Prenylated Dihydrochalcones from *Boronia bipinnata* that Inhibit the Malarial Parasite Enzyme Target Hemoglobinase II

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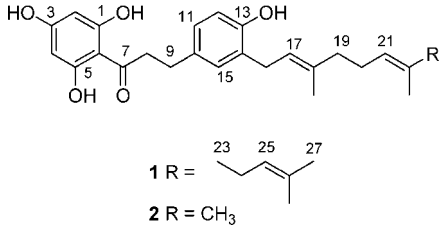
High-throughput screening of a plant and marine invertebrate extract library to find natural products that inhibit the malarial parasite enzyme target hemoglobinase II led to the isolation of two new active prenylated chalcones, bipinnatones A (**1**) and B (**2**), from aerial parts of the Queensland shrub *Boronia bipinnata*. Their structures were assigned from interpretation of 2D NMR and high-resolution ESIMS data. Compounds **1** and **2** inhibited hemoglobinase II with IC_{50} values of 64 and 52 μM, respectively.

Malaria is a serious disease in tropical regions of the world. An estimated two billion people live in areas at risk from the disease, and annually 1.7−2.5 million people die from malaria infections.[1] The disease is caused by the protozoan parasite *Plasmodium* spp. A combination of parasite drug resistance and vector resistance to insecticides has led to increased difficulty in combating this disease. Approaches that target inhibition of essential metabolic pathways within the *Plasmodium* parasite have been touted as vital new methods to treat this disease.[2] Plasmodium hemoglobinase II, also known as plasmepsin II, is an aspartic protease that is found in the human parasite *Plasmodium falciparum*.[3] The enzyme is involved in the digestion of hemoglobin from host cells within the acidic food vacuole of the parasite.[3] The enzyme has also been proposed to be involved in the cleavage of the cytoskeleton of infected erythrocytes.[4] As such, it is an essential enzyme for the survival of the parasite and an attractive target for drug discovery.

A high-throughput screen employing an enzyme assay in which the substrate AcKPIVFF(NO_2)RL was cleaved by recombinant pro-hemoglobinase to yield a colored product was used to find natural product extracts with potential antimalarial properties. Extracts from approximately 25 000 plants and marine invertebrates collected in Queensland were screened, and the CH_2Cl_2 extract of the aerial parts of *Boronia bipinnata* (Rutaceae) showed bioactivity in the assay. Bioassay-guided isolation of this extract led to the isolation of two new isoprenylated chalcones, bipinnatones A (**1**) and B (**2**). This paper reports the isolation, structure determination, and biological activity of **1** and **2**.



The aerial parts of *B. bipinnata* were exhaustively extracted with CH_2Cl_2, and the extract was chromatographed on Sephadex LH-20 eluting with MeOH. Fractions were pooled according to the ions observed in the (−) ESIMS for each fraction. Pooled fractions were tested for hemoglobinase activity, and two fractions with ions at *m/z* 477 and 409, respectively, were active. These fractions were the pure compounds **1** and **2**.

The (−) HRESIFTMS of **1** displayed a pseudomolecular ion peak at *m/z* 477.2653 $[M - H^+]^-$, allowing the molecular formula $C_{30}H_{38}O_5$ to be assigned. The ^{1}H NMR spectrum of **1** (see Experimental Section) contained signals for a 1,2,4-trisubstituted phenyl group [δ_H 6.65 (d, J = 8.0 Hz); 6.82 (dd, J = 2.4, 8.0 Hz); 6.85 (d, J = 2.4 Hz)], six deshielded methylene signals at δ_H 1.89, 1.97 (4H), 2.05, 2.73, 3.17, and 3.19, three double-bond proton triplets at δ_H 5.02, 5.08, and 5.25, four allylic methyl singlets at δ_H 1.52, 1,53, 1.59, and 1.64, three phenolic singlets at δ_H 8.99, 10.29, and 12.20 (2H), and a two-proton aromatic singlet at δ_H 5.80. Three head-to-tail isoprene linkages (a farnesyl group) were easily discernible from the COSY spectrum, as was a $-CH_2CH_2-$ moiety. HMQC and HMBC experiments provided enough correlations to determine the structure. A dihydrochalcone structure was indicated by correlations from the methylene protons at δ_H 2.73 (9-CH_2) to the aromatic carbons at δ_C 131.6 (C-10), 126.2 (C-11), and 129.1 (C-15) and to the ketone carbon at δ_C 204.2 (C-7). The ketone was attached to a symmetrical tetrasubstituted phloroglucinol since correlations were observed between the aromatic protons at δ_H 5.80 (H-2/H-4) and oxygenated aromatic carbons at δ_H 164.2 (C-1/C-5) and 164.6 (C-3), an upfield aromatic quaternary carbon at δ_C 103.8 (C-6), and a weak $^4J_{CH}$ correlation to the ketone carbon C-7. A HMBC correlation was also observed between 8-CH_2 and the aromatic carbon C-6. A strongly hydrogen bonded two-proton phenolic singlet at δ 12.20 supported the position of two phenols *ortho* to the ketone. The farnesyl group was placed at C-14 since correlations were observed between 16-CH_2 (δ_H 3.17) and carbon signals at δ_C 152.9 (C-13), 127.1 (C-14), and 129.1 (C-15). Two asymmetric double bonds in the farnesyl group each possessed *E* stereochemistry since the carbons of the methyl groups attached to these double bonds were observed upfield of 20 ppm. The structure **1** was assigned, and it was named bipinnatone A.

Accurate mass measurement of the pseudomolecular ion in the (−) HRESIMS at *m/z* 409.2031 allowed a molecular formula of $C_{25}H_{30}O_5$ to be assigned to **2**. The ^{1}H NMR spectrum of **2** was very similar to that of **1**. In fact, the only differences were the lack of signals for an allylic methyl, two allylic methylenes, and a double-bond proton when compared with the spectrum of **1**. COSY, HMQC, and HMBC experiments allowed all of the signals to be assigned and confirmed the structure as a geranyldihydrochalcone (**2**), which was named bipinnatone B.

A number of isoprenylated chalcones have been reported in the literature,[5] and four compounds, **3**−**6**, isolated from other Rutaceae plants *B. inconspicua*[6] and *Metrodorea nigra*[7] are the most similar in structure to **1** and **2**; however no biological activity has been reported for these compounds.

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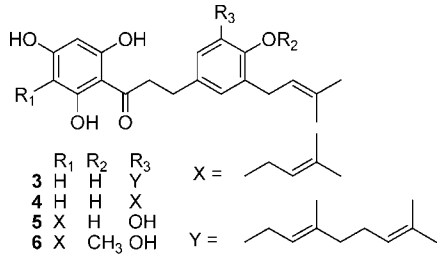

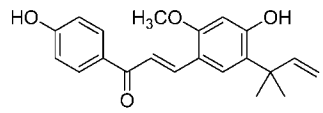

7

Bipinnatones A (**1**) and B (**2**) inhibited the enzyme hemoglobinase II with IC_{50} values of 64 and 51 μM, respectively. The IC_{50} value for the positive control pepstatin was 3 nM. Chalcones have been reported to possess a wide range of pharmacological activities including antileishmanial, anti-inflammatory, antimalarial, antibiotic, antimitotic, and modulation of P-glycoprotein-mediated multidrug resistance.[8–10] Licochalcone A (**7**), a 3′-prenylated chalcone isolated from the roots Chinese licorice, shows potent in vivo and in vitro activity against *P. falciparum* and *P. yoelii*.[11] It has been suggested that it and other chalcones act through inhibition of malarial cysteine protease.[12] The current study however suggests that prenylated chalcones probably inhibit hemoglobinase II since licochalcone (**7**) is similar in structure to bipinnatones A (**1**) and B (**2**). From previous structure−activity studies of prenylated chalcones it is anticipated that conversion of the dihydrochalcone in the bipinnatones to the corresponding chalcones would improve their potency.[13,14]

Experimental Section

General Experimental Procedures. All solvents used were Omnisolv HPLC grade. UV spectra were recorded on a CAMSPEC M501 UV/vis spectrophotometer, and IR spectra were recorded on a Bruker Tensor 27 spectrometer. NMR spectra were recorded on Varian Inova 600 and 500 MHz NMR spectrometers. Samples were dissolved in d_6-DMSO, and chemical shifts were calculated relative to the d_6-DMSO solvent peak (δ_H 2.49 and δ_C 39.5). 2D NMR spectra were recorded at 30 °C using standard Varian pulse sequences gCOSY, gHMQC, and gHMBC. ESIMS were measured on a Mariner Biospectrometry TOF workstation using negative electrospray ionization, mobile phase 1:1 MeOH−H_2O containing 0.1% formic acid, and HRESIMS were recorded on a Bruker Daltonics Apex III 4.7e Fourier-transform mass spectrometer. Sephadex LH-20 was used during purification.

Plant Material. Aerial parts of *B. bipinnata* were collected by D. A. Halford on October 7, 1993, from near Inglewood in South Eastern Queensland. A voucher specimen, AQ600060, is deposited at the Queensland Herbarium.

Extraction and Isolation. The ground aerial parts of *B. bipinnata* (10 g) were extracted with CH_2Cl_2 (800 mL), yielding a green gum (1.05 g), which was chromatographed on Sephadex LH-20 eluting with MeOH. Sixty fractions were collected, and these were pooled according to the ions observed in the (−) ESIMS for each fraction. Pooled fractions were tested for hemoglobinase activity, and two bioactive fractions were obtained. Pooled fractions 36−40 *m/z* 477 was pure bipinnatone A (**1**) (134.7 mg, 0.67%), and pooled fractions 44−54 *m/z* 409 was pure bipinnatone B (**2**) (84.1 mg, 0.42%).

Bipinnatone A (1): yellow gum; UV (MeOH) λ_{max} (log ε) 223 (4.31), 287 (4.25) nm; IR (KBr) ν_{max} 3178 br, 2972, 2929, 1626, 1603, 1509, 1497, 1451, 1301, 1257, 1169, 1046, 1024, 1001, 826 cm^{-1}; ^1H (600 MHz, d_6-DMSO) δ 1.52 (3H, br s, H-30), 1.53 (3H, br s, H-29), 1.59 (3H, br s, H-27), 1.64 (3H, br s, H-28), 1.89 (2H, d, J = 6.6 Hz, H-23), 1.97 (2H, t, J = 6.6 Hz, H-19), 1.97 (2H, q, J = 6.6 Hz, H-24), 2.05 (2H, q, J = 6.6 Hz, H-20), 2.73 (2H, t, J = 7.2 Hz, H-9), 3.17 (2H, d, J = 6.6 Hz, H-16), 3.19 (2H, t, J = 7.2 Hz, H-8), 5.02 (1H, t, J = 6.6 Hz, H-25), 5.08 (1H, t, J = 6.6 Hz, H-21), 5.25 (1H, t, J = 6.6 Hz, H-17), 5.80 (2H, s, H-2, H-4), 6.65 (1H, d, J = 8.0 Hz, H-12), 6.82 (1H, dd, J = 2.4, 8.0 Hz, H-11), 6.85 (1H, d, J = 2.4 Hz, H-15), 8.99 (1H, s, 13-OH), 10.29 (1H, s, 3-OH), 12.20 (2H, s, 1-OH, 5-OH); ^{13}C NMR (125 MHz, d_6-DMSO) δ 15.9 (q, C-29), 16.0 (q, C-28), 17.5 (q, C-30), 25.5 (q, C-27), 26.0 (q, C-24), 26.2 (q, C-20), 27.8 (t, C-16), 29.6 (t, C-9), 39.2 (t, C-19), 39.3 (t, C-23), 45.4 (t, C-8), 94.7 (d, C-2, C-4), 103.8 (s, C-6), 114.7 (d, C-12), 122.8 (d, C-17), 123.9 (d, C-21), 124.1 (d, C-25), 126.2 (d, C-11), 127.1 (s, C-14), 129.1 (d, C-15), 130.6 (s, C-26), 131.6 (s, C-10), 134.4 (s, C-18, C-22), 152.9 (s, C-13), 164.2 (s, C-1, C-5), 164.6 (s, C-3), 204.2 (s, C-7); (−)-HRESIMS *m/z* 477.2653 [M − H^+]$^-$ (calcd for $C_{30}H_{38}O_5$, 477.2647).

Bipinnatone B (2): yellow gum; UV (MeOH) λ_{max} (log ε) 227 (4.36), 288 (4.38) nm; IR (KBr) ν_{max} 3233 br, 2974, 2929, 1625, 1602, 1452, 1369, 1301, 1255, 1204, 1169, 1079, 1045, 1023, 998, 826 cm^{-1};. ^1H (600 MHz, d_6-DMSO) δ 1.53 (3H, br s, H-25), 1.60 (3H, br s, H-23), 1.64 (3H, br s, H-24), 1.97 (2H, t, J = 6.6 Hz, H-19), 2.04 (2H, q, J = 6.6 Hz, H-20), 2.73 (2H, t, J = 7.2 Hz, H-9), 3.16 (2H, d, J = 6.6 Hz, H-16), 3.19 (2H, t, J = 7.2 Hz, H-8), 5.08 (1H, t, J = 6.6 Hz, H-21), 5.25 (1H, t, J = 6.6 Hz, H-17), 5.79 (2-H, s, H-2, H-4), 6.65 (1H, d, J = 8.0 Hz, H-12), 6.82 (1H, dd, J = 2.4, 8.0 Hz, H-11), 6.85 (1H, d, J = 2.4 Hz, H-15), 9.00 (1H, s, 13-OH), 10.30 (1H, s, 3-OH), 12.19 (2H, s, 1-OH, 5-OH); ^{13}C NMR (125 MHz, d_6-DMSO) δ 15.9 (q, C-24), 17.7 (q, C-25), 25.7 (q, C-23), 26.3 (t, C-20), 28.0 (t, C-16), 29.7 (t, C-9), 39.3 (t, C-19), 45.5 (t, C-8), 94.8 (d, C-2, C-4), 103.7 (s, C-6), 114.7 (d, C-12), 122.6 (t, C-17), 124.1 (d, C-21), 126.2 (d, C-11), 127.2 (s, C-14), 129.2 (d, C-15), 130.7 (s, C-22), 131.6 (s, C-10), 134.6 (s, C-18), 152.8 (s, C-13), 164.2 (s, C-1, C-5), 164.6 (s, C-3), 204.1 (s, C-7); (−)-HRESIMS *m/z* 409.2031 [M − H^+]$^-$ (calcd for $C_{25}H_{30}O_5$, 409.2020).

Hemoglobinase II Assay. Recombinant prohemoglobinase II and the acetylated substrate (AcKPIVFF(NO_2)RL) were supplied by Astra Research Center, India. Prohemoglobinase was activated by diluting the proenzyme with 0.085 M citrate buffer, pH 4.7, and incubated for 1 h at rt. To each well containing either extract, fraction, pure compound, or pepstatin A were added 20 μL of enzyme and 40 μL of substrate (500 μM in citrate buffer). The plate was incubated for 60 min at rt. The reaction was stopped by addition of 50 μL of stop mix (0.54 M disodium phosphate, pH 9.0, 0.3 μM pepstatin A, and 0.028% 2,4,6-trinitrobenzene sulfonic acid) and the absorbance measured at 410 nm. After incubating for 120 min at rt, the final absorbance was read at 410 nm. IC_{50} values for the isolated compounds were obtained by testing three wells per concentration.

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