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BRIEF COMMUNICATION

A new antitrypanosomal alkaloid from the Red Sea marine sponge *Hyrtios* sp.

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Abstract

The antitrypanosomally active crude extract of the sponge *Hyrtios* sp. was subjected to metabolomic analysis using liquid chromatography coupled with high resolution electrospray ionization mass spectrometry (LC–HR-ESIMS) for dereplication purposes. As a result, a new alkaloid, hyrtiodoline A (**1**), along with other four known compounds (**2**–**5**) were reported. The structures of compounds **1**–**5** were determined by spectroscopic analyses, including 1D and 2D nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HRESI-MS) experiments, as well as comparison to the literature. We further investigated the antitrypanosomal activity of the five compounds, where compound **1** exhibited the most potent antitrypanosomal activity, with a half-maximal inhibitory concentration (IC_{50}) value of 7.48 μM after 72 h.

Marine sponges are a rich source of structurally novel chemical leads [1] that are interesting for drug discovery. In recent years, advances in chromatographic and spectroscopic techniques have facilitated the identification of structurally complex natural products [2] from sponges with >30% of all marine natural product discovered to date [3, 4]. Members of the genus *Hyrtios* (Demospongiae class, Dictyoceratida order, Thorectidae family) are known to produce alkaloids, sesterterpene, sesquiterpene, sterols, and macrolides [5–9], of which diverse activities are attributed such as cytotoxicity against murine P388 lymphocytic leukemia cells and various human tumor cell lines, antimalarial, antifungal, and antitrypanosomal activities [10, 11].

Dereplication of the secondary metabolites [12] from the antitrypanosomally active (90% growth inhibition) crude extract marine sponge *Hyrtios* sp. was achieved by high resolution Fourier transform mass spectrometry. Compounds identified and dereplicated from high-resolution mass spectral data sets of the marine sponge *Hyrtios* sp. by utilizing macros and algorithms that coupled MZmine with both in-house and commercial database MarinLit. The dereplicated compounds were showed in Fig. 1a, The crude extract of the Red Sea sponge *Hyrtios* sp. was then subjected to vacuum-liquid chromatography, followed by chromatography on silica gel and Sephadex LH-20, and finally, purification on semi-preparative high-pressure liquid chromatography (HPLC) to afford compounds **1–5** (Fig. 1b).

Compound **1** (2.5 mg) was isolated as a white powder. The molecular formula was determined to be $C_{12}H_{15}N_3O_2Na$, from its high-resolution electrospray ionization mass spectrometry, *m/z* calcd. 256.1064 for $C_{12}H_{15}N_3O_2Na$ (HRESI-MS). The ^{1}H-NMR spectrum (Table 1) revealed the presence of four proton resonances for the aromatic ABCD system, at δ_H 6.95 (1H, td, $J = 7.0$, 1.0 Hz; H-5), δ_H 7.03 (1H,td, $J = 8.1$, 1.1 Hz; H-6), δ_H 7.37 (1H, br d, $J = 7.2$ Hz; H-4), δ_H 7.45 (1H, br d, $J = 8.0$ Hz; H-7). In addition to two methylene protons at δ_H 2.82 (1H, dt, $J = 4.8$, 15.6; H-8a), δ_H 2.92 (1H, dt, $J = 6.6$, 15.6; H-

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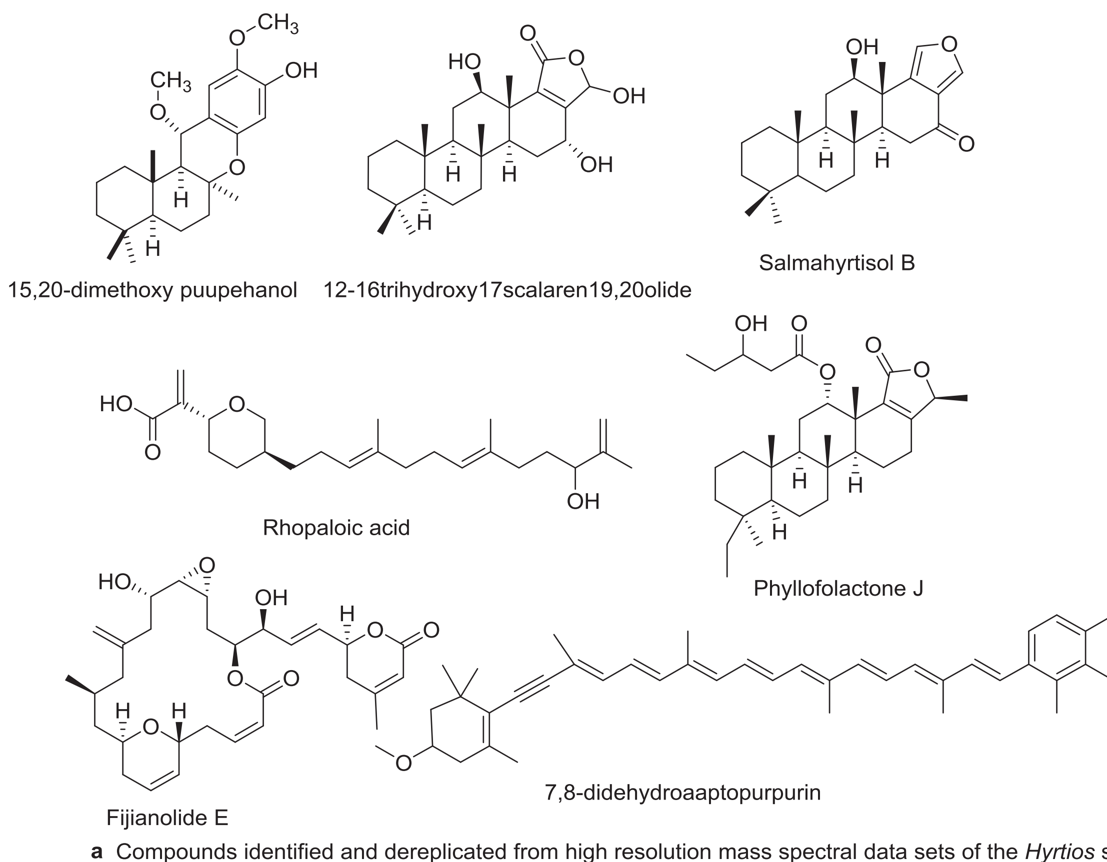
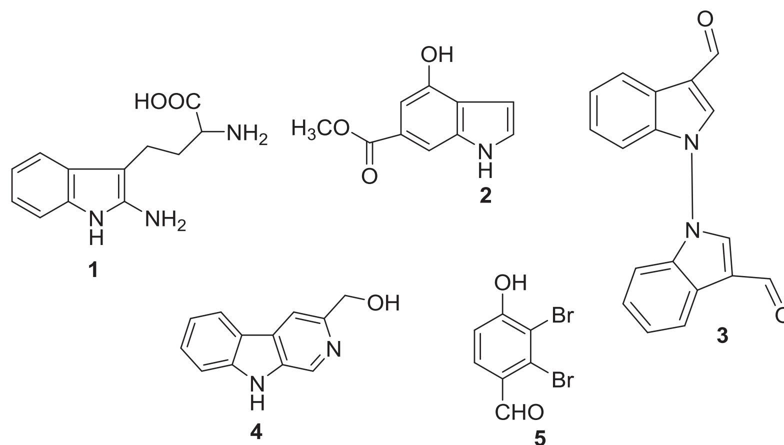
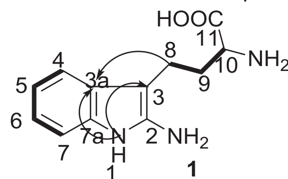




b Structures of the isolated compounds **1-5**.



c Significant HMBC and COSY correlations of compound 1

Fig. 1 a Compounds identified and dereplicated from high-resolution mass spectral data sets of the *Hyrtios* sp. **b** Structures of the isolated compounds **1–5**. **c** Significant HMBC and COSY correlations of compound **1**

8b), δ_H 3.27 (1H, m, H-9a), and δ_H 3.42 (1H, m; H-9b) overlapped with the water peak, in addition to a downfield broad singlet proton at δ_H 4.68 (1H, br s; H-10). Finally, a singlet peak at δ_H 10.68 (1H, s; NH-1) was inspected. On

Table 1 NMR-spectroscopic data of compound **1** in DMSO-d_6 (^{1}H: 600 MHz; ^{13}C: 150 MHz)

C/H no.	δ_H, Mult	COSY	δ_C, Mult	HMBC ($J = 8.3$ Hz)
1	—	—	—	—
2	—	—	125.8	—
3	—	—	104.2	—
3a	—	—	128.6	—
4	7.37 (1H, brd, $J =$ 7.2)	6.95	117.9	121.4, 136.0
5	6.95 (1H, td, $J =$ 7.0, 1.0)	7.37, 7.03	118.9	112.2, 128.6
6	7.03 (1H, td, $J =$ 8.1, 1.1)	6.95, 7.45	121.4	121.4, 136.0
7	7.45 (1H, brd, $J =$ 8.0)	7.03	112.2	118.9, 128.6
7a	—	—	136.0	—
8a	2.82 (1H, dt, $J =$ 4.8, 15.6)	—		128.6
8b	2.92 (1H, dt, $J =$ 6.6, 15.6)	—	18.1	128.6
9a	3.27 (1H, m)	—		
9b	3.42 (1H, m)	—	41.2	104.2, 165.5
		4.68		
10	4.68 (1H, brs)	—	55.3	—
11	—	—	165.5	—
NH	10.68 (1H, s)	—	—	120.5, 149.3

s singlet, *brs* broad singlet, *d* doublet, *brd* broad doublet, *t* triplet

the other hand, the ^{13}C NMR, DEPT spectra revealed the presence of 12 signals classified into five methine carbon signals at δ_C 117.9 (CH-4), δ_C 118.9 (CH-5), δ_C 121.4 (CH-6), δ_C 112.2 (CH-7), and δ_C 55.3 (CH-10); and five quaternary carbon signals at δ_C 125.8 (C-2), δ_C 104.2 (C-3), δ_C 128.6 (C-3a), δ_C 136.0 (C-7a), and δ_C 165.5 (C-11), in addition to two methylene carbons at δ_C 18.1 (CH_2-8) and δ_C 41.2 (CH_2-9). Investigation of the heteronuclear multiple bond correlation (HMBC) spectrum showed strong correlations between the δ_H 10.68 assignable to NH-1 and δ_C 104.2 (C-3); δ_C 128.6 (C-3a) confirmed the position of NH-1; and finally, the significant correlations between δ_H 2.82 for H-8a with δ_C 128.6 (C-3a), and between δ_H 2.92 corresponding to H-8b with δ_C 128.6 (C-3a),125.8 (C-2), confirmed the position of the aliphatic side chain at C-3. The HMBC spectrum showed the correlation between H-10 (δ_H 4.68) and the carboxylic carbon (C-11). The complete assignment of compound **1** was confirmed by investigation of heteronuclear single quantum coherence (HSQC), correlation spectroscopy (COSY), and HMBC as showed in Fig. 1c (Table 1). From the previous data, compound **1** (2-amino-4 (2-amino-1H-indole-3-yl) butanoic acid) was confirmed to be a new alkaloid, which we named Hyrtiodoline A.

Compounds **2–5** were identified based on HSQC, HMBC, COSY spectra, HRESI-MS and in comparison with the literature [9, 13–16] as 4-hydroxy-1*H*-indole-6-carboxylic acid methyl ester, synthetic known dimer of indole-3-carbaldehyde, 3-methylene hydroxy β-carboline alkaloid and 2,3-dibromo-4-hydroxybenzaldehyde, respectively. The five compounds were tested against *Trypanosoma brucei brucei*, and for cytotoxicity against J774.1 macrophages. Antitrypanosomal activity after 48 and 72 h was detected for compound **1** with IC_{50} values of 15.26 and 7.48 µM, respectively, with no cytotoxicity against J774.1 macrophages (IC_{50} of >200 µM). However, the other compounds showed no activity against *T. brucei*.

Material and methods

General experimental procedures

^{1}H, ^{13}C, COSY, HSQC, and HMBC NMR spectra were recorded on a Bruker Avance III HD 600 (Bruker Daltonics, Bremen, Germany) instrument. Accurate electrospray ionization mass spectra (ESI) were obtained by a micrOTOF focus (Bruker Daltonics, Bremen, Germany. TLC was performed on TLC plates precoated with silica gel F254 (Merck, Darmstadt, Germany). HPLC separation and purification were performed on a Agilent Technologies Series 1100 (ALS, UV Detect00719, USA) on a semi-preparative RP-C18 column (5 µm, 10 × 250 mm, Waters XBridge, city, Germany). MR 700 Microplate Reader (optical density measurement) (Dynatech Engineering Ltd., Willenhall, UK). The NMR spectral data of compound 1 were acquired using a 600 MHz instrument: ^{1}H, ^{13}C, ^{13}C-DEPT135, ^{1}H-^{1}H COSY, HSQC, and HMBC (optimized to $J = 8.3$ Hz) in DMSO-d_6 (Table 1).

Sponge material

The sponge material used in this work was collected by Safwat Ahmed (Suez Canal University) from the Egyptian coasts of the Red Sea at Sharm el-Sheikh, using scuba diving at a depth of 9.14 m. The collected material was immediately frozen and kept at −20 °C until investigation. The sponge biomass was identified by van Soest (Institute of Systematic Population Biology, Amsterdam University, The Netherlands). A voucher specimen was kept in the collections of the Zoological Museum of the University of Amsterdam, under registration number ZMAPOR19762. Another voucher sample was deposited under the number SAA-61 at the Pharmacognosy Department, Faculty of Pharmacy, Suez Canal University, Egypt.

Extraction and isolation

The freeze-dried sponge was extracted exhaustively with methanol–methylene chloride 1:1. The resulting crude extract was first partitioned between H_2O and EtOAc, followed by evaporation of the EtoAc layer and repartitioning of the aqueous fraction with butanol. The EtOAc-soluble material of *Hyrtios* (6 g) was subjected to vacuum-liquid ochrmatography on silica gel, using gradient elution with Pet-Ether:EtoAc then EtoAc 100% followed by methanol. The methanol fraction was subjected to silica gel column chromatography and eluted with a dichloromethane methanol (DCM–MeOH) gradient elution, followed by sephadex LH-20 (Merck, Bremen, Germany), and finally purified on semi-preparative HPLC using an acetonitrile (MeCN) and water solvent mixture complemented by 0.05% trifluoroacetic acid (10% MeCN/H_2O to 100% MeCN over 30 min at a flow rate of 5 mL/min), to yield compound **3** (Rt = 17.877 min) and compound **5** (Rt = 16.8 min); while the butanol fraction was subjected to sephadex LH-20 CC using a MeOH–H_2O gradient elution (10:100%), followed by final purification on semi-preparative HPLC using an acetonitrile (MeCN) and water solvent mixture complemented by 0.05% trifluoroacetic acid (10% MeCN/H_2O to 100% MeCN over 30 min at a flow rate of 5 mL/min), to afford the three compounds **1** (Rt = 18.1 min), **2** (Rt = 16.4 min), and **4** (Rt = 17.1 min).

Antitrypanosomal assay

Antitrypanosomal activity [17, 18] was tested, following the protocol of Huber and Koella [19, 20].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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