Antimalarial Activity of Lavandulyl Flavanones Isolated from the Roots of *Sophora flavescens*

Youn Chul KIM, a Hye-Sook KIM, b Yusuke WATAYA, b Dong Hwan SOHN, a Tai Hyun KANG, a Myung Soo KIM, a Yong Man KIM, d Geon-Mok LEE, e Jong-Duk CHANG, e and Hyun PARK a,d

a College of Pharmacy, Wonkwang University; Iksan 570–749, Korea; b Faculty of Pharmaceutical Sciences, Okayama University; Tsushima, Okayama, Okayama 700–8530, Japan; c Life Sciences Division, Korea Institute of Science and Technology; Seoul 130–650, Korea; d Department of Parasitology, College of Medicine, Wonkwang University; and e Department of the Third Medicine, Professional Graduate School of Oriental Medicine, WonKwang University; Iksan 570–749, Korea. Received October 23, 2003; accepted January 15, 2004

Four lavandulyl flavanones, (2S)-2’-methoxykurarinone (1), sophoraflavanone G (2), leachianone A (3), and (−)-kurarinone (4), which are isolated from the roots of *Sophora flavescens* have been tested for in vitro antimalarial activity against *Plasmodium falciparum*. Compounds 1—3 showed moderate antimalarial activities with EC50 values of 2.4 × 10−8, 2.6 × 10−8, and 2.1 × 10−8 M, respectively. These compounds did not show selective toxicity against *P. falciparum* in the toxicity test on mouse mammalian tumor cells, however, it is suggested that the position of methoxyl groups in flavanone skeleton plays an important role on antimalarial activity.

Key words *Plasmodium falciparum*; lavandulyl flavanone; *Sophora flavescens*; antimalarial

*Plasmodium falciparum*, the most widespread etiological agent of human malaria, is becoming increasingly resistant to conventional antimalarial drugs, which necessitates a continuous effort to search for new antimalarial drugs to control this disease.1,2) In the endemic area where malaria prevails, traditional herbal medicines are often used for antipyretic therapy. However, very little scientific information is available to assess the efficacy of these herbal remedies. Therefore, it is important to investigate the efficacy of the antimalarial activities of medicinal plants in order to determine their potential as sources in the development of new antimalarial drugs. Previous findings of antimalarial agents such as quinine and artemisinin from medicinal plants also encouraged the possibility of finding new antimalarial drugs from plant sources.

The root of *Sophora flavescens* Aiton (Leguminosae) is a Chinese herbal medicine well known to have antibacterial, anti-inflammatory, antipyretic, antiarrhythmic, antiasthmatic, antiulcerative, and antineoplastic effects and is used as an insecticide and for the treatment of diarrhea, gastrointestinal hemorrhage, and eczema.3) Phytochemical studies of *S. flavescens* have reported the isolation of quinolizidine alkaloids, flavonoids, and triterpenoids.4) Recently we isolated four lavandulyl flavanones, (2S)-2’-methoxykurarinone (1), sophoraflavanone G (2), leachianone A (3), and (−)-kurarinone (4), including two new compounds from this plant.5) Lavandulyl flavanones are known to be rarely distributed in the plant kingdom, and they are mainly found in *Sophora* species.

Based on both the established biological activities of the root of *S. flavescens* and the unique structures of its constituents, lavandulyl flavanones, we attempted to screen for antimalarial activity on four lavandulyl flavanones (1—4). The present paper reports on *in vitro* antimalarial activity of these lavandulyl flavanone compounds against *P. falciparum* strain FCR-3 and their cytotoxicity against mouse mammary FM3A cells, which serve as a host model.

**MATERIALS AND METHODS**

**Materials** Four lavandulyl flavanones, (2S)-2’-methoxykurarinone, sophoraflavanone G, leachianone A, and (−)-kurarinone, were isolated from the root of *Sophora flavescens* and their structures were elucidated. The procedures for isolation and identification of these compounds have been described elsewhere.3) The antimalarial agents, quinine hydrochloride, pyrimethamine and artemisinin, were purchased from Sigma (St. Louis, MO, U.S.A.). Mefloquine was a gift from F. Hoffman-La Roche LTD (Basel, Switzerland).

**Malaria Parasites** *P. falciparum* (ATCC 30932, FCR-3 strain) was used in this study. *P. falciparum* was cultivated by a modification of the method of Trager and Jensen6) using a 5% hematocrit of type A human red blood cells suspended in RPMI 1640 medium (Gibco, NY, U.S.A.), and supplemented with heat-inactivated 10% type A human serum. The plates were placed in a CO2–O2–N2 atmosphere (5% CO2, 5% O2, 90% N2 atmosphere) at 37 °C, and the medium was changed daily until 5% parasitemia (which means the existence of 5 parasite-infected erythrocytes in every 100 erythrocytes).

**Mammalian Cells** Mouse mammary tumor FM3A cells (wild-type, subclone F28-7)7) were supplied by the Japanese Cancer Research Resources Bank (JCRB). FM3A cells were maintained in a suspension culture at 37 °C in a 5% CO2 atmosphere in plastic bottles containing ES medium (Nissui Pharmaceuticals, Tokyo, Japan) supplemented with 2% heat-inactivated fetal bovine serum (Gibco, NY, U.S.A.).

**In Vitro Antimalarial Activity of Traditional Medicines**

The following procedures were used to assay antimalarial activity.8,9) Asynchronously cultivated *P. falciparum* was used. Various concentrations of compounds in dimethyl sulfoxide (DMSO) were prepared. Ten microliters of each solution was added to individual wells of a 24-well multi-dish. Erythrocytes with 0.3% parasitemia were added to each well containing 990 μl of culture medium to give a final hematocrit level of 3%. The plates were incubated at 37 °C for 72 h in a multigas incubator (5% CO2, 5% O2, 90% N2 atmosphere).
To evaluate the antimalarial activity of traditional medicines, we prepared thin blood films from each culture and stained them with Giemsa (Merck, Germany). A total of 10,000 erythrocytes per one thin blood film were examined under a microscope. All of the test compounds were assayed in duplicate at each concentration. Drug-free control cultures were run simultaneously. All data points represent the mean of three experiments. Parasitemia in the control reached between 4 and 5% at 72 h. The EC\textsubscript{50} value refers to the concentration of the compound necessary to inhibit by 50% the increase in parasite density at 72 h by 50% of the control.

**Toxicity against Mammalian Cell Line** FM3A cells grew with a doubling time of about 12 h. Prior to exposure to 
parasite density at 72 h by 50% of the control.

**RESULTS AND DISCUSSION**

Four lavandulyl flavanones (1—4) isolated from the root of *S. flavescens* were assessed for their antimalarial activities. The results of the *in vitro* antimalarial activity and cytotoxicity of tested compounds against *P. falciparum* and mammalian cells are summarized in Table 1. Compounds 1—3 showed moderate antimalarial activity with EC\textsubscript{50} values of 2.4, 2.6, and 2.1 \(\mu\text{M}\), respectively. The antimalarial agents, quinine, mefloquine, pyrimethamine, and artemisinin, were also tested for *in vitro* antimalarial activity (Table 1). Several positive controls exhibited potent antimalarial activities compared to lavandulyl flavanones. In the cytotoxicity test on mouse mammary tumor FM3A cells, compounds 1—3 showed a cytotoxic effect, indicating that these compounds have non-selective antimalarial activity.

In spite of the less beneficial antimalarial activity of lavandulyl flavanones, this shows some useful information about the structure—activity relationship. Sophoraflavanone G (2) possesses four hydroxyl groups in its flavanone skeleton (Chart 1). Methylation of hydroxyl group at C-2’ position in flavanone skeleton (compound 3) slightly increased antimalarial activity, however, methylation of hydroxyl group at C-5 position (compound 4) significantly decreased its antimalarial activity. It was also shown that decreasing antimalarial activity due to methylation of C-5 hydroxyl group was recovered by the additional methylation of hydroxyl group at C-2’ in the flavanone skeleton (compound 1). From these results, it is suggested that the position of methoxyl groups in flavanone skeleton plays an important role in antimalarial activity. On the other hand, selective cytotoxicity was not affected by the presence of methoxyl group. Even though it is hard to anticipate, there is still the possibility of increasing selectivity by structural modification. Therefore, it would be useful for the development of new antimalarial agents to modify the phenolic hydroxyl groups in the lavandulyl flavanone skeleton.

Although several types of flavonoids were known to possess antimalarial activity,\textsuperscript{9—13} this is first report on the antimalarial activity of lavandulyl flavanone.

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**REFERENCES**


**Table 1. In Vitro Antimalarial Activity and Cytotoxicity of Lavandulyl Flavanones**

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>P. falciparum</em> EC\textsubscript{50} ((\mu\text{M}))</th>
<th>FM3A EC\textsubscript{50} ((\mu\text{M}))</th>
<th>Selectivity\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2S)-2’-Methoxykurarinone (1)</td>
<td>2.4</td>
<td>3.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Sophoraflavanone G (2)</td>
<td>2.6</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Leachianone A (3)</td>
<td>2.1</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>(−)-Kurarinone (4)</td>
<td>&gt;20 (35\textsuperscript{b})</td>
<td>&gt;20 (25\textsuperscript{b})</td>
<td>1.0</td>
</tr>
<tr>
<td>Quinine</td>
<td>0.2</td>
<td>100.0</td>
<td>500</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>0.001</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>0.032</td>
<td>2.8</td>
<td>88</td>
</tr>
<tr>
<td>Artemisin</td>
<td>0.01</td>
<td>9.0</td>
<td>900</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Selectivity refers to the ratio of the EC\textsubscript{50} value for the FM3A cells and the EC\textsubscript{50} value for *P. falciparum*. \textsuperscript{b} Values in parenthesis show the growth inhibition (%) of each dose.

Chart 1. Structures of Lavandulyl Flavanones (1—4).