

SHORT COMMUNICATION

# 5-Lipoxygenase-inhibitory Constituents from *Schizandra fructus* and *Magnolia flos*

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**In order to establish the antiallergic properties of *Schizandra fructus* and *Magnolia flos*, several compounds isolated from these plants were tested for 5-lipoxygenase (5-LOX) inhibitory activity *in vitro*, for the first time. The compounds including schizandrins, schisandrols, gomisins, fargesin, eudesmin and liriorensinol B dimethyl ether, inhibited 5-LOX-catalysed leukotriene production from A23187-treated rat basophilic leukemia (RBL-1) cells at concentrations of 1–100  $\mu\text{M}$ . In particular, constituents such as schisandrol A and gomisins showed potent inhibitory activity ( $\text{IC}_{50}\text{s} < 10 \mu\text{M}$ ) on 5-LOX-catalysed leukotriene production, but were much less active on cyclooxygenase-2-catalysed prostaglandin  $\text{E}_2$  and inducible nitric oxide-catalysed NO production. These compounds have the potential to be developed as novel antiallergic agents and may contribute to the antiallergic pharmacological use of these plant materials in Chinese medicine. Copyright © 2009 John Wiley & Sons, Ltd.**

*Keywords:* *Schizandra fructus*; *Magnolia flos*; lipoxygenase; allergy.

## INTRODUCTION

Hydroperoxy-, hydroxyeicosatetraenoic acids and leukotrienes (LT) are synthesized from arachidonic acid (AA) by the action of several isoforms of lipoxygenases (LOX). Among them, 5-LOX is mainly responsible for the oxidation of the C-5 position of AA, eventually leading to the synthesis of LTs. Since LTs are deeply associated with several allergic disorders including bronchial asthma and psoriasis, 5-LOX inhibition may exert antiallergic activity. Some 5-LOX inhibitors and LT receptor antagonists have been used clinically to treat asthma (Riccioni *et al.*, 2007). Thus it may be beneficial to explore 5-LOX inhibitors from various plant constituents to establish their antiallergic property and to determine their potential for use as antiallergic agents.

*Schizandra fructus* (*Schizandra chinensis*, Schisandraceae) has been used traditionally in Korea, Japan and China for the treatment of coughs, dryness of the mouth, spontaneous sweating, dysentery, allergic disorders and insomnia (Chen and Li, 1993). *Magnolia flos* (*Magnolia obovata*, Magnoliaceae) has also been used for the treatment of gastrointestinal disorders, anxiety and allergic diseases including bronchial asthma in Korea, Japan and China (Fujita *et al.*, 1973). It was reported previously that the aqueous extract of *Schizandra fructus* inhibited cytokine release from mast cells (Kang *et al.*,

2006). In addition, an inhibition of histamine release by *Magnolia flos* was demonstrated previously (Shen *et al.*, 2008). These reports may explain partly some of the antiallergic properties of these plant materials. However, the mechanisms of the antiallergic activity of *Schizandra fructus* and *Magnolia flos* need to be elucidated further to establish clearly their pharmacological activities. During the preliminary screening procedure, methanol extracts of *Schizandra fructus* and *Magnolia flos* were found to show 5-LOX inhibitory activity. In the present study, several isolated compounds from these two plant materials were examined for 5-LOX inhibitory activity using rat basophilic leukemia cells (RBL-1). Their inhibitory activities on cyclooxygenase-2 (COX-2)-catalysed prostaglandin  $\text{E}_2$  ( $\text{PGE}_2$ ) production and inducible nitric oxide synthase (iNOS)-catalysed NO production were also examined in lipopolysaccharide (LPS)-treated RAW 264.7 cells.

## MATERIALS AND METHODS

**Chemicals.** Nordihydroguaiaretic acid (NDGA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lipopolysaccharide (LPS, *Escherichia coli* 0127:B8) were purchased from Sigma Chemicals (St Louis, MO). N-[2-cyclohexyloxy-4-nitrophenyl]methane sulfonamide (NS-398) was obtained from Biomol (Plymouth Meeting, PA). 2-Amino-5,6-dihydro-6-methyl-4H-1,3-thiazine hydrochloride (AMT) was purchased from Tocris Cookson Ltd (UK). Dulbecco's modified Eagle medium (DMEM) and other cell culture reagents including fetal bovine serum (FBS) were products of

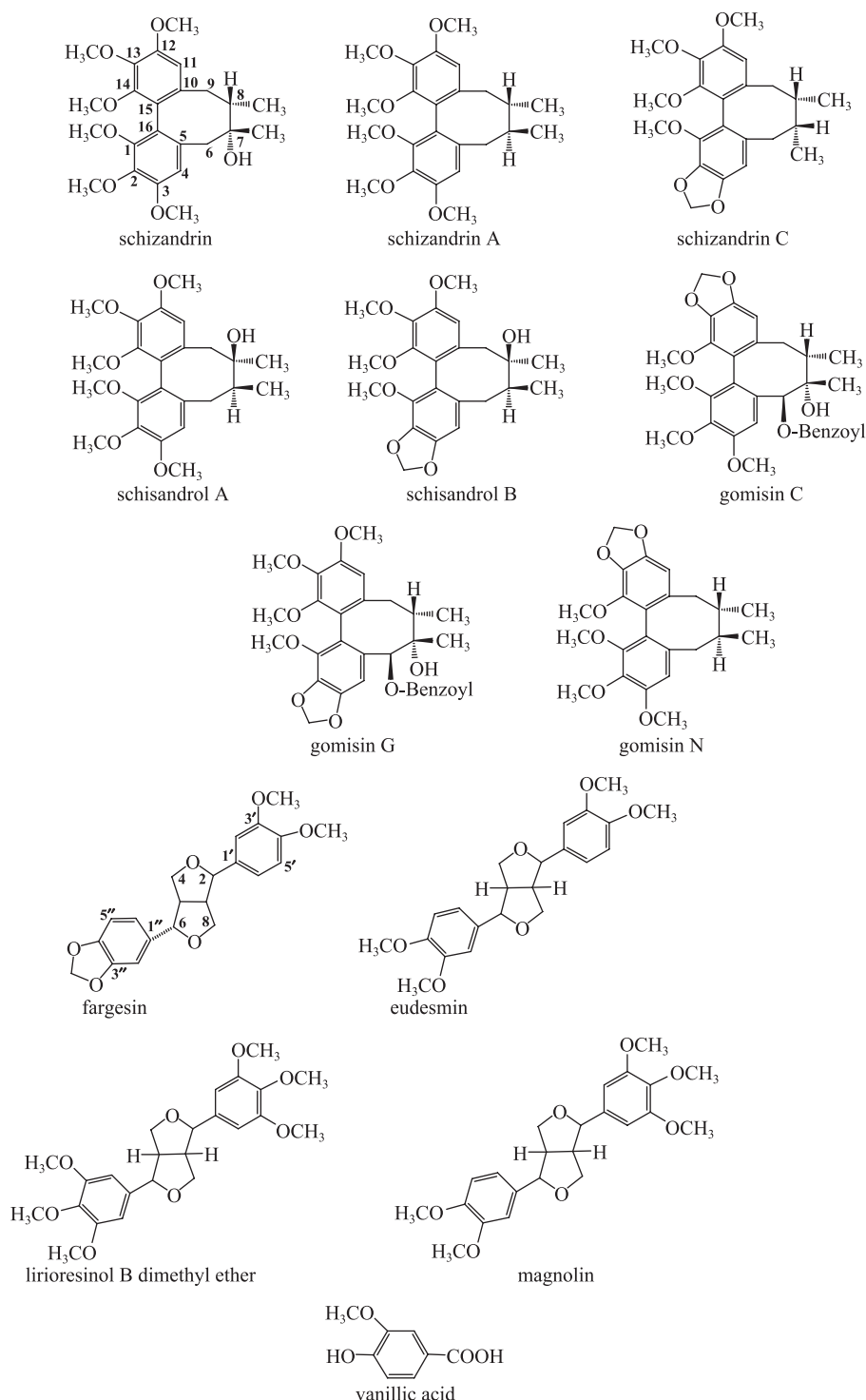
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Gibco BRL (Grand Island, NY). The protein assay kit was purchased from Bio-Rad Laboratories (Hercules, CA).

#### Isolation of the constituents from plant materials.

Schisandra fructus (*Schisandra chinensis* (Turz.) Baill., Schisandraceae) and Magnolia flos (*Magnolia ovobata*, Magnoliaceae) were purchased from a local market (Seoul, Korea) and authenticated by Professor K. Bae (Chungnam National University, Korea). Voucher specimens (CNU-1023, *Schisandra fructus*; CNU-594, *Magnolia flos*) were deposited in the herbarium of the

College of Pharmacy, Chungnam National University. From *Schisandra fructus*, the compounds were isolated, and structurally identified using previously described methods (Hung *et al.*, 2007) (Fig. 1). From the methanol extract (865 g) of *Magnolia flos* (10.45 kg), the  $\text{CH}_2\text{Cl}_2$  fraction (458.9 g) was obtained and chromatographically resolved on a silica gel using stepwise gradient elution with the solvents hexane–EtOAc, giving 15 fractions (MF-1–MF-15). Fraction MF-5 was recrystallized with MeOH to give fargesin. Silica gel column chromatography of fraction MF-10 eluted with hexane– $\text{CH}_2\text{Cl}_2$ –EtOAc (gradient) resulted in two sub-fractions



**Figure 1.** Chemical structures of the isolated compounds from *Schisandra fructus* and *Magnolia flos*.

(MF-10-1–MF-10-4). Sub-fraction MF-10-2 was recrystallized with MeOH to give eudesmin. Silica gel column chromatography of fraction MF-13 eluted with hexane–CH<sub>2</sub>Cl<sub>2</sub>–EtOAc (gradient) resulted in six sub-fractions (MF-13-1–MF-13-6). Sub-fraction MF-13-6 was recrystallized with MeOH to give liriorelinol B dimethyl ether. Silica gel column chromatography of fraction MF-15 eluted with hexane–CH<sub>2</sub>Cl<sub>2</sub>–EtOAc (gradient) resulted in seven sub-fractions (MF-15-1–MF-15-7). Sephadex LH-20 column chromatography of fraction MF-15-5 eluted with MeOH resulted in magnolin. Vanillic acid was obtained from the EtOAc fraction using Sephadex LH-20 column chromatography. The chemical structures of the compounds isolated were verified by comparison of the NMR data with those reported previously (Iida *et al.*, 1982; Ma and Han, 1995).

**Effects of the isolates on 5-LOX.** In order to evaluate the 5-LOX inhibitory activity, rat basophilic leukemia cells (RBL-1) purchased from American Type Culture Collection (ATCC, Rockville, MD) were cultured in RPMI 1640 with 10% FBS, 2 mM glutamine and 1% antibiotics under 5% CO<sub>2</sub> at 37 °C. The cells were plated in a 96-well plate for 2 h. The tested compounds were added and the cells were pre-incubated for 10 min. For 5-LOX activation, 3 μM of A-23187 (ionophore) was added and the cells were incubated for another 15 min using a slight modification of the previously described procedure (Tries *et al.*, 2002). The medium was collected and the concentration of 5-LOX products, cysteinyl leukotrienes (LTC<sub>4</sub>/D<sub>4</sub>/E<sub>4</sub>), was measured using an ELISA kit (Cayman Chemicals, Ann Arbor, MI) according to the manufacturer's recommended procedures. When cell viability was assessed by the MTT assay in all experiments, no significant cytotoxicity of the compounds tested was observed on RBL-1 cells at concentrations up to 100 μM.

**RAW 264.7 cell culture and measurement of NO and PGE<sub>2</sub> concentrations.** For examination of the COX-2- and iNOS-mediated PGE<sub>2</sub> and NO production, RAW 264.7 cells obtained from ATCC were cultured and activated with LPS based on previously described procedures (Chi *et al.*, 2001). The cells were plated in 96-well plates (2 × 10<sup>5</sup> cells/well). Test compounds and LPS (1 μg/mL) were added and the cells were incubated for 24 h. The PGE<sub>2</sub> concentration in the medium was measured using an ELISA kit for PGE<sub>2</sub> (Cayman Chemicals) according to the manufacturer's recommendations. The NO concentration was determined using Griess reagent. On RAW 264.7 cells, only schizandrin A was found to be cytotoxic at 100 μM by MTT assay.

## RESULTS AND DISCUSSION

It is well established that RBL-1 cells produce high amounts of LTs by 5-LOX when activated with A23187. In a typical experiment, RBL-1 cells synthesized cysteinyl LTs (1415.6 ± 180.9 pg/mL) in 15 min from the basal level of 6.1 ± 0.5 pg/mL by A23187 treatment. NDGA (LOX inhibitor) strongly inhibited LT synthesis as expected (92.7% inhibition at 1 μM). Under these conditions, compounds such as schizandrin considerably

inhibited 5-LOX-catalysed LT production concentration-dependently. Their IC<sub>50</sub> values are listed in Table 1. In particular, schisandrol A and gomisin showed the most potent inhibitory activity with IC<sub>50</sub> values less than 10 μM. Among the constituents from Magnolia flos, fargesin, eudesmin and liriorelinol B dimethyl ether strongly inhibited 5-LOX. In contrast, these compounds showed little or no inhibitory activity on COX-2-mediated PG and iNOS-mediated NO production in LPS-treated RAW 264.7 cells, a mouse macrophage-like cell line. Only schizandrin showed considerable iNOS inhibitory activity in these cells. In accordance with these results, the crude methanol extracts of these two plant materials also inhibited 5-LOX strongly, while far less inhibition was observed on PGE<sub>2</sub> and NO production.

Among the constituents from Schizandra fructus, the following structure–activity relationships were found. The 8-hydroxyl group increased 5-LOX inhibitory activity (schizandrin A vs schisandrol A and B). The 12,13-methylenedioxy group increased 5-LOX inhibition (schizandrin A vs gomisin C and N). 6-Benzoyl and 7-hydroxy residues also potentiated the inhibition (schizandrin A vs gomisin C and G). However, no consistent chemical structure to affect 5-LOX was found in the constituents of Magnolia flos.

**Table 1.** The IC<sub>50</sub> values of the isolated constituents against 5-LOX-catalysed LT, COX-2-catalysed PGE<sub>2</sub> and iNOS-catalysed NO production

Compound	IC <sub>50</sub> (μM or μg/mL) <sup>a</sup>		
	5-LOX	COX-2	iNOS
MeOH extract of Schizandra fructus	71.7	>50 <sup>b</sup>	>50
Schizandrin	39.7	>100 <sup>c</sup>	73.1
Schizandrin A	34.1	cyt. <sup>d</sup>	cyt.
Schizandrin C	36.8	– <sup>e</sup>	>100
Schisandrol A	7.6	>100	>100
Schisandrol B	20.1	>100	–
Gomisin C	4.1	>100	>100
Gomisin G	7.4	>100	–
Gomisin N	6.6	>100	>100
MeOH extract of Magnolia flos	18.6	>50	>50
Fargesin	25.5	–	>100
Eudesmin	43.9	–	>100
Liriorelinol B DME	16.2	–	>100
Magnolin	>100	–	>100
Vanillic acid	–	>100	>100
NDGA	0.2–1 <sup>f</sup>	NT	NT

NS-398 (selective COX-2 inhibitor) and AMT (NOS inhibitor) showed 98.8% and 97.7% inhibition of COX-2-catalysed PGE<sub>2</sub> and iNOS-catalysed NO production at 0.1 μM, respectively.

<sup>a</sup> The IC<sub>50</sub> values represented here were obtained from the inhibitory results of at least four different concentrations of the compounds (*n* = 3). The IC<sub>50</sub> values of all isolated and reference compounds were expressed in μM, while those of the MeOH extracts were expressed as μg/mL.

<sup>b</sup> At 50 μg/mL, these compounds showed less than 50% inhibition.

<sup>c</sup> At 100 μM, the compounds tested showed higher than 10% inhibition, but less than 50% inhibition.

<sup>d</sup> Cytotoxic at 100 μM.

<sup>e</sup> Less than 10% or no inhibition 100 μM.

<sup>f</sup> The ranges of IC<sub>50</sub> values from five independent experiments. NT, not tested.

Although there has been no previous report concerning the 5-LOX inhibition of any of the compounds tested in this study, several investigations of the antiallergic activity of these isolated compounds have been described previously. For instance, Schisandra lignans were demonstrated to inhibit passive cutaneous anaphylaxis reactions (Lee *et al.*, 2007). Interestingly, gomisin A was reported to inhibit the arachidonate cascade in macrophages. However, the authors claimed that the same compound did not inhibit 5-LOX activity (Ohkura *et al.*, 1990). In this study, gomisins C, G and N were found to be 5-LOX inhibitory. But it is not clear at present whether gomisin A is 5-LOX inhibitory or not, since gomisin A was not available for the present study. Among the constituents of *Magnolia flos*, eudesmin was demonstrated previously to inhibit the inflammatory cytokine release and T-cell proliferation (Cho *et al.*,

1999). To our knowledge, the present investigation is the first study demonstrating 5-LOX inhibitory activity of the compounds tested. From the results obtained, it is concluded that schizandrins, schisandrols, gomisins, fargesin, eudesmin and liriioresinol B dimethyl ether are 5-LOX inhibitors, being less active on COX-2 and iNOS. These 5-LOX inhibitory constituents may contribute at least in part to the antiallergic use of *Schisandra fructus* and *Magnolia flos* in Chinese medicine.

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