



## A novel sesquiterpene quinone from Hainan sponge *Dysidea villosa*

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### ABSTRACT

A new sesquiterpene quinone, 21-dehydroxybolinaquinone (**5**), together with two known related analogues, bolinaquinone (**6**) and dysidine (**7**), had been isolated from the Hainan sponge *Dysidea villosa*. The structure of the new compound **5** was elucidated on the basis of detailed analysis of spectroscopic data and by comparison with related model compounds. Compounds **5–7** were evaluated for the inhibitory activity against hPTP1B, a potential drug target for treatment of type-II diabetes and obesity, and cytotoxic activity against Hela cell line. The results showed that dysidine (**7**) had the strongest hPTP1B inhibitory activity with an  $IC_{50}$  value of 6.70  $\mu$ M and **6** had significant cytotoxic activity against Hela cell line with an  $IC_{50}$  value of 5.45  $\mu$ M. New compound **5** showed moderate PTP1B inhibitory activity and cytotoxicity with  $IC_{50}$  values of 39.50 and 19.45  $\mu$ M, respectively.

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Sesquiterpene quinones and related compounds represent a prominent class of biologically active metabolites.<sup>1</sup> These intriguing compounds are frequently encountered in the sponges of the genus *Dysidea* (Family Dysideidae).<sup>2–4</sup> Most sesquiterpene quinones have a bicyclic normal drimane skeleton, as exemplified by spongiaquinone (**1**),<sup>5</sup> or a rearranged drimane skeleton, as in 18-methoxyavarone (**2**),<sup>3</sup> even if several examples of monocyclic derivatives are also known.<sup>6</sup> A wide range of remarkable biological activity has been reported for many of these metabolites, ranging from antibacterial, cytotoxic, inhibitory activity against protein tyrosine kinase, to anti-HIV-I activity.<sup>3,7–9</sup> Among the more well-known members of this family are avarone (**3**) and avarol (**4**) that were first isolated from the Mediterranean sponge *Dysidea avara* in 1974.<sup>10</sup> Both compounds **3** and **4** exhibited significant antiviral activity against HIV-I<sup>11</sup> and their great pharmaceutical potential stimulated the continued interests of chemists and pharmacologists. A number of avarol derivatives<sup>12</sup> and related compounds<sup>13</sup> exhibiting interesting activity in enzyme assays measuring inhibition of various functions of HIV-I reverse transcriptase were discovered.

As part of our ongoing program devoted to the discovery of new bioactive metabolites from marine organisms collected in the Hainan Island, China,<sup>14–18</sup> we had the opportunity to study the chemical constituents of the sponge *D. villosa*. The Et<sub>2</sub>O-soluble and *n*-BuOH-soluble fractions from the Me<sub>2</sub>CO extract of the animals were separately subjected to a detailed chromatographic separation, which resulted in the isolation of one new sesquiterpene

quinone, named 21-dehydroxybolinaquinone (**5**), and one related analog, bolinaquinone (**6**),<sup>2</sup> from the Et<sub>2</sub>O extract, and one known metabolite, dysidine (**7**),<sup>19</sup> from the *n*-BuOH extract. This paper describes the isolation and structural elucidation of the new compound **5** and *in vitro* pharmacological evaluation of compounds **5–7**.

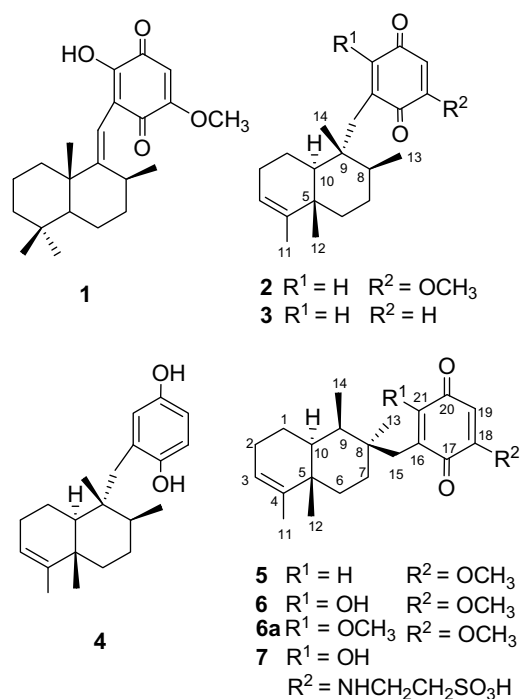
The lyophilized sponge *D. villosa* (dry weight 51.2 g) was extracted with acetone exhaustively. The acetone extract was partitioned consecutively between H<sub>2</sub>O and Et<sub>2</sub>O, H<sub>2</sub>O and *n*-BuOH. The Et<sub>2</sub>O-soluble fraction was subjected to silica gel and Sephadex LH-20 column chromatographies to afford compounds **5** (3.6 mg, 0.007% dry weight) and **6** (30.6 mg, 0.060% dry weight) respectively. The *n*-BuOH-soluble portion was purified by Sephadex LH-20 column chromatography to yield compound **7** (21.1 mg, 0.041% dry weight).

The known compounds were readily identified as bolinaquinone (**6**)<sup>2</sup> and dysidine (**7**),<sup>19</sup> respectively, by analysis of their NMR spectra and by comparison with the data reported in the literature.

21-Dehydroxybolinaquinone (**5**)<sup>20</sup> was obtained as a yellow oil. Its molecular formula C<sub>22</sub>H<sub>30</sub>O<sub>3</sub>, was established by HREIMS at  $m/z$  342.2192 (calcd. 342.2195,  $\Delta$  = −0.3 mmu), 16 mass units less than that of **6**. The same rearranged drimane skeleton as in bolinaquinone (**6**) was immediately inferred from 2D NMR data, mainly <sup>1</sup>H–<sup>1</sup>H COSY, HMQC, and HMBC measurements (Fig. 1). Particularly diagnostic for this assignment was the <sup>13</sup>C NMR chemical shifts of methyl groups 13 and 14 ( $\delta_c$  23.6 and 12.3, respectively) which were close to those reported for bolinaquinone (**6**) ( $\delta_c$  23.8 and 12.2) but very different from values reported for compounds containing the 4,9-friedodrimane skeleton [ca.  $\delta_c$  16.7 and 17.8 in 18-methoxyavarone (**2**)]<sup>3</sup> (Table 1). The localization of a substituted group at C-8 instead of commonly found substitution at

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C-9 was also supported by a <sup>1</sup>H–<sup>13</sup>C long range correlation observed in the HMBC spectrum between H<sub>2</sub>-15 (δ 2.39, d, J = 12.9 Hz; 2.49, d, J = 12.9 Hz) and C-7 (δ 28.7), C-8 (δ 38.0), and C-9 (δ 44.1), respectively. Careful comparison of NMR data of **5** with those of co-occurring bolinaquinone (**6**) (Table 1) revealed great similarities between them. In fact, the main differences of the <sup>13</sup>C NMR data of **5** and **6** happened at the “benzenoid” part (C-16–C-21) while the sesquiterpene moiety was almost identical except for C-15. These differences could be easily justified by the loss of the hydroxyl group at C-21 of **5**. Due to the deoxy effect, the <sup>13</sup>C NMR chemical shifts at C-18 and C-21 were significantly upfield shifted and the δ values of C-15, C-16, C-19 and C-20 were downfield shifted. Finally, the quinone moiety was unambiguously

**Table 1**  
<sup>1</sup>H and <sup>13</sup>C NMR data of **5**, and <sup>13</sup>C NMR data of **2** and **6**

No.	<b>5<sup>a</sup></b>		<b>2<sup>b</sup></b>	<b>6<sup>a</sup></b>
	δ <sub>H</sub> (mult., J, Hz)	δ <sub>C</sub>	δ <sub>C</sub>	δ <sub>C</sub>
1α	1.20 (m)	24.5 (t)	19.4 (t)	24.5 (t)
1β	1.72 (m)			
2	2.04 (m)	26.6 (t)	26.5 (t)	26.6 (t)
3	5.25 (br. s)	120.6 (d)	120.7 (d)	120.4 (d)
4		143.5 (s)	143.9 (s)	143.7 (s)
5		37.7 (s)	42.4 (s)	37.9 (s)
6α	1.29 (m)	31.9 (t)	36.1 (t)	31.9 (t)
6β	1.48 (m)			
7α	1.03 (m)	28.7 (t)	27.5 (t)	28.7 (t)
7β	1.59 (m)			
8		38.0 (s)	36.9 (d)	39.6 (s)
9	1.48 (m)	44.1 (d)	38.5 (s)	44.8 (d)
10	1.86 (m)	40.6 (d)	47.0 (d)	40.6 (d)
11	1.55 (d, 1.5 Hz)	17.6 (q)	18.0 (q)	17.6 (q)
12	0.96 (s)	19.7 (q)	20.0 (q)	19.7 (q)
13	0.89 (s)	23.6 (q)	16.7 (q)	23.8 (q)
14	0.96 (s)	12.3 (q)	17.8 (q)	12.2 (q)
15α	2.39 (d, 12.9 Hz)	38.0 (t)	35.2 (t)	33.8 (t)
15β	2.49 (d, 12.9 Hz)			
16		145.6 (s)	145.0 (s)	117.7 (s)
17		182.3 (s)	182.0 (s)	182.2 (s)
18		158.5 (s)	159.0 (s)	161.3 (s)
19	5.88 (s)	107.0 (d)	106.9 (d)	102.0 (d)
20		187.5 (s)	187.1 (s)	182.5 (s)
21	6.50 (s)	135.4 (d)	136.7 (d)	152.8 (s)
22	3.81 (s)	56.0 (q)	56.3 (q)	56.0 (q)

<sup>a</sup> Bruker DRX 400 spectrometer (400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C NMR) in CDCl<sub>3</sub>, chemical shifts (ppm) referred to CHCl<sub>3</sub> (δ<sub>H</sub>7.26) and to CDCl<sub>3</sub> (δ<sub>C</sub>77.0).

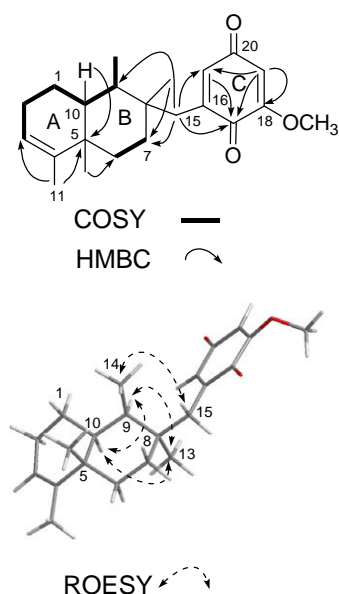
<sup>b</sup> Data reported in Ref. 3.

secured by comparison of the <sup>1</sup>H and <sup>13</sup>C NMR data (Table 1) with the corresponding part of model compound **2**. Thus, the planar structure of **5** was determined.

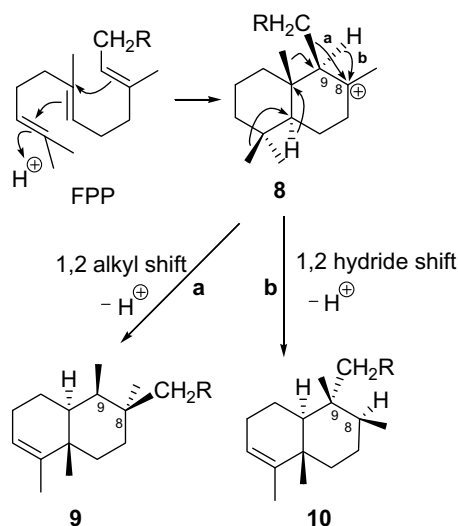
From a biogenetic point of view, the relative configurations around the rings A and B in **5** should be obviously the same as those of co-occurring compounds **6** and **7**. However, in order to confirm this deduction, ROESY experiment was performed on **5**. A series of ROESY correlations between H-9 and H-10, Me-13, between H-10 and Me-13, and between H<sub>2</sub>-15 and Me-14 (Fig. 1) clearly indicated that the rings A and B were *trans*-fused, and H-9, H-10, and Me-13 had the same α-orientation, while Me-12, Me-14, and CH<sub>2</sub>-15 were all β-oriented.

21-Dehydroxybolinaquinone (**5**) represents a new member of the family of sesquiterpene quinone derivatives with a further rearranged drimane skeleton characterized by the abnormal position of the quinone moiety. Compounds with this skeleton are quite rare compared to those showing 4,9-friedodrimane skeleton with a usual substitution at C-9. To the best of our knowledge, only a few of this kind of sesquiterpene quinones have been reported since the discovery of the first sesquiterpene quinone [bolinaquinone (**6**)] in 1998.<sup>2,4,19</sup> By analogy to the biogenesis of 4,9-friedodrimane-type sesquiterpene quinone (**10**),<sup>21,22</sup> a plausible biogenetic pathway as outlined in Scheme 1 could be proposed to explain the biogenetic origin of the sesquiterpene quinone derivatives with a further rearranged drimane skeleton. Thus, the cyclization of farnesyl pyrophosphate (FPP) takes place by an initial electrophilic attack at the *head* position of FPP giving rise to a concerted process leading to a bicyclic carbocationic intermediate (**8**), from which the final product, further rearranged drimane type (**9**), is formed through concerted process including 1,2 alkyl shift to C-8 (route **a**) whereas the 1,2 hydride shift (route **b**) will give 4,9-friedodrimane-type sesquiterpene quinone (**10**).

Human protein tyrosine phosphatase 1B (hPTP1B) is regarded as a key target for the treatment of Type-II diabetes and obesity because it could hydrolyze phosphotyrosines on the insulin receptor,



**Figure 1.** Selected NMR correlations of compound **5**.



**Scheme 1.** Plausible biogenetic pathway of formation of rearranged drimane skeletons.

deactivating it. For this reason, PTP1B has been the subject of intense study for the past few years<sup>23</sup> and a number of PTP1B inhibitors have been developed over the past decade in an effort to design potent and selective compounds as drug candidates. In our screening program to search for PTP1B inhibitors from the South China Sea marine invertebrates, we evaluated compounds **5–7** for the inhibitory activity against hPTP1B and the results showed that new compound **5** exhibited moderate PTP1B inhibitory activity with an  $\text{IC}_{50}$  value of  $39.50\ \mu\text{M}$  while **7** had the strongest activity with an  $\text{IC}_{50}$  value of  $6.70\ \mu\text{M}$ . More-in-depth pharmacology studies (unpublished data) reviewed that dysidine (**7**) is a novel slow-binding PTP1B inhibitor with moderate inhibition selectivity over other PTPs. Further cell based evaluation of dysidine (**7**) indicated that it could strongly activate insulin signaling pathway and promote membrane translocation of the glucose transporter 4 (GLUT4) in CHO-K1 and 3T3-L1 cells. Moreover, dysidine could also significantly increase glucose uptake in 3T3-L1 cells by 2.3 fold. Due to the potent PTP1B inhibitory activity and moderate specificity, dysidine is expected to be developed as a potential lead compound for anti-diabetes agent. The cytotoxicity against Hela cell line was also evaluated for compounds **5–7**. Among them, **6** exhibited the significant cytotoxic activity against Hela cell line with an  $\text{IC}_{50}$  value of  $5.45\ \mu\text{M}$  and new compound **5** showed a

moderate cytotoxicity with an  $\text{IC}_{50}$  value of  $19.45\ \mu\text{M}$ . Finally, in light of the promising anti-HIV-I activities of avarol, avarone, and their derivatives, the anti-HIV-I activities of **5–7** were also evaluated but unfortunately all of them were found inactive.

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