



Targeting PAR-2 in atopic dermatitis: Preclinical evaluation of the novel inhibitor E6795

Naomi Kitayama^a, Chisa Nakashima^{b,*}, Teruo Sawada^c, Nobuo Tanaka^{d,e},
Masatoshi Hagiwara^c, Atsushi Otsuka^b, Kenji Kabashima^{a,f}

^a Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan

^b Department of Dermatology, Kindai University Hospital, Osaka, Japan

^c Department of Drug Discovery Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

^d Department of Pharmacology, The Jikei University School of Medicine, Tokyo, Japan

^e Medical Research Support Center, Graduate School of Medicine, Kyoto University, Kyoto, Japan

^f A*STAR Skin Research Labs (A*SRL), Skin Research Institute of Singapore (SRIS), Singapore Immunology Network (SIgN), Agency for Science, Technology, and Research (A*STAR), 8A Biomedical Grove, IMMUNOS Building, Biopolis, 138648, Singapore

ARTICLE INFO

Keywords:

Atopic dermatitis
PAR-2 inhibitor
E6795
Inflammation
Pruritus
Skin barrier

ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by eczematous lesions, intense itching, and compromised skin barrier function. Despite the advent of new therapeutics, many individuals still face insufficient disease control, high costs, and relapse. Protease-activated receptor 2 (PAR-2), overexpressed in AD lesions, plays a central role in promoting inflammation, itch, and alterations in epidermal homeostasis. In this study, we present preclinical evidence for E6795, a small-molecule PAR-2 inhibitor designed to target these pathogenic processes. Kinase and pharmacological profiling confirmed that E6795 selectively inhibits PAR-2, demonstrating minimal off-target interactions across extensive kinase and pharmacological panels. Furthermore, E6795 improved AD-related endpoints across two murine models, including reduced inflammation and immune-cell infiltration; oral dosing reduced transepidermal water loss (TEWL), and the topical 2 % cream improved barrier-related histology and pruritus. Histological examination confirmed decreased inflammatory cell infiltration and enhanced barrier function following E6795 therapy. Our findings demonstrate that E6795 targets the three main pillars of AD pathophysiology—barrier dysfunction, inflammation, and itch—by selectively inhibiting PAR-2 signaling. This work underscores E6795's potential as a cost-effective, novel topical agent for AD, particularly beneficial for patients refractory to existing treatments.

1. Introduction

Atopic dermatitis (AD) is a chronic, recurrent inflammatory skin disease characterized by eczematous lesions and intense pruritus. AD is associated with impaired skin barrier function, allergy-driven inflammation, and severe pruritus. These intertwined factors form a positive feedback loop through complex interactions in AD pathogenesis [1].

The pathogenesis of AD involves multiple cytokines, and recent years have seen the development of novel treatments such as Janus kinase (JAK) inhibitors, interleukin (IL)-4/IL-13, anti-IL-31 receptor A, and anti-IL-13 antibodies. However, topical therapies, including corticosteroids, remain the cornerstone of AD management.

Protease-activated receptor 2 (PAR-2) is a cell surface receptor for

extracellular and environmental proteases that is notably abundant in the skin of patients with AD [2]. This receptor is widely expressed across various cell types and plays crucial roles in cutaneous biology, including the regulation of epidermal homeostasis, barrier integrity, immune responses, and nociception [3].

The relationship between AD and PAR-2 has been well documented [2,4], with recent studies highlighting its critical role in skin inflammation, pruritus, and barrier function [5]. These three interconnected pathogenic factors are fundamental to the development and progression of AD, driven by complex feedback mechanisms [1]. Despite its therapeutic potential, only a few PAR-2 inhibitors have demonstrated clear efficacy *in vivo* in skin inflammation models, leaving the clinical application of PAR-2-targeted therapies largely unrealized.

* Corresponding author.

E-mail address: chisa313@med.kindai.ac.jp (C. Nakashima).

<https://doi.org/10.1016/j.bbrc.2025.152590>

Received 16 June 2025; Received in revised form 25 August 2025; Accepted 2 September 2025

Available online 3 September 2025

0006-291X/© 2025 Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

E6795 (Fig. 1a; Patent numbers: JP6916799B2, US10227349B2) is a novel PAR-2 inhibitor synthesized by Eisai, identified through high-throughput screening (HTS) of the company's small molecule compound library. The primary HTS was performed using the PAR-2 agonist peptide SLIGKV-NH₂ (1 μ M) as a stimulant. Compounds exhibiting >40 % inhibitory activity at a concentration of 10 μ g/mL were initially selected as hits. A confirmatory assay was subsequently conducted at two compound concentrations (10 μ g/mL and 2 μ g/mL), and compounds that consistently showed >40 % inhibition at both concentrations were confirmed as final hits. Subsequent optimization of a lead compound resulted in the development of E6795. In this study, we evaluated the PAR-2 inhibitory activity of E6795 and investigated its therapeutic effects in a dermatitis model. We demonstrate that E6795 exhibits potent PAR-2 inhibitory activity *in vivo*, improving skin inflammation, pruritus, and barrier function in a mouse model of AD. Additionally, E6795 markedly reduced eosinophil and mast cell infiltration in the affected skin.

2. Materials and methods

2.1. Mice

Nc/Nga mice (male, 11–12 weeks old) were obtained from SLC Inc. (Shizuoka, Japan) and acclimatized for five days prior to the start of the experiment. The mice were bred in 55 $\pm$ 7 % humidity at 24 $\pm$ 1 $^{\circ}$ C in individually ventilated cages (IVCs) under specific pathogen-free (SPF) conditions tested positive for fur mites but free of all other pathogens with a 12-h light–dark cycle.

BALB/c mice (female, 6–14 weeks old) were purchased from Charles River Laboratories Japan (Kanagawa, Japan) and Orientalbio Co. Ltd. (Tokyo, Japan). These mice were bred in SPF facilities at Kyoto University. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Kyoto University Graduate School of Medicine (Kyoto, Japan).

2.2. Reagents and antibodies

We purchased MC903 from Tocris Bioscience (Bristol, United

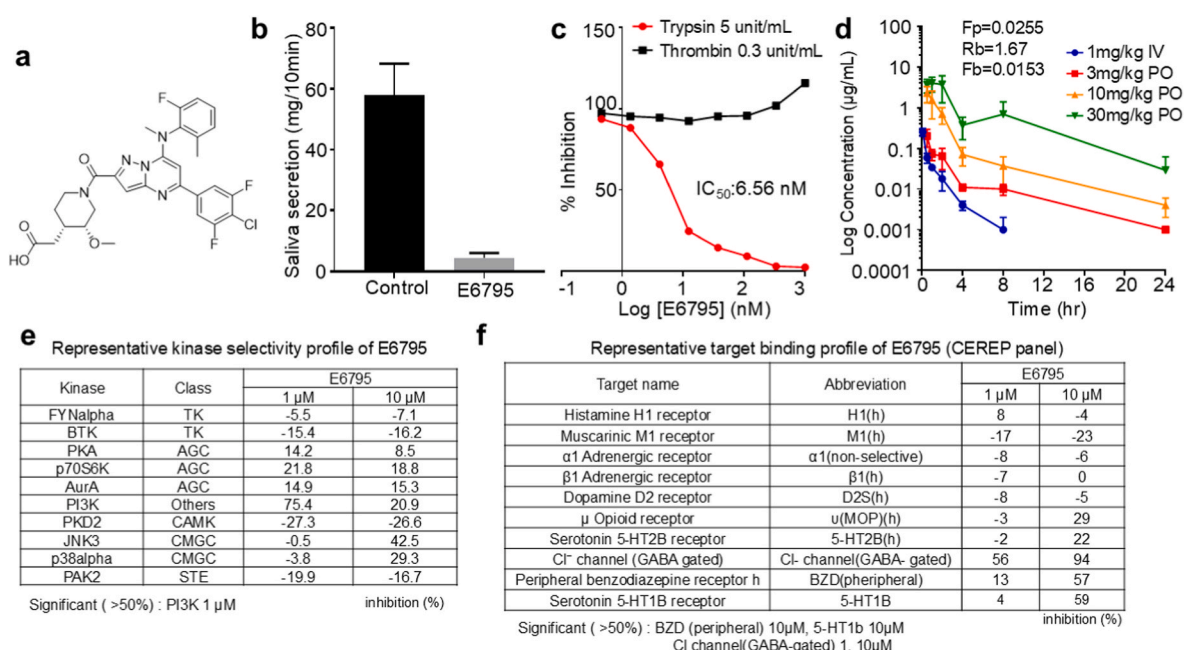


Fig. 1. Characterization of E6795 as a novel PAR-2 inhibitor.

(a) Chemical structure of E6795, a newly developed small-molecule PAR-2 inhibitor.

(b) Inhibition of PAR-2-mediated saliva secretion by E6795 in mice.

Saliva secretion was induced by administration of a PAR-2 activating peptide (PAR2-AP) and measured over a 10-min period. Oral administration of E6795 at a dose of 3 mg/kg significantly inhibited saliva secretion compared to the vehicle-treated control group (mean $\pm$ SEM; Control: 58.0 $\pm$ 10.3 mg/10 min, E6795: 4.5 $\pm$ 1.5 mg/10 min; n = 4 mice per group). Data represent means from independent experiments.

(c) Dose-response curves illustrating the inhibitory activity of E6795 on PAR-2 and PAR-1 in a cell-based calcium influx assay.

Cells were stimulated with trypsin (5 units/mL; PAR-2 agonist) or thrombin (0.3 units/mL; PAR-1 agonist) in the presence of various concentrations of E6795. IC₅₀ value for PAR-2 inhibition was determined as 6.56 nM. No significant inhibition of thrombin-induced PAR-1 activity was observed, confirming the selectivity of E6795 for PAR-2. Data are presented as mean percent inhibition from six independent experiments.

(d) Pharmacokinetic (PK) profile of E6795 in mice.

Pharmacokinetic analysis was performed to evaluate the systemic absorption and distribution characteristics of E6795. The free fraction in plasma (Fp), free fraction in blood (Fb), and blood-to-plasma ratio (Rb) were determined, indicating extensive plasma protein binding (~97.5 % bound), limited free fraction (~2.55 % in plasma, ~1.53 % in whole blood), and moderate distribution into red blood cells (Rb = 1.67).

(e) Representative kinase selectivity profile of E6795.

A subset of kinases was selected based on biological relevance to inflammatory signaling or observed inhibitory trends. PI3K showed apparent inhibition at 1 μ M (75.4 %) but not at 10 μ M (20.9 %), suggesting non-specific interaction or assay artifacts. All other kinases tested, including PKA, Aurora A, and BTK, showed less than 25 % inhibition, indicating minimal off-target activity and supporting the high selectivity of E6795 for PAR-2.

(f) Representative target binding profile of E6795 based on the CEREP panel.

Binding inhibition of E6795 at 1 μ M and 10 μ M was evaluated using the CEREP pharmacological panel across a range of neurotransmitter receptors, ion channels, and other molecular targets. The table shows selected targets representative of the broader screening panel. Binding activity is expressed as the percentage of inhibition relative to control at each concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Kingdom) and diluted it to 100 μ M in ethanol (EtOH).

E6795, a PAR-2 antagonist, was synthesized by Eisai Pharmaceuticals (Tokyo, Japan).

Naïve HEK293 (Human Embryonic Kidney 293 cells) and trypsin from porcine pancreas were obtained from Sigma-Aldrich (St. Louis, MO) and used for calcium-signaling assay.

Phycoerythrin (PE)-, PE-Cy7-, and Pacific Blue (PB, V450) conjugated anti-Siglec-F (E50-2440), anti-CD4 (RM4-5), and anti-CD45 (30-F11) mAbs were purchased from BD Biosciences (San Jose, Calif). Fluorescein isothiocyanate (FITC) and Brilliant Violet 605 (BV605) conjugated anti-CD117 (c-Kit; 2B8), and anti-CD11b (M1/70) mAbs were purchased from BioLegend (San Diego, CA). PE-conjugated anti-Fc ϵ R1 α (MAR-1) mAbs and Fixable Viability Dye eFluor 780 were purchased from eBioscience (San Diego, CA).

2.3. Calcium assay

2.3.1. Intracellular calcium concentration measurement

For calcium flux measurements, test compounds were first diluted in Hanks' balanced saline solution (HBSS) containing 20 mM HEPES (pH 7.4). Separately, the agonist trypsin (Sigma-Aldrich, T8816, EC 3.4.21.4) was freshly prepared at 5 U/mL (BAEE unit) in the same buffer and dispensed into a 384-well polypropylene reagent reservoir plate (Greiner). HEK293 cells, derived from human embryonic kidney tissue, were maintained in DMEM medium (Invitrogen) supplemented with 3 % fetal bovine serum (FBS) at 37 °C. Cells were harvested, resuspended at 2×10^5 cells/mL, and then seeded (25 μ L/well) onto type-I collagen-coated clear-bottom black 384-well plates (Greiner). Plates were incubated overnight at 37 °C in a humidified chamber with 5 % CO₂. On the assay day, cells were loaded with FLIPR Calcium Assay reagent (Molecular Devices) prepared in Hanks-HEPES buffer (20 mM, pH 7.4) at 25 μ L per well, and incubated for an additional hour at 37 °C. Test compounds (8 μ L/well) were subsequently added 30 min prior to calcium measurement. To initiate the calcium response, both the cell plate and trypsin reservoir plate were placed into an FDSS6000 fluorescence detection system (Hamamatsu Photonics K.K.). Using its automated dispensing system, trypsin reagent (22 μ L/well) was rapidly applied to the cells, triggering calcium influx. Real-time fluorescence intensities were monitored continuously for 120 s at 37 °C using the integrated CCD camera. IC₅₀ values were determined by nonlinear regression analysis (four-parameter logistic model with variable slope) of the fluorescence data. The resulting IC₅₀ values obtained as log₁₀ concentrations were converted back into actual concentrations by calculating the antilog (IC₅₀ = $10^{\log_{10} \text{IC}_{50}}$).

2.3.2. Measurement of PAR-2 agonist-induced salivation in mouse

Male C57BL/6J mice (Charles River), aged 6–10 weeks, were used to measure saliva secretion induced by the PAR-2 agonist peptide SLIGRL-NH₂ (SEQ ID NO: 2). To prepare dosing solutions, test compounds were diluted to a final concentration of 0.3 mg/mL in a mixture containing 0.5 % carboxymethyl cellulose and 2 % DMSO. SLIGRL-NH₂ was separately prepared at a concentration of 0.1 mg/mL. Initially, mice received oral administration of the prepared test compound solution (10 mL/kg) via an oral gavage using a syringe with an attached feeding tube. Forty-five minutes post-administration, mice were anesthetized by intraperitoneal injection of urethane (20 % w/v solution, 10 mL/kg). Fifteen minutes after anesthesia induction (60 min after the initial oral administration), the prepared SLIGRL-NH₂ solution (10 mL/kg) was administered intraperitoneally to induce salivation. Immediately after SLIGRL-NH₂ injection, saliva secretion was collected by inserting a pre-weighed cotton ball into the oral cavity of each mouse. Following a 10-min saliva collection period, the cotton balls were removed and weighed again to calculate the amount of saliva produced by each mouse.

2.3.3. MC903-induced murine AD model

MC903 was used to induce AD-like skin in mice. 100 μ M of MC903

was applied to BALB/c mice once a day topically to the ear skin for 8 consecutive days. MC903-treated mice received daily topical application of vehicle or E6795 hydrochloride dissolved in ethanol (5 mg/mL) or oral administration (10 mL/kg) of E6795 free body suspended in carboxymethyl cellulose (1 mg/mL) from day 4.

Ear thickness was measured daily using a thickness gauge (Teclock, Nagano, Japan). TEWL was measured with VAPOSCAN (AS-VT100RS, Asch Japan Co, Ltd, Tokyo, Japan) at days 0, 3, and 7. Skin samples were collected 24 h after the final application. Both ears were surgically removed and processed for hematoxylin and eosin staining or for FACS-based sorting of skin leukocytes.

2.3.4. Nc/Nga mice as a severe atopic dermatitis model

Nc/Nga mice that developed dermatitis were evenly divided into 5 groups, received daily topical application of 50 mg/mouse of E6795 cream (0 %, 2 %) for 7 days. Ear thickness was measured, and skin lesion severity was recorded daily. Skin lesion severity was scored based on five criteria: redness/bleeding, edema, scratching, tissue damage and crusts/dry area. On day 7, scratching behavior was assessed. Skin samples were collected from all mice on day 8. The ears were collected and processed for toluidine blue staining.

2.3.5. Measurement of scratching behavior frequency

After the final administration, scratching behavior frequency was measured in the animal housing room (set temperature: 24 °C, set humidity: 50 %, ventilation rate: 15–20 times/hour). The scratching behavior frequency at the time of group assignment was used as the pre-administration value.

Each animal was individually placed in a measurement cage (ϕ 11 cm $\times$ H 18 cm) and set in a measurement unit (NS-SCT10; NeuroScience Inc.). A dedicated water bottle containing 40–50 mL of drinking water was attached to the measurement cage, and 2 pellets of feed were provided per animal.

Measurements were conducted for 14 h from 17:00 to 07:00 the following day (with 17:00 to 19:00 designated as the acclimation period) using a scratch measurement system (MicroAct Ver.2.15A; NeuroScience Inc.). The scratching behavior frequency (Beat) was recorded for 12 h from 19:00 to 07:00, excluding the initial 2-h acclimation period. Additionally, hourly shaking behavior frequency was simultaneously calculated.

2.3.6. Histology and immunohistochemistry

For histological examination, mouse skin tissues were fixed with 10 % formalin in phosphate-buffered saline (PBS) and then embedded in paraffin. Sections with a thickness of 5 μ m were prepared and subjected to staining with hematoxylin and eosin (H&E). The identification of mast cells was performed with toluidine blue staining. The number of mast cells was counted in 5 fields (40 $\times$ objective). The slides were blinded, randomized, and re-read. For PAR-2 immunohistochemical analysis, tissue sections were immunostained as previously reported [6].

2.3.7. Human skin samples

Healthy control skin specimens were obtained from uninvolved regions adjacent to surgically excised benign skin tumors from patients. Lesional skin samples were acquired through biopsy from the affected skin of patients clinically diagnosed with atopic dermatitis at Kyoto University Hospital. All human skin samples were obtained with written informed consent, in accordance with the study protocol approved by the Kyoto University Graduate School of Medicine Ethics Committee (Approval No. R0743).

2.3.8. Flow cytometry

Mouse ear tissues collected for flow cytometric evaluation were first finely minced into small fragments. The minced skin samples were then enzymatically digested using complete RPMI 1640 medium (cRPMI), supplemented with 10 % heat-inactivated fetal bovine serum (FBS);

Invitrogen), 25 mmol/L HEPES buffer, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 0.05 mmol/L 2-mercaptoethanol, 1 mmol/L nonessential amino acids, 100 U/mL penicillin, 100 µg/mL streptomycin (RPMI 1640; Sigma-Aldrich), in addition to 1.6 mg/mL collagenase type II (Worthington Biochemical, Freehold, NJ) and 0.1 mg/mL DNase I (Sigma-Aldrich). Digestion was carried out at 37 °C for 60 min. Following enzymatic treatment, single-cell suspensions were prepared by passing the digested samples through a nylon filter with a pore size of 40 µm. The resulting single-cell preparations were labeled with fluorescently conjugated antibodies targeting specific surface markers. Flow cytometry analysis was conducted on a FACS Fortessa instrument (BD Biosciences). Subsequent analysis and quantification of individual cell populations were performed using FlowJo software (TreeStar, San Carlos, CA). Final cell counts for each cell population were normalized and presented as the absolute number of cells per square millimeter of ear skin.

2.3.9. Statistical analyses

In animal experiments, the indicated sample size ("n") represents the number of individual mice analyzed per group.

All statistical analyses were two-sided with an alpha level of 0.05. Data are presented as mean ± SEM. No imputation was performed; all analyses used available cases.

For endpoints with three groups (vehicle, E6795 oral, E6795 topical), we performed one-way ANOVA followed by Dunnett's multiple comparisons test versus the vehicle control to control the family-wise error rate across the two pre-specified contrasts (vehicle vs. E6795 oral; vehicle vs. E6795 topical). Adjusted $P < 0.05$ was considered statistically significant.

Ear thickness in the MC903 model was measured repeatedly in the same animals from day 4 to day 8. Given the small group sizes ($n = 4$ per group; oral day-8 $n = 3$) and occasional missing values, we analyzed each day as a pre-specified cross-sectional endpoint using one-way ANOVA followed by Dunnett's test (vs vehicle). This day-wise approach avoids additional modeling assumptions and keeps inference aligned with our prespecified readouts (with emphasis on days 7–8). We acknowledge that mixed-effects repeated-measures models would be preferable for larger, balanced datasets and plan to adopt such models in future studies.

For the Nc/Nga experiments that also comprised three groups (untreated, vehicle, E6795 cream 2 %), the same analysis strategy (one-way ANOVA followed by Dunnett's test versus the vehicle control) was applied; the untreated group was included to provide biological context.

For longitudinal outcomes (e.g., ear thickness in the MC903 model; skin score or scratching bouts in Nc/Nga), days/time windows were analyzed as pre-specified cross-sectional endpoints (e.g., day-wise analyses for ear thickness), and multiplicity was controlled using Dunnett's test as above.

Data were analyzed using GraphPad Prism Version 7.03 (GraphPad, La Jolla, CA).

3. Results

The newly developed compound E6795 demonstrates potent and selective inhibition of PAR-2.

In our pursuit of clinically viable PAR-2 inhibitors, we evaluated the novel compound E6795 (Fig. 1a) for its therapeutic potential in dermatological conditions. Previous studies have demonstrated that PAR-2 inhibitors can suppress trypsin-induced calcium influx [7]. To investigate the effects of E6795, we assessed the activation and inhibition of PAR-2 using calcium assays. Trypsin stimulation caused increased intracellular calcium influx, confirming PAR-2 activation. E6795 significantly inhibited PAR-2-mediated saliva secretion in an *in vivo* pharmacodynamic (PD) assay. Oral administration of E6795 at a dose of 3 mg/kg resulted in an approximately 92 % reduction in saliva secretion compared to vehicle-treated controls (mean saliva secretion:

58.0 ± 10.3 mg in control group, 4.5 ± 1.5 mg in E6795-treated group; Fig. 1b).

E6795 is an orally available PAR-2 inhibitor, exhibiting potent and selective inhibition with an IC_{50} of 6.56 nM in a cell-based calcium influx assay (Fig. 1c). IC_{50} values were calculated using nonlinear regression analysis with a variable-slope, four-parameter logistic model based on mean data obtained from six independent experiments. Notably, E6795 did not inhibit thrombin-induced PAR-1 activity, indicating selective inhibition of PAR-2 without affecting PAR-1.

3.1. Pharmacokinetic properties of E6795 in mice

To investigate the pharmacokinetic (PK) profile of E6795, we conducted a mouse PK study (Fig. 1d). The results demonstrated that E6795 is readily absorbed and distributed into systemic circulation following administration. The free fraction of E6795 in plasma (Fp) was approximately 2.55 %, indicating extensive plasma protein binding (~97.5 % bound). Moreover, the free fraction in whole blood (Fb) was approximately 1.53 %, with a blood-to-plasma ratio (Rb) of 1.67, suggesting distribution of the compound into red blood cells. Collectively, these pharmacokinetic characteristics demonstrate that although E6795 achieves systemic exposure, the fraction available as unbound, pharmacologically active compound is relatively low.

3.2. E6795 exhibits high selectivity for PAR-2 over broad kinase and pharmacological panels

To further assess the target specificity of E6795 and to evaluate potential off-target activities or unexpected toxicities, a kinase selectivity panel assay (Fig. 1e), and a pharmacological binding panel assay (CEREP panel; Fig. 1f) were performed at concentrations of 1 µM and 10 µM.

Among the tested kinases, PI3K exhibited notable inhibition at 1 µM (75.4 %), which was not sustained at 10 µM (20.9 %). This atypical concentration-response pattern suggests the possibility of non-specific interactions or assay-related artifacts, although the potential for off-target activity cannot be completely excluded. All other kinases, including those relevant to immune and inflammatory pathways—such as FYNα, BTK, PKA, p70S6K, Aurora A, PKD2, JNK3, p38α, and PAK2—showed no significant inhibition, with inhibition rates well below commonly accepted off-target thresholds (i.e., <30 %) at both concentrations.

In parallel, pharmacological screening against a broad array of receptors, ion channels, and neurotransmitter targets revealed minimal binding activity (<30 % inhibition at 10 µM) of E6795 against most targets, including histamine (H1), muscarinic acetylcholine (M1), adrenergic (α1, β1), dopamine (D2S), opioid (µ), and serotonin (5-HT2B) receptors. Notably, however, high binding inhibition was observed for GABA-gated chloride channels (56 % at 1 µM and 94 % at 10 µM), peripheral benzodiazepine receptor (57 % at 10 µM), and serotonin 5-HT1B receptor (59 % at 10 µM), suggesting potential off-target interactions requiring careful evaluation in further studies.

Collectively, these data strongly support the high selectivity of E6795 for PAR-2 across extensive kinase and pharmacological panels, while also identifying specific molecular interactions that merit additional investigation regarding their functional relevance and safety.

3.3. Increased PAR-2 expression in keratinocytes of AD patients

PAR-2 has been widely implicated in AD, a condition characterized by type 2 inflammation in the skin. It is reported that PAR-2 expression is elevated in keratinocytes from the skin of patients with AD compared to healthy controls [6,8]. However, few studies have demonstrated this increased expression in AD patients compared to healthy controls using immunohistochemical staining. PAR-2 is known to be expressed in the granular layer of keratinocytes. To assess whether the expression of

PAR-2 is increased in the granular and upper spinous layers of keratinocytes in AD, we performed immunohistochemical staining for PAR-2 in both healthy and AD human skin samples. In healthy skin, only a few PAR-2-positive cells were detected in the upper spinous to granular layers of keratinocytes. By contrast, in AD, numerous PAR-2-positive cells were observed in the granular and upper spinous layers of keratinocytes (Fig. 2). These findings suggest that the expression of PAR-2 in keratinocytes is increased in AD compared to healthy controls.

3.4. E6795 suppresses inflammation and immune cell infiltration in the MC903-induced AD mouse model

Next, to investigate the efficacy of E6795 in AD, a representative chronic skin disease characterized by type 2 inflammation, we examined its effects using several AD mouse models. First, we assessed the anti-inflammatory effects of E6795 in the MC903-induced AD mouse model. On day 4 following the initiation of MC903 topical application, the mice were divided into groups, and E6795 was administered either

topically or orally. On day 8, one-way ANOVA revealed a significant overall difference among the three groups ($P = 0.049$). Post hoc Dunnett's test indicated that topical E6795 significantly reduced ear thickness versus vehicle (adjusted $P = 0.036$), whereas oral E6795 did not reach statistical significance (adjusted $P = 0.138$). On day 7, oral E6795 showed a reduction trend versus vehicle (adjusted $P = 0.075$). We hypothesized that E6795 treatment would suppress the production of inflammatory mediators in keratinocytes and reduces the infiltration of inflammatory cells into the lesional skin via PAR-2 inhibition.

Histological analysis of the ear skin samples revealed decreased dermal inflammatory cell infiltration in both oral and topical E6795 treatment groups compared to vehicle-treated controls (Fig. 3b). To quantitatively evaluate inflammatory cell infiltration, fluorescence-activated cell sorting (FACS) was used to analyze eosinophil, neutrophil, $CD4^+$ T cell, and basophil populations in the ear skin. Both oral and topical E6795 treatment resulted in a significant reduction in eosinophils ($CD11b^+$, $SiglecF^+$). These findings suggest that E6795 treatment mitigates inflammatory cell infiltration and improves dermatitis in the MC903-induced AD mouse model.

3.5. E6795 administration resulted in an improvement in barrier function in the MC903 model

PAR-2 has been implicated in the regulation of skin barrier function, with evidence suggesting that its activation can impair barrier integrity [9]. Based on this, we hypothesized that PAR-2 inhibitors might improve skin barrier function. To investigate this, we evaluated the effects of E6795 on barrier function in the MC903 mouse model by measuring transepidermal water loss (TEWL). Oral administration of E6795 significantly reduced TEWL on day 7 compared to vehicle controls (Fig. 3c). Although it remains uncertain whether E6795 directly restores barrier integrity via PAR-2 blockade in keratinocytes or if these improvements occur secondarily through reduced skin inflammation, these results indicate that E6795 not only attenuates dermatitis and reduces dermal inflammatory cell infiltration but also improves epidermal barrier function directly or indirectly in the MC903 mouse model.

3.6. Topical 2 % E6795 cream reduces inflammation, epidermal thickening, and pruritus in Nc/Nga mice

Based on the anti-inflammatory effects observed with topical E6795 in the MC903 model (Fig. 3a), we concluded that this PAR-2 inhibitor demonstrates therapeutic efficacy through both systemic and local administration. These findings led us to develop a 2 % E6795 cream formulation (Supplementary Fig. 2) and evaluate its efficacy in the Nc/Nga mouse model of AD.

After 7 days of treatment, the clinical skin score showed a numerical reduction with E6795 cream versus vehicle; however, this did not reach statistical significance after multiplicity control (one-way ANOVA followed by Dunnett's test, adjusted $P = 0.136$; Fig. 4a). Hematoxylin and eosin (H&E) staining of ear samples from these mice revealed a significant reduction in epidermal thickness in the 2 % E6795 cream group (Fig. 4b), which was significant by ANOVA with Dunnett's post hoc test (adjusted $P = 3.7 \times 10^{-5}$; mean difference $-151.52 \mu m$, 95 % CI -180.82 to -122.22 ; Fig. 4c).

Because the itch associated with AD is triggered by mast cell tryptase activation of PAR-2 on sensory nerve endings [10], we measured scratching behavior in Nc/Nga mice treated with 2 % E6795 cream. Scratching behavior was assessed hourly starting at 7 p.m. on day 7, one week after treatment initiation. Using the prespecified primary endpoint (the mean across 19–24 h), E6795 cream significantly reduced scratching compared with vehicle (ANOVA $P = 0.045$; Dunnett-adjusted $P = 0.040$; Fig. 4d). Consistent with PAR-2-mediated pruritogenic signaling, dermal mast-cell infiltration was also significantly reduced by E6795 cream versus vehicle (adjusted $P = 0.000040$; mean difference -19.65 cells/HPF, 95 % CI -25.18 to -14.12 ; Fig. 4e and f). Together,

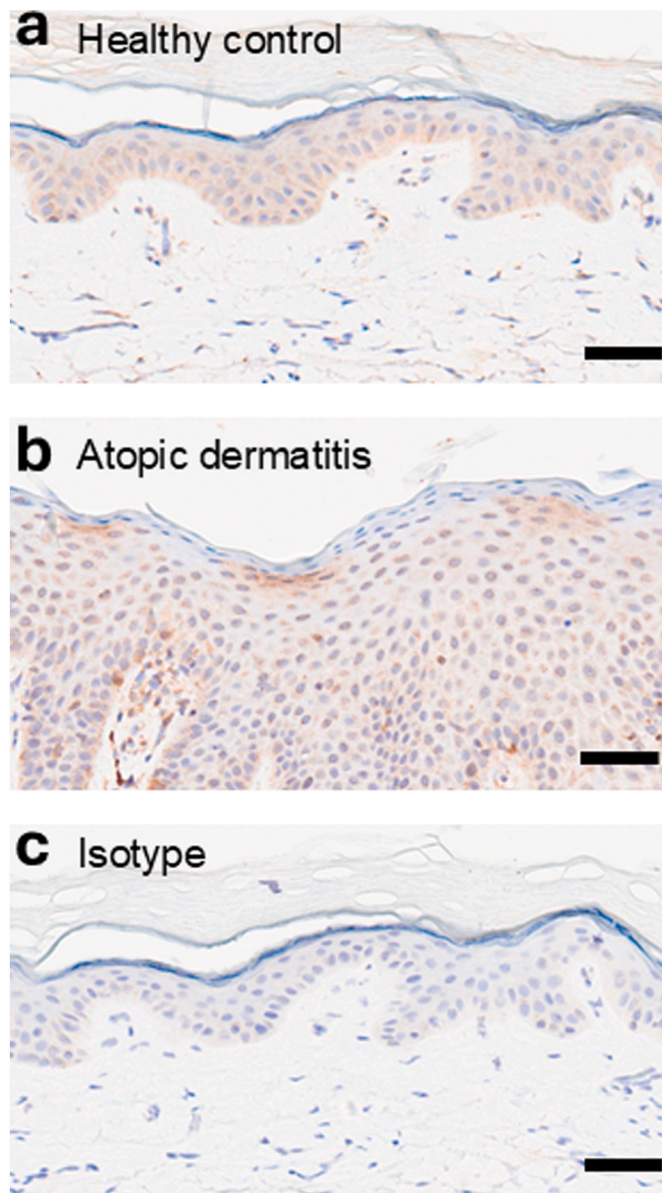


Fig. 2. PAR-2 expression in skin of healthy individuals and AD patients. Immunohistochemical staining of PAR-2 in skin samples from healthy individuals and AD patients. Scale bar: 50 μm .

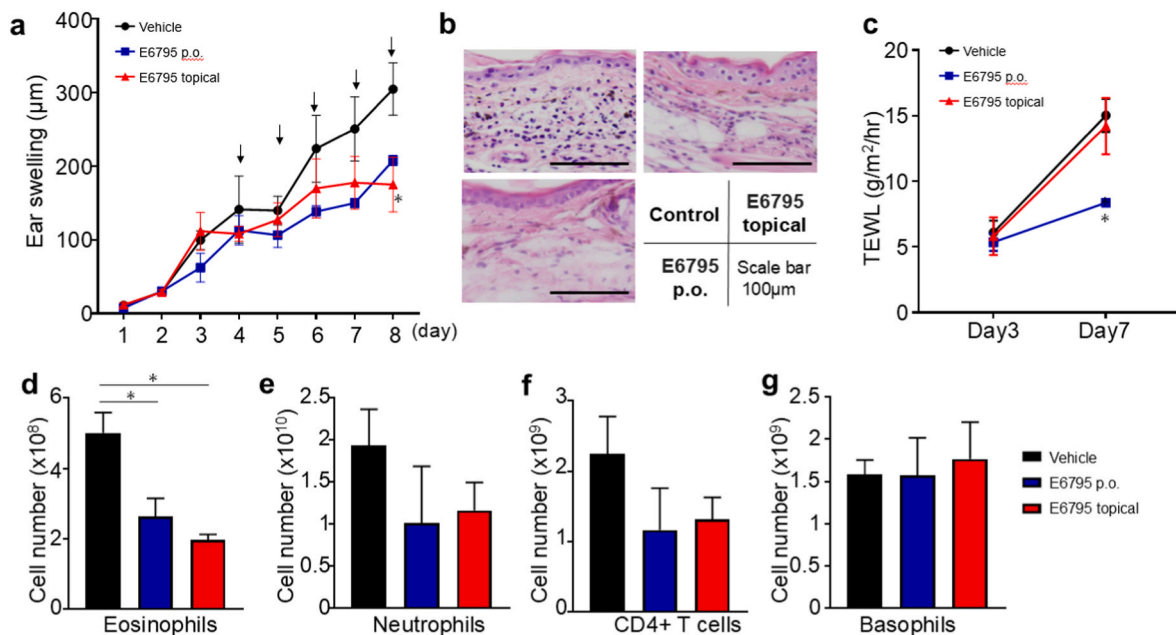


Fig. 3. Effects of E6795 in MC903-induced mouse AD model.

(a) Time course of ear thickness after MC903 application. Mice were treated with vehicle, topical, or oral E6795 from day 4. Arrows indicate the timing of treatment administration: each arrow corresponds to the day on which the respective treatment (oral or topical E6795) was applied. Topical E6795 significantly reduced ear thickness on day 8 compared to the vehicle control. Data are mean $\pm$ SEM ($n = 4$ per group except day 8 oral, $n = 3$). *Adjusted $P < 0.05$ vs vehicle (Dunnett's test). (b) Representative H&E staining of ear skin sections from each treatment group on day 8. Both oral and topical E6795 treatment reduced dermal inflammatory cell infiltration compared to vehicle controls. Scale bar: 100 μ m. (c) Transepidermal water loss (TEWL) measurements on days 3 and 7. Oral E6795 significantly reduced TEWL on day 7 compared to vehicle controls. Data are mean $\pm$ SEM ($n = 4$ per group except day 8 oral, $n = 3$). *Adjusted $P < 0.05$ vs vehicle (Dunnett's test). (d–g) Flow cytometric analysis of skin-infiltrating cells in ear tissue on day 8: (d) eosinophils (CD45+CD11b + SiglecF+), (e) neutrophils (CD45+CD11b + Ly6G+), (f) CD4⁺ T cells (CD45+CD4⁺), and (g) basophils (CD45+CD117-FcεRIα+). Both oral and topical E6795 treatment significantly reduced eosinophil infiltration. Data are mean $\pm$ SEM ($n = 4$ per group except day 8 oral, $n = 3$). *Adjusted $P < 0.05$ vs vehicle (Dunnett's test).

these data show that topical E6795 improves key functional and histological endpoints in the Nc/Nga model.

4. Discussion

In this study, we demonstrated that PAR-2 expression in keratinocytes is upregulated in the skin of patients with AD compared to healthy controls.

In addition to keratinocytes, PAR-2 is also known to be expressed in various cell types within the skin, such as fibroblasts, endothelial cells, mast cells, eosinophils, and other immune cells [4,6]. These cells play significant roles in inflammatory signaling, immune activation, and itch mechanisms associated with atopic dermatitis. Although the present study focused primarily on keratinocyte expression, the potential involvement of these other cell populations expressing PAR-2 warrants further investigation in future studies. Immunohistochemistry in this study was conducted under optimized and identical conditions for both healthy and AD skin samples, with appropriate negative controls (isotype control; Fig. 2c). PAR-2 staining was prominently observed in the granular and upper spinous layers of keratinocytes, consistent with previous reports describing increased PAR-2 expression in AD epidermis [4].

To assess the target specificity of the newly synthesized compound E6795 and to evaluate potential off-target activities or unexpected toxicities, we performed kinase and pharmacological profiling. Kinase profiling demonstrated that E6795 exhibits high selectivity for PAR-2, with minimal off-target inhibition of other kinases relevant to inflammation or immune regulation. While a modest interaction with PI3K was noted, its pharmacological relevance remains uncertain, as no systemic

immunosuppressive or metabolic effects were observed *in vivo*. These findings support the target specificity of E6795 for PAR-2, with a low likelihood of adverse kinase-related off-target effects [11,12].

Pharmacological profiling using the CEREP panel indicated that E6795 exhibits high selectivity, with minimal binding to most receptors, ion channels, and neurotransmitter targets. However, partial binding to GABA-gated chloride channels and the serotonin 5-HT1B receptor was observed, suggesting a potential for off-target effects in neurological contexts. While the therapeutic effects are likely mediated through selective PAR-2 inhibition, further studies are needed to clarify the relevance and safety implications of these interactions.

Given that both GABA and serotonin receptors have been implicated in itch transmission, the partial binding of E6795 to GABA-gated chloride channels and the 5-HT1B receptor may potentially influence pruritic responses. Although the anti-pruritic effects observed in our *in vivo* models are likely mediated through selective PAR-2 inhibition, we cannot entirely exclude the contribution of these off-target interactions. Further mechanistic studies will be necessary to clarify their role in the observed therapeutic outcomes.

Collectively, the kinase and pharmacological profiling data strongly support the high target specificity of E6795 for PAR-2, highlighting minimal off-target activities that would likely limit adverse pharmacological effects.

Pharmacokinetic characterization of E6795 in mice revealed a high plasma protein binding rate ($\sim 97.5\%$), resulting in a low free fraction ($\sim 2.55\%$ in plasma and $\sim 1.53\%$ in whole blood). Although the active, unbound fraction of E6795 is relatively low, the observed robust pharmacological efficacy *in vivo* suggests that even a small fraction of free drug is sufficient to exert meaningful therapeutic effects. These

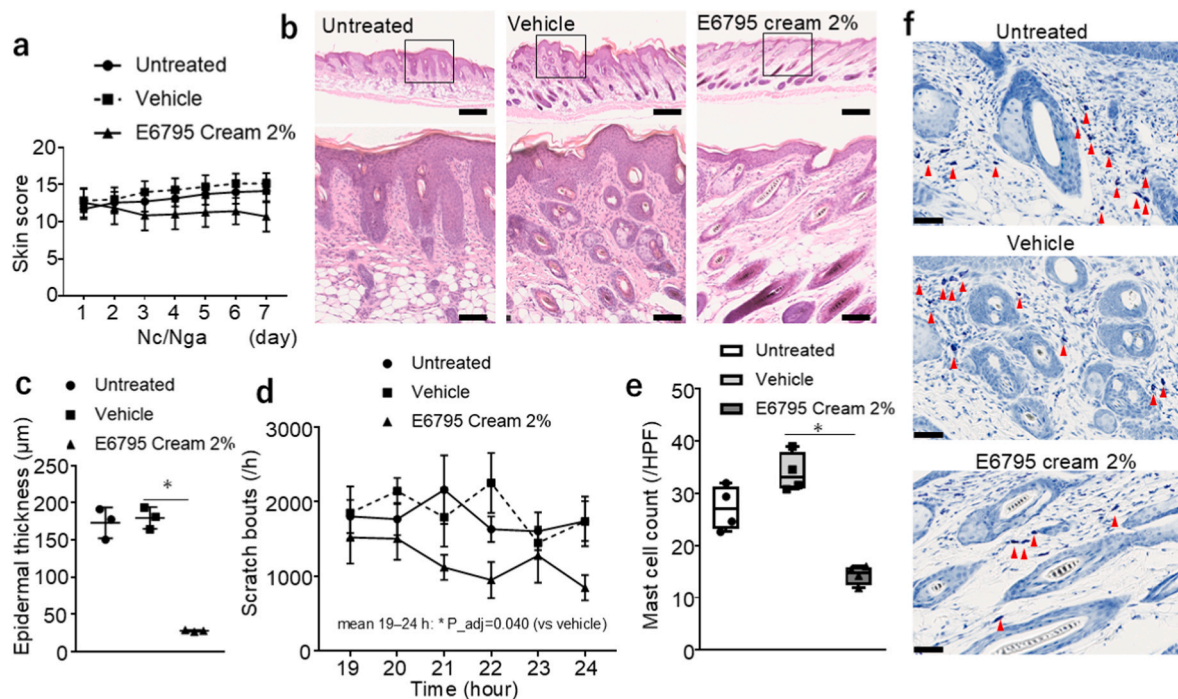


Fig. 4. Effects of 2 % E6795 cream in Nc/Nga mouse model of atopic dermatitis.

(a) Time course of skin score over 7 days of treatment. 2 % E6795 cream showed a numerical reduction versus vehicle but did not reach statistical significance after multiplicity control (adjusted $P = 0.136$, Dunnett's test; not statistically significant). Data are mean $\pm$ SEM ($n = 7$).

(b) Representative H&E staining of ear skin sections from each treatment group on day 8. 2 % E6795 cream reduced epidermal thickening and inflammatory cell infiltration. Scale bars: upper panels, 200 μ m; lower panels, 50 μ m.

(c) Quantification of epidermal thickness. 2 % E6795 cream significantly decreased epidermal thickness compared to the vehicle control. Data are mean $\pm$ SEM ($n = 7$). Adjusted $P < 0.05$ vs. vehicle (Dunnett's test).

(d) Evaluation of scratching behavior on day 7. 2 % E6795 cream primary endpoint (mean across 19–24 h) showed significant reduction. Data are mean $\pm$ SEM ($n = 7$). Adjusted $P = 0.040$ vs. vehicle (Dunnett's test).

(e) Quantification of mast cell numbers in the dermis. 2 % E6795 cream significantly decreased mast cell numbers compared to the vehicle control. Data are mean $\pm$ SEM ($n = 7$). Adjusted $P < 0.05$ vs. vehicle (Dunnett's test), averaged from 5 high-power fields (40 $\times$ objective) per sample.

(f) Representative toluidine blue staining of skin mast cells from each treatment group on day 8. 2 % E6795 cream reduced mast cell infiltration in the dermis. Scale bar: 50 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

pharmacokinetic properties should be considered in future clinical development and formulation strategies.

Following the confirmation of its target selectivity, we next evaluated the *in vivo* efficacy of E6795 in murine models of AD. E6795 exhibited potent anti-inflammatory effects by suppressing dermatitis in AD animal models. Additionally, this novel compound reduced eosinophil infiltration in the skin, scratching behavior, and mast cell infiltration, while improving skin barrier function in these models.

Upregulation and activation of PAR-2 have been implicated in several key aspects of AD pathophysiology [4,13], with strong associations between PAR-2 and inflammation, pruritus, and skin barrier function [5]. These three factors are characteristic hallmarks of AD, and are believed to interact in a complex manner, contributing to disease progression [1]. PAR-2 is widely expressed in various skin cell types and is thought to play a multifaceted role in epidermal homeostasis, barrier formation, immune response, and the sensation of pain and itch [3]. In keratinocytes and skin immune cells of AD patients, PAR-2 is highly expressed and induces inflammatory mediators such as thymic stromal lymphopoietin (TSLP) [2,13,14].

As a member of the protease-activated receptor family, PAR-2 is activated by specific serine and cysteine proteases, including tryptase, kallikrein, cathepsin, and house dust mite allergen proteases [3,15]. In AD patients, disruption of the epidermal barrier facilitates the penetration of external allergens and proteases, such as PAR-2 agonist proteases derived from house dust mites [16,17]. Barrier defects also enhance the activity of endogenous epidermal PAR-2 protease agonists, such as kallikreins-5 and -14, cathepsins, and mast cell tryptase [3,18].

These exogenous and endogenous proteases collectively activate PAR-2, leading to increased release of pruritogenic mediators from keratinocytes and mast cells. This activation stimulates pruriceptive sensory endings positive for transient receptor potential cation channel subfamily V member 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1), ultimately resulting in the sensation of itching [19,20]. Furthermore, recent studies have indicated that the induction of TSLP production and the pathways involved in skin barrier dysfunction are dependent on PAR-2 activation [4,17,21].

Although a few compounds have been reported as PAR-2 inhibitors with efficacy against dermatitis, most are limited to *in vitro* studies [22]. Among the PAR-2 inhibitors demonstrating clear efficacy *in vivo* in animal models of AD, only two have been highlighted in peer-reviewed studies: one from 2018 [23] and another from 2019 [10]. Additionally, there are reports suggesting that *Saussurea lappa* root and *Thuja orientalis* L., traditional herbal medicines used in Korea for the treatment of inflammatory disease such as asthma, rheumatism, coughs, tuberculosis and numerous other diseases—may exhibit PAR-2 inhibitory effects [24]. However, no PAR-2 inhibitor has yet been clinically applied as a pharmaceutical treatment.

Notably, previously reported PAR-2 inhibitors include peptides synthesized via solid-phase peptide synthesis (SPPS) [23] and lipidated peptides designed based on the intracellular loop domain of the receptor [10]. These peptides typically consist of multiple amino acids, with molecular weights ranging from approximately 1100 to 3000. In contrast, E6795 is a small molecule with a molecular weight of 602.1. This smaller size enhances its permeability across cell membranes and

reduces susceptibility to metabolic degradation, allowing it to reach the intracellular environment more effectively. These characteristics provide E6795 with greater flexibility and metabolic stability compared to earlier PAR-2 inhibitors, making it particularly suitable for topical applications.

Recent therapies for AD include biological treatment such as an IL-4/IL-13 inhibitor, an anti-IL-31 receptor A antibody, and an anti-IL-13 antibody. These treatments are primarily used for moderate to severe AD cases resistant to topical steroids [25]. However, they present challenges such as the need for injections and high costs. More affordable and minimally invasive topical treatments currently available include tacrolimus ointment (a topical calcineurin inhibitor), delgocitinib ointment (a JAK inhibitor), difamilast ointment (a phosphodiesterase 4 inhibitor), and tapinarof (an aryl hydrocarbon receptor modulator) [26]. Despite these options, cases resistant to these treatments remain common.

As a limitation, we did not quantify barrier proteins (filaggrin and loricrin) in the Nc/Nga experiments; future work will incorporate their assessment on paraffin-embedded sections to complement TEWL and histology. In addition, because pruritus and inflammation co-occur in the MC903 and Nc/Nga models, we could not isolate direct antipruritic effects; follow-up studies using an itch-specific model, such as the acetone-ether-water (AEW) model, are warranted.

As a statistical limitation, ear thickness was analyzed day-wise (ANOVA→Dunnett) rather than by a mixed-effects repeated-measures model because of small, unbalanced groups; future work will employ mixed-effects modeling when adequately powered.

In conclusion, our study demonstrates that E6795 effectively targets the core pathophysiological triad of AD—inflammation, pruritus, and skin barrier dysfunction—through selective PAR-2 inhibition. By showing robust anti-inflammatory and barrier-protective effects in multiple preclinical models, E6795 provides a promising alternative to existing agents such as JAK inhibitors, phosphodiesterase 4 inhibitors, and aryl hydrocarbon receptor modulators. This distinct mechanism of action not only broadens our therapeutic options but also has the potential to benefit patients who fail to respond adequately to current treatments. Given its demonstrated efficacy, favorable topical formulation profile, and presumed affordability, E6795 could emerge as a novel and clinically impactful addition to the AD treatment arsenal. Future clinical trials will be instrumental in translating these findings into practice, positioning E6795 as a significant step forward in the management of atopic dermatitis.

CRediT authorship contribution statement

Naomi Kitayama: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Chisa Nakashima:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Teruo Sawada:** Resources, Methodology. **Nobuo Tanaka:** Resources, Methodology. **Masatoshi Hagiwara:** Writing – review & editing, Supervision, Resources. **Atsushi Otsuka:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Kenji Kabashima:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Informed consent statement

Written informed consent was obtained from all individuals involved in the study.

Institutional review board statement

This study was approved by the ethics committee of the Graduate School of Medicine, Kyoto University (Kyoto, Japan) (approval number:

R0743).

Data availability statement

The data presented in this study are available upon reasonable request from the corresponding author.

Funding

This research was supported by a Grant-in-Aid for JSPS Fellows (grant number: JP18J20257 to N.K.), a grant from the Japan Agency for Medical Research and Development (AMED) (grant number: 18ek0410035h0003 to A.O.), and a collaborative research grant from Eisai Co., Ltd. (Tokyo, Japan).

Declaration of competing interest

This study was conducted in collaboration with Eisai Co., Ltd. (Tokyo, Japan). The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The authors declare no other conflicts of interest.

Acknowledgments

We acknowledge Kaori Tomari (Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan) and Masahiro Hirata (Department of Diagnostic Pathology, Kyoto University Hospital, Kyoto, Japan) for their technical assistance with immunohistochemical staining and experimental procedures.

We thank Richard S. J. Clark (Japan Section, Collaboration and Incubation Department, Deep Human Biology Learning, Eisai Co. Ltd.) for providing the compound and confirming data integrity at Eisai, Masahiro Kajita (Japan Section, Collaboration and Incubation Department, Deep Human Biology Learning, Eisai Co. Ltd.) for providing the compound, confirming data integrity at Eisai, and valuable scientific discussions, and Dr. Saki Izumi (Global Drug Metabolism and Pharmacokinetics, Biopharmaceutical Assessment, DHBL, Eisai Co. Ltd.) for performing mouse PK experiments.

We also acknowledge the support provided by the Department of Drug Discovery Medicine for Ectodermal Disorders, Kyoto University Graduate School of Medicine, a collaborative research program between Kyoto University and Eisai Co., Ltd.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2025.152590>.

References

- [1] K. Kabashima, New concept of the pathogenesis of atopic dermatitis: interplay among the barrier, allergy, and pruritus as a trinity, *J. Dermatol. Sci.* 70 (2013) 3–11.
- [2] S. Seeliger, C.K. Derian, N. Vergnolle, N.W. Bunnett, R. Nawroth, M. Schmelz, P.-Y. Von Der Weid, J. Buddenkotte, C. Sunderkötter, D. Metz, P. Andrade-Gordon, E. Harms, D. Vestweber, T.A. Luger, M. Steinhoff, Proinflammatory role of proteinase-activated receptor-2 in humans and mice during cutaneous inflammation in vivo, *FASEB J.* 17 (2003) 1871–1885.
- [3] S.E. Lee, S.K. Jeong, S.H. Lee, Protease and protease-activated receptor-2 signaling in the pathogenesis of atopic dermatitis, *Yonsei Med. J.* 51 (2010) 808–822.
- [4] M. Steinhoff, U. Neisius, A. Ikoma, M. Fartasch, G. Heyer, P.S. Skov, T.A. Luger, M. Schmelz, Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin, *J. Neurosci.* 23 (2003) 6176–6180.
- [5] T. Buhl, A. Ikoma, C. Kempkes, F. Cevikbas, M. Sulk, J. Buddenkotte, T. Akiyama, D. Crumrine, E. Camerer, E. Carstens, M.P. Schön, P. Elias, S.R. Coughlin, M. Steinhoff, Protease-activated receptor-2 regulates neuro-epidermal communication in atopic dermatitis, *Front. Immunol.* 11 (2020) 1740.
- [6] S. Frateschi, E. Camerer, G. Crisante, S. Rieser, M. Membrez, R.-P. Charles, F. Beermann, J.-C. Stehle, B. Breiden, K. Sandhoff, S. Rotman, M. Haftek, A. Wilson,

- S. Ryser, M. Steinhoff, S.R. Coughlin, E. Hummler, PAR2 absence completely rescues inflammation and ichthyosis caused by altered CAP1/Prss8 expression in mouse skin, *Nat. Commun.* 2 (2011) 161.
- [7] A.J. Kennedy, L. Sundström, S. Geschwindner, E.K.Y. Poon, Y. Jiang, R. Chen, R. Cooke, S. Johnstone, A. Madin, J. Lim, Q. Liu, R.-J. Lohman, A. Nordqvist, M. Fridén-Saxin, W. Yang, D.G. Brown, D.P. Fairlie, N. Dekker, Protease-activated receptor-2 ligands reveal orthosteric and allosteric mechanisms of receptor inhibition, *Commun. Biol.* 3 (2020) 782.
- [8] K. Stefansson, M. Brattsand, D. Roosterman, C. Kempkes, G. Bocheva, M. Steinhoff, T. Egelrud, Activation of proteinase-activated receptor-2 by human kallikrein-related peptidases, *J. Invest. Dermatol.* 128 (2008) 18–25.
- [9] M. Henehan, A. De Benedetto, Update on protease-activated receptor 2 in cutaneous barrier, differentiation, tumorigenesis and pigmentation, and its role in related dermatologic diseases, *Exp. Dermatol.* 28 (2019) 877–885.
- [10] T.P. Barr, C. Garzia, S. Guha, E.K. Fletcher, N. Nguyen, A.J. Wieschhaus, L. Ferrer, L. Covic, A. Kuliopulos, PAR2 pepducin-based suppression of inflammation and itch in atopic dermatitis models, *J. Invest. Dermatol.* 139 (2019) 412–421.
- [11] E. Robak, T. Robak, Bruton's kinase inhibitors for the treatment of immunological diseases: current status and perspectives, *J. Clin. Med.* 11 (2022) 2807.
- [12] I.-H. Huang, W.-H. Chung, P.-C. Wu, C.-B. Chen, JAK-STAT signaling pathway in the pathogenesis of atopic dermatitis: an updated review, *Front. Immunol.* 13 (2022) 1068260.
- [13] M. Steinhoff, C.U. Corvera, M.S. Thoma, W. Kong, B.E. McAlpine, G.H. Caughey, J. C. Ansel, N.W. Bunnett, Proteinase-activated receptor-2 in human skin: tissue distribution and activation of keratinocytes by mast cell tryptase, *Exp. Dermatol.* 8 (1999) 282–294.
- [14] A. Briot, C. Deraison, M. Lacroix, C. Bonnart, A. Robin, C. Besson, P. Dubus, A. Hovnanian, Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome, *J. Exp. Med.* 206 (2009) 1135–1147.
- [15] S. Seeley, L. Covic, S.L. Jacques, J. Sudmeier, J.D. Baleja, A. Kuliopulos, Structural basis for thrombin activation of a protease-activated receptor: inhibition of intramolecular liganding, *Chem. Biol.* 10 (2003) 1033–1041.
- [16] M.D. Chapman, S. Wüschmann, A. Pomés, Proteases as Th2 adjuvants, *Curr. Allergy Asthma Rep.* 7 (2007) 363–367.
- [17] T. Kato, T. Takai, T. Fujimura, H. Matsuoka, T. Ogawa, K. Murayama, A. Ishii, S. Ikeda, K. Okumura, H. Ogawa, Mite serine protease activates protease-activated receptor-2 and induces cytokine release in human keratinocytes, *Allergy* 64 (2009) 1366–1374.
- [18] J.-P. Hachem, E. Houben, D. Crumrine, M.-Q. Man, N. Schurer, T. Roelandt, E. H. Choi, Y. Uchida, B.E. Brown, K.R. Feingold, P.M. Elias, Serine protease signaling of epidermal permeability barrier homeostasis, *J. Invest. Dermatol.* 126 (2006) 2074–2086.
- [19] S.R. Wilson, L. Thé, L.M. Batia, K. Beattie, G.E. Katibah, S.P. McClain, M. Pellegrino, D.M. Estandian, D.M. Bautista, The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch, *Cell* 155 (2013) 285–295.
- [20] T. Liu, R.-R. Ji, Role of PAR-2 in neuroimmune communication and itch, in: *Itch*, CRC Press, 2014, pp. 215–234.
- [21] D. Redhu, K. Franke, V. Kumari, W. Francuzik, M. Babina, M. Worm, Thymic stromal lymphopoietin production induced by skin irritation results from concomitant activation of protease-activated receptor 2 and interleukin 1 pathways, *Br. J. Dermatol.* 182 (2020) 119–129.
- [22] Y.A. Joo, H. Chung, S. Yoon, J.I. Park, J.E. Lee, C.H. Myung, J.S. Hwang, Skin barrier recovery by protease-activated receptor-2 antagonist lobaric acid, *Biomol. Ther. (Seoul)* 24 (2016) 529–535.
- [23] B. Kim, H.-S. Kim, Novel peptide inhibits inflammation by suppressing of protease activated receptor-2, *Eur. J. Pharmacol.* 832 (2018) 25–32.
- [24] H.J. Yang, M.J. Kim, S. Kang, N.R. Moon, D.S. Kim, N.R. Lee, K.S. Kim, S. Park, Topical treatments of *Saussurea costus* root and *Thuja orientalis* L. synergistically alleviate atopic dermatitis-like skin lesions by inhibiting protease-activated receptor-2 and NF- κ B signaling in HaCaT cells and Nc/Nga mice, *J. Ethnopharmacol.* 199 (2017) 97–105.
- [25] M.J. Gooderham, H.C.-H. Hong, P. Eshtiaghi, K.A. Papp, Dupilumab: a review of its use in the treatment of atopic dermatitis, *J. Am. Acad. Dermatol.* 78 (2018) S28–S36.
- [26] S.J. Keam, Correction: tapinarof cream 1%: first approval, *Drugs* 82 (2022) 1433.