Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/etap

Cytotoxic effects of three new metabolites from Red Sea marine sponge, *Petrosia* sp.

Ahmed Abdel-Lateff[a,f], *Walied M. Alarif*[b,*], *Hany Z. Asfour*[c], *Seif-Eldin N. Ayyad*[d,g], *Alaa Khedr*[c], *Farid. A. Badria*[h], *Sultan S. Al-lihaibi*[b]

[a] *Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, PO Box 80260, Jeddah 21589, Saudi Arabia*
[b] *Department of Marine Chemistry, Faculty of Marine Sciences, King Abdulaziz University, PO. Box 80207, Jeddah 21589, Saudi Arabia*
[c] *Department of Pharmaceutical Chemistry and Phytochemistry, Faculty of Pharmacy, King Abdulaziz University, PO Box 80260, Jeddah 21589, Saudi Arabia*
[d] *Department of Chemistry, Faculty of Science, King Abdulaziz University, PO. Box 80203, Jeddah 21589, Saudi Arabia*
[f] *Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia 61519, Egypt*
[g] *Department of Chemistry, Faculty of Science, Dammietta University, New Dammietta, Egypt*
[h] *Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt*

ARTICLE INFO

Article history:
Received 28 October 2013
Received in revised form
2 March 2014
Accepted 5 March 2014
Available online 15 March 2014

Keywords:
Sponges
Sphingolipids
Sterols
Cytotoxicity
HepG2
MFC-7

ABSTRACT

Marine sponges represent an affluent source of biogenetically unprecedented array of biologically active compounds. This study revealed the isolation of ten compounds from marine sponge of *Petrosia* sp. Their chemical structures were determined by using 1D and 2D NMR, UV, IR and MS measurements. A polyoxygenated steroid (3β,7β,9α-trihydroxycholest-5-en (**1**), a purine-derivative (3,7-dimethyl-2-(methylamino)-3H-purin-6(7H)-one (**2**) and a sphingolipid (N-((3S,E)-1,3-dihydroxytetracos-4-en-2-yl)stearamide (**3**) proved to be new compounds. Meanwhile, seven known compounds; (**4**–**10**) were also identified. The cytotoxicity of the total extract and the isolated compounds were subjected to cytotoxicity evaluation employing two cancer cell lines; HepG2 and MCF-7. All tested compounds exhibited cytotoxic effect on both cancer cell lines with IC_{50} in range of 20-500 μM. The proposed mechanism of cytotoxic activities was examined through its molecular affinity to the DNA. Compound **5** showed the highest affinity to the DNA with IC_{50} 30 μg/mL.

* *Corresponding author*. Tel.: +966 0560352034.
E-mail address: walied1737@yahoo.com (W.M. Alarif).
http://dx.doi.org/10.1016/j.etap.2014.03.005
1382-6689/

1. Introduction

Genus *Petrosia* (Demospongiae, Petrosiidae) is a tropical marine sponge, was recognized for its veritable cornucopia metabolites; steroids, alkaloids, polyketides, terpenoids and polyacetylenes (Kim et al., 1998; Lim et al., 2001; Shen et al., 2004; Hoshino et al., 2003; Nukoolkarn et al., 2008; Giner et al., 1999; Cimino et al., 1989). These compounds possessed interesting pharmacological effects as anti-fungal, immunosuppressant, anti-inflammatory, anti-neoplastic, and cytotoxic compounds (Chelossi et al., 2004). For instance; polyacetylenetriol was isolated from mediterranean sponge *Petrosia* sp. and found to be a potent inhibitor of DNA polymerases (Loya et al., 2002). Moreover, 2-bromoamphimedine and petrosamine were isolated from the same genus and showed strong acetylcholinesterase inhibitory activities approximately six times higher than that of the reference galanthamine (Nukoolkarn et al., 2008).

The main objective of the current study is the isolation of bioactive metabolites from marine sponge, particularly, *Petrosia* sp. Extensive fractionation on NP-Silica led to the isolation of ten metabolites (**1–10**), (Fig. 1).

2. Experimental

2.1. General

Optical rotations were measured on ATAGO POLAX-L 2 polarimeter. GC-MS analyses were carried out using RTX-1 column (30 m, 0.25 mm). 1D and 2D NMR spectra were recorded on Bruker AVANCE III WM 600 MHz spectrometers and ^{13}C NMR at 150 MHz. Chemical shifts are given in δc (ppm) relative to TMS as internal standard. Thin layer chromatography was performed on silica gel (Kieselgel 60, F_{254}) of 0.25 mm layer thickness. Gel filtration was carried out using Sephadex LH-20. Spots were detected by using 50% ethanol/sulfuric acid as spray reagent.

2.2. Animal material

Marine sponge *Petrosia* sp. (Demospongiae, Petrosiidae) was collected from the North of Jeddah Saudi Arabia Red Sea coast (21°29′31″N 39°11′24″E), at a depth of 1–2 m, in June 2013. After collection of the fresh material of the sponge, it was squeezed by hand, then weighed and extracted with organic solvent. A voucher specimen (IP-2010-1) was deposited in the faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. *Petrosia* sp. was red–purple to brown in color. It has a compact, hard texture, with spherical oscula irregularly spread over the surface. It is similar to some extent to *Petrosia ficiformis*.

2.3. Extraction and isolation

The fresh *Petrosia* sp. (4.0 kg) was extracted with a mixture of $CHCl_3$:MeOH (1:1) (22 °C, 3 × 10 L) and yielded viscous brown extracts (6 g). The total extract was fractionated on NP-Silica ($\Phi = 50$, $L = 100$ cm, 100 mL each) employing gradient elution from *n*-hexane to EtOAc, and washed with methanol, 60 fractions were collected. The fractions were monitored by TLC pattern using UV_{254} lamp and 50%-sulfuric acid in methanol as spraying reagent. The fractions were combined into 6 pools (P001–P006). P001 (*n*-hexane:ether, 9:1) was further purified by fractionation on NP-silica gel ($\Phi = 30$, $L = 100$ cm, 10 mL each) employing flash column with fixed solvent system *n*-hexane: diethyl ether (9.5:0.5), 50 fractions, 20 mL each, were collected. The fractions (29 and 30) were promising according to the TLC pattern, were purified by employing PTLC to yield **5** (6 mg). P002 was fractionated on NP-silica using *n*-hexane:diethylether (7:3), was re-purified by PTLC, employing the same solvent system to give **8** (2 mg), **9** (2 mg) and **7** (7 mg) as polarity increased. P003 (*n*-hexane:ether 5:5) was re-fractionated on VLC-NP-silica using *n*-hexane:diethyl ether (5:5), 30 fractions were collected, 5 ml each, yielding **2**, **10** and **4** which were purified by PTLC employing the same solvent system giving 5, 8 and 1 mg, respectively. P005 (*n*-hexane:ethylacetate, 7:3) was fractionated on PTLC-NP silica using $CHCl_3$:MeOH:acetic acid (9:1:0.5), yielding **6** (1 mg), **3** (4 mg)and **1** (3 mg). Much effort had been paid to purify P004 and P006 but unfortunately, no isolates were obtained. Finally, all compounds were purified by employing Sephadex LH 20, using methanol as eluent.

2.3.1. 3β,7β,9α-Trihydroxycholest-5-en(1)

Amorphous powder, mp 221–223 °C (3 mg); $R_f = 0.15$, Benzene:ethyl acetate:MeOH (5:5:0.5), yellowish brown spot developed upon spraying with 50%-sulfuric acid in ethanol); IR υ_{max} (film) cm^{-1}: 3421, 1665, 2971, 2876; ESIMS *m/z* (rel. int.): 418 $[M]^+$ ($C_{27}H_{46}O_3$), 400 $[M–H_2O]^+$, 367 $[M–2H_2O–CH_3]^+$, 349 $[M–3H_2O–CH_3]^+$, 287 $[M–H_2O–C_8H_{17}]^+$, 269$[M–2H_2O–C_8H_{17}]^+$, 251 $[M–3H_2O–C_8H_{17}]^+$, 113 $[C_8H_{17}]$. HRESI-MS *m/z*: 418.3435 $[M]^+$ (calculated for $C_{27}H_{46}O_3$ 418.3447); ^1H NMR and ^{13}C NMR ($CDCl_3$) (Table 1).

2.3.2. 3,7-dimethyl-2-(methylamino)-3H-purin-6(7H)-one (2)

Amorphous powder, (5 mg); IR υ_{max} (film) cm^{-1}: 3120, 2876; ESIMS *m/z* (rel. int.): 194 $[(M+H]^+$, 163 $[M–CH_4N]^+$, 137 $[M–C_2H_4N_2]^+$, 108, 93. HRESIMS (pos.) *m/z*: 180.0880 $[(M+H)–CH_2]$(calculated for $C_7H_{10}N_5O$ 180.0885); ^1H NMR ($CDCl_3$, 600 MHz), δ (ppm): 3.41 (s, CH_3-2), 3.59 (s, CH_3-3), 3.99 (s, CH3-9), 7.51 (s, 1H-8); ^{13}C NMR ($CDCl_3$, 150 MHz), δ (ppm) 107.7 (C-5), 148.7(C-4), 151.8 (C-2), 141.4(C-8), 155.5 (C-6).

2.3.3. N-((3S,E)-1,3-dihydroxytetracos-4-en-2-yl)stearamide(3)

Yellowish brown viscous material (4 mg); IR υ_{max} (film) cm^{-1}: 3340, 3320 and 1640 cm^{-1}; HRESI-MS *m/z*: 649.6368 $[M]^+$(Calculated for $C_{42}H_{83}NO_3$ 649.6373); ^1H NMR and ^{13}C NMR ($CDCl_3$), (Table 1).

2.3.4. Dehydroepiandrosterone(4)

^1H NMR ($CDCl_3$), δ (ppm): 1.04 (s, 3H, CH_3-19), 0.98 (s, 3H, CH_3-18), 5.39 (brs, H-6), 3.55 (dddd, 10.8, 10.8, 6.6 and 4.8 Hz, 1H, H_α-3); ^{13}C NMR ($CDCl_3$), δ (ppm): 37.2 (C-1), 31.4 (C-2), 71.6 (C-3), 42.2 (C-4), 141.0 (C-5), 120.9 (C-6), 30.9 (C-7), 31.5 (C-8), 50.2 (C-9), 36.6 (C-10), 20.3 (C-11), 31.6 (C-12), 47.6 (C-13), 51.8 (C-14), 21.9 (C-15), 35.9 (C-16), 221.3 (C-17), 13.0 (C-18), 19.4 (C-19).