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## New diterpene alkaloids from the marine sponge *Agelas mauritiana*†

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Chemical investigation of an ethanol extract of the sponge *Agelas mauritiana* led to the isolation and characterization of five new diterpene alkaloids, namely, (–)-8′-oxo-agelasine B (**1**), (+)-agelasine B (**2**), (+)-8′-oxo-agelasine C (**3**), agelasine V (**4**), and (+)-8′-oxo-agelasine E (**5**), along with two known compounds, (–)-8′-oxo-agelasine D (**6**), and agelasine D (**7**). The structures of these compounds were determined by interpretation of spectroscopic data and comparison with literature properties. Compounds **1** and **3–5** are the second example of 8′-oxo-agelasine analogs. Compounds **2** and **7** not only exhibited moderate cytotoxicity toward the cancer cell lines PC9, A549, HepG2, MCF-7, and U937 with IC<sub>50</sub> values of 4.49–14.41 μM, but also showed potent antibacterial activities against a panel of methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates with MIC<sub>90</sub> values of 1–8 μg mL<sup>-1</sup>.

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## Introduction

Marine sponges are distinguished as excellent sources for marine natural products, with 283 new compounds reported in 2014 alone.<sup>1,2</sup> The *Agelas* genus is a prolific producer of secondary metabolites containing bromopyrrole derivatives,<sup>3,4</sup> sesqui- and diterpenoid alkaloids,<sup>5</sup> glycosphingolipids,<sup>6</sup> carotenoids,<sup>7</sup> steroids,<sup>8</sup> and fatty acids.<sup>9</sup> Particularly, terpenoid alkaloids from this genus are attractive compounds, which are characterized by a sesqui- or diterpene unit attached to different polar functional groups including 6-amino-5-(formylamino)-4-(methylamino)-1,3-diazine, guanidine, sulfone, and methyladeninum. Since the first sesquiterpenoid alkaloid, agelasidine A, was isolated from the sponge *Agelas* sp. in 1983, an array of terpenoid alkaloids have been reported to date.<sup>10</sup> These terpenoid derivatives are listed as agelasidines,<sup>10–15</sup> agelines,<sup>16</sup> agelasines,<sup>5,15,17–24</sup> agelasimines,<sup>25</sup> gelasines,<sup>18</sup> and axistatins,<sup>26</sup> which show interesting biological effects, such as cytotoxic,<sup>14,18,25,26</sup> anti-malarial,<sup>23</sup> anti-microbial,<sup>11–14,16,22,24,26</sup> anti-fouling,<sup>19</sup> antifungal,<sup>15</sup> as well as inhibitory effects on Na<sup>+</sup>/K<sup>+</sup>-ATPase.<sup>11,17,20</sup>

Our previous chemical investigation of the sponge *Agelas mauritiana* led to the isolation of four new antimicrobial alkaloids, in which (–)-8′-oxo-agelasine D was the first and the only

8′-oxo-agelasine reported to date.<sup>12</sup> As our continued exploration of this sponge in a search for structurally new products with promising bioactivities, we found that the CH<sub>2</sub>Cl<sub>2</sub>-soluble portion of an EtOH extract of the title sponge collected in March 2013, was cytotoxic against PC9, A549, and U937 cell lines in a Cell Counting Kit-8 (CCK-8) bioassay (8.5–10.5 μg mL<sup>-1</sup>) and showed moderate antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA 2010-210) with MIC<sub>90</sub> value of 1.0 μg mL<sup>-1</sup>. Further bioactivity-guided fractionation of this CH<sub>2</sub>Cl<sub>2</sub>-soluble portion led to the isolation of five new compounds (**1–5**), as well as two known compounds (**6** and **7**). Compounds **1** and **3–5** represent the second example of 8′-oxo-agelasine analogs. All isolated compounds (**1–7**) were evaluated for their cytotoxic and antibacterial activities. Herein, we describe the isolation, structure elucidation, and bioactivities of **1–7**.

## Results and discussion

Compound **1** was isolated as a pale yellow amorphous powder, and its molecular formula was deduced to be C<sub>26</sub>H<sub>39</sub>N<sub>5</sub>O from the HRESIMS ion at *m/z* 438.3232 [M + H]<sup>+</sup>. The IR bands at 3325, 1718 cm<sup>-1</sup> implied the presence of amino and carbonyl functionalities, respectively. UV absorption at 275 nm was in agreement with literature value<sup>23</sup> for purine moiety. The <sup>1</sup>H NMR spectrum of **1** displayed one deshielded aromatic siglet (δ<sub>H</sub> 8.17), two exchangeable siglets (δ<sub>H</sub> 7.36), one olefin siglet (δ<sub>H</sub> 5.13), one olefin triplet (δ<sub>H</sub> 5.28), four methyl siglets (δ<sub>H</sub> 1.79, 0.95, 0.68, and 3.46), and two methyl doublets (δ<sub>H</sub> 0.75 and 1.53). The <sup>13</sup>C NMR and HSQC spectra of **1** revealed 26 carbon signals corresponding to six methyls (including one nitrogen-bearing carbon at δ<sub>C</sub> 26.8), seven methylenes, five methines, and eight quaternary carbons (including one carbonyl carbon at

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$\delta_C$  152.6). The above-mentioned NMR data exhibited close resemblance to those of agelasine B,<sup>17</sup> except for the purine unit. The corresponding  $sp^2$  carbon in purine moiety at  $\delta_C$  141.7 in agelasine B<sup>17</sup> was replaced by a carbonyl carbon at  $\delta_C$  152.6 in **1**, which was established by the HMBC cross-peaks of the nitrogen methyl protons ( $\delta_H$  3.46) and C-4' ( $\delta_C$  148.3) and C-8' ( $\delta_C$  152.6), and of the aromatic proton ( $\delta_H$  8.17) and C-2' ( $\delta_C$  146.3), C-4' ( $\delta_C$  148.3), and C-5' ( $\delta_C$  105.4) (Fig. 2). Moreover, a  $^1H$ - $^{15}N$  HMBC experiment was conducted to confirm this 8-oxo-9-*N*-methyladenine moiety. The observed  $^1H$ - $^{13}N$  HMBC correlations of H-NCH<sub>3</sub>/N-9' ( $\delta_N$  127.8), H<sub>2</sub>-15 ( $\delta_H$  4.63)/N-7' ( $\delta_N$  118.2), H-2' ( $\delta_H$  8.17)/N-1' ( $\delta_N$  193.3), and N-3' ( $\delta_N$  224.3) further prove this point. The rest C<sub>20</sub>H<sub>33</sub> alkyl skeleton was assigned to have a clerodane skeleton by analysis of 2D NMR data (Fig. 2). In addition, HMBC correlations from two methylene protons ( $\delta_H$  4.63) to C-13 ( $\delta_C$  144.0), C-5' ( $\delta_C$  105.4), and C-8' ( $\delta_C$  152.6) suggested that the 9-*N*-methyladenine was attached to C-15 *via* N-7'.

The relative configuration of **1** was deduced from NOESY spectroscopic data (Fig. 3). The NOESY correlations of H<sub>2</sub>-15 ( $\delta_H$  4.63)/H<sub>3</sub>-16 ( $\delta_H$  1.79) and the chemical shift of C-16 ( $\delta_C$  16.9) indicated the 13*E*-configuration of the double bond  $\Delta$ .<sup>13,14</sup> Cross-peaks of H<sub>3</sub>-19 ( $\delta_H$  0.95)/H<sub>3</sub>-20 ( $\delta_H$  0.68) and H-6a ( $\delta_H$  1.66), H<sub>3</sub>-17 ( $\delta_H$  0.75)/H<sub>3</sub>-20, H-10 ( $\delta_H$  1.27)/H-11a ( $\delta_H$  1.48), H-12b ( $\delta_H$  1.84), and H-6b ( $\delta_H$  1.12), and H-6b/H-8 ( $\delta_H$  1.40) indicated that H<sub>3</sub>-17, H<sub>3</sub>-19, H-6a, and H<sub>3</sub>-20 were  $\alpha$ -oriented while H-8, H-6b, H-10, and H<sub>2</sub>-11 were  $\beta$ -oriented. Further comparison of the  $[\alpha]_D^{25}$  values of **1** ( $-33.9$ , MeOH) with that of agelasine B ( $-21.5$ , MeOH<sup>17</sup> and  $-27.2$ <sup>27</sup>) suggested the absolute configuration of **1** was probably identical to agelasine B since they had the same relative stereochemistry and the same sign of specific rotation. Thus, the structure of (-)-8'-oxo-agelasine B was concluded to be shown in Fig. 1.

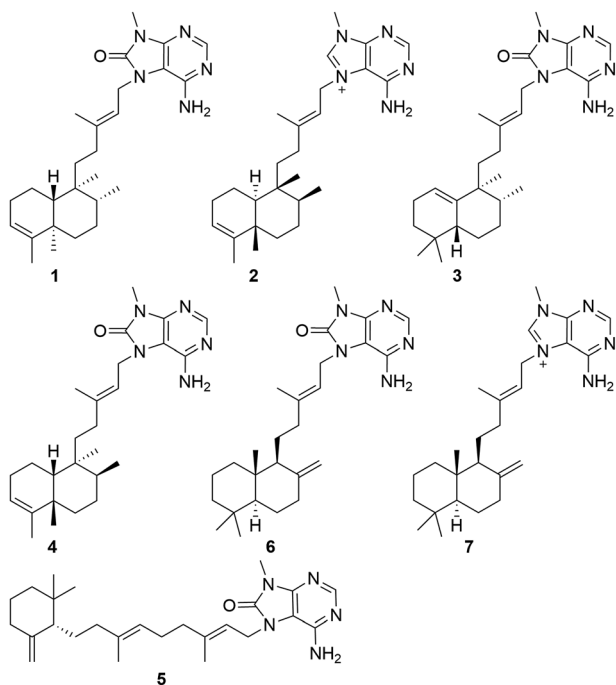


Fig. 1 The structures of compounds 1–7 from *Agelas mauritiana*.

Compound **2**, a white amorphous powder, possessed a molecular formula of C<sub>26</sub>H<sub>39</sub>N<sub>5</sub>, according to its  $^{13}C$  NMR and HRESIMS ( $m/z$  422.3280 [M + H]<sup>+</sup>, calcd for C<sub>26</sub>H<sub>40</sub>N<sub>5</sub>, 422.3284) data. Detailed analysis of the 1D and 2D NMR spectral data revealed that the planar structure of **2** was the same as agelasine B (Fig. 2).<sup>17</sup> The double bond between C-13 ( $\delta_C$  146.2) and C-14 ( $\delta_C$  114.9) possessed the *E*-geometry, which was established by the NOESY correlations of H<sub>2</sub>-15 ( $\delta_H$  5.16)/H<sub>3</sub>-16 ( $\delta_H$  1.79) and H-14 ( $\delta_H$  5.45)/H<sub>2</sub>-12 ( $\delta_H$  1.97,  $\delta_H$  1.87) and the chemical shift of C-16 ( $\delta_C$  16.7). Moreover, the  $\alpha$ -configurations of H-6b ( $\delta_H$  1.10) and H-10 ( $\delta_H$  1.29) were derived from NOESY correlations for H-6b/H-10, while the  $\beta$ -configurations of H-6a ( $\delta_H$  1.65), H<sub>3</sub>-17 ( $\delta_H$  0.78), H<sub>3</sub>-19 ( $\delta_H$  0.95), and H<sub>3</sub>-20 ( $\delta_H$  0.70) were implied from NOESY cross-peaks of H<sub>3</sub>-19/H-6a and H<sub>3</sub>-20 and H<sub>3</sub>-17/H<sub>3</sub>-20 (Fig. 4). The aforementioned data suggested **2** have identical

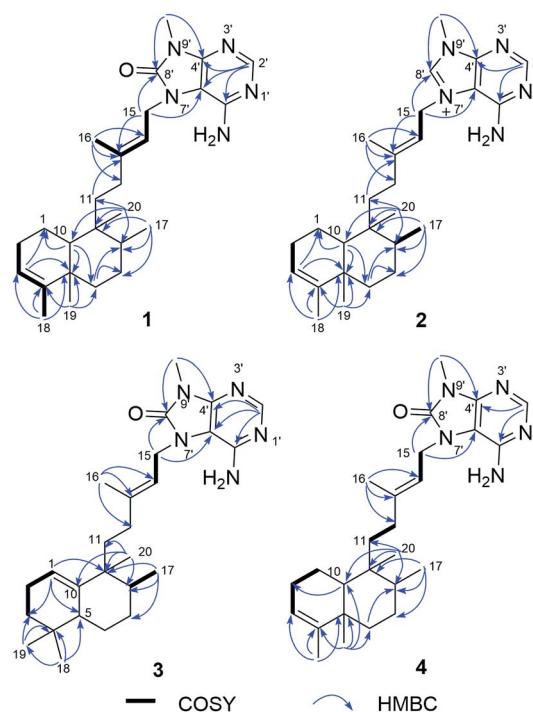


Fig. 2 Key  $^1H$ - $^1H$  COSY and HMBC correlations of compounds 1–4.

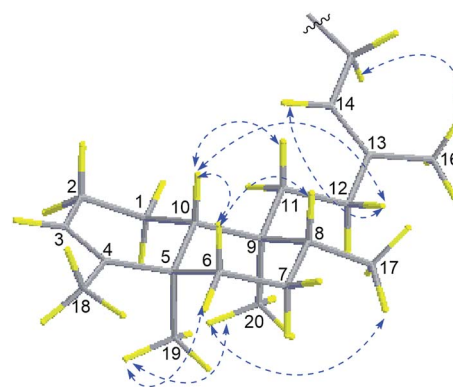


Fig. 3 Key NOESY correlations of compound **1**.



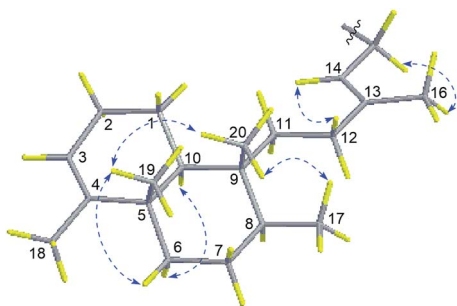


Fig. 4 Key NOESY correlations of compound 2.

relative configuration with agelasine B. However, the opposite optical rotation data for them [ $[\alpha]_D^{30} +22.7$ , MeOH], agelasine B [ $[\alpha]_D^{25} -21.5$ , MeOH<sup>17</sup> and  $[\alpha]_D^{25} -27.2$ <sup>27</sup>] suggest **2** differed in absolute configuration at chiral centers with agelasine B. Thus, the structure of **2** was established as a stereoisomer of agelasine B and named (+)-agelasine B.

Compound **3** showed a  $[M + H]^+$  ion peak at  $m/z$  438.3231 in the HRESIMS, corresponding to a molecular formula of  $C_{26}H_{39}N_5O$ . Comparison of the  $^1H$  and  $^{13}C$  NMR spectroscopic data (Tables 1 and 2) of compound **2** suggested a rearranged labdane skeleton for **3**, which was further supported by the

HMBC correlations from  $H_3-17$  ( $\delta_H$  0.83) to C-7 ( $\delta_C$  31.2), C-8 ( $\delta_C$  44.5), and C-9 ( $\delta_C$  42.5), from  $H_3-20$  ( $\delta_H$  1.00) to C-8, C-9, C-10 ( $\delta_C$  145.4), and C-11 ( $\delta_C$  29.6), from  $H_3-18$  ( $\delta_H$  0.84) to C-3 ( $\delta_C$  31.3), C-4 ( $\delta_C$  31.2), C-5 ( $\delta_C$  43.8), and C-19 ( $\delta_C$  27.8), from  $H_3-19$  ( $\delta_H$  0.84) to C-3, C-4, C-5, and C-18 ( $\delta_C$  27.8), from H-1 ( $\delta_H$  5.34) to C-3, C-5, and C-9, and from H-5 ( $\delta_H$  1.49) to C-1 ( $\delta_C$  117.6), C-3, C-7, C-10 ( $\delta_C$  145.4), C-18, and C-19 (Fig. 2). The 13*E*-configuration of the double bond  $\Delta^{13,14}$  was inferred from the NOESY correlations of  $H_2-15$  ( $\delta_H$  4.65)/ $H_3-16$  ( $\delta_H$  1.79) and H-14 ( $\delta_H$  5.27)/ $H_2-12$  ( $\delta_H$  1.81,  $\delta_H$  1.72) and the chemical shift of C-16 ( $\delta_C$  17.0). The NOESY correlations of  $H_3-17/H_3-20$  and H-6a ( $\delta_H$  1.78) indicated the  $\beta$ -orientation of  $H_3-17$ , H-6a, and  $H_3-20$ , while the cross-peaks of H-5/H-6b ( $\delta_H$  1.73) and H-8 ( $\delta_H$  1.28) suggested these protons are  $\alpha$ -oriented, establishing the relative configurations of **3** (Fig. 5), in consonance with those of the synthesis product (+)-agelasine C.<sup>28</sup> The absolute configurations of **3** was determined by comparison of its optical rotation [ $[\alpha]_D^{25} +29$  (MeOH)] and the synthesis product (+)-agelasine C [ $[\alpha]_D^{22} +25$  (MeOH)]. Therefore, the structure of (+)-8'-oxo-agelasine C (**3**) was defined as shown in Fig. 1.

Compound **4** had a molecular formula of  $C_{26}H_{39}N_5O$  based on the  $[M + H]^+$  ion at  $m/z$  438.3230 (calcd for  $C_{26}H_{40}N_5O$ , 438.3233) in the HRESIMS. Extensive analysis of HMBC and COSY correlations revealed that compound **4** shared the same planar structure as **1** (Fig. 2). The NOESY correlation of  $H_2-15$

Table 1  $^1H$  NMR spectroscopic data for compounds 1–5 ( $\delta$  in ppm, J in Hz)

Position	1 <sup>a,c</sup>	2 <sup>a,d</sup>	3 <sup>a,c</sup>	4 <sup>b,c</sup>	5 <sup>b,c</sup>
1a	1.38, m	1.54, m	5.34, m	1.83, m	2.00, m
1b				1.59, m	
2a	1.99, m	1.98, m	2.01, m	1.97, m	1.53, m
2b	1.92, m				
3a	5.13, s	5.13, s	1.38, m	5.34, m	1.46, m
3b			1.06, m		1.21, m
5			1.49, dd (12.6, 3.0)		1.67, m
6a	1.66, d (12.6)	1.65, dt (13.2, 3.0)	1.78, m	1.62, m	1.38, m
6b	1.12, m	1.10, m	1.73, m	1.48, m	
7a	1.92, m	1.46, m	1.41, dd (13.2, 3.6)	1.43, m	1.74, m
7b	1.37, m	1.38, m	1.09, m	1.34, m	
8	1.40, m	1.42, m	1.28, m	1.55, m	
9					5.03, s
10	1.27, m	1.29, d (12.6)		1.41, m	
11a	1.48, m	1.47, m	1.25, s	1.83, m	2.11, m
11b	1.29, m	1.35, m		1.24, m	
12a	1.92, m	1.97, m	1.81, m	2.12, td (13.2, 4.0)	2.11, m
12b	1.84, td (12.6, 4.8)	1.87, td (12.6, 4.8)	1.72, m	2.00, m	
14	5.28, t (6.0)	5.45, t (6.0)	5.27, t (6.0)	5.35, m	5.34, t (5.6)
15	4.63, d (6.0)	5.16, d (6.0)	4.65, d (6.0)	4.68, d (5.6)	4.67, d (5.6)
16	1.79, s	1.79, s	1.79, d (1.2)	1.83, s	1.82, s
17	0.75, d (5.4)	0.78, d (6.6)	0.83, s	0.85, d (7.2)	1.58, s
18	1.53, d (1.8)	1.54, d (1.2)	0.84, s	1.62, s	0.90, s
19	0.95, s	0.95, s	0.84, s	1.12, s	0.82, s
20a	0.68, s	0.70, s	1.00, s	1.01, s	4.74, s
20b					4.52, d (2.0)
2'	8.17, s	8.46, s	8.18, s	8.19	8.18, s
8'		9.54, s			
2'-NH <sub>2</sub>	7.36, br s	7.90, br s	7.62, br s	7.69, br s	7.66, br s
9'-CH <sub>3</sub>	3.46, s	3.89, s	3.50, s	3.51, s	3.50, s

<sup>a</sup> Measured at 600 MHz. <sup>b</sup> Measured at 400 MHz. <sup>c</sup> Measured in CDCl<sub>3</sub>. <sup>d</sup> Measured in DMSO-*d*<sub>6</sub>.



Table 2  $^{13}\text{C}$  NMR spectroscopic data for compounds 1–5 ( $\delta$  in ppm)

Position	1 <sup>a,c</sup>	2 <sup>a,d</sup>	3 <sup>a,c</sup>	4 <sup>b,c</sup>	5 <sup>b,c</sup>
1	18.3, CH <sub>2</sub>	17.8, CH <sub>2</sub>	117.6, CH	19.9, CH <sub>2</sub>	32.5, CH <sub>2</sub>
2	26.8, CH <sub>2</sub>	26.3, CH <sub>2</sub>	23.2, CH <sub>2</sub>	25.7, CH <sub>2</sub>	23.7, CH <sub>2</sub>
3	120.3, CH	120.2, CH	31.3, CH <sub>2</sub>	122.5, CH	36.3, CH <sub>2</sub>
4	144.3, C	143.6, C	31.2, C	141.8, C	34.9, C
5	38.1, C	37.6, C	43.8, CH	38.7, C	53.6, CH
6	36.7, CH <sub>2</sub>	36.2, CH <sub>2</sub>	30.1, CH <sub>2</sub>	32.1, CH <sub>2</sub>	24.8, CH <sub>2</sub>
7	27.4, CH <sub>2</sub>	27.0, CH <sub>2</sub>	31.2, CH <sub>2</sub>	27.2, CH <sub>2</sub>	38.2, CH <sub>2</sub>
8	36.2, CH	35.7, CH	44.5, CH	37.4, CH	137.0, C
9	38.6, C	38.2, C	42.5, C	38.7, C	122.5, CH
10	46.4, CH	45.9, CH	145.4, C	44.6, CH	149.3, C
11	36.4, CH <sub>2</sub>	35.8, CH <sub>2</sub>	29.6, CH <sub>2</sub>	36.0, CH <sub>2</sub>	26.1, CH <sub>2</sub>
12	33.0, CH <sub>2</sub>	32.5, CH <sub>2</sub>	33.9, CH <sub>2</sub>	33.7, CH <sub>2</sub>	39.5, CH <sub>2</sub>
13	144.0, C	146.2, C	144.7, C	144.7, C	143.3, C
14	119.5, CH	114.9, CH	119.3, CH	119.5, CH	119.8, CH
15	40.5, CH <sub>2</sub>	47.0, CH <sub>2</sub>	40.6, CH <sub>2</sub>	40.6, CH <sub>2</sub>	40.6, CH <sub>2</sub>
16	16.9, CH <sub>3</sub>	16.7, CH <sub>3</sub>	17.0, CH <sub>3</sub>	17.1, CH <sub>3</sub>	16.9, CH <sub>3</sub>
17	15.9, CH <sub>3</sub>	15.8, CH <sub>3</sub>	16.3, CH <sub>3</sub>	15.3, CH <sub>3</sub>	16.1, CH <sub>3</sub>
18	17.9, CH <sub>3</sub>	17.7, CH <sub>3</sub>	27.8, CH <sub>3</sub>	19.3, CH <sub>3</sub>	28.4, CH <sub>3</sub>
19	19.8, CH <sub>3</sub>	19.6, CH <sub>3</sub>	27.8, CH <sub>3</sub>	28.0, CH <sub>3</sub>	26.2, CH <sub>3</sub>
20	18.2, CH <sub>3</sub>	18.1, CH <sub>3</sub>	23.0, CH <sub>3</sub>	26.2, CH <sub>3</sub>	108.8, CH <sub>2</sub>
2'	146.3, CH	155.4, CH	145.8, CH	145.6, CH	145.6, CH
4'	148.3, C	148.9, C	148.3, C	148.3, C	148.3, C
5'	105.4, C	109.2, C	105.4, C	105.4, C	105.4, C
6'	143.6, C	152.3, C	143.5, C	143.4, C	143.3, C
8'	152.6, C	140.9, CH	152.6, C	152.6, C	152.6, C
9'-CH <sub>3</sub>	26.8, CH <sub>3</sub>	31.4, CH <sub>3</sub>	26.9, CH <sub>3</sub>	26.9, CH <sub>3</sub>	26.9, CH <sub>3</sub>

<sup>a</sup> Measured at 150 MHz. <sup>b</sup> Measured at 100 MHz. <sup>c</sup> Measured in CDCl<sub>3</sub>.

<sup>d</sup> Measured in DMSO-*d*<sub>6</sub>.

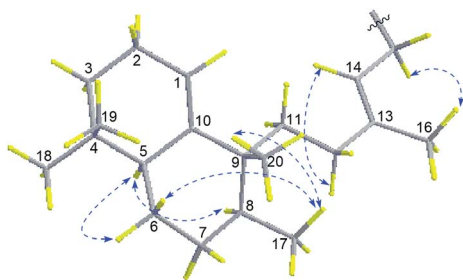


Fig. 5 Key NOESY correlations of compound 3.

( $\delta_{\text{H}}$  4.68)/H<sub>3</sub>-16 ( $\delta_{\text{H}}$  1.83) and H-14 ( $\delta_{\text{H}}$  5.35)/H<sub>2</sub>-12 ( $\delta_{\text{H}}$  2.12 and 2.00) and the chemical shift of C-16 ( $\delta_{\text{C}}$  17.1) suggested the *E*-geometry of the  $\Delta^{13,14}$  double bond. The NOESY cross-peaks of H<sub>3</sub>-17 ( $\delta_{\text{H}}$  0.85)/H-11b ( $\delta_{\text{H}}$  1.24) and H-10 ( $\delta_{\text{H}}$  1.41), H-10/H-12a ( $\delta_{\text{H}}$  2.12), H<sub>2</sub>-11 ( $\delta_{\text{H}}$  1.83 and 1.24), H<sub>3</sub>-19 ( $\delta_{\text{H}}$  1.12) and H-6a ( $\delta_{\text{H}}$  1.62), and H<sub>3</sub>-19/H-6a located H<sub>3</sub>-17, H-10, H-6a, and H<sub>3</sub>-19 on the same face, while NOESY correlation of H<sub>3</sub>-20 ( $\delta_{\text{H}}$  1.01)/H-8 ( $\delta_{\text{H}}$  1.55) positioned H<sub>3</sub>-20 and H-8 on the opposite face (Fig. 6). Thus, the structure of 4 was assigned as a stereoisomer of agelasine B and named agelasine V.

Compound 5, a pale yellow amorphous powder, had a molecular formula of C<sub>26</sub>H<sub>39</sub>N<sub>5</sub>O deduced from the  $^{13}\text{C}$  NMR and HRESIMS (438.3229 [M + H]<sup>+</sup>, calcd for C<sub>26</sub>H<sub>40</sub>N<sub>5</sub>O, 438.3233) data. Compound 5 possessed the same 8-oxo-9-*N*-methyladenine moiety according to the comparison of its 1D

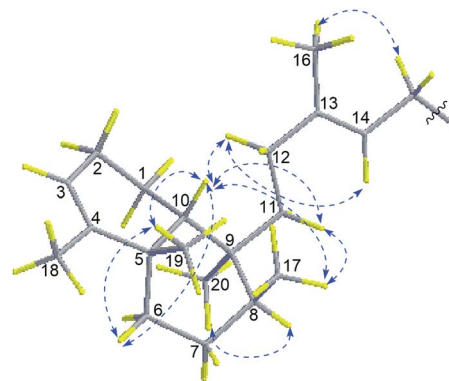
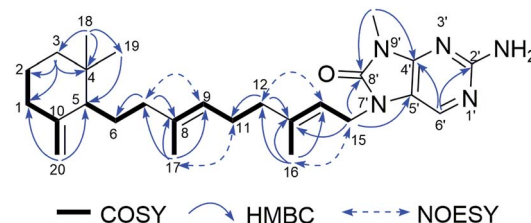


Fig. 6 Key NOESY correlations of compound 4.

Fig. 7 Key  $^1\text{H}$ - $^1\text{H}$  COSY, HMBC and NOESY correlations of compound 5.

NMR data (Tables 1 and 2) with those of compound 1. The rest 9, 10-*seco*-labdane skeleton was determined by analysis of 2D NMR data (Fig. 7). The configurations of the double bonds ( $\Delta^{9,10}$  and  $\Delta^{13,14}$ ) were established as *E* based on the NOESY correlations of H<sub>3</sub>-17 ( $\delta_{\text{H}}$  1.58)/H<sub>2</sub>-11 ( $\delta_{\text{H}}$  2.11), H-9 ( $\delta_{\text{H}}$  5.03)/H<sub>2</sub>-7 ( $\delta_{\text{H}}$  1.74), H<sub>3</sub>-16 ( $\delta_{\text{H}}$  1.82)/H<sub>2</sub>-15 ( $\delta_{\text{H}}$  4.67), and H<sub>2</sub>-12 ( $\delta_{\text{H}}$  2.11)/H-14 ( $\delta_{\text{H}}$  5.34) and the chemical shift of C-17 ( $\delta_{\text{C}}$  16.1) and C-16 ( $\delta_{\text{C}}$  16.9). The absolute configuration of 5 was assumed to be the same as (+)-trixagol, whose enantiomer was equate to the terpenoid side chain of (–)-agelasine E in that they both exhibit positive optical rotations [5 ([ $\alpha_{\text{D}}^{30}$  +30.6, MeOH), (+)-trixagol ([ $\alpha_{\text{D}}^{14}$  +14, CHCl<sub>3</sub>)<sup>29</sup> and agelasine E ([ $\alpha_{\text{D}}^{25}$  –17.1, MeOH)<sup>20</sup>]. Accordingly, The structure of (+)-8'-oxo-agelasine E (5) was proposed as shown Fig. 1.

All isolated compounds were assessed for their antibacterial activity against a methicillin-susceptible *S. aureus* (MSSA) strain H608 and four methicillin-resistant *S. aureus* (MRSA) strains 2010-260, 2010-210, 2010-292, and 2010-300. As shown in Table 3, compounds 2 and 7 exhibited potent activities against MRSA with MIC<sub>90</sub> values of 1–8  $\mu\text{g mL}^{-1}$  while other compounds showed no activity (MIC<sub>90</sub> > 64  $\mu\text{g mL}^{-1}$ ). The cytotoxic activities of individual compounds were evaluated against the PC9, A549, HepG2, MCF-7, and U937 cell lines using Cell Counting Kit-8 (CCK-8) bioassay (Table 3). Compounds 2 and 7 showed moderate activities against the five cancer cell lines with IC<sub>50</sub> values of 4.49–14.41  $\mu\text{M}$ . Other compounds showed no activity (IC<sub>50</sub> > 20  $\mu\text{M}$ ) except compound 6 show weak cytotoxicity against U937 cell line with IC<sub>50</sub> value of 16.89  $\mu\text{M}$ .





Table 3 Antibacterial and cytotoxic activities of compounds 1–7

Compounds <sup>a</sup>	Antibacterial activity against clinical MRSA <sup>d</sup> and MSSA <sup>e</sup> strains (MIC <sub>90</sub> , µg mL <sup>-1</sup> )					Cytotoxic activity (IC <sub>50</sub> , µM)				
	2010-260	2010-210	2010-292	2010-300	H608	PC9	A549	HepG2	MCF-7	U937
<b>2</b>	2	1	2	1	2	5.08	14.07	9.76	7.64	4.49
<b>6</b>	>64	>64	>64	>64	>64	>50	>50	>50	>50	16.89
<b>7</b>	4	4	8	8	1	4.49	14.41	10.07	5.47	6.86
Vancomycin <sup>b</sup>	1	1	1	0.5	2					
Doxorubicin <sup>c</sup>						0.22	0.49	0.20	0.38	0.05

<sup>a</sup> Compounds 1 and 3–5 were inactive in all assays. <sup>b</sup> Positive control for antibacterial assay. <sup>c</sup> Positive control for cytotoxic assay. <sup>d</sup> Clinical MRSA strains 2010-260, 2010-210, 2010-292, 2010-300. <sup>e</sup> Clinical MSSA strain H608.

## Conclusions

In conclusion, five new diterpene alkaloids, illustrated by (–)-8'-oxo-agelasine B (**1**), (+)-agelasine B (**2**), (+)-8'-oxo-agelasine C (**3**), agelasine V (**4**), (+)-8'-oxo-agelasine E (**5**), along with two known related metabolites, (–)-8'-oxo-agelasine D (**6**), agelasine D (**7**), were isolated from the marine sponge *Agelas mauritiana*. The structures of them were established by interpretation of spectroscopic data and comparison with literature properties. Isolation of compounds 1 and 3–5 provided new examples of 8'-oxo-agelasine analogs. Compounds 2 and 7 showed potent antibacterial and moderate cytotoxic activities. Analysis of the structures of the diterpene alkaloids (1–7) and their antibacterial and cytotoxic activities led to a preliminary summary of the structure–activity relationship (SAR): C-8'-carbonylated compounds (1 and 3–6) can provide lower antibacterial and cytotoxic activities than other analogs (2 and 7). Moreover, the interesting antibacterial activity of compounds 2 and 7 indicated that they could be possible lead candidates as promising anti-MRSA agents.

## Experimental section

### General experimental procedures

Optical rotation data were measured in MeOH on an Autopol I polarimeter (no. 30575, Rudolph Research Analytical) with a 10 cm length cell. UV were recorded on a Hitachi U-3010 spectrophotometer. IR (KBr) spectra were obtained on Jasco FTIR-400 spectrometer. 1D and 2D NMR spectra were recorded in DMSO-*d*<sub>6</sub> or CDCl<sub>3</sub> on a Bruker DRX-600 or on a Bruker DRX-400 MHz NMR spectrometers. HRESIMS data was recorded on a Q-TOF micro YA019 mass spectrometer. Column chromatography (CC) was carried out on Sephadex LH-20 (Amersham Pharmacia Biotech AB), silica gel (200–300 mesh, Qingdao Marine Chemical Inc. China), and reverse phase C18 silica gel (15 µm, Santai Technologies, Inc.). Analytical thin-layer chromatography was carried out using HSGF 254 plates and visualized by spraying with anisaldehyde-H<sub>2</sub>SO<sub>4</sub> reagent. Reversed-phase high-performance liquid chromatography (HPLC) was performed on Waters SunFire™ Prep C18 column (5 µm, 19 × 150 mm) with a Waters 1525 separation module equipped with a Waters 2998 photodiode array detector, and all solvents used for HPLC were of HPLC grade.

### Animal material

Samples of *Agelas mauritiana* were collected along the coast of Yongxing Island in the South China Sea on March 19, 2013. A voucher specimen (no. 13-7) has been deposited at Research Center for Marine Drugs, State Key Laboratory of Oncogenes and Related Genes, Shanghai Jiao Tong University.

### Extraction and isolation

The sponge (1.2 kg, wet weight) was cut and percolated with 95% EtOH at room temperature to afford the crude extract (15.2 g), which was suspended in H<sub>2</sub>O and extracted with EtOAc. The EtOAc-soluble extract (9.3 g) was concentrated under reduced pressure. Subsequently, the EtOAc-soluble extract was partitioned between 90% aqueous MeOH and petroleum ether to give 5.5 g petroleum ether-soluble fraction. The 90% aqueous MeOH fraction was diluted to 60% aqueous MeOH with H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> to afford a 4.5 g CH<sub>2</sub>Cl<sub>2</sub>-soluble fraction. This CH<sub>2</sub>Cl<sub>2</sub>-soluble extract was chromatographed on silica gel column eluting with a step gradient of CH<sub>2</sub>Cl<sub>2</sub>–MeOH (100 : 1 to 0 : 1) to give nine fractions (DA–DI). Fraction DH (1.1 g) was subjected to VLC over silica gel eluting with a CH<sub>2</sub>Cl<sub>2</sub>–EtOAc–MeOH–H<sub>2</sub>O system (10 : 5 : 1.5 : 0.2 and 0 : 0 : 1 : 0) to afford four subfractions (DH1–DH4). Subfraction DH3 (200.1 mg) was directly separated by reversed-phase HPLC (Waters SunFire™ Prep C18, 5 µm, 19 × 150 mm; 10.0 mL min<sup>-1</sup>; 210, 270 nm) eluting with a CH<sub>3</sub>CN–H<sub>2</sub>O–TFA system (50 : 50 : 0.1) to give (+)-agelasine B (**2**, 40.0 mg, *t*<sub>R</sub> 8.5 min) and agelasine D (**7**, 35.2 mg, *t*<sub>R</sub> 10.0 min). DH1 (163.6 mg) was subjected to silica gel column chromatography, using a gradient of CH<sub>2</sub>Cl<sub>2</sub>–EtOAc–MeOH–H<sub>2</sub>O solvent system (50 : 5:1 : 0.1, 45 : 5:1 : 0.1, 40 : 5:1 : 0.1, 25 : 5:1 : 0.1, 20 : 5:1 : 0.1, 20 : 5:1 : 0.1, 10 : 5:1.25 : 0.2, and 0 : 0:1 : 0) to give 5 subfractions (DH1A–DH1E). Subfraction DH1B (60.5 mg) was further purified by reversed-phase HPLC (Waters SunFire™ Prep C18, 5 µm, 19 × 150 mm; 9.0 mL min<sup>-1</sup>; 210, 270 nm) eluting with a CH<sub>3</sub>CN–H<sub>2</sub>O–TFA (55 : 45 : 0.1) system to give (–)-8'-oxo-agelasine B (**1**, 15.0 mg, *t*<sub>R</sub> 49.5 min), (–)-8'-oxo-agelasine D (**6**, 13.1 mg, *t*<sub>R</sub> 55.0 min), (+)-8'-oxo-agelasine C (**3**, 5.7 mg, *t*<sub>R</sub> 63.9 min), agelasine V (**4**, 2.8 mg, *t*<sub>R</sub> 52.0 min), (+)-8'-oxo-agelasine E (**5**, 2.9 mg, *t*<sub>R</sub> 70.0 min).

(–)-8'-Oxo-agelasine B (**1**). Pale yellow amorphous powder;  $[\alpha]_{D}^{25}$  –33.9 (*c* 0.11, MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 216 (4.07),



275 (3.64) nm; IR (KBr)  $\nu_{\max}$  3325, 2927, 2861, 1718, 1639, 1460, 1376, 1198, 1141  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; HRESIMS  $m/z$  438.3232  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{26}\text{H}_{40}\text{N}_5\text{O}$ , 438.3233).

(+)-Agelasine B (2). Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{20} +22.7$  ( $c$  0.10, MeOH); UV (MeOH)  $\lambda_{\max}$  ( $\log \epsilon$ ) 214 (4.16), 262 (3.63) nm; IR (KBr)  $\nu_{\max}$  3320, 2927, 2858, 1683, 1649, 1460, 1379, 1202, 1133, 798, 720  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; HRESIMS  $m/z$  422.3280  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{26}\text{H}_{40}\text{N}_5$ , 422.3284).

(+)-8'-Oxo-agelasine C (3). Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{25} +29.0$  ( $c$  0.08, MeOH); UV (MeOH)  $\lambda_{\max}$  ( $\log \epsilon$ ) 214 (4.00), 274 (3.50) nm; IR (KBr)  $\nu_{\max}$  3325, 2925, 2856, 1721, 1640, 1459, 1373, 1197, 1142  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; HRESIMS  $m/z$  438.3231  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{26}\text{H}_{40}\text{N}_5\text{O}$ , 438.3233).

Agelasine V (4). Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{20} +36.5$  ( $c$  0.08, MeOH); UV (MeOH)  $\lambda_{\max}$  ( $\log \epsilon$ ) 214 (3.62), 275 (3.12) nm; IR (KBr)  $\nu_{\max}$  3330, 2926, 2856, 1721, 1641, 1461, 1375, 1198, 1092, 802  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; HRESIMS  $m/z$  438.3230  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{26}\text{H}_{40}\text{N}_5\text{O}$ , 438.3233).

(+)-8'-Oxo-agelasine E (5). Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{30} +30.6$  ( $c$  0.11, MeOH); UV (MeOH)  $\lambda_{\max}$  ( $\log \epsilon$ ) 203 (3.58), 275 (3.03) nm; IR (KBr)  $\nu_{\max}$  3325, 2925, 2856, 1721, 1640, 1459, 1373, 1197, 1142  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; HRESIMS  $m/z$  438.3229  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{26}\text{H}_{40}\text{N}_5\text{O}$ , 438.3233).

### Antibacterial assay

The *in vitro* antibacterial assay was carried out as reported before.<sup>30</sup> Vancomycin was used as positive control and displayed MIC<sub>90</sub> values of 2, 1, 1, 1, and 0.5  $\mu\text{M}$  against methicillin-susceptible *S. aureus* strain H608 and methicillin-resistant *S. aureus* strains 2010-260, 2010-210, 2010-292, and 2010-300, respectively.

### Cytotoxicity assay

The effects of 1–7 on cell viability was determined using the Cell Counting Kit-8 (CCK-8). The human PC9, A549, HepG2, MCF-7, and U937 cell lines were obtained from the Institute of Biochemistry Cell Biology (Shanghai, China). Cells were seeded in 96 well plates ( $5 \times 10^3$  cells per well). After 24 h incubation, the cells were treated with various concentrations of 1–7 for 72 h. Then the CCK8 solution (10  $\mu\text{L}$ ) was added for additional 1 h incubation at 37 °C. The absorbance at 450 nm was measured in a microplate reader (spectra MAX190, Molecular Devices, USA). Independent experiments were performed in triplicate.

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