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# Bicyclic lactones and racemic mixtures of dimeric styrylpyrones from the leaves of *Milium velutinum*†

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A unique class of eight bicyclic lactones with a C<sub>18</sub> carbon architecture, named velutinones A–H (1–8), three new dimeric styrylpyrones, velutinindimers A–C (9–11), five known compounds, the kawapyrone, yangonin (12), three flavonoids (13–15), and an acetogenin, cananginone H (16) were isolated from the leaves of *Milium velutinum*. The absolute configurations of 2 and 5 were assigned by Mosher's method, whereas ECD, optical rotations, and X-ray crystallographic analysis indicated the racemic nature of compounds 10 and 11. Compounds 2–4 and 7–11 showed antimalarial activity with IC<sub>50</sub> values in the range of 5.4–10.0 μM. Moreover, 1–4 and 6–8 displayed cytotoxicity against the KB, MCF7, and NCI-H187 cancer cell lines and Vero cell lines with IC<sub>50</sub> values in the range of 4.0–24.1 μM.

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## 1. Introduction

*Milium velutinum* (Dunal) Hook. f. & Thomson belongs to the family Annonaceae. This plant is found widely in Thailand with local names “Khang hua mu” or “Kong kang”. A water decoction of the wood is used traditionally as a tonic and an aphrodisiac.<sup>1</sup> The genus *Milium* comprises ca. 50 species distributed from India, South East Asia, to Australia. At least 19 species of *Milium*, have been found in Thailand.<sup>2,3</sup> Eight of the *Milium* genera growing worldwide have been investigated for their phytochemistry and biological activities.<sup>4–19</sup> Among these species, a Thai medicinal plant, *M. velutinum*, has been shown to contain the acetogenin, goniiothalamusin,<sup>17</sup> an aporphine alkaloid, (+)-isocorydine  $\alpha$ -N-oxide,<sup>18</sup> and four alkaloids, reticuline, liriodenine, norcorydine, and isocorydine.<sup>19</sup> Recently, the isolation and characterization of the linear acetogenins, cananginones A–I from the stem bark of *M. velutinum* were reported.<sup>20,21</sup> In a continued investigation of this plant, the crude *n*-hexane and EtOAc extracts from the leaves of this plant were found to exhibit activity towards *Mycobacterium tuberculosis* with 99.6 and 98.9% inhibition at a concentration of 50 μg mL<sup>-1</sup>, respectively. Herein the isolation, structural identification, and bioactivities of eight new bicyclic lactones (1–8),

and three new cyclobutane dimers (9–11), as well as five known compounds (12–16) from the leaves of *M. velutinum* are discussed (Fig. 1).

## 2. Experimental section

### 2.1. General procedures

A Gallenkamp melting point apparatus (0–300 °C, 4 °C min<sup>-1</sup>, uncorrected) was used to measure melting points. Optical rotations were recorded on a JASCO P-1020 polarimeter and ECD spectra were recorded on a JASCO J-810 apparatus. UV spectra were recorded using an Agilent 8453 UV-visible spectrophotometer. FTIR spectra were recorded on a Bruker Tensor 27 spectrophotometer. The NMR spectra were acquired on a Varian Mercury Plus 400 spectrometer. HRESITOFMS spectra were recorded on a Micromass Q-TOF-2 mass spectrometer. Flash column chromatography (FCC) was performed on MERCK silica gel 60 (230–400 mesh) and LiChroprep® RP-18 (40–63 μm). Thin layer chromatography (TLC) was performed using precoated MERCK silica gel 60 PF<sub>254</sub> and RP-18 F<sub>254</sub>S.

### 2.2. Plant material

The leaves of *M. velutinum* were collected in Nam Som district, Udon Thani province, Thailand in November 2010. The identification of the plant was performed by Prof. Pranom Chantarnothai. A voucher specimen (S. Kanokmedhakul-17) was deposited at the herbarium of the Department of Biology, Faculty of Science, Khon Kaen University, Thailand. It should be noted that, in our previous report<sup>20</sup> on this plant, it was collected in different locations and was misidentified as *Cananga latifolia* because of incomplete material for species identification. It was identified based on the vegetative part (leaf and

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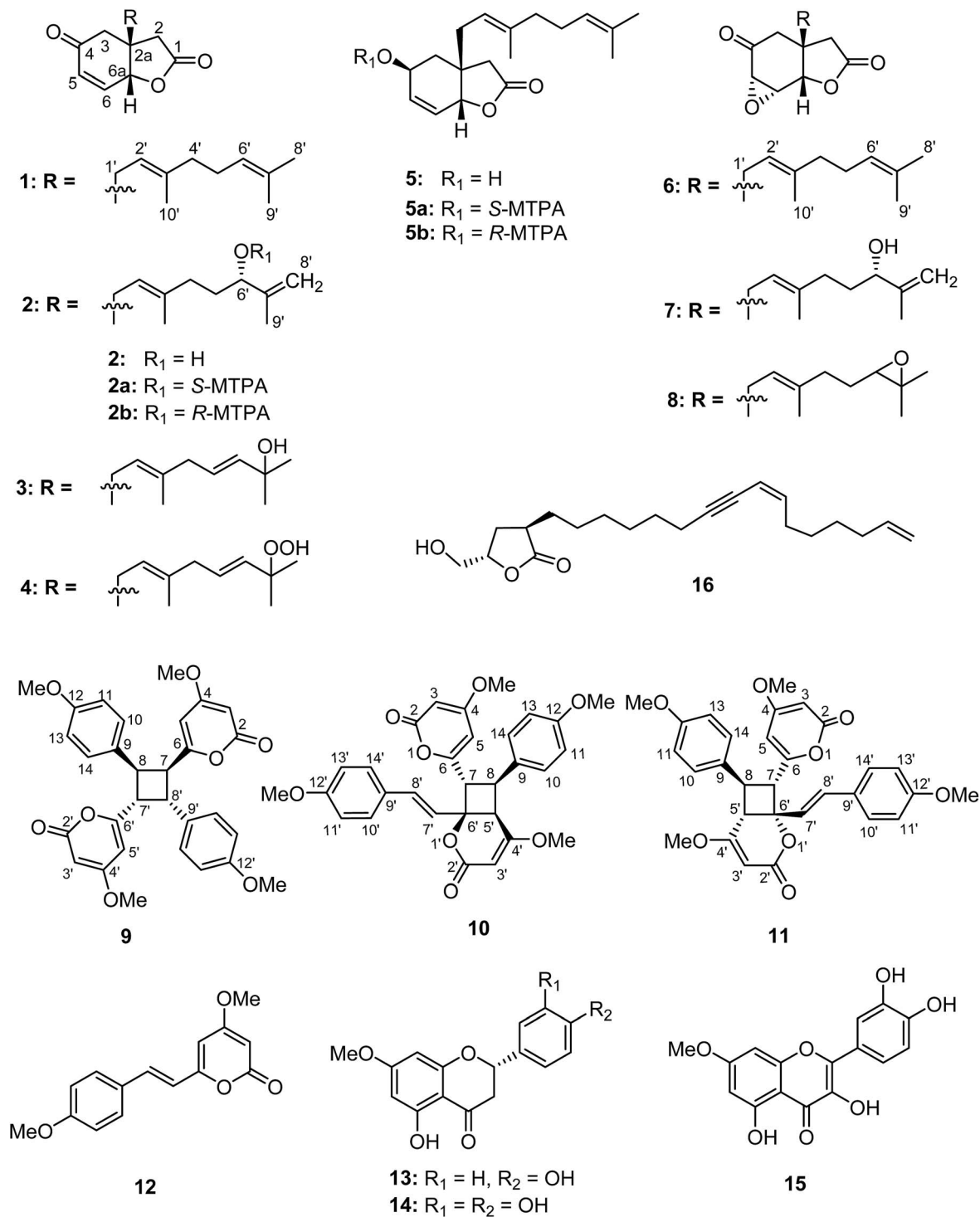


Fig. 1 Structures of isolated compounds 1–16.

stem). Later in 2015, plants with fruit from both locations were collected and the samples identified and confirmed as *Milium velutinum*, and this has been corrected as an erratum.<sup>21</sup> Since the work has been published for some time, to avoid any future confusion, the names of the new compounds have not been changed.

### 2.3. Extraction and isolation

The dried, milled leaves of *M. velutinum* (2.5 kg) were extracted with *n*-hexane (3 × 10 L) and EtOAc (3 × 10 L) to give 127 g (5.1%) and 93 g (3.7%) of *n*-hexane and EtOAc extracts, respectively. The *n*-hexane extract was separated using silica gel flash column chromatography (FCC), eluted with a gradient system of *n*-hexane–EtOAc (100 : 0, 90 : 10, 85 : 15, 70 : 30,



50 : 50, 40 : 60, 20 : 80, 0 : 100) and EtOAc–MeOH, EtOAc–MeOH (80 : 20, 50 : 50, 30 : 70, 15 : 85, 0 : 100) to afford 5 fractions, LH<sub>1</sub>–LH<sub>5</sub>. Fraction LH<sub>3</sub> (48.2 g) was chromatographed on silica gel FCC, eluting with *n*-hexane–acetone (4 : 1) to yield 3 subfractions, LH<sub>3,1</sub>–LH<sub>3,3</sub>. Subfraction LH<sub>3,2</sub> (32.8 g) was purified by LiChroprep RP-18 column chromatography, eluted with MeOH–H<sub>2</sub>O (4 : 1) to give 6 fractions, LH<sub>3,2,1</sub>–LH<sub>3,2,6</sub>. Further purification of subfraction LH<sub>3,2,1</sub> (0.48 g) by silica gel FCC, eluting with *n*-hexane–EtOAc (4 : 1) gave compounds **4** (53.8 mg) and **2** (23 mg) as colorless viscous liquids. Subfraction LE<sub>3,2,2</sub> (23.6 g) was separated on silica gel FCC, eluting with *n*-hexane–EtOAc (85 : 15) to give **1** (20.3 g) as a colorless viscous liquid. Subfraction LH<sub>3,2,3</sub> (1.7 g) was chromatographed on silica gel FCC, eluting with *n*-hexane–EtOAc (85 : 15) to give **1** (1.59 g) and **6** (57.3 mg) as colorless viscous liquids. Subfraction LH<sub>3,3</sub> (9.6 g) was purified by silica gel FCC, eluting with *n*-hexane–acetone (7 : 1) to afford 5 subfractions, LH<sub>3,3,1</sub>–LH<sub>3,3,5</sub>. Further purification of subfraction LH<sub>3,3,1</sub> (0.28 g) by silica gel FCC, eluted with *n*-hexane–EtOAc (3 : 1) gave **8** (49.8 mg) as a colorless viscous liquid. Subfraction LE<sub>3,3,2</sub> (0.49 g) was chromatographed on silica gel FCC, eluted with *n*-hexane–EtOAc (1 : 1) to afford **7** (408.5 mg) as a colorless viscous liquid. Subfraction LH<sub>3,4,4</sub> (0.68 g) was purified by silica gel FCC, eluting with *n*-hexane–EtOAc (75 : 35) to give an extra amount of **2** (551.7 mg). Subfraction LH<sub>3,4,5</sub> (0.56 g) was purified by silica gel FCC, eluting with *n*-hexane–EtOAc (1 : 1) to give **3** (507.4 mg) as a colorless viscous liquid. Fraction LH<sub>4</sub> (6.7 g) was purified by silica gel FCC, eluting with acetone–CH<sub>2</sub>Cl<sub>2</sub> (1 : 4) to yield 3 subfractions, LH<sub>4,1</sub>–LH<sub>4,3</sub>. Subfraction LH<sub>4,3</sub> (3.0 g) was chromatographed on silica gel FCC, eluted with *n*-hexane–acetone (7 : 3) to give 3 subfractions, LH<sub>4,3,1</sub>–LH<sub>4,3,3</sub>. Solid in subfraction LH<sub>4,3,3</sub> (1.6 g) was crystallized from CH<sub>2</sub>Cl<sub>2</sub>–*n*-hexane to give **12** (332 mg) as a yellow solid. Fraction LH<sub>5</sub> (1.9 g) was separated on silica gel FCC, eluted with *n*-hexane–acetone (7 : 3) to give 2 subfractions, LH<sub>5,1</sub>–LH<sub>5,2</sub>. Solid in subfraction LH<sub>5,2</sub> (0.62 g) was crystallized from MeOH–*n*-hexane to give **10** (30 mg) as colorless needles. The EtOAc extract was separated over silica gel FCC, using gradient elution with *n*-hexane–EtOAc (80 : 20 to 0 : 100) and EtOAc–MeOH (80 : 20 to 0 : 100) to afford 6 fractions, LE<sub>1</sub>–LE<sub>6</sub>. Fraction LE<sub>2</sub> (6.3 g) was then purified by silica gel FCC, eluting with *n*-hexane–acetone (4 : 1) to give 2 subfractions, LE<sub>2,1</sub>–LE<sub>2,2</sub>. Further purification of subfraction LE<sub>2,2</sub> (0.74 g) by silica gel FCC, eluting with *n*-hexane–EtOAc (4 : 1) gave **13** (552 mg) as a white solid. Fraction LE<sub>3</sub> (3.6 g) was purified by silica gel FCC, eluting with *n*-hexane–acetone (3 : 1) to give 2 subfractions, LE<sub>3,1</sub>–LE<sub>3,2</sub>. Subfraction LE<sub>3,2</sub> (0.31 g) was further separated by LiChroprep RP-18 column chromatography, eluting with MeOH–H<sub>2</sub>O (6 : 1) to give **16** (17.6 mg) as a colorless viscous liquid. Fraction LE<sub>4</sub> (12.0 g) was separated by silica gel FCC, eluting with *n*-hexane–acetone (7 : 3) to give 4 subfractions, LE<sub>4,1</sub>–LE<sub>4,4</sub>. Subfraction LE<sub>4,1</sub> (2.6 g) was purified by LiChroprep RP-18 column chromatography, eluting with MeOH–H<sub>2</sub>O (4 : 1) to give 2 subfractions, LE<sub>4,1,1</sub>–LE<sub>4,1,2</sub>. Subfraction LE<sub>4,1,1</sub> (1.8 g) was separated on silica gel FCC, eluting with MeOH–CH<sub>2</sub>Cl<sub>2</sub> (1 : 19) to give 3 subfractions, LE<sub>4,1,1,1</sub>–LE<sub>4,1,1,3</sub>. Solid in subfraction LH<sub>4,1,1,1</sub> was crystallized from MeOH to give **12** (21 mg) as a yellow solid. Subfraction LE<sub>4,1,1,2</sub>

(1.4 g) was purified by silica gel FCC, eluting with *n*-hexane–EtOAc (1 : 1) to give an additional amount of **2** (214 mg). Subfraction LE<sub>4,1,2</sub> (0.67 g) was purified by silica gel FCC, eluting with *n*-hexane–acetone (7 : 3) to give **5** (25.8 mg) as a colorless viscous liquid. Subfraction LE<sub>4,2</sub> (3.0 g) was purified by silica gel FCC, eluting with *n*-hexane–acetone (7 : 3) to give 2 subfractions, LE<sub>4,2,1</sub>–LE<sub>4,2,2</sub>. Solid in subfraction LH<sub>4,2,1</sub> was crystallized from MeOH to give an additional amount of **12** (105.3 mg). Solid in subfraction LH<sub>4,2,2</sub> (0.54 g) was crystallized from MeOH to give **14** (30 mg) as a white solid. Subfraction LE<sub>4,3</sub> (2.9 g) was purified by silica gel FCC, eluting with *n*-hexane–EtOAc (1 : 1), to give an additional amount of **3** (174 mg). Solid in subfraction LE<sub>4,4</sub> (0.12 g) was crystallized from DMF–CH<sub>2</sub>Cl<sub>2</sub> to give **15** (26 mg) as a yellow solid. Fraction LE<sub>5</sub> (8.4 g) was purified by silica gel FCC, eluting with MeOH–CH<sub>2</sub>Cl<sub>2</sub> (3 : 97) to give 2 subfractions, LE<sub>5,1</sub>–LE<sub>5,2</sub>. Solid in subfraction LE<sub>5,1</sub> (1.6 g) was crystallized from MeOH–CH<sub>2</sub>Cl<sub>2</sub> to give **10** (1.08 g) as colorless needles and the filtrate was further purified by silica gel FCC, eluting with EtOAc–CH<sub>2</sub>Cl<sub>2</sub> (15 : 85) to yield **9** (22 mg) as a white solid and **11** (75 mg) as colorless needles. Solids in subfractions LE<sub>5,2</sub> (6.2 g) and LE<sub>6</sub> (10.9 g) were crystallized from DMF–CH<sub>2</sub>Cl<sub>2</sub> to give **15** (931 mg) as a yellow solid.

**2.3.1 Velutinone A (1).** Colorless viscous liquid;  $R_f = 0.39$  (*n*-hexane–EtOAc, 7 : 3);  $[\alpha]_D^{23} -68.0$  ( $c$  0.20, CHCl<sub>3</sub>); ECD (80 μM, MeOH)  $\lambda_{max} (\Delta\epsilon)$  215 (–19.02) nm; IR (ATR)  $\nu_{max}$  2966, 2916, 2854, 1781, 1685, 1440, 1418, 1383, 1161, and 997 cm<sup>–1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 1; HRESITOFMS  $m/z$  311.1611 [M + Na]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>24</sub>O<sub>3</sub> + Na, 311.1618).

**2.3.2 Velutinone B (2).** Colorless viscous liquid;  $R_f = 0.34$  (*n*-hexane–EtOAc, 1 : 1);  $[\alpha]_D^{23} -61.2$  ( $c$  0.20, CHCl<sub>3</sub>); ECD (130 μM, MeOH)  $\lambda_{max} (\Delta\epsilon)$  215 (–28.90) nm; IR (ATR)  $\nu_{max}$  3468, 2920, 2857, 1777, 1683, 1447, 1385, 1165, 1065, 995, and 990 cm<sup>–1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 1; HRESITOFMS  $m/z$  305.1740 [M + H]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>24</sub>O<sub>4</sub> + H<sup>+</sup>, 305.1747).

**2.3.3 Velutinone C (3).** Colorless viscous liquid;  $R_f = 0.29$  (*n*-hexane–EtOAc, 1 : 1);  $[\alpha]_D^{23} -55.4$  ( $c$  0.20, CHCl<sub>3</sub>); ECD (50 μM, MeOH)  $\lambda_{max} (\Delta\epsilon)$  213 (–7.63) nm; IR (ATR)  $\nu_{max}$  3447, 2972, 2929, 1778, 1682, 1481, 1385, 1155, and 975 cm<sup>–1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 1; HRESITOFMS  $m/z$  327.1543 [M + Na]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>24</sub>O<sub>4</sub> + Na, 327.1572).

**2.3.4 Velutinone D (4).** Colorless viscous liquid;  $R_f = 0.39$  (*n*-hexane–EtOAc, 1 : 1);  $[\alpha]_D^{24} -58.4$  ( $c$  0.20, CHCl<sub>3</sub>); ECD (56 μM, MeOH)  $\lambda_{max} (\Delta\epsilon)$  213 (–9.74) nm; IR (ATR)  $\nu_{max}$  3393, 2978, 2931, 1778, 1682, 1417, 1384, 1165, and 996 cm<sup>–1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 1; HRESITOFMS  $m/z$  343.1472 [M + Na]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>24</sub>O<sub>5</sub> + Na, 343.1521).

**2.3.5 Velutinone E (5).** Colorless viscous liquid;  $R_f = 0.37$  (*n*-hexane–EtOAc, 1 : 1);  $[\alpha]_D^{23} -32.5$  ( $c$  0.20, CHCl<sub>3</sub>); ECD (62 μM, MeOH)  $\lambda_{max} (\Delta\epsilon)$  202 (–13.84) nm; IR (ATR)  $\nu_{max}$  3442, 3030, 2967, 2921, 2856, 1771, 1668, 1446, 1419, 1377, 1328, 1167, 1069, 1030, and 990 cm<sup>–1</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 2; HRESITOFMS  $m/z$  291.1935 [M + H]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>26</sub>O<sub>3</sub> + H<sup>+</sup>, 291.1955).

**2.3.6 Velutinone F (6).** Colorless viscous liquid;  $R_f = 0.50$  (*n*-hexane–EtOAc, 7 : 3);  $[\alpha]_D^{23} -29.5$  ( $c$  0.20, CHCl<sub>3</sub>); ECD (72 μM, MeOH)  $\lambda_{max} (\Delta\epsilon)$  207 (+4.83) nm; IR (ATR)  $\nu_{max}$  2967, 2917, 2855, 1789, 1719, 1423, 1377, 1347, 1161, 1035, 857, and 802 cm<sup>–1</sup>; for



Table 1  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data of 1–4 in  $\text{CDCl}_3$ 

No.	1		2		3		4	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)
1	174.2		174.3		174.2		174.4	
2	38.9	2.48, d (17.4), H $\alpha$ /2.39, d (17.4), H $\beta$	38.9	2.43, d (17.8), H $\alpha$ /2.39, d (17.8), H $\beta$	39.0	2.40, s	39.0	2.42, s
2a	44.7		44.6		44.5		44.5	
3	42.6	2.52, s	42.7	2.55, d (16.7), H $\alpha$ /2.50, d (16.7), H $\beta$	42.9	2.53, d (16.7), H $\alpha$ /2.48, d (16.7), H $\beta$	43.0	2.56, d (16.7), H $\alpha$ /2.50, d (16.7), H $\beta$
4	196.2		196.2		196.1		196.3	
5	131.2	6.17, dd (10.3, 1.2)	131.2	6.13, dd (10.3, 1.2)	131.2	6.12, dd (10.3, 1.2)	131.2	6.18, dd (10.3, 1.2)
6	141.4	6.75, dd (10.3, 3.3)	141.4 <sup>a</sup>	6.74, dd (10.3, 3.3)	141.3	6.74, dd (10.3, 3.3)	141.4	6.76, dd (10.3, 3.3)
6a	77.8	4.87, dd (3.3, 1.2)	77.9	4.87, d (3.3)	77.9	4.86, dd, (3.3, 1.2)	78.1	4.88, dd, (3.3, 1.2)
1'	36.0	2.31, dd (14.4, 7.7), H $\alpha$ /2.21, dd (14.4, 7.7), H $\beta$	36.2	2.31, dd (14.5, 7.7), H $\alpha$ /2.22, dd (14.5, 7.7), H $\beta$	36.6	2.30, dd (14.4, 7.7), H $\alpha$ /2.23, dd (14.4, 7.7), H $\beta$	36.8	2.32, dd (14.4, 7.7), H $\alpha$ /2.26, dd (14.4, 7.7), H $\beta$
2'	116.9	5.10, td (7.7, 1.4)	116.9	5.15, td (7.7, 1.4)	117.5	5.11, td (7.7, 1.4)	117.8	5.15, td (7.7, 1.4)
3'	141.5		141.4 <sup>a</sup>		140.3		140.0	
4'	39.9	2.10–2.02, m <sup>a</sup>	35.9	2.14–1.98, m	42.4	2.68, d (6.5)	42.5	2.73, d (5.5)
5'	26.2	2.10–2.02, m <sup>a</sup>	33.0	1.65–1.58, m	123.8	5.51, dt (15.6, 6.6)	128.0	5.62, dt (15.8, 6.0)
6'	123.7	5.01, t (5.4)	75.3	4.00, t (6.3)	140.2	5.60, d, (15.6)	135.9	5.56, d, (15.8)
7'	131.9		147.3		70.4		81.8	
8'	25.7	1.65, s	111.1	4.91, brs, H $\alpha$ /4.82, t (1.4), H $\beta$	29.7	1.28, s	24.3	1.31, s
9'	17.7	1.58, s	17.5	1.70, s	29.7	1.28, s	24.2	1.31, s
10'	16.3	1.60, s	16.4	1.61, s	16.5	1.58, s	16.6	1.60, s

<sup>a</sup> Overlap of the signals.Table 2  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data of 5–8 in  $\text{CDCl}_3$ 

No.	5		6		7		8	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)
1	176.2		173.8		173.9		173.7	
2	37.1 <sup>a</sup>	2.38, d (17.4), H $\alpha$ /2.19, d (17.4), H $\beta$	38.3	2.32, d (17.4), H $\alpha$ /2.28, d (17.4), H $\beta$	38.4	2.30–2.24, m <sup>a</sup>	38.4	2.30–2.24, m <sup>a</sup>
2a	42.3		47.5		47.4		47.4	
3	37.2 <sup>a</sup>	2.25–2.17, m <sup>a</sup> , H $\alpha$ /1.58–1.51, m, H $\beta$	39.9 <sup>a</sup>	2.86, d (13.9), H $\alpha$ /2.14, d (13.9), H $\beta$	39.9	2.82, d (13.9), H $\alpha$ /2.11, d (13.9), H $\beta$	39.9	2.84, d (13.9), H $\alpha$ /2.12, d (13.9), H $\beta$
4	63.7	4.31, m	204.6		204.5		204.5	
5	135.2	5.97, brd (10.2)	58.6	3.65, d (3.6)	58.5	3.62, d (3.6)	58.5	3.62, d (3.7)
6	125.2	6.75–5.65, m	54.9	3.37, d (3.6)	54.8	3.34, d (3.6)	54.8	3.34, d (3.7)
6a	80.1	4.62, m	76.6	4.71, d (3.6)	76.6	4.68, s	76.5	4.69, s
1'	36.5	2.25–2.09, m	36.9	2.29, dd (14.4, 7.7), H $\alpha$ /2.20, dd (14.4, 7.7), H $\beta$	36.8	2.25–2.09, m <sup>a</sup>	36.9 <sup>a</sup>	2.32–2.09, m <sup>a</sup>
2'	117.5	5.12, td (7.7, 1.4)	116.2	5.08, td (7.7, 1.4)	116.3	5.10, td (7.8, 1.4)	116.7	5.14, td (7.7, 1.4)
3'	140.2		141.8		141.6		141.1	
4'	39.8	2.08–1.98, m <sup>a</sup>	39.9 <sup>a</sup>	2.10–2.02, m <sup>a</sup>	35.8	2.01–1.96, m <sup>a</sup>	36.7 <sup>a</sup>	2.32–2.09, m <sup>a</sup>
5'	26.3	2.08–1.98, m <sup>a</sup>	26.2	2.10–2.02, m <sup>a</sup>	32.8			
6'	123.8	5.01, m	123.6	5.04, t (6.6)	75.1	1.64–1.55, m	27.2	1.69–1.53, m
7'	131.6		132.0		147.3	3.98, t (6.0)	63.7	3.65, t (6.7)
8'	25.6	1.63, s	25.7	1.68, s	111.0	4.79, brs, H $\alpha$ /4.89, t (0.8), H $\beta$	58.2	
9'	17.6	1.56, s	17.7	1.59, s	17.5	1.68, s	24.7	1.27, s
10'	16.2	1.58, s	16.4	1.61, s	16.4	1.60, s	18.7	1.23, s

<sup>a</sup> overlapping of the signals.

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data, see Table 2; HRESITOFMS  $m/z$  327.1562  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{18}\text{H}_{24}\text{O}_4 + \text{Na}$ , 327.1567).

**2.3.7 Velutinone G (7).** Colorless viscous liquid;  $R_f = 0.42$  (hexane–EtOAc, 1 : 3);  $[\alpha]_{\text{D}}^{23} -25.6$  ( $c$  0.20,  $\text{CHCl}_3$ ); ECD (94  $\mu\text{M}$ , MeOH)  $\lambda_{\text{max}} (\Delta\epsilon)$  205 (+11.89) nm; IR (ATR)  $\nu_{\text{max}}$  3481, 2939, 2858, 1783, 1718, 1651, 1422, 1347, 1164, 1033, and 902  $\text{cm}^{-1}$  for  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data, see Table 2; HRESITOFMS  $m/z$  321.1690  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{18}\text{H}_{24}\text{O}_5 + \text{H}^+$ , 321.1697).

**2.3.8 Velutinone H (8).** Colorless viscous liquid;  $R_f = 0.29$  ( $n$ -hexane–EtOAc, 7 : 3);  $[\alpha]_{\text{D}}^{24} -23.5$  ( $c$  0.20,  $\text{CHCl}_3$ ); ECD (75  $\mu\text{M}$ , MeOH)  $\lambda_{\text{max}} (\Delta\epsilon)$  215 (+11.84) nm; IR (ATR)  $\nu_{\text{max}}$  2962, 2924, 2855, 1785, 1719, 1423, 1378, 1163, and 1034  $\text{cm}^{-1}$ ; for  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data, see Table 2; HRESITOFMS  $m/z$  321.1690  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{18}\text{H}_{24}\text{O}_5 + \text{H}^+$ , 321.1697).

**2.3.9 Velutindimer A (9).** White solid; mp 201–203  $^\circ\text{C}$ ;  $R_f = 0.13$  ( $n$ -hexane–EtOAc, 1 : 1);  $[\alpha]_{\text{D}}^{28} + 0.08$  ( $c$  0.63, MeOH– $\text{CHCl}_3$ , 3 : 1); ECD (26  $\mu\text{M}$ , MeOH)  $\lambda_{\text{max}} (\Delta\epsilon)$  285 (0.00) nm; UV (MeOH)  $\lambda_{\text{max}} (\log \epsilon)$  226 (4.53), 285 (4.20); IR (ATR)  $\nu_{\text{max}}$  3088, 2944, 2837, 1715, 1643, 1611, 1564, 1513, 1455, 1409, 1249, 1180, 1034, and 818  $\text{cm}^{-1}$ ; for  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data, see Table 3; HRESITOFMS  $m/z$  539.1651  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{30}\text{H}_{28}\text{O}_8 + \text{Na}$ , 539.1682).

**2.3.10 Velutin dimer B (10).** Colorless needles; mp 205–207  $^\circ\text{C}$ ;  $R_f = 0.21$  ( $n$ -hexane–EtOAc, 1 : 1);  $[\alpha]_{\text{D}}^{28} + 0.08$  ( $c$  0.63,

MeOH– $\text{CHCl}_3$ , 5 : 3); ECD (33  $\mu\text{M}$ , MeOH)  $\lambda_{\text{max}} (\Delta\epsilon)$  265 (0.00) nm; UV (MeOH)  $\lambda_{\text{max}} (\log \epsilon)$  265 (4.45); IR (ATR)  $\nu_{\text{max}}$  2940, 2838, 1699, 1647, 1608, 1566, 1512, 1455, 1410, 1391, 1246, 1176, 1031 and 814  $\text{cm}^{-1}$ ; for  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data, see Table 3; HRESITOFMS  $m/z$  539.1666  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{30}\text{H}_{28}\text{O}_8 + \text{Na}$ , 539.1682).

**2.3.11 Velutin dimer C (11).** Colorless needles; mp 207–209  $^\circ\text{C}$ ;  $R_f = 0.18$  ( $n$ -hexane–EtOAc, 1 : 1);  $[\alpha]_{\text{D}}^{27} + 0.03$  ( $c$  0.23, MeOH– $\text{CHCl}_3$ , 9 : 1); ECD (22  $\mu\text{M}$ , MeOH)  $\lambda_{\text{max}} (\Delta\epsilon)$  268 (0.00) nm; UV (MeOH)  $\lambda_{\text{max}} (\log \epsilon)$  268 (4.59); IR (ATR)  $\nu_{\text{max}}$  2924, 2837, 1708, 1649, 1625, 1608, 1567, 1513, 1455, 1406, 1248, 1176, 1031, and 821  $\text{cm}^{-1}$ ; for  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data, see Table 3; HRESITOFMS  $m/z$  539.1664  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{30}\text{H}_{28}\text{O}_8 + \text{Na}$ , 539.1682).

## 2.4. Preparation of the (*R*)- and (*S*)- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl) phenyl acetate of 2

The determination of configuration for the stereogenic carbinol carbons was carried out following the method reported by Ohtani *et al.*<sup>22</sup> A solution of (*S*)-MPTA-Cl (10  $\mu\text{L}$ , 53.4  $\mu\text{mol}$ ) was added to a solution mixture of 2 (8 mg, 27.5  $\mu\text{mol}$ ) and DMAP (5 mg) in dry  $\text{CH}_2\text{Cl}_2$  (1 mL) and stirred under  $\text{N}_2$  at room temperature for 6 h. Then the solvent was removed *in vacuo*. The

Table 3  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data of 9–11 in  $\text{CDCl}_3$

No. position	9		10		11	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ ( <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ ( <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ ( <i>J</i> in Hz)
2	164.0		163.9		164.1	
3	87.7	5.21, d (2.2)	88.6	5.33, d (2.2)	88.9	5.45, d (2.2)
4	170.1		170.5		170.6	
5	101.3	5.71, d (2.2)	102.5	5.89, d (2.2)	102.0	5.99, d (2.2)
6	162.9		158.9		158.7	
7	43.0	4.35, dd (10.0, 7.6)	55.0	4.09, d (10.8)	54.5	3.64, d (10.3)
8	45.5	4.16, dd (10.0, 7.6)	38.7	4.26, dd (10.8, 9.9)	45.6	4.00, dd (10.3, 9.7)
9	129.4		127.7		131.4	
10	128.5	7.19, d (8.7)	128.6	7.16, d (8.7)	127.4	7.22, d (8.6)
11	113.9	6.82, d (8.7)	113.8	6.85, d (8.7)	114.2	6.89, d (8.6)
12	158.6		159.2		159.0	
13	113.9	6.82, d (8.7)	113.8	6.85, d (8.7)	114.2	6.89, d (8.6)
14	128.5	7.19, d (8.7)	128.6	7.16, d (8.7)	127.4	7.22, d (8.6)
2'	164.0		164.7		165.4	
3'	87.7	5.21, d (2.2)	91.7	5.29, s	89.2	5.19, s
4'	170.1		170.1		171.4	
5'	101.3	5.71, d (2.2)	45.8	3.55, d (9.9)	44.2	3.20, d (9.7)
6'	162.9		79.4		82.6	
7'	43.0	4.35, dd (10.0, 7.6)	122.2	6.42, d (15.8)	125.2	6.18, d (15.9)
8'	45.5	4.16, dd (10.0, 7.6)	130.8	6.86, d (15.8)	131.3	6.63, d (15.9)
9'	129.4		127.7		127.9	
10'	128.5	7.19, d (8.7)	128.1	7.34, d (8.7)	128.8	7.33, d (8.7)
11'	113.9	6.82, d (8.7)	114.1	6.85, d (8.7)	114.0	6.84, d (8.7)
12'	158.6		159.7		159.9	
13'	113.9	6.82, d (8.7)	114.1	6.85, d (8.7)	114.0	6.84, d (8.7)
14'	128.5	7.19, d (8.7)	128.1	7.34, d (8.7)	128.8	7.33, d (8.7)
4-OMe	55.7	3.75, s	55.8	3.69, s	55.8	3.76, s
12-OMe	55.2	3.67, s	55.3	3.79, s	55.27	3.79, s
4'-OMe	55.7	3.75, s	55.5	3.32, s	56.1	3.77, s
12'-OMe	55.2	3.67, s	55.3	3.78, s	55.30	3.80, s



residue was separated on preparative TLC (CH<sub>2</sub>Cl<sub>2</sub>) to give the (*R*)-ester (**2a**, 7 mg, 50%). The (*S*)-ester of **2** was prepared using the procedure described above [alcohol **2** (10 mg, 34.4 μmol), CH<sub>2</sub>Cl<sub>2</sub> (1 mL), dimethylaminopyridine (5 mg), and (*R*)-MPTA-Cl (10 μL, 53.4 μmol)] to yield (*S*)-ester (**2b**, 12 mg, 71%).

**2.4.1 Compound 2a.** Colorless viscous liquid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 6.67 (1H, dd, *J* = 10.3, 3.3 Hz, H-6), 6.19 (1H, dd, *J* = 10.3, 1.2 Hz, H-5), 5.37 (1H, dt, *J* = 12.0, 6.4, 6.4 Hz, H-6'), 5.07 (1H, dt, *J* = 7.6, 1.1 Hz, H-2'), 5.02 (brs, H-8'a), 4.98 (1H, brs, H-8'b), 4.87 (1H, dd, *J* = 3.3, 1.2, H-6a), 2.56 (1H, d, *J* = 16.7 Hz, H-3α), 2.51 (1H, d, *J* = 16.6 Hz, H-3β), 2.46 (1H, d, *J* = 17.4 Hz, H-2α), 2.39 (1H, d, *J* = 17.4 Hz, H-2β), 2.32 (1H, dd, *J* = 14.0, 7.7 Hz, H-1'a), 2.22 (1H, dd, *J* = 14.6, 7.6 Hz, H-1'b), 2.05–1.65 (4H, m, H-4' and 5'), 1.57 (3H, s, H-9'), 1.72 (3H, s, H-10'), [the α-methoxy-α-(trifluoromethyl)phenyl acetate portion exhibited δ 7.50 and 7.40 (5H, m, C<sub>6</sub>H<sub>5</sub>), 3.52 (3H, s, OCH<sub>3</sub>)].

**2.4.2 Compound 2b.** Colorless viscous liquid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 6.76 (1H, dd, *J* = 10.3, 3.5 Hz, H-6), 6.19 (1H, brs, *J* = 10.3 Hz, H-5), 5.37 (1H, ddd, *J* = 17.5, 6.7, 5.5 Hz, H-6'), 5.05 (1H, dt, *J* = 14.3, 7.7, 7.7 Hz, H-2'), 5.02 (1H, brs, H-8'a), 4.98 (1H, brs, H-8'b), 4.87 (1H, dd, *J* = 3.3, 1.2 Hz, H-6a), 2.56 (1H, d, *J* = 16.7 Hz, H-3α), 2.51 (1H, *J* = 16.7 Hz, H-3β), 2.46 (1H, d, *J* = 17.3, Hz, H-2α), 2.39 (1H, d, *J* = 17.3, Hz, H-2β), 2.30 (1H, dd, *J* = 14.2, 7.8 Hz, H-1'a), 2.22 (1H, dd, *J* = 14.2, 7.6 Hz, H-1'b), 2.09–1.90 (2H, m, H-4'), 1.90–1.65 (2H, m, H-5'), 1.72 (3H, s, H-10'), 1.59 (3H, s, H-9'), [the α-methoxy-α-(trifluoromethyl)phenyl acetate portion exhibited δ 7.50 and 7.41 (5H, m, C<sub>6</sub>H<sub>5</sub>), 3.52 (3H, s, OCH<sub>3</sub>)].

## 2.5. Preparation of the (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl) phenyl acetate of (**5**)

The esterification of **5** was carried out using the procedure described for the preparation of **2a** and **2b** to yield (*S*)-ester (**5a**, 7 mg, (50%)) and (*R*)-ester (**5b**, 12 mg, (71%)).

**2.5.1 Compound 5a.** Colorless viscous liquid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 6.03 (1H, d, *J* = 11.4 Hz, H-5), 5.97 (1H, dd, *J* = 11.4, 1.7 Hz, H-6), 5.65 (1H, t, *J* = 5.8 Hz, H-4), 5.07 (1H, t, *J* = 7.6 Hz, H-2'), 5.01 (1H, t, *J* = 6.6 Hz, H-6'), 4.64 (1H, brs, H-6a), 2.41 (1H, *J* = 17.4 Hz, H-2α), 2.35 (1H, *J* = 17.4 Hz, H-2β), 2.21 (1H, dd, *J* = 14.4, 8.0 Hz, H-1'a), 2.18 (1H, dd, *J* = 13.9, 4.8 Hz, H-3β), 2.14 (1H, dd, *J* = 14.4, 7.4 Hz, H-1'b), 2.08–1.98 (4H, m, H-4' and 5'), 1.71 (1H, dd, *J* = 13.9, 7.9 Hz, H-3α), 1.65 (3H, s, H-8'), 1.58 (3H, s, H-10'), 1.55 (3H, s, H-9'), [the α-methoxy-α-(trifluoromethyl)phenyl acetate portion exhibited δ 7.50 and 7.41 (5H, m, C<sub>6</sub>H<sub>5</sub>), 3.55 (3H, s, OCH<sub>3</sub>)].

**2.5.2 Compound 5b.** Colorless viscous liquid; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 5.95 (1H, d, *J* = 11.0 Hz, H-5), 5.92 (1H, d, *J* = 11.0 Hz, H-6), 5.66 (1H, t, *J* = 6.8 Hz, H-4), 5.12 (1H, t, *J* = 7.7 Hz, H-2'), 5.03 (1H, t, *J* = 6.6 Hz, H-6'), 4.63 (1H, brs, H-6a), 2.44 (1H, *J* = 17.4 Hz, H-2α), 2.35 (1H, *J* = 17.4 Hz, H-2β), 2.24 (1H, dd, *J* = 14.6, 5.5 Hz, H-1'a), 2.21 (1H, dd, *J* = 14.0, 4.3 Hz, H-3β), 2.18 (1H, dd, *J* = 14.6, 7.6 Hz, H-1'b), 2.09–2.02 (4H, m, H-4' and 5'), 1.82 (1H, dd, *J* = 14.0, 8.2 Hz, H-3α), 1.65 (3H, s, H-8'), 1.59 (3H, s, H-10'), 1.57 (3H, s, H-9'), [the α-methoxy-α-(trifluoromethyl)phenyl acetate portion exhibited δ 7.50 and 7.41 (5H, m, C<sub>6</sub>H<sub>5</sub>), 3.54 (3H, s, OCH<sub>3</sub>)].

## 2.6. X-ray crystallographic analyses of **10** and **11**

The reflection data were collected on a Bruker D8 Quest PHOTON100 CMOS detector with graphite-monochromated MoKα radiation using the APEX2 program.<sup>23</sup> Raw data frame integration was performed with SAINT,<sup>23</sup> which also applied correction for Lorentz and polarization effects. An empirical absorption correction using the SADABS program<sup>23</sup> was applied. The structure was solved by direct methods and refined by full-matrix least-squares method on *F*<sup>2</sup> with anisotropic thermal parameters for all non-hydrogen atoms using the SHELXTL software package.<sup>24</sup> All hydrogen atoms were placed in calculated positions and refined isotropically. Crystallographic data of **10** and **11** were deposited with the following Cambridge Crystallographic Data Centre codes: CCDC 1415288 and CCDC 1062125, respectively.

**2.6.1 Crystal data of 10.** C<sub>30</sub>H<sub>28</sub>O<sub>8</sub> (*M* = 516.52 g mol<sup>-1</sup>): orthorhombic, space group *Pna*2<sub>1</sub> (no. 33), *a* = 23.725(4) Å, *b* = 5.5211(10) Å, *c* = 39.408(7) Å, *V* = 5162.0(16) Å<sup>3</sup>, *Z* = 8, *T* = 293(2) K, μ(MoKα) = 0.096 mm<sup>-1</sup>, *D*<sub>calc</sub> = 1.329 g cm<sup>-3</sup>, 135 470 reflections measured (6.204° ≤ 2θ ≤ 52.798°), 10 518 unique (*R*<sub>int</sub> = 0.0929, *R*<sub>sigma</sub> = 0.0445) which were used in all calculations. The final *R*<sub>1</sub> was 0.0508 (*I* > 2σ(*I*)) and *wR*<sub>2</sub> was 0.1225 (all data).

**2.6.2 Crystal data of 11.** C<sub>31</sub>H<sub>29</sub>Cl<sub>3</sub>O<sub>8</sub>, MW = 635.89 g mol<sup>-1</sup>, triclinic, space group *P*1̄, *a* = 11.6555(5) Å, *b* = 12.1165(5) Å, *c* = 12.1203(5) Å, α = 113.9720(10), β = 102.4530(10), γ = 92.9570(10), *V* = 1508.54(11) Å<sup>3</sup>, *Z* = 2, *T* = 293(2) K, μ(MoKα) = 0.354 mm<sup>-1</sup>, *D*<sub>calc</sub> = 1.400 g cm<sup>-3</sup>, 61 914 reflections measured, 7472 unique (*R*<sub>int</sub> = 0.0262) which were used in all calculations. The final *R*<sub>1</sub> was 0.0642 (*I* > 2σ(*I*)) and *wR*<sub>2</sub> was 0.2211 (all data).

## 2.7. Antimalarial assay

Antimalarial activity was performed against *P. falciparum* (K1, multidrug resistant strain, see ESI†), using the method of Trager and Jensen.<sup>25</sup> 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.*<sup>26</sup> The inhibitory concentration (IC<sub>50</sub>) represents the concentration that causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [<sup>3</sup>H]-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin.

## 2.8. Antimycobacterial assay

Antimycobacterial activity was performed against *M. tuberculosis* H37Ra (purchased from ATCC) using the MicroplateAlamar Blue Assay (MABA).<sup>27</sup> The standard drug streptomycin was used as reference substance.

## 2.9. Cytotoxicity assay

Cytotoxicity assays against human epidermoid carcinoma (KB), human breast cancer (MCF7), and human small cell lung cancer (NCI-H187) cell lines human breast adenocarcinoma Resazurin microplate assay described by O'Brien and co-workers.<sup>28</sup> The reference substances were ellipticine and doxorubicin. Cytotoxicity test against primate cell line (Vero) was performed using



the green fluorescent protein detection method described by Hunt and co-workers.<sup>29</sup> The reference substances used were ellipticine and doxorubicin. All cells were purchased from ATCC.

### 3. Results and discussion

Chromatographic fractionation of *n*-hexane and EtOAc extracts yielded eight new bicyclic lactones, velutinones A–H (**1–8**), three cyclobutane dimers, velutinindimers A–C (**9–11**), and four known compounds (**12–15**), kawapyrone, yangonin (**12**),<sup>30</sup> three flavonoids, sakuranetin (**13**),<sup>31</sup> 7-*O*-methylerythrodityol (**14**)<sup>32</sup> and rhamnetin (**15**),<sup>33</sup> and an acetogenin, cananginone H (**16**)<sup>20,21</sup> (Fig. 1).

The IR spectra of **1–4** showed absorption bands of a  $\gamma$ -lactone moiety at (1789–1771  $\text{cm}^{-1}$ ) and a conjugated carbonyl functionality (1685–1862  $\text{cm}^{-1}$ ) similar to the absorption bands of a synthetic bicyclic cyclohexenone.<sup>34,35</sup>

Compound **1** possessed the molecular formula  $\text{C}_{18}\text{H}_{24}\text{O}_3$  based on the  $^{13}\text{C}$  NMR and HRESITOFMS ( $m/z$  311.1611  $[\text{M} + \text{Na}]^+$ ) data, indicating seven indices of hydrogen deficiency. The  $^1\text{H}$  NMR data (Table 1) had resonances at  $\delta$  2.48 (d,  $J = 17.4$  Hz, H-2 $\alpha$ ), 2.39 (d, 17.4 Hz, H-2 $\beta$ ), 2.52 (s, 2H, H-3), 6.17 (dd,  $J = 10.3, 1.2$  Hz, H-5), 6.75 (dd,  $J = 10.3, 3.3$  Hz, H-6), and 4.87 (dd,  $J = 3.3, 1.2$  Hz, H-6a). The  $^{13}\text{C}$  NMR data (Table 1), DEPT, and HMQC experiments indicated seven resonances which were associated with an  $\alpha, \beta$ -unsaturated carbonyl ( $\delta$  196.2/C-4), a lactone carbonyl ( $\delta$  174.2/C-1), two olefinic ( $\delta$  131.2/C-5 and 141.4/C-6), two methylene ( $\delta$  38.9/C-2 and 42.6/C-3), one methine ( $\delta$  77.8/C-6a), and one quaternary ( $\delta$  44.7/C-2a) carbons. Interpretation of the COSY and HMBC correlations (Fig. 2) indicated that **1** has a core structure of a five-membered lactone ring fused to an  $\alpha, \beta$ -unsaturated cyclohexenone ring. This arrangement is similar to that of a compound isolated from the fruit kernels of *Otoba parvifolia*<sup>35,36</sup> and from a total synthesis of its core structure,<sup>34</sup> except for the side chain at C-2a which was replaced by a geranyl moiety in **1**. This geranyl side chain ( $\text{C}_{10}\text{H}_{17}$ ) was evident from the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data (Table 1). The COSY spectrum showed the connectivity of the geranyl side chain by correlations between H-1' and H-2', and amongst H-4', H-5' and H-6'. The HMBC spectrum exhibited correlations of H-1' to C-2, C-3, C-2a, C-6a, C-2' and C-3'; H-2' to C-2a, C-1', C-3', C-4', and C-10'; H-4' to C-2', C-3', C-5', C-6' and C-10'; H-6' to C-4', C-5', C-8', and C-9' indicating that the geranyl group was linked to the stereogenic quaternary carbon C-2a (Fig. 2). The relative configuration at C-2a and C-6a was

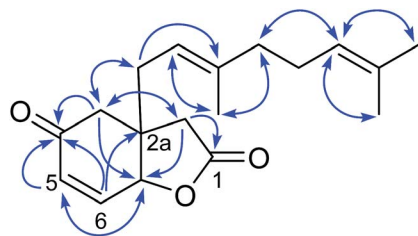


Fig. 2 Selected HMBC correlations of **1**.

established as *syn* from the NOESY correlation of H-6a and H-1'. Based on the above evidence, the structure of **1**, velutinone A, was defined as shown in Fig. 1.

Compound **2** had the molecular formula  $\text{C}_{18}\text{H}_{24}\text{O}_4$  derived from the  $^{13}\text{C}$  NMR and HRESITOFMS ( $m/z$  305.1740  $[\text{M} + \text{H}]^+$ ) data, demonstrating the same index of hydrogen deficiency, but having one additional oxygen atom compared to **1**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data of **2** (Table 1) were similar to those of **1**, except for the geranyl side chain being oxidized at C-6' and having a 7', 8' terminal double bond. The NMR spectroscopic data displayed resonances for an olefinic methylene protons at  $\delta_{\text{H}}$  4.82 (t,  $J = 1.4$  Hz, 4.91 (brs), H-8')/ $\delta_{\text{C}}$  111.1 and an oxymethine at  $\delta_{\text{H}}$  4.00 (t,  $J = 6.3$  Hz, H-6')/ $\delta_{\text{C}}$  75.3. The HMBC correlations of H-5' to C-3', C-4', C-6', and C-7'; H-6' to C-4', C-5', C-7', C-8', and C-9'; and H-8' to C-6', C-7', and C-9' confirmed the position of the terminal olefinic moiety and the hydroxy group in the side chain. The assignment of the (6'*S*) absolute configuration was done *via* the modified Mosher's ester method<sup>22</sup> (Fig. 3). Therefore, the structure of compound **2**, velutinone B, was defined as shown in Fig. 1.

Compound **3** had the molecular formula  $\text{C}_{18}\text{H}_{24}\text{O}_4$ , deduced from  $^{13}\text{C}$  NMR and HRESITOFMS ( $m/z$  327.1543  $[\text{M} + \text{Na}]^+$ ) data, implying the same index of hydrogen deficiency, but having one additional oxygen atom compared to **1**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data of **3** (Table 1) were similar to those of **1**, except for the appearance of the olefinic protons at  $\delta_{\text{H}}$  5.51 (dt,  $J = 15.6, 6.6$  Hz, H-5') and  $\delta_{\text{H}}$  5.60 (d,  $J = 15.6$  Hz, H-6'), and one additional oxygenated carbon signal at  $\delta_{\text{C}}$  70.4 (C-7'). The position of the hydroxy group at C-7' on the side chain was confirmed by the HMBC correlations of H-5' to C-3', C-4', C-6', and C-7'; and H-6' to C-4', C-5', C-7' and C-8'/C-9'. Therefore, the structure of compound **3**, velutinone C, was defined as shown (Fig. 1).

Compound **4** had the molecular formula  $\text{C}_{18}\text{H}_{24}\text{O}_5$  derived from the  $^{13}\text{C}$  NMR and HRESITOFMS ( $m/z$  343.1472  $[\text{M} + \text{Na}]^+$ ) data, demonstrating the same index of hydrogen deficiency, but having one additional oxygen atom compared to **3**. The NMR spectroscopic data of **4** (Table 1) was similar to that of **3**, except for the resonance of a C-7' hydroxy group. The  $^{13}\text{C}$  NMR spectrum revealed the unusual downfield oxygenated carbon signal at  $\delta_{\text{C}}$  81.8 for C-7', suggesting the presence of a hydroperoxy group.<sup>37</sup> Thus the structure of compound **4**, velutinone D, was determined as shown (Fig. 1).

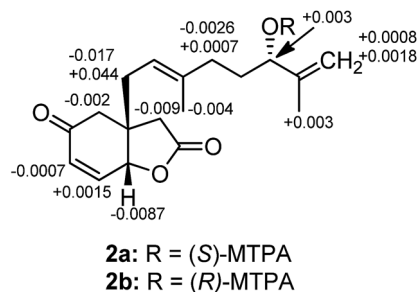


Fig. 3  $\Delta\delta$  values ( $\Delta\delta = \delta_{\text{S}} - \delta_{\text{R}}$  in ppm) obtained for MTPA esters **2a** and **2b**.



Compound **5** had the molecular formula  $C_{18}H_{26}O_3$  derived from the  $^{13}C$  NMR and HRESITOFMS ( $m/z$  291.1935  $[M + Na]^+$ ) data, implying six indices of hydrogen deficiency. The IR spectrum showed hydroxy ( $3442\text{ cm}^{-1}$ ) and  $\gamma$ -lactone carbonyl ( $1771\text{ cm}^{-1}$ ) functionalities. The NMR data of **5** (Table 2) corresponded to those of **1**, except that the ketone carbonyl resonance for C-4 was replaced by a resonance for an oxymethine group at  $\delta_H$  4.31 (m)/ $\delta_C$  63.7. The assignment of (4*S*) absolute configuration was done *via* the modified Mosher's ester method<sup>22</sup> (Fig. 4). The configurations of (2*aR* and 6*aR*) were assigned by NOESY and a molecular modeling study. NOESY correlations were observed between H-4 and H-2 $\alpha$ , H-6*a* and H-1'*a*, H-6*a* and H-3 $\beta$ , H-1'*b* and H-2 $\beta$ , and H-3 $\alpha$  and H-2' (Fig. 5). Therefore, the structure of compound **5**, velutinone E, was established as shown (Fig. 1).

Compound **6** had the molecular formula  $C_{18}H_{24}O_4$  derived from the  $^{13}C$  NMR and HRESITOFMS ( $m/z$  327.1562  $[M + Na]^+$ ) data, implying the same index of hydrogen deficiency, but having one more oxygen atom than **1**. The IR spectrum showed bands for  $\gamma$ -lactone ( $1789\text{ cm}^{-1}$ ) and a cyclohexanone ( $1719\text{ cm}^{-1}$ ) groups. The NMR data of **6** (Table 2) was similar to that of **1**, except for the resonances of the C-5/6 double bond which were replaced by those of an epoxide moiety [ $\delta_H$  3.65 (d,  $J = 3.6\text{ Hz}$ , H-5)/ $\delta_C$  58.6 and  $\delta_H$  3.37 (d,  $J = 3.6\text{ Hz}$ , H-6)/ $\delta_C$  54.9]. The relative configuration of the epoxide was assigned by the NOESY correlations between H-6 and H-6*a*. Hence, the structure of compound **6**, velutinone F, was defined as shown (Fig. 1).

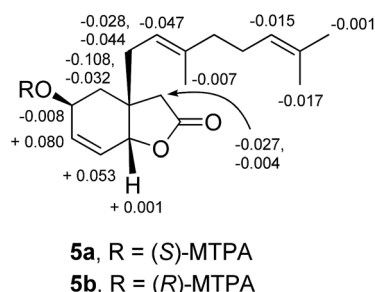


Fig. 4  $\Delta\delta$  values ( $\Delta\delta = \delta_S - \delta_R$  in ppm) obtained for MTPA esters **5a** and **5b**.

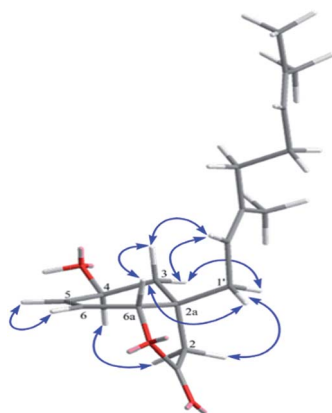


Fig. 5 Key NOESY correlations of **5**, energy minimized using MM2.

Compound **7** had the molecular formula  $C_{18}H_{24}O_5$ , established from the  $^{13}C$  NMR and HRESITOFMS ( $m/z$  321.1690  $[M + Na]^+$ ) data, having the same index of hydrogen deficiency as in **2**. The IR spectrum displayed an extra hydroxyl band at ( $3481\text{ cm}^{-1}$ ) which differed from that of **6**. The NMR data of **7** (Table 2) corresponded to that of **2**, except for the resonances of a C-5/6 double bond, which was replaced by resonances for an epoxide moiety [ $\delta_H$  3.62 (d,  $J = 3.6\text{ Hz}$ , H-5)/ $\delta_C$  58.5 and  $\delta_H$  3.34 (d,  $J = 3.6\text{ Hz}$ , H-6)/ $\delta_C$  54.8] as in that of **6**. The 6'*S* configuration was assigned by comparison of the NMR data to that of **2**. Thus the structure of **7**, velutinone G, was defined as shown (Fig. 1).

Compound **8** possessed the molecular formula  $C_{18}H_{24}O_5$  from the  $^{13}C$  NMR and HRESITOFMS ( $m/z$  321.1690  $[M + H]^+$ ) data, having seven indices of hydrogen deficiency as in **6**, but having one additional oxygen. The IR spectrum was also similar to that of **6**. The NMR data of **8** (Table 2) corresponded to that of **6**, except for the resonances for a C-6'/7' double bond which were replaced by resonances for an epoxide [ $\delta_H$  3.65 (t,  $J = 6.7\text{ Hz}$ , H-6'/ $\delta_C$  63.7 and  $\delta_C$  58.2, C-7')] at this position. The configuration at C-6' of **8** was proposed to be *S*, based on its ring opening to give **7**. Hence, structure of **8**, velutinone H, was designated as shown in Fig. 1.

There are only two closely related bicyclic lactones, panamonons A and B,<sup>38</sup> which have NMR data similar to those of compounds **1–8**. However, the relative configuration at the ring junction (C-2*a* and C-6*a*) of **1–8** were assigned as 2*aR* and 6*aR* which different from panamonons A and B (2*aR* and 6*aS*).<sup>38</sup> The ECD spectra of compounds **1–4**, containing an  $\alpha,\beta$ -unsaturated ketone, and compound **5**, showed negative Cotton effects in the range of 202–215 nm. While, compounds **6–8** contained a saturated ketone, exhibited positive Cotton effects in the range of 205–215 nm (Fig. 6).

To confirm the natural occurrence of **1–8**, the isolates velutinone A (**1**) with a geranyl side chain and velutinone H (**8**) with two epoxide rings were stirred with or without silica gel in EtOAc and MeOH for 4 days following the conditions of the separation process. No change on TLC was observed. We conclude that isolates **1–8** are natural occurring products not artefacts.

The putative biosynthetic pathway towards compounds **1–8** is shown in Fig. 7. The precursor, homogentisic acid,<sup>35,36</sup> could be prenylated by geranyl diphosphate to form intermediate **A**<sup>38</sup> which may be reduced and lactonized to form compound **1**. Reduction of **1** would produce **5**, while epoxidation of **1** would afford **6** or intermediate **B**. Oxidation of **5** would give **4** which could be reduced to give **3** or **B**, the latter *via* intermediate **C**. Protonation and deprotonation of **B** may give **2** and **3**. The hydroxy group of **3** could be oxidized to give **4**. Compound **8** could be derived from **6** or **B** *via* an oxidation reaction. Further protonation and deprotonation of **8** would give **7**.

Compound **9** showed an  $[M + Na]^+$  ion peak at  $m/z$  539.1651 in its HRESITOFMS, which in conjunction with the  $^{13}C$  NMR data indicated the molecular formula  $C_{30}H_{28}O_8$ , requiring seventeen indices of hydrogen deficiency. The IR spectrum showed bands for unsaturated lactone ( $1715\text{ cm}^{-1}$ ) and aromatic ( $1643\text{ cm}^{-1}$ ) groups. The UV spectrum also indicated an aromatic moiety ( $286\text{ nm}$ ). Since the NMR spectroscopic data





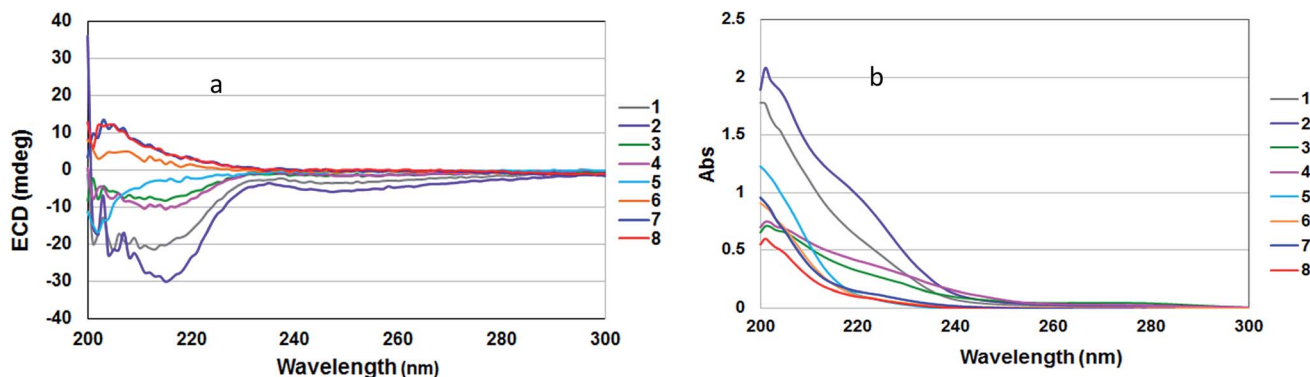


Fig. 6 ECD (a) and UV (b) spectra (in MeOH) of compounds 1–8.

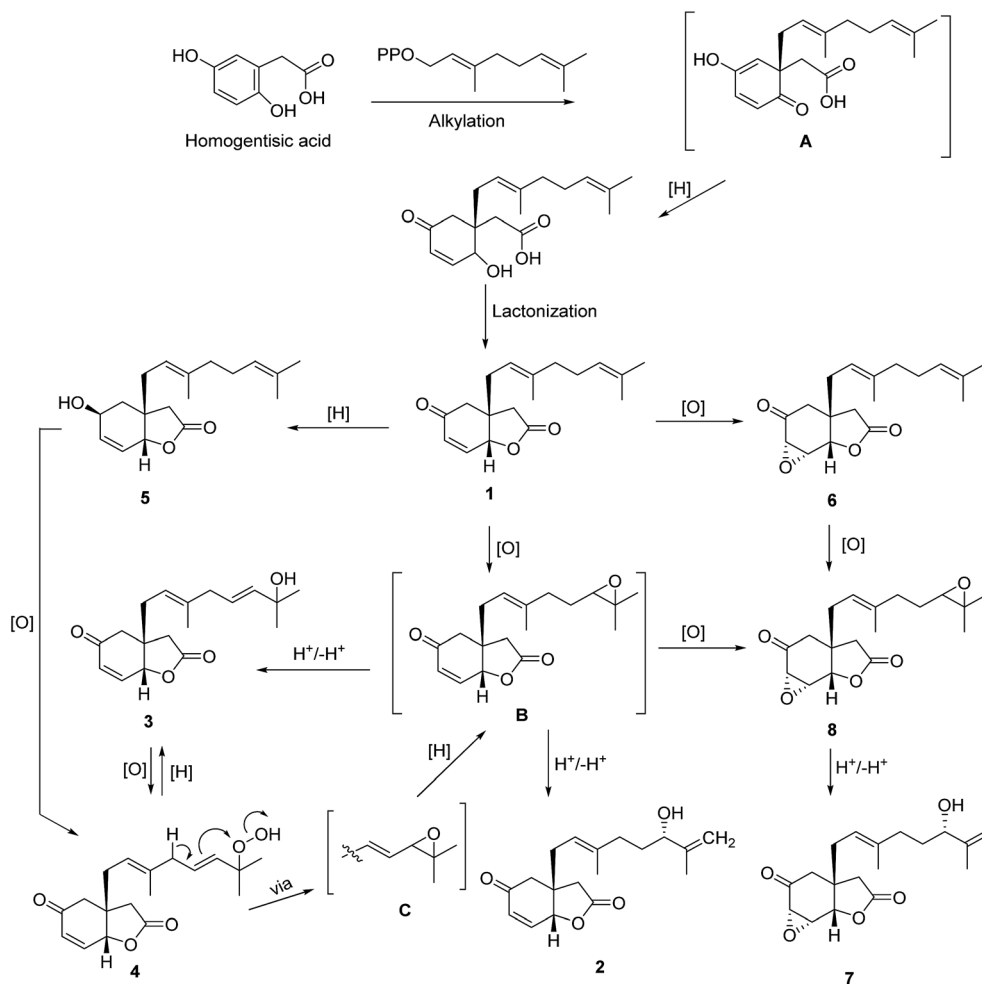


Fig. 7 Plausible biogenetic pathway of 1–8.

of 9 (Table 3) displayed half the number of resonance signals expected for 28 protons and 30 carbons, the structure should be a symmetrical dimer. These NMR resonances corresponded to those of the cyclobutane dimer, achyrodimer A,<sup>39</sup> except for hydroxy groups at C-12 and C-12' of the aromatic rings which were replaced by methoxy groups. The <sup>1</sup>H NMR spectroscopic data (Table 3) showed resonances for the *para*-substituted

benzene rings at  $\delta$  6.82 and 7.19 (each 4H, d,  $J = 8.7$  Hz), four methines of the cyclobutyl ring at  $\delta$  4.16 and 4.35 (each 2H, dd,  $J = 7.6, 10.0$  Hz), four olefinic protons for the two  $\alpha$ -pyrone moieties at  $\delta$  5.21 and 5.71 (each 2H, d,  $J = 2.2$  Hz), and four methoxy groups at  $\delta$  3.75 and 3.67 (each 6H, s). The <sup>13</sup>C NMR data (Table 3) showed resonances for the *para*-disubstituted benzene rings at  $\delta$  129.4 (C-9, 9'), 128.5 (C-10, 10' and C-14, 14'),



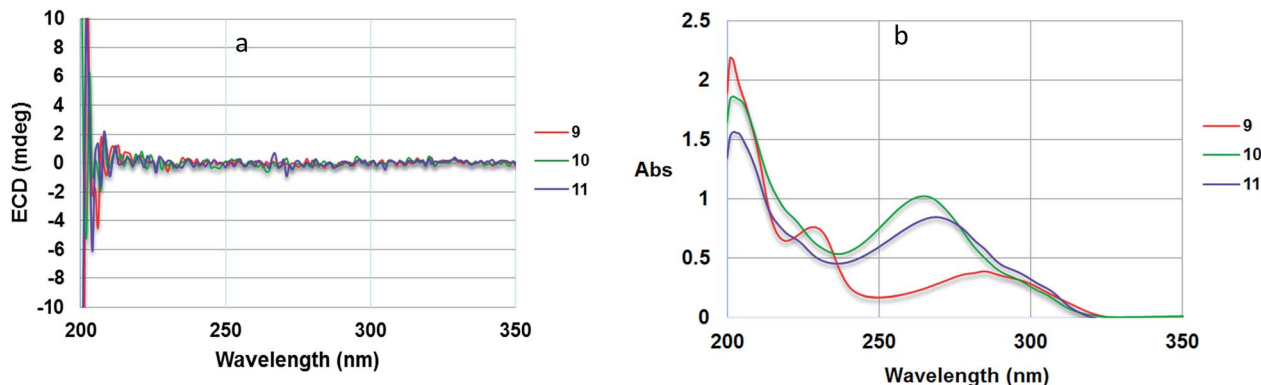


Fig. 8 ECD (a) and UV (b) spectra (in MeOH) of compounds **9**, **10** and **11**.

and 113.9 (C-11, 11'), the cyclobutyl methines at  $\delta$  43.0 (C-7, 7'), and 45.5 (C-8, 8') the  $\alpha$ -pyrone methines at  $\delta$  87.7 (C-3, 3'), 101.3 (C-5, 5') and methoxy groups at  $\delta$  55.7 (4, 4'-OMe) and 55.2 (12, 12'-OMe), and a carbonyl group at 162.9 (C-6, 6'). The correlations of H-7/7' to C-5/5', C-6/6', C-8/8', and C-9/9', and of H-8/8' to C-6/6', C-7/7', C-10/10', and C-14/14' from the HMBC spectrum revealed the connection of a cyclobutane ring to an  $\alpha$ -pyrone ring, and benzene rings at C-7/7' and C-8/8', respectively. Resonances for two sets of methoxy protons at  $\delta_{\text{H}}$  3.75 and 3.67 showed correlations with C-4/4' and C-12/12', respectively, confirming the location of methoxy groups at C-4/4' and C-12/12'. The correlations between H-7 (7') and H-8 (8') in the NOESY spectrum indicated the relative configuration on the cyclobutane ring as reported for achyrodimer A.<sup>39</sup> The specific rotation value of **9** was almost zero [ $+0.08$  ( $c$  0.63, MeOH-CHCl<sub>3</sub>; 3 : 1)] which was also the same as that reported for a symmetrical achyrodimer A.<sup>39</sup> Moreover, the ECD spectrum of **9** showed no signal for a Cotton effect (Fig. 8).<sup>40</sup> Based on this evidence the structure of compound **9** could contain a plane of symmetry. Hence, **9** was concluded to be a new symmetrical cyclobutane dimer of the isolated styrylpyrone, yangonin (**12**), and it was named velutinindimer A.

Compound **10** possessed the molecular formula C<sub>30</sub>H<sub>28</sub>O<sub>8</sub> from the <sup>13</sup>C NMR and HRESITOFMS ( $m/z$  539.1666 [M + Na]<sup>+</sup>) data, having the same index of hydrogen deficiency as **9**. The IR spectrum displayed bands for lactone (1699 cm<sup>-1</sup>) and aromatic (1647 cm<sup>-1</sup>) groups. The UV spectrum also supported an aromatic moiety (268 nm). The <sup>1</sup>H NMR data of **10** (Table 3) showed resonances for two *para*-disubstituted benzene rings at  $\delta$  6.85, 7.16, and 7.34 and 6.85 (each 2H, d,  $J$  = 8.7 Hz), an *E*-double bond at  $\delta$  6.42 and 6.86 (each 1H, d,  $J$  = 15.8 Hz),  $\alpha$ -pyrone ring at  $\delta$  5.33 and 5.89 (each 1H, d,  $J$  = 2.2 Hz, H-3 and H-5, respectively) and 5.29 (s, H-3'), three methine protons at  $\delta$  3.55 (d,  $J$  = 9.9 Hz, H-5'), 4.26 (dd,  $J$  = 10.8, 9.9 Hz, H-8), 4.09 (d,  $J$  = 10.8 Hz, H-7), and four methoxy groups at  $\delta$  3.32, 3.69, 3.78, and 3.79. The <sup>13</sup>C NMR spectrum, DEPT and HMQC experiments of **10** showed 30 resonances, including two sets of *p*-disubstituted benzene rings, one  $\alpha$ -pyrone ring, one olefinic, one cyclobutane ring, and four methoxy carbons. The HMBC spectrum displayed <sup>3</sup> $J$  correlations of H-3 to C-5; H-5 to C-3, and C-7; H-7 to C-5, C-9, C-5', and C-7'; H-8 to C-6, C-10, C-14, C-4',

and C-6'; H-10, 14 to C-8, and C-12; H-11, 13 to C-9; H-3' to C-5'; H-5' to C-7, C-9, C-3' and C-7'; H-7' to C-7, C-5', and C-9'; H-8' to C-6', C-10', and C-14'; H-10', 14' to C-8' and C-12'; H-11', 13' to C-9' and C-12'; 4-OMe to C-4; 12-OMe to C-12; 4'-OMe to C-4'; and 12'-OMe to C-12' confirming the structure of **10** (Fig. 9). The NMR data of **10** was comparable to the cyclobutane dimer achyrodimer D, reported from the aerial parts of *Achyrocline bogotensis*.<sup>39</sup> It was found that **10** was the methoxy derivative of achyrodimer D. The relative configuration of **10** was determined from the relatively large coupling constants (9.9–10.8 Hz) between H-7 and H-8, and H-8 and H-5', and the NOESY correlations between H-8 and H-5', H-7 and H-14, H-8 and H-10, H-5 and H-7, H-7' and H-10', and H-8' and H-14'. The magnitude of the coupling constant between H-7 and H-8 ( $J_{\text{trans}} = 10.8$  Hz), and

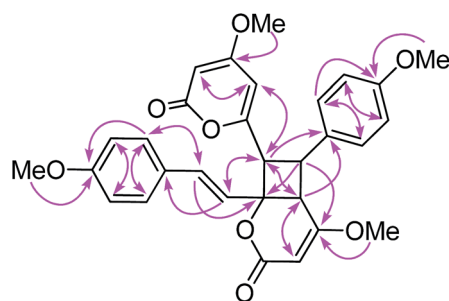


Fig. 9 Selected HMBC correlations of **10**.

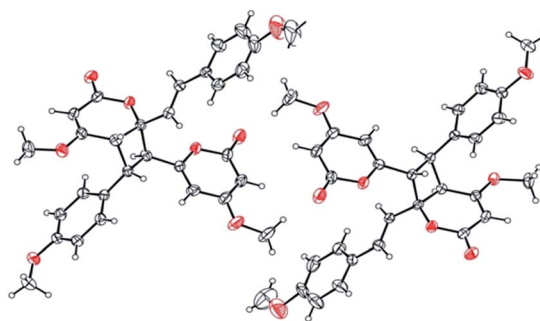


Fig. 10 ORTEP plot of the asymmetric units in **10**. The thermal ellipsoids are shown at 40% probability.



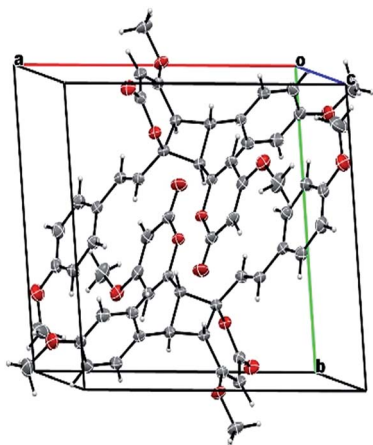


Fig. 11 Packing structure of **11** showing the inverted racemic mixture. The chloroform solvate molecule is omitted for clarity.

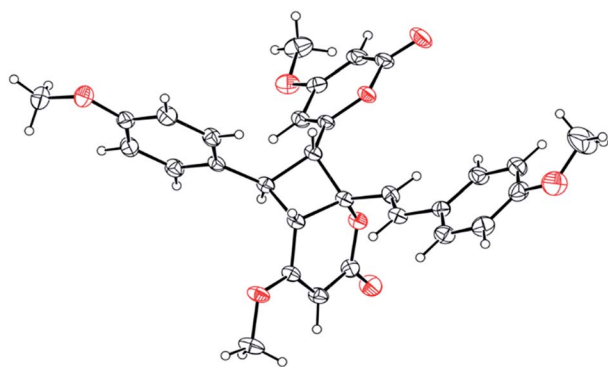


Fig. 12 ORTEP plot of the asymmetric unit in **11**. The thermal ellipsoids are shown at 30% probability level. The chloroform solvate molecule is omitted for clarity.

H-8 and H-5' ( $J_{\text{cis}} = 9.9$  Hz) could be correlated to the dihedral angle between those protons, which corresponded with the values from the Karplus equation for a four membered ring.<sup>41</sup>

The ECD measurement of **10** in MeOH showed no signal of the Cotton effect<sup>40</sup> (Fig. 8), and also the specific rotation value of **10** was almost zero (+0.08). These could suggest that compound **10** was a racemic mixture. Finally, the X-ray crystallographic analysis supported the structure of an isolated **10** containing asymmetric units of a racemic mixture (Fig. 10), and the one with the relative configuration (5'*S*, 6'*R*, 7*S*, and 8*S*) is shown in Fig. 1. Thus, the structure of **10** was an unsymmetrical cyclobutane dimer of the isolated yangonin (**12**), and it was named velutinindimer B.

Compound **11** exhibited an  $[M + Na]^+$  peak at  $m/z$  539.1664 in the positive HRESITOFMS corresponding to the molecular formula  $C_{30}H_{28}O_8Na$ , the same as that of **10**. The IR spectrum showed bands for a lactone moiety ( $1708\text{ cm}^{-1}$ ) and an aromatic ring ( $1649\text{ cm}^{-1}$ ). The UV spectrum indicated an aromatic moiety (273 nm). The NMR data (Table 3) and 2D NMR of **11** demonstrated a similar structure to a dimeric **10**. Nevertheless, slight differences in chemical shifts around compounds **11** and **10** in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data at positions 7, 8, 9, 5', 7' and 8' (Table 3) suggested different configurations at the cyclobutane ring between the two compounds. The large coupling constant of H-7 and H-8 ( $J_{\text{trans}} = 10.3$  Hz) and H-8 and H-5' ( $J_{\text{trans}} = 9.7$  Hz) could be explained in the same way as for **10**.<sup>41</sup> The NOESY spectrum displayed correlations of H-5' and H-7, H-7 and H-14, H-8 and H-10, H-7' and H-10', and H-8' and H-14', indicating the relative configuration of **11**. Compound **11** also showed no signal of the Cotton effect (Fig. 7)<sup>40</sup> and also its specific rotation value was almost zero (+0.3) suggesting that it should be a racemic mixture as compound **10**. The X-ray crystallographic analysis confirmed that the isolated compound **11** was a racemic mixture as in **10**, and the one with the relative configuration 5'*R*, 6'*S*, 7*R*, and 8*S* is shown in Fig. 1, 11 and 12. From the above evidence, the structure of **11** was determined to be another new dimeric styrylpyrone and it was named velutinindimer C.

To confirm the natural occurrence of styrylpyrone dimers, **9**–**11**, the isolated yangonin (**12**), was stirred with silica gel in EtOAc and MeOH for a week, following the conditions for our

Table 4 Biological activities of the isolated compounds

Compound	Antimalarial	Anti-TB	Cytotoxicity ( $\text{IC}_{50}$ , $\mu\text{M}$ )			
	( $\text{IC}_{50}$ , $\mu\text{M}$ )	(MIC, $\mu\text{M}$ )	KB <sup>a</sup>	MCF7 <sup>b</sup>	NCI-H187 <sup>c</sup>	Vero cell <sup>d</sup>
<b>1</b>	Inactive	43.4	4.0	4.8	4.2	5.8
<b>2</b>	9.6	82.1	9.6	12.9	6.5	8.8
<b>3</b>	10.0	Inactive	12.9	10.9	11.4	10.3
<b>4</b>	9.6	Inactive	10.5	15.2	8.7	11.7
<b>6</b>	Inactive	Inactive	14.5	20.7	11.5	11.2
<b>7</b>	9.8	Inactive	24.1	21.0	14.7	17.9
<b>8</b>	7.3	Inactive	10.5	11.9	6.8	18.2
<b>9</b>	6.4	Inactive	Inactive	Inactive	Inactive	Inactive
<b>10</b>	5.4	Inactive	Inactive	Inactive	Inactive	Inactive
<b>11</b>	5.8	Inactive	Inactive	Inactive	Inactive	Inactive
Dihydroartemisinin	0.004					
Streptomycin		0.3–0.5				
Ellipticine			0.8		5.6	3.1
Doxorubicin			0.3	0.1	0.01	

<sup>a</sup> Human oral epidermoid carcinoma. <sup>b</sup> Human breast cancer cells. <sup>c</sup> Human lung cancer cells. <sup>d</sup> Normal African green monkey kidney cells.



separation process. The formation of **9**, **10** and **11** was not observed. It is worth noting that isolated dimers **10** and **11** were racemic mixtures occurring from asymmetrical 2 + 2 cycloaddition of the isolated styrylpyrone, yangonin (**12**), while compound **9** has an axis of a symmetric dimer and so is not chiral.

The biological activities of the isolated compounds (purity > 95% from the NMR spectra) are shown in Table 4. Compounds **1** and **2**, with MIC values of 43.4 and 82.1  $\mu\text{M}$  respectively, should be responsible for the antimycobacterial activity against *M. tuberculosis* exhibited in the primary screening. From this result, the  $\alpha,\beta$ -unsaturated carbonyl in the core structure plays an important role against TB, while the hydroxy or peroxide functionalities at C-6' or C-7' reduce this activity. Since our previous work reported the antimalarial activity and cytotoxicity of compounds from *M. velutina*,<sup>20,21</sup> the compounds isolated herein have been further evaluated for their activities. Compounds **2–4** and **7–11** displayed antimalarial activity toward *P. falciparum* with IC<sub>50</sub> values in the range of 5.4–10.0  $\mu\text{M}$ . In addition, compounds **1–4** and **6–8** exhibited cytotoxicity against three cancer cell lines tested, with IC<sub>50</sub> values in the range of 4.0–24.1  $\mu\text{M}$ . Among these, **1**, **2** and **8** exhibited moderate cytotoxicity against NCI-H187 cell lines with IC<sub>50</sub> values of 4.2, 6.5 and 6.8  $\mu\text{M}$ , respectively, which were close to the standard drug, ellipticine (5.6  $\mu\text{M}$ ). Compounds **1–4** and **6–8** exhibited cytotoxicity towards the Vero cell line with IC<sub>50</sub> values in the range of 5.8–18.2  $\mu\text{M}$ . However, dimeric styrylpyrones **9–11** showed no cytotoxicity in the test.

## 4. Conclusions

Isolation of the leaves extracts of *M. velutina* yielded an unique class of eight bicyclic lactones with a C<sub>18</sub> carbons architecture, named velutinones A–H (**1–8**), three new dimeric styrylpyrones, velutinindimers A–C (**9–11**), five known compounds, the kawapryrone, yangonin (**12**), three flavonoids (**13–15**), and an acetogenin, cananginone H (**16**). Velutinindimers A–C (**9–11**) are dimers occurring from symmetrical and asymmetrical 2 + 2 cycloaddition of the isolated styrylpyrone, yangonin (**12**). The structures of velutinindimers B and C (**10** and **11**) were identified as mixtures which were confirmed by X-ray crystallographic, ECD and specific rotation analyses. Biological activity of the isolated compounds had been evaluated. Compounds **2–4** and **7–11** showed antimalarial activity with IC<sub>50</sub> values in the range of 5.4–10.0  $\mu\text{M}$ . Moreover, **1–4** and **6–8** displayed cytotoxicity against the KB, MCF7, and NCI-H187 cancer cell lines and Vero cell lines with IC<sub>50</sub> values in the range of 4.0–24.1  $\mu\text{M}$ .

## Acknowledgements

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