Research Article

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Cytotoxic ketosteroids from the Red Sea soft coral *Dendronephthya* sp.

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Abstract: A marine specimen of the Red Sea soft coral *Dendronephthya* sp. was extracted with a mixture of *n*-hexane, diethyl ether, and methanol. One new cytotoxic steroid dendronestadione (**1**), five known steroids: dendronesterones A-C (**2**–**4**), dendrotriol (**5**), and cholesterol (**6**) along with 4-oxo-pentanoic acid (**7**) and a polyhydroxy alkane hexitol (**8**) were isolated from the *Dendronephthya* sp. extract. The chemical structures of the isolated metabolites were elucidated by the application of several spectroscopic techniques (1D, 2D NMR, IR, and UV) and mass spectrometry. The antiproliferative effect of the isolated compounds was assessed against a panel of human cancer cell lines including HepG2, HT-29, and PC. The obtained results indicated that compounds **1**–**4** (dendronesterones A–C) exhibited a higher cytotoxic effect than that of the other co-isolated ones. Among all examined dendronesterones, dendronesterone C showed the highest IC_{50} values of 19.1 ± 1.81, 32.4 ± 2.84, and 7.8 ± 0.80 μM against the three cancer cells under investigation. Interestingly, all isolated ketosteroids showed potent effects against prostate cancer cells. These findings highlight the role of ketosteroids as an antiproliferative agent against the examined cells in this study.

Keywords: Octocorallia, Xeniidae, steroids, antiproliferation, prostate cancer

1 Introduction

The increasing evidence of human diseases like pathogenic bacteria or virus infection and cancer in the current decadal demand attention to discovering new treatment methods [1]. Chemotherapy, radiotherapy, and surgery are common treatment methods, while synthetic-antibiotic drugs are administered to cure microbial infection [2]. However, the administration of synthetic drugs shows side effects [3]. Natural products derived from plants or marine invertebrates have scientifically proven their merit with fewer side effects [4].

Soft corals inhabit competitive and extreme environments that produce secondary metabolites for their defense mechanism [5]. Until 2012, the metabolite from soft coral represented 22% of total new marine metabolites isolated from marine invertebrates [6]. Marine natural products from soft coral possess diverse molecular structures with pharmaceutical properties [7]. Many of them enter the preclinical and clinical studies due to their promising *in vivo* and *in vitro* evaluation [8]. The family of Xeniidae, Nephtheidae, Alcyoniidae, or Clavulariidae was the primary source of natural products [9]. Roughly 179 new steroids had been discovered from soft corals worldwide from 2015 to 2020, most of which belong to hydroxysteroids [10]. The noticeable bioactive activity from these newly discovered metabolites was anticancer, antibacterial, and anti-inflammatory [11].

Red Sea soft corals are prolific sources of secondary metabolites, including steroids, sesquiterpenoids, diterpenoids, triterpenoids, norterpenoids, ceramides, and several fatty acid derivatives [12–15]. Alcyonaceans of the genus *Dendronephthya* (family Nephtheidae) are common in the Indo-Pacific Ocean. Steroids are the most frequently isolated secondary metabolites from the species of *Dendronephthya* [16,17]. In this article, a specimen of *Dendronephthya* sp. was collected from the Red Sea waters of Jeddah city. The soft coral extract afforded one new cytotoxic steroid dendronestadione (**1**) along with seven known metabolites: dendronesterones A–C (**2**–**4**), dendrotriol (**5**), and cholesterol (**6**) along with 4-oxo-pentanoic acid (**7**) and the polyhydroxy alkane hexitol (**8**) (Figure 1).

2 Experimental

2.1 General

The instruments' specifications and solvents and materials used in this article are detailed elsewhere [17].

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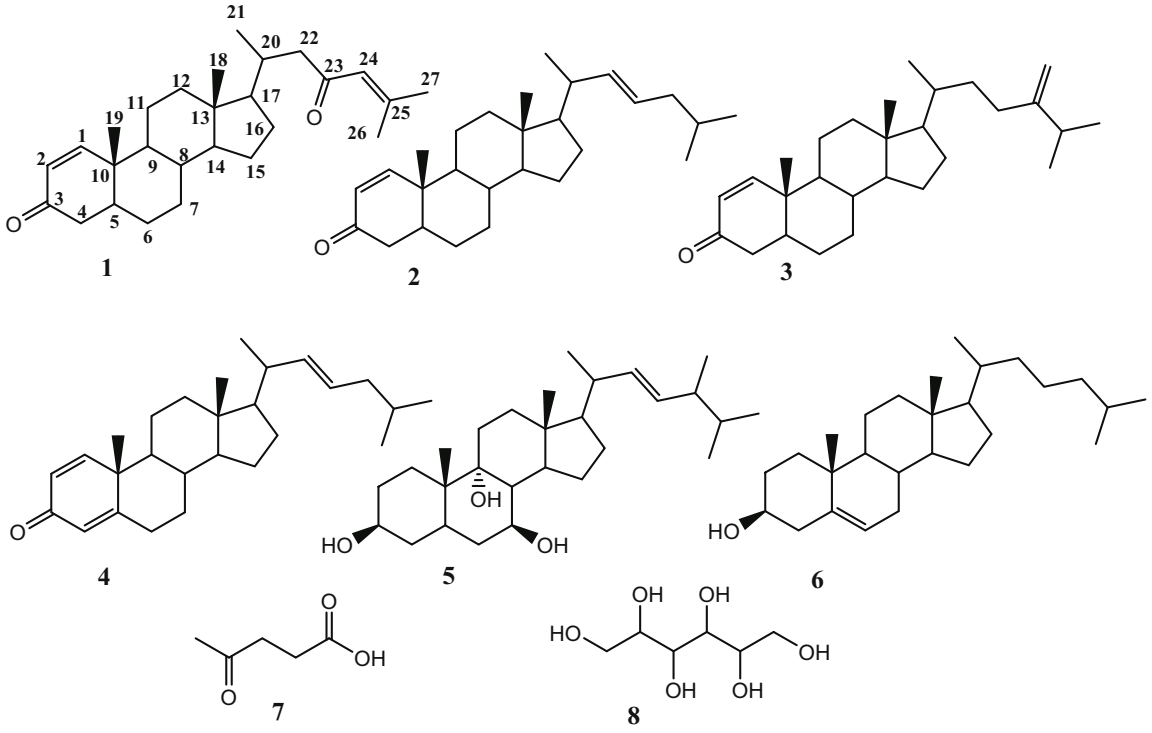


Figure 1: Molecular structures of compounds **1–8** isolated from *Dendronephthya* sp.

2.2 Animal material

A sample of a soft bodied coral *Dendronephthya* sp. animal was collected in April 2019 off Jeddah city coast, Saudi Arabia. A voucher specimen (DS19-01) was deposited at the Marine biology department, Faculty of Marine Sciences, KAU.

2.3 Extraction and isolation

The squeeze-dried *Dendronephthya* sp. sample (900 g) was macerated in acetone at room temperature (three times, 24 h each). The extract was completely dried to provide 37.3 g of solvent-free oil residue. 15.0 g of the residue was well mixed with a suitable amount of normal phase column chromatographic Si gel powder. The homogenized sample-silica gel paste was poured on the top of 1 m silica gel column (3.5 cm diameter). The fractionation process started with a non-polar solvent (*n*-hexane) and the polarity was increased gradually by adding increased percentages of chloroform in pet. ether and the EtOAc in pet. ether. A total of 678 fractions (25 ml each) were obtained. TLC plates were visualized with the aid of UV lamp (UV_{254}) and *p*-anisaldehyde-sulfuric acid reagent. PTLC glass plates were used for further purification. Chromatographic fractionation of the animal material was performed as previously described by Alassass et al. [15]. In brief, elution commenced with *n*-hexane, and then increased amounts of Et_2O were added to elevate the polarity and then the polarity increased by EtOAc. The initial fraction eluted with *n*-hexane was repurified with PTLC (100% *n*-hexane), to give a pink spot up on spraying with sulfuric acid reagent. This zone was collected (1.1 mg) and assigned as **7**. The fraction eluted with Et_2O/*n*-hexane (1:4) was applied to PTLC using the same previous composition, the spot at R_f 0.51 (reddish spot with sulfuric acid reagent) was collected (2.0 mg) and assigned as **6**. The fraction eluted with EtOAc/*n*-hexane (1.5:8.5) was applied to PTLC (22% EtOAc in *n*-hexane), and the spots appeared at R_f 0.73, 0.69, and 0.51 were collected and assigned to compounds **2** (1.0 mg), **3** (0.7 mg), and **4** (1.1 mg), respectively. The fraction eluted with EtOAc/*n*-hexane (1:4) was applied to PTLC (25% EtOAc in *n*-hexane), and the spot appeared at R_f 0.66 was collected to give compound **1** (0.9 mg). The fraction eluted with EtOAc/*n*-hexane (1:3) was applied to PTLC (35% EtOAc in *n*-hexane), and the spot appeared at R_f 0.49 was collected to give compound **5** (0.7 mg). The fraction eluted with MeOH/EtOAc/*n*-hexane (5:20:80) was applied to PTLC (15% MeOH in dichloromethane), and the spot appeared at R_f 0.66 was collected to give compound **8** (0.9 mg).

2.4 Characterization of the isolated compounds

2.4.1 Dendronestadione (1)

Gummy residue; $[\alpha]_D^{22}$ + 12.3 ($CHCl_3$, 0.02); UV (MeOH) λ_{max} 238 and 226 nm; IR ν_{max} 3,010, 2,930, 1,686, 1,675, 1,368, and 1,351 cm^{-1}; HR-ESI-MS *m/z* 396.3109 $[M+1]^+$(Calcd. for $C_{27}H_{41}O_2$, 397.3107); ^1H and ^{13}C NMR ($CHCl_3$) (Table 1).

Compounds **2–8** were characterized and identified by comparing their spectral data with the previously reported data in the literature. Those compounds were identified dendronesterones A–C (**2**–**4**), dendrotriol (**5**), and cholesterol (**6**) along with, 4-oxo-pentanoic acid (**7**) and the polyhydroxy alkane hexitol (**8**) [16,18].

2.5 Biological activities

2.5.1 Cytotoxic assay

HepG-2, HT-29, and PC-3 (human hepatocellular carcinoma, colorectal carcinoma, and prostate carcinoma) cells were obtained from American Type Culture Collection (ATCC) and cultured in RPMI1640 medium (Gibco, USA). The cytotoxicity test was carried out according to Mosdam [19].

Table 1: ^1H and ^{13}C NMR (500 and 125 MHz, respectively) spectral data of compound **1** in $CDCl_3$

C No.	δ_C	δ_H (*J* in Hz)	C No.	δ_C	δ_H (*J* in Hz)
1	158.6 (CH)	7.16, dd (10.0)	**16**	27.9 (CH_2)	1.68, m, 1.13, m
2	127.4 (CH)	5.85, d (10.0)	**17**	55.0 (CH)	1.66, m
3	200.3	—	**18**	12.1 (CH_3)	0.85, s
4	41.1 (CH_2)	2.37 dd (18.0, 14.0) 2.21 dd (18.0, 4.5)	**19**	13.0 (CH3)	1.01, s
5	44.4 (CH)	1.95, m	**20**	34.6 (CH)	1.95, m
6	28.5 (CH_2)	1.73, m 1.29, m	**21**	18.5 (CH_3)	1.02, d (7.0)
7	31.1 (CH_2)	1.71, m 0.98, m	**22**	52.9 (CH_2)	2.51, m 2.11, dd (15.0, 3.0)
8	35.6 (CH)	1.48, m	**23**	204.6 (CH_2)	—
9	50.0 (CH)	1.00, m	**24**	125.2 (CH)	6.19, br s
10	38.9 (C)	—	**25**	156.7 (C)	—
11	21.1 (CH_2)	1.80, m 1.53, m	**26**	21.0 (CH_3)	2.13, s
12	39.8 (CH_2)	2.07, m 1.18, m	**27**	27.5 (CH_3)	1.91, d (6.8)
13	43.0 (C)	—			
14	55.8 (CH)	1.19, m			
15	24.6 (CH_2)	1.57, m 1.14, m			

3 Results and discussion

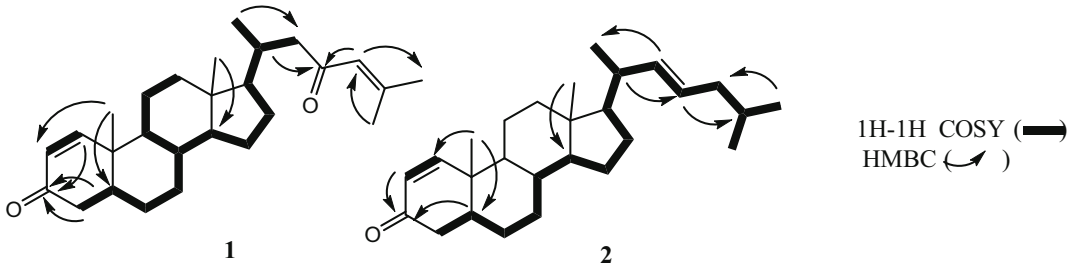
Sequential chromatographic fractionation and purification of the organic extract of the Red Sea soft coral resulted in the identification of eight secondary metabolites; six of them belong to steroids with C-27 and C-28 carbocyclic skeletons and the remaining compounds belong open chain alkanes.

Compound **1** was isolated as an optically active gummy substance. Its thin layer chromatography profile exhibited a positive response for steroids up on spraying with *p*-anisaldehyde-sulfuric acid reagent (blue turned brown color). Compound **1** showed absorption bands at 238 and 226 nm in the UV spectrum, indicating the presence of two a,b-unsaturated carbonyl functions. The molecular formula was found to be $C_{27}H_{40}O_2$ from HRESIMS (requiring eight unsaturation sites) supported by twenty-seven signals appeared in the ^{13}C NMR spectrum. No absorption due to hydroxyl function was found in the IR absorption spectrum; however, absorption bands due a,b-unsaturated carbonyls (1,686 and 1,675 cm^{-1}) and *gem*-dimethyl (1,368 cm^{-1}) were observed. The ^1H NMR spectrum indicated the presence of one signal of secondary methyl protons resonating at δ_H 1.02 ppm, four tertiary methyl protons resonating at δ_H 0.85, 1.01, 1.91, and 2.13 ppm, and three olefinic protons resonating at δ_H 7.16, 6.19, and 5.85 ppm (Table 1). The methylation pattern, the carbon number signals, the unsaturation degrees, and previous publications from the same genus call to the mind the probability of a steroid with a cholestane skeleton. The ^{13}C NMR spectrum of **1** showed the presence of signals due to carbonyl functions resonating at δ_C 204.6 and 200.3 ppm, four olefinic carbon signals resonating at 158.6, 156.7, 127.4, and 125.2 ppm. DEPT NMR experiments showed the presence of five unprotonated, nine methine, eight methylene, and five methyl carbons. HSQC NMR enabled the direct connection between all carbons and protons. Therefore, compound **1** contains no protonated hetero atoms. The presence of two carbonyls and two carbon–carbon double bonds accounted for three sites of unsaturation, which pointed out to the presence of a tetracyclic carbo-structure. The ^{13}C NMR signals at δ_C (204.6, 200.3), along with absorption bands in the IR and UV spectra suggested the presence of separate a,b-unsaturated carbonyls. ^1H–^1H COSY spectrum showed a distinct

proton sequence through the correlation observed between H-1 (δ_H 7.17) and H-2 (5.85). Other proton sequences were observed and clarified in Figure 2. The HMBC correlations observed from H-1 to the carbonyl carbon at 200.3 (C-3), 38.9 (C-10), and 13.0 (C-19) established the location of an a,b-unsaturated carbonyl function (C-1–C-3). The correlations observed from H-24 to the carbonyl carbon at 204.6 (C-23), and the *gem*-dimethyl function signals at 156.7, 27.5, and 21.0 established the presence of the second a,b-unsaturated carbonyl moiety at the forked tail (side chain). With the aid of ^{1}H–^{1}H COSY and HMBC NMR, the gross structure of **1** was concluded to be a cholestane skeleton with two carbonyl functions at C-3 and C-23 and two carbon–carbon double bonds at C-1 and C-24. A literature survey of chemical structures isolated from the genus *Dendronephthya* revealed that compound **1** was not previously isolated metabolite and was identified as dendronestadione (**1**).

Compound **2** was isolated as an optically active substance. It gave a positive response for steroids up on spraying with *p*-anisaldehyde-sulfuric acid reagent (blue turned brown color) on thin layer chromatography. Compound **2** showed an absorption band at 226 nm in the UV spectrum, indicating the presence of a,b-unsaturated carbonyl function. The molecular formula was found to be $C_{27}H_{42}O$, from HREIMS (required seven unsaturation sites) supported by 27 signals appeared in the ^{13}C NMR spectrum. No absorption due to hydroxyl function was found in the IR absorption spectrum; however, absorption bands due a,b-unsaturated carbonyl (1,688 cm^{-1}), carbon–carbon double bond (1,655 cm^{-1}), and *gem*-dimethyl (1,360 cm^{-1}) were observed. The ^{1}H NMR spectrum indicated the presence of three secondary methyl protons resonating at δ_H 1.00, 0.87, and 0.85 ppm, two tertiary methyl protons resonating at δ_H 1.01 and 1.00 ppm, and four olefinic protons resonating at δ_H 7.16, 5.85, 5.22, and 5.28 ppm. The ^{13}C NMR spectrum of **2** suggested the presence of a,b-unsaturated ketone (δ_C 200.6, 159.0, and 127.4) and carbon–carbon double bond (δ_H 138.0 and 126.5). DEPT NMR experiments showed the presence of three unprotonated, eleven methine, eight methylene, and five methyl carbons. HSQC NMR enabled the direct connection between carbons and protons. The presence of one carbonyl and two carbon–carbon double bonds accounted for three sites of unsaturation which pointed to the presence of a tetracyclic carbo-structure. With the aid of ^{1}H–^{1}H COSY and HMBC NMR, the gross structure of **2** was concluded to be a cholestane skeleton with a carbonyl function at C-3 and two carbon–carbon double bonds at C-1 and C-22. The geometry of the C-22–C-23 was proved to be *trans*-geometry due to the large coupling constant (J = 15.3 Hz). A literature survey of chemical structures isolated from the genus *Dendronephthya* revealed that compound **2** was previously isolated from *Dendronephthya gigantea* and was identified as dendronesterone A [16]. Compounds **3**, **4**, **7**, and **8** were identified by the comparison of their spectral data with those reported in the literature [18]. Compound **5** was identified by the comparison of its spectral data with that reported in the literature [18].

In this work, eight compounds were isolated from the Res Sea animal, *Dendronephthya* sp. The antiproliferative action of these metabolites was investigated against a panel of three cancer cell lines, HepG2, HT-29, and PC. Compound **1** showed the highest cytotoxic effects, where it had IC_{50} values of 19.1 ± 1.81, 32.4 ± 2.84, and 7.8 ± 0.80 μM in the treatment of HepG2, HT-29, and PC-3 cells respectively. Compound **2** showed good cytotoxic activity against PC-3 cells with an IC_{50} value of 43.7 ± 3.12 μM. By contrast to compounds **2** and **3**, compound **4** showed reasonable activity against all examined cells (Table 2). The data in Table 2 strictly clarified the following points: all examined steroids are more active toward PC-3 cells; decoration of the side chain with α,β-unsaturated ketone might affect the bioactivity exerted by the examined steroids; ketosteroids are more cytotoxic than the co-isolated fatty acid derivatives and the 3-hydroxy steroids.



1 2

Figure 2: Selected ^{1}H–^{1}H COSY and HMBC correlations of compounds **1** and **2**.

Table 2: Cytotoxicity of compounds **1–8** isolated from *Dendronephthya* sp.

Compound No.	IC_{50} (μM)*		
	HepG2	HT-29	PC-3
1	19.1 ± 1.81	32.4 ± 2.84	7.8 ± 0.80
2	89.0 ± 4.22	>100	43.7 ± 3.12
3	>100	>100	91.0 ± 6.44
4	81.0 ± 7.91	94.1 ± 9.00	66.7 ± 6.75
5	>100	>100	>100
6	>100	>100	>100
7	>100	>100	>100
8	>100	>100	>100
Doxorubicin	1.9 ± 0.03	1.8 ± 0.01	3.1 ± 0.15

*Four replicates were used for each treatment. Doxorubicin = positive control.

4 Conclusion

The Red Sea soft coral animal *Dendronephthya* sp. is a rich source of the ketosteroids of C-27 and C-28 carbocyclic skeletons in addition to keto-fatty acids. All ketosteroids showed antiproliferative effects with varying degrees of activity. Compound **1**, which was characterized by the presence of two α,β-unsaturated carbonyls, showed the highest potency among all compounds. It is worth noting that the maximum antiproliferative effect of all ketosteroids was observed against prostate adenocarcinoma (PC-3) cells.

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Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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