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Bicyclic lactones and racemic mixtures of dimeric styrylpyrones from the leaves of *Miliusa velutina*†

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A unique class of eight bicyclic lactones with a C_{18} 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 *Miliusa velutina*. 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_{50} 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_{50} values in the range of 4.0–24.1 μM.

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1. Introduction

Miliusa velutina (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.[1] The genus *Miliusa* comprises *ca.* 50 species distributed from India, South East Asia, to Australia. At least 19 species of *Miliusa*, have been found in Thailand.[2,3] Eight of the *Miliusa* genera growing worldwide have been investigated for their phytochemistry and biological activities.[4–19] Among these species, a Thai medicinal plant, *M. velutina*, has been shown to contain the acetogenin, goniothalamusin,[17] an aporphine alkaloid, (+)-isocorydine α-*N*-oxide,[18] and four alkaloids, reticuline, liriodenine, norcorydine, and isocorydine.[19] Recently, the isolation and characterization of the linear acetogenins, cananginones A–I from the stem bark of *M. velutina* were reported.[20,21] 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^{-1}, 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. velutina* are discussed (Fig. 1).

2. Experimental section

2.1. General procedures

A Gallenkamp melting point apparatus (0–300 °C, 4 °C min^{-1}, 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_{254} and RP-18 $F_{254}S$.

2.2. Plant material

The leaves of *M. velutina* were collected in Nam Som district, Udon Thani province, Thailand in November 2010. The identification of the plant was performed by Prof. Pranom Chantaranothai. 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[20] 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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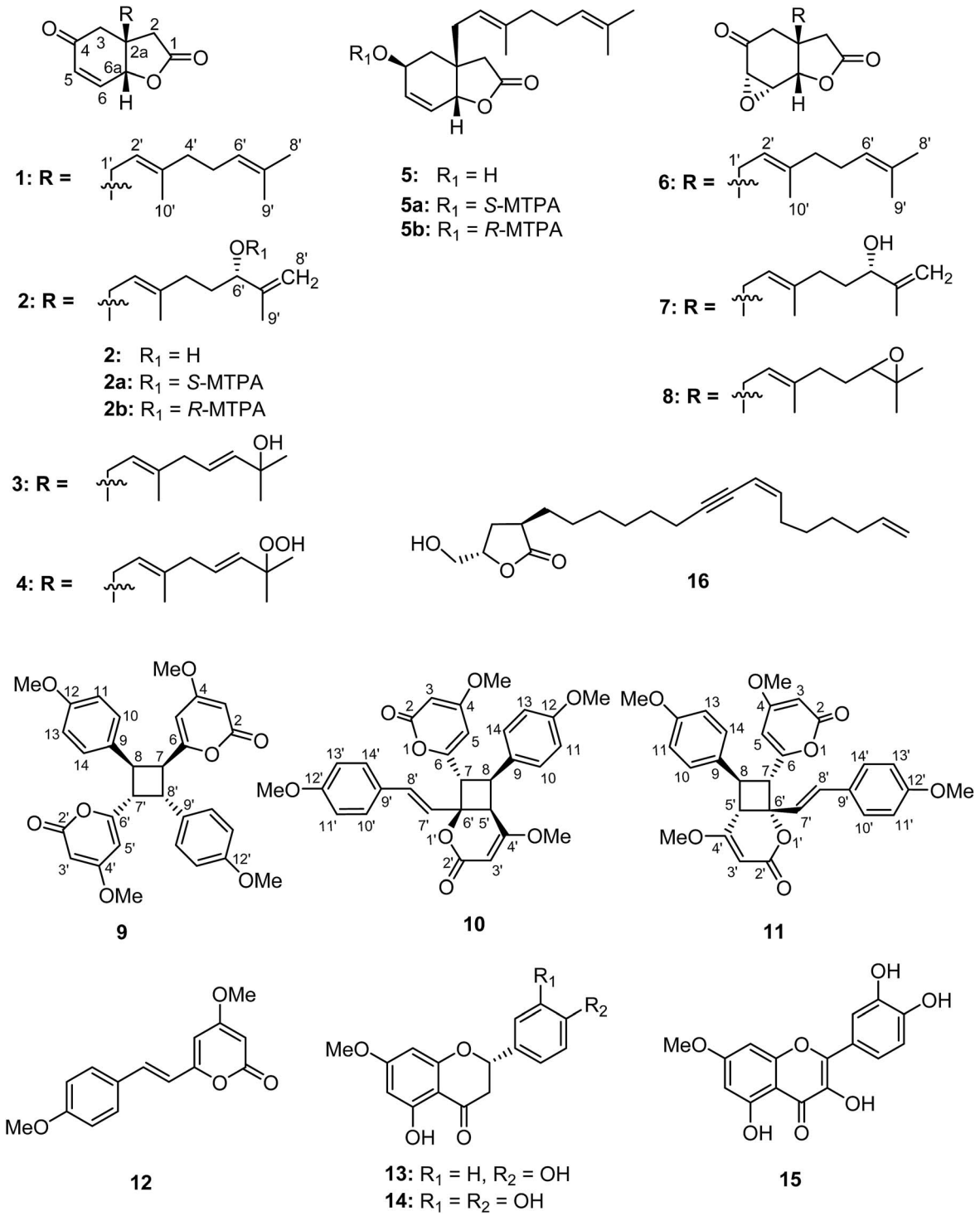

Fig. 1 Structures of isolated compounds **1–16**.

stem). Later in 2015, plants with fruit from both locations were collected and the samples identified and confirmed as *Miliusa velutina*, and this has been corrected as an erratum.[21] 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. velutina* (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,

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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_1–LH_5. Fraction LH_3 (48.2 g) was chromatographed on silica gel FCC, eluting with *n*-hexane–acetone (4 : 1) to yield 3 subtractions, $LH_{3.1}$–$LH_{3.3}$. Subfraction $LH_{3.2}$ (32.8 g) was purified by LiChroprep RP-18 column chromatography, eluted with MeOH–H_2O (4 : 1) to give 6 fractions, $LH_{3.2.1}$–$LH_{3.2.6}$. Further purification of subfraction $LH_{3.2.1}$ (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 $LE_{3.2.2}$ (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 $LH_{3.2.3}$ (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 $LH_{3.3}$ (9.6 g) was purified by silica gel FCC, eluting with *n*-hexane–acetone (7 : 1) to afford 5 subfractions, $LH_{3.3.1}$–$LH_{3.3.5}$. Further purification of subfraction $LH_{3.3.1}$ (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 $LE_{3.3.2}$ (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 $LH_{3.4.4}$ (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 $LH_{3.4.5}$ (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_4 (6.7 g) was purified by silica gel FCC, eluting with acetone-CH_2Cl_2 (1 : 4) to yield 3 subfractions, $LH_{4.1}$–$LH_{4.3}$. Subfraction $LH_{4.3}$ (3.0 g) was chromatographed on silica gel FCC, eluted with *n*-hexane–acetone (7 : 3) to give 3 subfractions, $LH_{4.3.1}$–$LH_{4.3.3}$. Solid in subfraction $LH_{4.3.3}$ (1.6 g) was crystallized from CH_2Cl_2-*n*-hexane to give **12** (332 mg) as a yellow solid. Fraction LH_5 (1.9 g) was separated on silica gel FCC, eluted with *n*-hexane–acetone (7 : 3) to give 2 subfractions, $LH_{5.1}$–$LH_{5.2}$. Solid in subfraction $LH_{5.2}$ (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_1–LE_6. Fraction LE_2 (6.3 g) was then purified by silica gel FCC, eluting with *n*-hexane–acetone (4 : 1) to give 2 subfractions, $LE_{2.1}$–$LE_{2.2}$. Further purification of subfraction $LE_{2.2}$ (0.74 g) by silica gel FCC, eluting with *n*-hexane–EtOAc (4 : 1) gave **13** (552 mg) as a white solid. Fraction LE_3 (3.6 g) was purified by silica gel FCC, eluting with *n*-hexane–acetone (3 : 1) to give 2 subfractions, $LE_{3.1}$–$LE_{3.2}$. Subfraction $LE_{3.2}$ (0.31 g) was further separated by LiChroprep RP-18 column chromatography, eluting with MeOH–H_2O (6 : 1) to give **16** (17.6 mg) as a colorless viscous liquid. Fraction LE_4 (12.0 g) was separated by silica gel FCC, eluting with *n*-hexane–acetone (7 : 3) to give 4 subfractions, $LE_{4.1}$–$LE_{4.4}$. Subfraction $LE_{4.1}$ (2.6 g) was purified by LiChroprep RP-18 column chromatography, eluting with MeOH–H_2O (4 : 1) to give 2 subfractions, $LE_{4.1.1}$–$LE_{4.1.2}$. Subfraction $LE_{4.1.1}$ (1.8 g) was separated on silica gel FCC, eluting with MeOH–CH_2Cl_2 (1 : 19) to give 3 subfractions, $LE_{4.1.1.1}$–$LE_{4.1.1.3}$. Solid in subfraction $LH_{4.1.1.1}$ was crystallized from MeOH to give **12** (21 mg) as a yellow solid. Subfraction $LE_{4.1.1.2}$ (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 $LE_{4.1.2}$ (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 $LE_{4.2}$ (3.0 g) was purified by silica gel FCC, eluting with *n*-hexane–acetone (7 : 3) to give 2 subfractions, $LE_{4.2.1}$–$LE_{4.2.2}$. Solid in subfraction $LH_{4.2.1}$ was crystallized from MeOH to give an additional amount of **12** (105.3 mg). Solid in subfraction $LH_{4.2.2}$ (0.54 g) was crystallized from MeOH to give **14** (30 mg) as a white solid. Subfraction $LE_{4.3}$ (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 $LE_{4.4}$ (0.12 g) was crystallized from DMF–CH_2Cl_2 to give **15** (26 mg) as a yellow solid. Fraction LE_5 (8.4 g) was purified by silica gel FCC, eluting with MeOH–CH_2Cl_2 (3 : 97) to give 2 subfractions, $LE_{5.1}$–$LE_{5.2}$. Solid in subfraction $LE_{5.1}$ (1.6 g) was crystallized from MeOH–CH_2Cl_2 to give **10** (1.08 g) as colorless needles and the filtrate was further purified by silica gel FCC, eluting with EtOAc–CH_2Cl_2 (15 : 85) to yield **9** (22 mg) as a white solid and **11** (75 mg) as colorless needles. Solids in subfractions $LE_{5.2}$ (6.2 g) and LE_6 (10.9 g) were crystallized from DMF-CH_2Cl_2 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, $CHCl_3$); ECD (80 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 215 (−19.02) nm; IR (ATR) ν_{max} 2966, 2916, 2854, 1781, 1685, 1440, 1418, 1383, 1161, and 997 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESITOFMS *m*/*z* 311.1611 $[M + Na]^+$ (calcd for $C_{18}H_{24}O_3$ + Na, 311.1618).

2.3.2 Velutinone B (2). Colorless viscous liquid; $R_f = 0.34$ (*n*-hexane–EtOAc, 1 : 1); $[\alpha]_D^{24}$ −61.2 (*c* 0.20, $CHCl_3$); ECD (130 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 215 (−28.90) nm; IR (ATR) ν_{max} 3468, 2920, 2857, 1777, 1683, 1447, 1385, 1165, 1065, 995, and 990 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESITOFMS *m*/*z* 305.1740 $[M + H]^+$ (calcd for $C_{18}H_{24}O_4 + H^+$, 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, $CHCl_3$); ECD (50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 213 (−7.63) nm; IR (ATR) ν_{max} 3447, 2972, 2929, 1778, 1682, 1481, 1385, 1155, and 975 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESITOFMS *m*/*z* 327.1543 $[M + Na]^+$ (calcd for $C_{18}H_{24}O_4$ + 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, $CHCl_3$); ECD (56 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 213 (−9.74) nm; IR (ATR) ν_{max} 3393, 2978, 2931, 1778, 1682, 1417, 1384, 1165, and 996 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESITOFMS *m*/*z* 343.1472 $[M + Na]^+$ (calcd for $C_{18}H_{24}O_5$ + 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, $CHCl_3$); ECD (62 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−13.84) nm; IR (ATR) ν_{max} 3442, 3030, 2967, 2921, 2856, 1771, 1668, 1446, 1419, 1377, 1328, 1167, 1069, 1030, and 990 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 2; HRESITOFMS *m*/*z* 291.1935 $[M + H]^+$ (calcd for $C_{18}H_{26}O_3 + H^+$, 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, $CHCl_3$); ECD (72 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 207 (+4.83) nm; IR (ATR) ν_{max} 2967, 2917, 2855, 1789, 1719, 1423, 1377, 1347, 1161, 1035, 857, and 802 cm^{-1}; for

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Table 1 ^{1}H and ^{13}C NMR spectroscopic data of **1–4** in $CDCl_3$

	1		**2**		**3**		**4**	
No.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	174.2		174.3		174.2		174.4	
2	38.9	2.48, d (17.4), Hα/2.39, d (17.4), Hβ	38.9	2.43, d (17.8), Hα/2.39, d (17.8), Hβ	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), Hα/2.50, d (16.7), Hβ	42.9	2.53, d (16.7), Hα/2.48, d (16.7), Hβ	43.0	2.56, d (16.7), Hα/2.50, d (16.7), Hβ
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[a]	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), Ha/2.21, dd (14.4, 7.7), Hb	36.2	2.31, dd (14.5, 7.7), Ha/2.22, dd (14.5, 7.7), Hb	36.6	2.30, dd (14.4, 7.7), Ha/2.23, dd (14.4, 7.7), Hb	36.8	2.32, dd (14.4, 7.7), Ha/2.26, dd (14.4, 7.7), Hb
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[a]		140.3		140.0	
4′	39.9	2.10–2.02, m[a]	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[a]	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, Ha/4.82, t (1.4), Hb	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

[a] Overlap of the signals.

Table 2 ^{1}H and ^{13}C NMR spectroscopic data of **5–8** in $CDCl_3$

	5		**6**		**7**		**8**	
No.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	176.2		173.8		173.9		173.7	
2	37.1[a]	2.38, d (17.4), Hα/2.19, d (17.4), Hβ	38.3	2.32, d (17.4), Hα/2.28, d (17.4), Hβ	38.4	2.30–2.24, m[a]	38.4	2.30–2.24, m[a]
2a	42.3		47.5		47.4		47.4	
3	37.2[a]	2.25–2.17, m[a], Hα/1.58–1.51, m, Hβ	39.9[a]	2.86, d (13.9), Hα/2.14, d (13.9), Hβ	39.9	2.82, d (13.9), Hα/2.11, d (13.9), Hβ	39.9	2.84, d (13.9), Hα/2.12, d (13.9), Hβ
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), Ha/2.20, dd (14.4, 7.7), Hb	36.8	2.25–2.09, m[a]	36.9[a]	2.32–2.09, m[a]
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[a]	39.9[a]	2.10–2.02, m[a]	35.8	2.01–1.96, m[a]	36.7[a]	2.32–2.09, m[a]
5′	26.3	2.08–1.98, m[a]	26.2	2.10–2.02, m[a]	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, Ha/4.89, t (0.8), Hb	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

[a] overlapping of the signals.

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^{1}H and ^{13}C NMR spectroscopic data, see Table 2; HRESITOFMS *m*/*z* 327.1562 $[M + Na]^+$ (calcd for $C_{18}H_{24}O_4$ + Na, 327.1567).

2.3.7 Velutinone G (7). Colorless viscous liquid; R_f = 0.42 (hexane–EtOAc, 1 : 3); $[\alpha]_D^{23}$ −25.6 (*c* 0.20, $CHCl_3$); ECD (94 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 205 (+11.89) nm; IR (ATR) ν_{max} 3481, 2939, 2858, 1783, 1718, 1651, 1422, 1347, 1164, 1033, and 902 cm^{-1} for ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; HRESITOFMS *m*/*z* 321.1690 $[M + H]^+$ (calcd for $C_{18}H_{24}O_5 + H^+$, 321.1697).

2.3.8 Velutinone H (8). Colorless viscous liquid; R_f = 0.29 (*n*-hexane–EtOAc, 7 : 3); $[\alpha]_D^{24}$ −23.5 (*c* 0.20, $CHCl_3$); ECD (75 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 215 (+11.84) nm; IR (ATR) ν_{max} 2962, 2924, 2855, 1785, 1719, 1423, 1378, 1163, and 1034 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; HRESITOFMS *m*/*z* 321.1690 $[M + H]^+$ (calcd for $C_{18}H_{24}O_5 + H^+$, 321.1697).

2.3.9 Velutindimer A (9). White solid; mp 201–203 °C; R_f = 0.13 (*n*-hexane–EtOAc, 1 : 1); $[\alpha]_D^{28}$ + 0.08 (*c* 0.63, MeOH–$CHCl_3$, 3 : 1); ECD (26 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 285 (0.00) nm; UV (MeOH) λ_{max} (log ε) 226 (4.53), 285 (4.20); IR (ATR) ν_{max} 3088, 2944, 2837, 1715, 1643, 1611, 1564, 1513, 1455, 1409, 1249, 1180, 1034, and 818 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; HRESITOFMS *m*/*z* 539.1651 $[M + Na]^+$ (calcd for $C_{30}H_{28}O_8$ + Na, 539.1682).

2.3.10 Velutinin dimer B (10). Colorless needles; mp 205–207 °C; R_f = 0.21 (*n*-hexane–EtOAc, 1 : 1); $[\alpha]_D^{28}$ +0.08 (*c* 0.63, MeOH–$CHCl_3$, 5 : 3); ECD (33 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 265 (0.00) nm; UV (MeOH) λ_{max} (log ε) 265 (4.45); IR (ATR) ν_{max} 2940, 2838, 1699, 1647, 1608, 1566, 1512, 1455, 1410, 1391, 1246, 1176, 1031 and 814 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; HRESITOFMS *m*/*z* 539.1666 $[M + Na]^+$ (calcd for $C_{30}H_{28}O_8$ + Na, 539.1682).

2.3.11 Velutinin dimer C (11). Colorless needles; mp 207–209 °C; R_f = 0.18 (*n*-hexane–EtOAc, 1 : 1); $[\alpha]_D^{27}$ +0.03 (*c* 0.23, MeOH–$CHCl_3$, 9 : 1); ECD (22 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 268 (0.00) UV (MeOH) λ_{max} (log ε) 268 (4.59); IR (ATR) ν_{max} 2924, 2837, 1708, 1649, 1625, 1608, 1567, 1513, 1455, 1406, 1248, 1176, 1031, and 821 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; HRESITOFMS *m*/*z* 539.1664 $[M + Na]^+$ (calcd for $C_{30}H_{28}O_8$ + Na, 539.1682).

2.4. Preparation of the (*R*)-and (*S*)-α-methoxy-α-(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.*[22] A solution of (*S*)-MPTA-Cl (10 μL, 53.4 μmol) was added to a solution mixture of **2** (8 mg, 27.5 μmol) and DMAP (5 mg) in dry CH_2Cl_2 (1 mL) and stirred under N_2 at room temperature for 6 h. Then the solvent was removed *in vacuo*. The

Table 3 ^{1}H and ^{13}C NMR spectroscopic data of **9–11** in $CDCl_3$

	9		**10**		**11**	
No. position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* 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

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residue was separated on preparative TLC (CH_2Cl_2) 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_2Cl_2 (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; ^{1}H NMR ($CDCl_3$, 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_6H_5), 3.52 (3H, s, OCH_3)].

2.4.2 Compound 2b. Colorless viscous liquid; ^{1}H NMR ($CDCl_3$, 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′), 1.90–165 (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_6H_5), 3.52 (3H, s, OCH_3)].

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; ^{1}H NMR ($CDCl_3$, 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_6H_5), 3.55 (3H, s, OCH_3)].

2.5.2 Compound 5b. Colorless viscous liquid; ^{1}H NMR ($CDCl_3$, 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_6H_5), 3.54 (3H, s, OCH_3)].

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.[23] Raw data frame integration was performed with SAINT,[23] which also applied correction for Lorentz and polarization effects. An empirical absorption correction using the SADABS program[23] was applied. The structure was solved by direct methods and refined by full-matrix least-squares method on F^2 with anisotropic thermal parameters for all non-hydrogen atoms using the SHELXTL software package.[24] 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. $C_{30}H_{28}O_8$ (M = 516.52 g mol^{-1}): orthorhombic, space group $Pna2_1$ (no. 33), a = 23.725(4) Å, b = 5.5211(10) Å, c = 39.408(7) Å, V = 5162.0(16) Å^3, Z = 8, T = 293(2) K, μ(MoKα) = 0.096 mm^{-1}, D_{calc} = 1.329 g cm^{-3}, 135 470 reflections measured (6.204° ≤ 2Θ ≤ 52.798°), 10 518 unique (R_{int} = 0.0929, R_{sigma} = 0.0445) which were used in all calculations. The final R_1 was 0.0508 ($I > 2\sigma(I)$) and wR_2 was 0.1225 (all data).

2.6.2 Crystal data of 11. $C_{31}H_{29}Cl_3O_8$, MW = 635.89 g mol^{-1}, triclinic, space group $P\bar{1}$, 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^3, Z = 2, T = 293(2) K, μ(MoKα) = 0.354 mm^{-1}, D_{calc} = 1.400 g cm^{-3}, 61 914 reflections measured, 7472 unique (R_{int} = 0.0262) which were used in all calculations. The final R_1 was 0.0642 ($I > 2\sigma(I)$) and wR_2 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.[25] 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.*[26] The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}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).[27] 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.[28] The reference substances were ellipticine and doxorubicin. Cytotoxicity test against primate cell line (Vero) was performed using

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the green fluorescent protein detection method described by Hunt and co-workers.[29] 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**),[30] three flavonoids, sakuranetin (**13**),[31] 7-*O*-methyleriodictyol (**14**)[32] and rhamnetin (**15**),[33] and an acetogenin, cananginone H (**16**)[20,21] (Fig. 1).

The IR spectra of **1–4** showed absorption bands of a γ-lactone moiety at (1789–1771 cm^{-1}) and a conjugated carbonyl functionality (1685–1862 cm^{-1}) similar to the absorption bands of a synthetic bicyclic cyclohexenone.[34,35]

Compound **1** possessed the molecular formula $C_{18}H_{24}O_3$ based on the ^{13}C NMR and HRESITOFMS (*m*/*z* 311.1611 $[M + Na]^+$) data, indicating seven indices of hydrogen deficiency. The ^1H NMR data (Table 1) had resonances at δ 2.48 (d, $J = 17.4$ Hz, H-2α), 2.39 (d, 17.4 Hz, H-2β), 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}C NMR data (Table 1), DEPT, and HMQC experiments indicated seven resonances which were associated with an α,β-unsaturated carbonyl (δ 196.2/C-4), a lactone carbonyl (δ 174.2/C-1), two olefinic (δ 131.2/C-5 and 141.4/C-6), two methylene (δ 38.9/C-2 and 42.6/C-3), one methine (δ 77.8/C-6a), and one quaternary (δ 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 α,β-unsaturated cyclohexanone ring. This arrangement is similar to that of a compound isolated from the fruit kernels of *Otoba parvifolia*[35,36] and from a total synthesis of its core structure,[34] except for the side chain at C-2a which was replaced by a geranyl moiety in **1**. This geranyl side chain ($C_{10}H_{17}$) was evident from the ^1H and ^{13}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 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 $C_{18}H_{24}O_4$ derived from the ^{13}C NMR and HRESITOFMS (*m*/*z* 305.1740 $[M + H]^+$) data, demonstrating the same index of hydrogen deficiency, but having one additional oxygen atom compared to **1**. The ^1H and ^{13}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 δ_H 4.82 (t, $J = 1.4$ Hz, 4.91 (brs), H-8′)/δ_C 111.1 and an oxymethine at δ_H 4.00 (t, $J = 6.3$ Hz, H-6′)/δ_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[22](-Fig. 3). Therefore, the structure of compound **2**, velutinone B, was defined as shown in Fig. 1.

Compound **3** had the molecular formula $C_{18}H_{24}O_4$, deduced from ^{13}C NMR and HRESITOFMS (*m*/*z* 327.1543 $[M + Na]^+$) data, implying the same index of hydrogen deficiency, but having one additional oxygen atom compared to **1**. The ^1H and ^{13}C NMR spectroscopic data of **3** (Table 1) were similar to those of **1**, except for the appearance of the olefinic protons at δ_H 5.51 (dt, $J = 15.6$, 6.6 Hz, H-5′) and δ_H 5.60 (d, $J = 15.6$ Hz, H-6′), and one additional oxygenated carbon signal at δ_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 $C_{18}H_{24}O_5$ derived from the ^{13}C NMR and HRESITOFMS (*m*/*z* 343.1472 $[M + Na]^+$) 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}C NMR spectrum revealed the unusual downfield oxygenated carbon signal at δ_C 81.8 for C-7′, suggesting the presence of a hydroperoxy group.[37] Thus the structure of compound **4**, velutinone D, was determined as shown (Fig. 1).

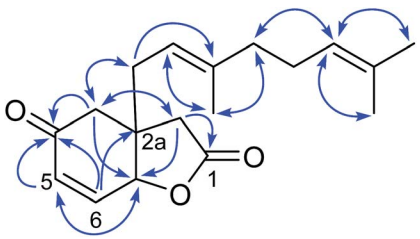
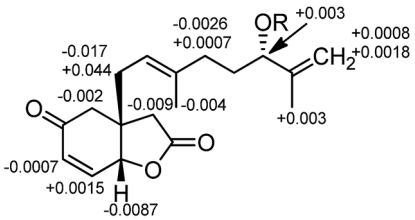


Fig. 2 Selected HMBC correlations of **1**.



2a: R = (*S*)-MTPA
2b: R = (*R*)-MTPA

Fig. 3 Δδ values ($\Delta\delta = \delta_S - \delta_R$ in ppm) obtained for MTPA esters **2a** and **2b**.

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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 cm^{-1}) and γ-lactone carbonyl (1771 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 δ_H 4.31 (m)/δ_C 63.7. The assignment of (4*S*) absolute configuration was done *via* the modified Mosher's ester method[22] (Fig. 4). The configurations of (2a*R* and 6a*R*) were assigned by NOESY and a molecular modeling study. NOESY correlations were observed between H-4 and H-2α, H-6a and H-1′a, H-6a and H-3β, H-1′b and H-2β, and H-3α 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 γ-lactone (1789 cm^{-1} and a cyclohexanone (1719 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 [δ_H 3.65 (d, J = 3.6 Hz, H-5)/δ_C 58.6 and δ_H 3.37 (d, J = 3.6 Hz, H-6)/δ_C 54.9)]. The relative configuration of the epoxide was assigned by the NOESY correlations between H-6 and H-6a. Hence, the structure of compound **6**, velutinone F, was defined as shown (Fig. 1).

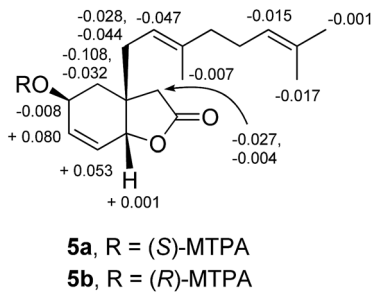

Fig. 4 Δδ values ($\Delta\delta = \delta_S - \delta_R$ in ppm) obtained for MTPA esters **5a** and **5b**.

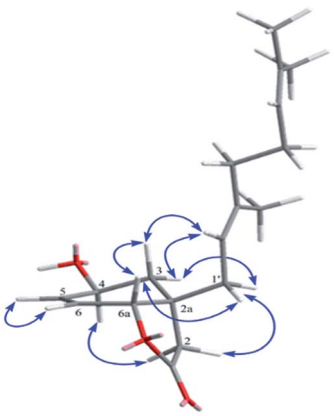

Fig. 5 Key NOESY correlations of **5**, energy minimized using MM2.

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 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 [δ_H 3.62 (d, J = 3.6 Hz, H-5)/δ_C 58.5 and δ_H 3.34 (d, J = 3.6 Hz, H-6)/δ_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 [δ_H 3.65 (t, J = 6.7 Hz, H-6′/δ_C 63.7 and δ_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,[38] which have NMR data similar to those of compounds **1–8**. However, the relative configuration at the ring junction (C-2a and C-6a) of **1–8** were assigned as 2a*R* and 6a*R* which different from panamonons A and B (2a*R* and 6a*S*).[38] The ECD spectra of compounds **1–4**, containing an α,β-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,[35,36] could be prenylated by geranyl diphosphate to form intermediate **A** [38] 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 cm^{-1}) and aromatic (1643 cm^{-1}) groups. The UV spectrum also indicated an aromatic moiety (286 nm). Since the NMR spectroscopic data

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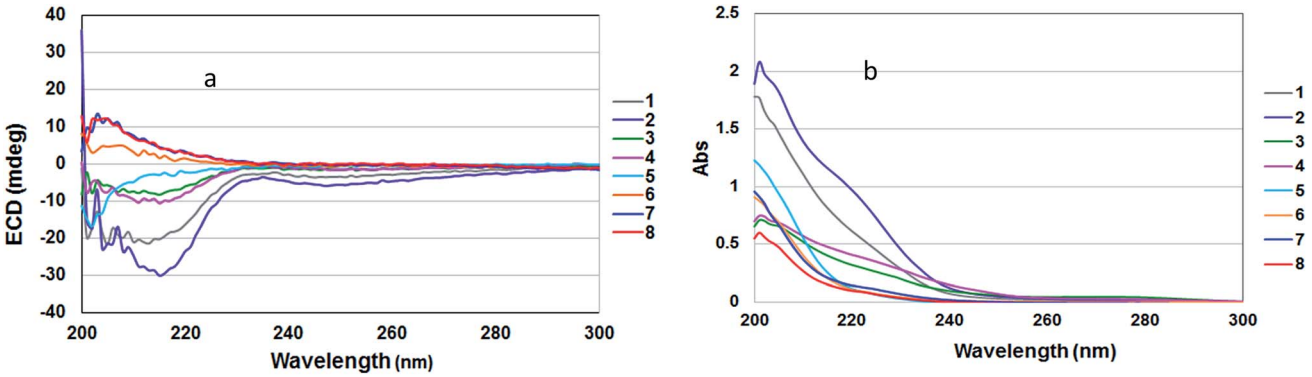

Fig. 6 ECD (a) and UV (b) spectra (in MeOH) of compounds **1–8**.

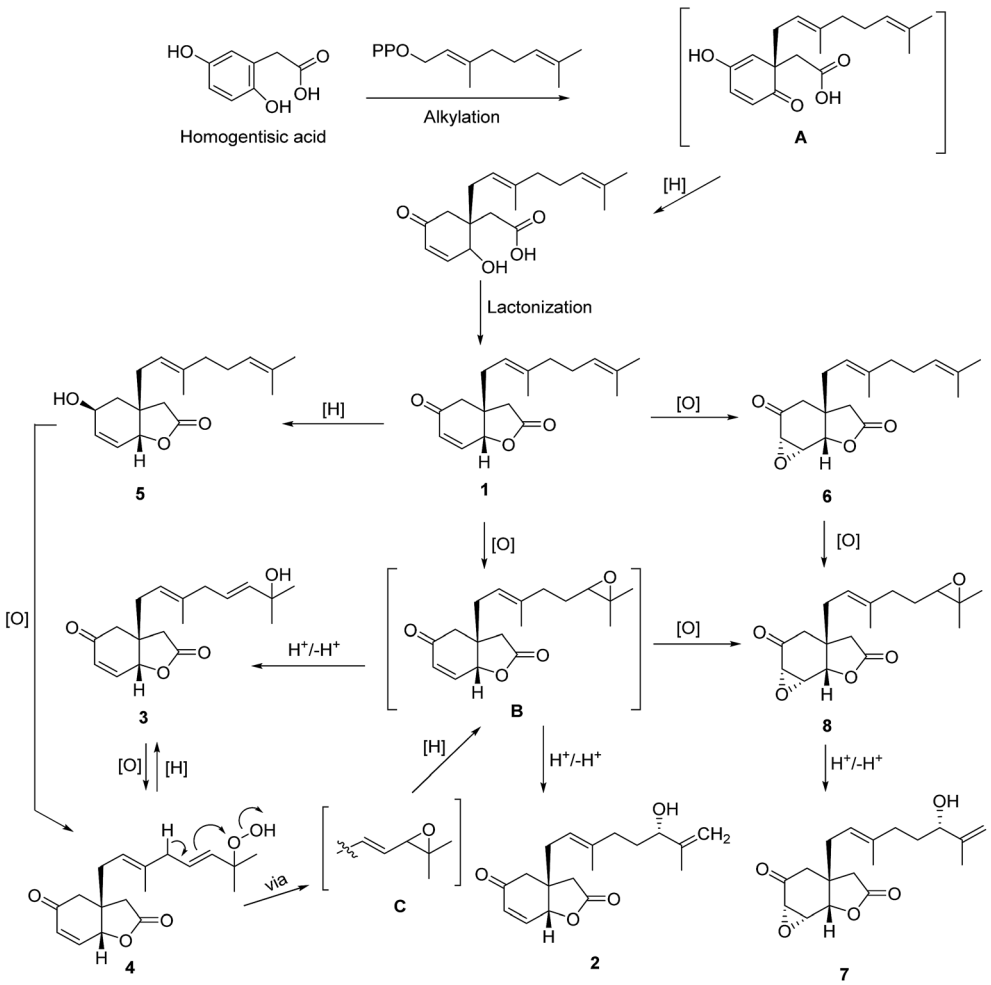

Fig. 7 Plausible biogenetic pathway of **1–8**.

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,[39] except for hydroxy groups at C-12 and C-12′ of the aromatic rings which were replaced by methoxy groups. The ^{1}H NMR spectroscopic data (Table 3) showed resonances for the *para*-substituted benzene rings at δ 6.82 and 7.19 (each 4H, d, J = 8.7 Hz), four methines of the cyclobutyl ring at δ 4.16 and 4.35 (each 2H, dd, J = 7.6, 10.0 Hz), four olefinic protons for the two α-pyrone moieities at δ 5.21 and 5.71 (each 2H, d, J = 2.2 Hz), and four methoxy groups at δ 3.75 and 3.67 (each 6H, s). The ^{13}C NMR data (Table 3) showed resonances for the *para*-disubstituted benzene rings at δ 129.4 (C-9, 9′), 128.5 (C-10, 10′ and C-14, 14′),

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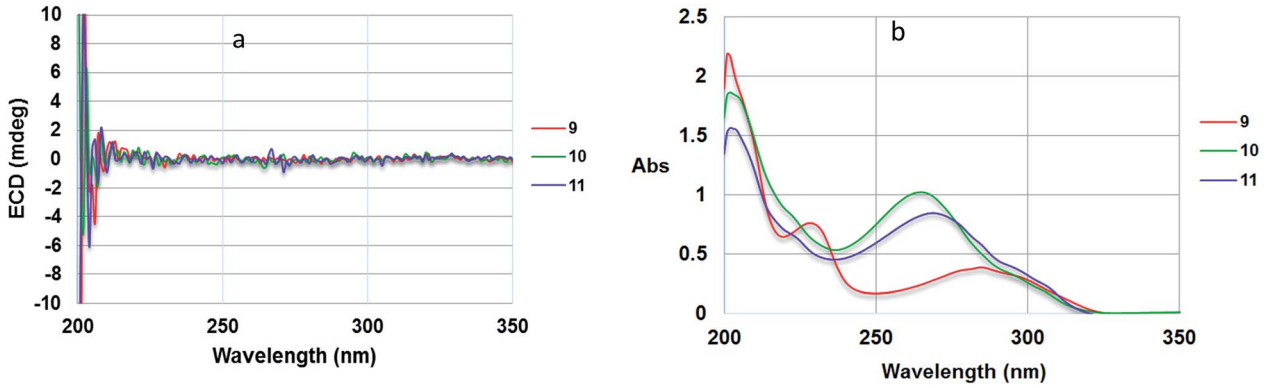

Fig. 8 ECD (a) and UV (b) spectra (in MeOH) of compounds **9**, **10** and **11**.

and 113.9 (C-11, 11′), the cyclobutyl methines at δ 43.0 (C-7, 7′), and 45.5(C-8, 8′) the α-pyrone methines at δ 87.7 (C-3, 3′), 101.3 (C-5, 5′) and methoxy groups at δ 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 α-pyrone ring, and benzene rings at C-7/7′ and C-8/8′, respectively. Resonances for two sets of methoxy protons at δ_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.[39] The specific rotation value of **9** was almost zero [+0.08 (*c* 0.63, MeOH–$CHCl_3$; 3 : 1)] which was also the same as that reported for a symmetric achyrodimer A.[39] Moreover, the ECD spectrum of **9** showed no signal for a Cotton effect (Fig. 8).[40] 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 $C_{30}H_{28}O_8$ from the ^{13}C NMR and HRESITOFMS (*m*/*z* 539.1666 $[M + Na]^+$) data, having the same index of hydrogen deficiency as **9**. The IR spectrum displayed bands for lactone (1699 cm^{-1}) and aromatic (1647 cm^{-1}) groups. The UV spectrum also supported an aromatic moiety (268 nm). The ^1H NMR data of **10** (Table 3) showed resonances for two *para*-disubstituted benzene rings at δ 6.85, 7.16, and 7.34 and 6.85 (each 2H, d, J = 8.7 Hz), an *E*-double bond at δ 6.42 and 6.86 (each 1H, d, J = 15.8 Hz), α-pyrone ring at δ 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 δ 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 δ 3.32, 3.69, 3.78, and 3.79. The ^{13}C NMR spectrum, DEPT and HMQC experiments of **10** showed 30 resonances, including two sets of *p*-disubstituted benzene rings, one α-pyrone ring, one olefinic, one cyclobutane ring, and four methoxy carbons. The HMBC spectrum displayed ^3J 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*.[39] 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_{trans} = 10.8 Hz), and

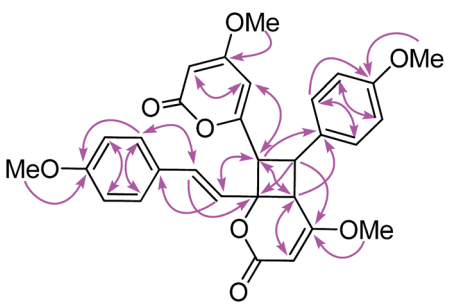

Fig. 9 Selected HMBC correlations of **10**.

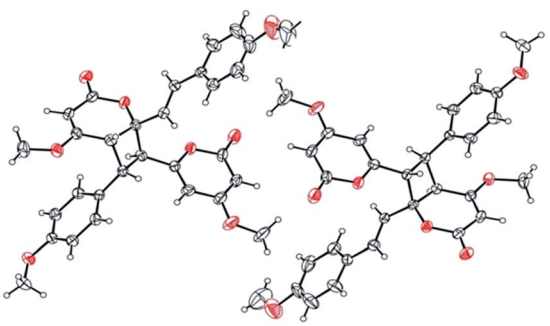

Fig. 10 ORTEP plot of the asymmetric units in **10**. The thermal ellipsoids are shown at 40% probability.

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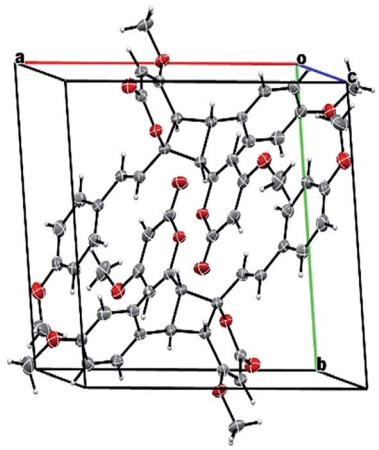

Fig. 11 Packing structure of **11** showing the inverted racemic mixture. The chloroform solvate molecule is omitted for clarity.

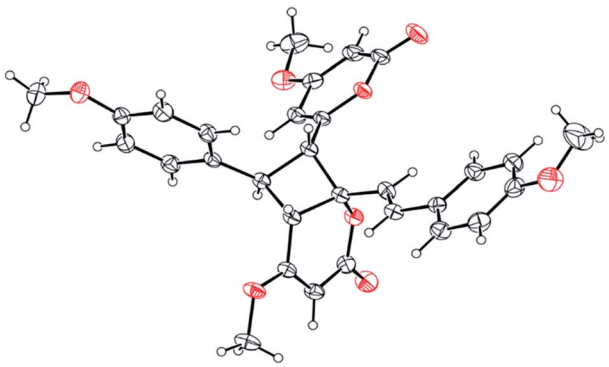

Fig. 12 ORTEP plot of the asymmetric unit in **11**. The thermal ellipsoids are shown at 30% probability level. The chloroform solvate molecule is omitted for clarity.

H-8 and H-5′ (J_{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.[41]

The ECD measurement of **10** in MeOH showed no signal of the Cotton effect[40] (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 cm^{-1}) and an aromatic ring (1649 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 ^1H and ^{13}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_{trans} = 10.3 Hz) and H-8 and H-5′ (J_{trans} = 9.7 Hz) could be explained in the same way as for **10**.[41] 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)[40] 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

	Antimalarial	Anti-TB	Cytotoxicity (IC_{50}, μM)			
Compound	(IC_{50}, μM)	(MIC, μM)	KB[a]	MCF7[b]	NCI-H187[c]	Vero cell[d]
1	Inactive	43.4	4.0	4.8	4.2	5.8
2	9.6	82.1	9.6	12.9	6.5	8.8
3	10.0	Inactive	12.9	10.9	11.4	10.3
4	9.6	Inactive	10.5	15.2	8.7	11.7
6	Inactive	Inactive	14.5	20.7	11.5	11.2
7	9.8	Inactive	24.1	21.0	14.7	17.9
8	7.3	Inactive	10.5	11.9	6.8	18.2
9	6.4	Inactive	Inactive	Inactive	Inactive	Inactive
10	5.4	Inactive	Inactive	Inactive	Inactive	Inactive
11	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	

[a] Human oral epidermoid carcinoma. [b] Human breast cancer cells. [c] Human lung cancer cells. [d] Normal African green monkey kidney cells.

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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 μM respectively, should be responsible for the antimycobacterial activity against *M. tuberculosis* exhibited in the primary screening. From this result, the α,β-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*,[20,21] 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_{50} values in the range of 5.4–10.0 μM. In addition, compounds **1–4** and **6–8** exhibited cytotoxicity against three cancer cell lines tested, with IC_{50} values in the range of 4.0–24.1 μM. Among these, **1**, **2** and **8** exhibited moderate cytotoxicity against NCI-H187 cell lines with IC_{50} values of 4.2, 6.5 and 6.8 μM, respectively, which were close to the standard drug, ellipticine (5.6 μM). Compounds **1–4** and **6–8** exhibited cytotoxicity towards the Vero cell line with IC_{50} values in the range of 5.8–18.2 μM. 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_{18} carbons 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**). Velutinindimers A–C (**9–11**) are dimmers 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_{50} 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_{50} values in the range of 4.0–24.1 μM.

Acknowledgements

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