New antimicrobial biscembrane hydrocarbon and cembranoid diterpenes from the soft coral *Sarcophyton trocheliophorum*

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**Abstract:** A new tetracyclic biscembrane hydrocarbon, trocheliane (1), along with two new cembranoid diterpenes, sarcotrocheldiol A (2) and B (3), and the known diterpene cembrene-C (4), were isolated from the Red Sea soft coral *Sarcophyton trocheliophorum*. The structures and relative stereochemistry of the compounds were elucidated by interpretation of MS, 1D NMR, and 2D NMR experiments. The sensitivity of some pathogenic bacteria used as test organisms to the new compounds 1–3 was determined. 1 showed appreciable antimicrobial activity with the diameter of inhibition zones ranging from 11 to 18 mm. 1 was active against the two multidrug resistant bacteria *Acinetobacter baumannii* and *Staphylococcus aureus*. Minimal inhibitory concentrations (MICs) of compound 1 were recorded for all the tested bacteria using the fluorescein diacetate method and the recorded MIC values ranged from 4 to 6 \(\mu\)M.

**Key words:** Soft coral, biscembranes, diterpenoids, antibacterial activity

1. Introduction

Common, prolific, and offering potentially novel and biologically active secondary metabolites are the characteristics of the species of the soft bodied coral *Sarcophyton* (phylum, Cnidaria; class, Anthozoa; subclass, Octocorallia; order, Alcyonacea; family, Alcyoniidae).\(^1\) The most frequently isolated metabolites are terpenoids. Among terpenoids, diterpene dimers or biscembranoids are extremely rare compounds and are almost exclusive to four species of the genus *Sarcophyton*, i.e. *S. glaucum*, *S. elegans*, *S. tortuosum*, and *S. latum*.\(^2,3\) Several cembranoid-type diterpenes have been shown to exhibit various pharmacological activities, such as antifeedant\(^4\) and anti-inflammatory activities,\(^5\) and cytotoxicity.\(^6\) We have previously reported several cytotoxic cembranoids from *Sarcophyton glaucum* and *S. trocheliophorum* collected from the Saudi Arabian Red Sea.\(^7-9\)

2. Results and discussion

Our further investigation of the CH\(_2\)Cl\(_2\)-soluble material of the CHCl\(_3\):MeOH extract of the soft bodied organism *S. trocheliophorum* led to the isolation of several metabolites: 1 (5.3 mg, 0.0067%), 2 (3.5 mg, 0.0044%), 3 (3.6 mg, 0.0046%), and 4 (15.0 mg, 0.0190%) (Figure 1).

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Compound 1 was isolated as gummy material. 1 was analyzed for C_{40}H_{58} on the basis of HREIMS at m/z 538.4528 (M)^+ and $^{13}$C NMR spectra. The IR (neat) spectrum displayed absorptions due to (C–H) 3050–2700, (C=C) 1630, (aromatic ring) 1510, and (terminal methylene) 925 functionalities cm$^{-1}$. The presence of a substituted benzene ring was concluded from the UV absorption maximum ($\lambda_{max}$) at 221 nm, supported by the IR absorption at 1510 cm$^{-1}$. The $^{13}$C NMR spectrum of 1 (cf. exp.) showed resonances for 40 carbons, differentiated by DEPT NMR experiment into 8 methyl, 12 methylene, 10 methine, and 10 quaternary carbons. Eight of the twelve elements of unsaturation as indicated by the molecular formula of 1 are attributed to eight C=C double bonds evidenced from 16 signals between $\delta_C$ 110.1 and 149.3; thus, the molecule is a tetracarbocyclic skeleton. The $^1$H NMR signals at 7.02 (br s), 6.94 (br d, $J = 7.8$ Hz), and 7.11 (br d, $J = 7.8$ Hz) together with the aromatic methyl signal at $^1H$/C$_2$.32 (s)/21.1 revealed the presence of 1,3,4-trisubstituted benzene. Moreover, $^1$H, $^{13}$C, and HSQC NMR spectral data featured the following: three olefinic methines resonating at $\delta_H$/δC 4.98 (ddd, $J = 12.0, 6.0, 1.2$ Hz)/125.9, 5.06 (ddd, $J = 12.0, 6.6, 1.2$ Hz)/121.8, and 5.19 (ddd, $J = 12.0, 7.2, 1.2$ Hz)/124.0; exocyclic methylene protons at 4.65 (d, $J = 2.4$ Hz) and 4.71 (d, $J = 2.4$ Hz)/110.1; three doublet methyls at 0.71 (d, $J = 6.6$ Hz)/17.3, 0.99 (d, $J = 6.6$ Hz)/21.3, and 1.26 (d, $J = 6.6$ Hz)/22.3, and four singlet methyls at 1.56/18.0, 1.57/15.5, 1.59/15.3, and 1.0/16.6. The $^1$H–$^1$H COSY and HSQC analyses led to assignment of the following proton sequences:
CH₂–CH₂–CH= (C-36–C-2–C-4), CH₂–CH₂–CH= (C-6–C-8), CH₂–CH₂ (C-10–C-11), CH₃–CH–CH₃ (C-15–C-17; isopropyl moiety) =CH–CH₂ (C-13–C-14), CH–CH₂ (C-21–C-22), =CH–CH= (C-28–C-29), and CH₃–CH–CH₂–CH₂ (C-40–C-31–C-33). Extensive study of the HMBC correlations established the following connectivities: a) the correlations observed between H-15 and the quaternary carbon C-12 (δC 149.3), C-13 (121.8), the CH₂ carbon C-11 (33.9), and the methyl carbon C-17 (21.3) established the attachment of the isopropyl function to C-12. The correlations between H-13 and the quaternary carbon C-1 (39.9) and C-11, between H-8 and the methyl carbon C-18 (15.5) and C-10 (28.1), and between H-4 and C-19 (15.3), C-6 (32.4), and the methine carbon C-2 (45.9), together with those between H₃-20 and C-14 (39.4), C-21 (43.7), C-2, and C-1 established the 14-membered ring, which is very similar to the data of cembrene-C (5). b) For the remaining part of the structure we expect a tricyclic partial structure containing a 3,4-disubstituted toluene, which was unambiguously determined from the HMBC’s correlations: H-21 with C-2, C-35 (δC 133.4), C-23 (150.3), and C-1; H₃-37 with C-34 (128.7) and C-36; H-24 with C-30 (134.8), C-38 (110.1), and C-26 (128.7); and finally H-29 with C-31 (32.5) and C-27 (134.4). Hence, the gross structure can be constructed as in Figure 1, and the trivial name trocheliane (1) was given. The relative stereochemistry of the asymmetric carbons (C-1, C-2, and C-31) and the geometry of the double bonds were deduced from the NOESY spectrum and studying the chemical shift (δC) values of the methylene carbons allylic to the carbon–carbon double bond.¹⁰ The cross peak between H-2 and H₃-20 implies cis fusion between the 14-membered ring moiety and the cyclohexene ring. These protons are also correlated with protons of Me-18, Me-19, Me-40, and H-13, also indicating cofacial orientation of these groups, and the E configuration for C-4/C-5, C-8/C-9, and C-12/C-13 double bonds as well. The downfield chemical shift value of the allylic CH₂ group δC is higher than 30 ppm, supporting the E configuration of these double bonds.

Compound 2 was obtained as gummy material. 2 was analyzed for C₂₀H₃₄O₃ on the basis of HREIMS at m/z 322.2500 (M)⁺ and ¹³C NMR spectra, implying four degrees of unsaturation. The ¹³C (cf. exp.) and DEPT NMR experiments allowed the determination of 20 resonances attributable to five Me carbons (δC 17.6, 20.5, 20.7, 23.8, and 29.3), five CH₂ carbons (42.5, 39.9, 22.8, 34.1, and 18.6), and four sp³ CH carbons including two oxygen-bearing (72.0 and 75.8), three sp² CH (124.4, 128.2, and 135.7), and three quaternary C-atoms including two O-bearing (73.6 and 74.9). The ¹H NMR and COSY NMR of 2 displayed three Me singlet signals (δH 1.83, 1.33, 1.04), one ¹Pr [δH 1.22 (m, 1H), 0.88 (d, J = 6.6 Hz, 3H) and 0.76 (d, J = 6.6 Hz, 3H)], three olefinic protons [δH 5.28 (d, J = 10.8 Hz), 5.85 (ddd, J = 15.6, 10.8, 4.2 Hz) and 5.41 (br d, J = 15.6 Hz)], and two O-bearing CH protons [δH 4.62 (dd, J = 10.8, 4.8 Hz), 3.48 (d, J = 9.6 Hz)]. The NMR data of 2 displayed great similarity to those of the known compound sarcotrocheliol (5).⁹ The main difference was signals at δC 128.2 and 135.7, suggesting the presence of a disubstituted double bond system in 2, instead of a trisubstituted double bond system in 5, and a signal at 73.6, indicating an extra oxygenated quaternary carbon. The ¹³C NMR signals at δC 124.4 and 141.2 indicated a nonconjugated trisubstituted double bond belonging to C-3–C-4 based on the HMBC correlation with Me-18, also ¹³C NMR signals at δC 128.2 and 135.7 assigned as nonconjugated disubstituted double bonds belonging to C-6–C-7 by HMBC correlation between Me-19 and C-7. Moreover, ¹H–¹H COSY spectral analysis established three partial structures of consecutive proton spin systems from H-5 to H-7 through H-6; from H-9 to H-11 through H-10; and finally from H-3 to H-13 through H-2, H-1 (H-15), H-14, and H-13. These data, together with the HMBCs correlation, from H-16 to C-15, C-17, and C-1; from H-18 to C-3, C-4, and C-5; from H-19 to C-7, C-8, and C-9; and from H-20 to C-12, C-11, and C-13 established the connectivity within the 14-membered ring. The relative configuration of 2 was assigned
mainly from the NOESY spectrum and the coupling constant values \( (J) \). The existence of a cross peak between the \( H_3-18/H-6 \) and \( H_3-19 \), together with the absence of the correlation \( H_3-18/H-3 \), as well as the presence of a cross peak between \( H-3 \) and \( H-7 \), led to the \( E \) geometry for the \( C-3-C-4 \) double bond. This \( (E) \) geometry was also supported by the downfield chemical shift of the allylic \( CH_2 \) group of \( C-5 \) at \( \delta_C 42.5 \) (i.e. \( \delta_C > 30 \)) and the \( J \) value \( (10.8 \text{ Hz}) \).\(^{10}\) The \( E \) geometry of \( C-6=C-7 \) was concluded from the large coupling constant value \( (J = 15.6 \text{ Hz}) \), and the NOE correlation \( H-7/H-3 \). The cross peak between \( H-1 \) and \( H-5a \) \( (m, 2.75-2.80) \) and \( H_3-20 \) allowed us to assign them as \( \beta \)-oriented and \( OH-11 \) as \( \alpha \)-oriented, owing to the fact that the \( ^4 \text{Pr} \) group must be \( \alpha \)-oriented (cembranes from the order Alcyonaceae possess an \( \alpha \)-oriented isopropyl group at \( C-1 \)).\(^{11}\) Analysis of all the NMR spectroscopic data confirmed \( 2 \) to be a cembranoid derivative, and the trivial name sarcotrocheldiol A was given.

Compound \( 3 \) was found to have the same molecular formula as \( 2 \), \( C_{20}H_{34}O_3 \), as indicated from HREIMS and \( ^{13} \text{C} \) NMR spectra \((cf. \ exp.)\). Comparison of the \( ^1 \text{H} \) and \( ^{13} \text{C} \) NMR data of \( 3 \) with those of \( 2 \) revealed that \( 3 \) is analogous to those of \( 2 \) except for the replacement of a disubstituted double bond (\( C-6=C-7 \)) in \( 2 \) by the substitution of a hydroxyl group at \( C-7 \) resonating at \( \delta_C 67.8 \) in \( 3 \), and the appearance of an exocyclic double bond at \( C-8 \) \( \delta_C 147.5 \). The structure of \( 3 \) was established by analyses of HMBC and COSY NMR spectra. Moreover, the similar splitting pattern and \( J \) values of \( H-3 \) \( (10.8 \text{ Hz}) \) in both \( 2 \) and \( 3 \), together with the significant NOE interaction between \( H_3-18 \) and \( H-6a \), revealed the \( E \) geometry of the trisubstituted double bond at \( C-3=C-4 \). Therefore, the structure of \( 3 \) was identified as sarcotrocheldiol B.

Compound \( 4 \) was identified by comparing its spectral data with those in the literature.\(^{12}\) The sensitivity of some pathogenic bacteria including \textit{Acinetobacter baumannii}, \textit{Escherichia coli}, \textit{Klebsiella pneumonia}, \textit{Pseudomonas aeruginosa}, \textit{Staphylococcus aureus}, \textit{S. epidermidis}, and \textit{Streptococcus pneumoniae} to the purified fractions \( 1-3 \) was determined by agar well diffusion.\(^{13}\) To compare the antibacterial activity results, ampicillin was used as positive control due to its broad spectrum of bactericidal activity against both gram-positive and gram-negative pathogens. \( 1 \) showed appreciable antimicrobial activity against the tested bacteria with diameter of inhibition zones ranging from 12 to 18 mm. \( 1 \) was active against the two multidrug resistant bacteria \textit{A. baumannii} and \textit{S. aureus}. Very weak antibacterial activity was recorded against all the tested pathogenic bacteria for compounds \( 2 \) and \( 3 \).

Minimal inhibitory concentrations (MICs) of compound \( 1 \) were recorded for all the tested bacteria using a fluorescein diacetate assay.\(^{14}\) The recorded MICs ranged from 4 to 6 \( \mu \text{M} \) (Table).

**Table.** The antibacterial activity (diameter of inhibition zone, mm) of the tested compounds (1–3) and the control (5 \( \mu \text{g/mL} \), and MIC values of compound 1.

<table>
<thead>
<tr>
<th>Tested bacteria</th>
<th>Mean diameter of the inhibition zone (mm) ± SD</th>
<th>MIC (( \mu \text{M} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Acinetobacter baumannii}</td>
<td>( 18 \pm 3.2 )</td>
<td>20 ( \pm 1.2 )</td>
</tr>
<tr>
<td>\textit{Escherichia coli}</td>
<td>( 13 \pm 1.7 )</td>
<td>15 ( \pm 1.3 )</td>
</tr>
<tr>
<td>\textit{Klebsiella pneumonia}</td>
<td>( 15 \pm 1.2 )</td>
<td>24 ( \pm 2.2 )</td>
</tr>
<tr>
<td>\textit{Pseudomonas aeruginosa}</td>
<td>( 16 \pm 2.2 )</td>
<td>19 ( \pm 3.0 )</td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus}</td>
<td>( 18 \pm 1.4 )</td>
<td>28 ( \pm 1.4 )</td>
</tr>
<tr>
<td>\textit{S. epidermidis}</td>
<td>( 16 \pm 1.4 )</td>
<td>24 ( \pm 1.7 )</td>
</tr>
<tr>
<td>\textit{Streptococcus pneumoniae}</td>
<td>( 13 \pm 1.5 )</td>
<td>28 ( \pm 1.4 )</td>
</tr>
</tbody>
</table>

NF: Not found, Ampicillin: positive control
3. Conclusions
Herein, we report the first isolation of an unprecedented biscembranoid dimer hydrocarbon (trocheliane, 1) and two new furan-containing cembranoid diterpenes (2, 3) from the Red Sea coral S. trocheliophorum. Product 1 is a promising compound due to its activity against the two multidrug resistant bacteria A. baumannii and S. aureus. The MICs were determined using a fluorescein diacetate assay. The recorded MICs ranged from 4 to 6 μM (Table). Trocheliane (1) could be the addition adduct of two cembrene-C (4) isomers (Figure 2).

![Figure 2. Hypothetical formation of trocheliane (1).]

4. Experimental
4.1. General
Silica gel (SiO₂; Kieselgel 60 F₅₀) of 0.25 mm layer thickness. 1 D and 2 D NMR: Bruker AVANCE III WM at 600 MHz, and ¹³C NMR at 150 MHz; δ in ppm rel. to Me₄Si as internal standard, J in Hz. High resolution mass spectra were recorded on a Finnigan FTMS 2001 instrument.

Soft coral S. trocheliophorum was collected (May, 2013) from the Red Sea coast (21° 29' 31" N 39° 11' 24" E), north of Jeddah, Saudi Arabia, at a depth of 5–10 m. It was identified by Dr Yahia Folos (Marine Biology Department, Faculty of Marine Sciences, KAU). A voucher sample (JAD 09060) was deposited at the Chemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, KSA.

Bacterial isolates: Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, S. epidermidis, and Streptococcus pneumoniae were taken from the culture collection of the Microbiology Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

4.2. Extraction and isolation
Coral material was washed with water and dried in the shade at room temperature. The dried material (79.00 g) was exhaustively extracted with equal volumes of CHCl₃/MeOH (2 × 6 L, 24 h for each batch) at room temperature. The residue (20.25 g) was partitioned between CH₂Cl₂ and water; the CH₂Cl₂ soluble
material was dried (9.18 g) and chromatographed by NP (Merck, 60G) column chromatography employing n-hexane/CH$_2$Cl$_2$, followed by EtOAc/MeOH mixtures with increasing polarity. Fractions of ~100 mL were collected. TLC was carried out by employing silica gel chromatoplates, an appropriate solvent system, and 50% H$_2$SO$_4$ in MeOH as spraying reagent. Fractions containing a single compound were combined and further purified by preparative TLC of glass-supported silica gel plates (20 cm × 20 cm) of 250 μm thickness.

Fraction A, eluted with n-hexane:methylene chloride (9.5:0.5, 37.00 mg), was purified by preparative TLC using the solvent system n-hexane:methylene chloride (9:1). The band with R$_f$ = 0.75 (pink color with sulfuric acid/methanol) was taken to give compound 4 as a colorless oil (15.00 mg). Fraction B, eluted with n-hexane (9:1, 36.00 mg), was purified by preparative TLC using the solvent system n-hexane:methylene chloride (8:2). The band with R$_f$ = 0.70 (violet appearance under UV $\lambda_{254}$, and brown color with H$_2$SO$_4$/MeOH) was taken to give compound 1 (Figure 1) as a colorless oil (5.3 mg, 0.0067 dry weight). Fraction H, eluted with CH$_2$Cl$_2$:MeOH (9:1, 35.00 mg), was purified by preparative TLC using the solvent system n-hexane:EtOAc (2:8) to give two bands. The first band, with R$_f$ = 0.38 (brown color with H$_2$SO$_4$/MeOH), was taken to give colorless oil (3.5 mg) of compound 2. The second band, with R$_f$ = 0.35 (brown color with H$_2$SO$_4$/MeOH), was taken to give colorless oil (3.6 mg) of compound 3.

Trocheliane (1): Gummy material; [α]$_D^{20}$ $-$22.0 (c 0.02, C$_6$H$_6$); IR (film) cm$^{-1}$: 3050–2700 (CH), 1630, 1620 (C=C), 1510, 925, 740; $^1$H NMR (C$_6$D$_6$, 600 MHz): 2.01–2.05 (1H, m, H-2), 2.15–2.20 (1H, m, H-3a), 2.10–2.15 (1H, m, H-3b), 4.98 (1H, ddd, $J$ = 12.0, 6.0, 1.2 Hz, H-4), 1.98–2.04 (1Hm, H-6a), 1.56–1.58 (1H, m, H-6b), 1.92–1.94 (1H, m, H-7a), 1.32–1.36 (1H, m, H-7b), 5.19 (1H, ddd, $J$ = 15.0, 7.2, 1.2 Hz, H-8), 1.64–1.70 (1H, m, H-10a), 1.30–1.33 (1H, m, H-10b), 1.92–1.98 (1H, m, H-11a), 1.76–1.80 (1H, m, H-11b), 5.06 (1H, ddd, $J$ = 12.6, 6.6, 1.2 Hz, H-13), 2.01–2.10 (1H, m, H-14a), 1.44–1.48 (1H, m, H-14b), 2.20–2.26 (1H, m, H-15), 0.71 (3H, $J$ = d, 6.6 Hz, H-16), 0.99 (3H, d, $J$ = 6.6 Hz, H-17), 1.57 (3H, s, H-18), 1.59 (3H, s, H-19), 1.00 (3H, s, H-20), 2.68–2.69 (1H, m, H-21), 1.81–1.86 (1H, m, H-22a), 1.57–1.61 (1H, m, H-22b), 2.12 (1H, d, $J$ = 6.6 Hz, H-24a), 2.06 (1H, d, $J$ = 6.6 Hz, H-24b), 7.02 (1H, s, H-26), 6.94 (1H, br d, $J$ = 7.8, H-28), 7.11 (1H, $J$ = br d, 7.8, H-29), 2.75 (1H, hex, H-31), 1.31–1.39 (1H, m, H-32a), 1.21–1.29 (1H, m, H-32b), 1.26–1.30 (1H, m, H-33a), 1.30–1.32 (1H, m, H-33b), 2.20–2.23 (1H, m, H-36a), 2.16–2.20 (1H, m, H-36b), 1.56 (3H, s, H-37), 4.65 (1H, d, $J$ = 2.4, H-38a), 4.70 (1H, d, $J$ = 2.4, H-38b), 2.32 (3H, s, H-39), 1.26 (3H, d, 6.6, H-40),$^{13}$C NMR (C$_6$D$_6$, 150 MHz): 39.9 (C, C-1), 45.9 (CH, C-2), 38.9 (CH$_2$, C-3), 125.9 (CH, C-4), 133.9 (C, C-5), 32.4 (CH$_2$, C-6), 30.8 (CH$_2$, C-7), 124.0 (CH, C-8), 140.0 (C, C-9), 28.1 (CH$_2$, C-10), 33.9 (CH$_2$, C-11), 149.3 (C, C-12), 121.8 (CH, C-13), 39.4 (CH$_2$, C-14), 31.8 (CH, C-15) 17.3 (CH$_3$, C-16), 21.3 (CH$_3$, C-17), 15.5 (CH$_3$, C-18), 15.3 (CH$_3$, C-19), 16.6 (CH$_3$, C-20), 43.7 (CH, C-21), 21.4 (CH$_2$, C-22), 150.3 (C, C-23), 23.7 (CH$_2$, C-24), 139.9 (C, C-25), 128.7 (CH, C-26), 134.4 (C, C-27), 126.1 (CH, C-28), 126.7 (CH, C-29), 134.8 (C, C-30), 32.5 (CH, C-31), 29.6 (CH$_2$, C-32), 22.7 (CH$_2$, C-33), 128.7 (C, C-34), 133.4 (C, C-35), 24.8 (CH$_2$, C-36), 18.0 (CH$_3$, C-37), 110.1 (CH$_2$, C-38), 21.1 (CH$_3$, C-39), 22.3 (CH$_3$, C-40); HRESIMS data $m/z$ 538.4528 [M]+ (Calculated 538.4539 for C$_{40}$H$_{58}$).

Sarcotrocheldiol A (2): Colorless oil; [α]$_D^{20}$ 62.4 (c 0.012, CHCl$_3$); IR (film) cm$^{-1}$: 3423 (OH), 3180 (OH), 2937 (C–H), 1645 (C=C), 1378, 1221, 1045; $^1$H NMR (CDCl$_3$, 600 MHz): 1.34–1.36 (1H, m, H-1), 4.62 (1H, d, $J$ = 10.8, 4.8, H-2), 5.28 (1H, d, $J$ = 10.8, H-3), 2.77 (1H, dd, $J$ = 12.0, 10.8 Hz, H-5a), 2.56 (1H, dd, $J$ = 12.0, 4.2 Hz, H-5b), 5.87 (1H, ddd, $J$ = 15.6, 10.8, 4.2 Hz, H-6), 5.41 (1H, br d, $J$ = 15.6 Hz, H-7), 1.86–1.88 (1H, m, H-9a), 1.54–1.56 (1H, m, H-9b), 1.74–1.76 (1H, m, H-10a), 1.26–1.30 (1H, m, H-10b), 3.48
(1H, d, 9.6 Hz, H-11), 1.62–1.64 (1H, m, H-13a), 1.35–1.36 (1H, m, H-13b), 2.28–2.32 (1H, m, H-14a), 1.20–1.22 (1H, m, H-14b), 1.21–1.23 (1H, m, H-15), 0.88 (3H, d, J = 6.6 Hz, H-16), 0.76 (3H, d, J = 6.6 Hz, H-17), 1.83 (3H, s, H-18), 1.33 (1H, s, H-19), 1.04 (1H, s, H-20),

$^{13}$C NMR (CDCl$_3$, 150 MHz): 45.6 (CH, C-1), 72.0 (CH, C-2), 124.4 (CH, C-3), 141.1 (C, C-4), 42.5 (CH$_2$, C-5), 128.2 (CH, C-6), 135.7 (C, C-7), 73.6 (C, C-8), 39.9 (CH$_2$, C-9), 22.8 (CH$_2$, C-10), 75.8 (CH$_2$, C-11), 74.9 (CH$_2$, C-12), 18.6 (C, C-13), 34.1 (CH, C-14), 29.4 (CH, C-15), 20.7 (CH$_3$, C-16), 20.5 (CH$_3$, C-17), 17.6 (CH$_3$, C-18), 29.3 (CH$_3$, C-19), 23.8 (CH$_3$, C-20);

HRESIMS data $m/z$ 322.2501 [M]+ (Calculated 322.2508 for C$_{20}$H$_{34}$O$_3$).

Sarcotrocheldiol B (3): Colorless oil $[\alpha]_D^{20}$ 89.1 ($c$ 0.010, CHCl$_3$); IR (film) cm$^{-1}$: 3383 (OH), 3180 (OH), 2937 (C–H), 1645 (C=C), 1378, 1221, 1045;

$^1$H NMR (CDCl$_3$, 600 MHz): 1.29–1.32 (1H, m, H-1), 4.52 (1H, dd, J = 10.8, 5.4, H-2), 5.23 (1H, d, J = 10.8, H-3), 2.13–2.15 (1H, m, H-5a), 2.17–2.19 (1H, m, H-5b), 1.88–1.90 (1H, m, H-6a), 2.12–2.15 (1H, m, H-6b), 3.88 (1H, dd, J = 10.8, 1.2 Hz, H-7), 2.48–2.51 (1H, m, H-9a), 2.28–2.32 (1H, m, H-9b), 1.95–1.97 (1H, m, H-10a), 1.28–1.30 (1H, m, H-10b), 3.76 (1H, d, 9.6 Hz, H-11), 1.62–1.64 (1H, m, H-13a), 1.38–1.40 (1H, m, H-13b), 2.26–2.28 (1H, m, H-14a), 1.26–1.28 (1H, m, H-14b), 1.18–1.20 (1H, m, H-15), 0.86 (3H, d, J = 6.6 Hz, H-16), 0.71 (3H, d, J = 6.6 Hz, H-17), 1.73 (3H, s, H-18), 5.07 (1H, br d, J = 1.2 Hz, H-19a), 5.06 (1H, br d, J = 1.2 Hz, H-19b), 1.05 (1H, s, H-20), $^{13}$C NMR (CDCl$_3$, 150 MHz): 45.9 (CH, C-1), 70.4 (CH, C-2), 125.3 (CH, C-3), 137.7 (C, C-4), 36.4 (CH$_2$, C-5), 30.1 (CH$_2$, C-6), 67.8 (CH, C-7), 147.5 (C-8), 30.9 (CH$_2$, C-9), 27.1 (CH$_2$, C-10), 70.6 (CH, C-11), 74.6 (C, C-12), 18.6 (CH$_2$, C-13), 33.7 (CH$_2$, C-14), 29.0 (CH$_3$, C-15), 20.7 (CH$_3$, C-16), 20.3 (CH$_3$, C-17), 14.2 (CH$_3$, C-18), 112.4 (CH$_3$, C-19), 23.5 (CH$_3$, C-20); HRESIMS data $m/z$ 322.2501 [M]+ (Calculated 322.2508 for C$_{20}$H$_{34}$O$_3$).

4.3. Antibacterial activity assay

The growth inhibition of the isolated compounds against several pathogenic bacteria was carried out using agar well diffusion. First 0.1 mL of suspended bacterium in sterile medium ($1.5 \times 10^8$ CFU/mL) was spread on Mueller–Hinton agar. Then 50 µL of each sample (10 g/mL) was poured into the wells (6-mm diameter). For yeast and fungi, cell and spore suspensions ($2 \times 10^4$ CFU/mL) were spread on potato dextrose agar. All plates were left for 1 h at 48 °C and then incubated for 24 h at 37 °C for bacteria and 4 days at 25 °C for fungi. Inhibition zone diameters formed around the well were measured and the mean diameter of three replicates was calculated. DMSO was used as a negative control and ampicillin as a positive control.

MICs were determined by a modified version of the method described by Chand et al. and Aly and Gumgumji. First 175 µL of an exponentially growing culture ($10^6$–$10^7$ CFU/mL) was poured into each well of the microtiter plate along with 20 µL of solution of each concentration of the isolated compounds. The appropriate solvent was used as control. Then 5 µL of a solution of fluorescein diacetate (FDA) 0.2% (w/v) in acetone was added to the microtiter plate after incubation for 40 min, and incubation was continued for 90 min. The green color produced from the hydrolysis of FDA was measured at 490 nm (MR7000 automatic ELISA tray reader) along with the blanked wells and control wells containing microbial cultures.

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References