elucidated by extensive spectroscopic analyses including MS and 1D and 2D NMR data. One metabolite, 6-*O*-methyl-7-chloroaveratin (**2**), displayed inhibition activity against three human tumor cell lines, SF-268, MCF-7, and NCI-H460, with IC_{50} values of 7.11, 6.64, and 7.42 μM, respectively.



Natural halogenated compounds possess a variety of antibacterial, antifungal, antiviral, anti-inflammatory, antiproliferative, cytotoxic, antifouling, antifeedant, ichthyotoxic, and insecticidal activities[1] and are widely used in medicine and agriculture. For example, the prominent chlorinated antibiotic vancomycin is clinically used to treat infections that are resistant to all other antibiotic drugs. Halogenated secondary metabolites are generally found in marine organisms such as mollusks, sponges, bryozoa, cyanobacteria, macroalgae, and marine microorganisms due to the abundance of halogen ions in ocean water.[2−4] To date, more than 5000 halogenated natural products have been discovered.[2]

Recently, marine-derived fungi have proven to be new sources of natural organohalides.[5] As part of our program to discover new natural products from fungi originating from the South China Sea, we isolated cytochalasins from the marine-derived fungus *Xylaria* sp. SCSIO 156,[6] immunosuppressive mycophenolic acid derivatives from the marine-derived *Penicillium* sp. SOF07,[7] and cytotoxic cycloheptapeptides from the marine-derived *Acremonium persicinum* SCSIO 115.[8] Recently, the fungus *Aspergillus* sp. SCSIO F063 was isolated from a marine sediment sample. Metabolomic and subsequent chemical investigations of this strain cultivated in potato dextrose broth (PDB) supplemented with 3% sea salt led to the isolation and identification of seven new chlorinated anthraquinones (**1**–**7**). To determine whether the biosynthetic machinery has the potential to produce other halogenated compounds, the fungus was fermented in PDB supplemented with 3% sodium bromide. As expected, two new brominated anthraquinones (**8**, **9**) were obtained from methanolic extracts of the fungal mycelia, together with a new nonhalogenated anthraquinone (**10**). In addition, five known anthraquinones (**11**–**15**) were also obtained from this fungus. We report herein the fermentation, isolation, structure elucidation, and biological activities of these marine-derived fungal compounds.

RESULTS AND DISCUSSION

The fungus *Aspergillus* sp. SCSIO F063 was fermented in PDB containing 3% sea salt on an 8 L scale. The culture was extracted successively with EtOAc and acetone, and the organic extract was subjected to silica gel column chromatography (CC) followed by Sephadex LH-20 CC and semipreparative HPLC to yield seven new chlorinated anthraquinones (**1**–**7**) and five known compounds (**11**–**15**). The fungus was cultivated once again in PDB supplemented with 3% sodium bromide. Accordingly, two new brominated anthraquinones (**8**, **9**) and a new anthraquinone (**10**) were obtained from MeOH extracts of the fungal mycelia. The known compounds averantin (**11**),[9−11] 1′-*O*-methylaverantin (**12**),[9] 6-*O*-methylaverantin (**13**),[12] averantin-1′-butyl ether (**14**),[13] and averythrin (**15**)[14] were identified on the basis of comparison of their 1D NMR with previously reported data (Supporting Information, Tables S1−S5). The specific rotation of **11** showed a negative value ($[\alpha]^{25}_{D}$ −140 (*c* 0.05, MeOH)), which is in agreement with that of (*S*)-(−)-averantin ($[\alpha]^{22}_{579}$ −138 (*c* 0.37, EtOH)).[15] Further comparisons of the CD spectra for **11**

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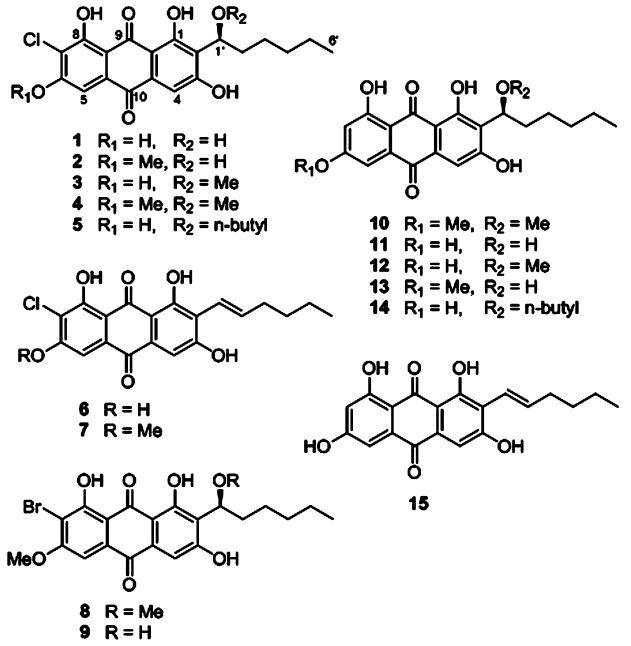

and **13** with that of (*S*)-(−)-averantin previously reported confirmed that **11** and **13** possess the *S* configuration (Figure 2).[16]

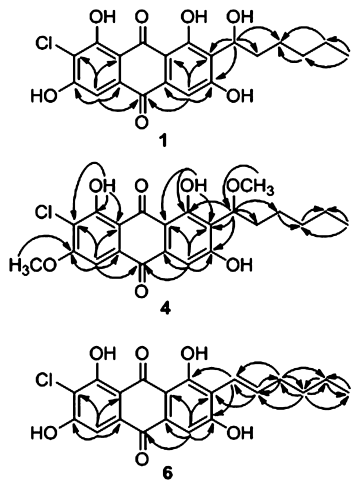

Figure 1. Summary of HMBC correlations for compounds **1**, **4**, and **6**.

Compound **1** was isolated as a yellow powder, and its molecular formula was found to be $C_{20}H_{19}ClO_7$ on the basis of HRESIMS; **1** was thus determined to possess 11 degrees of unsaturation. The presence of chlorine in **1** was based on the ca. 1:3 ratio of isotopic peak intensities at *m*/*z* 407.0722 $[M - H + 2]^-$ and the quasimolecular peak at *m*/*z* 405.0742 $[M - H]^-$ identified in the HRESIMS spectrum. The ^1H NMR spectrum of **1** (Table 1) was characterized by resonances consistent with two hydrogen-bonded phenol moieties at δ_H 12.45 and 12.69 (br s, OH-1, 8), two aromatic methine protons at δ_H 6.99 and 7.19 (s, H-4, 5), one oxygen-bearing methine proton at δ_H 5.11 (t, H-1′), four methylene groups between δ_H 1.26 and 1.78, and a methyl group at δ_H 0.84 (t, H_3-6′). The ^{13}C NMR spectrum (Table 2) revealed signals corresponding to two carbonyls (δ_C 188.5 and 180.3, C-9, 10), two aromatic methine carbons, and 10 aromatic quaternary carbons with chemical shifts attributable to a highly substituted anthraquinone scaffold, together with six aliphatic carbon signals (an oxygen-bearing carbon at δ_C 66.9, four methylene carbons at δ_C 35.3, 31.0, 24.7, 22.0, and a methyl carbon at δ_C 13.9) assignable to an alkyl side chain. The two phenolic groups of **1** appear to associate intramolecularly with the carbonyl moiety at δ_C 188.5, on the basis of their chemical shift values, suggesting that **1** is a 1,8-dihydroxy anthraquinone. In the HMBC spectrum, both isolated aromatic protons (H-4, H-5) showed correlations to the carbonyl moiety at δ_C 180.3 (Figure 1), indicating the connectivity of these protons to C-4 and C-5, respectively. Further HMBC correlations from H-4 to C-2, C-3, C-4a, C-9a and from H-5 to C-6, C-7, C-8a, C-10a established a 2,7-disubstituted-1,3,6,8-tetrahydroxy anthraquinone. Subsequent HMBC correlations of H-1′/C-2′, C-3′; H-4′/C-3′; H-5′/C-3′; and H_3-6′/C-4′, C-5′ refined the structure elucidation of the side chain for **1**; correlations from H-1′ to C-2 and C-3 revealed the location of the side chain at C-2. Chlorination of C-7 was found to be consistent with the C-7 chemical shift at δ_C 112.9. The absolute configuration of the stereogenic center at C-1′ was assigned as *S* on the basis that the CD spectrum for **1** was in agreement with that of (*S*)-(−)-averantin (**11**) (Figure 2).[16] Ultimately, the structure of new compound **1** was elucidated as (1′*S*)-7-chloroaverantin.

Compound **2** was obtained as a red powder. It showed a quasimolecular ion peak at *m*/*z* 419.0899 $[M - H]^-$ and an isotopic peak at *m*/*z* 421.0880 $[M - H + 2]^-$ in the HRESIMS spectrum, representing a 14 mass unit gain relative to **1** and a molecular formula of $C_{21}H_{21}ClO_7$. The ^1H and ^{13}C NMR spectroscopic data of **2** were very similar to those of **1** (Tables 1 and 2), except that methoxy signals were observed at δ_H 3.99 and δ_C 57.0 in **2**. Moreover, the ^{13}C NMR signal of C-5 was shifted upfield from δ_C 107.8 in **1** to δ_C 102.8 in **2**, indicating the 6-OH in **1** is replaced in **2** with a methyl ether. Further HMBC correlations from the methoxy protons to C-6 confirmed the presence of the C-6 methyl ether, which differentiates this structure from that of **1**. Accordingly, compound **2** was established as 6-*O*-methyl-7-chloroaverantin.

HRESIMS revealed that compound **3** possesses the same molecular formula, $C_{21}H_{21}ClO_7$, as that for **2**. The ^1H and ^{13}C NMR spectroscopic data of **3** resembled those of **1** (Tables 1 and 2), aside from signals consistent with the presence of a methoxy group at δ_H 3.38 and δ_C 57.9 in **3**. Additionally, ^{13}C NMR data revealed that a chemical shift for the signal representative of the oxygen-bearing methine changed from δ_C 66.9 in **1** to δ_C 79.2 in **3**, suggesting C-1′ connectivity to a methoxy group in **3** instead of the hydroxy group encountered at the same position in compound **1**. On the basis of these data and comparative analyses relative to **1** and **2**, compound **3** was identified as 1′-*O*-methyl-7-chloroaverantin.

Compound **4** was obtained as a yellow powder. Its molecular formula was determined to be $C_{22}H_{23}ClO_7$ on the basis of HRESIMS, which was two CH_2 units greater than that of **1**. The ^1H and ^{13}C NMR spectra of **4** showed a high degree of similarity to those of compounds **1**−**3**, inferring that **4** had the identical anthraquinone backbone to that seen in compounds **1**−**3**. Comparing the NMR data of **4** to those of **1** revealed signals for two additional methoxy groups (δ_H 4.07, δ_C 57.0, 6-OMe; δ_H 3.45, δ_C 58.2, 1′-OMe). These methoxy groups were linked to C-6 and C-1′, as determined by HMBC correlations from the protons of 6-OMe to C-6 and from the protons of 1′-OMe to C-1′ (Figure 1). In addition, the ^{13}C NMR data of C-5 and C-1′ in **4** shifted upfield and downfield to δ_C 103.2 and 79.5, respectively, in the same fashion as that in **2** and **3**, further

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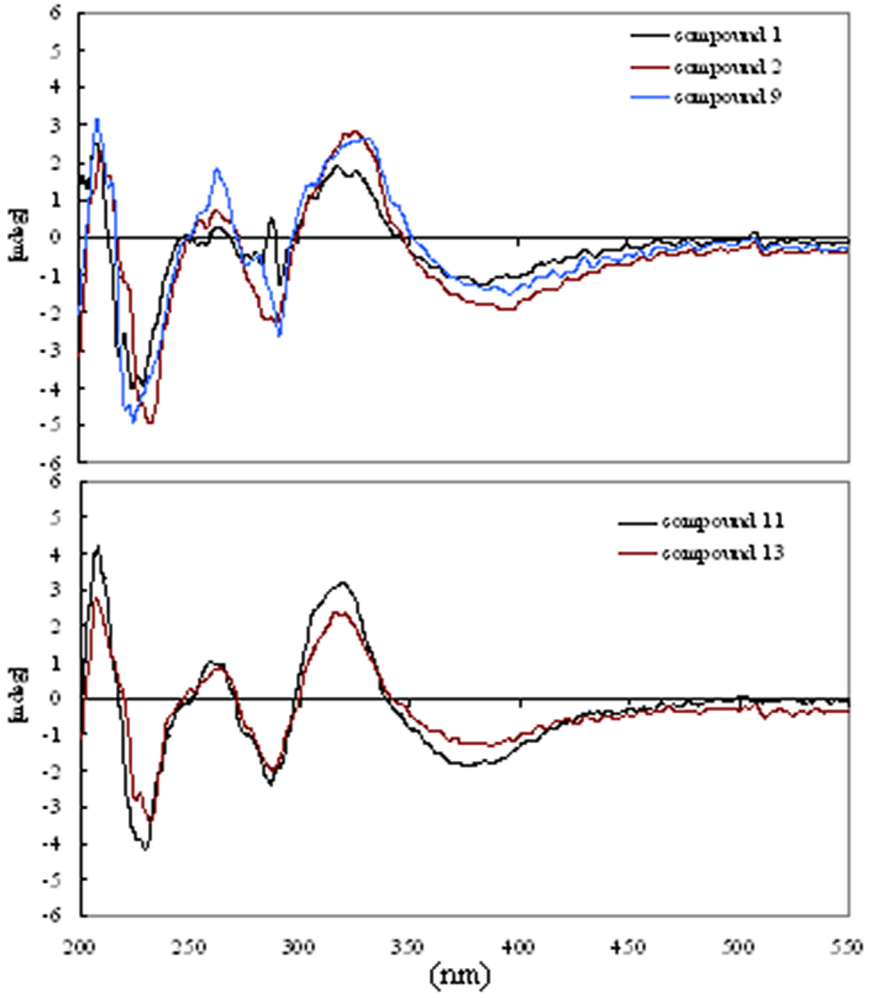

Figure 2. CD spectra of compounds **1**, **2**, **9**, **11**, and **13** in MeOH.

Table 1. Summary of ^{1}H (500 MHz) NMR Spectroscopic Data for Compounds 1−10

	1[a]	**2**[a]	**3**[b]	**4**[c]	**5**[d]	**6**[a]	**7**[c]	**8**[d]	**9**[b]	**10**[d]
position	δ_H mult. (*J* in Hz)	δ_H mult. (*J* in Hz)	δ_H mult. (*J* in Hz)	δ_H mult. (*J* in Hz)	δ_H mult. (*J* in Hz)	δ_H mult. (*J* in Hz)	δ_H mult. (*J* in Hz)	δ_H mult. (*J* in Hz)	δ_H mult. (*J* in Hz)	δ_H mult. (*J* in Hz)
4	6.99, s	7.02, s	7.12, s	7.28, s	7.29, s	7.16, s	7.26, s	7.29, s	7.14, s	7.29, s
5	7.19, s	7.18, s	7.19, s	7.41, s	7.46, s	7.21, s	7.31, s	7.37, s	7.26, s	7.37, d (2.5)
7										6.67, d (2.5)
1′	5.11, t (5.5)	5.12, dd (7.5, 4.5)	4.84, t (5.0)	4.91, dd (7.5, 4.5)	5.00, dd (8.3, 4.7)	6.48, ddd (16.0, 2.8, 1.3)	6.57, dd (16.0, 1.3)	4.93, dd (8.0, 5.0)	5.24, t (5.5)	4.93, dd (8.0, 5.0)
2′	1.78, m;	1.80, m;	1.76, m;	1.83, m;	1.74, m;	6.81, m	6.87, dt	1.85, m;	1.77, m;	1.85, m ;
	1.72, m	1.72, m	1.69, m	1.74, m	1.84, m		(16.0, 7.0)	1.77, m	1.71, m	1.77, m
3′	1.41, m;	1.42, m;	1.42, m;	1.48, m;	1.41, m;	2.18, dd	2.21, dd	1.52, m;	1.43, m;	1.53, m ;
	1.32, m	1.34, m	1.32, m	1.38, m	1.52, m	(14.0, 7.0)	(14.0, 7.0)	1.43, m	1.33 m	1.40, m
4′	1.26, m	1.28, m	1.22, m	1.28, m	1.31, m	1.41, m	1.42, m	1.32, m	1.24, m	1.32, m
5′	1.26, m	1.27, m	1.22, m	1.23, m	1.31, m	1.33, m	1.34, m	1.31, m	1.23, m	1.30, m
6′	0.84, t (5.0)	0.86, t (6.5)	0.79, t (7.0)	0.86, t (6.5)	0.90, t (7.0)	0.90, t (7.0)	0.88, t (7.0)	0.89, t (6.5)	0.90, t (6.5)	0.90, t (6.5)
1-OH	12.45, br s		12.53, br s	12.54, br s	12.53, br s	12.72, br s	12.73, br s	12.54, br s	12.45, br s	12.34, br s
8-OH	12.69, br s		12.86, br s	12.79, br s	13.00, br s	12.79, br s	12.77, br s	12.96, br s	12.93, br s	12.74, br s
6-OMe		3.99, s		4.07, s			4.02, s	4.08, s	4.01, s	3.93, s
1′-OMe			3.38, s	3.45, s				3.48, s		3.47, s
1″					3.57, m					
2″					1.61, m					
3″					1.41, m					
4″					0.93, t (7.5)					

[a]Recorded in DMSO-d_6. [b]Recorded in $CDCl_3$/CD_3OD. [c]Recorded in $CDCl_3$/DMSO-d_6. [d]Recorded in $CDCl_3$.

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Table 2. Summary of ^{13}C (125 MHz) NMR Spectroscopic Data for Compounds 1−10

	1[a]	2[a]	3[b]	4[c]	5[d]	6[a]	7[c]	8[d]	9[b]	10[d]
position	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C
1	160.7, C	161.0, C,	162.0, C	162.1, C	161.9, C	162.1, C	162.2, C	162.3, C	161.2, C	165.0, C
2	122.3, C	122.2, C	118.8, C	118.8, C	119.5, C	117.1, C	117.4, C	118.9, C	121.8, C	118.6, C
3	163.4, C	163.8, C	163.2, C	163.8, C	164.0, C	161.8, C	162.3, C	163.9, C	164.3, C	166.2, C
4	109.1, CH	109.4, CH	110.1, CH	111.1, CH	108.2, CH	108.5, CH	109.1, CH	110.8, CH	110.8, CH	110.3, CH
4a	132.8, C	132.4, C	133.6, C	133.6, C	132.7, C	130.9, C	132.1, C	133.7, C	133.3, C	135.2, C
5	107.8, CH	102.8, CH	108.2, CH	103.2, CH	110.9, CH	107.6, CH	102.5, CH	103.2, CH	102.9, CH	108.0, CH
6	160.8, C	160.4, C	161.7, C	161.1, C	159.7, C	160.4, C	160.3, C	162.1, C	162.0, C	163.5, C
7	112.9, C	114.3, C	114.4, C	116.1, C	113.7, C	112.8, C	114.6, C	110.9, C	110.9, C	107.0, CH
8	159.2, C	158.1, C	160.3, C	159.3, C	158.3, C	159.2, C	158.4, C	160.5, C	160.5, C	162.0, C
8a	108.6, C	110.5, C	109.4, C	110.7, C	110.4, C	108.9, C	110.5, C	108.8, C	108.4, C	109.1, C
9	188.5, C	188.7, C	189.3, C	189.8, C	189.4, C	188.7, C	189.2, C	189.7, C	189.3, C	189.9, C
9a	108.1, C	108.0, C	108.8, C	108.8, C	108.8, C	108.1, C	107.6, C	106.9, C	106.5, C	110.2, C
10	180.3, C	180.0, C	181.6, C	181.1, C	180.9, C	180.2, C	180.0, C	181.2, C	181.4, C	181.6, C
10a	131.3, C	131.9, C	131.9, C	132.4, C	133.7, C	131.9, C	130.5, C	133.5, C	132.9, C	134.1, C
1′	66.9, CH	66.8, CH	79.2, CH	79.5, CH	77.7, CH	118.6, CH	118.4, CH	79.6, CH	69.1, CH	79.6, CH
2′	35.3, CH_2	35.3, CH_2	34.3, CH_2	34.5, CH_2	34.6, CH_2	138.0, CH	138.3, CH	34.6, CH_2	36.0, CH_2	34.6, CH_2
3′	24.7, CH_2	24.7, CH_2	24.8, CH_2	24.9, CH_2	25.2, CH_2	33.9, CH_2	33.9, CH_2	24.9, CH_2	24.8, CH_2	25.0, CH_2
4′	31.0, CH_2	31.0, CH_2	31.4, CH_2	31.5, CH_2	31.6, CH_2	30.9, CH_2	30.8, CH_2	31.5, CH_2	31.5, CH_2	31.5, CH_2
5′	22.0, CH_2	22.0, CH_2	22.3, CH_2	22.5, CH_2	22.8, CH_2	21.7, CH_2	21.6, CH_2	22.5, CH_2	22.4, CH_2	22.5, CH_2
6′	13.9, CH_3	13.9, CH_3	13.8, CH_3	14.0, CH_3	14.0, CH_3	13.8, CH_3	13.5, CH_3	14.0, CH_3	13.8, CH_3	14.0, CH_3
6-OMe		57.0, CH_3		57.0, CH_3			56.5, CH_3	57.1, CH_3	56.9, CH_3	56.0, CH_3
1′-OMe			57.9, CH_3	58.2, CH_3				58.2, CH_3		58.1, CH_3
1″					70.9, CH_2					
2″					31.6, CH_2					
3″					19.4, CH_2					
4″					14.3, CH_3					

[a]Recorded in DMSO-d_6. [b]Recorded in $CDCl_3$/CD_3OD. [c]Recorded in $CDCl_3$/DMSO-d_6. [d]Recorded in $CDCl_3$.

substantiating the above conclusion. Thus, compound **4** was established as 6,1′-*O*,*O*-dimethyl-7-chloroaverantin.

Compound **5** had a molecular formula of $C_{24}H_{27}ClO_7$, as established by HRESIMS, displaying a quasimolecular ion peak at *m*/*z* 461.1378 $[M - H]^-$ and an isotopic peak at *m*/*z* 463.1357 $[M - H + 2]^-$. Compound **5** had the same anthraquinone core structure as found in compounds **1**−**4**, which was deduced by comparison of the ^1H and ^{13}C NMR spectroscopic data of **5** (Tables 1 and 2) with those of **1**−**4**. Detailed analyses of the 1D NMR spectroscopic data of **5** showed four more aliphatic carbon signals at δ_C 70.9, 31.6, 19.4, and 14.3 compared to **1**, suggesting **5** possesses another alkyl chain (C-1″−C-4″) in the molecule. These NMR data were almost in agreement with those of the known compound averantin-1′-butyl ether (**14**, Supporting Information, Table S4),[13] except the ^1H NMR signal of H-7 was absent in **5**, confirming the substitution of a chlorine atom at C-7. Thus, the structure of compound **5** was determined to be 7-chloroaverantin-1′-butyl ether.

Compound **6** was isolated as a red powder. The molecular formula of **6** was determined to be $C_{20}H_{17}ClO_6$ by HRESIMS, indicating 12 degrees of unsaturation, one greater than found previously for **1**. The ^1H and ^{13}C NMR spectroscopic data of **6** were slightly different from those of the known averythin (**15**), which contains a double bond between C-1′ and C-2′ on the side chain. Comparing the NMR data of **6** with those of averythin revealed that the ^1H NMR signal of H-7 was missing, and the ^{13}C resonance of C-7 was shifted downfield ($\Delta\delta_C$ +4.5, Supporting Information, Table S5), confirming the presence of C-7 chloro substitution in **6**. Consequently, compound **6** was determined to be 7-chloroaverythrin.

The molecular formula $C_{21}H_{19}ClO_6$ of compound **7** determined by HRESIMS represents a 14 mass unit gain relative to that of 7-chloroaverythrin (**6**). The ^1H and ^{13}C NMR spectroscopic data were similar to those of **6**, except for the presence of methyl ether signals at δ_H 4.02 and δ_C 56.5 (6-OMe). These data implied that **7** was a methylated product of 7-chloroaverythrin. Moreover, the ^{13}C NMR resonance for C-5 shifted from δ_C 107.6 in **6** to δ_C 102.5 in **7**, suggesting C-6 connectivity for the methoxy group. This assignment was confirmed by the observation of an HMBC correlation from the methoxy protons to C-6. Hence, compound **7** was assigned as 6-*O*-methyl-7-chloroaverythrin.

Compound **8** was acquired as a yellow powder, and its ESIMS spectrum displayed a quasimolecular ion peak at *m*/*z* 477.0 $[M - H]^-$ and an isotopic peak at *m*/*z* 479.0 $[M - H + 2]^-$. The relative abundance of the two peaks was ca. 1:1, revealing the presence of bromide substitution. Subsequent HRESIMS experiments established the molecular formula $C_{22}H_{23}BrO_7$ for **8**. The ^1H and ^{13}C NMR spectra of **8** were nearly identical with those of **4**, indicating that the structure of **8** was highly related to that of **4**. The only difference was that the chlorine at C-7 in **4** was replaced by a bromine. Therefore, compound **8** was established as 6,1′-*O*,*O*-dimethyl-7-bromoaverantin.

The molecular formula of compound **9** was measured as $C_{21}H_{21}BrO_7$ by HRESIMS. The ^1H and ^{13}C NMR spectra of **9** were almost identical with those of **2**, suggesting that the chlorine atom of **2** was substituted by a bromine atom in **9**. Accordingly, compound **9** was elucidated to be 6-*O*-methyl-7-bromoaverantin.

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Compound **10**, displaying the lowest polarity of the compounds identified herein, was isolated, and its molecular formula was determined by HRESIMS to be $C_{22}H_{24}O_7$. The ^1H and ^{13}C NMR spectra for **10** resembled those of **4** and **8** with the exception that a pair of *meta*-coupled aromatic protons were observed at δ_H 7.37 and 6.67 (J = 2.2 Hz, H-5,7) in the ^1H NMR spectrum of **10**. Additionally, when considering the ^{13}C NMR data for **10**, a new aromatic carbon signal (δ_C 107.0) was apparent instead of the halogenated quaternary carbon signal characteristic of **4** and **8**. These data indicated that the halogen at C-7 in **4** and **8** was replaced by a hydrogen atom in **10**. Accordingly, compound **10** was established as 6,1′-*O*,*O*-dimethylaverantin.

All of these anthranquinones have a stereogenic carbon at C-1′ except for compounds **6**, **7**, and **15**. As mentioned earlier, the absolute configurations of the new compound **1** and the known compounds **11** and **13** were assigned as *S* by comparing their CD spectra with that previously published for (*S*)-(−)-averantin. Similarly, we have determined the *S* configuration for the asymmetric C-1′ of the new compounds **2** and **9** (Figure 2). However, the absolute configurations of the other compounds **3**, **4**, **5**, **8**, **10**, **12**, and **14**, which have a methoxy or butoxy substitution at C-1′, could not be established through the CD spectra (Supporting Information, Figure S42). The specific rotations of **3**, **4**, **5**, **8**, **10**, **12**, and **14** did show negative values, which were consistent with those of compounds **1**, **2**, **9**, **11**, and **13** (Experimental Section and Supporting Information). Therefore, compounds **3**, **4**, **5**, **8**, **10**, **12**, and **14** were proposed to bear the same *S* configuration on the basis of the specific rotation data, as well as the shared biosynthetic pathway.

So far, only a few bromoanthraquinones have been reported from nature. Compounds **8** and **9** are structurally related to proisocrinins A−F, the multiply brominated anthraquinones discovered recently from a stalked crinoid;[17] both of them have an alkyl side chain at the C-2 position. We also added sodium iodide to the PDB medium, but no iodinated secondary metabolites were detected in the fermentation culture. It is speculated that the fungus *Aspergillus* sp. SCSIO F063 harbors a halogenase, which is capable only of catalyzing chlorination and bromination of the anthraquinone substrate.

Halogenated natural products have a wide range of biological and ecological functions. In this study, both new halogenated anthraquinones and the known compounds were evaluated for their cytotoxic activities against human breast adenocarcinoma MCF-7, human glioblastoma SF-268, and human large-cell lung carcinoma NCI-H460 cell lines using the previously reported SRB method.[18] Although compounds **1**−**15** possess similar structural features, their cytotoxicities are significantly different. Among these compounds, 6-*O*-methyl-7-chloroaverantin (**2**) exhibited the strongest cytotoxic activity against the tumor cell lines SF-268, MCF-7, and NCI-H460 with IC_{50} values of 7.11, 6.64, and 7.42 μM, respectively. The results of the cytotoxic bioassays are presented in Table 3. Compounds **1**−**15** were also tested for their antibacterial activities against three Gram-positive bacteria, *Staphylococcus aureus* ATCC 29213, *Bacillus thuringiensis* ATCC 39765, and *Bacillus subtilis* ATCC 6633, using a disk diffusion method,[19] but none of them showed an inhibition zone at a concentration of 10 μg/6 mm paper disk.

Table 3. *In Vitro* Cytotoxicities (IC_{50}, μM) of the Metabolites 1−15 against Tumor Cells SF-268, MCF-7, and NCI-H460 (n = 3)

compound[a]	SF-268	MCF-7	NCI-H460
1	>50	36.41 ± 1.08	>50
2	7.11 ± 0.14	6.64 ± 0.36	7.42 ± 0.14
3	34.06 ± 2.98	26.09 ± 1.65	37.19 ± 1.95
5	>50	49.53 ± 0.72	>50
7	>50	24.38 ± 0.81	>50
9	24.69 ± 0.72	25.62 ± 0.72	18.91 ± 1.43
11	>50	45.47 ± 0.47	>50
12	33.59 ± 1.08	35.31 ± 0.54	44.22 ± 0.27
14	47.19 ± 1.08	40.47 ± 1.43	>50
15	>50	29.69 ± 6.47	>50
cisplatin[b]	4.59 ± 0.12	10.23 ± 0.41	1.56 ± 0.13

[a]IC_{50} of compounds **4**, **6**, **8**, **10**, and **13** were >50 μM. [b]Positive control.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained with an MCP-300 polarimeter (Anton Paar). UV spectra were recorded on an UV-1750 spectrometer (Shimadzu). CD spectra were measured with a J-810 spectrophotometer (JASCO). NMR spectra were recorded with an Avance 500 spectrometer (Bruker) at 500 MHz for ^1H nuclei and 125 MHz for ^{13}C nuclei. Chemical shifts (δ) are given with reference to TMS. Coupling constants (J) are given in Hz. ESIMS spectra were detected with an LCQ DECA XP spectrometer (Thermo) and an Esquire 3000 Plus spectrometer (Bruker). HRESIMS spectra were recorded with Q-TOF-Micromass and Xevo G2 TOF spectrometers (Waters) and a 6520 Q-TOF mass spectrometer (Agilent). Column chromatography was performed using silica gel (100−200 mesh, Yantai Jiangyou Silica Gel Development Co., Ltd.) and Sephadex LH-20 (Amersham Pharmacia). Medium-pressure liquid chromatography (MPLC) was performed on a CHEETAH 100 automatic flash chromatography (Bonna-Agela). Semipreparative HPLC was operated with two 210 solvent delivery modules with a 335 PDA detector (Varian) and an ODS-A column (10 × 250 mm, 5 μm, YMC). Natural sea salt is a commercial product (Guangdong Province Salt Industry Group Co., Ltd.).

Fungal Material. The fungal strain SCSIO F063 in this study was isolated from a marine sediment sample collected in the South China Sea (17°14.285′ N; 110°26.478′ E) at a depth of 1451 m, in August 2008. The fungus was identified using a molecular biological protocol calling for DNA amplification and sequencing of the ITS-5.8S rDNA region.[20] The BLAST sequenced data have been deposited at GenBank (accession no. JQ308207). A voucher strain of this fungus was preserved at the RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, in Guangzhou, China.

Fermentation and Isolation Using PDB with Sea Salt. The fungus *Aspergillus* sp. SCSIO F063 was maintained on potato dextrose agar supplemented with 3% sea salt. The agar plugs containing the fungal strain were inoculated in 250 mL Erlenmeyer flasks, each containing 25 mL of potato dextrose broth supplemented with 3% sea salt. Flask cultures were incubated at 28 °C on a rotary shaker at 200 rpm for 2 days as seed culture. Each of the seed cultures (25 mL) was transferred into 1000 mL Erlenmeyer flasks containing 250 mL of PDB supplemented with 3% sea salt. These flasks were incubated at 28 °C on a rotary shaker at 200 rpm for 8 days. After fermentation, the culture (8 L) was centrifuged to yield the supernatant and a mycelial cake. The supernatant was extracted with an equal volume of EtOAc three times, the extracts were combined, and solvent was removed under reduced pressure. The mycelial cake was extracted with 1 L of acetone three times, the organic layers were combined, and the solvent was removed under reduced pressure. The two organic extracts were combined to give 7.3 g of residue. The extract was subjected to silica gel CC using gradient elution with a mixture of $CHCl_3$/MeOH (100:0, 98:2, 96:4, 94:6, 92:8, 90:10, 85:15, 80:20, 50:50, v/v) to give nine fractions (Fr.1−Fr.9), respectively. Fr.4 was purified repeatedly by silica gel CC eluting with petroleum ether (PE) and EtOAc mixtures

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(100:0, 90:10, 80:20, 70:30 v/v) to afford Fr.4-1 to Fr.4-13. Fr.4-5 was purified by semipreparative HPLC with an ODS column using an elution system consisting of solvent A (0.1% HOAc/15% CH_3CN in H_2O) and solvent B (0.1% HOAc/85% CH_3CN in H_2O). Elution was done at 2.5 mL/min with 80% B for 15 min, followed by a linear gradient from 80% to 100% B over the course of 5 min, and then elution with 100% B for 15 min to give **12** (13.1 mg), **13** (12.6 mg), and **14** (5.5 mg). UV detection was at 290 nm. Fr.4-7 to Fr.4-13 were combined and isolated by MPLC with an ODS column, eluting with CH_3CN/H_2O (30/70 to 100/0 over 40 min, 15 mL/min), to give Fr.4-(7-13)-1 to Fr.4-(7-13)-3. Fr.4-(7-13)-1 and Fr.4-(7-13)-3 were purified by semipreparative HPLC to yield **2** (8.3 mg), **3** (4.4 mg), and **5** (7.8 mg), **7** (5.9 mg), respectively. Fr.6 to Fr.9 were combined and chromatographed repeatedly by silica gel CC eluting with PE and EtOAc mixtures (100:0, 90:10, 80:20, 70:30 v/v) to afford Fr.(6-9)-1 to Fr.(6-9)-12. Fr.(6-9)-8 and Fr.(6-9)-11 were purified by semipreparative HPLC to yield **1** (11.3 mg), **11** (6.4 mg), **15** (6.8 mg), and **4** (5.2 mg), **6** (4.5 mg), respectively.

Fermentation and Isolation Using PDB Supplemented with Sodium Bromide. The PDB for the seed culture and large-scale fermentation was supplemented with 3% NaBr. After fermentation, the culture (4 L) was separated into the supernatant and a mycelial cake. The mycelial cake was extracted three times with 500 mL of MeOH, the extracts were combined, and the organic solvent was removed under reduced pressure to afford 2.8 g of residue for further purification. This residue was subjected to silica gel CC using gradient elution with PE and EtOAc mixtures (95:5, 90:10, 85:15, 80:20, 70:30, 60:40, 50:50, 0:100, v/v). Subfractions were analyzed by TLC and grouped into eight fractions (Fr.1–Fr.8). Fr.1 was further isolated by silica gel CC eluting with PE/EtOAc (95:5) to obtain **10** (6.1 mg). Fr.4, eluted by PE/EtOAc (80:20), was separated by silica gel CC eluting with a mixture of $CHCl_3$/MeOH (98:2, 94:6, 90:10, v/v) to give Fr.4-1, **11** (56.1 mg), and **12** (44.3 mg). Fr.4-1 was further purified by semipreparative HPLC with an ODS column using an elution system consisting of solvent A (0.1% HOAc in H_2O) and solvent B (0.1% HOAc/75% CH_3CN in H_2O). Elution was done at 2.5 mL/min with 90% B for 15 min, followed by a linear gradient from 90% to 100% B over the course of 2 min, and then elution with 100% B for 15 min to give **9** (3.1 mg) and **13** (15.3 mg). UV detection was at 290 nm. Fr.7, which was eluted by PE/EtOAc (70:30), was isolated using semipreparative HPLC under the same chromatography conditions as described above to afford **8** (3.8 mg) and **15** (8.3 mg).

*(1′S)-7-Chloroaverantin (**1**):* yellow powder; $[\alpha]^{25}_D$ −70 (*c* 0.10, $CHCl_3$/MeOH, 5:1); UV (MeOH) λ_{max} (log ε) 222 (4.01), 263 (3.83), 316 (4.13), 474 (3.41) nm; CD (0.000 042 M, MeOH), λ_{max} ($\Delta\varepsilon$) 397 (−0.77), 317 (1.36), 283 (−0.54), 262 (0.19), 223 (−2.90), and 207 (1.80) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; (−)-ESIMS *m*/*z* 405.2 $[M - H]^-$, 407.3 $[M - H + 2]^-$; (−)-HRESIMS *m*/*z* 405.0742 $[M - H]^-$ (calcd for $C_{20}H_{18}ClO_7$, 405.0741), 407.0722 $[M - H + 2]^-$.

*(1′S)-6-O-Methyl-7-chloroaverantin (**2**):* red powder; $[\alpha]^{25}_D$ −166 (*c* 0.11, $CHCl_3$/MeOH, 5:1); UV (MeOH) λ_{max} (log ε) 225 (4.03), 273 (3.89), 292 (4.03), 449 (3.56) nm; CD (0.000 053 M, MeOH), λ_{max} ($\Delta\varepsilon$) 396 (−1.36), 321 (1.95), 289 (−1.61), 262 (0.51), 228 (−3.15), and 209 (1.60) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; (−)-ESIMS *m*/*z* 419.1 $[M - H]^-$, 421.0 $[M - H + 2]^-$; (−)-HRESIMS *m*/*z* 419.0899 $[M - H]^-$ (calcd for $C_{21}H_{20}ClO_7$, 419.0898).

*(1′S)-1′-O-Methyl-7-chloroaverantin (**3**):* yellow powder; $[\alpha]^{25}_D$ −10 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 222 (4.05), 262 (4.65), 315 (4.12), 503 (3.43) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; (−)-ESIMS *m*/*z* 419.4 $[M - H]^-$, 421.0 $[M - H + 2]^-$; (−)-HRESIMS *m*/*z* 419.0895 $[M - H]^-$ (calcd for $C_{21}H_{20}ClO_7$, 419.0898).

*(1′S)-6,1′-O,O-Dimethyl-7-chloroaverantin (**4**):* yellow powder; $[\alpha]^{25}_D$ −20 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 (4.08), 284 (4.09), 325 (3.76), 449 (3.53) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; (−)-ESIMS *m*/*z* 433.4 $[M - H]^-$, 435.2 $[M - H + 2]^-$; (−)-HRESIMS *m*/*z* 433.1050 $[M - H]^-$ (calcd for $C_{22}H_{22}ClO_7$, 433.1054).

*(1′S)-7-Chloroaverantin-1′-butyl ether (**5**):* red powder; $[\alpha]^{25}_D$ −59 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 222 (4.03), 264 (3.80), 316 (4.00), 469 (3.35) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; (−)-ESIMS *m*/*z* 461.2 $[M - H]^-$, 463.3 $[M - H + 2]^-$; (−)-HRESIMS *m*/*z* 461.1378 $[M - H]^-$ (calcd for $C_{24}H_{26}ClO_7$, 461.1373).

*7-Chloroaverythrin (**6**):* red powder; UV (MeOH) λ_{max} (log ε) 232 (4.12), 320 (4.40), 444 (3.78) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; (−)-ESIMS *m*/*z* 387.1 $[M - H]^-$, 389.3 $[M - H + 2]^-$; (−)-HRESIMS *m*/*z* 387.0636 $[M - H]^-$ (calcd for $C_{20}H_{16}ClO_6$, 387.0641).

*6-O-Methyl-7-chloroaverythrin (**7**):* red powder; UV (MeOH) λ_{max} (log ε) 224 (4.11), 296 (4.05), 453 (3.61) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; (−)-ESIMS *m*/*z* 401.4 $[M - H]^-$, 403.5 $[M - H + 2]^-$; (−)-HRESIMS *m*/*z* 401.0789 $[M - H]^-$ (calcd for $C_{21}H_{18}ClO_6$, 401.0792).

*(1′S)-6,1′-O,O-Dimethyl-7-bromoaverantin (**8**):* yellow powder; $[\alpha]^{25}_D$ −31 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 224 (3.94), 285 (3.94), 326 (3.51), 447 (3.39) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; (−)-ESIMS *m*/*z* 477.0 $[M - H]^-$, 479.0 $[M - H + 2]^-$, 399.1 $[M - Br]^-$; (−)-HRESIMS *m*/*z* 477.0566 $[M - H]^-$ (calcd for $C_{22}H_{22}BrO_7$, 477.0549).

*(1′S)-6-O-Methyl-7-bromoaverantin (**9**):* red powder; $[\alpha]^{25}_D$ −87 (*c* 0.32, $CHCl_3$/MeOH, 5:1); UV (MeOH) λ_{max} (log ε) 224 (4.41), 289 (4.41), 320 (4.14), 445 (3.89) nm; CD (0.000 043 M, MeOH), λ_{max} ($\Delta\varepsilon$) 396 (−1.06), 330 (1.88), 291 (−1.84), 263 (1.30), 225 (−3.49), and 208 (2.26) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; (−)-ESIMS *m*/*z* 463.5 $[M - H]^-$, 465.3 $[M - H + 2]^-$; (−)-HRESIMS *m*/*z* 463.0411 $[M - H]^-$ (calcd for $C_{21}H_{20}BrO_7$, 463.0392).

*(1′S)-6,1′-O,O-Dimethylaverantin (**10**):* yellow powder; $[\alpha]^{25}_D$ −110 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 220 (4.01), 290 (3.68), 449 (3.17) nm; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; (−)-ESIMS *m*/*z* 399.1 $[M - H]^-$; (−)-HRESIMS *m*/*z* 399.1460 $[M - H]^-$ (calcd for $C_{22}H_{23}O_7$, 399.1444).

Cytotoxicity Assay. The metabolites **1**–**15** were evaluated for their cytotoxic activities against SF-268, MCF-7, and NCI-H460 cell lines with the SRB method.[18] Cells (180 μL) with a density of 3×10^4 cells/mL of media were seeded onto 96-well plates and incubated for 24 h at 37 °C, 5% CO_2. Various concentrations of compounds (20 μL) were added to the plate wells, and the plates were further incubated for 72 h. After incubation, cell monolayers were fixed with 50% (w/v) trichloroacetic acid (50 μL) and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by washing repeatedly with 1% acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution (200 μL) for OD determination at 570 nm using a microplate reader. Cisplatin was used as a positive control, possessing potent cytotoxic activity. All data were obtained in triplicate and are presented as means ± SD; IC_{50} values were calculated with the SigmaPlot 10.0 software using a nonlinear curve-fitting method.

Antibacterial Activity Assay. Compounds **1**–**15** were tested for antibacterial activity using a disk diffusion assay.[19] Seed cultures of three bacteria, *Staphylococcus aureus* ATCC29213, *Bacillus thuringiensis* ATCC39765, and *Bacillus subtilis* ATCC6633, were cultivated in LB broth medium by incubating the organisms for 10 h at 37 °C. Then the seed cultures were added to LB agar medium to make plates. Sterile filter disks (6 mm diameter) infused with 5 μL of test solution (2 μg/μL DMSO) and positive control (5 μg/μL DMSO ampicillin) were added to the plates. The plates were placed in an incubator at 37 °C. After 12 h, the diameters of the zones of growth inhibition around each disk were recorded.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HRESIMS and NMR spectra of compounds **1**–**10**. These data are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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