Introduction

Infections caused by methicillin-resistant Staphylococcus aureus (MRSA) in compromised hosts pose a serious problem all over the world, because MRSA strains are resistant to numerous antibiotics and can be transmitted from patient to patient via transiently colonized hands of hospital personnel (Brumfitt and Hamilton-Miller, 1989; Mulligan et al. 1993). Although vancomycin (VCM) is the most effective antibiotic for MRSA infections, clinical use often results in unexpected side effects and development of infections by VCM.

Antibacterial properties of a new isoflavonoid from Erythrina poeppigiana against methicillin-resistant Staphylococcus aureus

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Summary

A new isoflavonoid, together with four known isoflavonoids, was isolated from the roots of Erythrina poeppigiana. The chemical structure was determined by extensive spectroscopic studies, and then its antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA) was investigated. The new isoflavonoid was identified as 3,9-dihydroxy-10-\(\gamma,\gamma\)-dimethylallyl-6a,11a-dehydropterocarpan (compound 1). Compound 1 inhibited bacterial growth most potently of the five isolates, and had a minimum inhibitory concentration (MIC) of 12.5 \(\mu\)g/ml against thirteen MRSA strains. Inhibitory activity was based on bactericidal action and viable cell number reduced by approximately 1/10,000 after 4 h incubation with compound 1. Despite intense bactericidal action against MRSA, compound 1 never resulted in leakage of 260 nm-absorbing substances from bacterial cells. Compound 1 (12.5 \(\mu\)g/ml) completely inhibited incorporation of radio-labeled thymidine, uridine and leucine into MRSA cells. Although glucose incorporation was also markedly inhibited by the compound, the amount of glucose incorporated by bacterial cells increased gradually with incubation time. These findings suggest that compound 1 exhibits anti-MRSA activity by interfering with incorporation of metabolites and nutrients into bacterial cells or by affecting the nucleic acids of MRSA cells. Furthermore, this new compound could be a potent phytotherapeutic agent for treating MRSA infections.

Key words: Erythrina poeppigiana, Isoflavonoid, MRSA, Growth inhibition, Metabolite incorporation
resistant enterococci (VRE) (Bailie and Neal, 1988; Schouten et al. 2000). For these reasons, substantial attention has been focused on exploration and utilization of secondary metabolites of plants (phytochemicals) as an alternative to and/or in combination with traditional antibiotics for treating MRSA infections (Inumasa et al. 1994; Sato et al. 1995a; Tanaka et al. 2002). Among the phytochemicals, flavonoids seem to be the most potentially useful candidates because they are widely distributed in edible plants and possess broad pharmacological activity (Pathak et al. 1991). Plants belonging to Leguminosae are known to contain abundance of flavonoids. The genus Erythrina (Leguminosae) is distributed in tropical and subtropical regions of the world and comprises over 100 species. Erythrina poeppigiana is widely distributed in Central and South America, and has been used as a shade tree as well as an ornamental plant. In the course of investigating secondary metabolites in the roots of this plant, we isolated a new isoflavonoid together with four known compounds.

In the present study, the inhibitory action and antibacterial characteristics of the newly isolated isoflavonoid were investigated against MRSA.

Materials and Methods

Plant material and isolation of phytochemicals

Roots of E. poeppigiana were collected in Okinawa Prefecture, Japan, in April 2001. A voucher specimen was preserved in the Department of Natural Product Chemistry, Faculty of Pharmacy, Meijo University, Japan. Finely powdered roots (3.2 kg) were macerated in acetone (36 l) and the solvent was removed to yield a residue, which was divided into n-hexane-, methylenechloride (CH2Cl2), and ethyl acetate (EtOAc)-soluble fractions. The CH2Cl2-soluble fraction (20 g) was applied to silica gel column and eluted with chloroform (CHCl3)-acetone (10:1→1:1) and CHCl3-methanol (10:1) (400 ml for each fraction) to give 18 fractions (frs. 1–18). Frs. 10–15 (8.6 g) were subjected to silica gel column chromatography and eluted with CHCl3-acetone (10:1→3:1→1:1) (20 ml for each fraction) to give 70 fractions (frs. 19–88). Frs. 40 contained eryvarin D (18.5 mg). Frs. 50–55 (119 mg) were again subjected to silica gel column chromatography with benzene-EtOAc (10:1) to give compound 1 (36.4 mg). Frs. 56–64 (301.4 mg) were purified by silica gel column chromatography eluting with benzene-EtOAc (5:1) to give cristacarpin (66.9 mg). Frs. 85–88 (1.2 g) were again subjected to silica gel column chromatography with benzene-EtOAc (10:1) to yield demethylmedicarpin (15 mg) and erysubin F (5 mg).

Structural determination of phytochemicals

The chemical structure of compound 1 was determined by spectral analyses, including UV, IR, 1H NMR, 13C NMR, EIMS and HREIMS. The known compounds, cristacarpin, demethylmedicarpin, erysubin F and eryvarin D were identified by comparison with authentic samples or reported values (Mitscher et al. 1984; Ingham and Tahara 1985; Tanaka et al. 1996; Tanaka et al. 2001).

Bacterial strains and determination of minimum inhibitory concentration

The thirteen strains of MRSA used were the laboratory stock cultures of the Department of Oral Pathology, Asahi University School of Dentistry, Japan. They were defined as MRSA based on their resistance to methicillin and oxacillin according to the guidelines of the National Committee for Clinical Laboratory Standards (1990). All strains were aerobically grown in Mueller Hinton Broth (Difco, Detroit, MI, USA) for 24 h at 37 °C. After incubation, they were diluted with the same medium to give a concentration of approximately 106 colony forming units (CFU)/ml. Isolated compounds were dissolved in dimethyl sulfoxide (DMSO) and 2-fold serial dilutions were made, and added to Mueller Hinton agar plates (1% v/v; final concentration range of 1.56–100 µg/ml). Bacterial cell suspensions were inoculated onto the plates using a bacterial planter (10 µl). The final number of CFU inoculated onto the agar plates was 104 for all strains. Agar plates were aerobically incubated at 37 °C for 24 h. MIC was defined as the lowest concentration at which no colonies were observed after incubation. The agar plate containing only DMSO (1% v/v) served as a control.

Effect of compound 1 on bacterial viability

MRSA G47 strain was suspended in phosphate buffered saline (PBS; pH 7.0, 0.07M) to give a concentration of 6.8 × 108 CFU/ml. Compound 1 in DMSO (final concentration of 12.5 µg/ml) was added to the suspension (1% v/v) and stirred at room temperature. At specified time intervals (1, 2 and 4 h), aliquots (0.5 ml) were removed from the suspension and 10-fold serial dilutions in PBS were made, and diluted (0.1 ml) were then streaked onto Mueller Hinton agar plates. Plates were aerobically incubated at 37 °C for 24 h. Changes in viable cell number were estimated by counting the colonies formed on agar plates. As a control, only DMSO (1% v/v) was added to the cell suspension.

Leakage of cellular substances from bacterial cells

Leakage of 260 nm-absorbing substances from bacterial cells was measured as previously reported (Sato et al. 1997). Compound 1 in DMSO was added to
MRSA (G47) cell suspension in PBS (1 × 10^8 CFU/ml) to give a final concentration of 12.5 µg/ml. Cell suspensions were stirred at room temperature, and 1.0 ml aliquots were filtered (pore size of 0.22 µm) at 1, 2, 4 or 24 h after incubation. Filtrate was extracted in an equal volume of chloroform, and absorbance of the aqueous phase was then measured at 260 nm using a spectrophotometer (U-2000, Hitachi, Tokyo, Japan), and compared with that of the control (filtrate obtained from cell suspension containing only DMSO at 1% v/v).

**Incorporation of radio-labeled materials into bacterial cells**

Incorporation of several metabolites as well as leucine and glucose into bacterial cells was investigated according to a previously reported method (Sato et al. 1996). [6-3H] Thymidine was purchased from Amersham Bioscience Corp. (Piscataway, NJ, USA). [5,6-^3H]-Uridine and [1-3H]-glucose-D were obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA) and [4,5-3H]-leucine was purchased from Moravek Biochemicals, Inc. (Brea, CA, USA). Compound 1 (final concentration of 12.5 µg/ml) and radiolabeled compound (final radioactive concentration of 37 kBq/ml for thymidine and uridine; 18.5 kBq/ml for leucine and glucose) were added to MRSA (G47) cell suspension (5 × 10^8 CFU/ml) in PBS. Mixtures were stirred at room temperature. At specified time intervals (10, 20, 40 or 60 min for thymidine and uridine; 1, 5, 15 or 30 min for leucine and glucose), a 0.5 ml aliquot of each cell suspension was mixed with 5.0 ml of ice-cold 10% (w/v) trichloroacetic acid, and then filtered (pore size of 0.22 µm) to collect bacterial cells. Bacterial cells were washed twice with 5.0 ml of 5% trichloroacetic acid. Filters were then subjected to an automatic sample combustion system (ASC-113, Aloka, Tokyo, Japan). Radioactivity recovered in

![Chemical structures of isolated compounds.](image)

**Fig. 1.** Chemical structures of isolated compounds.  
Compound 1 – 3,9-dihydroxy-10-γ,γ-dimethylallyl-6a,11a-dehydropterocarpan  
Cristacarpin – 3,6a-dihydroxy-9-methoxy-10-γ,γ-dimethylallylpterocarpan  
Demethylmedicarpin – 3,9-dihydroxypterocarpan  
Erysubin F – 7,4′-dihydro-8,3′-di(γ,γ-dimethylallyl)isoflavone  
Eryvarin D – 3-hydroxy-9-methoxy-10-γ,γ-dimethylallyl- 6a,11a-dehydropterocarpan
Aquasol-2 (Packard, Meriden, CT, USA) was measured by a liquid scintillation counter (LSC-5100, Aloka). Cell suspensions containing individual radiolabeled material and DMSO (without compound 1) served as controls.

## Results

Compound 1 was obtained as amorphous powder. The molecular formula was determined to be C_{20}H_{18}O_{4} ([M]+ m/z 322.1211) by HREI mass spectrum. UV spectrum and the characteristic signal of C-6 in the 1H NMR spectrum showed that compound 1 has a pterocarpan skeleton (Prasad et al. 1985). The 1H NMR spectrum included signals of ABX type aromatic protons (δ6.44, 6.46 and 7.35), a set of ortho coupled aromatic protons (δ6.76 and 7.03), and a prenyl group (δ1.77, 1.89, 3.68 and 5.39). The placement of the prenyl group on the C-10 position was confirmed from the HMBC spectrum, revealing that methylene protons at C-1′ correlated with the sp² quaternary carbons at C-9, C-10 and C-10a (spectral data available on demand). UV (MeOH) λ_{max} nm (log ε): 351 (4.24), 333 (4.33), 288 sh (3.80), 241 (4.10), 208 (4.43); IR (KBr) ν_{max} cm⁻¹: 3400, 1615; EIMS m/z (rel. int.): 322 ([M]+, 82), 266 (100), 237 (8), 152 (8); 1H NMR (600 MHz, CDCl₃) δ: 1.77, 1.89 (6H, each s, H-4′, 5′), 3.68 (2H, d, J = 7.3 Hz, H-1′), 4.97 (1H, br s, OH), 5.39 (1H, t, J = 7.3 Hz, H-2′), 5.53 (2H, s, H-6), 6.44 (1H, d, J = 2.2 Hz, H-4), 6.46 (1H, dd, J = 8.1, 2.2 Hz, H-2), 6.76 (1H, d, J = 8.8 Hz, H-8), 7.03 (1H, d, J = 8.8 Hz, H-7), 7.35 (1H, d, J = 8.1 Hz, H-1); 13C NMR (150.8 MHz, CDCl₃) δ: 156.9 (C-3), 155.1 (C-4a), 154.5 (C-10a), 151.9 (C-9), 147.0 (C-11a), 153.1 (C-3′), 121.2 (C-2′), 121.0 (C-1), 119.1 (C-6b), 116.0 (C-7), 112.5 (C-8), 111.3 (C-10), 110.0 (C-11b), 108.4 (C-2), 106.1 (C-6a), 103.9 (C-4), 65.6 (C-6), 25.8 (C-5′), 23.1 (C-1′), 17.9 (C-4′). The NMR spectra were accomplished from the 2D-NMR experiments (¹H-¹H COSY, HSQC and HMBC). From elucidation of the spectral data, the structure of compound 1 was characterized as 3,9-dihydroxy-10-γ,γ-dimethylallyl-6a,11a-dehydropterocarpan. The chemical structures of compound 1 and four known isoflavonoids are shown in Fig. 1.

The MIC values of the five compounds as well as methicillin and oxacillin against the thirteen MRSA strains are summarized in Table 1. They were highly resistant to both antibiotics, while VCM inhibited the growth at a concentration range of 0.78–3.13 µg/ml (data not shown). Compound 1 exhibited the highest inhibitory activity among the five compounds, having a MIC value of 12.5 µg/ml, followed by eryvarin D and demethylmedicarpin. Changes in viable cell number caused by compound 1 are shown in Fig. 2. The number of viable cells was scarcely reduced after 4 h incubation in the control, while it was reduced approximately 1/1,000 at 2 h and 1/10,000 at 4 h incubation with compound 1. Absorbance in the supernatant at 260 nm did not increase after exposing MRSA cells to compound 1 and these values were equivalent to those of the control at all experimental time points (Table 2). Table 3 shows the incorporation of thymidine and uridine into MRSA cells. In the controls, incorporation of both metabolites increased with incubation time. In contrast, incorpora-
Antibacterial properties of a new isoflavonoid from *Erythrina poeppigiana*

Leucine incorporation was also completely inhibited by compound 1 (Table 4). Incorporation of glucose was strongly reduced in the presence of compound 1, however, some increase in glucose incorporation was noticed after incubation for 5 to 30 min. The observed increase was statistically significant (p < 0.01).

### Discussion

In a previous report, we used sixteen isoflavonoids from *Erythrina variegata* to demonstrate that pterocarps possessing \(\gamma, \gamma\)-dimethylallyl group at the C-2 and/or C-10 position and hydroxyl groups at the C-3 and/or C-9 position show anti-MRSA activity and that methyl ethers of the C-9 position reduce the activity

### Table 1. Minimum inhibitory concentrations of isoflavonoids against MRSA.

<table>
<thead>
<tr>
<th>MRSA</th>
<th>Compound 1</th>
<th>Eryvarin D</th>
<th>Erysubin F</th>
<th>Cristacarpin</th>
<th>Demethylmedicarpin</th>
<th>Methicillin</th>
<th>Oxacillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6</td>
<td>12.5(^1)</td>
<td>12.5</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>G19</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>G31</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>G47</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>G71</td>
<td>12.5</td>
<td>12.5</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>G73</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>G86</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>G89</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>G115</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>118 M</td>
<td>12.5</td>
<td>12.5</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>103 H</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>145 M</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>208</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>25</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

\(^1\) µg/ml

### Table 2. Leakage of 260 nm absorbing substance from MRSA cells.

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(^1)</td>
<td>0.022 ± 0.001(^2)</td>
<td>0.097 ± 0.001</td>
<td>0.170 ± 0.004</td>
<td>0.345 ± 0.006</td>
<td>0.741 ± 0.002</td>
</tr>
<tr>
<td>Compound 1 (12.5 µg/ml)(^2)</td>
<td>0.022 ± 0.001</td>
<td>0.110 ± 0.003</td>
<td>0.181 ± 0.003</td>
<td>0.326 ± 0.002</td>
<td>0.839 ± 0.007</td>
</tr>
</tbody>
</table>

\(^1\) Cell suspension containing DMSO (1% v/v)
\(^2\) Cell suspension containing compound 1 in DMSO (1% v/v)

### Table 3. Incorporation of radio-labeled thymidine and uridine into MRSA cells.

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymidine(^1)</td>
<td>Control(^2)</td>
<td>64810 ± 2057(^4)</td>
<td>76560 ± 2751</td>
<td>87026 ± 4730</td>
</tr>
<tr>
<td></td>
<td>Compound 1 (12.5 µg/ml)(^3)</td>
<td>2428 ± 932</td>
<td>1615 ± 235</td>
<td>1456 ± 166</td>
</tr>
<tr>
<td>Uridine(^1)</td>
<td>Control</td>
<td>4005 ± 291</td>
<td>4226 ± 108</td>
<td>5100 ± 351</td>
</tr>
<tr>
<td></td>
<td>Compound 1 (12.5 µg/ml)(^4)</td>
<td>478 ± 67</td>
<td>455 ± 72</td>
<td>613 ± 43</td>
</tr>
</tbody>
</table>

\(^1\) Radio-labeled thymidine and uridine were added at a final radioactive concentration of 37 kBq/ml
\(^2\) Cell suspension containing thymidine or uridine plus DMSO (1% v/v)
\(^3\) Cell suspension containing thymidine or uridine plus compound 1 in DMSO (1% v/v)
\(^4\) Mean of 4 separate determinations ± S.D. (d.p.m.)
A new isoflavonoid, 3,9-dihydroxy-10-γ,γ-dimethylallyl-6a,11a-dehydroxypterocarpan, that satisfies this requirement showed the highest anti-MRSA activity. Eryvarin D (3-hydroxy-9-γ,γ-dimethylallyl-6a,11a-dehydroxypterocarpan) which possesses a methyl ether at the C-9 position, and demethylmedicapin (3,9-dihydroxypterocarpan) which lacks a γ,γ-dimethylallyl group demonstrated reduced antibacterial activity. Cristacarpin (3,6a-dihydroxy-9-methoxy-10-γ,γ-dimethylallylpterocarpan) and erysubin F (7,4-dihydroxy-8,3′-di(γ,γ-dimethylallyl)isoflavone) showed much lower activity.

Compound 1 inhibited the growth of thirteen MRSA strains at 12.5 µg/ml. This MIC value was slightly higher than those of previously reported anti-MRSA flavonoids (Inumano et al. 1994; Sato et al. 1995b; Tanaka et al. 2002). However, the compound possessed an intense bactericidal action against MRSA, and also inhibited growth of a human-isolated VRE strain at the same concentration (data not shown). These antibacterial qualities would be advantageous for treatment and prophylaxis of MRSA infections by reducing the risk of VRE infections. Despite the strong bactericidal action against MRSA cells, compound 1 never resulted in leakage of 260 nm-absorbing substances from MRSA cells. Measurement of absorbance in supernatant at this wave-length is frequently used to detect leakage of materials such as nucleotide and proteins from microbial cells (Amin et al. 1988). The compound does not therefore seem to exert its bactericidal action by altering the permeability of the cellular membrane or by damaging its structure and/or functions. Although Tsuchiya and Inumano (2000) have reported that sophoraflavanone G with anti-MRSA activity reduced membrane fluidity using model membranes, the biological significance of their results with regard to antibacterial activity remains unknown. We further investigated the effect of compound 1 on incorporation of thymidine, uridine, leucine and glucose into MRSA cells and found that the compound completely inhibits incorporation of thymidine, uridine and leucine. Glucose incorporation was also strongly suppressed, but some increase in glucose incorporation occurred during the experimental period. These results may indicate two possible modes of action of compound 1 against MRSA cells. One is that the protoplasmic membrane is the operative target and compound 1 exhibits antibacterial activity by interfering with incorporation of metabolites and nutrients into bacterial cells. Compound 1 showed a broad spectrum of antibacterial activity on Gram-positive bacteria including streptococci, Actinomycyes and Lactobacillus species, however, it failed to inhibit Gram-negative bacteria such as Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae even at 100 µg/ml (data not shown), suggesting that the outer membrane of Gram-negative bacteria interferes with the accessibility of the compound to the protoplasmic membrane. The second possibility is that compound 1 affects the nucleic acids in MRSA cells. In addition to complete inhibition of metabolite incorporation of bacterial cells, the observed intense bactericidal action of compound 1 bears some resemblance to that induced by 4-quinolone anti-microbial agents which inhibit DNA synthesis (Russell and Chopra, 1990). Further investigation regarding the mode of action of compound 1, as well as its toxicity, would be required before clinical application.

It is well known that nasal carriage of MRSA is an important risk factor for transmission of the bacterium in community hospitals (Mulligan et al. 1993). Mupirocin ointment applied to the nares can eliminate both nasal carriage and hand colonization in hospital personnel and colonized patients (Hill et al. 1988), however, resistance to mupirocin with long-term, intermittent usage has been documented (Smith and Kennedy, 1988; Cookson, 1990). The combination of

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### Table 4. Incorporation of radio-labeled glucose and leucine into MRSA cells.

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>1</th>
<th>5</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Glucose†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control‡</td>
<td>19695 ± 2472†</td>
<td>46062 ± 3325</td>
<td>54873 ± 7098</td>
<td>64207 ± 1579</td>
</tr>
<tr>
<td>Compound 1 (12.5 µg/ml)</td>
<td>1889 ± 1202</td>
<td>1231 ± 112</td>
<td>1652 ± 83</td>
<td>2451 ± 80</td>
</tr>
<tr>
<td>Leucine†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control‡</td>
<td>4107 ± 344</td>
<td>14374 ± 691</td>
<td>33852 ± 724</td>
<td>55400 ± 2984</td>
</tr>
<tr>
<td>Compound 1 (12.5 µg/ml)</td>
<td>1521 ± 309</td>
<td>1305 ± 56</td>
<td>1684 ± 181</td>
<td>1088 ± 34</td>
</tr>
</tbody>
</table>

† Radio-labeled glucose and leucine were added at a final radioactive concentration of 18.5 kBq/ml
‡ Cell suspension containing glucose or leucine plus DMSO (1% v/v)
phytochemicals with antibiotics would be useful for prophylaxis of bacterial infections with respect to reducing MIC values, which may result in enhancing antibacterial potency, lowering side effects and preventing development of resistant mutants (Liu et al. 2001). A study investigating the efficacy of combining the present compound with mupirocin is currently underway. The prominent antibacterial action of compound 1 would make the isoflavonoid useful in the treatment and prophylaxis of MRSA infections.

**References**


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