



# Minor Prenylated Flavonoids from the Twigs of *Macaranga adenantha* and Their Cytotoxic Activity

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**Abstract** Three new minor prenylated flavonoids, named macadenanthins A–C (**1–3**), together with three known ones (**4–6**), were isolated from the twigs of *Macaranga adenantha*. Their structures were elucidated on the basis of extensive spectroscopic analysis including NMR, UV and MS. The prenyl moieties in compounds **1–3** were further modified by cyclization and hydroxylation. The new compounds were tested for their cytotoxicity against four cancer cell lines (MCF-7, Hep G2, Hela and P388) and showed IC<sub>50</sub> values in the range of 13.76–22.27 μM.

**Keywords** *Macaranga adenantha* · Prenylated Flavonoids · Macadenanthins A–C · Cytotoxicity

## 1 Introduction

Prenylated flavonoids are attracting great attention from the scientific community due to their structural uniqueness and

remarkable biological activities [1]. The different prenylation position, various lengths of prenyl chain and further modifications on the prenyl moiety such as cyclization and hydroxylation resulted in the chemical diversity of the prenylated products, and which also made them exhibit promising biological activities. Prenylated flavonoids have a relatively narrow distribution in the plant kingdom and an overview of the literature indicated that prenylated flavonoids are the typical secondary metabolites of the genus *Macaranga* [2].

*Macaranga adenantha* Gagnep (Euphorbiaceae) is an arbor distributed in the tropical rainforests, previous studies showed that it contained triterpenoids, steroids and phenolic compounds [3–5]. As part of our program to discover new anticancer prenylated aromatic products from the genus *Macaranga* [6–8], a phytochemical investigation on this plant led to the isolation and characterization of three minor new prenylated flavonoids, named macadenanthins A–C (**1–3**), along with three known ones, including glyasperin A (**4**) [9], broussonflavonol F (**5**) [10], and macarangin (**6**) [11]. The new compounds **1–3** were evaluated for their cytotoxicity against a small panel of cancer cell lines. Described herein are the isolation, structure elucidation and cytotoxicity of these compounds (Fig. 1).

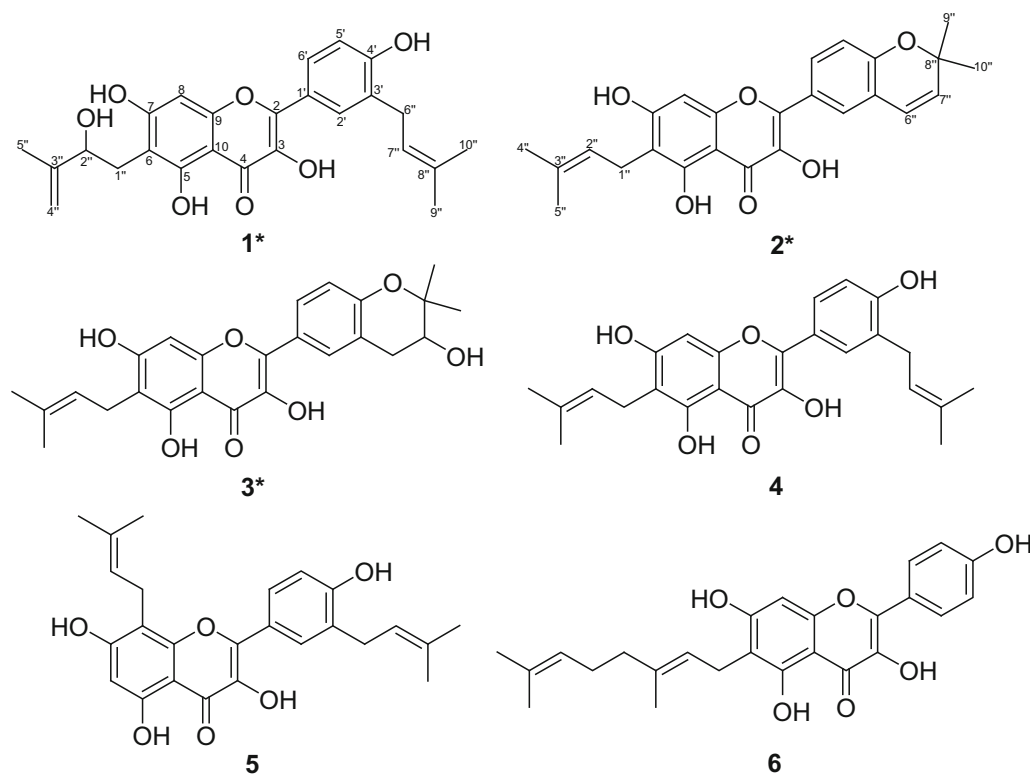
Da-Song Yang and Shuang-Mei Wang authors contributed equally to this work.

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**Fig. 1** Structures of compounds 1–6

## 2 Results and Discussion

Macadenanthin A (**1**) was obtained as optically active yellow powder ( $[\alpha]_D^{21} -1.9$ ,  $c$  0.12, MeOH). The molecular formula  $C_{25}H_{26}O_7$  was deduced from the HRESIMS ( $m/z$  437.1609  $[M-H]^-$ ), requiring 13° of unsaturation. The IR spectrum showed absorption bands for OH ( $3425\text{ cm}^{-1}$ ), carbonyl ( $1648\text{ cm}^{-1}$ ) and aromatic ring ( $1604$  and  $1488\text{ cm}^{-1}$ ) moieties. The UV spectrum showed absorption maxima at 270 and 380 nm, which indicated the presence of a flavonol skeleton [12]. Analysis of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR (Table 1) data of **1** aided by HSQC revealed resonances for a hydrogen-bonded hydroxy group ( $\delta_{\text{H}}$  12.61, s); a 1,3,4-trisubstituted benzene ring ( $\delta_{\text{H}}$  7.00, 1H, d,  $J = 8.5$  Hz; 7.98, 1H, dd,  $J = 8.5, 2.2$  Hz; 8.07, 1H, d,  $J = 2.2$  Hz); a 6-substituted kaempferol [ $\delta_{\text{C}}$  136.5 (s), 176.5 (s), 95.0 (d);  $\delta_{\text{H}}$  6.52 (1H, s)] [9, 10]; a prenyl [ $\delta_{\text{C}}$  29.1 (t), 123.2 (d), 133.1 (s), 25.9 (q), 17.9 (q);  $\delta_{\text{H}}$  3.40 (2H, d,  $J = 7.4$  Hz), 5.38 (1H, t,  $J = 7.4$  Hz), 1.74 (3H, s), 1.75 (3H, s)] [9] and a 2-hydroxy-3-methyl-3-butenyl group [ $\delta_{\text{C}}$  29.5 (t), 76.4 (d), 148.2 (s), 110.4 (t), 18.3 (q);  $\delta_{\text{H}}$  3.07 (1H, dd,  $J = 14.4, 3.6$  Hz), 2.92 (1H, dd,  $J = 14.4, 7.9$  Hz), 4.42 (1H, dd,  $J = 7.9, 3.6$  Hz), 4.92 (1H, s), 4.76 (1H, s), 1.83 (3H, s)] [12]. These signals indicated that **1** should be a diprenylated kaempferol and the attachment of

2-hydroxy-3-methyl-3-butenyl and prenyl groups to C-6 and C-3' were deduced from HMBC correlations of H-1''/C-5, C-6, C-7; H-2''/C-6; H-6''/C-2', C-3', C-4' (Fig. 2). The COSY correlation of H-1''/H-2'' and the HMBC correlations of H-1''/C-3''; H-4''/C-2'', C-5''; and Me-5''/C-2'' revealed that the terminal double bond and oxygenated methine were belong to 6-prenyl moiety. Therefore, the structure of **1** was elucidated as 6-(2-hydroxy-3-methyl-3-butenyl)-3'-prenyl kaempferol.

Macadenanthin B (**2**) possessed a molecular formula of  $C_{25}H_{24}O_6$  as deduced from its HREIMS ( $m/z$  420.1576  $[M]^+$ ), suggesting 14° of unsaturation. The IR spectrum of **2** indicated the characteristic bands of hydroxyl ( $3423\text{ cm}^{-1}$ ) and conjugated carbonyl ( $1648\text{ cm}^{-1}$ ) groups. Comparing the NMR spectral data (Table 1) of **2** with those of **1** revealed that it shared the same molecular-skeleton with **1** and the major differences between them could be rationalized to the signals corresponding to the 2-hydroxy-3-methyl-3-butenyl group in **1** was replaced by a 2,2-dimethylpyran ring [ $\delta_{\text{C}}$  122.6 (d), 132.3 (d), 77.9 (s), 28.3  $\times$  2 (q);  $\delta_{\text{H}}$  6.45 (1H, d,  $J = 9.9$  Hz), 5.78 (1H, d,  $J = 9.9$  Hz), 1.41 (6H, s)] [13] in **2**, which was consistent with its molecular formula. This deduction was further supported by the COSY correlation of H-6''/H-7'' and the HMBC correlations of H-6''/C-8''; Me-9''/C-7'', C-10''; and

**Table 1**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data for compounds **1–3** in acetone- $d_6$ 

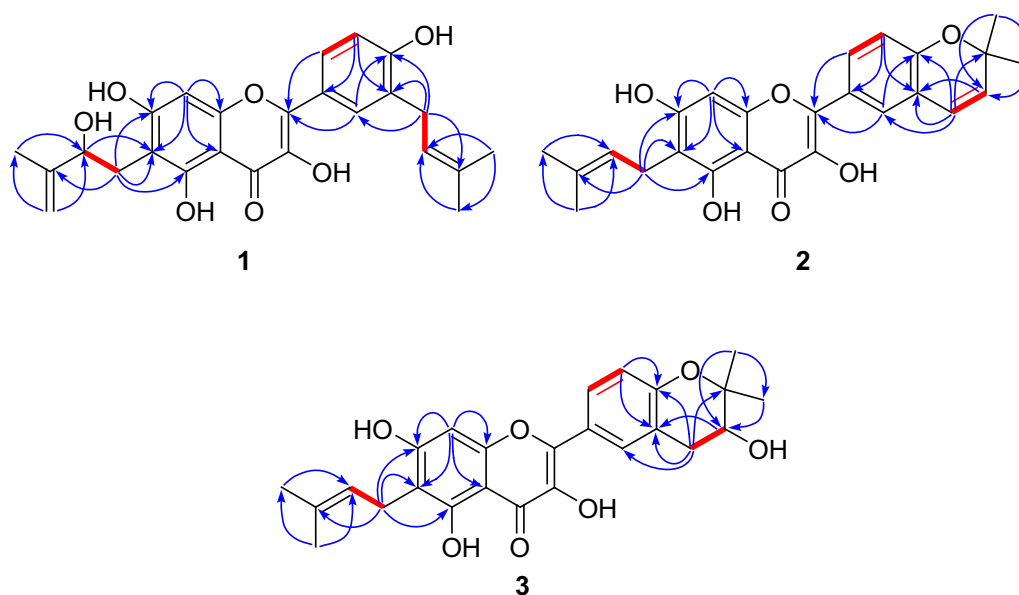
No	<b>1</b> <sup>a</sup>		<b>2</b> <sup>b</sup>		<b>3</b> <sup>a</sup>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)
2	148.2 s		146.4 s		146.4 s	
3	136.5 s		137.0 s		136.7 s	
4	176.5 s		176.7 s		176.6 s	
5	159.5 s		158.7 s		158.8 s	
6	109.6 s		111.7 s		111.8 s	
7	164.2 s		162.9 s		163.6 s	
8	95.0 d	6.52, s	93.6 d	6.49, s	94.0 d	6.61, s
9	156.1 s		155.7 s		155.9 s	
10	103.8 s		104.0 s		103.7 s	
1'	123.4 s		124.8 s		115.8 s	
2'	130.3 d	8.07, d (2.2)	126.8 d	7.88, d (2.0)	130.4 d	7.98, br s
3'	129.0 s		122.0 s		121.4 s	
4'	157.8 s		155.5 s		155.7 s	
5'	115.8 d	7.00, d (8.5)	117.1 d	6.84, d (8.6)	117.7 d	6.87, d (9.3)
6'	128.0 d	7.98, dd (8.5, 2.2)	129.8 d	7.97, dd (8.6, 2.0)	127.9 d	7.98, br s
1''	29.5 t	3.07, dd (14.4, 3.6)	22.0 t	3.31, d (7.1)	22.0 t	3.35, d (7.2)
		2.92, dd (14.4, 7.9)				
2''	76.4 d	4.42, dd (7.9, 3.6)	123.2 d	5.23, t (7.1)	123.4 d	5.27, t (7.2)
3''	148.2 s		131.7 s		131.4 s	
4''	110.4 t	4.92, s	25.8 q	1.61, s	25.9 q	1.64, s
		4.76, s				
5''	18.3 q	1.83, s	17.8 q	1.74, s	17.9 q	1.77, s
6''	29.1 t	3.40, d (7.4)	122.6 d	6.45, d (9.9)	32.2 t	3.10, dd (16.5, 5.9)
						2.80, overlap
7''	123.2 d	5.38, t (7.4)	132.3 d	5.78, d (9.9)	69.5 d	3.86, dd (7.9, 5.9)
8''	133.1s		77.9 s		78.7 s	
9''	25.9 q	1.74, s	28.3 q	1.41, s	26.0 q	1.37, s
10''	17.9 q	1.75, s	28.3 q	1.41, s	21.1 q	1.29, s
5-OH		12.61, s				12.40, s

<sup>a</sup> Recorded at 600 MHz<sup>b</sup> Recorded at 400 MHz

Me-10''/C-7'' (Fig. 2). The observed HMBC correlations of H-1''/C-5, C-6, C-7; H-6''/C-2', C-3', C-4'; and H-7''/C-3' indicated the prenyl and 2,2-dimethylpyran moieties were connected with C-6 and C-3', respectively. As a result, the structure of **2** was determined as 6-prenyl-3',4'-(2,2-dimethylpyrano) kaempferol.

Macadenanthin C (**3**) was obtained as optically active yellow powder ( $[\alpha]_{\text{D}}^{21} -3.3$ ,  $c$  0.12, MeOH) and the molecular formula was deduced to be  $\text{C}_{25}\text{H}_{26}\text{O}_7$  based on its HREIMS ( $m/z$  438.1675  $[\text{M}]^+$ ), suggesting 13° of unsaturation. The UV spectrum showed the characteristic absorbances for a flavonol [ $\lambda_{\text{max}}$  (log  $\epsilon$ ) 270 (4.47) and 376 (4.27) nm] [12]. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **3** (Table 1) were similar with those of **2** and the differences between

them could be rationalized to the 2,2-dimethylpyran ring in **2** were replaced by a 2,2-dimethyl-3-hydroxydihydropyrano ring [ $\delta_{\text{C}}$  32.2 (t), 69.5 (d), 78.7 (s), 26.0 (q), 21.1 (q);  $\delta_{\text{H}}$  3.10 (1H, dd,  $J = 16.5, 5.9$  Hz), 2.80 (1H, overlap), 3.86 (1H, dd,  $J = 7.9, 5.9$  Hz), 1.37 (3H, s), 1.29 (3H, s)] [14] in **3**. This deduction was confirmed by the COSY correlation of H-6''/H-7'' and the HMBC correlations of H-6''/C-8''; Me-9''/C-7'', C-10''; and Me-10''/C-7'' (Fig. 2). The observed HMBC correlations of H-1''/C-5, C-6, C-7; H-6''/C-2', C-3', C-4'; H-7''/C-3' (Fig. 2) demonstrated that prenyl and 2,2-dimethyl-3-hydroxydihydropyrano groups were located at C-6 and C-3', respectively. Therefore, the structure of **3** was determined as 6-prenyl-3',4'-(2,2-dimethyl-3-hydroxydihydropyrano) kaempferol.



**Fig. 2** Selected HMBC (↷) and COSY (—) correlations of compounds 1–3

Since prenylated flavonoids are reported to have modest or strong anticancer activities [1, 2, 6], the cytotoxicity of new compounds 1–3 were evaluated against human breast adenocarcinoma (MCF-7), human hepatocellular (Hep G2), human cervical carcinoma (Hela), mouse leukemia (P388) cell lines by MTT method, with 5-FU used as a positive control [15]. The results showed that compounds 2 and 3 exhibit cytotoxicity against Hep G2 cell line with  $IC_{50}$  values of 13.76 and 22.27  $\mu\text{M}$  ( $IC_{50}$  of 5-FU was 102.01  $\mu\text{M}$ ), respectively. Compound 2 exhibit cytotoxicity against Hela cell line with  $IC_{50}$  value of 16.18  $\mu\text{M}$  ( $IC_{50}$  of 5-FU 106.47  $\mu\text{M}$ ).

### 3 Experimental Section

#### 3.1 General Experimental Procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter. CD spectra were obtained on an automated circular dichroism spectrometer (Applied Photo-physics). UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained on a Bruker Tenor 27 spectrometer with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400, DRX-500 or AV III-600 spectrometers with TMS as an internal standard. ESIMS were recorded using a Finnigan MAT 90 instrument and HRESIMS was performed on an API QSTAR time-of-flight spectrometer, HREIMS were recorded on a Waters AutoSpec Premier P776 instrument.

Column chromatography was performed on Sephadex LH-20 (Amersham Biosciences, Piscataway, USA), silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), RP-18 gel (LiChroprep, 40–63  $\mu\text{m}$ ; Merck, Darmstadt, Germany), and MCI gel CHP20P (75–150  $\mu\text{m}$ , Mitsubishi Chemical Corporation, Tokyo, Japan). Semi-preparative HPLC was performed on a Agilent 1200 (column: Zorbax SB-C18, 250  $\times$  9.4 mm; DAD detector). Fractions were monitored by TLC, visualized by heating silica gel plates sprayed with 15 %  $\text{H}_2\text{SO}_4$  in EtOH.

#### 3.2 Plant Material

The twigs of *M. adenantha* were collected from Malipo County of Yunnan province, P. R. China, in June 2013. A voucher specimen (Yangyp-20130619) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, which was identified by one of the authors (Prof. Yong-Ping Yang).

#### 3.3 Extraction and Isolation

The dried and powdered twigs of *M. adenantha* (9.5 kg) were extracted three times with 90 % EtOH (25 L) for 24 h at room temperature and filtrated. The filtrate was concentrated and partitioned between  $\text{H}_2\text{O}$  and EtOAc and then the EtOAc portion was decolorized on MCI gel (eluting with 95 % EtOH). The residue (380 g) was chromatographed on silica gel ( $\text{CHCl}_3$ –MeOH 1:0 to 1:1) to afford fractions A–F. Fraction B was purified over a Sephadex LH-20 CC ( $\text{CHCl}_3$ –MeOH 1:1) and then

fractionated by RP-18 gel (MeOH-H<sub>2</sub>O 40 to 100 %) to provide subfractions B1-B4. B1 was subjected to silica gel CC (CHCl<sub>3</sub>-EtOAc 4:1) and further purified by Sephadex LH-20 CC (CHCl<sub>3</sub>-MeOH 1:1) to afford **6** (1.0 mg). B2 was subjected to silica gel CC eluting with CHCl<sub>3</sub>-EtOAc (3:1) and further purified by semipreparative HPLC (MeOH - H<sub>2</sub>O, 65:35) to afford **2** (2.5 mg), **4** (0.8 mg) and **5** (1.2 mg). B3 was repeatedly chromatographed on silica gel and Sephadex LH-20 CC, followed by preparative TLC with CHCl<sub>3</sub>-EtOAc (4:1) to furnish **1** (1.9 mg) and **3** (1.3 mg).

### 3.4 Macadenanthin A (1)

Yellow powder;  $[\alpha]_D^{21}$  -1.9 (*c* 0.12, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 205 (4.70), 270 (4.34), 384 (4.13), 426 (4.14) nm; IR (KBr)  $\nu_{\max}$  3425, 2969, 2924, 2855, 1648, 1625, 1604, 1562, 1488, 1367, 1318, 1279, 1191, 1091, 902, 820, 583 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1; ESIMS *m/z* 437 [M-H]<sup>-</sup>, 875 [2M-H]<sup>-</sup>; HRESIMS *m/z* 437.1609 (calcd for C<sub>25</sub>H<sub>25</sub>O<sub>7</sub> [M-H]<sup>-</sup>, 437.1600).

### 3.5 Macadenanthin B (2)

Yellow powder;  $[\alpha]_D^{21}$  -2.5 (*c* 0.34, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 211 (4.50), 236 (4.41), 361 (3.98), 430 (4.21) nm; IR (KBr)  $\nu_{\max}$  3423, 2974, 2924, 1648, 1624, 1602, 1564, 1483, 1362, 1320, 1278, 1195, 1157, 1135, 1091, 1042, 960, 807 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1; ESIMS *m/z* 419 [M-H]<sup>-</sup>, 839 [2M-H]<sup>-</sup>; HREIMS *m/z* 420.1576 (calcd for C<sub>25</sub>H<sub>24</sub>O<sub>6</sub> [M]<sup>+</sup>, 420.1573).

### 3.6 Macadenanthin C (3)

Yellow powder;  $[\alpha]_D^{21}$  -3.3 (*c* 0.12, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 204 (4.83), 270 (4.47), 376 (4.27), 426 (4.21) nm; IR (KBr)  $\nu_{\max}$  3428, 2925, 2855, 1648, 1625, 1612, 1485, 1367, 1321, 1268, 1190, 1155, 1090, 1044, 822, 604, 543 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1; ESIMS *m/z* 437 [M-H]<sup>-</sup>; HREIMS *m/z* 438.1675 (calcd for C<sub>25</sub>H<sub>26</sub>O<sub>7</sub> [M]<sup>+</sup>, 438.1679).

### 3.7 Cytotoxicity Bioassays

Compounds **1-3** were tested for their cytotoxicity against human breast adenocarcinoma (MCF-7), human hepatocellular (Hep G2), human cervical carcinoma (Hela), mouse leukemia (P388) by the MTT method, and 5-FU was used as a positive control. Briefly, 100  $\mu$ L cell suspension (1  $\times$  10<sup>5</sup> cells/mL) was seeded into 96-well microtiter plates and cultured for 24 h before the compound was added. Then, different concentrations of the compounds

were added to the plates, the cells were cultivated for 48 h, and 10  $\mu$ L of MTT (5 mg/mL) was added to each well. After 4 h, the culture medium was removed and the formazan crystal was completely dissolved with 150  $\mu$ L DMSO to each well by vigorously shaking the plate. Finally, formazan absorbance was assessed by a BioRad microplate reader at 570 nm.

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**Conflict of interest** The authors declare no conflict of interest.

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