



## Walsogynes H–O from *Walsura chrysoogyne*

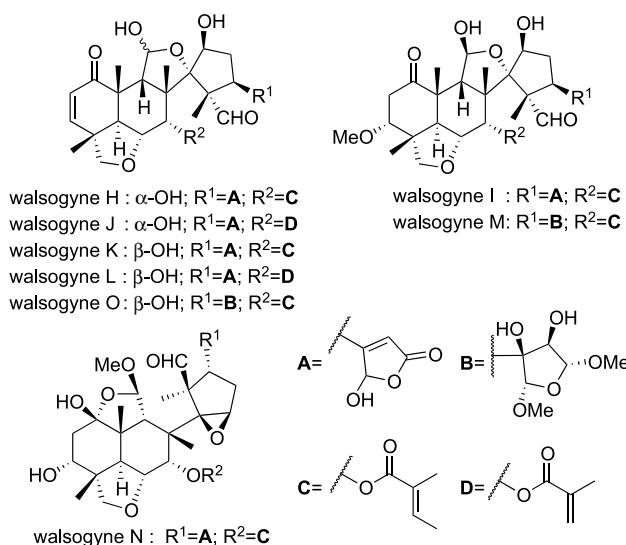
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### Abstract

Eight new limonoids, walsogynes H–O (1–8) were isolated from the barks of *Walsura chrysoogyne*, and their structures were determined on the basis of the 1D and 2D NMR data. Walsogynes H–M (1–6) and O (8) were concluded to be 11,12-*seco* limonoids with a dodecahydro-1*H*-naphtho[1,8-*bc*:3,4-*c'*]difuran skeleton, and walsogyne N (7) to be 11,12-*seco* limonoid sharing a unique dodecahydronaphtho[1,8-*bc*:5,4-*b'**c'*]difuran skeleton. Walsogynes H–O (1–8) exhibited potent antimalarial activity against *Plasmodium falciparum* 3D7 strain with IC<sub>50</sub> value of 2.5, 2.6, 1.6, 2.5, 1.5, 2.6, 2.1, and 1.1 μM, respectively.

### Graphic abstract



**Keywords** Limonoids · *Walsura chrysoogyne* · Antimalarial activity

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## Introduction

*Walsura*, a genus belonging to Meliaceae, is composed of about 16 species distributed from Sri Lanka to the Himalaya and Indochina through Malaysia, Indonesia to New Guinea, and *Walsura chrysogyne* is distributed in Peninsular Malaysia [1]. The plants of this genus have been reported to produce limonoids, dammarane, tirucallane and apotirucallane triterpenoids [2–7]. In our search for new bioactive compounds [8–27], we have reported the isolation of new limonoids and triterpenoids from plants of this genus [3, 4, 19], and alkaloids showing antimalarial activity [22, 26, 27]. Continuation of the bioassay-guided investigation on the extract of *W. chrysogyne* bark led to the isolation of eight new limonoids, walsogynes H–O (1–8) showing antimalarial activity (Fig. 1). Structure elucidation of 1–8 and their antimalarial activity of a series of walsogynes are reported herein.

## Results and discussions

Compounds 1–8 were obtained as optically active white amorphous solids. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Tables 1 and 2) suggested the identity of 1–8 as walsogynes derivatives. Based on the  $^{13}\text{C}$  NMR data, 1–6 and 8 were assumed to be derivatives of walsogyne A [2], and 7 was assumed to be a derivative of walsogyne B [4].

Compounds 1 and 4 were determined to have the same molecular formula,  $\text{C}_{31}\text{H}_{38}\text{O}_{11}$ , based on the HR-ESIMS

data. Their  $^1\text{H}$  and  $^{13}\text{C}$  NMR data are highly similar and the differences are similar to the differences of walsogynes F and G [4], indicating the structure of 1 as 11-epi-4. Furthermore, except for the signal associated with the  $\alpha,\beta$ -unsaturated- $\gamma$ -lactone moiety (C-20–C-23), the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of 4 and walsogyne A were highly similar. Thus, the structures of 1 and 4 were deduced to be as shown in Fig. 1. Analysis of the 2D NMR data further supported the proposed structures.

Compounds 3 and 5 were also determined to possess the same molecular formula,  $\text{C}_{30}\text{H}_{36}\text{O}_{11}$ . Furthermore, their NMR data are highly similar to each other and to 1 and 4. However, the signals associated with CH-3' and CH<sub>3</sub>-4' of the tiglate moiety in 1 and 4 are not observed in 3 and 5, and  $\text{sp}^2$  methylene signals ( $\delta_{\text{C}}$  127.2,  $\delta_{\text{H}}$  5.61 and 6.49, and  $\delta_{\text{C}}$  125.9,  $\delta_{\text{H}}$  5.67 and 5.92 for 3 and 5) are observed instead. Therefore, 3 and 5 should have a methacrylate moiety instead of a tiglate moiety. Analysis of the 2D NMR data supported the structures of 3 and 5 to be as shown in Fig. 1. Specifically, the presence of a methacrylate moiety was supported by the HMBC correlations of H<sub>3</sub>-4' to C-1', C-2' and C-3'.

Compound 2 was revealed to have the molecular formula  $\text{C}_{32}\text{H}_{42}\text{O}_{12}$  by HRESITOFMS. Its NMR data are highly similar to 4. However, the signals for H-2 and H-3 in 1 are not observed in 2, and a methoxy signal ( $\delta_{\text{H}}$  3.34) and three aliphatic signals ( $\delta_{\text{H}}$  2.66, d, 17.5;  $\delta_{\text{H}}$  3.14, d, 17.5; and  $\delta_{\text{H}}$  3.58, br s) are observed instead. Based on the chemical shifts and the multiplicity patterns, 2 was proposed to be 2,3-dihydro-3 $\alpha$ -methoxy-4. The proposed structure was also confirmed through analysis of the 2D NMR data. In particular, the HMBC correlations of H<sub>3</sub>-29 and the methoxy to C-3, and H-3 to C-1, and the NOESY correlation of H-3/H<sub>3</sub>-29 confirmed the position and the configuration of the methoxy at C-3.

By HRESITOFMS, 6 and 8 were revealed to have the molecular formula  $\text{C}_{34}\text{H}_{50}\text{O}_{14}$  and  $\text{C}_{33}\text{H}_{46}\text{O}_{13}$ , respectively. Their NMR data are also highly similar to 4 and 2, respectively, differing only on the signals assigned to the  $\alpha,\beta$ -unsaturated- $\gamma$ -lactone in 2 and 4. Analysis of the NMR data revealed that the furan moiety in both 6 and 8 were highly oxidized. The planar structure of the modified furan moiety in 6 was deduced from the  $^1\text{H}$ - $^1\text{H}$  COSY correlation of H-22 and H-23, and the HMBC correlations of H-21 to C-17 and C-20, H-22 to C-17, H-23 to C-21, a methoxy ( $\delta_{\text{H}}$  3.07) to C-21 and a methoxy ( $\delta_{\text{H}}$  3.38) to C-23 (Fig. 2). The relative configuration of the furan moiety was then deduced from the ROESY correlations in pyridine- $d_6$  to be as shown in Fig. 3 since only the proposed configuration in Fig. 1 will fulfill the conditions set by the ROESY correlations shown in Fig. 3. Based on the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of 6 and 8, the furan moiety in 8

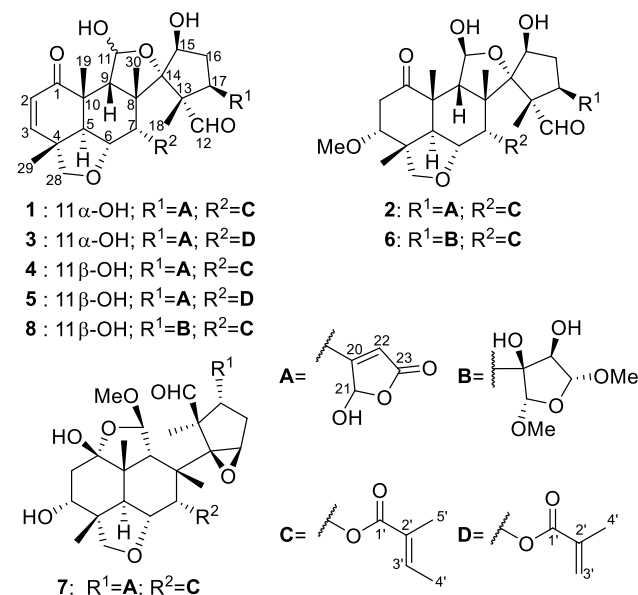


Fig. 1 Structures of 1–8

**Table 1**  $^1\text{H}$  NMR data of **1–8** in  $\text{CD}_3\text{OD}$  (\* in  $\text{DMSO}-d_6$ )

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
2a	5.91 (1H, d, 9.8)	2.66 (1H, d, 17.5)	5.90 (1H, d, 9.8)	5.97 (1H, d, 9.8)	5.96 (1H, d, 9.7)
2b		3.14 (1H, d, 17.5)			
3	7.05 (1H, d, 9.8)	3.58 (1H, brs)	7.05 (1H, d, 9.8)	7.20 (1H, d, 9.8)	7.20 (1H, d, 9.8)
5	3.53 (1H, d, 12.9)	3.00 (1H, d, 12.5)	3.53 (1H, d, 13.0)	2.65 (1H, d, 12.6)	2.65 (1H, d, 12.6)
6	4.38 (1H, dd, 12.9, 3.2)	4.41 (1H, d, 12.5)	4.40 (1H, dd, 13.2, 3.0)	4.46 (1H, dd, 12.6, 2.9)	4.47 (1H, dd, 12.7, 2.7)
7	5.45 (1H, d, 3.2)	5.32 (1H, brs)	5.46 (1H, d, 3.0)	5.37 (1H, d, 3.0)	5.38 (1H, d, 2.9)
9	2.75 (1H, d, 6.7)	2.75 (1H, d, 6.4)	2.76 (1H, d, 6.6)	2.85 (1H, d, 7.1)	2.84 (1H, d, 7.0)
11	5.51 (1H, d, 6.7)	5.24 (1H, d, 6.4)	5.51 (1H, d, 6.6)	5.27 (1H, d, 7.1)	5.25 (1H, d, 7.1)
12	9.67 (1H, s)	9.58 (1H, s)	9.72 (1H, s)	9.58 (1H, s)	9.64 (1H, m)
15	4.31 (1H, d, 4.7)	4.91 (1H, d, 4.5)	4.31 (1H, d, 4.8)	4.91 (1H, m)	4.91 (1H, d, 5.3)
16a	1.77 (1H, m)	1.79 (1H, m)	1.77 (1H, m)	1.82 (1H, m)	1.82 (1H, m)
16b	2.68 (1H, ddd, 16.3, 11.1, 5.3)	2.79 (1H, m)	2.68 (1H, m)	2.78 (1H, ddd, 19.0, 11.0, 5.4)	2.77 (1H, ddd, 15.6, 10.6, 5.1)
17	3.40 (1H, m)	3.48 (1H, m)	3.41 (1H, m)	3.48 (1H, m)	3.47 (1H, m)
18	1.23 (3H, s)	1.22 (3H, s)	1.23 (3H, s)	1.22 (3H, s)	1.23 (3H, m)
19	1.33 (3H, s)	1.43 (3H, s)	1.33 (3H, s)	1.40 (3H, s)	1.38 (3H, m)
21	5.45* (1H, brs)	5.37* (1H, m)	5.34* (1H, m)	5.45* (1H, m)	5.45* (1H, m)
22	6.07* (1H, brs)	6.13* (1H, m)	6.28* (1H, m)	6.25* (1H, m)	6.26* (1H, m)
28a	3.27 (1H, m)	3.44 (1H, d, 7.0)	3.31 (1H, m)	3.30 (1H, d, 7.2)	3.34 (1H, m)
28b	3.60 (1H, d, 6.9)	3.67 (1H, d, 7.0)	3.61 (1H, d, 7.0)	3.67 (1H, d, 7.2)	3.69 (1H, d, 7.3)
29	1.25 (3H, s)	1.32 (3H, s)	1.25 (3H, s)	1.29 (3H, s)	1.29 (3H, m)
30	1.66 (3H, s)	1.64 (3H, s)	1.66 (3H, s)	1.67 (3H, s)	1.67 (3H, m)
3'a	5.61 (1H, qd, 7.1, 1.3)	6.79 (1H, q, 6.5)	5.61 (1H, s)	6.72 (1H, q, 6.4)	5.67 (1H, m)
3'b			6.49 (1H, s)		5.92 (1H, m)
4'	1.76 (3H, d, 7.1)	1.84 (3H, d, 6.5)	1.89 (3H, m)	1.81 (3H, d, 6.4)	
5'	1.79 (3H, brs)	1.84 (3H, s)		1.82 (3H, s)	1.92 (3H, m)
3-OMe		3.34 (3H, s)			

	<b>6</b>	<b>7</b>	<b>8</b>
	In $\text{CD}_3\text{OD}$	In $\text{C}_5\text{D}_5\text{N}$	In $\text{DMSO}-d_6$
2a	2.62 (1H, d, 12.6)	2.71 (1H, d, 17.7)	1.92 (1H, m)
2b	2.62 (1H, d, 12.6)	3.12 (1H, d, 17.7)	1.98 (1H, m)
3	3.57 (1H, m)	3.43 (1H, brs)	3.57 (1H, m)
5	2.97 (1H, d, 12.4)	3.37 (1H, d, 12.2)	2.44 (1H, d, 12.8)
6	4.40 (1H, dd, 12.4, 2.7)	4.57 (1H, dd, 12.2, 2.8)	4.11 (1H, dd, 12.6, 2.9)
7	5.26 (1H, d, 2.7)	5.87 (1H, d, 2.8)	5.18 (1H, d, 2.9)
9	2.72 (1H, d, 6.5)	3.23 (1H, d, 6.6)	2.41 (1H, m)
11	5.18 (1H, d, 6.5)	5.81 (1H, dd, 6.6, 2.4)	5.31 (1H, d, 5.8)
12	9.31 (1H, s)	10.03 (1H, s)	9.76 (1H, m)
15	4.82 (1H, m)	5.55 (1H, m)	3.58 (1H, brs)
16a	2.11 (1H, m)	3.01 (1H, m)	1.97 (1H, m)
16b	2.54 (1H, ddd, 16.5, 10.6, 5.7)	3.16 (1H, m)	2.19 (1H, m)
17	2.93 (1H, m)	3.57 (1H, m)	2.77 (1H, d, 8.0)
18	1.43 (3H, s)	2.09 (3H, s)	1.05 (3H, m)
19	1.42 (3H, s)	1.33 (3H, s)	1.05 (3H, m)
21	4.54 (1H, s)	5.24 (1H, s)	5.59 (1H, m)
22	3.85 (1H, d, 3.6)	4.55 (1H, dd, 7.3, 3.7)	6.25 (1H, m)
23	4.72 (1H, d, 3.6)	5.28 (1H, d, 3.7)	
28a	3.43 (1H, d, 6.5)	3.58 (1H, d, 6.7)	3.22 (1H, m)
28b	3.65 (1H, d, 6.5)	3.92 (1H, d, 6.7)	3.62 (1H, m)
			In $\text{CD}_3\text{OD}$
			5.95 (1H, d, 9.8)
			7.18 (1H, d, 9.8)
			2.63 (1H, d, 12.5)
			4.44 (1H, dd, 12.5, 3.0)
			5.32 (1H, d, 3.0)
			2.82 (1H, d, 7.1)
			5.21 (1H, d, 7.1)
			9.32 (1H, m)
			4.82 (1H, m)
			2.10 (1H, m)
			2.54 (1H, ddd, 16.6, 10.8, 5.7)
			2.93 (1H, m)
			1.43 (3H, s)
			1.38 (3H, s)
			4.51 (1H, m)
			3.84 (1H, d, 3.6)
			4.72 (1H, d, 3.6)
			3.29 (1H, m)
			3.66 (1H, d, 7.2)

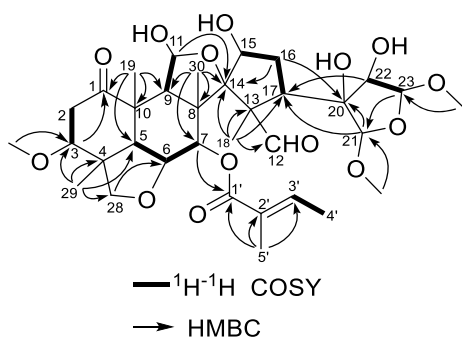
**Table 1** (continued)

	6		7		8
29	1.31 (3H, s)	1.25 (3H, s)	1.32 (3H, m)		1.28 (3H, s)
30	1.60 (3H, s)	1.86 (3H, s)	0.94 (3H, m)		1.64 (3H, s)
3'	6.75 (1H, q, 7.0)	7.03 (1H, q, 9.7)	6.75 (1H, q, 7.1)		6.69 (1H, q, 7.0)
4'	1.84 (3H, d, 7.0)	1.65 (3H, d, 7.1)	1.78 (3H, d, 7.1)		1.83 (3H, d, 7.0)
5'	1.82 (3H, s)	2.10 (3H, s)	1.82 (3H, s)		1.80 (3H, s)
3-OMe	3.32 (3H, s)	3.15 (3H, s)			
11-OMe			3.38 (3H, s)		
21-OMe	3.07 (3H, s)	3.33 (3H, s)			3.06 (3H, s)
23-OMe	3.38 (3H, s)	3.39 (3H, s)			3.38 (3H, s)

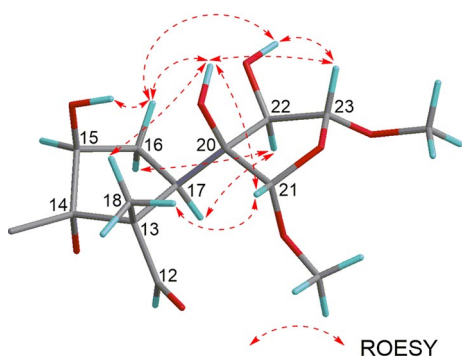
**Table 2**  $^{13}\text{C}$  NMR data of 1–8

	1 <sup>a</sup>	2 <sup>a</sup>	3 <sup>a</sup>	4 <sup>a</sup>	5 <sup>a</sup>	6 <sup>a</sup>	6 <sup>b</sup>	7 <sup>c</sup>	8 <sup>a</sup>
1	205.4	213.7	205.4	202.6	202.5	214.1	212.3	106.7	203.0
2	131.1	40.4	131.1	131.2	131.2	40.2	40.0	37.2	131.0
3	152.8	81.8	152.8	154.6	154.6	81.9	80.6	69.2	155.0
4	42.6	44.5	42.7	42.7	42.7	44.5	43.4	42.5	42.6
5	46.2	42.1	46.3	47.1	47.1	42.0	40.8	40.0	46.9
6	74.7	73.9	74.6	74.4	74.4	74.1	73.0	71.6	74.6
7	73.0	74.1	73.3	73.5	73.8	73.9	72.7	74.4	73.4
8	52.8	56.5	52.8	56.5	56.6	56.7	55.8	43.5	56.8
9	59.2	65.0	59.3	63.6	63.7	64.7	64.2	58.4	63.3
10	46.1	50.3	46.2	46.3	46.3	50.3	49.2	46.8	46.3
11	96.6	99.4	96.6	98.8	98.8	99.1	98.5	106.5	98.5
12	202.6	201.3	203.0	201.6	201.9	202.9	200.9	N.D	203.0
13	59.6	60.2	59.7	60.1	60.2	60.2	59.5	58.5	60.2
14	98.4	100.3	98.5	99.9	100.0	100.7	100.0	72.0	100.0
15	80.7	80.6	80.7	80.8	80.8	80.2	79.6	60.2	80.4
16	40.2	39.4	40.2	39.4	39.5	33.1	33.0	29.5	33.2
17	41.3	41.2	42.1	41.2	41.2	46.8	46.4	N.D	46.8
18	12.7	13.1	12.8	13.1	13.1	14.2	14.3	13.0	14.3
19	22.3	22.3	22.3	22.4	22.4	22.2	21.6	18.3	22.5
20	148.6	148.6	148.6	148.6	148.6	82.6	N.D	148.6	82.5
21	98.9	98.9	98.9	98.9	98.9	109.0	108.4	98.9	109.0
22	120.4	120.4	120.4	120.4	120.4	82.5	81.8	120.4	82.6
23	171.5	171.5	171.5	171.5	171.5	111.7	111.3	171.5	112.0
28	80.7	77.7	80.7	80.4	80.4	77.5	76.4	76.8	80.3
29	20.3	19.3	20.4	20.4	20.5	19.3	18.6	18.9	20.4
30	25.8	24.1	25.8	23.8	23.9	23.6	23.7	23.9	23.4
1'	169.2	168.4	168.6	168.0	167.4	168.4	166.4	164.9	168.0
2'	130.8	130.7	138.8	130.9	138.9	130.8	130.5	128.1	131.0
3'	139.7	139.0	127.2	138.6	125.9	138.3	135.9	137.4	138.0
4'	14.5	12.1	18.4	14.6	18.5	12.1	14.1	14.4	12.3
5'	12.0	14.6		12.3		14.6	14.3	12.3	14.6
3-OMe		58.1				58.1	57.5		
11-OMe								56.1	
21-OMe						54.3	54.1		54.3
23-OMe						56.4	55.6		56.4

<sup>a</sup>In CD<sub>3</sub>OD; <sup>b</sup> in C<sub>5</sub>D<sub>5</sub>N; <sup>c</sup>in DMSO-*d*<sub>6</sub>



**Fig. 2** Selected 2D NMR correlations of **6**



**Fig. 3** Selected ROESY correlations of **6**

should have the same relative configuration as in **6**. Thus, the structure of **8** was proposed to be as shown in Fig. 1.

Compound **7** was revealed to have the molecular formula  $C_{32}H_{42}O_{12}$  by HRESITOFMS. Its  $^1H$  and  $^{13}C$  NMR data are highly similar to walsogyne B. However, the NMR data suggested that the furan moiety in walsogyne B was oxidized to a lactone moiety similar to the one found in **1**. Furthermore, the signals for H-2 and H-3 in walsogyne B are also not observed in **7**, and three aliphatic signals ( $\delta_H$  1.92,  $\delta_H$  1.98 and  $\delta_H$  3.57) are observed instead. Finally, the HMBC correlations of H<sub>3</sub>-29 to C-3 and H-3 to C-1 and the NOESY correlation of H-3/H<sub>3</sub>-29 confirmed the position and the  $\alpha$  orientation of the hydroxy at C-3.

Considering that **1–8** were isolated from the same extract as walsogynes B–G [4], their absolute configurations were assumed to be similar to walsogynes B–G based on the biogenetic relationships.

### Antimalarial activity

Walsogynes H–O (**1–8**) were tested for the antimalarial activity against *Plasmodium falciparum* 3D7 strain. The assay showed that **1–8** had potent in vitro antimalarial activity [the half-maximal (50%) inhibitory concentration

( $IC_{50}$ ) = 2.5, 2.6, 1.6, 2.5, 1.5, 2.6, 2.1, and 1.1  $\mu M$ , respectively] (Table 3).

From *Walsura* spp., one of limonoid peroxide has been reported to show antimalarial activity [28]. We also reported some limonoids, ceramicines A–D isolated from the barks of *C. ceramicus*, exhibited antimalarial activity against *P. falciparum* 3D7 in vitro [29]. However, the skeleton of these limonoids was different from that of walsogynes. A series of walsogynes H–O (**1–8**) and walsogynes B, D, and E ( $IC_{50}$  = 2.4, 2.6, and 2.6  $\mu M$ , respectively) had more potent antimalarial activity than these known limonoids (Table 3). The activity might be depending on their unique 11,12-*seco* limonoid skeleton but not influenced by their substituent patterns.

## Experimental section

### General experimental procedures

Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were recorded on a Shimadzu UVmini-1240 spectrophotometer and IR spectra on a JASCO FT/IR-4100 spectrophotometer. High-resolution ESI MS were obtained on a JMS-T100LP (JEOL).  $^1H$  and 2D NMR spectra were measured on a 400 MHz or 600 MHz spectrometer at 300 K, while  $^{13}C$  NMR spectra were on a 100 MHz or 150 MHz spectrometer. The residual solvent peaks were used as internal standards ( $\delta_H$  7.26 and  $\delta_C$  77.0 for  $CDCl_3$ ,  $\delta_H$  3.31 and  $\delta_C$  49.0 for  $CD_3OD$ ).

### Material

The barks of *W. chrysogyne* were collected in Mersing, Malaysia in October 2000. The botanical identification was made by Mr. Teo Leong Eng, Faculty of Science, University of Malaya. Voucher specimens (Herbarium No. 4957)

**Table 3** Antimalarial activity of **1–8** and walsogynes B, D, and E against *Plasmodium falciparum* 3D7 strain

	$IC_{50}$ ( $\mu M$ )
<b>1</b>	2.5
<b>2</b>	2.6
<b>3</b>	1.6
<b>4</b>	2.5
<b>5</b>	1.5
<b>6</b>	2.6
<b>7</b>	2.1
<b>8</b>	1.1
Walsogyne B	2.4
Walsogyne D	2.6
Walsogyne E	2.6

are deposited in the Herbarium of Chemistry Department, University of Malaya.

## Extraction and isolation

The dried ground barks of *W. chrysogyne* (440 g) were extracted successively with MeOH and 54 g of extract were obtained. The total extract was successively partitioned with *n*-hexane, EtOAc, *n*-BuOH, and water. The EtOAc-soluble materials (11.5 g) were separated with a silica gel column (CHCl<sub>3</sub>/MeOH, 1:0 → 0:1) to obtain 10 fractions (E-1 ~ E-10). Fraction E-5 was further separated to five fractions (E-5-1 ~ E-5-5) with an LH-20 column (CHCl<sub>3</sub>/MeOH, 1:1). Fraction E-5-3 was further separated with a silica gel column (CHCl<sub>3</sub>/MeOH, 1:0 → 0:1) to obtain 13 fractions (E-5-3-1 ~ E-5-3-13). Fraction E-5-3-9 was further separated by HPLC (Shiseido C18 MGII, H<sub>2</sub>O/MeCN, 70:30) to obtain impure **1–5**. Impure **1** and **3–5** were purified using HPLC (Nacalai tesque Cholesterol, H<sub>2</sub>O/MeCN, 80:20) to obtain pure **1** (1.3 mg, 0.0003%, *t*<sub>R</sub> 70 min), **3** (1.3 mg, 0.0003%, *t*<sub>R</sub> 58 min), **4** (0.5 mg, 0.0001%, *t*<sub>R</sub> 46 min) and **5** (1.8 mg, 0.0004%, *t*<sub>R</sub> 38 min). In addition, impure **2** was purified using HPLC (Nacalai tesque Cholesterol, H<sub>2</sub>O/MeCN, 75:25) to obtain pure **2** (1.8 mg, 0.0004%, *t*<sub>R</sub> 48 min). Fraction E-5-3-8 was further separated by HPLC (Shiseido C18 MGII, H<sub>2</sub>O/MeCN, 70:30) to obtain **6** (1.3 mg, 0.0003%, *t*<sub>R</sub> 26 min), **7** (2.4 mg, 0.0005%, *t*<sub>R</sub> 20 min), **8** (0.5 mg, 0.0001%, *t*<sub>R</sub> 22 min).

*Walsogyne H (1)* white amorphous solid.  $[\alpha]_D^{28} - 28$  (*c* 1.0, MeOH). IR (film)  $\nu_{\max}$  3411, 2928, 1746, 1710, 1671 cm<sup>-1</sup>. UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 216 (4.32) nm. CD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 205 (12.50), 228 (− 1.79), 250 (2.29), 300 (− 1.01) nm. ESIMS *m/z* 609 (M + Na)<sup>+</sup>. HRESIMS *m/z* 609.2335 [calcd for C<sub>31</sub>H<sub>38</sub>O<sub>11</sub>Na (M + Na)<sup>+</sup>: 609.2312].

*Walsogyne I (2)* white amorphous solid.  $[\alpha]_D^{28} - 10$  (*c* 1.0, MeOH). IR (film)  $\nu_{\max}$  3435, 2928, 1743, 1721, 1711 cm<sup>-1</sup>. UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 213 (4.52) nm. CD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 206 (4.13), 226 (− 2.08), 247 (1.36), 297 (0.25) nm. ESIMS *m/z* 641 (M + Na)<sup>+</sup>. HRESIMS *m/z* 641.2571 [calcd for C<sub>32</sub>H<sub>42</sub>O<sub>12</sub>Na (M + Na)<sup>+</sup>: 641.2574].

*Walsogyne J (3)* white amorphous solid.  $[\alpha]_D^{28} - 40$  (*c* 0.41, MeOH). IR (film)  $\nu_{\max}$  3411, 2920, 1715, 1670 cm<sup>-1</sup>. UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 211 (4.04) nm. CD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 205 (5.98), 225 (0.67), 243 (− 1.70), 304 (− 0.51) nm. ESIMS *m/z* 595 (M + Na)<sup>+</sup>. HRESIMS *m/z* 595.2160 [calcd for C<sub>30</sub>H<sub>36</sub>O<sub>11</sub>Na (M + Na)<sup>+</sup>: 595.2155].

*Walsogyne K (4)* white amorphous solid.  $[\alpha]_D^{28} - 12$  (*c* 0.7, MeOH). IR (film)  $\nu_{\max}$  3444, 2926, 1747, 1714, 1681 cm<sup>-1</sup>. UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 215 (4.09) nm. CD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 208 (8.78), 228 (− 1.41), 247 (1.77), 296 (− 0.63), 349 (0.29) nm. ESIMS *m/z* 609 (M + Na)<sup>+</sup>. HRESIMS *m/z* 609.2309 [calcd for C<sub>31</sub>H<sub>38</sub>O<sub>11</sub>Na (M + Na)<sup>+</sup>: 609.2312].

*Walsogyne L (5)* white amorphous solid.  $[\alpha]_D^{28} - 1.5$  (*c* 0.58, MeOH). IR (film)  $\nu_{\max}$  3418, 2957, 2922, 1715 cm<sup>-1</sup>. UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 209 (4.53) nm. CD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 208 (4.26), 220 (0.30), 242 (2.30), 297 (− 0.55), 350 (0.29) nm. ESIMS *m/z* 595 (M + Na)<sup>+</sup>. HRESIMS *m/z* 595.2183 [calcd for C<sub>30</sub>H<sub>36</sub>O<sub>11</sub>Na (M + Na)<sup>+</sup>: 595.2155].

*Walsogyne M (6)* white amorphous solid.  $[\alpha]_D^{18} - 21$  (*c* 0.92, MeOH). IR (film)  $\nu_{\max}$  3446, 2931, 1731, 1716, 1698 cm<sup>-1</sup>. UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 213 (4.54) nm. CD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 204 (9.84), 239 (0.038), 296 (0.97) nm. ESIMS *m/z* 705 (M + Na)<sup>+</sup>. HRESIMS *m/z* 705.3121 [calcd for C<sub>34</sub>H<sub>50</sub>O<sub>14</sub>Na (M + Na)<sup>+</sup>: 705.3109].

*Walsogyne N (7)* white amorphous solid.  $[\alpha]_D^{18} - 14$  (*c* 1.00, MeOH). IR (film)  $\nu_{\max}$  3393, 2919, 1716 cm<sup>-1</sup>. UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 211 (4.42) nm. CD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 209 (4.94), 258 (0.78), 304 (− 1.34) nm. ESIMS *m/z* 619 (M + H)<sup>+</sup>. HRESIMS *m/z* 619.2802 [(M + H)<sup>+</sup> calcd for C<sub>32</sub>H<sub>43</sub>O<sub>12</sub> (M + H)<sup>+</sup>: 619.2755].

*Walsogyne O (8)* white amorphous solid.  $[\alpha]_D^{18} - 32$  (*c* 0.33, MeOH). IR (film)  $\nu_{\max}$  3401, 2947, 1715, 1652 cm<sup>-1</sup>. UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 216 (4.51) nm. CD (MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 210 (15.32), 240 (− 1.04), 287 (− 0.50) nm. ESIMS *m/z* 673 (M + Na)<sup>+</sup>. HRESIMS *m/z* 673.2816 [calcd for C<sub>33</sub>H<sub>46</sub>O<sub>13</sub>Na (M + Na)<sup>+</sup>: 673.2836].

*Parasite strain culture P. falciparum* laboratory strain 3D7 was obtained from Prof. Masatsugu Kimura (Osaka City University, Osaka, Japan). For the assessment of anti-malarial activity of the compounds in vitro, the parasites were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 0.5 g/L L-glutamine, 5.96 g/L HEPES, 2 g/L sodium bicarbonate (NaHCO<sub>3</sub>), 50 mg/L hypoxanthine, 10 mg/L gentamicin, 10% heat-inactivated human serum, and red blood cells (RBCs) at a 3% hematocrit in an atmosphere of 5% CO<sub>2</sub>, 5% O<sub>2</sub>, and 90% N<sub>2</sub> at 37 °C as previously described [30]. Ring-form parasites were collected using the sorbitol synchronization technique [31]. Briefly, the cultured parasites were collected by centrifugation at 840 g for 5 min at room temperature, suspended in a fivefold volume of 5% D-sorbitol (Nacalai Tesque, Kyoto, Japan) for 10 min at room temperature, and then they were washed twice with RPMI 1640 medium to remove the D-sorbitol. The utilization of blood samples of healthy Japanese volunteers for the parasite culture was approved by the institutional review committee of the Research Institute for Microbial Diseases (RIMD), Osaka University (approval number: 22–3).

*Antimalarial activity* Ring-form-synchronized parasites were cultured with compounds **1–8** and walsogynes B, D, and E at sequentially decreasing concentrations (50, 15, 5, 1.5, 0.5, and 0.15 μM) for 48 h for the flow cytometric analysis using an automated hematology analyzer, XN-30. The XN-30 analyzer was equipped with a prototype algorithm for cultured falciparum parasites [prototype; software version:



01-03, (build 16)] and used specific reagents (CELLPACK DCL, SULFOLYSER, Lysercell M, and Fluorocell M) (Sysmex, Kobe, Japan) [32, 33]. Approximately 100  $\mu$ L of the culture suspension diluted with 100  $\mu$ L phosphate-buffered saline was added to a BD Microtainer MAP Microtube for Automated Process K<sub>2</sub> EDTA 1.0 mg tube (Becton Dickinson and Co., Franklin Lakes, NJ, USA) and loaded onto the XN-30 analyzer with an auto-sampler as described in the instrument manual (Sysmex). The parasitemia (MI-RBC%) was automatically reported [32]. Then 0.5% DMSO alone or containing 5  $\mu$ M artemisinin used as the negative and positive controls, respectively. The growth inhibition (GI) rate was calculated from the MI-RBC% according to the following equation:

$$\text{GI}(\%) = 100 - (\text{test sample} - \text{positive control}) / (\text{negative control} - \text{positive control}) \times 100$$

The IC<sub>50</sub> was calculated from GI (%) using GraphPad Prism version 5.0 (GraphPad Prism Software, San Diego, CA, USA) [34].

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