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Antiproliferative homoscalarane sesterterpenes from two Madagascan sponges★

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Abstract

Dereplication of the antiproliferative ethyl acetate fraction of the Madagascan sponge *Carteriospongia* sp. led to the detection and isolation of the two known homoscalarane-type sesterterpenes **1** and **2**. Investigation of a similar sponge containing closely related compounds afforded the four new antiproliferative homoscalarane sesterterpenes (**3**, **5**–**7**). The structures of all isolated compounds were elucidated by spectroscopic methods, including UV, IR and 1D- and 2D-NMR. Compounds **1**, **3** and **5** displayed submicromolar antiproliferative activity against the A2780 ovarian cell line with IC₅₀ values of 0.65, 0.26 and 0.28 μM, respectively, while compounds **6** and **7** showed moderate activity (4.5 and 8.7 μM, respectively). Compounds **3** and **5** also displayed anti-proliferative activity against the H522-T1 non-small cell lung and A2058 human melanoma cancer cell lines.

Keywords

Marine natural products; Sponge; *Carteriospongia*; Homoscalarane; Sesterterpenes; Antiproliferative activity; Nuclear magnetic resonance

1. Introduction

Despite the improvement of pharmaceuticals and good responses to initial chemotherapy, surgery and radiation therapy, epithelial ovarian cancer is still one of the leading causes of gynecological cancer mortality. In the United States alone, 22,240 new cases and 14,030 deaths are estimated for 2013.¹ One of the reasons for the increase of these numbers is

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Supplementary data Copies of ¹H spectra of compounds **3**, **5**–**7**, and of the ¹³C NMR spectra of compounds **3**, **6**, and **7**. This information can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.xxx>. These data include MOL files of compounds **3**, **5**–**7**.

chemoresistance, and so the search for new and selective pharmaceuticals is as important as it is challenging. One of the aims of the Madagascar International Cooperative Biodiversity Group (ICBG) program is the discovery of potential anticancer natural products from Madagascar. Sponges are well known to contain bioactive secondary metabolites, and the recent introduction of eribulin mesylate (Halaven™, Eisai) into clinical use demonstrates that even a synthetically challenging drug can be developed successfully. Eribulin is a synthetic analogue of the sponge derived compound halichondrin B, and it has been approved by the Food and Drug Administration for treatment of patients with metastatic breast cancer who have failed at least two prior rounds of anthracycline and taxane-based chemotherapy.² Madagascan sponges (*Lendenfeldia* sp., *Phyllospongia* sp.) are rich sources of a variety of scalarane-type sesterterpenes which have a broad range of biological activities, including anticancer activity.³

2. Results and discussion

2.1. Isolation and Structure Elucidation of Bioactive Sesterterpenes

In the expectation of finding bioactive ingredients, we have been carrying out chemical investigations of ethanol extracts from a variety of Madagascan marine organisms. Preliminary bioassay of the extracts received using the A2780 human ovarian cell line demonstrated that extracts of a sponge identified as a *Carteriospongia* sp. and of an unidentified marine sponge both showed good antiproliferative activity with IC₅₀ values of 3.3 and 3 µg/mL, respectively. The activity of the extract of the *Carteriospongia* sp. was found to be concentrated in the EtOAc fraction (IC₅₀ 2.4 µg/mL) obtained from a liquid-liquid partition. Chromatography of the EtOAc fraction on a size exclusion column gave an active fraction with improved activity (IC₅₀ 1.3 µg/mL). About 2 mg of this active fraction was subjected to dereplication using HPLC followed by off-line bioassay, Electrospray Ionization Mass Spectrometry, ¹H NMR and analysis using the ¹H NMR search features of MarinLit and the Dictionary of Natural Products. These studies identified the known compound 12α-acetoxy-22-hydroxy-24-methyl-24-oxoscalar-16-en-25-al (**1**) as the most active constituent (IC₅₀ 0.65 µM), together with the weakly active 16β,22-dihydroxy-24-methyl-24-oxoscalar-25,12β-olactone (**2**).⁴ HPLC/UV analysis of the bioactive EtOAc soluble fraction (IC₅₀ 0.45 µg/mL) of the EtOH extract of the second sponge extract showed a very similar HPLC profile to that of the *Carteriospongia* sp. extract, suggesting that this sponge was also a *Carteriospongia* sp. Size exclusion chromatography of this second extract on Sephadex LH-20 followed by further purification by HPLC and silica gel column chromatography of the most active fraction afforded the four bioactive homoscalarane compounds **3** and **5–7**.

Compound **3** had the molecular formula C₂₆H₄₀O₄ as determined by positive-ion high resolution electrospray ionization (HRESI) mass spectrometry [*m/z* 439.2819 [M+Na]⁺ (calcd for C₂₆H₄₀O₄Na, 439.2824)], which displayed a quasi-molecular ion peak at *m/z* 439.2819 [M+Na]⁺. Its IR spectrum showed absorption bands at 3436, 1713, and 1653 cm⁻¹, suggestive of hydroxyl, saturated carbonyl and α,β-unsaturated carbonyl functions. The ¹H NMR spectroscopic data of **3** (Table 1) exhibited signals for five methyl singlets, four on *sp*³ carbons (δ 0.80, 0.86, 0.90, 1.11) and one on an *sp*² carbon (δ 2.27), one hydroxymethylene group (δ 3.82 and 4.00, each d, *J* = 11.9 Hz), one oxygen-bearing methine (δ 3.30, overlapped with the solvent protons), one olefin methine (δ 7.30, dd, *J* = 4.8, 2.8 Hz) and an aldehyde proton (δ 9.89, d, *J* = 2.4 Hz). Inspection of the ¹³C NMR spectrum revealed signals for quaternary methyl groups, aldehyde (δ_C 200.3, C-25) and methyl ketone (δ_C 205.5, C-24) carbonyl groups, two olefin carbons at δ_C 136.8 and 146.1 ppm (C-17 and C-16), a C-22 hydroxymethylene group (δ_C 62.2), and an oxymethine carbon at δ_C 77.6; this carbon was coupled to the C-12 proton at δ 3.30 as observed in an HSQC spectrum. The above data suggested that compound **3** was a type II homoscalarane

sesterterpene which was oxygenated at C-12, C-22, C-24 and C-25.⁵ The NMR data of **3** were very similar to those of the homoscalarane sesterterpene **4** previously isolated from a *Lendenfeldia* species.⁶ Comparison of the ¹H- and ¹³C NMR data of **3** with those of **4** indicated that the difference between the two compounds was only in their stereochemistry at C-12 and/or C-18. 2D-NMR experiments were then carried out to confirm the planar structure of **3** and to clarify the orientation of the substitutions at C-12 and C-18. In an HMBC spectrum, long-range correlations were observed from the oxymethylene proton signals at δ 3.82 and 4.00 and C-1, C-5, C-9 and C-10, the oxygen-bearing methine at δ 3.30 and C-9, C-14, and C-23, the aldehyde proton signal at δ 9.89 and C-13, C-17, and C-18; the olefin methine signal at δ 7.30 and C-14, C-18 and the methyl ketone at C-24 (δ 205.5). The α -orientations of the hydroxyl group at C-12 and the aldehyde at C-18 were determined by interpretation of a NOESY spectrum. As illustrated in Figure 1, NOESY correlations were observed between H-12 (δ 3.30) and CH₃-19 (δ 1.11), between CH₃-19 and CH₃-23 (δ 0.90), between CH₃-23 and H-18 (δ 3.70) and between H-18 and CH₃-26 (δ 2.27). The relative stereostructure of **3** was thus determined to be 12 α ,22-dihydroxy-24-methyl-24-oxoscalar-16-en-25 α -al.

Compound **5** had the same molecular formula as **3** (C₂₆H₄₀O₄) as deduced by positive-ion HRESIMS (m/z 439.2819 [M+Na]⁺, requires 439.2824). Similarly to **3**, the IR spectrum showed absorption bands for hydroxyl, saturated carbonyl and α,β -unsaturated carbonyl functions. In addition the ¹H and ¹³C NMR data of **5** (Table 1) were almost superimposable with those of **3**, indicating that compounds **3** and **5** must share the same planar structure. Comparison of the ¹³C NMR data of **3** with those of **5** revealed that the major differences between the two compounds were the chemical shifts of C-12, C-18 and C-23, signals at δ_C 83.0, 63.0 and 10.1 (C-12, C-18 and C-23, respectively) were observed in **5** instead of δ_C 77.6, 57.3 and 16.1 in **3**. Interestingly, the ¹H and ¹³C NMR chemical shifts of the aldehyde group of **5** were the same as those of **3**, and the chemical shift of H-12 appeared at δ 3.40 as a doublet of doublets (J = 13.8, 6.7 Hz), suggesting that the proton at C-12 was axially oriented. Thus, the only possible differences between the two compounds must be the orientation of the hydroxyl group at C-12 and/or the aldehyde group at C-18. The assignment of the carbons in the structure of **5** and the determination of the orientation of the substitutions at C-12 and C-18 were made by 2D-NMR experiments including HSQC, HMBC and NOESY. The planar structure was confirmed to be 12,22-dihydroxy-24-methyl-24-oxoscalar-16-en-25-al by interpretation of the HSQC, COSY and HMBC spectra, which displayed similar correlations with those observed in the spectra of **3**. The β -orientations of the hydroxyl group at C-12 and the aldehyde at C-18 were determined by the interpretation of the NOESY spectrum. As depicted in Figure 2, NOESY correlations were observed between H-12 (δ 3.40) and H-18 (δ 3.68), between H-19 (δ 1.13) and H-23 (δ 0.77), between H-23 and the aldehyde proton H-25 (δ 9.87), and between H-25 and H-26 (δ 2.24). The relative stereostructure of **5** was thus assigned as 12 β ,22-dihydroxy-24-methyl-24-oxoscalar-16-en-25 β -al.

The molecular formula of compound **6** was determined to be C₂₆H₃₈O₃ by positive-ion HRESIMS analysis (m/z 399.2916 [M+H]⁺, requires 399.2899). Its IR spectrum displayed absorption bands of hydroxyl group at 3432 cm⁻¹ and a carbonyl group at 1693 cm⁻¹, consistent with a cyclopentenone with additional conjugation. The ¹H NMR spectrum had signals for four singlet methyl groups at δ 0.56, 0.81, 0.88, and 1.09, assigned to H-23, -20, -21 and H-19, respectively, one hydroxymethylene group (δ 3.86 and 4.01, each a doublet, J = 11.9 Hz); an axially oriented proton of an oxygen-bearing methine (δ 3.44, dd, J = 11.5, 4.4 Hz); and three olefinic methine protons (δ 6.65, brs, H-16, δ 8.11, ddd, J = 6.1, 2.2, 1.0 Hz, H-25 and δ 6.24, ddd, J = 6.0, 2.3 Hz, H-26). The ¹³C NMR data of **6** (Table 2) revealed the presence of 26 resonances, including four methyls (δ 9.0, 16.3, 22.2, 34.4; C-23, -19, -20, and -21, respectively); eight methylenes (one of which is attached to an oxygen

function; δ 62.5, C-22); eight methines; one carbonyl (δ 198.7); and five quaternary carbons, ascribable to a homoscalarane-type sesterterpene. The planar structure of **6** as well as the allocation of the functionalities was determined by HSQC, COSY, HMBC and NOESY experiments. In the COSY spectrum, the spin systems: CH₂-CH₂-CH₂ (H-1 through H-3), CH-CH₂-CH₂ (H-5 through H-7), CH-CH₂-CH (H-9 through H-12), CH-CH₂-CH (H-14 through H-16), and CH-CH-CH (H-18 through H-26) were observed. The presence of a cyclopentenone ring at C-17 and C-18 and the location of the carbonyl group and the two double bonds were determined by the observation of long range HMBC cross peaks from H-25 (δ 8.11) to C-13, and to C-17 and to the carbonyl (δ 198.7); from the methyl proton signal at δ 0.56 (H-23) to C-18 and to C-14; from H-26 (δ 6.24) to C-18, and C-17; from H-16 (δ 6.64) to C-14, and C-18, and the carbonyl group. These assignments were supported by the downfield shift of the ¹H and ¹³C resonances of the proton and carbon at the 25-position (δ _H 8.11 ppm and δ _C 165.8 ppm, respectively). The presence of a hydroxyl group at C-12 was substantiated by long range correlations between H-12 and C-9, C-11, C-13, C-23 and C-14. The location of the hydroxymethylene at C-22 was confirmed by the HMBC correlation between H-22 and C-1, C-10, C-5 and C-9.

The ¹H and ¹³C NMR data of **6** were very similar to those of phyllofenone B (**8**), previously isolated from the sponge *Phyllospongia foliascens*,⁷ except for the signals due to the oxygen-bearing carbon at C-12 (δ 83.2), the hydroxyl group at C-22, and the methyl group at C-21. The β -orientation of the hydroxyl group at C-12 and the configuration of C-18 were deduced from the NOESY cross peaks between H-22 and H-21, H-22 and H-19, H-19 and H-23, H-23 and H-25, and H-12 and H-18 (Figure 3). From the above data the relative stereostructure of **6** was assigned as 12 β ,22-dihydroxy-24-oxo-24-homoscalara-16,25(26)-diene. The 12-epimer of 22-deoxygenated **6**, phyllofenone D, has been isolated from the Chinese sponge *Phyllospongia foliascens*.⁸ Biosynthetically **6** can be seen as the product of an aldol condensation between the methyl ketone and the aldehyde carbonyl group of **5**.

The molecular formula of compound **7** was established as C₂₆H₄₀O₅ by positive-ion HRESIMS (m/z 433.2958 [M+H]⁺, requires 433.2954). The presence of hydroxyl groups and an α,β -unsaturated lactone in **7** was suggested by the IR absorption bands at 3331 cm⁻¹ and at 1715 cm⁻¹ and 1664 cm⁻¹, respectively. Inspection of the ¹H and ¹³C NMR data (Table 2) showed that **7** is a pentacyclic scalarane-type sesterterpene with α,β -unsaturated lactone and acetal functions. The ¹H NMR spectrum displayed five singlet methyl resonances (δ 0.81, 0.88, 1.08, 1.12, 1.54) in addition to the signals of the hydroxymethylene (δ 3.83 and 4.00, each doublet, J = 12.1 Hz) and the axial proton of an oxygen-bearing methine (δ 3.47, dd, J = 10.8, 4.7 Hz). The ¹³C NMR data indicated the presence of an α,β -unsaturated lactone (δ 166.5, 136.7 and 175.2), and an acetal group (δ 107.5 ppm), together with signals for a 22- and 12 β -hydroxy-substituted scalarane. Examination of the ¹H and ¹³C NMR data of **7** revealed a close similarity to those of 22-hydroxy-24-methyl scalarolide (**9**)⁹ except for the presence of the acetal carbon signal at C-24 (δ 107.5) in **7**. The planar structure of **7**, the allocation of the hydroxyl groups at C-12, C-22, and C-24, the methyl group at C-24 as well as the presence of an α,β -unsaturated lactone ring at C-17 and C-18 were corroborated by interpretation of the HSQC, COSY and HMBC spectra. Long range HMBC cross peaks were observed from the signal due to the methyl group at δ 1.08 (CH₃-23), to C-12 (δ 78.0), C-13 (δ 43.5), C-14 (δ 56.6) and to C-18 (δ 136.7). Moreover, the proton signal at δ 1.54 showed long range correlations with C-24 (δ 107.5) and C-17 (δ 166.5) while the ¹H signal of H-16 (δ 2.46, dd, J = 19.8, 5.2 Hz) displayed HMBC correlations with C-17, C-18 and C-14 (δ 56.6). The location of the lactone carbonyl at C-25 was substantiated by the abnormally low frequency of the IR absorption band of the lactone carbonyl group, due to the interaction of the hydroxyl group at C-12.⁹ From the above data, the structure of **7** was deduced to be 12 β ,22,24e-trihydroxy-24-methylscalar-17-ene-18,24-carbolactone. This structure is closely related to

that of phyllolactone E (**10**), a bishomoscalarane from the marine sponge *Phyllospongia lamellose*.¹⁰ Biosynthetically the hydroxylactone can be formed by oxidation of the aldehyde group of **5** to a carboxyl group, followed by intramolecular lactone formation with the C-25 carbonyl group. Since hydroxylactone formation is reversible the configuration of C-24 was not determined, but the stereochemistry at C-24 of the related compound **10** was reported to be *S* by a NOESY experiment. Although the proton and carbon chemical shifts arising from the lactone- and the D-ring of the present compound are superposable with those of **10**, the overlapping of the signals of H-15 and H-16b (1.98 and 1.99, respectively) which had NOESY correlations with H-14 (1.45, m) did not allow firm conclusions on the stereochemistry at C-24 to be drawn.

2.2. Biological Activities

Alkylated scalarane-type sesterterpenes or homoscalaranes are reported to be chemical markers of sponges belonging to the order Dictyoceratida and their nudibranch associates.⁵ Although the taxonomy of one of the sponges that is the subject of this study has not been determined, the similarity of its secondary metabolites to those from a sponge of the *Carteriospongia* genus, together with the similarity of the isolated compounds (**3**, **5**–**7**) with those obtained from a member of the *Phyllospongia* genus, which is a synonym of the *Carteriospongia* genus, suggests that it is also a member of this genus. Both sponges belong to the Dictyoceratida order of homoscalarane-rich sponges.

The present bioassay guided isolation work provided compounds **1**, **3** and **5** as the compounds with the strongest antiproliferative activities from the two marine samples (Table 3). Compounds **1**, **3** and **5** displayed activity against the A2780 human ovarian cancer cell line with IC₅₀ values of 0.65, 0.26 and 0.28 μ M, respectively, while compounds **6** and **7** displayed only moderate activity (IC₅₀ 4.5 and 8.7 μ M respectively). The IC₅₀ values of compounds **1**, **3** and **5** with aldehyde groups and an α,β -unsaturated ketone were very similar and were an order of magnitude lower than the values for **6** and **7**, which have α,β -unsaturated ketone or lactone groups but not aldehyde groups. It thus appears that the presence of the aldehyde group in compounds **1**, **3** and **5** is a key structural factor in the higher potency of these compounds.

Compounds **3** and **5**–**7** were also tested against the H522-T1 non-small cell lung and human A2058 melanoma cancer cell lines. Compound **5** showed good activity against H522-T1 cells, with an IC₅₀ of 0.61 μ M. Compound **5** is more active than **3** in this cell line, suggesting that the stereochemistry at C-12 and C-18 plays a role in this activity.

3. Experimental

3.1. General Experimental Procedures

Optical rotations were recorded on a JASCO P-2000 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. ¹H and ¹³C NMR spectra were recorded on a JEOL Eclipse 500 and Bruker 600 spectrometers in CD₃OD and referenced to residual CH₃OH (83.31 and 49.0 ppm resonances for ¹H and ¹³C NMR spectra, respectively). Mass spectra were obtained on JEOL JMS-HX-110, Agilent 6220 LC-TOF-MS, or Finnigan LTQ LC/MS instruments. Preparative HPLC was performed using Shimadzu LC-10AT pumps coupled with a semipreparative Varian Dynamax C-8 column (5 μ m, 250 $\times$ 10 mm), a Shimadzu SPD M10A Diode Array Detector (DAD) and a SCL-10A system controller.

3.2. Antiproliferative Bioassays

The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported.¹¹ The A2780 cell line is a drug-sensitive ovarian cancer cell line.¹² Assays against H522-T1 non-small cell lung, human A2058 melanoma cancer cell lines were carried out at Eisai, Inc., as previously described for similar cell lines.¹³

3.3. Marine organisms

The sponges were collected using SCUBA off the coast of Nosy Be, Madagascar (13°11' 13" S 049°42' 40" E, Northern Madagascar). The identification of the *Carteriospongia* sp. (NB-04-05-44) organism was made by Dr. Jean Maharavo. The voucher specimen of the second sponge, designated NB-04-06-17, decomposed due to a power failure, and attempts to recollect it failed. In addition, the photograph of this sponge was of poor quality and was not suitable for identification purposes. Duplicate voucher specimens of NB-04-05-44 were deposited at the Centre National d'Application des Recherches Oceanographique (CNRO), Nosy Be, Madagascar.

3.4. Extraction of the Sponges

Fresh *Carteriospongia* sp. sponge NB-04-05-44 (250 g), was soaked for 24 h in 1:1 EtOH:seawater to fix the organism. The aqueous EtOH supernatant was decanted off, and the sponge cut into small pieces and soaked in EtOH (approximately 10-fold excess) for 24 h. The EtOH was decanted off and the process repeated with a second batch of EtOH. The two EtOH fractions were combined and evaporated to yield 6.0 g of crude extract. A sample of 1.80 g of this extract was made available to Virginia Tech. The same process with NB-04-06-17 yielded 6.5 g of extract from 270 g of wet sponge, and 1.88 g of this extract were made available to Virginia Tech.

3.5. Fractionation of *Carteriospongia* sp. sponge extract NB-04-05-44

About 100 mg of the *Carteriospongia* sp. extract NB-04-05-44 was dissolved in MeOH (100 mL) to remove salts. The MeOH supernatant was removed, evaporated, and resuspended in H₂O (100 mL) before extracting it with EtOAc (2 × 100 mL). The EtOAc fraction showed significant antiproliferative activity with an IC₅₀ value of 2.4 µg/mL. This active extract was then subjected to size exclusion chromatography on a column of Sephadex LH-20 (1.8 × 39 cm) with elution by CH₂Cl₂:MeOH, 1:1, to give four fractions, of which the second fraction (Fr-2) was the most active (1.3 µg/mL).

3.6. Dereplication

The active Fr-2 was dissolved in 1 mL of MeOH and 100 µL of the solution was injected onto a semipreparative Varian Dynamax C-8 column (5 µm, 250 × 10 mm) and eluted with a solvent gradient from H₂O:CH₃CN 50:50 for 20 min, to 40:60 from 20 to 25 min, to 30:70 from 25 min to 35 min and to 0:100 from 35 min to 37 min, ending with 100% CH₃CN at 60 min. Eleven fractions (0–9.5 min; 9.6–10.2 min; 10.3–11.9 min; 12.0–12.5 min; 12.6–13.2 min; 13.3–13.8 min; 13.9–14.47 min; 14.49–14.8 min; 14.9–15.6 min; 15.7–16.2 min and from 16.3 min till the end of the run) were collected, evaporated and submitted to bioassay. The sixth fraction (13.3–13.8 min) was the most active (IC₅₀ = 0.3 µg/mL). A second HPLC run under the same conditions was performed to purify this fraction (13.3–13.8 min, peak) for ¹H NMR and MS analysis. The resulting active compound **1** (*m/z* 481.2938 [M+Na]⁺, C₂₆H₄₂O₅Na requires 481.2930) exhibited six methyl signals in its ¹H NMR spectrum (δ 0.75, 0.87, 0.95, 1.15, 2.16, 2.33, each singlet, 3-H) and a signal for an aldehyde proton (δ 9.41, d, *J* = 3.9 Hz). These characteristics identified it as 12α-acetoxy-22-hydroxy-24-methyl-24-oxoscalar-16-en-25-al, previously isolated from a sponge of the genus

Lendenfeldia.⁴ Compound **1** was reisolated on a larger scale to confirm its stereochemistry, and the less active compound **2** (IC₅₀ = 23 μ M) was also isolated during this process.

3.7. Fractionation of Sponge Extract NB-04-06-17

The EtOH extract NB-04-06-17 (1.5 g) was dissolved in MeOH (250 mL) to remove salts, and the MeOH-soluble fraction was evaporated and suspended in H₂O (250 mL) before extraction with EtOAc (3 $\times$ 250 mL). The EtOAc fraction showed significant antiproliferative activity to the A2780 ovarian cancer cell line, with an IC₅₀ value of 0.45 μ g/mL. This active extract was then subjected to size exclusion column chromatography on a Sephadex LH-20 (2.5 $\times$ 47 cm) with elution by CH₂Cl₂:MeOH, 1:1, to give five fractions. All five fractions showed antiproliferative activity, with fractions 3 and 4 displaying the lowest IC₅₀ values of 0.72 and 0.73 μ g/mL. Fraction 4 was subjected to HPLC on a semipreparative Varian Dynamax C-8 column (25 $\times$ 1.0 cm) with a solvent gradient from H₂O:CH₃CN 50:50 for 20 min, to 40:60 from 20 to 25 min, to 30:70 from 25 min to 35 min and to 0:100 from 35 min to 37 min, ending with 100% acetonitrile at 37 min to give compound **6** (2.8 mg, *t_R*: 17.53 min). The peaks at *t_R* 15.05 and 18.90 min were further purified by chromatography on a silica gel column (2.0 $\times$ 22 cm, solvent system hexane:EtOAc, 4:6) to give compounds **3** (0.9 mg) and **7** (0.92 mg). Fraction 3 was subjected to HPLC on a semipreparative Varian Dynamax C-8 column (25 $\times$ 1.0 cm) using the same solvent system as above to afford compound **5** (1.2 mg, *t_R*: 15 min).

3.8. 12 α ,22-dihydroxy-24-methyl-24-oxoscalar-16-en-25 α -al (**3**)

Amorphous powder, [α]_D -47.5 (*c* 0.2, CH₃OH); IR (film, MeOH) 3436, 1713, 1653, 1209 cm⁻¹; UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 232 (2.8), 210 (2.6); ¹H NMR and ¹³C NMR spectral data, see Table 1; positive ion HRESIMS *m/z* 439.2819 [M+Na]⁺ (calcd for C₂₆H₄₀O₄Na, 439.2824).

3.9. 12 β ,22-dihydroxy-24-methyl-24-oxoscalar-16-en-25 β -al (**5**)

Amorphous powder; [α]_D +11.5 (*c* 0.4, CH₃OH), IR (film) 3436, 1713, 1653, 1209cm⁻¹; UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 235 (2.4), 211 (2.3); ¹H NMR and ¹³C NMR spectra, see Table 1; positive ion HRESIMS *m/z* 439.2819 [M+Na]⁺ (calcd for C₂₆H₄₀O₄Na, 439.2824).

3.10. 12 β ,22-dihydroxy-24-oxo-24-homoscalara-16,25(26)-diene (**6**)

Amorphous powder; [α]_D +60 (*c* 0.4, CH₃OH), IR (film) 3432, 2927, 1693, 1660 and 1465cm⁻¹; UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 252 (3.1); ¹H NMR and ¹³C NMR spectra, see Table 2; positive ion HRESIMS *m/z* 399.2916 [M+H]⁺ (calcd for C₂₆H₃₉O₃, 399.2899).

3.11. 12 β ,22,24 ϵ -trihydroxy-24-methylscalar-17-ene-18,24-carbolactone (**7**)

Amorphous powder, [α]_D²⁹ -22.3 (*c* 0.2, CH₃OH); IR (film) 3431, 2928, 1715, 1663 and 1443cm⁻¹; UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 218 (3.1); ¹H and ¹³C NMR see Table 2; positive ion HRESIMS *m/z* 433.2958 [M+H]⁺ (calcd for C₂₆H₄₁O₅, 433.2954).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
NOESY Correlations observed in **3**.

Figure 2.
NOESY Correlations observed in **5**.

Figure 3.
NOESY Correlations observed in **6**.

Table 1

¹H and ¹³C NMR (500 and 125 MHz, respectively) data for compounds 3–5 in CD₃OD

position	3		4 ⁶	5	
	δ_{H} (J in Hz)	δ_{C}	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1	0.72 td ($J = 11.0, 3.6$) 2.33 m	35.6	34.3	0.73 td ($J = 11.0, 3.6$) 2.32 m	35.6
2	1.52 m	18.9	17.7	1.52 m	19.0
3	1.42 m	43.1	41.6	1.42 m	43.3
	1.81 dt ($J = 12.9, 2.9$)			1.84 dt ($J = 12.9, 2.9$)	
4		33.9	32.8		33.9
5	0.94 dd ($J = 12.7, 2.6$)	58.2	56.7	0.90 m	58.2
6	1.42 m	19.5	18.3	1.42 m	19.5
	1.67 m			1.67 m	
7	0.95 m	43.5	41.9	0.95 m	43.1
	1.20 m			1.20 m	
8		38.5	37.3		38.5
9	0.83 brd ($J = 12.5$)	60.3	58.5	0.88 brd ($J = 12, 5$)	60.2
10		43.0	42.0		43.5
11	1.88 brd ($J = 11.9$)	30.9	29.8	1.89 m	31.4
	1.98 brt ($J = 11.9$)			1.90 brt ($J = 11.9$)	
12	3.30 overlapped	77.6	81.8	3.40 dd ($J = 13.8, 6.7$)	83.0
13		44.9	43.9		43.1
14	1.32 dd ($J = 11.1, 5.9$)	49.8	52.9	1.32 dd ($J = 11.1, 5.9$)	49.8
15ab	2.47 dt ($J = 20.0, 5.2$)	25.0	23.2	2.45 m	25.2
16	7.30 dd ($J = 4.8, 2.8$)	146.1	143.0	7.17 brs	145.0
17		136.8	138.6		136.8
18	3.70 brs	57.3	61.3	3.68 (brs)	63.0
19	1.11 s	16.8	16.4	1.13 s	17.1
20	0.86 s	34.4	33.7	0.86 s	34.4
21	0.80 s	22.3	25.0	0.80 s	22.3
22	3.82 d ($J = 11.9$)	62.6	62.6	3.82 d ($J = 11.9$)	62.6
	4.00 d ($J = 11.9$)			3.99 d ($J = 11.9$)	
23	0.90 s	16.1	9.6	0.77 s	10.1
24		205.5	205.5		205.3
25	9.89 d ($J = 2.4$)	200.3	200.3	9.87 d ($J = 2.6$)	200.3
26	2.27 s	24.8	24.8	2.24 s	24.4

Table 2¹H and ¹³C NMR Spectroscopic data (500 and 125 MHz, respectively) for compounds **6** and **7** in CD₃OD

position	6		7	
	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H}	δ_{C}
1	0.75 td (<i>J</i> = 11.0, 3.6) 2.35 brd (<i>J</i> = 13.6)	35.7	0.75 td (<i>J</i> = 12.0, 3.5) 2.33 brd (<i>J</i> = 12.0)	35.4
2ab	1.54 m	19.0	1.55 m	19.1
3	1.45 m 1.81 dt (<i>J</i> = 12.7, 2.5)	43.1	1.45 m 1.70 m	43.2
4		33.9		33.9
5	0.99 dd (<i>J</i> = 12.7, 2)	58.2	0.97 m	58.4
6	1.42 m	19.5	1.43 m	19.6
7	0.98 m 1.22 m	43.5	0.89 m 1.20 m	43.3
8		38.9		38.5
9	1.05 brd (<i>J</i> = 12,5)	60.6	1.00 m	60.0
10		43.1		43.2
11	1.95 dm (<i>J</i> = 13.8) 2.02 m	31.8	1.97 m 2.01 m	29.6
12	3.44 dd (<i>J</i> = 11.5, 4.4)	83.2	3.47 dd (<i>J</i> = 10.8, 4.7)	78.0
13		42.6		43.5
14	1.40 dd (<i>J</i> = 11.2, 5.9)	55.2	1.45 m	56.6
15a	2.45 m	25.5	1.98 overlapped	17.4
15b	2.48 m		1.98 overlapped	
16a	6.65 brs	132.8	2.46 dd (<i>J</i> = 19.8, 5.2)	24.2
16b			1.99 overlapped	
17		137.9		166.5
18	3.07 brs	58.2		136.7
19	1.09 s	16.3	1.12 s	17.2
20	0.81 s	22.2	0.81 s	22.2
21	0.88 s	34.4	0.88 s	33.9
22	3.86 d (<i>J</i> = 11.9) 4.01 d (11.9)	62.5	3.83 d (<i>J</i> = 12.1) 4.00 d (<i>J</i> = 12.1)	62.4
23	0.56 s	9.0	1.08 s	16.7
24		198.7		107.5
25	8.11 ddd (<i>J</i> = 6.1, 2.2, 1.0)	165.8		175.2
26	6.24 dd (<i>J</i> = 6.0, 2.3)	135.9	1.54 s	23.7

Table 3

Antiproliferative activities of compounds 1, 3, 5–7

Compound	Cancer cell line (IC ₅₀ μ M)		
	A2780	A2058	H522-T1
1	0.65	NT	NT
3	0.26	1.83	1.70
5	0.28	0.37<x<1.11	0.61
6	4.5	4.17	5.28
7	8.7	>10	>10
Taxol	0.012	0.0047	0.0081