Cytotoxic neviotane triterpene-type from the red sea sponge *Siphonochalina siphonella*

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ABSTRACT

Background: *Siphonochalina siphonella* is a marine sponge collected from saudi Red Sea water and scare study from this region. **Objective:** To isolate the anticancer triterpenes with potent cytotoxicity from marine sponge, *Siphonochalina siphonella* and state the mode of action in cancer cell lines. **Materials and Methods:** The sponge material was collected, extracted with organic solvent, and fractionated on different adsorbents. The structure of the pure metabolites were elucidated employing different spectroscopic techniques including 1D (^{1}H and ^{13}C) and 2D (COSY, HMQC and HMBC) NMR, and MS spectroscopy. **Results:** A new Neviotine-C (1) was obtained, along with four known metabolites (2-5). All compounds, except 5, were tested towards MCF-7, PC-3 and A549 and showed effects with IC_{50} in range 7.9-87 μM, whilst, 3 showed potent anti-proliferative activity against PC-3 and A549 with IC_{50} = 7.9 ± 0.120 and 8.9 ± 0.010 μM, respectively. **Conclusion:** Compounds (1-4) showed significant cytotoxic activities, while 3 showed potent effect. The antiproliferative of 3 was attributed to significant S-phase cell cycle arrest.

Key words: Marine, sponge, *Siphonochalina*, triterpenes and anticancer

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INTRODUCTION

The oceans cover more than 70% of the whole earth and have more than 29 phylums and half million of marine organisms.[1-3] The Red Sea sponge belongs to genus *Siphonochalina* (Phylum: Porifera; Class: Demospongiae; Order: Haplosclerida; Family: Callyspongiidae), are known as a potential source of unique triterpenoidal metabolites. Up-to-date the published compounds were belonging to four skeletons out of thirty, namely; sipholane, siphonellane, neviotane, and dahabane.[4-6] In continuation of our projects on searching for bioactive metabolites,[7] a sponge identified as *Siphonochalina siphonella,* was collected from Red Sea. The total extract was fractionated on NP-Silica, purified with PTLC and yielded five triterpenoidal derivatives [1-5, Figure 1].

RESULTS AND DISCUSSION

MTT assay was used to assess the antiproliferative effect of the isolated compounds against three different cell lines (PC-3, A549, and MC-7) in comparison to standard anticancer drug (doxorubicin). All compounds showed selective anti-proliferative activities against PC-3 and A549 (IC_{50} ranges from 7.9-87 μM) while 3 showed effects against PC-3 and A549 with IC_{50} = 7.9 ± 0.120 and 8.9 ± 0.010, respectively. The profile of cells treated with (3) indicates possible cell phase (S-phase) specific effect of these compounds [Figure 2].

Compounds (1 and 2) were obtained as a mixture; different chromatographic techniques were applied, led to isolation of the two compounds in pure form. A few weeks later that 1 was transformed into 2. This phenomena was observed due to the slightly changes in color and approved by the NMR measuring. It was axiomatic that the work was repeated several times. Unfortunately, 1 was rapidly changed to 2.

Compound 1, was isolated as glassy oil with molecular formula $C_{30}H_{50}O_6$ based on the HRESIMS (Negative mode) m/z = 487.70 [M^+-H-H_2O]. Its structure was assigned by interpretation of the measured NMR chemical shifts (proton and carbon) and Nuclear Overhauser Effect (2D NOE) spectral data. The NMR spectra of 2 were measure in $CDCl_3$ to enable the comparison of NMR data of 1 and 2, while the published data of 2 was

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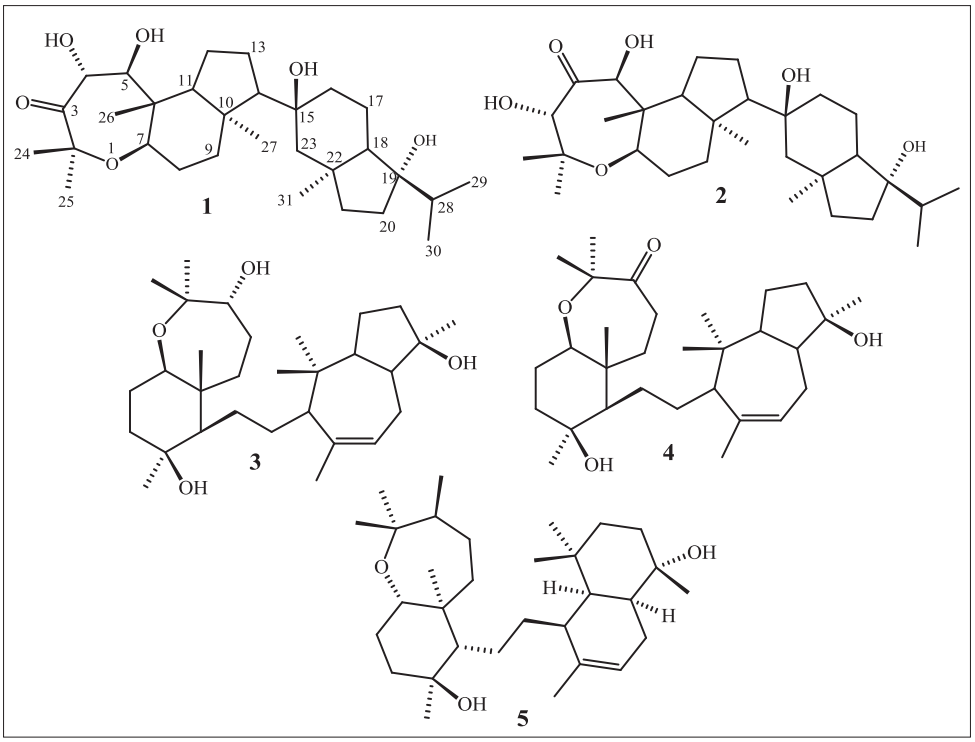

Figure 1: Compounds (1-5) isolated from *Siphonochalina siphonella*

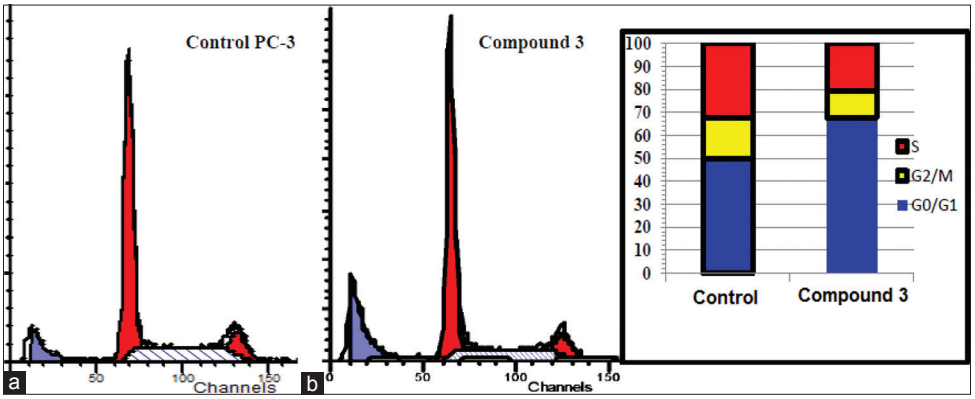

Figure 2: DNA cytometry analysis of compound 3 Cells were exposed to 3 (b) for 24 h and compared to negative control cells (a). Cell cycle distribution was determined using DNA cytometry analysis and different cell phases were plotted as percent of total events (*n*=3)

measured in a mixture of $CDCl_3$ and CD_3OD. The ^1H NMR spectral data showed the presence of seven methyl groups at δ_H 0.88 (d), 0.89 (d), 1.00 (s), 1.16 (s), 1.25 (s), 1.30 (s), 1.41 (s), ppm, three downfield protons 4.66 (dd, 9.0, 7.8), 3.46 (d, 9.0) and 3.34 (dd, 12.0, 1.2) and two hydroxyl signals which are interchangeable 3.37, 2.84. The ^{13}C NMR spectral data (^1H decoupled and DEPT) of 1, indicated the presence of 30 (6 unsaturation) signals, assigned to be seven methyles, nine methylenes, seven methines and seven quaternary carbons. As the ^1H and ^{13}C NMR data enabled all but two of the hydrogen atoms within 1 to be accounted for, it was evident that the remaining two protons were present as part of hydroxyl functions, plus two additional OH protons which are interpreted from HSQC spectral data. The conclusion was supported by IR absorption at λ_{max} 3485 (OH), 1717 (CO) cm^{-1}. After association of all the protons with directly bonded carbons *via* 2D NMR (HSQC) spectral measurements, it was possible to deduce the planer structure of 1 by interpretation of the ^1H–^1H COSY and ^1H–^{13}C HMBC NMR spectra. ^1H NMR spectrum has three downfield signals at δ_H 4.66 (dd, 9.0, 7.8), 3.46 (d, 9.0) and 3.34 (dd, 12.0, 1.2) ppm attached by carbon atoms from HSQC at δ_C 76.3 (C-4) and 79.1 (C-5) and 76.3 (C-7), respectively. Further investigation of ^1HNMR spectrum showed two signals appeared as doublets coupled to two hydroxyl doublets at δ_H 3.01 (d, J = 8 Hz) and 3.42 (d, J = 4 Hz) ppm.

The ^{13}C NMR spectrum showed the two quaternary oxygenated carbons at δ 74.39 (C-15) and δ 88.2 (C-19)

ppm signals indicated the presence of another two tertiary hydroxyl groups. After assigning six of unsaturation which appeared in the molecular formula of 1 (a carbonyl group and absence of double bonds), 1 has five rings. Two methyl groups out of seven in 1 are assigned as a part of isopropyl radical CH_3-29 (δ_H 0.89, d, J = 7.2; δ_C 17.5 ppm) and CH_3-30 (δ_H 0.88, d, J = 7.2; δ_C 16.9 ppm). The remaining five methyl groups were appeared singlet in ^{1}H NMR spectrum, which indicated their attachment with quaternary carbons.

Extensive study of the ^{1}H and ^{13}C NMR spectral data and calculated the $\Delta\delta$ difference showed significant difference in the assignments than those in 2 especially those from C-2 to C-7 [Table 1]. The remaining data are coincided with those of 2. The position of the carbonyl group δ_C 215.3 ppm in 1 is assigned as C-3 instead of δ_C 212.3 ppm in 2, which assigned as C-4, this deduction based on the HMBC correlations from the geminal two quaternary methyles at C-2 (CH_3-24 and CH_3-25) with the carbonyl group and also absence of their correlation with any oxymethine carbon. The location of the two secondary hydroxyls of 1 in positions α and β to a ketone, was suggested by the coupling constant between hydroxyl group and methine protons of C-3 (δ_H 4.66, dd, 9.0, 7.8; δ_C 79.1) and C-5 (δ_H 3.46, d, 9.0; δ_C 76.3). There are three possible substitution pattern could be occurred in the heptacyclic ring, one of them is published in 2. The remaining two possibility are 3-oxa-4, 5–dihdroxy and 4, 4–dihdroxy 5-oxa. HMBC spectral data of 1, showed correlations between CH_3-24 and CH_3-25 to C-3 (C = O) and absence the correlation between CH_3-27 and C-3 (C = O).

The relative configuration of the secondary hydroxyl groups was deduced by calculating the coupling constant. The large *J*-value (9Hz) of doublets of hydroxymethine protons at δ_H 4.66 and 3.45 ppm, indicated that the trans orientation relationship, therefore, their correspondent hydroxyl group should be β and α for OH-3 and OH-4, respectively. This deduction was supported by the 2D NOESY correlations between H_3-27 and H-5; correlations between H_3-24 and H-4; correlation also between H_3-24 and H_3-26. Finally, NOESY correlation between H-7 and H_3-27. On these bases, the junction between the heptacyclic ring and the adjacent hexacyclic ring was trans.

Compound 1 is an isomer of 2 which was isolated in the current study and was published previously.[5] The arguments that explain this conclusion are published in the following discussion. Proton and carbon NMR data for both 1 and 2 are presented in table 1. A computer survey including Science Finder, indicated 1 is a new iso-neviotine-A (Neviotine-C).

Table 1: ^{1}H [$CDCl_3$, 600 MHz] and ^{13}C [$CDCl_3$, 150 MHz] NMR spectral data 1 and 2[a]

No	Neviotine-C (1)		Neviotine-C (2)	
	δC[a]	δ_H [b, c]	δC	δ_H
2	83.2		76.2	
3	215.3		84.4	4.24 (1H d, *J*=4.8)
4	76.3	4.66 (1H dd, *J*=9.0, 7.8)	212.3	
5	79.1	3.46 (1H d, *J*=9.0)	75.5	5.07 (1Hd, *J*=7.2)
6	43.1		44.4	
7	76.3	3.34 (1H dd, *J*=12.0, 1.2)	68.9	4.88 (1H dd, *J*=13.2, 3.6)
8	26.6	1.50 (1H m) 1.88 (1H m)	26.5	1.47 (1H m) 1.75 (1Hm)
9	36.9	1.06 (1H m) 1.59 (1H m)	36.9	1.07 (1H, m) 1.61 (1H m)
10	42.8		43.1	
11	55.6	1.55 (1H m)	55.1	1.56 (1H m)
12	22.9	1.72 (1H m) 1.86 (1H m)	23.2	1.70 (1H m) 1.88 (1H m)
13	21.9	1.62 (1H m) 1.70 (1H m)	22.0	1.71 (1H m) 1.74 (1H m)
14	62.3	1.36 (1H m)	62.4	1.40 (1H m)
15	74.3		74.4	
16	36.7	1.69 (1H m) 1.83 (1H m)	36.7	1.09 (1H m) 1.62 (1H m)
17	20.9	1.34 (1H m) 1.44 (1H m)	20.9	1.37 (1H m) 1.44 (1H m)
18	54.8	1.34 (1H m)	54.8	1.40 (1H m)
19	88.2		88.2	
20	36.3	1.77 (1H m) 1.86 (1H m)	36.6	1.85 (1H m) 1.90 (1H m)
21	35.6	1.46 (1H m) 2.28 (1H, br dt, *J*=12.0, 5.4)	35.6	1.48 (1H m) 2.31 (1H, br dt, *J*=12.0, 5.4)
22	42.0		42.0	
23	47.3	1.39 (1H, br d) 1.72 (1H, br d)	47.3	1.43 (1H br d, *J*=14.4) 1.79 (1H br d, *J*=14.4)
24	21.6	1.30 (3H s)	22.3	1.33 (3H s)
25	26.5	1.41 (3H s)	26.8	1.22 (3H s)
26	15.5	1.16 (3H s)	14.6	0.69 (3H s)
27	21.5	1.00 (3H s)	20.4	1.31 (3H s)
28	32.9	1.78 (1H m)	32.9	1.79 (1H br s)
29	17.5	0.89 (3H d, *J*=7.2)	17.5	0.90 (1H d, *J*=7.2)
30	16.9	0.88 (3H d, *J*=7.2)	16.9	0.89 (3H d, *J*=7.2)
31	35.0	1.25 (3H s)	35.0	1.26 (3H s)
OH (3)				2.84 (1H d, *J*=4.8)
OH (4)		3.42 (1H d, *J*=7.8)		
OH (5)		3.01 (1H s)		3.36 (1H d, *J*=7.8)

[a]All assignments are based on 1D and 2D measurements (HMBC, HSQC, COSY), [b]Implied multiplicities were determined by DEPT (C=s, CH=d, CH2=t). [a]J in Hz

Compound 2 has molecular formula $C_{30}H_{50}O_6$ based on the HRESIMS (negative mode) m/z = 488.50 [M^+-H_2O]. The ^{1}H NMR spectral data showed the presence of nine methyl groups at δ_H 1.34 (s), 1.31 (s), 1.27 (s), 1.22 (s), 0.67 (s), 0.90 (d), 0.89 (d) ppm and five downfield protons 5.07, 4.89, 4.25, 3.37, 2.84 two of which were exchangeable 3.37, 2.84 plus two additional OH protons which are not

detectable. The ^{13}C NMR spectral data (^{1}H decoupled and DEPT) of 2 showed 30 (6 unsaturation) signals assigned indicated the presence of seven methyles, nine methylenes, seven methines and seven quaternary carbons. As the ^{1}H and ^{13}C NMR data enabled all but four of the hydrogen atoms within 2 to be accounted for, it was evident that the remaining four protons were present as part of hydroxyl functions, the conclusion was supported by IR absorption at λ_{ma} × 3485 (OH), 1717 (CO) cm^{-1}. After association of all the protons with directly bonded carbons *via* 2D NMR (HSQC) spectral measurements, it was possible to deduce the planer structure of 2 by interpretation of the ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC NMR spectra. ^{1}H NMR spectrum showed two downfield signals at δ_H 4.24 (d, J = 4.8 Hz) ppm and 5.07 (d, J = 7.2 Hz) ppm attached by carbon atoms from HSQC at δ 84.5 (C-3) and 75.5 (C-5) indicated that two of them were hydroxylated secondary methine groups. Further investigation of ^{1}HNMR spectrum showed two signals appeared as doublets coupled to two hydroxyl doublets at δ_H 2.83 (d, J = 4.8 Hz) and 3.36 (d, J = 7.8 Hz) ppm. The location of the two secondary hydroxyls of 2 in positions α and α' to a ketone, was suggested by the coupling constant between hydroxyl group and methine protons of C-3 and C-.[5] The ^{13}C NMR spectrum showed the two quaternary oxygenated carbons at δ 74.39 and δ 88.21 ppm signals indicated the presence of another two tertiary hydroxyls groups. After assigning six of unsaturation which appeared in the molecular formula of 2 (a carbonyl group and absence of double bonds) compound 2 has five rings. Two methyl groups out of seven in 2 are assigned as a part of isopropyl radical CH_3-29 (δ_H 0.90, d, J = 7.2; δ_C 17.5ppm) and CH_3-30 (δ_H 0.89, d, J = 7.2; δ_C 16.9ppm). The remaining five methyl groups were appeared singlet in ^{1}H NMR spectrum, which indicated their attachment with quaternary carbons.

The 2D NOESY spectrum showed correlation between the hydroxyl group of C-5 with CH_3-26 and assigned as β-configuration. The absence of the correlation between the hydroxyl groups of C-3, indicated it is to be having α-configuration. All the other CH correlations are in full agreement with the suggested structure and the literature. Compound 2 was identified as Neviotine-A which was previously published.[5]

Compound 2 was found to have higher energy content based on the molecular mechanics calculations 13 kcal/mol. The force field (MMFF94) identified the stability difference which is attributed to electrostatic factors [Table 4 and Figure 3]. Visual inspection of the 3D structures of the two compounds indicated that the more stable isomer is able to form an effective intramolecular hydrogen bonding with one of the flanking α-oriented hydroxyl group that is attending an equatorial configuration. The same hydrogen bonding is not energetically feasible in 2 since the α-hydroxyl is attending an axial configuration. There is a difference between distance between the OH and the O of the carbonyl (2.0 vs. 3.5 Å), that makes the H-bond is much more effective for 2. The mechanism for conversion of 1 to 2 can be explained by simple keto-enol tautomerization, a common phenomenon in aliphatic ketones. The tautomerization occurs, probably under effect of moisture or any other source of protons and facilitated by stability driving force.

Along with the compound 1, four known compounds (2-5) [Table 2] were obtained and their structure elucidation was established by comparison with the published data.[4,5,8-10]

The antiproliferative effects of all compounds, except 5, were tested towards MCF-7, PC-3 and A549 by employing MTT assay. They showed effects with IC_{50} in range 7.9-87 μM, whilst, 3 showed potent antiproliferative activity against PC-3 and A549 with IC_{50} = 7.9 ± 0.120 and 8.9 ± 0.010 μM, respectively [Table 3].

DNA flow-cytometry was used to evaluate the effect of the most potent isolated compound (3) on the cell cycle distribution of PC-3 cell lines [Figure 2]. Compound (3) significantly decreased cell population in S-phase from 32.57 ± 1.1% to 20.43 ± 0.7%. Compound (3) induced significant compensatory increase in the non-proliferating cell fraction (G_0/G_1-phase) from 49.40 ± 0.7% to 67.72 ± 0.5%.

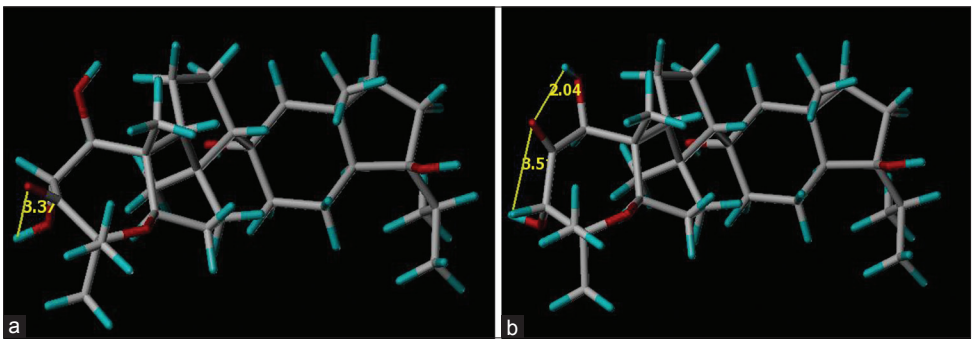

Figure 3: 3D Minimum energy conformation of 1 and 2 (a) Compound 1, (b) Compound 2

Table 2: ^{1}H [$CDCl_3$, 600 MHz] and ^{13}C [$CDCl_3$, 150 MHz] NMR spectral data of 3-5[a]

C.	Sipholenone-A (4)		Sipholenol-A (3)		Sipholenol-L (5)	
	δ_C[b]	δ_H[c]	δ_C	δ_H	δ_C	δ_H
1	41.9		42.6		42.7	
2	39.5	1.87 (1H m) 1.21 (1H t, 13.8)	25.1	2.02 (1H m) 1.72 (1H m)	34.2	1.54 (1H m) 1.46 (1H m)
3	34.9	3.22 (1H ddd, *J*=13.8, 10.8, 3) 2.13 (1H ddd, *J*=6.6, 4.2, 1.8)	33.7	1.97 (1H m) 1.77 (1H m)	25.1	2.01 (1H dd, *J*=12.6, 2.4) 1.71 (1H m)
4	218.2		77.0	3.82 (1H d, *J*=6.6)	77.0	3.81 (1H d, *J*=6.6)
5	82.4		77.8		77.8	
7	81.1	2.93 (1H dd, *J*=12, 4.2)	76.4	3.51 (1H dd, *J*=12.0, 4.2)	76.4	3.51 (1H dd, *J*=12, 4.2)
8	26.4	1.90 (1H m) 1.46 (1H m)	26.6	1.71 (1H m) 1.38 (1H m)	26.6	1.72 (1H m) 1.39 (1H m)
9	39.0	1.66 (1H m) 1.44 (1H m)	39.1	1.61 (1H m) 1.46 (1H m)	39.2	1.62 (1H m) 1.50 (1H m)
10	72.2		72.4		72.4	
11	55.5	0.72 (1H dd, *J*=4.2, 1.8)	55.7	0.86 (1H brdd, *J*=2.4, 1.8)	56.6	0.92 (1H m)
12	26.8	1.55 (1H m) 1.16 (1H m)	26.8	1.52 (1H m) 1.20 (1H m)	27.5	1.51 (1H m) 1.49 (1H m)
13	24.9	1.90 (1H m) 1.81 (1H dt, *J*=12.6, 4.2)	24.9	1.99 (1H m) 1.94 (1H m)	36.3	1.78 (1H m) 1.72 (1H m)
14	57.4	1.61 (1H m)	57.5	1.62 (1H m)	47.0	2.45 (1H m)
15	142.9		143.1		135.6	
16	121.4	5.47 (2H brdd, *J*=6.0, 1.8)	121.2	5.46 (1H brdd, *J*=9.6, 4.8)	121.7	5.26 (1H br d, *J*=9.6)
17	24.7	2.00 (1H m) 1.77 (1H m)	24.7	2.06 (1H m) 1.81 (1H m)	28.9	2.10 (1H m) 1.86 (1H m)
18	52.6	2.49 (1H dd, 6.0 *J*=1.8)	52.7	2.48 (1H ddd, *J*=12.6, 8.4)	47.6	1.90
19	82.1		82.1		72.9	
20	33.7	1.96 (1H m) 1.71 (1H m)	33.8	1.58 (1H m) 1.45 (1H dt, *J*=13.8, 4.8)	29.7	1.72 (1H m) 1.25 (1H m)
21	37.1	1.65 (1H m) 1.58 (1H m)	37.1	1.66 (1H m) 1.59 (1H m)	39.6	1.73 (1H m) 1.06 (1H m)
22	48.7	1.79 (1H m)	48.8	1.80 (1H m)	34.8	
23	35.4		35.4		41.9	2.06 (1H dd, 4.2, 3.6)
24	12,1	1.15 (3H s)	12.9	1.00 (3H s)	13.2	1.00 (3H s)
25	20.4	1.31 (3H s)	21.4	1.27 (3H s)	29.1	1.13 (3H s)
26	26.6	1.27 (3H s)	29.1	1.13 (3H s)	21.4	1.27 (3H s)
27	29.9	1.10 (3H s)	29.8	1.08 (3H s)	30.3	1.15 (3H s)
28	30.2	1.76 (H brs)	30.2	1.76 (H br s)	21.9	1.77 (H br s)
29	25.6	1.25 (3H s)	25.6	1.26 (3H s)	28.5	1.17 (3H s)
30	29.4	1.02 (3H s)	29.5	1.03 (3H s)	35.5	1.08 (3H s)
31	31.6	1.09 (3H s)	31.7	1.09 (3H s)	24.7	0.89 (3H s)

[a]All assignments are based on 1D and 2D measurements (HMBC, HSQC, COSY). [b]Implied multiplicities were determined by DEPT (C=s, CH=d, CH2=t). [a]J in Hz

Subsequent led to decrease in S- in G_2/M-phase from 17.63 ± 0.8 to 11.85 ± 0.3%. The influence of 3, on cell cycle progression of PC-3 decreased the S-phase cell population with reciprocal increase in the non-proliferating cell fraction (G_0/G_1-phase). Herein, the profile of PC-3 cells which was treated with 3, indicates its effect by decreasing G2-/M and S-phases by 12.14% and 5.78% respectively while it increases the G0-G1 phase by 17.92%.

EXPERIMENTAL

General

Silica gel GF 254 (Merck, Darmstadt, Germany) was used for analytical thin layer chromatography (TLC). Preparative thin layer chromatography (PTLC) was performed on aluminum oxide plates (20 × 20cm) of 250μm thickness. Electron impact mass spectra were

Table 3: The IC50 (μM) of compounds 1-4 against different tumor cell lines

Compounds	PC-3 [a, b]	A549	MCF-7
1	53.6±0.17	87.2±1.34	45.5±0.06
2	71.2±0.34	76.3±0.35	46.3±0.06
3	07.9±0.12	08.9±0.01	56.3±0.17
4	53.9±0.25	24.8±0.22	36.2±0.13
Doxorubicin	00.93±1.2	0.41±0.11	01.2±0.03

[a]MCF-7 (Human breast carcinoma), PC-3 (Human prostate cancer) and A549 (Lung cancer; adenocarcinoma human alveolar basal epithelial cells). [b]Values are means of 3 replicates±SD

Table 4: Energy calculation by molecular modeling of 1 and 2

Kind of energy[a]	Compound 1	Compound 2
Bond stretching energy	18.283	17.556
Angle bending energy	43.463	39.244
Torsional energy	48.416	44.258
Str-bend energy	01.161	01.620
Out of plane bending energy	00.171	00.008
1-4 van der Waals energy	59.838	61.435
Van der Waals energy	11.709	11.245
1-4 electrostatic energy	49.964	66.802
Electrostatic energy	−00.417	−23.233
Total energy	232.589	218.933

[a]Energy calculated by kcals/mol

determined at 70 ev on a Kratos (Manchester, UK) MS-25 instrument. 1D and 2D NMR spectra were recorded in $CDCl_3$ on Bruker (Karlsruhe, Germany) AVANCE III WM 600 MHz spectrometers and ^{13}C NMR at 150 MHz. Tetramethylsilane (TMS) was used as internal standard. Plates were sprayed with 50%-sulphuric acid in methanol and heated at 100°C for 1-2 minutes.

Sponge sample

The sponge *Siphonochalina siphonella* was collected from deep water (5 meter) of Sharm Obhur (21°29'31"N 39°11'24"E), Jeddah, Saudi Arabia, and was identified by Dr. Yahya Floos (Faculty of Marine Sciences, King Abdulaziz University). A Voucher sample (JAD 04012) has been deposited at the Chemistry Department, Faculty of Science, King Abdulaziz University.

Extraction and isolation of compounds

The freeze-dried sponge (94.9g) was extracted two times with 6 L of a mixture of CH_2Cl_2-MeOH (1:1, v/v) for 24 hours at 22°, and viscous dark reddish oil was obtained (22.7g). This extract was fractionated on NP-Silica (5 × 75cm, 500g, Merck 7739), employing gradient elution from Pet. ether to EtOAc. The fraction (F-A) which eluted with Pet. ether -EtOAc (6:4, v/v, 117mg, F-A) was further purified by PTLC employing Benzene -EtOAc (7:3) yielded 3 (R_f = 0.38, 17mg), 1 (R_f = 0.38, 8mg), and 2 (R_f = 0.47, 18mg) The fraction (F-B) which eluted with Pet. ether -EtOAc (5:5, v/v, 10 mg was purified by PTLC employing Benzene -EtOAc (5:5, v/v), yielded 4 (R_f = 0.6, 10 mg), and 5 (R_f = 0.5, 1mg).

Molecular modeling method

Modeling work was performed using SYBYL-X2.1 (Tripos Inc., a Celera Co., St Louis, MS, USA) molecular modeling package (licensed to King Abdulaziz University). The compounds were sketched on chemical drawing program and saved as sdf file. The sdf file was optimized with Prepare Ligand commands in SYBYL-X as Quick 3D Job. The generated 3D structures were further optimized using Minimize Energy computational commands (Force Filed: MMFF94; charges: MMFF94; Iterations: until conversions; Termination: Gradient at 0.01 kcal/ml; initial optimization: None; dielectric Function: Constant; Rest of Parameters: SYBYL-X Default). The final structures were considered as the global minimum conformers as the compounds contain no rotatable bonds. The molecular mechanics experiment resulted in Energy Terms in the energy calculation equation as shown in table 4.

Biological assays

MTT assay for cytotoxicity assay

The assay was performed as published by Abdel-Wahab *et al*.[11] The cell lines were obtained from the American Type Culture Collection (ATTC) and cultured in RPMI1640, supplemented with 10% heat-inactivated fetal calf serum and antibiotics (penicillin, 100units/mL; streptomycin sulfate, 100μg/mL) at 37°C, in an atmosphere of 95% air and 5% CO_2 under humidified condition. All chemicals were purchased from Sigma, unless otherwise indicated. RPMI1640 and fetal calf serum (FCS) were purchased from Gibco. A stock solution (10 μM) of samples was prepared in dimethylsulfoxide (DMSO) and diluted with various concentrations with serum-free culture medium. The *in vitro* antitumor activity of compounds 1-4 and 5-Fu were determined by MTT assay method. Briefly, exponentially growing cells were seeded in 96-well plates (5000 to each well) and allowed to attach overnight. After 24 h, the cells were treated with indicated concentrations of samples for 48 h, and then MTT (100 μL, 1mg/mL) was added. After incubation for 4 h at 37°C, the MTT solution was removed and the crystals of viable cells were dissolved with DMSO (150 μL) in each well; 4000/well exponentially growing cells were seeded in 96-well plates and treated with indicated concentrations of samples for 48 h, and then MTT (10 mg/mL, 10 μL) was added. After incubation for 4 h at 37°C the crystals of viable cells were dissolved overnight with SDS (sodium dodecylsulfonate, 10%, 100 μL) in each well. The absorbance spectra were measured on an ELISA Processor II Microplate Reader at a wavelength of 570nm. The percentage of cytotoxicity was defined with

treated and untreated cell lines. The 50% cytotoxic activity dose (IC_{50}) was defined as the concentration of samples that reduced the absorbance of the treated cells by 50%.

Statistical analysis

All experiments were conducted three times. The results of experiments were expressed as means ± S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used to determine significance when compared to the control group. Graph Pad Prism5 was used for statistical calculations (Graph pad Software, San Diego California, U.S.A.).

Analysis of cell cycle distribution[12]

To assess the effect of the column fractions on cell cycle distribution, cells were treated with the pre-determined IC_{50} for 24 h and collected by trypsinization, washed with ice-cold PBS and re-suspended in 0.5ml of PBS (Skehan *et al.*, 1990). Ten milliliter of 70% ice-cold ethanol was added gently while vortexing, and cells were kept at 4°C for 1 h and stored at -20°C until analysis. Upon analysis, fixed cells were washed and re-suspended in 1 ml of PBS containing 50 µg/ml RNase A and 10 µg/ml propidium iodide (PI). After 20 min incubation at 37°C, cells were analyzed for DNA contents by FACSVantageTM (Becton Dickinson Immunocytometry Systems). For each sample, 10,000 events were acquired. Cell cycle distribution was calculated using CELLQuest software (Becton Dickinson Immunocytometry Systems).

Neviotine-C (1)

Glassy oil (8 mg, 0.0084%), IR λ_{max} (film) cm^{-1}: 3423, 2929, 2874, 1702, 1465, 1378, 1294, 1253, 1175, 1141, 1090, 1060, 1040, 996, 970, 912, 887, 828, 757; ^{13}C NMR ($CDCl_3$, 150 MHz) and ^{1}H NMR ($CDCl_3$, 600 MHz) spectroscopic data [Table 1]; HRESIMS (positive-ion mode), m/z = 487.70 [M^+-H-H_2O] (Calculated m/z = 506.7144 for $C_{30}H_{50}O_6$).

Neviotine-A (2)

Amorphous powder (18 mg, 0.0198%), IR λ_{max} (film) cm^{-1}: 3485, 2932, 2874, 1717, 1464, 1378, 1286, 1176, 1122, 1062, 1033, 977, 887; ^{13}C NMR ($CDCl_3$, 150 MHz) and ^{1}H NMR ($CDCl_3$, 600 MHz) spectroscopic data, see table 1; HRESIMS (negative mode) m/z = 488.50 [M^+-H_2O]. (Calculated m/z = 506.7144 for $C_{30}H_{50}O_6$).

Sipholenol-A (3)

White amorphous powder (10 mg, 0.010%), IR λ_{max} (film) cm^{-1}: 3615, 3599, 3422, 2967, 2933, 2861, 1462, 1439, 1374, 1291, 1250, 1160, 1128, 1077, 1050, 987, 931, 909, 840, 753, 663; ^{13}C NMR ($CDCl_3$, 150 MHz) and ^{1}H NMR ($CDCl_3$, 600 MHz) spectroscopic data [Table 2]; HRFABMS m/z = 497.3755 [M^++Na]. (Calculated m/z = 499.3763 for $C_{30}H_{52}0_4Na$).

Sipholenone-A (4)

Colorless oil (17 mg, 0.0179%), IR λ_{max} (film) cm^{-1}: 3457, 2860, 2932, 2865, 1709, 1463, 1375, 1294, 1253, 1169.73, 1129, 1082, 1044, 911, 842, 815, 751; ^{13}C NMR ($CDCl_3$, 150 MHz) and ^{1}H NMR ($CDCl_3$, 600 MHz) spectroscopic data [Table 2]; HRFABMS m/z = 497.3474 [M^++Na], (Calculated m/z = 497.3607 for $C_{30}H_{50}0_4Na$).

Sipholenol-L (5)

Pale orange oil (1 mg, 0.0010%), IR λ_{max} (film) cm^{-1}: 3424.23, 2925.39, 2853.17, 1461.16, 1374.94, 1292.64, 1160.45, 1125.68, 1081.35, 1045.06, 908.88, 756.92.; ^{13}C NMR ($CDCl_3$, 150 MHz) and ^{1}H NMR ($CDCl_3$, 600 MHz) spectroscopic data [Table 2]; HRFABMS m/z = 497.3734 [M^++Na]. (Calculated m/z = 499.3763 for $C_{30}H_{52}0_4Na$).

CONCLUSION

A new natural triterpene Neviotane-C (1) and four known triterpenes (2-5) were isolated from *Siphonochalina siphonella* collected from Saudi water and identified by spectroscopic data. All compounds, except 5, were tested against PC-3, A549 and MCF-7. The isolated compounds showed considerable anti-proliferative activity selectively against PC-3 and A549 cell lines (IC_{50} ranges from 7.9-87 µM). Compound 3 showed potent anticancer activity towards PC-3 and A549 with IC_{50} = 7.9 ± 0.120 and 8.9 ± 0.010, respectively.

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