The Orally Available Spleen Tyrosine Kinase Inhibitor 2-[7-(3,4-Dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino]-nicotinamide Dihydrochloride (BAY 61-3606) Blocks Antigen-Induced Airway Inflammation in Rodents

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ABSTRACT

Spleen tyrosine kinase (Syk) tyrosine kinase plays essential roles in receptors for Fc portion of immunoglobulins and B cell receptor complex signaling in various inflammatory cells; therefore, inhibitors of Syk kinase may show potential as antiasthmatic/allergic therapeutics. We identified 2-[7-(3,4-dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino]-nicotinamide dihydrochloride (BAY 61-3606), a potent (IC₅₀ values between 5 and 46 nM) and selective inhibitor of Syk kinase. BAY 61-3606 inhibited not only degranulation but also lipid mediator and cytokine synthesis in mast cells. BAY 61-3606 was highly efficacious in basophils obtained from healthy human subjects (IC₅₀ = 10 nM) and seems to be at least as potent in basophils obtained from atopic (high serum IgE) subjects (IC₅₀ = 8.1 nM). B cell receptor activation and receptors for Fc portion of IgG signaling in eosinophils and monocytes were also potently suppressed by BAY 61-3606. Oral administration of BAY 61-3606 to rats significantly suppressed antigen-induced passive cutaneous anaphylactic reaction, bronchoconstriction, and bronchial edema at 3 mg/kg. Furthermore, BAY 61-3606 attenuated antigen-induced airway inflammation in rats. Based on these anti-inflammatory effects of BAY 61-3606 both in vitro and in vivo, it was demonstrated that Syk may play a very critical role in the pathogenesis of allergic reactions.

Spleen tyrosine kinase (Syk) is a cytosolic 72-kDa protein tyrosine kinase that plays an essential role in high-affinity IgE receptor (FceRI)-mediated signaling in mast cells and basophils (Beaven and Baumgartner, 1996). Mast cells developed from Syk-deficient mice conclusively demonstrated the essential role of Syk in FceRI signaling not only for degranulation but also for lipid mediator synthesis and cytokine production (Costello et al., 1996). Mast cells and basophils produce cytokines important for the late phase allergic reaction (Costello et al., 1996; Shichijo et al., 1999). Mast cell-deficient mice did not exhibit airway inflammation (Kung et al., 1995) or hyperresponsiveness (Kobayashi et al., 2000). These experimental results suggest that mast cells play important roles not only in early but also in late phase allergic reactions and that Syk inhibitors would prevent both phases.

In addition to the critical role of Syk in FceRI signaling, it has been reported that Syk is essential in signaling from receptors for IgG (FcγR). Syk-deficient macrophages and neutrophils failed to phagocytose IgG-coated antigen through FcγR (Crowley et al., 1997; Kiefer et al., 1998). Antigen presentation mediated by antibody and FcγR (FcγR and FcεRI) was demonstrated to be around 100-fold more efficient than that in the absence of the antibody (Sallusto and Lanzavecchia, 1994; Maurer et al., 1996). In fact, an antigen/IgE immune complex more efficiently induced airway inflammation than the antigen alone (Zuberi et al., 2000). The bronchoalveolar lavage (BAL) fluid from the ovalbumin (OVA)-challenged mice contained significant amounts of antigen-
specif IgE and IgE-OVA immune complexes. These observations suggest the importance of FcR-mediated phagocytosis/antigen presentation for the deterioration of inflammation and thereby the signaling through Syk to facilitate phagocytosis in maintaining chronic inflammation by repeated and effective antigen presentation.

Furthermore, recent in vivo experiments, in which syk antisense oligodeoxynucleotide treatment inhibited airway inflammation in rats, directly suggest an important role of Syk in pulmonary inflammation (Stenton et al., 2000).

These literature data strongly suggest that Syk is an important enzyme in various inflammation pathways relevant to respiratory diseases and therefore a key target for a novel antiasthmatic therapy. We have recently identified an orally available Syk kinase inhibitor, BAY 61-3606, and in this study, we have characterized the pharmacological profiles of BAY 61-3606 both in vitro and in vivo.

### Materials and Methods

**Chemicals, Antibodies, and Kits.** $2\{7\{3,4\{Dimethoxyphenyl\}imida\}zol[1,2-\{p\}pyrimidin-5-ylamino\}-nicotinamide dihydrochloride (BAY 61-3606) was synthesized by the Department of Chemistry (Bayer Yakuhi, Ltd., Kyoto, Japan). The structure of BAY 61-3606 is shown in Fig. 1. Peptide substrates for Syk (biotin-KISDFGLSKALRADE-NYYKAQTHGKWPVK W) and Lyn (biotin-Ahx-KVEKIGEGTYGV- Fig. 1. Peptide substrates for Syk (biotin-KISDFGLSKALRADE-

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**Human Cultured Mast Cell (HCMC) Assays.** HCMCs were developed from cord blood stem cells by culturing with 80 ng/ml stem cell factor and 50 ng/ml IL-6 for more than 12 weeks according to the method of Saito et al. (1995). HCMCs were sensitized with 1 μg/ml human IgE overnight. After washing cells with Hank’s balanced salt solution with 0.1% BSA, HCMCs were treated with a test compound for 15 min and then challenged with 4 μg/ml anti-human IgE for 30 min (for histamine and PGD2). For cytokine measurements, cells were resuspended with culture medium and stimulated for 6 h. Supernatants were recovered after centrifugation and kept at –20°C until ELISA assays for histamine, PGD2, LTC4/D4/E4, and GM-CSF. Released tryptase was measured by the enzyme assay with Boc-Ala-Gly-Pro-Arg-MCA as a substrate.

**Human Basophil Degranulation Assay.** Peripheral blood samples were obtained from atopic and nonatopic volunteers. Leukocytes were purified by dextran sedimentation. Leukocytes were resuspended in Hanks’ balanced salt solution with 0.1% BSA and seeded into 96-well plates (5 × 104 cells/well). After incubation with a test compound for 15 min, cells were stimulated with 4 μg/ml anti-human IgE antibody for 30 min at 37°C. Supernatants were recovered after centrifugation and stored at –20°C until use. Released histamine was measured by ELISA.

**Mouse Eosinophil Superoxide Production Assay.** Spleens were obtained from IL-5 transgenic mice (kind gift from Dr. Tomi- nagas, Department of Medical Biology, Kochi Medical School, Japan) (Tominaga et al., 2002). Mouse eosinophils were purified by negative selection with magnetic beads-labeled with anti-Thy1.2 and anti-B220 monoclonal antibodies by using MACS (Miltenyi Biotec Inc., Auburn, CA). After purification, cells were cultured overnight in RPMI 1640 medium containing 10% fetal calf serum and an-

**Rats for in Vivo Studies.** Male Wistar rats for acute models and male Brown Norway rats for a chronic model (both 6 week old or older) (Charles River Japan, Yokohama, Japan) were used.

**Passive Cutaneous Anaphylaxis (PCA) Reactions.** Rats were passively sensitized by s.c. injection of 5 ng of anti-DNP IgE in dorsal skin. One day later, a test compound in saline containing 10% cremo- phor was administered 5 min (i.v.) or 60 min (p.o.) before DNP-BSA administration (1 mg in saline containing 0.5% Evans blue, i.v.). Thirty minutes later, rats were sacrificed, and Evans blue in the sites of sensitization was extracted by formamide overnight at 65°C. Absorbency at 620 nm was measured to determine the amount of Evans blue.

**Chemical Structure of BAY 61-3606.** 2H2N

![Fig. 1. Chemical structure of BAY 61-3606.](image-url)
Bronchoconstriction and Bronchial Edema Models in Rats. Rats were passively sensitized by i.v. injection of 10 μg of SPE-7 1 day before experiments. After urethane anesthesia, main bronchi were exposed and cannulated to measure the change in pulmonary pressure. BAY 61-3606 was administered (p.o.) 60 min before injection of the antigen (1.5 μg of DNP-BSA in saline containing 0.5% Evans blue, i.v.). Change in pulmonary pressure was monitored for 10 min after antigen exposure. Thirty minutes after the challenge, rats were sacrificed and lungs were perfused with 20 ml of phosphate-buffered saline. Evans blue in main bronchus was extracted by formamide and measured as described above.

Airway Inflammation Model in Rats. Rats were immunized by i.p. injection of OVA in Al(OH)₃ suspension on days 0 and 14. On days 20 and 21, an aerosol of 1% OVA in saline was administered by inhalation. BAL fluid was collected, and cell number and differential counts were determined. BAY 61-3606 was administered (p.o.) from days 0 to 21 (b.i.d.). Dexamethasone was administered (p.o.) from days 0 to 9 and days 18 to 21 (b.i.d.).

Results

Biochemical Characterization. We have identified an orally available Syk kinase inhibitor, BAY 61-3606 (Fig. 1), from a series of imidazopyrimidine analogs. BAY 61-3606 inhibited kinase activity of Syk in a concentration-dependent manner with an IC₅₀ value of 10 nM (Fig. 2a). Lineweaver-Burk analysis confirmed competitive inhibition against ATP (Fig. 2b), and the Kᵢ value was determined as 7.5 nM. BAY 61-3606 was a highly selective inhibitor of Syk kinase. Other selected tyrosine kinases, Lyn, Fyn, Src, Itk, and Btk, were not inhibited by BAY 61-3606 in concentrations up to 4.7 μM (Table 1).

Inhibition of Cellular Function. In functional assays to measure FcRI-mediated degranulation in mast cells, BAY 61-3606 inhibited the release of various inflammatory mediators in a concentration-dependent manner. The IC₅₀ values for the FcRI-mediated hexosaminidase release from a rat basophilic leukemia cell line, RBL-2H3 (Fig. 3a), and serotonin release from rat peritoneal mast cells (Table 2) were found to be 46 and 17 nM, respectively. In RBL-2H3 cells, phosphorylation of Syk was also attenuated (data not shown). None of the reference compounds, dexamethasone (glucocorticoid), disodium cromoglycate (DSCG, mast cell stabilizer), and montelukast (LT antagonist), were found to inhibit hexosaminidase release from RBL-2H3 cells at the concentrations evaluated in this study (Fig. 3a).

In a manner similar to its effect on the degranulation of RBL-2H3 cells and rat peritoneal mast cells, BAY 61-3606 inhibited FcεRI-mediated histamine and tryptase release from HCMCs with IC₅₀ values of 5.1 and 5.5 nM, respectively (Fig. 3, b and c). In addition to the effects on the degranulation, BAY 61-3606 inhibited FcεRI-mediated lipid mediator release (PGD₃ and LTC₄/D₄/E₄) and de novo synthesis of the cytokine GM-CSF in HCMCs (IC₅₀ = 5.8, 3.3, and 200 nM, respectively; Fig. 3, d–f). Montelukast (IC₅₀ = 6.8 μM for histamine and 7.0 μM for tryptase) and DSCG (IC₅₀ = 860 μM for histamine) inhibited degranulation from HCMCs only very weakly, and dexamethasone showed no effect up to 30 μM (Fig. 3, b and c). The potency of reference compounds was weak for lipid mediator synthesis (montelukast, IC₅₀ = 6.8 μM for LT; Fig. 3, d and e). Dexamethasone showed higher potency (IC₅₀ = 6 nM) than BAY 61-3606 for inhibition of cytokine production (Fig. 3f).

BAY 61-3606 was also found to inhibit the degranulation of human freshly isolated basophils. Leukocyte fractions including basophils were isolated from peripheral blood of both high- (>280 U/ml) and low (<280 U/ml)-serum IgE donors. The expression level of FcεRI in leukocytes from high-serum IgE donors was higher than that from low-serum IgE donors as demonstrated by flow cytometry (data not shown). The leukocytes from both groups responded to anti-IgE stimulation by releasing histamine. BAY 61-3606 inhibited histamine release from leukocytes in high and low IgE groups equipotently, giving IC₅₀ values of 8.1 and 10 nM, respectively (Fig. 4).

BAY 61-3606 was also found to inhibit B cell receptor (BCR)-mediated signaling. The IC₅₀ values for BCR-stimulated increases in intracellular calcium concentration in the

Table 1: Selectivity profile of BAY 61-3606 in six tyrosine kinase assays

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Ki (nM)</th>
</tr>
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<tbody>
<tr>
<td>Syk</td>
<td>7.5 ± 2.5</td>
</tr>
<tr>
<td>Lyn</td>
<td>&gt;5,400</td>
</tr>
<tr>
<td>Fyn</td>
<td>&gt;12,500</td>
</tr>
<tr>
<td>Src</td>
<td>&gt;6,250</td>
</tr>
<tr>
<td>Itk</td>
<td>&gt;4,700</td>
</tr>
<tr>
<td>Btk</td>
<td>&gt;5,000</td>
</tr>
</tbody>
</table>

Kᵢ values were shown as mean ± S.E. of two independent experiments.

![Fig. 2. Syk kinase assay. The kinase reaction was conducted for 30 min at room temperature in the presence of the indicated concentrations of compounds, 30 μM ATP, and 3 μM biotinylated peptide substrate, which corresponded to the activation loop domain of Syk kinase itself. After termination of the reaction with the addition of the EDTA-containing stop buffer, reaction mixtures were transferred into streptavidin-coated plates to trap biotinylated substrate. After washing, phosphorylation of the substrate was detected by the addition of europium-labeled anti-phosphotyrosine monoclonal antibody (4G10) and the measurement with the multilabel counter ARVO (Wallac Oy, Turku, Finland). a, inhibition curve by BAY 61-3606. b, Lineweaver-Burk plot analysis. Each point indicates mean ± S.D. of two independent experiments.](https://doi.org/10.1289/ehp.1176)
Ramos human B cell line and for BCR-induced mouse splenic B cell proliferation were 81 and 58 nM, respectively (Table 2). Phosphorylation of Syk in Ramos cells was concentration dependently reduced (data not shown). Dexamethasone showed a similar potent inhibition of B cell growth (IC50/H1100530 nM) as BAY 61-3606, whereas DSCG and montelukast showed no effect up to 10/H9262M (data not shown).

Furthermore, BAY 61-3606 was found to block FcγR-mediated activation of monocytes effectively. BAY 61-3606 inhibited FcγR-mediated superoxide production from a human monocytic cell line, U937 (Fig. 5b) and human monocytes freshly isolated from peripheral blood (Table 2) (IC50 = 52 and 12 nM, respectively). The reference compounds were without effect up to 10 μM on the respiratory burst from U937 triggered by FcγRI-aggregation (Fig. 5b). We also examined effects of BAY 61-3606 on the respiratory burst in eosinophils by FcγRI stimulation. In mouse, immobilized IgG elicited superoxide production, and it was suppressed by the pretreatment of cells with anti-FcγRIII/II monoclonal antibody 2.4G2 (Fig. 5a). BAY 61-3606 inhibited respiratory burst in a concentration-dependent manner with an IC50 value of 35 nM (Table 2), and it completely suppressed su-

**Fig. 3.** Effects of BAY 61-3606 and reference compounds on the activation of mast cells. a, DNP-BSA-induced degranulation of rat basophilic leukemia cell line, RBL-2H3 cells, which were sensitized with anti-DNA IgE. Hexosaminidase activity in the supernatant was measure by an enzyme assay. Each point indicates mean ± S.E. of four independent experiments. b–f, human cultured mast cells sensitized with human IgE were challenged with anti-human IgE for 30 min (a–d) or 6 h (e). Supernatants were recovered and tryptase activity was measured by an enzyme assay (b). ELISA assays were performed for histamine (a), PGD2 (c), LTC4/D4/E4 (d), and GM-CSF (e). Each point indicates mean ± S.E. of three to four independent experiments.

**TABLE 2**

Summary of IC50 values of BAY 61-3606 in various cellular assays

<table>
<thead>
<tr>
<th>Cell</th>
<th>Receptor</th>
<th>Readout</th>
<th>IC50 (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBL-2H3</td>
<td>FcεRI</td>
<td>Hexosaminidase</td>
<td>46 ± 19 (4)</td>
</tr>
<tr>
<td>Rat peritoneal mast cells</td>
<td>FcεRI</td>
<td>Serotonin</td>
<td>17 ± 14 (4)</td>
</tr>
<tr>
<td>HCMC</td>
<td>FcεRI</td>
<td>Histamine</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>HCMC</td>
<td>FcεRI</td>
<td>Tryptase</td>
<td>5.5 ± 1.5</td>
</tr>
<tr>
<td>HCMC</td>
<td>FcεRI</td>
<td>PGD2</td>
<td>5.8 ± 1.3</td>
</tr>
<tr>
<td>HCMC</td>
<td>FcεRI</td>
<td>LTs</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Human leukocytes from high IgE donors</td>
<td>FcεRI</td>
<td>Histamine</td>
<td>8.1 ± 6.1</td>
</tr>
<tr>
<td>Human leukocytes from low IgE donors</td>
<td>FcεRI</td>
<td>Histamine</td>
<td>10.2 ± 5.2</td>
</tr>
<tr>
<td>Ramos</td>
<td>BCR</td>
<td>[Ca2+]</td>
<td>81 ± 26 (6)</td>
</tr>
<tr>
<td>Mouse splenic B cells</td>
<td>BCR</td>
<td>Proliferation</td>
<td>58 ± 26 (4)</td>
</tr>
<tr>
<td>Mouse eosinophils</td>
<td>FcγR</td>
<td>Superoxide</td>
<td>35 ± 14 (3)</td>
</tr>
<tr>
<td>U937</td>
<td>FcγRI</td>
<td>Superoxide</td>
<td>52 ± 14 (4)</td>
</tr>
<tr>
<td>Human fresh monocytes</td>
<td>FcγR</td>
<td>Superoxide</td>
<td>12 ± 7 (4)</td>
</tr>
</tbody>
</table>
paperoxide production at 1 μM, whereas the efficacy of other reference compounds was none or weak at the concentrations used in this study (Fig. 5a).

In Vivo Characterization. PCA assay was carried out to investigate the effect of BAY 61-3606 on mast cell-mediated type-I allergic reactions in rats. Oral administration of BAY 61-3606 dose dependently inhibited the PCA reaction with an ED₅₀ value of 8 mg/kg (statistical inhibition at 3 mg/kg; p < 0.05) (Fig. 6a). Although DSCG (30 mg/kg i.v.) showed 60% inhibition of dye leakage by the PCA reaction (Fig. 6b), this compound was not active with oral administration (data not shown). Neither montelukast (10 mg/kg p.o.) nor dexamethasone (0.3 mg/kg p.o.) showed any inhibitory activity (Fig. 6, c and d).

The effects of BAY 61-3606 on antigen-induced asthmatic models were investigated. In a bronchoconstriction model, BAY 61-3606 dose dependently inhibited the DNP-BSA-induced increase in pulmonary pressure, and the dose of 3 mg/kg showed statistically significant suppression (Fig. 7). As shown in Fig. 8, BAY 61-3606 also significantly attenuated DNP-BSA-induced bronchial edema at and over dosages of 3 mg/kg. Furthermore, BAY 61-3606 significantly inhibited eosinophil accumulation in the BAL fluid at dose of 30 mg/kg to the same level as dexamethasone at a dose of 0.3 mg/kg (Fig. 9). The inhibitory effect of BAY 61-3606 on the total cell number in BAL fluid was also observed at 30 mg/kg, but it was not statistically significant (data not shown). Body weight of dexamethasone-treated rats decreased gradually during the course of the experiment (starting from 140 g, −20 g at the end of the experiment); however, those of BAY 61-3606-treated groups at all dosages were almost as same as the vehicle group (−40 to 60 g at the end of the experiment) (data not shown).

Discussion

BAY 61-3606 potently inhibited recombinant Syk kinase activity in an ATP-competitive manner (Kᵢ = 7.5 nM; Fig. 2). Furthermore, more than 625-fold selectivity against several other tyrosine kinases, such as Lyn, Fyn, Src, Itk, and Btk, was demonstrated (Table 1).

The efficacy of BAY 61-3606 on antigen-induced degranulation was confirmed both in RBL-2H3 cells (IC₅₀ = 46 nM; Fig. 3a) and freshly isolated rat mast cells (IC₅₀ = 17 nM; Table 2). Also in vivo, BAY 61-3606 was effective in suppressing PCA reactions in skin (Fig. 6). Oral dosing over 3 mg/kg was significantly effective and inhibition was dose-dependent. Furthermore, effects of BAY 61-3606 on lung mast cells were confirmed in two acute asthmatic models; DNP-BSA-induced bronchoconstriction (Fig. 7) and bronchial edema (Fig. 8). Statistic significance was obtained over 3 mg/kg p.o. in both assays. Also in mice, oral administration of BAY 61-3606 suppressed PCA reaction dose dependently (data not shown). This in vivo evidence suggests that BAY 61-3606 may be an effective orally available antiallergy medicine.

Although RBL-2H3 and rat peritoneal mast cells have been used frequently to study the effect of compounds on mast cells, compounds effective on these cells were often less active in human mast cells (Pearce et al., 1982). We thus studied the efficacy of BAY 61-3606 on cord blood stem cell-derived HMCs. HMCs have been recognized to show similar pharmacological characteristics to human lung mast cell (Shichijo et al., 1998), which is one of the primary target cells for asthma therapy. Similarly to rat cells, BAY 61-3606 blocked activation of HMCs by FceRI-aggregation (Fig. 3, b–f). The efficacy was not only on degranulation but also on lipid mediators and cytokine production. These results are consistent with the phenotypes observed in Syk-deficient mast cells (Costello et al., 1996). Relatively smaller IC₅₀ values in rapid mediator release/synthesis in HMCs compared with those in rat mast cells would indicate heterogeneity of mast cells. The relatively high IC₅₀ value of BAY 61-3606 in GM-CSF production might be due to longer incubation time in this assay or to difference in sensitivity to Syk inhibition between readouts. Tryptase has been considered to cause the remodeling in the airway (Sommernoff, 2001). Reticular basement membrane thickness occurs early in the asthma process even in childhood (Jeffery, 2001). Therefore, effective inhibition of tryptase release by BAY 61-3606 might be effective for the airway remodeling as a long-term efficacy.

We extended the efficacy study to freshly isolated human cells, including basophils, which express FceRI. BAY 61-3606 showed almost similar efficacy on the degranulation of cells from high-serum IgE (IC₅₀ = 8.1 nM) and low-serum IgE (IC₅₀ = 10.2 nM) donors (Fig. 4), indicating its efficacy in humans, including atopic patients. Based on these mast cell and basophil data, BAY 61-3606 is a potent inhibitor of human mast cell/basophil activation by antigen.

BAY 61-3606 also suppressed BCR signaling. The inhibition of BCR engagement-induced calcium mobilization in a human B cell line, Ramos, by BAY 61-3606 (IC₅₀ = 81 nM; Table 2) is consistent with the phenotype of Syk-deficient DT40 cells (Takata et al., 1994). Purified splenic B cells responded to anti-IgM antibody to show proliferation. BAY 61-3606 attenuated this cell growth (IC₅₀ = 58 nM; Table 2). This is the first pharmacological demonstration, as far as we know, that inhibition of Syk results in the prevention of a B cell function. These B cell data imply that clonal expansion after BCR-engagement by antigen and further maturation could be attenuated by Syk kinase inhibitors.

BAY 61-3606 concentration dependently inhibited FceRI-mediated respiratory burst not only in mouse eosinophils (IC₅₀ = 35 nM; Table 2) but also in a human monocyte cell line, U937 (IC₅₀ = 52 nM; Fig. 5b) and freshly isolated human monocytes (IC₅₀ = 12 nM; Table 2). These data are consistent with the previous publications using syk antisense or cells derived from knockout mice (Matsuda et al., 1996;
Crowley et al., 1997; Kiefer et al., 1998; Lach-Trifilieff et al., 2000) and indicate one aspect of anti-inflammatory profiles of BAY 61-3606.

To confirm an outcome of inhibitory actions of BAY 61-3606 in various types of inflammatory cells, we examined the efficacy of BAY 61-3606 at 30 mg/kg p.o., b.i.d., greatly suppressed accumulation of eosinophils in BAL fluid (Fig. 9). The inhibition was 70% and comparable with that of dexamethasone (0.3 mg/kg p.o., b.i.d.). Thus, not only mast cell-stabilizing activity but also anti-inflammatory activity of BAY 61-3606 was confirmed in vivo. The requirement of higher dose in the chronic model might be related to the pharmacokinetic profile of BAY 61-3606. When rats were treated with a single dose of 10 mg/kg p.o. BAY 61-3606, a maximal concentration of 0.167 mg/l (360 nM) was reached 2 h after administration. Due to relatively fast elimination ($t_{1/2} = 1.78$ h), we administered the compound twice a day. It should be noted that dexamethasone reduced the increase in body weight of rats, but BAY 61-3606 had no effect during the course of this experiment (data not shown).

The potency of BAY 61-3606 in mast cell and basophil assays was superior to that of DSCG, a widely used mast cell stabilizer as an inhalant (Figs. 3 and 6). Moreover, dexamethasone and montelukast showed little or no effect in many assays selected for this study (Figs. 3, 5, and 6). This difference in efficacy profiles between BAY 61-3606 and other widely used drugs

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shown to be essential in collagen-induced activation, which is important for the clotting reaction (Watson et al., 2000). In breast cancer, Syk has been implicated as an important inhibitor of cancer cell growth and metastasis (Coopman et al., 2000). In natural killer cells, expression of dominant-negative Syk attenuated natural cytotoxicity (Brumbaugh et al., 1997). In addition, the expression of Syk was reported in several other nonhematopoietic cells (Yanagi et al., 2001). All of these concerns should be clarified in detail in future safety toxicological studies.

In conclusion, BAY 61-3606 is an orally available Syk-selective kinase inhibitor, which exhibits a variety of actions on mast cells, basophils, B cells, eosinophils, and antigen-presenting cells. BAY 61-3606 would have benefits in the treatment of asthma by preventing antigen-induced bronchoconstriction and airway inflammation.

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