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Xanthones with growth inhibition against HeLa cells from *Garcinia xipshuanbannaensis*

Quan-Bin Han [a], Nian-Yun Yang [a], Hong-Lei Tian [b], Chun-Feng Qiao [a], Jing-Zheng Song [a], Donald C. Chang [c], Shi-Lin Chen [d], Kathy Q. Luo [b,*], Hong-Xi Xu [a,*]

[a] *Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine, Shatin, Hong Kong, PR China*
[b] *Bioengineering Graduate Program, Department of Chemical Engineering, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, PR China*
[c] *Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, PR China*
[d] *Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing 100094, PR China*

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ABSTRACT

Eight prenylated xanthones, bannaxanthones A–H (**1**–**8**), together with seven known compounds, were isolated from the acetone extract of the twigs of *Garcinia xipshuanbannaensis*. Their structures were elucidated by spectroscopic data interpretation. The cytotoxic activities of these compounds were evaluated using the MTT method. The results showed that xanthones with an unsaturated prenyl group had stronger cytotoxic activity against cancer cells, whereas those with hydroxylated prenyl groups had none.

1. Introduction

The medicinal plants of the genus *Garcinia* (Clusiaceae) are one of the research focuses in the view of natural medicinal chemists. As rich natural resources of xanthones, biflavones, and benzophenones (Sordat-Diserens et al., 1989), *Garcinia* plants show various biological properties including antibacterial activity (Rukachaisirikul et al., 2000), antimalarial activity (Likhitwitayawuid et al., 1998), and cytotoxicity (Asano et al., 1996). The most notable lead compound derived from this generous genus is gambogic acid (CAS No. 2752-65-0), which has attracted the interest of many chemists and pharmacologists (Cardillo and Merlini, 1967; Lin et al., 1993; Weakley et al., 2001; Tisdale et al., 2004; Tseng et al., 2004; Zhao et al., 2004; Wu et al., 2004; Zhang et al., 2004; Pandey et al., 2007; Lu et al., 2007). As a prominent anticancer drug candidate, this bioactive compound has been studied in phase I clinical trial in China (Zhou and Wang, 2007). To identify more active compounds, we have conducted a series of studies and analysis on the cytotoxicity (Han et al., 2006, 2007; Yang et al., 2007), stability (Han et al., 2005), and quantitative contents (Song et al., 2007) of the prenylated xanthones isolated from *Garcinia* plants. Herein, we report the isolation, structure elucidation and bioactivity evaluation of eight new and seven known prenylated xanthones from *Garcinia xipshuanbannaensis* which is only distributed in Yunnan province of China and has not been chemically studied before.

2. Results and discussion

The twig barks of *G. xipshuanbannaensis* were extracted with acetone at room temperature for 2 days and the acetone extract was partitioned with $CHCl_3$ and water. The $CHCl_3$-soluble fraction was subjected to a series of chromatographic separations including silica gel liquid chromatography, Sephadex LH-20 column chromatography, and reversed-phase preparative HPLC (Alltima C-18) to obtain eight new prenylated xanthones named bannaxanthones A–H (**1**–**8**), along with seven known compounds (Fig. 1): guttiferone E (**9**) (Roux et al., 2000), xanthochymol (**10**) (Roux et al., 2000), garcinone E (**11**) (Sakai et al., 1993), allanxanthone C (**12**) (Azebaze et al., 2006), isojacareubin (**13**) (Ishiguro et al., 1993), garcinone C (**14**) (Sen et al., 1982), γ-mangostin (**15**) (Sakai et al., 1993).

Bannaxanthone A (**1**) is 1,3,6,7-tetrahydroxy-2-(3-hydroxy-3-methylbutyl)-8-(3-methylbut-2-enyl)xanthone. It was obtained as a yellow amorphous powder with the molecular formula

* Corresponding authors. Address: Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine, Shatin, Hong Kong, PR China. Tel.: +852 3406 2873; fax: +852 3551 7333 (H.-X. Xu); Tel.: +852 2358 8434; fax: +852 3106 4857 (K.Q. Luo).
E-mail addresses: qluo@ust.hk (K.Q. Luo), xuhongxi@hkjcicm.org (H.-X. Xu).

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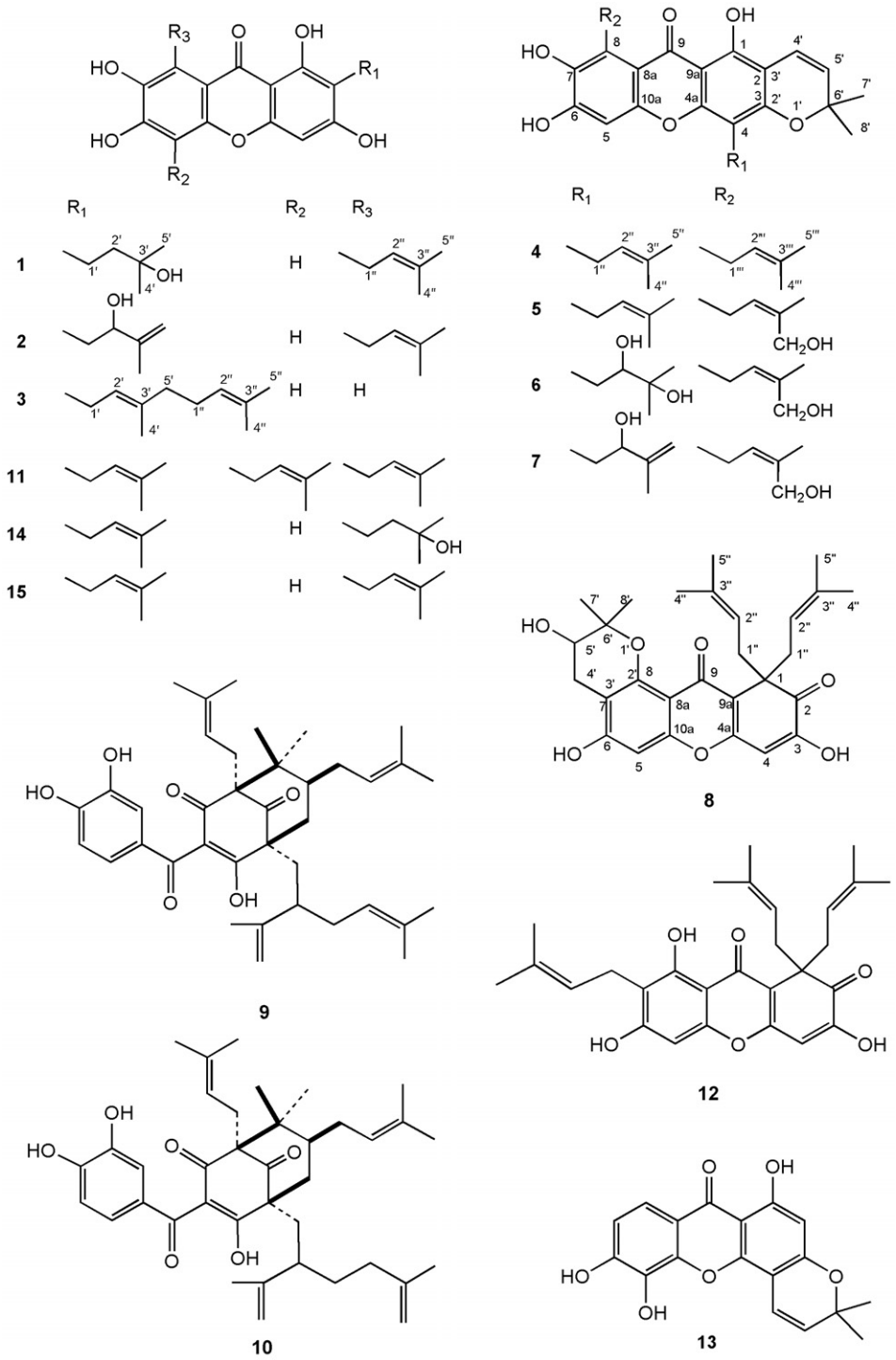

Fig. 1. The structures of compounds **1**–**15**.

$C_{23}H_{26}O_7$, and a $[M-H]^-$ ion peak at *m/z* 413.1589 in its HRESIMS. The IR spectrum showed the presence of hydroxyl groups (3353 cm^{-1}), a conjugated carbonyl group (1647 cm^{-1}), and benzene rings (1616, 1577, and 1542 cm^{-1}). The UV spectrum had absorption maxima at 263 and 322 nm. The ^1H NMR spectroscopic data (Table 1) was very similar to that of a known compound, garcinone C (**14**) (Sen et al., 1982), which had the same molecular formulae and was also isolated at this time. The only significant difference in their ^1H NMR spectra was that the proton signals at δ 3.46 and 3.35 (each 2H) in **14** shifted to δ 4.20 and 2.77 (each 2H) in **1**. The characteristic quaternary carbon signal at δ 70.3 also suggested a 3-hydroxy-3-methylbutyl group in **1**. Therefore, compound **1** was elucidated as an isomer of garcinone C (**14**). The prenyl group was located at C-8 by the HMBC correlations between H-1″ with C-7 and C-8a, and that between H-2″ with C-8. The 3-hydroxy-3-methylbutyl group was linked to C-2 according to the HMBC correlations: 1-OH with C-1 and C-2; H-1′ with C-1, C-3 and C-3′; H-2′ with C-2, C-4′/5′; and H-4′/5′ with C-3′, C-2′. The assigned structure was further confirmed by a detailed HMBC analysis (Fig. 2).

Table 1
^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compounds **1**–**3** [in $(CD_3)_2CO$, δ ppm]

Position	**1**		**2**		**3**	
	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C
1	–	161.4	–	161.4	–	161.3
2	–	111.8	–	108.2	–	111.0
3	–	162.6	–	163.8	–	163.0
4	6.35 *s*	92.9	6.31 *s*	93.7	6.52 *s*	93.7
4a	–	155.4	–	155.9	–	156.1
5	6.81 *s*	100.8	6.83 *s*	100.7	–	132.8
6	–	152.1	–	151.9	–	151.5
7	–	141.4	–	141.2	6.98 *d* (8.7)	131.2
8	–	128.8	–	128.7	7.64 *d* (8.7)	117.1
8a	–	111.8	–	111.6	–	114.5
9	–	182.9	–	182.7	–	180.8
9a	–	103.4	–	103.2	–	102.6
10a	–	153.2	–	153.2	–	146.5
1′	2.77 *m*	17.5	3.12 *dd* (14.4, 1.7) 2.90 *dd* (14.4, 8.0)	29.0	3.38 *d* (7.2)	21.5
2′	1.73 *m*	42.9	4.41 *dd* (8.0, 1.7)	76.2	5.31 *t* (7.2)	122.9
3′	–	70.3	–	147.9	–	134.9
4′	1.26 *s*	29.0	4.95, 4.77 *s*	109.9	1.80 *s*	15.9
5′	1.26 *s*	29.0	1.84 *s*	18.0	1.97 *t* (8.3)	40.1
1″	4.20 *d* (6.8)	26.1	4.19 *d* (6.8)	26.0	2.06 *m*	27.0
2″	5.32 *t* (6.8)	124.1	5.32 *d* (6.8)	124.0	5.08 *t* (7.4)	124.8
3″	–	131.0	–	130.9	–	131.2
4″	1.65 *s*	25.7	1.64 *s*	25.6	1.60 *s*	25.4
5″	1.84 *s*	18.0	1.84 *s*	18.0	1.55 *s*	17.3
1-OH	13.94 *s*	–	14.17 *s*	–	13.46 *s*	–

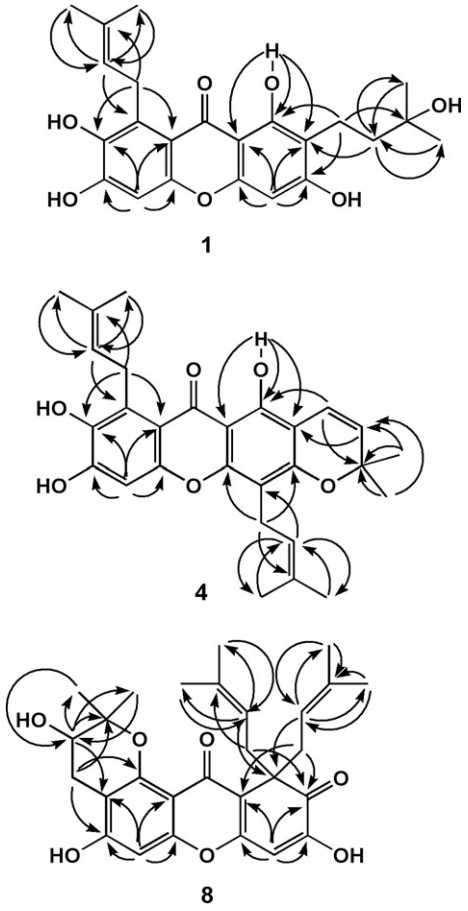

Fig. 2. HMBC correlations of compounds **1**, **4**, and **8**.

Similarly, compound **2** was elucidated to be a close analogue of **1**, having the molecular formula $C_{23}H_{24}O_7$ with two less protons. The difference was readily found from the ^{1}H NMR spectra (Table 1). The signals due to the 3-hydroxy-3-methylbutyl group in **1** were replaced by those of a non-oxygenated methylene (δ 3.12, *dd*, *J* = 14.4, 1.7 Hz, and δ 2.90, *dd*, *J* = 14.4, 8.0 Hz, H-1′), an oxygenated methine (δ 4.41, 1H, *dd*, *J* = 8.0, 1.7 Hz, H-2′), a terminal double bond (δ 4.95 and 4.77, each 1H, brs, H-4′), and a methyl group (δ 1.84, 3H, s, H-5′). These resonances suggested a 2-hydroxy-3-methylbut-3-enyl group. This group was confirmed and located at C-2 by the HMBC correlations: H-4′ with C-5′, C-3′, and C-2′; H-5′ with C-4′, C-3′, and C-2′; H-2′ with C-2, C-4′, and C-5′; 1-OH with C-1 and C-2; H-1′ with C-1, C-3 and C-3′. Therefore, compound **2** was established to be 1,3,6,7-tetrahydroxy-2-(2-hydroxy-3-methylbut-3-enyl)-8-(3-methylbut-2-enyl)xanthone, and named bannaxanthone B. All the ^{1}H and ^{13}C NMR spectroscopic data were unambiguously assigned by HMBC analyses.

Bannaxanthone C (**3**), 1,3,5,6-tetrahydroxy-2-(3,7-dimethyloct-2,6-dienyl)xanthone, was obtained as a yellow amorphous powder, having the molecular formula $C_{23}H_{24}O_6$ according to MS analysis. By comparing the ^{1}H NMR spectrum of the new compound (Table 1) with that of the previously isolated compounds from our group, compound **3** was deduced to be an isomer of 1,2,5,6-tetrahydroxy-7-geranylxanthone (Han et al., 2007). The 3,7-dimethyloct-2,6-dienyl (geranyl) group was confirmed and located at C-2 by the HMBC correlations of: 1-OH, H-1′ and H-2′ with C -2; H-5′ with C-2′, C-3′, C-4′, C-1″ and C-2″; H-1″ with C-5′, C-3′, C-2″ and C-3″; H-2″ with C-5′ and C-1″. A couple of adjacent aromatic protons (δ 7.64, 6.98, each 1H, *d*, *J* = 8.7 Hz) were assigned as H-7 and H-8, respectively, by the HMBC interaction of H-8 with the significant carbonyl carbon C-9 (δ 180.8). Another singlet aromatic proton signal at δ 6.52 was indicated to be H-4 by its HMBC correlations with C-2, C-3, C-4a, and C-9a. Based on the above observations, the structure of **3** was established as 1,3,5,6-tetrahydroxy-2-geranylxanthone, and named bannaxanthone C. The HMBC spectrum was analyzed in full detail and thereby all the ^{1}H and ^{13}C NMR spectroscopic data were clearly assigned.

Bannaxanthone D (**4**) was 1,6,7-trihydroxy-6′,6′-dimethyl-*2H*-pyrano(2′,3′:3,2)-4,8-di(3-methylbut-2-enyl)xanthone. It was obtained as a yellow amorphous powder with the molecular formula $C_{28}H_{30}O_6$, showing a $[M+Na]^+$ ion peak at *m/z* 485.1939 in its HRESIMS. The IR spectrum established the presence of hydroxyl groups (3355 cm^{-1}), a conjugated carbonyl group (1651 cm^{-1}), and benzene rings (1616, 1574, and 1542 cm^{-1}). The UV spectrum displayed absorption maximum at 299 nm. The ^{1}H NMR spectrum (Table 2) exhibited signals of a chelated hydroxyl proton 1-OH at δ 13.92, three aromatic protons H-5 at δ 6.91 (1H, *s*), H-4′ at δ 6.70 (1H, *d*, *J* = 10.0 Hz), and H-5′ at δ 5.68 (1H, *d*, *J* = 10.0 Hz). Also observed were a singlet resonance at δ 1.48 (6H, *s*) due to two tertiary methyl groups and the characteristic signals of two 3-methylbut-2-enyl groups: δ 5.31 (1H, *t*, *J* = 6.7 Hz), δ 5.23 (1H, *t*, *J* = 7.3 Hz), δ 4.19 (2H, *d*, *J* = 6.7 Hz), δ 3.43 (2H, *d*, *J* = 7.3 Hz), δ 1.64, 1.65, 1.84, 1.87 (each 3H, *s*). The ^{13}C NMR spectrum displayed resonances for 28 carbons (Table 1), including one carbonyl carbon, two aromatic rings with oxygenated carbons, and three C_5 groups, corresponding to a triprenylated and tetrahydroxylated xanthone. One of the C_5 group was proved to be attached at C-2, forming a 6′,6′-dimethyl-*2H*-pyrano(2′,3′:3,2)-xanthone skeleton, by the HMBC correlations between C-2 (δ 104.7) with the chelated 1-OH and the *cis*-coupled H-4′ and H-5′, and those between H-5′ with the oxygenated quaternary carbon C-6′ and the tertiary methyl

Table 2
^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compounds **4**–**8** [in $(CD_3)_2CO$, δ ppm]

Position	**4**		**5**		**6**		**7**		**8**	
	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C
1	–	156.9	–	156.3	–	156.5	–	156.6	–	56.8
2	–	104.7	–	103.7	–	104.2	–	104.1	–	201.6
3	–	157.7	–	157.4	–	158.0	–	157.9	–	153.0
4	–	106.9	–	106.6	–	105.4	–	104.2	6.43 *s*	109.3
4a	–	154.4	–	154.0	–	154.9	–	154.8	–	156.6
5	6.91 *s*	101.3	6.88 *s*	101.2	6.86 *s*	101.3	6.87 *s*	101.2	6.44 *s*	94.1
6	–	152.6	–	153.3	–	153.6	–	153.6	–	159.8
7	–	141.8	–	141.3	–	141.2	–	141.4	–	105.9
8	–	129.2	–	127.3	–	127.2	–	127.1	–	155.3
8a	–	111.9	–	111.0	–	111.0	–	110.9	–	108.5
9	–	183.5	–	183.1	–	183.2	–	183.2	–	173.6
9a	–	104.2	–	103.3	–	103.8	–	103.6	–	118.7
10a	–	153.7	–	153.6	–	153.6	–	153.6	–	157.2
4′	6.70 *d* (10.0)	116.5	6.69 *d* (10.0)	116.1	6.71 *d* (10.0)	116.1	6.70 *d* (10.0)	116.0	2.58, 2.96 *m*	26.9
5′	5.68 *d* (10.0)	127.9	5.69 *d* (10.0)	127.6	5.70 *d* (10.0)	127.6	5.69 *d* (10.0)	127.4	3.82 *m*	68.5
6′	–	78.5	–	78.1	–	78.3	–	78.3	–	78.3
7′	1.48 *s*	28.4	1.48 *s*	28.0	1.49 *s*	28.1	1.50 *s*	28.1	1.42 *s*	25.7
8′	1.48 *s*	21.9	1.48 *s*	28.0	1.49 *s*	28.1	1.50 *s*	28.1	1.28 *s*	20.2
1″	3.43 *d* (7.3)	26.3	3.42 *d* (7.4)	21.5	2.94 *m*	25.4	3.00 *m*	29.5	2.67 *dd* (7.0, 13.3) 3.50 *dd* (7.0, 13.3)	38.2
2″	5.23 *t* (7.3)	123.4	5.22 *t* (7.4)	122.9	3.68 *m*	78.3	4.39 *t* (6.9)	75.3	4.72 *t* (7.0)	119.5
3″	–	131.3	–	131.1	–	72.5	–	148.5	–	134.2
4″	1.65 *s*	25.9	1.65 *s*	25.4	1.30 *s*	25.4	4.62, 4.70 *s*	110.0	1.45 *s*	25.5
5″	1.87 *s*	18.0	1.87 *s*	17.7	1.30 *s*	25.4	1.87 *s*	17.4	1.47 *s*	17.7
1‴	4.19 *d* (6.7)	26.3	4.25 *d* (7.6)	26.0	4.25 *d* (7.5)	26.0	4.24 *d* (7.6)	26.0	–	–
2‴	5.31 *t* (6.7)	124.4	5.56 *t* (7.6)	126.5	5.56 *t* (7.5)	126.5	5.56 *t* (7.6)	126.5	–	–
3‴	–	131.4	–	134.7	–	134.7	–	134.8	–	–
4‴	1.64	25.9	1.74 *s*	22.2	1.74 *s*	22.2	1.74 *s*	22.2	–	–
5‴	1.84	18.2	4.34 *s*	61.9	4.34 *s*	61.9	4.34 *s*	62.0	–	–
1-OH	13.92 *s*	–	13.87 *s*	–	13.91 s	–	13.95 *s*	–	–	–

carbons C-7′ and C-8′. The other two 3-methylbut-2-enyl groups were also located at C-4 and C-8, respectively, according to the HMBC correlations between H-2″ with C-4, and H-2″ with C-8. The remaining aromatic proton was indicated to be H-5 by its HMBC correlations with C-6, C-7, C-8a, and C-10a. Based on above structure analysis, compound **4** was 1,6,7-trihydroxy-6′,6′-dimethyl-*2H*-pyrano(2′,3′:3,2)-4,8-di(3-methylbut-2-enyl)xanthone, and named bannaxanthone D. The assigned structure was confirmed further by analyzing the detailed HMBC correlations (Fig. 2).

Bannaxanthone E (**5**) was 1,6,7-trihydroxy-6′,6′-dimethyl-*2H*-pyrano(2′,3′:3,2)-4-(3-methylbut-2-enyl)-8-(4-hydroxy-3-methylbut-2-enyl)xanthone. The IR and UV spectra suggested an analogue of compound **4**. Its molecular formula was determined as $C_{28}H_{30}O_7$ by the HRESIMS (*m/z* 501.1883, $C_{28}H_{30}O_7Na$), with one more oxygen atom than that of **4**. The ^{1}H and ^{13}C NMR spectroscopic data were very similar to those of **4**, except that one methyl signal of the latter was replaced by the signal due to a hydroxymethyl group (δ 4.34, 2H, *s*; 61.9, *t*). The extra group was assigned to C-4‴ by the HMBC correlations between the hydroxymethyl proton with C-2‴, C-3‴, and C-5‴, and the NOE effect between H-2‴ and H-5″. The remaining HMBC correlations resembled those of **4** and confirmed the assigned structure.

Compounds **6** and **7** were another two analogues of **4** and **5**. In the same way, they were identified as 1,6,7-trihydroxy-6′,6′-dimethyl-*2H*-pyran(2′,3′:3,2)-4-(2,3-dihydroxy-3-methylbutyl)-8-(4-hydroxy-3-methylbut-2-enyl)xanthone (**6**) and 1,6,7-trihydroxy-6′,6′-dimethyl-*2H*-pyrano(2′,3′:3,2)-4-(2-hydroxy-3-methylbut-3-enyl)-8-(3-hydroxymethylbut-2-enyl)xanthone (**7**), and named bannaxanthones F and G, respectively. This elucidation was supported by the complete HMBC analysis, and all the ^{1}H and ^{13}C NMR spectroscopic data were unambiguously assigned as shown in Table 2.

Bannaxanthone H (**8**) was 1,2-dihydro-3,6-dihydroxy-1,1-di(3-methylbut-2-enyl)-6′,6′-dimethyl-5′-hydroxy-*2H*-pyrano(2′,3′:8,7)-xanthene-2,9-dione. It was also obtained as a yellow amorphous powder. The molecular formula was determined as $C_{28}H_{32}O_7$ according to its HRESIMS, which showed the $[M-H]^-$ ion peak at *m/z* 479.2055. The IR spectrum established the presence of hydroxyl groups (3394 cm^{-1}), a conjugated carbonyl group (1670 cm^{-1}), and a xanthone carbonyl group (1653 cm^{-1}). Different from the other polyprenylated xanthones, the ^{1}H NMR spectrum of **8** displayed a set of noticeable signals at δ 4.72 (2H, *t*, *J* = 7.0 Hz), 2.67 and 3.50 (each 2H, *dd*, *J* = 7.0, 13.3 Hz), 1.45 and 1.47 (each 6H, *s*). These resonances were typical of a *gem* bis(3-methylbut-2-enyl) group linked to a sp^3 carbon atom (δ 56.8, C-1) (Iinuma et al., 1997). The additional carbonyl signal at δ 201.6 suggested a xanthenedione skeleton of this compound. Further comparison with the isolated compounds confirmed that this was an analogue of a known xanthenedione named allanxanthone C (**12**) (Azebaze et al., 2006), having similar substituents on the same xanthenedione skeleton. Compared to compound **12**, this new compound did not exhibit signals of the third prenyl group. The substituted group was a structural moiety of 6′,6′-dimethyl-5′-hydroxy-*2H*-pyran ring, showing signals at δ 2.58 (1H, *m*, H-4′a), and 2.96 (1H, *m*, H-4′b), 3.82 (1H, *m*, H-5′), 1.42 and 1.28 (each 3H, *s*, H-7′ and H-8′) in the ^{1}H NMR spectrum. The corresponding resonances in the ^{13}C NMR spectrum were due to a non-oxygenated methylene carbon at δ 26.9 (C-4′), an oxygenated methine carbon at δ 68.5 (C-5′), an oxygenated quaternary carbon at δ 78.3 (C-6′), and two methyl carbons at δ 25.7 (C-7′) and 20.2 (C-8′). To unequivocally corroborate the substitution pattern of **8**, the HMBC spectrum was also analyzed in full detail as shown in Fig. 2. The complete HMBC analysis confirmed the assigned structure. All of the ^{1}H and ^{13}C NMR spectroscopic data were clearly indicated as presented in Table 2 according to the detailed HMBC and HMQC correlations.

The cytotoxic activities of isolated xanthones were evaluated using the MTT assay. To quantitatively compare the cytotoxic potency of the tested compounds, three parameters including GI_{50}, TGI, and LC_{50} were calculated as described in Section 4. The results showed that compounds **4**, **11**, and **15** are the most effective

Table 3
GI_{50}, TGI, and LC_{50} of isolated compounds ($n = 3$)

Compounds[a]	GI_{50} (μM)	TGI (μM)	LC_{50} (μM)
2	12.1 ± 0.8	>100	>100
4	20.8 ± 3.9	24.7 ± 0.6	26.2 ± 0.2
11	8.2 ± 2.7	19.3 ± 0.9	25.6 ± 1.5
14	29.7 ± 13.2	47.2 ± 1.7	59.4 ± 9.9
15	10.8 ± 4.7	18.4 ± 7.0	27.0 ± 3.9
Camptothecin	4.8 ± 2.4	41.1 ± 10.4	>200
Etoposide	17.0 ± 4.0	91.3 ± 5.3	>200

[a] Compounds **1, 3, 5, 6, 7, 8, 12,** and **13** are inactive with GI_{50} beyond 30 μM.

compounds in inhibiting cancer cell growth and promoting cancer cell death with lower TGI and LC_{50} (Table 3) than two clinically used anticancer drugs, camptothecin and etoposide. It is interesting to note that all of these three active compounds contain unsaturated hydrophobic prenyl group. In comparison, compounds **1**, **2**, **3**, **5**, **6**, **7**, **8**, **12**, **13**, and **14**, which have a hydroxylated prenyl group, were less potent in killing cancer cells. The unsaturated hydrophobic prenyl group may be therefore essential for the interaction between this group of compounds and the drug target. We also found that the number of unsaturated prenyl substitutes can significantly affect the cytotoxic activity of xanthone compounds: the more prenyl groups, the more potent effect, which had been found in our previous study (Han et al., 2007). These results suggested that increasing the polarity of the prenyl group may actually reduce the cytotoxic activity of the prenylated xanthones.

3. Concluding remarks

Prenylated xanthones and related benzophenones are two major chemical groups that commonly occur together in *Garcina* species. The prenylated xanthones can be further classified into two types: caged xanthones such as gambogic acid, and normal xanthones with unfolded side chains such as bannaxanthones. We concluded from this study and literature research that caged xanthones never occur alongside either normal xanthones or benzophenones. This suggests that while gambogic acid can be synthesized from normal xanthone (Ollis et al., 1965), there must be two strictly different biogenesis pathways in nature: one that leads to gambogic acids and one that leads to similar caged benzophenones and simple xanthones.

Another important finding from our study is that the greater the number of prenyl substituents the higher was the cytotoxic potency. This could contribute to the generation of more potent cytotoxic compounds, in which complex moiety constructed with more prenyls can be synthesized.

4. Experimental

4.1. General experimental procedures

Ultraviolet absorption spectra were recorded using a Perkin–Elmer Lambda L14 spectrometer, whereas infrared spectra were obtained with a Perkin–Elmer 577 spectrometer. ^1H and ^{13}C NMR spectra were measured on a Bruker AV-400 spectrometer with TMS as the internal standard. The electrospray ionization mass spectra were measured using an Esqurie 2000 instrument. The high resolution mass spectra were measured by an Esqurie 4000 instrument. Column chromatography (CC) was performed with silica gel 60 (200–300 mesh, Merck), Sephadex LH-20, and reversed-phase C-18 silica gel (250 mesh, Merck). Pre-coated TLC sheets of silica gel 60 GF_{254} were used. Agilent 1100 series equipped with an Alltima C-18 column (4.6 × 250 mm) was used for HPLC analysis and a preparative Alltima C-18 column (22 × 250 mm) was used in sample preparation.

4.2. Plant material

Twigs of *G. xipshuanbannaensis* H.Y. Li were collected in Bubang of Xishuangbanna prefecture, Yunnan Province, China in 2006. The plant material was identified by Dr. Chunfeng Qiao. A herbarium sample (CMED-0471) is deposited in the Hong Kong Jockey Club Institute of Chinese Medicine.

4.3. Extraction and isolation

Air-dried and powdered twigs (3.0 kg) were extracted with acetone (3 × 3 L) at room temperature for six days. The extracted solution was evaporated under reduced pressure to yield a dark green residue (168 g). An aqueous suspension (1 L) of this extract was extracted with $CHCl_3$ (3 × 1000 mL). The $CHCl_3$ fraction was evaporated in vacuo to give a residue (43 g), which was subjected to silica gel cc eluted with a $CHCl_3/CH_3OH$ system (100:0–10:1, v/v) gradient. Five fractions (I–V) were obtained on the basis of TLC analysis. Fraction I was separated by HPLC on a Alltima C-18 column and eluted with a gradient of CH_3CN in 0.1% AcOH (0.1% AcOH/CH_3CN, 10:90) to yield **9** (3 mg) and **10** (5 mg). Fraction II was applied to a Sephadex LH-20 column to give two major subfractions. The first subfraction was separated by HPLC on a semi-preparative Alltima C-18 column and eluted with 0.1% AcOH/CH_3CN (20:80) to afford **11** (130 mg), **12** (1.05 g), and **5** (160 mg), respectively. The second subfraction was further separated in a similar way, eluted with 0.1% AcOH/CH_3CN (10:90) to give **4** (90 mg). Fraction III was separated using reversed-phase C-18 silica gel and eluted with a CH_3CN/H_2O system (60:40–40:60) gradient to afford five subfractions. Subfraction 1 was separated by HPLC on a semi-preparative Alltima C-18 column, eluted with 0.1% AcOH/CH_3CN (60:40) to give **6** (11 mg), **1** (6 mg), and **13** (3 mg). Subfraction 2 was further separated by the same method, using 0.1% AcOH/CH_3CN (55:45) as the eluting solvent to produce **2** (18 mg) and **14** (21 mg). Eluting with 0.1% AcOH/CH_3CN (50:50), the semi-preparative HPLC of subfraction 3 yielded compound **7** (21 mg). Compound **3** (3 mg) was isolated from subfraction 4 after repeated HPLC purification on an Alltima C-18 column, eluted with 0.1% AcOH/CH_3CN (45:55). By the same method, using 0.1% AcOH/CH_3CN (40:60) as eluting solvent, compound **15** (83 mg) was isolated from subfraction 5. Fraction IV was separated by semi-preparative HPLC on an Alltima C-18 column and eluted with 0.1% AcOH/CH_3CN (65:35) to produce **8** (14 mg).

4.3.1. Bannaxanthone A (**1**)

Yellow amorphous solid, UV λ_{max}^{MeOH} nm (log ε): 263 (4.68), 322 (4.49); IR ν_{max}^{KBr} cm^{-1}: 3353, 2970, 2360, 1647, 1616, 1577, 1542, 1458, 1288, 1195, 1157, 1076, 1041, 817; For ^1H and ^{13}C NMR [$(CD_3)_2CO$] spectroscopic data, see Table 1; negative ESIMS *m/z*: 413 $[M-H]^-$ (100); positive ESIMS *m/z*: 437 $[M+Na]^+$ (87); HR-ESIMS *m/z*: $[M-H]^-$ 413.1589 (calcd. for $C_{23}H_{25}O_7$, 413.1595).

4.3.2. Bannaxanthone B (**2**)

Yellow amorphous solid, UV λ_{max}^{MeOH} nm (log ε): 264 (4.72), 322 (4.50); IR ν_{max}^{KBr} cm^{-1}: 3352, 2923, 2360, 1647, 1616, 1458, 1284, 1196, 1164, 1095, 1045, 821; For ^1H and ^{13}C NMR [$(CD_3)_2CO$] spectroscopic data, see Table 1; negative ESIMS *m/z*: 411 $[M-H]^-$ (100), positive ESIMS *m/z*: 435 $[M+Na]^+$ (76); HR-ESIMS *m/z*: $[M-H]^-$ 411.1424 (calcd. for $C_{23}H_{23}O_7$, 411.1438).

4.3.3. Bannaxanthone C (**3**)

Yellow amorphous solid, UV λ_{max}^{MeOH} nm (log ε): 256 (4.49), 330 (4.18); IR ν_{max}^{KBr} cm^{-1}: 3337, 2932, 1652, 1575, 1491, 1467, 1297, 1260, 1232, 1182, 1153, 1115, 1075; For ^1H and ^{13}C NMR [$(CD_3)_2CO$] spectroscopic data, see Table 1; negative ESIMS *m/z*:

395 $[M-H]^-$ (100), positive ESIMS *m/z*: 419 $[M+Na]^+$ (34); HR-ESIMS *m/z*: $[M-H]^-$ 395.1484 (calcd. for $C_{23}H_{23}O_6$, 395.1489).

*4.3.4. Banaxanthone D (**4**)*

Yellow amorphous solid, UV λ_{max}^{MeOH} nm (log ε): 299 (4.60); IR ν_{max}^{KBr} cm^{-1}: 3355, 2974, 2360, 1651, 1616, 1574, 1542, 1458, 1434, 1280, 1195, 1153, 1122, 1056, 995, 837, 721; For ^1H and ^{13}C NMR $[(CD_3)_2CO]$ spectroscopic data, see Table 2; negative ESIMS *m/z*: 461 $[M-H]^-$ (100); positive ESIMS *m/z*: 485 $[M+Na]^+$ (100); HR-ESIMS *m/z*: 485.1939 $[M+Na]^+$ (calcd. for $C_{28}H_{30}O_6Na$, 485.1940).

*4.3.5. Bannaxanthone E (**5**)*

Yellow amorphous solid, UV λ_{max}^{MeOH} nm (log ε): 300 (4.56); IR ν_{max}^{KBr} cm^{-1}: 3355, 2974, 2360, 1647, 1604, 1554, 1458, 1434, 1280, 1153, 1118, 1056, 979, 837, 721; For ^1H and ^{13}C NMR $[(CD_3)_2CO]$ spectroscopic data, see Table 2; negative ESIMS *m/z*: 477 $[M-H]^-$ (100); positive ESIMS *m/z*: 501 $[M+Na]^+$ (100); HR-ESIMS *m/z*: 501.1883 $[M+Na]^+$ (calcd. for $C_{28}H_{30}O_7Na$, 501.1889).

*4.3.6. Bannaxanthone F (**6**)*

Yellow amorphous solid, UV λ_{max}^{MeOH} nm (log ε): 300 (4.55); IR ν_{max}^{KBr} cm^{-1}: 3355, 2974, 2360, 1651, 1612, 1574, 1458, 1377, 1288, 1153, 1118, 1060, 991, 837; For ^1H and ^{13}C NMR $[(CD_3)_2CO]$ spectroscopic data, see Table 2; negative ESIMS *m/z*: 511 $[M-H]^-$ (100), positive ESIMS *m/z*: 535 $[M+Na]^+$ (100); HR-ESIMS m/z: $[M-H]^-$ 511.1951 (calcd. for $C_{28}H_{31}O_9$, 511.1963).

*4.3.7. Bannaxanthone G (**7**)*

Yellow amorphous solid, UV λ_{max}^{MeOH} nm (log ε): 300 (4.55); IR ν_{max}^{KBr} cm^{-1}: 3463, 2974, 2360, 1651, 1612, 1570, 1438, 1365, 1276, 1211, 1188, 1153, 1118, 1060, 987, 829, 721; For ^1H and ^{13}C NMR $[(CD_3)_2CO]$ spectroscopic data, see Table 2; negative ESIMS *m/z*: 493 $[M-H]^-$ (100), positive ESIMS *m/z*: 517 $[M+Na]^+$ (95); HR-ESIMS *m/z*: $[M-H]^-$ 493.1847 (calcd. for $C_{28}H_{29}O_8$, 493.1857).

*4.3.8. Bannaxanthone H (**8**)*

Yellow amorphous solid, UV λ_{max}^{MeOH} nm (log ε): 296 (4.19); IR ν_{max}^{KBr} cm^{-1}: 3394, 2974, 2927, 2360, 2341, 1670, 1653, 1612, 1454, 1377, 1265, 1192, 1130, 1083, 1057, 821; For ^1H and ^{13}C NMR $[(CD_3)_2CO]$ spectroscopic data, see Table 2; negative ESIMS *m/z*: 479 $[M-H]^-$ (100); positive ESIMS *m/z*: 503 $[M+Na]^+$ (61); HR-ESIMS *m/z*: $[M-H]^-$ 479.2055 (calcd. for $C_{28}H_{31}O_7$, 479.2064).

4.4. Cell culture, MTT assay and calculation of GI_{50}, TGI and LC_{50}

The MTT assay was carried out according to the previous protocol (Tian et al., 2007). The value of GI_{50}, TGI and LC_{50} were determined using the method described by NCI (Monks et al., 1991) with some modifications. First 7500 HeLa cells were seeded in each well of a 96-well plate. After overnight culture, the tested compounds at different concentrations were added into 96-well plates. The concentrations applied were 100, 50, 25, 12.5, 6.25, and 3.125 μM, respectively. The optical density (OD) of the control group at 0 h (C_0) and 48 h (C_{48}) together with the compound treated groups at 48 h (T_i) were measured using the MTT assay. Three dose response parameters were calculated for each compound using the following definitions. GI_{50} is the concentration of a compound inhibiting 50% of the cell growth. At GI_{50}, the OD of the tested compound = $[(T_i - C_0)/(C_{48} - C_0)] \times 100 = 50$. TGI is the concentration of a compound completely inhibits the cell growth at 48 h. At TGI, the OD of the tested compound = C_0. LC_{50} is defined as the concentration at which the tested compound kill 50% of the cells at 48 h. At IC_{50} the OD of the tested compound = $[(T_i - C_0)/C_0] \times 100 = -50$. Anticancer drugs, camptothecin (Calbiochem, USA) and etoposide (Sigma, USA) were used as positive controls.

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