Cytotoxic Isoprenoids from the Red Sea Soft Corals *Sarcophyton glaucum* and *Xenia umbellata*

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Abstract. Three cytotoxic cembranoids, *sarcophytolol* (1), *sarcophytolide B* (2) and *sarcophytolide C* (3) were identified from the Et₂O-soluble fraction derived from the crude extract of the soft coral *Sarcophyton glaucum*, along with three known aromadendrane-type sesquiterpenoids, *aromadendrene* (4), *palustrol* (5) and *viridiflorol* (6) were isolated from *Xenia umbellata* collected from the Red Sea water, Jeddah. Chemical structures were determined by analyzing their NMR and MS data. Compounds 1-6 were evaluated for potential cytotoxic activities, employing five cancer panels; HepG2, MCF-7, A549, PC-3 and VERO. Interestingly, compounds 1 and 3 exhibited selective cytotoxic effects on HepG2 and showed no toxicity to the other cell lines. However, 2 showed selective cytotoxic effect on MCF-7 with no toxicity on other cell lines. Meanwhile compounds 4-6 showed cytotoxic activity against both MCF-7 and HepG2 with no toxicity on normal VERO cells.

Keywords: Red Sea, soft coral, Alcyoniidae, terpenoids, cytotoxicity

1. Introduction

Cancer is one of the major causes of death, with approximately 12.7 million cancer cases, 60% of them died in 2008 alone ^[1]. This makes cancer drug discovery more urgent and deep needs ^[2]. Natural products

remain important despite the current trend toward rational drug designs^[3]. Water is covering two-thirds of the earth's surface and home to nearly one million multicellular (plants and animals) and one billion unicellular (Distributed under 100 different phyla) organisms^[4]. Living in harsh environment makes the marine organisms struggling to survive, which is accruing through chemical defense mechanism. Thus, marine organisms represent a generator for natural products with diversity of chemical structures and biological activities^[5]. The Red Sea represents the tropical and subtropical climatic region with a variety of the endemic biota. Up-to-date *ca.* 180 soft corals species were identified world-wide. The majority of them are native to the Red Sea^[5]. Soft corals are known for the production of terpenoidal metabolites especially cembrane diterpenes and sesquiterpenes^[6]. The course of our research program aims at the discovery of bioactive and/or new metabolites from marine organisms. We investigated the constituents of the shallow water Red Sea soft corals *Xenia umbellata* and *Sarcophyton galucum* (Family Alcyoniidae, Order Alcyonacea, subclass Octocorallia, class Anthozoa, phylum coelenterata). We have isolated six metabolites (Fig. 1) and their cytotoxicity against five cancer panels; HepG2, MCF-7, A549, PC-3 and VERO were studied.

2. Materials and Methods

2.1 General experimental procedure

Optical rotations were measured on ATAGO POLAX-L 2 polarimeter. EI/MS analyses were carried out on a Shimadzu-QP 2010. GC/MS analyses were carried out using RTX-1 column (30 m, 0.25mm). The 1D and 2D NMR spectra were recorded on Bruker AVANCE III WM 600 MHz spectrometers and ¹³C NMR at 150 MHz. Chemical shifts are given in δ_c (ppm) relative to TMS as internal standard.

Fig. 1: Compounds isolated from *Sarcophyton glaucum* (1-3) and *Xenia umbellata* (4-6)

Chemical shifts are given in ppm relative to TMS as internal standard. Thin layer chromatography was performed on silica gel (Kieselgel 60, F254) of 0.25 mm layer thickness. Gel filtration was carried out using Sephadex LH-20. Spots were detected by using ethanol /sulfuric acid as spray reagent.

2.2 Animal materials

Soft coral *S. glaucum* (Order Alcyonacea, Family Alcyoniidae) was collected from the Red Sea coast (21°29'31"N 39°11'24"E) in Jeddah, at a depth of 5 m, in May, 2012. Collection of this material was immediately subjected to the extraction. A voucher specimen (SC-2012-8) was deposited in the Faculty of Marine Science, King Abdulaziz University, Jeddah, Saudi Arabia.

The marine invertebrate *X. umbellata* (Alcyonaceae, Xeniidae) had been collected by Snorkeling in the winter on 21 November 2012 along the cost of Red sea in Saudi Arabia, Jeddah (in protected reef slopes - Obhor) at a depth of 3-5 m. A voucher specimen (Sx-2012-11) was

deposited in the Faculty of Marine Science, King Abdulaziz University, Jeddah, Saudi Arabia. The specimens were immediately

frozen until dryness and then extracted.

2.3 Extraction and isolation

Sarcophyton gaucum

The fresh Soft coral *S. glaucum* (3.0 kg) was minced and extracted by diethyl ether at room temperature (22 °C, 3 X 5 Liters). The yielded viscous blackish residue (20 g), was fractionated on NP-Silica employing gradient elution from *n*-hexane to EtOAc, followed by dichloromethane to acetone (Φ = 50, L= 100 cm, 100 mL each). Twenty fractions were collected. The fractions were investigated by ¹H-NMR and TLC pattern using UV₂₅₄ lamp and/or 50%-sulfuric acid in methanol as spraying reagent. All compounds were purified by PTLC and re-purified by employing Sephadex LH-20 (Φ = 5, L= 10 cm, 5 mL each) using a mixture of MeOH: CHCl₃ (9:1). Fraction 5 was purified by RP-18 HPLC (MeOH/H₂O, 85:15) to afford yield **1** (8.0 mg) and **2** (9.0 mg). Fraction 8 was purified by RP-18 HPLC (MeOH/H₂O, 65:35) to yield **3** (10 mg).

Sarcophytolol, (1)

Colorless oil (8.0 mg, 0.0084%); $[\alpha]_D^{22} + 10.0$ (c 0.02, CHCl₃); IR $\lambda_{\max}$ (film) cm⁻¹: 3713, 3423, 2937, 1445, 1378, 1221, 1045; ¹³C NMR (CDCl₃, 150 MHz) and ¹H NMR (CDCl₃, 600 MHz) spectroscopic data (Table 1); HRESI-MS (positive mode) $m/z = 307.2632$ [M+H]⁺ (Calculated $m/z = 306.2559$ for C₂₀H₃₄O₂).

Sarcophytolide B, (2)

Colorless oil (10.0 mg); $[\alpha]_D^{22} - 90.0$ (c 0.02, CHCl₃); IR $\lambda_{\max}$ (film) cm⁻¹: 3472, 2925, 1748, 1454, 1392, 1090, 771; ¹³C NMR (CDCl₃, 150 MHz) and ¹H NMR (CDCl₃, 600 MHz) spectroscopic data, (Table 1); HRESI-MS (Positive-ion-mode) m/z 317.2118 [M+H]⁺ (Calculated $m/z = 316.2380$ for C₂₀H₂₈O₃).

Sarcophytolide C, (3)

Colorless oil (9.0 mg); $[\alpha]_D^{22} - 60.0$ (c 0.015, CHCl₃); IR $\lambda_{\max}$ (film) cm⁻¹: 3472, 2925, 1748, 1450, 1388, 1086, 763; ¹³C NMR (CDCl₃, 150 MHz) and ¹H NMR (CDCl₃, 600 MHz) spectroscopic data (Table 1);

HRESI-MS (Positive-ion-mode) m/z 317.2118 $[M+H]^+$ (Calculated m/z = 316.2380 for $C_{20}H_{28}O_3$).

Xenia umbellata

The residue (14 g) was homogenized with suitable amount of silica gel and then subjected to Si-gel open column (500 g, 75×2 cm) for separation. *n*-hexane was used as eluent and the polarity was increased by adding increasing amounts of EtOAc. Fractions were collected (50 ml). The course of fractionation was traced using TLC technique with the aid of both UV light and spray reagent. For impure fractions preparative TLC (PTLC) was applied to isolate a single compound.

Aromadendrene, (4)

The fraction eluted with *n*-hexane, R_f = 0.98 (16.1 mg) was re-purified by PTLC. The violet color band (with spray reagent) R_f = 0.90 gave pale yellow oil (9.3 mg). IR $\nu_{\max}$ (neat) cm^{-1} : 3080, 1635, 1460, 1377, and 895; GC-MS m/z (rel. int.): 204 (M^+), m/z 41 (base peak). ^1H NMR (CDCl_3 , 600 MHz): δ = 0.24 (1H, dd, J = 10.9, 9.3 Hz, H-6), 0.55 (1H, ddd, J = 10.9, 9.3, 5.9 Hz, H-7), 0.94 (3H, d, J = 6.7 Hz, H₃-4), 0.96 (3H, s, H₃-12), 1.01 (3H, s, H₃-13), 4.74 (1H, s, H_a-14), 4.71 (1H, s, H_b-14); ^{13}C NMR (CDCl_3 , 150 MHz): δ = 152.3 (s, C-10), 109.8 (CH₂, C-14), 50.8 (CH, C-1), 42.2 (CH, C-5), 37.9 (CH, C-4), 35.8 (CH₂, C-9), 31.3 (CH₂, C-3), 28.7 (CH₃, C-13), 28.3 (CH₂, C-2), 24.9 (CH, C-7), 23.6 (CH, C-6), 22.20 (CH₂, C-8), 17.2 (C, C-11), 16.4 (CH₃, C-15), 15.9 (q, C-12).

Palustrol, (5)

The fraction eluted by *n*-hexane/ ethylacetate (9:1), R_f = 0.80 (240.9 mg) was re-purified by PTLC using the solvent system *n*-hexane/ ethylacetate (8:2). The brown color band (with spray reagent) R_f = 0.74 gave colorless oil (124.5 mg). IR $\nu_{\max}$ (neat) cm^{-1} : 3326, 2954, 2868, 1471, 1375, 1264, 992, 940, 890; GCMS m/z (rel. int.): : 222 (M^+ , 2%), 204 (19), 189 (12), 165 (12), 161 (28), 147 (21), 122(75), 111(100), 109(43), 107(48). ^1H NMR (CDCl_3 , 600 MHz): δ = 1.84 (1H, m, H_a-2), 1.37 (1H, m, H_b-2), 1.82 (2H, m, H₂-3), 2.15 (1H, m, H-4), 1.60 (1H, dd, J = 10.2, 10.2 Hz, H-5), 0.51 (1H, dd, J = 10.8, 9 Hz, H-6), 0.68 (1H, ddd, J = 10.8, 9, 6.6 Hz, H-7), 1.88 (1H, m, H_a-8), 1.06 (1H, m, H_b-8), 1.47 (1H, m, H_a-9), 1.35 (1H, m, H_b-9), 1.27 (1H, m, H-10), 1.03 (3H, s, H₃-12), 1.06 (3H, s, H₃-13), 0.98 (3H, d, J = 6.6 Hz, H₃-14), 0.92 (3H, d,

$J = 6.6$ Hz, H₃-15); ^{13}C NMR (CDCl₃, 150 MHz): $\delta = 85.7$ (CH, C-1), 47.2 (CH, C-5), 46.1 (CH, C-10), 38.9 (CH₂, C-2), 35.0 (CH, C-4), 32.8 (CH₂, C-3), 32.3 (CH₂, C-9), 28.9 (CH₃, C-13), 27.1 (CH, C-7), 24.5 (CH₂, C-8), 22.0 (CH, C-6), 19.9 (C, C-11), 18.7 (CH₃, C-14), 17.7 (CH₃, C-15), 15.6 (CH₃, C-12).

Viridiflorol, (6)

The fraction eluted by *n*-hexane/ ethylacetate (9:1), $R_f = 0.49$ (240.9 mg) was re-purified by PTLC using the solvent system *n*-hexane/ ethylacetate (8:2). The brown color band (with spray reagent) $R_f = 0.56$ gave white crystalline material (22.7 mg). IR ν_{max} (neat) cm^{-1} : 3350, 2925, 2866, 1466, 1376, 1112, 990, 941, 887, 682; GCMS m/z (rel. int.): 204 [M^+] (7), 189 (6), 161 (19), 147 (14), 133 (10), 122 (44), 109 (53), 95 (30), 81 (36), 69 (44), 55 (28), 43 (100); ^1H NMR (CDCl₃, 600 MHz): $\delta = 0.33$ (dd, $J = 10.2, 9.0$ Hz, H-6), 0.73 (ddd, $J = 10.8, 9.0, 6$ Hz, H-7), 0.94 (3H, d, $J = 7.2$ Hz, H₃-15), 0.98 (3H, s, H₃-12), 1.04 (3H, s, H₃-13), 1.14 (3H, s, H₃-14); ^{13}C NMR (CDCl₃, 150 MHz): $\delta = 74.6$ (C, C-10), 53.8 (CH, C-1), 40.8 (CH₂, C-9), 39.2 (CH, C-5), 38.4 (CH, C-4), 30.8 (CH₂, C-3), 30.5 (CH₃, C-14), 28.7 (CH₂, C-8), 25.0 (CH₂, C-2), 24.6 (CH, C-6), 23.4 (CH₃, C-12), 20.3 (CH, C-7), 19.20 (CH, C-11), 16.0 (CH₃, C-13), 15.4 (CH₃, C-15).

2.4 Cell cytotoxicity assay

Cell culture:

HepG2, MCF-7, A549 and PC-3 cell lines were grown in RPMI-1640. VERO and A549 cells were grown in DMEM. All culture media were supplemented with 10% heat inactivated FBS, 100 units/ml of penicillin and 100 mg/mL of streptomycin and maintained at 37 °C in a humidified atmosphere containing 5% CO₂. The cells were maintained as “monolayer culture” by serial subculturing.

MTT Cytotoxicity assay:

The cytotoxic activity of the compounds was tested with five different mammalian cell lines HepG2, MCF-7, A549, PC-3 and VERO. The cell lines were obtained from American Type Culture Collection (ATCC) (Mosmann, 1983). The cells were cultivated at 37°C and 10% CO₂ in RPMI-1640 (Lonza, 12-702F) medium supplemented with 10% fetal bovine serum (FBS, Lonza, Cat. No.14-801E), 100 IU/ml penicillin

and 100 µg/ml streptomycin (Lonza, 17-602E). Inhibition of proliferation was measured in 96-well plate. 60 µL of serial dilutions of the test compounds were given to 120 µL of the suspended cells (50,000/mL) in wells of 96-well plates. After 5 days of growth each well was judged visually under the microscope (Mosman, 1983). The metabolic activity of the cells was measured by an MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium ·bromide) assay ERVA, Cat. No. 20395.01) was added to each well and incubated for 4 hours at 37°C and 10% CO₂. After that the medium was removed and formazan particles were dissolved by adding 100 µl Isopropanol/HCl solution. The plates were then incubated for 15 min on shaker (600 r.p.m/ min) to dissolve the formazan crystals. The intensity of the purple color was measured at λ 540 nm by microplate reader (ELx800 Absorbance Microplate Reader, BioTek). The cytotoxicity was recorded at the concentrations that gave 50 % growth inhibition (IC₅₀).

3. Results and Discussion

Compound **1** was isolated as a faint yellow oil (8.0 mg) with $[\alpha]_D^{22} + 10.0$ (*c* 0.02, CHCl₃). The structure elucidation commenced when the molecular formula of **1**, C₂₀H₃₄O₂ was established by LCESI-MS (negative mode) *m/z* = 305.2 [M-H]⁺, 287.1 [M-H₂O]⁺, 269.1 [M-2H₂O]⁺. This result was validated by HRESI-MS (positive mode) *m/z* = 307.2632 [M+H]⁺. The ¹³C (¹H decoupled) and DEPT NMR spectra of **1**, showed 20 resonances attributable to five methyl, six methylenes, six methines and three quaternary carbons (Table 1).

Two of the four elements of unsaturation, as indicated by the molecular formula of **1**, are attributed to two C=C double bonds (Table 1); thus, the molecule is bicyclic. As the ¹H and ¹³C NMR data enabled all but one of the hydrogen atoms within **1** to be accounted for, it was evident that the remaining proton was present as part of a hydroxyl. function, a deduction supported by the OH stretching in IR spectral data (λ_{max} 3423 cm⁻¹). After association of all protons with directly bonded carbons *via* HSQC spectral measurement, it was possible to deduce the planar structure of **1** by interpretation of the ¹H-¹H COESY and ¹H-¹³C HMBC spectra.

Table 1. ^1H [CDCl_3 , 600 MHz] and ^{13}C NMR [CDCl_3 , 150 MHz] NMR spectral data of **1-3**^a

1			2		3	
	$\delta^{13}\text{C}^b$	$\delta^1\text{H}^c$	$\delta^{13}\text{C}$	$\delta^1\text{H}$	$\delta^{13}\text{C}^b$	$\delta^1\text{H}$
1	46.6 (d)	1.32 (1H, m)	162.2 (s)	-	162.9 (s)	-
2	71.1 (d)	4.53 (1H, dd, 10.2, 5.4)	37.4 (t)	2.40 (2H, ddd, 10.8, 4.2, 3.0)	36.9 (t)	2.40 (2H, ddd, 10.8, 4.2, 3.0)
3	125.4 (d)	5.27 (1H, d, 10.2)	25.2 (t)	1.90 (1H, m) 1.70 (1H, m)	25.8 (t)	1.90, m 1.70, m
4	138.6 (s)	-	61.4 (d)	2.68 (1H, dd, 4.8, 4.2)	61.8 (d)	2.66 (1H, dd, 6.6, 6.0)
5	39.9 (t)	2.19 (1H, m) 1.97 (1H, m)	59.9 (s)	-	60.2 (s)	-
6	25.2 (t)	2.32 (1H, m) 2.11 (1H, m)	39.0 (t)	1.10 (2H, m)	38.4 (t)	1.10 (2H, m)
7	124.1 (d)	5.00 (1H, dd, 10.2, 5.4)	23.3 (t)	2.13 (2H, m)	23.0 (d)	2.13 (2H, m)
8	136.0 (s)	-	124.9 (d)	5.17 (1H, dd, 9.6, 5.4)	124.6 (d)	5.11 (1H, dd, 8.4, 7.8)
9	33.7 (t)	2.40 (1H, m) 1.29 (1H, m)	144.0 (s)	-	145. (s)	-
10	18.7 (t)	1.77 (1H, m) 1.29 (1H, m)	36.4 (t)	2.01 (1H, ddd, 13.2, 10.8, 2.4) 2.20 (1H, ddd, 18.0, 12.6, 9.0)	35.1 (t)	2.05 (1H, m) 2.20 (1H, m)
11	31.9 (t)	1.77 (1H, m) 1.28 (1H, m)	27.6 (t)	2.10 (1H, ddd, 13.2, 5.4, 2.4) 2.78, (1H, ddd, 18.0, 9.6, 7.8)	26.8 (t)	2.10 (1H, m) 2.69 (1H, m)
12	35.2 (t)	2.13 (1H, m) 2.00 (1H, m)	135.6 (s)	-	134.5 (s)	-
13	75.0 (s)	-	121.0 (d)	5.05 (1H, dd, 10.2, 1.2)	123.1 (s)	4.99 (1H, dd, 9.6, 1.2)
14	71.9 (d)	3.88 (1H, d, 9.6)	78.8 (d)	5.57 (1H, dd, 10.2, 1.2)	79.9 (d)	5.45 (1H, dd, 9.6, 1.2)
15	29.0 (d)	1.18 (1H, m)	120.6 (s)	-	119.1 (s)	-
16	20.8 (q)	0.88 (3H, d, 6.6)	174.0 (s)	-	175.0 (s)	-
17	20.3 (q)	0.73 (3H, 6.6)	15.4 (q)	1.65 (3H, s)	15.5 (q)	1.60 (3H, s)
18	24.2 (q)	1.65 (3H, s)	17.1 (q)	1.28 (3H, s)	18.1 (q)	1.27 (3H, s)
19	17.3 (q)	1.61 (3H, s)	16.1 (q)	1.91 (3H, s)	16.7 (q)	1.93 (3H, s)
20	15.1 (q)	1.02 (3H, s)	8.92	1.88 (3H, s)	9.2 (q)	1.86 (3H, s)

1			2		3	
	$\delta^{13}\text{C}^b$	$\delta^1\text{H}^c$	$\delta^{13}\text{C}$	$\delta^1\text{H}$	$\delta^{13}\text{C}^b$	$\delta^1\text{H}$
1	46.6 (d)	1.32 (1H, m)	162.2 (s)	-	162.9 (s)	-
2	71.1 (d)	4.53 (1H, dd, 10.2, 5.4)	37.4 (t)	2.40 (2H, ddd, 10.8, 4.2, 3.0)	36.9 (t)	2.40 (2H, ddd, 10.8, 4.2, 3.0)
3	125.4 (d)	5.27 (1H, d, 10.2)	25.2 (t)	1.90 (1H, m) 1.70 (1H, m)	25.8 (t)	1.90, m 1.70, m
4	138.6 (s)	-	61.4 (d)	2.68 (1H, dd, 4.8, 4.2)	61.8 (d)	2.66 (1H, dd, 6.6, 6.0)
5	39.9 (t)	2.19 (1H, m) 1.97 (1H, m)	59.9 (s)	-	60.2 (s)	-
6	25.2 (t)	2.32 (1H, m) 2.11 (1H, m)	39.0 (t)	1.10 (2H, m)	38.4 (t)	1.10 (2H, m)
7	124.1 (d)	5.00 (1H, dd, 10.2, 5.4)	23.3 (t)	2.13 (2H, m)	23.0(d)	2.13(2H, m)
8	136.0 (s)	-	124.9 (d)	5.17 (1H, dd, 9.6, 5.4)	124.6 (d)	5.11 (1H, dd, 8.4, 7.8)
9	33.7 (t)	2.40 (1H, m) 1.29 (1H, m)	144.0 (s)	-	145.(s)	-
10	18.7 (t)	1.77 (1H, m) 1.29(1H, m)	36.4 (t)	2.01 (1H, ddd, 13.2, 10.8, 2.4) 2.20 (1H, ddd, 18.0, 12.6, 9.0)	35.1 (t)	2.05 (1H, m) 2.20 (1H, m)
11	31.9 (t)	1.77(1H, m) 1.28(1H, m)	27.6 (t)	2.10 (1H, ddd, 13.2, 5.4, 2.4) 2.78,(1H, ddd, 18.0, 9.6, 7.8)	26.8 (t)	2.10 (1H, m) 2.69 (1H, m)
12	35.2 (t)	2.13(1H, m) 2.00(1H, m)	135.6 (s)	-	134.5 (s)	-
13	75.0 (s)	-	121.0 (d)	5.05 (1H, dd, 10.2, 1.2)	123.1(s)	4.99 (1H, dd, 9.6, 1.2)
14	71.9 (d)	3.88 (1H, d, 9.6)	78.8 (d)	5.57 (1H, dd, 10.2, 1.2)	79.9 (d)	5.45 (1H, dd, 9.6, 1.2)
15	29.0 (d)	1.18 (1H, m)	120.6 (s)	-	119.1 (s)	-
16	20.8 (q)	0.88 (3H, d, 6.6)	174.0 (s)	-	175.0 (s)	-
17	20.3 (q)	0.73 (3H, 6.6)	15.4 (q)	1.65 (3H, s)	15.5 (q)	1.60 (3H, s)
18	24.2 (q)	1.65 (3H, s)	17.1 (q)	1.28 (3H, s)	18.1 (q)	1.27 (3H, s)
19	17.3 (q)	1.61 (3H, s)	16.1 (q)	1.91 (3H, s)	16.7 (q)	1.93 (3H, s)
20	15.1 (q)	1.02 (3H, s)	8.92 (s)	1.88 (3H, s)	9.2 (q)	1.86 (3H, s)

^a All assignments are based on 1D and 2D measurements (HMBC, HSQC, COESY). ^b Implied multiplicities were determined by DEPT (C = s, CH = d, CH₂ = t). ^c J in Hz.

From the ^1H - ^1H COESY spectrum of **1**, the coupling between H₃-16 resonating at δ_{H} 0.88 (d, $J = 6.6$) and H-15 at δ_{H} 1.18 (m), as well as this signal and the H₃-17 at δ_{H} 0.73 (d, 6.6) indicated the presence of isopropyl moiety. The presence of this moiety and its connection to C-1 were established by long-range C-H correlations observed between the resonances of H₃-16 and those of C-1 (δ_{C} 46.6), C-15 (δ_{C} 29.0), and C-17 (δ_{C} 20.3), between H₃-17 and C-1, C-15, and C-16 (δ_{C} 20.8). The existence of a furan ring in which C-1 is part, was concluded by studying the correlations observed between H-1 resonating at δ_{H} (1.32, m) and both H-2 at δ_{H} 4.53 (dd, 10.2, 5.4) and H-14 at δ_{H} 3.88 (d, 9.6), in addition to the long-range correlations between H-1 with C-2 (δ_{C} 71.1), C-3 (δ_{C} 125.4), C-13 (δ_{C} 75.0), C-14 (δ_{C} 71.9), C-15 (δ_{C} 29.0), C-16 (δ_{C} 20.8) and C-17 (δ_{C} 20.3). Furthermore, HMBC correlation between H-2 and C-1 (δ_{C} 46.6), C-2 (δ_{C} 71.1), C-13 (δ_{C} 75.0) and C-14 (δ_{C} 71.9); also correlation between H₃-20 (δ_{H} 1.02, s) and C-13 and C-14 indicated the connection between C-13 and C-14 and positioning of CH₃-20 on C-13, thus the five membered ring was closed by ether link between C-2 and C-13. On these bases the positioning of the isopropyl group to be attached to C-1 and then the furan ring was established. The presence of a furan ring, in addition to two carbon-carbon double bonds together with the absence of any other unsaturation imply the existence of one more ring. The nature of the second ring was proved to be 14-membered ring based on the following: a) ^{13}C NMR signals δ_{C} 125.4 (C-3), 138.6 (C-4), 124.1 (C-7) and 136.0 (C-8) indicated the presence of two trisubstituted double bonds without conjugation, a deduction supported by absence of characteristic absorption in UV spectrum, b) ^1H - ^1H COESY spectral data indicated the possibility to establish three partial structures of consecutive proton systems extending from H₂-5 (δ_{H} 1.97, m and 2.19, m) to H₂-7 (δ_{H} 5.00, dd, 10.2, 5.4) through H₂-6 (δ_{H} 2.11, m and 2.32, m); from H₂-9 (δ_{H} 2.4, m and 1.29, m) to H₂-12 (δ_{H} 2.13, m, and 2.00, m) through H₂-10 (δ_{H} 1.29, m and 1.77, m) and H₂-11 (δ_{H} 1.28, m and 1.77, m), and finally from H-1 to H-3 (δ_{H} 5.27, d, 10.2) through H-2, c) the connectivities of these partial structures were further established by interpretation of the HMBC correlations (Fig. 2) between the carbons of the three parts as observed from H₃-16 to C-15, C-17, C-1, C-2, C-3, C-4; from H₃-18 (δ_{H} 1.56, s) to C-3, C-4, C-5 (δ_{C} 39.9), C-6 (δ_{C} 25.2), C-7, and C-8; from H₃-19 (δ_{H} 1.61, s) to C-8 (δ_{C} 136.0), C-9 (δ_{C} 33.7), C-10 (δ_{C} 18.7), C-11 (δ_{C} 31.9), C-12 (δ_{C} 35.2), C-13 (δ_{C} 75.0), and C-14 (δ_{C} 71.9). The relative configuration of **1** assigned by 2D

NOESY spectral data, which indicated the presence of cross peaks between the vinylic CH₃-18 and H₂-6 (δ_{H} 2.32, m and 2.11, m) suggested the *E* geometry for the C-3/C-4 double bond, which was also identified by the chemical shift of H-3 at δ_{H} 5.25 (d, 10.2). The geometry of the trisubstituted olefin at C-3/C-4 is *E* oriented owing to the values of chemical shifts of allylic methylene $\delta_{\text{C}} > 30$ and the *J* values. Cross peaks were assigned between the vinylic CH₃-19 (δ_{H} 1.61, s) and H₂-6 (δ_{H} 1.62, m and 1.42, m, ppm) suggested the *E* geometry for the C-7/C-8 double bond, which was also identified by the chemical shift of H-7 at δ_{H} 5.00 (d, 10.2, 5.4). The geometry of the trisubstituted olefin at C-7/C-8 was assigned as *E* based on the higher field chemical shift of the olefinic methyl signal for C-19 (δ_{C} 17.3). Furthermore, the NOE correlations between H-2/H-1, Me-18/H-1 (δ_{H} 1.34), Me-19/H₂-10, Me-20/H-3, Me-18/ Me-16, and Me-18/H₂-12, demonstrated the 2*S**, 7*S**, and 8*S** configurations as depicted in Figure 2. A careful analysis of all the NMR spectroscopic data (COESY, HSQC, HMBC, and NOESY) confirmed that (**1**) is sarcophytol^[7]

Fig. 2: selected 1H-1H COESY (—) and HMBC (→) correlations of compounds **1** and **2**

Compound **2** was isolated as faint yellowish oil (10.0 mg) with $[\alpha]_{\text{D}}^{22} - 90.0$ (c 0.02, CHCl₃). The LCESI-MS (Negative-ion-mode) of **2**, exhibited m/z 315.18 $[\text{M}-\text{H}]^+$, consistent with molecular formula C₂₀H₂₈O₃ (Seven degrees of unsaturation) and validated by HRESI-MS (Positive-ion-mode) m/z 317.2118 $[\text{M}+\text{H}]^+$. The IR spectrum indicated the presence of an α , β -unsaturated- γ -lactone (1750 cm⁻¹), an olefin (1669 cm⁻¹), and an epoxide (1256 cm⁻¹) functions. ¹³C (¹H decoupled) and DEPT NMR spectra (Table 1) exhibited 20 carbon signals establishing: four methyls, six methylenes, four methines (two are oxygenated), six quaternary carbons, one of which is a carbonyl carbon. Careful examination of the ¹³C NMR data revealed clear evidences for the presence of the following figures: a) trisubstituted epoxy ring δ_{C} 61.4 (C-4, d) and 59.9 (C-5, s) assigned for C-4 and C-5, respectively, b) an α ,

β -unsaturated- γ -lactone based on characteristic signals resonating at δ_C 174.0 (C-16, s), 120.6 (C-15, s) and 162.2 (C-1, s) supported by IR absorption at $\lambda_{\max}$ 1750 cm^{-1} , c) two trisubstituted C=C bonds resonating at δ_C 124.9 (C-8, d), 144.0 (C-9, s), 135.6 (C-12, s) and 121.0 (C-13, d) and d) an oxymethine carbon which appeared at δ_C 78.8 (C-14). The ^1H NMR spectrum featured three olefinic methyl protons resonating at δ_H 1.65 (CH₃-17, s), 1.91 (CH₃-19, s) and 1.88 (CH₃-20, s); a methyl protons at 1.28 (CH₃-18, s); two oxymethine protons at δ_H 2.68 (dd, 4.8, 4.2; H-14) and at δ_H 5.57 (dd, J = 10.2, 1.2 Hz; H-14) and two olefinic protons at δ_H 5.15 (dd, 9.6, 5.4 Hz; H-8) and δ_H 5.05 (dd, 10.2, 1.2 Hz; H-13). After association of all protons with directly bonded carbons *via* HSQC spectral measurement, the role of both ^1H - ^1H COESY and ^1H - ^{13}C HMBC spectra started in structure elucidation. The ^1H - ^1H COSY spectrum established four proton sequences. The coupling between the methine proton signal resonating at δ 2.68 (H-4) and the methylenes protons at 1.90 and 1.70 (H-3), as well as these signals and the CH₂ protons at δ 2.40 (H-2) established the connectivity of the H-4/H-3/H-2 fragment (Fig. 2). The second sequence was established from the correlations between the olefinic proton resonating at δ 5.15 (H-8) and the CH₂ protons at 2.13 (H-7), which in turn are correlated with signal of CH₂ protons at δ 1.10 (H-6) confirm the connectivity of the H-8/H-7/H-6 fragment. The third one was established from the coupling between the CH₂ protons resonating at δ 2.01 and 2.20 (H-10) with the CH₂ protons at 2.78 and 2.10 (H-11) led to the connectivity of the H-11/H-10 part. Finally, the fourth represents an oxymethine proton H-14 in correlation with an olefinic proton H-13, led to the connectivity of the H-14/H-13 fragment. The HMBC correlations between the methyl protons H₃-17 with C-16, C-15 and C-1 and that of the oxymethine proton H-14 with C-2 and C-13 led to establishing a trisubstituted furenone fragment. The methyl protons signal at δ_H 1.28 (s, H-18) indicates a proximal oxygen functionality identified from ^{13}C NMR to be an epoxide (Table 1). The location of the epoxide ring at C4/C-5 was detected from HMBC correlations (Fig. 2), as there are clear correlations between H-4 with C-3, C-2 and C-5, also correlation between H₃-18 with C-5, C-4 and C-6, and between H-3 and C-1 established the connections from C-1 to C-6. The olefinic methyl proton at H-19, also shows an HMBC correlation with C-8, C-9 and C-10, while the olefinic proton H-8 is correlated to both C-7 and C-9, prove the connection from C-7 to C-10. The last connection from C-11 to C-14 was concluded from the correlation

between the olefinic methyl proton H-20 with C-11, C-12 and C-13. Surveying the soft coral literature especially sarcophyton, led to the assumption of a cembranoid-skeleton backbone. The previous data strongly indicated a cembranoid molecular framework containing position of the epoxide moiety is shifted to be C-4/C-5. The vicinal coupling constant of 10.2 Hz between H-13 and H-14 as well as 2D-NMR NOESY correlation of H-14 with H₃-20 established a *trans* configuration between the γ -lactone (H-14) and the olefinic proton (H-13). A computer survey was done employing Science Finder Data bases indicated **2** is sarcophytolide B ^[7].

Compound **3** was obtained as colorless oil (9.0 mg) with $[\alpha]_D^{22}$ -60.0 (c 0.015, CHCl₃). The negative mode of LCMS exhibited a $[M-H]^+$ m/z 315.180, indicating a molecular formula of C₂₀H₂₈O₃ and validated by HRESI-MS (Positive-ion-mode) m/z 317.2118 $[M+H]^+$. The seven degrees of unsaturation were supported by NMR data. The IR spectrum indicated the presence of α , β -unsaturated- γ -lactone (1750 cm⁻¹), an olefin (1669 cm⁻¹), and an epoxide (1256 cm⁻¹) functions. ¹³C and DEPT NMR spectral data (Table 1) exhibited 20 carbon signals establishing: four methyls, six methylenes, four methines (including two oxygenated), and six quaternary carbons (including one carbonyl and sp³ carbon). The ¹H and ¹³C NMR spectral data of **3** are closely similar to that of **2**, although during separation process they are quietly differed in the retention factor (R_f), which tempted us to check the difference in stereochemistry. 2D NOESY NMR spectral data was employed for assigning the relative configuration of **3** (Figure 2). The presence of a NOESY cross peak between the vinylic H-8 and H-13, cross peaks between CH₃-20 and H-12, CH₃-19 and H-11, indicated the *Z* geometry of both C-13/C-14 and C-9/C-10 double bonds. This deduction supported by the chemical shift of H-14 at δ_H 4.99 (dd, 9.6, 1.2). Furthermore, the conclusive NOE correlations between H-14 and H-1; between H-14 and CH₃-20; between H-3 and H-, confirmed the *Z* geometry of the two vinylic double bonds. The absence of cross peaks between CH₃-18 and H-5 indicated the *trans* configuration. After survey employing different data bases includes Science Finder, indicates **3** is sarcophytolide C ^[7].

Cytotoxicity of the isolated compounds (**1-6**) was evaluated employing five cancer panels; HepG2, MCF-7, A549, PC-3 and VERO (Table 2). 5-fluorouracil was used as a positive control. Interestingly, compounds (**1** and **3**) exhibited selective cytotoxic effects on HepG2 and showed no toxicity on the other cell lines. However, **2** showed selective

cytotoxic effect on MCF-7 with no toxicity on other cell lines. Meanwhile, **4-6** showed cytotoxic activity against both MCF-7 and HepG2 with no toxicity on normal Vero cells.

Table 2. Cytotoxic activity of the compounds **1-6**, towards five cancer cell lines

Comp. No.	IC ₅₀ (μg/mL)				
	HpG2 ^a	MCF-7	A549	PC-3	VERO
1	20±0.032	ND ^b	196.7±0.032	31.5±0.032	ND
2	NT	25±0.164	73.8±0.042	216.5±0.164	ND
3	20±0.153	NT	200.7±0.164	112.4±0.032	ND
4	20±0.068	20±0.054	98.4±0.120	09.3±0.164	ND
5	25±0.089	30±0.131	88.4±0.135	155.3±0.171	ND
6	26±0.061	33±0.075	100.4±0.092	17.3±0.125	ND

^a Human hepatocellular liver carcinoma (HepG2), human breast adenocarcinoma (MCF-7), Lung carcinoma (A549) and normal adult African green monkey kidney (VERO). Prostate cancer (PC-3)

^bND: not detected.

Acknowledgment

Grateful to King Abdulaziz university, Jeddah, Saudi Arabia, for supporting this work.

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أيزوبرينات سامة من مرجان البحر الاحمر ساركوفيتون جلوكوم و زينيا أمبيلاتا

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المستخلص. ثلاثة سيمبرانويدات سامة هم: ساركوفيتولول (1) و ساركوفيتوليد ب (2) و ساركوفيتوليد سي (3) تم تعريفهم من الجزء الذائب في الاثير ثنائى إيثيل من المستخلص العضوى للمرجان الناعم من نوع ساركوفيتون جلوكوم و مصدره ساحل البحر الاحمر السعودى، بالإضافة الى ثلاثة سسكوترينات من جنس الاروماندندرين تم تعريفهم من مستخلص المرجان الناعم زينيا امبيلاتا و هي: اروماديندين (4) و بالوسترول (5) و فيريديفلورول (6). تم إثبات التركيب الكيميائى باستخدام الطرق الطيفية المختلفة مثل الرنين النووى المغناطيسى و طيف الاشعة فوق البنفسجية و تحت الحمراء بالإضافة الى مطياف الكتلة كما تم إجراء اختبار قياس السمية على المركبات المفصولة كمضادات لسرطان الكبد وسرطان الثدي و سرطان الرئة و سرطان البروستاتا و كذلك سرطان كلى القرد الإفريقيى الناضج الأخضر. ووجد أن المركبين رقم 1 و 3 لهما سمية اختيارية نحو سرطان الكبد و مركب رقم 2 له سمية اختيارية نحو سرطان الثدي كما وجد ان المركبات رقم -6 و 4 لها سمية اختيارية لسرطان الكبد وسرطان الثدي.