Inhibitory Effect of the 4-Aminotetrahydroquinoline Derivatives, Selective Chemoattractant Receptor-Homologous Molecule Expressed on T Helper 2 Cell Antagonists, on Eosinophil Migration Induced by Prostaglandin D₂

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ABSTRACT

Prostaglandin (PG) D₂, a major cyclooxygenase metabolite generated from immunologically stimulated mast cells, is known to induce activation and chemotaxis in eosinophils, basophils, and T helper 2 (Th2) lymphocytes via a newly identified PGD₂ receptor, chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2). CRTH2 is hypothesized to play an important role in the outcome of allergic responses. However, the absence of selective CRTH2 antagonists has prevented the elucidation of the role of CRTH2 in pathogenesis of allergic diseases. We now report compounds discovered as selective CRTH2 antagonists, (2'R,4'S)-(N-(1-benzoyl-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)-N-phenylisobutryamide (K117) and (2'R,4'S)-(N-(1-benzoyl-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)-N-phenylcyclopropenecarboxamide (K604). K117 and K604 have inhibitory effects on human CRTH2 with Kᵢ values of 5.5 and 11 nM, respectively. The effect of these compounds is CRTH2-specific with no cross-reactivity against 15 other receptors and four arachidonic acid-metabolizing enzymes. K117 and K604 has no effect on the basal Ca²⁺ level and inhibited the Ca²⁺ response induced by PGD₂ in 293EBNA cells expressing human CRTH2. Also, K117 and K604 inhibit PGD₂-induced human eosinophil chemotaxis with IC₅₀ values of 7.8 and 42.2 nM, respectively, but they do not inhibit the CC-chemokine receptor 3 agonist eotaxin-induced chemotaxis. These results indicate that K117 and K604 are highly potent and selective antagonists for human CRTH2. These compounds have possibilities to become useful tools to explore CRTH2 functions in allergic diseases.

Prostaglandin (PG) D₂ is a major cyclooxygenase metabolite produced by mast cells responding to IgE-dependent stimuli (Lewis et al., 1982). In patients with allergic rhinitis, bronchial asthma, and atopic dermatitis, PGD₂ levels increase dramatically after allergen challenge in nasal and bronchial lavage fluids, and skin chamber fluids, respectively (Naclerio et al., 1983, 1985; Murray et al., 1986; Charlesworth et al., 1991).

PGD₂ exerts a variety of actions in various tissues and cells via two G protein-coupled receptors, prostaglandin D₂ receptor (DP) and chemoattractant receptor-homologous molecule expressed on T helper type 2 cells (CRH2T2) (Boie et al., 1995; Hirai et al., 2001). CRTH2 was cloned as a Th2-specific marker by differential display (Nagata et al., 1999). CRTH2-mediated signals enhance the IL-2, IL-4, IL-5, and IL-13 production by Th2 cells and cause up-regulation of CD11b and CD40L in resting Th2 cells (Tanaka et al., 2004). CRTH2 is expressed not only on Th2 cells but also on eosinophils and basophils and induces their migration (Hirai et al., 2001).

ABBREVIATIONS: PG, prostaglandin; DP, prostaglandin D₂ receptor; CRTH2, chemoattractant receptor-homologous molecule expressed on T helper 2 cells; Th2, T helper 2; IL, interleukin; TP, thromboxane-type prostanoid; K376, (2'R,4'S)-(3-benzazepine; SQ29548, 1R,3S)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; S029548, [1S-[1α,2α(Z),3α,4α]]-7-[3-[(2-[[phenylamino][carbonyl][hydrzino][methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; HBSS, Hank’s balanced salt solution; DMSO, dimethyl sulfoxide.
Gervais et al. (2001) have demonstrated that PGD₂ induces degranulation of eosinophil via CRTH2 stimulation. Since PGD₂ has been shown to enhance the release of leukotriene C₄ in response to the calcium ionophore A23187 in eosinophils (Raible et al., 1992), this phenomenon may be through the activation of CRTH2. These reports have strongly suggested the critical role of PGD₂ and CRTH2 in allergic diseases. The pharmacological blockade of PGD₂-mediated events at CRTH2 receptors may then reduce the allergic pathogenesis caused by activated Th2 cells, basophils, and eosinophils. However, the importance of CRTH2 in allergic pathogenesis still remains to be clarified. To elucidate the implications of CRTH2 in these pathological conditions, one of the best tools is a selective ligand. Recently, ramatroban (BAY u3405), a thromboxane-type prostanoid (TP) receptor antagonist, has been revealed to be a CRTH2 antagonist (Sugimoto et al., 2003), and indomethacin, a cyclooxygenase inhibitor, has been identified as a CRTH2 agonist (Hirai et al., 2002). The use of these compounds is, however, limited due to the other activities they have in addition to their effects on CRTH2. Therefore, we have explored molecules specific for CRTH2. We now report a small molecule agonist K376 and antagonists K117 and K604, which are selective for CRTH2 and the effects of these compounds on eosinophil migration.

Materials and Methods

Chemicals. The sources of materials used in this study were as follows: [5,6,8,9,12,14,15(α)]-3H]prostaglandin D₂ ([3H]PGD₂, 192 Ci/mm) was from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK); PGD₂, BW245c, and 13,14-dihydro-15-keto-PGD₂ (DK-PGD₂) were from Cayman Chemical (Ann Arbor, MI); indomethacin was from Sigma-Aldrich (St. Louis, MO); Fura 2-acetoxyethyl ester (Fura 2/AM) was from Dojindo (Kumamoto, Japan); human recombinant eotaxin was from Strathmann Biotech (Hanover, Germany); and fetal bovine serum was from Serologicals Corp. (Norcross, GA). K117, K376, and K604 were synthesized in Research Laboratories of Kyowa Hakko Kogyo. All other chemicals and solvents were used in their analytical pure forms.

Construction of pAMoh-hCRTH2 Mammalian Expression Vector. The human CRTH2 cDNA was generated by reverse transcription-polymerase chain reaction using a human leukocyte total RNA as a template and the following primers: 5'-CCAAGCTTCCACCATGTCGGCCAACGCCACACTG-3' (sense) and 5'-TAGGTACCTAAGTACCTACTCGAGGTGCTGCTCAGC-3' (antisense). The amplified DNA fragment was cloned into pCR-Blunt vector using Zero Blunt polymerase chain reaction cloning kit (Invitrogen, Carlsbad, CA). The nucleotide sequence of cloned cDNA was confirmed to be identical to the reported sequence (GenBank accession no. AY507142). The human CRTH2 cDNA was subsequently subcloned into HindIII-Asp718 sites in the mammalian episomal expression vector pAMoh (the hygromycin B resistant-type of pAMo vector; Sasaki et al., 1993) for generation of a cell line stably expressing human CRTH2.

Cells and Culture. The cell line 293EBNA was purchased from Invitrogen and maintained in culture in a humidified atmosphere at 37°C in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 250 μg/ml G418 (for EBNA selection). Stable expression of human CRTH2 in 293EBNA cells was achieved by transfection of the pAMoh-hCRTH2 plasmid using FuGENE 6 reagent (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions. Cells were maintained in culture for 48 h post-transfection and then grown in the presence of 300 μg/ml hygromycin B (Wako Junyaku, Osaka, Japan) for 2 weeks to select for resistant cells expressing the human CRTH2 receptor (293EBNA/hCRTH2).

Fig. 1. Chemical structures of 4-aminotetrahydroquinoline derivatives and effect of compounds on [3H]PGD₂ binding to the membranes prepared from CRTH2-expressing cells. A, Scatchard plot of [3H]PGD₂ binding to the membranes prepared from CRTH2-expressing cells. B, chemical structures of K376 (a), K117 (b), and K604 (c). C, inhibitory effect of compounds on the [3H]PGD₂ binding to the membranes prepared from CRTH2-expressing cells. The membranes were incubated with various concentrations of compounds (K376, closed circles; K117, closed squares; and K604, closed triangles) and 1 nM [3H]PGD₂. The concentration-response curves were obtained from assays performed in duplicate at four concentrations of each drug.
Receptor Binding Assay. Cultured 293EBNA/hCRTH2 cells were homogenized in ice-cold assay buffer (10 mM Hepes-KOH, pH 7.4, 1 mM EDTA, and 10 mM MnCl₂) using a Polytron homogenizer (Kinetica, Luzern, Switzerland). The homogenate was centrifuged at 40,000g for 20 min at 4°C, and then the pellet was suspended in assay buffer and stored frozen at −80°C until use.

[14C]PGD₂ binding to the membrane fraction of 293EBNA/hCRTH2 was measured by a modification of the method described by Wright et al. (1998). Briefly, the reaction mixture containing an assay buffer, the membrane suspension (20 μg of 293EBNA/hCRTH2), 0.5% bovine serum albumin, and 1 nM [14C]PGD₂ was incubated for 120 min at 25°C in a volume of 0.2 ml. Nonspecific binding was determined in the presence of 1 μM PGD₂. Under these conditions, specific binding (total minus nonspecific) of the radioligand to the receptor reached equilibrium. The binding reactions were terminated by filtration through GF/B filters (Whatman, Maidstone, UK) under reduced pressure using a MT-24 cell harvester (Brandel Inc., Gaithersburg, MD). The filters were washed three times with ice-cold assay buffer and placed in scintillation vials, and the bound radioactivity was determined using a liquid scintillation counter (LS6500; Beckman Coulter, Fullerton, CA). The concentration-response curves were obtained from assays performed in duplicate at four concentrations of each drug.

Selectivity Panel. The binding assays for 15 receptors and the enzymatic assays for four arachidonic acid metabolizing enzymes were run by MDS Pharma Services (Bothell, WA). The target runs were adrenergic receptors α₁ (nonselective), α₂ (nonselective), and β (nonselective); dopamine D₁ and D₂ receptors; histamine H₁ and H₂ receptors; muscarinic receptor M₃; serotonin receptors 5-HT₁A and 5-HT₂ (nonselective); leukotriene D₄ receptor (Cys-LT₁); leukotriene B₄ receptor (LTB₄); platelet-activating factor (PAF) receptors; thromboxane A₂ receptor (TXA₂); and thromboxane (TX) synthase. All assays were run using recombinant human receptors and enzymes, except where noted. The full methods and references can be found on the MDS Pharma Services Web site (http://www.mdsps.com/). The assays were run at 1 μM of all test compounds, and the percentage of inhibition is given as the average of two experiments performed in duplicate.

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<th>Source of Target</th>
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<tr>
<td>Rat brain</td>
<td>0.25 nM [3H]prazosin</td>
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<td>Rat cerebral cortex</td>
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The chemoattractants in chemotaxis assay buffer (0.5 ml) were placed in the lower chamber. In the upper chamber, purified eosinophils (1 × 10^5 cells) were loaded in a volume of 0.1 ml. The Transwells were then incubated for 3 h at 37°C in a 5% CO2 humidified atmosphere. The migrated cells into the lower chamber were recovered and counted by a flow cytometry (BD Biosciences, San Jose, CA). For evaluation of agonistic activity on human CRTH2, the test compounds in chemotaxis assay buffer were placed in the lower chamber without any chemoattractants, and the cells were loaded into the upper chamber. For evaluation of antagonistic activity on human CRTH2, the test compounds were placed in the lower chamber with chemoattractants, and the cells incubated with the test compound for 15 min at 37°C were loaded into the upper chamber. For the control wells, chemotaxis assay buffer with 0.1% DMSO was used. As the chemoattractants, 100 nM PGD2 and 10 ng/ml human recombinant eotaxin were used as a CRTH2 agonist and a CCR3 agonist, respectively. All experiments were performed in triplicate.

**Statistical Analysis.** Data are expressed as mean ± S.E.M. The statistical significance was determined by one-way analysis of variance, followed by William's test for multiple comparisons using Statistical Analysis System software (SAS Institute, Cary, NC). When comparing only two groups, the difference in means was analyzed by the Aspin-Welch test using SAS software.

**Results**

**Competition for [3H]PGD2-Specific Binding to Membrane of 293EBNA/hCRTH2 Cells.** The analysis of [3H]PGD2 binding to human CRTH2 and the Scatchard transformation were shown in Fig. 1A. As reported previously (Sawyer et al., 2002), [3H]PGD2 bound with high affinity (K_d = 1.4 ± 0.035 nM, B_max = 1758 ± 19 fmol/mg membrane protein) to the membranes prepared from 293EBNA/hCRTH2. We identified three low molecular weight compounds, K376, K117, and K604 (Fig. 1B), having potent inhibitory effects in this equilibrium competition assay. Then, further evaluation was performed to reveal their ability to compete with [3H]PGD2-specific binding. These compounds inhibited the binding of [3H]PGD2 to human CRTH2 in a concentration-dependent manner (Fig. 1C). The K_i values of K376, K117, and K604 for hCRTH2 were 91, 5.5, and 11 nM, respectively.

**Fig. 2.** Responses induced by K376 in 293EBNA cells stably expressing CRTH2. K376 was added at 0.1, 1, and 10 μM to Fura 2/AM-loaded cells and intracellular Ca^2+ -dependent fluorescence changes were recorded. A, vector-transfected 293EBNA cells did not show any responsiveness to K376. B, dose response of 293EBNA/CRTH2 cells to K376. K376 showed CRTH2 mediated agonistic activity. Arrows indicate the time points of compound addition. The vertical axis shows the 340:380 ratios. Studies are representatives of three separate experiments.
radioligands for \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptors, \( \beta \)-adrenoceptors, dopamine \( D_1 \) and \( D_2 \) receptors, histamine \( H_1 \) and \( H_2 \) receptors, muscarinic \( M_1 \) receptors, serotonin 5-HT\(_{1A}\) and 5-HT\(_{2}\) receptors, Cys-LT\(_1\) receptors, BLT\(_1\) receptors, PAF receptors, thromboxane \( A_2 \) receptors, and prostanoid DP receptors and on the enzymatic activities of arachidonic acid-metabolizing enzymes such as COX-1, COX-2, 5-LO, and TX synthase (Table 1).

**Effects of K376, K117, and K604 on Ca\(^{2+}\) Mobilization in 293EBNA/hCRTH2 Cells.** PGD\(_2\) induced Ca\(^{2+}\) mobilization in 293EBNA/hCRTH2 cells at nanomolar concentrations, but it did not in mock-transfected 293EBNA/Vec cells even at 10 \( \mu \)M (data not shown). K376 induced Ca\(^{2+}\) mobilization in 293EBNA/hCRTH2 cells at submicromolar concentrations with around 1 order of magnitude lower potency than that of PGD\(_2\) (Fig. 2B); however, K376 induced no significant Ca\(^{2+}\) mobilization in the mock-transfected 293EBNA/Vec cells even at 10 \( \mu \)M (Fig. 2A). Having once responded to PGD\(_2\), 293EBNA/hCRTH2 cells did not show any responses to K376 in the Ca\(^{2+}\) mobilization assay and vice versa (data not shown).

On the other hand, although K117 and K604 showed no effect on the Ca\(^{2+}\) mobilization in 293EBNA/hCRTH2 cells, K117 and K604 inhibited the Ca\(^{2+}\) mobilization induced by 3 nM PGD\(_2\) in a concentration-dependent manner (Fig. 3, A and B, respectively).

**Agonistic Effect of K376 on Human Eosinophil Chemotaxis.** Consistent with the previous observation (Hirai et al., 2001), human eosinophils exhibited chemotactic responses to either CRTH2 agonist PGD\(_2\) or DK-PGD\(_2\), but not to DP agonist BW245c (Fig. 4A). Also, a cyclooxygenase inhibitor, indomethacin, which has been reported to have an agonistic activity on human CRTH2 (Hirai et al., 2002), induced a chemotactic activity of human eosinophils (Fig. 4B). K376, a compound showing agonistic activity on human CRTH2 in the Ca\(^{2+}\) mobilization assay, induced the weak human eosinophil chemotaxis at the concentration of 10 \( \mu \)M (Fig. 4C).

**Antagonistic Effects of K117 and K604 on PGD\(_2\)-Induced Human Eosinophil Chemotaxis.** The optimal PGD\(_2\) concentration of 100 nM and eotaxin concentration of 10 ng/ml were used to evaluate the antagonistic activity of the compounds. Both K117 and K604, which showed antagonistic activity on human CRTH2 in the Ca\(^{2+}\) mobilization assay, inhibited the PGD\(_2\)-induced human eosinophil chemotaxis in a concentration-dependent manner (Fig. 5, A and B). The inhibitory concentration 50% (IC\(_{50}\)) values were 7.8 and 42.2 nM, respectively. On the other hand, neither K117 nor K604 influenced eosinophil chemotaxis induced by eotaxin, the CCR3 agonist, at 1 \( \mu \)M (Fig. 5C).

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**Fig. 3.** Effects of K117 and K604 on PGD\(_2\)-induced Ca\(^{2+}\) mobilization in 293EBNA cells stably expressing CRTH2. K117 (A) and K604 (B) were added at 0.1, 0.3, and 1 \( \mu \)M to Fura 2/AM-loaded cells, and then PGD\(_2\) was added at 3 nM. Intracellular Ca\(^{2+}\)-dependent fluorescence changes were recorded. Intracellular Ca\(^{2+}\) mobilizations induced by PGD\(_2\) were antagonized by K117 and K604. Arrows indicate the time points of compound addition. The vertical axis shows the 340:380 ratios. Studies are representatives of three separate experiments.
CRTH2, identified as one of the two PGD₂ receptors, has been speculated to have a critical role in allergic diseases (Hirai et al., 2001). However, the precise role of CRTH2 in allergic pathogenesis still remains to be clarified. One of the best tools for probing the function of this receptor is a CRTH2-specific ligand. Ramatroban, a TP receptor antagonist, has recently been revealed to be a CRTH2 antagonist (Sugimoto et al., 2003). However, the use of ramatroban as a CRTH2 antagonist is limited due to its TP antagonistic activity, although its CRTH2 antagonistic activity is more potent than its TP antagonistic activity. The object of this study is to identify a potent and selective CRTH2 antagonist.

To explore the CRTH2 antagonists, we used a PGD₂ binding assay with 293EBNA cell membranes expressing human CRTH2. Saturation analysis of [³H]PGD₂-specific binding to human CRTH2 revealed the presence of a specific binding site with an equilibrium dissociation constant (Kₐ) of 1.4 nM. This value is 1 order of magnitude lower than the value (31.3 nM) reported previously by Hirai et al. (2001), in which a whole cell binding assay was performed. This dissociation may reflect the differences in experimental settings such as buffer constitution, incubation time, reaction temperature, and human CRTH2 expression system. It has also been reported that saturation analysis of [³H]PGD₂-specific binding to human embryonic kidney 293 (EBNA) cell membranes expressing CRTH2 reveals the presence of high-affinity binding sites with a Kₐ value of 2.5 nM, although there also exists low-affinity binding sites (Sawyer et al., 2002).

Using an equilibrium competition binding assay, we have identified 4-aminotetrahydroquinoline derivatives having potent inhibitory activity on human CRTH2. The Kᵢ values of K376, K117, and K604 are 91, 5.5, and 11 nM, respectively.

**Discussion**

CRTH2, identified as one of the two PGD₂ receptors, has been speculated to have a critical role in allergic diseases (Hirai et al., 2001). However, the precise role of CRTH2 in allergic pathogenesis still remains to be clarified. One of the best tools for probing the function of this receptor is a CRTH2-specific ligand. Ramatroban, a TP receptor antagonist, has recently been revealed to be a CRTH2 antagonist (Sugimoto et al., 2003). However, the use of ramatroban as a CRTH2 antagonist is limited due to its TP antagonistic activity, although its CRTH2 antagonistic activity is more potent than its TP antagonistic activity. The object of this study is to identify a potent and selective CRTH2 antagonist.

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Using an equilibrium competition binding assay, we have identified 4-aminotetrahydroquinoline derivatives having potent inhibitory activity on human CRTH2. The Kᵢ values of K376, K117, and K604 are 91, 5.5, and 11 nM, respectively.
K117 and K604 are more potent than ramatroban because calculated from the reported IC\textsubscript{50} value (Sugimoto et al., 2003), K\textsubscript{i} value of ramatroban is 86 nM. The selectivity of our compounds for CRTH2 is demonstrated by the lack of binding to other representative G protein-coupled receptors, including DP receptors (Table 1).

We have further evaluated these compounds by Ca\textsuperscript{2+} mobilization assay to identify whether these compounds are antagonists. When PGD\textsubscript{2} is added, 293EBNA/CORTH2 cells show intracellular Ca\textsuperscript{2+} mobilization, but the vector-transfected 293EBNA cells do not show any responses to PGD\textsubscript{2}, even at a concentration of 10 nM. Therefore, intracellular Ca\textsuperscript{2+} mobilization in 293EBNA/CORTH2 induced by PGD\textsubscript{2} can be the specific response via CRTH2. This result is consistent with a previous report that revealed that CRTH2 can induce Ca\textsuperscript{2+} influx (Hirai et al., 2001). In our assay system, K117 and K604 do not influence the basal Ca\textsuperscript{2+} level in 293EBNA/CORTH2 cells by themselves, but they do inhibit intracellular Ca\textsuperscript{2+} mobilization induced by 3 nM PGD\textsubscript{2} in a concentration-dependent manner (Fig. 3). The concentration of 1 nM is sufficient to completely ablate the response. Therefore, these results suggest that K117 and K604 have an antagonistic activity on human CRTH2 without any agonistic activity. On the other hand, K376 induces intracellular Ca\textsuperscript{2+} mobilization in a concentration-dependent manner in 293EBNA/CORTH2 cells, even though the potency is weaker than PGD\textsubscript{2} and DK-PGD\textsubscript{2}. CRTH2 full agonists. Since the potency of K376 in inducing eosinophil chemotaxis is also comparatively weak in spite of its potent activity in the binding assay (Figs. 1C and 4C), K376 may be a partial agonist, not a full agonist of human CRTH2. The structures of K376, K117, and K604 are very similar. Therefore, it can be possible to manipulate the structure to convert an agonist to an antagonist and vice versa. However, more information about the structure-activity relationship is required, which we are now investigating.

CRTH2 is expressed on human eosinophils, and once activated by PGD\textsubscript{2}, a chemoattractant response is induced (Hirai et al., 2001). On the other hand, DP is also expressed on human eosinophils, and DP has been considered to negatively regulate the action of other mediators (Monneret et al., 2005). According to previous studies, PGD\textsubscript{2} induces the chemotactic response of eosinophils in our assay system. DK-PGD\textsubscript{2}, a metabolite of PGD\textsubscript{2} and selective CRTH2 agonist, also induces eosinophil migration, but BW245c, a selective DP agonist, does not. Therefore, it has been confirmed that eosinophils show a chemoattractant response via CRTH2. Since both K117 and K604 inhibit eosinophil migration induced by 100 nM PGD\textsubscript{2}, but not by eotaxin, an agonist of CCR3 chemokine receptor, these compounds selectively inhibit the response via CRTH2 (Fig. 5, A and B). K117 is a more potent antagonist than K604 because an IC\textsubscript{50} value of K117 (7.8 nM) is lower than that of K604 (42.2 nM). This result is consistent with the results from the competitive binding assay and Ca\textsuperscript{2+} mobilization assay (Figs. 1C and 3). Furthermore, K117 and K604 are more potent compounds than ramatroban because it has been reported that ramatroban has an IC\textsubscript{50} value of 170 nM in the eosinophil migration assay in which 1 nM PGD\textsubscript{2} is used as a chemoattractant (Sugimoto et al., 2003).

In allergic responses, there are two phases of reactions. One is the immediate type reaction induced by mast cell-activated mediators, including histamine and leukotrienes (Naclerio, 1988). The other is late reaction induced by migrated inflammatory cells, such as eosinophils and lymphocytes especially the Th2 phenotype, several hours after an immediate reaction (Frew and Kay, 1990). PGD\textsubscript{2} is well known as an important mediator of the immediate reaction in addition to histamine and leukotrienes because plenty of PGD\textsubscript{2} is immediately released after the antigen-antibody reaction from mast cells (Schleimer et al., 1985; Murray et al., 1986). It has also been indicated that PGD\textsubscript{2} is continuously released several hours after the immediate reaction (Reddy and Herschman, 1997). Therefore, PGD\textsubscript{2} may work as a mediator in the late allergic reaction through activation of CRTH2 expressed on eosinophils and Th2 cells. To reveal the roles of CRTH2 in the allergic pathology, the evaluation with the CRTH2 antagonists in the animal allergic models is a beneficial approach. K117 and K604, potent and selective CRTH2 antagonists, can be suitable tools in this approach.

In conclusion, we have identified low molecular weight compounds K117 and K604 as selective and potent CRTH2 antagonists. These compounds can be useful tools to explore the CRTH2 functions in allergic diseases in the future.

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