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Structure -Activity Relationships of Lignans from *Schisandra chinensis* as PAF Antagonists

Hyeong Kyu Lee,* Im Seon Lee, Keun Young Jung

Korea Research Institute of Bioscience and Biotechnology, P.O.Box 115, Yusong, Taejon 305-600, Korea

INTRODUCTION

The fruits of *Schisandra chinensis* with a sweet-sour taste are known as 'omija' in Korea, 'gomishi' in Japan and 'wuweizi' in China. They are classified as *Schisandra chinensis* Turcz., or northern *Schisandra* and *Schisandra sphenanthera* Rehd. et Wils, or southern *Schisandra*. The former is larger, thicker, and less brownish in appearance than the latter. The latter is effective for stopping coughs, but has much less enriching and tonifying effects than the former.[1)]

From *Fructus Schisandrae*, the major known compounds are sesquicarene, citral, β-bisabolene, β-chamigrene, α-ylangene, stigmasterol, schisandrins, schisandrols, deoxyschisandrins, gomisins, vitamine C and vitamin E.[2-13)]

The various pharmacological and clinical effects of the compounds have been reported. The prominent liver-protective actions were found with schisandrin C and schisantherin D in CCl_4-treated cultures, and deoxygomisin A, gomisin N, schisandrin C and schisantherin D were effective in preventing GalN-induced cell damage. Schisantherin A, B, C and D had a reducing effect on the transaminase.[14-15)] Schisandrol A and schisandrin B showed an inhibitory effect on the central nervous system.[16-17)] Stressed-induced gastric secretion was prevented by schisandrol A and B.[18)] Schisandrol B showed effectively to

prolong hexobarbital-induced sleeping time in mice and inhibits tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in mouse skin.[18-19] Besides, the extract showed antibacterial action, adaptogen-like action, vasodilation and therapeutic effect on neurologic diseases.[20-23]

However, the compounds from *S. chinensis* had not been studied about PAF antagonistic activity. Therefore, we have reported active compounds from *S.chinensis* as PAF antagonists.[24-25] In this study, we discuss activity-structure relationships of lignans and their derivatives as PAF antagonists.

MATERIALS AND METHODS

Extraction and Isolation of Compounds from *S. chinensis* B. The crushed fruits (6 kg) were extracted 3 times with MeOH (15 l) at room temp. The combined MeOH extracts were evaporated at 40℃ under reduced pressure to give a brown residue (200 g). This was partitioned with *n*-hexane and water. Seven fractions were separated from hexane extract (180 g) by silica gel chromatography (*n*-hexane-EtOAc, 100:0→0:100, step gradient). From the second fraction (50.3 g), schisandrin A (**1**), schisandrin C (**2**), γ-schisandrin (**3**, 15 mg) and gomisin N (**4**, 49 mg) were isolated by silica gel chromatography (Hexane-EtOAc, 24:1) and the method of Ikeya *et al.*[5] From the third fraction (6.8 g), anwulignan (**5**, 40 mg) was first isolated from *S. chinensis* by silica gel chromatography (Hexane-EtOAc, 24:1). Gomisin M_1 (**6**, 31 mg), M_2 (**7**, 22 mg), benzoylisogomisin O (**8**, 11 mg) and gomisin E (**9**, 168 mg) were isolated by silica gel chromatography (Hexane-EtOAc, 12:1) from the fourth fraction (3.7 g). The treatment of **6** (8 mg) and **7** (6 mg) with diazomethane yielded gomisin M_1 methyl ether (**10**, 4.5 mg) and gomisin M_2 methyl ether (**11**, 5 mg), respectively. By the method of Li *et* al,[12] hydrolysis of **8** (4 mg) yielded isogomisin O (**12**, 3 mg). From the fifth fraction (2.6 g), pregomisin (**13** 68 mg), gomisin G (**14**, 18 mg), gomisin K_1 (**15**, 26 mg) and gomisin K_3 (**16**, 47 mg) were isolated by silica gel chromatography (Hexane-EtOAc, 5:1). By treatment with diazomethane, compound **13** (16 mg), **15** (6 mg) and **16** (5 mg)

yielded pregomisin mono methyl ether (**17**, 6 mg) and pregomisin dimethyl ether (**18**, 6 mg), gomisin K_1 methyl ether (**19**, 5 mg) and gomisin K_3 methyl ether (**20**, 3 mg), respectively. The acetylation of **15** (5 mg) and **16** (8 mg) with acetic anhydride and pyridine yielded gomisin K_1 acetate (**21**, 4 mg) and gomisin K_3 acetate (**22**, 6 mg), respectively. Gomisin B (**23**, 33 mg), gomisin C (**24**, 27 mg), schisandrol A (**25**, 2.85 g) and schisandrol B (**26**, 1.3 g) were isolated by silica gel chromatography (Hexane-EtOAc, 3:1) from the sixth fraction (16.6 g). By dehydration of **25** (200 mg) and **26** (180 mg) with $POCl_3$,[12-13] 6(7)-dehydroschisandrol A (**27**, 116 mg), 7(18)-dehydroschisandrol A (**28**, 41 mg), 6(7)-dehydroschisandrol B (**29**, 87 mg) and 7(18)-dehydroschisandrol B (**30**, 35 mg) were yielded, respectively. Identification of lignan structures, as shown in chart 1, were confirmed by the related references (**1**,[3] **2**,[4] **3-4**, **6-7**, **10-11**,[5] **5**,[6] **8**,[7] **9**,[8] **13**,[9] **14**, **23**, **24**,[10] **15-16**, **19-21**,[11] **25-26**,[3] **27-30** [12-13]).

Assay of ^{3}[H] PAF Receptor Binding Inhibition on Washed Rabbit Platelets PAF antagonistic activity was determined as described in literature [26-27] with some modifications. The reaction mixture consisted of 100 $\mu\ell$ of rabbit platelet suspension (4×108 cell/mℓ), 90 $\mu\ell$ of [^{3}H]PAF (0.9 nM, 70,000 dpm) with or without unlabelled PAF (500-fold of radioactive form), and 60 $\mu\ell$ of sample or control solution. The reaction mixture was incubated at room temperature for 30 min. The free PAF was separated from bound PAF by filtration of the reaction mixture and radioactivity was then measured. The difference between total radio activities of bound [^{3}H]PAF in the absence and presence of excess unlabeled PAF is defined as specific binding of the radio labeled ligand. In a set of experiments, [^{3}H]PAF was incubated with different concentrations of samples and the antagonistic effect of samples on the specific binding was expressed as percentage inhibition of the control. The IC_{50} value was defined as the final concentration of the inhibitor required to block 50 % of the specific [^{3}H]PAF binding to rabbit platelet receptors. Triplicated tests were carried on each concentration, and IC_{50} values were calculated from 4 concentrations at least.

Statistical analysis Statistical significance was evaluted by students's t-test.

RESULTS AND DICUSSION

The active compounds (**1-7**, **15-16**, **25-26**) and less active compounds (**2**, **8-9**, **14**, **23-24**) were isolated by a bioactivity-guided separation. They have dibenzocyclooctene structures except for **5** (IC_{50}, 2.6×10^{-4} M) which is first isolated from *S. chinensis* and **13**. It led us to investigate how chemical structures of the active compounds and their derivatives contribute to inhibition of binding the substrate to the PAF receptor.

1. Effect of esterification of the hydroxyl at C-6

In lignans, the esterification of hydroxyl at C-6 (**8-9**, **14**, **23-24**) did not show PAF antagonistic activity independent of angeloyl or benzoyl ester (IC_{50}, $>10^{-3}$ M). But the activity of **12**, hydrolyzed product of **8**, was increased (IC_{50}, 5.9×10^{-4} M). Thus, the introduction of ester at C-6 tends to decrese activity.

2. Effect of biphenyl configuration

Lignans with dibenzocylooctene struture are classified as *R* (*R*-form) and *S* biphenyl configuration (*S*-form) by cotton effect.[28] Compounds **3** and **4**, with methylene dioxy moiety, have *R* and *S*-form, respectively. Compared with **4** (IC_{50}, 1.3×10^{-4} M), activity of **3** (IC_{50}, 7.7×10^{-5} M) was high though the difference was not significant. However, in **1** and **19** without methylene dioxy moiety, the activity of **1** (IC_{50}, 1.6×10^{-5} M) with *R*-form was much higher than that of **19** (IC_{50}, 2.2×10^{-4} M) with *S*-form with high significance level ($p<0.01$). These results suggest that activity of *R*-form is higher than *S*-form.

3. Effect of methylene dioxy moiety

Among compounds with *R*-form, the activities of **1** and **25** (IC_{50}, 2.5×10^{-4} M) without methylene dioxy moiety were significantly higher ($p < 0.01$) than those of **3** and **26** (IC_{50}, 5.9×10^{-4} M) with methylene dioxy moiety, respectively. In *S*-form compounds, the activity of **2** (IC_{50}, $>10^{-3}$ M) with two methylene dioxy moieties was weaker than that of **4** with one methylene dioxy moiety. Thus, the more methylene dioxy moiety it has, the less activity is shown.

4. Effect of substituents on the phenyl nucleus

Compounds **15** and **16** without methylene dioxy moiety have a hydroxyl group at C-12 and C-14, respectively. To compare activities of substituents on the phenyl nucleus, these compounds were acetylated or methylated. Compared to **15** (IC_{50}, 2.6×10^{-4} M) with *S*-form, the activities of **19** and **21** (IC_{50}, $>10^{-3}$ M) did not increase by substitution on the phenyl nucleus. However, compared to **16** (IC_{50}, 1.6×10^{-4} M) with *R*-form, compound **20** (IC_{50}, 1.7×10^{-5} M) showed remarkable increment ($p < 0.05$), whereas no apparent influence was observed in **22** (IC_{50}, 1.5×10^{-4} M). However, with *R*-form and methylene dioxy moiety, the activity of **11** (IC_{50}, 6.3×10^{-5} M) was similar to that of **7** (IC_{50}, 4.8×10^{-5} M). These results indicate that activity of *R*-form without methylene dioxy moiety is increased by methylation on the phenyl nucleus.

Besides lignans with *R*-form or *S*-form, compounds **6** with the mixture of *R,S*-form and **13** without dibenzocyclooctene structure were methylated. The activity of **6** (IC_{50}, 5.2×10^{-5} M) with methylene dioxy moiety was similar to that of **10** (IC_{50}, 7.5×10^{-5} M). However, compared to **13** (IC_{50}, 6.2×10^{-5} M) without methylene dioxy moiety, the activities **17** (IC_{50}, 3.3×10^{-5} M) and **18** (IC_{50}, 2.0×10^{-5} M) were increased at significant level ($p < 0.05$), respectively. Compound **18** has the cleaved structure between carbons at C-15 and C-16 of compound **1**. But their activities were similar.

5. Effect of hydroxyl at C-7

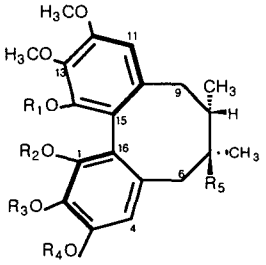
With exception of hydroxyl at C-7, the structures of **25** and **26** are the same with **1** and **3**, respectively. But activities of **1** and **3**, were significantly higher than those of **25** and **26** with hydroxyl at C-7 ($p < 0.01$), respectively. Thus, the introduction of hydroxyl at C-7 tends to decrease activity.

6. Effect of dehydration at C-7

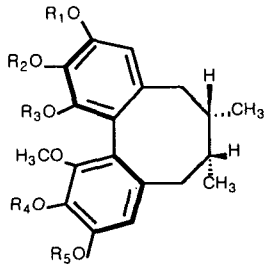
Because of hydroxyl at C-7, the activities of **25** and **26** were decreased. When they were dehydrated at C-7, the activities of **28** (IC_{50}, 1.3×10^{-4} M) and **30** (IC_{50}, 1.2×10^{-4} M) with exocyclic methylene moiety were similar to those of **25** and **26**, respectively. However, the activities of **27** (IC_{50}, 2.1×10^{-6} M) and **29** (IC_{50}, 4.2×10^{-5} M) with endocyclic double bond were significantly higher than those of **25** and **26** ($p < 0.01$), respectively. These findings indicate that the endocyclic double bond at C-7 is more effective than exocyclic methylene moiety as the partial structure of PAF antagonists.

Therapeutic considerations

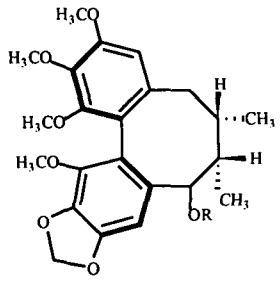
Fruits of *S. chinensis* contains about 2% lignans by weight. Among them, six compounds(**1**, **2**, **3**, **4**, **25**, **26**) are the main constituents.[29] The intense or mild PAF antagonistic activities, in present study, were found with major lignans (**1**, **4**, **25**, **26**) and their derivatives (**27-30**) as well as minor lignans. This fact suggests that these lignans may be responsible for the therapeutic efficacy of the crude drug in PAF-related inflammation, *e.g.* asthma, allergy, atopy's dermatitis and other inflammatory diseases. Futhermore, the activity-structure relationships, as PAF antagonists, will provide useful information for the interaction between the PAF receptor and its ligands.



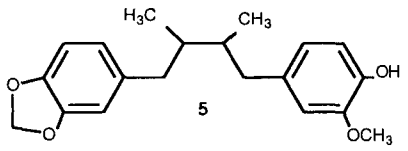
1, 20 : R_1=R_2=R_3=R_4 =CH_3, R_5=H
3, 11 :R_1=R_2=CH_3, R_3+R_4 =CH_2, R_5=H
7 :R_1=CH_3, R_2=H, R_3+R_4 =CH_2, R_5=H
16:R_1=H, R_2=R_3=R_4=CH_3, R_5=H
22: R_1=Ac, R_2=R_3=R_4=CH_3, R_5=H
25 :R_1=R_2=R_3=R_4=CH_3, R_5=OH
26 : R_1=R_2=CH_3, R_3+R_4 =CH_2, R_5=OH



2: R_1+R_2=CH_2, R_3=CH_3, R_4+R_5 =CH_2
4 : R_1+R_2=CH_2, R_3= R_4=R_5 =CH_3
6 : R_1=R_2=CH_3, R_3=H, R_4+R_5 =CH_2
10, 19 : R_1= R_2=R_3=R_4=R_5=CH_3
15: R_1=H, R_2=R_3=R_4=R_5=CH_3
21: R_1=Ac, R_2=R_3=R_4=R_5=CH_3



8:R=Bz
12:R=H



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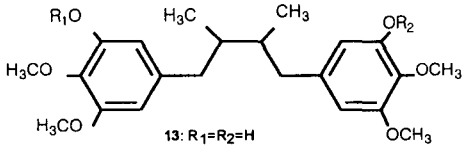
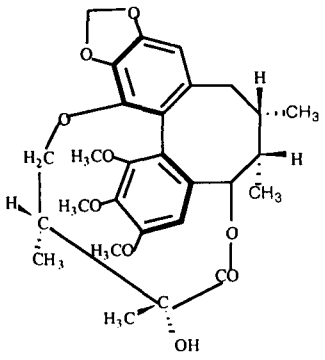
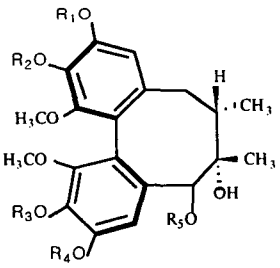
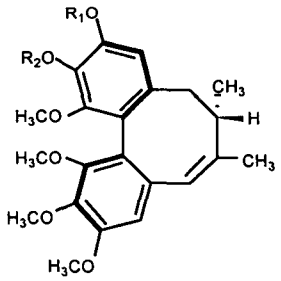


13: R_1=R_2=H
17: R_1 or R_2=H, R_1 or R_2=CH_3
18 :R_1=R_2=CH_3

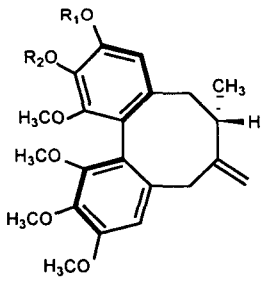




14 : R_1= R_2=CH_3, R_3+R_4=-CH_2, R_5=Bz
23: R_1+R_2=CH_2, R_3=- R_4=CH_3, R_5=Ang
24: R_1+R_2=CH_2, R_3=- R_4=CH_3, R_5=Bz



27 : R_1=R_2=CH_3
29 : R_1+R_2=CH_2



28 : R_1=R_2=CH_3
30 : R_1+R_2=CH_2

Chart 1: Lignans isolated from the fruits of S. chinensis

Compounds **6** and **10** are *R* and *S*-mixtures of biphenyl configuration.
Ang= Angeroyl, Bz= Benzoyl.

Table 1. PAF Antagonistic Activities of Lignans and Their Derivatives from *S. chinensis*

Compound	IC_{50} value	Compound	IC_{50} value
Schisandrin A (**1**)	1.6×10^{-5} M	Gomisin K_3 (**16**)	1.6×10^{-4} M
Schisandrin C (**2**)	$>10^{-3}$ M	Pregomisin mono methyl ether (**17**)	3.3×10^{-5} M
γ -Schisandrin (**3**)	7.7×10^{-5} M	Pregomisin dimethyl ether (**18**)	2.0×10^{-5} M
Gomisin N (**4**)	1.3×10^{-4} M	Gomisin K_1 methyl ether (**19**)	2.2×10^{-4} M
Anwulignan (**5**)	2.6×10^{-4} M	Gomisin K_3 methyl ether (**20**)	1.7×10^{-5} M
Gomisin M_1 (**6**)	5.2×10^{-5} M	Gomisin K_1 acetate (**21**)	$>10^{-3}$ M
Gomisin M_2 (**7**)	4.8×10^{-5} M	Gomisin K_3 acetate (**22**)	1.5×10^{-4} M
Benzoylisogomisin O (**8**)	$>10^{-3}$ M	Gomisin B (**23**)	$>10^{-3}$ M
Gomisin E (**9**)	$>10^{-3}$ M	Gomisin C (**24**)	$>10^{-3}$ M
Gomisin M_1 methyl ether (**10**)	7.5×10^{-5} M	Schisandrol A (**25**)	2.5×10^{-4} M
Gomisin M_2 methyl ether(**11**)	6.3×10^{-5} M	Schisandrol B (**26**)	5.9×10^{-4} M
Isogomisin O (**12**)	5.9×10^{-4} M	6(7)-Dehydro schisandrol A (**27**)	2.1×10^{-6} M
Pregomisin (**13**)	6.2×10^{-5} M	7(18)-Dehydro schisandrol A (**28**)	1.3×10^{-4} M
Gomisin G (**14**)	$>10^{-3}$ M	6(7)-Dehydro schisandrol B (**29**)	4.2×10^{-5} M
Gomisin K_1 (**15**)	2.6×10^{-4} M	7(18)-Dehydro schisandrol B (**30**)	1.2×10^{-4} M

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