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NEW BROMOINDOLE ALKALOID ISOLATED FROM THE MARINE SPONGE *HYRTIOS ERECTUS*

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Abstract – 5'-[(6-Bromo-1*H*-indol-3-yl)methyl]-3'-methylimidazolidine-2',4'-dione (**1**), a new bromoindole alkaloid was derived from the marine sponge *Hyrtios erectus* (order Dictyoceratida) collected in the Red Sea. Additionally, two known indole alkaloids (**2**–**3**) were also isolated. The chemical structures of the isolated compounds were elucidated on the basis of detailed spectroscopic analysis as well as comparison with published data. Compound **1** showed weak antiproliferative activity against colorectal carcinoma (HCT-116), breast adenocarcinoma cells (MCF-7) and hepatocellular carcinoma (HepG2). Additionally, at concentration of 3 mg/mL, compound **1** displayed moderate antibacterial activity against *S. aureus* and *E. coli*.

Sponges have proven to be rich source for new bioactive marine derived compounds.[1] Sesquiterpenes and indole alkaloids are the common secondary metabolites that have been isolated from the marine sponges belonging to the order Dictyoceratida, family Thorectidae.[2-15] Other metabolites including, sesterterpenes,[16-20] macrolides,[21,22] and *β*-carboline alkaloids.[9,15,23] The genus *Hyrtios* displayed diverse biological activities.[9,10,13,16-18,21,22,24,25] Indole alkaloid derivatives display a range of interesting type of biological activities including anticancer, anti-inflammatory, antiviral,[26] antioxidant,[27] antimalarial,[4] antimicrobial,[3,4,8] and antidepressant.[28] In our previous work,[29,30] we reported the isolation of several biologically active scalarane sesterterpenes including three new ones from the title sponge. In the course of our ongoing efforts to identify drugs from sea, we have investigated the extract of the Red Sea marine sponge *Hyrtios erectus*. We report herein the purification, structure elucidation and biological evaluation

including antiproliferative and antibacterial activities of three indole alkaloids including the new compound 5'-[(6-bromo-1*H*-indol-3-yl)methyl]-3'-methylimidazolidine-2',4'-dione (**1**), together with the previously reported compounds, 5-hydroxy-1*H*-indole-3-carboxylic acid methyl ester (**2**)[10,31] and 5-hydroxyindole-3-aldehyde (**3**).[32] The isolated compounds exhibit weak antiproliferative activity against several cancer cell lines.

Successive chromatographic fractionation of the VLC fraction, (MeOH-$CHCl_3$, 1:3), obtained from the methanolic extract of the sponge using silica gel column chromatography, sephadex LH-20 and finally purification on semipreparative reversed phase HPLC column gave the new bromoindole alkaloid 5'-[(6-bromo-1*H*-indol-3-yl)methyl]-3'-methylimidazolidine-2',4'-dione (**1**), together with the previously reported compounds, 5-hydroxy-1*H*-indole-3-carboxylic acid methyl ester (**2**) and 5-hydroxyindole-3-aldehyde (**3**) (Figure 1). All of the known compounds were identified by extensive study of their spectral data, including ESIMS, 1D and 2D NMR data, as well as by comparison with published data.

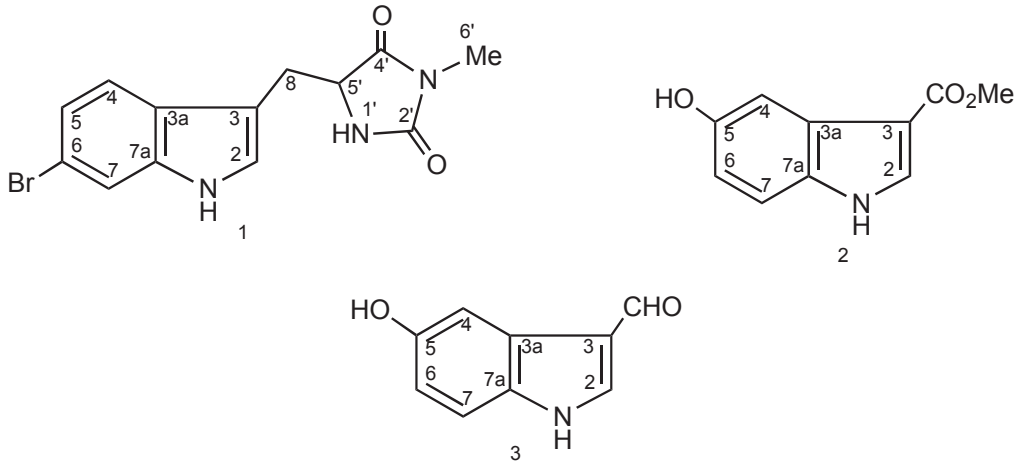

Figure 1. Structures of isolated compounds **1**–**3**

Compound **1** (Figure 1) was isolated as yellow amorphous solid. The molecular formula, $C_{13}H_{12}N_3O_2Br$, was established from ESIMS molecular ion peak at *m*/*z* 322.1 $[M+H]^+$ as well as from ^{13}C NMR data. The ESIMS of compound **1** displayed two equally intense pseudo-molecular ion peaks at *m*/*z* 322 and 324 $[M+H]^+$ (supplementary materials, Figure S1), indicating the presence of one bromine atom in the molecule. Of the 9 degree of unsaturation indicated by the molecular formula of compound **1**, six were present as double bonds (4xC=C, and 2xC=O), as deduced from the ^1H and ^{13}C NMR data (Table 1) and the molecule must be a tricylic. The ^1H NMR data (Table 1) revealed two labile protons (NH) at δ_H 8.13 (s) and 5.24 (s), four aromatic protons at δ_H 7.25 (1H, d, H-7), 7.54 (1H, br d, H-5), 7.45 (1H, d, H-4), and

7.06 (1H, s, H-2), an *N*-methyl group at δ_H 2.96, one methylene group (δ_H 3.41, dd, *J*=14.5, 2.6 Hz, and 2.98, m), and one methine proton at δ_H 4.27, m. (supplementary materials, Figure S2).

Table 1. NMR data and HMBC correlations of compound **1** ($CDCl_3$)

Position	δ_C	δ_H (m, *J* in Hz)	HMBC (H→C)[a]
NH-1		8.13, s	C-3, C-3a
2	123.5, CH	7.06, s	C-3, C-7a, C-8
3	110.1, qC		
3a	125.8, qC		
4	119.8, CH	7.45, d (8.5)	C-3, C-6, C-7a
5	114.3, CH	7.54, br d	C-3a, C-6, C-7
6	116.2, qC		
7	123.4, CH	7.25, d	C-3a, C-5, C-7a
7a	137.0, qC		
8	28.0, CH_2	3.41, dd (14.45, 2.55); 2.98, m	C-2, C-3, C-3a, C-4', C-5'
NH-1'		5.24, s	C-2', C-4', C-5'
2'	157.0, qC		
4'	173.3, qC		
5'	57.8, CH	4.27, m	C-3, C-8, C-2', C-4'
6'	24.5, CH_3	2.96, s	C-2', C-4'

[a] HMBC correlations are from proton(s) stated to the indicated carbons.

Interpretation of the NMR spectroscopic data [^{1}H NMR, ^{13}C NMR, HSQC, HMBC and ^{1}H-^{1}H COSY, (Table 1, supplementary materials, Figures S2-S6)] supported the presence of disubstituted indole moiety. The HMBC correlations of H-2 (δ_H 7.06) to C-3, C-3a, C-7a, and C-8; H-8 (δ_H 3.41 and 2.97) to C-2, C-3a, C-5', and C-4'; and N*CH*$_3$ (δ_H 2.96) to C-4', and C-2' suggested the presence of *N*-methylimidazolidine-2',4'-dione (two C=O bonds and one ring) connected to indole moiety through C-3 (Figure 2). The ^{1}H-^{1}H COSY correlation between (H-4 & H-7) and H-5 and lack of correlation with H-6 supported the presence of bromine atom at C-6.

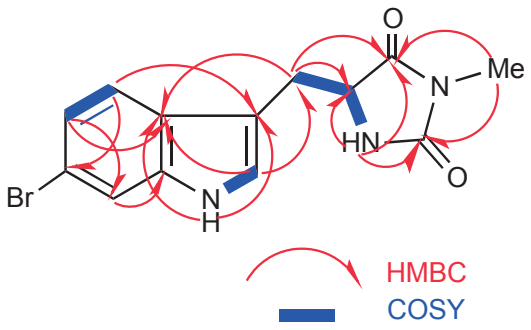

Figure 2. Selected COSY and HMBC correlations of compound **1**

Extensive study of these spectral data, including ESIMS, 1D and 2D NMR data and comparison with the published data demonstrated that spectral data of compound **1** were consistent with the data for 5'-[(5,6-dibromo-1*H*-indol-3-yl)methyl]-3'-methylimidazolidine-2',4'-dione, previously reported metabolites from *Smenospongia* sp. (order Dictyoceratida, family Thorectidae),[33] with the exception that compound **1** contains one bromine atom. No optical rotation was observed, $[\alpha]_D^{25}$ 0.0, indicated that **1** was the racemic mixture of S and R-isomer. Thus, compound **1** was identified as a racemic mixture of (S&R) of 5'-[(6-bromo-1*H*-indol-3-yl)methyl]-3'-methylimidazolidine-2',4'-dione. Compound **1** represents a further example of bromoindole alkaloids[33-36] isolated from marine origin. From the above results and discussion, compound **1** was assigned as depicted in Figure 1.

The other known compounds **2**–**3** (Figure 1) were identified by extensive study of their spectral data as well as by comparison with the published data (See the Supporting Information for the complete spectral data of compounds **1**–**3**). Thus, the compounds were identified as 5-hydroxy-1*H*-indole-3-carboxylic acid methyl ester (**2**)[10,31] and 5-hydroxyindole-3-aldehyde (**3**).[32] Although carbonated compounds had been previously prepared synthetically, they also reported and isolated from a natural source,[10,31,32] but it cannot be excluded that it may be an artifact due to extraction procedures.

Antiproliferative effects of compounds **1**–**3** against three different tumor cell lines were evaluated using SRB-U assay over concentration range 0.01–100 μM. Compounds **1**–**3** displayed weak antiproliferative activity (IC_{50} >100 μM) against all cell lines under investigation (HCT-116, MCF-7 and HepG2). Doxorubicin was used as positive cytotoxic control and exhibited IC_{50} of 0.11, 0.41 and 0.85 μM against HCT-116, MCF-7 and HepG2 cell lines, respectively. Additionally, in the antibacterial assay, at concentration of 3 mg/mL, compounds **1** showed inhibition zones of 14 mm against *S. aureus* compared to 20 mm displayed by erythromycin and inhibition zones of 21 mm against *E. coli* compared to 20 mm displayed by chloramphenicol. Furthermore, compound **1** was inactive against *B. subtilis* and *P. aeruginosa*. Compounds **2**–**3** were inactive in the antibacterial assay.

In conclusion, our search for isolation of biologically active compounds from marine invertebrates has led to the chemical investigation of the marine sponge *Hyrtios erectus* collected from the Red Sea. Compounds (**1**–**3**) including new one (**1**), were purified and their chemical structures were determined using spectroscopic studies. The isolated compounds belong to the class of indole alkaloids. Biological evaluation of the isolated compounds showed that compounds **1** displayed moderate antimicrobial and weak antiproliferative activities.

EXPERIMENTAL

General. Optical rotation was measured on the automatic high-speed laboratory polarimeter P3000 (A.KRUSS Optronic GmbH, Hamburg, Germany). UV spectra were measured on a Hitachi 300

Spectrophotometer (Hitachi High-Technologies Corporation, Kyoto, Japan). High-resolution ESIMS data were recorded with an Ultra-High Resolution (UHR) TOF spectrometer (Impact, Bruker, Bremen, Germany). NMR spectra were obtained in $CDCl_3$ on a Bruker Avance DRX 600-MHz spectrometer (Bruker, Bremen, Germany) at 600-MHz for ^1H NMR and 150 MHz for ^{13}C NMR. NMR chemical shifts were expressed in parts per million (ppm) referenced to residual $CDCl_3$ solvent signals (δ_H 7.26 for ^1H and δ_C 77.0 for ^{13}C). Precoated SiO_2 60 F_{254} plates (Merck, Darmstadt, Germany) were used for TLC. For column chromatography, SiO_2 (70–230 mesh, Merck, Darmstadt, Germany) was used. HPLC purifications were performed on HPLC column (5 μm ZORBAX Eclipse XDB-C18, 250 × 4.6 mm, Agilent, Santa Clara, CA, USA).

Biological Materials. The Red Sea marine sponge used in this study was collected from Egypt, using scuba diving. The sponge material was immediately frozen after collection and kept at −20 °C until investigation. The sponge specimen was identified as *Hyrtios erectus* (class: Demospongiae, order: Dictyoceratida, family: Thorectidae) by Dr. R. van Soest (Institute of Systematic Population Biology, Amsterdam University, The Netherlands). A voucher specimen (ZMAPOR19761) is kept in the Zoological Museum of the University of Amsterdam, The Netherlands.

Purification of Compounds 1–3. A frozen sponge sample (wet wt., 0.9 kg) of *Hyrtios erectus* was extracted at room temperature in MeOH until exhaustion. The combined extracts were concentrated under reduced pressure to obtain the organic crude extract (85 g). The total crude extract was subjected to silica gel column using VLC (vacuum liquid chromatography) gradient elution (*n*-hexane-$CHCl_3$-MeOH) to afford fractions 1–9. Fraction 6 (3.55 g) (MeOH-$CHCl_3$, 1:3) was chromatographed on silica gel column with $CHCl_3$-EtOAc-MeOH gradient elution to give 12 sub-fractions. Fraction 6-4 (134 mg) was fractionated over a sephadex LH-20 column chromatography (CH_2Cl_2/MeOH 1:1) to afford 8 sub-fractions. Fraction 6-4-5 (11 mg) was then finally purified by HPLC (ODS XDP-Zorbax column, 5 μm, 250 × 4.6 mm, 75% MeCN/H_2O, 1.5 mL/min flow rate and 220 nm UV detection) to yield compounds **1** (3.3 mg) and **2** (1.8 mg). Fraction 6-5 (108 mg) was fractionated over a sephadex LH-20 column chromatography (CH_2Cl_2/MeOH 1:1) to afford 9 sub-fractions. Fraction 6-4-6 (15 mg) was then finally purified by HPLC (ODS XDP-Zorbax column, 5 μm, 250 × 4.6 mm, 75% MeCN /H_2O, 1.5 mL/min flow rate and 220 nm UV detection) to yield compounds **3** (10.1 mg).

Compound (1): Yellow amorphous solid (3.3 mg); $[\alpha]_D^{25}$ 0.0 (*c* 0.1, MeOH); UV (λ_{max}, MeOH) (log ε): 226 (4.3), 231 (4.6), 285 (2.5) nm; NMR data: see Table 1; ESI-MS: *m/z* 322.1 $[M + H]^+$. HRESIMS: *m/z* 322.0194 (calculated for $C_{13}H_{12}N_3O_2Br$ $[M + H]^+$, 322.0191).

Evaluation of Antiproliferative Activity. Colorectal adenocarcinoma cells (HCT-116), human breast adenocarcinoma cells (MCF-7) and human hepatocellular carcinoma cells (HepG2) were obtained from the VACSERA (Giza, Egypt). HCT-116 cells were maintained in RPMI-1640 media while HepG2 and MCF-7

cells were maintained in DMEM media. All media were supplemented with 10% heat-inactivated fetal bovine serum 100 μg/mL, 100 units/mL and penicillin streptomycin. Cells were propagated in a humidified incubator with 5% (*v/v*) CO_2 atmosphere at 37 °C. The antiproliferative activity of the compounds **1**–**3** on colorectal carcinoma (HCT-116), breast adenocarcinoma (MCF-7) and hepatocellular carcinoma cells (HepG2) were evaluated using the sulforhodamine B (SRB) assay as previously described.[37,38]

Evaluation of Antibacterial Activity. The *in vitro* antibacterial activity testing was carried out using the standard disk diffusion assay[39] against pathogenic bacteria (*Staphylococcus aureus* strain ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* strain ATCC 25922 and *Pseudomonas aeruginosa* strain ATCC 27853). Sterile filter disks, each 6 mm in diameter, were saturated with compound **1** (at concentration of 3 mg/mL) and placed on agar plates that had been inoculated with the test pathogen. After 24 h incubation at 37 °C, the antibacterial activity was evaluated as diameter of the inhibition zone.

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