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Chlorinated metabolites with antibacterial activities from a deep-sea-derived *Spiromastix* fungus†

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Chromatographic separation of the solid cultures of a deep-sea-derived *Spiromastix* fungus (MCCC 3A00308) resulted in the isolation of eight compounds. Their structures were identified on the basis of the spectroscopic data. Compounds 1–8 are classified as depsidone-type (1–4), isocoumarin-type (5 and 6), and benzothiazole-type (7 and 8), of which 1–7 are new compounds and 1–3 along with 5 and 6 are chlorinated. Compound 3 is characterized by trichlorination and shows potent activities against Gram-positive pathogenic bacteria including *Staphylococcus aureus* ATCC 25923, *Bacillus thuringiensis* ATCC 10792, and *Bacillus subtilis* CMCC 63501, with minimum inhibitory concentration (MIC) values ranging from 0.5 to 1.0 $\mu\text{g mL}^{-1}$. This study extends the chemical diversity of chlorinated natural products from marine-derived fungi and provides a promising lead for the development of antibacterial agents.

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1. Introduction

Marine-occurring halogenated compounds are widely distributed in macroorganisms (algae,^{1–3} sponges,^{4–7} corals,^{8,9} and tunicates^{10,11}) and microorganisms (fungi and bacteria)^{12–19} that inhabit extreme marine environments. Halometabolites play a crucial role in pharmaceutical and agriculture applications. It is estimated that approximately 25% of clinically used medicines are halogenated, demonstrating a significant contribution of halogen atoms to bioactivity. Well-known halogenated medicines include the antibiotics chloramphenicol,²⁰ vancomycin,²¹ and rebeccamycin,²² which are clinically used for their antibacterial and antitumor properties. Compared to those from marine macroorganisms, bioactive halometabolites from marine microorganisms are relatively unexplored. Nevertheless, a number of brominated and chlorinated resorcylics, *i.e.*, resorcylic acid lactones, with structural novelty have been isolated from marine-derived fungus.²³ In addition, marine-derived bacteria are a promising source for the production of halometabolites, as exemplified by the napyradiomycins obtained from a marine-derived actinomycete strain that show induction of apoptosis in the colon tumor cell line HCT-116,²⁴ and the chlorinated bisindole alkaloids (indimicins) obtained

from a deep-sea-derived *Streptomyces* sp. that show antitumor activities in cell levels.²⁵ Halogenated compounds in nature are catalyzed by the relevant halogenases, including haloperoxidases, nonheme Fe(II)- α -ketoglutarate-dependent halogenases, *S*-adenosyl-L-methionine-dependent halogenases and flavin-dependent halogenases to incorporate with fluorine, chlorine, bromine, and iodine donors.^{26,27} In our previous study, a group of chlorodepsidones, namely, spiromastixones B–P, were obtained from a deep-sea *Spiromastix* sp. fungus; these compounds display selective activity against a panel of Gram-positive bacteria, including potent inhibition against methicillin-resistant and vancomycin-resistant pathogenic bacteria.^{14,28} In addition to known chlorodepsidones, the LC-MS total ion chromatograph of the EtOAc extract of the fermented *Spiromastix* sp. strain provided the ion peaks for a number of unknown chlorinated compounds. To discover new chlorinated metabolites from the same fungal strain, the minor components from the EtOAc fraction of the solid cultures were subjected to chromatographic separation, resulting in the isolation of four new depsidones (spiromastixones P–S, 1–4), three new isocoumarins (spiromastimelleins A (5) and B (6)), and spiromastibenzothiazole A (7)), together with the known benzothiazole 8,²⁹ of which 1–3 and 5 and 6 were chlorinated (Fig. 1). Herein, the structural elucidation and antibacterial activities of all compounds are described.

2. Experimental section

2.1 General procedure

UV spectra were recorded using a Cary 300 spectrometer. IR data were recorded using a Thermo Nicolet Nexus 470 FT-IR

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Fig. 1 Chemical structures of 1–8.

spectrometer. A Bruker Avance-400 FT NMR spectrometer was used to measure NMR spectroscopic data, and DMSO- d_6 was used as the solvent with the reference peaks at δ_H 2.50 and δ_C 39.9 ppm. A Xevo G2 Q-TOF mass spectrometer was used to record the HRESIMS spectra. Semipreparative HPLC was performed using an Alltech instrument coupled with a YMC-packed reversed-phase C18 column (5 μ m, 10 mm $\times$ 250 mm). Column chromatography (CC) was carried out using silica gel (100–200 and 200–300 mesh), octadecyl silica (ODS, 50 μ m, YMC, Japan), and Sephadex LH-20 (18–110 μ m, Uppsala, Sweden). TLC analysis was performed using precoated silica gel plates (Merck, Kieselgel 60 F254). All solvents used for chromatography were of analytical grade.

2.2 Fungal material

The fungus *Spiromastix* sp. MCCC 3A00308 was isolated from South Atlantic Ocean sediment at a depth of 2869 m. The protocols for taxonomy and fermentation were described in the previous study.¹⁴

2.3 Isolation and purification

The fermented broth was extracted with EtOAc, and the EtOAc extract was fractionated into ten fractions (FA–FJ) using vacuum liquid chromatography on silica gel, with petroleum ether (PE)–acetone (50 : 1 to 1 : 1) as the eluent. Previously, fractions FF and FH have been chemically examined.^{14,28} In this study, fraction FI was selected for further examination. FI (186.2 mg) was fractionated on a Sephadex LH-20 column eluted with MeOH to yield 4 (5.6 mg) and 1 (4.5 mg), along with subfractions FI5–FI8. FI5 (32.1 mg) was separated *via* semipreparative HPLC with MeCN–H₂O (13 : 7) as the mobile phase to obtain 2 (2.3 mg) and 3 (3.8 mg). FI6 (56.5 mg) was purified *via* semipreparative HPLC using MeOH–H₂O (7 : 3) as the mobile phase to yield 5 (8.5 mg) and 6 (7.3 mg). FI7 (26.6 mg) was separated *via* semipreparative HPLC with MeCN–H₂O (3 : 17) to yield 7 (4.4 mg) and 8 (3.8 mg).

Spiromastixone P (1): white powder; UV (MeOH) λ_{max} (log ϵ) 225 (4.26), 262 (3.86) nm; IR (KBr) ν_{max} 3400, 2934, 1653, 1613, 1454, 1385, 1251 cm^{-1} ; 1H and ^{13}C NMR data, see Table 1; HRESIMS m/z 361.0843 $[M - H]^-$ (calcd for C₁₉H₁₈ClO₅, 361.0843).

Spiromastixone Q (2): white powder; UV (MeOH) λ_{max} (log ϵ) 223 (4.32), 266 (3.63) nm; IR (KBr) ν_{max} 3391, 1726, 1595, 1431,

1241 cm^{-1} ; 1H and ^{13}C NMR data, see Table 1; HRESIMS m/z 395.0449 $[M - H]^-$ (calcd for C₁₉H₁₇Cl₂O₅, 395.0453).

Spiromastixone R (3): white powder; UV (MeOH) λ_{max} (log ϵ) 223 (4.25), 266 (3.71) nm; IR (KBr) ν_{max} 3381, 1729, 1594, 1428, 1240 cm^{-1} ; 1H and ^{13}C NMR data, see Table 1; HRESIMS m/z 429.0053 $[M - H]^-$ (calcd for C₁₉H₁₆Cl₃O₅, 429.0063).

Spiromastixone S (4): white powder; IR (KBr) ν_{max} 3259, 1718, 1607, 1451, 1251, 1221 cm^{-1} ; 1H and ^{13}C NMR data, see Table 1; HRESIMS m/z 371.1126 $[M - H]^-$ (calcd for C₂₀H₁₉O₇, 371.1131).

Spiromastimellein A (5): colorless oil; $[\alpha]_D^{25} + 223$ (c 1.07, CH₂Cl₂); UV (MeOH) λ_{max} 220 (3.15), 269 (2.65), 313 (1.74) nm; ECD (MeOH) λ_{max} ($\Delta\epsilon$): 244 (+33.09), 264 (+36.11), 315 (−4.47) nm; IR (KBr) ν_{max} 3188, 1692, 1658, 1617, 1469, 1216 cm^{-1} ; 1H and ^{13}C NMR data, see Table 2; HRESIMS m/z 239.0118 $[M - H]^-$ (calcd for C₁₁H₈ClO₄, 239.0111).

Spiromastimellein B (6): colorless oil; $[\alpha]_D^{25} + 165$ (c 0.65, CH₂Cl₂); UV (MeOH) λ_{max} 218 (3.17), 269 (2.45), 313 (1.82) nm; ECD (MeOH) λ_{max} ($\Delta\epsilon$): 245 (+24.15), 264 (+26.34), 318 (−2.48) nm; IR (KBr) ν_{max} 3419, 2962, 1767, 1710, 1690, 1663, 1461, 1243 cm^{-1} ; 1H and ^{13}C NMR data, see Table 2; HRESIMS m/z 272.9718 $[M - H]^-$ (calcd for C₁₁H₇Cl₂O₄, 272.9721).

Spiromastibenzothiazole A (7): colorless oil; UV (MeOH) λ_{max} 220 (2.25), 240 (4.15), 273 (3.55) nm; 1H and ^{13}C NMR data, see Table 2; HRESIMS m/z 208.0070 $[M - H]^-$ (calcd for C₉H₆NO₃S, 208.0068).

2.4 Antibacterial test

Antibacterial effects against Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus thuringiensis* ATCC 10792, and *Bacillus subtilis* CMCC 63501) and a Gram-negative bacterium (*Escherichia coli* ATCC 25922) were detected using a protocol in the literature.²⁸ Chloroamphenicol was used as a positive control.

3. Results and discussion

Spiromastixone P (1) was obtained as a white powder. Its molecular formula was established as C₁₉H₁₉ClO₅ on the basis of the HRESIMS data (m/z 361.0843, $[M - H]^-$), indicating ten degrees of unsaturation. The IR absorptions at 3400, 1653, and 1613 cm^{-1} suggested the presence of hydroxy, carbonyl, and phenyl functionalities. The 1H NMR data showed three

Table 1 ^1H and ^{13}C NMR data of 1–4 in $\text{DMSO}-d_6$

	1		2		3		4	
Position	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)
1	111.4, C		119.5, C		119.9, C		111.5, C	
2	163.2, C		160.9, C		160.7, C		162.5, C	
3	105.1, CH	6.62, br s	105.9, CH	6.85, s	105.9, CH	6.88, s	105.1, CH	6.59, d (2.2)
4	162.7, C		158.5, C		158.7, C		163.2, C	
5	115.6, CH	6.63, br s	112.7, C		112.6, C		115.5, CH	6.62, d (2.2)
6	149.7, C		145.5, C		145.8, C		149.6, C	
7	162.6, C		161.9, C		161.3, C		162.8, C	
8	35.5, CH_2	2.67, t (7.5)	33.0, CH_2	2.83, t (7.4)	33.1, CH_2	2.83, t (7.5)	35.5, CH_2	2.68, t (7.5)
9	24.7, CH_2	1.49, m	22.7, CH_2	1.54, m	22.7, CH_2	1.54, m	24.7, CH_2	1.49, m
10	14.3, CH_3	0.85, t (7.2)	14.1, CH_3	0.86, t (7.4)	14.1, CH_3	0.86, t (7.3)	14.3, CH_3	0.85, t (7.3)
1'	141.6, C		142.2, C		142.6, C		141.2, C	
2'	143.1, C		145.5, C		140.4, C		145.3, C	
3'	105.7, CH	6.75, s	109.0, C		111.8, C		105.9, CH	6.61, s
4'	151.4, C		151.7, C		148.3, C		153.6, C	
5'	116.9, C		112.9, CH	6.69, s	119.2, C		119.7, C	
6'	134.1, C		133.5, C		132.1, C		134.0, C	
7'	29.9, CH_2	2.85, t (7.8)	31.2, CH_2	2.67, t (7.7)	29.9, CH_2	2.85, t (7.8)	29.8, CH_2	2.78, t (7.8)
8'	22.7, CH_2	1.54, m	23.6, CH_2	1.57, m	22.5, CH_2	1.56, m	24.3, CH_2	1.55, m
9'	14.6, CH_3	1.04, t (7.3)	14.3, CH_3	0.98, t (7.3)	14.5, CH_3	1.05, t (7.4)	14.8, CH_3	0.99, t (7.3)
10'							169.0, C	
4-OH		10.78, s		11.65, br s		11.75, br s		10.72, br s
4'-OH		10.53, s		10.50, s		10.45, br s		11.93, br s

aromatic protons, two methyl group triplets, four methylene groups, and two phenol protons (Table 1). Diagnostic ^{13}C NMR and HSQC data revealed a total of 19 carbon resonances, from which 12 aromatic resonances for two phenyl groups, a carbonyl carbon at δ_{C} 162.6, four methylene and two methyl carbons were identified. These NMR data were characteristic of a depsidone, and closely resembled those of spiromastixone I,¹⁴ a co-isolated analog from the same fungal strain. The 2D NMR data revealed

that the partial structure of ring A was identical to that of the known analog, but the absence of an aromatic proton and the presence of an additional quaternary carbon in aromatic ring C were recognized. The HMBC correlations from H-3' to C-1' (δ_{C} 141.6), C-2', C-4' (δ_{C} 151.4), and C-5' (δ_{C} 116.9), OH-4' (δ_{H} 10.53, s) to C-3' (δ_{C} 105.7), C-4', and C-5', and from H₂-7' to C-1', C-5', and C-6' (δ_{C} 134.1), clarified that C-5' was chlorinated. An ether bond connecting rings A and C across C-2 to C-1' was evident

Table 2 ^1H and ^{13}C NMR data of 5–8 in $\text{DMSO}-d_6$

	5		6		7		8	
No.	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)
1	164.8, C		165.3, C					
2					152.2 ^a , CH	9.06, s	152.4 ^a , CH	9.07, s
3	156.1, C		155.7, C					
3a					146.5, C		147.0, C	
4	35.0, CH	4.08, q (7.0)	34.8, CH	4.11, q (7.0)	130.9, C		125.2, CH	7.84, s
4a	142.8, C		140.3, C					
5	109.6, C		110.9, C		117.3, CH	6.92, d (2.3)	123.3, C	
6	161.3 ^a , C		157.5, C		155.9, C		154.6, C	
7	102.6, CH	6.49, s	108.3, C		105.8, CH	7.32, d (2.3)	106.3, CH	7.46, s
7a					7.32, d (2.3)		133.6, C	
8	161.9 ^a , C		157.8, C		37.7, CH_2	3.98, s	36.2, CH_2	3.62, s
8a	99.2, C		99.4, C					
9	97.2, CH_2	4.86, br s 4.80, br s	97.8, CH_2	4.90, d (1.8) 4.85, d (1.8)	172.7, C		173.0, C	
10	21.5, CH_3	1.34, d (7.0)	21.5, CH_3	1.36, d (7.0)				
6-OH		11.74, br s			9.78, s			10.0, s
8-OH		10.74, s		11.34, s				
COOH					12.32, br s			12.17, br s

^a Assignment in the column can be interchanged.

from the NOE correlation between H-3 and H₂-7' (Fig. 2). Thus, **1** was identified as a 5'-chlorinated spiromastixone I.

Spiromastixone **Q** (**2**) had a molecular formula of C₁₉H₁₈Cl₂O₅ as determined by the HRESIMS data (m/z 395.0449 [M – H][–]). A comparison of the NMR data (Table 1) revealed that **2** was an analog of spiromastixone **K** with the same partial structure of ring A. The difference was found in ring C, where a quaternary carbon was observed at C-3' (δ_C 109.0) instead of the methine group of the known analogue. The HMBC correlations from 4'-OH (δ_H 10.50, s) to C-3', C-4' (δ_C 151.7), and C-5' (δ_C 112.9), and from H-5' (δ_H 6.69, s) to C-3', C-1' (δ_C 142.2), and C-7' (δ_C 31.2) suggested a chlorine substitution at C-3'. Thus, **2** was identified as a 3'-chlorinated spiromastixone **K**.¹⁴

The molecular formula of spiromastixone **R** (**3**) was determined as C₁₉H₁₇Cl₃O₅ from the HRESIMS (m/z 429.0053, [M – H][–]) and ¹³C NMR data. The similar NMR data of **2** and **3**, except for the quaternary carbon at C-5' (δ_C 119.2) for **3**, in association with the molecular composition identified **3** to be a 5'-chlorinated analog of **2**. The NOE interaction between H-3 (δ_H 6.88) and H₂-7' confirmed the location of the ether bond.

The HRESIMS data (m/z 371.1126 [M – H][–]) indicated the molecular formula of spiromastixone **S** (**4**) to be C₂₀H₂₀O₇. Its NMR data resembled those of spiromastixone **I**, but a carboxylic group (δ_C 169.0) and the loss of H-5' were recognized. The HMBC correlations from H-3' (δ_H 6.61) to C-1' (δ_C 141.2) and C-5' (δ_C 119.7) and from H₂-7' (δ_H 2.78) to C-1', C-5', and C-6' (δ_C 134.0), along with the NOE interaction between H-3 (δ_H 6.59) and H₂-7', allowed the assignment of **4** as an analog of spiromastixone **I** with a carboxylic group at C-5'.

Spiromastimellein **A** (**5**) has a molecular formula of C₁₁H₉ClO₄ as determined from the HRESIMS and NMR data, requiring seven degrees of unsaturation and containing a chlorine atom. The IR absorptions at 3188 and 1658 cm^{–1} suggested the presence of hydroxy and carbonyl functionalities. The ¹H NMR spectrum exhibited resonances including a methyl doublet, an exocyclic methylene group, an sp³ methine, an aromatic singlet, and two D₂O-exchangeable protons (Table 2). The ¹³C NMR spectrum showed 11 carbon resonances, including six aromatic carbons corresponding to a phenyl unit, a carbonyl carbon, two olefinic carbons, an sp³ methine, and a methyl carbon (Table 2). The aromatic proton H-7 (δ_H 6.49, s) showed HMBC correlations with C-5 (δ_C 117.3), C-6 (δ_C 161.3), C-8 (δ_C 161.9), and C-8a (δ_C 99.2), which, in conjunction with the NOE relationships of H-7 with the phenol protons OH-6 (δ_H

11.74, br) and OH-8 (δ_H 10.74, s), indicated an aromatic ring with OH groups at C-6 and C-8. The long-range W correlation of H-7 with C-1 (δ_C 164.8) suggested the presence of a carbonyl group at C-8a. The presence of a butene unit was established from the COSY relationship between H-4 (δ_H 4.08, q) and H₃-10 (δ_H 1.34, d) together with the HMBC correlations between H₂-9 (δ_H 4.86, 4.80)/C-4 (δ_C 35.0) and H₃-10/C-3 (δ_C 156.1). The linkage of the butane unit to C-4a (δ_C 142.8) was deduced from the HMBC correlations between H₃-10 and C-4a and from H-4 to C-4a, C-5 and C-8a. According to the molecular unsaturation, the one remaining unsaturation was contributed by the formation of a δ -lactone across C-1 and C-3, while a chlorine atom was substituted at C-5. Thus, **5** was determined to be a chlorinated isocoumarin with an exomethylene group at C-3.³⁰ Compound **5** and clearanol C^{30–32} both possess a δ -lactone ring substituted with a methylene group at the C-3 position and a methyl group at the C-4 position. Since both compounds have similar optical rotations and ECD spectra ([α]_D²⁵ + 223 and positive Cotton Effects (CEs) at 244 and 264 nm for **5**; [α]_D²⁵ + 215.0 (MeOH) and positive CEs at 237 and 267 nm for clearanol C,³⁰ Fig. 3 and S8-2†), they are both assigned the absolute configuration of *S* at C-4.

The molecular formula of spiromastimellein **B** (**6**) was determined to be C₁₁H₈Cl₂O₄ based on the HRESIMS and NMR data, containing two chlorine atoms. The NMR data of **6** (Table 2) were closely related to those of **5**, except that the aromatic carbon C-7 was substituted. The HMBC correlations of OH-8 (δ_H 11.34) with the nonprotonated aromatic carbons C-7 (δ_C 108.3), C-8 (δ_C 157.8), and C-8a (δ_C 99.4) supported the conclusion that **6** was a 7-chlorinated analog of **5**. The same sign of optical rotation and the similar ECD data (Fig. 3) suggested that both **5** and **6** had the same configuration.

Spiromastimellein **C** (**7**) has a molecular formula of C₉H₇NO₃S as determined from the HRESIMS data (m/z 208.0070 [M – H][–]), containing seven degrees of unsaturation. The ¹H NMR spectrum exhibited *meta*-coupled aromatic H-5 (δ_H 6.92, d, J = 2.3 Hz) and H-7 protons (δ_H 7.32, d, J = 2.3 Hz), an olefinic H-2 proton (δ_H 9.06, s), methylene protons (δ_H 3.98, s), and D₂O-exchangeable OH-6 (δ_H 9.78, s) and COOH protons (δ_H 12.32, br) (Table 2). A *tetra*-substituted phenyl ring was established from the HMBC correlations from H-5 and H-7 to C-3a (δ_C 146.5) and from OH-6 to C-5 (δ_C 117.3), C-6 (δ_C 155.9), and C-7 (δ_C 105.8), indicating the presence of a phenolic group at C-6. The presence of an acetic acid unit at C-7 was evident from the HMBC

Fig. 2 Key COSY, HMBC and NOE correlations of **1**, **5** and **7**.

Fig. 3 Experimental ECD spectra of **5** and **6** in MeOH.

correlations from H₂-8 to carboxylic carbon C-9 (δ_{C} 172.7) as well as to C-5 and C-3a. The remaining unit containing a nitrogen atom and a sulfur atom was suggested to be a thiazole moiety fused to the phenyl ring, which was supported by the HMBC correlations from H-2 to C-4a and C-8a.

In addition, a known compound identical to M4582 (**8**)³⁰ was isolated, which was identified as an analog of **7** with an alternative substitution of the acetic acid unit at C-5 on the basis of the NMR data.

Compounds **1–8** were tested for antibacterial activity against the Gram-positive bacterium strains *Staphylococcus aureus* ATCC 25923, *Bacillus thuringiensis* ATCC 10792, and *Bacillus subtilis* CMCC 63501, as well as against Gram-negative bacterium *Escherichia coli* ATCC 25922. Compounds **1–6** showed significant inhibitory activities against three Gram-positive bacteria with minimum inhibitory concentration (MIC) values ranging from 0.5–32 $\mu\text{g mL}^{-1}$. However, all compounds exhibited weak activity against *E. coli*, implying selective inhibition toward Gram-positive bacteria (Table 3). Comparison of

the activities revealed that the capabilities of depsidones to produce antibacterial effects was related to the number of chlorine atoms in the backbone. Among them, **3**, which was trichlorinated, was the most active. Dichlorinated analogue **2** showed more activity than **1**; the latter incorporated one chlorine atom. Compound **4** without chlorine substitution showed weaker activity than **1–3**. Similarly, dichlorinated isocoumarin **6** showed more activity than the monochlorinated analogue **5**.

4. Conclusion

In summary, six new chlorinated metabolites were obtained from a deep-sea-derived *Spiromastix* sp. fungus. These findings enriched the chemical diversity of marine-fungus-derived chlorinated metabolites, and implied that marine-derived fungi are a potential source to discover halometabolites. The antibacterial activities of **3** were comparable to that of the positive control chloroamphenicol, suggesting that **3** was a promising lead for the development of an antibacterial agent.

Table 3 Antibacterial effects of **1–8**

Compound	MIC ($\mu\text{g mL}^{-1}$)			
	<i>Staphylococcus aureus</i> ATCC 25923	<i>Bacillus thuringiensis</i> ATCC 10792	<i>Bacillus subtilis</i> CMCC 63501	<i>Escherichia coli</i> ATCC 25922
1	8	4	8	>128
2	4	2	2	>128
3	1	1	0.5	>128
4	32	16	16	>128
5	32	32	16	>128
6	16	4	4	>128
7	>128	>128	>128	>128
8	>128	>128	>128	>128
CP ^a	1	1	1	4

^a CP: chloroamphenicol used as a positive control.

The different scaffolds of 3 and chloroamphenicol implied that 3 may have a distinct mode of action, but this requires further investigation.

Author contributions

SN and DL isolated all compounds; ZS and JH did the bioassay; AF and WL elucidated the structures and edited the article.

Conflicts of interest

There are no conflicts to declare.

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