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Antiplasmodial β-triketones from the flowers of the Australian tree *Angophora woodsiana*

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ABSTRACT

Chemical investigations of the MeOH extract of air dried flowers of the Australian tree *Angophora woodsiana* (Myrtaceae) yielded two new β-triketones, woodsianones A and B (**1**, **2**) and nine known β-triketones (**3**–**11**). Woodsianone A is a β-triketone-sesquiterpene adduct and woodsianone B is a β-triketone epoxide derivative. The structures of the new and known compounds were elucidated from the analysis of 1D/2D NMR and MS data. The relative configurations of the compounds were determined from analysis of ^{1}H–^{1}H coupling constants and ROESY correlations. All compounds (**1**–**11**) had antiplasmodial activity against the chloroquine sensitive strain 3D7. The known compound rhodomyrtone (**5**) and new compound woodsianone B (**2**) showed moderate antiplasmodial activities against the 3D7 strain (1.84 μM and 3.00 μM, respectively) and chloroquine resistant strain Dd2 (4.00 μM and 2.53 μM, respectively).

Malaria is a vector borne disease caused by protozoan parasites of five species of the genus *Plasmodium*; *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and the simian *P. knowles*.[1] Malaria is a significant public health problem that mainly affects communities in the developing world including Africa, South-East Asia, Central and South America and the Eastern Mediterranean region. The emerging resistance to currently used drugs is now compounding the problem further. According to the World Health Organization (WHO) 438,000 deaths from malaria occurred in 2015, of which 90% occurred in Africa and 10% occurred in South-East Asia and the Eastern Mediterranean.[2]

There are a limited number of drugs available to treat malaria. The most widely used drugs include quinine and its derivatives, antifolate combination drugs and artemisinin and its derivatives.[3,4] Although effective drugs are available to treat malaria the development of resistance to anti-malarial drugs by the *Plasmodium* parasite is widespread.[5] Resistance to chloroquine, (discovered in 1930) had developed in Thailand by 1957 and later independently developed in South America. By 1980 chloroquine resistance was prevalent in Sub-Saharan Africa, leading to a sharp increase in the number of deaths in this region. In 1973 sulfadoxine-pyrimethamine (SP) was introduced as a first-line treatment but by 1980 the parasite had developed resistance in Thailand and subsequently resistance was discovered in Asia and Africa. Subsequently, within the span of only 7 years (1985–1992) resistance also developed against Mefloquine.[6] The discovery and development of artemisinin, a potently active sesquiterpene extracted from the Chinese plant *Artemisia annua*[7] led to artemisinin based therapies being the treatment of choice in regions where chloroquine resistance has emerged. Artemisinin and its derivatives including artesunate, artemether and dihydroartemisinin are highly effective and rapidly suppress the asexual stage of parasitemia ($t1/2 \sim 1$ h). However, when artemisinin derivatives and many other drugs are used as monotherapies, they are generally required to be taken repeatedly over seven days for complete elimination of parasite but for many patients compliance is problematic since many terminate treatment before seven days and this results in recrudescence and resistance of drugs to the parasite.[8,9] Initially, resistance to artemisinin was found in the Pailin province of Western Cambodia where artemisinins are predominantly used as oral monotherapies. In January 2014, WHO reported artemisinin resistance in Lao People's Republic, Myanmar, Thailand and Vietnam.[10,11] Today there is a concern that artemisinin resistance may spread beyond the Greater Mekong sub-region, similar to

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chloroquine and sulfadoxine-pyrimethamine.[11] Termination of artemisinin monotherapy was recommended by WHO in 2007 and re-confirmed in 2011 and 2013.[12] Today, artemisinin and derivatives are only recommended for use in combination with a partner drug (amodiaquin, mefloquine, lumefantrine, amodiaquine, sulfadoxine-pyrimethamine, piperaquine) with a different mechanisms and speed of action.[13] Artemisinin combination therapies (ACT) result in a rapid reduction in the parasite biomass following prolonged drug exposure with a partner drug with a longer half-life.[14] The emergence of artemisinin resistance in recent years is of grave concern for ACTs. Emergence of multi drug resistance (MDR) parasites would have a devastating effect on global malaria control and millions of deaths due to malaria would become unavoidable.[15] There is therefore an urgent need to discover new anti-malarial drugs with different mechanisms of action to those currently in use. We have previously shown that natural products containing β-triketone moieties show promising antiplasmodial activity and therefore new compounds from this structure class could provide further insights enabling development of these structures as antimalarial drug leads.

The Australian endemic genus *Angophora* from the angiosperm family Myrtaceae is represented by 16 species of "gum" or Eucalypt trees.[16] The genus has been shown through molecular and morphological evidence to be closely related to two other Eucalypt genera, *Eucalyptus* and *Corymbia*[17] and we have previously shown that the flowers of *Corymbia* species are a rich source of β-triketones some of which show moderately potent antiplasmodial activity.[18–20] Since *Angophora* species are very closely related to *Corymbia* species, we anticipated that their flowers may also contain β-triketones although no previous studies have investigated their chemistry. In this study the flowers of *Angophora woodsiana* were chemically explored. Locally known as the smudgy apple, *Angophora woodsiana* is a medium sized rough barked tree of about 20 m height that flowers profusely between December and January.[21]

Methanol extracts from the flowers were purified by reverse phase and normal phase HPLC to yield two new compounds, woodsianone A (**1**) and B (**2**) and the nine known compounds, rhodomyrtosone A (**3**), rhodomyrtosone D (**4**), rhodomyrtone (**5**), tomentodione A (**6**), tomentodione B (**7**) 4*S*-ficifolidione (**8**), kunzeanone A (**9**), watsonianone A (**10**) and watsonianone B (**11**). The known compounds were identified by ^{1}H and 2D NMR analysis and through comparison with literature data. All of the compounds contain β-triketone moieties corroborating our hypothesis that the genus should be an additional source of this structure class. Structures of the two new compounds were elucidated from analysis of 1D and 2D NMR data and antiplasmodial activities of all compounds were evaluated. This provided further evidence that β-triketones are new scaffolds for malaria drug discovery.

The MeOH extract (8.0 g) of *A. woodsiana* was partitioned between hexane and MeOH to yield a hexane soluble fraction (1.8 g). The remaining MeOH soluble fraction was further partitioned with dichloromethane (DCM) to yield a DCM soluble fraction (3.44 g). One gram of the hexane soluble fraction was further purified by diol bonded silica gel HPLC (21 mm × 150 mm) and eluted with a 60 min gradient from hexane to DCM at 9 mL/min and then further eluted for 10 min with DCM to yield 70 fractions. This was repeated three times and fractions were analyzed by ^{1}H NMR. Fractions containing compounds of interest, as judged by their ^{1}H NMR signals were combined yielding four fractions. Each of these fractions was further purified by preparative (21 mm × 250 mm) phenyl bonded silica gel HPLC with a 60 min gradient from 100% H_2O to 100% MeOH at 9 mL/min and further eluted for 10 min with MeOH, yielding woodsianone A (**1**), and nine known compounds rhodomyrtosone A (**3**) tomentodione A (**6**), tomentodione B (**7**) 4*S*-ficifolidione (**8**), kunzeanone A (**9**), watsonianone A (**10**) and watsonianone B (**11**) (Fig. 1). The DCM fraction was purified by diol bonded HPLC (21 mm × 150 mm) eluting with a 60 min gradient from hexane to DCM at 9 mL/min. Early eluting fractions contained the new compound woodsianone B (**2**). The crude MeOH extract (23 g) was purified using medium pressure liquid chromatography (MPLC) with a gradient from 100% H_2O to 100% MeOH. Further purification of MPLC fractions 11 and 12 was achieved by diol bonded HPLC (21 mm × 150 mm) eluting with a 60 min gradient from hexane to DCM at 9 mL/min yielding rhodomyrtosone D (**4**) and rhodomyrtone (**5**).[22]

Woodsianone A (**1**), was isolated as a colorless amorphous solid and its molecular formula was determined to be $C_{30}H_{46}O_3$ from analysis of (+) HRESIMS data (*m*/*z* MH^+ 455.3530 calculated 455.3520).[23] The IR spectrum for **1** showed absorption bands at 1610 cm^{-1} and 1380 cm^{-1} and indicating carbonyls were present in the molecule. The UV spectrum displayed UV absorption maxima at 267 nm and 204 nm. The ^{1}H NMR spectrum (800 MHz, $CDCl_3$) of **1** (Table 1) had resonances assigned to five secondary (δ_H 0.78, 0.91, 0.94, 0.94, 1.00) and five quaternary aliphatic methyl groups (δ_H 1.26, 1.27, 1.30, 1.31, 1.26) four aliphatic methylene groups (δ_H 1.85/1.29, 1.43/1.37, 1.64/1.68, 1.05/1.94) and eight methine groups (δ_H 1.69, 1.71, 1.86, 1.91, 1.97, 2.40, 2.95, 5.54). The ^{13}C NMR spectrum contained 30 carbon resonances, eight of which were quaternary; two ketone carbonyls (δ_C 213.7, 198.7); one conjugated oxygenated enol carbon (δ_C 167.2), three olefinic carbons (δ_C 109.6, 126.9, 145.8), two aliphatic quaternary carbons (δ_C 55.9, 48.1) and one oxygenated quaternary carbon (δ_C 77.4).

An edited HSQC spectrum revealed that **1** had ten up-field methyl signals, signals for four methylene groups and eight methine groups. HMBC correlations from four of the quaternary methyl resonances (δ_H 1.26, 1.27, 1.30, 1.31) to downfield quaternary carbons at δ_C 167.2, 48.1, 55.9, 213.7, 198.7, 109.6 (C-1a, C-2, C-3, C-4, C-5, C-5a respectively) established the β-diketone moiety. The COSY correlations between δ_H 2.95 (H-6), δ_H 1.91 (H-7) and δ_H 1.05 (H-1α'); δ_H 1.91 (H-7) and δ_H 1.43 (H-16); δ_H 1.43 (H-16) and δ_H 2.40 (H-15); δ_H 2.40 (H-15) and δ_H 1.97 (H-14), δ_H 1.97 (H-14) and δ_H 0.91 (H_3-21) established the H-1′/H-6/H-7/H-16/H-15/H-14/H-21 partial structure within the molecule. Similarly, COSY correlations from δ_H 1.86 (H-18) to δ_H 0.78 (H_3-19) and δ_H 0.94

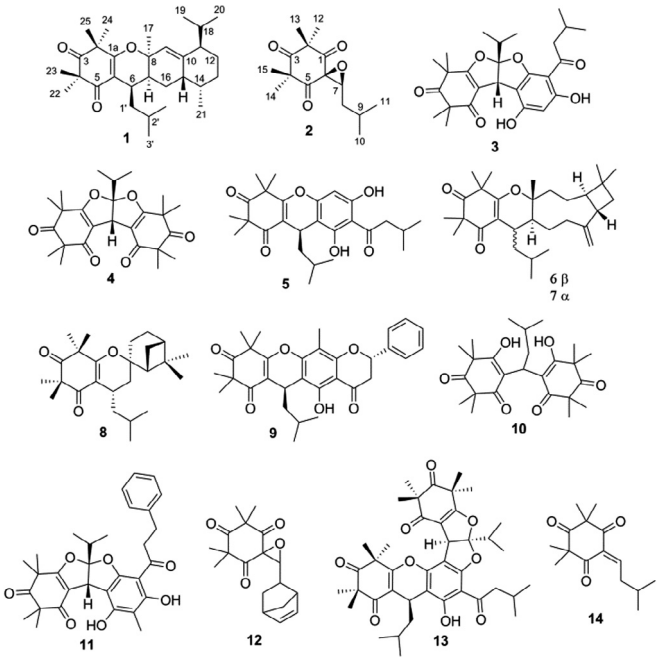

Fig. 1. β-triketones isolated from *Angophora woodsiana*.