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Research Article

Two New Cytotoxic Candidaspongiolides from an Indonesian Sponge

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Marine sponges have been recognized as potentially rich sources of various bioactive molecules. In our continuing search for new secondary metabolites from Indonesian marine invertebrates, we collected a sponge, whose extract showed cytotoxicity against cultured cells at 0.1 μg/mL. Purification of the extract yielded two new macrolides **2** and **3** along with known candidaspongiolide (**1**). The structures for compounds **2** and **3** were elucidated by spectral analysis (^{1}H, ^{13}C, COSY, HMQC, HMBC) and by comparison of their NMR data with those of **1**. Compounds **2** and **3** exhibited a little more potent cytotoxicity (IC_{50} 4.7 and 19 ng/mL) than that (IC_{50} 37 ng/mL) of candidaspongiolide (**1**) against NBT-T2 cells.

1. Introduction

Sponges, a group of sedentary organisms, cannot move and escape from predators. Most sponges are filter feeders pumping water to its body to obtain foods and oxygen and to expel wastes and may be threatened by microorganisms during filtering seawater rich in bacteria and fungi [1, 2]. In order to defend themselves against predators, pathogens and competitors, sponges may have developed to produce or accumulate secondary metabolites during their long evolution, such as feeding deterrent, antimicrobial, antifungal, and antifouling molecules. Interestingly, some of the compounds have also shown remarkable potency as drug candidates against various human diseases as discussed elsewhere [3–9].

In 1984, Schmitz and coworkers isolated tedanolide from the Caribbean marine sponge *Tedania ignis* [10]. Tedanolide is a unique 18-membered macrolide where lactonization occurs at a primary hydroxyl group instead of a common secondary one, and this class of macrolide has been reported to exhibit strong cytotoxicity at pico to nanomolar range [10, 11]. The unique structure in combination with promising biological activity leads tedanolide as an intriguing target for formal and total syntheses [12–14]. More recently, Meragelman and coworkers reported a macrolide named candidaspongiolide related to tedanolide with modification at C-11 to C-15 from the marine sponge *Candidaspongia sp*. Candidaspongiolide exhibited potent cytotoxicity in NCI 60 cells panel with GI_{50} of 14 ng/mL [15], protein synthesis inhibition, and apoptosis induction [16].

In our continuing search for potential drug leads from Indonesian marine invertebrates [17, 18], we obtained a sponge whose extract showed cytotoxicity at 0.1 μg/mL against NBT-T2 cells in a screening process. Purification of the extract provided candidaspongiolide (**1**) along with two new analogs **2** and **3**, which are the subject of this paper.

2. Materials and Methods

2.1. Chemicals and Equipments. Methanol (MeOH) used for extraction was of technical grade. Reagent grade solvents were used for isolating compounds **1**–**3**. Merck Si-60 (70–230 mesh) was used for silica gel column chromatography, while Merck Si-60 F_{254} for analytical TLC. HPLC was performed either on a Waters 510 pump with a Waters 486 UV detector and a Shodex RI-101 or on a Hitachi L-6000 pump with a Hitachi L-4000 UV detector and a Shodex RI-101 using a Mightysil Si-60 (10 × 250 mm)

column. Optical rotations were measured on a Jasco 1010 polarimeter using a cell with 3.5 mm aperture. IR spectra were recorded on a Jasco FT/IR-6100 instrument, whereas HRESIMS was measured on a Jeol JMS-T100L spectrometer using reserpine or sodium trifluoroacetate as an internal standard. Most of ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$, while those of compound **3** were measured in CD_3OD with TMS as an internal standard on Jeol A500 and/or a Bruker AVANCE III-500 in $CDCl_3$. The ^{1}H and ^{13}C chemical shifts were given in ppm, while coupling constants were in Hz.

2.2. Sponge. Specimens of the sponge tagged K09-02 was collected by hand using SCUBA at 15–25 m depth at Kupang, West Timor, East Nusa Tenggara, Indonesia on August 2009. By comparing underwater images of our specimen with that of the specimen of NCI group [15], it is likely to be the same sponge. The specimen was kept frozen until extraction. The sponge K09-02 may be an endemic species to this region. The colonies are grey in color and stand.

2.3. Extraction and Isolation. After cutting into small pieces, the sponge (653 g, wet) was soaked in MeOH for 24 h for three times. Then, the solution was concentrated under vacuum to obtain a crude extract. The methanolic extract (17.0 g) was triturated with ethyl acetate (EtOAc) to provide a lipophilic fraction (2.[illegible], which killed NBT-T2 cells at 0.1 μg/mL. This fraction was subjected to a silica gel column eluting with stepwise gradient solvents (hexane : EtOAc = 2 : 1, 1 : 1, 1 : 2, 0 : 1, EtOAc : MeOH = 10 : 1) to afford ten fractions. Fraction 5 (126.0 mg) was purified by repetitive Si-60 HPLC using hexane-EtOAc mixtures to provide candidaspongiolide **1** (15.4 mg). Fraction 6 (70.8 mg) was also [illegible]ified by Si-60 HPLC using a solvent system hexane : EtOAc = 2 : 1 to afford compound **2** (9.8 mg). Fraction 9 (107.1 mg) was also subjected to repetitive Si-60 HPLC using hexane : EtOAc = 1 : 6, EtOAc : CH_2Cl_2 : MeOH = 20 : 20 : 1, and EtOAc : CH_2Cl_2 : MeOH = 10 : 20 : 1 as solvent systems sequentially, to give compound **3** (21.8 mg). Isolation of these compounds was guided by cytotoxicity testing and NMR spectra.

2.4. Compound 1. Colorless glass, $[\alpha]_D^{25}$ +69 (*c* 0.55, MeOH). IR ν_{max} (neat) 3419, 2925, 2854, 1742, 1715, 1456, 1372, 1234, 1086 cm^{-1}. ^{1}H and ^{13}C NMR; see Tables 1 and 2. HR-ESIMS $[M+Na]^+$ *m/z* 945.55514, 959.57079, 973.58884 (calcd for $C_{50}H_{82}NaO_{15}^+$ 945.55459 (Δ +0.58 ppm), $C_{51}H_{84}NaO_{15}^+$ 959.57024 (+0.57 ppm), and $C_{52}H_{86}NaO_{15}^+$ 973.58589 (+3.0 ppm)).

2.5. Compound 2. Colorless glass, $[\alpha]_D^{25}$ +72 (*c* 0.75, MeOH). IR ν_{max} (neat) 342[illegible]925, 2854, 1748, 1715, 1456 cm^{-1}. ^{1}H and ^{13}C NMR; see Tables 1 and 2. HR-ESIMS $[M+Na]^+$ *m/z* 903.54964, 917.56023, 931.57576, 945.60036 and 959.61056 (calcd for $C_{48}H_{80}NaO_{14}^+$ 903.54403 (Δ +6.2 ppm), $C_{49}H_{82}NaO_{14}^+$ 917.55968 (+0.60 ppm), $C_{50}H_{84}NaO_{14}^+$ 931.57533 (+0.46 ppm), $C_{51}H_{86}NaO_{14}^+$ 945.59098 (+9.9 ppm), and $C_{52}H_{88}NaO_{14}^+$ 959.60663 (+4.1 ppm)).

2.6. Compound 3. Yellow glass, $[\alpha]_D^{25}$ +97 (*c* 0.35, MeOH). IR ν_{max} ([illegible]at) 3418, 2925, 2854, 1715, 1457, 1373, 1244, 1084, 995 cm^{-1}. ^{1}H and ^{13}C NMR; see Tables 1 and 2. HR-ESIMS $[M+Na]^+$ *m/z* 665.31522 (calcd for $C_{32}H_{50}NaO_{13}^+$ 665.31436 (+1.3 ppm)).

2.7. Acetylation. Compound **1** (0.2 mg) was dissolved in pyridine (50 μL) and acetic [illegible]hydride (50 μL). The mixture was stirred for three days under a nitrogen atmosphere at room temperature. After removal of excess reagents with nitrogen flow and vacuum, the reaction product **4** was checked with ^{1}H NMR and ESIMS. Compound **2** was similarly treated to give **4**.

2.[illegible] Compound 4 from 1. ^{1}H NMR: δ5.65 [illegible] (*J* = 2.7, 9.5 Hz), 5.52 m, 5.51 d (*J* = 10.1 Hz), 5.36 [illegible], 5.34 dd (*J* = 2.2, 9.1 Hz), 5.28 dt (*J* = 2.3, 10.5 Hz), 5.07 d (*J* = 11.0 Hz), 4.79 d (*J* = 6.6 H[illegible]4.54 dd (*J* = 2.0, 11.3 Hz), 4.25 dd (*J* = [illegible], 8.1 Hz), 4.13 dd (*J* = 6.3, 11.5 Hz), [illegible]3 s (3H), 3.34 dq (*J* = 10.0, 6.7 Hz), 3.29 dq (*J* = 10.[illegible]0 Hz), 3.01 dd (*J* = 9.2, 18.4 Hz), 2.88 d (*J* = 9.3 [illegible]), 2.60 dd (*J* = 2.6, 18.4 Hz), 2.35–2.4 m, 2.32 dd (*J* = [illegible]9, 9.3 Hz), 2.21 s (3H), 2.10 s (3H), 2.09 s (3H[illegible]2.02 s (3H), 1.69 d (*J* = 1.1 Hz, 3H), 1.66 dd (*J* = 1.7, 6.8 Hz, 3H), 1.44 d (*J* = 7.1 Hz, 3H), 1.37 s (3H), 1.17 d (*J* = 7.1 Hz, 3H), 1.08 d (*J* = 6.6 Hz, 3H), 0.90 t (*J* = 6.1 Hz, 3H). HR-ESIMS *m/z* 1072.60225, 1086.60997, 1099.62465 (calcd for $^{12}C_{55}{}^{13}CH_{88}NaO_{18}^+$ 1072.59019 (+11.24 ppm), $^{12}C_{56}{}^{13}CH_{90}NaO_{18}^+$ 1086.60584 (+3.8 ppm), and $C_{58}H_{92}NaO_{18}^+$ 1099.61814 (+5.9 ppm)).

2.9. Compound 4 fro[illegible]2. ^{1}H NMR: δ 5.65 dd (*J* = 2.6, 9.8 Hz), 5.52 m, 5.51 d (*J* = 10.[illegible]z), 5.37 m, 5.34 dd (*J* = 1.7, 9.1 Hz), 5.28 dt (*J* = [illegible], 10.5 Hz), 5.07 d (*J* = 10.9 Hz), 4.78 d (*J* = 6.3 Hz), 4.54 dd (*J* = 3.1, [illegible] Hz), 4.25 dd (*J* = 2.3, 8.1 Hz), 4.13 dd (*J* = [illegible], 11.3 Hz), 3.43 s (3H), 3.34 dq (*J* = 10.1, 5.7 Hz), 3.29 dq (*J* = 10.[illegible]2 Hz), 3.01 dd (*J* = 9.4, 18.6 Hz), 2.88 d (*J* [illegible].2 Hz), 2.60 dd (*J* = 2.5, 18.6 Hz), 2.35–2.4 m, 2.32 ([illegible] 1.9, 9.3 Hz), 2.22 s (3H), 2.10 s (3H), 2.09 s ([illegible]), 2.02 s (3H), 1.70 d (*J* = 1.1 Hz, 3H), 1.62 dd (*J* = 1.7, 6.8 Hz, 3H), 1.44 d (*J* = 7.1 Hz, 3H), 1.37 s (3H), 1.17 d (*J* = 7.1 Hz, 3H), 1.08 d (*J* = 6.6 Hz, 3H), 0.90 t (*J* = 6.1 Hz, 3H). HR-ESIMS $[M+Na^+]$ *m/z* 1071.58258, 1085.60199, 1099.61165 (calcd for $C_{56}H_{88}NaO_{18}^+$ 1071.58629 (−4 ppm), $C_{57}H_{90}NaO_{18}^+$ 1085.60194 (−.5 ppm), and $C_{58}H_{92}NaO_{18}^+$ 1099.61759 (−5.9 ppm)).

2.10. Screening Process. NBT-T2 cells were purchased f[illegible] Riken and used for cytotoxicity testing. NBT-T2 is a cell line derived from chemically induced rat bladder carcinoma cells [19]. The sponge extract was tested at 0.1, 1, and 10 μg/mL in triplicate, while fractions were done at 0.01, 0.1, and 1 μg/mL. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with Sigma antibiotic-antimycotic, Biowest fetal bovine serum, Gibco MEM nonessential amino acid in a Falcon 24-well plate or 48-well plate. After adding the extract or a fraction, cells were incubated for 24 h under 5% CO_2 at 36°C [16].

Table 1: ^{13}C NMR data for compounds **1**, **2**, and **3**.

C no.	**1**[15,a]	**1**[a]	**2**[a]	**3**[b]
1	171.3 qC	171.3 qC	171.3 qC	171.3 qC
2	70.7 CH	70.8 CH	70.8 CH	72.9 CH
3	83.4 CH	83.3 CH	83.1 CH	84.7 CH
4	47.8 CH	47.7 CH	47.9 CH	49.5 CH
5	214.6 qC	214.6 qC	216.2 qC	217.6 qC
6	48.5 CH	48.5 CH	49.8 CH	51.1 CH
7	80.1 CH	80.1 CH	79.2 CH	79.9 CH
8	131.6 qC	132.0 qC	136.2 qC	139.0 qC
9	132.0 CH	131.7 CH	129.4 CH	129.8 CH
10	46.2 CH	46.1 CH	45.9 CH	46.5 CH
11	211.5 qC	211.7 qC	211.9 qC	212.7 qC
12	42.6 CH_2	42.7 CH_2	42.5 CH_2	44.3 CH_2
13	68.9 CH	68.8 CH	69.0 CH	69.3 CH
14	81.6 qC	81.6 qC	81.6 qC	85.0 qC
15	210.8 qC	211.0 qC	211.2 qC	216.5 qC
16	46.9 CH	47.0 CH	46.9 CH	46.4 CH
17	77.8 CH	77.7 CH	77.7 CH	78.4 CH
18	62.7 qC	62.7 qC	62.7 qC	63.9 qC
19	67.1 CH	67.0 CH	67.0 CH	67.1 CH
20	31.1 CH	31.1 CH	30.9 CH	32.4 CH
21	129.7 CH	129.4 CH	129.6 CH	131.6 CH
22	125.5 CH	125.5 CH	125.4 CH	126.2 CH
23	13.5 CH_3	13.5 CH_3	13.3 CH_3	13.6 CH_3
24	14.6 CH_3	14.5 CH_3	14.4 CH_3	15.3 CH_3
25	14.7 CH_3	14.5 CH_3	15.0 CH_3	15.6 CH_3
26	10.8 CH_3	10.7 CH_3	10.2 CH_3	10.5 CH_3
27	16.3 CH_3	16.2 CH_3	16.4 CH_3	15.7 CH_3
28	63.5 CH_2	63.5 CH_2	63.5 CH_2	65.7 CH_2
29	63.2 CH_2	63.2 CH_2	62.8 CH_2	64.1 CH_2
30	11.1 CH_3	11.0 CH_3	10.9 CH_3	11.5 CH_3
31	18.6 CH_3	18.4 CH_3	18.3 CH_3	18.7 CH_3
32	60.3 CH_3	60.3 CH_3	60.3 CH_3	60.3 CH_3
33	169.5 qC	169.9 qC	—	—
34	21.6 CH_3	21.5 CH_3	—	—
35	173.5 qC	173.7 qC	173.6 qC	—
36	34.2 CH_2	34.1 CH_2	34.0 CH_2	—
37	29.0 CH_2	29.0 CH_2	29.1 CH_2	—

[a]Measured in $CDCl_3$. [b]Measured in CD_3OD.

Then, the cells were observed under a microscope to evaluate viability of cells whether the fractions were cytotoxic or not.

2.11. MTT Assay. Cultured cells were inoculated to a 96-well plate with approximate cell density of 1×10^4 cells/mL in DMEM. After 24 h incubation, a series of DMSO solution of compounds **1**–**3** were applied to each well and the final concentrations were adjusted as 0, 1, 12.5, 25, 37.5, 50, 62.5, to 75 ng/mL. Cells were incubated for another 24 h, and the media were replaced with 20 μL of 5 g/mL MTT solution in PBS and incubated for 3.5 h. After removal of PBS solution, an amount of 150 μL of DMSO was added to each well and the cells were reincubated for 15 min prior to measurement with a Tecan microplate reader at 590 nm with reference filter at 620 nm [20, 21].

3. Results and Discussion

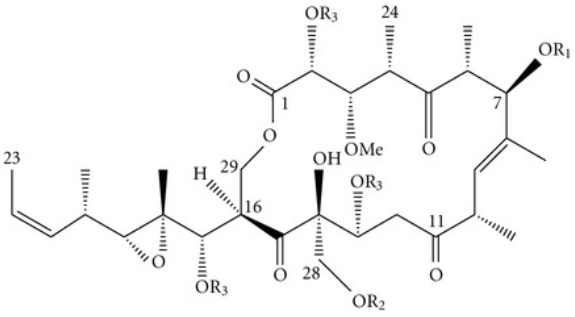
As an EtOAc soluble portion of a methanolic extract of the sponge K09-02 showed potent cytotoxicity against cultured NBT-T2 cells, the portion was separated repetitively on a silica gel column followed by Si-60 HPLC affording three compounds **1**, **2**, and **3** as shown in Figure 1.

By inspecting ^{1}H and ^{13}C NMR spectra of compound **1** together with database search (Tables 1 and 2, Figures S1 and S2 (Supplementary Materials available online

Table 2: ^{1}H NMR data for compounds **1**, **2**, and **3** (J in Hz).

C no.	**1**[15,a]	**1**[a]	**2**[a]	**3**[b]
1	—	—	—	—
2	3.96 dd (1.0, 7.3)	3.96 dd (1.3, 7.5)	3.98 dd (1.3, 7.8)	3.76 d (2.2)
3	3.64 dd (1.3, 7.8)	3.67 dd (1.3, 8.0)	3.67 dd (1.3, 8.4)	3.81 dd (2.2, 9.8)
4	3.12 m	3.13 dd (8.0, 7.1)	3.10 dq (8.4, 7.3)	3.16 dq (9.8, 7.1)
5	—	—	—	—
6	3.18 dq (10.7, 7.3)	3.22 dq (10.7, 6.8)	3.04 dq (9.8, 6.8)	3.16 dq (10.0, 7.1)
7	5.39 d (10.7)	5.41 d (10.7)	4.12 d (10.0)	4.03 d (10.0)
8	—	—	—	—
9	5.60 d (9.3)	5.62 d (9.6)	5.48 d (10.5)	5.33 d (9.3)
10	3.38 dq (9.3, 6.8)	3.41 dq (9.6, 7.0)	3.49 dq (10.5, 7.1)	3.36 dq (9.3, 6.8)
11	—	—	—	—
12	2.66 dd (9.8, 16.1) 2.49 dd (2.4, 16.1)	2.69 dd (9.8, 16.1) 2.49 dd (2.5, 16.1)	2.72 dd (9.8, 16.1) 2.51 dd (2.0, 16.1)	2.75 dd (9.5, 17.6) 2.23 dd (2.0, 17.6)
13	4.40 m	4.42 m	4.39 dt (2.0, 9.8)	4.44 dd (2.9, 9.5)
14	—	—	—	—
15	—	—	—	—
16	4.02 dt (3.9, 10.9)	4.03 ddd (3.9, 10.5, 11.5)	4.09 dt (3.9, 11.0)	4.08 ddd (3.9, 10.8, 11.4)
17	3.12 m	3.13 m	3.20 dd (11.0)	3.20 d (10.7)
18	—	—	—	—
19	2.56 d (9.3)	2.59 d (9.3)	2.58 d (9.8)	2.62 d (9.3)
20	2.44 m	2.47 m	2.47 m	2.48 m
21	5.23 dt (1.5, 10.7)	5.25 ddd (0.7, 10.2, 10.9)	5.24 dt (1.5, 10.5)	5.31 m
22	5.48 dq (10.7, 6.8)	5.51 dq (10.9, 6.8)	5.49 dq (10.5, 6.8)	5.51 dq (10.7, 6.8)
23	1.59 dd (1.5, 6.8)	1.62 dd (1.2, 6.8)	1.62 dd (1.5, 6.8)	1.62 dd (1.7, 6.8)
24	1.18 d (6.8)	1.21 d (7.1)	1.21 d (7.3)	1.23 d (7.1)
25	1.13 d (7.3)	1.16 d (6.8)	1.28 d (6.8)	1.26 d (7.1)
26	1.54 brd (1.0)	1.59 d (0.8)	1.63 s	1.65 d (1.5)
27	1.07 d (6.8)	1.09 d (7.0)	1.10 d (7.1)	1.03 d (6.8)
28	4.44 d (11.7) 4.19 d (11.7)	4.46 d (11.5) 4.22 d (11.5)	4.45 d (11.5) 4.21 d (11.5)	3.75 d (10.5) 3.76 d (10.5)
29	4.17 dd (3.7, 9.8) 4.10 dd (10.2, 10.9)	4.20 dd (3.7, 10.2) 4.12 dd (10.2, 11.2)	4.24 dd (3.0, 9.5) 4.08 m	4.35 dd (3.9, 10.5) 3.91 dd (10.5, 11.4)
30	1.38 s	1.42 s	1.42 s	1.35 s
31	1.11 d (6.4)	1.13 d (6.3)	1.14 d (6.6)	1.11 d (6.6)
32	3.28 s	3.31 s	3.30 s	3.39 s
33	—	—	—	—
34	2.01 s	2.04 s	—	—
35	—	—	—	—
36	2.24 t (7.6)	2.27 t (7.6)	2.27 t (11.8)	
37	1.53 brs	1.59 brs	1.25 brs	
OH-2	2.85 d (7.3)	2.92 d (7.5)	2.99 d (7.8)	2.92 d (7.5)
OH-13	—		4.71 s	

[a]Measured in $CDCl_3$. [b]Measured in CD_3OD.



(1) R_1 = Ac, R_2 = $CO(CH_2)_{14-16}CH_3$, R_3 = H

(2) R_1 = R_3 = H, R_2 = $CO(CH_2)_{14-19}CH_3$

(3) R_1 = R_2 = R_3 = H

(4) R_1 = R_3 = Ac, R_2 = $CO(CH_2)_{14-16}CH_3$

FIGURE 1: Structures of compounds (1)–(4).

at doi:10.5402/2011/852619)), we could readily identify that it is a member of candidaspongiolide, a series of 18-membered cytotoxic macrolide retaining one of fatty acid moieties from C_{14} to C_{18} at C-28 [15]. HR-ESIMS of our material exhibited molecular-related ions at *m/z* 945.55514, 959.57079, 973.58884 $[M+Na]^+$ indicating that compound **1** is candidaspongiolide esterified with the homologs of three saturated fatty acids (palmitic, margaric, and stearic acids).

Compound **2** was obtained as colorless glass with $[\alpha]_D^{25}$ +72. After elucidation of its ^1H and ^{13}C NMR spectra, compound **2** was found to be an analog of **1**. However, the ^{13}C NMR spectrum showed two carbonyl carbons at δ_C 171.3 q (C-1) and 173.1 (C-35) instead of three in **1** (Table 1, Figure S3). As the signals for an acetoxy group (δ_H 2.04 s, δ_C 21.5 q) in **1** are missing in **2**, it was suggested that **2** is a deacetyl derivative of **1**. The lack of the acetyl group is in a good agreement with ^1H NMR spectrum and COSY analysis showing that H-7 proton signal (δ_H 4.12 d, J = 10.0 Hz) in **2** shifted to higher field than that (δ_H 5.41 d, J = 10.7 Hz) in **1** (Table 2). HR-ESIMS of **2** showed a series of sodiated ions $[M+Na]^+$ at *m/z* 903.54964, 917.56023, 931.57576, 945.60036, and 959.61056 corresponding to the presence of C_{16} to C_{20} esters. For structural confirmation, compound **2** was acetylated to give tetraacetate **4**, which showed signals identical with **4** obtained from **1** (Figure S4). Compound **4** exhibited four acetyl signals at δ_H 2.22 s, 2.10 s, 2.09 s, and 2.02 s and molecular-related ions corresponding to macrolide esters with C_{16} to C_{18} fatty acids.

Compound **3** was isolated as a yellowish glass with $[\alpha]_D^{25}$ +97. Its molecular formula was established as $C_{32}H_{50}O_{13}$ by observing a molecular related ion at *m/z* 665.31522 $[M+Na]^+$ in HR-ESIMS. ^1H and ^{13}C NMR spectra (Tables 1 and 2, Figures S5 and S6) revealed that compound **3** has a similar macrolide structure to that of compound **1** except for the lack of a fatty acid ester moiety and an acetate found in **1**. Higher field chemical shifts observed for H-7 (δ_H 4.03) and H-23 (δ_H 3.75) indicated that **3** is devoid of acyl groups. Close similarity of ^1H and ^{13}C NMR data of **3** to **1** (Table 2) indicated that the macrolide core structure of compound **3** is identical to compound **1**.

All of natural compounds **1**–**3** exhibited potent cytotoxicity, IC_{50} 37, 4.7, and 19 ng/mL, against NBT-T2 cells. The result is not in good agreement with those reported by Meragelman and coworkers, that is, candidaspongiolide (**1**) showed stronger growth inhibition (GI_{50}14 ng/mL) than the core compound (42 ng/mL) [15]. Additionally Paul et al. paperd the importance of a linear carbon chain on the cytotoxicity in the case of amphidinol [22]. The difference may be explained either by the number of cell lines or by different sensitivity of NBT-T2 cells.

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