Kim *et al. Applied Biological Chemistry* (2025) 68:1
https://doi.org/10.1186/s13765-025-00979-x

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE Open Access

3-bromo-4-(2-hydroxyethyl)-7-methoxy-2H-chromen-2-one inhibits allergic reactions in IgE-mediated RBL-2H3 cells and a passive cutaneous anaphylaxis mouse model

Moonsu Kim[1], Huiji Kim[1], Sun Young Moon[1], Seong-Ah Shin[1], Minji Kim[1], Seyeon Choi[1], Sugeun Gwon[1], Jun Hyuck Lee[2,3], Hyun Ho Park[4] and Chang Sup Lee[1*]

Abstract

Allergic reactions occur when the immune system overreacts to generally harmless substances, leading to both acute and chronic diseases, which can be fatal. Mast cells are critical mediators of allergic reactions as they bind allergens and trigger the release of inflammatory mediators. In this study, we investigated the anti-allergic effects of the coumarin derivative 3-bromo-4-(2-hydroxyethyl)-7-methoxy-2H-chromen-2-one in rat basophilic leukemia (RBL)-2H3 cells sensitized to dinitrophenyl (DNP)-immunoglobulin E (IgE) and human serum albumin (HSA). Our results demonstrated that 3-bromo-4-(2-hydroxyethyl)-7-methoxy-2H-chromen-2-one effectively reduces the release of β-hexosaminidase and histamine, inhibiting mast cell degranulation. Additionally, 3-bromo-4-(2-hydroxyethyl)-7-methoxy-2H-chromen-2-one suppressed the production of allergy-related pro-inflammatory cytokines (IL-4, IL-13, and TNF-α) and inhibited key signaling pathways, including MAPK, AKT, and NF-κB. Furthermore, in a passive cutaneous anaphylaxis (PCA) mouse model, 3-bromo-4-(2-hydroxyethyl)-7-methoxy-2H-chromen-2-one reduced ear edema and Evans blue infiltration, further confirming its anti-allergic effects. Collectively, these findings suggest that 3-bromo-4-(2-hydroxyethyl)-7-methoxy-2H-chromen-2-one is a promising candidate for the development of anti-allergic therapeutics.

Keywords Allergy, Mast cells, 3-Bromo-4-(2-Hydroxyethyl)-7-Methoxy-2H-Chromen-2-One, Anti-Allergic therapeutics, AKT pathway, MAPK pathway

Introduction

Allergies occur when the immune system identifies a normally harmless substance, including pollen, food, and animal hair, as a threat and overreacts to it [1–3]. Allergic reactions lead to chronic conditions such as asthma, allergic rhinitis, and atopic dermatitis, as well as acute conditions like anaphylaxis, which can be life-threatening in severe cases [4, 5].

Allergens trigger a response primarily mediated by mast cells [6–8]. Mast cells play a critical role in the immune system and are predominantly found near the blood vessels, skin, and epithelial surfaces. While mast cells regulate immune and inflammatory responses, their excessive activation leads to allergic reactions [9, 10].

*Correspondence:
Chang Sup Lee
changsup@gnu.ac.kr
[1] Collage of Pharmacy and Research Institute of Pharmaceutical Sciences, Gyeongsang National University, Jinju 52828, Republic of Korea
[2] Research Unit of Cryogenic Novel Material, Korea Polar Research Institute, Incheon 21990, Republic of Korea
[3] Department of Polar Sciences, University of Science and Technology, Incheon 21990, Republic of Korea
[4] College of Pharmacy, Chung-Ang University, Seoul 06974, Republic of Korea

When an allergen enters the body, antigen-presenting cells, such as dendritic cells or macrophages, present the allergen to naïve T cells. These T cells then proliferate and interact with the major histocompatibility complex (MHC) class II molecules on B cells to produce IL-4 and IL-13. This process induces a class switch recombination; it is the process by which B cells switch from IgM to IgE expression, and is a critical step in the allergic response. The secreted IgE binds to the allergen and attaches to the FcεRI receptors on mast cells. Allergen-IgE binding triggers the activation of a cascade of intracellular signaling pathways (namely, the mitogen-activated protein kinase (MAPK) and AKT pathways) within the mast cell [7, 11]. The MAPK pathway comprises three major classes: extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 [12]. These pathways are key regulators of inflammation, cell proliferation, and differentiation [13]. The AKT pathway contributes to allergic reactions by activating the nuclear factor-kappa B (NF-κB) pathway, producing pro-inflammatory cytokines [14, 15]. These signaling cascades collectively produce cytokines such as IL-4, IL-13, and TNF-α, which initiate and amplify the allergic response [16–19]. The regulation of these pathways is crucial in inhibiting the onset of allergic reactions and alleviating symptoms associated with allergic diseases.

Allergic diseases are often caused by immune dysfunctions. To manage these conditions, patients typically use antihistamines or corticosteroids to target the affected tissue [20, 21]. However, these drugs are associated with side effects, including lethargy, skin atrophy, erythema, and, in severe cases, cardiotoxicity [22, 23]. Therefore, there is an urgent need to develop novel anti-allergic modulators that exert minimal side effects. Coumarin compounds exhibit significant anti-cancer, anti-inflammatory, anti-oxidant, and anti-allergic properties, and are generally associated with relatively few side effects [24–26]. However, the antiallergic effects of the coumarin derivative, 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one, remain unknown. Therefore, in this study, we aimed to determine the anti-allergic effects of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one.

Material & methods

Materials

(Minimum essential medium) alpha (α-MEM) and fetal bovine serum (FBS) were purchased from Corning (NY, USA). Penicillin–streptomycin-glutamine (PSQ) was purchased from Gibco (Waltham, MA, USA). Reagents including 3-(4, 5-dimethylthiazol-2-yl)−2, 5-diphe nyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), anti-dinitrophenyl antibody (anti-DNP-IgE), dinitrophenyl-human serum albumin (DNP-HSA), ketotifen, toluidine blue, 4-nitrophenyl N-acetyl-β-D-glucosaminide, histamine, and *o*-phthalaldehyde were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's phosphate-buffered saline (DPBS) was purchased from WELGENE Inc. (Gyeongsan, Korea). Primers for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) were purchased from Macrogen (Seoul, Korea). Primary antibodies (p-ERK, p-JNK, p-p38, p-AKT, p-p65 and β-actin) were obtained from Cell Signaling Technology (Danvers, MA, USA). Secondary antibodies (goat anti-mouse and goat anti-rabbit IgG) were purchased from SeraCare (Gaith ersburg, MD, USA).

Cell line

Rat Basophilic Leukemia (RBL)−2H3 cells were purchased from Korean Cell Line Bank (Seoul, Korea). RBL-2H3 cells were cultured in α-MEM supplemented with 1% PSQ and 10% fetal bovine serum (FBS) at 37 ℃ and 5% CO_2.

Animals

Male BALB/c mice (7 weeks old) were purchased from Koatech (Jinju, Korea). All mice were acclimated to their surroundings 1 week prior to experimentation. The room temperature and humidity were maintained at 25 ± 2 ℃ and 55 ± 5%, respectively. The experimental protocol was approved by the Gyeongsang National University Institute of Animal Care and Use Committee (2022–064).

MTT assay

Cell viability was measured using the MTT assay. Briefly, RBL-2H3 cells were seeded in 96-well plates and allowed to stabilize for 17 h. The cells were incubated with various concentrations (25 μM, 50 μM, and 100 μM) of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one and then treated with MTT (3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide, dissolved in α-MEM to 1 mg/ml) for 2 h. Subsequently, the MTT solution was removed and DMSO was added to dissolve the formazan crystal. The absorbance was measured at 570 nm using a Varioskan LUX microplate reader (Thermo Fisher Scientific, Waltham, USA).

Beta-hexosaminidase assay

RBL-2H3 cells (5×10^5 cells/well) were seeded into a 12-well plate and treated with anti- dinitrophenyl (DNP)-IgE (0.2 μg/ml) for 17 h. Following incubation, the cells were washed three times with PBS and treated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one for 1 h. Then, DNP-HSA was

added to the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one or ketotifen-treated wells, and the cells were incubated for a further 6 h. The supernatant was transferred into a 96-well plate and 1 mM β-hexosaminidase substrate (4-nitrophenyl N-acetyl-β-D-glucosaminide dissolved in 0.2 M citrate buffer pH 4.5) was added to each well. After incubation at 37 ℃ for 1 h 50 min, a stop solution (0.1 M Na_2CO_3/$NaHCO_3$ pH 10.0) was added to each well. Finally, absorbance was measured at 405 nm using a microplate reader (Varioskan LUX microplate reader).

Histamine assay

RBL-2H3 cells (5×10^5 cells/well) were seeded into a 12-well plate and treated with anti-DNP-IgE (0.2 μg/ml) in each well for 17 h. After incubation, the cells were washed three times with PBS and treated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one for 1 h. Then, DNP-HSA was added to the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one or ketotifen-treated wells for 6 h. The supernatant was separated into Eppendorf tubes, and 0.1 N HCl and 60% percholic acid was added to the supernatant. After 20 min of centrifugation at 12,000 × g, 5 N NaOH, 5 M NaCl, and n-butanol were added to the solution and the mixture was vortexed. The organic phase was collected, mixed with 0.1 N HCl and n-heptane, and vortexed. Histamine in the aqueous phase was added to a 96-well plate and assayed using o-phthalaldehyde (OPA). The fluorescence intensity (Ex: 360 nm, Em: 440 nm) was measured using a fluorescent plate reader (Varioskan LUX microplate reader).

Toluidine blue staining

RBL-2H3 cells (5×10^5 cells/well) were seeded into 12-well plate and sensitized with anti-DNP-IgE (0.2 μg/ml) for 17 h to visualize the degranulation of mast cells. After IgE stimulation, the cells were washed three times with PBS and treated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one for 1 h. DNP-HSA was then added to 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one- and ketotifen-treated cells for 6 h. After the supernatant was removed, the cells were washed three times with PBS. Cells were fixed with absolute methanol for 30 min. After fixation, cells were washed three times with PBS and stained with toluidine blue (0.1% w/v in 1% NaCl solution, pH 2.5) for 20 min. Cells were rewashed three times, and images of the stained cells were captured using an inverted microscope (IMT i-Solution, Inc, 400X).

qRT-PCR assay

Total RNA was extracted using TRIzol solution. RBL-2H3 cells (5×10^5 cells/well) were seeded into a 12-well plate and incubated with anti-DNP-IgE (0.2 μg/ml) for 17 h. After incubation, the cells were washed three times with PBS and treated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one for 1 h, and DNP-HSA for 1 h. After treatment, the cells were washed 3 times with cold PBS and TRIzol solution was added and mixed via pipetting. After incubation for 5 min, chloroform as added, and the mixture was vortexed and incubated again for 2–3 min. Next, the cells were centrifuged for 15 min at 12,000 × g and 4 ℃. The aqueous phase was transferred to a new Eppendorf tube and isopropanol as added.After incubation for 10 min, cells were pelleted at 12,000 × g and 4 ℃ for 10 min. After centrifugation, the supernatant was discarded, and the cells were resuspended in 75% ethanol. After brief vortexing, samples were centrifuged for 5 min at 12,000 × g and 4 ℃, and the supernatant was discarded. After air-drying for 5–10 min, the samples were resuspended in RNase-free water. To determine the RNA quantity, RNA samples were quantified using a NanoDrop (Thermo Fisher Scientific). To synthesize cDNA, samples were prepared using the amfiRivert cDNA Synthesis Platinum Master Mix (GenDEPOT). RNA denaturation and synthesis were performed using a Thermocycler (SimpliAmp Thermal Cycler (Applied Biosystems). cDNA samples were quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific). qRT-PCR analysis was performed using a PowerUp™ SYBR™ Green Master Mix kit (Applied biosystems), according to the manufacturer's instructions. The StepOnePlus Real-time PCR System (Applied biosystems). Relative mRNA expression was calculated using the ΔΔCt method.

Western blot

RBL-2H3 cells (2.7×10^6 cells/well) were seeded into a 60 mm dish and sensitized with anti-DNP-IgE (0.2 μg/ml) for 17 h. After sensitization, cells were treated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one for 1 h. Cells were treated with DNP-HSA for 30 min. The cells were washed three times with cold PBS and collected in Eppendorf tubes using a cell scraper. To isolate total protein, cells were lysed with RIPA buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100), protease inhibitor (1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mg/ml aprotinin, leupeptin, and pepstatin), and phosphatase inhibitor (30 mM NaF, 1.5 mM Na_3VO_4). Proteins were separated by SDS-PAGE and transferred onto nitrocellulose (NC) membranes. After blocking the membrane with 5% skim milk

in Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBS-T) for 30 min, the membrane was incubated with primary antibodies overnight at room temperature. The membranes were washed with TBS-T every 5 min for 30 min. The membrane was then incubated with secondary antibodies for 1 h. After incubation, the membranes were washed with TBS-T every 5 min for 30 min. Finally, the membrane was incubated with an ECL solution to detect signal intensity using a Chemidoc (iBright™ CL1500, Invitrogen).

Immunocytochemistry

RBL-2H3 cells (5×10^5 cells/well) were seeded into a 12-well plate with cover glass and sensitized with anti-DNP-IgE (0.2 μg/ml) for 17 h. After incubation, the cells were washed three times with PBS and treated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one for 1 h. The cells were washed again with PBS and treated with DNP-HSA for 30 min and 1 h. After incubation, the coverslips were transferred onto new 12-well plates and washed with PBS. The cells were treated with 4% paraformaldehyde for 20 min without light. Next, the cells were permeabilized with permeabilizing solution (0.2% Triton X-100, 0.1% Citrate in PBS) for 5 min, washed with PBS, and blocked with blocking solution (2% BSA in PBS) for 30 min with shaking. After blocking, the cells were treated with the primary antibody for 2 h at room temperature. After 2 h, the cells were washed thrice with PBS and incubated with a secondary antibody for 1 h. After incubation, the cells were washed with PBS and treated with Hoechst solution for 2 min in the dark. Cells were washed with PBS every 15 min for 5 min, mounted on glass slides, and fluorescence intensity was measured using a fluorescence microscope (Zeiss, Germany).

Passive cutaneous anaphylaxis assay

To confirm the anti-allergic response of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one in vivo, passive cutaneous anaphylaxis (PCA) was performed. BALB/c mice (male, 8 weeks old) were intradermally injected with anti-DNP-IgE in their ears. After 24 h, 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one was intraperitoneally injected (PBS with control group), and 1 h later, mice were intravenously treated with 2% Evans blue containing DNP-HSA. After 30 min, the mice were sacrificed and ear thickness was measured using Vernier Calipers. Ear tissues were punched for Evans blue extraction. Punched ear tissues were soaked in 1 M KOH and a mixture of 0.6 N phosphoric acid and acetone solution, and incubated at 37 °C overnight. The supernatants were transferred to a 96-well plate and the absorbance was measured at 620 nm using a microplate reader (Varioskan LUX microplate reader).

Statistical analysis

Data are presented as mean ± standard deviation (SD) of three independent replicates. Differences between groups were analyzed using the Student's t-test. Statistical significance was set at $p<0.05$.

Results

3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one is not cytotoxic to RBL-2H3 cells

Prior to evaluating the anti-allergic effects of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one (Fig. 1A), we assessed its potential cytotoxicity using the MTT assay. The assay was performed with various concentrations of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one (25 μM, 50 μM, 70 μM, and 100 μM).

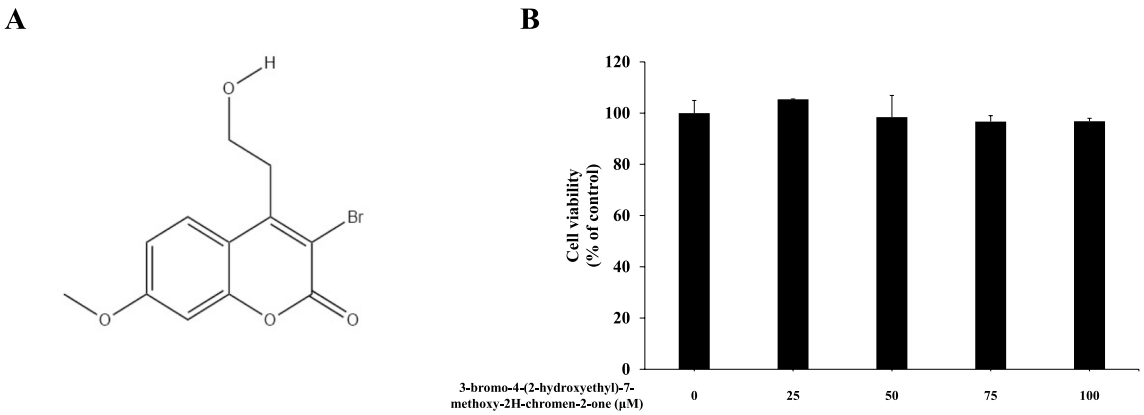

Fig. 1 Effects of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one on RBL-2H3 viability. **a** Chemical structure of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one. **b** The cells were treated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one (0, 25, 50, 75, and 100 μM) for 7 h, and cell viability was measured using MTT assay. The data are presented as the means of ± standard deviation (SD). The experiments were performed in triplicate, and representative data are presented

As shown in Fig. 1B, cytotoxicity was not observed, regardless of dose. These results indicate that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one does not induce cytotoxic effects, even at concentrations as high as 100 μM.

3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one inhibits DNP-IgE/human serum albumin(HSA)-induced degranulation in RBL-2H3

Upon allergen exposure, IgE-allergen complexes bind to FcεRI receptors on mast cells, triggering degranulation and the release of various mediators, such as β-hexosaminidase, histamine, pro-inflammatory cytokines, and other inflammatory molecules, stored in their granules [27, 28]. Therefore, we conducted β-hexosaminidase and histamine release assays to evaluate the effect of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one on DNP-IgE/HSA-induced degranulation in RBL-2H3 cells. Additionally, a toluidine blue assay, which stains mast cell granules, was conducted to visualize degranulation and further confirm the anti-allergic effect of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one [29]. As shown in Fig. 2A, degranulation in the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one treated group was lower than that in the DNP-IgE/HSA-treated group. Moreover, β-hexosaminidase and histamine release in the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one group were significantly lower than that in the DNP-IgE/HSA group (Fig. 2B and 2C). These findings indicate that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one strongly inhibits β-hexosaminidase and histamine degranulation.

3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chrome n-2-one suppresses mRNA pro-inflammatory cytokine expressions in DNP-IgE/HSA-induced RBL-2H3

During the allergic response, mast cells secrete inflammatory cytokines that intensify inflammation. Notably, the mRNA expression levels of cytokines such as IL-4, IL-13, and TNF-α are significantly increased [30–32]. Therefore, we evaluated the effect of

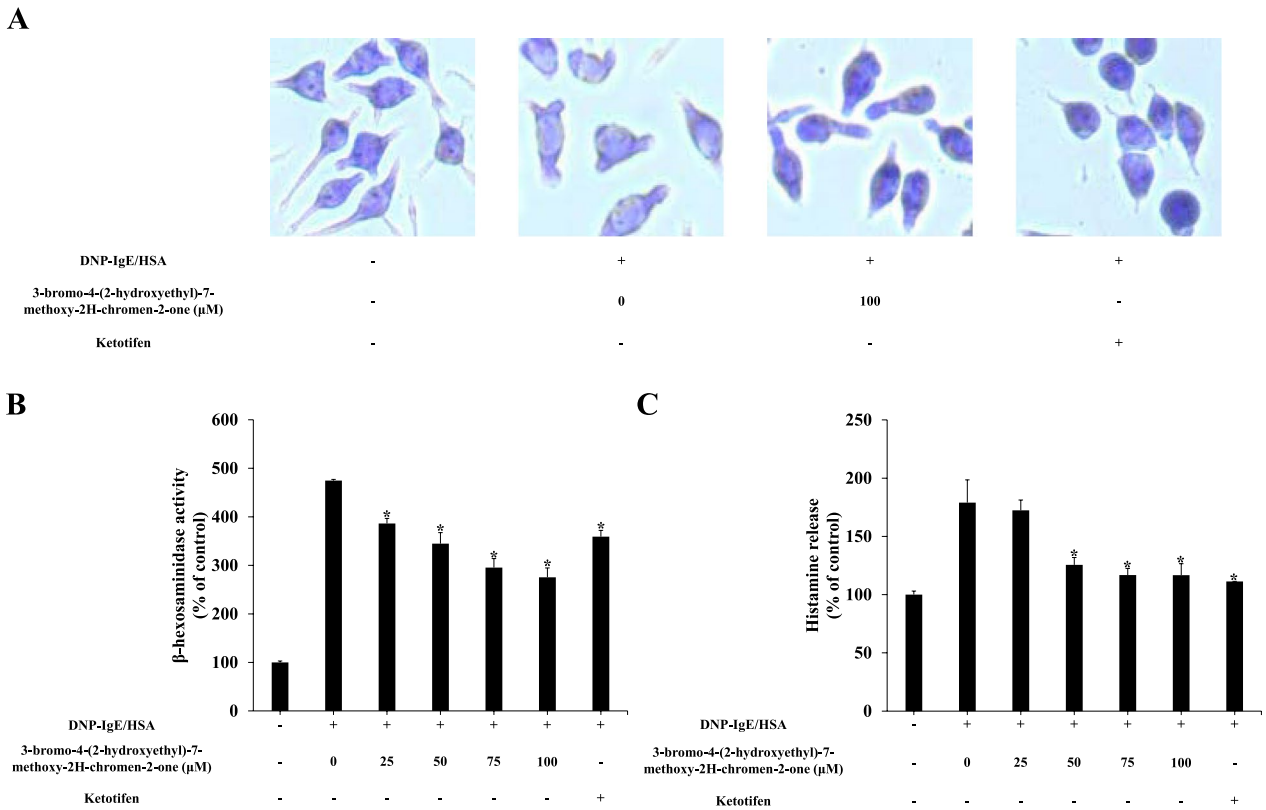

Fig. 2 Effects of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one in DNP-IgE/HSA-induced degranulation in RBL-2H3 RBL-2H3 cells were seeded with anti-DNP/IgE (0.2 μg/mL) and incubated for 17 h. The cells were pretreated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one (0, 25, 50, 75, and 100 μM) or ketotifen, followed by DNP-HSA (0.4 μg/mL) treatment for 6 h. (**a**) Toluidine blue staining of RBL-2H3 cells. (**b**, **c**) β-hexosaminidase (b) and histamine release (c). Data are presented as means of ± standard deviation (SD). Three independent experiments were performed and representative data are presented. *$p < 0.05$

3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one on the expression of pro-inflammatory cytokines. As shown in Fig. 3A–C, the DNP-IgE/HSA-treated group exhibited elevated levels of allergy-related pro-inflammatory cytokines compared to the control group, whereas the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one-treated group demonstrated a reduction in these cytokines relative to the DNP-IgE/HSA-treated group. These findings suggest that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one exerts anti-allergic effects by inhibiting the expression of allergic-inflammatory cytokines.

3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one inhibits the phosphorylation of the MAPK and AKT pathways in DNP-IgE/HSA-induced RBL-2H3 cells

MAPK signaling pathways, including ERK, JNK, and p38 MAPKs, are activated by external stimuli such as allergens. In mast cells and basophils, MAPK activation is triggered by IgE receptor (FcεRI) cross-linking upon allergen exposure. Therefore, inhibition of MAPK activation is crucial for preventing allergic reactions [13, 33, 34]. Accordingly, we investigated whether 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one affects the MAPK pathway. As shown in Fig. 4A, MAPK phosphorylation in the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one-treated group was lower than that in the DNP-IgE/HSA group. In mast cells and basophils, AKT phosphorylation is triggered by the cross-linking of FcεRI upon allergen exposure, initiating downstream signaling cascades [35, 36]. Phosphorylated AKT promotes the release of granules containing histamine and other inflammatory mediators, while enhancing the production of cytokines such as IL-4 and IL-13, which intensify the allergic response [37, 38]. Accordingly, we investigated whether 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one decreases AKT phosphorylation, which was elevated by DNP-IgE/HSA exposure. 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one inhibited AKT phosphorylation (Fig. 4B). These

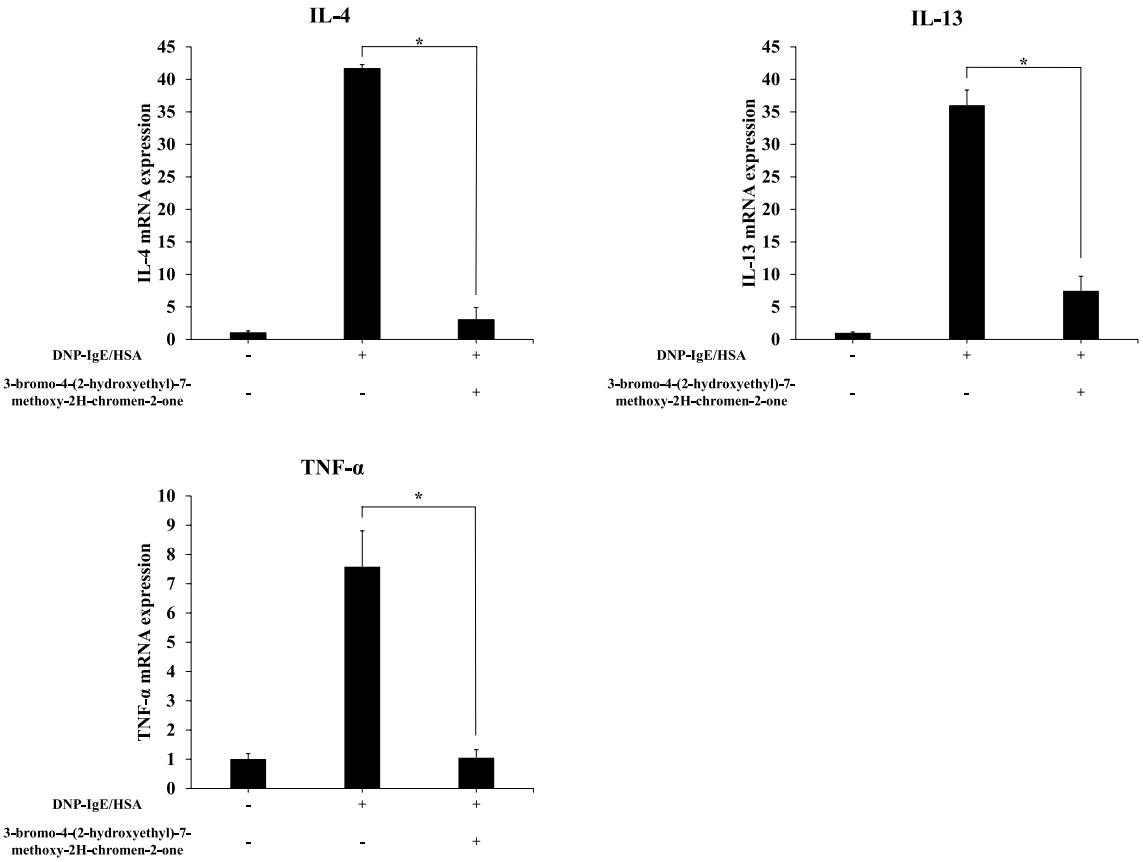

Fig. 3 Effects of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one on the mRNA expression of pro-inflammatory cytokines in DNP-IgE/HSA-induced RBL-2H3 RBL-2H3 cells were seeded with anti-DNP/IgE (0.2 μg/mL) and incubated for 17 h. The cells were pretreated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one (100 μM), followed by treatment with DNP-HSA (0.4 μg/mL) for 1 h. (**a**) IL-4, (**b**) IL-13, and (**c**) TNF-α mRNA expression measured using qRT-PCR. *$p < 0.05$. Data are presented as mean ± standard deviation (SD). The experiments were performed in triplicate and representative data are presented.

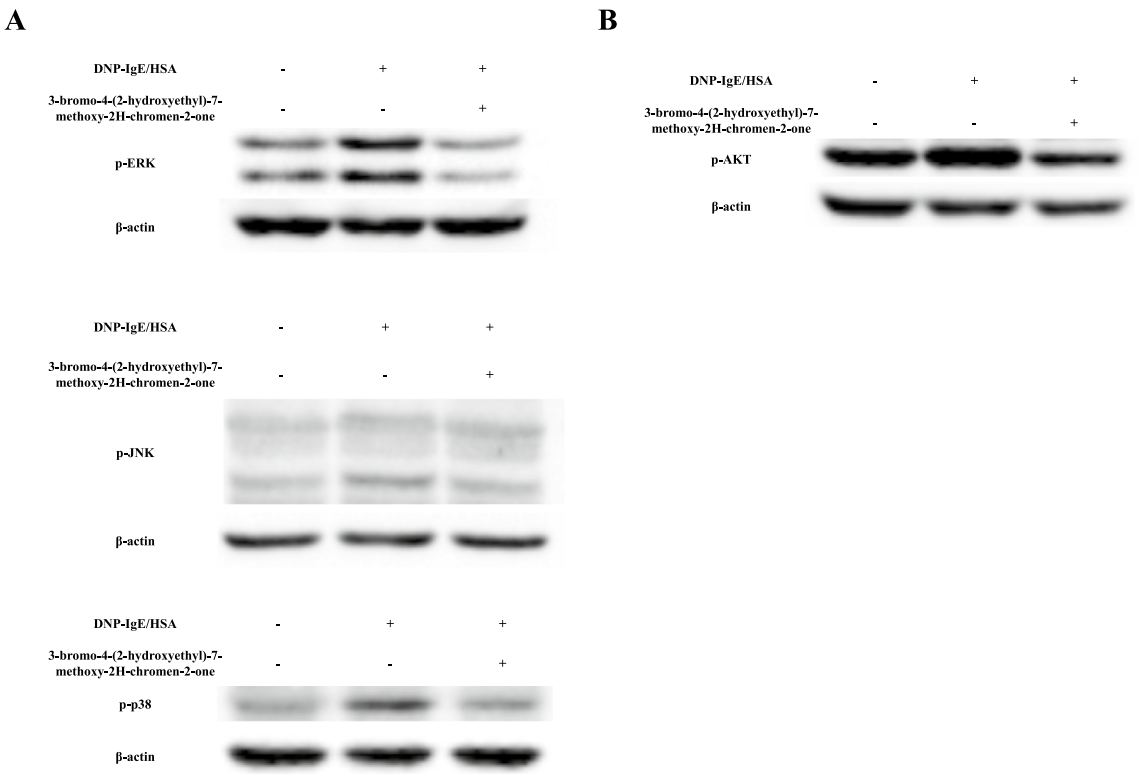

Fig. 4 Effects of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one on the phosphorylation levels of MAPK, AKT pathways in DNP-IgE/HSA-induced RBL-2H3 RBL-2H3 cells were seeded with anti-DNP/IgE (0.2 μg/mL) and incubated for 17 h. The cells were pretreated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one (100 μM), followed by treatment with DNP-HSA (0.4 μg/mL) for 30 min. (**a**) ERK, JNK, p38, and (**b**) AKT phosphorylation levels were measured by western blot analysis. Data are presented as mean ± standard deviation (SD). The experiments were performed in triplicate and representative data are presented.

findings suggest that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one may attenuate the IgE-mediated activation of RBL-2H3 cells by modulating the MAPK and AKT signaling pathways.

3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one suppresses NF-κB pathway phosphorylation in DNP-IgE/HSA-induced RBL-2H3

Phosphorylation of p65, a key subunit of the NF-κB complex, is a crucial step in activating the NF-κB signaling pathway. This activation promotes the transcription of pro-inflammatory cytokines and mediators central to the allergic response. Once phosphorylated, p65 translocates to the nucleus, where it drives the expression of cytokines such as IL-4, IL-13, and TNF-α, which play significant roles in the amplification of allergic inflammation [39–41]. Therefore, we investigated the effects of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one on p65 translocation and phosphorylation. As illustrated in Fig. 5A, the translocation of p65 to the nucleus in the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one-treated group was lower than that in the DNP-IgE/HSA-treated group. Furthermore, the phosphorylation of p65 was significantly attenuated in the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one-treated group relative to that in the DNP-IgE/HSA-treated group (Fig. 5B).

3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one inhibits DNP-IgE-HSA mediated PCA in BALB/c mice

The PCA mouse model extensively investigates the early stages of allergic reactions, including the activation of FcεRI, mast cell degranulation, and the associated inflammatory responses. Thus, this model is valuable for examining the underlying mechanisms of allergy and testing potential therapeutic agents [42, 43]. Therefore, we conducted a PCA assay to determine whether 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one exerts the observed activity in vivo. As shown in Fig. 6A, the DNP-IgE/HSA-treated group was stained with blue, indicating that Evans blue infiltration occurred because of vascular dilation caused by mast cell activation. In contrast, the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one-treated group exhibited reduced Evans

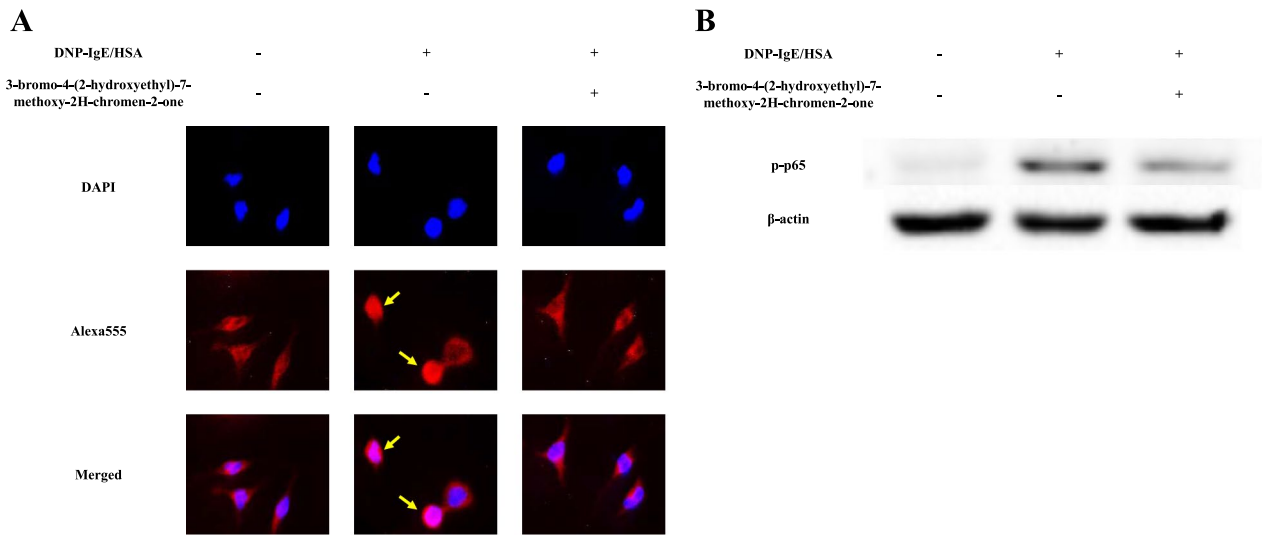

Fig. 5 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one inhibition of NF-κB translocation in DNP-IgE/HSA-induced RBL-2H3. RBL-2H3 cells were seeded with anti-DNP/IgE (0.2 μg/mL) and incubated for 17 h. The cells were pretreated with 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one (100 μM), followed by treatment with DNP-HSA (0.4 μg/mL) for 1 h. **a** p65 translocation was visualized using immunofluorescence. **b** p-p65 phosphorylation levels were measured by western blot analysis. Data are presented as mean ± standard deviation (SD). The experiments were performed. In triplicate and representative data are presented.

blue staining, suggesting the inhibition of mast cell activation. Furthermore, ear thickness in the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one group was thinner than that in the DNP-IgE/HSA-treated group (Fig. 6B). In the Evans blue extraction (Fig. 6C), the DNP-IgE/HSA-treated group showed a two-fold increase in infiltration compared to the control group. However, the 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one-treated group demonstrated a reduction in Evans blue infiltration, similar to that in the control group, indicating that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one may inhibit mast cell degranulation and, in turn, reduce immune cell infiltration. In this study, 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one reduced extravasation and alleviated allergic symptoms. These findings suggest that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one exerts anti-allergic effects in vivo.

Discussion

Allergy is a immune system dysfunction triggered by normally non-hazardous substances such as pollen, food, and animal dander. Allergic reaction is primarily mediated by mast cells, which are activated by the interaction between allergens and allergen-specific IgE antibodies, leading to uncontrolled immune responses [1, 4, 44]. To mitigate allergic reactions, such as anaphylactic shock, antihistamines, corticosteroids, and epinephrine are commonly used; however, these medications are often associated with adverse side effects [45–47]. Consequently, there is a critical need for the development of novel therapeutic agents that offer effective treatment with reduced side effects.

In this study, we investigated the anti-allergic effects of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one on RBL-2H3 cells using a PCA model. During an allergic response, mast cell degranulation releases granules containing histamine, β-hexosaminidase, pro-inflammatory cytokines, and other inflammatory molecules, which subsequently triggers symptoms such as sneezing, itching, and vasodilation [48]. 3-Bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one reduced the release of β-hexosaminidase and histamine in DNP-IgE-sensitized and HSA-stimulated RBL-2H3 cells. These findings suggest that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one effectively inhibits mast cell degranulation.

The observed inhibition of mast cell degranulation underscores the potential anti-allergic mechanism of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one. IL-4 and IL-13 are key mediators that drive inflammatory responses in allergic reactions, highlighting the importance of suppressing these cytokines to mitigate allergic inflammation. To assess the effects of the compounds on pro-inflammatory cytokine expression, qRT-PCR was performed. The results showed that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one significantly reduced the expression of IL-4, IL-13, and TNF-α, effectively attenuating the allergic response. The results demonstrated

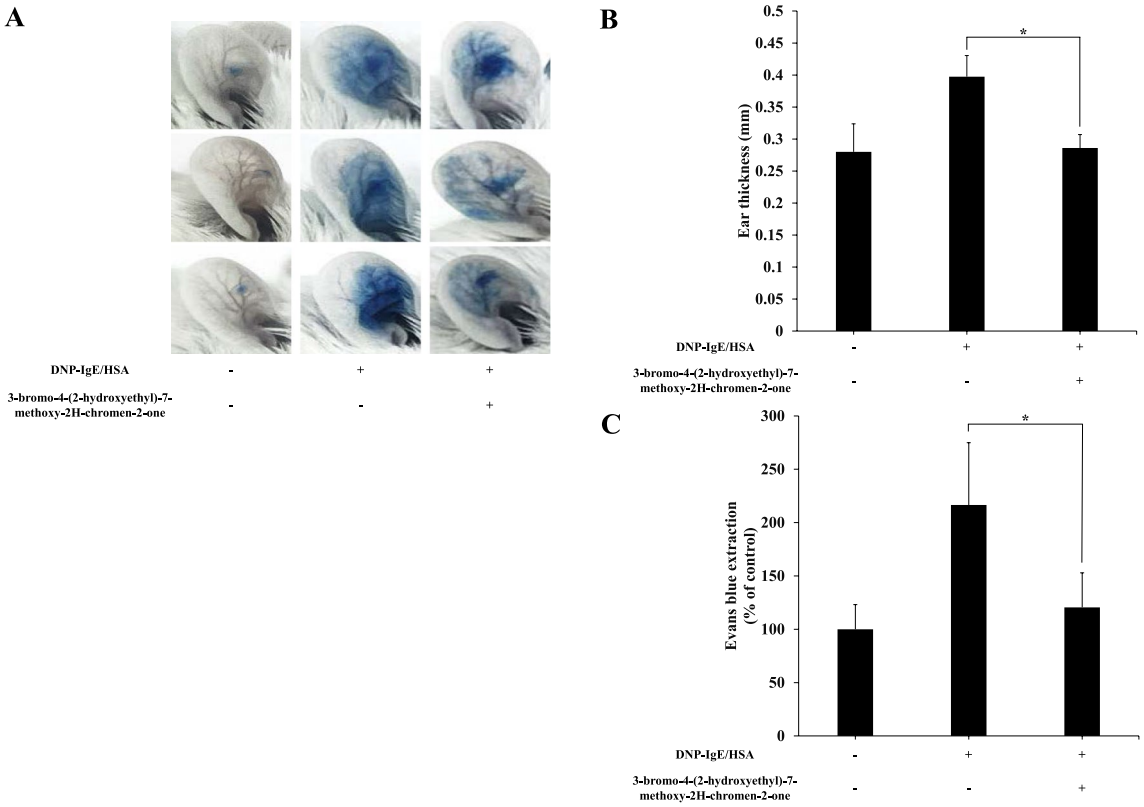

Fig. 6 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one inhibits passive cutaneous anaphylaxis in BALB/c mice. BALB/c mice (male, 8 weeks old) were intradermally injected with anti-DNP-IgE into the ears. After 24 h, 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one was intraperitoneally injected with 25 mg/kg (PBS with control group), and 1 h later, mice were intravenously treated with 2% Evans blue containing DNP-HSA. After 30 min, the mice were sacrificed. **a** Images of Evans blue infiltration in the ears. **b** Ear thickness measured using Vernier Calipers. **c** Evans blue-stained ears were punched for infiltrated Evans blue extraction. Punched ear tissues were soaked in 1 M KOH and a mixture of 0.6 N phosphoric acid and acetone solution and incubated for Evans blue extraction at 37 °C overnight. The supernatants were transferred to a 96-well plate, and the absorbance was measured using a microplate reader.

that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one significantly reduced the expression of IL-4, IL-13, and TNF-α, suggesting that the compound attenuates allergic responses through the modulation of allergic cytokines. The inhibition of MAPK pathways, including ERK, JNK, and p38, as well as the AKT signaling pathway, activated through the cross-linking of IgE receptors (FcεRI) upon allergen exposure, is critical for preventing allergic reactions[49, 50]. To investigate this, we performed western blot analysis, which demonstrated that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one inhibited the phosphorylation of both MAPK and AKT pathways. These findings suggest that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one may attenuate IgE-mediated activation of RBL-2H3 cells by modulating the MAPK and AKT signaling pathways. NF-κB is a crucial transcription factor that amplifies allergic inflammation by promoting the expression of cytokines such as IL-4, IL-13, and TNF-α [51, 52]. To evaluate the effects of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one, we investigated its impact on the phosphorylation and nuclear translocation of p65, a key subunit of the NF-κB complex, in DNP-IgE/HSA-sensitized RBL-2H3 cells. The results demonstrated that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one inhibited the phosphorylation of p65 and reduced its translocation into the nucleus.

To evaluate the anti-allergic effects of 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one in vivo, we performed a PCA assay in BALB/c mice, a model that enables the confirmation of allergic reactions in DNP-IgE-sensitized tissues [53, 54]. The results showed that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one inhibited vasodilation in anti-DNP-IgE-HSA-sensitized ear tissue, effectively reducing ear edema. Furthermore, its anti-allergic effects were validated using Evans blue dye extraction, which quantitatively measures dye penetration into tissues. These findings confirmed that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one mediates

anti-allergic responses by suppressing allergic reactions in sensitized tissues. In anaphylactic shock, a severe acute hypersensitivity response occurs due to allergen-IgE reactions. Epinephrine is used as the primary treatment, while bronchodilators, antihistamines, and glucocorticoids are administered as secondary treatments to relieve symptoms [47, 55]. Based on the results from our in vivo PCA model, 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one may have potential as a treatment for anaphylactic shock.

In conclusion, we demonstrated that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one exerts anti-allergic effects by inhibiting mast cell degranulation both in vitro and in vivo. These findings indicated that 3-bromo-4-(2-hydroxyethyl)−7-methoxy-2H-chromen-2-one is a potential candidate for the development of anti-allergic therapeutics.

Abbreviations

RBL-2H3	Rat basophilic leukemia-2H3
NF-κB	nuclear factor-kappa B
ERKs	Extracellular signal-regulated kinases
MAPK	Mitogen-activated protein kinase
MHC	Major histocompatibility complex
OPA	O-phthalaldehyde
TBS	Tris-buffered saline
PCA	Passive cutaneous anaphylaxis
FcεRI	IgE receptor

Acknowledgements
Not applicable.

Author contributions
M. K., H. K., S. Y. M., S-A. S., M. K., S. C., and S. G. performed the experiments. M. K., S. Y. M., and C.S.L. wrote the manuscript with guidance from C.S.L. J. H. L. and H. H. P. provided an intellectual contribution to this study. All authors have read and agreed to the published version of the manuscript.

Funding
This research was supported by the Korea Polar Research Institute (KOPRI) grant funded by the Ministry of Oceans and Fisheries (KOPRI Project No. * PE21900).

Availability of data and materials
The datasets that support the findings of this study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests. Chang Sup Lee is an Associate Editor of Applied Biological chemistry. Associate Editor status has no bearing on editorial consideration.

Received: 18 December 2024 Accepted: 1 January 2025
Published online: 07 January 2025

References

1. Tewtrakul S, Subhadhirasakul S (2007) Anti-allergic activity of so me selected plants in the Zingiberaceae family. J Ethnopharmacol 109(3):535–538
2. Konradsen JR et al (2015) Allergy to furry animals: new insights, diagnostic approaches, and challenges. J Allergy Clin Immunol 135(3):616–625
3. Sampson HA, Ho D (1997) Clinical aspects of allergic disease: relationship between food-specific IgE concentration and the risk of positive food challenges. J Allergy Clin Immunol 100:444–451
4. Babina M et al (2018) Allergic Fcε RI-and pseudo-allergic MRGPRX 2-triggered mast cell activation routes are independent and inversely regulated by SCF. Allergy 73(1):256–260
5. Kay AB (2001) Allergy and allergic diseases. N Engl J Med 344(1):30–37
6. Liu F-T, Goodarzi H, Chen H-Y (2011) IgE, mast cells, and eosinophils in atopic dermatitis. Clin Rev Allergy Immunol 41:298–310
7. Galli SJ, Tsai M (2012) IgE and mast cells in allergic disease. Nat Med 18(5):693–704
8. Komi DEA, Wöhrl S, Bielory L (2020) Mast cell biology at molecular level: a comprehensive review. Clin Rev Allergy Immunol 58(3):342–365
9. Amin K (2012) The role of mast cells in allergic inflammation. Respir Med 106(1):9–14
10. Stone KD, Prussin C, Metcalfe DD (2010) IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 125(2):S73–S80
11. Krystel-Whittemore M, Dileepan KN, Wood JG (2016) Mast cell: a multi-functional master cell. Front Immunol. https://doi.org/10.3389/fimmu.2015.00620
12. Alam R, Gorska MM (2011) Mitogen-activated protein kinase signalling and ERK1/2 bistability in asthma. Clin Exp Allergy 41(2):149–159
13. Motojima H et al (2013) Acteoside inhibits type **I** allergy through the down-regulation of Ca/NFAT and JNK MAPK signaling pathways in basophilic cells. J Nat Med 67:790–798
14. Bai D, Ueno L, Vogt PK (2009) Akt-mediated regulation of NFκB and the essentialness of NFκB for the oncogenicity of PI3K and Akt. Int J Cancer 125(12):2863–2870
15. He X et al (2022) Astragalus polysaccharide relieves inflammatory responses in guinea pigs with allergic rhinitis via ameliorating NF-kB-mediated Treg/Th17 imbalance. Am J Rhinol Allergy 36(5):638–648
16. Gilfillan AM, Tkaczyk C (2006) Integrated signalling pathways for mast-cell activation. Nat Rev Immunol 6(3):218–230
17. Lianto P et al (2018) Inhibitory effects of quail egg on mast cells degranulation by suppressing PAR2-mediated MAPK and NF-kB activation. Food Nutr Res. https://doi.org/10.2921/fnr.v62.1084
18. Kim S-H, Shin T-Y (2009) Anti-inflammatory effect of leaves of Eriobotrya japonica correlating with attenuation of p38 MAPK, ERK, and NF-κB activation in mast cells. Toxicol In Vitro 23(7):1215–1219
19. Min Y-D et al (2007) Quercetin inhibits expression of inflammatory cytokines through attenuation of NF-κB and p38 MAPK in HMC-1 human mast cell line. Inflamm Res 56:210–215
20. Simons FER (2003) H1-antihistamines: more relevant than ever in the treatment of allergic disorders. J Allergy Clin Immunol 112(4):S42–S52
21. Charman C, Williams H (2003) The use of corticosteroids and corticosteroid phobia in atopic dermatitis. Clin Dermatol 21(3):193–200
22. Church DS, Church MK (2011) Pharmacology of antihistamines. World Allergy Organ J 4:S22–S27
23. Uva L et al (2012) Mechanisms of action of topical corticosteroids in psoriasis. Int J Endocrinol 2012(1):561018
24. Keri RS et al (2015) Recent progress in the drug development of coumarin derivatives as potent antituberculosis agents. Eur J Med Chem 100:257–269
25. Detsi A, Kontogiorgis C, Hadjipavlou-Litina D (2017) Coumarin derivatives: an updated patent review (2015–2016). Expert Opin Ther Pat 27(11):1201–1226
26. Witaicenis A et al (2014) Antioxidant and intestinal anti-inflammatory effects of plant-derived coumarin derivatives. Phytomedicine 21(3):240–246
27. Yodsaoue O et al (2009) Anti-allergic activity of principles from the roots and heartwood of caesalpinia sappan on antigen-induced β-hexosaminidase release. Phytotherapy Res 23(7):1028–1031
28. Fukuishi N et al (2014) Does β-hexosaminidase function only as a degranulation indicator in mast cells? The primary role of β-hexosaminidase in mast cell granules. J Immunol 193(4):1886–1894
29. Sridharan G, Shankar AA (2012) Toluidine blue: a review of its chemistry and clinical utility. J oral Maxillofac Pathol 16(2):251
30. McLeod JJ, Baker B, Ryan JJ (2015) Mast cell production and response to IL-4 and IL-13. Cytokine 75(1):57–61

31. Brown MA et al (1987) B cell stimulatory factor-1/interleukin-4 mRNA is expressed by normal and transformed mast cells. Cell 50(5):809–818
32. Hide I (2003) Mechanism of production and release of tumor necrosis factor implicated in inflammatory diseases Nihon Yakurigaku Zasshi. Folia Pharmacol Jpn 121(3):163–173
33. Ye J et al (2017) Polydatin inhibits mast cell-mediated allergic inflammation by targeting PI3K/Akt, MAPK, NF-κB and Nrf2/HO-1 pathways. Sci Rep 7(1):11895
34. Xiong X et al (2021) Qingxue jiedu formulation ameliorated DNFB-induced atopic dermatitis by inhibiting STAT3/MAPK/NF-κB signaling pathways. J Ethnopharmacol 270:113773
35. Class I PI3K-mediated Akt and ERK signals play a critical role in FcεRI-induced degranulation in mast cells. 2013
36. Kambayashi T, Koretzky GA (2007) Proximal signaling events in Fc**ε**RI-mediated mast cell activation. J Allergy Clin Immunol 119(3):544–552
37. Kim M-S, Rådinger M, Gilfillan AM (2008) The multiple roles of phosphoinositide 3-kinase in mast cell biology. Trends Immunol 29(10):493–501
38. Ekoff M et al (2006) Coaggregation of FcεRI with FcγRIIB inhibits degranulation but not induction of Bcl-2 family members A1 and Bim in mast cells. Allergy Asthma Clin Immunol 2:1–11
39. Lertnimitphun P et al (2021) Safranal alleviated OVA-induced asthma model and inhibits mast cell activation. Front Immunol 12:585595
40. Giridharan S, Srinivasan M (2018) Mechanisms of NF-κB p65 and strategies for therapeutic manipulation. J Inflamm Res. https://doi.org/10.2147/JIR.S140188
41. Lee HP et al (2022) Inhibitory effects of Grewia tomentosa Juss on IgE-mediated allergic reaction and DNCB-induced atopic dermatitis. Plants. https://doi.org/10.3390/plants11192540
42. Yoo J-M, Sok D-E, Kim MR (2014) Anti-allergic action of aged black garlic extract in RBL-2H3 cells and passive cutaneous anaphylaxis reaction in mice. J Med Food 17(1):92–102
43. Kaul S, Hoffmann A (2001) [Mediator Release Assay of rat basophil leukemia cells as alternative for passive cutaneous anaphylaxis (PCA)][Article in German]: LINZ 2000. Altern Animal Exp 18(1):55–58
44. Wernersson S, Pejler G (2014) Mast cell secretory granules: armed for battle. Nat Rev Immunol 14(7):478–494
45. Li L et al (2022) Pharmacogenomics for the efficacy and side effects of antihistamines. Exp Dermatol 31(7):993–1004
46. Matura M, Goossens A (2000) Contact allergy to corticosteroids. Allergy. https://doi.org/10.1034/j.1398-9995.2000.00121.x
47. Chaudhuri K et al (2008) Anaphylactic shock in pregnancy: a case study and review of the literature. Int J Obstet Anesth 17(4):350–357
48. White MV (1990) The role of histamine in allergic diseases. J Allergy Clin Immunol 86(4):599–605
49. Athari SS (2019) Targeting cell signaling in allergic asthma. Signal Transduct Target Ther 4(1):45
50. Ma B et al (2021) PI3K/AKT/mTOR and TLR4/MyD88/NF-κB signaling inhibitors attenuate pathological mechanisms of allergic asthma. Inflammation 44:1895–1907
51. Mitsuta, K., et al. Production of TNF-[alpha] by Peripheral Blood Mononuclear Cells through Activation of Nuclear Factor [kappa] B by Specific Allergen Stimulation in Patients with Atopic Asthma. Allergy and asthma proceedings. 2003 OceanSide Publications.
52. Ahmad S et al (2018) The key role of TNF-TNFR2 interactions in the modulation of allergic inflammation: a review. Front Immunol 9:2572
53. Zabel BA et al (2008) Mast cell–expressed orphan receptor CCRL2 binds chemerin and is required for optimal induction of IgE-mediated passive cutaneous anaphylaxis. J Exp Med 205(10):2207–2220
54. Bryce PJ et al (2016) Humanized mouse model of mast cell–mediated passive cutaneous anaphylaxis and passive systemic anaphylaxis. J Allergy Clin Immunol 138(3):769–779
55. LoVerde D et al (2018) Anaphylaxis. Chest 153(2):528–543

Publisher's Note