

Studies on the Red Sea Sponge *Haliclona* sp. for its Chemical and Cytotoxic Properties

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ABSTRACT

Background: A great number of novel compounds with rich chemical diversity and significant bioactivity have been reported from Red Sea sponges. **Objective:** To isolate, identify, and evaluate the cytotoxic activity of the chemical constituents of a sponge belonging to genus *Haliclona* collected from the Eastern coast of the Red Sea. **Materials and Methods:** The total ethanolic extract of the titled sponge was subjected to intensive chromatographic fractionation and purification guided by cytotoxic bioassay toward various cancer cell lines. The structures of the isolated compounds were elucidated using spectroscopic techniques including one-dimension and two-dimension nuclear magnetic resonance, mass spectrometry, ultraviolet, and infrared data, as well as comparison with the reported spectral data for the known compounds. X-ray single-crystal structure determination was performed to determine the absolute configuration of compound 4. The screening of antiproliferative activity of the compounds was carried on three tumor cell lines, namely the human cervical cancer (HeLa), human hepatocellular carcinoma (HepG2), and human medulloblastoma (Daoy) cells using MTT assay. **Results:** This investigation resulted in the isolation of a new indole alkaloid, 1-(1H-indol-3-yloxy) propan-2-ol (1), with the previously synthesized pyrrolidine alkaloid, (2*R*, 3*S*, 4*R*, 5*R*) pyrrolidine-(1-hydroxyethyl)-3,4-diol hydrochloride (4), isolated here from a natural source for the first time. In addition, six known compounds tetillapyrone (2), nortetillapyrone (3), 2-methyl maleimide-5-oxime (5), maleimide-5-oxime (6), 5-(hydroxymethyl) dihydrofuran-2 (3H)-one (7), and ergosta-5,24 (28)-dien-3-ol (8) were also identified. Most of the isolated compounds exhibited weak cytotoxic activity against HepG-2, Daoy, and HeLa cancer cell lines. **Conclusion:** This is the first report of the occurrence of the indole and pyrrolidine alkaloids, 1-(1H-indol-2-yloxy) propan-2-ol (1), and the - (1-hydroxyethyl)-3,4-diol hydrochloride (4), in the Red Sea *Haliclona* sp.

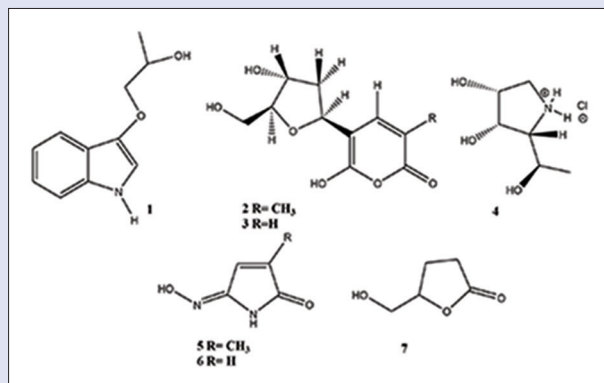
Key words: 5-(hydroxymethyl) dihydrofuran-2 (3H)-one, *Haliclona* sp., indole alkaloid, maleimide-5-oxime, pyrrolidine alkaloid

SUMMARY

- From the Red Sea *Haliclona* sp. two alkaloids with indole and pyrrolidine nuclei, 1-(1H-indol-2-yloxy) propan-2-ol (1) and pyrrolidine-(1-hydroxyethyl)-3,4-diol

hydrochloride (4) were isolated and fully characterized; in addition to six known compounds (2, 3, 5-8)

- The absolute configuration and the three-dimension stereo-molecular structure of compound 4 were determined by X-ray crystallography
- The different extracts and isolated compounds showed weak cytotoxic activity against HepG-2, Daoy, and HeLa cancer cell lines.



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INTRODUCTION

The Red Sea is characterized by a great diversity of living organisms.^[1] Its coral reefs, which extend about 2000 km, sustain more than 200 species of sponge, yet only a few of them have been studied.^[2] During the last two decades, a great number of novel compounds with rich chemical diversity and significant bioactivity have been reported from Red Sea sponges.^[3] Previous chemical studies of marine sponges belonging to the genus *Haliclona* (family Chalinidae) led to the isolation of a variety of bioactive secondary metabolites including alkaloids,^[4,5]

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macrolides,^[6] polyacetylenes,^[7] polyketides,^[8] steroids,^[9] peptides, and halogenated derivatives.^[10-12] Numerous bioactivities were reported for these metabolites such as anticancer, anti-inflammatory, antifouling, antidiabetic, and antimicrobial activities.^[5,10,13-15] Many of these interesting secondary metabolites became a target for chemical synthesis and the optimization of lead compounds.^[6,16] For instance, the isoquinoline alkaloid mimosamycin, isolated from the *Haliclona* sponge, was found to be cytotoxic against melanoma and ovarian tumor cell lines in humans, with an IC_{50} of approximately 10 $\mu\text{g}/\text{mL}$.^[4]

In the course of our ongoing research activities toward the isolation of biologically active compounds from marine and terrestrial sources, we had the opportunity to work on the ethanolic extract of a sponge belonging to the genus *Haliclona* collected from the Eastern coast of the Red Sea in Jeddah, Saudi Arabia. We herein present the isolation and structural elucidation of eight compounds, two of which are reported for the first time from a natural source. In addition, the antiproliferative activity of the extracts and the isolated compounds was also checked. Our data are a contribution to the exploration of the structural diversity of secondary metabolites from Red Sea organisms.

MATERIALS AND METHODS

General experimental procedures

Optical rotations (α_D^{25}) were measured on a Perkin-Elmer Model 341 LC polarimeter (Perkin-Elmer, Waltham, MA, the USA). The ultraviolet (UV) and infrared (IR) spectra were recorded on Hitachi-UV-3200 and JASCO 320-A spectrometers. The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at the NMR Unit at the College of Pharmacy, Sattam Bin Abdulaziz University, on an UltraShield Plus 500 MHz (Bruker) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values are reported in δ (ppm) relative to the internal standard TMS or residual solvent peak; the coupling constants (J) are reported in Hertz (Hz). Two dimension-NMR experiments (correlation spectroscopy [COSY], heteronuclear single quantum coherence [HSQC], heteronuclear multiple bond correlation [HMBC], and nuclear overhauser effect spectroscopy) were obtained using a standard Bruker program. A high-resolution mass spectrophotometer, Jeol JMS-700, was used for accurate mass determination. Electron impact mode of ionization was used, keeping ionization energy at 70 eV. Resolution was set up to 10 K direct probe was used with temperature ramp setting, initial temperature 50°C rise with rate of 32°C/min and final temperature set up to 350°C. X-ray was measured using a Bruker APEX-II D8 Venture diffractometer at 100 K. Data collection was performed on a Bruker Smart Apex II D8 Venture system, using Mo $K\alpha$ radiation, with a graphite monochromator, fine-focus micro tube. Thin-layer chromatography (TLC) was performed on precoated silica gel F254 plates (E. Merck, Darmstadt, Germany); detection was done at 254 nm and by spraying with p-anisaldehyde/ H_2SO_4 reagent. Centrifugal preparative TLC (CPTLC) was performed on a chromatotron (Harrison Research, Palo Alto, CA, the USA). Plates coated with 2 mm of silica gel 60, 0.04–0.06 mm, were used. All chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA). The absorbance at 549 nm was read on a microplate reader (ELX 800, Bio-Tek Instruments, Winooski, VT, the USA).

Animal material

The marine sponge *Haliclona* sp. was collected, in 2012, by scuba divers from SharmObhur, Jeddah, on the Saudi Arabian Red Sea coast, and was identified by Dr. Yahia Folos, Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. The sponge was deep-frozen immediately after collection and then freeze-dried to provide the dry material.

Extraction and isolation

The freeze-dried sponge (350 g) was extracted with 70% ethanol ($3 \times 1 \text{ L}$) at room temperature. The combined alcohol extract was filtered and evaporated under reduced pressure using a rotatory evaporator at 38°C to produce 25 g of the alcohol extract. The residue was suspended in water (200 mL) and successively partitioned with *n*-hexane ($3 \times 300 \text{ mL}$), dichloromethane ($3 \times 400 \text{ mL}$), ethyl acetate ($3 \times 300 \text{ mL}$), and *n*-butanol ($3 \times 200 \text{ mL}$). The *n*-hexane and dichloromethane fractions, combined due to the similarity on TLC (1.9 g), were subjected to chromatographic purification using a chromatotron with an *n*-hexane/EtOAc gradient, followed by EtOAc/methanol up to 20% to give 84 fractions of 5 ml each. Fractions were monitored by TLC silica gel, and similar fractions were pooled together to obtain four main fractions, designated as fractions A – D. Fraction A (10–23, 25 mg), eluted with 10% EtOAc/*n*-hexane, was further purified by CPTLC (1% MeOH/ CHCl_3) to afford 5 mg of 8. Fraction B (26–65, 15 mg), eluted by 50% EtOAc/*n*-hexane, was further purified using CPTLC (5% MeOH/ CHCl_3) to afford 1 (3 mg). A similar treatment of fractions C (76–78, 20 mg) and D (79–84, 50 mg) afforded 7 (10 mg) from C and 5 (20 mg) and 6 (19 mg) from D, using MeOH/ CHCl_3 (0.5 and 2%, respectively).

The combined ethyl acetate and *n*-butanol fractions (2.2 g) were applied onto the top of a silica gel packed column, eluted with $\text{CHCl}_3/\text{MeOH}$ in gradient elution mode to obtain three main fractions (E – G). Fraction E (75 mg), eluted with 5% MeOH/ CHCl_3 , was subjected to CPTLC to afford 5 and 6 from fractions 16 to 21. Fraction 31 (19 mg) was subjected to CPTLC purification (5% MeOH/ CHCl_3) to afford 10 mg of 3. Conversely, fraction F, eluted with 15% MeOH/ CHCl_3 , afforded two main sub-fractions after CPTLC purification. CPTLC chromatography of the subfractions 32–33 (14 mg), using 5% MeOH/ CHCl_3 , afforded 5 mg of 2. Subfractions 46–49 (47 mg) were subjected to Sephadex LH-20 CC eluted with 10% $\text{H}_2\text{O}/\text{MeOH}$ to give 30 mg of 4.

Crystal structure determination

Slow evaporation of chloroform solution of pure compound 4 yielded colorless crystals. A crystal of dimensions, 0.47 mm \times 0.35 mm \times 0.14 mm was selected for X-ray diffraction analysis. Data collection of 4 was performed on a Bruker Smart Apex II D8 Venture system, using Mo $K\alpha$ radiation from a fine focus microtube equipped with a graphite monochromator. The selected crystal was kept at 100 K under a stream of cooled nitrogen gas from a KRYO-FLEX low-temperature device. Data collection, indexing, and initial cell refinements were all carried out using APEX II software (Inc.: Analytical X-ray systems, In APEX II, B. A., Ed. 5465 East Cheryl Parkway, Madison WI 53711-5373, 2005). Frame integration and final cell refinements were done using SAINT software (SAINT, I., Analytical X-ray Systems. In version 6.45A; Bruker AXS, Ed. 5465 East Cheryl Parkway, Madison WI 53711-5373, 2003). Structure solution, refinement, graphics, and generation of publication materials were performed using SHELXTL software (SHELXTL, Bruker AXS, 5 In Systems, I. A. X.-r., Ed. 465 East Cheryl Parkway, Madison WI 53711-5373, 2002).^[17]

Cytotoxic activity

Three tumor cell lines were utilized in this study, namely the human cervical cancer (HeLa), human hepatocellular carcinoma (HCC HepG2), and human medulloblastoma (Daoy) cells. The cervical cancer cell line HeLa was obtained from American Type Culture Collection (Rockville, MD, USA). The HCC HepG2 cells were a kind gift from Dr. Ayman El-Kadi (University of Alberta, Edmonton, Alberta, Canada). The medulloblastoma cell line Daoy was a kind gift from Dr. Abdelilah Aboussekhra (Department of Molecular Oncology, King Faisal Specialist

Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia). HeLa and HepG2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)/high glucose supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 1% penicillin/streptomycin. Daoy was cultured in DMEM/F12 supplemented with 10% FBS, 2 mM L-glutamine, and 1% penicillin/streptomycin.

Screening of antiproliferative activity by MTT assay

The isolated compounds were evaluated at the Cell Culture Laboratory, College of Pharmacy, King Saud University, in a primary three-cell line-one concentration (50 µg/ml) anticancer assay against the previously mentioned cell lines. The cytotoxicity was assessed by testing the capacity of the reducing enzymes present in viable cells to convert MTT to formazan crystals as described by Al-Salahi *et al.*^[18]

The cytotoxic activity of the anticancer drug dasatinib, a potent, multitargeted kinase inhibitor of BCR-ABL and SRC family kinases,^[19] against the three cell lines was measured at the same concentrations of tested compounds and utilized as a standard for comparative purposes.

RESULTS AND DISCUSSION

A combination of different chromatographic techniques as CPTLC and gel filtration of the ethanolic extract of the marine sponge *Haliclona* sp. afforded eight compounds (1–8) [Figure 1], from which two were identified from a natural source for the 1st time (1, 4).

Structure elucidation of the new compounds (1, 4)

1-(1H-indol-2-yloxy) propan-3-ol (1) was isolated as a viscous liquid. Its high-resolution electron impact mass spectrometry (HREI-MS) showed an odd molecular ion peak at m/z 191.0946 [M]⁺, calculated for C₁₁H₁₃NO₂ with six degrees of unsaturation. A broad absorption band at 3435 cm⁻¹ with intense bands at 1595, 1500, and 1220 cm⁻¹ in the IR spectrum indicated the presence of OH and/or NH in addition to an aromatic ring. The ¹³C spectrum of 1 [Table 1] revealed the presence of 11 carbon signals, which were assigned, with the aid of DEPT experiments, to one methyl, one *sp*3 oxygenated methylene, one *sp*3 oxygenated methine, five *sp*2 methines, and three *sp*2 quaternary carbons. The ¹H NMR spectrum showed the resonance of four coupled aromatic

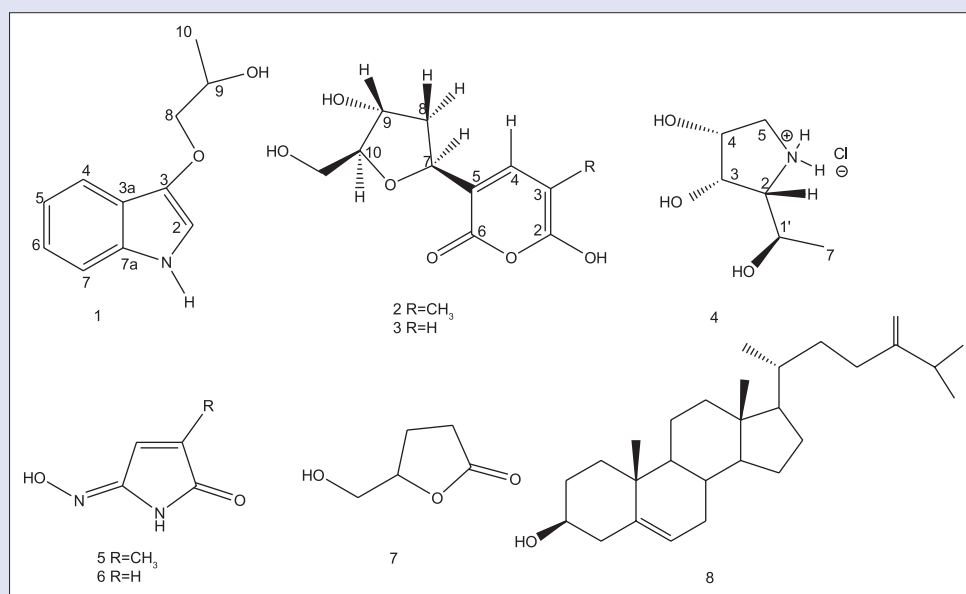


Figure 1: Structures of isolated compounds from *Haliclona* sp.

Table 1: ¹H and ¹³C-NMR data of compounds (1) and (4) (500 MHz for ¹H- and ¹³C-NMR, (1) in CD₃OD and (4) in DMSO-*d*₆)

Position	Compound (1)		Compound (4)	
	δ_H^*	δ_C	δ_H^*	δ_C
1	-	-	2.51 brs	-
2	7.95 brs	133.6, CH	3.10 dd, $J=9.1, 2.3$ Hz	66.0, CH
3	-	110.0, qC	4.03 t, $J=3.0$ Hz	69.1, CH
3a	-	128.0, qC		
4	8.13 brd, $J=7.8$ Hz	122.2, CH	4.19 ddd, $J=8.8, 3.5, 3.0$ Hz	69.9, CH
5	7.16 dt, $J=7.6, 1.3$ Hz	122.1, CH	α 2.86 dd, $J=11.1, 8.4$ Hz	46.4, CH ₂
6/1'	7.20 dt, $J=7.6, 1.3$ Hz	123.4, CH	β 3.26 dd, $J=11.1, 8.4$ Hz	61.7, CH
7/2'	7.45 brd, $J=7.6$ Hz	112.8, CH	3.96 dq, $J=9.1, 6.3$ Hz	20.4, CH ₃
7a	-	138.2, qC	1.11 d, $J=6.3$ Hz	
8	3.44 m	68.5, CH ₂		
9	3.80 m	69.2, CH		
10	1.15 d, $J=6.4$ Hz	19.6, CH ₃		
OH				
NH			2.51 brs	

*Multiplicities were deduced by DEPT and HSQC; CH₃: Methyl; CH₂: Methylene; CH: Methine; qC: Quaternary carbon

protons at δ_{H} 8.13 (1H, brd, $J = 7.8$ Hz), 7.45 (1H, brd, $J = 7.6$ Hz), 7.20 (1H, dt, $J = 7.6, 1.3$ Hz), and 7.16 (1H, dt, $J = 7.6, 1.3$ Hz), assigned for H-4, H-7, H-6, and H-5, respectively. Moreover, a broad singlet at δ_{H} 7.95 (H-2) was observed, indicating an ABCD aromatic spin system of an indole derivative with a C-3 substitution.^[20] The ^1H NMR spectrum also showed the presence of a methyl doublet at δ_{H} 1.15 ($J = 6.4$ Hz), an oxygenated methine at δ_{H} 3.80, and an oxygenated methylene at δ_{H} 3.44, diagnostic for an alkyl moiety. Its identity was deduced from the clear ^1H - ^1H COSY correlation signals between the oxygenated proton at δ_{H} 3.80 (1H, m, H-9) and both proton signals at δ_{H} 3.44 (2H, m, H-8), as well as the methyl doublet at δ_{H} 1.15 (3H, d, $J = 6.4$, H₃-10).

In addition, 2J and 3J HMBC correlations between the methyl protons at δ_{H} 1.15 (H₃-10) and the carbon signals at δ_{C} 69.2 (C-9) and 68.5 (C-8) confirmed the side chain identity as 2-hydroxy propane. The attachment of the 2-hydroxy propane moiety was confirmed to be at position 3 and not 2 by the appearance of two significant cross-peak correlations in the HMBC spectrum between both protons at δ_{H} 8.13 (brd, H-4) and 3.44 (2H, m, H-8) and the quaternary carbon at δ_{C} 132.5 (C-3) [Figure 2]. Based on the above discussion, compound 1 was proved to be 1-(1H-indol-3-yloxy) propan-2-ol, isolated here for the 1st time from a natural source. Several indole alkaloid derivatives have been reported from different sponges, including *Hyrtios*, *Sarcotragus*, *Tedania*, and *Dracmacidon*.^[20-23] Lately, the indole series of alkaloids have become an attractive research field for the development of new pharmacological lead compounds due to their interesting and diverse biological activities.^[24]

(2*R*, 3*S*, 4*R*, 5*R*) pyrrolidine-(1-hydroxyethyl)-3,4-diol hydrochloride (4) was obtained as white needle crystals. The HREI-MS showed an odd M^+ at m/z 147.0895, calculated for $\text{C}_6\text{H}_{13}\text{NO}_3$ with one degree of unsaturation. A monocyclic structure was suggested by the absence of a double bond or carbonyl carbon signals in the ^{13}C -NMR. The IR spectrum showed absorption bands at 3392 cm^{-1} (OH and/or NH) and 1640 cm^{-1} (N-H bending). The ^{13}C -NMR and DEPT experiment of 4 showed a total of six protonated carbons ascribed as one methyl, one methylene, and four downfield methine carbons. The ^1H -NMR spectrum showed a methyl doublet at δ_{H} 1.11 and a broad singlet at δ_{H} 2.51, assigned for NH [Table 1]. The two protons at δ_{H} 2.86 and 3.26 and correlated to a carbon signal at δ_{C} 46.4 (CH₂) in the HSQC spectrum were assigned for CH₂-5. COSY correlations of protons enabled the assignment of the initial structure of 4 as shown in Figure 2. The HMBC experiment was useful to prove the final structure of 4, where cross-peak correlations

were observed from the methyl protons at δ_{H} 1.10 to C-1' (δ_{C} 61.7) and C-2 (δ_{C} 66.0); from the proton signal at δ_{H} 3.96 (H-1') to carbon C-2 (δ_{C} 66.0); from the proton signal at δ_{H} 4.03 (H-3) to C-2 (δ_{C} 66.0); from H-5 β at δ_{H} 3.26 to C-3 at δ_{C} 69.1; and from the proton signal at 2.86 (H-5 α) to carbon at δ_{C} 69.9 (C-4). The unambiguous orientation of the hydroxyl groups at C-1', C-3, and C-4 and the configuration at the asymmetric centers of positions 1', 2, 3, and 4 were confirmed by X-ray diffraction. Fortunately, quality crystals for X-ray diffraction were obtained by slow evaporation of a solution of 4 in CHCl_3 , confirming the proposed structure and relative configuration [Figure 3]. The absolute configuration of all four chiral centers was established as 2*R*, 3*S*, 4*R*, and 5*R*. The Flack absolute configuration parameter was 0.04,^[25] where a value close or equal to zero represents the correct structure.

The polyhydroxylated pyrrolidine derivative 4 is apparently an isomer of the synthetic compound 6-deoxy-1,4-dideoxy-1,4-imino-D-mannitol (DIM).^[26] DIM and 6-deoxy-DIM are known as powerful amino-sugar glycosidase inhibitors, which act by mimicking the natural substrates. They are used for studying the mechanism of action of these enzymes and tested as potential drugs to treat a variety of carbohydrate-mediated diseases, such as diabetes mellitus.^[27] In addition, several isomers of 4 were previously synthesized, along with some pyrrolidine analogues of galactofuranose, from carbohydrate lactones and reported to be the first known inhibitor of *Escherichia coli* K12 UDP-Gal mutase and mycobacterial galactan biosynthesis.^[28]

Through comparison with data from previous literature, the structures of the known compounds were determined to be tetillapyrone (2), nortetillapyrone^[3,29,30] 2-methyl maleimide-5-oxime (5),^[31] Maleimide-5-oxime (6),^[29] 5-(hydroxymethyl) dihydrofuran-2 (3H)-one (7),^[32,33] and Ergosta-5,24 (28)-dien-3-ol (8), also known as chalinasterol or ostreasterol.^[34] It is worth noting that compound 5 is reported here for the 1st time from the genus *Haliclona*, while compound 7 is not yet been reported as occurring in marine organisms.

Spectral data of the known compounds (2, 3, 5-8)

Tetillapyrone (2)

^1H NMR (CD_3OD , 500 MHz): δ_{H} 7.83 (1H, s, H-4), 6.30 (1H, t, 6.7 Hz, H-7), 2.20 (1H, m, H-8a), 2.25 (1H, m, H-8b), 4.42 (1H, m, H-9), 3.93 (1H, q, 4.0 Hz, H-10), 3.83 (1H, ddd, 12, 5.0, 3.5 Hz, H-11a), 3.81 (1H, ddd, 12, 5.0, 3.5 Hz, H-11b), 1.90 (3H, br s, CH₃).

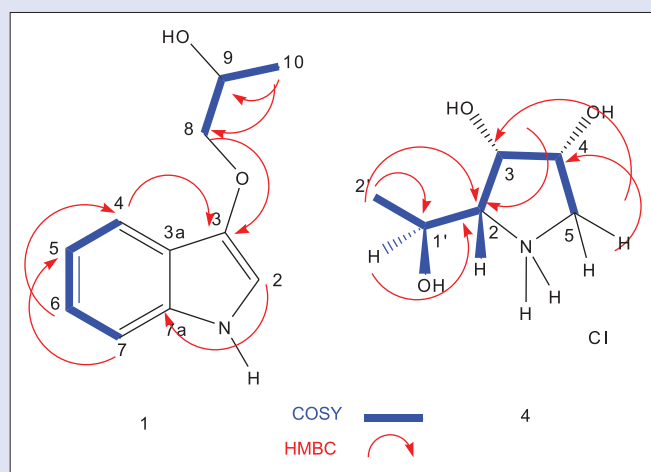


Figure 2: Key correlation spectroscopy and heteronuclear multiple bond correlation correlations of 1 and 4

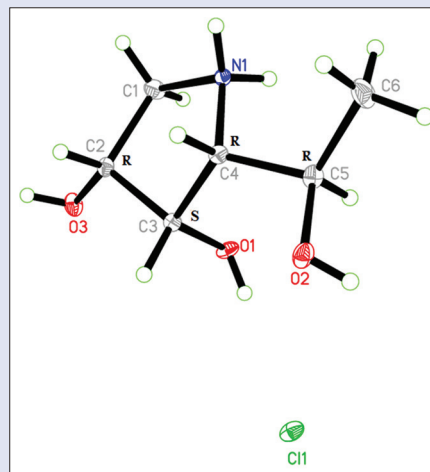


Figure 3: ORTEP projection of 4, with the displacement ellipsoids drawn at the 50% probability level

¹³C NMR (CD₃OD, 125 MHz): δ_C 152.4 (C-2), 111.6 (C-3), 138.2 (CH-4), 111.6 (C-5), 166.5 (C-6), 86.3 (CH-7), 41.2 (CH₂-8), 72.2 (C-9), 88.8 (C-10), 62.7 (C-11), 12.55 (CH₃).

Nortetillapyrone (3)

¹H NMR (CD₃OD, 500 MHz): δ_H 5.73 (1H, d, 8.1 Hz, H-3), 7.99 (1H, d, 8.1 Hz, H-4), 6.28 (1H, t, 6.6 Hz, H-7), 2.31 (1H, m, H-8a), 2.22 (1H, m, H-8b), 4.41 (1H, dq, 4.0, 3.5 Hz, H-9), 3.94 (1H, q, 3.5 Hz, H-10), 3.85 (1H, m, H-11a), 3.80 (1H, m, H-11b).

¹³C NMR (CD₃OD, 125 MHz): δ_C 152.3 (C-2), 102.8 (CH-3), 142.6 (CH-4), 102.8 (C-5), 166.3 (C-6), 86.7 (CH-7), 41.3 (CH₂-8), 72.3 (C-9), 89.0 (C-10), 62.9 (C-11).

2-Methyl maleimide-5-oxime (5)

¹H NMR (DMSO-d₆, 500 MHz): δ_H 11.00 brs (-NH), 10.59 brs (=N-OH), 7.24 s (1H, H-4),

1.72 (3H, CH₃); ¹³C NMR (DMSO-d₆, 125 MHz): δ_C 164.93 (CO-2), 151.47 (C-5), 137.70 (CH-4), 107.67 (C-3), 11.75 (CH₃).

Maleimide-5-oxime (6)

¹H NMR (DMSO-d₆, 500 MHz): δ_H 11.00 brs (-NH), 10.59 brs (=N-OH), 7.24 s (H-4), 1.72 (CH₃); ¹³C NMR (DMSO-d₆, 125 MHz): δ_C 164.93 (CO-2), 151.47 (C-5), 137.70 (CH-4), 107.67 (C-3).

Dihydrofuran-2 (3H)-one (7)

¹H NMR (CDCl₃, 500 MHz): δ_H 2.47 (2H, H-3), 2.16 (1H, H-3a), 1.95 (2H, H-3b), 4.52 (1H, H-5), 3.58 (2H, H-6), 5.06 (OH).

¹³C NMR (CDCl₃, 125 MHz): δ_C 177.5 (C-2), 28.1 (CH₂-3), 23.0 (CH₂-4), 80.6 (CH₂-5), 62.7 (CH₂-6).

Ergosta-5,24 (28)-dien-3-ol (8)

¹H NMR (CDCl₃, 500 MHz): δ_H 1.80 (2H, d, 5.1 Hz, H-1), 1.51 (2H, H-2), 3.53 (1H, m, H-3), 2.27 (2H, m, H-4), 5.35 (1H, brd, 5.7, H-6), 1.95 (2H, m, H-7), 0.66 (3H, s, H-18), 1.00 (3H, s, H-19), 0.95 (2H, d, 6.6 Hz, H-21), 1.03 (3H, d, 2.3 Hz, H-26), 1.02 (3H, d, 2.3 Hz, H-27), 4.66 (1H, s, H-28), 4.71 (1H, s, H-28).

¹³C NMR (CDCl₃, 125 MHz): δ_C 37.3 (CH₂-1), 31.7 (CH₂-2), 71.8 (CH-3), 42.3 (CH₂-4), 140.8 (C-5), 121.7 (CH-6), 31.0 (CH₂-7), 31.9 (CH-8), 50.1 (CH-9), 36.6 (C-10), 21.1 (CH₂-11), 39.8 (CH₂-12), 42.3 (C-13), 56.8 (CH-14), 24.3 (CH-15), 28.2 (CH₂-16), 56.0 (CH-17), 11.9 (CH₂-18), 19.4 (CH₃-19), 35.8 (CH-20), 18.7 (CH₃-21), 23.9 (CH₂-22), 34.7 (CH₂-23), 156.9 (C-24), 33.8 (CH-25), 22.0 (CH₃-26), 28.1 (CH₃-27), 105.9 (C-28).

Crystal data for compound 4

The three-dimension (3D) stereo-molecular structure presented herein was elucidated from a single colorless crystal suitable to X-ray diffraction, obtained from a batch of crystals obtained by slow evaporation of chloroform solvent. Data were collected using a Bruker APEX-II D8 Venture diffractometer at 100 K. Crystal data of compound 1: C₆H₁₃NO₃·HCl, M_r = 183.63, colorless needle, 0.71 mm × 0.12 mm × 0.06 mm, orthorhombic, space group *P*2₁2₁2₁, a = 5.1645 (3) Å, b = 12.4218 (7) Å, c = 13.0547 (7) Å, V = 837.49 (8) Å³, Z = 4, r_{calcd} = 1.456 Mg m⁻³, F (000) = 392, Mo Kα radiation, λ = 0.71073 Å, θ_{max} = 30.6, -7 ≤ h ≤ 7, -17 ≤ k ≤ 17, -18 ≤ l ≤ 18, 22465 measured reflections, 2563 independent, R (int) = 0.084, completeness to θ max = 99.9%, μ = 0.42 mm⁻¹, 156 parameters were refined against all reflections, R (F² > 2 σ [F²]) = 0.034, wR (F²) = 0.075, Flack parameter = 0.04 (3), Δρ_{max} = 0.38 e Å⁻³, Δρ_{min} = -0.32 e Å⁻³, GOF = 1.02 based on F₂. After extensive refinement and software calculations using likelihood methods, the compound is proven to be 100% enantiopure as (2*R*, 3*S*, 4*R*, 5*R*) [Figure 3].

Crystallographic data reported in this paper, have been deposited with The Cambridge Crystallographic Data Centre, deposit No.

CCDC 684583. Copies of these data can be obtained, free of charge on application to the Director, CCDC 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; or E-mail: deposit@ccdc.cam.ac.uk).

Cytotoxic activity

The cytotoxicity was tested against the three cancer cell lines HepG-2 (human liver cancer cell line), Daoy (human medulloblastoma), and HeLa (human cervical cancer cell line) using MTT assay,^[18] with dasatinib as a reference drug. As shown in Table 2, the *n*-hexane/CHCl₃ extract displayed the highest cytotoxic activity toward Daoy cell line (42.19% inhibition), while the two other extracts were weakly cytotoxic against HepG-2, Daoy, and HeLa cancer cell lines (11.28–29.83% inhibition). All isolated compounds showed weak cytotoxic activities against the tested cell lines. The strongest cytotoxic activity was exhibited by compound 8 against Daoy and HeLa cells (35.66 and 33.78% inhibition, respectively), followed by compound 6 (30.24% inhibition against Daoy cells). All compounds displayed very weak activity against the HeLa cell line, with inhibition percentages from 0% to 20%. In fact, the present findings for cytotoxic activity of compound 8 are consistent with results demonstrated for similar steroidal compounds obtained from various marine natural sources.^[35,36] A previous study had shown that ergosta-5,24 (28)-dien-3-ol (8), reported from several *Haliclona* sponges, possess moderate cytotoxicity against the human foreskin fibroblast cell line (Hs27 cells) with an IC₅₀ value of 58 μM.^[34] On the other hand, a recent study revealed that alkaloids, isolated from roots of *Zanthoxylum nitidum*, with mannopyranoside indole nucleus, possessed significant cytotoxic activities against selected tumor cell lines with IC₅₀ of <30 μM. Structural variations of attached functional groups might explain the lack of activity in compound 1.^[37] On the basis of these results, further lead optimization and structure-activity relationship studies are recommended to synthesize derivatives with potent antitumor activities.

CONCLUSION

Two alkaloids from the Red Sea *Haliclona* sp. with indole and pyrrolidine nuclei, 1-(1*H*-indol-2-yl)oxy) propan-2-ol (1) and pyrrolidine-(1-hydroxyethyl)-3,4-diol hydrochloride (4), were isolated and fully characterized; in addition to six known compounds (2, 3, 5-8). Furthermore, the absolute configuration and the 3D stereo-molecular structure of compound 4 were determined by X-ray crystallography. The different extracts and isolated compounds showed weak cytotoxic activity against HepG-2, Daoy, and HeLa cancer cell lines.

Table 2: Cytotoxic activities of *Haliclona* sp. extracts and isolated compounds

Cell line extract	Percentage inhibition*		
	HeLa	HepG-2	Daoy
<i>n</i> -Hex/CHCl ₃ extract	27.26	22.21	42.19
Ethyl acetate and <i>n</i> -butanol	23.65	21.43	20.12
Total extract	25.84	29.83	11.28
Compound 1	20.53	24.39	16.15
Compound 2	4.07	25.19	14.46
Compound 3	0	5.57	25.48
Compound 4	10.09	9.16	8.31
Compound 5	0	6.75	13.54
Compound 6	11.43	20.6	30.24
Compound 7	7.74	6.3	7.65
Compound 8	33.78	24.49	35.66
Dasatinib	56.4	89.67	90.36

*NT: Not tested; *Percentage inhibition of cell survival at a concentration of 50 μg/ml, relative to control

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Lira NS, Montes RC, Tavares JF, da Silva MS, da Cunha EV, de Athayde-Filho PF, *et al.* Brominated compounds from marine sponges of the genus *Aplysina* and a compilation of their ¹³C NMR spectral data. *Mar Drugs* 2011;9:2316-68.
- Qian PY, Wang Y, Lee OO, Lau SC, Yang J, Lafi FF, *et al.* Vertical stratification of microbial communities in the Red Sea revealed by 16S rDNA pyrosequencing. *ISME J* 2011;5:507-18.
- Blunt JW, Copp BR, Munro MH, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep* 2005;22:15-61.
- Rashid MA, Gustafson KR, Boyd MR. A new isoquinoline alkaloid from the marine sponge *Haliclona* species. *J Nat Prod* 2001;64:1249-50.
- Mol VP, Raveendran TV, Parameswaran PS. Antifouling activity exhibited by secondary metabolites of the marine sponge, *Haliclona exigua* (Kirkpatrick). *Int Biodeterior Biodegradation* 2009;63:67-72.
- Liu YH, Wang B, Liu DY, Li LD, Fei LN. Chemical and biological activities of marine sponge genus *Haliclona*. *JTO* 2008;27:70-82.
- Alarif WM, Abdel-Latef A, Al-Lihaibi SS, Ayyad SE, Badria FA. A new cytotoxic brominated acetylenic hydrocarbon from the marine sponge *Haliclona* sp. with a selective effect against human breast cancer. *Z Naturforsch C* 2013;68:70-5.
- Kennedy J, Codling CE, Jones BV, Dobson AD, Marchesi JR. Diversity of microbes associated with the marine sponge, *Haliclona simulans*, isolated from Irish waters and identification of polyketide synthase genes from the sponge metagenome. *Environ Microbiol* 2008;10:1888-902.
- Santalova EA, Makarieva TN, Ponomarenko LP, Denisenko VA, Krasokhin VB, Mollo E, *et al.* Sterols and related metabolites from five species of sponges. *Biochem Syst Ecol* 2007;35:439-46.
- Randazzo A, Bifulco G, Giannini C, Bucci M, Debitus C, Cirino G, *et al.* Halipeptins A and B: two novel potent anti-inflammatory cyclic depsipeptides from the Vanuatu marine sponge *Haliclona* species. *J Am Chem Soc* 2001;123:10870-6.
- Faulkner DJ. Marine natural products. *Nat Prod Rep* 2001;18:1-49.
- Rashid MA, Gustafson KR, Boswell JL, Boyd MR. Haligramides A and B, two new cytotoxic hexapeptides from the marine sponge *Haliclona nigra*. *J Nat Prod* 2000;63:956-9.
- Charan RD, Garson MJ, Brereton IM, Willis AC, Hooper JN. Haliclonacyclamines A and B, cytotoxic alkaloids from the tropical marine sponge *Haliclona* sp. *Tetrahedron* 1996;52:9111-220.
- Trianto A, Hermawan I, de Voogd NJ, Tanaka J. Halioxepine, a new meroditerpene from an Indonesian sponge *Haliclona* sp. *Chem Pharm Bull (Tokyo)* 2011;59:1311-3.
- Nuzzo G, Ciavatta ML, Villani G, Manzo E, Zanfardino A, Varcamonti M, *et al.* Fulvynes, antimicrobial polyoxygenated acetylenes from the Mediterranean sponge *Haliclona fulva*. *Tetrahedron* 2012;68:754-60.
- Sagar S, Kaur M, Minneman KP. Antiviral lead compounds from marine sponges. *Mar Drugs* 2010;8:2619-38.
- Sheldrick GM. A short history of SHELX. *Acta Crystallogr A* 2008;64:112-22.
- Al-Salahi R, Marzouk M, Ashour AE, Alswaidan I. Synthesis and antitumor activity of 1,2,4-triazolo[1,5-a]quinazolines. *Asian J Chem* 2014;26:2173-6.
- Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, *et al.* Discovery of N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem* 2004;47:6658-61.
- Ashour MA, Ehab S, Elkhayat ES, Ebel R, Edrada R, Proksch P. Indole alkaloid from the red sea sponge *Hyrtios erectus*. *Arcivoc* 2007;xv: 225-31.
- Dillman RL, Cardellina JH. Aromatic secondary metabolites from the sponge *Tedania ignis*. *J Nat Prod* 1991;54:1056-61.
- Mancini I, Guella G, Pietra F, Debitus C, Waikedre J. From inactive nortopsentin D, a novel bis (indole) alkaloid isolated from the Axinellid sponge *Dracmacidon* sp. from deep waters south of new Caledonia, to a strongly cytotoxic derivative. *Helv Chim Acta* 1996;79:2075-82.
- Liu Y, Jung JH, Zhang S. Indole alkaloids from a sponge *Sarcotragus* species. *Biochem Syst Ecol* 2006;34:453-6.
- Kochanowska-Karamyan AJ, Hamann MT. Marine indole alkaloids: Potential new drug leads for the control of depression and anxiety. *Chem Rev* 2010;110:4489-97.
- Flack HD. On enantiomorph-polarity estimation. *Acta Cryst* 1983;39:876-81.
- Bashyal BP, Fleet GW, Gough MJ, Smith PW. Synthesis of the α -mannosidase inhibitors swainsonine [(1S, 2R, 8R, 8aR)-1,2,8-trihydroxyoctahydroindolizine] and 1,4-dideoxy-1,4-imino-d-mannitol from mannose. *Tetrahedron* 1987;43:3083-93.
- Winchester B, al Daher S, Carpenter NC, Cenci di Bello I, Choi SS, Fairbanks AJ, *et al.* The structural basis of the inhibition of human α -mannosidases by azafuranose analogues of mannose. *Biochem J* 1993;290:743-9.
- Lee RE, Smith MD, Nash RJ, Griffiths RC, McNeil M, Grewal RK, *et al.* Inhibition of UDP-gal mutase and mycobacterial galactan biosynthesis by pyrrolidine analogues of galactofuranose. *Tetrahedron Lett* 1997;38:6733-6.
- Wattanadilok R, Sawangwong P, Rodrigues C, Cidade H, Pinto M, Pinto E, *et al.* Antifungal activity evaluation of the constituents of *Haliclona baeri* and *Haliclona cymaeformis*, collected from the Gulf of Thailand. *Mar Drugs* 2007;5:40-51.
- Watanadilok R, Sonchaeng P, Kijjoo A, Damas AM, Gales L, Silva AM, *et al.* Tetillapyrone and nortetillapyrone, two unusual hydroxy-pyran-2-ones from the marine sponge *Tetilla japonica*. *J Nat Prod* 2001;64:1056-8.
- Kijjoo A, Bessa J, Wattanadilok R, Sawangwong P, Nascimento MS, Pedro M, *et al.* Dibromotyrosine derivatives, a maleimide, aplysamine-2 and other constituents of the marine sponge *Pseudoceratina purpurea*. *Z Naturforsch* 2005;60b: 904-8.
- Hamamoto H, Suzuki Y, Takahashi H, Ikegami S. A new solid-phase reaction system utilizing a temperature-responsive catalyst: Oxidative cyclization with hydrogen peroxide. *Adv Synth Catal* 2007;349:2685-9.
- Yu Z, Liu J, Gong Y, Fan W, Ma J, Zhou N, *et al.* Chemical constituents of non-saponin from *Pulsatilla cernua*. *Zhongcaoyao* 2013;44:3264-9.
- Viegelmann C, Parker J, Ooi T, Clements C, Abbott G, Young L, *et al.* Isolation and identification of antitrypanosomal and antimycobacterial active steroids from the sponge *Haliclona simulans*. *Mar Drugs* 2014;12:2937-52.
- Sun Y, Tian L, Huang J, Li W, Pei YH. Cytotoxic sterols from marine-derived fungus *Penicillium* sp. *Nat Prod Res* 2006;20:381-4.
- Byju K, Anuradha V, Vasundhara G, Nair SM, Kumar NC. *In vitro* and *in silico* studies on the anticancer and apoptosis-inducing activities of the sterols identified from the soft coral, *Subergorgia reticulata*. *Pharmacogn Mag* 2014;10 Suppl 1:S65-71.
- Hu J, Shi X, Mao X, Chen J, Li H. Cytotoxic mannopyranosides of indole alkaloids from *Zanthoxylum nitidum*. *Chem Biodivers* 2014;11:970-4.



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