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Anti-Tumor Activity of the Farnesyl-Protein Transferase Inhibitors Arteminolides, Isolated from *Artemisa*

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Abstract—Members of the *Artemisia* genus are important medicinal plants found throughout the world. Arteminolides A–D (**1**–**4**), isolated from the aerial parts of *Artemisia,* have an inhibitory activity on farnesyl-protein transferase (FPTase; EC 2.5.1.29) in in vitro assay. This study was carried out with the purpose of validating anti-tumor effects of the compounds in human tumor cells and mouse xenograft model. The arteminolides inhibited tumor cell growth in a dose-dependent manner. Furthermore, arteminolide C (**3**) blocked in vivo growth of human colon and lung tumor xenograft without the loss of body weight in nude mice.

Introduction

The genus *Artemisia*, one of the largest genera belonging to the Compositae family consisting of more than 350 species, is predominantly distributed in the world.[1] *Artemisia* species are frequently utilized for the treatment of diseases such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses.[2,3] This genus is receiving growing attention presumably due to: (1) the diversified biology and chemistry of the constituents, (2) the frequent application in traditional medical practice, and (3) the rich source of the plant material. According to literature, over 260 *Artemisia* species have been investigated to reveal that they contain many classes of secondary metabolites including sesquiterpenoids, flavonoids, coumarins, glycosides, sterols, and polyacetylenes.[4–8]

Isolated compounds, arteminolides, are belong to a sesquiterpene lactone, which exhibits a wide range of biological activities.[9] It is well known that the exomethylene group on the sesquiterpene lactone is an essential group for biological activities including cytotoxicity against tumor cells. The chemical studies showed that various cytotoxic sesquiterpene lactones react with thiols such as cysteine by rapid Michael-type addition.[10,11]

Farnesyl-protein transferase (FPTase) inhibitors have been shown to inhibit the growth of human tumors in mouse xenograft models and, more dramatically, in transgenic mouse models. Recent work has demonstrated that specific inhibitors of the FPTase might be interesting chemical leads to develop effective therapeutic agents for the treatment of cancer.[12]

Arteminolides, isolated from the aerial parts of *Artemisia argyi*, were reported to have inhibitory activity on farnesyl-protein transferase (FPTase) with IC_{50} values of 0.7∼1.0 μM.[13,14] To prove the anti-tumor effects of the arteminolides, potent FPTase inhibitors, we carried out tumor growth assay against human cancer cells and in human tumor xenograft experiments.

Results and Discussion

Inhibition of tumor cell growth of arteminolides

An activity of arteminolide C (**3**) on the cell proliferation of the 26 tumor cell lines was examined using cell

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proliferation assay kit.[15] Compound **3** exhibited a dose-dependent inhibition of cell growth in a broad range of concentrations and the GI_{50} value of **3** for in vitro growth inhibition was approximately 1.3∼8.1 μ/mL and the average concentration of the compound for in vitro growth inhibition by 50% (GI_{50}) was calculated to be 5.36 μg/mL against 26 kinds of human tumor cells. The panel of human tumor cell lines tested is summarized in Table 1. Some cell lines, such as HCT15 (colon), MDA-MB-435s (breast), and SNB75 (CNS), were relatively sensitive for the compound, exhibiting GI_{50}s 1.30, 1.76, and 1.35 μg/mL, respectively. However, one of renal tumor cell line A498 is very resistant against the compound **3** with $GI_{50} > 30$ μg/mL (Fig. 1).

Growth inhibitory activity of the other compounds was also determined against a couple of human tumor cell lines including SW620 (colon), MDA-MB-231 (breast), HCT116 (colon), and MCF7 (breast). In Table 2, we summarized the cytotoxicity against four human tumor cell lines to see the structure–activity relationship.

These four compounds have very similar anti-tumor profiles against the tested human tumor cell lines and the only structural differences among them are acyl groups at C-8. Therefore, the results suggest that the acyl side chains at C-8 would not significantly contribute the anti-tumor effects.

Table 1. Growth inhibitory activity of arteminolid C (**3**) against human tumor cell lines[a]

Organ	Cell line	GI_{50} (μg/mL)
Lung	A549	4.06±0.21[b]
	NCI-H23	2.95±0.13
	NCI-H226	4.99±0.25
Colon	HCT116	6.54±0.23
	HCT15	1.30±0.07
	COLO#205	4.17±0.17
	HCC2998	4.07±0.26
	SW620	4.58±0.25
	HT29	5.14±0.27
	DLD-1	6.54±0.28
Melanoma	SK-MEL-2	2.68±0.16
	SK-MEL-5	2.76±0.18
	LOXIMVI	6.48±0.27
	RPMI 7951	2.41±0.19
Breast	MDA-MB-231	7.96±0.31
	MDA-MB-435-s	1.76±0.06
	MCF7	4.03±0.28
	MCF7/ADR	3.18±0.19
Prostate	DU145	3.30±0.21
	PC-3	4.77±0.25
Renal	CAKI-1	8.12±0.27
	ACHN	3.37±0.19
	A498	>30
CNS	SNB19	7.91±0.34
	SNB75	1.35±0.05
	Mean GI	5.38±0.22

[a]Anti-tumor agent, adriamycin was used as control of cytotoxicity evaluation (GI_{50}, μg/mL).
[b]Results are expressed as mean±SD.

Inhibition of tumor cell growth of hydrogenated arteminolides

It was generally accepted that sesquiterpene lactones have antitumor activity, because they have an α-methylene-γ-lactone group.[12] To see whether α-methylene-γ-lactone group of arteminolide was involved in the inhibition of tumor cell growth, hydrogenation of compounds **2** and **3** was performed using H_2/Pd/C in THF and arteminolide B′(**5**) and C′(**6**) were purified by HPLC. Structure of the hydrogenated compound was established by comparison of spectroscopic data with that of **2** and **3**.[8] The molecular formula $C_{35}H_{44}O_8$ of **5** was obtained from the analyses of HRFABMS ($[M+H]^+$ 593.2868) for the hydrogenated compound **5**, which means that two double bonds are reduced in this reaction. In ^1H NMR of **5**, it was found that the signals of α-methylene-γ-lactone (H-13′ at δ_H 5.32 and 6.06) and H-3 at δ_H 6.19 were disappeared. Two new doublet methyl peaks were observed at δ_H 1.15 (J=6.9 Hz) and 1.10 (J=6.9 Hz). Stereochemistry of the compound was assigned by the NOE experiments.

Table 2. Growth inhibitory activity of arteminolid **1**–**4** against human tumor cell lines[a]

Cell line	Compd	GI_{50} (μg/mL)
Colon (SW620)	**1**	6.01±0.41[b]
	2	6.58±0.55
	3	4.99±0.25
	4	5.98±0.65
Colon (HCT116)	**1**	3.54±0.33
	2	3.40±0.57
	3	6.54±0.33
	4	3.57±0.36
Breast (MDA-MB-231)	**1**	4.34±0.25
	2	3.42±0.21
	3	7.96±0.31
	4	6.61±0.49
Breast (MCF7)	**1**	4.8±0.37
	2	4.12±0.66
	3	4.03±0.28
	4	3.90±0.27

[a]Anti-tumor agent, adriamycin was used as control of cytotoxicity evaluation (GI_{50}, μg/mL).
[b]Results are expressed as mean±SD.

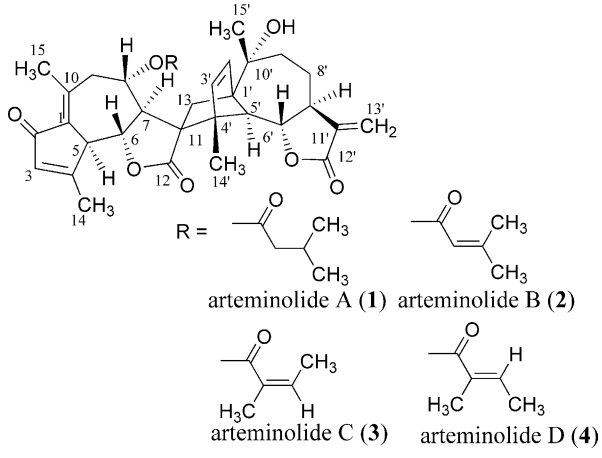

Figure 1. Structure of arteminolide **1**–**4**.

We already reported that the hydrogenated arteminolide C′ (**6**) inhibited the enzymatic activity of FPTase with IC_{50} value of 0.8 μM, which means that α-methylene-γ-lactone did not effect on inhibition of FPTase activity.[8]

To determine the inhibitory activity of tumor cell growth of hydrogenated arteminolides, compounds **5** and **6** (Fig. 2) were treated a couple of human tumor cell lines including SW620 (colon), MDA-MB-231 (breast), HCT116 (colon), and MCF7 (breast) and was summarized in Table 3. As shown Table 3, growth inhibitory activity of hydrogenated arteminolides is about 5 times less than that of arteminolides with exocyclic methylene group (see Fig. 3). The results are very consistent with the published paper their considerable cytotoxity belonging to the presence of α-methylene-γ-lactone group.

In vivo effect of arteminolide C(3) on tumor cell growth

SW620 (colon) and NCI H23 (lung) tumor xenograft model of nude mice were used to investigate inhibitory activity of **3** on tumor growth. SW620 and NCI-H23 cells were implanted subcutaneously into the right flank of nude mice and compound was intraperitoneously administered at a concentration of 50 mg/kg per day for 17 days. To determine the toxicity of the compound, the body weight of tumor-bearing animals was measured. On day 17, the mice were sacrificed and the sizes of tumors were measured. On 17 days after implantation, tumor volume was decreased by 30.3% in NCI-H23 human lung adenocarcinoma cells xenografted nude mice and also 39.5% in SW620 compared to control mice (Fig. 4).[16] Body weight loss was not observed in mice implanted with SW620 and NCI-H23 cells (Fig. 5).

In summary, low antiproliferative activity of hydrogenated arteminolides (**5** and **6**) indicates that α-methylene-γ-lactone group of sesquiterpene lactones significantly contributed the growth inhibition of tumor cells. An anti-tumor activity of arteminolide **3** in human tumor xenografted nude mice studies of the arteminolides

Table 3. Growth inhibitory activity of hydrogenated arteminolid **5** and **6** against human tumor cell lines[a]

Cell line	Compound	GI_{50} (μg/mL)
Colon (SW620)	**5**	25.2±1.21[b]
	6	21.2±0.93
Colon (HCT116)	**5**	25.4±0.83
	6	28.0±1.17
Breast (MDA-MB-231)	**5**	45.2±2.16
	6	36.2±0.98
Breast (MCF7)	**5**	19.8±0.81
	6	17.6±0.90

[a]Anti-tumor agent, adriamycin was used as control of cytotoxicity evaluation (GI_{50}, μg/mL).
[b]Results are expressed as mean±SD.

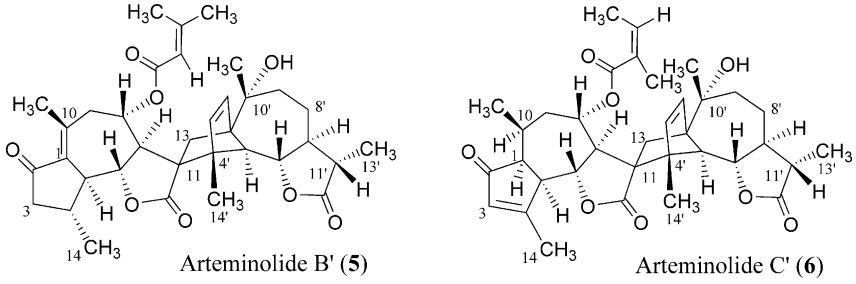

Figure 2. Structure of hydrogenated arteminolides **5** and **6**.

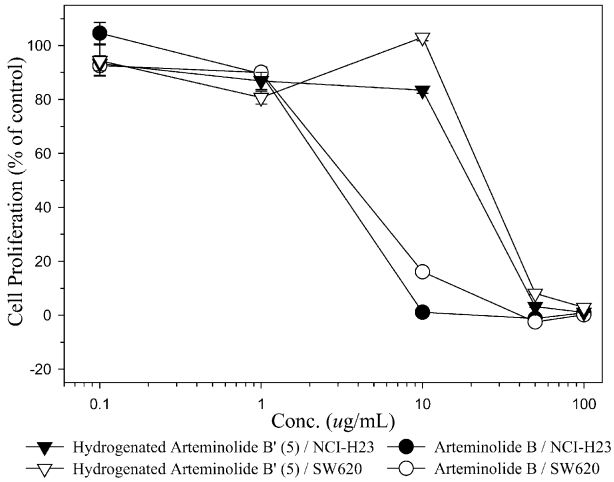

Figure 3. Growth inhibition of human tumor cell lines by compounds **2** and **5**.

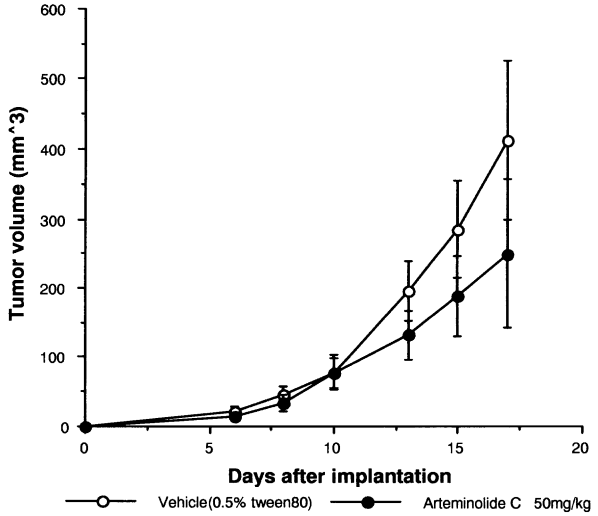

Figure 4. Tumor volume change of SW620 xenografted nude mice treated with arteminolide C(**3**).

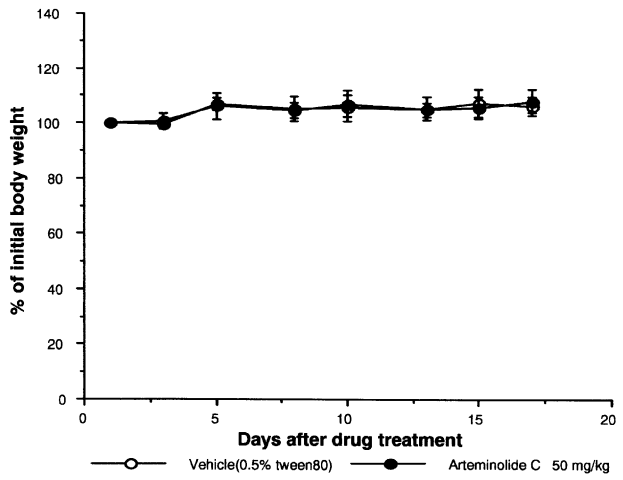

Figure 5. Body weight changes of SW620 xenografted nude mice treated with arteminolide C(**3**).

support that the compounds will be a good lead molecules for the development of anti-tumor drug.

Experimental

Isolation

Aerial parts of *A. argyi* were collected in 2001, near Incheon city, Korea, and identified by Professor K. Bae, School of Pharmacy, Chungnam National University. Aerial parts of *A. argyi* (20 kg) were extracted with $CHCl_3$ (3×30 L). The combined extract was concentrated, and the dark residue was subjected to silica gel flash chromatography with $CHCl_3$/MeOH solvent pairs. Fractions were monitored by FPTase inhibition activity and silica gel TLC ($CHCl_3$–MeOH, 9:1). The active fractions ($CHCl_3$–MeOH, from 9:1 to 8:2) were subjected to C18 column chromatography with aqueous MeOH. The two fractions eluted with 70 and 80% MeOH were shown to have strong inhibition activity against rat FPTase and were collected and further purified by chromatography on a Sephadex LH-20 column eluting with MeOH. Final purification of arteminolides A–D (**1**–**4**) was accomplished by ODS-HPLC with 80% MeOH to yield compounds.

Hydrogenation of arteminolide B(2). For reduction of the α-methylene-γ-lactone group, arteminolide B(**2**) was dissolved in THF and stirred at room temperature. Then, palladium on charcoal was added to the solution and stirred at room temperature under a hydrogen atmosphere for 30 min. The resulting mixture was concentrated in vacuum evaporator and purified by HPLC to give arteminolide B′(**5**).

Arteminolide B′(5). Mp 167–168 °C; UV (MeOH) λ_{max} 216 nm; $[\alpha]_D^{25}$ +28° (*c* 0.22, MeOH); ^{1}H NMR in $CDCl_3$ δ 2.46, 2.10 (3-H, m), 2.01 (4-H, m), 2.92 (5-H, m), 4.24 (6-H, t, *J* = 10.2 Hz), 2.59 (7-H, t, *J* = 10.1 Hz), 4.85 (8-H, dt, *J* = 10.2, 2.9 Hz), 2.33, 2.42 (9-H, m), 1.88 (13-H, d, *J* = 11.4 Hz), 2.57 (13-H, d, *J* = 11.4 Hz), 1.15 (14-H, d, *J* = 6.9 Hz), 2.21 (15-H, s), 5.77 (2′-H, d, *J* = 5.4 Hz), 5.84 (3′-H, d, *J* = 5.4 Hz), 2.93 (5′-H, d, *J* = 10.2 Hz), 3.95 (6′-H, t, *J* = 9.9 Hz), 2.38 (7′-H, m), 1.25, 2.01 (8′-H, m), 1.81 (9′-H, m), 2.65 (11′-H, q, *J* = 6.9 Hz), 1.01 (13′-H, d, *J* = 6.9 Hz), 1.52 (14′-H, s), 1.26 (15′-H, s), 5.49 (2″-H, bs), 2.32 (4″-H, d, *J* = 2.4 Hz), 2.21 (5″-H, s); ^{13}C NMR in $CDCl_3$ δ 132.04 (C-1), 205.03 (C-2), 48.04 (C-3), 42.74 (C-4), 48.90 (C-5), 79.60 (C-6), 58.54 (C-7), 67.02 (C-8), 45.53 (C-9), 149.07 (C-10), 61.42 (C-11), 179.02 (C-12), 40.07 (C-13), 12.89 (C-14), 20.34 (C-15), 62.69 (C-1′), 136.23 (C-2′), 137.58 (C-3′), 58.32 (C-4′), 66.47 (C-5′), 77.21 (C-6′), 46.30 (C-7′), 24.73 (C-8′), 35.32 (C-9′), 72.67 (C-10′), 28.88 (C-11′), 179.14 (C-12′), 16.10 (C-13′), 16.82 (C-14′), 29.55 (C-15′), 164.55 (C-1″), 115.84 (C-2″), 158.64 (C-3″), 29.55 (C-4″), 27.47 (C-5″).

Cell culture

The cell lines used were obtained originally from ATCC and were maintained in RPMI 1640 (Gibco/BRL) or DMEM supplemented with 10% heat-inactivated FBS (Gibco/BRL) and 25 mM Hepes. Cell cultures were maintained at 37 °C under a humidified atmosphere of 5% CO_2 in an incubator.[15]

Cells (5000 cells) were seeded into 96-well plates in RPMI 1640, or DMEM containing 10% FBS. After 20–24 h, cells were replenished with fresh complete medium containing either a test compound or 0.1% DMSO. After incubation for 48 h, cell proliferation reagent WST-1 (Roche, Germany) was added to each well. The amount of WST-1 formazan produced was measured at 450 nm using an ELISA Reader (Bio-Rad, CA, USA).

Nude-mouse xenograft assay

To evaluate compound **3** for antitumor activity in vivo, SW620 human colon adenocarcinoma cells (3×10^7 cells/mL) and NCI H23 human lung cells (5×10^7 cells/mL) were implanted subcutaneously into right flank of nude mice on day 0. Compound was dissolved in 0.5% Tween 80 and was intraperitoneously administered at a concentration of 50 mg/kg per day for 17 days. The amount dosage was 0.2 mL of the solution per 20 g body weight animals. On day 17, the mice were sacrificed and the sizes of tumors were measured. Tumor volumes were estimated as; length (mm)×width (mm)×height (mm)/2.[16] To determine toxicity of the compounds, the body weight of tumor-bearing animals was also measured. Statistical significance between the control and treatment groups was evaluated using Student's *t*-test. Animal experiments were performed under the permission according to 'Institutional Guideline of Animal Experiments' of Korea Research Institute of Bioscience & Biotechnology.

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References and Notes

1. Marco, J. A.; Barbera, O. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1990; Vol. 7A, p 201.
2. Robles, M.; Aregullin, M.; West, J.; Rodriguez, E. *Planta Med*. **1995**, *61*, 199.
3. Tang, H. Q.; Hu, J.; Yang, L.; Tan, R. X. *Planta Med*. **1998**, *64*, 295.
4. Tan, R. X.; Lu, H.; Wolfender, J. L.; Yu, T. T.; Zheng, W. F.; Yang, L.; Gafner, S.; Hostettmann, K. *Planta Med*. **1999**, *65*, 64.
5. Tan, R. X.; Tang, H. Q.; Hu, J.; Shuai, B. *Phytochemistry* **1998**, *49*, 157.
6. Valant-Vetschera, K. M.; Wollenweber, E. Z. *Naturforsch* **1995**, *50c*, 353.
7. Kim, J. H.; Kim, H. K.; Jeon, S. B.; Son, K. H.; Kim, E. H.; Kang, S. K.; Sung, N. D.; Kwon, B. M. *Tetrahedron Lett*. **2002**, *43*, 6205.
8. Seo, J. M.; Kang, H. M.; Son, K. H.; Kim, J. H.; Lee, C. W.; Kim, H. M.; Chang, S. I.; Kwon, B. M. *Planta Med*. **2003**, *69*, 218.
9. Robles, M.; Aregullin, M.; West, J.; Rodriguez, E. *Planta Med*. **1995**, *61*, 199.
10. Schmidt, T. J. *Current Org. Chem*. **1999**, *3*, 577.
11. Heilmann, J.; Wasescha, M. R.; Schmidt, T. *J. Bioorg. Med. Chem*. **2001**, *9*, 2189.
12. Gibbs, J. B.; Oliff, A. *Ann. Rev. Pharmacol. Toxicol*. **1997**, *37*, 143.
13. Lee, S. H.; Kim, H.K; Kang, H. M.; Seo, J. M.; Son, K. H.; Lee, H. S.; Kwon, B. M. *J. Org. Chem*. **2002**, *67*, 7670.
14. Lee, S. H.; Kang, H. M.; Song, H. C.; Lee, H.; Lee, U. C.; Son, K. H.; Kim, S. H.; Kwon, B. M. *Tetrahedron* **2000**, *56*, 4711.
15. Kim, H. M.; Han, S. B.; Kim, S. M.; Kang, J. S.; Oh, G. T.; Hong, D. H. *Toxicol. Pharmacol. Meth*. **1996**, *36*, 163.
16. Sun, J.; Qian, Y.; Hamilton, A. D.; Sebti, S. M. *Oncogene* **1998**, *16*, 1467.