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Three bioactive sesquiterpene quinones from the Fijian marine sponge of the genus Hippospongia

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SHORT COMMUNICATION

Three bioactive sesquiterpene quinones from the Fijian marine sponge of the genus *Hippospongia*

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A sesquiterpenoid quinone, epi-ilimaquinone (**1**), and two sesquiterpene amino quinones, smenospongine (**2**) and glycinylilimaquinone (**3**), were isolated from the Fijian marine sponge *Hippospongia* sp. The structures of these compounds were determined by spectroscopic analysis. Compounds **1** and **3** were reported for the first time in this study from the sponge of the genus *Hippospongia*. Compound **1** displayed potent cytotoxic activity and showed antibacterial activity against methicillin-resistant *Staphylococcus aureus*, wild type *S. aureus* and vancomycin-resistant *Enterococcus faecium* and displayed antifungal activity against amphotericin-resistant *Candida albicans* while compounds **2** and **3** showed moderate cytotoxic activity. However, compound **1** did not show appreciable antifungal activity against wild type *C. albicans*, *Cryptococcus neoformans*, *Aspergillus niger*, *Penicillium* sp., *Rhizopus sporangia* or *Sordaria* sp.

Keywords: marine sponge; sesquiterpene quinones; cytotoxicity; *Hippospongia* sp.

1. Introduction

Marine sponges have been a promising resource for discovering a huge diversity of secondary metabolites. A number of unusual quinones and hydroquinones (de Guzman et al., 1998) linked to a sesquiterpenoid skeleton have been isolated from the sponges of order Dictyoceratida (Sladić & Gašić, 2006). These play a defensive role and exhibit interesting biological activities (Piao et al., 2011). Sesquiterpenoid quinones including epi-ilimaquinone (**1**) and glycinylilimaquinone (**3**) previously reported from various genera belonging to Dictyoceratida such as *Fenestraspongia* (Carte, Rose, & Faulkner, 1985), *Dactylospongia* (Mitome, Nagasawa, Miyaoka, Yamada, & van Soest, 2002) and *Petrosaspongia* (Kwak, Schmitz, & Kelly, 2000) are also biologically active. However, compounds **1** and **3** are reported for the first time from the genus *Hippospongia* in this study. Further, few reports have been published on symbiotic microbes from Dictyoceratida such as bacteria (Müller et al., 2004; Thakur & Müller, 2005) and cyanobacteria (Hentschel et al., 2001) for the production of secondary metabolites particularly piperazines, brominated phenols and quinone compounds with diverse pharmaceutical relevance. However, there are no previous reports of associated microbes from the genus *Hippospongia*. In this study, a sesquiterpenoid quinone, epi-ilimaquinone (**1**), two sesquiterpene amino quinones, smenospongine (**2**), and glycinylilimaquinone (**3**),

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were isolated from Fijian marine sponge *Hippospongia* sp. This article describes the isolation, purification, structure elucidation and biological activities of sesquiterpene quinones.

2. Results and discussion

The collective crude MeOH and DCM extract of a *Hippospongia* sp. was subjected to solvent partitioning and yielded MeOH, DCM, hexane and BuOH soluble fractions. All the fractions were analysed for cytotoxicity and the active fractions (hexane, DCM and MeOH) were separately subjected to flash column chromatography and semi-preparative RP-HPLC to afford three cytotoxic compounds (**1**–**3**) (Figure 1).

The major compound epi-ilimaquinone (**1**) was isolated as bright yellow crystals. The molecular formula was established as $C_{22}H_{31}O_4$ on the basis of its HRESIMS (m/z 359.2215 is for $[M+H]^+$) and supported by NMR data. The ^{1}H-NMR spectrum illustrated three methyl signals: two singlet (s), tertiary methyls (3H) and one doublet (d) secondary methyl (3H) at 0.90, 1.07 and 0.95 ppm, respectively; one singlet methoxy group (OCH_3) at 3.89 ppm; a singlet at 5.89 ppm for a olefinic proton; a doublet (d) at 4.71/4.68 ppm for two protons; a broad singlet (br s) for the hydroxyl group (OH) and a AB quartet at 2.61/2.50 ppm for the two methylene protons. The ^{13}C-NMR showed two carbonyl signals (182.9 and 182.6 ppm) and six olefinic carbon atoms (161.8, 153.6, 153.4, 117.8, 105.8 and 102.1 ppm), which accounted for five out of eight degrees of unsaturation which indicated a tricyclic compound. The NMR data together with the physical characteristics of compound **1**, such as colour change in base from yellow to violet and oxidation in air, suggested that compound **1** is a benzoquinone. The alkyl ^{1}H-NMR signals indicated that the substitution on the benozquinone was sesquiterpenoid. The AB quartet at 2.61/2.50 ppm was assigned to the benzylic methylene which connected the benzoquinone to the sesquiterpene skeleton. The chemical shifts at 0.90, 1.07 and 0.95 ppm were attributed to the methyls of the sesquiterpene moiety. The LC–MS and NMR data implied that **1** is ilimaquinone. The ^{1}H-NMR spectrum contained signals expected for ilimaquinone except for the chemical shifts for the exocyclic methylene, which were downfield at 4.68/4.71 ppm. This suggested that compound **1** is a stereoisomer of

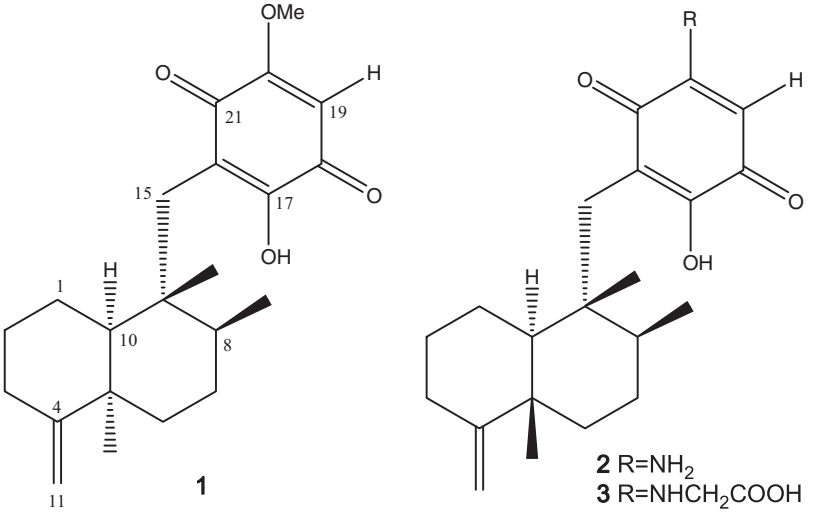

Figure 1. Structures of compounds **1**–**3**.

ilimaquinone, epi-ilimaquinone, which possesses the drimane skeleton which is known as *cis*-4,9-friedodrim-4(15)-ene.

Smenospongine (**2**) was obtained as a purple solid and the molecular formula $C_{21}H_{30}O_3N$ (*m/z* 344.2295, $[M+H]^+$) was deduced using HRESIMS and NMR data. The ^{1}H- and ^{13}C-NMR data of **2** were similar to epi-ilimaquinone except for the absence of the methoxy group which was replaced by an amino group. The absence of the methoxy signal and the upfield shift (113.7 ppm) of C-20 were consistent with the placement of NH_2 on C-20. The olefinic proton at H-11, 4.44 ppm was due to the exocyclic methylene group. This chemical shift together with the HMBC correlations suggested that **2** possessed a rearranged drimane skeleton belonging to the group *trans*-4,9-friedodrim-4(15)-ene.

Glycinylilimaquinone (**3**) was purified as a red solid and the molecular formula was determined as $C_{23}H_{31}NO_5$ (*m/z* 402.2351 $[M+H]^+$). The ^{1}H- and ^{13}C-NMR data of **3** were similar to epi-ilimaquinone and smenospongine, however, an extra carbonyl and a methylene signal were observed in place of the methoxy group on the benzoquinone moiety. These signals (H-22, 3.98, C-22, 44.4 and C-23, 170.5 ppm) are due to the glycine residue. The methylene proton (H-22, 3.98 ppm) signal showed two- and three-bond HMBC correlations to two quaternary carbon atoms, at C-23, 170.5 ppm and C-20, 151.5 ppm, respectively. These correlations suggested that the glycine residue is linked to the quinone ring through the nitrogen and resulted in glycinylilimaquinone (**3**). Similar to **2**, glycinylilimaquinone also possessed a rearranged drimane skeleton belonging to the group *trans*-4,9-friedodrim-4(15)-ene.

Compound **1** exhibited significant cytotoxic activity with LD_{50} of 18 ppm against brine shrimp. However, compounds **2** and **3** showed the lethality at $LD_{50} = 188$ and $LD_{50} = < 500$ ppm, respectively. Furthermore, compound **1** displayed potent antibacterial activity against methicillin-resistant *Staphylococcus aureus*, wild type *S. aureus*, vancomycin-resistant *Enterococcus faecium* and amphotericin-resistant *Candida albicans* with the minimum inhibitory concentrations of 62.5, 31.3, 15.6 and 125 $\mu g\,mL^{-1}$, respectively. Compounds **2** and **3** were not analysed for antimicrobial activities due to insufficient quantity. Compound **1** failed to show significant antifungal activity against wild type *C. albicans, Cryptococcus neoformans, Aspergillus niger, Penicillium* sp., *Rhizopus sporangia* or *Sordaria* sp.

3. Conclusion

We conclude that a sesquiterpenoid quinone, epi-ilimaquinone (**1**), and two sesquiterpene amino quinones, smenospongine (**2**) and glycinylilimaquinone (**3**), were isolated from the Fijian marine sponge *Hippospongia* sp. Compounds **2** and **3** showed moderate cytotoxic activity, but compound **1** displayed promising cytotoxic, and antimicrobial activity.

Supplementary material

Experimental details, NMR spectra and ESI–LCMS data for the compounds 1–3 are available as supplementary material.

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