Arch Pharm Res Vol 34, No 2, 217-222, 2011
DOI 10.1007/s12272-011-0206-0

Archives of Pharmacal Research
www.springer.com/12272

In Vitro Sortase A Inhibitory and Antimicrobial Activity of Flavonoids Isolated from the Roots of *Sophora flavescens*

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(Received August 16, 2010/Revised October 5, 2010/Accepted November 1, 2010)

A series of flavonoids (**1**-**14**) was isolated from the roots of *Sophora flavescens*. We evaluated their ability to inhibit both microbial growth and sortase A, an enzyme that plays a key role in cell wall protein anchoring and virulence in *Staphylococcus aureus*. Most prenylated flavonoids (**7**-**13**) displayed potent inhibitory activity against gram-positive and gram-negative bacteria except *E. coli*, with minimum inhibitory concentrations values ranging from 4.40 to 27.7 μM, and weak or no activity against fungal strains tested. Kurarinol (**6**) was a potent inhibitor of sortase A, with an IC_{50} value of 107.7 ± 6.6 μM. A preliminary structure-activity relationship, including essential structural requirements, is described.

Key words: *Sophora flavescens*, Antibacterial activity, Sortase A, Flavonoids, Kurarinol

INTRODUCTION

The dried root of *Sophora flavescens* Aiton (Leguminosae) is a Chinese herbal medicine well known to have anti-inflammatory, antiarrhythmic, antipyretic, antiasthmatic, and antiulcerative effects and is used for the treatment of diarrhea, gastrointestinal hemorrhage, and eczema (Zheng et al., 1999). Alkaloids and flavonoids are known constituents of Sophorae radix (Kuroyanagi et al., 1999). Recently, *S. flavescens* was also identified as a rich source of flavonoids, ones that possess a wide range of biological activities, including anticancer, anti-inflammatory, and antibacterial properties (Zheng et al., 1999; Kang et al., 2000; Kim et al., 2002; Cha et al., 2009).

Gram-positive pathogenic bacteria display surface proteins that play important roles in their adhesion to specific organ tissues, invasion of host cells, or the evasion of host-immune responses (Cossart and Jongquires, 2000). These virulence-associated proteins are covalently anchored to bacterial cell wall peptidoglycans through a general sorting mechanism catalyzed by a superfamily of membrane-associated transpeptidases termed sortases (Maresso and Schneewind, 2008). Two sortase isoforms, sortase A (SrtA) and sortase B (SrtB), have been identified in *Staphylococcus aureus* (Mazmanian et al., 1999, 2003). The SrtA isoform plays a critical role in the pathological effects of gram-positive bacteria by modulating the ability of the bacterium to adhere to host tissue via the covalent anchoring of adhesion molecules and other virulence-associated proteins to cell wall peptidoglycans. *S. aureus* mutants lacking sortase fail to display surface proteins and are defective in the establishment of infections but microbial viability is not affected (Mazmanian et al., 2000). There have only been a few reports in the literature describing inhibitors of sortase, due in part to the fact that the importance of sortase as a new target has only recently been acknowledged (Maresso and Schneewind, 2008; Oh et al., 2010). Therefore, inhibitors of SrtA might be promising candidates for the treatment and/or prevention of gram-positive bacterial infections.

During the course of our search for SrtA inhibitors from natural products, we encountered Sophorae radix whose crude extract exhibited significant inhibitory

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activity (IC_{50}= 65 μg/mL) toward *S. aureus* SrtA. In this study, the active principles isolated from *S. flavescens* were characterized by spectroscopic analysis and their inhibitory effect against SrtA and minimum inhibitory concentrations (MICs) against several bacteria and fungi were determined.

MATERIALS AND METHODS

General experimental procedures

NMR spectra were recorded on Bruker AMX-500 and Varian Gemini 2000 spectrometers in DMSO-d_6. Mass spectra were obtained at the Korea Basic Science Institute on a JEOL JMS 700 high-resolution mass spectrometer. UV absorption spectra were recorded on a Hitachi U-3010 spectrophotometer; IR absorption was recorded on a JASCO 300E FT-IR spectrometer. For chromatographic procedures, silica gel PF 254 and silica gel 230-400 mesh or 60-230 mesh (Merck) were used. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA.

Plant material

The roots of *Sophora flavescens* were purchased from the Kyungdong-Mart, Seoul, Korea, in March 2009. A voucher specimen is on deposit at the Natural Products Research Institute, College of Pharmacy, Seoul National University.

Extraction and isolation of active compounds

The dried roots of *S. flavescens* (5.0 kg) were extracted repeatedly with dichloromethane (10 L × 3) and methanol (10 L × 3). The combined crude extract (328.7 g) was partitioned between H_2O (163.4 g) and *n*-butanol (145.3 g) and then the latter was repartitioned between *n*-hexane (13.3 g) and 10% aqueous MeOH (118.2 g). The 10% aqueous MeOH layer was repartitioned between H_2O (40.9 g) and EtOAc (69.6 g). An aliquot of the EtOAc layer (8.0 g) was subjected to reversed-phase vacuum flash chromatography using sequential mixtures of H_2O and MeOH as eluants (elution order: 50%, 40%, 30%, 20%, 10% aqueous MeOH, and 100% MeOH), 100% EtOAc, and 100% acetone.

Based on bioactivity tests and TLC analysis, the fraction (1.4 g) eluted with 30% aqueous MeOH from flash chromatography was separated by C_{18} reversed-phase HPLC (YMC ODS-A column, 30% aqueous MeOH) to yield, in order of elution, compounds **1**, **6**, **2**-**5**, **11**, **10**, and **7**. Final purification was then acomplished by reversed-phase HPLC (35% aqueous MeOH) to afford 7.1, 69.5, 10.0, 36.7, 3.5, 137.7, 315.3, 100.3, and 81.2 mg of **1**-**7**, **10**, and **11**, respectively. The fraction (209.6 mg) eluted with 20% aqueous MeOH from flash chromatography was separated by reversed-phase HPLC (25% aqueous MeOH) to afford 104.9, 44.2, and 5.5 mg of **7**, **8**, and **9**, respectively. The fraction (245.7 mg) eluted with 10% aqueous MeOH from flash chromatography was separated by reversed-phase HPLC (15% aqueous MeOH) to yield, in order of elution, compounds **8**, **9**, **13**, **14**, and **12**. Final purification was then acomplished by reversed-phase HPLC (25% aqueous MeOH) to afford 2.0, 13.6, 5.5, 35.6, and 9.5mg of **8**, **9**, and **12**-**14**, respectively.

Sortase A (SrtA) activity assay

Staphylococcus aureus ATCC 6538p was the source of the *srtA* gene. Expression and purification of recombinant $SrtA_{\Delta 24}$ were carried out according to a previously published procedure (Oh et al., 2004). The ability of compounds **1**-**14** to inhibit SrtA was assayed by using a fluorescent peptide (Dabcyl-QALPETGEE-Edans). *p*-Hydroxymecuribenzoic acid (pHMB) was used as a positive control. All reported values are the means of triplicate assays.

Antimicrobial activity assay

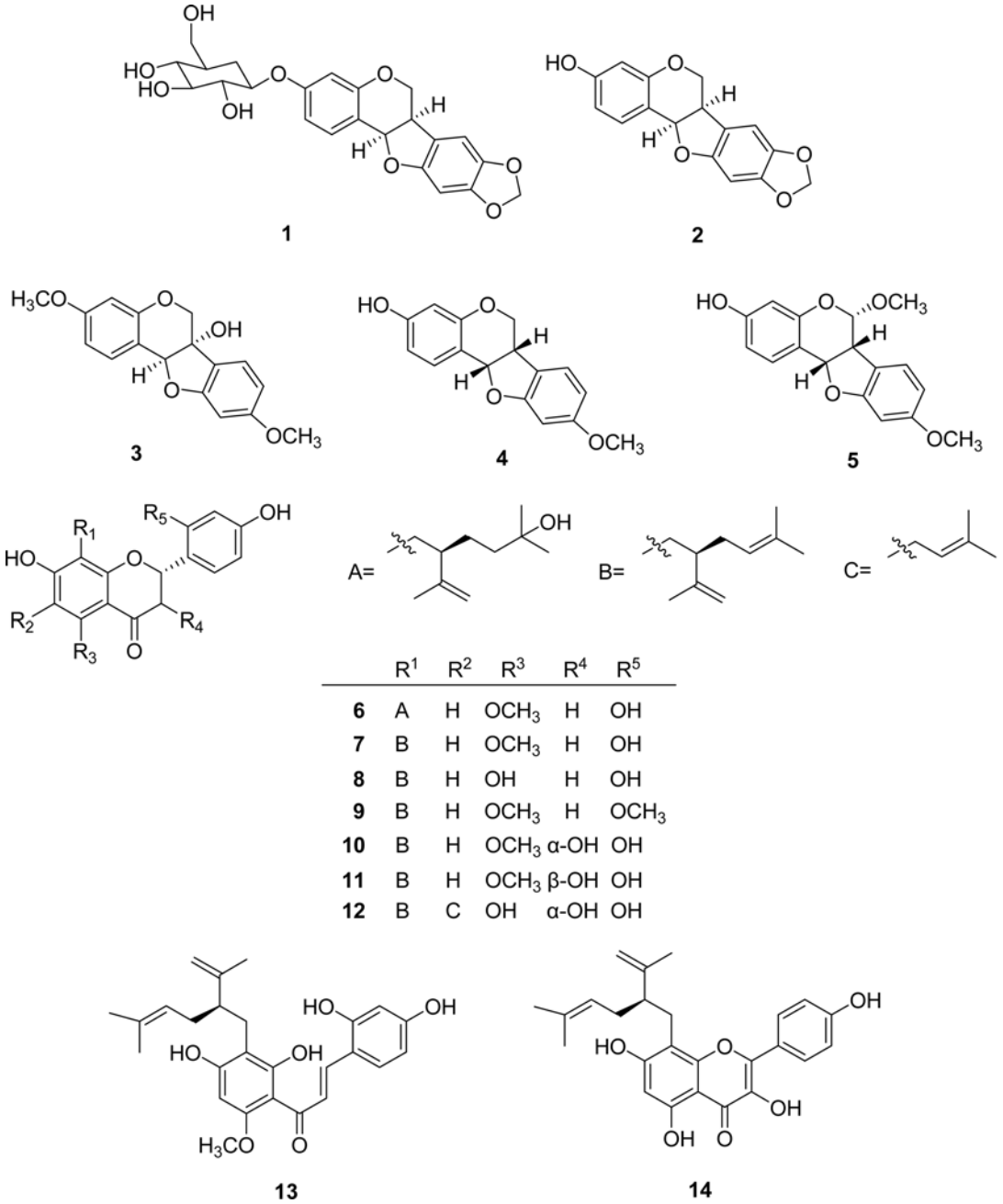
Antimicrobial activity was evaluated by calculating the MIC, determined by a microdilution method (Oh et al., 2008). The organisms to be tested were grown in Standard Method (SM) broth (Difco Co.) at 37°C for the bacteria, and YPD broth (1% yeast extract, 2% peptone, and 2% dextrose) at 28°C for the fungi. Stock solutions of the series of compounds were prepared in DMSO. In each well of a 96-well plate we mixed 90 μL of cells (10^4 cells/mL) with 10 μL of a 10 times concentrated test compound solution in 5% DMSO. This gave a final DMSO concentration of 0.5%. For bacteria, culture plates were incubated for 24 h at 37°C. For fungi, *C. albicans* and *T. mentagrophytes* were incubated for 48 h at 28°C and 72 h at 28°C, respectively. Culture with DMSO (0.5%) was used as solvent controls, and culture supplemented with ampicillin or amphotericin B was used as a positive control. The MIC was defined as the lowest concentration able to inhibit any visible microorganism growth.

RESULTS AND DISCUSSION

Fractination guided by SrtA inhibitory activity and repeated column chromatography of the combined crude extract from the roots of *S. flavescens* yielded fourteen compounds. Based on the results of combined spectral analyses and comparison of spectral data with those of known compounds, the isolated compounds were identified as trifolirhizin (**1**), (-)-maackiain

(**2**) (Park et al., 2003), (-)-variabiin (**3**) (Ingham and Markham, 1980), (+)-medicarpin (**4**) (Miller et al., 1988), (-)-sophoracarpan A (**5**) (Kinoshita et al., 1990), kurarinol (**6**), kurarinone (**7**) (Jung et al., 2004), sophoraflavanone G (**8**), (2S)-2'-methoxykurarinone (**9**) (Kang et al., 2000), kushenol I (**10**), kushenol N (**11**), kushenol M (**12**) (Ryu et al., 1996), kuraridin (**13**) (Ryu et al., 1997), and 8-lavandulylkaempferol (**14**) (Mizuno et al., 1988) (Fig. 1). Spectral data for these compounds were consistent with data reported previously.

Compounds **1**-**14** were evaluated for inhibitory activity against SrtA from *S. aureus* ATCC 6538p. The inhibitory potencies, expressed as IC_{50} values, of the tested compounds are shown in Table I and are compared to that of a synthetic known SrtA inhibitor, pHMB (IC_{50} = 130 ± 6.1 μM). Nearly all of these compounds had SrtA inhibitory activity at concentrations > 200 μM. Among the compounds tested, kurarinol (**6**)



	R^1	R^2	R^3	R^4	R^5
6	A	H	OCH_3	H	OH
7	B	H	OCH_3	H	OH
8	B	H	OH	H	OH
9	B	H	OCH_3	H	OCH_3
10	B	H	OCH_3	α-OH	OH
11	B	H	OCH_3	β-OH	OH
12	B	C	OH	α-OH	OH

Fig. 1. Chemical structures of trifolirhizin (**1**), (-)-maackiain (**2**), (-)-variabiin (**3**), (+)-medicarpin (**4**), (-)-sophoracarpan A (**5**), kurarinol (**6**), kurarinone (**7**), sophoraflavanone G (**8**), (2S)-2'-methoxykurarinone (**9**), kushenol I (**10**), kushenol N (**11**), kushenol M (**12**), kuraridin (**13**) and 8-lavandulylkaempferol (**14**)

exhibited the most potent inhibitory activity against SrtA, with an IC_{50} value of 107 ± 6.6 μM and was significantly more active than kurarinone (**7**), which possesses the B-type side chain at C-8 (Fig. 1). These results suggest that the A-type side chain possessing a hydroxyl group at C-8 is required for potent inhibitory activity against SrtA. Sortase inhibitors should act as anti-infective agents and disrupt the pathogenesis of bacterial infections without affecting microbial viability (Mazmanian et al., 2000). Therefore, we also determined the MICs of our isolated compounds against *S. aureus* ATCC 6538p. As shown in Table II, kurarinol (**6**) was weakly active against *S. aureus* with an MIC value of 219 μM. Taken together, kurarinol (**6**) is a good starting candidate for SrtA inhibitor design. Recently, some plant-derived compounds have been

Table I. Inhibitory effects of flavonoids (**1**-**14**) on SrtA activity

Compound	SrtA inhibitory rate (%)[a]			SrtA IC_{50} (μM)
	25 μg/mL	50 μg/mL	100 μg/mL	
pHMB[b]	10.3 ± 1.1	53.5 ± 2.3	99.7 ± 3.5	130 ± 6.1
Trifolirhizin (**1**)	4.9 ± 0.3	14.7 ± 0.7	25.8 ± 1.7	> 224
(-)-Maackiain (**2**)	6.3 ± 0.1	18.4 ± 1.3	26.0 ± 1.2	> 352
(-)-variabiin (**3**)	3.9 ± 0.1	13.4 ± 0.9	23.1 ± 2.0	> 333
(+)-medicarpin (**4**)	0.4 ± 0.2	8.3 ± 0.8	12.6 ± 1.5	> 369
(-)-sophoracarpan A (**5**)	0.3 ± 0.1	2.5 ± 0.2	10.6 ± 0.8	> 333
Kurarinol (**6**)	30.5 ± 1.6	51.1 ± 3.2	96.5 ± 4.1	107 ± 6.6
Kurarinone (**7**)	3.1 ± 0.1	6.5 ± 0.3	38.0 ± 2.7	> 228
Sophoraflavanone G (**8**)	17.0 ± 2.4	21.3 ± 1.4	32.5 ± 1.4	> 235
(2S)-2-Methoxykurarinone (**9**)	7.8 ± 0.5	14.3 ± 1.6	32.2 ± 2.0	> 221
Kushenol I (**10**)	17.8 ± 1.8	28.3 ± 2.3	43.8 ± 1.9	> 220
Kushenol N (**11**)	25.1 ± 0.7	36.8 ± 2.9	47.2 ± 2.1	> 220
Kushenol M (**12**)	0.8 ± 0.3	1.2 ± 0.1	8.4 ± 0.2	> 196
Kuraridin (**13**)	11.2 ± 0.4	22.8 ± 1.4	32.2 ± 3.0	> 282
8-Lavandulylkaempferol (**14**)	0.5 ± 0.2	3.7 ± 0.2	13.2 ± 0.9	> 236

[a]Inhibitory rates are means ± S.D. (n = 3), [b]pHMB: *p*-hydroxymecuribenzoic acid.

Table II. MICs of *S. flavescens* flavonoids (**1**-**14**) against different bacteria and fungi

Compound	MIC (μM)						
	Sa	Bs	St	Pv	Ec	Ca	Tm
Ampicillin	4.46	4.46	8.93	4.46	17.89	-	-
Amphotericin B	-	-	-	-	-	17.89	8.93
Trifolirhizin (**1**)	> 224	> 224	> 224	> 224	> 224	> 224	> 224
(-)-Maackiain (**2**)	> 352	> 352	> 352	> 352	> 352	> 352	> 352
(-)-variabiin (**3**)	> 333	> 333	> 333	> 333	> 333	333	333
(+)-medicarpin (**4**)	368	368	368	> 368	368	368	368
(-)-sophoracarpan A (**5**)	> 333	> 333	333	> 333	> 333	> 333	> 333
Kurarinol (**6**)	219	219	219	109	> 219	> 219	> 219
Kurarinone (**7**)	7.12	7.12	6.25	14.27	> 228	> 228	114
Sophoraflavanone (**8**)	7.36	7.36	7.36	3.68	> 235	> 235	> 235
(2S)-2-Methoxykurarinone (**9**)	27.7	55.3	27.7	27.7	> 221	> 221	> 221
Kushenol I (**10**)	27.5	13.8	27.5	27.5	> 220	220	220
Kushenol N (**11**)	27.5	27.5	27.5	27.5	> 220	> 220	> 220
Kushenol M (**12**)	24.6	12.3	12.3	6.14	> 196	> 196	> 196
Kuraridin (**13**)	8.81	17.7	8.81	4.40	> 282	141	141
8-Lavandulylkaempferol (**14**)	236	118	236	118	> 236	> 236	> 236

Microorganisms: *Sa*, *Staphylococcus aureus* ATCC 6538p; *Bs*, *Bacillus subtilis* ATCC 6633; *St*, *Salmonella typhimurium* ATCC 14028; *Pv*, *Proteus vulgaris* ATCC 3851; *Ec*, *Escherichia coli* ATCC 25922; *Ca*, *Candida albicans* ATCC 10231; *Tm*, *Trichophyton mentagrophytes* IFO 40996.

reported to be active against SrtA including β-sitosterol-3-*O*-glucopyranoside (IC_{50} = 18.3 μg/mL) from *Fritillaria verticillata* (Kim et al., 2003), berberine chloride (IC_{50} = 8.7 μg/mL) from *Coptis chinensis* and related isoquinoline alkaloids such as beberine sulfate (IC_{50} = 11.5 μg/mL), palmatine chloride (IC_{50} = 12.7 μg/mL), and β-hydrastine (IC_{50} > 80 μg/mL) (Kim et al., 2004). Kurarinol (**6**) from *S. flavescens* has activity that compares favorably to previously reported constituents.

A variety of bioactive compounds have been isolated from *S. flavescens* for the treatment of inflammation, antioxidant, cancer, and cardiovascular disorders (Zheng et al., 1999; Kang et al., 2000; Kim et al., 2002), but knowledge about the antimicrobial potential of these compounds is limited (Kuroyanagi et al., 1999; Chen et al., 2005; Cha et al., 2009). Among the isolated flavonoids in this study, compounds **7**, **8**, **13** had the more potent antibacterial activity, and **6** and **14** had weaker activity than the other compounds **9**-**12** (Table II). These results indicate that the lavandulyl or isoprenyl moieties at C-8 contribute to the activity of prenylflavonone derivatives, while the A-type side chain possessing a hydroxyl group (Fig. 1) noticeably reduces antibacterial activity. A hydroxyl group at C-3 also decreased antibacterial activity (Kuroyanagi et al., 1999). In contrast, all of these compounds had weak or no antifungal activity against *C. albicans* ATCC 10231 and *T. mentagrophytes* IFO 40996.

In summary, our results revealed the SrtA inhibitory activity of flavonoids from *S. flavescens* and the importance to SrtA inhibitory activity of a prenyl group attached to the flavonoid molecule. Among the fourteen tested compounds, kurarinol exhibited potent inhibitory activity against SrtA and may represent a good starting candidate for the design of SrtA inhibitors. Considering their strong antibacterial activity, prenylated flavonoids such as kurarinone, sophoraflavanone G and kuraridin could be used to treat microbial infections.

ACKNOWLEDGEMENTS

This work was supported by a grant from the BioGreen 21 Program (20090601-060-003-001-03-00) of the Rural Development Administration, Republic of Korea.

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