

Inhibitory Effects of Panduratin A on Allergy-Related Mediator Production in Rat Basophilic Leukemia Mast Cells

Yuri Choi,¹ Myung Suk Kim,¹ and Jae-Kwan Hwang^{1,2}

Abstract—Immediate-type hypersensitivity is characterized by elevated levels of immunoglobulin E (IgE) and activated mast cell plays a crucial role by releasing granule contents, lipid-derived mediators, cytokines, and chemokines. To evaluate the antiallergic effects of panduratin A isolated from *Boesenbergia pandurata* Roxb., we determined its effects on calcium (Ca^{2+}) influx, degranulation, and inflammatory mediators in calcium ionophore A23187 and phorbol 12-myristate 13-acetate (PMA)-stimulated rat basophilic leukemia (RBL-2H3) cells. Panduratin A (20 μM) inhibited secretion of β -hexosaminidase ($46.69 \pm 9.6\%$), histamine ($34.32 \pm 2.1\%$), and Ca^{2+} influx (43.84%). Panduratin A reduced the production of prostaglandin E_2 (PGE_2 , $47.58 \pm 3.4\%$), leukotriene B_4 (LTB_4 , $98.15 \pm 1.6\%$), and the mRNA expression of cyclooxygenase-2, 5-lipoxygenase, interleukin (IL)-4, IL-13, and tumor necrosis factor- α . Furthermore, panduarin A attenuated phosphorylation of Akt, the mitogen-activated protein kinases (MAPK) extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK) expression. These results indicate that panduratin A might be useful as an agent against immediate-type hypersensitivity.

KEY WORDS: panduratin A; *Boesenbergia pandurata* Roxb.; IgE; inflammatory mediator; mast cell RBL-2H3.

INTRODUCTION

The prevalence of allergic diseases such as atopic dermatitis, allergic rhinitis, and asthma is a serious health problem worldwide. Allergic diseases are traditionally referred to as immediate or immediate-type hypersensitivity reactions, with immunoglobulin E (IgE) as a critical factor [1].

Mast cell activation mediated by the high-affinity receptor for IgE (Fc RI) is considered as a key event in the allergic inflammatory response [2]. Upon allergen provocation, cross-linkage of IgE bound to its high affinity receptor on mast cells triggers complex biochemical reactions including calcium (Ca^{2+}) influx, cytoskeletal reorganization, and activation of protein tyrosine kinase, phospholipase, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) [3].

Following this signaling process, the mast cell secretes three classes of biologically active products: those stored in cytoplasmic granules, lipid-derived mediators, and newly synthesized cytokines and growth factors as well as other products [4]. The preformed granule content histamine is a hallmark of allergic reactions and induces vascular permeability. Lipid-derived mediators, prostaglandins (PGs), and leukotrienes (LTs) promote vasodilation and erythema [5, 6]. Activated mast cells also release a broad range of newly synthesized cytokines, chemokines, and growth factors which have the potential to recruit other immune cells either directly or indirectly. Thus, mast cells contribute to chronic inflammation as well as acute inflammation [4].

Rat basophilic leukemia (RBL-2H3) cells are mucosal mast cells with functions similar to primary mast cells and normal basophils; therefore, RBL-2H3 cells are a pertinent model for the study of allergic inflammation [5, 6]. Calcium ionophore A23187 and phorbol 12-myristate 13-acetate (PMA) promote mast cell activation by upregulating Ca^{2+} influx, the critical functional component in the mast

¹Department of Biotechnology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, South Korea

²To whom correspondence should be addressed at Department of Biotechnology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, South Korea. E-mail: jkhwang@yonsei.ac.kr

cell degranulation, and activating PKC. It is well-known that A23187/PMA upregulate secretion of allergy-related factors from mast cells [7]. Modulation of mast cell-mediator production may be a major target for effective antiallergic drugs; therefore, in the present study, the inhibitory effects of panduratin A on these inflammatory mediators in RBL-2H3 cells were evaluated.

Boesenbergia pandurata Roxb. is a tropical plant that has been used as a spice, food ingredient, and a traditional medicine for the treatment of dental caries, colic, fungal infections, dry cough, rheumatism, and muscular pain [8]. Rhizomes of *B. pandurata* contain pinostrobin, cardamonin, boesenbergin, 5,7-dihydroxyflavone, 1,8-cineole, hydroxypanduratin, and panduratin A [9]. Of these, panduratin A (Fig. 1) is a major compound among the constituents of *B. pandurata* and a natural chalcone compound that has demonstrated anticancer, antioxidant, anti-inflammatory, antibacterial, and antibiofilm activities [10–14]. However, the effects of panduratin A on allergic disease have not yet been examined. Although many researchers have assessed the therapeutic effects of traditional medicines or foods in allergic diseases, the potent antiallergic effects of natural compounds have rarely been reported. In the present study, we evaluated the possible antiallergic activity of panduratin A by determining its effect on the production of inflammatory mediators in RBL-2H3 cells stimulated with A23187/PMA.

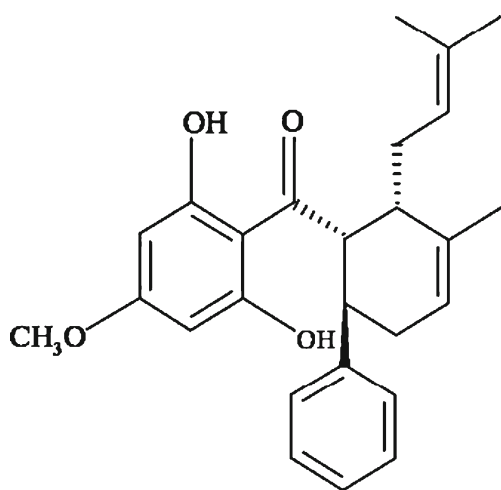


Fig. 1. Chemical structure of panduratin A.

MATERIALS AND METHODS

Chemicals

Panduratin A was dissolved in DMSO and further diluted in piperazine-N,N'-bis-(2-ethanesulfonic acid) (PIPES) buffer, which consisted of 119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM MgCl₂, 1 mM CaCl₂, 2.5 mM PIPES (Sigma-Aldrich, St. Louis, MO, USA), 0.1 % bovine serum albumin, and 40 mM NaOH (pH 7.2). The final concentration of DMSO was up to a maximum of 0.1 %. A23187 and PMA were purchased from Sigma-Aldrich. Antibodies against Akt, extracellular signal-regulated kinase (ERK), p38, c-Jun N-terminal kinase (JNK), phosphorylated ERK, phosphorylated Akt, phosphorylated p38, and phosphorylated JNK were purchased from Cell Signaling Technology (Tokyo, Japan).

Preparation of Panduratin A

Dried rhizomes from *B. pandurata* Roxb. were collected in Jakarta, Indonesia. A voucher specimen was deposited in the Department of Biotechnology, Yonsei University (Seoul, Korea). The ground *B. pandurata* Roxb. (100 g) was extracted with 95 % ethanol (400 ml), and the extract (11.95 g) was further fractionated with ethyl acetate (2×200 ml). The ethyl acetate fraction was applied to a silica gel column (5×45 cm, 650 g of silica gel; 70–230 mesh; Merck KGaA, Darmstadt, Germany) and eluted with *n*-hexane:chloroform:ethyl acetate 15:5:1.5 (2 l, v/v/v) to give seven fractions (fraction 1 to fraction 7). Fraction 3 (0.9–1 l, 1.51 g) was further separated with *n*-hexane:ethyl acetate:methanol 18:2:1 (1.5 l, v/v/v), yielding fraction 3-B (1.1 g). Fraction 3-B was eluted with 100 % methanol (0.8–0.9 ml) using a recycling preparative HPLC [(JAIGEL W-252 column, 20 mm i.d. × 500 mm l; Japan Analytical Industry Co., Ltd., Tokyo, Japan). Detection was performed by UV absorption at 365 nm, and the mobile phase was 100 % MeOH at a flow rate 3 ml/min. Compound 3-B (300–400 ml, 0.9 g) was obtained as a single compound. Careful comparison of several spectral data of compound 3-B including ¹³C-NMR, ¹H-NMR, ¹³C-DEPT, ¹H-¹H COSY, ¹H-¹³C HSQC, ¹H-¹³C HMBC, and FAB-MS with previously reported data [11] suggested that the chemical structure was panduratin A (99 %, Fig. 1) or (4-methoxy-2,6-dihydroxyphenyl)-[3'-methyl-2'-(3''-methybut-2''-enyl)-6'-phenylcyclohex-3'-enyl] methanone. Optical rotation was measured with a

PerkinElmer 241 polarimeter (PerkinElmer Life and Analytical Sciences, Inc., Massachusetts, USA) as $[\alpha] + 0.0066$ ($c=0.1$, CHCl_3). The isolated panduratin A was dissolved in dimethyl sulfoxide (DMSO).

Cell Viability Assay

Cell viability was determined with the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (Sigma-Aldrich, St. Louis, MO, USA). RBL-2H3 cells were grown in 24-well plates (2×10^5 cells/ml) for 24 h. After treating the cells with various concentrations of panduratin A for 24 h, the cells were incubated with both 5 μM A23187 and 50 nM PMA, or 10 μM A23187 alone for 30 min in serum-free medium. The cell medium was then replaced with 100 μl MTT (1 mg/ml) and incubated for an additional 4 h. After washing the cells, the insoluble formazan products were dissolved in 200 μl DMSO. Absorbance was determined by spectrophotometry at 550 nm with a VERSAmax tunable microplate reader (Molecular Devices, Inc., Sunnyvale, CA, USA).

Measurement of Intracellular Ca^{2+}

Cells were grown to confluence in black-wall clear-bottom 96-well plates (Corning Costar, Acton, MA, USA) for 24 h. Before the assay, cells were incubated in serum-free medium containing 5, 10, and 20 μM of panduratin A for 3 h. All assays were performed with the Calcium Plus assay kit (Molecular Devices Inc.) according to the manufacturer's instructions. Calcium assay solution was dissolved in Hanks' Balanced Salt Solution (HBSS) (Invitrogen, Carlsbad, CA, USA) containing 2.5 mM probenecid and 20 mM HEPES. RBL-2H3 cells were incubated with 100 μl loading buffer for 1 h at 37 °C. After incubation, cells were stimulated with A23187 (10 μM in HBSS), and intracellular Ca^{2+} levels were determined at 25 °C (485 nm excitation/525 nm emission) with a FlexStation (Molecular Devices, Inc.). Fluorescence intensity was recorded every 2 s for the first 5 min after adding the A23187.

Measurement of β -Hexosaminidase Release

Mast cell supernatants (30 μl) were incubated with an equal volume of 1 mM 4-nitrophenyl-*N*-acetyl- β -D-glucosaminide (Sigma-Aldrich) in citrate buffer (0.1 M, pH 4.5) for 1 h at 37 °C. The enzyme reaction was terminated with 100 μl stopping buffer (0.1 M Na_2CO_3 , NaHCO_3 [pH 10.0]), and absorption at 405 nm was

measured with a VERSAmax tunable microplate reader. Results were expressed as the percentage of total cellular β -hexosaminidase content by comparison with the absorption of lysates of cells treated with 5 % Triton X-100 (Sigma-Aldrich), according to the following equation: % total enzyme activity = $([\text{secreted} - \text{spontaneous}] / \text{total content}) \times 100 \%$.

Measurement of Histamine Release

Histamine released into the cell culture medium was determined with a commercially available enzyme-linked immunosorbent assay kit (Oxford Biochemical Research Inc., Oxford, MI, USA) according to the manufacturer's protocol. Results were expressed as the percentage of total cellular histamine content by comparison with the absorption of lysates of cells treated with 5 % Triton X-100 (Sigma-Aldrich), according to the following equation: % total histamine = $([\text{secreted} - \text{spontaneous}] / \text{total content}) \times 100 \%$.

Reverse Transcription-Polymerase Chain Reaction

RNA was extracted with TRIzol reagent (Invitrogen) according to the manufacturer's protocol and quantified by spectrophotometry at 260 nm. The cDNA was synthesized in a 20 μl reaction containing 1 μg total RNA, oligo(dT), and reverse transcriptase premix (Elpis Biotech., Inc., Taejeon, Korea). Reverse transcription (RT) was initiated at 70 °C for 5 min, followed by incubation at 42 °C for 60 min and was terminated at 94 °C for 5 min. Amplification of the cDNA products (5 μl) by polymerase chain reaction (PCR) was performed with a PCR premix (Elpis Biotech.) and the following primer pairs: cyclooxygenase (COX)-2 forward 5'-TGC CAC CAA CGC TGC CAC AA-3', COX-2 reverse 5'-TGG GGT GGG CTT CAG CGG TA-3' (262 bp); 5-lipoxygenase (5-LO) forward 5'-CGT GTG GGA AGC TAT CCA GT-3', 5-LO reverse 5'-GAA GAT CAC CAC CGT CAG GT-3' (205 bp); interleukin (IL)-4 forward 5'-ACC TTG CTG TCA CCC TGT TC-3', IL-4 reverse 5'-TTG TGA GCG TGG ACT CAT TC-3' (351 bp); IL-13 forward 5'-GCT CTC GCT TGC CTT GGT GG-3', IL-13 reverse 5'-CAT CCG AGG CCT TTT GGT TA-3' (274 bp); tumor necrosis factor (TNF)- α forward 5'-CAA GGA GCA GAA GTT CCC AA-3', TNF- α reverse 5'-CGG ACT CCG TGA TGT CTA AG-3' (500 bp); β -actin forward 5'-AGC CAT GTA CGT AGC CAT CC-3', β -actin reverse 5'-TCT CAG CTG TGG TGG TGA AG-3' (227 bp). Before PCR amplification, primers for the cytokines and β -actin were denatured at 94 °C for

5 min. Amplification consisted of 25 cycles of denaturation at 94 °C for 30 s, annealing for 1 min (62 °C for COX-2, IL-4, and IL-13; 60 °C for TNF- α , 5-LO, and β -actin), and extension at 72 °C for 1 min, followed by a final 5-min extension at 72 °C. PCR was performed in a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA). PCR products were separated by 1.5 % agarose gel electrophoresis and visualized by ethidium bromide staining and UV illumination. β -Actin was used as an internal control. A densitometer was used for RT-PCR on DIG chemiluminescent film (volume of all markers/volume of β -actin).

Measurement of Prostaglandin E₂ and Leukotriene B₄

Cell culture medium was collected, and the secretion of prostaglandin E₂ (PGE₂) and leukotriene B₄ (LTB₄) was determined with a competitive radioimmunoassay kit according to the manufacturer's protocol (Sapphire Bioscience Pty. Ltd., Redfern, NSW, Australia).

Western Blot Analysis

RBL-2H3 cells were lysed in RIPA buffer containing protease inhibitors and then held on ice for 10 min. The lysate protein concentrations were determined with the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of protein (30 μ g) in each sample were loaded, separated by 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Whatman GmbH, Dassel, Germany). Blotted membranes were blocked with 5 % skim milk in Tris-buffered saline containing 0.1 % Tween 20 for 1 h and then incubated with primary antibodies for 16 h at 4 °C. After three washes in Tris-buffered saline containing 0.1 % Tween 20, the membranes were incubated with horseradish peroxidase-linked secondary antibodies for 2 h. Proteins were detected with enhanced chemoluminescence (Amersham Biosciences, Little Chalfont, UK) and visualized with a G:BOX Chemi transilluminator (Syngene, Synoptics Ltd., Cambridge, UK).

Statistical Analysis

Each experiment was performed at least three times. Results are expressed as mean $\pm$ standard deviation (SD). The vehicle (DMSO) and treated groups were compared by one-way analysis of variance fol-

lowed by Scheffe's test (SPSS 17.0, Chicago, IL, USA). ^{##} $P < 0.01$, $*P < 0.05$, and $***P < 0.01$ were considered statistically significant.

RESULTS

Cytotoxicity of Test Substances

The viability of RBL-2H3 cells treated with panduratin A was evaluated by the colorimetric MTT assay. It was found that RBL-2H3 cells treated with panduratin A (1–20 μ M) for 24 h and stimulated with both 5 μ M A23187 and 50 nM PMA, or 10 μ M A23187 alone for 30 min did not cause any cytotoxicity (Fig. 2a–c).

Inhibitory Effects of Panduratin A on β -Hexosaminidase and Histamine Release in A23187-Stimulated RBL-2H3 Cells

To determine the effect of panduratin A on mast cell degranulation, its ability to inhibit the release of β -hexosaminidase was examined. Panduratin A inhibited β -hexosaminidase release in RBL-2H3 cells treated with A23187 (10 μ M) at concentrations of 5, 10, and 20 μ M in a dose-dependent manner. At a concentration of 20 μ M, the level of inhibition reached 46 % (Fig. 3a). In addition, the effect of panduratin A on histamine release was evaluated to determine its antiallergic activity. Panduratin A also inhibited histamine release in RBL-2H3 cells treated with A23187 (10 μ M) in a dose-dependent manner. At a concentration of 20 μ M, the level of inhibition reached 51 % (Fig. 3b).

Effect of Panduratin A on Calcium Mobilization in RBL-2H3 Cells

To determine the suppression mechanism of degranulation by panduratin A, intracellular free Ca²⁺ concentrations in A23187-stimulated RBL-2H3 cells was examined. In RBL-2H3 cells pretreated with 5, 10, or 20 μ M of panduratin A before A23187 (10 μ M) stimulation, the intracellular Ca²⁺ concentration was lower than that of the vehicle group treated with A23187 (Fig. 4). This result indicates that panduratin A suppressed various intracellular signals, which are associated with the elevation of intracellular Ca²⁺.

Panduratin A Suppresses Allergic Response in RBL-2H3 Cells

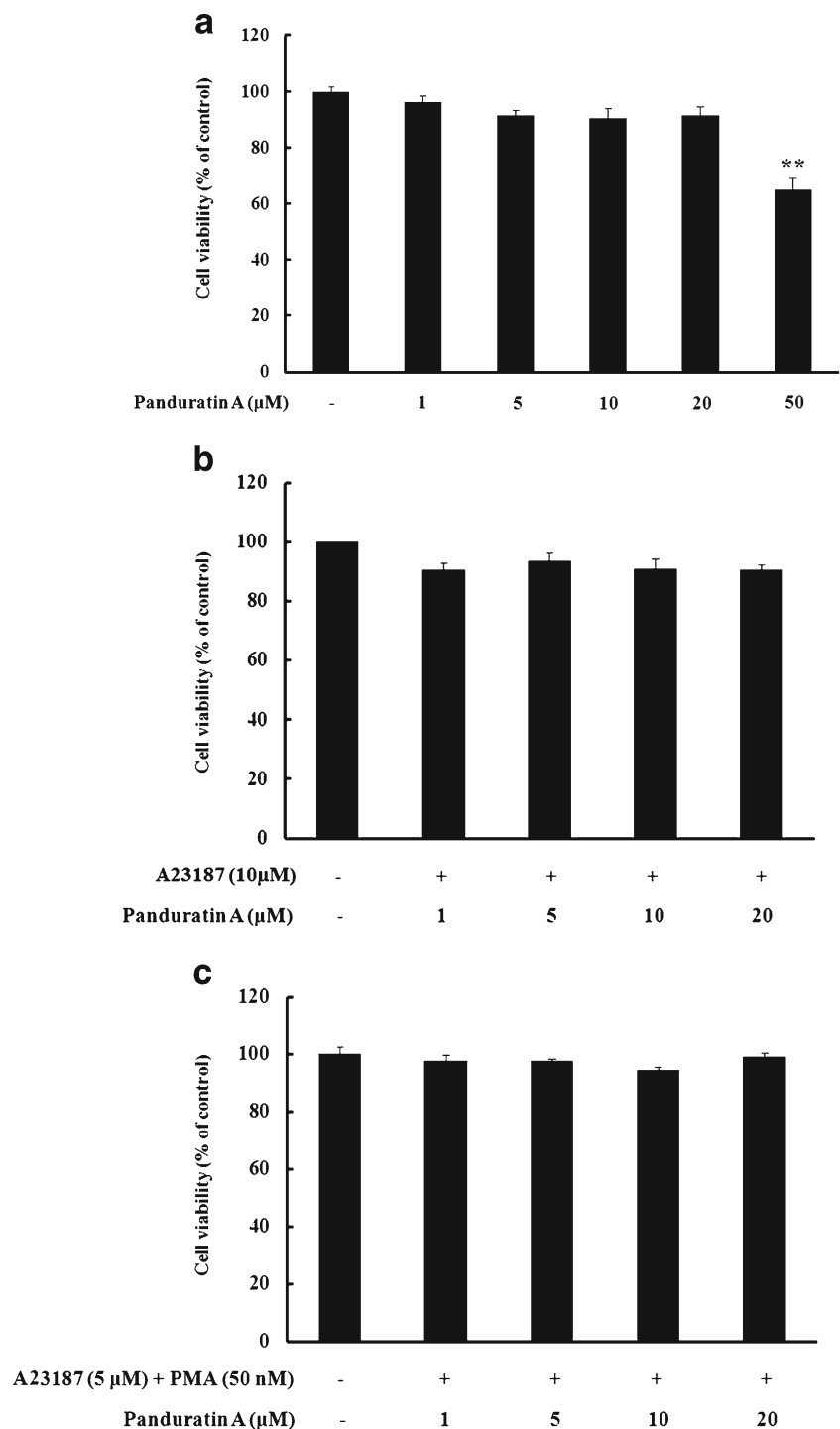


Fig. 2. Effects of panduratin A, A23187, and PMA on cell viability. **a** RBL-2H3 cells were treated with panduratin A (1–50 μM), **b** panduratin A with A23187 (10 μM) alone, or **c** panduratin A with A23187 (5 μM) and PMA (50 nM) for 24 h. The cell viability was measured by MTT assay. Results are expressed as the mean ± SD of three independent experiments. ** $P < 0.01$ compared to the vehicle (DMSO).

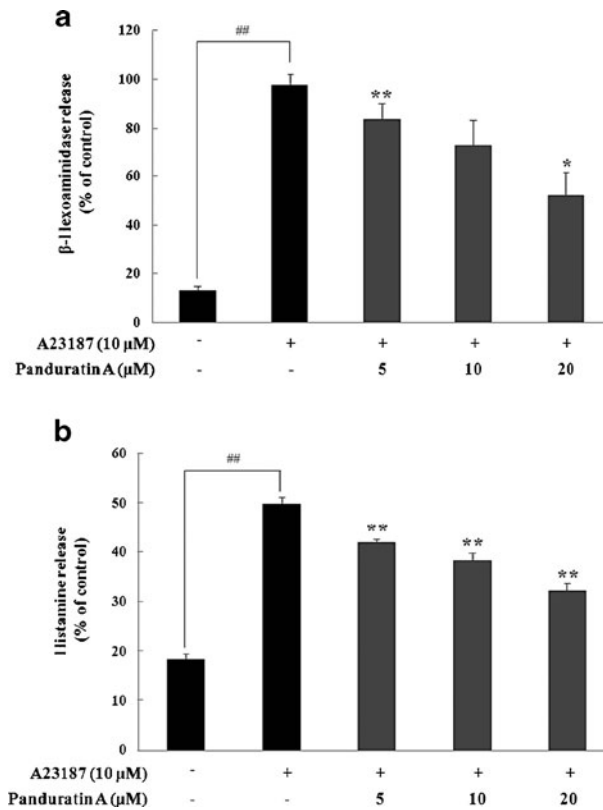


Fig. 3. Effects of panduratin A on β -hexosaminidase and histamine release. RBL-2H3 cells were treated with panduratin A (5, 10, or 20 μ M) for 3 h and stimulated with A23187 (10 μ M) for 30 min. **a** β -Hexosaminidase release was measured by β -hexosaminidase assay and **b** histamine release was determined with enzyme-linked immunosorbent assay kit. Results are expressed as the mean $\pm$ SD of three independent experiments. * P <0.05, ** P <0.01 compared with A23187 alone.

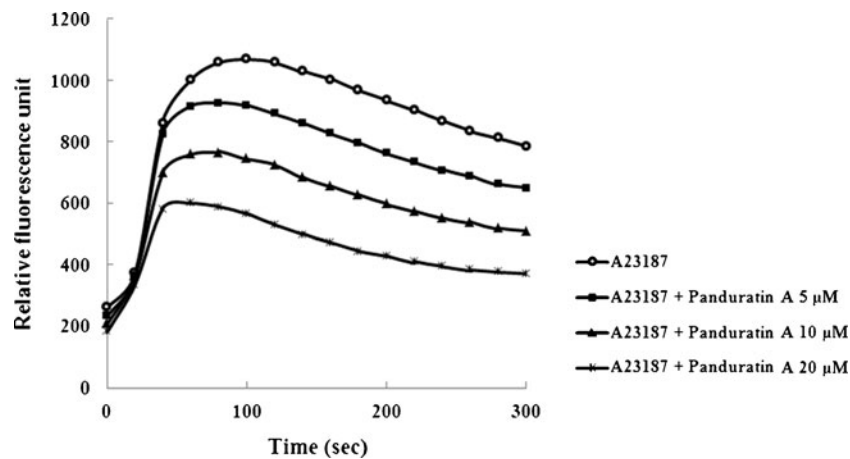


Fig. 4. Effect of panduratin A on A23187-induced Ca^{2+} mobilization in RBL-2H3 cells. RBL-2H3 cells were seeded overnight in a 96-well poly-D-lysine-coated black-well clear-bottom plate. Cells were incubated with Calcium Plus Assay kit and panduratin A (5, 10, or 20 μ M) for 30 min. After incubation, the assay plate was directly transferred to the FlexStation and data were analyzed using SoftMax[®] Pro.

Inhibitory Effects of Panduratin A on COX-2 mRNA Expression and PGE₂ Production in A23187/PMA-Stimulated RBL-2H3 Cells

COX-2 is involved in the metabolism of arachidonic acids to PGs [15]. The effect of panduratin A on COX-2 gene expression in RBL-2H3 cells stimulated with A23187/PMA was, therefore, assessed by RT-PCR. COX-2 mRNA levels were elevated in stimulated RBL-2H3 cells. However, treatment with 5, 10, or 20 μ M of panduratin A-attenuated COX-2 gene expression (Fig. 5a). Further, panduratin A significantly inhibited

PGE₂ secretion in stimulated RBL-2H3 cells in a dose-dependent manner. At a concentration of 20 μ M, the level of inhibition reached 48 % (Fig. 5b).

Inhibitory Effects of Panduratin A on 5-LO mRNA Expression and LTB₄ Production in A23187/PMA-Stimulated RBL-2H3 Cells

The effect of panduratin A on the expression of 5-LO, an enzyme that produces LTs from arachidonic acid, was also evaluated by RT-PCR. A23187/PMA treatment

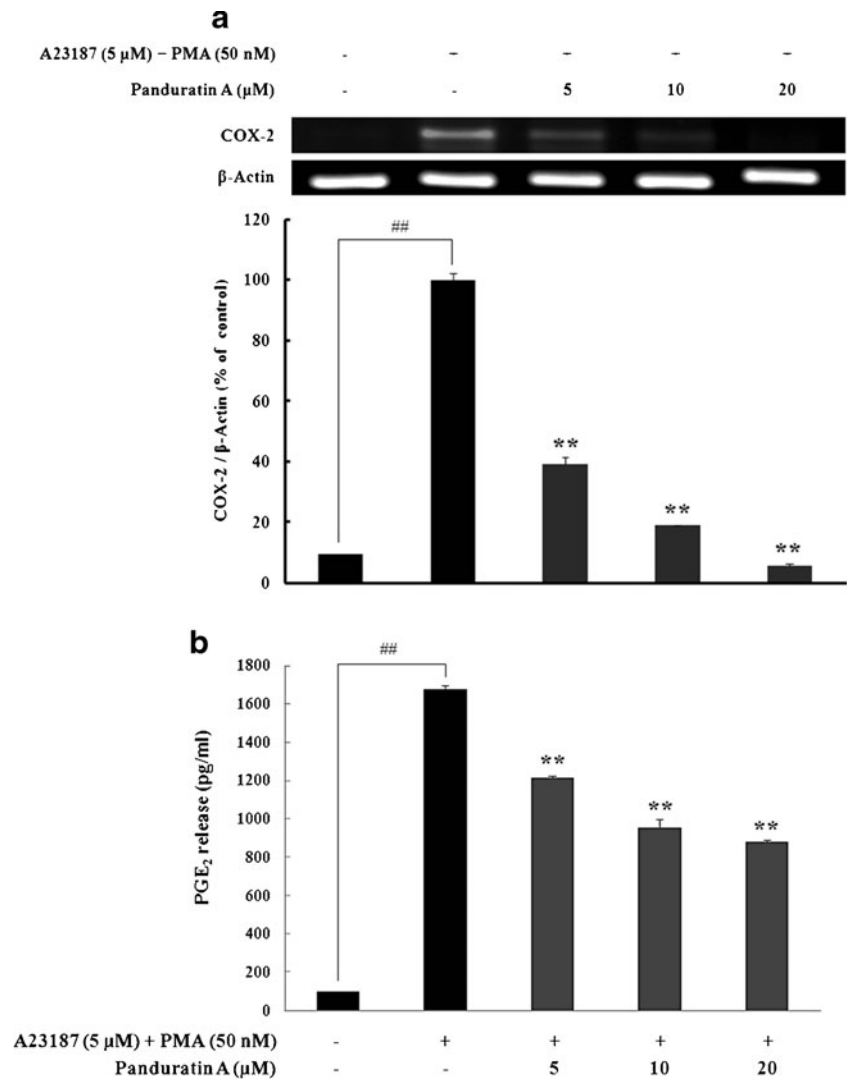


Fig. 5. Effects of panduratin A on COX-2 mRNA expression and PGE₂ secretion. RBL-2H3 cells were treated with panduratin A (5, 10, or 20 μ M) for 3 h and stimulated with A23187 (5 μ M) and PMA (50 nM). **a** COX-2 mRNA level was determined with RT-PCR. **b** PGE₂ secretion was determined by enzyme-linked immunosorbent assay kit. Results are expressed as the mean $\pm$ SD of three independent experiments. ^{##}*P*, ^{**}*P*<0.01 compared with cotreatment with A23187 and PMA.

induced 5-LO gene expression in RBL-2H3 cells. However, treatment with 5, 10, or 20 μM of panduratin A attenuated the A23187/PMA-induced 5-LO upregulation (Fig. 6a). Likewise, panduratin A significantly reduced LTB_4 levels in RBL-2H3 cells stimulated with A23187/PMA in a dose-dependent manner. At a concentration of 20 μM , the level of inhibition reached 98 % (Fig. 6b).

Inhibitory Effect of Panduratin A on Proinflammatory Cytokine Gene Expression in A23187/PMA-Stimulated RBL-2H3 Cells

The ability of panduratin A to inhibit proinflammatory cytokine (IL-4, IL-13, and $\text{TNF-}\alpha$) expression induced by A23187/PMA in RBL-2H3 cells was assessed by RT-PCR. The combination of A23187 and PMA increased the mRNA levels of IL-4, IL-13, and

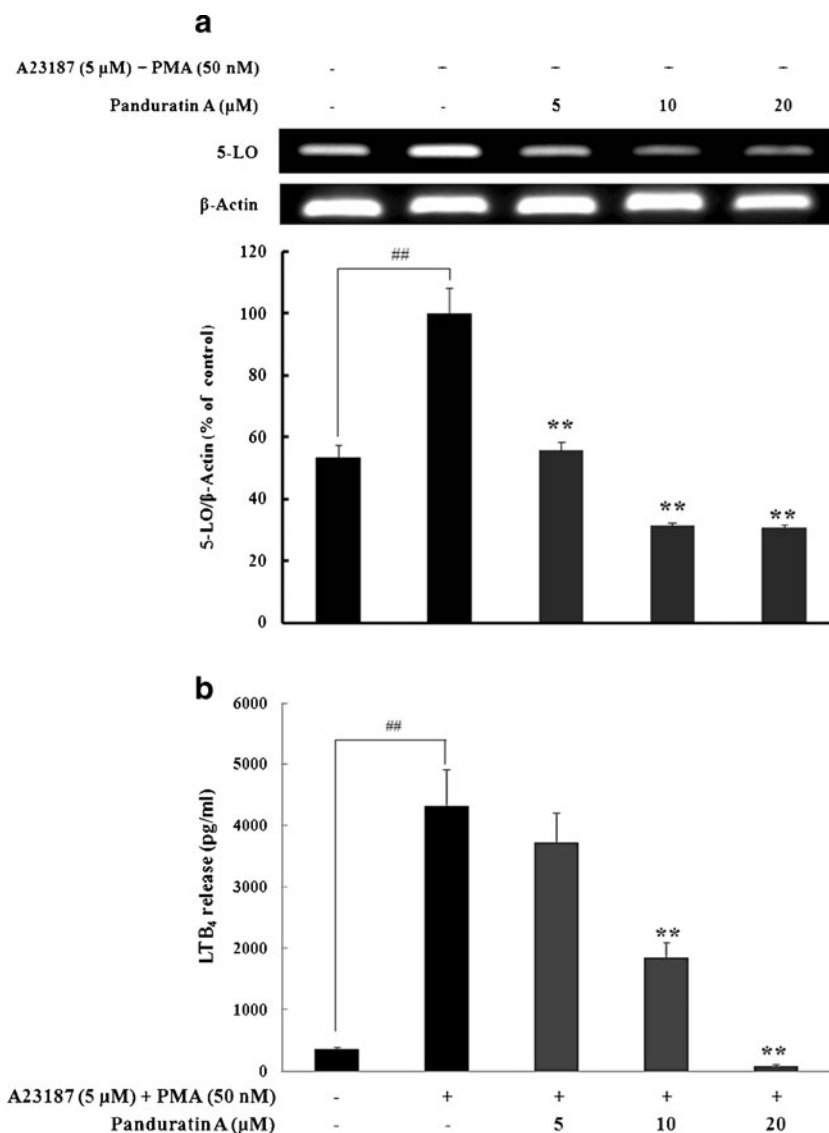


Fig. 6. Effects of panduratin A on 5-LO mRNA expression and LTB_4 secretion. RBL-2H3 cells were treated with panduratin A (5, 10, or 20 μM) for 3 h and stimulated with A23187 (5 μM) and PMA (50 nM). **a** 5-LO mRNA level was determined with RT-PCR. **b** LTB_4 secretion was determined by enzyme-linked immunosorbent assay kit. Results are expressed as the mean $\pm$ SD of three independent experiments. $^{##}P$, $^{**}P < 0.01$ compared with cotreatment with A23187 and PMA.

Panduratin A Suppresses Allergic Response in RBL-2H3 Cells

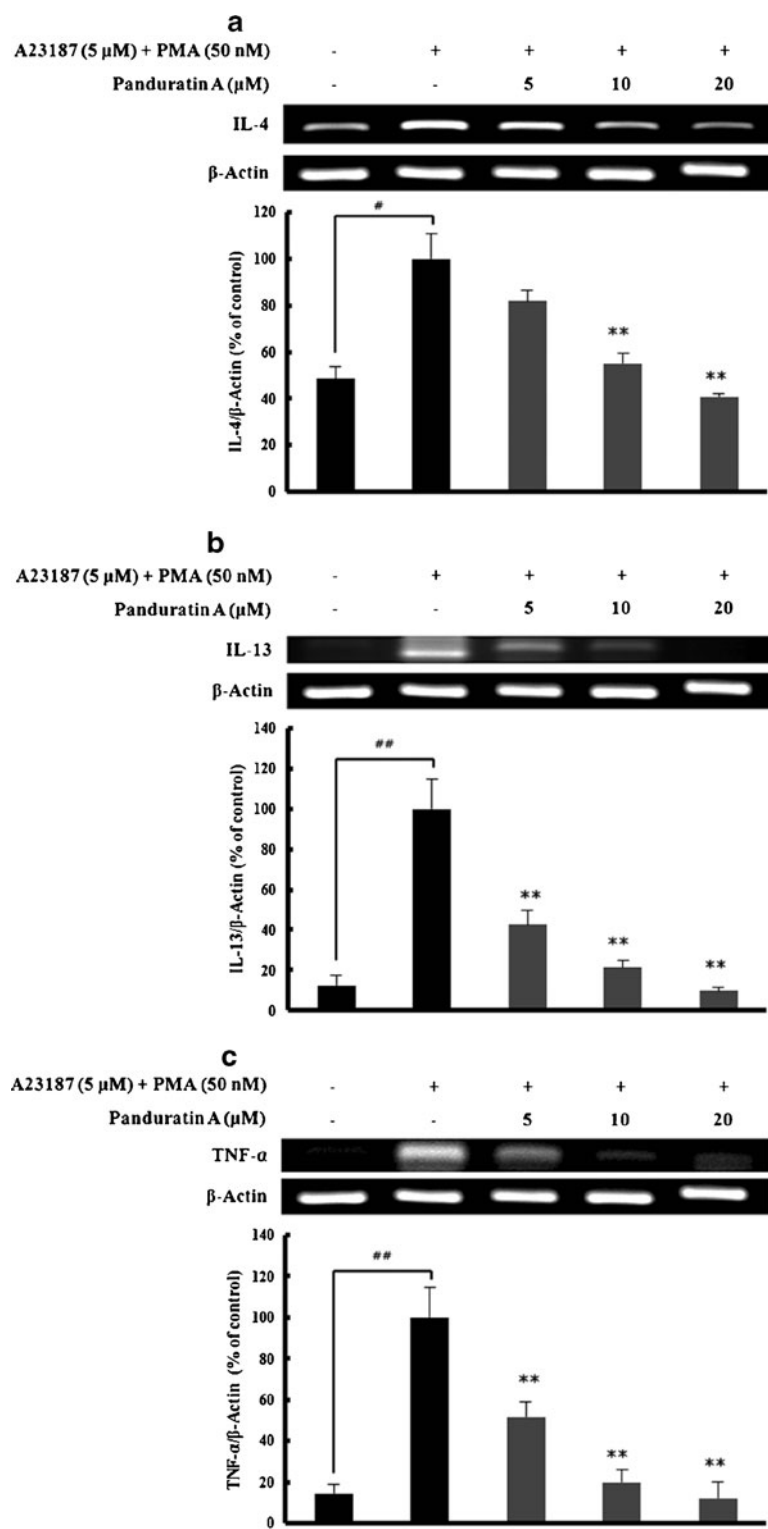


Fig. 7. Effects of panduratin A on IL-4, IL-13, and TNF- α expression. RBL-2H3 cells were treated with panduratin A (5, 10, or 20 μ M) for 3 h and stimulated with A23187 (5 μ M) and PMA (50 nM). **a** IL-4, **b** IL-13, and **c** TNF- α mRNA levels were determined with RT-PCR. Results are expressed as the mean $\pm$ SD of three independent experiments. # P <0.05, ## P <0.01 compared with cotreatment with A23187 and PMA.

TNF- α in RBL-2H3 cells. However, treatment with 5, 10, or 20 μ M of panduratin A attenuated the mRNA levels of IL-4, IL-13, and TNF- α in RBL-2H3 cells (Fig. 7a–c).

Effect of Panduratin A on Akt, ERK, p38, and JNK Signaling

To determine the signaling mechanism of panduratin A on the activation of RBL-2H3 cell, phosphorylation of Akt, ERK, p38, and JNK was examined by Western blot. A23187/PMA stimulation in RBL-2H3 cells led to phosphorylation of Akt, ERK, p38, and JNK. Akt, ERK, p38, and JNK were phosphorylated 10 min after stimulation, and phosphorylation peaked at 30 min. However, in panduratin A-pretreated RBL-2H3 cells, phosphorylation of Akt and the three MAPKs was suppressed at 10 min. Further, panduratin A down-regulated phosphorylation of Akt, ERK, p38, and JNK in a dose-dependent manner (Fig. 8).

DISCUSSION

Immediate-type hypersensitivity is initiated and amplified by inflammatory mediators (histamines, LTs, and PGs) and cytokines (TNF- α , IL-4, and IL-13) from immune cells such as mast cells, eosinophils, and basophils. In particular, the mast cells are crucial players in the regulation of the allergic inflammatory responses

by virtue of their abilities to produce proinflammatory mediators including histamines [16]. A recent study with mast cell-deficient mice demonstrated that particular mast cell-derived mediators (histamines, cytokines, and proteases) have an important role in the allergic inflammation condition [17]. Therefore, studies on allergic diseases have elicited information about specific mediators of mast cell and have searched for compounds able to regulate these inflammatory mediators [3, 18, 19].

Chalcones are naturally occurring plant flavonoids found to be in rich in vegetables and fruits, and they have various physiological properties such as anticancer, antioxidant, and anti-inflammatory [20–22]. Moreover, chalcones are also known to suppress allergy-related mediators. For example, licochalcones, isolated from the root of *Glycyrrhiza inflata*, suppress degranulation by decreasing the intracellular Ca^{2+} level and tyrosine phosphorylation of ERK in RBL-2H3 cells [18]. Isoliquiritigenin and E-7-O-methylpangamol, isolated from the aerial parts of *Cissus sicyoides*, also suppress degranulation [23]. Panduratin A is a plant flavonoid that harbors a variety of beneficial effects including anticancer, antioxidant, anti-inflammatory, antibacterial, and antibiofilm activities [10–14]. Also, among chalcone compounds contained in *B. pandurata*, panduratin A showed strongest activity against NO and PGE₂ with IC₅₀ values of 5.3 μ M and 10.5 μ M, respectively, and a moderate effect on TNF- α (IC₅₀=60.3 μ M) in RAW264.7 cells [24]. However, the effects of panduratin A on allergic disease have not yet been

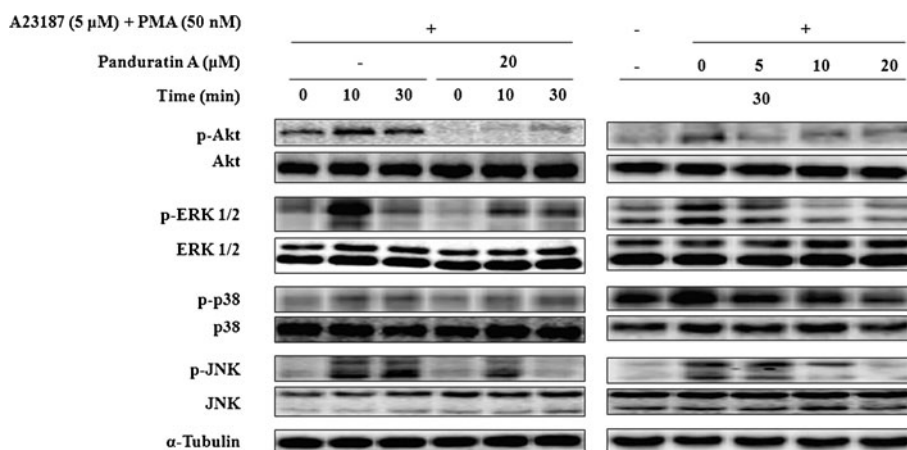


Fig. 8. Effect of panduratin A on Akt and MAPK phosphorylation. RBL-2H3 cells were treated with panduratin A (20 μ M) for 3 h and stimulated with A23187 (5 μ M) and PMA (50 nM) for 0, 10, 30 min. Phosphorylation of Akt, ERK, p38, and JNK was determined with Western blot analysis. RBL-2H3 cells were treated with panduratin A (5, 10 or 20 μ M) for 3 h and stimulated with A23187 (5 μ M) and PMA (50 nM) for 30 min. Phosphorylation of Akt, Erk, p38, and JNK was determined with Western blot analysis.

examined. In the present study, we evaluated the inhibitory effects of panduratin A, natural chalcone compound, isolated from *B. pandurata* Roxb. on allergy-related mediators in RBL-2H3 cells, an *in vitro* model for mast cell function.

IgE and A23187/PMA are known to mediate the release of granule contents and expression of proinflammatory genes (*IL-4*, *IL-13*, *TNF- α* , *5-LO*, and *COX-2*) from mast cells by uptaking extracellular Ca^{2+} and activating PKC [25, 26]. A recent study showed that activation of mast cells by A23187/PMA increased proinflammatory cytokine levels [27].

In the present study, panduratin A diminished degranulation of preformed granule contents from RBL-2H3 cells, as assessed by the release of β -hexosaminidase and histamine (Fig. 3). These results indicate that the antiallergic effects of panduratin A are due to the suppression of histamine release, which causes vascular permeability, smooth-muscle contraction, and inflammation. To demonstrate the mechanism of degranulation, we also found that panduratin A inhibited A23187-stimulated Ca^{2+} influx (Fig. 4). This suggests that panduratin A inhibits degranulation and other inflammatory responses by blocking intracellular Ca^{2+} release.

Panduratin A also reduced the secretion of inflammatory mediators PGE_2 and LTB_4 , the arachidonic acid metabolites, via the COX and LO pathways [28]. Furthermore, panduratin A inhibited the mRNA expression of 5-LO and COX-2, key enzymes in these pathways (Figs. 5 and 6). These results indicate that panduratin A inhibits synthesis of inflammatory mediators by suppressing the COX and LO pathways.

We also found that panduratin A significantly reduced the mRNA expression of the proinflammatory cytokines IL-4, IL-13, and TNF- α (Fig. 7). IL-4, IL-13, and TNF- α play a major role in triggering and sustaining the allergic reaction in mast cells. IL-4 and IL-13 induce T cell development and switch B cells to the IgE isotype. TNF- α also activates physiological immune response by promoting leukocyte infiltration and tissue fibrosis, and it is thought to be an initiator of cytokine-related inflammatory states via stimulation of cytokine production [29]. Although inflammation is the physiological immune defense system against pathogens, overexpression of these cytokines can also lead to serious inflammatory disease like atopic dermatitis and asthma. Our result of attenuating the level of IL-4, IL-13, and TNF- α suggests that panduratin A may allay allergic conditions.

MAPK pathways are major target mechanisms for allergic disease therapy [30]. Therefore, to investigate

the mechanism by which panduratin A blocked activation of RBL-2H3 cells, we evaluated phosphorylation of Akt and important MAPKs (ERK, p38, and JNK). In this study, panduratin A blocked phosphorylation of Akt, ERK, p38, and JNK, suggesting that panduratin A attenuates mast cell activation by regulating Akt and MAPK signaling (Fig. 8).

In conclusion, we found that panduratin A significantly suppressed degranulation, production of lipid-derived mediators, and expression of proinflammatory gene by blocking the activation of IgE-mediated signal transduction. These findings indicate that panduratin A shows potential as a natural remedy for immediate-type hypersensitivity.

ACKNOWLEDGMENTS

This work was supported, in part, by the Yonsei Biomolecule Research Initiative of the Two-step Brain Korea 21 Project.

REFERENCES

1. Rosenwasser, L.J. 2011. Mechanisms of IgE Inflammation. *Current Allergy Asthma and Reports* 11: 178–183.
2. Gilfillan, A.M., and C. Tkaczuk. 2006. Integrated signaling pathways for mast-cell activation. *Nature Reviews Immunology* 6: 218–230.
3. Itoh, T., K. Ohguchi, M. Inuma, Y. Nozawa, and Y. Akao. 2008. Inhibitory effects of polymethoxy flavones isolated from *Citrus reticulata* on degranulation in rat basophilic leukemia RBL-2H3: Enhanced inhibition by their combination. *Bioorganic & Medicinal Chemistry* 16: 7592–7598.
4. Galli, S.J., M. Tsai, and A.M. Piliponsky. 2008. The development of allergic inflammation. *Nature* 454: 445–454.
5. Chen, H.J., C.K. Shin, H.Y. Hsu, and W. Chiang. 2010. Mast cell-dependent allergic responses are inhibited by ethanolic extract of adlay (*Coix lachrymal-jobi* L. var. *ma-yuen* Steff) testa. *Journal of Agricultural Food Chemistry* 58: 2596–2601.
6. Kim, K., Y. Kim, H.Y. Kim, J.Y. Ro, and D. Jeong. 2008. Inhibitory mechanism of anti-allergic peptides in RBL-2H3 cells. *European Journal of Pharmacology* 581: 191–203.
7. Shim, J.U., P.S. Oh, and K.T. Lim. 2009. Anti-inflammatory activity of ethanol extract from *Geranium sibiricum* Linne. *Journal of Ethnopharmacology* 126: 90–95.
8. Trakontavakorn, G., K. Nakahara, H. Shinmoto, M. Takenaka, M. Onishi-Kameyama, and H. Ono. 2001. Structural analysis of a novel antimutagenic compound, 4-hydroxypanduratin A, and the antimutagenic activity of flavonoids in a Thai spice, fingerroot (*Boesenbergia pandurata* Schult.) against mutagenic heterocyclic amines. *Journal of Agricultural Food Chemistry* 49: 3046–3050.
9. Mahidol, C., P. Tantiwachwuttikul, V. Reutrakul, and W.C. Taylor. 1984. Constituents of *Boesenbergia pandurata* (syn. *Kaempferia pandurata*). III Isolation and synthesis of (+)-boesenbergin B. *Australian Journal of Chemistry* 37: 1739–1745.

10. Yun, J.M., M.H. Kweon, H. Kwon, J.K. Hwang, and H. Mukhtar. 2006. Induction of apoptosis and cell cycle arrest by a chalcone panduratin A isolated from *Kaempferia pandurata* in androgen-independent human prostate cancer cells PC3 and DU145. *Carcinogenesis* 27: 1454–1464.
11. Yun, J.M., H. Kwon, and J.K. Hwang. 2003. *In vitro* anti-inflammatory activity of panduratin A isolated from *Kaempferia pandurata* in RAW264.7 cells. *Planta Medica* 69: 1102–1108.
12. Sohn, J.H., K.L. Han, S.H. Lee, and J.K. Hwang. 2005. Protective effects of panduratin A against oxidative damage of tert-butylhydroperoxide in human HepG2 cells. *Biological & Pharmaceutical Bulletin* 28: 1083–1106.
13. Rukayadi, Y., S. Han, D. Yong, and J.K. Hwang. 2010. *In vitro* antibacterial activity of panduratin A against enterococci clinical isolates. *Biological & Pharmaceutical Bulletin* 33: 1489–1493.
14. Yanti, Y. Rukayadi, K.H. Lee, and J.K. Hwang. 2009. Activity of panduratin A isolated from *Kaempferia pandurata* Roxb. against multi-species oral biofilms *in vitro*. *Journal of Oral Science* 51: 87–95.
15. Giuliano, F., and T.D. Warner. 2002. Origins of prostaglandin E₂. Involvements of cyclooxygenase (COX)-1 and COX-2 in human and rat systems. *Journal of Pharmacology and Experimental Therapeutics* 303: 1001–1006.
16. Galli, S.J., J. Kalesnikoff, M.A. Grimaldeston, A.M. Piliponsky, C.M. Williams, and M. Tsai. 2005. Mast cells as “tunable” effector and immunoregulatory cells: recent advances. *Annual Review of Immunology* 23: 749–786.
17. Wershil, B.K. 2000. Mast cell-deficient mice and intestinal biology. *American Journal of Physiology—Gastrointestinal and Liver Physiology* 278: G343–G348.
18. Tanifuji, S., E. Aizu-Yokota, M. Funakoshi-Tago, Y. Sonoda, H. Inoue, and T. Kasahara. 2010. Licochalcones suppress degranulation by decreasing the intracellular Ca²⁺ level and tyrosine phosphorylation of ERK in RBL-2H3 cells. *International Immunopharmacology* 10: 769–776.
19. Westcott, J.Y., S.E. Wenzel, and S.C. Dreskin. 1996. Arachidonate-induced eicosanoid synthesis in RBL-2H3 cells: stimulation with antigen or A23187 induces prolonged activation of 5-lipoxygenase. *Biochimica et Biophysica Acta* 1303: 74–81.
20. Katsori, A.M., and D. Hadjipavlou-Litina. 2009. Chalcones in cancer: understanding their role in terms of QSAR. *Current Medicinal Chemistry* 16: 1062–1081.
21. Sahu, N.K., S.S. Balbhadra, J. Choudhary, and D.V. Kohli. 2012. Exploring pharmacological significance of chalcone scaffold: a review. *Current Medicinal Chemistry* 19: 209–225.
22. Yadav, V.R., S. Prasad, B. Sung, and B.B. Aggarwal. 2011. The role of chalcones in suppression of NF-κB-mediated inflammation and cancer. *International Immunopharmacology* 11: 295–309.
23. Xu, F., H. Matsuda, H. Hata, K. Sugawara, S. Nakamura, and M. Yoshikawa. 2009. Structures of new flavonoids and benzofuran-type stilbene and degranulation inhibitors of rat basophilic leukemia cells from the Brazilian herbal medicine *Cissus sicyoides*. *Chemical & Pharmaceutical Bulletin (Tokyo)* 57: 1089–1095.
24. Supinya, T., S. Sanan, K. Chatchanok, P. Chanita, and C. Sarot. 2009. Anti-inflammatory effects of compounds from *Kaempferia parviflora* and *Boesenbergia pandurata*. *Food Chemistry* 115: 534–538.
25. Kempuraj, D., B. Madhappan, S. Christodoulou, W. Boucher, and J. Cao. 2005. Papadopolou, N. Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. *British Journal of Pharmacology* 145: 934–944.
26. Pernas-Sueiras, O., A. Alfonso, M.R. Vieytes, and L.M. Botana. 2006. PKC and cAMP positively modulate alkaline-induced exocytosis in the human mast cell line HMC-1. *Journal of Cell Biochemistry* 99: 1651–1663.
27. Park, H.H., S. Lee, J.M. Oh, M.S. Lee, K.H. Yoon, and B.H. Park. 2007. Anti-inflammatory activity of fisetin in human mast cells (HMC-1). *Pharmacological Research* 55: 31–37.
28. Gonzalez-Periz, A., and J. Claria. 2007. New approaches to the modulation of the cyclooxygenase-2 and 5-lipoxygenase pathways. *Current Topics in Medicinal Chemistry* 7: 297–309.
29. Bradding, P. 1996. Human mast cell cytokines. *Clinical & Experimental Allergy* 26: 13–19.
30. Abdel-Raheem, I.T., I. Hide, Y. Yanase, Y. Shigemoto-Mogami, N. Sakai, and Y. Shirai. 2005. Protein kinase C-α mediates TNF release process in RBL-2H3 mast cells. *British Journal of Pharmacology* 145: 415–423.