Phospholipase Cy1 Inhibitory Principles from the Sarcotestas of Ginkgo biloba

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Ten phenolic compounds were isolated from the CHCl₃ extract of *Ginkgo biloba* sarcotestas (Ginkgoaceae) as a new class of phosphatidylinositol-specific phospholipase $C\gamma 1$ (PI-PLC $\gamma 1$) inhibitors. The substances without the long chain were ineffective. On the other hand, the activities of these compounds were dramatically decreased by acetylation of aromatic hydroxyl groups of cardanol, phenolic acid, and bilobol and by methylation of the aromatic carboxyl group of phenolic acid. The unsaturated long chain as well as the aromatic hydroxyl and carboxyl groups might play a key role for the PI-PLC $\gamma 1$ inhibitory activity. These compounds also inhibited the growth of a number of human cancer cell lines, but were less cytotoxic against a human normal colon cell line.

Phosphatidylinositol-specific phospholipase C (PI-PLC) plays a central role in the intracellular signal transduction by hydrolyzing phosphatidylinositol 4,5-biphosphate to give two second messengers, inositol 1,4,5-triphosphate and diacylglycerol, which lead to a series of events that culminate in DNA synthesis and cell proliferation.^{1,2} PI-PLC is known to have isozymes such as β_1 , β_2 , γ_1 , γ_2 , δ_1 , and δ_2 .^{3,4}

Recent reports suggested that an inhibitor of PI-PLC, especially the γ isoform, would be a useful tool for development of anticancer agents.⁵ Microinjection of PI-PLC γ into quiescent NIH3T3 cells induced DNA synthesis and transformation.⁶ On the other hand, specific mutation-restoration of PI-PLC γ binding to a tyrosine mutated platelet-derived growth factor (PDGF) receptor is sufficient to confer a mitogenic response to PDGF.⁷ Specifically, the overexpression of PI-PLC γ 1 in human cancers such as those of lung,⁸ bladder,⁸ breast,⁹ glia,¹⁰ and colorectum^{11,12} as compared to normal tissues, have suggested a role for the PI-PLC γ 1 isoform in the generation of mitogenic signals associated with tumor progression.

We have studied natural products as PI-PLC $\gamma 1$ inhibitors for development of anticancer agents.^{13,14} In the course of screening of the Korean medicinal plants, the CHCl₃ extract of *Ginkgo biloba* sarcotestas showed potent inhibitory activity against PI-PLC $\gamma 1$. *G. biloba* principles on this enzyme activity have not previously been reported. Thus, this paper describes the isolation of inhibitory principles, their effects, and the modes of inhibitory action on PI-PLC $\gamma 1$. In addition, growth inhibitory effects on normal and human cancer cell lines were studied.

Results and Discussion

The CHCl₃ extract of *G. biloba* L. (Ginkgoaceae) sarcotestas was found to exhibit potent inhibitory activ-

ity against PI-PLC γ 1. We isolated 10 PI-PLC γ 1 inhibitors by bioassay-guided separation of the CHCl₃ extract. This extract inhibited activity of PI-PLC γ 1 by 82.38% at 125 μ g/mL, and fractions 1–6 obtained by Si gel column chromatography produced inhibitions of 3.51, 95.02, 87.95, 92.58, 76.32, and 74.02% at the same concentration, respectively. Compounds 1–10 were purified from the fractions 2 and 4. The chemical structures of these compounds were identified as cardanols (1–4), phenolic acids (5–7), and bilobols (8–10) (Figure 1) from their physical and spectral data in comparison with those of published values.

These phenolic compounds from *G. biloba* ($C_{15:1}$, $C_{17:1}$, and $C_{13:0}$) have been reported as antitumor principles,¹⁵ and those from *Anacardium occidentale* ($C_{15:0}$, $C_{15:1}$, $C_{15:2}$, and $C_{15:3}$) have been known as molluscicides,¹⁶ antibacterial agents,¹⁷ cytotoxic agents in BT-20 (breast carcinoma) and Hela (epithelioid cervix carcinoma) cells,¹⁸ prostaglandin synthetase inhibitors,^{19,20} lipoxygenase inhibitors,²¹ antiacne agents,²² and tyrosinase inhibitors.²³

Compounds 1–10 were evaluated for their ability to inhibit PI-PLCy1 in vitro, and showed concentrationdependent inhibitory effects (Table 1). These phenolic compounds are a new class of PI-PLC γ 1 inhibitors. Among them, phenolic acid $C_{17:1}$ {6-[10' (Z)-heptadecenyl]salicylic acid, (6)} showed the most powerful activity, with an IC₅₀ of 0.83 μ g/mL (2.22 μ M), whereas bilobol $C_{13:0}$ [5-tridecyl resorcinol, (10)] was the least active inhibitor, with an IC₅₀ of 26.63 μ g/mL (72.32 μ M). Activities of these compounds were either greater or similar in comparison with the previously reported inhibitory substances against PI-PLCy1 from bovine, pig, and rat brain; amentoflavone (IC₅₀; 29 μ M, bovine brain), prenylated flavonoids (7.5 -35μ M, bovine brain), Q12723 (8.8 μ M, bovine brain), vinaxanthone (5.4 μ M, rat brain), hispidospermidin (16 μ M, rat brain), and caloporoside $(18-31 \mu M, pig brain)$.^{13,24–28} Specifically, phenolic acids bearing unsaturated long-chain substituents (5, 6) were very potent inhibitors of PI-PLC γ 1. Structurally related 6-alkenyl salicylic acids were previ-

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Figure 1. Chemical structures of compounds 1–10 isolated from the sarcotestas of *G. biloba* and their derivatives.

Table 1. Inhibitory Effects of Compounds **1–10** Isolated from the Sarcotestas of *G. biloba* and Their Derivatives against PI-PLC $\gamma 1^a$

compound	inhibitory effects			
cardanol C _{15:1} (1)	$3.90\pm 0.16^{b}(12.91^{c})$			
cardanol C _{17:1} (2)	3.21 ± 0.17 (9.73)			
cardanol C _{13:0} (3)	15.83 ± 0.81 (57.85)			
cardanol C _{15:0} (4)	$17.62 \pm 1.05 \ (57.96)$			
cardanol C _{15:1} acetate (1a)	>250			
<i>m</i> -cresol	>250			
phenolic acid $C_{15:1}$ (5)	1.96 ± 0.10 (5.66)			
phenolic acid $C_{17:1}$ (6)	0.83 ± 0.05 (2.22)			
phenolic acid C _{13:0} (7)	5.20 ± 0.25 (16.27)			
phenolic acid C _{15:1} methyl ester (5a)	>250			
methyl phenolic acid C _{15:1} acetate (5b)	>250			
salicylic acid	120.53 ± 5.32			
bilobol C _{15:1} (8)	9.55 ± 0.48 (30.03)			
bilobol C _{17:1} (9)	10.58 ± 0.45 (30.57)			
bilobol C _{13:0} (10)	26.63 ± 1.52 (72.32)			
bilobol C _{15:1} acetate (8a)	>250			
5-methyl resorcinol	>250			

^{*a*} Data were expressed as mean \pm SE of three experiments. ^{*b*} IC₅₀ value (μ g/mL). ^{*c*} IC₅₀ value (μ M).

ously isolated as molluscicides from the leaves and stems of *Spondias mombin*, and the highest activities were also found for the unsaturated phenolic acids ($C_{15:3} > C_{15:2} > C_{17:3}$, $C_{17:2} > C_{15:1}$).²⁹

When PI-PLC γ 1 activities of these compounds were compared according to differences of the aromatic moiety, phenolic acids (5-7), cardanols (1-4), and bilobols (8-10) exhibited inhibitory activities of the order 5>1>8, 6>2>9, 7>3>10. Phenolic acids, which possess a carboxyl group, were more active than the corresponding cardanols (5>1, 6>2, 7>3), whereas bilobols possessing an additional hydroxyl group were less active than corresponding cardanols (1>8, 2>9), **4**>**10**). On the other hand, the mode of PI-PLC γ 1 inhibition by cardanol $C_{15:1}$ (1) was competitive with respect to the PI substrate in kinetic studies (Figure 2A). However, phenolic acid $C_{15:1}$ (5) and bilobol $C_{15:1}$ (8) showed noncompetitive modes of inhibition (Figure 2B and 2C). Thus, these changes of activity caused by additional carboxyl and hydroxyl groups seemed to result from the differences of reaction sites of phenolic acids and bilobols with that of cardanol and suggested that the carboxyl and hydroxyl groups are important for noncompetitive inhibition.

Cardanols with unsaturated long carbon chains (1, 2) showed more potent activity than those with saturated long chains (3, 4). For example, the IC₅₀ (3.90 μ g/mL) of cardanol C_{15:1} (1) was about 4.5 times higher than that (17.62 μ g/mL) of cardanol C_{15:0} (4). Phenolic



Figure 2. Lineweaver–Burk plot for the inhibition of PI-PLC γ 1 by compounds **1**, **5**, and **8** in the presence of various concentrations of PI [10 (\diamond), 2 (\blacksquare), and 0 (\triangle) μ g/mL]. The figure is represented as a double reciprocal plot. A; cardanol C_{15:1} (**1**); B, phenolic acid C_{15:1} (**5**); C, bilobol C_{15:1} (**8**). Data were expressed as mean of triplicate. Extent of error is less than 5%.

 Table 2.
 Growth Inhibitory Effects of Compounds 1–10 Isolated from the Sarcotestas of *G. biloba* on Human Cancer Cell Lines and a Normal Colon Cell Line

compound	A-549 ^a	MCF-7 ^a	HCT-15 ^a	SKOV-3 ^a	HT-1197 ^a	CCD-18-Co ^a
cardanol $C_{15:1}$ (1)	9.09	5.56	29.90	12.90	18.81	54.58
cardanol C _{17:1} (2)	11.80	7.19	32.90	NT^b	20.84	66.42
cardanol C _{13:0} (3)	25.90	7.04	29.70	>50	14.56	73.24
cardanol C _{15:0} (4)	11.90	8.71	31.90	NT^b	41.81	NT^b
phenolic acid $C_{15:1}$ (5)	31.30	6.44	12.70	>50	46.26	97.14
phenolic acid $C_{17:1}$ (6)	29.20	5.06	8.39	>50	28.14	NT^b
phenolic acid C _{13:0} (7)	>50	22.67	>50	20.40	44.64	59.11
bilobol C _{15:1} (8)	>50	5.68	14.88	23.40	8.43	33.63
bilobol C _{17:1} (9)	NT^b	NT^b	NT^b	NT^b	NT^b	NT^b
bilobol C _{13:0} (10)	>50	1.91	15.32	>50	2.20	>100

^a IC₅₀ value (µg/mL). ^b Not tested.

acids (5, 6) and bilobols (8, 9) also exhibited similar trends. In addition, the substances without the longchain substituent, *m*-cresol, salicylic acid, and 5-methyl resorcinol were evaluated for the inhibitory activity. Salicylic acid was a very weak inhibitor (120.53 μ g/mL), and *m*-cresol and 5-methyl resorcinol were ineffective at 250 μ g/mL. On the other hand, the activities of compounds 1, 5, and 8 were dramatically decreased by acetylation of the aromatic hydroxyl group (1a, 5b, and 8a) and by methylation of the aromatic carboxyl group (5a). These results suggested that unsaturated long-chain substituents and aromatic hydroxyl and carboxyl groups may be important for PI-PLC γ 1 inhibitory activity. These studies are the first with *G. biloba* principles on this enzyme activity.

Recent reports indicate that PI-PLC γ 1 plays a key role in proliferating and progression of human cancer.^{5–12} Therefore, the growth inhibitory effects of these principles were examined on several human cancer cell lines and a normal cell line (Table 2). All compounds inhibited the growth of human cancer cells such as HCT-15 (colon), MCF-7 (breast), A-549 (lung), HT-1197 (bladder), and SKOV-3 (ovary). Interestingly, these compounds were less cytotoxic on the normal colon cell line (CCD-18-Co) than on the corresponding colon carcinoma (HCT-15). Therefore, these inhibitory principles may be beneficial not only as references in cellsignaling studies but also as candidates, anticancer agents being less toxic against normal tissues.

Experimental Section

General Experimental Procedures. IR spectra were obtained with a Perkin–Elmer 1710 spectrometer.

The NMR spectra were taken on either a JEOL GSX 400 (¹H, 400 MHz; ¹³C, 100 MHz) or a JEOL LA 300 (¹H, 300 MHz; ¹³C, 75 MHz) spectrometer. MS were obtained on a VG Trio-2 spectrometer. Radioactivity of [³H] PI for PI-PLC γ 1 assay was measured on a Wallac 1409 liquid scintillation counter. TLC was carried out on Si gel 60 F₂₅₄ and RP-18 F₂₅₄ plates (Merck, Darmstadt, Germany). Column chromatography was performed over Si gel 60 (Merck, particle size 230–400 mesh or 15 μ m) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Solvents for HPLC and column chromatography were obtained from Merck and Duksan Chemical Co. (Seoul, Korea), respectively.

Plant Material. The fresh sarcotestas of *Ginkgo biloba* L. (Ginkgoaceae) were collected from the ginkgo trees grown in the Kwanak Campus of Seoul National University, Seoul, Korea in September 1996, and identified by Dr. Dae Suk Han, emeritus professor, College of Pharmacy, Seoul National University. A voucher specimen has been deposited in the herbarium of our institute.

Enzyme and Chemicals. $PLC\gamma 1$ was purified to homogeneity (over 95% purity) from bovine cerebellum through DE-52, matrix green gel affinity, phenyl 5-PW, and MONO O column chromatography.³⁰ L-3-Phosphatidyl inositol (PI), HEPES, EGTA, and sodium deoxycholate (SDC) were purchased Sigma Chemical Co. (St. Louis, MO). [³H-inositol] PI and cocktail solution were obtained from Amersham (Buckinghamshire, UK). Amentoflavone, which was isolated previously,¹³ was used as a positive control in the PI-PLC $\gamma 1$ assay. Salicylic acid, 5-methyl resorcinol, and *m*-cresol for the structure–activity relationships were purchased from Sigma Chemical Co.

Extraction and Isolation. The fresh sarcotestas (3 kg) were extracted three times with $CHCl_3$ in an ultrasonic apparatus for 3 h and, upon removal of the solvent in vacuo, yielded the $CHCl_3$ extract (80 g). Separation of PI-PLC γ 1 inhibitory principles from the CHCl₃ extract was carried out by bioassay-guided separation; it was fractionated by column (6 \times 65 cm) chromatography over Si gel (800 g, 230-400 mesh) using CHCl₃–MeOH mixtures with increasing polarity $(100:1\rightarrow2:1)$ to give six fractions, of which fractions 2 and 4 showed potent activity. Fraction 2 (34 g) was subjected to column chromatography over Si gel (5.2 imes40 cm, 230-400 mesh, 350 g, n-hexanes-EtOAc 10:1) and Sephadex LH-20 (3.4×49 cm, *n*-hexane-CH₂Cl₂-MeOH 5:5:1) to give four subfractions. A semipreparative HPLC (YMC-Pack C₁₈, 4 μ m, 10 \times 250 mm, CH₃CN-MeOH 10:1, 254 nm, 2 mL/min) was used for further purification of subfraction 2, which led to the isolation of compounds 1 (52 mg, t_R 28.28 min), 2 (45 mg, $t_{\rm R}$ 46.25 min), **3** (15 mg, $t_{\rm R}$ = 30.72 min), and **4** (13 mg, $t_{\rm R}$ 52.72 min). Semipreparative HPLC was carried out twice over Phenomenex C₁₈ (5 μ m, 10 imes 250 mm, 254 nm, 2 mL/min, solvent a MeOH-OHAc 99:1; solvent b MeOH-AcOH-H₂O 99:1:10) for subfraction 3, to provide compounds 5 (16 mg, b; $t_{\rm R}$ 113.10 min), 6 (18 mg, a; $t_{\rm R}$ 30.99 min), and 7 (15 mg, b; $t_{\rm R}$ 104.14 min). From fraction 4 (20 g), compounds 8 (200 mg, $t_{\rm R}$ 102.32 min), 9 (50 mg, $t_{\rm R}$ 85.00 min), and 10 (13 mg) were separated using vacuum column chromatography (7 \times 20 cm, 270 g, 15 μ m, *n*-hexane-Me₂CO 10:1 \rightarrow 3:1), Sephadex LH-20 (3.4 \times 49 cm, *n*-hexane-CH₂Cl₂-MeOH 5:5:1), and semipreparative HPLC (Phenomenex C_{18} , 5 μ m, 10 \times 250 cm, 254 nm, 2 mL/min, MeOH-H₂O 6:1).

In Vitro **PI-PLC***γ***1 Assay**. The PI-PLC*γ***1** assay was performed by the method of Rhee et al.³⁰ In brief, the enzyme solution was dissolved in 50 mM HEPES/NaOH (pH 7.0), 3 mM CaCl₂, and 1 mM EGTA, and a reaction mixture contained 0.02 μ Ci [³H-inositol] PI, 10 nM PI ,and 0.1%-SDC. The enzyme reaction was initiated by adding enzyme at 37 °C and terminated after 10 min by adding 1 mL of CHCl₃-MeOH-concentrated HCl (50:50:0.3, by volume) and 0.3 mL of 1 N HCl containing 3 mM EGTA. Samples were vortexed and centrifuged at 2000 rpm for 10 min, and 0.5 mL of the aqueous phase was placed in a scintillation vial, and the radioactivity of [3H-inositol] IP was counted. Each inhibitor was tested in triplicate at each point. Amentoflavone was used as a positive control. The inhibitory modes of action were kinetically analyzed by changing the concentration of the PI (2–50 μ M) in the presence of various concentrations of the inhibitors (0, 2, and 10 μ g/mL).

Cell Lines and Culture Media. Cell lines (A-549, MCF-7, HCT-15, SKOV-3, CCD-18Co, and HT-1197) were obtained from the Korean Cell Line Bank, Seoul National University, Seoul, Korea. Media (DMEM and RPMI-1640), and antibiotics (penicillin-streptomycin) were purchased from Gibco-BRL (Grand Island, NY). Fetal bovine serum was obtained from Hyclon (Logan, UT).

Growth Inhibition Assay of Cancer Cell. A549 (human lung carcinoma), MCF-7 (human breast adeno-

carcinoma), HCT-15 (human colon adenocarcinoma), and SKOV-3 (human ovary adenocarcinoma) were grown in RPMI-1640 medium supplemented with 10% heatinactivated fetal bovine serum plus 1% antibiotics. CCD-18Co (human normal colon) and HT-1197 (human bladder carcinoma) were grown in DMEM medium with the same supplements. Cells were cultured in T-flasks in CO₂ incubator supplied with 5% CO₂ and 95% humid air at 37 °C. Cells were maintained $5-10 \times 10^4$ cells/ mL and subcultured 2–3 times per week; 0.05% trypsin– EDTA was used for dissociating monolayer cells. Cell suspension $(1-8 \times 10^4 \text{ cells/well})$ of log phase was added to each well of a 96-well plate to determine the human cancer cell growth inhibition of compounds and incubated in a CO₂ incubator at 37 °C. After day 1, compounds were treated, and cells were cultured for two additional days. The final DMSO concentration was adjusted to 0.5% in all samples. Each experiment was performed in triplicate. Viable cells were counted by the SRB method.³¹ Cell growth inhibition index was determined as IC₅₀; the drug concentration resulting in 50% growth inhibition was compared to the untreated control. Adriamycin was used as a positive control.

Identification of Compounds 1–10. The structures of compounds 1–10 (Figure 1) were identified, respectively, as cardanols {3-[8' (Z)-pentadecenyl]phenol (1), 3-[10' (Z)-heptadecenyl]phenol (2), 3-tridecyl phenol (3) and 3-pentadecyl phenol (4); phenolic acids {6-[8' (Z)-pentadecenyl]salicylic acid (5), 6-[10' (Z)-heptadecenyl]salicylic acid (6), and 6-tridecyl phenol (7)}; and bilobols {5-[8' (Z)-pentadecenyl]resorcinol (8), 5-[10' (Z)heptadecenyl]resorcinol (9), and 5-tridecyl resorcinol (10) by IR, ¹H NMR, ¹³C NMR, and MS. These structures were confirmed by comparison with those of literature data.^{15,19,32–33} The positions of double bonds in compounds 1, 2, 5, 6, 8, and 9 were determined from product ions by collisionally activated dissociation of [M -H + 2Li]⁺ precursor ions, which were generated by FABMS using a Li⁺ containing matrix.³³

Preparation of Compounds 1, 5, and 8 Derivatives. Cardanol $C_{15:1}$ (1) 3 mg and bilobol $C_{15:1}$ (8) 5 mg were acetylated by Ac₂O in pyridine at room temperature, and yielded *O*-acetyl 3-[8' (*Z*)-pentadecenyl]phenol (cardanol $C_{15:1}$ acetate, 1a) 2 mg and 1, 3-*O*diacetyl 5-[8' (*Z*)-pentadecenyl]resorcinol (bilobol $C_{15:1}$ diacetate, 8a) 3.5 mg. Phenolic acid $C_{15:1}$ (5) 10 mg was esterified by CH₂N₂ in Et₂O in an ice bath, to give methyl 6-[8' (*Z*)-pentadecenyl]salicylate (phenolic acid $C_{15:1}$ methyl ester, 5a) 12 mg. Phenolic acid C $_{15:1}$ methyl ester (5a) 5 mg was acetylated by the method described previously, and methyl *O*-acetyl 6-[8' (*Z*)-pentadecenyl]salicylate (methyl phenolic acid C_{15:1} acetate, 5b) 3.5 mg was obtained.

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References and Notes

- (1) Nishizuka, Y. Science 1992, 258, 607-614.
- (2) Berridge, M. J. Nature **1993**, 361, 315–325.
- (3) Suh, P. G.; Ryu, S. H.; Moon, K. H.; Suh, H. W.; Rhee, S. G. *Proc. Natl. Acad. Sci. (USA)* 1988, *85*, 5419–5423.
- (4) Suh, P. G.; Ryu, S. H.; Moon, K. H.; Suh, H. W.; Rhee, S. G. Cell 1988, 54, 161–169.

- (5) Hill, S. R.; Bonjouklian, R.; Powis, G.; Abraham, R. T.; Ashendel, C. L.; Zalkow, L. H. Anti-Cancer Drug Design 1994, 9, 353-361
- Smith, M. R.; Ryu, S. H.; Suh, P. G.; Rhee; S. G.; Kung, H. F. (6)(b) Shifti, N. R., Rya, S. H., Shi, T. Sui, T. B., Killey, S. G., Kung, H. F. Proc. Natl. Acad. Sci. (USA) 1989, 86, 3859–3663.
 (7) Valius, M.; Kazlankas, A. Cell 1993, 73, 321–334.
 (8) Robertson, J. B.; Hurd, S. D.; Koh, M. O.; Arteaga, C. L. Proc. 10, 1000

- Am. Assoc. Cancer Res. **1992**, *33*, 89. Arteaga, C. L.; Johnson, M. D.; Todderud, G.; Coffey, R. J.; Carpenter, G.; Page, D. L. Proc. Natl. Acad. Sci. (USA) **1991**, *88*, 10435–10439. (9)
- (10) Haas, N.; McDanel, H.; Godwin, A.; Humphrey, P.; Bigner, S.; Wong, A. *Proc. Am. Assoc. Cancer Res.* **1991**, *32*, 17.
 (11) Yeatman, T. J.; Mao, W. G.; Karl, R. C.; Djeu, J. Y. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 1043–1049.
 (20) M. D. Y. L. K. W. Kim, S. G.; Kim, V. L. Puri, S. H.; Sub, P.
- Noh, D. Y.; Lee, Y. H.; Kim, S. S.; Kim, Y. I.; Ryu, S. H.; Suh, P. (12)G.; Park, J. G *Cancer* **1994**, *73*, 36–41. (13) Lee, H. S.; Oh, W. K.; Kim, B. Y.; Ahn, S. C.; Kang, D. O.; Shin,
- D. I.; Kim, J.; Mheen, T. I.; Ahn, J. S. Planta Med. 1996, 62, 293 - 296
- (14) Lee, J. S.; Park, S.-Y.; Kim, J. Y.; Oh, W. K.; Lee, H. S.; Ahn, J. S.; Kim, J. Seoul Natl. Univ. J. Pharm. Sci. 1996, 21, 30-42.
- (15) Itokawa, H.; Totsuka, N.; Nakahara, K.; Takeya, K.;, Lepoittevin, J.-P.; Asakawa, Y. Chem. Pharm. Bull. 1987, 35, 3016–3020.
- (16) Kubo, I.; Komatsu, S.; Ochi, M. J. Agric. Food Chem. 1986, 34, 970-973.
- (17) Himejima, M.; Kubo, I. J. Agric. Food Chem. 1991, 39, 418-421.
- Kubo, I.; Ochi, M.; Vieira, P. C.; Komatsu, S. J. Agric. Food (18)Chem. 1993, 41, 1012-1015.
- (19) Kubo, I.; Kim, M.; Naya, K.; Komatsu, S.; Yamagiwa, Y.; Ohashi, K.; Sakamoto, Y.; Hirakawa, S.; Kamikawa, T. Chem. Lett. 1987, 1101 - 1104.

- (20) Grazzini, R.; Hesk, D.; Heininger, E.; Hildenbrandt, G.; Reddy, C. C.; Cox-Foster, D.; Medford, J. Craig, R.; Mumma, R. O. Biochem. Biophys. Res. Commun. **1991**, *176*, 775–780.
- (21)Shobha, S. V.; Ramadoss, C. S.; Ravindranath, B. J. Nat. Prod. 1994, 57, 1755-1757,
- (22) Kubo, I.; Muroi, H.; Kubo, A. J. Nat. Prod. 1994, 57, 9-17.
- (23) Kubo, I.; Kinst-Hori, I.; Yokokawa, Y. J. Nat. Prod. 1994, 57, 545 - 551
- Lee, H. S.; Ko, H. R.; Ryu, S. Y.; Oh, W. K.; Kim, B. Y.; Ahn, S. C.; Mheen, T. I.; Ahn, J. S. *Planta Med.* **1997**, *63*, 266–268. Ogawara, H.; Higashi, K.; Manita, S.; Hidaka, M.; Kato, H.; (24)
- (25)Takenawa, T. *Biochim. Biophys. Acta* **1993**, *1175*, 289–292. Aoki, M.; Itezono, Y.; Shirai, H.; Nakayama, N.; Sakai, A.;
- (26)Tanaka, Y.; Yamaguchi, A.; Shimma, N.; Seto, H.; Yokose, K. *Tetrahedron Lett.* **1991**, *32*, 4737–4740.
- Ohtsuka, T.; Itezoro, Y.; Nakayama, N.; Sakai, A.; Shimma, N.; Yokose, K.; Seto, H. J. Antibiotics **1994**, 47, 6–15. (27)
- (28)Weber, W.; Schu, P.; Anke, T.; Velten, R.; Steglich, W. J. Antibiotics 1994, 47, 1188-1194
- (29)Corthout, J.; Pieters, L.; Claeys, M.; Geerts, St.; Vanden Berghe, D.; Vlietinck, A. Planta Med. 1994, 60, 460-463.
- Rhee, S. G.; Ryu, S. H.; Lee, K. Y.; Cho, K. S. Methods Enzymol. (30)**1991**, 197, 502–511.
- (31) Skehan, P.; Storeng, R.; Scudiero, D. A.; Monks, A.; McMahon, J.; Waren, J.; Bokesch, H.; Kenney, S.; Boyd, M. R. J. Natl. Cancer Inst. 1990, 82, 1107-1112.
- (32) Rossi, R.; Carpita, A.; Quirici, M. G.; Veracini, C. A. Tetrahedron **1982**, *38*, 639–644.
- Claeys, M.; Van den Heuvel, H.; Claereboudt, J.; Corthout, J. (33)Pieters, L.; Vlietinck, A. J. Biol. Mass Spectrom. 1993, 22, 647-653.

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