Organic & Biomolecular Chemistry

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Watsonianone A–C, anti-plasmodial β-triketones from the Australian tree, *Corymbia watsoniana*†

Cite this: *Org. Biomol. Chem.*, 2013, **11**, 453

Anthony R. Carroll,*[a,b] Vicky M. Avery,[c] Sandra Duffy,[c] Paul I. Forster[d] and Gordon P. Guymer[d]

Received 2nd October 2012,
Accepted 22nd November 2012

DOI: 10.1039/c2ob26931g

www.rsc.org/obc

Three new β-triketones, watsonianones A–C, and the known compound corymbone B were isolated from the flowers of the Australian eucalypt *Corymbia watsoniana*. Watsonianone A is the first naturally occurring methylene bridged bis-tetramethylcyclohexatrione, watsonianone B is only the fourth fused bisfurano β-triketone and watsonianone C is the first 4,4a,9,9a-tetrahydro-2*H*-xanthene-1,3,5,7(6*H*,8*H*)-tetraone to be reported in the literature. MS and NMR analysis established the structures of the new compounds. All three new compounds showed anti-plasmodial activity against chloroquine resistant (Dd2) and sensitive strains (3D7) of the parasite, *Plasmodium falciparum*, responsible for malarial infections. Watsonianone B was the most potent inhibitor (IC_{50} 0.289 μM *vs. Pf* 3D7) demonstrating significant selectivity against the human cell line, HEK 293 (>400 ×). Stage specificity studies indicate that watsonianone B is predominantly active against young ring stages of *P. falciparum*.

Introduction

Plants from the family Myrtaceae have yielded a number of β-triketone natural products with diverse structure and biological activity.[1] Australia has a very rich diversity of plants from this family including the rainforest genera *Syzygium*, *Rhodomyrtus*, *Pilidiostigma* and the drier sclerophyll and woodland genera *Melaleuca*, *Callistemon*, *Leptospermum* and *Eucalyptus*.[2] The very large genus *Eucalyptus* contains over 600 species and comprises trees and shrubs that are widespread and mainly endemic to Australia.[3] Over a decade ago morphological analysis of the floristic features of a group of species belonging to the bloodwood, ghost gum and spotted gum groups within this genus led to these species being reassigned to a new genus *Corymbia* Hill and Johnson.[4] Subsequent analysis of sRNA demonstrated that the genera *Eucalyptus* and *Corymbia* differed at the molecular level as well.[5] The genus *Corymbia* comprises >100 species that are widespread in tropical to warm temperate savannah vegetation in northern Australia.[6] Our interest in the *Corymbia* genus was sparked by the observation of marked thyrotropin releasing hormone receptor binding activity in extracts we obtained from the flowers and seeds of a number of species.[7,8] This led us to isolate a series of β-triketone derivatives named corymbone A and B and myrtucommulones A and F–I as the active compounds.[7,8] These β-triketones ionize strongly by negative electrospray MS and show characteristic and extremely downfield (12–18 ppm) sharp singlets in NMR spectra acquired in $CDCl_3$. MS and NMR analysis of extracts from flower and seed samples obtained from a number of other *Corymbia* species indicated that they too contained β-triketone derivatives but unique molecular ion signals in their (−) ESIMS suggested that different compounds from those previously isolated were present in some of these samples. A flower sample from *Corymbia watsoniana* subsp. *capillata* (Brooker and A. R. Bean), collected from Expedition Range in Central Queensland was highlighted for further analysis because it had $[M - H^+]^-$ ions at *m*/*z* 431, 517, 521 and 537 in the (−) ESI MS. Extraction and purification of extracts from this species resulted in four β-triketones, watsonianones A–C and corymbone B, being isolated (Fig. 1). Watsonianone A is the first naturally occurring methylene bridged bis-tetramethylcyclohexatrione to be identified, watsonianone B contains the rare fused bisfurano β-triketone moiety and watsonianone C is the first 4,4a,9,9a-tetrahydro-2*H*-xanthene-1,3,5,7(6*H*,8*H*)-tetraone to be reported in the literature. We are currently assessing natural products for their ability to inhibit or kill the blood bourne parasite *Plasmodium flaciparum* that is responsible for malaria infections, with the aim to identifying novel therapeutic starting points.[9–12] The compounds identified in this study were found to be active and highly selective against the malaria parasite. Herein

[a]*Environmental Futures Centre, Griffith University, Gold Coast, QLD 4222, Australia. E-mail: a.carroll@griffith.edu.au; Fax: +61 7 55529047; Tel: +61 7 55529187*
[b]*Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia*
[c]*Discovery Biology, Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia*
[d]*Queensland Herbarium, Brisbane Botanic Gardens, Toowong, QLD 4006, Australia*
†Electronic supplementary information (ESI) available: 1D and 2D NMR spectra for watsonianone A–C. See DOI: 10.1039/c2ob26931g

the isolation, structure determination and biological activity of these novel compounds are reported.

Results and discussion

Purification of the CH_2Cl_2 extract from the flowers of *C. watsoniana* by HPLC on C_{18} silica gel eluting with a linear gradient from H_2O to MeOH over 60 min. yielded watsonianone A, (**1**) impure watsonianone B (**2**), watsonianone C (**3**) and corymbone B (**4**). Watsonianone B was further purified by HPLC on C_{18} silica gel eluting with a gradient from 30% H_2O/70% MeOH to 100% MeOH over 60 min.

Watsonianone A (**1**) was obtained as a yellow gum. Analysis of (−) HRESIFTMS data at *m/z* 431.24389 (*Δ* 0.05 ppm) allowed a molecular formula $C_{25}H_{36}O_6$ to be assigned to **1**. Absorption bands at 3400 and 1718 cm^{-1} in the IR spectrum suggested that the molecule contained alcohol, and carbonyl functionalities. The ^1H NMR spectrum of **1** (Table 1) contained a methyl doublet at δ_H 0.85 (6H), three methyl singlets at δ_H 1.37 (6H), 1.38 (6H) and 1.43 (12H), a methine multiplet at δ_H 1.40 (1H), a methylene triplet at δ_H 1.83 (2H), a doubly allylic methine triplet at δ_H 4.10 (1H) and a sharp hydrogen bonded OH proton signal at δ_H 13.30 (2H). The ^{13}C NMR spectrum contained 14 resonances including a ketone carbonyl at δ_C 212.3, two carbons at δ_C 191.4, 191.6 that could be assigned to enol and carbonyl carbons from the enol form of a β-diketone, an olefinic carbon at δ_C 114.1 and two quaternary aliphatic carbons at δ_C 51.5 and 52.1. The remaining eight signals were upfield of 40 ppm and included five methyls at δ_C 22.4 (2C), 24.0 (2C), 24.9 (2C), 25.2 (2C) and 25.9 (2C), two methine carbons at δ_C 26.9 and 29.5 and a methylene carbon at δ_C 37.9 as determined from edited HSQC correlations. The combined ^1H, ^{13}C NMR and HSQC data suggested that **1** contained elements of symmetry. HMBC correlations from methyl protons H_3-7/H_3-7″ and H_3-8/H_3-8″ to downfield quaternary carbons at δ_C 191.6 and 212.3 and the quaternary aliphatic carbon δ_C 52.1 and from H_3-9/H_3-9″ and H_3-10/H_3-10″ to downfield quaternary carbons at δ_C 191.4 and 212.3 and the quaternary aliphatic carbon δ_C 51.5 suggested the molecule contained two identical 2,2,4,4-tetramethylcyclohexatrione moieties (syncarpic acid). This was further supported by HMBC correlations from the downfield exchangeable proton at δ_H 13.30 to δ_C 191.4, 114.1 and 51.5. COSY correlations from the methyl doublet δ_H 0.85 to the methine multiplet at δ_H 1.40, from this methine proton to the methylene protons at δ_H 1.83 and from these methylene protons to the methine proton at δ_H 4.10 indicated that the molecule contained an isopentyl moiety. HMBC correlations from H_2-2′ and H-1′ to the olefinic carbons at δ_C 114.1 (C-6 and C-6″) and from H-1′ to the downfield quaternary carbons at δ_C 191.4 and 191.6 indicated that the isopentyl group was attached directly to the two syncarpic acids at C-1′ and therefore the structure of **1** was determined to be 4,4′-(2-methylbutylidene)bis(5-hydroxy-2,2,6,6-tetramethylcyclohex-4-ene-1,3-dione). This compound has previously been proposed to be a key intermediate in the biogenesis of rhodomyrtosone D, a compound isolated from the Thai plant *Rhodomyrtus tomentosa* but the compound has not previously been isolated or synthesised.[13] A related lower homologue has been made by reacting syncarpic acid with isobutyraldehyde.[14]

Watsonianone B (**2**) was also isolated as a yellow gum and (+) HRESIFTMS data (*m/z* 541.21727 $[M + Na]^+$, *Δ* 4.4 ppm) established a molecular formula of $C_{31}H_{34}O_7$ for **2**. ^1H and COSY NMR analysis (Table 2) clearly demonstrated that **2** contained a monosubstituted phenyl group, five methyl singlets, an isopropyl group, an ethyl group, an allylic methine singlet and two downfield exchangeable protons. The ^{13}C NMR spectrum contained four quaternary signals downfield of δ_C 179, three oxygenated aromatic quaternary carbons, six additional

Table 1 ^1H and ^{13}C NMR Data for watsonianone A (**1**) in $CDCl_3$

Position	δ_C[a]	δ_H[b] (mult, *J* in Hz, int.)	HMBC
1,1″	191.6	—	—
2,2″	52.1	—	—
3,3″	212.3	—	—
4,4″	51.5	—	—
5,5″	191.4	—	—
5-OH,5″-OH		13.30 (s, 2H)	C-4, C-4″, C-5, C-5″, C6, C-6″
6,6″	114.1	—	—
7,7″	24.0	1.38 (s, 6H)	C-1, C-1″, C-2, C-2″, C-3, C-3″, C-8, C-8″
8,8″	25.9	1.43 (s, 6H)	C-1, C-1″, C-2, C-2″, C-3, C-3″, C-7, C-7″
9,9″	24.9	1.43 (s, 6H)	C-3, C-3″, C-4, C-4″, C-5, C-5″, C-10, C-10″
10,10″	25.2	1.37 (s, 6H)	C-3, C-3″, C-4, C-4″, C-5, C-5″, C-9, C-9″
1′	29.5	4.10 (t, 6.9, 1H)	C-1, C-1″, C-5, C-5″, C-6, C-6″, C-2′, C-3′
2′	37.9	1.83 (t, 6.9, 2H)	C-6, C-6″, C-1′, C-3′, C-4′, C-5′
3′	26.9	1.40 (m, 1H)	—
4′,5′	22.4	0.85 (d, 6.0, 6H)	C-2′, C-3′, C-4′, C-5′

[a] 125 MHz. [b] 600 MHz.

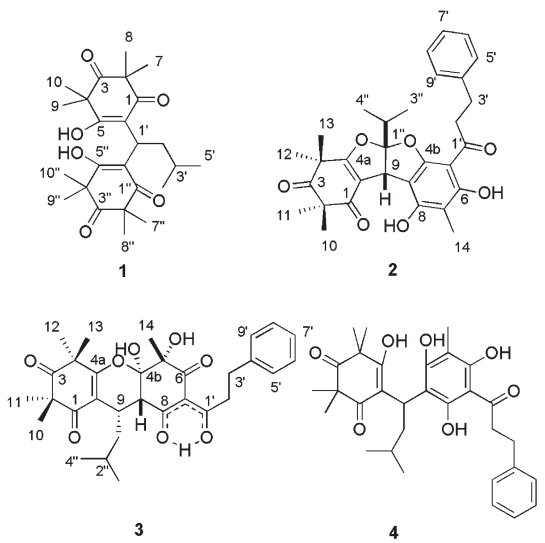

Fig. 1 β-Triketones isolated from *Corymbia watsoniana*.

Table 2 ^{1}H and ^{13}C NMR data for watsonianone B and C (**2–3**) in $CDCl_3$

	Watsonianone B (**2**)			Watsonianone C (**3**)		
Position	δ_C [a]	δ_H [b] (mult, *J* in Hz, int.)	HMBC	δ_C [a]	δ_H [b] (mult, *J* in Hz, int.)	HMBC
1	198.3	—		197.2	—	
2	55.1	—		56.1	—	
3	211.1	—		211.9	—	
4	45.6	—		47.3	—	
4a	179.6	—		166.5	—	
4b-OH					4.20 (s, 1H)	C-4b, C-5, C-8a
4b	157.7	—		98.8	—	
5	100.9	—		78.3	—	
5-OH					4.10 (bs, 1H)	—
6	163.8	—		194.8	—	
6-OH	—	13.44 (s, 1H)	C-5, C-6, C-7			
7	107.3	—		108.9	—	
8	157.4	—		197.2	—	
8-OH	—	9.88 (s, 1H)	C-7, C-8, C-8a		18.50 (s, 1H)	C-7, C-8, C-8a, C-1′, C-2′
8a	103.5	—		44.1	3.28 (d, 1.0, 1H)	C-4b, C-8, C-9a
9	45.3	4.51 (s, 1H))	C-4a, C-4b, C-8a, C-9a, C-2″	28.7	3.75 (ddd, 1.0, 3.4, 11.1, 1H)	C-1, C-4a, C-4b, C-8a, C-9a, C-1″, C-2″
9a	113.2	—		111.2	—	
10	23.2	1.35 (s, 3H)	C-1, C-2, C-3, C-11	21.2	1.25 (s, 3H)	C-1, C-2, C-3, C-11
11	25.8	1.41 (s, 3H)	C-1, C-2, C-3, C-10	26.8	1.33 (s, 3H)	C-1, C-2, C-3, C-10
12	24.3	1.42 (s, 3H)	C-3, C-4, C-4a, C-13	24.4	1.05 (s, 3H)	C-3, C-4, C-4a, C-13
13	24.2	1.51 (s, 3H)	C-3, C-4, C-4a, C-12	24.7	1.35 (s, 3H)	C-3, C-4, C-4a, C-12
14	7.5	2.06 (s, 3H)	C-6, C-7, C-8	21.8	1.42 (s, 3H)	C-4b, C-5, C-6
1′	202.6	—		202.7		
2′	44.3	3.28 (dt, 16.3, 7.7, 1H)	C-1′, C-4′, C-3′	40.3	3.30 (ddd, 6.0, 9.4, 15.4, 1H)	C-1′, C-3′, C-4′
		3.39 (dt, 16.3, 7.7, 1H)	C-1′, C-4′, C-3′		3.22 (ddd, 6.9, 9.4, 15.4, 1H)	C-1′, C-3′, C-4′
3′	30.7	3.02 (t, 7.7, 1H)	C-1′, C-2′,C-4′, C-5′, C-9′	30.9	2.95 (ddd, 6.0, 9.4, 14.6, 1H)	C-1′, C-4′, C-5′, C-9′
					2.86 (ddd, 6.9, 9.4, 14.6, 1H)	C-1′, C-4′, C-5′, C-9′
4′	141.2	—		140.0	—	
5′	128.3	7.27 (d, 7.2, 1H)	C-3′, C-7′, C-9′	128.3	7.27 (t, 7.7, 1H)	C-3′, C-7′, C-9′
6′	128.4	7.31 (t, 7.2, 1H)	C-4′, C-8′	128.5	7.20 (d, 7.7, 1H)	C-4′, C-8′
7′	126.0	7.22 (t, 7.2, 1H)	C-5′, C-9′	126.4	7.19 (t, 7.7, 1H)	C-5′, C-9′
8′	128.4	7.31 (t, 7.2, 1H)	C-4′, C-6′	128.5	7.20 (d, 7.7, 1H)	C-4′, C-6′
9′	128.3	7.27 (d, 7.2, 1H)	C-3′, C-5′, C-7′	128.3	7.27 (t, 7.7, 1H)	C-3′, C-5′, C-7′
1″	129.3	—		40.1	1.92 (ddd, 3.4, 11.1, 13.7, 1H)	C-8, C-9, C-9a, C-2″, C-3″, C-4″
					1.39 (m, 1H)	C-8, C-9, C-9a, C-2″, C-3″, C-4″
2″	35.3	2.38 (hept, 6.9, 1H)	C-9, C1″, C-3″, C-4″	25.6	1.79 (m, 1H)	C-1″, C-3″, C-4″
3″	15.6	1.04 (d, 6.9, 3H)	C-1″, C-2″, C-4″	20.6	1.10 (d, 6.9, 3H)	C-1″, C-2″, C-4″
4″	15.7	1.06 (d, 6.9, 3H)	C-1″, C-2″, C-3″	23.7	0.98 (d, 6.9, 3H)	C-1″, C-2″, C-3″

[a] 125 MHz. [b] 600 MHz.

quaternary carbons between δ_C 100.9 and 141.2, three aromatic methine signals, two upfield quaternary signals at δ_C 45.3 and 55.1 and eleven aliphatic signals upfield of δ_C 45.3. HSQC correlations established the chemical shift for all pairs of carbon and hydrogen signals associated with protonated carbons. This combined data suggested that **2** also contained a syncarpic acid group. Correlations observed in a HMBC spectrum confirmed this assignment since characteristic correlations from the methyl singlets at δ_H 1.35 and 1.41 to two carbonyl carbons at δ_C 198.3 and 211.1 and the aliphatic quaternary carbon at 55.1 and between methyl singlets at δ_H 1.42 and 1.51 and the carbonyl carbon at δ_C 211.1, a conjugated oxygenated enol carbon at δ_C 179.6 and the quaternary carbon at 45.6 were observed. The presence of a 3-phenylpropionyl group was deduced from HMBC correlations observed between the phenyl doublet at δ_H 7.27 and the ethyl carbon at δ_C 30.7 and between the ethyl protons at δ_H 3.02, 3.28, 3.39 and the ketone carbonyl carbon at δ_C 202.6. A 1,3-dihydroxy-2-methylphenyl group was suggested from correlations between the two phenolic protons 6-OH and 8-OH and the aromatic methyl singlet H_3-14 and oxygenated carbons at δ_C 163.8 and 157.4 and an upfield quaternary carbon at δ_C 107.3. This partial structure could be extended to a fully substituted phloroglucinol since the two phenolic protons also correlated to additional upfield aromatic quaternary carbons at δ_C 100.9 and 103.5. The chemical shift of these carbons dictated that they must each be *ortho* to two oxygens. The syncarpic acid was linked to the phloroglucinol by a methine bridge since correlations were observed between the allylic methine proton H-9 and C-4a, C-4b, C-8a and C-9a. This methine

carbon was also linked to the isopropyl group *via* a quaternary carbon bridge, since HMBC correlations were observed between H-9 and C-2″ and between H-2″ and C-9 while the isopropyl methyl and methine protons each correlated to a quaternary carbon at δ_C 129.3. The chemical shift of this carbon suggested that it was either sp^2 hybridised or doubly oxygenated and since all of the atoms that are present in the molecule as deduced from HRESIMS analysis were accounted for, this carbon must be linked by two oxygen atoms to both C-4a and C-4b and is therefore a ketal. Finally the phenylpropionyl group was attached at the only unassigned position, C-5 and since the phenolic proton 6-OH was significantly shifted downfield (δ_H 13.44), this fitted well for a molecule with an intramolecular hydrogen bond between the carbonyl at C-1′ and the phenol 6-OH. Watsonianone B (**2**) therefore contains a bisfurano group linked to a β-triketone and a phloroglucinol and is only the fourth compound containing this rare moiety to be isolated.[13,15] Watsonianone B contains two stereogenic centres and analysis of ROESY correlations was used to deduce the relative configuration of these centres. Intense correlations between H-9 and H-2″, H_3-3″ and H_3-4″ indicated that H-9 and the isopropyl group were on the same face of the molecule. This relative configuration is the same as that reported previously for the three related compounds, rhodomyrtosone A, and tomentosones A and B.[15] Watsonianone B is related most closely to rhodomyrtosone A since replacement of its isopropyl side chain with a benzyl group would yield watsonianone B.

Watsonianone C (**3**) was isolated as an optically active yellow gum. The pseudomolecular ion in the HRESIFTMS of **3** analyzed for a molecular formula, $C_{31}H_{38}O_8$ (*m*/*z* 561.24589, Δ 0.1 ppm, $C_{31}H_{38}O_8Na^+$). Watsonianone C contained the same number of carbons as both watsonianone B (**2**) and corymbone B (**4**) suggesting it was probably structurally related. Prominent features of the ^{1}H NMR spectrum of **3** were the presence of five methyl singlets, two methyl doublets and signals associated with a phenylethyl group and this further supported the conclusion that **3** was closely related to **2** and **4**. An unusual feature of the ^{1}H NMR spectrum was the presence of a sharp singlet at δ_H 18.50. The ^{13}C NMR spectrum of **3** contained characteristic signals for a syncarpic acid moiety and a phenylpropionyl group, but unlike **2** no aromatic signals were observed that could be assigned to a phloroglucinol. Instead, two additional downfield quaternary carbons at δ_C 194.8 and 197.2, two upfield quaternary carbons at δ_C 108.9 and 98.8, an oxygenated quaternary carbon at δ_C 78.3 and an aliphatic methine carbon at δ_C 44.1 were observed and this suggested that the phloroglucinol was oxidised in **3**. Correlations observed in the HSQC and COSY spectra indicated that **3** also contained an isopentyl group and two hydroxyl groups attached to quaternary carbons. HMBC correlations from the methyl singlets at δ_H 1.05, 1.25, 1.33 and 1.35 to quaternary carbons at δ_C 211.9, 197.2, 166.5, 56.1 and 47.3 were consistent with a syncarpic acid moiety being present. The methine proton at δ_H 3.75 from the isopentyl group exhibited HMBC correlations to two of these downfield quaternary carbons (δ_C 166.5 and 197.2) indicating that the isopentyl group was directly attached to the syncarpic acid moiety. The presence of the phenylpropionyl group was confirmed from HMBC correlations from the methylene protons H_2-2′ and H_2-3′ to a carbonyl carbon at δ_C 202.7. The phenylpropionyl group was attached to a quaternary olefinic carbon since a HMBC correlation was also observed between the methylene proton at δ_H 3.22 and δ_C 108.9. The fifth methyl singlet at δ_H 1.42 showed HMBC correlations to carbons at δ_C 194.8, 98.8 and 78.3, while the hydroxy proton at δ_H 4.20 correlated to δ_C 98.8, 78.3 and 44.1. This indicated that **3** contained a hemiketal that was adjacent to both an aliphatic methine (C-8a), and a tertiary alcohol carbon substituted by methyl and ketone groups. The proton signal at δ_H 3.30 (H-8a) showed HMBC correlations to the ketal carbon C-4b as well as to the isopentyl carbons, C-9 and C-1″, the syncarpic acid carbon C-9a and the only remaining unassigned carbon, a downfield quaternary carbon at δ_C 197.2. With all of the carbon resonances accounted for by these correlations it was logical to link the two downfield quaternary carbons, C-6 and C-8, to C-7. The extremely downfield exchangeable proton at δ_H 18.50 correlated to five carbon resonances. This was somewhat unexpected since enol or phenolic protons generally only correlate to the three carbons that are within three bonds. Three of the correlations (δ_C 44.1, 197.2 and 108.9) suggested that this proton was attached to an oxygen at C-8, however the remaining two correlations (δ_C 202.7 and 40.3) suggested that the OH group was attached at C-1′. It could therefore be concluded that this proton was a intramolecular hydrogen bonded hydroxyl proton from the enol form of a β-diketone. The proton was equally hydrogen bonded to both O-8 and O-1′ indicating that a resonance stabilised structure in which the π electrons from the two carbonyl carbons were shared between C-7, C-8 and C-1′. This provided an explanation for the extreme downfield shift of this proton since the electron density around this proton would be extremely low.[16] Furthermore, these HMBC correlations firmly established the bond between C-7 and C-8. An ether linkage between the hemiketal carbon C-4b and the syncarpic acid carbon C-4a was proposed to complete the structural assignment for **3**, since this fulfilled the required degrees of unsaturation as defined from MS analysis. ROESY correlations were used to determine the relative configuration of the four stereogenic centres in **3**. An intense correlation between H_3-14 and H-8a demonstrated that these protons were 1,3-diaxial. An additional intense correlation between H-8a and H-9 indicated that both of these protons were on the β face of the molecule. This was further supported by the observation of a very small mutual coupling (1.0 Hz) between these protons indicating that they were almost 90° to each other. The hydroxyl proton 4b-OH correlated to the isopentyl proton H-1″b indicating that this hydroxyl group was located on the α face.

Watsonianone C (**3**) is the first example of a 4,4a,9,9a-tetrahydro-2*H*-xanthene-1,3,5,7(6*H*,8*H*)-tetraone to be reported in the literature.

Compounds **1–3** were tested for their ability to inhibit the growth of chloroquine sensitive (3D7) and resistant (Dd2) strains of the malarial parasite, *Plasmodium falciparum*.

Human cell cytotoxicity was assessed using the mammalian cell line HEK-293. Watsonianone B (**2**) was most active against both the Dd2 and 3D7 strains displaying IC_{50} values of 0.44 and 0.29 μM, respectively. Watsonianone C (**3**) was the next most active compound displaying IC_{50} values of 1.18 μM (Dd2) and 1.07 μM (3D7) and **1** was the least active compound displaying IC_{50} values of 8.8 μM (Dd2) and 5.3 μM (3D7). Watsonianone A showed cytotoxic activity at significantly higher doses, reaching 42% inhibition only at 120 μM against HEK-293 (SI > 22), whereas watsonianone B and C reached 85% and 83% activity at 120 μM (SI > 400 and SI > 100, respectively). The watsonianones were investigated further to determine their mode of action against *Plasmodium falciparium*. All three compounds were shown to be more active against the ring stage of *P. falciparum*, with watsonianone B exhibiting activity predominantly at the very young ring stages. It is interesting to note that tomentosone A that we reported recently and which differs from watsonianone B by the addition of a second alkyl syncarpic acid and the replacement of the benzyl group with an isopropyl group was 2× and 5× less active against the 3D7 and Dd2 strains of *P. falciparum* respectively.[11] This suggests that either the second syncarpic acid group reduces activity or alternatively that replacement of the isopropyl with a benzyl group enhances antimalarial activity. The tomentosones and watsonianones represent the first reported β-triketone inhibitors of *P. falciparum*.

Conclusions

In summary, four β-triketones have been isolated from the flowers of the Australian tree *C. watsoniana*. Two of the compounds, watsonianone A and C, possess structural motifs not previously encountered in nature and watsonianone B contains the rarely encountered bisfuranophlorglucinol β-triketone tetracyclic ring system. Moderately potent antimalarial activity was obtained for the three new compounds, with watsonianone B exhibiting the most potent and selective activity profile, demonstrated to be predominant ring stage specific in effect. Our discovery that watsonianones and tomentosones show significant antimalarial activity suggests that the β-triketone pharmacophore represents a new *Plasmodium* inhibitor class. Studies are currently ongoing to discover additional β-triketone inhibitors from Australian and Thai plants from the family Myrtaceae.

Experimental

General procedures

NMR spectra were recorded at 30 °C on a Varian 600 MHz spectrometer equipped with a triple resonance cold probe. The ^1H and ^{13}C chemical shifts were referenced to the solvent peak for $CDCl_3$ at δ_H 7.26 and δ_C 77.0. LRESIMS were recorded on a Applied Biosystems Mariner Biospectrometry TOF workstation using negative electrospray ionization, mobile phase 1 : 1 MeOH : H_2O. HRESIFTMS were recorded on a Bruker Daltronics Apex III 4.7e Fourier-transform mass spectrometer. IR and UV spectra were recorded on a Bruker Tensor 27 spectrometer and a Shimadzu UV-1800 UV spectrophotometer, respectively. Optical rotations were measured on a JASCO P-1020 polarimeter and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Alltech Davisil 30–40 μm 60 Å C_{18} bonded silica was used to adsorb the flower extract prior to HPLC separation. A Merck Hitachi L7100 pump equipped with a Merck Hitachi L7455 PDA detector and a Merck Hitachi L7250 autosampler were used for HPLC. A Betasil C_{18} 5 μm 120 Å column (21.2 mm × 150 mm) was used for semi-preparative HPLC separations. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade, and the H_2O was Millipore Milli-Q PF filtered.

Collection and identification of the plant material

The flowers from the plant sample *Corymbia watsoniana* subsp. *capillata* (Brooker and A. R. Bean) was collected from Expedition Range in Central Queensland. A voucher specimen, PIF18936, has been deposited at the Queensland Herbarium, Brisbane.

Extraction and isolation

The air-dried ground flowers of *C. watsoniana* (12.5 g) were extracted with CH_2Cl_2 (4 × 300 mL), yielding a yellow/brown gum (1.85 g). The CH_2Cl_2 extract was adsorbed onto C_{18} silica gel (1.3 g) and the extract impregnated gel was placed in a HPLC pre-column cartridge (10 mm × 20 mm), connected in series to a C_{18}-bonded silica HPLC column (21 mm × 150 mm) and eluted with a gradient from H_2O to MeOH over 60 min at a flow rate of 9 mL min^{-1}. The column was then eluted with MeOH for a further 10 min. Fractions were collected every minute and an aliquot from each was analysed by negative electrospray mass spectrometry. Those fractions that contained only one molecular ion were further analyzed by ^1H NMR spectroscopy to determine purity. Fractions 47–50 were pure watsonianone C (113.5 mg, 0.9%), fractions 53–54 were pure watsonianone A (44.3 mg, 0.35%) and fraction 59 was pure corymbone B (15.2 mg, 0.12%). Fractions 51–52 were impure and were further purified by C18 HPLC with a gradient from 30% H_2O/70% MeOH to MeOH over 60 min at a flow rate of 9 mL min^{-1}. Sixty fractions were collected and fractions 55–56 was pure watsonianone B (36.5 mg, 0.29%).

Watsonianone A (1). Yellow gum; UV λ_{max} (MeOH)/nm (log ε) 217 (4.23), 264 (3.98), 291 (3.81); IR ν_{max} (film)/cm^{-1} 3400, 2978, 2937, 2872, 1718, 1589, 1458, 1385; ^1H and ^{13}C NMR data ($CDCl_3$) see Table 1; (−)-HRESIFTMS *m*/*z* 431.24389 (calcd for $C_{25}H_{35}O_6$, 431.24392).

Watsonianone B (2). Yellow gum; $[\alpha]_D^{23}$ +5.0 (*c* 0.17, MeOH); UV λ_{max} (MeOH)/nm (log ε) 215 (4.25), 224 (4.24), 268 (4.11), 288 (4.11); IR ν_{max} (film)/cm^{-1} 3400, 2936, 2872, 1718, 1623, 1496, 1454, 1385, 1162; ^1H and ^{13}C NMR data ($CDCl_3$) see Table 2; (+)-HRESIFTMS *m*/*z* 541.21727 (calcd for $C_{31}H_{34}O_7Na$, 541.21968).

Watsonianone C (3). Yellow gum; $[\alpha]_D^{23}$ +10.2 (*c* 0.09, MeOH); UV λ_{max} (MeOH)/nm (log ε) 234 (4.46), 261 (4.43); IR ν_{max}

(film)/cm^{-1} 3420, 2955, 2871, 1716, 1632, 1456, 1386; ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 2; (+)-HRESIFTMS *m*/*z* 561.24589 (calcd for $C_{31}H_{38}O_8Na$, 561.24595).

Malaria imaging assay

Anti-malarial activity was determined using the method described by Duffy and Avery, 2012.[17] Briefly, compounds were incubated in the presence of 2 or 3% parasite (3D7 or Dd2) and 0.3% hematocrit in a total assay volume of 50 μL, for 72 h at 37 °C and 5% CO_2, in Poly-D-lysine coated CellCarrier Imaging plates. After incubation plates were stained with DAPI (4′,6-diamidino-2-phenylindole) in the presence of Saponin and Triton X-100 and incubated for a further 5 h at RT in the dark before imaging on the OPERA™ HTS confocal imaging system. The digital images obtained were then analyzed using the PerkinElmer Acapella spot detection software where spots which fulfill the criteria established for a stained parasite are counted. The % inhibition of parasite replication was calculated using DMSO and Artemisinin control data.

Cytotoxicity assay

Compounds were added to assay wells containing adherent cells (HEK293) seeded at the appropriate densities according to their respective growth rates in 384-well black/clear tissue culture treated plates (Falcon, BD Biosciences) in an assay volume of 45 μL. The plates were incubated for 72 h at 37 °C and 5% CO_2. For the HEK-293 cell line, the supernatant was removed after incubation, and 40 μL of 10% Presto Blue substrate (Sigma Aldrich) in DMEM plus 2 mM Glutamax was added to each well. The plates were incubated for a further 3 h and measured on the Perkin-Elmer EnVision at 530/595 nm. The percent inhibition of growth was calculated in relation to the maximum and minimum inhibition of fluorescence caused by >10% DMSO or 20 μM Puromycin (100% inhibition) and 0.4% DMSO (no inhibition). All experiments were performed in triplicate, $n = 3$. IC_{50} values were obtained by plotting % inhibition against log dose using Prizm4 graphing package using non-linear regression with variable slope plot.

Acknowledgements

We thank Dr H. Vu for providing HRESIFTMS analysis of the compounds. Australian red cross for blood supply.

Notes and references

1 I. P. Singh and S. P. Bharate, *Nat. Prod. Rep.*, 2006, **23**, 558–591.
2 B. S. Briggs and L. A. S. Johnson, *Proc. Linn, Soc. N.S.W.*, 1979, **102**, 157–256.
3 G. M. Chippendale, *Flora of Australia*, Australian Government Publishing Service, Canberra, 1988, vol. 19.
4 K. D. Hill and L. A. S. Johnson, *Telopea*, 1995, **6**, 185–504.
5 C. Parra-O, M. J. Bayly, F. Udovicic and P. Y. Ladiges, *Taxon*, 2006, **55**, 653–663.
6 P. Ladiges, C. Parra-O, A. Gibbs, F. Udovicic, G. Nelson and M. Bayly, *Cladistics*, 2011, **27**, 29–41.
7 A. R. Carroll, S. Urban, J. Lamb, R. Moni, G. P. Guymer, P. I. Forster and R. J. Quinn, *J. Nat. Prod.*, 2008, **71**, 881–883.
8 A. R. Carroll, J. Lamb, R. Moni, G. P. Guymer, P. I. Forster and R. J. Quinn, *J. Nat. Prod.*, 2008, **71**, 1564–1568.
9 L. S. Fernandez, M. S. Buchanan, A. R. Carroll, Y. J. Feng, R. J. Quinn and V. M. Avery, *Org. Lett.*, 2009, **11**, 329–332.
10 A. R. Carroll, S. Duffy and V. M. Avery, *J. Org. Chem.*, 2010, **75**, 8291–9294.
11 A. R. Carroll, S. Duffy, M. Sykes and V. M. Avery, *Org. Biomol. Chem.*, 2011, **9**, 604–609.
12 A. R. Carroll, B. D. Nash, S. Duffy and V. M. Avery, *J. Nat. Prod.*, 2012, **75**, 1206–1209.
13 A. Hiranrat and W. Mahabusarakam, *Tetrahedron*, 2008, **64**, 11193–11197.
14 W. D. Crow, T. Osawa, K. M. Platz and D. S. Sutherland, *Aust. J. Chem.*, 1976, **29**, 2525–2531.
15 A. Hiranrat, W. Mahabusarakam, A. R. Carroll, S. Duffy and V. M. Avery, *J. Org. Chem.*, 2012, **77**, 680–683.
16 D. R. Clark, J. Emsley and F. Hibbert, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1299–1301.
17 S. Duffy and V. M. Avery, *Am. J. Trop. Med. Hyg.*, 2012, **86**, 84–92.

