Chemical investigation of the soft coral *Sarcophyton glaucum* collected from the Red Sea led to isolation of 11 isoprenoidal metabolites (1–11). A new sesquiterpenoid, 6-oxo-germacra-4(15),8,11-triene (1), a new natural cembranoid, sarcophinediol, along with two known sesquiterpenoids (2 and 3) and seven known cembranoids (5–11) was obtained. The structures of the compounds were established based on their NMR, MS, IR and UV spectral data. All compounds were evaluated for their cytotoxicity employing three cancer cell lines (HepG2, MCF-7 and HCT116). Compounds 4 and 6 showed significant cytotoxicity towards HepG2 with IC\(_{50}\) values of 18.8 ± 0.07 and 19.9 ± 0.02 μM, respectively. Compounds 5–7 exhibited potent cytotoxicity against MCF-7 cells with IC\(_{50}\) values of 9.9 ± 0.03, 2.4 ± 0.04 and 3.2 ± 0.02 μM, respectively. Compounds 1, 4 and 5 showed significant activities towards HCT116 cells with IC\(_{50}\) values of 29.4 ± 0.03, 19.4 ± 0.02 and 25.8 ± 0.03 μM, respectively.

**Keywords:** *Sarcophyton glaucum*; cembranes; cytotoxic; HepG2; MCF-7

1. Introduction

The genus *Sarcophyton* is known for its richness in cembrane-type diterpenoids, biscembranoids, sesquiterpenoids and steroids. Up to date, more than 300 natural cembranoid derivatives were isolated (Wahlberg & Eklund 1992; Cheng et al. 2008; Cheng, Chuang, et al. 2010, Cheng, Wang, et al. 2010). Cembranoid diterpenes are a common class of natural metabolites in marine milieu (Hegazy et al. 2012). The basic chemical structural patterns typically featured a common 14-membered carbocyclic nucleus, festooned with carbon–carbon double bond(s) and methyl groups; this framework is easily exposed to oxidation, photochemical, rearrangement and enzymatic processes leading to the formation of cyclic ether, lactone, pyran or furan moieties around the cembrane skeleton (Fahmy et al. 2004, 2006; Szymanski et al. 2012). Unconventional cembranoids containing 1,2- or 1,3- or 1,4-membered variants were also identified. The cembranoid derivatives play an important role in the biomedical perspective (Fahmy et al. 2006; Huang et al. 2006; Cheng, Chuang, et al. 2010; Cheng, Wang, et al. 2010; Szymanski et al. 2013).
2. Results and discussion

In continuation of a project for isolation of the antiproliferative principles, a soft coral *Sarcophyton glaucum* was collected from the Saudi territorial water. Extensive fractionation of the organic extract yielded 11 terpenoidal metabolites (I–11). A new sesquiterpene, 6-oxo-germacra-4(15),8,11-triene, (I), a new natural product, sarcophinedioli (4), which was previously prepared semi-synthetically, along with two known sesquiterpenoids: 10(14)-aromadendrene (2) and palustrol (3) and six known cembranoids: deoxosarcophine (5), sarcotrocheliol acetate (6), sarcotrocheliol (7), sarcophytolide B (8), sarcophytolol (9), cembrene-C (10) and Sarcophine (11) was isolated (Figure 1).

Compound 1 was isolated as colourless oil with $[\alpha]_D^{22} + 5.0$ (c = 0.05, CHCl$_3$). Structural elucidation commenced when the molecular formula of 1, C$_{15}$H$_{22}$O, was established by LC-ESI-MS (positive-ion-mode) $m/z$ = 278.8 [M – H + CH$_3$COO]$^+$, and validated by HR-ESI-MS (positive mode) $m/z$ = 219.1738 [M + H]$^+$. The UV spectrum showed characteristic absorption for the hydroxyl (321.2420 [M+H]$^+$). The IR spectrum revealed the presence of C–O (1708 cm$^{-1}$) and C–C (1641 cm$^{-1}$) functionalities. The absence of conjugation was confirmed by the UV spectrum. The $^{13}$C NMR spectrum of 1 (see Supplementary Material) showed resonances for 15 carbons, differentiated by DEPT NMR experiment into two methyl, six methylene, four methine and three quaternary carbons. Four of the five elements of unsaturation, as indicated by the molecular formula of 1, are attributed to three C–C double bonds and a carbonyl carbon; thus, the molecule is monocyclic. $^1$H NMR data of 1 showed one methyl singlet, one methyl doublet and four exomethylene proton signals. All the spectral data are in agreement with the germacrane-type sesquiterpene skeleton with carbonyl group and disubstituted carbon–carbon double bond. Regarding the germacrane-carbon skeleton, the isopropyl group linked to C-7 should have been converted into isopropenyl group owing to the absence of correlation of the two doublet methyl signals in $^1$H NMR and COSY spectra, and the appearance instead of one singlet methyl signal. The HMBC correlations observed between the singlet methyl protons signal resonating at $\delta_H$ 1.60 ppm and the exomethylene protons signals resonating at $\delta_H$ 4.74 and 4.72 ppm on one side and the methine carbon signal appearing at $\delta_C$ 52.7 on the other side establish unambiguously the position of the isopropenyl moiety. Extensive interpretation of the COSY spectrum indicated the presence of the following correlations sequence: the doublet methyl protons resonating at $\delta_H$ 0.84, the aliphatic methine proton signal at $\delta_H$ 1.78, the olefinic proton at $\delta_H$ 4.95, the olefinic proton signal at $\delta_H$ 4.95 and the methine proton at $\delta_H$ 2.46, this sequence in turn establishes the position of the double bond to be in position six or eight depending on the germacrane numbering system. The HMBC correlations between H-7 signal with the carbonyl–carbon resonating at $\delta_C$ 204.3, the isolated methylene carbon at $\delta_C$ 37.3 and the olefinic carbon signal at $\delta_C$ 132.6 indicated the position of the keto group at C-6 and the exocyclic methylene protons at $\delta_H$ 5.23 and 5.08 ppm.

The relative stereochemistry of the asymmetric centres C-7 and C-10 was established on the basis of the NOESY experiments. The absence of NOE between Me-14 and H-10 suggested the cofacial orientation of H-7 and H-10. The geometry of the double bond between C-8 and C-9 was found to be cis owing to the low value of coupling constant (J). Accordingly, the relative configuration of 1 was deduced as (8Z,7R*,10S*). From the previous discussion, compound 1 can be identified as 6-oxo-germacra-4(15),8,11-triene.
DEPT measurement into four methyl, seven methylene, four methine and five quaternary carbons. Three of the five elements of unsaturation, as indicated by the molecular formula of 4, are attributed to two C=C double bonds; thus, the molecule is bicyclic. Careful investigation of the $^{13}$C NMR spectral data indicated the presence of two upfield shifted oxygenated carbons for a trisubstituted epoxy ring resonating at $\delta_C$ 62.29 and 59.98. Three trisubstituted C=C bonds

Figure 1. Structures of compounds 1–11 isolated from S. glaucum.
resonate at $\delta_C$ 143.98, 120.03, 120.99, 138.01, 125.33 and 135.65 ppm, in addition to an oxygenated methylene carbon resonating at $\delta_C$ 76.23 ppm. $^1$H NMR spectral data indicated the presence of two olefinic methyl protons resonating at $\delta_H$ 1.65 (3H, s) and 1.80 (3H, s); methyl protons at 1.25 (3H, s) and 1.33 (3H, s) and three olefinic proton signals at $\delta_H$ 5.96 (1H, d, $J = 1.8, 10.8$ Hz), $\delta_H$ 6.43 (1H, d, 10.8 Hz) and $\delta_H$ 5.12 (1H, dd, 12.6, 6.6 Hz). After association of all protons with directly bonded carbons via HSQC spectral measurement, it was possible to deduce the planar structure of 4 by interpretation of the $^1$H–$^1$H COSY and $^1$H–$^{13}$C HMBC spectra. $^1$H–$^1$H COSY spectral data indicated the correlation between the methine proton signal resonating at $\delta_H$ 2.86 (1H, dd, 4.8, 6.6 Hz, H-7) and the methylene protons at $\delta_H$ 1.90 (m) and 1.66 (m) which showed spin correlation with CH 2 protons at $\delta_H$ 1.30 (m) and 2.02 (m), thus establishing the connectivity of H-5/H-6/H-7. Further correlations were observed between the methine proton resonating at $\delta_H$ 5.12 (H-9) and the two protons appearing at $\delta_H$ 2.33 (1H, m) and 2.20 (1H, m), which in turn are correlated to the signal of CH 2 protons at $\delta_H$ 1.81 (s, H-18) and C-3, C-4 and C-5; correlations between H-20 ($\delta_H$ 1.60) with C-11, C-12 and C-13; correlations between H-19 ($\delta_H$ 1.25) with C-7, C-8 and C-9 indicated that H-17, H-18, H-19 and H-20 are positioned on C-15, C-4, C-8 and C-12, respectively. An extensive computer survey on soft corals indicated that Sarcophyton is abundantly produced cembranoids. Careful investigation of these literature data and comparing it with the obtained data indicated that 4 has C-14 cembrane skeleton (Fahmy et al. 2006; Huang et al. 2006; Cheng, Chuang, et al. 2010, Cheng, Wang, et al. 2010; Lu et al. 2010; Szymanski et al. 2013). Compound 4 was obtained as a mixture of two epimers (4 and 4a), which proved to be inseparable. $^{13}$C and $^1$H NMR spectra of the mixture showed duplicated signals and the difference between them ranged from $\pm 0.01$ to 0.05 ppm. The optical rotation value $[^\alpha]_D^{22} = +1.0$ ($c = 0.01, \text{CHCl}_3$) confirms that they are diasteroisomers. Extensive study of the NMR spectral data indicated that the orientation of two positions can be assigned; the oxiran ring and the hydroxyl group at C-15. The former is proved by $^{13}$C, $^1$H and 2D NOESY NMR assignments. Thus, 4 has 15$^S*$ and 15$^R*$. Extensive literature survey revealed that compound 4 is a new natural product which was previously published as semi-synthetic (Sarcophoninediol), which was published without NMR data (Fahmy et al. 2004; 2006; Szymanski et al. 2012).

Compounds 5–11 were identified by comparison of their recorded spectral data with the published data (Warmers & König 1999; Fahmy et al. 2006; Huang et al. 2006; Cheng, Chuang, et al. 2010, Cheng, Wang, et al. 2010; Lu et al. 2010; Szymanski et al. 2013; Al-Footy, et al. 2014; Al-Lihaibi et al. 2014).

The antiproliferative activity of compounds 1–11 was evaluated against three cancer cell lines (HepG2, MCF-7 and HCT116) with reference to the standard anticancer drug (doxorubicin), using Sulforhodamine B assay. The data in Table S1 highlight considerable antiproliferative activity. The tested compounds showed observable activity towards HepG2 cells in the range of 18.8–734.3 $\mu$M. Compounds 4 and 6 showed significant cytotoxicity towards HepG2 with IC$_{50}$ values of 18.8 ± 0.07 and 19.9 ± 0.02 $\mu$M, respectively. The compounds showed activity against MCF-7 cells in the range 1.88–57.0 $\mu$M. It is noteworthy to report that compounds 5, 6 and 7 exhibited potent activity against MCF-7 cells with IC$_{50}$ values of 9.9 ± 0.03, 2.4 ± 0.04 and 3.2 ± 0.02 $\mu$M, respectively. Furthermore, the compounds showed cytotoxic activity towards HCT116 cells in a concentration range of 21.8–86.7 $\mu$M, whilst compounds 1, 4 and 5 showed significant activity towards HCT116 with IC$_{50}$ values of 29.4 ± 0.03, 19.4 ± 0.02 and 25.8 ± 0.03 $\mu$M, respectively.
3. Materials and methods

3.1. General
Silica gel GF 254 (Merck, Darmstadt, Germany) was used for analytical thin layer chromatography (TLC). Preparative thin layer chromatography was performed on aluminium oxide plates (20 cm × 20 cm) of 250 μm thickness. 1D and 2D NMR spectra were recorded in CDCl₃ on Bruker (Karlsruhe, Germany) AVANCE III WM 600 MHz spectrometers and ¹³C NMR spectra at 150 MHz. Tetramethylsilane was used as internal standard. Plates were sprayed with 50% sulphuric acid in methanol and heated at 100°C for 1–2 min.

3.2. Animal material
Soft coral *S. glaucum* (Order Alcyonacea, Family Alcyoniidae) was collected from the north of Jeddah Saudi Arabia Red Sea coast (21°29′31″N 39°11′24″E) in Jeddah, at a depth of 5–10 m, in January 2014. This collection was done by SCUBA divers. After collection, this material was immediately subjected to extraction. A voucher specimen (SC-2014-10) has been deposited in the faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. *S. glaucum* is called elephant ear coral or green toadstool coral. It has a thick smooth, single stalk with a flared smooth mushroom-shaped top that can be folded or shaped into a funnel. The flesh is firm and soft, yet can be easily torn. The polyps can retract all the way, giving them a smooth look.

3.3. Extraction and isolation
The fresh soft coral *S. glaucum* (5.0 kg) was minced and exhaustively extracted with a mixture of CH₂Cl₂:MeOH (2:1 v/v, 24 h for each batch, 22°C, 10 L × 3), and then the combined extracts were concentrated under vacuum to yield viscous blackish residue. The residue was partitioned between diethyl ether and water; the organic layer was dried (30 g) and then fractionated on normal phase silica gel (NP-Silica), eluted stepwise with *n*-hexane containing increasing amounts of diethyl ether and then increasing polarity with EtOAc. A total of 100 fractions (F 1–100) were collected. The fractions were investigated by TLC pattern using UV lamp and/or 50%-sulfuric acid in methanol as spraying reagent. Fraction F 3 eluted with *n*-hexane:diethyl ether (9.5:0.5, 300.00 mg) was purified by preparative TLC using the solvent system *n*-hexane:diethyl ether (9.5:0.5). The band with *R*ₘ = 0.96 (violet-red colour with sulphuric acid–methanol) was taken to give compound 2 as colourless oil (22.00 mg); the band with *R*ₘ = 0.88 (pink colour with sulphuric acid–methanol) was taken to give compound 1 as colourless oil (1.50 mg) and the band with *R*ₘ = 0.80 (UV active and exhibited dark violet colour with sulphuric acid–methanol) was taken to give compound 11 as colourless oil (9.00 mg). Fraction F 13 eluted with *n*-hexane:diethyl ether (8:2, 125.00 mg) was purified by preparative TLC using the solvent system *n*-hexane:diethyl ether (8:2) to give two bands. The first band with *R*ₘ = 0.71 (violet colour with sulphuric acid–methanol) was taken to give colourless oil (16 mg), compound 6; the second band with *R*ₘ = 0.50 (violet colour with sulphuric acid–methanol) was taken to give colourless oil (4.50 mg), compound 3. Fraction F 41 eluted with *n*-hexane:EtOAc (9:1, 123.00 mg) was purified by preparative TLC using the solvent system *n*-hexane:ethyl acetate (8:2). The band with *R*ₘ = 0.25 (pink colour with sulphuric acid–methanol) was taken to give colourless oil (12.00 mg), compound 5 and the band with *R*ₘ = 0.69 (violet colour with sulphuric acid–methanol) was taken to give colourless oil (11.50 mg), compound 9. Fraction F 50 eluted with *n*-hexane:EtOAC (8:2, 70.00 mg) was purified by RP-18 HPLC (MeOH–H₂O, 65:35) which yielded 8 (3.00 mg) and 11 (2.00 mg). Fraction F 52 eluted with *n*-hexane:EtOAc (8:2, 70.00 mg) was purified by RP-18 HPLC (MeOH–H₂O, 65:35) which yielded 4 (8.00 mg).
3.4. Spectral data

3.4.1. 6-Oxo-germacra-4(15),8,11-triene (1)

Yellowish oil (1.5 mg, 0.0084%); [α]D22 +5.0 (c = 0.015, CHCl3); IR λmax (film) cm⁻¹: 2937 (C=H), 1708 (C=C), 1378, 1221, 1045; ¹H NMR (CD6D6, 600 MHz): 1.79 (1H, m, H-1a), 1.40 (1H, m, H-1b), 1.98 (1H, ddd, 3.6, 10.8, 13.8 Hz, H-2), 1.50 (1H, ddd, 3.6, 10.8, 13.8 Hz, H-2), 2.81 (1H, dd, 13.2, 12.0 Hz, H-3a), 2.08 (1H, dd, 12.0, 4.8 Hz, H-3b); IR νmax (film) cm⁻¹: 2937 (C=H), 1708 (C=H), 1378, 1221, 1045; ¹HN M (CD6D6, 600 MHz): 1.79 (1H, dq, 6.6, 4.2, 4.2 Hz, H-10), 4.95 (1H, d, 4.2, 4.2 Hz, H-8), 4.95 (1H, d, 4.2, 4.2 Hz, H-9), 1.78 (1H, dd, 6.6, 4.2, 4.2 Hz, H-10), 4.74 (1H, s, H-12), 4.72 (1H, s, H-12), 1.6 (3H, s, H3-13), 0.84 (3H, d, 6.6 Hz, H3-14); ¹CN (CD6D6, 150 MHz): 37.2 (C-1), 37.5 (C-2), 204.3 (C-6), 52.7 (C-7), 139.6 (C-9), 148.6 (C-11), 108.8 (C-12), 21.3 (C-13), 20.4 (C-14), 118.4 (C-15); HR-ESI-MS (positive mode) m/z = 219.1738 [M + H]+ (calculated m/z = 218.3346 for C20H34O2).

3.4.2. Sarcophine diol (4)

Colourless oil (8.0 mg, 0.0084%); [α]D22 +1.0 (c = 0.02, CHCl3); IR λmax (film) cm⁻¹: 3713 (O=H), 2937 (C=H), 1645 (C=C), 1378, 1221, 1045; ¹H NMR (CDCl3, 600 MHz): 6.43 (1H, d, 10.8 Hz, H-1), 5.96 (1H, dd, 10.8, 1.8 Hz, H-3), 2.02 (1H, m, H-5), 2.33 (1H, m, H-5), 1.92 (2H, m, H-13), 2.30 (1H, m, H-14), 3.66 (1H, d, 7.8 Hz, H-7), 3.46 (1H, d, 7.8 Hz, H-7), 3.36 (3H, s, H3-17), 3.18 (3H, s, H3-18), 1.36 (3H, s, H3-17), 1.29 (3H, s, H3-19), 1.60 (3H, s, H3-20); ¹CN (CDCl3, 150 MHz): δ = 144.18 (s, C-1), 120.03 (d, C-2), 120.95 (d, C-3), 138.01 (s, C-4), 38.46 (t, C-5), 23.18 (t, C-6), 62.29 (d, C-7), 59.98 (s, C-8), 25.85 (t, C-9), 26.26 (t, C-10), 125.33 (d, C-11), 135.65 (s, C-12), 35.60 (d, C-13), 41.22 (t, C-14), 76.23 (s, C-15), 68.91 (t, C-16), 16.10 (q, C-17), 17.83 (q, C-18), 24.30 (q, C-19), 17.03 (q, C-20); HR-ESI-MS (positive-ion-mode) m/z = 321.2420 [M + H]+ (calculated m/z = 320.2351, for C20H32O3).

4. Conclusion

Investigation of Red Sea S. glaucum led to isolation of 11 isoprenoidal metabolites (1–11). A new sesquiterpenoid, 6-oxo-germacra-4(15),8,11-triene (1), a new natural cembranoid, sarcophine diol, along with two known sesquiterpenoids (2 and 3) and seven known cembranoids (5–11) was obtained. Compounds 4 and 6 showed significant cytotoxicity towards HepG2 with IC₅₀ values of 18.8 ± 0.07 and 19.9 ± 0.02 μM. Compounds 1, 4 and 5 showed significant activity towards HCT116 cells with IC₅₀ values of 29.4 ± 0.03, 19.4 ± 0.02 and 25.8 ± 0.03 μM, respectively. The observed potential cytotoxic activity warrants further investigations.
Supplementary material

Supplementary material relating to this paper is available online.

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