Tetrahedron 71 (2015) 577–583

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Scalarane sesterterpenes from the Egyptian Red Sea sponge *Phyllospongia lamellosa*

Marwa H.A. Hassan [a], Mostafa E. Rateb [a,b], Mona Hetta [a], Tarek A. Abdelaziz [c], Mohamed A. Sleim [d], Marcel Jaspars [b,*], Rabab Mohammed [a,*]

[a] *Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, 62514, Egypt*
[b] *Marine Biodiscovery Centre, Department of Chemistry, University of Aberdeen, AB24 3UE Scotland, UK*
[c] *Marine Invertebrates, National Institute of Oceanography and Fisheries, Red Sea Branch, Hurghada 84511, Egypt*
[d] *Pharmacognosy Dept., Faculty of Pharmacy, Cairo University, Cairo, 11787, Egypt*

ARTICLE INFO

Article history:
Received 1 October 2014
Received in revised form 25 November 2014
Accepted 9 December 2014
Available online 13 December 2014

Keywords:
Scalarane sesterterpenes
Phyllospongia lamellosa
Phyllospongin
Cytotoxic
Antibacterial

ABSTRACT

Biology and HRESIMS-guided screening of the dichloromethane fraction of the marine sponge *Phyllospongia lamellosa* collected from the Red Sea resulted in the isolation and characterization of five new scalarane sesterterpenes; phyllospongins A–E (**1**–**5**), in addition to four known derivatives, 12α-acetoxy-20,24-dimethyl-25-norscalar-16-en-24-one (**6**), 12α-acetoxy-13β,18β-cyclobutan-20,24-dimethyl-24-oxoscalar-16-en-25β-ol (**7**), 12α-acetoxy-24,25-epoxy-24-hydroxy-20,24-dimethylscalarane (**8**), and scalardysin-A (**9**) that were previously isolated from *Carteriospongia* sp. and *Dysidea* sp. The structures of the isolated compounds were fully characterized using NMR spectroscopic techniques and mass spectrometric analysis. All the isolated compounds were tested for their cytotoxic activity against human cancer cell lines (HePG-2, MCF-7, and HCT-116) and antibacterial activity against some Gram-positive and Gram-negative strains.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Marine ecosystems comprise an enormous resource of chemical structures with diverse biological activities that may lead to potential new drug candidates.[1] Marine sponges are known as rich source of secondary metabolites with potent biological activities. Most of these metabolites have different carbon skeletons to those of terrestrial origin.[2,3] By 2012, around 25,000 marine natural products were discovered.[4] Some marine-derived metabolites or their semi-synthetic derivatives have already been approved for clinical use such as Prialt® (ziconotide; potent analgesic), Yondelis® (trabectedin or ET-743; antitumor), and eribulin mesylate was approved for use against breast cancer[5] while others are in advanced clinical trials, such as aplidine and kahalalide F.[1] The discovery of new bioactive compounds from natural sources requires biology-guided fractionation, chemical profiling of the metabolites using HRLCMS, and dereplication using available natural product databases.[6–10]

Sesterterpenoids are a group of pentaprenyl terpenoids derived from geranylfarnesyl diphosphate.[11] Although a small group, they are isolated from diverse sources like terrestrial fungi, lichens, higher plants, insects, and various marine organisms, especially sponges.[11] To date, more than one thousand sesterterpenoids have been isolated, which may play a variety of antagonistic and beneficial roles in the interactions between organisms.[11] Scalaranes are an important class of sesterterpenes and are a major constituent in *Dictyoceratida* sponges,[12] e.g., *Cacospongia* sp.,[13] *Hyrtios* sp.,[14] and *Phyllospongia* sp.[15] Different scalarane structure types have been classified as C_{25} (scalarane), C_{26} (homoscalarane), and C_{27} (bishomoscalarane), together with structures containing tetra- and pentacyclic skeletons[16] as recently outlined by Crews et al.[17] Scalarane sesterterpenes exhibit diverse biological activities including anti-inflammatory,[18] antimicrobial,[19] anti-platelet aggregation,[20] and cytotoxicity.[21] They are also reported to have antifeedant,[22] anti-fouling,[16] and ichthyotoxic[23] activities as potential defensive mechanisms in sponges. Regarding the chemistry of *Phyllospongia lamellosa* (synonym: *Phyllospongia madagascarensis*),[24] norscalaranes, tetracyclic and bishomoscalarane sesterterpenes, respectively, were previously isolated.[15,25] Together with screening of sterol contents of *P. madagascarensis*.[26]

Herein, cytotoxicity-guided screening together with LC–HRESIMS analysis allowed for the isolation, structure characterization, and biological screening of biologically active sesterterpenes from *P. lamellosa* collected from Hurghada, Egypt on the Red Sea in 2012.

* Corresponding authors. Tel.: +44 1224 272 895; fax: +44 1224 272 921 (M.J.); tel.: +20 822362211; fax: +20 822319397 (R.M.); e-mail addresses: m.jaspars@abdn.ac.uk (M. Jaspars), rmwork06@yahoo.com (R. Mohammed).

http://dx.doi.org/10.1016/j.tet.2014.12.035
0040-4020/© 2014 Elsevier Ltd. All rights reserved.

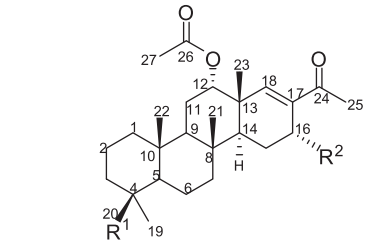
The cytotoxic dichloromethane fraction was further purified using different chromatographic techniques, which led to the isolation of compounds **1**–**9**. Using a combination of different NMR experiments in addition to accurate mass analysis indicated that five out of the nine isolated compounds were new natural products. The structures of the isolated compounds are described in detail together with their cytotoxic activity against different cancer cell lines as well as their antibacterial activity.

2. Results and discussion

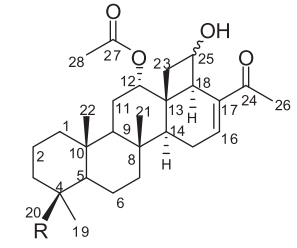
After collection from Hurghada, Egypt, the sample *P. lamellosa* was frozen and extracted with methanol. After concentration of the organic extract, the residue was suspended in water and partitioned using a modified Kupchan partition scheme[27] to give five main fractions, which were screened for their cytotoxic effect against different cancer cell lines. Dereplication using liquid chromatography–high-resolution electrospray ionization mass spectrometry (LC–HRESIMS) analysis together with ^{1}H NMR spectral data confirmed the presence of new compounds in the bioactive dichloromethane fraction, further chromatographic purification of that fraction over silica gel followed by Sephadex LH-20 and preparative C-18 HPLC (CH_3CN/H_2O) resulted in the isolation and characterization of nine scalarane sesterterpenes of which five were new (Fig. 1).

Compound **1** was obtained as a colorless solid, its molecular formula was established as $C_{29}H_{44}O_5$ based on the high-resolution electrospray ionization mass spectrometry (HRESIMS) that showed an $[M+H]^+$ ion at *m*/*z* 473.3254 (calcd *m*/*z* 473.3262). The ^{1}H NMR spectrum (Table 1) together with the multiplicity-edited HSQC showed resonances attributed to (i) eight singlet methyl groups at [δ_H 0.87 (H-19), 0.82 (H-20), 0.82 (H-21), 0.86 (H-22), 1.04 (H-23), 2.21 (H-25), 2.09 (H-27), 2.03 (H-29)], (ii) seven methylene groups, three methines, and two oxymethines in addition to a trisubstituted double bond. The ^{13}C NMR spectrum (Table 1) showed resonances, which indicate; (i) two acetate moieties at [δ_C 170.9 (C-26), 21.5 (C-27) and δ_C 170.3 (C-28), 21.4 (C-29)], which were further confirmed by the HMBC correlations (Fig. 2) of H_3-27/C-26 and H_3-29/C-28, respectively, (ii) a methyl ketone moiety at δ_C 197.5 (C-24) and 25.9 (C-25), which was confirmed by the HMBC correlation of H_3-25/C-24, (iii) a tri-substituted olefin at δ_C 135.0 (C-17) and $\delta_{C/H}$ 153.6/6.70 (C-18). These data indicated a normal C_{25} scalarane sesterterpene skeleton.[28] The 1D and 2D NMR spectral data of compound **1** showed a close similarity to the reported spectroscopic data of known compound flabelliferin B, previously isolated from *Carteriospongia flabellifera*.[3] The only difference between the two compounds was the additional resonances in NMR spectral data for an acetate moiety [δ_C 170.3 (C-28), $\delta_{C/H}$ 21.4/2.03 (C-29)] replacing the OH group at position-16 in flabelliferin B, which was confirmed by the HMBC correlations of H-16/C-28, H-16/C-18, and H-15/C-17 and the COSY correlation of H-15/H-16 (Fig. 2). The double bond at position-17/18 was supported by HMBC correlations of H-18 to C-13, C-14, C-17, and C-24. The relative configuration of **1** was assigned by comparison of its ^{1}H and ^{13}C NMR data with flabelliferin B,[3] phyllofenone C;[29] isolated from the closely related Indonesian sponge *Strepsichordia aliena*, 20,24-bishomo-25-norscalaranes isolated from another Indonesian marine sponge *Carteriospongia foliascens*[30] and by analysis of its ROESY data (Fig. 3). To determine the configuration at C-16, the ROESY correlations between H-16 to both H-15β and H-15α and no observed correlation with H-14 was consistent with molecular mechanics simulation (Fig. 3), together with comparison of coupling constant for H-16 (δ_H 5.74, dd, *J*=4.2, 1.4 Hz) (Table 1) with previously related compounds phyllofenone C (δ_H 5.75, dd, *J*=4.2, 1.5 Hz)[29] and 20,24-bishomo-25-norscalaranes (δ_H 4.54, d, *J*=4.1 Hz)[30] indicated an α-configuration of the acetate moiety at position-16. Therefore, compound **1** was assigned the name 12α,16α-diacetoxy-24-methyl-25-norscalar-17-en-24-one (phyllospongin A).

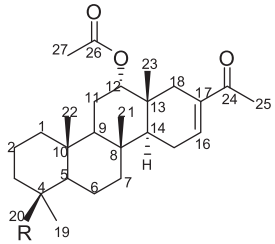
The molecular formula of compound **2** was deducted as $C_{30}H_{46}O_5$ based on the HRESIMS analysis that gave $[M+H]^+$ ion at *m*/*z* 487.3423 (calcd *m*/*z* 487.3418). The direct comparison of ^{1}H and ^{13}C NMR spectral data (Table 1) together with the 2D spectral data of compound **2** showed close similarity to that of **1** with the replacement of the methyl singlet at δ_H 0.82 (H-20) in **1** with an ethyl group at the same position in **2**. This was confirmed by the COSY correlations of H_2-20 (δ_H 1.52 and δ_H 1.18) to the methyl triplet at δ_H 0.75 (H_3-30) and the HMBC correlations of H_2-20 to C-4, C-3, C-5,



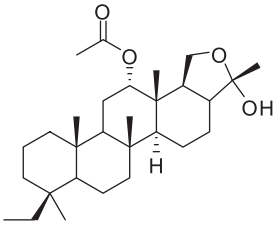
Phyllospongin A (**1**, R^1= CH_3, R^2= α-CH_3COO)
Phyllospongin B (**2**, R^1=CH_2CH_3, R^2 = α-CH_3COO)



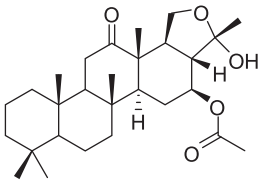
Phyllospongin D (**4**, R= CH_3, β-OH)
Phyllospongin E (**5**, R= CH_3, α-OH)
Compound **7** (**7**, R= CH_2CH_3, β-OH)



Phyllospongin C (**3**, R=CH_3)
Compound **6** (**6**, R= CH_2CH_3)



Compound (**8**)



Sclaradysin A (**9**)

Fig. 1. Structures of compounds **1**–**9**.

Table 1
Summary of ^{1}H and ^{13}C NMR spectroscopic data for compounds **1**–**3**[a,b]

Position	Phyllospongin A (**1**)		Phyllospongin B (**2**)		Phyllospongin C (**3**)	
	δ_C/ppm, type	δ_H/ppm (mult, *J* in Hz)	δ_C/ppm, type	δ_H/ppm (mult, *J* in Hz)	δ_C/ppm, type	δ_H/ppm (mult, *J* in Hz)
1	39.8, CH_2	0.75 (m) 1.57 (m)	40.2, CH_2	0.69 (m) 1.58 (m)	39.9, CH_2	0.74 (m) 1.59 (m)
2	18.6, CH_2	1.40 (m) 1.56 (m)	18.4, CH_2	1.36 (m) 1.48 (m)	18.6, CH_2	1.40 (m) 1.58 (m)
3	42.2, CH_2	1.13 (m) 1.37 (m)	36.8, CH_2	0.86 (m) 1.67 (m)	42.2, CH_2	1.13 (m) 1.36 (m)
4	33.5, C		36.3, C		33.4, C	
5	56.7, CH	0.90 (m)	58.7, CH	0.96 (m)	56.7, CH	0.84 (m)
6	18.1, CH_2	1.42 (m) 1.58 (m)	18.0, CH_2	1.45 (m) 1.56 (m)	18.2, CH_2	1.38 (m) 1.54 (m)
7	40.8, CH_2	0.93 (m) 1.72 (m)	41.1, CH_2	0.92 (m) 1.73 (m)	41.5, CH_2	1.01 (m) 1.73 (dt, 12.5, 9.1)
8	37.1, C		37.2, C		37.0, C	
9	53.1, CH	1.34 (dd, 13.0, 2.6)	53.5, CH	1.34 (m)	52.7, CH	1.27 (m)
10	36.9, C		36.9, C		37.5, C	
11	22.4, CH_2	1.75 (m) 1.80 (m)	22.3, CH_2	1.75 (m) 1.79 (m)	22.4, CH_2	1.68 (ddt, 14.6, 12.3, 1.9) 1.77 (dt, 14.5, 2.4)
12	76.5, CH	5.06 (t, 2.7)	76.5, CH	5.05 (t, 2.8)	77.3, CH	4.78 (t, 2.5)
13	41.3, C		41.3, C		35.9, C	
14	44.1, CH	1.77 (m)	44.1, CH	1.76 (m)	47.9, CH	1.54 (m)
15	24.2, CH_2	1.61 (m) 1.87 (m)	24.2, CH_2	1.61 (m) 1.86 (m)	24.0, CH_2	2.17 (m) 2.32 (m)
16	65.0, CH	5.74 (dd, 4.2, 1.4)	65.0, CH	5.74 (dd, 4.3, 1.5)	139.7, CH	6.85 (m)
17	135.0, C		135.0, C		137.8, C	
18	153.6, CH	6.70 (s)	153.6, CH	6.70 (s)	35.5, CH_2	1.94 (d, 16.6) 2.25 (m)
19	33.4, CH_3	0.87 (s)	28.6, CH_3	0.82 (s)	33.4, CH_3	0.85 (s)
20	21.5, CH_3	0.82 (s)	24.6, CH_2	1.18 (m) 1.52 (m)	21.5, CH_3	0.80 (s)
21	16.1, CH_3	0.82 (s)	16.9, CH_3	0.85 (s)	16.1, CH_3	0.91 (s)
22	17.2, CH_3	0.86 (s)	17.1, CH_3	0.84 (s)	16.4, CH_3	0.82 (s)
23	20.0, CH_3	1.04 (s)	20.0, CH_3	1.04 (s)	20.0, CH_3	0.86 (s)
24	197.5, C		197.5, C		199.2, C	
25	25.9, CH_3	2.21 (s)	25.9, CH_3	2.21 (s)	25.4, CH_3	2.28 (s)
26	170.9, C		170.9, C		170.4, C	
27	21.5, CH_3	2.09 (s)	21.5, CH_3	2,09 (s)	21.6, CH_3	2.07 (s)
28	170.3, C		170.4, C			
29	21.4, CH_3	2.03 (s)	21.4, CH_3	2.03 (s)		
30			8.8, CH_3	0.75 (t, 7.0)		

[a] ^{1}H and ^{13}C assignments were based on COSY, HMBC, HSQC, and ROESY spectroscopic data.
[b] ^{1}H NMR data were measured at 600 MHz and ^{13}C NMR data were measured at 150 MHz in $CDCl_3$.

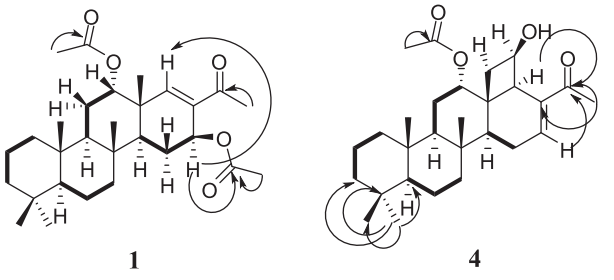


Fig. 2. Selected COSY (—) and HMBC (⌒) correlations of the new compounds exemplified by **1** and **4**.

and C-19 and the HMBC correlations of H_3-30 to C-4 in compound **2**. This difference was further supported by the 14 mass unit differences between **1** and **2**. The relative configuration of **2** is identical to that of **1** based on identical ROESY correlations, molecular mechanics simulation (data not shown), and identical coupling constant analysis deduced typical α-configuration of the acetate moiety at position-16. Compound **2** is a new homologue of **1** for which the name 12α,16α-diacetoxy-20,24-dimethyl-25-norscalar-17-en-24-one (phyllospongin B) is suggested.

Compound **3** was obtained as colorless solid, HRESIMS analysis resulted in *m*/*z* 415.3209 $[M+H]^+$ ion (calcd 415.3207) giving a molecular formula of $C_{27}H_{42}O_3$. The 1D NMR spectral data (Table 1) together with the multiplicity-edited HSQC spectrum showed resonances indicating (i) one acetate moiety [δ_C 170.4 (C-26), δ_C 21.6 (C-27)], which was confirmed by the HMBC correlation of H_3-27/C-26, (ii) methyl ketone moiety at [δ_C 199.2 (C-24), δ_C 25.4 (C-25)] that was confirmed by the HMBC correlation of H_3-25/C-24, (iii) a trisubstituted olefin at 137.8 (C-17) and $\delta_{C/H}$ 139.7/6.85 (C-16); the position of this double bond was confirmed by the COSY correlations of H-16 to H_2-15 and the HMBC correlations of H-16 to C-14, C-24, and C-18 and both H_3-25 and H_2-18 to C-17. These spectral data revealed a remarkable similarity between **3** and the known compound **6**[31], which was also isolated in this study (Table 1, Supplementary data). The only difference between **3** and **6** was the replacement of the ethyl group in **6** with a methyl singlet at δ_H 0.80 (H_3-20) in **3**, which was further confirmed by HMBC correlations of H_3-20 to C-19, C-4, C-3, and C-5. Our conclusion was also corroborated with the 14 mass unit differences from **6**. The relative configuration of compound **3** was established based on the close similarity of its ROESY correlations to the previously isolated compound **6**.[31] The *all-trans* configuration of rings A/B/C/D was the same as in **6** based on coupling constants of H-5, H-9, and H-14 together with ^{13}C NMR shifts for the attached methyl moieties at C-4, C-8, C-10, and C-13 (Table 1 and Table 1, Supplementary data). Therefore compound **3** is a new sesterterpenoid for which the name 12α-acetoxy-24-methyl-25-norscalar-16-en-24-one (phyllospongin C) is proposed.

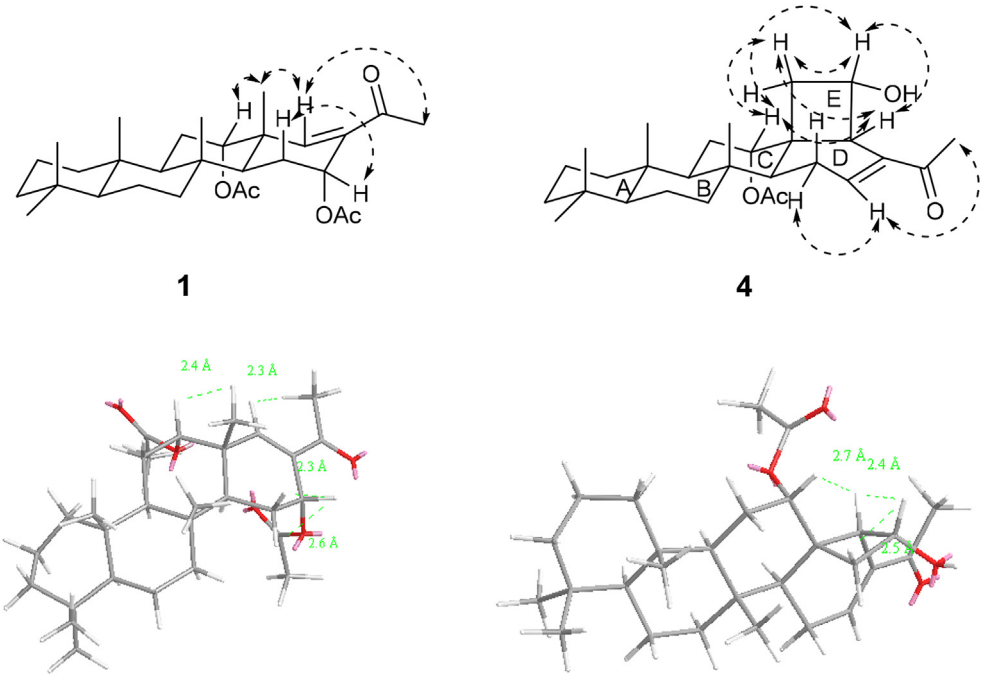


Fig. 3. Selected ROESY (↷) correlations and molecular mechanics models of the new compounds exemplified by **1** (left) and **4** (right).

Compound **4** was obtained as colorless solid, its molecular formula was deduced as $C_{28}H_{42}O_4$ based on the HRESIMS $[M+H]^+$ at *m*/*z* 443.3163 (calcd 443.3156). The ^1H and ^{13}C NMR spectral data (Table 2) together with the multiplicity-edited HSQC indicated the following diagnostic moieties: (i) four methyl singlets at [δ_H 0.85 (H-19), δ_H 0.80 (H-20), δ_H 0.77 (H-21), and δ_H 0.79 (H-22)], (ii) a methyl ketone [δ_C 202.2 (C-24), $\delta_{C/H}$ 25.6/2.35 (C/H_3-26)] placed at position-17 through the HMBC correlations of H_3-26 to C-17 and both H-16 and H-18 to C-24 (Fig. 2), (iii) eight methylene groups, four methines, and two oxymethines, (iv) a tri-substituted olefin at δ_C 137.3 (C-17) and $\delta_{C/H}$ 142.7/7.16 (C-16) where the HMBC correlations of H-16/C-24 and H_3-26/C-17 confirmed its position, (v) an acetate [δ_C 170.8 (C-27), δ_C 21.5 (C-28)], which was confirmed by HMBC correlation of H_3-28/C-27. Searching AntiMarine for these structural features indicated a close similarity to the known bishomoscalarane 12α-acetoxy-13β,18β-cyclobutane-20,24-dimethyl-24-oxoscalar-16-en-25β-ol; compound **7** previously isolated from *Phyllospongia papyracea*,[17] and in this study (Table 1, Supplementary data) with the replacement of the ethyl moiety attached to position-4 in **7** with a methyl singlet at δ_H 0.80 (H_3-20), which was confirmed by HMBC correlations of H_3-20 to C-4, C-3, C-5, and C-19. Direct comparison of ^1H, ^{13}C NMR, optical rotation, and ROESY data of **4** (Fig. 3) to those of the known isolated compound **7**[17] indicated an identical relative configuration. To determine the configuration at C-25, molecular mechanics simulation was carried out with 25 β-OH (Fig. 3), and the proximity between H-25 and H-18 was confirmed by ROESY correlations of H-12 and H-25 to both H-23α and H-18 together with comparison of coupling constant of H-12 (δ_H 5.10, t, *J*=2.6 Hz), H-25 (δ_H 4.63, q, *J*=7.8 Hz) (Table 2) with that of previously related compounds; H-12 (δ_H 5.12, dd, *J*=2.5, 2.0 Hz), H-25 (δ_H 4.65, qd, *J*=8.0, 3.0 Hz),[17] indicated an α-configuration of the acetate moiety at C-12 and β-configuration of the OH group at position-25. Therefore, compound **4** was assigned as a new natural product to which we gave the name 12α-acetoxy-13β,18β-cyclobutane-24-methyl-24-oxoscalar-16-en-25β-ol (phyllospongin D).

The molecular formula of compound **5** was assigned as $C_{28}H_{42}O_4$, based on the HRESIMS $[M+H]^+$ at *m*/*z* 443.3161 (calcd, 443.3156), obtained as colorless solid. The same chemical formula and the almost identical 1D and 2D NMR spectral features (Table 2) indicated that **5** is an isomer of **4**. The main difference between **5** and **4** appeared in ^{13}C NMR spectrum data where the resonance at C-25 in **5** was shifted 6.3 ppm downfield in comparison with that of **4**. Moreover, minor changes in ^1H NMR about (>±0.25 ppm) were observed for resonances in ring E, including H-13, H-18, and H-23.[17] In addition, a difference in the H-25 chemical shift (0.96 ppm) and splitting pattern was observed compared to that of **4**, **7** and the previously isolated compound 12α-acetoxy-13β,18β-cyclobutane-20,24-dimethyl-24-oxoscalar-16-en-25α-ol.[17] On that basis molecular mechanics simulation was carried out with 25 α-OH (data not shown), and the proximity between H-25 and H-18 was confirmed by the ROESY correlations between H-12 to both H-23α and H-18, H-25/H-23β while no ROESY correlation was observed between H-25 and H-18 in addition to the large decrease in optical rotation when compared with **4**. Comparison of coupling constant analysis of H-12 (δ_H 5.65, t, *J*=2.6 Hz), H-25 (δ_H 3.61, d, *J*=7.0 Hz) (Table 2) with that of previously related compounds; H-12 (δ_H 5.66, t, *J*=3.0 Hz), H-25 (δ_H 3.63, d, *J*=7.0 Hz).[17] These data confirmed the α-configuration of both the acetate moiety at C-12 and the −OH group at position C-25. Compound **5** was given the name 12α-acetoxy-13β,18β-cyclobutane-24-methyl-24-oxoscalar-16-en-25α-ol (phyllospongin E).

The known compounds 12α-acetoxy-20,24-dimethyl-25-norscalar-16-en-24-one (**6**),[31] 12α-acetoxy-13β,18β-cyclobutane-20,24-dimethyl-24-oxoscalar-16-en-25β-ol (**7**),[17] 12α-acetoxy-24,25-epoxy-24-hydroxy-20,24-dimethylscalarane (**8**),[23] and scalardysin-A (**9**)[32,33] were isolated for the first time from the species *P. lamellosa* and were completely characterized by comparison of their NMR spectral data with that previously reported (Table 1, Supplementary data).

When screened for their cytotoxic activity (Table 3), all compounds had activity against the three cancer cell lines used except compound **9**, which was inactive at the highest concentration used (10 μg/mL). Compounds **2**–**5** and **8** showed cytotoxic activity against HCT-116 with compound **4** as potent as doxorubicin. Compound **5** showed cytotoxic activity against MCF-7 comparable

Table 2
Summary of ^{1}H and ^{13}C NMR spectroscopic data for compounds **4** and **5**[a,b]

Position	Phyllospongin D (**4**)		Phyllospongin E (**5**)	
	δ_C/ppm, type	δ_H/ppm (mult, *J* in Hz)	δ_C/ppm, type	δ_H/ppm (mult, *J* in Hz)
1	40.0, CH_2	0.61 (td, 13.8, 4.0) 1.56 (m)	40.0, CH_2	0.62 (td, 12.9, 2.8) 1.58 (m)
2	18.6, CH_2	1.39 (m) 1.60 (m)	18.6, CH_2	1.39 (m) 1.61 (m)
3	40.5, CH_2	1.07 (td, 13.0, 3.5) 1.71 (dt, 12.6, 3.3)	40.6, CH_2	1.05 (td, 13.2, 4.0) 1.71 (dt, 15.2, 2.3)
4	33.4, C		33.4, C	
5	56.7, CH	0.82 (m)	56.7, CH	0.82 (m)
6	18.2, CH_2	1.33 (m) 1.53 (m)	18.2, CH_2	1.33 (m) 1.54 (m)
7	42.1, CH_2	1.12 (m) 1.36 (m)	42.1, CH_2	1.12 (td, 13.5, 4.0) 1.36 (m)
8	36.9, C		37.9, C	
9	52.7, CH	1.19 (dd, 13.0, 2.0)	52.7, CH	1.19 (dd, 13.0, 2.2)
10	36.9, C		37.0, C	
11	22.2, CH_2	1.64 (m) 1.82 (m)	21.4, CH_2	1.66 (td, 15.2, 2.3) 1.85 (dt, 14.3, 2.7)
12	76.2, CH	5.10 (t, 2.6)	77.1, CH	5.65 (t, 2.6)
13	37.9, C		40.3, C	
14	43.1, CH	1.31 (m)	44.2, CH	1.31 (dd, 12.0, 4.5)
15	23.1, CH_2	2.22 (m) 2.37 (dt, 19.0, 6.0)	22.6, CH_2	2.12 (m) 2.36 (m)
16	142.7, CH	7.16 (dd, 6.6, 1.9)	140.8, CH	6.98 (dd, 6.8, 2.0)
17	137.3, C		139.2, C	
18	43.2, CH	3.00 (m)	45.7, CH	2.70 (m)
19	33.4, CH_3	0.85 (s)	33.4, CH_3	0.85 (s)
20	21.5, CH_3	0.80 (s)	21.5, CH_3	0.79 (s)
21	15.0, CH_3	0.77 (s)	15.3, CH_3	0.77 (s)
22	16.4, CH_3	0.79 (s)	16.4, CH_3	0.81 (s)
23	35.3, CH_2	1.85 (dd, 11.9, 7.7) 1.98 (m)	34.1, CH_2	1.56 (m) 2.27 (m)
24	202.2. C		198.6, C	
25	67.1, CH	4.63 (q, 7.8)	73.4, CH	3.61 (d, 7.0)
26	25.6, CH_3	2.34 (s)	25.4, CH_3	2.30 (s)
27	170.8, C		170.9, C	
28	21.5, CH_3	2.05 (s)	21.5, CH_3	2.05 (s)

[a] ^{1}H and ^{13}C assignments were based on COSY, HMBC, HSQC, and ROESY spectroscopic data.
[b] ^{1}H NMR data were measured at 600 MHz and ^{13}C NMR data were measured at 150 MHz in $CDCl_3$.

to doxorubicin. All the isolated compounds were less active against the HePG-2 cell line when compared to doxorubicin.

On the other hand, all compounds showed antibacterial activity when screened against Gram-positive strains with compounds **4**, **5**, and **7** showing the most potent activity (~1.7–3.3 μg/mL) in comparison with the other compounds, which showed weak activity (Table 4). However, all screened compounds didn't exhibit antibacterial effect against Gram-negative strains represented by

Table 3
Results of in vitro cytotoxic assay for compounds **1**–**9** (IC_{50}, μg/mL)[a]

Sample	MCF-7	HCT-116	HePG-2
1	2.00±0.15	1.16±0.11	1.30±0.15
2	2.14±0.14	0.59±0.04	1.32±0.12
3	ND[b]	0.77±0.08	1.82±0.14
4	1.07±0.18	0.29±0.02	2.10±0.09
5	0.61±0.09	0.78±0.07	2.08±0.13
6	ND	ND	1.83±0.17
7	1.33±0.11	1.35±0.04	1.73±0.11
8	1.86±0.20	0.81±0.09	1.52±0.10
9	ND	ND	ND
Adriamycin[c]	0.7±0.02	0.69±0.03	0.6±0.02

[a] Cytotoxic data are the average of two independent replicates.
[b] ND: not detected at the highest measured concentration of 10 μg/mL.
[c] Adriamycin (Doxorubicin) used as positive control, MCF-7 [human Caucasian breast adenocarcinoma], HCT-116 [colon cell line] and HePG-2 [human hepatocellular carcinoma cell line].

Table 4
Antibacterial activity of compounds **1**–**9**

Average MIC[a] (μg/mL)	Bacterial strains			
	S. aureus	*B. subtilis*	*E. coli*	*V. parahemolyticus*
1	6.8	10.3	>20	18.6
2	5.7	8.2	>20	15.5
3	16.6	18.9	>20	>20
4	1.9	1.7	>20	6.8
5	2.5	3.3	>20	9.8
6	15.8	17.5	>20	>20
7	1.8	1.7	>20	7.0
8	17.5	13.2	>20	>20
9	15.0	12.3	>20	>20
Ampicillin	0.3	0.7	4.1	2.1

[a] Antibacterial data are the average of two independent replicates.

Escherichia coli using highest measured concentration of 20 μg/mL while compounds **4**, **5**, and **7** showed moderate to weak activity (~6.8–9.8 μg/mL) when tested against *Vibrio parahemolyticus*.

3. Conclusion

Biology-guided screening together with LC–HRESIMS analysis proved efficient tools for rapid dereplication and selection of biologically interesting extracts, which could potentially include new secondary metabolites. In this study, five new scalarane sesterterpenes (**1**–**5**) together with another four known scalaranes sesterterpenes (**6**–**9**) were isolated based on cytotoxicity-guided screening and LC–HRESIMS analysis of *P. lamellosa* collected from the Red Sea, Hurghada, Egypt. The structures of these compounds were fully characterized using NMR and HRESIMS analyses. Cytotoxic screening indicated compounds **2**–**5** and **8** had potent cytotoxic activity against HCT-116 with **4** as potent as doxorubicin while **5** showed cytotoxic activity against MCF-7 comparable to doxorubicin. Re-collection of this sponge in large quantities for further isolation of these potent cytotoxic metabolites will allow further in-depth study of their mechanism of action and other chemical modifications. On the other hand, some of the screened compounds showed moderate antibacterial activity against Gram-positive microorganisms.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured in methanol on a Perkin–Elmer 241 instrument at the sodium D line (589 nm). The UV spectra were acquired in methanol using Thermo Scientific NanoDrop 2000C UV–vis spectrophotometer. IR spectra were measured using JASCOFT/IR-300E spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 25 °C with a Varian VNMRS 600 MHz NMR spectrometer. High-resolution mass spectra were acquired with a Thermo scientific LTQ/XL Orbitrap, specifications; analyzer: FTMS, mass range: normal full ms 100–2000, resolution: 30,000. For LC–ESIMS, gradient separation was achieved using a SunFire C-18 analytical HPLC column (5 μm, 4.6×150 mm, Waters) with a mobile phase of 0–100% MeOH over 30 min at a flow rate of 1 mL/min. HPLC was performed on Agilent 1260 Infinity preparative HPLC system with an Agilent Eclipse XDB-C18 column (5 μ, 10×250 mm, Agilent technologies, USA). All chemical reagents were purchased from Sigma–Aldrich and used without further purification.

4.2. Biological materials

4.2.1. The marine sample collection and identification. P. lamellosa Esper, 1794 (order Dictyoceratida) previously known as *P. madagascarensis* Hyatt, 1877[24] was collected from Shaab Saad area at

13 km northern Hurghada along the Red Sea Coast at depth of 5–7 m in March 2012. A voucher specimen (NIOF203/2012) was reserved at the National Institute of Oceanography and Fisheries, Red Sea Branch, Invertebrates Department.

4.2.2. The human tumor cell lines. Human breast cancer cell line (MCF-7), human colon cancer cell line (HCT-116), and human liver cancer cell line (HePG-2) were supplied by the Bioassay-Cell Culture Laboratory, National Research Center, El-Tahrir St., Dokki, Cairo 12622, Egypt.

4.3. Extraction and isolation

P. lamellosa (1.8 kg wet weight) was soaked in methanol three times and the methanol extract was collected and further evaporated under reduced pressure to give a total extract of semisolid brown residue (50 g). The methanol extract was partitioned using a modified Kupchan's scheme and then concentrated under reduced pressure and screened for their cytotoxic effect. The active dichloromethane fraction (25 g) was further fractionated on silica gel using an *n*-hexane/ethyl acetate/methanol gradient to give 75 fractions (codes Fr1–Fr75). Fractions were monitored by TLC using different solvents as *n*-hexane, dichloromethane, and ethyl acetate and similar fractions were pooled. NMR-guided analysis revealed the following fractions 24, 33, and 39 were of interest. Fr24 was chromatographed on Sephadex LH-20 using *n*-hexane/dichloromethane in a ratio (80:20) to afford 22 fractions. Sub-fractions (Sub-Fr13–Fr22) were combined and further purified on Agilent 1260 Infinity preparative HPLC using MeCN in H_2O (70–100%) for 20 min followed by 100% MeCN for 10 min at 20 mL/min flow rate. These conditions afforded: compounds **1** (3.4 mg), **2** (3.2 mg), **3** (5 mg), and **6** (2 mg). Fr33 was chromatographed on column Sephadex LH-20 using the same solvent system to afford 22 sub-fractions. Sub-fractions (Sub-Fr12–Fr22) were combined and further purified on Sephadex LH-20 using *n*-hexane/dichloromethane in a ratio (90:10) to afford compound **8** (3 mg). Fr39 was chromatographed on Sephadex LH-20 using the same solvent system to afford 32 sub-fractions and sub-Fr25–Fr32 afforded compound **9** (17 mg). Sub-fractions (Fr5–Fr22) were combined and further purified on Agilent 1260 Infinity preparative HPLC using MeCN in H_2O (70–100%) for 20 min followed by 100% MeCN for 10 min at 20 mL/min flow rate to afford compounds **4** (14.6 mg), **5** (2.2 mg), and **7** (8 mg).

*4.3.1. Phyllospongin A (**1**).* 12,16α-Diacetoxy-24-methyl-25-norscalar-17-en-24-one; colorless solid; $[\alpha]_D^{20}$ +4.1 (*c* 0.80, MeOH); UV (MeOH) λ_{max} (log ε) at 253 (3.8) nm; IR (CH_2Cl_2) ν_{max} 3829, 3429, 2927, 1732, 1677, 1378, 1246, 1121, 1029, 705 cm^{-1}; for complete NMR data see Table 1; HRESIMS *m*/*z* 473.3254 $[M+H]^+$ (calculated for $C_{29}H_{45}O_5$, 473.3262).

*4.3.2. Phyllospongin B (**2**).* 12,16α-Diacetoxy-20,24-dimethyl-25-norscalar-17-en-24-one; colorless solid; $[\alpha]_D^{20}$ +3.9 (*c* 0.73, MeOH); UV (MeOH) λ_{max} (log ε) at 254 (4.3) and 351 (2.7) nm; IR (CH_2Cl_2) ν_{max} 3436, 2928, 1729, 1675, 1460, 1247, 1127, 1026, 967, 744 cm^{-1}; for complete NMR data see Table 1; HRESIMS *m*/*z* 487.3423 $[M+H]^+$ (calculated for $C_{30}H_{47}O_5$, 487.3418).

*4.3.3. Phyllospongin C (**3**).* 12α-Acetoxy-24-methyl-25-norscalar-16-en-24-one; colorless solid; $[\alpha]_D^{20}$ +8.4 (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ε) at 252 (4.0) nm; IR (CH_2Cl_2) ν_{max} 3434, 2929, 2354, 1731, 1651, 1438, 1244, 1028, 878 cm^{-1}; for complete NMR data see Table 1; HRESIMS *m*/*z* 415.3209 $[M+H]^+$ (calculated for $C_{27}H_{43}O_3$, 415.3207).

*4.3.4. Phyllospongin D (**4**).* 12α-Acetoxy-13β,18β-cyclobutane-24-methyl-24-oxoscalar-16-en-25β-ol; colorless solid; $[\alpha]_D^{20}$ +124.2 (*c* 0.49, MeOH); UV (MeOH) λ_{max} (log ε) at 254 (4.2) nm; IR (CH_2Cl_2) ν_{max} 3832, 3431, 2930, 1733, 1661, 1454, 1245, 1068, 820, 610 cm^{-1}; for complete NMR data see Table 2; HRESIMS *m*/*z* 443.3163 $[M+H]^+$ (calculated for $C_{28}H_{43}O_4$, 443.3156).

*4.3.5. Phyllospongin E (**5**).* 12α-Acetoxy-13β,18β-cyclobutane-24-methyl-24-oxoscalar-16-en-25α-ol; colorless solid; $[\alpha]_D^{20}$ +52.5 (*c* 0.45, MeOH); UV (MeOH) λ_{max} (log ε) at 252 (3.9) nm; IR (CH_2Cl_2) ν_{max} 3433, 2929, 2351, 1731, 1642, 1447, 1248, 1031, 617 cm^{-1}; for complete NMR data see Table 2; HRESIMS *m*/*z* 443.3161 $[M+H]^+$ (calculated for $C_{28}H_{43}O_4$, 443.3156).

4.4. Cytotoxicity assay

The cytotoxic effect of the nine isolated compounds **1**–**9** was evaluated in vitro against three human cancer cell lines: human breast cancer cell line (MCF-7), human colon cancer cell line (HCT-116), and human liver cancer cell line (HePG-2) using MTT assay[34,35] in two independent replicates (Table 3) where Adriamycin (Doxorubicin) [M.wt.=579.9] was used as standard.

4.5. Antibacterial screening

The antibacterial activity of compounds **1**–**9** was evaluated against the Gram-positive strains *Staphylococcus aureus* ATCC25923, and *Bacillus subtilis* NCTC2116 and the Gram-negative strains *E. coli* ATCC25922 and *V. parahemolyticus* NCTC10441, using the agar diffusion method.[36] Filter paper disks containing ampicillin (10 μg), oxolinic acid (2 μg), or oxytetracycline (30 μg) were used as positive controls. MICs against species were calculated using the method described before with minor modifications.[36] In brief, tested strains were grown in Müller–Hinton (MH) broth[37] to early stationary phase and then diluted to an OD600=0.005. The assays were performed in a 96-well microtiter plate format in duplicate, with two independent cultures for each strain. All compounds were dissolved in DMSO (Sigma) and added to the cultures in wells so that the final concentration of DMSO was 10%, a concentration that did not affect the growth of any of the tested strains. The effect of different dilutions of the compounds (800.08 μg mL) on the growth was assessed after 18 h incubation at 37 °C using a Labsystems iEMS reader MF plate reader at OD620. The MIC was determined as the lowest concentration showing no growth compared to the MH broth control.

Acknowledgements

The authors would like to thank the Bioassay-Cell Culture Laboratory, National Research Centre, El-Tahrir St., Dokki, Cairo, Egypt for performing cytotoxic activity testing on different human cancer cell lines, Michelle Kelly, Department of Pharmacognosy, Pharmacology, Chemistry and Biochemistry and National Center for Natural Products Research, The University of Mississipi for primary identification of *Phyllospongia lamellosa* and the Finance and Development Unit of research projects, University of Beni-Suef for supporting our project No. 12/11.

Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2014.12.035.

References and notes

1. Ebada, S. S.; Lin, W. H.; Proksch, P. *Mar. Drugs* **2010**, *8*, 313.
2. Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2014**, *31*, 160.

3. Diyabalanage, T.; Ratnayake, R.; Bokesch, H. R.; Ransom, T. T.; Henrich, C. J.; Beutler, J. A.; McMahon, J. B.; Gustafson, K. R. *J. Nat. Prod.* **2012**, *75*, 1490.
4. MarinLit: A database of marine natural product literature, The Royal Society of Chemistry, UK, http://pubs.rsc.org/marinlit/.
5. Menis, J.; Twelves, C. *Breast Cancer Targets Ther.* **2011**, *3*, 101.
6. Cordell, G. A.; Shin, Y. G. *Pure Appl. Chem.* **1999**, *71*, 1089.
7. Wolf, D.; Siems, K. *Chimia* **2007**, *61*, 339.
8. Bradshaw, J.; Butina, D.; Dunn, A. J.; Green, R. H.; Hajek, M.; Jones, M. M.; Lindon, J. C.; Sidebottom, P. J. *J. Nat. Prod.* **2001**, *64*, 1541.
9. Konishi, Y.; Kiyota, T.; Draghici, C.; Gao, J.-M.; Yeboah, F.; Acoca, S.; Jarussophon, S.; Purisima, E. *Anal. Chem.* **2007**, *79*, 1187.
10. Genilloud, O.; Gonzalez, I.; Salazar, O.; Martin, J.; Tormo, J. R.; Vicente, F. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 375.
11. Wang, L.; Yang, B.; Lin, X.; Zhou, X.; Liu, Y. *Nat. Prod. Rep.* **2013**, *30*, 455.
12. Kamel, H. N.; Kim, Y. B.; Rimoldi, J. M.; Fronczek, F. R.; Ferreira, D.; Slattery, M. *J. Nat. Prod.* **2009**, *72*, 1492.
13. Fattorusso, E.; Mango, S.; Santacroce, C.; Sica, D. *Tetrahedron* **1972**, *28*, 5993.
14. Pettit, G. R.; Cichacz, Z. A.; Tan, R.; Hoard, M. S.; Melody, N.; Pettit, R. K. *J. Nat. Prod.* **1998**, *61*, 13.
15. Ponomarenko, L. P.; Kalinovsky, A. I.; Stonik, V. A. *J. Nat. Prod.* **2004**, *67*, 1507.
16. Sera, Y.; Adachi, K.; Shizuri, Y. *J. Nat. Prod.* **1999**, *62*, 152.
17. Li, H.-J.; Amagata, T.; Tenny, K.; Crews, P. *J. Nat. Prod.* **2007**, *70*, 802.
18. (a) Alvi, K. A.; Crews, P. *J. Nat. Prod.* **1992**, *55*, 859; (b) Fontana, A.; Mollo, E.; Ortea, J.; Gavagnin, M.; Cimino, G. *J. Nat. Prod.* **2000**, *63*, 527.
19. (a) Hochlowski, J. E.; Faulkner, D. J.; Bass, L. S.; Clardy, J. *J. Org. Chem.* **1983**, *48*, 1738; (b) Wonganuchitmeta, S.; Yuenyong-sawad, S.; Keawpradub, N.; Plubrukarn, A. *J. Nat. Prod.* **2004**, *67*, 1767.
20. Nakagawa, M.; Hamamoto, Y.; Ishihama, M.; Hamasaki, S.; Endo, M. *Tetrahedron Lett.* **1987**, *28*, 431.
21. (a) Rueda, A.; Zubia, E.; Ortega, M.; Carballo, J. L.; Salva, J. *J. Org. Chem.* **1997**, *62*, 1481; (b) Jaspers, M.; Jackson, E.; Lobkovsky, E.; Clardy, J.; Diaz, M. C.; Crews, P. *J. Nat. Prod.* **1997**, *60*, 556; (c) Paloma, L. G.; Randazzo, A.; Minale, L.; Debitus, C.; Roussakis, C. *Tetrahedron* **1997**, *53*, 10451; (d) Jimenez, J. I.; Yoshida, W. Y.; Scheuer, P. J.; Lobkovsky, E.; Clardy, J.; Kelly, M. *J. Org. Chem.* **2000**, *65*, 6837; (e) Song, J.; Jeong, W.; Wang, N.; Lee, H.; Sim, C. J.; Oh, K.; Shin, J. *J. Nat. Prod.* **2008**, *71*, 1866.
22. Walker, R. P.; Thompson, J. E.; Faulkner, D. *J. Org. Chem.* **1980**, *45*, 4976.
23. Braekman, J. C.; Daloze, D.; Kaisin, M.; Moussiauz, B. *Tetrahedron* **1985**, *41*, 4603.
24. Barnes, D. K. A.; Bell, J. J. *Afr. J. Ecol.* **2002**, *40*, 337.
25. Chang, L. C.; Otero-Quintero, S.; Nicholas, G. M. *Tetrahedron* **2001**, *57*, 5731.
26. Santalova, E. A.; Makarieva, T. N.; Ponomarenko, L. P.; Denisenko, V. A.; Krasokhin, V. B.; Mollo, E.; Cimino, G.; Stonik, V. A. *Biochem. Syst. Ecol.* **2007**, *35*, 439.
27. Houssen, W. E.; Jaspars, M. In *Natural Products Isolation*, 2nd ed.; Sarker, S. D., Latif, Z., Gray, A. I., Eds.; Humana Press: Totowa, NJ, USA, 2006; pp 353–390.
28. Fu, X.; Zeng, L.; Su, J.; Schmitz, F. J. *J. Nat. Prod.* **1999**, *62*, 644.
29. Jiménez, J. I.; Yoshida, W. Y.; Scheuer, P. J.; Kelly, M. *J. Nat. Prod.* **2000**, *63*, 1388.
30. Williams, D. E.; Hollander, I.; Feldberg, L.; Frommer, E.; Mallon, R.; Tahir, A.; Soest, R.; Andersen, R. J. *J. Nat. Prod.* **2009**, *72*, 1106.
31. Quinn, R. J.; Tucker, D. J. *Aust. J. Chem.* **1989**, *42*, 751.
32. Zviely, M.; Kashman, Y. *Tetrahedron Lett.* **1979**, *40*, 3879.
33. Roy, M. C.; Tanaka, J.; Voogd, N.; Higa, T. *J. Nat. Prod.* **2002**, *65*, 1838.
34. Omar, H. A.; Sargeant, A. M.; Weng, J. R.; Wang, D.; Kulp, S. K.; Patel, T.; Chen, C. S. *Mol. Pharmacol.* **2009**, *76*, 957.
35. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
36. Kronvall, G. *J. Clin. Microbiol.* **1983**, *17*, 975.
37. Müller, H. J.; Hinton, J. *Proc. Soc. Exp. Biol. Med.* **1941**, *48*, 330.