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 (1H, 13C, COSY, HMQC, HMBC) and by comparison of their NMR data with those of 1. Compounds 2 and 3 exhibited a little more potent cytotoxicity (IC50 4.7 and 19 ng/mL) than that (IC50 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 (1) 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 GI50 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 F254 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 P-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-T100LP spectrometer using reeserine 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\(_2\)OD with TMS as an internal standard on a 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 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 (2.7 g), which killed NBT-T2 cells at 10.0 mg/L and/or a lipophilic fraction (2.7 g), which killed NBT-T2 cells at 10.0 mg/L, while those of compound 3 were measured in CD\(_2\)OD with TMS as an internal standard. Most of \(^1\)H and \(^{13}\)C NMR spectra were measured in CDCl\(_3\) whereas HRESIMS was measured on a Jeol JMS-T100LP spectrometer using reeserine 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\(_2\)OD with TMS as an internal standard on a 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.

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

| C no. | 1$^{a}$ | 1$^{a}$ | 2$^{b}$ | 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 | 214.9 qC | 216.2 qC | 217.6 qC |
| 6     | 48.5 CH  | 48.3 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  | 62.6 qC  | 63.9 qC |
| 19    | 67.1 CH  | 67.0 CH  | 67.0 CH  | 67.4 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$_3$ | 13.4 CH$_3$ | 13.3 CH$_3$ | 13.3 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$_2$ | 64.8 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$_3$OD.

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 $1 \times 10^4$ 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 $\mu$L of 5 g/mL MTT solution in PBS and incubated for 3.5 h. After removal of PBS solution, an amount of 150 $\mu$L 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. | $^1$ | $^2$ | $^3$ |
|-------|------|------|------|
| 1     |      |      |      |
| 2     | 3.96 dd (1.0, 7.3) | 3.96 dd (1.3, 7.5) | 3.98 dd (1.3, 7.8) |
| 3     | 3.64 dd (1.3, 7.8) | 3.67 dd (1.3, 8.0) | 3.67 dd (1.3, 8.4) |
| 4     | 3.12 m | 3.13 dd (8.0, 7.1) | 3.10 dq (8.4, 7.3) |
| 5     |      |      | 3.16 dq (9.8, 7.1) |
| 6     | 3.18 dq (10.7, 7.3) | 3.22 dq (10.7, 6.8) | 3.04 dq (9.8, 6.8) |
| 7     | 5.39 d (10.7) | 5.41 d (10.7) | 4.12 d (10.0) |
| 8     |      |      | 4.03 d (10.0) |
| 9     | 5.60 d (9.3) | 5.62 d (9.6) | 5.48 d (10.5) |
| 10    | 3.38 dq (9.3, 6.8) | 3.41 dq (9.6, 7.0) | 3.49 dq (10.5, 7.1) |
| 11    |      |      | 3.36 dq (9.3, 6.8) |
| 12    | 2.66 dd (9.8, 16.1) | 2.69 dd (9.8, 16.1) | 2.72 dd (9.8, 16.1) |
| 13    | 4.40 m | 4.42 m | 4.39 dt (2.0, 9.8) |
| 14    |      |      | 4.44 dd (2.9, 9.5) |
| 15    |      |      |      |
| 16    | 4.02 dt (3.9, 10.9) | 4.03 dd (3.9, 10.5, 11.5) | 4.09 dt (3.9, 11.0) |
| 17    | 3.12 m | 3.13 m | 3.20 dd (11.0) |
| 18    |      |      | 3.20 d (10.7) |
| 19    | 2.56 d (9.3) | 2.59 d (9.3) | 2.58 d (9.8) |
| 20    | 2.44 m | 2.47 m | 2.47 m |
| 21    | 5.23 dt (1.5, 10.7) | 5.25 dd (0.7, 10.2, 10.9) | 5.24 dt (1.5, 10.5) |
| 22    | 5.48 dq (10.7, 6.8) | 5.51 dq (10.9, 6.8) | 5.49 dq (10.5, 6.8) |
| 23    | 1.59 dd (1.5, 6.8) | 1.62 dd (1.2, 6.8) | 1.62 dd (1.5, 6.8) |
| 24    | 1.18 d (6.8) | 1.21 d (7.1) | 1.21 d (7.3) |
| 25    | 1.13 d (7.3) | 1.16 d (6.8) | 1.28 d (6.8) |
| 26    | 1.54 brd (1.0) | 1.59 d (0.8) | 1.63 s |
| 27    | 1.07 d (6.8) | 1.09 d (7.0) | 1.10 d (7.1) |
| 28    | 4.44 d (11.7) | 4.46 d (11.5) | 4.45 d (11.5) |
| 29    | 4.19 d (11.7) | 4.22 d (11.5) | 4.21 d (11.5) |
| 30    | 4.17 d (3.7, 9.8) | 4.20 dd (3.7, 10.2) | 4.24 dd (3.0, 9.5) |
| 31    | 4.10 dd (10.2, 10.9) | 4.12 dd (10.2, 11.2) | 4.08 m |
| 32    | 1.38 s | 1.42 s | 1.42 s |
| 33    | 1.11 d (6.4) | 1.13 d (6.3) | 1.14 d (6.6) |
| 34    | 3.28 s | 3.31 s | 3.30 s |
| 35    |      |      | 3.39 s |
| 36    |      |      |      |
| 37    | 2.24 t (7.6) | 2.27 t (7.6) | 2.27 t (11.8) |
| OH-2 | 1.53 brs | 1.59 brs | 1.25 brs |
| OH-13| 2.85 d (7.3) | 2.92 d (7.5) | 2.99 d (7.8) |

$^a$Measured in CDCl$_3$. $^b$Measured in CD$_3$OD.
Compound 4 is a deacetyl derivative of 1 in a good agreement with 1H NMR spectrum and COSY showed signals identical with 959.57079, 973.58884 [M+Na]+ indicating that compound material exhibited molecular-related ions at [M+Na]+ in HR-ESIMS. 1H and 13C NMR spectra (Table 1, Figure S3) revealed that compound 4 is candidaspongiolide esterified with the homologs of three saturated fatty acids (palmitic, margaric, and stearic acids).

Compound 2 was obtained as a colorless glass with [α]125
+72. After elucidation of its 1H and 13C NMR spectra, compound 2 was found to be an analog of 1. However, the 13C NMR spectrum showed two carbonyl carbons at δC 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 (931.57576, 945.60036, and 959.61056 corresponding to the δC 21.5 q) in 1 shifted to higher field than that (δC 10.0 Hz) in 2 shifted to higher field than that (δH 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 C16 to C30 esters. For structural elucidation, 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 δH 2.22 s, 2.10 s, 2.09 s, and 2.02 s and molecular-related ions corresponding to macrolide esters with C16 to C18 fatty acids.

Compound 3 was isolated as a yellowish glass with [α]125
+97. Its molecular formula was established as C32H50O13 by observing a molecular-related ion at m/z 665.31522 [M+Na]+ in HR-ESIMS. 1H and 13C 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 acetyl found in 1. Higher field chemical shifts observed for H-7 (δH 4.03) and H-28 (δH 3.75) indicated that 3 is devoid of acyl groups. Close similarity of 1H and 13C 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, IC50 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 (GL50 14 ng/mL) than the core compound (42 ng/mL) [15]. Additionally Paul et al. papered the importance of a linear carbon chain on the cytotoxicity in the case of amphibinol [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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