Biologically active compounds from the red sea sponge *Suberea* sp.

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Abstract: Investigation of the cytotoxic fraction of the extracts of the Red Sea sponge *Suberea* sp. resulted in the identification of two new compounds, 1-(hydroxy(1*H*-pyrrol-2-yl)methyl)guanidine and 4-(2-amino-3-methylbut-3-en-1-yl)phenol (1 and 2) together with the previously reported 2-(3,5-dibromo-4-hydroxyphenyl)acetamide (3), subereaphenol C (4), dibromoverongiaquinol (5) and bromochloroverongiaquinol (6). The compounds were assigned by interpretation of their one- and two-dimensional NMR and MS spectral data. The cytotoxic activities of the compounds against two cancer cell lines were evaluated. In addition, the antimicrobial activities of the compounds were discussed.

Keywords: Red Sea sponge; *Suberea* species*;* pyrrole derivatives, phenolic and halogenated compounds; cytotoxic and antimicrobial activities.

INTRODUCTION

Marine sponges are considered as the richest source of bioactive secondary metabolites (Blunt *et al*., 2017). Members of the Verongida are excellent producer of bromotyrosine-derived alkaloids (Encarnacion *et al*., 2000; Lee *et al*., 2013; Buchanan *et al*., 2008; Badr *et al.,* 2008). These compounds displayed different biological activities including antimicrobial (Encarnacion *et al*., 2000), cytotoxic (Lee *et al*., 2013; Buchanan *et al*., 2008) as well as enzyme inhibitory activity (Buchanan *et al*., 2008). As a part of our continuous interest to identify bioactive leads from members of the Verongida from the Red Sea (Shaala *et al*., 2012; Shaala *et al*., 2011; Abou-Shoer *et al*., 2008; Shaala *et al*., 2008; Badr *et al*., 2008; Shaala *et al*., 2015), we have investigated the organic extract of the sponge *Suberea* sp. Bioactive secondary metabolites of the genus *Suberea* include derivatives of dibromotyrosine, alkaloids, halogenated compounds as well as terpenoidal derivatives (Blunt *et al*., 2017; Buchanan *et al*., 2008; Shaala *et al*., 2012; Shaala *et al.,* 2011; Abou-Shoer *et al*., 2008; Shaala *et al*., 2008; Debitus *et al*., 1998; Tsuda *et al*., 2001; Hirano *et al*., 2000).

Here, we report about the identification of two new compounds, amino(1*H*-pyrrol-2-yl)methyl carbamimidate and 4-(2-amino-3-methylbut-3-en-1-yl)phenol (1 and 2) together with the known compounds, 2-(3,5-dibromo-4-hydroxyphenyl)acetamide (3) (Chib *et al*., 1978), subereaphenol C (4) (Abou-Shoer *et al*., 2008; Shaala *et al*.; 2015), dibromoverongiaquinol (5) (Debitus *et al*., 1998), and bromochloroverongiaquinol (6) (Debitus *et al*., 1998) (fig. 1) from the sponge *Suberea* sp. Extensive examination of the spectral data of the compounds supported and secured the structural determination of the compounds. The compounds showed variable cytotoxic, antiproliferative and antimicrobial activities. In this paper, the structure assignment, the cytotoxic and antimicrobial activities of the compounds are reported.

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MATERIALS AND METHODS

General experimental procedures

Experiments were carried out as reported before (Shaala *et al*., 2012; Shaala *et al*., 2011; Abou-Shoer *et al*., 2008; Shaala *et al*., 2008).

Biological materials

The sponge *Suberea* species was identified by Prof. Rob van Soest and was previously described before (Shaala *et al.,* 2015).

Fractionation of the extracts and purification of 1-6

The dried sponge material (330g) was macerated in methanol (3 × 1000mL) at room temperature. The resulted extracts were dried, and the residue was dissolved in 1000 mL of 90% methanol. After that, the mixture was defatted by *n*-hexane (3 × 300mL). The mother liquor was diluted with H_2O and the mixture was extracted with CH_2Cl_2 (3 × 300mL) and the CH_2Cl_2 extracts were dried. The CH_2Cl_2 residue (6.3g) was subjected to VLC on SiO_2 using *n*-hexane-CH_2Cl_2-MeOH to give six subfractions (A–F). Fraction B (1.3g) was partitioned on a LH-20 resin using MeOH giving five subfractions (B1–B5). B-4 (174mg) was chromatographed on Sephadex LH-20 eluted with methanol and the major subfraction (55mg) was subjected to purification on RP C18 HPLC column using 50% acetonitrile in water to afford 1 (5.5mg), 2 (7.0mg) and 5 (2.3mg). Similarly, fraction B-5 (170mg) was fractionated on Sephadex LH-20 eluted with methanol and the major fraction (95mg) was subjected to purification on RP C18 HPLC column with 45% acetonitrile in water to give 3 (19mg) and 4 (11mg) and 6 (4.2mg).

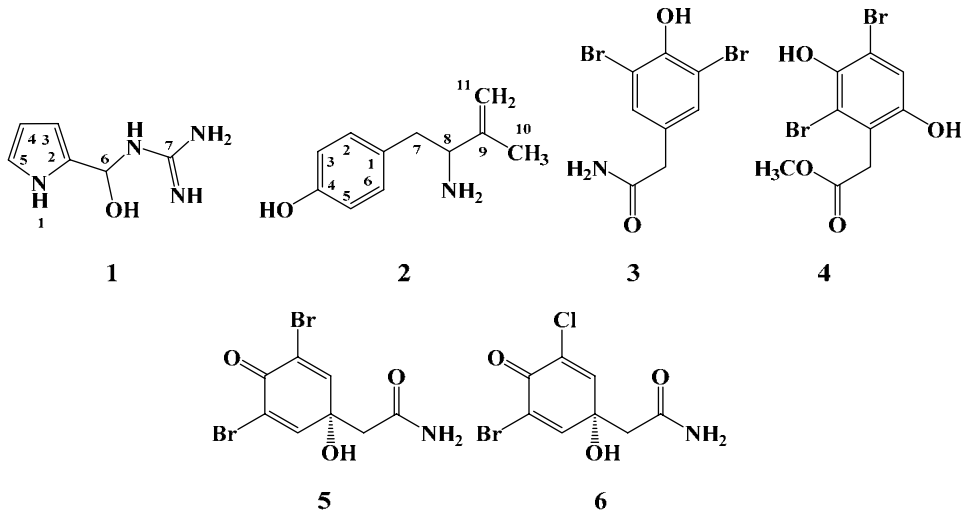

Fig. 1: Structures of compounds 1-6.

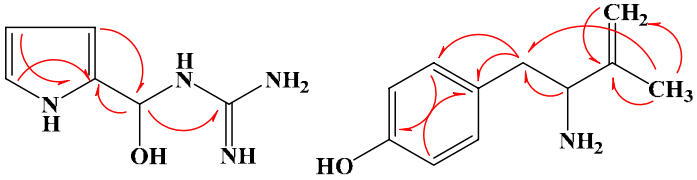

Fig. 2: Selected HMBC correlations for 1 and 2.

Biological activities of the compounds

Cytotoxicity evaluation of 1-6

The cytotoxic activities of compounds 1-6 against colorectal carcinoma (HCT 116) and HeLa cells were evaluated using sulforhodamine assay as previously described (Skehan *et al*.; 1990).

Antibacterial evaluation of 1-6

The antimicrobial activity of the compounds were determined as previously described (Kiehlbauch *et al*., 2000) with replication (*n=3*). *Candida albicans* and *E. coli* were served as target models for fungi and bacteria. A 100-µg of each compound was loaded onto 6-mm sterile filter circular paper disc. The paper discs were left to dry in the air. The dried paper discs were placed onto the nutrient agar plate that had already been inoculated with a lawn of target microorganisms (*C. albicans* and *E. coli*, separately). After 48 h of incubation at 30°C (for *C. albicans*) and 24 h of incubation at 37°C (for *E. coli*), the antimicrobial activities of the compounds were calculated.

RESULTS

Investigation of the cytotoxic fraction of the organic extract of the Red Sea sponge *Suberea* sp. led to the isolation of six compounds (1-6) including two new ones (1 and 2). The compounds were identified as follows: 1-(hydroxy(1*H*-pyrrol-2-yl)methyl)guanidine (1), 4-(2-amino-3-methylbut-3-en-1-yl)phenol (2), 2-(3,5-dibromo-4-hydroxyphenyl)acetamide (3) (Chib *et al*., 1978), subereaphenol C (4) (Abou-Shoer *et al*., 2008; Shaala *et al*.; 2015), dibromoverongiaquinol (5) (Debitus *et al*., 1998) and bromochloroverongiaquinol (6) (Debitus *et al*., 1998). The structure determination of the compounds was based on interpretation of their NMR and MS spectral data and by comparison with data in the literatures.

Characterization

1-(Hydroxy(1*H*-pyrrol-2-yl)methyl)guanidine (1):
Yellowish amorphous powder; NMR data: table 1; HRESIMS *m/z* 155.0935 (calcd for $C_6H_{11}N_4O$, $[M + H]^+$, 155.0933).

4-(2-Amino-3-methylbut-3-en-1-yl)phenol (2): colorless powder; NMR data: table 2; HRESIMS *m/z* 178.1236 (calcd for $C_{11}H_{16}NO$, $[M + H]^+$, 178.1232).

DISCUSSION

Determination of the structures of 1-6

Compound 1 (fig. 1) with a molecular formula of $C_6H_{10}N_4O$ is deduced from the HRESIMS. The NMR signals (table 1) at $\delta_{H/C}$ 134.1 (qC, C-2), 106.8/6.29 (d, J = 2.4 Hz) (CH, C-3/H-3), 118.5/6.47 (t, J=2.4 Hz) (CH, C-4/H-4), and 113.8/7.12 (d, J=2.4 Hz) (CH, C-5/H-6) supported the assignment of substituted pyrrole moiety (Iwai *et al*., 2014; Tsuda *et al*., 1999). The side chain at C-2 was assigned as 1-hydroxymethylguanidine. The signals are at $\delta_{H/C}$ 77.3/6.40 (br.s.) (CH, C-6/H-6) and 157.5 (qC, C-7) supported this assignment. The chemical shift of the

Table 1: NMR data and HMBC correlations of compound 1 (CD_3OD).

Position	δ_C (mult.)	δ_H [mult., *J* (Hz)]	HMBC (H→C#)
2	134.1 (qC)		H-4, H-5, H-6
3	106.8 (CH)	6.29 (d, 2.4)	
4	118.5 (CH)	6.47 (t, 2.4)	H-5
5	113.8 (CH)	7.12 (d, 2.4)	H-4
6	77.3 (CH)	6.40 (br.s.)	H-3
7	157.5 (qC)		H-6

Table 2: NMR data and HMBC correlations of compound 2 (CD_3OD).

Position	δ_C (mult.)	δ_H [mult., *J* (Hz)]	HMBC (H→C#)
1	127.0 (qC)		H-3, H-5, H_2-7
2,6	132.7 (CH)	6.98 (d, 8.5)	
3,5	116.4 (CH)	6.70 (d, 8.5)	
4	158.0 (qC)		H-2, H-6
7	39.2 (CH_2)	3.21 (m), 2.82 (m)	H-8, H_3-10
8	57.6 (CH)	4.22 (m)	
9	147.8 (qC)		H_3-10
10	23.0 (CH_3)	1.73 (s)	
11	111.5 (CH_2)	4.88 (s)	H_3-10

guanidine moiety at 157.5 is in good agreement with reported value for a terminal guanidine (Wright *et al*., 2017). In addition, the HMBC cross peak from H-6 (δ_H 6.40) to C-7 (δ_C 151.1) supported the placement of the guanidine moiety to C-6 (fig. 2). Additionally, the COSY experiment secured the 2-substuituted pyrrole moiety through the consecutive COSY correlations between H-3 and H-4 and between H-4 and H-5 and HMBC cross-peaks from H-4 and H-5 to C-2 and from H-3 to C-6 (fig. 2). Accordingly, 1 was assigned as 1-(hydroxy(1*H*-pyrrol-2-yl)methyl)guanidine and is reported here as a new compound.

Compound 2 (fig. 1) had a molecular formula of $C_{11}H_{15}NO$. Its NMR data (table 2) showed signals for a para-substituted benzene moiety. The HSQC experiment displayed four aromatic methines, one aliphatic methine, two methylenes including an exocyclic methylene and one methyl together with three quaternary carbons. The $^1H/^{13}C$ signals at 127.0 (qC, C-1), 6.98/132.7 (CH, H-2,4/C-2,4), 6.7/116.4 (CH, H-3,5/C-3,5) and 158.0 (C-4) are assigned as para-substituted phenol. The side chain at C-1 was assigned from two-dimensional NMR experiments (COSY, HSQC and HMBC) (fig. 2). The COSY experiment showed a geminal coupling between the protons at C-7 (CH_2, 3.21 and 2.88) as well as vicinal couplings to H-8 (CH, 4.22). In the HSQC experiment the protons of H_2-7 and H-8 were corroborated to the ^{13}C signals at 39.2 (CH_2, C-7) and 57.6 (CH, C-8), suggesting the attachment of C-8 to NH_2 moiety. The COSY coupling system was interrupted by an exomethylene moiety attached to a terminal methyl as assigned from the $^1H/^{13}C$ NMR signals at 147.8 (qC, C-9), 4.88/111.5 (CH_2, H_2-11/C-11) and 1.73/23.0 (CH_3, H_3-10/C-10). This assignment was secured by HMBC correlations of H_3-10/C-11, H_3-10/C-9 and H_2-11/C-9 (fig. 2). Thus, 2 was assigned as 4-(2-amino-3-methylbut-3-en-1-yl)phenol and is considered as a new compound.

Compounds 3–6 (fig. 1) were identified as 2-(3,5-dibromo-4-hydroxyphenyl)acetamide (3) (Chib *et al*., 1978), subereaphenol C (4) (Abou-Shoer *et al.,* 2008; Shaala *et al.*; 2015), dibromoverongiaquinol (5) (Debitus *et al*., 1998), and bromochloroverongiaquinol (6) (Debitus *et al*., 1998) by analysis of their NMR data and by comparison with the literatures.

The isolated compounds were evaluated for their cytotoxic and antiproliferative activities against HCT 116 and HeLa cell lines using sulforhodamine assay as previously described (Skehan *et al*.; 1990). Compound 6 was the most active one. It showed IC_{50} of 4.5 and 10 μg/mL against HeLa and HCT 116 cell lines, respectively. On the other hand, compound 1 showed weak cytotoxic activity against HCT-116 and HeLa cell line with IC_{50} of 25 and 30μg/mL, while 2 was weakly active with IC_{50} of 20 and 27μg/mL, respectively. In the antimicrobial screen, 2 showed inhibition zone of 15mm against *C. albicans*, while 1 and 6 showed inhibition zones against *E. coli* with 8 and 12mm, respectively.

CONCLUSION

Investigation of the sponge *Suberea* sp. resulted in the identification of two new compounds, 1-(hydroxy(1*H*-pyrrol-2-yl)methyl)guanidine and 4-(2-amino-3-methylbut-3-en-1-yl)phenol (1 and 2) together with 2-

(3,5-dibromo-4-hydroxyphenyl)acetamide (3), subereaphenol C (4), dibromoverongiaquinol (5) and bromochloroverongiaquinol (6). The structure determination of the compound was based on interpretation of the spectral data of the compounds. Compound 6 showed potent activity against HCT 116 and HeLa cell line, while compounds 2 and 4 were moderately active against these cells. Compound 2 showed moderate antifungal activity against *C. albicans*, while 1 and 6 were moderately active against *E. coli.*

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