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# The Rhizomes of *Acorus gramineus* and the Constituents Inhibit Allergic Response *In vitro* and *In vivo*

**Hyun Lim[1], Seung Young Lee[2], Kang Ro Lee[2], Yeong Shik Kim[3] and Hyun Pyo Kim[1,*]**

*[1]College of Pharmacy, Kangwon National University, Chunchon 200-701, [2]School of Pharmacy, Sungkyunkwan University, Suwon 440-746, [3]College of Pharmacy, Seoul National University, Seoul 110-460, Republic of Korea*

## Abstract

The rhizomes of *Acorus gramineus* have frequently been used in traditional medicine mainly for sedation as well as enhancing brain function. In this study, the anti-allergic activity of *A. gramineus* was investigated. The 70% ethanol extract of the rhizomes of *A. gramineus* was found to inhibit the allergic response against 5-lipoxygenase (5-LOX)-catalyzed leukotriene (LT) production from rat basophilic leukemia (RBL)-1 cells and β-hexosaminidase release from RBL-2H3 cells with $IC_{50}$'s of 48.9 and >200 μg/ml, respectively. Among the 9 major constituents isolated, β-asarone, (2R,3R,4S,5S)-2,4-dimethyl-1,3-bis (2',4',5'-trimethoxyphenyl) tetrahydrofuran (AF) and 2,3-dihydro-4,5,7-trimethoxy-1-ethyl-2-methyl-3-(2,4,5-trimethoxyphenyl)indene (AI) strongly inhibited 5-LOX-catalyzed LT production in A23187-treated RBL-1 cells, AI being the most potent ($IC_{50}$=6.7 μM). Against β-hexosaminidase release by antigen-stimulated RBL-2H3 cells, only AI exhibited strong inhibition ($IC_{50}$=7.3 μM) while β-asarone and AF showed 26.0% and 39.9% inhibition at 50 μM, respectively. In addition, the ethanol extract of *A. gramineus* showed significant inhibitory action against the hapten-induced delayed hypersensitivity reaction in mice by oral administration at 200 mg/kg. Therefore, it is suggested that *A. gramineus* possesses anti-allergic activity and the constituents including β-asarone and AI certainly contribute to the anti-allergic activity of the rhizomes of *A. gramineus*.

**Key Words:** *Acorus gramineus*, β-Asarone, 2,3-Dihydro-4,5,7-trimethoxy-1-ethyl-2-methyl-3-(2,4,5-trimethoxyphenyl)indene, 5-Lipoxygenase, β-Hexosaminidase, Anti-allergy

## INTRODUCTION

Humans suffer from various allergic disorders in their life time including asthma, systemic allergic disorders and some skin disorders. It is difficult to completely cure these disorders. Many different kinds of small molecular weight-drugs and recently several biologics such as anti-TNF-α monoclonal antibody are used clinically (Walsh, 2011). However, there is a continual need for plant alternative medicine since they are relatively safe and cost-effective, and especially the cumulative record of their use over thousands of years exists. In this respect, the effect of many plant extracts were examined for their anti-allergic activity in our screening procedure, and the rhizomes of *Acorus gramineus* were found to possess some anti-allergic activity *in vitro*.

The rhizome of *Acorus gramineus* (Araceae) is a well-known Chinese traditional medicine. This plant material has been used widely as antipyretic, memory enhancement, analgesia, sedative and digestive in China, Japan and Korea (Liao *et al.*, 1998). The major constituents are β-asarone and phenylpropenes (Park *et al.*, 2011). There have been many investigations concerning the effects of this plant material on the improvement of brain function (Liao *et al.*, 1998; Chun *et al.*, 2008), and an anti-allergic property has not been elucidated to date. In our preliminary experiment, the ethanol extract of the rhizomes of *A. gramineus* (AGE) were found to possess an anti-allergic action although the potency was not strong. Therefore, the anti-allergic activity of *A. gramineus* and the constituents was investigated using an *in vitro* and *in vivo* animal model of allergic responses in the present investigation.

## MATERIALS AND METHODS

### Chemicals

A23187 was obtained from Biomol (Plymouth Meeting, PA, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), nordihydroguaiaretic acid (NDGA), prednisolone, quercetin, carboxymethylcellulose (CMC), dinitrophenyl (DNP)-BSA, anti-DNP mouse IgE and p-nitrophenyl-N-acetyl-

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***Corresponding Author**

E-mail: hpkim@kangwon.ac.kr
Tel: +82-33-250-6915, Fax: +82-33-255-7865

β-D-glucosaminide were purchased from Sigma Chem (St. Louis, MO, USA). DMEM and other cell culture reagents including FBS were products of Gibco BRL (Grand Island, NY, USA). A protein assay kit was purchased from Bio-Rad (Hercules, CA, USA).

## Animals

Male ICR mice (4 weeks old, specific pathogen-free) were obtained from Orient-Bio Co. (Korea). The animals were maintained in the animal facility (KNU) at 20-22°C under 40-60% relative humidity and a 12 h/12 h (light/dark) cycle for at least 7 days prior to the experiment. The experimental design using the animals was approved by the local committee for animal experimentation, KNU (KIACUC-11-0007), and the animals were handled according to the guidelines described in the KFDA Guide for the Care and Use of Laboratory Animals.

## Preparation of the extracts of the rhizomes of *A. gramineus* and isolation of the constituents

The rhizomes of *A. gramineus* were collected in Jeju Island, Korea in March 2009, and identified by one of authors (Dr. K. R. Lee). A voucher specimen (SKKU-NPL-0910) was deposited at the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea. The dried and chopped rhizomes of *A. gramineus* (150.0 g) were extracted with water and 70% EtOH under reflux and then filtered. The filtrates were evaporated under reduced pressure to give water extract and EtOH extract. These are used for the pharmacological assays. For an isolation of active constituents, the rhizome parts of *A. gramineus* (15 kg) were extracted at room temperature with 80% MeOH to give a MeOH extract (1.2 kg). The isolation and identification of β-asarone, asaraldehyde, propioveratrone, acoraminol A, acoraminol B, 7S,8R-dihydrodiconiferyl alcohol (AA), 2,4,5-trimethoxyallylbenzene (AB), (2R,3R,4S,5S)-2,4-dimethyl-1,3-bis (2',4',5'-trimethoxyphenyl)tetrahydrofuran (AF), 2,3-dihydro-4,5,7-trimethoxy-1-ethyl-2-methyl-3-(2,4,5-trimethoxyphenyl)indene (AI) from the MeOH extract of *A. gramineus* were reported previously (Fig. 1) (Park *et al.*, 2011).

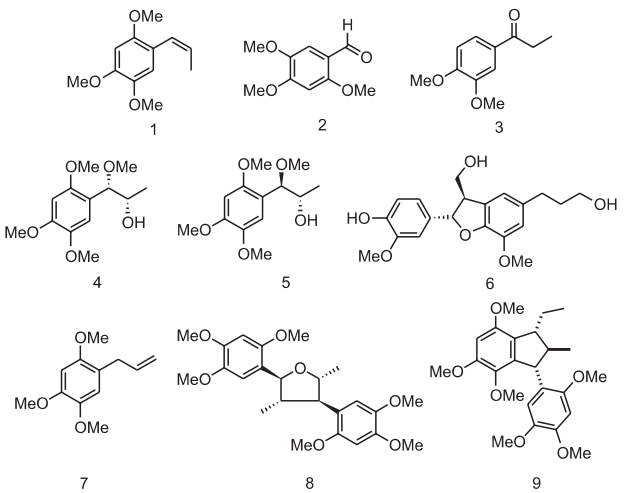


**Fig. 1.** Chemical structures of the compounds isolated from the rhizomes of *A. gramineus*. 1: β-asarone, 2: asaraldehyde, 3: propioveratrone, 4: acoraminol A, 5: acoraminol B, 6: (7S,8R)-dihydrodiconiferyl alcohol (AA), 7: 2,4,5-trimethoxyallylbenzene (AB), 8: (2R,3R,4S,5S)-2,4-dimethyl-1,3-bis(2',4',5'-trimethoxyphenyl) tetrahydrofuran (AF), 9: 2,3-dihydro-4,5,7-trimethoxy-1-ethyl-2-methyl-3-(2,4,5-trimethoxyphenyl)indene (AI).

## Rat basophilic leukemia-1 (RBL-1) cell culture and measurement of leukotriene (LT) concentration

To evaluate the 5-lipoxygenase (5-LOX) inhibitory activity, RBL-1 cells purchased from the American Type Culture Collection (ATCC, Rockville, VA, USA) were cultured in RPMI 1640 with 10% FBS under 5% $CO_2$ at 37°C. The test compounds were dissolved in DMSO and diluted to appropriate concentrations with serum-free DMEM. The final concentration of DMSO was adjusted to 0.1% (v/v). The cells were preincubated with the test compounds for 10 min. Then, A23187 (3 μM) was added to activate the 5-LOX and the cells were further incubated for 15 min as previously described, with slight modification (Tries *et al.*, 2002). The media was then collected and the concentration of the 5-LOX product, cysteinyl leukotrienes ($LTC_4/D_4/E_4$), was measured using an ELISA kit (Enzo Life Sciences) as recommended by the manufacturer. The cell viability was assessed using an MTT assay as previously described (Mossman, 1983).

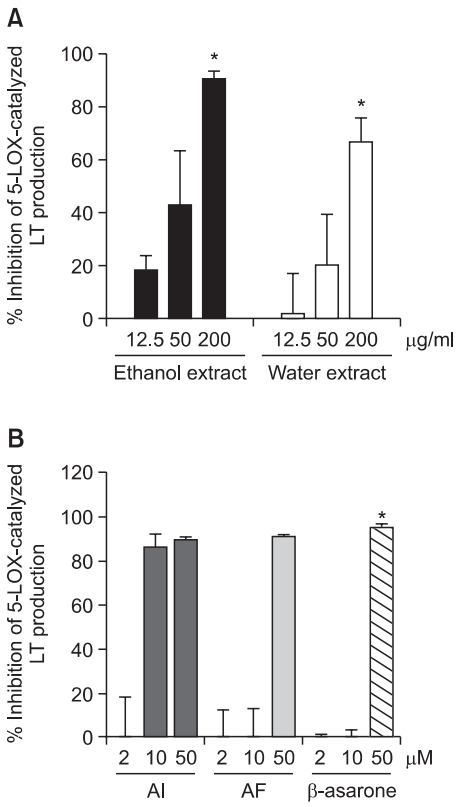


**Fig. 2.** Inhibition of 5-LOX-catalyzed LT production in A23187-treated RBL-1 cells by the extracts of *A. gramineus* and the constituents. (A) Effects of the extracts of *A. gramineus* on LT production. (B) Effects of the selected constituents on LT production. Water extract (white), ethanol extract (black), β-asarone (stripe), AF (light grey), AI (dark grey). *$p$<0.05, Significantly different from A23187-treated control group (n=3). % inhibition = (A23187-treated control − sample treatment w/ A23187)/(A23187-treated control − control w/o A23187) ×100.

### RBL-2H3 cell culture and antigen-induced degranulation of β-hexosaminidase

Sensitization and degranulation procedures were followed by the previously described procedure (Choi *et al.*, 1996). In brief, anti-DNP mouse IgE was added to RBL-2H3 cells (ATCC) for sensitization and incubated overnight. Twenty four hours later, the cells were washed with siraganian buffer (pH 7.2). The test compounds dissolved in DMSO were added and the cells were incubated for 30 min. Then, DNP-BSA (1 μg/ml) was added, and after 10 min incubation, the reaction was stopped by cooling in an ice bath. The supernatant was obtained by centrifugation. The substrate (1 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide) was added and incubated for 1 h at 37°C. The reaction was stopped by adding 0.1 M $Na_2CO_3$/$NaHCO_3$ (200 μl/well). The absorbance was measured at 405 nm.

### Picryl chloride-induced delayed hypersensitivity in mice

For measuring inhibitory activity against delayed type hypersensitivity (DTH, type IV hypersensitivity), 7% picryl chloride (Nacalai Tesque Inc., Japan) in acetone (100 μl/mouse) was smeared to the abdomen of mice to sensitize the animals for the elicitation phase group. For the animals of the control group, acetone (100 μl) was applied instead of picryl chloride. Seven days later, the elicitation phase of delayed hypersensitivity was induced by application of 1% picryl chloride in acetone (20 μl/ear) to right ears of the sensitized mice. For obtaining the induction phase reaction, 1% picryl chloride (20 μl/ear) was applied to right ears of acetone-treated mice. For the control group, only acetone (20 μl) was applied to right ears of acetone-treated mice. After 24 h, ear thickness was measured. Test compounds dissolved in 0.4% CMC were orally administered 1 h after initial treatment of sensitizer or acetone. The same amounts of test compounds in vehicle were treated again 1 h after final picryl chloride or acetone treatment.

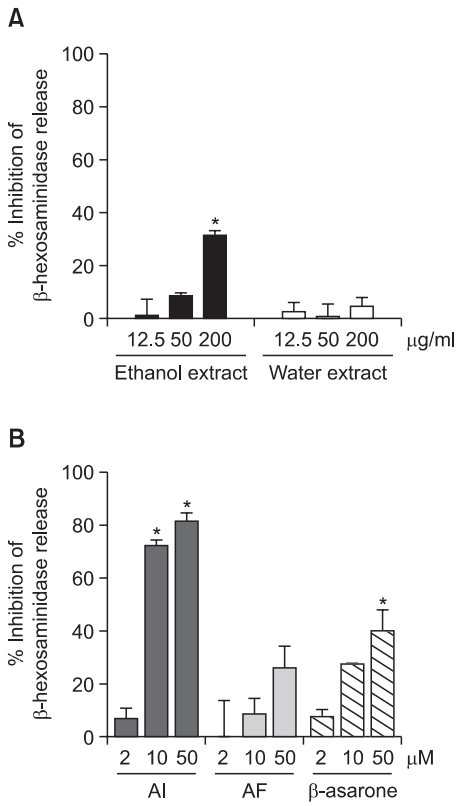


**Fig. 3.** Inhibition of β–hexosaminidase degranulation in antigen-treated RBL-2H3 cells by the extracts of *A. gramineus* and the constituents. (A) Effects of the extracts of *A. gramineus* on β–hexosaminidase degranulation. (B) Effects of the selected constituents on β–hexosaminidase degranulation. Water extract (white), ethanol extract (black), β-asarone (stripe), AF (light grey), AI (dark grey), **p*<0.05, Significantly different from antigen-treated control group (n=3). % inhibition = (antigen-treated control − sample treatment w/ antigen)/(antigen-treated control − control w/o antigen) ×100.

### Statistical analysis

All data were represented as arithmetic mean ± SD. One-way analysis of variance (ANOVA), followed by Dunnett's test was used to determine the statistical significance.

## RESULTS

In A23187-treated RBL-1 cells, cysteinyl-LTs (1,811.7 ± 134.5 pg/ml) were synthesized by 5-LOX for 15 min. The basal level of the cysteinyl-LTs without A23187 treatment was 50.3 ± 18.9 pg/ml (n=3). Under these conditions, the water and ethanol extracts considerably inhibited LT production at 12.5-200 μg/ml (Fig. 2A). The $IC_{50}$ values for the water and ethanol extracts were 120.0 and 48.9 μM, respectively. When the constituents were tested, β-asarone, AF and AI showed a strong inhibitory action (Fig. 2B), while AB showed weak inhibition. Their $IC_{50}$ values were represented in Table 1. NDGA (LOX inhibitor, 1 μM) used as a reference strongly inhibited LT production (99.3%).

In antigen-treated RBL-2H3 cells, β-hexosaminidase was degranulated and released into the media for a 10 min incubation period (from 0.0 ± 3.2% to 100.0 ± 1.2%, n=3). Under these conditions, the ethanol extract of *A. gramineus* significantly inhibited the degranulation reaction at 50-200 μg/ml (Fig. 3A). The ethanol extract showed 31.4% inhibition at 200

**Table 1.** Inhibition of the constituents of the rhizomes of *A. gramineus* against 5-LOX catalyzed LT production and β-hexosaminidase degranulation

| Compounds | % inhibition at 50 μM[a] | |
|---|---|---|
| | 5-LOX | β-hexosaminidase |
| β-Asarone | 94.9 (31.1)[b] | 39.9 |
| Asaraldehyde | -[c] | 12.9 |
| Propioveratrone | - | - |
| Acoraminol A | - | -[ct] |
| Acoraminol B | - | -[ct] |
| AA | 5.2 | -[ct] |
| AB | 29.9 | 8.4 |
| AF | 90.8 (32.0) | 26.0 |
| AI | 89.3 (6.7) | 81.5 (7.3) |

[a]All values are arithmetic mean of % inhibition at 50 μM, n=3, [b]The values of the parenthesis are $IC_{50}$ vales in μM. [c]not active, [ct]cytotoxic at 2-50 μM by MTT assay.

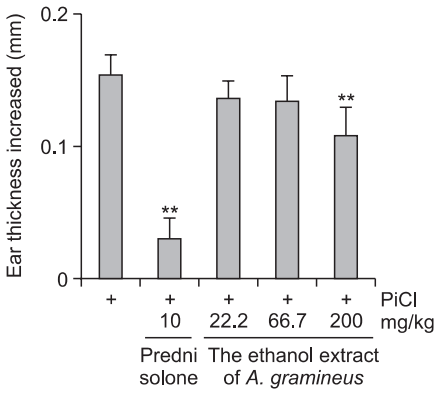


**Fig. 4.** Inhibition of delayed-type hypersensitivity response in mice. Picryl chloride was applied to abdomen of mice to sensitize and, one week later, applied again to ears of the sensitized mice. One day later, ear thickness was measured. Data represented the arithmetic mean ± S.D. (n=6). ***p*<0.01, Significantly different from the picryl chloride-treated control group. % inhibition = (picryl chloride control − sample treatment w/ picryl chloride)/(picryl chloride-treated control − control w/o picryl chloride) ×100.

μg/ml, while the water extract did not show significant inhibitory action at 50-200 μg/ml. When the inhibitory action of the constituents was examined, only AI showed strong inhibitory activity (Fig. 3B, Table 1). On the other hand, β-asarone, asaraldehyde and AF showed weak inhibitory action at 50 μM (less than 50% inhibition). The reference agent, quercetin (degranulation inhibitor, 20 μM), strongly inhibited β-hexosaminidase release (92.2%).

In addition, the inhibitory action of *A. gramineus* on a delayed-type hypersensitivity in mice was evaluated. A treatment of hapten, picryl chloride, to the sensitized mice produces strong ear edema of delayed-type hypersensitivity. In this condition, when orally administered at 22.2-200 mg/kg, the ethanol extract of *A. gramineus* significantly inhibited the DTH response (29.9%) only at 200 mg/kg as shown in Fig. 4. The reference drug, prednisolone, potently inhibited DTH (80.5%) at 10 mg/kg. Therefore, it is suggested that *A.gramineus* possesses anti-allergic activity against the immediate-type as well as against the delayed-type hypersensitivity.

## DISCUSSION

In the present investigation, inhibitions of 5-LOX inhibition and β-hexosaminidase release were studied for investigating anti-allergy activity *in vitro.* Leukotrienes (LT) are produced from arachidonic acid by 5-LOX. LTs are deeply involved in several allergic disorders such as asthma and atopic dermatitis (Rubin and Mollison, 2007). Thus 5-LOX inhibitory activity was examined using a mast cell line (RBL-1). And it is well known that the immediate hypersensitivity reaction is provoked at least in part by histamine released from antigen-stimulated mast cells and basophils. Along with a histamine release, β-hexosaminidase is also degranulated from the antigen-stimulated cells, which is frequently used as a biomarker of an immediate-type allergic response (Schuwartz *et al.*, 1981; Marquadt and Wasserman, 1983). Thus, RBL-2H3 cells were used and the amounts of β-hexosaminidase release were checked. On these parameters, *A. gramineus* and its constituents showed anti-allergic activity.

Many pharmacological studies have revealed that *A. gramineus* and its major constituent, β-asarone, could protect brain damage, alleviate memory function and inhibit Alzheimer's symptoms (Liao *et al.*, 1998; Chun *et al.*, 2008; Geng *et al.*, 2010; Pages *et al.*, 2010; Zou *et al.*, 2011). However, the anti-allergic and anti-inflammatory activities of this plant material and its constituents including β-asarone were rarely demonstrated. There have been reports of cyclooxygenase-1 inhibitory action of trans-asarone (Momin *et al.*, 2003) and passive cutaneous anaphylaxis inhibition of γ-asarone (Hashimoto *et al.*, 1994). To our best knowledge, this is the first report of the anti-allergic property of *A. gramineus* and the constituents.

In conclusion, the rhizomes of *A. gramineus* and its several constituents possess anti-allergic activity *in vitro* against 5-LOX-catalyzed LT production and antigen-induced β-hexosaminidase release from mast cell lines. In particular, 2,3-dihydro-4,5,7-trimethoxy-1-ethyl-2-methyl-3-(2,4,5-trimethoxyphenyl)indene (AI) showed strong inhibitory activity against these two parameters. β-Asarone, a major constituent of A. *gramineus*, also showed anti-allergic action. Moreover, *A. gramineus* showed inhibitory activity on DTH reaction although the potency was not strong. It is suggested that β-asarone and 2,3-dihydro-4,5,7-trimethoxy-1-ethyl-2-methyl-3-(2,4,5-trimethoxyphenyl)indene (AI) certainly contribute to the anti-allergic property of *A. gramineus*.

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## REFERENCES

Choi, O. H., Kim, J. H. and Kinet, J. P. (1996) Calcium mobilization via sphingosine kinase in signaling by the FcεRI antigen receptor. *Nature* **380**, 634-636.

Chun, H. S., Kim, J. M., Choi, E. H. and Chang, N. (2008) Neuroprotective effects of several Korean medicinal plants traditionally used for stroke remedy. *J. Med. Food* **11**, 246-251.

Geng, Y., Li, C., Liu, J., Xing, G., Zhou, L., Dong, M., Li, X. and Niu, Y. (2010) β-Asarone improves cognitive function by suppressing neuronal apoptosis in the β-amyloid hippocampus injection rats. *Biol. Pharm. Bull.* **33**, 836-843.

Hashimoto, K., Yanagisawa, T., Okui, Y., Ikeya, Y., Maruno, M. and Fujita, T. (1994) Studies on anti-allergic compounds in the roots of *Asiasarum sieboldi. Planta. Med.* **60**, 124-127.

Liao, J. F., Huang, S.Y., Jan, Y. M., Yu, L. L. and Chen, C. F. (1998) Central inhibitory effects of water extract of *Acori graminei* rhizome in mice. *J. Ethnopharmacol.* **61**, 185-193.

Marquardt, D. L. and Wasserman, S. I. (1983) Modulation of rat serosal mast cell biochemistry by *in vivo* dexamethasone administration. *J. Immunol.* **131**, 934-939.

Momin, R. A., De Witt, D. L. and Nair, M. G. (2003) Inhibition of cyclooxygenase (COX) enzymes by compounds from *Daucus carota* L. seeds. *Phytother. Res.* **17**, 976-979.

Mossman, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. *J. Immunol. Methods* **65**, 55-63.

Pages, N., Mauois, P., Delplanque, B., Bac, P., Stables, J. P., Tamariz,

J., Chamorro, G. and Vamecq, J. (2010) Activities of α-asarone in various animal seizure models and in biochemical assays might be essentially accounted for by antioxidant properties. *Neurosci. Res.* **68**, 337-344.

Park, C. H., Kim., K. H., Lee, I. K., Lee, S. Y., Choi, S. U., Lee, J. H. and Lee, K. R. (2011) Phenolic constituents of *Acorus gramineus. Arch. Pharm. Res.* **34**, 1289-1296.

Rubin, P. and Mollison, K. W. (2007) Pharmacotherapy of diseases mediated by 5-lipoxygenase pathway eicosanoids. *Prostaglandins Other Lipid Mediat.* **83**, 188-197.

Schwartz, L. B., Lewis, R. A., Seldin, D. and Austen, K. F. (1981) Acid hydrolases and tryptase from secretory granules of disrupted human ling mast cells. *J. Immunol.* **126**, 1290-1294.

Tries, S., Neupert, W. and Laufer, S. (2002) The mechanism of action of the new anti-inflammatory compound ML3000: inhibition of 5-LOX and COX-1/2. *Inflamm. Res.* **51**, 135-143.

Walsh, G. M. (2011) Novel cytokine-directed therapies for asthma. *Discov. Med.* **11**, 283-291.

Zou, D. J., Wang, G., Liu, J. C., Dong, M. X., Li, X. M., Zhang, C., Zhou, L., Wang, R. and Niu, Y. C. (2011) β-Asarone attenuates β-amyloid-induced apoptosis through the inhibition of the activation of apoptosis signal-regulating kinase 1 in SH-SY5Y cells. *Pharmazie* **66**, 44-51.