Antimalarial Principles from *Artemisia indica*

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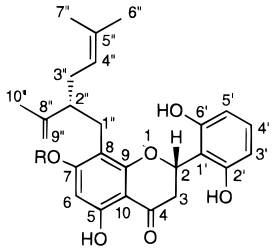
Activity-guided investigation of *Artemisia indica* Willd. has led to isolation of exiguaflavone A, exiguaflavone B, maackiain, and 2-(2,4-dihydroxyphenyl)-5,6-methylenedioxybenzofuran. Exiguaflavones A and B exhibit in vitro antimalarial activities of 4.60×10^{-6} and 7.05×10^{-6} g/mL, respectively, against *Plasmodium falciparum*.

The prevalence of malaria in many regions of the world, together with the lack of vaccine and the emergence of strains resistant to antimalarial drugs in use, makes it necessary to continue to search for new synthetic and naturally occurring antimalarials.[1] Artemisinin, a sesquiterpene lactone bearing an endoperoxide moiety isolated from *Artemisia annua* L., and its semisynthetic derivatives form a group of such new drugs currently under development and already in use in some countries.[2−5] As a part of a multidisciplinary research program on antimalarial natural products,[6,7] we came across the alleged antimalarial activity of *Artemisia indica* Willd. (Compositae) reported in the guidelines of Thai medicinal plants used in primary health care.[8] The crude MeOH extract of the stems of *A. indica* Willd.[9] shows reasonably high potency (EC_{50} 6.60×10^{-6} g/mL) in a biological assay against *Plasmodium falciparum*.[10,11] We therefore undertook a systematic study employing the same bioassay to guide the isolation and purification procedures.

Activity-guided isolation and purification of the crude MeOH extract of the stems of *A. indica* Willd. (EC_{50} 6.60×10^{-6} g/mL) by column chromatography (Si gel 60H, MeOH−CH_2Cl_2) followed by crystallization provided exiguaflavanone B (**1**), exiguaflavanone A (**2**), maackiain (**3**), and 2-(2,4-dihydroxyphenyl)-5,6-methylenedioxybenzofuran (**4**) in 0.0051%, 0.0084%, 0.002%, and 0.0005% yields, respectively, from the dried plant material.

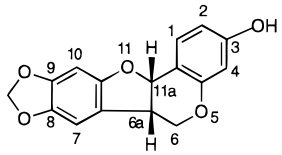
Although compounds **3** and **4** show moderate activities against *P. falciparum*, with EC_{50} values of 4.70×10^{-5} g/mL (1.65×10^{-4} M) and 2.70×10^{-5} g/mL (1.00×10^{-4} M), exiguaflavanones A and B exhibit stronger effects. Their respective activities against *P. falciparum* K1 strain in vitro are in the order of 4.6×10^{-6} g/mL (1.08×10^{-5} M) and 7.05×10^{-6} g/mL (1.60×10^{-5} M), respectively.

The physical properties and spectroscopic data of all four compounds, **1**−**4**, isolated from *A. indica* Willd. agreed well with those already reported in the literature; exiguaflavanones A and B from *S. exigua* Craib.,[12] maackiain from *M. amurensis* Rupr.,[13] and 2-(2,4-dihydroxyphenyl)-5,6-methylenedioxybenzofuran from *S. tomentosa* Linn.[14] The fact that hydroxyflavanones



1, R = CH_3

2, R = H



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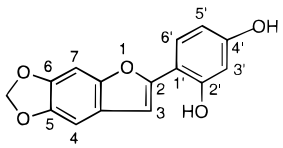

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1 and **2** exhibit in vitro antimalarial activity is very interesting because it has already been reported that certain flavanoids, such as artemitin and casticin, potentiated the in vitro activity of artemisinin against *P. falciparum*.[15] Also, the hydroxybenzyl-substituted dihydrochalcones, uvaretin and diuvaretin, isolated from *Uvaria* species displayed the in vitro EC_{50} values of 3.49 and 4.20×10^{-6} g/mL, respectively, against the same parasite.[16]

Experimental Section

General Experimental Procedure.[17] ^1H, ^{13}C, multiplicity determinations (DEPTs), NOEDF, and 2D NMR (H−H COSY, HMQC, and C−H COSY) experiments were performed on a JNM-A500 NMR spectrometer. Chemical shifts are given in δ (ppm) from TMS as internal standard. MS were collected on a Finnigan MAT INCOS 50 mass spectrometer. IR spectra were measured on a Perkin−Elmer 2000 NIR FT infrared spectrophotometer. UV spectra were recorded on a JASCO Uvidex-650 double beam spectrophotometer. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. Melting points were taken on an electrothermal melting point apparatus and are uncorrected. Si gel 60H, 5−40 μm (E. Merck, cat. no. 107736) was used for column chromatography.

Plant Material. The air-dried stems of *A. indica* Willd. were purchased from Thai traditional dispensary,

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Bangkok, Thailand. Authentication was done by comparison with a herbarium specimen (specimen BKF NO 82247) at Royal Forest Department Herbarium, Bangkok, Thailand.

Extraction and Isolation. The air-dried stems of *A. indica* Willd. (2 kg) were ground and extracted by soaking in *n*-hexane (4 L) at room temperature for 3 days, then filtered. The process was repeated twice, and the filtrates were then combined and evaporated under vacuum to dryness to give a brown residue. The plant material was then further extracted with MeOH (2 × 4 L). Filtration and evaporation of the solvent provided a dark brown viscous liquid (106 g, 5.30%, EC_{50} 6.60 × 10^{-6} g/mL).

The MeOH crude extract was subjected to Si gel column chromatography (4 cm × 60 cm) using a mixture of CH_2Cl_2 and MeOH as the mobile phase with gradient elution and was separated into 11 fractions (AR1−AR11). The antimalarial activities of these fractions were determined by their EC_{50} values against *P. falciparum*, K1 strain, from which fractions AR-3 (18.9845 g), AR-4 (3.4715 g), and AR-8 (17.0979 g) were selected for further study. AR-3 (EC_{50} 1.20 × 10^{-5} g/mL) was subjected to further column chromatography using a mixture of *n*-hexane and EtOAc as eluent with gradient elution to afford exiguaflavanone B (**1**) 0.1015 g, as a colorless viscous oil.[12]

Upon standing for several days, the fraction AR-4 (EC_{50} 1.15 × 10^{-5} g/mL) gave white crystals, which, after recrystallization from MeOH−CH_2Cl_2, provided white needles of maackiain (**3**) 0.0396 g, mp 181−182 °C [lit. mp 178.5−179 °C (aqueous MeOH), mp 179−181 °C (MeOH−H_2O)].[13]

The last fraction, AR-8 (EC_{50} 3 × 10^{-6} g/mL), was further separated by column chromatography using a mixture of CH_2Cl_2 and EtOAc as eluent with gradient elution to yield, after crystallization from MeOH−CH_2Cl_2, 2-(2,4-dihydroxyphenyl)-5,6-methylenedioxy-benzofuran (**4**) 0.0102 g, as an off-white solid (MeOH−CH_2Cl_2); mp 226.5−227 °C [lit. mp 235−237 °C (MeOH−H_2O)][14] and exiguaflavanone A (**2**) 0.1684 g, as white crystals; mp 177.5−178 °C (MeOH−CH_2Cl_2) [lit. mp 178−179 °C (C_6H_6)].[12]

Antimalarial Assay. Continuous in vitro cultures of asexual erythrocytic stages of *P. falciparum* (K1, multidrug-resistant strain) were maintained following the method of Trager and Jensen.[10] Quantitative assessment of antimalarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins.[11] Effective concentration (EC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. An EC_{50} value of 1.60 × 10^{-7} g/mL (3.10 × 10^{-7} M) was observed for the standard sample, chloroquine diphosphate, in the same test system.

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