The Journal of Applied Pharmacology, **13**, 263-266(2005)

Cytotoxic Phenylpropanoids from the Rhizomes of *Alpinia galanga*

Joo-Won Nam, Sun-Jack Kim, Ah-Reum Han, Sang Kook Lee, and Eun-Kyoung Seo*

College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

(Received September 7, 2005; Accepted October 12, 2005)

Abstract – A bioassay-guided fractionation of the *n*-hexane and chloroform extracts of the rhizomes of *Alpinia galanga* led to the isolation of two active compounds, 1'*S*-1'-acetoxychavicol acetate (**1**) and *p*-coumaryl alcohol γ-*O*-methyl ether (**2**). 1'*S*-1'-acetoxychavicol acetate (**1**) exhibited significant cytotoxicity against all human cancer cell lines tested (A549; IC_{50} 8.14, SNU638; 1.27, HCT116; 1.77, HT1080; 1.2, HL60; IC_{50} 2.39 μg/ml), whereas *p*-coumaryl alcohol γ-*O*-methyl ether (**2**) showed selective cytotoxicity against the SNU638 cell (IC_{50} = 1.62 μg/ml).

Key words ☐ *Alpinia galanga*, Zingiberaceae, cytotoxicity, 1'*S*-1'-acetoxychavicol acetate, *p*-coumaryl alcohol γ-*O*-methyl ether

INTRODUCTION

Alpinia galanga (L.) Swartz. (Zingiberaceae) is the ginger that has been widely cultivated in South and Southeast Asia. This plant has been traditionally used for flatulence, dyspepsia, vomiting, and sickness at stomach (Grieve, 1994). Previous phytochemical investigations on this plant have resulted in the isolation of various types of phenylpropanoids, lignans, and terpenoids (Morikawa *et al.*, 2005, Akhtar *et al.*, 2004, Matsuda *et al.*, 2003a, Matsuda *et al.*, 2003b, Jirovetz *et al.*, 2003, Mitsui *et al.*, 1976). Some of these isolates have been reported to exhibit anti-allergic (Matsuda *et al.*, 2003a), gastroprotective, (Matsuda *et al.*, 2003b), antifungal (Janssen and Scheffer, 1985), anti-ulcer activities (Mitsui *et al.*, 1976), and nitric oxide production inhibitory activity (Morikawa *et al.*, 2005).

As a part of our research program to discover potential anticancer agents from higher plants, *A. galanga* was selected for phytochemical investigation since *n*-hexane extract and $CHCl_3$ extracts exhibited significant cytotoxicity with the IC_{50} values of 15.09 and 12.69 μg/ml (A549) during preliminary screening. The bioassay-guided fractionation using the cytotoxicity assay against several human cancer cell lines, led to isolation of two active phenylpropanoids. Herein, we describe the isolation and the cytotoxic activity of compounds **1** and **2** against five human cancer cell lines tested in the present study.

*Corresponding author
Tel: +82-2-3277-3047, Fax: +82-2-3277-3051
E-mail: Yuny@ewha.ac.kr

MATERIALS AND METHODS

Plant materials

The rhizomes of *A. galanga* were collected in Surabaya, Indonesia, in 2001 and were identified by professor Tri Windono (University of Surabaya, Indonesia). A voucher specimen (NO. 07/DT/XI/2001) has been deposited at University of Surabaya, JL. Raya Kalirungkut, Surabaya 60293, Indonesia.

General experimental procedures

Optical rotations were measured with a P-1010 polarimeter (Jasco, Japan) at 25°C. UV and IR spectra were recorded on a U-3000 spectrophotometer (Hitachi, Japan) and a FTS 135 FT-IR spectrometer (Bio-Rad, CA), respectively. NMR experiments were conducted on a Unity INOVA 400 MHz FT-NMR (Varian, CA, USA), and TMS was used as an internal standard. EIMS was obtained on a JMS 700 Mstation HRMS spectrometer (JEOL, Japan). TLC analysis was performed on Kieselgel 60 F_{254} (Merck, Germany) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich) followed by charring at 110 °C for 5-10 min. Silica gel (230-400 mesh, Merck, Germany) was used for column chromatography. All solvents used for chromatographic separations were distilled before use.

Extraction and isolation

The dried rhizomes of *A. galanga* (500 g) were extracted with methanol (MeOH; 2 l × 3) for 24 hrs by percolation. The MeOH extracts (90.5 g) were concentrated *in vacuo*, suspended in water, and partitioned with *n*-hexane (400 ml × 4), $CHCl_3$ (400 ml × 4) and *n*-BuOH (400 ml × 2), successively. The *n*-hexane and $CHCl_3$ extracts were combined due to their similar TLC pattern and then the combined extract (10.6 g) was subjected to a silica gel flash column chromatography using *n*-hexane-EtOAc (gradient, 20:1 → 1:2 v/v) as a solvent system, to produce 18 fractions. Compound **1** (3 g, 0.6 w/w%) was obtained from fraction 11 eluted with *n*-hexane-EtOAc (20:1 → 15:1 v/v) from the column chromatography. Fraction 14 eluted with *n*-hexane-EtOAc (9:1 → 8:1 v/v) from the column chromatography afforded crystals of compound **2** (0.1 g, 0.02 w/w%).

1'*S*-1'-Acetoxychavicol acetate (**1**): Yellow oil, $[\alpha]_D^{25}$: -38.6° (*c* 0.2, MeOH); IR (film) ν_{max} (cm^{-1}): 1740, 1608, 1372, 1197; UV λ_{max} MeOH (log ε): 267.0 nm (2.80), 217.5 nm (3.91); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.36 (2H, d, *J* = 8.6 Hz, H-2 and H-6), 7.07 (2H, d, *J* = 8.6 Hz, H-3 and H-5), 6.26 (1H, d, *J* = 6.0 Hz, H-1′), 5.98 (1H, ddd, *J* = 17.1, 10.4, 6.0 Hz, H-2′), 5.29 (1H, dt, *J* = 17.1, 1.2 Hz, H-3′ *trans*), 5.24 (1H, dt, *J* = 10.4, 1.2 Hz, H-3′ *cis*), 2.28 (3H, s, $OCOC\underline{H}_3$-4), 2.09 (3H, s, $OCOC\underline{H}_3$-1); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 170.0 (s, $O\underline{C}OCH_3$-1′), 169.4 (s, $O\underline{C}OCH_3$-4), 150.6 (s, C-9), 136.6 (s, C-8), 136.2 (d, C -2′), 128.5 (d, C-2), 121.8 (d, C-3), 117.2 (t, C-3’), 75.6 (d, C-1′), 21.3 (q, $OCO\underline{C}H_3$-4), 21.2 (q, $OCO\underline{C}H_3$-1′); EIMS *m/z* (% rel. int.) 234 ([M]+, 2), 192 (77), 174 (10), 150 (93), 132 (100).

p-Coumaryl alcohol γ-*O*-methyl ether (**2**): Physical and spectral data were comparable to the literature values (Ly *et al.*, 2003). White needle crystals, IR (film) ν_{max} (cm^{-1}): 3281, 1611, 1377; UV λ_{max} MeOH (log ε): 262.5 nm (4.31); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.25 (2H, d, *J* = 8.6 Hz, H-2 and H-6), 6.77 (2H, d, *J* = 8.6 Hz, H-3 and H-5), 6.53 (1H, d, *J* = 15.8 Hz, H-1′), 6.12 (1H, dt, *J* = 16.8, 6.4 Hz, H-2′), 4.09 (2H, q, *J* = 6.4, 1.2 Hz, H-3′), 3.40 (3H, s, $OC\underline{H}_3$-3); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 155.7 (s, C-4), 132.8 (d, C-1′), 129.6 (s, C-1), 128.1 (d, C-2 and C-6), 123.5 (d, C-2′), 115.7 (d, C-3 and C-5), 73.6 (t, C-3′), 58.0 (q, $O\underline{C}H_3$-3); EIMS *m/z* (% rel. int.) 164 ([M]+, 100), 132 (45), 103 (11), 77 (7).

Chemicals

All chemicals and reagents used were of highest purity. Trichloroacetic acid (TCA) and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Minimal essential medium with Earles salt (MEME), fetal bovine serum (FBS), non-essential amino acid solution (10 mM, 100×), trypsin-EDTA solution (1×) and antibiotic-antimycotic solution (PSF) were from GIBCO-BRL (Grand Island, NY, USA).

In vitro cytotoxicity assay

Cytotoxic potential was determined as described previously (Lee *et al.,* 1998). Briefly, cells (in log growth phase) were counted, diluted to 5×10^4 cells/mL with fresh medium, and added to 96-well microtiter plates (190 μL/well) containing test materials (10 μL in 10% aqueous DMSO). Test plates were incubated for 3 days at 37°C in CO_2 incubator. For zero day controls, cells were incubated for 30 mins at 37°C in CO_2 incubator. All treatments were performed in triplicate. After the incubation periods, cells were fixed by the addition of 50 μL of cold 50% aqueous trichloroacetic acid (4°C for 30 min), washed 4-5 times with tap water, and air-dried. The fixed cells were stained with sulforhodamine B (SRB) (0.4% w/v SRB in 1% aqueous acetic acid) for 30 mins Free SRB solution was then removed by rinsing with 1% acetic acid. The plates were then air-dried, the bound dye was solubilized with 200 μL of 10 mM tris-base (pH 10.0), and absorbance was determined at 515 nm using an ELISA plate reader. Finally, the absorbance values obtained with each of the treatment procedures were averaged, and the averaged value obtained with the zero day control was subtracted. These results were expressed as a percentage, relative to solvent-treated control incubations, and IC_{50} values were calculated using non-linear regression analyses (percent survival versus concentration).

RESULTS AND DISCUSSION

Two active compounds **1** and **2** were isolated from the combined *n*-hexane and $CHCl_3$ extracts of the rhizomes of *A.*

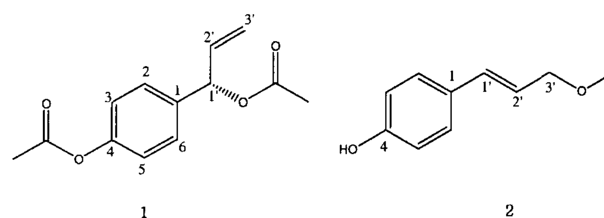

Fig. 1. Structures of compounds **1** and **2** isolated from *A. galanga*

galanga by bioassay-guided fractionation using the cytotoxicity assay against several human cancer cell lines. These compounds were evaluated for their cytotoxic activities against a panel of human cancer cell lines as shown in Table I. Compound **1** exhibited potent cytotoxicity against all the human cancer cell lines tested, whereas compound **2** showed selective cytotoxicity against the SNU-638 stomach cancer cell with IC_{50} value of 1.62 μg/ml. Structures of **1** and **2** were identified as 1'*S*-1'-acetoxychavicol acetate (**1**) and *p*-coumaryl alcohol γ-*O*-methyl ether (**2**), respectively, by analysis of their NMR data as well as by comparison of their physical and spectral data with those of literature values (Jaki *et al.*, 2004; Ly *et al.*, 2003). In particular, the configuration at C-1' of **1** was determined to be "*S*" according to the optical rotation value of $[\alpha]_D^{25}$ -38.6° (*c* 0.2, MeOH, lit. -53°) (Noro *et al.*, 1988).

1'*S*-1'-acetoxychavicol acetate (**1**) has been found as a major constituent of the rhizomes of *A. galanga* and showed the diverse biological activity such as inhibitory effects on NO production (Matsuda *et al.,* 2005; Ando *et al.*, 2005) and NF-κB (Ito *et al.*, 2005), anti-allergic activity (Matsuda *et al.*, 2003a), gastroprotective effect (Matsuda *et al.*, 2003b), anticarcinogenic effect (Moffatt *et al.*, 2000), and cancer chemopreventive activity (Miyauchi *et al.*, 2000). In the present study, its cytotoxic activities against the human cancer cell lines of A549, SNU-638, HCT-116, and HT1080 are reported for the first time although the cytotocixity on leukemia HL-60 cell line has been reported previously (Ito *et al.*, 2004). The cytotoxic activity on the colon cancer cell lines of compound **1** was also reported previously, however, HCT116 cell line was never been used.

p-Coumaryl alcohol γ-*O*-methyl ether (**2**) which was found as an antioxidant compound (Ly *et al.*, 2003) previously, was isolated from *A. galanga* for the first time in the present study. To the best of our knowledge, this is also the first report on the cytotoxic activity of compound **2**.

Table I. The cytotoxicity of isolates from *A. galanga*[a]

Compound	Cell lines[b]				
	A549	SNU-638	HCT116	HT1080	HL-60
1	8.14	1.27	1.77	1.20	2.39
2	12.53	1.62	16.84	17.18	13.94
Ellipticine[c]	0.05	0.81	0.38	0.56	0.35

[a]Results are expressed as IC_{50} values in μM.
[b]Cell lines: A549 = Human Lung Cancer Cell Line; SNU-638 = Human Stomach Cancer Cell Line; HCT116 = Human Colon Cancer Cell Line; HT1080 = Human Fibro Sarcoma Cell Line; HL-60 = Human Leukemia Cell Line
[c]Ellipticine was used as a positive control.

ACKNOWLEDGMENTS

This study was supported by Research Institute of Pharmaceutical Sciences, College of Pharmacy, Ewha Womans University

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