Structure–Activity Relationships of Lignans from *Schisandra chinensis* as Platelet Activating Factor Antagonists

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We studied the structure–activity relationships of lignans from *Schisandra chinensis* and their derivatives as platelet activating factor (PAF) antagonists. Strong activity was shown in lignans without an ester group at C-6, a hydroxyl group at C-7 or a methylene dioxy moiety and with an *R*-biphenyl configuration. 6(7)-Dehydroschisandrol A, a derivative of schisandrol A, showed the highest activity (IC_{50} 2.1×10^{-6} M) in this study.

Key words *Schisandra chinensis*; platelet activating factor antagonist; dibenzocyclooctadiene; lignan

Platelet activating factor (PAF) with the chemical structure of a 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine is a product of IgE-sensitized basophils.¹⁾ PAF exerts a myriad of physiological and pathological roles, such as the aggregation, degranulation and chemotaxis of neutrophils, asthma, bronchoconstriction, increased vascular permeability, hypotension, decreased cardiac output, altered liver metabolism, allergic skin disease and transplanted organ rejection.²⁾

Recently, we have examined PAF antagonistic activity on the extracts of various herbs and reported the active substances,^{3,4)} schisandrin A, B, pregomisin and chamigrenal from *Schisandra chinensis* which is used in tonic and cough remedies.

In a continuing fractionation, we isolated other active lignans which have not been reported as PAF antagonists, and we separated two isomers, γ -schisandrin and gomisin N, from schisandrin B fraction by the method of Ikeya *et al.*⁵⁾

Therefore, we report the revised PAF activity of schisandrin B and the structure–activity relationships of lignans and their derivatives as PAF antagonists.

MATERIALS AND METHODS

Analytical Instruments and Materials For the isolation of compounds and bioassay, analytical instruments and materials were prepared as reported previously.⁴⁾

Isolation and Preparation of Compounds from *S. chinensis* Hexane extract (200 g) was separated into seven fractions and schisandrin A (1), B and C (2) were isolated from the second fraction as in the previous study.³⁾ But γ -schisandrin (3, 15 mg) and gomisin N (4, 49 mg) were separated from schisandrin B (70 mg) by 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 M₁ (6, 31 mg), M₂ (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 M₁ methyl ether (10, 4.5 mg) and gomisin M₂ methyl ether (11, 5 mg), respectively. By the method of Li *et al.*,⁶⁾ the hydrolysis of 8 (4 mg) yielded isogomisin O (12, 3 mg). From the fifth fraction (2.6 g) where pregomisin (13) had been isolated,⁴⁾ gomisin G (14, 18 mg), gomisin K₁ (15, 26 mg) and

K₃ (16, 47 mg) were isolated by silica gel chromatography (hexane–EtOAc, 5 : 1). By treatment with diazomethane, compounds 13 (16 mg), 15 (6 mg) and 16 (5 mg) yielded pregomisin mono methyl ether (17, 6 mg), pregomisin dimethyl ether (18, 6 mg), gomisin K₁ methyl ether (19, 5 mg) and gomisin K₃ methyl ether (20, 3 mg), respectively. The acetylation of 15 (5 mg) and 16 (8 mg) with acetic anhydride and pyridine yielded gomisin K₁ acetate (21, 4 mg) and gomisin K₃ 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 POCl₃,^{6,7)} the former yielded 6(7)-dehydroschisandrol A (27, 116 mg) and 7(18)-dehydroschisandrol A (28, 41 mg) and the latter yielded 6(7)-dehydroschisandrol B (29, 87 mg) and 7(18)-dehydroschisandrol B (30, 35 mg), respectively. The identification of lignan structures, as shown in Chart 1, was confirmed by the related references (1,⁸⁾ 2,⁹⁾ 3, 4, 6, 7, 10, 11,⁵⁾ 5,¹⁰⁾ 8,¹¹⁾ 9,¹²⁾ 13,¹³⁾ 14, 23, 24,¹⁴⁾ 15, 16, 19 to 21,¹⁵⁾ 25, 26,⁸⁾ 27 to 30^{6,7)}).

Assay of [³H]PAF Receptor Binding Inhibition on Washed Rabbit Platelets The procedure was described in a previous paper.^{3,4)} Triplicated tests were carried out at each concentration, and IC_{50} values were calculated from at least 4 concentrations. Ginkgolide and magnolin were used as the positive controls.

Statistical Analysis Statistical significance was evaluated by students's *t*-test.

RESULTS AND DISCUSSION

The active compounds (3 to 7, 15, 16, 25, 26) and less active compounds (8, 9, 14, 23, 24) were isolated by a continuing bioactivity-guided separation. They have dibenzocyclooctene structures except for 5 (IC_{50} 2.6×10^{-4} M), which was first isolated from *S. chinensis*. This led us to investigate the structure–activity relationships of lignans as PAF antagonists.

1. *Effect of Esterification of the Hydroxyl at C-6*; As in the lignans with an angeloyl or a benzoyl ester group, the esterification of a hydroxyl group at C-6 (8, 9, 14, 23, 24) did not show PAF antagonistic activity (IC_{50} $> 10^{-3}$ M). However, the activity of 12, the hydrolyzed product of 8, was increased

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Chart 1. Lignans from *S. chinensis*

6 and 10 have a biphenyl configuration of the *R*, *S*-mixture. Ang=angeloyl, Bz=benzoyl.

(IC_{50} , 5.9×10^{-4} M). Thus, the introduction of an ester group at C-6 tends to decrease activity.

2. *Effect of Biphenyl Configuration*; Lignans with a dibenzocyclooctene structure are classified as either *R* (*R*-form) or *S* biphenyl configurations (*S*-form) by the cotton effect.¹⁶⁾ Compounds 3 and 4, with a methylene dioxy moiety, have the *R* and *S*-form, respectively. Compared with 4 (IC_{50} , 1.3×10^{-4} M), the activity of 3 (IC_{50} , 7.7×10^{-5} M) was high. However, in 1 and 19 without a methylene dioxy moiety, the activity of 1 (IC_{50} , 1.6×10^{-5} M) with the *R*-form was much higher than that of 19 (IC_{50} , 2.2×10^{-4} M) with the *S*-form at a high significance level ($p < 0.01$). These results suggest that the activity of the *R*-form is more potent than that of the *S*-

form.

3. *Effect of Methylene Dioxy Moiety*; Among compounds with the *R*-form, the activities of 1 and 25 (IC_{50} , 2.5×10^{-4} M) without a methylene dioxy moiety were significantly higher ($p < 0.01$) than those of 3 and 26 (IC_{50} , 5.9×10^{-4} M) with a 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 introduction of a methylene dioxy moiety tends to decrease the activity.

4. *Effect of Substituents on the Phenyl Nucleus*; Compounds 15 and 16 without a methylene dioxy moiety have hydroxyl groups at C-12 and C-14, respectively. To compare

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

Positive control: ginkgolide (IC₅₀, 1.9×10⁻⁷ M), magnolin (IC₅₀, 2.3×10⁻⁵ M).

the activities of the substituents on the phenyl nucleus, these compounds were acetylated or methylated. Compared to **15** (IC₅₀, 2.6×10⁻⁴ M) with an *S*-form, the activities of **19** and **21** (IC₅₀, >10⁻³ M) did not increase by substitution on the phenyl nucleus. However, compared to **16** (IC₅₀, 1.6×10⁻⁴ M) with an *R*-form, **20** (IC₅₀, 1.7×10⁻⁵ M) showed remarkable increase (*p*<0.05), whereas no apparent influence was observed in **22** (IC₅₀, 1.5×10⁻⁴ M). However, with an *R*-form and methylene dioxy moiety, the activity of **11** (IC₅₀, 6.3×10⁻⁵ M) was similar to that of **7** (IC₅₀, 4.8×10⁻⁵ M). These results indicate that the activity of the *R*-form without a methylene dioxy moiety is increased by methylation on the phenyl nucleus.

Besides lignans with an *R*-form or *S*-form, **6** with the mixture of *R,S*-form and **13** without a dibenzocyclooctene structure were methylated. The activity of **6** (IC₅₀, 5.2×10⁻⁵ M) with a methylene dioxy moiety was similar to **10** (IC₅₀, 7.5×10⁻⁵ M). However, compared to **13** (IC₅₀, 6.2×10⁻⁵ M) without a methylene dioxy moiety, the activities of **17** (IC₅₀, 3.3×10⁻⁵ M) and **18** (IC₅₀, 2.0×10⁻⁵ M) were increased at a significant level (*p*<0.05), respectively. Compound **18** has a cleaved structure between carbons at C-15 and C-16 of **1**. However, their activities were similar.

5. Effect of Hydroxyl at C-7; With the exception of a hydroxyl group at C-7, the structures of **25** and **26** are the same as **1** and **3**, respectively. But the activities of **1** and **3** were

significantly higher than those of **25** and **26** with a hydroxyl group at C-7 (*p*<0.01), respectively. Thus, the introduction of a hydroxyl group at C-7 tends to decrease activity.

6. Effect of Dehydration at C-7; Because of a hydroxyl group at C-7, the activities of **25** and **26** were decreased. When they were dehydrated at C-7, the activities of **28** (IC₅₀, 1.3×10⁻⁴ M) and **30** (IC₅₀, 1.2×10⁻⁴ M) with an exocyclic methylene moiety were similar to **25** and **26**, respectively. However, the activities of **27** (IC₅₀, 2.1×10⁻⁶ M) and **29** (IC₅₀, 4.2×10⁻⁵ M) with an 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 an exocyclic methylene moiety as a partial structure of PAF antagonists.

Therapeutic Considerations; The Fruits of *S. chinensis* contains about 2% lignans by weight. Among them, six compounds (**1**, **2**, **3**, **4**, **25**, **26**) are the main constituents.¹⁷⁾ The intense or mild PAF antagonistic activities, in the present study, were found in major lignans (**1**, **4**, **25**, **26**) and their derivatives (**27** to **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, atopic dermatitis and other inflammatory diseases. Furthermore, the activity-structure relationships, as PAF antagonists, will provide useful information on the interaction between the PAF receptor and its ligands.

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