JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 1227–1236

Received December 18, 2007
Accepted May 16, 2008
DOI 10.1211/jpp.60.9.0016
ISSN 0022-3573

Inhibitory activities of prenylated flavonoids from *Sophora flavescens* against aldose reductase and generation of advanced glycation endproducts

Hyun Ah Jung, Na Young Yoon, Sam Sik Kang, Yeong Shik Kim and Jae Sue Choi

Abstract

Important targets for the prevention and treatment of diabetic complications include aldose reductase (AR) inhibitors (ARIs) and inhibitors of advanced glycation endproduct (AGE) formation. Here we evaluate the inhibitory activities of prenylated flavonoids isolated from *Sophora flavescens*, a traditional herbal medicine, on rat lens AR (RLAR), human recombinant AR (HRAR) and AGE formation. Among the tested compounds, two prenylated chalcones – desmethylanhydroicaritin (**1**) and 8-lavandulylkaempferol (**2**) – along with five prenylated flavanones – kurarinol (**8**), kurarinone (**9**), (*2S*)-2′-methoxykurarinone (**10**), (*2S*)-3β,7,4′-trihydroxy-5-methoxy-8-(γ,γ-dimethylally)-flavanone (**11**), and kushenol E (**13**) were potent inhibitors of RLAR, with IC50 values of 0.95, 3.80, 2.13, 2.99, 3.77, 3.63 and 7.74 μM, respectively, compared with quercetin (IC50 7.73 μM). In the HRAR assay, most of the prenylated flavonoids tested showed marked inhibitory activity compared with quercetin (IC50 2.54 μM). In particular, all tested prenylated flavonols, such as desmethylanhydroicaritin (**1**, IC50 0.45 μM), 8-lavandulylkaempferol (**2**, IC50 0.79 μM) and kushenol C (**3**, IC50 0.85 μM), as well as a prenylated chalcone, kuraridin (**5**, IC50 0.27 μM), and a prenylated flavanone, (*2S*)-7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylally)-flavanone (**12**, IC50 0.37 μM), showed significant inhibitory activities compared with the potent AR inhibitor epalrestat (IC50 0.28 μM). Interestingly, prenylated flavonoids **1** (IC50 104.3 μg mL^{-1}), **2** (IC50 132.1 μg mL^{-1}), **3** (IC50 84.6 μg mL^{-1}) and **11** (IC50 261.0 μg mL^{-1}), which harbour a 3-hydroxyl group, also possessed good inhibitory activity toward AGE formation compared with the positive control aminoguanidine (IC50 115.7 μg mL^{-1}). Thus, *S. flavescens* and its prenylated flavonoids inhibit the processes that underlie diabetic complications and related diseases and may therefore have therapeutic benefit.

Division of Food Science and Biotechnology, Pukyong National University, Busan 608-737, South Korea

Hyun Ah Jung, Na Young Yoon, Jae Sue Choi

Department of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, South Korea

Sam Sik Kang, Yeong Shik Kim

Correspondence: Jae Sue Choi, Division of Food Science and Biotechnology, Pukyong National University, Busan 608-737, South Korea. E-mail: choijs@pknu.ac.kr

Funding: This study was supported financially by the Pukyong National University under the 2007 Post-Doc. Programme. This study was also supported by a grant from the Food & Drug Administration, South Korea (2005).

Introduction

According to a report from the US National Health and Nutrition Examination Survey 1999–2004, nearly 60% of patients with diabetes have more than one complication caused by chronic diabetes (AACE 2007). Moreover, diabetic complications, including retinopathy, neuropathy, nephropathy and cataracts, are leading causes of morbidity and death in diabetic patients. There is therefore growing interest in drugs that alleviate the various symptoms of diabetic complications.

Several studies have suggested that hyperglycaemia may have important roles in the pathogenesis of diabetic complications by several mechanisms, including increased aldose reductase (AR)-related polyol pathway flux, increased formation of advanced glycation endproducts (AGE), activation of protein kinase C (PKC) isoforms, increased hexosamine pathway flux and overproduction of superoxide (Brownlee 2001). There is much evidence for increased formation of AGE and the overexpression of AGE receptors (RAGE), as well as AR-related polyol pathway flux under conditions of chronic hyperglycaemia in-vivo (Peyrou & Sternberg 2006). We have investigated two important targets to evaluate their potential for the prevention and treatment of diabetic complications: one is to search for AR inhibitors (ARIs) and the other is to search for inhibitors of AGE formation.

AR is an NADPH-dependent oxidoreductase, one of the important enzymes in the polyol pathway that catalyses the reduction of various sugars to sugar alcohols, such as glucose to

sorbitol, which is followed by fructose production catalysed by NADH-dependent sorbitol dehydrogenase. Under normal conditions, the affinities of cell-based AR for glucose and galactose are low; however, in the diabetic condition, a surge in the rate of the AR-related polyol pathway augments intracellular concentrations of sorbitol and its metabolites (i.e. fructose), followed by accumulation in cells due to their poor penetration across membranes and inefficient metabolism; this results in the development of diabetic complications (Kador et al 1980; de la Fuente et al 2003; Kawanishi et al 2003). Excess glucose is metabolized into sorbitol by the NADPH-AR pathway, followed by fructose formation induced by NADH-sorbitol dehydrogenase, leading to the formation of reactive dicarbonyl species, which are highly related to formation of AGE (Ahmed & Thornalley 2007). Although several synthetic ARIs exhibit potent effects, either their use is limited, or they have been withdrawn from clinical trials because of relatively low efficacy, poor pharmacokinetics and unsatisfactory safety (Kawanishi et al 2003; Manzanaro et al 2006; Peyrou & Sternberg 2006). In the case of one potent AGE formation inhibitor, aminoguanidine, there is still relatively little human data; side-effects in human trials included abnormalities in liver function, gastrointestinal disturbances, nausea and headaches, despite very low toxicity in both human and animal trials (Thornalley 2003). Although these synthetic agents have been proposed as prototypes for new promising drugs, pharmaceutical companies and many researchers have tried to find new potent and safe ARIs and AGE formation inhibitors from natural materials (Nakagawa et al 2002; de la Fuente et al 2003; Kawanishi et al 2003). There is growing interest in the benefits of dietary supplements as nutraceuticals, as well as traditional herbal medicines as pharmaceuticals that lack toxic and/or side-effects.

Sophora flavescens Ait. (Leguminosae) is a perennial shrub that occurs in the wild and is also cultivated throughout North East Asia. The dried root of *S. flavescens*, Sophorae Radix, is an important herbal medicine that is used in folk medicine as an antipyretic, analgesic, anthelmintic and stomachic, and is used for the treatment of gastrointestinal haemorrhage, diarrhoea and eczema (Tang & Eisenbrand 1992; Huang 1993). *S. flavescens* is reported to harbour quinolizidine alkaloids, triterpenoid saponins and prenylated flavonoids (Tang & Eisenbrand 1992), which are responsible for its various biological and pharmacological properties, including anti-diabetic (Jung et al 2006; Kim et al 2006), anti-apoptotic (Jiang et al 2007), anti-bacterial (Chen et al 2005), anti-inflammatory (Kim et al 2002), antioxidant (Jung et al 2005a,b; Piao et al 2006), anti-viral (Ding et al 2006) and cytotoxic effects (Kang et al 2000; Ko et al 2000). On the basis of their quantitative and qualitative aspects, many researchers have focused on the matrine-type alkaloids and prenylated flavonoids (Chen et al 2005; Zhang et al 2007). In particular, there is growing interest in the prenylated flavonoids, which are implicated in the anti-inflammatory (Kim et al 2002), antioxidant (Jung et al 2005a,b; Piao et al 2006), anti-diabetic (Kim et al 2006), monoamine oxidase inhibitory (Hwang et al 2005) and cytotoxic effects (Kang et al 2000; Ko et al 2000) of *S. flavescens*. Among these flavonoids, sophoraflavanone G and kurarinone have been reported to have potent cytotoxic activity (Kang et al 2000; De Naeyer et al 2004) and radical scavenging activity (Piao et al 2006), as well as inhibitory activities against tyrosinase (Kim et al 2003; Son et al 2003), α-glucosidase and β-amylase (Kim et al 2006). Also, kuraridin, kurarinone and sophoraflavanone G have been reported to inhibit cyclo-oxygenases and lipoxygenases (Chi et al 2001; Kim et al 2002). We have previously demonstrated the antioxidant properties of desmethylanhydroicaritin and 8-lavandulylkaempferol from *S. flavescens* as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and peroxynitrite ($ONOO^-$) scavengers in vitro (Jung et al 2005a,b).

As mentioned above, *S. flavescens* and its isolated prenylated flavonoids have exhibited anti-diabetic activities in several enzymatic systems (Kim et al 2006) and inhibit the Na^+-glucose cotransporter (Sato et al 2007), implicated in diabetes. Also, Jung et al (2006) have previously shown that *Sophora* species exhibit antioxidant-capacity-related anti-diabetic effects in rats with streptozotocin-induced diabetes. However, comprehensive examinations of *S. flavescens*-derived prenylated flavonoids and their effects on diabetic complications mediated via different enzymes have not been done. Moreover, there has been limited investigation of the AR inhibitory activities of prenylated flavonoids, despite numerous studies on various flavonoids functioning as ARIs. The aims of our research were therefore to evaluate the inhibitory effects of *S. flavescens* and fourteen prenylated flavonoids against rat lens AR (RLAR), human recombinant AR (HRAR) and formation of AGE. The flavonoids included three flavonols: desmethylanhydroicaritin (**1**), 8-C-lavandulylkaempferol (**2**) and kushenol C (**3**); three chalcones: kuraridinol (**4**), kuraridin (**5**) and xanthohumol (**6**); and eight flavanones: sophoraflavanone G (**7**), kurarinol (**8**), kurarinone (**9**), (2S)-2′-methoxykurarinone (**10**), (2S)-3β,7,4′-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**11**), (2S)-7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**12**), kushenol E (**13**) and leachianone G (**14**).

Materials and Methods

The ^{1}H- and ^{13}C-NMR spectra were determined using a JEOL JNM ECP-400 spectrometer (Tokyo, Japan) at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR in DMSO-d_6. Column chromatography was performed using silica gel 60 (70–230 mesh, Merck, Darmstadt, Germany), RP-18 Lichroprep (40–63 μM, Merck) and Sephadex LH-20 (20–100 μM, Sigma, St Louis, MO, USA). Thin-layer chromatography was conducted on precoated Kieselgel 60 F254 plates (20 × 20 cm, 0.25 mm; Merck) and RP-18 F_{254s} plates (5 × 10 cm; Merck), using 50% H_2SO_4 as a spray reagent.

Chemicals

Bovine serum albumin, aminoguanidine hydrochloride, D-(−)-fructose, D-(+)-glucose, NADPH, quercetin, kaempferol, and DL-glyceraldehyde dimer were purchased from Sigma. Sodium azide was purchased from Junsei Chemical Co. (Tokyo, Japan) and and HRAR (0.4 units) was from Wako Chemicals (Osaka, Japan). Epalrestat was generously donated by Dr J. S. Kim from the Korea Institute of Oriental Medicine. All solvents used in the column chromatography were of reagent grade, and were purchased from commercial sources.

Plant materials

The *S. flavescens* Aiton roots were collected from Yeong-Cheon, Kyeong Buk Province, Korea, in March 2004, and were authenticated by Professor J. H. Lee (Dong Guk University, Seoul, Korea). A voucher specimen (no. 20040320) was deposited in the author's laboratory (J. S. Choi).

Extraction, fractionation and isolation

The dried roots of *S. flavescens* (10 kg) were refluxed with methanol (MeOH) for 3 h (3×10 L). The total filtrate was then concentrated to dryness in vacuo at 40°C in order to render the MeOH extract (2.2 kg). This extract was suspended in distilled water and then successively partitioned with methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc) and *n*-butanol (BuOH) to yield CH_2Cl_2 (230 g), EtOAc (250 g) and *n*-BuOH (610 g) fractions, respectively, as well as an aqueous residue (1110 g). Repeated chromatography of the CH_2Cl_2 fraction over a silica-gel column and Sephadex LH20 yielded desmethylanhydroicaritin (**1**, 20 mg), xanthohumol (**6**, 15 mg), (2*S*)-2′-methoxykurarinone (**10**, 50 mg), (2*S*)-3β,7,4′-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**11**, 35 mg) and (2S)-7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**12**, 50 mg).

The EtOAc fraction (250 g) was subject to successive chromatography using silica gel, RP-18 gel and Sephadex LH20 to obtain 8-C-lavandulylkaempferol (**2**, 25 mg), kushenol C (**3**, 80 mg), kuraridinol (**4**, 1.08 g), kuraridin (**5**, 20 mg), sophoraflavanone G (**7**, 30 mg), kurarinol (**8**, 3.20 g), kurarinone (**9**, 107 mg), kushenol E (**13**, 20 mg) and leachianone G (**14**, 15 mg) (Jung et al 2005a,b, 2008).

All isolated compounds were characterized and identified by spectroscopic methods, including ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz), as well as by comparisons with published data (i.e. three flavonols: desmethylanhydroicaritin (**1**), 8-C-lavandulylkaempferol (**2**) and kushenol C (**3**); three chalcones: kuraridinol (**4**), kuraridin (**5**) and xanthohumol (**6**); and eight flavanones: sophoraflavanone G (**7**), kurarinol (**8**), kurarinone (**9**), (2S)-2′-methoxykurarinone (**10**), (2S)- 3β,7,4′-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**11**), (2S)-7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**12**), kushenol E (**13**) and leachianone G (**14**) (Ryu et al 1997; Wu et al 1985; Jung et al 2004, 2005a,b). The structures of the prenylated flavonoids are shown in Figure 1.

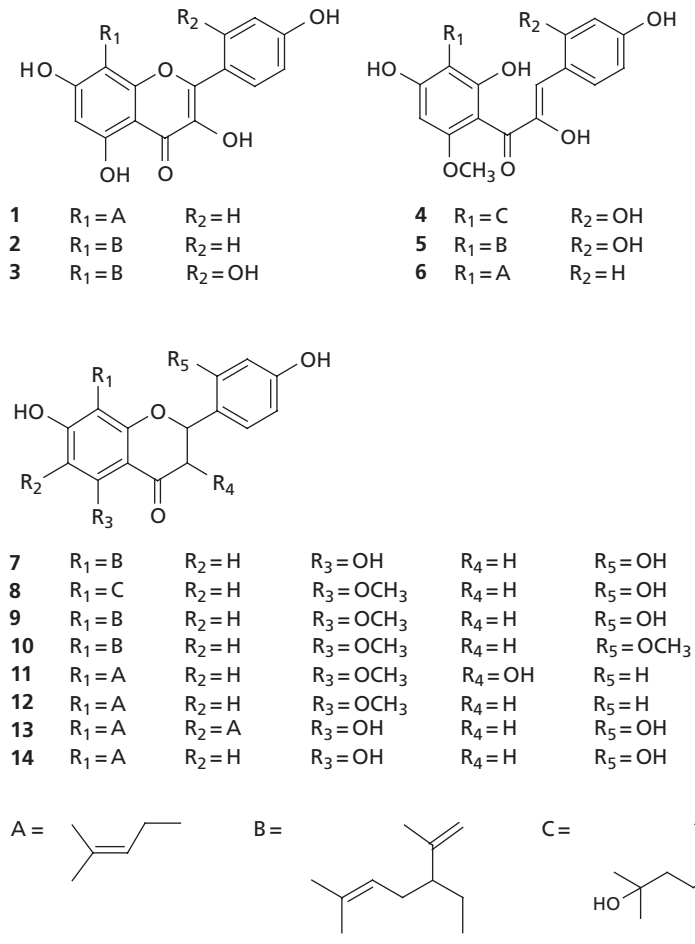

Figure 1 Structures of prenylated flavonoids from *S. flavescens*.

Assay for RLAR inhibitory activity

In these experiments we followed The Guidelines for Care and Use of Laboratory Animals as approved by Pukyong National University. Rat lens homogenate was prepared according to the modified method of Hayman & Kinoshita (1965). Briefly, the lenses were removed from the eyes of Sprague–Dawley rats (Samtako BioKorea, Inc., Osan, Korea) weighing 250–280 g. The lenses were homogenized in sodium phosphate buffer (pH 6.2), which was prepared from dibasic sodium phosphate ($Na_2HPO_4 \cdot H_2O$, 0.66 g) and monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$, 1.27 g) in 100 mL of double-distilled water. The supernatant was obtained by centrifugation of the homogenate at 10000 rev min^{-1} at 4°C for 20 min and was frozen until use. A crude AR, with a specific activity of 6.5 U mg^{-1}, was used in the evaluations of enzyme inhibition. The partially purified material was separated into 1.0 mL aliquots and stored at −40°C. Each 1.0 mL cuvette contained equal units of enzyme, 100 mM sodium phosphate buffer (pH 6.2) and 1.6 mM NADPH, both with and without 50 mM of the substrate, DL-glyceraldehyde, and an inhibitor (final concentration ~1–100 $\mu g\,mL^{-1}$ for the extract and fractions, and ~0.4–10 $\mu g\,mL^{-1}$ for the compounds, dissolved in DMSO). The AR activity was determined by measuring the decrease in NADPH absorption at 340 nm over 4 min, using a Ultrospec2100pro UV/Visible spectrophotometer with SWIFT II Applications software (Amersham Biosciences, New Jersey, USA). Quercetin and epalrestat, which are well known ARIs, along with kaempferol were used as references. The inhibition percentage was calculated as $[1-(\Delta A \text{ sample} - \Delta A \text{ blank}) / (\Delta A \text{ control} - \Delta A \text{ blank})] \times 100$, where ΔA represents the change in absorbance in 4 min with the test sample plus substrate (glyceraldehyde), or control (100% DMSO + glyceraldehyde). The concentration that produced 50% inhibition (IC50) is expressed as the mean ± s.e.m.

Assay for HRAR inhibitory activity

Inhibition of HRAR was determined according to the method of Nishimura et al (1991). The reaction mixture was prepared as follows: 100 μL 0.15 mM NADPH, 100 μL 10 mM DL-glyceraldehyde as a substrate, 5 μL RHAR and various concentrations of the samples (final concentration ~0.04–5 $\mu g\,mL^{-1}$, dissolved in DMSO) in a total volume of 1.0 mL 100 mM sodium phosphate buffer (pH 6.2). The AR activity was determined by measuring the decrease in NADPH absorption at 340 nm over 1 min. Quercetin and epalrestat, well known ARIs, along with kaempferol, were used as references. The percentage inhibition was calculated as for the RLAR assay above, except that ΔA sample min^{-1} represents the decrease in absorbance in 1 min with the test samples and substrate. The IC50 values are means from triplicate experiments.

Inhibition of AGE formation

Inhibition of AGE formation was determined according to the modified method of Vinson & Howard (1996). To prepare the AGE reaction solution, 10 $mg\,mL^{-1}$ bovine serum albumin in 50 mM sodium phosphate buffer (pH 7.4), with 0.02% sodium azide to prevent bacterial growth, was added to 0.2 M fructose and 0.2 M glucose. The reaction mixture (950 μL) was then mixed with various concentrations of the samples (50 μL, final concentration ~4–200 $\mu g\,mL^{-1}$ for the extracts, fractions and compounds) dissolved in 10% DMSO. After incubating at 37°C for 7 days, the fluorescence intensity of the reaction products was determined using a spectrofluorometric detector (FLx800 microplate fluorescence reader, Bio-Tek Instruments, Inc., Winooski, VT, USA), with excitation and emission wavelengths at 350 nm and 450 nm, respectively. The percentage inhibition of AGE formation was determined from a graphical plot of the data and is expressed as the mean ± s.e.m. (triplicate experiments). The nucleophilic hydrazine aminoguanidine hydrochloride was used as a reference in the AGE assay.

Statistical analysis

The Kruskal–Wallis and Mann–Whitney *U* tests were used to determine the statistical significance of differences between values for various experimental and control groups. Data are expressed as the mean (determined graphically) ± s.e.m. performed in triplicate.

Results

Inhibition of aldose reductase

To evaluate the likely effects of the roots of *S. flavescens* on diabetic complications, inhibition of RLAR activity by the MeOH extract and its fractions were measured by the modified method of Hayman and Kinoshita (1965). As shown in Table 1, the MeOH *S. flavescens* extract and its CH_2Cl_2 and EtOAc fractions exhibited potent inhibitory activities in the RLAR assay, with mean IC50 values of 8.24, 6.21 and 6.92 $\mu g\,mL^{-1}$, respectively, compared with the positive

Table 1 Inhibitory activities of the MeOH extract and its fractions from *S. flavescens* against rat lens aldose reductase

Test sample	Concn ($\mu g\,mL^{-1}$)[a]	Inhibition (%)			IC50 ($\mu g\,mL^{-1}$)[b]
Quercetin	5	68.67	68.67	67.47	2.16 ± 0.06
	1	43.37	42.17	42.17	
MeOH	10	56.63	55.42	56.63	8.24 ± 0.16
extract	1	27.71	22.89	21.69	
CH_2Cl_2	10	62.32	71.01	71.01	6.21 ± 0.35
fraction	1	24.64	27.54	24.64	
EtOAc	10	60.87	66.67	66.67	6.92 ± 0.03
fraction	1	28.99	18.84	17.39	
n-BuOH	100	46.88	48.44		112.26 ± 4.09
fraction	10	29.69	31.25		
Aqueous	100	26.56	29.69		261.42 ± 15.75
fraction	10	15.62	15.63		

[a]Final concentrations of test samples were 1–100 $\mu g\,mL^{-1}$ for the extract and fractions and 1–10 $\mu g\,mL^{-1}$ for quercetin, dissolved in DMSO. [b]The concentration that caused 50% inhibition (IC50) is given as the mean ± s.e.m. of triplicate experiments.

control, quercetin (IC50 2.16 $\mu g mL^{-1}$). On the other hand, two polar fractions, including the *n*-BuOH and aqueous fractions, showed marginal inhibitory activities, even at higher concentrations (IC50 values 112.26 and 261.42 $\mu g mL^{-1}$, respectively).

The inhibitory activities of the fourteen prenylated flavonoids isolated from the CH_2Cl_2 and EtOAc fractions of *S. flavescens* against RLAR are shown in Table 2. On the whole, the prenylated flavonols and prenylated flavanones showed higher activities than the prenylated chalcones in the RLAR assay. Among the tested compounds, two prenylated flavonols – desmethylanhydroicaritin (**1**) and 8-lavandulylkaempferol (**2**) – exhibited remarkable inhibition of RLAR activities, with IC50 values of 0.95 μM and 3.80 μM, respectively, compared with IC50 values of 7.73 μM and 0.28 μM for quercetin and epalrestat, respectively, two well known ARIs. Also, most of the prenylated flavanones, kurarinol (**8**), kurarinone (**9**), (2*S*)-2′-methoxykurarinone (**10**), (2*S*)-3β,7,4′-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**11**) and kushenol E (**13**), were potent inhibitors of RLAR, with IC50 values of 2.13, 2.99, 3.77, 3.63 and 7.74 μM, respectively. Xanthohumol (**6**) and leachianone G (**14**) showed good inhibition of RLAR, with IC50 values of 10.73 and 12.97 μM, respectively, followed by two prenylated chalcones **4** and **5**, with IC50 values of 22.14 and 21.60 μM, respectively. The prenylated flavonoids **7** and **12** showed marginal inhibitory capacity (IC50 values 59.58 and 32.69 μM, respectively).

The inhibition of HRAR by the prenylated flavonoids isolated from *S. flavescens* is shown in Table 3. Prenylated

Table 2 Inhibitory activities of prenylated flavonoids from *S. flavescens* against rat lens aldose reductase

Flavonoid	Concn ($\mu g mL^{-1}$)[a]	Inhibition (%)		IC50[b]	
				$\mu g mL^{-1}$	μM
Quercetin	5	71.95	73.17	2.61 ± 0.10	7.73 ± 0.29
	2.5	47.56	50.24		
Kaempferol	5	65.00	65.00	2.77 ± 0.06	9.67 ± 0.13
	1	37.50	38.75		
Epalrestat	0.1	55.56	59.26	0.09 ± 0.00	0.28 ± 0.01
	0.01	–6.17	–4.94		
Flavonols					
1	0.4	60.29	55.88	0.34 ± 0.03	0.95 ± 0.04
	0.08	20.59	11.76		
2	2	63.79	56.60	1.61 ± 0.13	3.80 ± 0.23
	0.4	22.41	15.22		
3	10	60.87	55.43	8.12 ± 0.34	18.54 ± 0.55
	2	21.74	27.17		
Chalcones					
4	10	53.66	47.56	10.10 ± 1.51	22.14 ± 2.34
	2	32.93	35.37		
5	10	52.44	51.22	9.46 ± 0.19	21.60 ± 0.31
	2	25.61	23.17		
6	10	74.39	75.61	3.80 ± 0.20	10.73 ± 0.40
	2	43.90	41.46		
Flavanones					
7	50	70.73	70.73	25.26 ± 0.45	59.58 ± 0.75
	10	36.59	37.80		
8	2	69.51	62.20	0.97 ± 0.19	2.13 ± 0.29
	0.4	43.90	39.02		
9	2	65.85	62.20	1.31 ± 0.05	2.99 ± 0.08
	0.4	31.71	31.71		
10	2	57.81	58.49	1.70 ± 0.00	3.77 ± 0.00
	0.4	15.62	13.04		
11	2	62.50	60.38	1.34 ± 0.14	3.63 ± 0.27
	0.4	37.50	28.26		
12	10	51.72	41.67	11.57 ± 4.24	32.69 ± 8.47
	2	46.55	30.19		
13	10	74.67	73.33	3.29 ± 0.29	7.74 ± 0.47
	2	44.00	46.67		
14	10	66.67	64.00	4.62 ± 0.62	12.97 ± 1.23
	2	38.67	45.33		

[a]Final concentrations of test samples were 0.4–10 $\mu g mL^{-1}$ for the compounds, which were dissolved in DMSO. [b]The concentration giving 50% inhibition (IC50, in $\mu g mL^{-1}$ and μM) were calculated from the log dose inhibition curve and expressed as the mean ± s.e.m of duplicate experiments.

flavonols, including desmethylanhydroicaritin (**1**), 8-C-lavandulylkaempferol (**2**) and kushenol C (**3**), showed marked inhibitory activities, with IC50 values of 0.45, 0.79 and 0.85 μM, respectively, compared with an IC50 value of 2.54 μM for quercetin. Among the prenylated chalcones tested, xanthohumol (**6**) with a 4′-hydroxyl group, had no inhibitory activity, whereas kuraridinol (**4**) and kuraridin (**5**), with a 2′,4′-dihydroxyl group and 5-methoxyl group, exhibited marked inhibitory activities (IC50 1.32 and 0.27 μM, respectively). Interestingly, one prenylated flavonol (compound **3**) and two prenylated flavanones (compounds **7** and **12**) showed poor inhibitory activities in the RLAR assay (IC50 18.54, 59.58, and 32.69 μM, respectively) but showed potent inhibitory activities in the HRAR assay, with IC50 values of 0.85, 1.42 and 0.37 μM, respectively. In particular, kuraridin (**5**) and (2S)-7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**12**) were potent inhibitors of HRAR compared with the ARI epalrestat (IC50 0.28 μM). Inhibition of RLAR by the prenylated flavanones **13** and **14** was moderate (IC50 7.74 and 12.97 μM) but these compounds had similar potency to quercetin against HRAR (IC50 2.09 μM for **13**, 2.49 μM for **14** vs 2.54 μM for quercetin). Even though prenylated flavanones **8–11** showed significant inhibitory activities in the RLAR assay, they had moderate activities in the HRAR assay, with IC50 values of 4.39, 3.81, 11.06 and 4.50 μM, respectively.

Table 3 Inhibitory activities of prenylated flavonoids from *S. flavescens* against recombinant human aldose reductase

Flavonoid	Concn (μg mL^{-1})[a]	Inhibition (%)[b]	IC50[c]	
			μg mL^{-1}	μM
Quercetin	2.5	80	0.86	2.54
	0.5	50		
	0.1	30		
Kaempferol	2.5	53	2.30	8.04
	0.5	20		
Epalrestat	0.05	83	0.09	0.28
	0.125	8		
Flavonols				
1	0.2	62	0.16	0.45
	0.04	23		
2	1	75	0.33	0.79
	0.2	45		
3	1	87	0.37	0.85
	0.2	40		
Chalcones				
4	1	65	0.60	1.32
	0.2	35		
5	5	70	0.12	0.27
	0.2	65		
	0.04	35		
6	10	–	–	–
Flavanones				
7	1	65	0.60	1.42
	0.2	35		
8	5	65	2.00	4.39
	1	45		
9	5	75	1.67	3.81
	1	45		
10	5	50	5.00	11.06
	1	45		
11	5	75	1.67	4.50
	1	45		
12	5	90	0.13	0.37
	0.2	65		
	0.04	30		
13	5	80	0.89	2.09
	0.2	45		
14	5	80	0.89	2.49
	0.2	45		

[a]Final concentrations were 0.04–5 μg mL^{-1} for the compounds, in DMSO. [b]The % inhibition and [c]concentration giving 50% inhibition (IC50) are means from triplicate experiments.

Inhibition of AGE formation

We also examined the inhibitory activities of the *S. flavescens* derived-prenylated flavonoids against AGE formation. Most of the prenylated flavonoids exhibited no inhibitory effects in the AGE assay at the higher concentration (200 μg mL^{-1}). However, desmethylanhydroicaritin (**1**), 8-C-lavandulylkaempferol (**2**), kushenol C (**3**), and (2S)-3β,7,4′-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**11**) possessed good inhibitory activities against AGE formation with IC50 values of 104.3, 132.1, 84.6 and 261.0 μg mL^{-1}, respectively, compared with the positive control, aminoguanidine (IC50 115.7 μg mL^{-1}) (Table 4). In particular, the prenylated flavonoids **1–3** and **11** were more potent inhibitors of AGE formation than aminoguanidine, with IC50 values of 294.6, 313.1, 193.1 and 705.4 μM, respectively, compared with 1051.5 μM for aminoguanide.

Discussion

AR and AGE formation have been implicated in the onset of many diabetic complications, including atherosclerosis, cardiac dysfunction, retinopathy, neuropathy and nephropathy. In particular, AGE formation and the AR-related polyol pathway are highly connected with each other, and to hyperglycaemia (Kaneko et al 2005; Peyrou & Sternberg 2006). Several ARIs and AGE formation inhibitors have been proposed for preventing and treating various diabetic complications but had undesirable side-effects (de la Fuente et al 2003; Thornalley 2003). We have therefore investigated the inhibitory effects of *S. flavescens*, a traditional medicine, on these two activities that contribute to diabetic complications. We have evaluated the inhibitory activities of fourteen prenylated flavonoids, along with the MeOH extract and its fractions from *S. flavescens*, on AR (RLAR and HRAR) and AGE formation.

As shown in Table 1, the CH_2Cl_2 and EtOAc fractions were predominantly loaded with components that were bioactive in the RLAR system. Interestingly, most prenylated flavonoids are reported as being isolated mainly from the CH_2Cl_2 and EtOAc fractions (Ryu et al 1997; Kim et al 2003; Sato et al 2007; Zhang et al 2007), indicating that the

Table 4 Inhibitory activities of prenylated flavonoids from *S. flavescens* against formation of advanced glycation endproducts

Flavonoids	Concn (μg mL^{-1})[a]	Inhibition (%)[b]			IC50[c]	
					μg mL^{-1}	μM
1	200	83.14	83.33	83.38	104.3 ± 0.20	294.6 ± 0.57
	100	49.24	49.95	49.31		
	20	19.40	19.59	20.18		
2	200	66.99	67.16	67.11	132.1 ± 0.23	313.1 ± 0.53
	100	47.82	48.15	47.92		
	20	11.23	12.39	11.91		
3	100	56.87	56.82	56.91	84.6 ± 0.02	193.1 ± 0.04
	20	21.90	22.16	21.95		
	4	10.40	9.86	10.31		
11	200	38.85	39.02	38.97	261.0 ± 0.32	705.4 ± 0.87
	100	26.29	26.67	26.39		
	20	9.27	9.98	9.11		
AG[d]	200	71.53	71.20	71.46	115.7 ± 0.16	1051.5 ± 1.41
	100	51.06	50.94	50.92		
	20	20.08	20.48	19.47		

[a]Final concentrations of test samples were 4–200 μg mL^{-1} for the compounds, which were dissolved in 10% DMSO. [b]The % inhibition and [c]concentration giving 50% inhibition (IC50) were determined for triplicate experiments. [d]Aminoguanidine (AG) was used as a positive control.

prenylated flavonoids may be, at least in part, associated with the inhibitory activities of *S. flavescens* against AR. In our previous research on the antioxidant capacities of *S. flavescens* and its prenylated flavonoids, we demonstrated that the CH_2Cl_2 and EtOAc fractions showed powerful antioxidant activities in the DPPH assay (Jung et al 2005a,b). The previous and present findings on several fractions of *S. flavescens* support the proposal that the EtOAc fraction must contain the bioactive components that possess the effects against diabetic complications, in which the antioxidant system has been implicated. Although the data are not shown, two alkaloids, matrine and oxymatrin, and one ptercarpan, trifolirhizin, were major compounds of *S. flavescens*, but exhibited no inhibitory activity against RLAR at a higher concentration (50 μg mL^{-1}), indicating that these major components do not contribute to the effects against diabetic complications.

Repeated column chromatography of the active EtOAc fraction yielded three flavonols: desmethylanhydroicaritin (**1**), 8-C-lavandulylkaempferol (**2**) and kushenol C (**3**); three chalcones: kuraridinol (**4**), kuraridin (**5**) and xanthohumol (**6**); and eight flavanones: sophoraflavanone G (**7**), kurarinol (**8**), kurarinone (**9**), (2S)-2′-methoxykurarinone (**10**), (2S)-3β,7,4′-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**11**), (2S)-7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**12**), kushenol E (**13**) and leachianone G (**14**) (Figure 1). Although there have been numerous studies on various flavonoids functioning as ARIs, the inhibitory activities of prenylated flavonoids against AR have not been studied in detail. We therefore examined the AR inhibitory activities of the prenylated flavonoids, and further evaluated their relevance to specific structures via the RLAR and HRAR assays.

In the RLAR assay, prenylated flavonols **1** and **2**, as well as prenylated flavanones **8–11** and **13**, exhibited marked inhibitory activities against RLAR compared with quercetin (Table 2). In particular, desmethylanhydroicaritin (**1**) was the most potent RLAR inhibitor, showing eight-fold greater inhibition than quercetin. Taking into account the detailed structure–activity relationships of the prenylated flavonols and prenylated chalcones, compounds **1, 2**, and **6**, bearing a 4′-hydroxyl group, showed relatively potent inhibitory activities in the RLAR assay compared with compounds **3–5**, which bear a 2′,4′-dihydroxyl group. Rastelli et al (2000) have also shown that the 4′-hydroxyl group of the chalcones may play an important role in the inhibition of AR. When it comes to prenylated flavanones **8** and **9**, which contain a 2′,4′-dihydroxyl group, a 5-methoxyl group and an 8-lavandulyl group, both had more potent inhibitory effects in the RLAR assay than their counterparts, prenylated chalcones **4** and **5**. However, with regard to xanthohumol (**6**) and (2S)-7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**12**), both of which possess a 4′-hydroxyl group, a 5-methoxyl group and an 8-prenyl group, the former chalcone was three-fold more potent than the latter flavanone, suggesting that this prenylated chalcone might exhibit more activity than its counterpart prenylated flavanone. Most studies on flavonoids, in the absence of the prenyl and lavandulyl groups, have reported that 2′,4′-dihydroxyl chalcones exert the most potent inhibitory effects, and chalcones have usually exhibited higher activities than flavanones in the RLAR assay (Lim et al 2001; Kawanishi et al 2003). However, our present results on prenylated flavonoids revealed that the prenylated chalcones, which contain a 4′-hydroxyl group rather than a 2′,4′-hydroxyl group, and the prenylated flavanones rather than prenylated chalcones exhibited superior inhibitory activities in the RLAR assay, indicating that the prenylated and lavandulyl groups at the C-8 position might be the main contributors to the augmentation and/or modification of their effects. Matsuda et al (2002) suggested that the C2–C3 double bond increases RLAR inhibitory effects; our studies have yielded inconsistent results, which may have resulted from the functional groups, such as the prenyl and lavandulyl

groups of the prenylated flavonoids. Some evidence has also suggested that certain flavanones possess strong RLAR inhibitory activities (Kadota et al 1994; Yoshikawa et al 1998; Matsuda et al 2002). Considering our results, by and large the prenylated flavanones and prenylated flavonols showed higher activities than the prenylated chalcones in the RLAR assay. These apparently contradictory results for the flavonoids and prenylated flavonoids remain to be resolved, but the prenyl and lavandulyl groups at the C-8 position of the flavonoid skeleton may be responsible for the contrasting results. To elaborate on the differences between the prenylated flavonoids and flavonoids, the inhibitory effects of keampferol, quercetin and prenylated flavonols **1–3** against RLAR were measured, and their IC50 values were in the order of desmethylanhydroicaritin (**1**, 0.95 μM) > 8-C-lavandulylkaempferol (**2**, 3.80 μM) > quercetin (7.73 μM) > kaempferol (9.67 μM) > kushenol C (**3**, 18.54 μM). By comparison with kaempferol, the RLAR inhibitory activities of prenylated flavonols **1** and **2** were ten times and three times greater than that of kaempferol, respectively, indicating that the prenyl and lavandulyl groups may play important roles in the RLAR inhibitory activity. The important structural characteristics of ARI may be harboured within the polar portion as well as the hydrophobic ring system (Peyrou & Sternberg 2006; El-Kabbani & Podjarny 2007). The hydrophobic ring systems of the ARIs are tightly bound adjacent to the anion-binding site of AR, and the polar systems are bound in an anion-binding site adjacent to the nicotinamide ring of the coenzyme (El-Kabbani & Podjarny 2007). As mentioned, prenylated flavanones, which possess the C-ring of a flavonoid unit as the hydrophobic ring system, may be deemed reasonable ARIs in the RLAR system.

As shown in Table 3, most of the prenylated flavonoids tested, including prenylated flavonols **1–3**, prenylated chalcones **4** and **5** and some prenylated flavanones (**7** and **12–14**), exerted significant inhibitory activity against HRAR.

The results suggest that *S. flavescens*-derived prenylated flavonoids may be promising therapeutic agents and have benefits applicable to humans. Although the AR enzymes from several sources, including muscle, blood and lens, have similar structures, there are several reports of differences in their activities and the effects of different agents (Kador et al 1980; Nishimura et al 1991). Unlike with RLAR, the prenylated chalcones and prenylated flavonols exhibited more potent activity than the prenylated flavanones in the HRAR assay. There are a few reports on the inhibitory activity of chalcones, indicating that 2′,4′-dihydroxychalcones exhibited strong activity against HRAR (Iwata et al 1999). Both the prenylated flavonols **1–3** and the prenylated chalcones **4** and **5** exhibited marked inhibitory activities, with IC50 values of approximately 0.27–1.32 μM compared with quercetin (IC50 2.54 μM) and epalrestat (IC50 0.28 μM). In particular, the remarkably active prenylated flavonols **1–3** contain the kaempferol moiety with a 3,5,7,4′-tetrahydroxy group, suggesting that the kaempferol-like hydroxyl functional groups may participate in their HRAR inhibitory activities. To confirm the relevance of the prenyl group in prenylated flavonoids, the HRAR inhibitory effects of kaempferol, quercetin and prenylated flavonols **1–3** were measured, and their IC50 values were in the order of desmethylanhydroicaritin (**1**, 0.45 μM) > 8-C-lavandulylkaempferol (**2**, 0.79 μM) > kushenol C (**3**, 0.85 μM) > quercetin (2.54 μM) > kaempferol (8.04 μM). Kaempferol is also reported to have comparable inhibitory activity to the potent ARI quercetin (Lim et al 2006). As for prenylated flavonols **1** and **2**, differences in their HRAR inhibitory activity (IC50 0.45 μM for **1** and IC50 0.79 μM for **2**) may have resulted from the presence of a prenyl group or a lavandulyl group at the C-8 position. Kato et al (2006) suggested that the methoxyl group and the side alkyl chain length in the aromatic ring may be key characteristics for enzyme recognition and binding. Among three of the prenylated flavonols, the HRAR inhibitory activity of compound **1** was double that of compounds **2** and **3**, indicating that a prenyl group rather than a lavandulyl group at the C-8 position contributed to their effects. Among the tested prenylated flavonoids, kuraridin (**5**), with a lavandulyl group at the C-8 position and a 2′,4′-dihydroxyl group, was the most potent HRAR inhibitor (IC50 0.27 μM), followed by (2S)-7,4′-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**12**; IC50 0.37 μM), compared with epalrestat (IC50 0.28 μM).

According to our results, there were remarkable differences in the inhibitory potencies of the compounds against HRAR and RLAR. Overall, the prenylated flavanones and prenylated flavonols showed higher activities than the prenylated chalcones in the RLAR assay, whereas the prenylated chalcones and prenylated flavonols exhibited greater activity than the prenylated flavanones in the HRAR assay (Tables 2 and 3). In particular, prenylated flavonol **3** and the two prenylated chalcones **4** and **5** had remarkable HRAR inhibitory activity but exhibited marginal inhibitory activity in the RLAR assay. The prenylated flavanones **7, 12**, and **14** possessed significant inhibitory activities in the HRAR assay, but not in the RLAR assay. The results clearly demonstrate that the differences in potency can be attributed to the chemical structures of the ARIs, as well as to different AR sources, such as the species, organ and tissue (Brownlee 2001). We found that the susceptibility of AR to various ARIs can exhibit striking differences, depending on human and animal sources, which may occur, at least partly, as a result of different degrees of bulk tolerance for the various enzymes (Kador et al 1980; Nishimura et al 1991).

We also examined the inhibitory activities of *S. flavescens* derived-prenylated flavonoids against AGE formation, given the high degree of connection between AGE formation, AR and diabetic complications. Most of the prenylated flavonoids had no effect in the AGE assay. However, prenylated flavonols **1–3** and the prenylated flavanone **11** possessed good inhibitory activities against AGE formation compared with the standard aminoguanidine (Table 4). All active prenylated flavonoids have a hydroxyl group at the 3 position, indicating that the 3-hydroxyl group may be one of the structural requirements for inhibition of AGE formation. Matsuda et al (2003) proposed that the number of hydroxyl groups at the 3′, 4′, 5 and 7 positions may play an important role in the potency of AGE formation inhibitors. Auercetin and kaempferol are more potent inhibitors of AGE formation than aminoguanidine, with IC50 values of approximately 200 μM (Wirasathien et al 2007). Similar to the structure of quercetin, kushenol C (**3**), with four hydroxyl groups at the 2′, 4′, 5

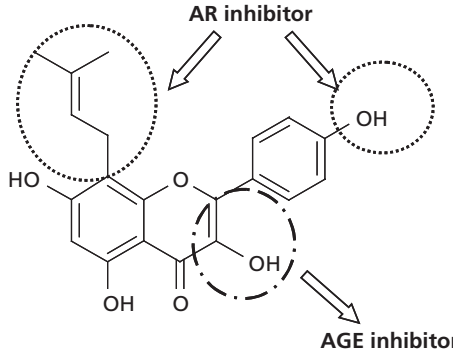

Figure 2 Key functional groups of desmethylanhydroicaritin (**1**) from *S. flavescens* that relate to prevention of diabetic complications. AGE, advanced glycation endproducts; AR, aldose reductase.

and 7 positions, was the most potent inhibitor of AGE formation.

S. flavescens-derived prenylated flavonoids may therefore have a promising role as therapeutic agents for diabetic complications, based on their simultaneous activities against AR and AGE formation. With regard to desmethylanhydroicaritin (**1**), a prenyl group at the C-8 position, along with a 3,4′-dihydroxyl group, may contribute, at least in part, to the overall effects against diabetic complications, including the inhibition of RLAR, HRAR and AGE formation (Figure 2).

Conclusion

S. flavescens and its prenylated flavonoids may exert comprehensive inhibitory effects against diabetic complications via the AR-polyol pathway and AGE formation systems, which are highly implicated in oxidative stress. Prenylated flavonol **2** and prenylated flavanone **11** exhibited antioxidant activities in our antioxidant assays, including the DPPH and $ONOO^-$ systems (Jung et al 2005a,b). Our previous and current results suggest that prenylated flavonols and prenylated chalcones may possess good inhibitory activities against diabetic complications, implicated by their significant antioxidant activity. Also, most of the prenylated flavonoids exerted potent inhibitory activity in the RLAR as well as the HRAR assays, clearly suggesting their potential for use in the development of therapeutic or preventive agents for diabetic complications and related diseases.

References

AACE (American Association of Clinical Endocrinologists). State of Diabetes in America: A comprehensive report issued by the American Association of Clinical Endocrinologists. In: *Summary Report on National Business Coalition on Health Activities Related to Diabetes, May 2007, Summary of Activities Related to Diabetes*, NBCH, 2007

Ahmed, N., Thornalley, P. J. (2007) Advanced glycation endproducts: what is their relevance to diabetic complications? *Diabetes Obes. Metab*. **9**: 233–245

Brownlee, M. (2001) Biochemistry and molecular cell biology of diabetic complications, *Nature* **414**: 813–820

Chen, L., Cheng, X., Shi, W., Lu, Q., Go, V. L., Heber, D., Ma, L. (2005) Inhibition of growth of *Streptococcus mutans*, methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant *enterococci* by kurarinone, a bioactive flavonoid isolated from *Sophora flavescens. J. Clin. Microbiol*. **43**: 3574–3575

Chi, Y. S., Jong, H. G., Son, K. H., Chang, H. W., Kang, S. S., Kim, H. P. (2001) Effects of naturally occurring prenylated flavonoids on enzymes metabolizing arachidonic acid: cyclooxygenases and lipoxygenases. *Biochem. Pharmacol*. **62**: 1185–1191

de la Fuente, J. A., Manzanaro, S., Martín, M. J., de Quesada, T. G., Reymundo, I., Luengo, S. M., Gago, F. (2003) Synthesis, activity, and molecular modeling studies of novel human aldose reductase inhibitors based on a marine natural product. *J. Med. Chem*. **46**: 5208–5221

De Naeyer, A., Vanden Berghe, W., Pocock, V., Milligan, S., Haegeman, G.., De Keukeleire, D. (2004) Estrogenic and anticarcinogenic properties of kurarinone, a lavandulyl flavanone from the roots of *Sophora flavescens. J. Nat. Prod*. **67**: 1829–1832

Ding, P. L., Liao, Z. X., Huang, H., Zhou, P., Chen, D. F. (2006) (+)-12α-Hydroxysophocarpine, a new quinolizidine alkaloid and related anti-HBV alkaloids from *Sophora flavesencs. Bioorg. Med. Chem. Lett*. **16**: 1231–1235

El-Kabbani, O., Podjarny, A. (2007) Selectivity determinants of the aldose and aldehyde reductase inhibitor-binding sites. *Cell Mol. Life Sci*. **64**: 1970–1978

Hayman, S., Kinoshita, J. H. (1965) Isolation and properties of lens aldose reductase. *J. Biol. Chem*. **240**: 877–882

Huang, K. C. (1993) *The pharmacology of Chinese herbs*, CRC press Inc., Boca Raton: FL, pp 63–66

Hwang, J. S., Lee, S. A., Hong, S. S., Lee, K. S., Lee, M. K., Hwang, B. Y., Ro, J. S. (2005) Monoamine oxidase inhibitory components from the roots of *Sophora flavescens. Arch. Pharm. Res*. **28**: 190–194

Iwata, S., Nagata, N., Omae, A., Yamaguchi, S., Okada, Y., Shibata, S., Okuyama, T. (1999) Inhibitory effect of chalcone derivatives on recombinant human aldose reductase. *Biol. Pharm. Bull*. **22**: 323–325

Jiang, H., Hou, C., Zhang, S., Xie, H., Zhou, W., Jin, Q., Cheng, X., Qian, R., Zhang, X. (2007) Matrine upregulates the cell cycle protein E2F-1 and triggers apoptosis via the mitochondrial pathway in K562 cells. *Eur. J. Pharmacol*. **559**: 98–108

Jung, M. J., Kang, S. S., Jung, H. A., Kim, G. J., Choi, J. S. (2004) Isolation of flavonoids and a cerebroside from the stem bark of *Albizzia julibrissin. Arch. Pharm. Res*. **27**: 593–599

Jung, H. J., Kang, S. S., Hyun, S. K., Choi, J. S.(2005a) In vitro free radical and $ONOO^-$ scavengers from *Sophora flavescens. Arch. Pharm. Res*. **28**: 534–540

Jung, H. J., Kang, S. S., Woo, J. J., Choi, J. S. (2005b) A new lavandulylated flavonoid with free radical and $ONOO^-$ scavenging activities from *Sophora flavescens. Arch. Pharm. Res*. **28**: 1333–1336

Jung, C. H., Zhou, S., Ding, G. X., Kim, J. H., Hong, M. H., Shin, Y. C., Kim, G. J., Ko, S. G. (2006) Antihyperglycemic activity of herb extracts on streptozotocin-induced diabetic rats. *Biosci. Biotechnol. Biochem*. **70**: 2556–2559

Jung, H. A., Jeong, D. M., Chung, H. Y., Lim, H. A., Kim, J. Y., Yoon, N. Y., Choi, J. S. (2008) Re-evaluation of the antioxidant prenylated flavonoids from the roots of *Sophora flavescens. Biol. Pharm. Bull*. **31**: 908–915

Kador, P. F., Kinoshita, J. H., Tung, W. H., Chylack, L. T. Jr. (1980) Differences in the susceptibility of various aldose reductases to inhibition. II. *Invest. Ophthalmol. Vis. Sci*. **19**: 980–982

Kadota, S., Basnet, P., Hase, K., Namba, T. (1994) Matteuorienate A and B, two new and potent aldose reductase inhibitors from

Matteuccia orientalis (Hook.) Trev. *Chem. Pharm. Bull*. **42**: 1712–1714

Kaneko, M., Bucciarelli, M., Hwang, Y. C., Lee, L., Yan, S. F., Schmidt, A. M., Ramasamy, R. (2005) Aldose reductase and AGE-RAGE pathway: key players in myocardial ischemic injury. *Ann. NY Acad. Sci*. **1043**: 702–709

Kang, T. H., Jeong, S. J., Ko, W. G., Kim, N. Y., Lee, B. H., Inagaki, M., Miyamoto, T., Higuchi, T., Kim, Y. C. (2000) Cytotoxic lavandulyl flavanones from *Sophora flavescens. J. Nat. Prod*. **63**: 680–681

Kato, A., Higuchi, Y., Goto, H., Kizu, H., Okamoto, T., Asano, N., Hollinshead, J., Nash, R. J., Adachi, I. (2006) Inhibitory effects of *Zingiber officinale* Roscoe derived components on aldose reductase activity *in vitro* and *in vivo. J. Agric. Food Chem*. **54**: 6640–6644

Kawanishi, K., Ueda, H., Moriyasu, M. (2003) Aldose reductase inhibitors from nature. *Curr. Med. Chem*. **10**: 1353–1374

Kim, D. W., Chi, Y. S., Son, K. H., Chang, H. W., Kim, J. S., Kang, S. S., Kim, H. P. (2002) Effects of sophoraflavanone G, a prenylated flavonoid from *Sophora flavescens*, on cyclooxygenase-2 and *in vivo* inflammatory response. *Arch. Pharm. Res*. **25**: 329–335

Kim, S. J., Son, K. H., Chang, H. W., Kang, S. S., Kim, H. P. (2003) Tyrosinase inhibitory prenylated flavonoids from *Sophora flavescens. Biol. Pharm. Bull*. **26**: 1348–1350

Kim, J. H., Ryu, Y. B., Kang, N. S., Lee, B. W., Heo, J. S., Jeong, I. Y., Park, K. H. (2006) Glycosidase inhibitory flavonoids from *Sophora flavescens. Biol. Pharm. Bull*. **29**: 302–305

Ko, W. G., Kang, T. H., Kim, N. Y., Lee, S. J., Kim, Y. C., Ko, G. I., Ryu, S. Y., Lee, B. H. (2000) Lavandulylflavonoids: a new class of in vitro apoptogenic agents from *Sophora flavescens. Toxicol. In Vitro* **14**: 429–433

Lim, S. S., Jung, S. H., Ji, J., Shin, K. H., Keum, S. R. (2001) Synthesis of flavonoids and their effects on aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rat tissues. *J. Pharm. Pharmacol*. **53**: 653–668

Lim, S. S., Jung, Y. J., Hyun, S. K., Lee, Y. S., Choi, J. S. (2006) Rat lens aldose reductase inhibitory constituents of *Nelumbo nucifera* stamens. *Phytother. Res*. **20**: 825–830

Manzanaro, S., Salva, J., de la Fuente, J. (2006) A. Phenolic marine natural products as aldose reductase inhibitors. *J. Nat. Prod*. **69**: 1485–1487

Matsuda, H., Morikawa, T., Toguchida, I., Yoshikawa, M. (2002) Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. *Chem. Pharm. Bull*. **50**: 788–795

Matsuda, H., Wang, T., Managi, H., Yoshikawa, M. (2003) Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorg. Med. Chem*. **11**: 5317–5323

Nakagawa, T., Yokozawa, T., Terasawa, K., Shu, S., Juneja, L. R. (2002) Protective activity of green tea against free radical- and glucose-mediated protein damage. *J. Agric. Food Chem*. **50**: 2418–2422

Nishimura, C., Yamaoka, T., Mizutani, M., Yamashita, K., Akera, T., Tanimoto, T. (1991) Purification and characterization of the recombinant human aldose reductase expressed in baculovirus system. *Biochim. Biophys. Acta* **1078**: 171–178

Peyrou, J., Sternberg, M. (2006) Advanced glycation endproducts (AGEs): Pharmacological inhibition in diabetes. *Pathol. Biol*. **54**: 405–419

Piao, X L., Piao, X. S., Kim, S. W., Park, J. H., Kim, H. Y., Cai, S. Q. (2006) Identification and characterization of antioxidants from *Sophora flavescens. Biol. Pharm. Bull*. **29**: 1911–1915

Rastelli, G., Antolini, L., Benvenuti, S., Costantino, L. (2000) Structural bases for the inhibition of aldose reductase by phenolic compounds. *Bioorg. Med. Chem*. **8**: 1151–1158

Ryu, S. Y., Lee, H. S., Kim, Y. K., Kim, S. H. (1997) Determination of isoprenyl and lavandulyl positions of flavonoids from *Sophora flavescens* by NMR experiment. *Arch. Pharm. Res*. **20**: 491–495

Sato, S., Takeo, J., Aoyama, C., Kawahara, H. (2007) Na^+-glucose cotransporter (SGLT) inhibitory flavonoids from the roots of *Sophora flavescens. Bioorg. Med. Chem*. **15**: 3445–3449

Son, J. K., Park, J. S., Kim, J. A., Kim, Y., Chung, S. R., Lee, S. H. (2003) Prenylated flavonoids from the roots of *Sophora flavescens* with tyrosinase inhibitory activity. *Planta Med*. **69**: 559–561

Tang, W., Eisenbrand, G. (1992) *Chinese drugs of plant origin: chemistry, pharmacology, and use in traditional and modern medicine*. Springer-Verlag, Berlin: Germany, pp 931–943

Thornalley, P. J. (2003) Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. *Arch. Biochem. Biophys*. **419**: 31–40

Vinson, J. A., Howard III, T. B. (1996) Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients. *J. Nutr. Biochem*. **7**: 659–663

Wirasathien, L., Pengsuparp, T., Suttisri, R., Ueda, H., Moriyasu, M., Kawanishi, K. (2007) Inhibitors of aldose reductase and advanced glycation end-products formation from the leaves of *Stelechocarpus cauliflorus* R.E. Fr. *Phytomedicine* **14**: 546–550

Wu, L., Miyase, T., Akira, U., Kuroyanagi, M., Noro, T. (1985) Studies on the constituents of *Sophora flavescens. Chem. Pharm. Bull*. **33**: 3231–3236

Yoshikawa, M., Shimada, H., Nishida, N., Li, Y., Toguchida, I., Yamahara, J., Matsuda, H. (1998) Antidiabetic principles of natural medicines. II. Aldose reductase and alpha-glucosidase inhibitors from Brazilian natural medicine, the leaves of *Myrcia multiflora* DC. (Myrtaceae): structures of myrciacitrins I and II and myrciaphenones A and B. *Chem. Pharm. Bull*. **46**: 113–119

Zhang, L., Xu, L., Xiao, S. S., Liao, Q. F., Li, Q., Liang, J., Chen, X. H., Bi, K. S. (2007) Characterization of flavonoids in the extract of *Sophora flavescens* Ait. by high-performance liquid chromatography coupled with diode-array detector and electrospray ionization mass spectrometry. *J. Pharm. Biomed. Anal*. **44**: 1019–1028