Arch Pharm Res Vol 32, No 6, 857-862, 2009
DOI 10.1007/s12272-009-1607-1

Archives of Pharmacal Research
www.springer.com/12272

Bile Acid Derivatives from a Sponge-Associated Bacterium *Psychrobacter* sp.

Huayue Li[1,*], Pramod B. Shinde[1,*], Hye Ja Lee[2], Eun Sook Yoo[2], Chong-O. Lee[3], Jongki Hong[4], Sang Ho Choi[5], and Jee H. Jung[1]

[1]College of Pharmacy, Pusan National University, Busan 609-735, Korea, [2]College of Medicine, Cheju National University, Jeju 690-756, Korea, [3]Pharmaceutical Screening Center, Korea Research Institute of Chemical Technology, Daejon 305-343, Korea, [4]College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea, and [5]Department of Agricultural Biotechnology, Seoul National University, Seoul 151-747, Korea

(Received April 3, 2009/Revised May 25, 2009/Accepted June 9, 2009)

In our search for bioactive metabolites from a marine sponge-associated bacterium *Psychrobacter* sp., a new bile acid derivative (**1**), which was assumed to be an artifact, were isolated along with six known (**2**-**7**) compounds by bioactivity-guided fractionation. Elucidation of the structure of the new compound was done using a combination of NMR (^{1}H, ^{13}C, HMBC, HSQC, and COSY) and MS spectroscopy. Compound **1** exhibited moderate suppressive effects on both NO and IL-6 production at a concentration of 200 μM (87.3 μg/mL) without significant cytotoxicity against cells. Compounds **2**-**5** and **7** showed selective inhibitory activity against several human pathogenic bacterial strains at the low concentration of 30 μg/well. In a cytotoxicity evaluation, only compound **7** showed mild cytotoxicity against five human solid tumor cell lines (A-549, SK-OV-3, SK-MEL-2, XF-498, and HCT-15) with ED_{50} values in the range of 11-14 μg/mL.

Key words: Sponge-associated, *Psychrobacter* sp., Bile acid, Anti-inflammatory, Antibacterial, Cytotoxicity

INTRODUCTION

In recent years, much attention has been given to marine sponge-associated microorganisms, which have emerged to be a rich source of novel and biologically active secondary metabolites (Piel, 2004). A bacterial strain that exhibited good antibacterial activity was isolated from a marine sponge *Stelletta* sp., and was classified as the genus *Psychrobacter* (Li et al., 2008). *Psychrobacter* spp., belonging to gram-negative gamma-proteobacteria, have been isolated from several kinds of marine sponges including *Stelletta tenui*, *Halichondria rugosa*, and *Dysidea avara* (Li et al., 2007). Members of the genus *Psychrobacter*, discovered at both poles, in deep sea waters, and in other diverse marine environments, are psychrotolerant or psychrophilic and halotolerant (Bowman et al., 1996).

In our previous investigation of the sponge-associated bacterium, *Psychrobacter* sp., we isolated sixteen cyclic dipeptides (Li et al., 2008). In a continuing study of the same bacterium, we isolated seven bile acid derivatives (**1**-**7**). The current paper deals with the isolation, structural elucidation, and bioactivity of these bile acid derivatives.

MATERIALS AND METHODS

General experimental procedures

^{1}H and 2D NMR (HSQC, HMBC, and COSY) spectra were recorded at 500 MHz using a Varian INOVA 500 spectrometer, and ^{13}C NMR spectra were recorded on a Varian UNITY 400 spectrometer. Chemical shifts were reported with reference to the respective solvent peaks and residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FABMS data were obtained on a JEOL JMS SX-102A spectrometer. HPLC was done on a Gilson 370 pump with a YMC ODS-H80 column (250

*These authors equally contributed to this work
Correspondence to: Jee H. Jung, College of Pharmacy, Pusan National University, Busan 609-735, Korea
Tel. 82-51-510-2803, Fax. 82-51-513-6754
E-mail: jhjung@pusan.ac.kr

× 10 mm i.d., 4 μm, 80 Å) using an RI-101 detector.

Bacterial isolation and culture conditions

The sponge *Stelletta* sp. was collected by hand using SCUBA gear (20 m depth) in 2005 off the coast of Geoje Island, Korea. Following a rinse with sterile sea water, small pieces (1×1 cm^3) of the surface and inner tissue of the sponge were homogenized and then inoculated on Zobell 2216 marine agar (5 g/L of peptone, 1 g/L of yeast extract, 1 mg/L of $FePO_4 \cdot 4H_2O$, and 15 g/L of agar in 75% aged sea water; pH was adjusted to 7.4 with 1 M NaOH) at 25°C for 3 days. From this process, 12 pure bacterial strains (J05B1-1~J05B1-12) were isolated from the sponge *Stelletta* sp. Among these 12 bacterial strains, the EtOAc extract of J05B1-11 (200 μg/well) showed the highest inhibitory effect against *Staphylococcus aureus* 503 (ATCC) (inhibition zone: 16 mm) and *Escherichia coli* TEM (ATCC) (inhibition zone: 8 mm) in radial diffusion assays. Comparative analyses of the 16S rDNA sequence (1,390 nt) of strain J05B1-11 with those of representative members of the major lines of descent within the domain *Bacteria* revealed that the isolate was phylogenetically affiliated with the genus *Psychrobacter* (Li et al., 2008).

Single colonies of the bacterium *Psychrobacter* sp. were subcultured in 500 mL Erlenmeyer flasks each containing 200 mL of Zobell 2216 broth medium (5 g/L of peptone, 1 g/L of yeast extract, 1 mg/L of $FePO_4 \cdot 4H_2O$ in 75% aged sea water; pH 7.4) and incubated at 25°C for 4 days on a rotary shaker (160 rpm). To provide cultures for metabolite isolation, 8 mL of a subculture was transferred to a 2 L Erlenmeyer flask containing 800 mL of the same medium, and incubated for 7 days under similar conditions.

Extraction and isolation

The combined culture broth (80 L) was extracted with EtOAc at room temperature, and the EtOAc extract (4.5 g) was partitioned between 90% MeOH and *n*-hexane to yield 3.3 g and 1.0 g of residues, respectively. The aqueous MeOH layer, which exhibited good antibacterial properties against *Staphylococcus aureus* 503 (inhibition zone: 15 mm) and *Escherichia coli* TEM (inhibition zone: 5 mm), was subjected to a stepped-gradient MPLC (ODS-A, 120 Å, S-30/50 mesh) eluting with 20-100% MeOH to yield 18 fractions. Compounds **1-3** and **7** (4.1, 1.6, 43.2, and 27.9 mg, respectively) were obtained by separation of fraction 13 (inhibition zone: 18 mm against *S. aureus*) on a reversed-phase HPLC (YMC ODS-H80 column) eluting with 85% MeOH (1 mL/min). Compounds **4-6** (5.6, 5.0, and 1.1 mg, respectively) were isolated from fraction 8 (inhibition zone: 16 mm against *S. aureus*) eluting with 75% MeOH + 0.2% HCOOH (v/v) (1 mL/min). The structures of compounds **1-7** are shown in Fig. 1.

3-Dimethoxy-12α-hydroxycholanic acid (1)

Colorless oil; ^{1}H NMR (CD_3OD, 500 MHz) δ 3.95 (1H, br t, J = 2.5 Hz, H-12), 3.17 (3H, s, H-25), 3.11 (3H, s, H-26), 2.31 (1H, m, H-23a), 2.20 (1H, m, H-23b), 1.77 (1H, m, H-22a), 1.31 (1H, m, H-22b), 0.99 (3H, d, J = 6.0, H-21), 0.93 (3H, s, H-19), 0.70 (3H, s, H-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 178.4 (C-24), 102.5 (C-3), 74.0 (C-12), 49.2 (C-14), 48.2 (C-17), 47.7 (C-25), 47.6 (C-26), 47.5 (C-13), 41.1 (C-5), 37.2 (C-8), 36.7 (C-20), 35.5 (C-10), 34.4 (C-4), 34.2 (C-9), 34.0 (C-1), 32.4 (C-22), 32.3 (C-23), 30.1 (C-11), 28.3 (C-7), 27.9 (C-6), 27.3 (C-2), 26.7 (C-16), 24.8 (C-15), 23.6 (C-19), 17.6 (C-21), 13.2 (C-18). LRFABMS m/z 435.36 $[M - H]^-$; HRFABMS m/z 435.3114 $[M - H]^-$ (calc. for $C_{26}H_{43}O_5$ 435.3189).

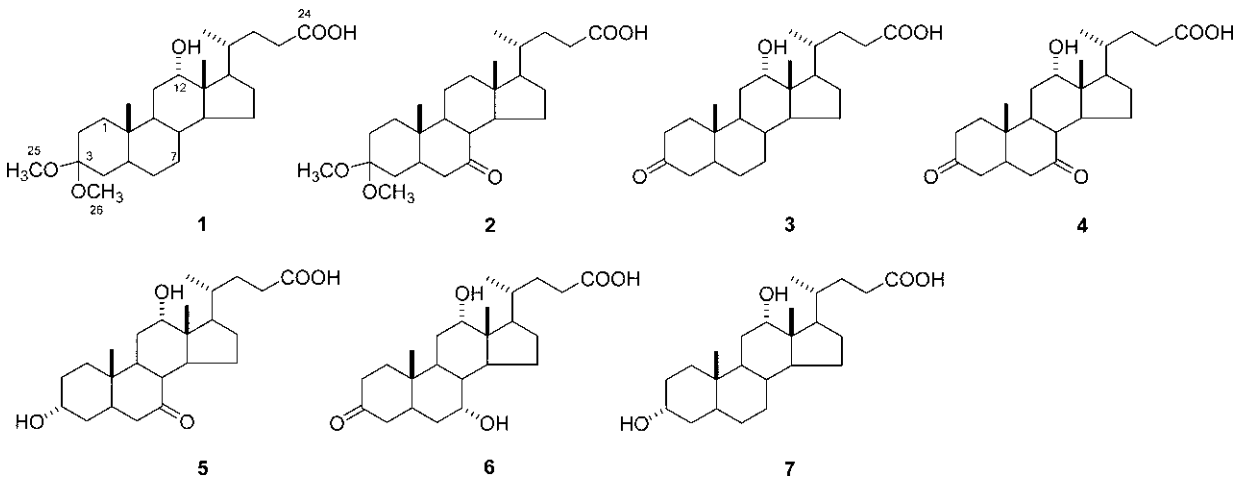

Fig. 1. Structures of bile acid derivetives **1-7** isolated from *Psychrobacter* sp.

3-Dimethoxy-7-ketocholanic acid (2)
Yellow oil; ^{1}H NMR (CD_3OD, 500 MHz) δ 3.14 (3H, s, H-25), 3.10 (3H, s, H-26), 2.97 (1H, dd, *J* = 13.0, 6.5 Hz, H-6a), 2.54 (1H, d, *J* = 11.5, H-8), 2.31 (1H, m, H-23a), 2.18 (1H, m, H-23b), 1.98 (1H, m, H-6b), 1.80 (1H, m, H-22a), 1.30 (1H, m, H-22b), 1.23 (3H, s, H-19), 0.95 (3H, d, *J* = 7.0, H-21), 0.70 (3H, s, H-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 215.2 (C-7), 178.7 (C-24), 101.6 (C-3), 56.3 (C-17), 50.7 (C-8), 50.3 (C-14), 47.8 (C-25), 47.7 (C-26), 46.0 (C-6), 45.8 (C-5), 44.2 (C-9), 43.8 (C-13), 40.3 (C-12), 36.6 (C-20), 36.5 (C-10), 35.6 (C-4), 33.1 (C-1), 32.5 (C-22), 32.5 (C-23), 29.3 (C-16), 28.0 (C-2), 25.8 (C-15), 23.4 (C-19), 23.0 (C-11), 18.8 (C-21), 12.5 (C-18). LRFABMS *m/z* 457.37 $[M + Na]^+$.

12α-Hydroxy-3-ketocholanic acid (3)
Colorless oil; ^{13}C NMR (CD_3OD, 100 MHz) δ 216.2, 178.3, 73.9, 49.2, 48.2, 47.7, 45.9, 43.2, 38.0, 37.9, 37.0, 36.7, 35.6, 34.8, 32.3, 32.1, 30.1, 28.6, 27.7, 26.7, 24.8, 22.7, 17.8, 13.2 LRFABMS *m/z* 389.37 $[M - H]^-$; HRFABMS *m/z* 389.2725 $[M - H]^-$ (calc. for $C_{24}H_{37}O_4$ 389.2770).

12α-Hydroxy-3,7-diketocholanic acid (4)
Yellow oil; ^{13}C NMR (CD_3OD, 100 MHz) δ 213.8, 213.2, 178.2, 72.9, 50.7, 49.0, 47.7, 47.3, 45.8, 43.8, 41.9, 37.4, 37.3, 36.6, 36.3, 36.1, 32.3, 32.0, 30.8, 28.7, 25.3, 22.3, 17.6, 13.2.

3 α,12α-Dihydroxy-7-ketocholanic acid (5)
Yellow oil; ^{13}C NMR (CD_3OD, 100 MHz) δ 216.0, 178.9, 72.9, 71.6, 50.7, 47.6, 47.5, 47.3, 46.3, 41.9, 38.3, 37.5, 36.6, 35.9, 35.1, 32.3, 32.0, 30.6, 30.5, 28.7, 25.3, 23.2, 17.6, 13.2.

7 α,12α-Dihydroxy-3-ketocholanic acid (6)
Colorless oil; ^{13}C NMR (CD_3OD, 100 MHz) δ 216.6, 178.8, 73.8, 68.8, 48.1, 47.8, 46.6, 44.9, 43.0, 41.0, 37.8, 37.6, 36.8, 36.2, 35.0, 32.4, 32.2, 29.9, 28.7, 28.4, 24.2, 22.1, 17.6, 13.0.

3 α,12α-Dihydroxycholanic acid (7)
Yellow oil; ^{13}C NMR (CD_3OD, 100 MHz) δ 178.3, 74.0, 72.5, 49.3, 48.1, 47.5, 43.6, 37.4, 37.2, 36.7, 36.4, 35.3, 34.8, 32.3, 32.0, 31.1, 29.9, 28.6, 28.4, 27.5, 24.9, 23.7, 17.6, 13.2.

Anti-inflammatory activity

Evaluation of anti-inflammatory activity was performed at Cheju National University.

Cell culture: The murine macrophage RAW 264.7 cell line was purchased from ATCC (Rockville, MD) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-activated fetal bovine serum, streptomycin (100 μg/mL), and penicillin (100 U/mL) at 37°C in a 5% CO_2 atmosphere.

Cytotoxicity assay: Cell viability was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, MTT was added to cells for the designed time. After 4 h, the formazan crystals were dissolved by adding DMSO. Metabolic activity was quantified by measuring light absorbance at 540 nm.

Measurement of NO production: Nitrite, the endpoint of NO generation by activated macrophage, was measured by a colorimetric assay (Ryu et al., 2000). Briefly, 100 μL of Griess reagent (1% sulfanilamide and 0.1% *N*-[1-naphthyl]-ethylenediamine dihydrochloride in 5% phosphoric acid) was added to 100 μL samples of medium. The concentration of NO_2^- was calculated by comparison with a standard curve prepared using $NaNO_2$.

Measurement of the production of the pro-inflammatory cytokine interleukin-6 (IL-6): Compounds were dissolved in EtOH and diluted with DMEM. The final concentration of chemical solvents did not exceed 0.1% in the culture medium, a concentration which does not change IL-6 production. Before stimulation with lipopolysaccharide (LPS) (1 μg/mL) and test materials, RAW 264.7 cells were incubated for 18 h in 24-well plates under the same conditions. The medium was used for IL-6 assay using a mouse ELISA kit (BD Biosciences, CA, USA).

Antibacterial activity

Antibacterial activities were tested by a radial diffusion assay adapted from the method of Lehrer et al. (1991) with some modifications. Bacteria were grown overnight at 37°C in LB media and diluted to 1/100. A gel solution containing 2.5% (w/v) of powdered LB medium, and 1.5% agar was prepared and autoclaved. Then, 0.15 mL of the diluted bacterial culture was added to 15 mL of the gel solution at 40-50°C. Once the bacteria were adequately dispersed, the gel was poured into a Petri dish (90 × 15 mm). After solidification, wells were made using a 2 mm punch. Ten μL of each sample (20 mg/mL for crude samples; 3 mg/mL for pure compounds) were added to each well, and the plates were incubated for 18 h at 37°C. Tetracycline (≥ 98% purity, 30 μg/well) was used as a positive control. The diameters of the inhibition zones surrounding the wells were measured in millimeters.

Cytotoxicity against human cancer cell lines

Evaluation of cytotoxicity was performed at the Korean Research Institute of Chemical Technology. Rapidly growing cells (A-549, human lung cancer; SK-

OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF-498, human CNS cancer; HCT-15, human colon cancer) were harvested, counted, and inoculated at the appropriate concentrations ((1-2) × 10^4 cells/well) into 96-well microtiter plates. After incubation for 24 h, the compounds dissolved in culture medium (RPMI 1640, Gibco; 10% FBS, Gibco) were applied to the culture wells in triplicate followed by incubation for 48 h at 37°C under a 5% CO_2 atmosphere. The culture was fixed with cold TCA, and was stained by 0.4% SRB (sulforhodamine B, Sigma) dissolved in 1% acetic acid. After solubilizing the bound dye with 10 mM unbuffered Tris base using a gyrotatory shaker, the absorbance at 520 nm was measured with a microplate reader (Dynatech Model MR 700). The fifty percent inhibitory concentration (IC_{50}) was defined as the concentration that reduced absorbance by 50% compared to the control level in untreated wells.

RESULTS AND DISCUSSION

Compound **1** was isolated as a white, amorphous solid. The molecular formula of **1** was established as $C_{26}H_{44}O_5$ on the basis of HRFABMS data. The exact mass of the $[M - H]^-$ ion (*m/z* 435.3114) matched well with the expected formula of $C_{26}H_{43}O_5$ (δ + 0.4 mmu). Although **1** was collected as a single peak in an HPLC separation (85% MeOH, 1 mL/min), the ^1H NMR spectrum of **1** showed the presence of two sets of proton signals, one of which was the same with that of **3**. Both **1** and **3** were isolated from fraction 13, which were eluted at the retention times of 68 min and 33 min, respectively. To make sure that **1** was not carelessly mixed with **3** during the separating process, we repeatedly purified **1** under the same HPLC conditions; however, in each purification, two peaks were observed in the HPLC chromatogram, of which the retention times were a complete match with those of **1** and **3**. In addition, the 1D and 2D NMR spectra of the final HPLC collection **1** still exhibited two sets of signals (**1** and **3**). Therefore, it was speculated that **1** was not stable in its pure form, and spontaneously converted into **3**. According to the integrations in the ^1H NMR spectra of **1**, the conversion reached an equilibrium of a 1 : 1 ratio. By comparing the ^1H and ^{13}C NMR spectra with those of reported data (Bettarello et al., 2000), **3** was identified as 12α-hydroxy-3-ketocholanic acid. A detailed comparison of the 1D and 2D NMR data of **1** with those of **3** showed that the signals were very similar to each other, except for two methoxyl groups at δ_H 3.11 (H-26) and δ_H 3.17 (H-25), and the ^{13}C chemical shifts of C-1 to C-5 (δ_C 34.0, 27.3, 102.5, 34.4, and 41.1, respectively, in **1**; δ_C 38.0, 37.9, 216.2, 43.2, and 45.9, respectively, in **3**). A quaternary carbon signal at δ_C 102.5, showed correlations with H-25 and H-26 in HMBC data (Fig. 2), indicating that both methoxyl groups were attached to this carbon. And considering the chemical shift changes of C-1 to C-5, the location of the dimethoxylated carbon (δ_C 102.5) was assigned as C-3. The configuration of the hydroxyl group at C-12 of **1** was defined as 'α-OH' by comparison of the NMR data with those of **3**. Therefore, the structure of **1** was determined as 3-dimethoxy-12α-hydroxycholanic acid. Ketals are formed by treatment of ketones with alcohols in the presence of acid catalysts, and this reaction is reversible. Fantin et al. (1992) reported that 3-keto groups in 3,7-, 3,12-, and 3,7,12-ketocholanic acids spontaneously converted into the corresponding 3-dimethyl ketals in MeOH. In the ^1H NMR spectra, we also observed that the isolated compounds containing 3-keto groups (**3**, **4**, and **6**) were partly converted into corresponding 3-dimethyl ketals with equilibrium ratio of 11 : 1, 2.5 : 1, and 8 : 1 (ketone: ketal), respectively. Considering the amount ratio (1 : 11) of **1** (4.1 mg) and **3** (43.2 mg), **1** is speculated to be the conversion product of **3** in MeOH during the isolation process. Thereafter, purified **1** returns to **3** with 1 : 1 ratio equilibrium.

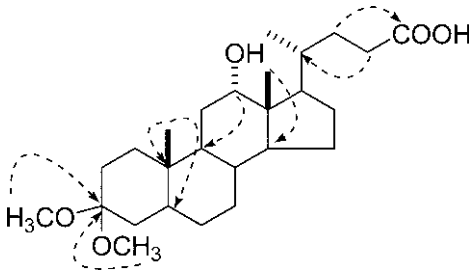

Fig. 2. Key HMBC correlations for compound **1**

Compound **2** was identified as 3-dimethoxy-7-ketocholanic acid by comparison of NMR (^1H, ^{13}C, COSY, HSQC, and HMBC) and MS data with those of reported (Fantin et al., 1992). Compound **2** was also a ketal form, but it was substantially stable. As reported in the literature (Fantin et al., 1992), compound **2** might be formed from complete conversion of 3,7-diketocholanic acid in MeOH, which is the original metabolite of *Psychrobacter* sp. The structural difference between the stable ketal form (**2**) and other analogues (**3**, **4**, and **6**) is the absence of a hydroxyl group at C-12. Therefore, it was assumed that the instability of **1** was due to the hydroxyl group at C-12. According to literatures (Fantin et al., 1992; Fantin et al., 1993) as well as the results of our experiments, 3-keto group was much more reactive than 7-keto (**2**, **4**, and **5**) or 12-keto groups in MeOH solvent, which is

easy to be converted to ketal group. However, in the presence of hydroxyl groups at C-7 (**6**) or C-12 (**3**, **4**, and **6**), the conversion of 3-keto group to ketal group was significantly suppressed.

Bile acids are the final products of the metabolism of cholesterol and are essential for the solubilization and transport of dietary lipids (Hofmann and Hagey, 2008). As prokaryotic cells are believed neither to have the biosynthetic pathway to cholesterol nor to produce bile acids from cholesterol (Maneerat et al., 2005), it is unusual to isolate bile acids from bacterial culture. However, in recent years, it has been reported that some bacteria are capable of producing bile acids when cultured in the cholesterol containing media (Park et al., 1995; Maneerat et al., 2005; Kim et al., 2007). These reported bile acidproducing bacteria and the *Psychrobacter* sp. in this study are not taxonomically related, but all of them are halo-tolerant. Thus, it is assumed that some halo-tolerant bacteria might be capable of producing bile acids by using cholesterol in the media as a precursor.

According to the literatures, biological activities of the bile acids are mainly focused on liver diseases (Hofmann and Hagey, 2008). Chenodeoxycholic acid decreased the cholesterol saturation of bile as well as efficiency of cholesterol absorption (Danzinger et al., 1972). Ursodeoxycholic acid was shown to induce dissolution of cholesterol gallstones (Howard and Fromm, 1999). 6α-Ethyl-chenodeoxycholic acid was shown to have antifibrotic effects and to protect against acute cholestasis (Fiorucci et al., 2007). However, the activities of bile acid derivatives against other human diseases have scarcely been reported. In our investigation for bioactivities of these bacterial bile acids, anti-inflammatory, antibacterial, and cytotoxic effects were evaluated. Anti-inflammatory activity was determined by the evaluation of the inhibitory effects of the compounds (**1**, **3**-**5**, and **7**) on the major pathophysiological mediators (NO and IL-6) in murine macrophage cells (Fig. 3 and 4). Compounds **1** and **3** exhibited similar suppressive effects on NO production at the concentration of 200 μM without notable cytotoxicity to the RAW 264.7 cells. Compound **7** inhibited NO production in a dose-dependent manner (50-200 μM); however, it also showed cytotoxicity against the cells, indicating that the suppressive effect on NO production might be due to the cytotoxic effect. On the production of IL-6, only **1** exhibited inhibitory activity at the concentration of 200 μM without notable cytotoxicity. Again, compound **7** showed the highest inhibition of IL-6 production. The antibacterial activity of compounds **2**-**7** was determined by radial diffusion method against six human pathogenic bacterial strains. Compounds

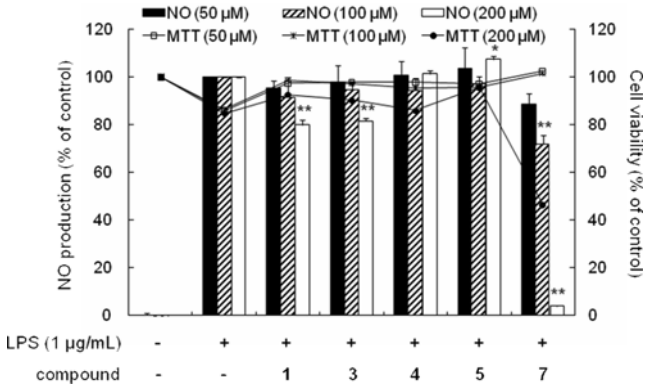

Fig. 3. Effects of compounds **1**, **3**-**5**, and **7** on the nitric oxide (NO) production in LPS-stimulated RAW264.7 cells. RAW264.7 cells were stimulated with LPS (1 μg/mL) in the presence of sample (50, 100, 200 μM) for 24 h. NO_2^- was determined by Griess reagent methods. Cell viability was determined by MTT assay. The data represent the mean ± S.D. of triplicate experiments. *p<0.05, **p<0.01 compared with that of LPS alone.

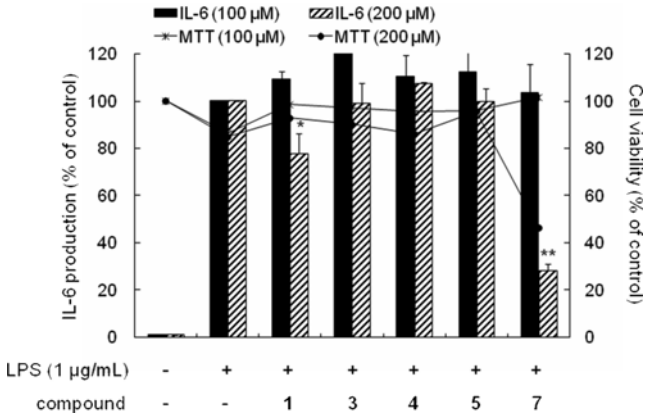

Fig. 4. Effects of compounds **1**, **3**-**5**, and **7** on the interleukin-6 (IL-6) production in LPS-stimulated RAW264.7 cells. RAW264.7 cells were stimulated with LPS (1 μg/mL) in the presence of sample (100, 200 μM) for 24 h. IL-6 was determined by ELISA methods. Cell viability was determined by MTT assay. The data represent the mean ± S.D. of triplicate experiments. *p<0.05, **p<0.01 compared with that of LPS alone.

2-**5** and **7** showed selective inhibitions of several bacterial strains at a low concentration of 30 μg/well (Table I). In the cytotoxicity test against five human solid tumor cell lines (A-549, SK-OV-3, SK-MEL-2, XF-498, and HCT-15), compound **7** demonstrated weak activity (Table II).

ACKNOWLEDGEMENTS

This study was supported by a grant from Marine Biotechnology Program funded by Ministry of Land,

Table I. Antibacterial activity of compounds **2**-**7** (30 μg/well)

bacterial strain	inhibition zone (mm)						
	2	**3**	**4**	**5**	**6**	**7**	tetracycline
Staphylococcus aureous 503 (+)	8	8	-	-	-	9	30
Escherichia coli TEM (-)	-	-	-	-	-	-	20
Pseudomonas aeruginosa 9027 (-)	6	6	5	5	-	7	13
Salmonella typhimurium (-)	-	-	-	-	-	-	22
Klebsiella aerogenes 1522 E (-)	-	-	-	-	-	-	23
Enterobacter cloacae P 99 (-)	-	6	5	7	-	6	7

-, inactive.

Table II. Cytotoxicity of compounds **2**-**7**[a] against human solid tumor cell lines

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
2	26.38	>30	27.05	27.61	23.16
3	25.71	24.04	28.41	>30	23.83
4	>30	>30	>30	>30	>30
5	>30	>30	>30	>30	>30
6	>30	>30	>30	>30	>30
7	12.30	13.94	10.86	12.75	10.81
doxobubicin	0.003	0.012	0.003	0.035	0.039

[a] Data expressed in ED_{50} values (μg/mL). A-549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF-498, human CNS cancer; HCT-15, human colon cancer.

Transport, and Maritime Affairs, Korea. Pramod B. Shinde was supported by Post-Doc. Program of Pusan National University.

REFERENCES

Bettarello, L., Bortolini, O., Fantin, G., and Guerrini, A., Mixed oxo-hydroxy bile acids as actual or potential impurities in ursodeoxycholic acid preparation: a ^{1}H and ^{13}C NMR study. *Il Farmaco*, 55, 51-55 (2000).

Bowman, J. P., Cavanagh, J., Austin, J. J., and Sanderson, K., Novel *Psychrobacter* species from Antarctic ornithogenic soils. *Int. J. Syst. Bacteriol.*, 46**,** 841-848 (1996).

Danzinger, R. G., Hofmann, A. F., Schoenfield, L. J., and Thistle, J. L., Dissolution of cholesterol gallstones by chenodeoxycholic acid. *N. Engl. J. Med.* 286, 1-8 (1972).

Fantin, G., Fogagnolo, M., Medici, A., Pedrini, P., and Cova, U., Spontaneous ketalization of 3-keto-5b-cholanic acids in methanol. *Tetrahedron Lett.*, 33, 3235-3236 (1992).

Fantin, G., Fogagnolo, M., Medici, A., Pedrini, P., and Cova, U., Synthesis of 7- and 12-hydroxy- and 7,12-dihydroxy-3-keto-5fl-cholan-24-oic acids by reduction of 3,7-, 3,12- and 3,7,12-oxo derivatives. *Steroids*, 58, 524-526 (1993).

Fiorucci, S., Rizzo, G., Donini, A., Distrutti, E., and Santucci, L., Targeting farnesoid X receptor for liver and metabolic disorders. *Trends Mol. Med.* 13, 298-309 (2007).

Hofmann, A. F. and Hagey, L. R., Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. *Cell. Mol. Life Sci.*, 65, 2461-2483 (2008).

Howard, D. E. and Fromm, H., Nonsurgical management of gallstone disease. *Gastroenterol. Clin. North Am.* 28,133-144 (1999).

Kim, D., Lee, J. S., Kim, J., Kang, S. J., Yoon, J. H., Kim, W. G., and Lee, C. H., Biosynthesis of bile acids in a variety of marine bacterial taxa. *J. Microbiol. Biotechnol.*, 17, 403-407 (2007).

Lehrer, R. I., Rosenman, M., Harwig, S. S. S. L., Jackson, R., and Eisenhauer, P., Ultrasensitive assays for endogenous antimicrobial activity. *J. Immunol. Methods*, 137, 167-173 (1991).

Li, H., Lee, B. C., Kim, T. S., Bae, K. S., Hong, J., Choi, S. H., Bao, B., and Jung, J. H., Bioactive cyclic dipeptides from a marine sponge-associated bacterium, *Psychrobacter* sp. *Biomol. Ther.*, 16, 356-363 (2008).

Li, Z., Hu, Y., Liu, Y., Huang, Y., He, L., and Miao, X., 16S rRNA clone library-based bacterial phylogenetic diversity associated with three South China Sea sponges. *World J. Microbiol. Biotechnol.*, 23, 1265-1272 (2007).

Maneerat, S., Nitoda, T., Kanzaki, H., and Kawai, F., Bile acids are new products of a marine bacterium, *Myroides* sp. strain SM1. *Appl. Michrobiol. Biotechnol.*, 67, 679-683 (2005).

Park, S. C., Kim, C. J., Uramoto, M., Yun, H. I., Yoon, K. H., and Oh, T. K., Antibacterial substance produced by *Streptococcus faecium* under anaerobic culture. *Biosci. Biotech. Biochem.*, 59, 1966-1967 (1995).

Piel, J., Metabolites from symbiotic bacteria. *Nat. Prod. Rep.*, 21, 519-538 (2004).

Ryu, S. Y., Oak, M. H., Yoon, S. K., Cho, D. I., Yoo, G. S., Kim, T. S., and Kim, K. M., Anti-allergic and anti-inflammatory triterpenes from the herb of *Prunella vulgaris*. *Planta Med.*, 66, 358-360 (2000).