J Food Sci Nutr
Vol 10, p 257~261 (2005)

J Food Science and Nutrition

Cytotoxic Effects of Furanosesterterpenes, Cyclitol Derivatives, and Bromotyrosine Derivative Isolated from Marine Sponges

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Abstract

Marine sponges are known to produce a number of cytotoxic secondary metabolites. In the course of searching for cytotoxic metabolites from marine organisms, we have evaluated cytotoxic activities of six marine secondary metabolites isolated from various sponges. The cytotoxic compounds 1~6 were isolated by the application of various chromatographic methods, including column chromatography and HPLC. The molecular structures were mostly determined using mass spectrometry (MS) and Nuclear Magnetic Resonance (NMR) Spectroscopy. Furanosestererpenes (compounds 1~3) from *Psammocinia* sp., cyclitol derivatives (compounds 4 and 5) from *Sarcotragus* sp., and bromotyrosine-type compound (6) from an association of two sponges *Jaspis wondoensis* and *Poecillastra wondoensis* were evaluated for their cytotoxic activity against three cancer cell lines; Hep G2, HeLa, and MCF-7. All tested compounds exhibited cyctoxicity at concentrations ranging from 5 μg/mL to 25 μg/mL. Particularly, among the tested compounds, compound 6 showed the highest potency displaying at least 80% of cytotoxicity at 5 μg/mL level against all three cancer cell lines.

Key words: sponges, cytotoxicity, furanosesterterpenes, cyclitol derivatives, bromotyrosine, human cancer cell lines

INTRODUCTION

Among various cancer therapies - chemotherapy, radiotherapy, and surgery - chemotherapy is now considered the most effective method of cancer treatment (1). Drugs derived from natural products have been playing a dominant role in pharmaceutical therapeutics, especially in the treatment of cancer (as exemplified by Taxol®, Oncovin®, and Hycamtin®), all of which are developed from plant-derived natural products. Although plant-derived drugs play a dominant role in contemporary chemotherapy, the potential of marine-derived natural products as prolific sources of new drugs, such as anticancer agents, has been frequently recognized (2,3). The two major reasons that the marine environment is considered to be potential sources of biologically active metabolites are: 1) the immense biological diversity in the ocean and 2) the existence of many sedentary marine organisms which are believed to be ecologically evolved to produce potent biologically active secondary metabolites as chemical means of defense. According to a recent review (3), more than 40 chemical entities derived from marine environments have entered into clinical and preclinical trials for a wide range of pharmaceutical effects such as cancer, anti-infectives, analgesia, Alzheimer's disease, inflammation, and immunomodulation. Among the various life forms from the ocean, sponges produce a number of bioactive compounds, and represent the single best source of marine bioactive compounds with pharmaceutical potential (4,5). Sponges (phylum Porifera) are primitive multi-celled animals that have existed for 700~800 million years. Of the approximately 15,000 sponge species, most occur in marine environments. In addition, sponges can live in a poor nutrient environment and it evolved with a unique chemical defense strategies against potential predators or for competing for space (6). Potentially therapeutic compounds identified in sponges include anticancer agents and immunomodulators (7).

In our quest for cytotoxic metabolites from marine organisms, we evaluated cytotoxic activities of six marine secondary metabolites isolated from various sponges [3:1 mixture of (8*Z*, 13*Z*, 18*R*, 20*Z*)-strobilinin and (7*Z*, 13*Z*, 18*R*, 20*Z*)-felixinin **(1)**, 1:1 mixture of (8*E*, 13*Z*, 18*R*, 20*Z*)-strobilinin and (7*E*, 13*Z*, 18*R*, 20*Z*)-felixinin **(2)**, variabilin **(3)**, sarcotride A **(4)**, sarcotride B **(5)**, and psammaplin A **(6)**] against three cancer cell lines (Hep G2, HeLa, and MCF-7).

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MATERIALS AND METHODS

Animal materials

The sponge producing furanosesterterpenes (**1**~**3**) was collected by hand through scuba diving to a depth of 20 m in October 2001, off Ulleung Island, Korea. The specimen was identified as *Psammocinia* sp. by Prof. C. J. Sim of Hannam University. A voucher specimen of the sponge (registry No. Spo. 42) was deposited at the Natural History Museum, Hannam University, Daejeon, Korea (8).

The sponge producing cyclitol derivatives **(4, 5)** was collected in July 1998 (15~25 m depth), off Cheju Island, Korea. This specimen was identified as *Sarcotragus* sp. by Prof. Chung Ja Sim of Hannam University. A voucher specimen (J98J-5) of the sponge (registry No. Por. 33) was deposited in the Natural History Museum, Hannam University, Daejeon, Korea (9).

The sponges producing psammaplin A **(6)** were collected in July 1999 (15~25 m depth), off the coast of Gomun Island, Korea. Prof. C. J. Sim of Hannam University, identified the specimen as an association of two sponges, *Jaspis wondoensis* Sim & Kim (family Jaspidae) and *Poecillastra wondoensis* Sim & Kim (family Pachastrellidae). The voucher specimen (J99K-1) of the sponge was deposited in the Natural History Museum, Hannam University, Daejeon, Korea (10).

Extraction and isolation

Three furanosestererpenes (**1**~**3**) were isolated from the MeOH extract of frozen *Psammocinia* sp. using various chromatographic methods. The structures of compounds **1**~**3** were identified by comparison with their spectral data with those reported in the literature (11). ^{1}H and ^{13}C NMR spectra were recorded on a DMX600 instrument. Chemical shifts are reported with reference to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). EIMS analyses were done on a JEOL JMS-SX-102A while FABMS data were obtained using a JEOL JMS-HX110/110A. Full details of the isolation and characterization are described elsewhere (8).

Sarcotride A **(4)** and sarcotride B **(5)** were isolated from the MeOH extract of frozen sponge *Sarcotragus* sp. using various chromatographic methods.

The structures of compounds **4** and **5** were identified by analysis of various spectroscopic methods such as MS and NMR. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC200 and a Varian Inova 500 spectrometer. Chemical shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FABMS data were obtained on a JEOL JMS-SX-102A double-focusing instrument. Full details of the isolation and characterization are described elsewhere (9).

Psammaplin A **(6)** was isolated from the MeOH extract of an association of two sponges *Jaspis wondoensis* and *Poecillastra wondoensis* using various chromatographic methods. The structure of compound 6 was identified by comparison with its spectral data with those reported in the literature, and analysis of various spectroscopic methods such as MS and NMR. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200 and Varian Inova 500 instruments. Chemical shifts were reported with reference to the respective solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FABMS data were obtained on a JEOL JMS-SX-102A double focusing MS. ESIMS data were obtained using a Finnigan DecaXP. MALDI-TOFMS data were obtained on an Applied Biosystems Voyager-DESTR. Full details of the isolation and characterization are described elsewhere (10).

Chemicals

3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), NP-40 and menadione were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Flavin adenine dinucleotide (FAD), dicumarol, and glucose-6-phosphate dehydrogenase were purchased from Amresco (USA). All other chemicals were purchased from commercial sources and were of the highest purity available.

Cell lines and culture

Hep G2 (human hepatocellular carcinoma), HeLa (human cervicse adenocarcinma) and MCF-7 (human breast adenocarcinoma pleual effusion) were obtained from Korean Cell Line Bank (KCLB). All cell lines in this study were maintained at 37°C in a 5% CO_2 air mixture in DMEM media containing 10% fetal bovine serum and 1% penicillium streptomycin (100 units/mL).

Cell viability assay

Cell toxicity was assessed by the MTT assay based on the metabolic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (12). Briefly, the cells were sub-cultured into a 24-well plate with approximately 10^5 cells per well in 1 mL of medium. After 24 h of incubation, the agents being tested were added to triplicate wells at final concentrations of 1, 10, 25, or 50 μg/mL; 0.4% DMSO was used as the control. The plates were then incubated in a 37°C humidified incubator with 5% CO_2 for 48 h. At the end of the incubation, 100 μL of MTT solution in PBS (3 mg/mL) was added to each well. The culture was then incubated for 4 h to convert MTT to formazan. Thereafter, the supernatant was aspirated and 1 mL of 1:1 mixture of DMSO-EtOH was added to dissolve the formazan. Plates

were agitated on a plate shaker to ensure a homogeneous solution, and the optical densities (OD) were read at 570 nm. Data were expressed in terms of relative viable cell number % [(OD of treated cells/OD of control cells) × 100].

RESULTS AND DISCUSSION

Cytotoxic potentials of compounds **1**~**6** (Fig. 1) on Hep G2, HeLa, and MCF-7 cells were assessed by MTT assay, which is based on the reduction of MTT by mitochondrial dehydrogenase of intact cells to a purple formazan product. As shown in Fig. 2, compounds **1**~**6** exhibited cytotoxic activity against Hep G2 cells at concentrations of 5 μg/mL, 10 μg/mL, 15 μg/mL, 20 μg/mL, and 25 μg/mL, respectively. Among the tested compounds, compound 6 showed the highest potency, exhibiting 89% cytotoxicity at 5 μg/mL level. Compound

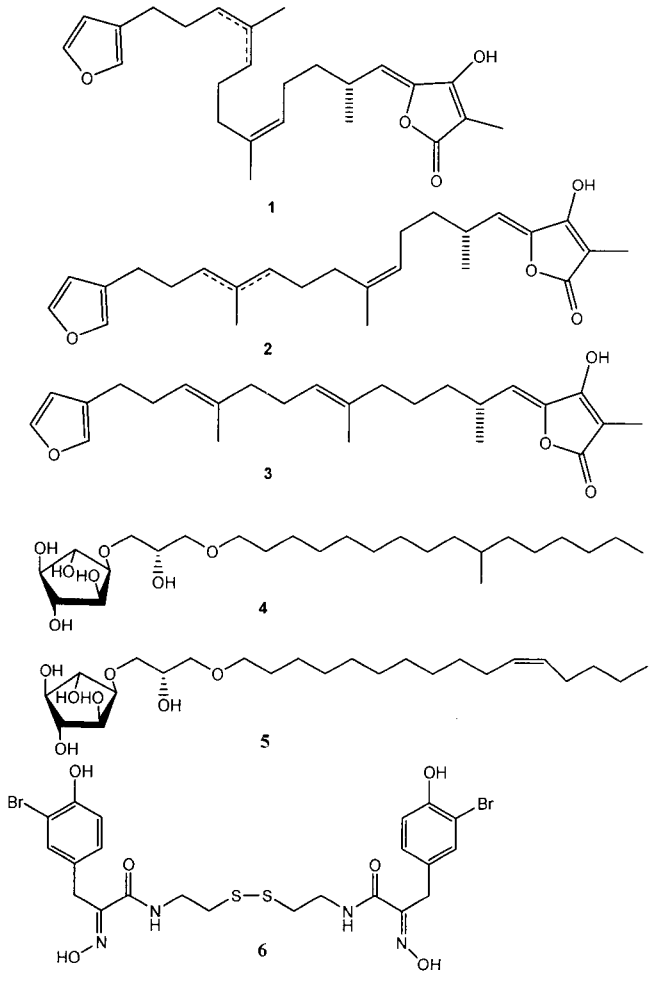

Fig. 1. Chemical structures of [3:1 mixture of (8*Z*, 13*Z*, 18*R*, 20*Z*)-strobilinin and (7*Z*, 13*Z*, 18*R*, 20*Z*)-felixinin (**1**), 1:1 mixture of (8*E*, 13*Z*, 18*R*, 20*Z*)-strobilinin and (7*E*, 13*Z*, 18*R*, 20*Z*)-felixinin (**2**), variabilin (**3**), sarcotride A (**4**), sarcotride B (**5**), and psammaplin A (**6**).

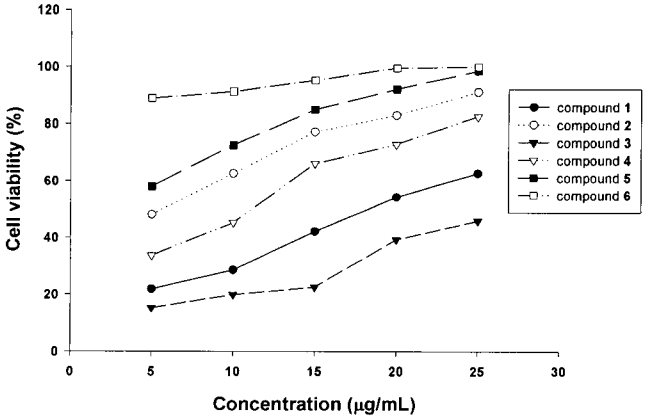

Fig. 2. Cytotoxic effects of compounds **1**~**6** on Hep G2 cells. Cells were incubated for 48 hr in the presence of various concentrations of compounds **1**~**6**, and then cell viability was assessed using the MTT test.

5 exhibited a similar magnitude of cytotoxicity to compound 6 at higher concentrations (85% at 15 μg/mL). On the other hand compounds **1**~**4** displayed weaker cytotoxicities as compared to those of compound **5** and **6**, yet the compounds exhibited dose-dependent activity at the tested concentration levels. Cytotoxic activities of compounds **1**~**6** against HeLa cell showed similar pattern as observed against Hep G2 cells (Fig. 3). At concentration of 5 μg/mL, compound **6** exhibited strong activity displaying 93% cytotoxicity. At the same concentration level, the cytotoxicities of compounds **1**~**5** were 22, 69, 6, 51, and 43%, respectively. Cytotoxic activities of compounds **1**~**6** against the MCF-6 cell line also showed similar trend to those against Hep G2 and HeLa cell lines (Fig. 4).

Marine sponges of the order Dictyoceratida are known to contain various linear furanosesterterpene derivatives such as compounds **1**~**3**, which are characterized by a furan group and a terminal tetronic acid moiety (8).

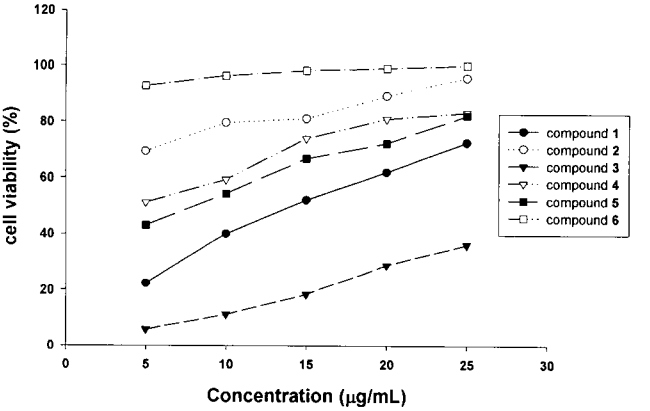

Fig. 3. Cytotoxic effects of compounds **1**~**6** on HeLa cells. Cells were incubated for 48 hr in the presence of various concentrations of compounds **1**~**6**, and then cell viability was assessed using the MTT test.

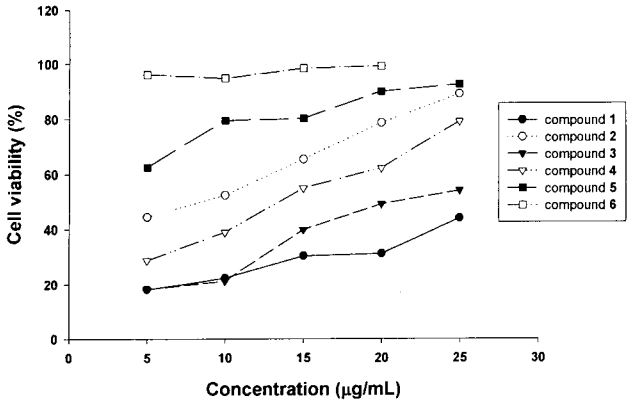

Fig. 4. Cytotoxic effects of compounds **1**~**6** on MCF-7 cells. Cells were incubated for 48 hr in the presence of various concentrations of compounds **1**~**6**, and then cell viability was assessed using the MTT test.

These furanosesterterpenes have been reported to have various bioactivities including: antiviral, antibacterial, antiinflammatory, antitumor, and protein phosphatase inhibitory activity and toxicity to sea urchin and starfish eggs (11,13,14).

Cyclitol derivatives analogous to **4** and **5** have been isolated from various sponges such as Caribbean sponges *Pseudoceratina crassa* (15), *Verongula gigantea*, *Aplysina fistularis fulva*, *Aplysina cauliformis*, *Neofibularia nolitangere* (16), Okinawan sponges *Luffariella* sp., *Biemna* sp., *Xestopongia* sp. (17), and a Korean sponge *Petrosia* sp. (18). Therefore, it has been suggested that this class of metabolites might be ubiquitously present in sponges and play some significant role in the life of the organisms. Recently, compounds **4** and **5** have been shown to exhibit moderate to significant cytotoxicity against a small panel of five human tumor cell lines (9). In addition, antifeedant activity and nerve growth factor stimulatory activity of this class of compounds have been reported (9).

Psammaplin A (**6**) belongs to a bromotyrosine family of compounds, which is considered to be a chemotaxonomic marker of sponges of the order Verongida. The structural diversity of bromotyrosine derivatives covers from simple bromotyrosine monomers represented by aeroplysinin to more complex bastadins. Among these metabolites, psammaplins and related compounds comprise a small group of metabolites biosynthesized by linear connection of bromotyrosines and modified cysteines (10). Various biological activities of these compounds were reported, and especially for psammaplin A, bioactivities such as antimicrobial activity (19), cytotoxicity against the leukemia cell line P388, (20) and inhibition of DNA topoisomerase (21) have been reported.

In summary, cytotoxic potentials of three different classes of marine metabolites, i. e., furanosesterterpenes (**1**~**3**), cyclitol derivatives (4, 5), and bromotyrosine derivative (6), were evaluated against three cancer cell lines Hep G2, HeLa, and MCF-7. Cytotoxic effects against the three tumor cell lines showed similar trends, and compound **6** exhibited the strongest activity with more than 85% cytotoxicity at the 5 μg/mL level against all three cell lines. Recently, correlation between psammaplin A (**6**)-induced cytotoxicity and its inhibition of DNA replication has been suggested (22). Likewise, further studies on the mechanism of cytotoxicity exerted by compounds **1**~**5** need to be conducted. In addition, further pharmacological evaluations of these compounds as potential anticancer agent would be fruitful.

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(Received July 1, 2005; Accepted August 26, 2005)