Supporting Information

Bioactive diterpenes from the aerial parts of *Anisochilus harmandii*

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Extraction and isolation

Air-dried aerial parts of *A. hamandii* (0.8 kg) were ground into powder and then extracted successively with hexane (3 x 3 L), EtOAc (3 x 3 L), and MeOH (3 x 3 L). Removal of solvents from each extract under reduced pressure gave hexane (21.8 g, 2.75 %), EtOAc (11.8 g, 1.47 %), and MeOH (12.2 g, 1.52 %) extracts, respectively. The hexane extract (20.0 g) was separated on silica gel (230-400 mesh, 180 g) flash column chromatography (FCC, 8 x 30 cm), eluted with a gradient system of hexane-EtOAc and EtOAc-MeOH to give 6 fractions, HF1- HF6 [HF1 (200 mL), HF2 (400 mL), HF3 (400 mL), HF4 (600 mL), HF5 (600 mL), HF6 (800 mL)]. Fraction HF2 (hexane-EtOAc, 60:40, 400 mL, 3.5 g,) was purified by silica gel (230-400 mesh, 60 g) FCC (4 x 50 cm) and gradually eluted with hexane-EtOAc and EtOAc-MeOH to give 4 subfractions, HF2.1-HF2.4. Subfraction HF2.2 (hexane-EtOAc, 50:50, 100 mL, 2.4 g) was purified by preparative TLC using hexane-EtOAc (80:20) as eluent to yield colorless crystals of 3 (Rf = 0.44, 30.2 mg). Subfraction HF2.4 (hexane-EtOAc, 10:90, 100 mL, 163 mg) was further purified by preparative TLC using CH2Cl2-MeOH (95:5) as eluent to give a white solid of 4 (Rf = 0.44, 33.9 mg). Fraction HF3 (hexane-EtOAc, 50:50, 400 mL, 2.4 g) was purified on silica gel (70-230 mesh, 40 g) column chromatography (CC, 2.5 x 60 cm), gradually eluted with hexane-EtOAc and EtOAc-MeOH to give 5 subfractions, HF3.1-HF3.5. Subfraction HF3.4 (hexane-EtOAc, 70:30, 100 mL, 156 mg) was purified by preparative TLC, using CH2Cl2-EtOAc (80:20) as eluent to yield a white amorphous solid of 5 (Rf = 0.35, 26.7 mg). Subfraction HF3.5, (hexane-EtOAc, 40:60, 100 mL, 83 mg) was purified by preparative TLC, using hexane-CH2Cl2-MeOH (28:70:3) as eluent (developed x 3) to yield a white amorphous solid of 6 (Rf = 0.33, 14.0 mg) and 1 (Rf = 0.22, 9.2 mg). Fraction HF4 (hexane-EtOAc, 30:70, 600 mL, 1.0 g) was purified on silica gel (70-230 mesh, 30 g) CC (2.5 x 60 cm), gradually eluted with hexane-EtOAc and EtOAc-MeOH to give 6 subfractions, HF4.1-HF4.6. Subfraction HF4.2 (hexane-EtOAc, 70:30, 200 mL, 298 mg) was
separated on silica gel (70-230 mesh, 15 g) CC (1.5 x 60 cm), gradually eluted with CH$_2$Cl$_2$-MeOH and MeOH to give 4 subfractions, HF$_{4.2.1}$-HF$_{4.2.4}$. Subfraction HF$_{4.2.2}$ (CH$_2$Cl$_2$-MeOH, 95:5, 300 mL, 113 mg) afforded a white amorphous solid of 7 [$R_f = 0.31$ (CH$_2$Cl$_2$-MeOH, 94:6), 6.7 mg]. Subfraction HF$_{4.2.4}$ (CH$_2$Cl$_2$-MeOH, 93:7, 100 mL, 41.1 mg) afforded an additional amount of compound 1 (4.3 mg). Subfraction HF$_{4.4}$ (hexane-EtOAc, 60:40, 200 mL, 213 mg) was purified on silica gel 15g CC (1.5 x 60), gradually eluted with CH$_2$Cl$_2$-MeOH to give a white amorphous solid of 8 [$R_f = 0.24$ (CH$_2$Cl$_2$-MeOH, 94:6), 5.0 mg]. Subfraction HF$_{4.5}$ (CH$_2$Cl$_2$-MeOH, 94:6, 100 mL) was further purified by preparative TLC using EtOAc-MeOH (98:2) as eluent to give an additional amount of 1 ($R_f = 0.33$, 43.9 mg).

Fraction HF$_5$ (hexane-EtOAc, 70:30, 400 mL, 0.6 g) was purified by preparative TLC, using hexane-EtOAc-MeOH (50:49:1) as eluent to yield an amorphous solid of 3 ($R_f = 0.48$, 9.7 mg). The EtOAc extract (11.0 g) was separated on silica gel (230-400 mesh, 180 g) FCC (8 x 30 cm), eluted with a gradient system of hexane-EtOAc and EtOAc-MeOH to give 6 fractions, EF$_1$-EF$_6$. Fraction EF$_4$ (hexane-EtOAc, 40:60, 100 mL, 172 mg) was subjected to silica gel CC, eluted with gradient systems of hexane-EtOAc and EtOAc-MeOH to yield 6 subfractions designated as EF$_{4.1}$-EF$_{4.6}$. Subfraction EF$_{4.4}$ was purified by preparative TLC using hexane-EtOAc (30:70) as eluent to yield a white amorphous solid of 9 ($R_f = 0.55$, 4.5 mg). Fraction EF$_{4.5}$ (hexane-EtOAc, 30:70, 100 mL, 124 mg) was further purified by preparative TLC using hexane-EtOAc (30:70) as eluent to give a white amorphous solid of 2 ($R_f = 0.46$, 69.1 mg). Fraction EF$_6$ (hexane-EtOAc, 20:80, 400 mL, 2.4 g) was chromatographed on silica gel (230-400 mesh, 30 g) FCC (2.5 x 60 cm), eluted with gradient systems of hexane-EtOAc and EtOAc-MeOH to give 4 subfractions, EF$_{6.1}$-EF$_{6.4}$. Subfraction EF$_{6.3}$ (hexane-EtOAc, 30:70, 100 mL, 126.5 mg) was purified by preparative TLC, using hexane-EtOAc (30:70) as eluent to yield a white amorphous solid of 10 ($R_f = 0.37$, 36.1 mg).
Bioassays:

Antiplasmodial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain), using the method of Trager and Jensen [12]. Quantitative assessment of malarial activity *in vitro* was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al. [13]. The inhibitory concentration (IC₅₀) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* incorporation of [³H]-hypoxanthine by *P. falciparum*. The standard compound dihydroartemisinin (99%) was in-house supplied by Dr. Bongkoch Tarnchompoo, BIOTEC (Table 2).

The antimycobacterial activity was assessed against *M. tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA) [14]. Standard drugs, isoniazid (> 99%) and kanamycin sulfate (> 95%), were obtained from Sigma (Table 2).

The cytotoxic assays against human epidermoid carcinoma (KB), human small cell lung cancer (NCI-H187), and human breast cancer (MCF-7) cell lines were performed employing the colorimetric method as described by Skehan et al. [15]. The reference substances were ellipticine (> 95% ) and doxorubicin (> 95%) from Sigma and Ebewe, respectively (Table 2).