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Anti-*Helicobacter*, Antitubercular and Cytotoxic Activities of Scalaranes from the Red Sea Sponge *Hyrtios erectus*[†]

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Abstract: The Red Sea specimen of the marine sponge *Hyrtios erectus* (order Dictyoceratida) was found to contain scalarane-type sesterterpenes. 12-*O*-deacetyl-12,19-di-*epi*-scalarin (**14**), a new scalarane sesterterpenoid, along with fourteen previously-reported scalarane-type sesterterpenes (**1–13** and **15**) have been isolated. The chemical structures of the isolated compounds were elucidated on the basis of detailed 1D and 2D NMR spectral data and mass spectroscopy, as well as by comparison with reported data. The anti-*Helicobacter pylori*, antitubercular and cytotoxic activities of all fifteen compounds were evaluated to reveal the potency of Compounds **1**, **2**, **3**, **4**, **6**, **7** and **10**. Amongst these, Compounds **1**, **3**, **4**, **6** and **10** displayed a promising bioactivity profile, possessing potent activities in the antitubercular and anti-*H. pylori* bioassay. Compounds **2** and **7** showed the most promising cytotoxic profile, while Compounds **1** and **10** showed a moderate cytotoxic profile against MCF-7, HCT-116 and HepG2 cell lines.

Keywords: sponges (Porifera); *Hyrtios erectus*; scalarane sesterterpenoids; 12-*O*-deacetyl-12,19-di-*epi*-scalarin; *Helicobacter pylori*; antitubercular; cytotoxic

1. Introduction

Marine sponges of the genus *Hyrtios*, order Dictyoceratida, family Thorectidae, occur often in tropical and subtropical sea waters and frequently form the dominant habitat on coral reefs. Previous biological and chemical investigations of the genus *Hyrtios* and their derived microbial symbionts have showed the presence of numerous natural products possessing a variety of important biological activities and unique structure including scalarane-type sesterterpenes [1–21], sesquiterpenes [22–24], acyclic triterpenoids [25], macrolides [26–29], steroids [30], indole and β -carboline alkaloids [24,31–38]. Many sesterterpenoids of the scalarane class showed a variety of pharmacological activities. Among

these is the sesterterpene heteronemin [14,39], which exhibited antimycobacterial activity against *M. tuberculosis* H₃₇Rv with a MIC value of 6.25 µg/mL [40]. Other biological activities of scalaranes include cytotoxic [3–5,13,18–20,41,42], antifeedant [43,44], antitubercular [6], antimicrobial [45,46], ichthyotoxic [47], anti-inflammatory [14,48], platelet aggregation inhibition [49,50] and nerve growth factor synthesis-stimulation [21,51].

In the course of our ongoing efforts to identify drugs from the sea [2,3,52], we have studied and investigated the extract of the Red Sea marine sponge *Hyrtios erectus* (Figure 1). We report herein the characterization, anti-*H. Pylori*, antitubercular and cytotoxic activities of fifteen scalarane-type sesterterpenes including the new compound 12-*O*-deacetyl-12,19-di-*epi*-scalarin (14), together with the previously-reported compounds, sesterstatin 7 (1) [6], heteronemin (2) [39], scalarolide (3) [43], 12-*epi*-24-deoxyscalarin (4) [53], scalarolide acetate (5) [46], 19 acetylsesterstatin 3 (6) [4], 12-deacetyl-12,18-di-*epi*-scalaradial (7) [43], 12-deacetyl-12-*epi*-scalaradial (8) [14], sesterstatin 3 (9) [20], 12β,20α-dihydroxy-16β-acetoxy-17-scalaren-19,20-olide (10) [54], 12-*O*-acetyl-16-*O*-methylhyrtiolide (11) [55], 12β-acetoxy,16β-methoxy,20α-hydroxy-17-scalaren-19,20-olide (12) [3], 24-methoxypetrosaspongia C (13) [2] and 12-acetoxy,16-*epi*-hyrtiolide (15) [3]. The previously-known compounds exhibited diverse cytotoxic activity against several cancer cell lines.



Figure 1. Red Sea sponge *Hyrtios erectus* (morphology before methanol extraction).

2. Results and Discussion

2.1. Purification of Compounds 1–15

Successive fractionation of the lipophilic fraction obtained from the methanolic extract of the sponge using silica gel column chromatography followed by final purification on semipreparative reversed phase HPLC column afforded fifteen pure isolated compounds (1–15) containing a scalarane-type framework, of which Compound 14 was assigned as a new scalarane sesterterpenoid.

2.2. Structural Elucidation of Compounds 1–15

Compound 14 (Figure 2) was isolated and purified as an amorphous powder. The molecular formula, C₂₅H₃₈O₄, was established from the positive ESIMS, as well as from ¹³C-NMR data. In the mass spectrum of Compound 14, the molecular ion peak was of low abundance or absent, but strong peaks corresponding to losses of water were observed at *m/z* 385.3 [C₂₅H₃₆O₃ (M – H₂O)⁺] and *m/z* 367.3 [C₂₅H₃₅O₂ (M + H – 2H₂O)⁺] (Supplementary Materials, Figure S1). The ¹H and ¹³C-NMR spectra measured in CDCl₃ (Table 1), as well as correlations in the HMBC (Table 1, Figure 3) suggested that Compound 14 was also a scalarane-type sesterterpenoid. Thus, five singlets at δ_H 0.81, 0.83, 0.85, 0.86 and 0.93 were assigned to the five methyl groups of a scalarane skeleton. In the ¹³C-NMR

spectrum of **14** (Table 1), the carbon signals in the low-field region at δ_C 98.5 (C-19), 127.3 (C-17), 136.3 (C-16) and 166.9 (C-20) were reminiscent of those of scalarin [56] (Figure 2).

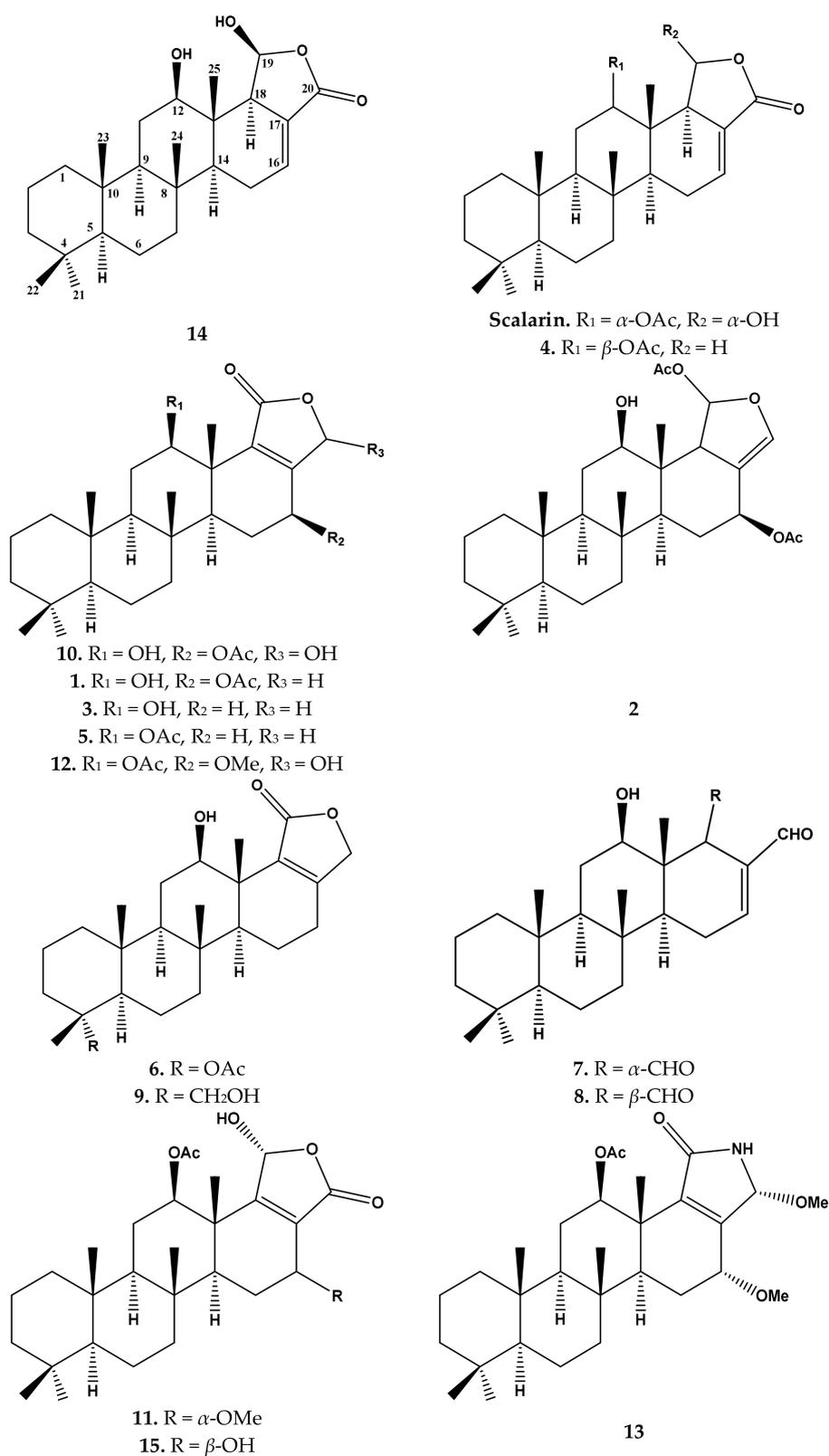
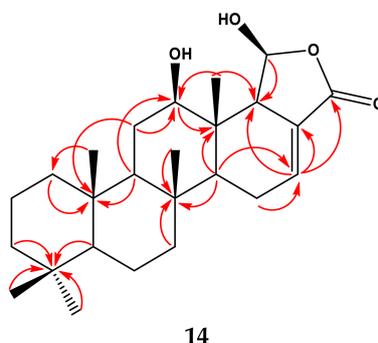


Figure 2. Scalarane Sesterterpenes 1–15.

Table 1. NMR data and HMBC correlations of Compound 14 (CDCl₃).

Position	δ_C	δ_H (m, J in Hz)	HMBC (H→C) ^a
1	39.8, CH ₂	1.73, 0.83 (m)	C-10
2	18.5, CH ₂	1.63, 1.46 (m)	C-4, C-10
3	42.0, CH ₂	1.39, 1.15 (m)	C-4
4	33.2, C	-	-
5	56.4, CH	0.83 (m)	C-4
6	18.0, CH ₂	1.58, 1.42 (m)	
7	41.4, CH ₂	1.74, 0.95 (m)	C-8
8	37.4, C	-	-
9	58.8, CH	0.93 (m)	C-10, C-12
10	37.4, C	-	-
11	26.1, CH ₂	1.79, 1.50 (m)	C-10, C-12
12	80.4, CH	3.57 (dd, 11.05, 4.25)	C-9, C-11, C-18, C-25
12-OH		4.19 (s)	
13	39.9, C	-	-
14	52.7, CH	1.25 (m)	C-8, C-9, C-13, C-16, C-18
15	23.5, CH ₂	2.17, 2.37 (m)	C-16
16	136.3, CH	6.87 (dd, 6.80, 3.40)	C-14, C-15, C-18, C-20
17	127.3, C	-	-
18	58.7, CH	2.54 (m)	C-12, C-13, C-25
19	98.5, CH	5.74 (d, 5.10)	C-18
20	166.9, C	-	-
21	21.3, CH ₃	0.81 (s)	C-4
22	33.2, CH ₃	0.83 (s)	C-4
23	16.5, CH ₃	0.85 (s)	C-1, C-5, C-9, C-10
24	16.7, CH ₃	0.93 (s)	C-7, C-8, C-9, C-14
25	9.1, CH ₃	0.86 (s)	C-12, C-13, C-14, C-18

^a HMBC correlations are from proton(s) stated for the indicated carbons.

**Figure 3.** Selected HMBC correlations observed for Compound 14.

The NMR spectra of Compound 14 (Supplementary Materials, Figures S2–S5) were quite similar to those of scalarin [56] (Figure 2). However, several diagnostic differences were observed. The most evident were the absence of the signals due to the acetyl group, the upfield shift of H-12 at δ_H 3.57, the chemical shift of CH-18 at δ_C 58.7, δ_H 2.54 (δ_C 50.8, δ_H 3.14, in scalarin), and the ¹³C chemical shift of CH-19 at δ_C 98.5 (δ_C 98.9 in scalarin).

The relative configuration of H-12 and H-19 was detected by coupling constants (for H-12) and confirmed by interpreting the NOESY spectrum (Supplementary Materials, Figure S6). The α -configuration of H-12 was deduced on the basis of the diaxial coupling of H-12 (δ_H 3.57; dd, J = 11.05 and 4.25 Hz) with H-11 and cross-peaks with α -oriented H-9 and H-14 in NOESY (Figure 4). Finally, the signal assigned to H-19 was correlated in the NOESY spectrum with H-18, which in turn was correlated with H-14, and H-12 indicated the α orientation of H-19 (Figure 4);

therefore, the configuration at the C-12/C-19 carbons was opposite to that of scalarin (Figure 2). To the best of our knowledge, Compound **14** has not been reported before in the literature; therefore, it is considered as a new scalarane sesterterpene analogue. The name 12-*O*-deacetyl-12, 19-*di-epi*-scalarin was assigned for this compound.

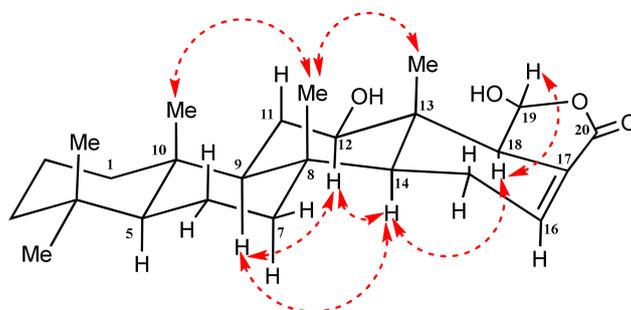


Figure 4. Important NOESY NMR correlations observed for Compound **14**.

The other known Compounds **1–13** and **15** (Figure 2) were identified by extensive study of their spectral data, including ESIMS, 1D and 2D NMR data, as well as by comparison with the published data.

2.3. Biological Activities of the Isolated Compounds 1–15

The isolated Compounds **1–15** were evaluated for their antitubercular, anti-*H. pylori* and cytotoxic activities (Table 2). Anti-*H. pylori* compounds are reported herein for the first time for scalarane sesterterpene analogues. Among these, Compounds **1, 3, 4, 6** and **10** displayed a promising bioactivity profile, possessing potent activities in the antitubercular and anti-*H. pylori* bioassays. Compound **7** displayed an interesting bioactivity profile, possessing potent antitubercular and cytotoxic activities and being practically slightly active in the anti-*H. pylori* bioassay.

Table 2. Anti-*H. pylori*, antitubercular and cytotoxic activities of Compounds **1–15** in μM .

Compound	Anti- <i>H. pylori</i> (MIC)	Anti-TB (MIC)	Cytotoxic ($\text{IC}_{50} \pm \text{SEM}$)		
			MCF-7	HCT-116	HepG2
1	4.39	0.54	24.6 ± 2.3	25.5 ± 3.3	19.8 ± 1.4
2	NA	16.00	1.2 ± 0.1	0.4 ± 0.1	1.1 ± 0.1
3	10.10	5.05	NA	NA	NA
4	9.11	1.12	25.9 ± 1.9	17.5 ± 1.3	24.7 ± 4.8
5	146.02	9.13	22.0 ± 0.4	15.2 ± 2.0	15.3 ± 1.1
6	8.78	4.39	-	-	-
7	20.23	5.05	1.6 ± 0.1	1.4 ± 0.05	1.6 ± 0.5
8	80.95	10.12	32.7 ± 3.2	34.5 ± 7.5	23.5 ± 1.8
9	77.73	19.42	NA	NA	NA
10	8.47	4.23	12.4 ± 0.9	2.9 ± 0.7	5.5 ± 0.9
11	263.71	16.47	55.6 ± 2.1	17.8 ± 1.9	20.1 ± 1.5
12	32.97	8.24	37.3 ± 5.5	22.8 ± 2.2	34.9 ± 6.1
13	16.03	8.02	54.2 ± 3.3	26.5 ± 1.1	26.6 ± 3.8
14	81.38	20.33	NA	NA	NA
15	33.97	16.97	34.9 ± 4.9	48.6 ± 7.2	27.3 ± 3.3
isoniazid	—	0.87	—	—	—
clarithromycin	1.31	—	—	—	—
doxorubicin	—	—	0.41 ± 0.1	0.11 ± 0.04	0.85 ± 0.1

NA: No activity within the range of concentration used; data are presented as the mean \pm SD; $n = 3$.

In the cytotoxic assay, the compounds tested against various cell lines (MCF-7, HCT-116 and HepG2) showed variable cytotoxic activity. Amongst these, Compounds **2** and **7** showed the most promising cytotoxic profile with IC_{50} values ranging from 0.4 ± 0.1 – 1.2 ± 0.1 μM and from 1.4 ± 0.05 – 1.6 ± 0.1 μM against the cell lines under investigation, respectively. Compounds **1, 5** and **10** showed a moderate cytotoxic profile with IC_{50} values less than, or approximately, 20 μM against

all cell lines under investigation. Other compounds showed weak to no activity against the cell lines under investigation (Table 2).

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotation was measured on the automatic high-speed laboratory polarimeter P3000 (A.KRUSS Optronic GmbH, Hamburg, Germany). UV spectra were measured on a Hitachi 300 Spectrophotometer (Hitachi High-Technologies Corporation, Kyoto, Japan). High-resolution ESIMS data were recorded with an ultra-high resolution (UHR) TOF spectrometer (Impact, Bruker, Bremen, Germany). NMR spectra were obtained in CDCl₃ on a Bruker Avance DRX 600-MHz spectrometer (Bruker, Bremen, Germany) at 600 MHz for ¹H-NMR and 150 MHz for ¹³C-NMR. NMR chemical shifts were expressed in parts per million (ppm) referenced to residual CDCl₃ solvent signals (δ_{H} 7.26 for ¹H and δ_{C} 77.0 for ¹³C). Precoated SiO₂ 60 F₂₅₄ plates (Merck, Darmstadt, Germany) were used for TLC. For column chromatography, SiO₂ (70–230 mesh, Merck, Darmstadt, Germany) was used. HPLC purifications were performed on an HPLC column (5- μm ZORBAX Eclipse XDB-C18, 250 \times 4.6 mm, Agilent, Santa Clara, CA, USA).

3.2. Biological Materials

The marine sponge specimens used in this study (Figure 1) were collected from the Red Sea, Egypt, by scuba diving. The sponge material was immediately frozen after collection and kept at -20 °C until investigation. The sponge specimen was later identified to be *Hyrtios erectus* (class: Demospongiae, order: Dictyoceratida, family: Thorectidae) by Dr. Rob van Soest (Institute of Systematic Population Biology, Amsterdam University, The Netherlands). A voucher specimen was preserved at the Zoological Museum of the University of Amsterdam, under Registration Number ZMAPOR19761.

3.3. Purification of Compounds 1–15

The collected sponge specimen (0.90 kg, wet wt.) was cut into small pieces and was macerated exhaustively at room temperature in MeOH. The combined extracts were concentrated under reduced pressure to yield the organic crude extract (85 g). The total crude extract was subjected to silica gel column using VLC (vacuum liquid chromatography) gradient elution (*n*-hexane–CHCl₃–MeOH) to afford Fractions 1–9.

Fraction 4 (*n*-hexane–CHCl₃, 1:3) was chromatographed on silica gel column with *n*-hexane–CHCl₃–MeOH gradient elution to give 8 further fractions. Of these, Fraction 3 (700 mg) was fractionated over a silica gel column chromatography (*n*-hexane–CHCl₃), then finally purified by HPLC (ODS XDP-Zorbax column, 5 μm , 250 \times 4.6 mm, 80% CH₃CN/H₂O, 1.5-mL/min flow rate and 220-nm UV detection), and Compounds 1–8 were obtained (4.6, 14, 3.4, 3, 4, 2.9, 2.3 and 3.5 mg, respectively). In turn, Fraction 5 (137 mg) was further chromatographed on silica gel column chromatography followed by final purification on HPLC the same as Fraction 3, and compounds 9–14 were obtained (2.2, 4, 3, 2.3, 2.7 and 1.3 mg, respectively). Finally, Fraction 6 (55 mg) was also chromatographed and purified as Fraction 3 to obtain Compound 15 (3.5 mg).

Compound 14: Amorphous solid (1.3 mg); $\alpha_{\text{D}}^{25} +112.0$ (*c* 0.1, CHCl₃); UV (λ_{max} , MeOH) (log ϵ): 226 (4.31), 285 (2.54) nm; NMR data: see Table 1; ESI-MS: *m/z* 385.3 [M – H₂O]⁺. HRESIMS: *m/z* 403.2851 (calculated for C₂₅H₃₉O₄ [M + H]⁺, 403.2848).

3.4. Biological Activity of Compounds 1–15

3.4.1. Anti-*Helicobacter pylori* Activity Assessment

The anti-*H. pylori* activity was assessed against a strain of *Helicobacter pylori* (American Type Culture Collection, H.b., ATCC 700392) using a micro-well dilution method as previously described [57]. Clarithromycin was used as a positive control and exhibited an MIC of 1.31 μM .

3.4.2. Antitubercular Activity Assessment

A nonvirulent strain *Mycobacterium tuberculosis* (ATCC 25177, H37Ra) was obtained from the American Type Culture Collection. The antitubercular activity was assessed according to the protocol described by Franzblau [58,59] using the microplate Alamar blue assay (MABA). Isoniazid was used as positive control and exhibited an MIC of 0.87 μM .

3.4.3. Cytotoxic Activity Assessment

3.4.3.1. Cell Culture

Human hepatocellular carcinoma cells (HepG2), colorectal adenocarcinoma cells (HCT-116) and human breast adenocarcinoma cells (MCF-7) were obtained from the VACSERA (Giza, Egypt). HCT-116 cells were maintained in Roswell Park Memorial Institute Media (RPMI-1640), while HepG2 and MCF-7 cells were maintained in Dulbecco Modified Eagle's Media (DMEM). All culture media were supplemented with heat-inactivated fetal bovine serum (10% *v/v*), penicillin-G (100 units/mL) and streptomycin sulfate (100 $\mu\text{g}/\text{mL}$). Cells were passaged in a humidified chamber (37 °C and 5% (*v/v*) CO_2).

3.4.3.2. Trypan-Blue Exclusion Assay

Cell viability was measured prior to seeding using the trypan-blue exclusion method. Briefly, exponentially-growing cells were trypsinized by 0.25% trypsin-EDTA solution. Aliquots of cell suspensions were mixed with trypan blue solution (0.4% *w/v*), and blue color (positive cells) was determined. Cells were not seeded unless viability was greater than 95%.

3.4.3.3. Cytotoxic Assessment

The cytotoxic effects of Compounds 1–15 on breast (MCF-7), colorectal (HCT-116) and liver cancer cells (HepG2) were evaluated using the sulforhodamine B (SRB) method as previously described [60]. Briefly, mid-exponentially-proliferating cells were trypsinized by trypsin-EDTA (0.25% *w/v*) and seeded in 96-well plates (1000–2000 cells/well). Cells were treated with serial concentrations of the compounds under investigation for 72 h and subsequently fixed with TCA (10% *w/v*) for 1 h at 4 °C. After washing three times, cells were stained with 0.4% SRB solution for 10 min in a dark place and then washed with glacial acetic acid (1% *v/v*). After drying, Tris-HCl (50 mM, pH 7.4) was used to dissolve the SRB-stained cells, and color intensity was measured at 540 nm. The dose response relationship of test compounds was analyzed using the E_{max} model (Equation (1)).

$$\% \text{ cell viability} = (100 - R) \times \left(1 - \frac{[D]^m}{K_d^m + [D]^m} \right) + R \quad (1)$$

where (R) is the residual unkilld fraction (the resistance fraction), (D) is the drug concentration used, (K_d) or IC_{50} is the drug concentration needed to produce 50% viability reduction and (m) is the Hill-type coefficient. IC_{50} is defined as the drug concentration required to reduce maximum absorbance by 50% compared to untreated control cells [61].

3.4.4. Statistical Analysis

Data are presented as the mean \pm SEM using GraphPad prism™ software Version 5.00 (GraphPad software Inc., La Jolla, CA, USA) for Windows Version 10.00. Analysis of variance (ANOVA) with the Tukey–Kremer post hoc test was used to calculate the significance using SPSS® for Windows, Version 17.0.0. $p < 0.05$ was taken as a cut-off value for significance.

4. Conclusions

Chemical investigation of the bioactive extract of the sponge *Hyrtios erectus*, collected in the Red Sea, Egypt, yielded fifteen compounds of scalarane-type sesterterpenes. Compounds 1–15 including a new one (14) were purified, and their chemical structures were characterized using spectroscopic studies. The isolated compounds belong to the class of scalarane sesterterpenes and displayed diverse biological activities including anti-*H. pylori*, antitubercular and cytotoxic. Anti-*H. pylori* compounds are reported herein for the first time for scalarane sesterterpene analogues. Among these, Compounds 1, 3, 4, 6 and 10 displayed a promising bioactivity profile, possessing potent activities in the antitubercular and anti-*H. pylori* bioassays. Compound 7 displayed an interesting bioactivity profile, possessing potent antitubercular and cytotoxic activities and being practically slightly active in the anti-*H. pylori* bioassay. Moreover, Compounds 2 and 7 showed the most promising cytotoxic profile, while Compounds 1, 5 and 10 showed a moderate cytotoxic profile against MCF-7, HCT-116 and HepG2 cell lines. Other compounds showed weak to no activity against the cell lines under investigation.

Supplementary Materials: The supplementary materials are available online.

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Author Contributions: S.S.E. and S.A.A. designed the experiments. S.A.A. collected the sponge specimen. S.S.E. performed the experiments. A.O.N., A.M.A.-A. and M.A.E. performed the biological activity study. S.S.E., H.Z.A. and S.A.A. analyzed the data. A.M.A. and S.S.E. wrote and edited the manuscript.

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