



Inhibitory effects of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate on allergic inflammatory responses in rat basophilic leukemia cells



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ABSTRACT

Mast cells play crucial roles in the initiation of allergic inflammatory responses by releasing various mediators such as histamines, cytokines, and leukotrienes. In addition, signaling cascade pathways, such as the mitogen-activated protein kinase (MAPK) pathway, contribute to the regulation of mast cell degranulation. Accordingly, different research strategies have been pursued to develop anti-inflammatory and anti-allergic drugs by regulating these signaling pathways. The development of new drugs that inhibit mast cell degranulation may help in the treatment of allergies. In this study, we investigated the effects of coumarin derivatives on mast cell degranulation. The effect of coumarin derivatives on degranulation in rat basophilic leukemia (RBL)-2H3 cells was determined by a β -hexosaminidase assay and histamine assay. A coumarin derivative 1 (C1), 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate, inhibited degranulation in a dose-dependent manner and demonstrated maximum therapeutic effect when used at 25 μ M. Additionally, these compounds inhibited the phosphorylation of the extracellular signal-regulated kinase (ERK) pathway. Taken together, these results indicate that 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate inhibits mast cell degranulation by suppressing the activation of the ERK pathway and this inhibitory effect suggests potential therapeutic strategies towards the prevention of allergic disorders.

1. Introduction

Allergy is a hypersensitive reaction of the immune system caused by a foreign antigen (allergen) in a harmless environment [1]. The main symptoms of asthma, atopic dermatitis, and allergic rhinitis such as itching, fever, and pain occur throughout allergic responses, and these symptoms are generated by the excessive activation of mast cells [2].

Mast cells play crucial roles in the initiation of allergic responses and mast cell activation is closely related to the specific immunoglobulin E (IgE) antibody as the typical activator [3]. IgE binds to the high-affinity IgE receptor Fc epsilon receptor 1 (FcεRI) expressed on the surfaces of mast cells [4]. The crosslinking of FcεRI on mast cells with antigens triggers intracellular signaling cascades, leading to the activation of downstream mediators such as Lyn, Syk, protein kinase C (PKC), and mitogen activated protein kinase (MAPK) as well as increases in the concentration of intracellular Ca^{2+} (calcium ion) [5]. In addition, the aggregation of IgE-FcεRI leads to the exocytosis of preformed granules including histamine, chemokines, cytokines, and serine proteases and stimulates the *de novo* synthesis of leukotrienes and prostaglandins [6]. These inflammatory mediators initiate diverse pathophysiological events

in allergic reactions including the contraction of airway smooth muscles, secretion of mucus, and recruitment of other inflammatory cells [7]. Accordingly, the regulation of inflammatory mediator production is critical for the control of allergic reactions.

Rat basophilic leukemia (RBL)-2H3 cells are rodent mucosal-type mast cells that secrete inflammatory mediators [8]. RBL-2H3 cell line is not totally representative of primary mast cells and basophils, however, RBL-2H3 cell line shares some characteristics of both and is capable of simple subculture. The major feature of mast cells is the secretion of histamine. It is concerned in allergy and inflammatory responses, involving an important role in mediating interactions between other cells. These histamines are also secreted in RBL-2H3 cells [9]. Thus, RBL-2H3 cells have been typically used in *in vitro* studies on allergic responses [10,11]. Phorbol myristate acetate (PMA), an activator of PKC, and A23187, a calcium ionophore, increase intracellular calcium ion levels and induce the activation of signaling pathways in RBL-2H3 cells [12,13]. Accordingly, in this study, we used a combination of PMA and A23187 to test the anti-allergic effects of coumarin derivatives in RBL-2H3 cells.

Specifically, we investigated the effect of coumarin derivatives on

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promoting the activation of intracellular signaling cascades leading to degranulation in RBL-2H3 cells stimulated with PMA and A23187. Among the coumarin derivatives, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate strongly inhibited the degranulation of RBL-2H3 cells. Furthermore, we observed that 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate suppressed the activation of the MAPK pathway.

2. Materials and methods

2.1. Materials

Coumarin derivatives were synthesized as described previously [14–16]. Coumarin derivatives were dissolved in dimethyl sulfoxide (DMSO) and diluted in PIPES buffer (25 mM PIPES, 119 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 0.4 mM MgCl₂, 5.6 mM glucose, 0.1% bovine serum albumin, pH 7.2). The final concentration of DMSO was 0.125% (v/v) and the same concentration of DMSO was used as a control. PMA (phorbol myristate acetate), A23187 (calcium ionophore) and o-phthalaldehyde (OPT) were purchased from Sigma Aldrich (St. Louis, MO, USA). Specific primary antibodies used for total and phosphorylated p38, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase kinases 3 (MKK3), MAP kinase kinases 1/2 (MEK1/2) or MKK4 were purchased from Cell Signaling Technology (Beverly, MA, USA). Finally, anti-β-actin antibody was purchased from Abcam (Cambridge, UK).

2.2. Cell culture

RBL-2H3 cells and RAW264.7 cells were cultured in Dulbecco's modified Eagle's medium [DMEM] (Hyclone Logan, UT, USA) supplemented with 10% heat inactivated fetal bovine serum (FBS), 1% penicillin, and 1% streptomycin (Hyclone). MOLT-4 cells were cultured in RPMI-1640 medium (Hyclone Logan, UT, USA) supplemented with 10% heat inactivated fetal bovine serum (FBS), 1% penicillin, and 1% streptomycin (Hyclone). These cells were incubated in a humidified atmosphere containing 5% CO₂ at 37 °C.

2.3. Degranulation

To determine the effect of chemicals on degranulation, we measured the levels of β-hexosaminidase release. This enzyme is contained within granules in mast cells and has been used as a granule marker [17]. RBL-2H3 cells (2 × 10⁵ cells/mL) were cultured in 24-well plates for 24 h. Next, the medium was removed and the cells were incubated with different concentration of chemicals diluted in PIPES buffer for 1 h at 37 °C. After incubation, the cells were washed with PIPES buffer two times and stimulated with PMA (50 nM) and A23187 (1 μM) for 30 min at 37 °C. Then, 20 μL of supernatant was allowed to react with 80 μL of substrate buffer (2 mM 4-*p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide in 0.05 M sodium citrate buffer, pH 4.5) for 30 min at 37 °C. The reaction was stopped by the addition of 200 μL of stop buffer (0.1 M NaHCO₃, pH 10). The absorbance was measured at 405 nm using an Epoch microplate spectrophotometer (Biotek, Santa Barbara, CA, USA).

The assay for histamine secretion was performed. RBL-2H3 cells (2 × 10⁵ cells/mL) were cultured in 24-well plates for 24 h. The treatment of chemicals and stimulants was performed with the same procedure as described above for β-hexosaminidase release. The released histamine was measured by the OPT spectrofluorometric procedure. Briefly, histamine reacts with OPT to form a fluorophore. In this assay, 1 mL of supernatant, 0.2 mL of 1 N NaOH, and 0.1 mL of 1% OPT were mixed and incubated at room temperature for 5 min. The reaction was stopped by adding 0.1 mL of 3 N HCl. The fluorescence intensity was then measured at excitation and emission wavelengths of 360 nm and 450 nm, respectively, using a Synergy H1 hybrid multi-mode microplate reader (Biotek, Santa Barbara, CA, USA).

2.4. Cell viability assay

The viability of RBL-2H3 cells, RAW264.7 cells and MOLT-4 cells was measured with an MTT assay. Each cell was cultured in 24-well plates for 24 h at 37 °C. Following this, the cells were treated with chemicals diluted to various concentrations in medium. RBL-2H3 cells and RAW264.7 cells were incubated for 24 h at 37 °C and MOLT-4 cells were incubated for 12 h at 37 °C. After treating with chemicals, the supernatant was discarded and the cells were then incubated with MTT solution (5 mg/mL). The supernatant was again discarded, and water-insoluble formazan compounds formed within the cells were dissolved in DMSO. Then, the solution was shifted to 96-well plates and the absorbance was measured at 550 nm using an Epoch microplate spectrophotometer (Biotek, Santa Barbara, CA, USA).

2.5. Nitrite assay

Nitrite levels were determined using the Griess method. RAW264.7 cells (5 × 10⁴ cells/mL) were cultured in 24-well plates for 24 h. The cells were treated with C1 and activated for 24 h with 1 μg/mL of LPS for the measurement of pro-inflammatory effects. Also, the cells were pretreated with C1 and N-iminoethyl-L-lysine (L-NIL) for 1 h and activated for 24 h with 1 μg/mL of LPS for the measurement of anti-inflammatory effects. L-NIL, a selective inhibitor of inducible nitric oxide synthesis (iNOS), was used as an assay positive control. After that, 100 μL of supernatant was allowed to react with 50 μL of the Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in distilled water) for 10 min at room temperature. The absorbance of the mixture was analyzed at 550 nm using an Epoch microplate spectrophotometer (Biotek, Santa Barbara, CA, USA).

2.6. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Total cellular RNA was isolated from MOLT-4 cells using X-zol RNA reagent (PhileKorea, Seoul, Republic of Korea), and the RNA concentration was measured with an Epoch microplate spectrophotometer (Biotek, Santa Barbara, CA, USA). To analyze the gene expression levels of interferon-γ (IFN-γ) and interleukin-4 (IL-4) mRNAs, 500 ng of total RNA was reverse-transcribed to cDNA using ReverTra Ace qPCR RT Master Mix (TOYOBO, Osaka, Japan). The relative gene expression was measured with THUNDERBIRD SYBR qPCR Mix (TOYOBO), and hypoxanthine guanine phosphoribosyltransferase (HPRT), the house-keeping gene, was used for normalization. The primer pairs for INF-γ (sense, 5'-CCAACCTAAGCAAGATCCCA-3' and antisense, 5'-GGGTC-ACCTGACACATTCAA-3'), IL-4 (sense, 5'-CACCTTACAGGAGATCAT-CAAACT-3' and antisense, 5'-TCCTTCTCAGTTGTGTTCTTGG-3') and HPRT (sense, 5'-CCTGGCGTCGTGATTAGTG-3' and antisense, 5'-TGAGGAATAAACACCCCTTCCA-3') were used to analyzed gene expression level. qRT-PCR was performed using a StepOne Plus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA).

2.7. Western blotting

RBL-2H3 cells were collected by centrifugation and lysed in radio-immunoprecipitation assay (RIPA) buffer (Sigma-Aldrich) containing protease and phosphatase inhibitors (Quartett Immunodiagnostika und Biotechnologie Vertriebs GmbH, Berlin, Germany). To quantify total proteins from each lysate, the bicinchoninic acid assay was performed. Subsequently, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with equal protein amounts (30 μg) of samples in a 10% acrylamide gel. Proteins isolated in the acrylamide gel were transferred to polyvinylidene difluoride membranes (Amersham Hybond-P; GE Healthcare, Buckinghamshire, UK). Immediately following the transfer, membranes were submerged into 1% skim milk in Tris

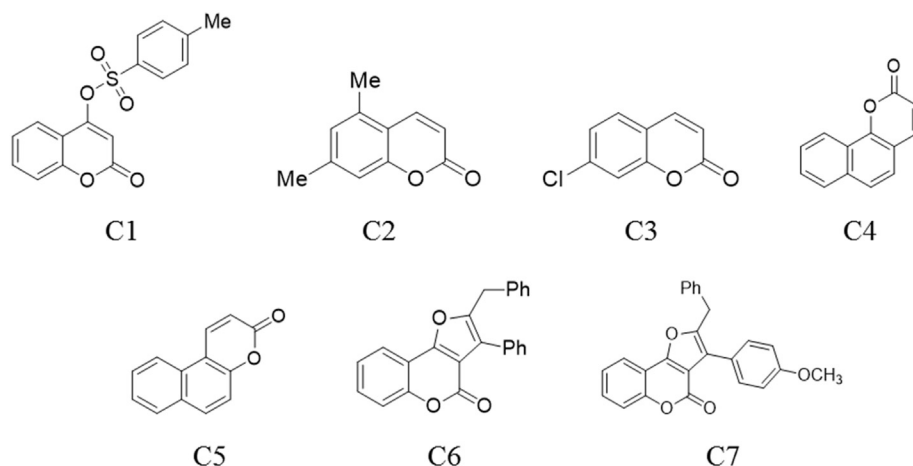


Fig. 1. The structures of coumarin derivatives. C1: 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate. C2: 5,7-dimethyl-2H-chromen-2-one. C3: 7-chloro-2H-chromen-2-one. C4: 2H-benzo[h]chromen-2-one. C5: 3H-benzo[f]chromen-3-one. C6: 2-benzyl-3-phenyl-4H-furo[3,2-c]chromen-4-one. C7: 2-benzyl-3-(4-methoxyphenyl)-4H-furo[3,2-c]chromen-4-one.

buffered saline (TBS) containing 0.05% Tween 20 (TBS-T) for 1 h. The membranes were then incubated with primary antibodies at 4 °C for 16 h. After three washes in TBS-T, the membranes were incubated with horseradish peroxidase-linked secondary antibodies at room temperature for 1 h. Next, the membranes were washed in TBS-T again and the visualization of the protein bands was accomplished using WesternBright Peroxide chemiluminescent detection reagent (Advansta, Menlo Park, CA, USA). Finally, the membranes were incubated in stripping solution (10% SDS, 0.5 M Tris-Cl, 0.8% β -mercaptoethanol) for 10 min at 60 °C and reused.

2.8. Statistical analysis

All the data in this study have been presented as mean $\pm$ standard deviation (SD) of more than three experiments. Statistical differences among groups were assessed by one-way ANOVA followed by Bonferroni's test using SPSS (v.21, for Windows). $^{**}P < 0.05$ was considered significant.

3. Results

3.1. Inhibitory effects of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate on degranulation in RBL-2H3 cells

In this study, we first performed the β -hexosaminidase assay to determine the effects of coumarin derivatives on degranulation in RBL-2H3 cells (Fig. 1). RBL-2H3 cells were respectively treated with seven coumarin derivatives at a concentration of 25 μ M and then stimulated with PMA and A23187 (Fig. 2A). We observed that 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate reduced the release of β -hexosaminidase by approximately 50% (Fig. 2A), while the other derivatives did not affected the cells. The viability of RBL-2H3 cells was measured with an MTT assay (Fig. 2B). Interestingly, we observed that the viability of RBL-2H3 cells was not affected by coumarin derivatives at a concentration of 25 μ M. Thus, these results indicated that coumarin derivatives did not have cytotoxic effects on this cell line and that only 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate inhibited degranulation without exhibiting cytotoxicity (Fig. 2B). Subsequently, we investigated the dose-dependent effects of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate on degranulation and cytotoxicity in RBL-2H3 cells (Fig. 3A). Different concentrations of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate significantly inhibited degranulation in a concentration-dependent manner compared with that in the untreated controls

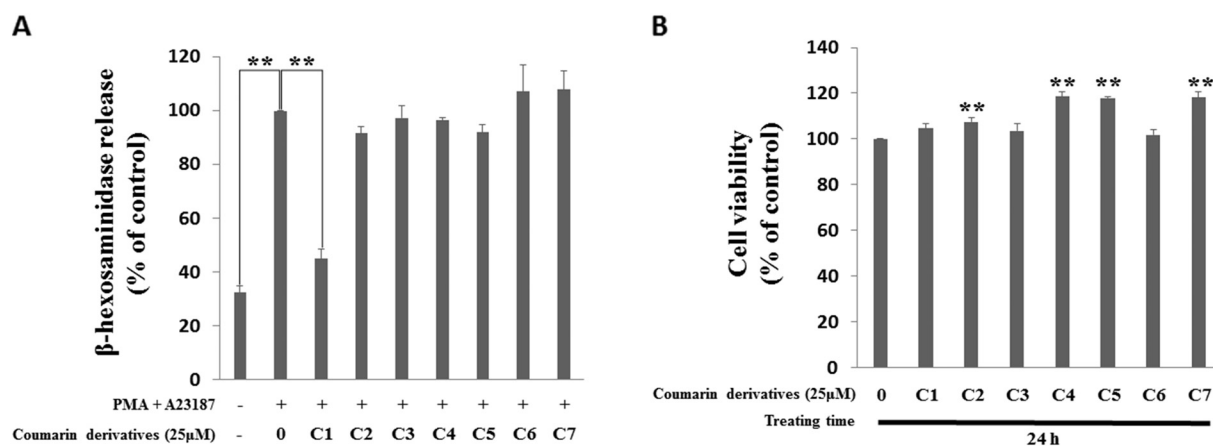


Fig. 2. Effect of coumarin derivatives on β -hexosaminidase release and cell viability in RBL-2H3 cells. A: β -hexosaminidase release in RBL-2H3 cells that were treated with 25 μ M of coumarin derivatives for 1 h and stimulated with PMA (50 nM) and A23187 (1 μ M). B: Cell viability of RBL-2H3 cells treated with coumarin derivatives for 24 h. Results are presented as the mean $\pm$ standard deviation (SD) of three experiments. $^{**}P < 0.05$. C1: 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate. C2: 5,7-dimethyl-2H-chromen-2-one. C3: 7-chloro-2H-chromen-2-one. C4: 2H-benzo[h]chromen-2-one. C5: 3H-benzo[f]chromen-3-one. C6: 2-benzyl-3-phenyl-4H-furo[3,2-c]chromen-4-one. C7: 2-benzyl-3-(4-methoxyphenyl)-4H-furo[3,2-c]chromen-4-one.

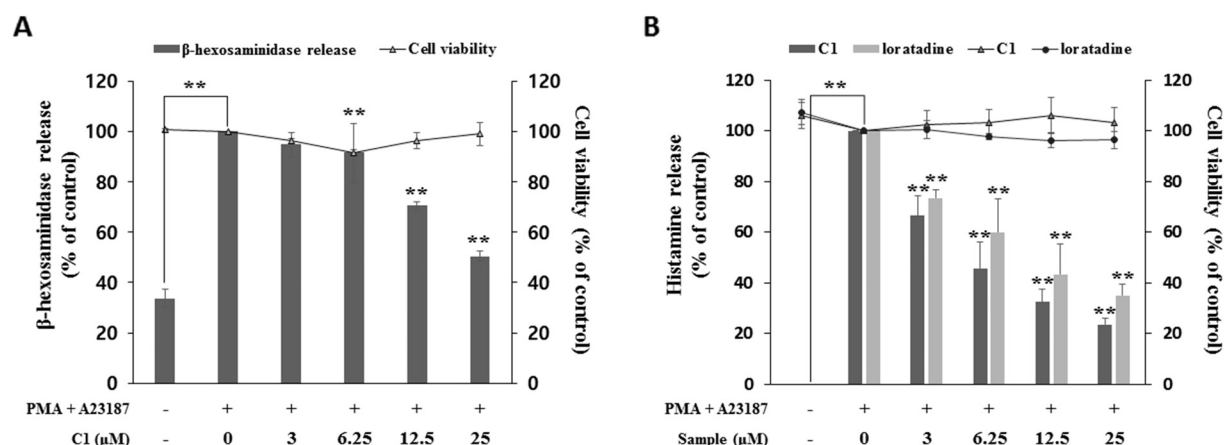


Fig. 3. Effects of C1, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate, on cell viability, β -hexosaminidase release and histamine release in RBL-2H3 cells. **A:** β -hexosaminidase release in RBL-2H3 cells that were treated with 3, 6.25, 12.5, or 25 μ M of C1 for 1 h and stimulated with PMA (50 nM) and A23187 (1 μ M). **B:** Histamine release in RBL-2H3 cells that were treated with same concentration gradient with C1 and loratadine for 1 h and stimulated with PMA and A23187. Cell viability of RBL-2H3 cells treated with samples followed by stimulation. Loratadine was used as a positive control. Results are presented as the mean $\pm$ SD of three experiments. **P < 0.05 compared with stimulated RBL-2H3 cells.

(Fig. 3A). It is worth noting that at the concentrations used in this study, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate did not demonstrate any cytotoxic effects (Fig. 3A).

In addition, we measured the histamine release by comparing 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate with loratadine, a selective H1 receptor antagonist [18]. As a result, it was confirmed that both 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate and loratadine inhibit histamine release in a dose-dependent manner without cytotoxicity (Fig. 3B).

3.2. Effects of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate in RAW264.7 cells and MOLT-4 cells

We measured the effects of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate in macrophages and T cells (Fig. 4). RAW264.7 cells and MOLT-4 cells as a representative cell line were used, respectively [19,20]. We first performed the nitrite assay to determine the effects of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate on pro-inflammatory or anti-inflammatory effects in RAW264.7 cells. RAW264.7 cells

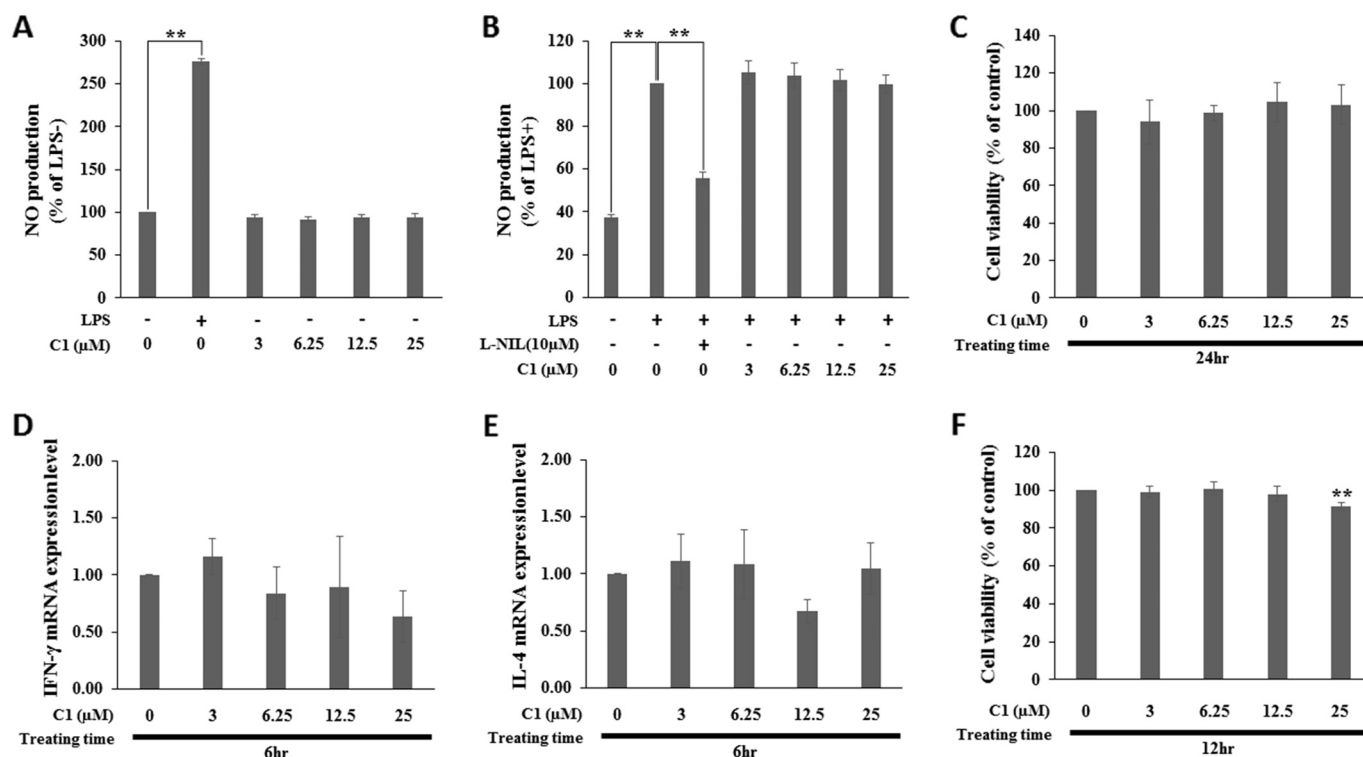


Fig. 4. Effects of C1, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate, on LPS-induced NO production in RAW264.7 cells and on cytokine expression in MOLT-4 cells. **A–C:** LPS-induced NO production and cell viability in RAW264.7 cells treated with indicated concentrations of C1. **A:** Cells were treated for 24 h with C1 and activated for 24 h with 1 μ g/mL of LPS for the measurement of pro-inflammatory effects. **B:** Cells were pretreated for 1 h with C1 and activated for 24 h with 1 μ g/mL of LPS for the measurement of anti-inflammatory effects. **C:** Cell viability of RAW264.7 cells treated with C1 for 24 h. **D–F:** Cytokine expression level and cell viability in MOLT-4 cells treated with indicated concentrations of C1. **D:** mRNA expression level of IFN- γ . **E:** mRNA expression level of IL-4. **F:** Cell viability of MOLT-4 cells treated with C1 for 12 h. Data are expressed as the percentage of activity relative to the control. Results are presented as the mean $\pm$ SD of three experiments. **P < 0.05 compared with stimulated RBL-2H3 cells.

were treated with a serial diluted 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate and stimulated with LPS. As a result, RAW264.7 cells stimulated by LPS increased NO production, however, we could not observe any effect on 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate in RAW264.7 cells (Figs. 4A–B). We next investigated cytokines related with T cells in MOLT-4 cells after being treated with 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate by qRT-PCR. However, no significant changes were observed in mRNA expression levels of IFN- γ and IL-4, and this result was not statistically significant (Figs. 4D–E). In addition, no cytotoxicity was observed in the 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate treated cells (Figs. 4C, F).

3.3. Effects of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate on the MAPK activation pathway in RBL-2H3 cells

We determined that 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate inhibited the degranulation of RBL-2H3 cells that had been stimulated with PMA along with A23187. Therefore, to evaluate the mechanisms of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate on pro-inflammatory cytokine expression, we next examined the effect of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate on the activation of MAPKs in RBL-2H3 cells (Fig. 5). Treatment with PMA and A23187 induced the activation of all three types of MAPKs namely p38, JNK, and ERK in RBL-2H3 cells. In the present study, RBL-2H3 cells were treated with various concentrations of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate and stimulated with PMA and A23187. Three major MAPKs (ERKs, JNKs, p38) were activated by PMA and A23187. However, interestingly, only the phosphorylation of ERK1/2 was suppressed in cells treated with 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate, in a dose-dependent manner (Fig. 5A). Meanwhile, phosphorylation of p38 and JNK were not inhibited compared with that in untreated controls. In addition, we demonstrated that 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate exhibited inhibitory effects upstream of the MAPK pathway. When RBL-2H3 cells were stimulated with PMA and A23187, we observed that MKKs were activated. However, we showed that 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate did not inhibit the phosphorylation of MKKs in a dose-dependent manner (Fig. 5B). These results indicated that 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate suppressed the phosphorylation of ERK1/2 pathway and 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate and at least, in part, inhibited mast cell activation *via* regulation of the MAPK pathway.

4. Discussion

The prevalence of allergic disorders such as allergic rhinitis, asthma, and atopic dermatitis pose serious health concerns worldwide [21]. However, the causes of allergic disorders are unclear and the current therapeutic strategies in place to combat them are insufficient. Therefore, establishing a precise understanding of their pathophysiologies is critical for the development of new therapeutic agents against allergic diseases.

Mast cells are granulocytes containing preformed mediators such as histamine, heparin, and proteases and are widely distributed in the connective tissue [22]. Mast cell degranulation is induced by diverse stimuli and the released mediators play important roles in triggering allergic inflammation [23]. The mast cell activation is regulated by the aggregation of IgE receptors (Fc ϵ RI) on mast cells [3,4,24]. Also, the addition of PMA and A23187 induces biochemical and ionic events leading to the activation of PKC and the increase in intracellular calcium influx [12,13,25]. The increase in intracellular calcium levels subsequently induces degranulation leading to the secretion of inflammatory mediators such as histamine, serotonin, heparin, proteases, and cytokines [6]. Overall, various biochemical reactions involving mast cells result in allergic symptoms [26].

In this study, we examined the effects of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate on mast cell degranulation by measuring the release of β -hexosaminidase. We first performed the β -hexosaminidase assay to measure the effects of coumarin derivatives on degranulation in RBL-2H3 cells. Among the derivatives used, the degranulation inhibitory effect of C1, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate, was the highest at a concentration of 25 μ M (Fig. 2A). Also, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate inhibited degranulation in a dose-dependent manner in RBL-2H3 cells (Fig. 3A). Notably, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate demonstrated inhibitory effects on degranulation without inducing cytotoxicity, as compared to controls (Fig. 3A). In addition, we performed the histamine release assay to verify that RBL-2H3 cells are an appropriate pathophysiological model for this study (Fig. 3B). Loratadine was used as a positive control in this assay. In previous studies, loratadine has been proven effective as a selective H1 receptor antagonist that inhibits the degranulation in mast cells [27,28]. As a result, both 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate and loratadine effectively inhibited histamine release in a dose-dependent manner in RBL-2H3 cells (Fig. 3B). Therefore, it has been experimentally demonstrated that

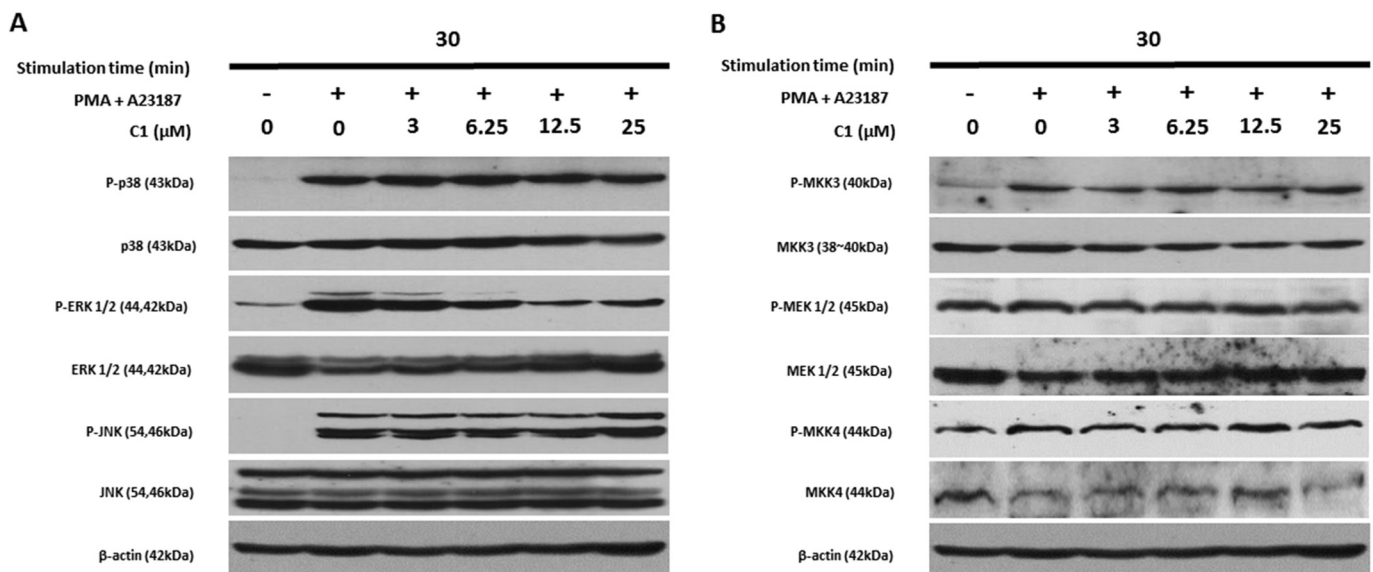


Fig. 5. Effects of C1, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate, on the phosphorylation of MAPKs in RBL-2H3 cells. A: Immunoblot analysis of MAPK phosphorylation. B: Immunoblot analysis of MAPKK phosphorylation.

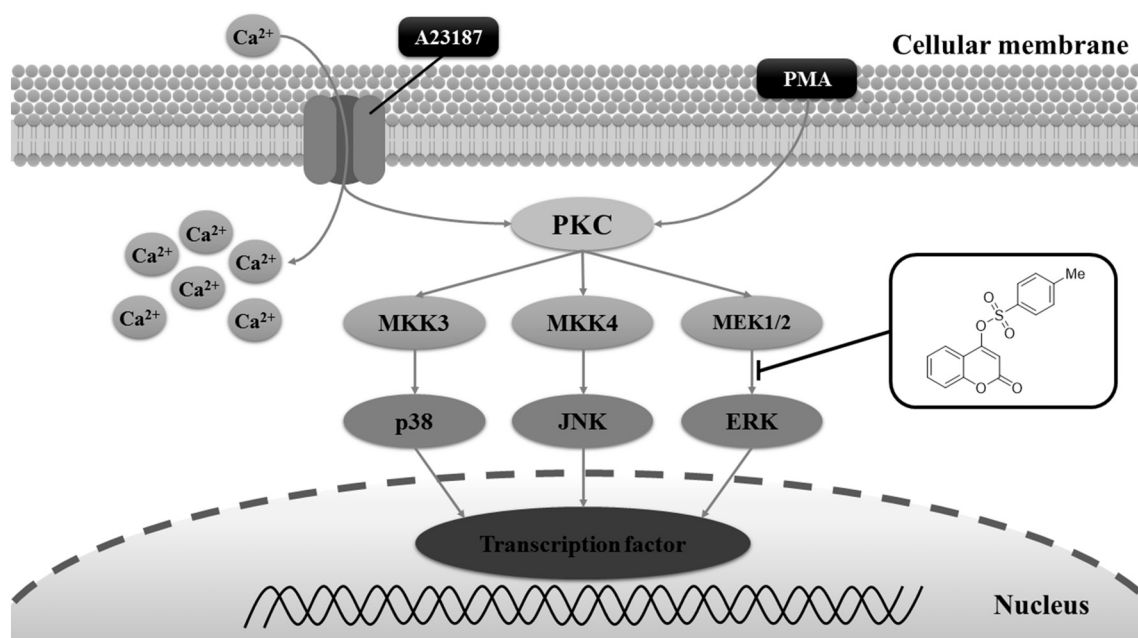


Fig. 6. Schematic diagram of MAPK pathways suppressed by C1, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate, in RBL-2H3 cells.

RBL-2H3 cells can be used as a degranulation model.

We also treated 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate with other immune cells, RAW264.7 cells and MOLT-4 cells, but could not observe any significant effect (Fig. 4). Thus, we concluded that 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate has a specific effect on RBL-2H3 cells among various immune cells.

The MAPK cascade is a key signaling pathway involved in immune reactions [29,30]. Because of their critical roles in mediating immune signaling, the MAPK cascade including p38, ERK, and JNK pathways represents appropriate targets to study inflammatory responses [29,31,32]. The addition of PMA and A23187 to mast cells induces the phosphorylation of Syn and MAPKs as well as the increase in calcium influx. In addition, the expression of cytokines is activated by stimulation [5,33]. To further examine the effects of chemical treatment on the signaling cascade, the inhibitory activities of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate against MAPK pathways were assessed. Our findings established that 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate significantly reduced the phosphorylation of the ERK pathway (Fig. 5).

We investigated whether the coumarin derivative, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate, contributes to the regulation of responses elicited by PMA and A23187 stimulation in RBL-2H3 cells. In conclusion, this study illustrates that the inhibitory effect of 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate on RBL-2H3 degranulation occurs via inhibition of ERK phosphorylation (Fig. 6). Therefore, 2-oxo-2H-chromen-4-yl 4-methylbenzenesulfonate can be used as a therapeutic agent in allergic inflammatory diseases.

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