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## Antibacterial Compounds from *Glycyrrhiza uralensis*

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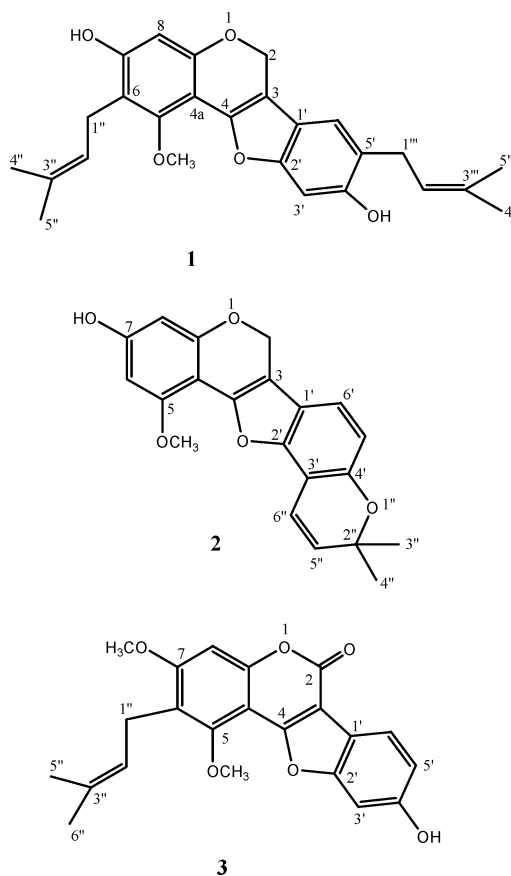
From the roots of *Glycyrrhiza uralensis*, two new pterocarpenes, glycyrrhizol A (**1**) and glycyrrhizol B (**2**), along with four known isoflavonoids, 5-*O*-methylglycyrol (**3**), isoglycyrol (**4**), 6,8-diisoprenyl-5,7,4'-trihydroxyisoflavone (**5**), and gancaonin G (**6**), were isolated using a bioassay-guided fractionation method. The structures of the new compounds (**1** and **2**) were elucidated by spectroscopic data interpretation. The known compounds (**3**–**6**) were identified by comparison of their spectroscopic data with reported values in the literature. Glycyrrhizol A (**1**) and 6,8-diisoprenyl-5,7,4'-trihydroxyisoflavone (**5**) exhibited potent antibacterial activity against *Streptococcus mutans* with minimum inhibitory concentrations of 1 and 2  $\mu\text{g/mL}$ , respectively, while glycyrrhizol B (**2**) and gancaonin G (**6**) showed more moderate activity.

The genus *Glycyrrhiza* consists of about 30 species,<sup>1</sup> of which some have been used by humans for over 4000 years.<sup>2</sup> Licorice is the name applied to the roots and stolons of *Glycyrrhiza* species (Fabaceae). *Glycyrrhiza uralensis* Fisch. ex DC. (Chinese name “Gancao”, or Chinese licorice) is one of the most frequently used traditional medicines in mainland China and in some other countries as well. Over the past decades, many research groups have investigated its chemical constituents<sup>3–8</sup> and biological activities.<sup>1,9–13</sup> Previous chemical studies have led to the identification of about 100 phenolic compounds, many of which are isoprenoid-substituted phenols.<sup>4,5</sup> Most of these isoprenoid-substituted flavonoids are isoflavans with oxygen substituted at the C-5 position. Some of these flavonoids have shown inhibitory activities against bacterial growth.<sup>10–12</sup>

*Streptococcus mutans* (*S. mutans*) is an oral pathogen responsible for the initiation and progression of dental caries.<sup>14</sup> There is an urgent need for new antimicrobial compounds that can inhibit *S. mutans* effectively.<sup>14,15</sup> In our previous studies, we have screened over 1000 Chinese medicinal herbs for inhibitory activities against *S. mutans* and other pathogens.<sup>16,17</sup> Recently, we have found that an ethanolic extract from the roots of *Glycyrrhiza uralensis* exhibited strong antibacterial activity against *S. mutans*. Following a bioassay-guided isolation procedure, two new pterocarpenes were identified, namely, glycyrrhizol A (**1**) and glycyrrhizol B (**2**), along with the previously known isoflavonoids 5-*O*-methylglycyrol (**3**),<sup>8</sup> isoglycyrol (**4**),<sup>6</sup> 6,8-diisoprenyl-5,7,4'-trihydroxyisoflavone (**5**),<sup>18</sup> and gancaonin G (**6**).<sup>8</sup> The antibacterial activity of compounds **1**–**6** was evaluated against *S. mutans* quantitatively.

Extraction of the air-dried ground roots of *G. uralensis* with 95% ethanol followed by solvent–solvent partitioning resulted in the localization of the active components in the  $\text{CHCl}_3$  fraction. Chromatography of this fraction on a Sephadex LH-20 column followed by column chromatography over silica gel and preparative TLC furnished six compounds (**1**–**6**). The structures of the known compounds (**3**–**6**) were determined by comparison of their spectroscopic data with reported values in the literature.<sup>6,8,18</sup>

Glycyrrhizol A (**1**) was obtained as a pale orange solid with a molecular formula of  $\text{C}_{26}\text{H}_{28}\text{O}_5$ , as determined from its HRMS, APCIMS, and  $^{13}\text{C}$  NMR data. The molecular formula indicated 13 degrees of unsaturation within the molecule. Moreover, two 3,3-dimethylallyl substituents were observed from four  $\text{sp}^2$ -hybridized

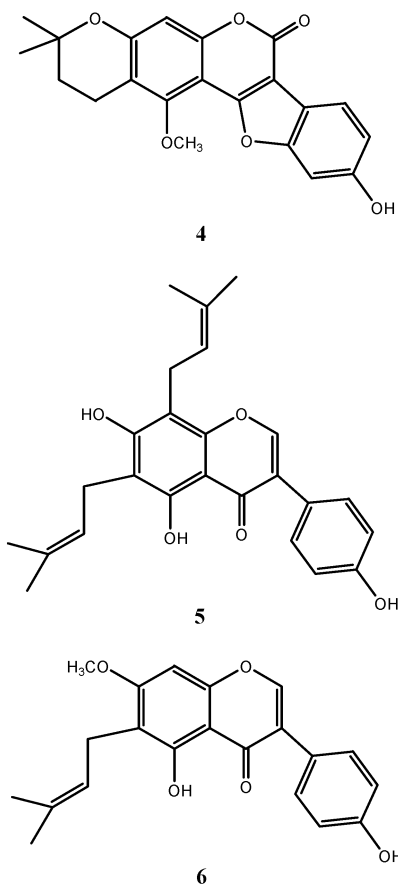


carbons, along with two methylene and four methyl signals in the NMR spectra ( $^1\text{H}$ ,  $^{13}\text{C}$  NMR, HMBC, and HMQC, see Experimental Section). The UV absorptions at 338 and 354 nm, an oxygenated methylene at  $\delta$  5.45 (2H, s, C-2) in the  $^1\text{H}$  NMR spectrum, and carbon signals at  $\delta$  65.0 (C-2), 106.7 (C-3), and 145.7 (C-4) were similar to those of 3-hydroxy-9-methoxy-10-(3,3-dimethylallyl)-pterocarpene.<sup>6,8,18</sup> These data, together with the HMBC correlations of H-2 with C-8a, C-3, C-4, and C-1', and correlations between H-2 and H-6' in a NOESY experiment unequivocally suggested a pterocarpene skeleton.<sup>3,19–21</sup> Furthermore, the presence of two hydroxyls, one methoxy, and two 3,3-dimethylallyl substituents was evident from the MS data and NMR spectra (see Experimental Section). Three aromatic proton singlets at  $\delta$  7.10, 7.08, and 6.31 appeared in the  $^1\text{H}$  NMR spectrum, together with the biogenetically

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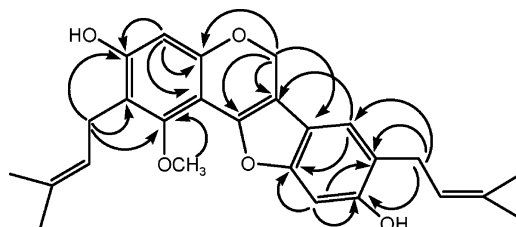
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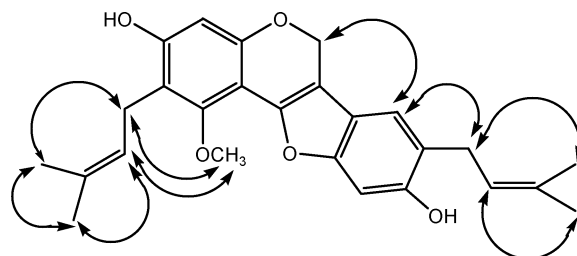


expected oxygenation at C-5, C-7, and C-4',<sup>1,7,19,21,22</sup> indicating that the 3,3-dimethylallyl groups could be placed at C-6, C-8, or C-5'. This was resolved from the HMBC NMR spectrum, which showed correlations of the H-1'' at  $\delta$  3.41 with C-5 ( $\delta$  153.0) and C-7 ( $\delta$  156.2); H-8 at  $\delta$  6.31 with C-4a ( $\delta$  104.6), C-6 ( $\delta$  114.3), C-7 ( $\delta$  156.2), and C-8a ( $\delta$  153.8); and H-2 at  $\delta$  5.45 with C-8a, allowing for the location of one 3,3-dimethylallyl group located at C-6. Additionally, the NOESY NMR correlations of the methoxy protons with H-1'' and H-2'' and the HMBC correlation of the OCH<sub>3</sub> signal with C-5 were used to assign the substitution of this methoxy group at C-5. Finally, HMBC correlations between the methylene protons of H-1''' with C-4' and C-6' and the NOESY correlations between H-2 and H-6' and between H-6' and H-1''' revealed the connectivity of the second 3,3-dimethylallyl group. Thus, on the basis of these spectroscopic data, the structure of glycyrrhizol A (1) was determined as 7,4'-dihydroxy-5-methoxy-6,5'-di(3,3-dimethylallyl)-pterocarpene.

The molecular formula of glycyrrhizol B (2) was determined to be C<sub>21</sub>H<sub>18</sub>O<sub>5</sub> by analysis of its HREIMS and <sup>13</sup>C NMR data. The <sup>1</sup>H NMR spectrum in deuteriochloroform showed three sets of protons: four aromatic doublets inclusive of two *ortho*- and two *meta*-coupled protons at  $\delta$  7.05, 6.74, 6.12, and 6.11; two *cis*-olefinic doublets at  $\delta$  6.89 and 5.73; and three aliphatic singlets, with one methylene at  $\delta$  5.44, one methoxy at  $\delta$  3.93, and two methyls at  $\delta$  1.47. These data, along with the UV absorption bands and on biogenetic grounds,<sup>7</sup> suggested a skeleton of 2 similar to that of 1. Furthermore, the NOESY correlation between H-2 and H-6', as well as HMBC cross-peaks between H-6'' at  $\delta$  6.89 and C-2' ( $\delta$  151.2), C-3' ( $\delta$  106.9), and C-4' ( $\delta$  149.9), and between H-5'', C-3', and C-2'' ( $\delta$  76.3) enabled the substitution pattern of the B ring and the 2,2-dimethylpyran ring to be determined. This was confirmed by HMBC correlations between H-6', C-2', and C-4' and between H-5', C-1', C-3', and C-4'. The substitution and assignments of the A ring of 2 resulted from the careful comparison of its NMR data with those in the literature,<sup>3,5,23–27</sup> combined with



**Figure 1.** Selected HMBC correlations observed for 1 (from H to C).



**Figure 2.** Key NOE correlations observed for 1.

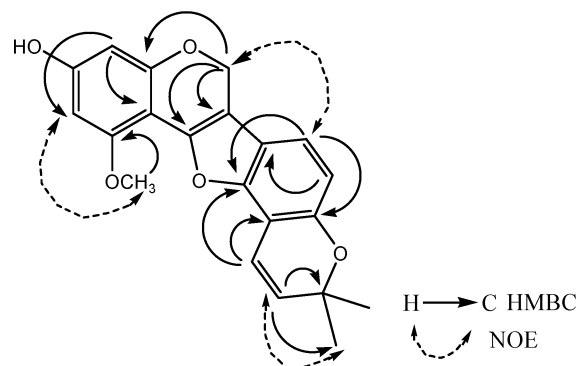
an analysis of the NOESY and HMBC spectra. A cross-peak shown in the NOESY spectrum between OCH<sub>3</sub> and H-6 and HMBC correlations observed between H-6 and C-7 and between H-8 and C-7, as well as the weak HMBC correlations of H-2 and C-8a and of H-8 and C-8a, suggested the substitution of the A ring, as shown. Accordingly, the structure of glycyrrhizol B (2) was assigned as 7-hydroxy-5-methoxy-2'',2''-dimethyl-2H-pyrano[3',4',5'',6'']-pterocarpene.

The antibacterial activity of all isolated compounds was tested against the oral Gram-positive bacterium *S. mutans*. The MIC (minimal inhibitory concentration) value determined for each compound was as follows: glycyrrhizol A (1), 1  $\mu$ g/mL; glycyrrhizol B (2), 32  $\mu$ g/mL; 5-*O*-methylglycyrol (3), 500  $\mu$ g/mL; isoglycyrol (4), 500  $\mu$ g/mL; 6,8-diisoprenyl-5,7,4'-trihydroxyisoflavone (5), 2  $\mu$ g/mL; and gancaonin G (6), 125  $\mu$ g/mL.

Licorice products are used worldwide as flavoring and sweetening agents in tobaccos, chewing gums, candies, beverages, and toothpaste. In some countries, *Glycyrrhiza* species including *Glycyrrhiza uralensis* are some of the oldest and most frequently employed traditional herbal medicines. *Glycyrrhiza* species produce a number of phytochemicals including essential oils, triterpenoids, alkaloids, phenols, polyamines, and polysaccharides, which may contribute to their many pharmaceutical effects such as anti-inflammatory, antiviral, antiulcer, and anticarcinogenic activities.<sup>2</sup> In addition to these effects, flavonoids isolated from licorice were recently reported to have antimicrobial activities against methicillin-sensitive *Staphylococcus aureus* (MSSA), *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.<sup>9</sup> In vitro inhibition of clarithromycin and amoxicillin-resistant *Helicobacter pylori* strains with licorice was also reported.<sup>28</sup> Moreover, several isoflavones (flavonoids, pterocarpanes, and pterocarpenes) from the plant were reported to have antibacterial activities against vancomycin-resistant enterococci (VRE) and methicillin-resistant *S. aureus* (MRSA).<sup>9,10,12</sup> The present study provides further evidence that some of the isoflavones in the roots of *G. uralensis* possess antibacterial activities.

## Experimental Section

**General Experimental Procedures.** NMR spectra were recorded in CDCl<sub>3</sub> on a Bruker Avance-500 NMR spectrometer, with chemical shifts given in  $\delta$  (ppm) with residual CDCl<sub>3</sub> as internal reference and coupling constants in Hz. ESIMS and APCIMS were performed on an IonSpec Ultima 7.0 T spectrometer. HREIMS were obtained on a VG Analytical AutoSpec spectrometer. Sephadex LH-20 (Sigma) was used for column chromatography. TLC was performed on silica gel 60 F<sub>254</sub> aluminum TLC sheets (E. Merck) and visualized under short (254 nm) and long (366 nm) wavelength UV light and by spraying



**Figure 3.** Selected HMBC and NOE correlations for **2**.

with 1% anisaldehyde in  $\text{H}_2\text{SO}_4$  followed by heating on a hot plate for 5 min. All solvents used were HPLC grade (Fisher).

**Plant Material.** Roots of *Glycyrrhiza uralensis* were collected in Jiangying, Jiansu Province, People's Republic of China, in June, 2003. The air-dried and powdered roots of *G. uralensis* was prepared by Jiangyin Herbal Extract Production Company and shipped to Los Angeles with appropriate permissions. Dr. Lili Ma, Professor of Zhejiang University of Traditional Chinese Medicine, authenticated the plant collection. A voucher specimen (GS-Jiangying-002) is deposited in the School of Dentistry and Dental Research Institute, University of California, Los Angeles.

**Extraction and Isolation.** The air-dried and powdered roots of *G. uralensis* (3.1 kg) were percolated with 95% EtOH to afford 152.5 g of brown extract. A portion of the extract (146.5 g) was suspended in water (400 mL) and further extracted with EtOAc ( $600 \times 6$  mL) to afford a brown semisolid (73.0 g). The crude EtOAc extract (72.0 g) was suspended in aqueous MeOH (80% MeOH, 400 mL) and extracted with *n*-hexane ( $500 \times 5$  mL). The antibacterial aqueous layer was diluted to 50% by adding water and further extracted with  $\text{CHCl}_3$  ( $600 \times 3$  mL). The dried residue (ca. 50 g) obtained from the  $\text{CHCl}_3$  extract was found to be the most active, and a portion of the extract (25 g) was fractionated over Sephadex LH-20 (60 g) eluting with *n*-hexane– $\text{CH}_2\text{Cl}_2$  (1:4, 800 mL), then  $\text{CHCl}_3$ –acetone (3:2, 1000 mL), and finally MeOH (1000 mL), to furnish three fractions,  $\text{A}_1$ – $\text{A}_3$ , of which fractions  $\text{A}_1$  (3.43 g) and  $\text{A}_2$  (6.75 g) were found to be active. Fractions  $\text{A}_1$  and  $\text{A}_2$  were then combined, and a 10 g aliquot was chromatographed on a silica gel column (200 g), eluting with petroleum ether–acetone (4:1), to give fractions  $\text{B}_1$ – $\text{B}_9$ . The most active fraction,  $\text{B}_3$ , was further purified by gel permeation over Sephadex LH-20 (100 g), eluting with MeOH, to afford compound **1** (10.0 mg) and a fraction  $\text{C}_1$ , which was further purified by repeated preparative TLC (RP-18,  $20 \times 20$  cm) eluted with 90% MeOH to yield compounds **2** (3.0 mg,  $R_f$  0.43), **3** (6.3 mg,  $R_f$  0.28), **4** (12.3 mg,  $R_f$  0.34), (**5**) (10.0 mg,  $R_f$  0.34), and **6** (3.1 mg,  $R_f$  0.34).

**Glycyrrhizol A (1):** pale orange solid; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 209 (4.73), 251 (4.31), 338 (4.50), 354 (4.41) nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.10 (1H, s, H-3'), 7.08 (1H, s, H-6'), 6.31 (1H, s, H-8), 5.48 (1H, s, OH), 5.45 (2H, s, H-2), 5.44 (1H, s, OH), 5.34 (1H, m, H-2''), 5.27 (1H, m, H-2''), 3.87 (3H, s,  $\text{OCH}_3$ ), 3.43 (2H, d,  $J$  = 4.0 Hz, H-1'''), 3.41 (2H, d,  $J$  = 4.3 Hz, H-1''), 1.84 (3H, s,  $\text{H}_3$ -4''), 1.80 (3H, s,  $\text{H}_3$ -4'''), 1.79 (3H, d,  $J$  = 1.0 Hz,  $\text{H}_3$ -5'''), 1.76 (3H, d,  $J$  = 1.0 Hz,  $\text{H}_3$ -5'');  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  156.2 (C, C-7), 155.1 (C, C-2'), 153.8 (C, C-8a), 153.0 (C, C-5), 152.2 (C, C-4'), 145.7 (C, C-4), 135.0 (C, C-3''), 134.8 (C, C-3''), 123.7 (C, C-5'), 122.1 (C, C-2''), 122.1 (C, C-2''), 118.8 (C, C-1'), 118.2 (C, C-6'), 114.3 (C, C-6), 106.7 (C, C-3), 104.6 (C, C-4a), 101.2 (C, C-8), 99.2 (C, C-3'), 65.0 (C, C-2), 62.4 ( $\text{OCH}_3$ ), 30.1 (C, C-1'''), 25.9 (C, C-5''), 25.8 (C, C-5''), 22.4 (C, C-1'), 17.9 (C, C-4''), 17.9 (C, C-4''); APCIMS (+)-ve mode  $m/z$  421  $[\text{M} + \text{H}]^+$ ; HREIMS  $m/z$  420.1921  $[\text{M}]^+$  (calcd for  $\text{C}_{26}\text{H}_{28}\text{O}_5$ , 420.1937).

**Glycyrrhizol B (2):** yellow amorphous powder; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 210 (4.75), 228 (4.71), 290 (4.48), 326 (4.37), 342 (4.34) nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.05 (1H, d,  $J$  = 8.5 Hz, H-6'), 6.89 (1H, d,  $J$  = 10.0 Hz, H-6''), 6.74 (1H, s, H-8), 6.74 (1H, d,  $J$  = 8.5 Hz, H-5'), 6.12 (1H, d,  $J$  = 2.0 Hz, H-8), 6.11 (1H, d,  $J$  = 2.0 Hz, H-6), 5.73 (1H, d,  $J$  = 10.0 Hz, H-5''), 5.44 (2H, s, H-2), 3.93 (3H, s,  $\text{OCH}_3$ ), 1.47 (3H, s,  $\text{H}_3$ -3''), 1.47 (3H, s,  $\text{H}_3$ -4'');  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  157.1 (C, C-7), 155.9 (C, C-5), 155.9 (C, C-8a), 151.2

(C, C-2'), 149.9 (C, C-4'), 146.6 (C, C-4), 130.7 (C, C-5''), 118.9 (C, C-1'), 117.1 (C, C-6'), 116.3 (C, C-6''), 112.7 (C, C-5'), 106.9 (C, C-3'), 105.9 (C, C-3), 100.4 (C, C-4a), 97.1 (C, C-6), 93.4 (C, C-8), 76.3 (C, C-2''), 65.1 (C, C-2), 56.2 ( $\text{OCH}_3$ ), 27.5 (C, C-3''), 27.5 (C, C-4''); APCIMS (+)-ve mode  $m/z$  351  $[\text{M} + \text{H}]^+$ ; HREIMS  $m/z$  350.1148  $[\text{M}]^+$  (calcd for  $\text{C}_{21}\text{H}_{18}\text{O}_5$ , 350.1154).

**Antibacterial Assay.** The in vitro antibacterial activity of compounds **1**–**6** was determined against the Gram-positive oral bacteria *Streptococcus mutans* according to the National Committee of Clinical Laboratory Standards (NCCLS) recommended minimum inhibitory concentration (MIC) protocol<sup>29</sup> with modifications.<sup>16,17</sup> Briefly, 2-fold dilution series were made from all tested antibacterial agents starting from 1000  $\mu\text{g/mL}$  in a 96-well plate (Microtest 96 microtiter plate). *S. mutans* strain ATCC 25175 was grown at 37 °C under anaerobic conditions (10%  $\text{H}_2$ , 10%  $\text{CO}_2$ , 80%  $\text{N}_2$ ) in brain heart infusion (BHI) broth media (Becton Dickinson, Sparks, MD). An aliquot of 50  $\mu\text{L}$  of bacterial suspension at a concentration of  $10^6$  colony-forming units/mL was added to 50  $\mu\text{L}$  of antibacterial dilution. Chlorhexidine (0.12%) was used as positive control and the untreated suspension as negative control. The MIC is defined as the lowest concentration of the test agent that visibly inhibited bacterial growth after incubation at 37 °C for 16–20 h.

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## References and Notes

- Nomura, T.; Fukai, T.; Akiyama, T. *Pure Appl. Chem.* **2002**, *74*, 1199–1206.
- Wang, Z. Y.; Nixon, D. W. *Nutr. Cancer* **2001**, *39*, 1–11.
- Shibano, M.; Henmi, A.; Matsumoto, Y.; Kusano, G.; Miyase, T.; Hatakeyama, Y. *Heterocycles* **1997**, *45*, 2053–2060.
- Hatano, T.; Takagi, M.; Ito, H.; Yoshida, T. *Phytochemistry* **1998**, *47*, 287–293.
- Hatano, T.; Aga, Y.; Shintani, Y.; Ito, H.; Okuda, T.; Yoshida, T. *Phytochemistry* **2000**, *55*, 959–963.
- Fukai, T.; Wang, Q. H.; Nomura, T. *Heterocycles* **1989**, *29*, 1369–1378.
- Fukai, T.; Nishizawa, J.; Yokoyama, M.; Nomura, T. *Heterocycles* **1993**, *36*, 2565–2576.
- Kiuchi, F.; Xing, C.; Tsuda, Y. *Heterocycles* **1990**, *31*, 629–636.
- Fukai, T.; Marumo, A.; Kaitou, K.; Kanda, T.; Terada, S.; Nomura, T. *Fitoterapia* **2002**, *73*, 536–539.
- Fukai, T.; Oku, Y.; Hano, Y.; Terada, S. *Planta Med.* **2004**, *70*, 685–687.
- Tsukiyama, R.; Katsura, H.; Tokuriki, N.; Kobayashi, M. *Antimicrob. Agents Chemother.* **2002**, *46*, 1226–1230.
- Hatano, T.; Shintani, Y.; Aga, Y.; Shiota, S.; Tsuchiya, T.; Yoshida, T. *Chem. Pharm. Bull.* **2000**, *48*, 1286–1292.
- Hsieh, T. C.; Wu, J. M. *Int. J. Oncol.* **2002**, *20*, 583–588.
- Johnson, N. W. In *Risk Markers for Oral Diseases*; Cambridge University Press: Cambridge, U.K., 1991.
- Satcher, S. G. *Oral Health in America: A Report of the Surgeon General Executive Summary*; U.S. Department of Health and Human Services: Washington, D.C., 2000 (<http://www.surgeongeneral.gov/library/oralhealth/>).
- Chen, L.; Ma, L.; Park, N. H.; Shi, W. *J. Clin. Microbiol.* **2001**, *39*, 3009–3012.
- Chen, L.; Cheng, X.; Shi, W.; Lu, Q.; Go, V. L.; Heber, D.; Ma, L. *J. Clin. Microbiol.* **2005**, *43*, 3574–3575.
- Sekine, T.; Inagaki, M.; Ikegami, F.; Fujii, Y.; Ruangrunsi, N. *Phytochemistry* **1999**, *52*, 87–94.
- Yenesew, A.; Derese, S.; Irungu, B.; Midiwo, J. O.; Waters, N. C.; Liyala, P.; Akala, H.; Heydenreich, M.; Peter, M. G. *Planta Med.* **2003**, *69*, 658–661.
- Yenesew, A.; Midiwo, J. O.; Guchu, S. M.; Heydenreich, M.; Peter, M. G. *Phytochemistry* **2002**, *59*, 337–341.
- Prasad, A. V. K.; Kapil, R. S.; Popli, S. P. *Indian J. Chem. (Sect. B)* **1985**, *24*, 236–239.
- Miyase, T.; Sano, M.; Nakai, H.; Muraoka, M.; Nakazawa, M.; Suzuki, M.; Yoshino, K.; Nishihara, Y.; Tanai, J. *Phytochemistry* **1999**, *52*, 303–310.
- Brinkmeier, E.; Geiger, H.; Zinsmeister, H. D. *Phytochemistry* **1999**, *52*, 297–302.
- Ingham, J. L.; Dewick, P. M. *Phytochemistry* **1979**, *18*, 1711–1714.
- Tanaka, H.; Oh-Uchi, T.; Etoh, H.; Sako, M.; Sato, M.; Fukai, T.; Tateishi, Y. *Phytochemistry* **2003**, *63*, 597–602.

- (26) Atindehou, K. K.; Queiroz, E. F.; Terreaux, C.; Traore, D.; Hostettmann, K. *Planta Med.* **2002**, 68, 181–182.
- (27) Preston, N. W. *Phytochemistry* **1977**, 16, 2044–2045.
- (28) Fukai, T.; Marumo, A.; Kaitou, K.; Kanda, T.; Terada, S.; Nomura, T. *Life Sci.* **2002**, 71, 1449–1463.
- (29) *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard*, 6th Ed.; NCCLS: Wayne, PA, 2003.

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