Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron
Tetrahedron 62 (2006) 8850–8859

Bioactive prenylated xanthones and anthraquinones from *Cratoxylum formosum* ssp. *pruniflorum*

Nawong Boonnak,[a] Chatchanok Karalai,[a,*] Suchada Chantraprommma,[a] Chanita Ponglimanont,[a] Hoong-Kun Fun,[b] Akkharawit Kanjana-Opas[c] and Surat Laphookhieo[d]

[a]*Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand*
[b]*X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia*
[c]*Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand*
[d]*School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand*

Received 31 March 2006; revised 16 May 2006; accepted 1 June 2006
Available online 25 July 2006

Abstract—Ten new compounds (**1–10**), pruniflorone A–J, together with 21 known compounds (**11–31**) were isolated from the roots and barks of *Cratoxylum formosum* ssp. *pruniflorum*. Their structures were determined by spectroscopic methods. Compounds **1** and **11** were also confirmed by X-ray diffraction data. In addition, antibacterial and cytotoxic activities of the isolates were also evaluated.
© 2006 Elsevier Ltd. All rights reserved.

1. Introduction

Cratoxylum belongs to the family Guttiferae, which is distributed in several Southeast Asian countries. Six species are found in Thailand;[1] *Cratoxylum arboresens*, *Cratoxylum cochinchinense*, *Cratoxylum maingayi*, *Cratoxylum sumatranum* ssp. *neriifolium*, *Cratoxylum formosum* ssp. *formosum* (Jack) Dyer and *Cratoxylum formosum* (Jack) Dyer ssp. *pruniflorum* (Kurz) Gogel. The last two species, which are subspecies of *C. formosum*, can be differentiated through the young twigs, leaves, pedicels and sepals. Those of *C. formosum* ssp. *formosum* are glabrous, whereas *C. formosum* ssp. *pruniflorum* are densely villous.[2] Some species of this genus have been used for the treatment of diuretic, stomachic and tonic effects,[3] as well as for diarrhoea and flatulence,[4] and for food poisoning and internal bleeding.[5] They produce various types of secondary metabolites, including xanthones,[6a–c] triterpenoids[6b,7] and flavonoids.[3] We have previously isolated a number of xanthones from the roots of *C. formosum* ssp. *formosum*.[8] As a continuation of our study on this genus, we report herein nine new xanthones (**1–9**): pruniflorones A–I, and nine known xanthones (**11–19**) from the roots, a new anthraquinone (**10**): pruniflorone J, six known anthraquinones (**20–25**) and six known xanthones (**26–31**) from the barks of *C. formosum* ssp. *pruniflorum*. In addition, the antibacterial and cytotoxic activities of selected compounds are also reported.

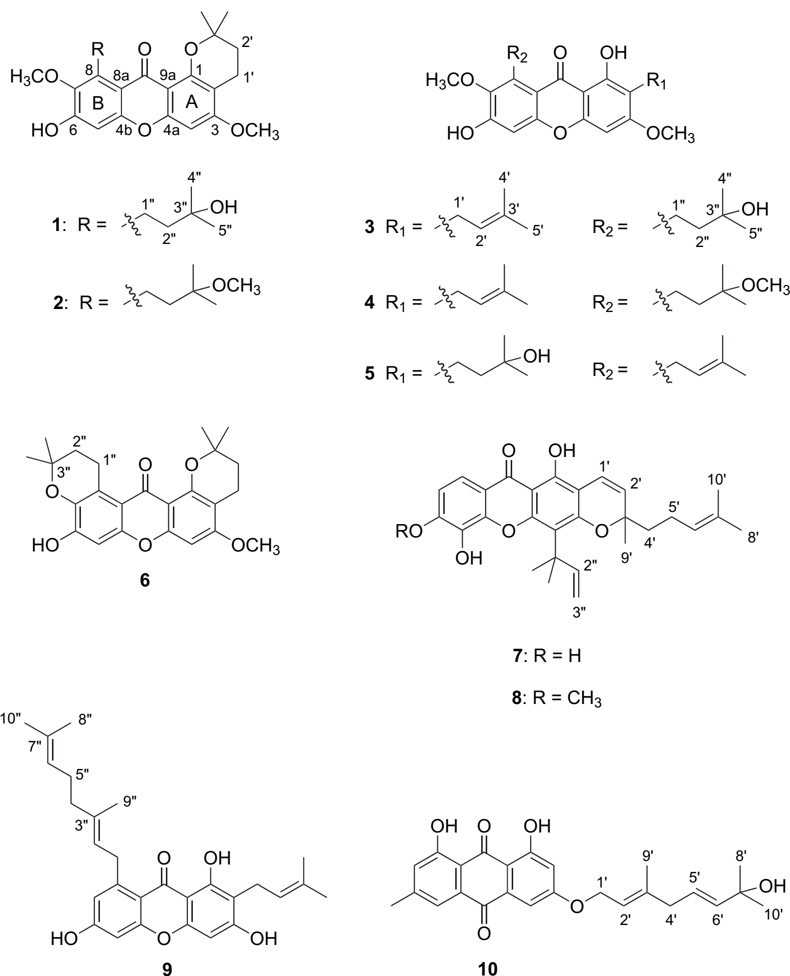
2. Results and discussion

The CH_2Cl_2 extracts of the roots and barks of *C. formosum* ssp. *pruniflorum* were subjected to column chromatography to give nine new xanthones (**1–9**) and a new anthraquinone (**10**). All isolated new xanthones gave characteristic signals in the UV spectrum, showing absorption bands in the range of 236–261 and 312–380 nm. The IR spectra also exhibited characteristic conjugated carbonyl and hydroxyl functionalities in the range of 1632–1646 and 3170–3414 cm^{-1}, respectively. Moreover, the ^1H and ^{13}C NMR spectral data suggested that the isolated new xanthones **1–6** had the 1,3,6,7-oxygenated xanthone skeleton, whereas xanthones **7–8** and **9** were 1,3,5,6- and 1,3,6-oxygenated xanthones, respectively.

Pruniflorone A (**1**) was isolated as a pale yellow powder, which was further recrystallized from $CHCl_3$–MeOH (4:1, v/v) to yield pale yellow single crystals. The X-ray structure (Fig. 1) confirmed a molecular structure with a prenylated xanthone skeleton and a molecular formula $C_{25}H_{30}O_7$. Its structure was supported by ^1H and ^{13}C NMR spectral data (Tables 1 and 2). The ^1H NMR spectral data of **1** (Table 1) showed two aromatic protons at δ 6.29 (s, H-4) and 6.72 (s, H-5), and two methoxyl groups at δ 3.90 (s, 3-OMe) and 3.84 (s, 7-OMe). In addition, the ^1H NMR spectral data also exhibited a dimethylchromane ring[9] at δ 2.73 (2H, br t,

Keywords: *Cratoxylum formosum* ssp. *pruniflorum*; Pruniflorone; Xanthone; Anthraquinone; Antibacterial activity; Cytotoxic activity.
* Corresponding author. Tel.: +66 7428 8444; fax: +66 7421 2918; e-mail addresses: chatchanok.k@psu.ac.th; chatchanok_k@yahoo.com

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.06.003



Structures of pruniflorone A-J

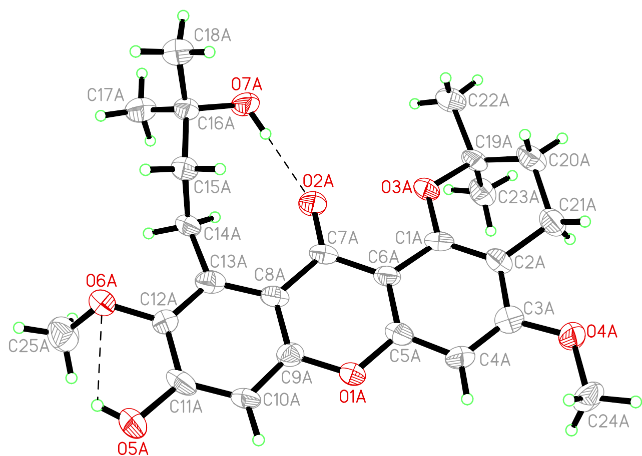


Figure 1. The ORTEP plot of **1**. There are two molecules in asymmetric unit of **1**, only one molecule was shown for clarity.

J=7.5 Hz, H-1′), 1.72 (2H, br t, J=7.5 Hz, H-2′) and 1.34 (6H, s, H-4′ and H-5′). A 3-hydroxyl-3-methylbutyl group[9] was evident from signals at δ 3.38 (2H, m, H-1″), 1.78 (2H, m, H-2″) and 1.30 (6H, s, H-4″ and H-5″). From these data, the structure of pruniflorone A was deduced to be **1**.

Pruniflorone B (**2**) was obtained as a yellow powder with a molecular ion peak at m/z 456.2116 $[M]^+$ in the HREIMS, corresponding to a molecular formula of $C_{26}H_{32}O_7$. The UV, ^1H and ^{13}C NMR spectral data of **2** (Tables 1 and 2) were similar to those of **1**, indicating the presence of a xanthone skeleton. However, an additional methoxyl singlet signal was apparent in both the ^1H+^{13}C spectra (δ_H 3.35, δ_C 49.2). This methoxyl group was located at C-3″ from the HMBC correlation with C-3″ (75.1). The completed HMBC correlations were summarized in Table 3. Thus, the structure of pruniflorone B was assigned as **2**.

Pruniflorone C (**3**) showed a molecular formula of $C_{25}H_{30}O_7$ by HREIMS. The ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 2) were similar to those of **1**, except for the presence of signals for a prenyl group at δ_H 3.33 (2H, d, J=7.2 Hz, H-1′), δ_C 21.1; δ_H 5.21 (1H, br t, J=7.2, H-2′), δ_C 122.1; δ_H 1.79 (3H, s, H-4′), δ_C 17.5; δ_H 1.68 (3H, s, H-5′), δ_C 25.6 and δ_C 131.6 (C-3′) instead of the dimethylchromane ring present in **1**. HMBC data confirmed the position of the prenyl group in **3** (Table 3) at C-2 by the ^2J correlation of H-1′ with C-2, and the ^3J correlations of H-1′ with C-1 and C-3. The structure of pruniflorone C was therefore assigned as **3**.

Pruniflorone D (**4**) showed a molecular formula of $C_{26}H_{32}O_7$ by HREIMS. The ^1H NMR spectral data (Table 1) of **4** were similar to those of **3**, except for an additional methoxyl singlet signal at δ_H 3.32, δ_C 49.2. This group replaced the hydroxyl group at C-3″ in **3**. HMBC correlations between

Table 1. ^{1}H NMR spectral data of **1–6** (δ in ppm, multiplicities, *J* in Hz)

Position	**1**[a]	**2**[b]	**3**[c]	**4**[b]	**5**[c]	**6**[a]
4	6.29 s	6.33 s	6.32 s	6.34 s	6.38 s	6.35 s
5	6.72 s	6.76 s	6.75 s	6.82 s	6.78 s	6.74 s
1′	2.73 br t (7.5)	2.63 br t (7.0)	3.33 d (7.2)	3.36 d (7.5)	2.72 m	2.64 t (6.9)
2′	1.72 br t (7.5)	1.83 br t (7.0)	5.21 br t (7.2)	5.23 br t (7.5)	1.71 m	1.82 t (6.9)
4′	1.34 s	1.41 s	1.79 s	1.81 s	1.29 s	1.46 s
5′	1.34 s	1.41 s	1.68 s	1.69 s	1.29 s	1.46 s
1″	3.38 m	3.37 m	3.39 m	3.38 m	4.12 d (6.9)	3.58 t (6.9)
2″	1.78 m	1.79 m	1.77 m	1.77 m	5.26 m	1.84 t (6.9)
4″	1.30 s	1.31 s	1.32 s	1.30 s	1.85 s	1.36 s
5″	1.30 s	1.31 s	1.32 s	1.30 s	1.69 s	1.36 s
1-OH			[d]	13.60 s	[d]	
3-OMe	3.90 s	3.89 s	3.90 s	3.91 s	3.92 s	3.90 s
7-OMe	3.84 s	3.83 s	3.84 s	3.86 s	3.80 s	
3″-OMe		3.35 s		3.32 s		

[a] Recorded at 300 MHz in $CDCl_3$.
[b] Recorded at 500 MHz in $CDCl_3$.
[c] Recorded at 300 MHz in $CD_3OD/CDCl_3$.
[d] Exchangeable with CD_3OD.

3″-OMe and δ_C 74.9 (C-3″), and H-2″ with δ_C 22.2 (C-1″) and 74.9 (C-3″) (Table 3) proved this assignment. Thus, the structure of pruniflorone D was assigned as **4**.

Pruniflorone E (**5**) was isolated as a yellow gum, which showed a molecular ion peak at *m/z* 442.2000 $[M]^+$ in the HREIMS, corresponding to a molecular formula of $C_{25}H_{30}O_7$. The UV and IR spectra of **5** exhibited the same patterns as those of **3** and **4**. Extensive 1D and 2D NMR analysis of **5** showed that this xanthone has the same substituents as **3**; a 3-hydroxyl-3-methylbutyl, a prenyl and two methoxyl groups (Tables 1 and 2). However, the ^{1}H NMR spectral data of **5** (Table 1) and **3** exhibited different chemical shifts for the 3-hydroxyl-3-methylbutyl and prenyl moieties. From HMBC spectral data (Table 3), the methylene protons of H-1′ of a 3-hydroxyl-3-methylbutyl group showed correlations with δ_C 163.3 (C-3), 159.8 (C-1) and 103.6 (C-2), and the methoxyl group at δ_H 3.92 showed a cross peak with δ_H 6.38 (H-4) in the NOESY spectrum (Table 4). It was therefore apparent there that the 3-hydroxyl-3-methylbutyl and methoxyl substituent groups were located at C-2 and C-3 in ring A, respectively. In addition, the signal for H-1″ of the prenyl group (δ 4.12, 2H, d, J=6.9 Hz) in **5** appears further downfield than the expected values for this functionality (ca. δ 3.5–3.3).[10] This can be explained by the fact that H-1″ is in a deshielding region of the carbonyl functionality. The HMBC experiment was also used to confirm the position of attachment of the prenyl group in **5** (Table 3) at C-8 by the ^{2}J correlation of H-1″ with C-8. Thus, the structure of pruniflorone E was assigned as **5**, a constitutional isomer of **3**.

Table 2. ^{13}C NMR (75 MHz) spectral data of **1–6** in $CD_3OD/CDCl_3$

Position	**1**	**2**[a]	**3**[a]	**4**[a,b]	**5**[a]	**6**[b]
1	155.2	155.3	159.1	159.8	159.8	155.6
2	105.6	105.4	111.3[c]	111.5	103.6	105.2
3	162.0	161.4	163.3	163.5	163.3	161.3
4	89.6	89.6	88.7	88.8	88.9	89.7
5	101.3	100.8	101.7	101.4	101.7	99.7
6	154.6	154.5	156.0	154.5	155.1	150.1
7	143.3	142.3	143.1	142.6	143.0	137.7
8	138.4	138.5	138.4	138.8	137.2	122.0
9	177.5	176.4	181.8	182.0	181.9	177.4
4a	157.1	156.9	155.1	155.2	155.4	157.2
4b	155.2	152.9	155.6	155.8	155.6	151.7
8a	113.8	115.5	111.2[c]	112.5	112.0	114.1
9a	107.2	107.9	103.5	103.8	111.9	107.8
1′	16.9	17.1	21.1	21.4	16.9	17.1
2′	31.3	31.4	122.1	122.3	42.1	31.5
3′	75.7	75.2[c]	131.6	131.7	71.1	75.2[c]
4′	26.1	26.5	17.5	17.8	28.9	26.6
5′	26.1	26.5	25.6	25.8	28.9	26.6
1″	21.8	21.8	21.8	22.2	26.4	22.6
2″	43.9	39.7	44.0	39.9	123.2	33.1
3″	70.7	75.1[c]	70.8	74.9	131.9	75.3[c]
4″	28.7	25.3	28.7	25.2	18.1	26.5
5″	28.7	25.3	28.7	25.2	25.8	26.5
3-OMe	55.6	55.7	55.3	55.8	55.8	55.7
7-OMe	60.9	62.0	61.2	62.2	61.4	
3″-OMe		49.2		49.2		

[a] Recorded at 125 MHz.
[b] Recorded in $CDCl_3$.
[c] May be interchangeable.

Pruniflorone F (**6**), a pale yellow powder, was deduced as $C_{24}H_{26}O_6$ from an exact mass measurement. The ^{1}H and ^{13}C NMR spectral data of **6** (Tables 1 and 2) were closely related to those of **1**. The major difference was the replacement of the ^{1}H NMR signals for the methoxyl and 3-hydroxyl-3-methylbutyl groups at C-7 and C-8, respectively, of **1** with a dimethylchromane ring at δ 3.58 (2H, t, J=6.9 Hz, H-1″), 1.84 (2H, t, J=6.9 Hz, H-2″) and 1.36 (6H, s, H-4″ and H-5″) in **6**. The observed HMBC correlations (Table 3) confirmed the assignment of this structure.

Pruniflorone G (**7**), a brown powder, was deduced as $C_{28}H_{30}O_6$ from an exact mass measurement. The ^{1}H NMR spectral data of **7** (Table 5) were similar to those of gerontoxanthone I (29),[11] except for the appearance of the signal of a chromene ring bearing a methyl group and six-carbon side chain (Table 5) instead of the prenyl moiety present in gerontoxanthone I. The proposed structure was further supported by the appearance of an abundant fragment ion *m/z* 379 ($[M]^+$ −83), resulting from loss of the 4-methylpent-3-enyl moiety. The location of a chromene ring was confirmed by HMBC (Table 6), in which the methine proton H-1′ at δ_H 6.83 was correlated with δ_C 81.1 (C-3′), 105.2 (C-2), 156.8 (C-1) and 159.2 (C-3), while the methine proton H-2′ at δ_H 5.58 was correlated with δ_C 26.9 (C-9′), 41.8

Table 3. HMBC (300 MHz) spectral data of **1–6** in $CDCl_3$

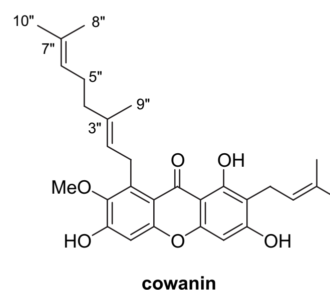
Position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[a]	**6**
4	C-2, C-3, C-9, C-4a, C-9a	C-2, C-3, C-9, C-4a, C-9a	C-2, C-3, C-9, C-4a, C-9a	C-2, C-3, C-9, C-4a, C-9a	C-2, C-3, C-9, C-4a, C-9a	C-3, C-9, C-4a, C-9a
5	C-7, C-8, C-9, C-4b, C-8a	C-6, C-7, C-9, C-4b, C-8a	C-6, C-7, C-8, C-4b, C-8a, C-9	C-6, C-9, C-4b, C-8a	C-6, C-7, C-9, C-4b, C-8a	C-6, C-7, C-9, C-4b, C-8a
1′	C-1, C-2, C-3, C-2′, C-3′	C-1, C-2, C-3, C-2′, C-3′	C-1, C-2, C-3, C-2′, C-3′	C-1, C-2, C-3, C-2′, C-3′	C-1, C-2, C-3, C-2′	C-1, C-2, C-3, C-2′, C-3′
2′	C-2, C-1′, C-3′, C-4′, C-5′	C-2, C-1′, C-3′, C-4′, C-5′		C-2, C-4′, C-5′	C-3′, C-4′, C-5′	C-2, C-1′, C-3′, C-4′, C-5′
4′	C-1, C-1′, C-2′	C-2′, C-3′	C-2′, C-3′, C-5′	C-2′, C-3′	C-2′, C-3′	C-2′, C-3′
5′	C-1, C-1′, C-2′	C-2′, C-3′	C-2′, C-3′, C-4′	C-2′, C-3′	C-2′, C-3′	C-2′, C-3′
1″	C-7, C-8, C-8a, C-3″	C-7, C-8, C-8a, C-2″, C-3″	C-7, C-8, C-8a, C-3″	C-7, C-8, C-8a, C-2″	C-8	C-7, C-8, C-8a, C-2″, C-3″
2″	C-8, C-3″, C-4″, C-5″	C-8, C-1″, C-3″, C-4″, C-5″	C-8, C-3″	C-1″, C-3″, C-4″, C-5″		C-8, C-1″, C-3″, C-4″, C-5″
4″	C-1″, C-2″, C-3″	C-2″, C-3″	C-1″, C-2″, C-3″	C-2″, C-3″	C-2″, C-3″, C-5″	C-2″, C-3″
5″	C-1″, C-2″, C-3″	C-2″, C-3″	C-1″, C-2″, C-3″	C-2″, C-3″	C-2″, C-3″, C-4″	C-2″, C-3″
1-OH				C-1, C-2, C-9a		
3-OMe	C-3	C-3	C-3	C-3	C-3	C-3
7-OMe	C-7	C-7	C-7	C-7	C-7	
3″-OMe		C-3″		C-3″		

[a] Recorded in $CD_3OD/CDCl_3$.
[b] Recorded at 500 MHz.

(C-4′), 81.1 (C-3′), 105.2 (C-2) and 159.2 (C-3), respectively. From these data, the structure of pruniflorone G was assigned as **7**.

Pruniflorone H (**8**) was obtained as a yellow powder, which showed a molecular ion peak at *m/z* 476.2215 $[M]^+$ in the HREIMS corresponding to a molecular formula of $C_{29}H_{32}O_6$. The ^1H and ^{13}C NMR spectral data of **8** (Table 5) showed characteristics similar to those of **7**, except that an additional signal of a methoxyl group was observed at δ_H 3.32, δ_C 56.6 in **8**, this methoxyl group was located at C-6 due to the correlation with δ_C 149.0 (C-6) from HMBC experiment (Table 6). Thus, the structure of pruniflorone H was assigned as **8**.

Pruniflorone I (**9**) showed a molecular formula of $C_{28}H_{32}O_5$ by HREIMS. The ^1H and ^{13}C NMR spectral data of **9** (Table 7) showed characteristics similar to those of cowanin,[12] except for the appearance of an aromatic proton at δ_H 7.18 (s, H-7), δ_C 123.7 instead of the methoxyl group at δ_H 3.80 (s, 7-OMe) present in cowanin. Therefore, the structure of pruniflorone I was deduced as **9**.



cowanin

Pruniflorone J (**10**) was isolated as orange viscous oil, which was assigned as $C_{25}H_{26}O_6$ from an exact mass measurement. The UV spectrum of **10** exhibited absorption maxima at 269, 283, 366 and 440 nm, suggesting an anthraquinone as a basic structure.[13] IR absorption bands at 1673 and 1625 $cm^{-1}$and ^{13}C NMR chemical shifts at 190.8 and 182.0 also indicated the presence of carbonyl and chelated carbonyl groups, respectively. Chelated hydroxyl protons were shown at δ_H 12.30 (1H, s) and 12.13 (1H, s). The ^1H and ^{13}C NMR spectral data of **10** (Table 8) showed characteristics similar to those of 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone,[14] except for the appearance of *trans*-olefinic protons at δ_H 5.62 (1H, dd, J=6.5, 15.5 Hz, H-5′) and 5.69 (1H, d, J=15.5 Hz, H-6′) in **10** instead of methylene protons at C-5′ and an olefin at C-6′. The chemical shift of the methylene protons at C-4′ was shifted downfield (δ_H 2.79 (2H, d, J=6.5 Hz, H-4′)) compared to δ_H 2.11,[14] due to the double allylic status of these protons. The location of H-4′ at C-4′ was supported by HMBC correlations. The chemical shift of C-7′ (δ 70.8) suggested an oxy-quarternary carbon, whose position was confirmed by HMBC correlations with H-5′ and H-6′. Thus, the structure of pruniflorone J was assigned as **10**.

The following known compounds were also isolated from the roots and barks of *C. formosum* ssp. *pruniflorum*: dulxisxanthone F (**11**),[15] β-mangostin (**12**),[16] α-mangostin (**13**),[16] formoxanthone A (**14**),[8] 3-isomangostin (**15**),[16] 3,4-dihydro-5,9-dihydroxy-8-methoxy-7-(3-methoxy-3-methylbutyl)-2,2-dimethyl-2*H*,6*H*-pyrano-[3,2-*b*]xanthen-6-one (**16**),[17]

Table 4. NOESY (300 MHz) spectral data of **1–6** in $CDCl_3$

Position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[a]	**6**
4	3-OMe	3-OMe	3-OMe	3-OMe	3-OMe	3-OMe
5						
1′	H-2′	H-2′	H-2′, H-4′	H-2′		H-2′
2′	H-1′, H–4′, H-5′	H-1′, H-4′, H-5′	H-1′, H-5′	H-1′, H-5′	H-4′, H-5′	H-1′, H-4′, H-5′
4′	H-2′	H-2′	H-1′		H-2′	H-2′
5′	H-2′	H-2′	H-2′	H-2′	H-2′	H-2′
1″	7-OMe, H-2″	H-2″	H-2″	H-2″		H-2″
2″	H-1″, H-4″, H-5″	H-4″, H-5″, 3″-OMe	H-1″, H-4″, H-5″	H-1″, H-4″, H-5″, 3″-OMe	H-5″	H-1″, H-4″, H-5″
4″	H-2″	H-2″	H-2″	H-3″		H-2″
5″	H-2″	H-2″	H-2″	H-3″	H-2″	H-2″
1-OH						
3-OMe	H-4	H-4	H-4	H-4		H-4
7-OMe						
3″-OMe				H-4″, H-5″, 3″-OMe		

[a] Recorded in $CD_3OD/CDCl_3$.
[b] Recorded at 500 MHz.

3,4-dihydro-5,9-dihydroxy-7-(3-hydroxy-3-methylbutyl)-8-methoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (**17**),[17] isocudraniaxanthone B (**18**),[18] 10-*O*-methylmacluraxanthone (**19**),[19] 3-geranyloxy-6-methyl-1,8-dihydroxy-anthraquinone (**20**),[14] 11-hydroxy-5-methoxy-2,2,9-trimethyl-2*H*-anthra-[1,2-*b*]pyran-7,12-dione (**21**),[20] vismiaquinone A (**22**),[21] madagascin (**23**),[22] physcion (**24**),[23] emodin (**25**),[24] formoxanthone B (**26**),[8] macluraxanthone (**27**),[25] xanthone V_1 (**28**),[26] gerontoxanthone I (**29**),[11] 6-deoxyjacareubin (**30**)[27] and 3,4-dihydrojacareubin (**31**).[28] These compounds were identified by comparison of their spectroscopic data with those reported in the literature. In addition, the X-ray structure of **11** was reported here for the first time (Fig. 2).

Table 5. ^1H and ^{13}C NMR spectral data of **7** and **8** in $CDCl_3$

Position	**7**		**8**	
	^1H (J in Hz)[a]	^{13}C (δ)[b]	^1H (J in Hz)[a]	^{13}C (δ)[b]
1		156.8		156.6
2		105.2		104.9
3		159.2		159.3
4		112.7		113.0
5		131.0		133.4
6		149.0		151.4
7	6.96 d (9.0)	112.8	6.97 d (9.0)	108.2
8	7.69 d (9.0)	117.5	7.75 d (9.0)	116.8
9		180.7		181.0
4a		154.1		154.8
4b		144.5		144.3
8a		113.7		114.2
9a		102.9		103.0
1′	6.83 d (9.9)	116.7	6.81 d (9.9)	116.7
2′	5.58 d (9.9)	125.6	5.56 d (9.9)	125.6
3′		81.1		81.1
4′	1.91 m[c]	41.8	1.89 m[c]	41.7
	1.72 m[c]		1.70 m[c]	
5′	2.14 m	23.2	2.10 m	23.3
6′	5.13 br t (7.2)	123.7	5.12 br t (7.5)	123.7
7′		132.1		132.0
8′	1.60 s	17.6	1.59 s	17.6
9′	1.47 s	26.9	1.45 s	26.9
10′	1.69 s	25.7	1.68 s	25.6
1″		41.4		41.3
2″	6.75 dd (10.8, 17.7)	156.7	6.66 dd (10.5, 17.4)	154.9
3″	5.05 dd (1.2, 10.8)	103.3	5.04 dd (1.2, 10.5)	104.5
	5.23 dd (1.2, 17.7)		5.18 dd (1.2, 17.4)	
4″	1.66 s	28.0	1.66 s	28.4
5″	1.66 s	28.4	1.66 s	28.4
1-OH	13.50 s		13.50 s	
6-OMe			3.32 s	56.6

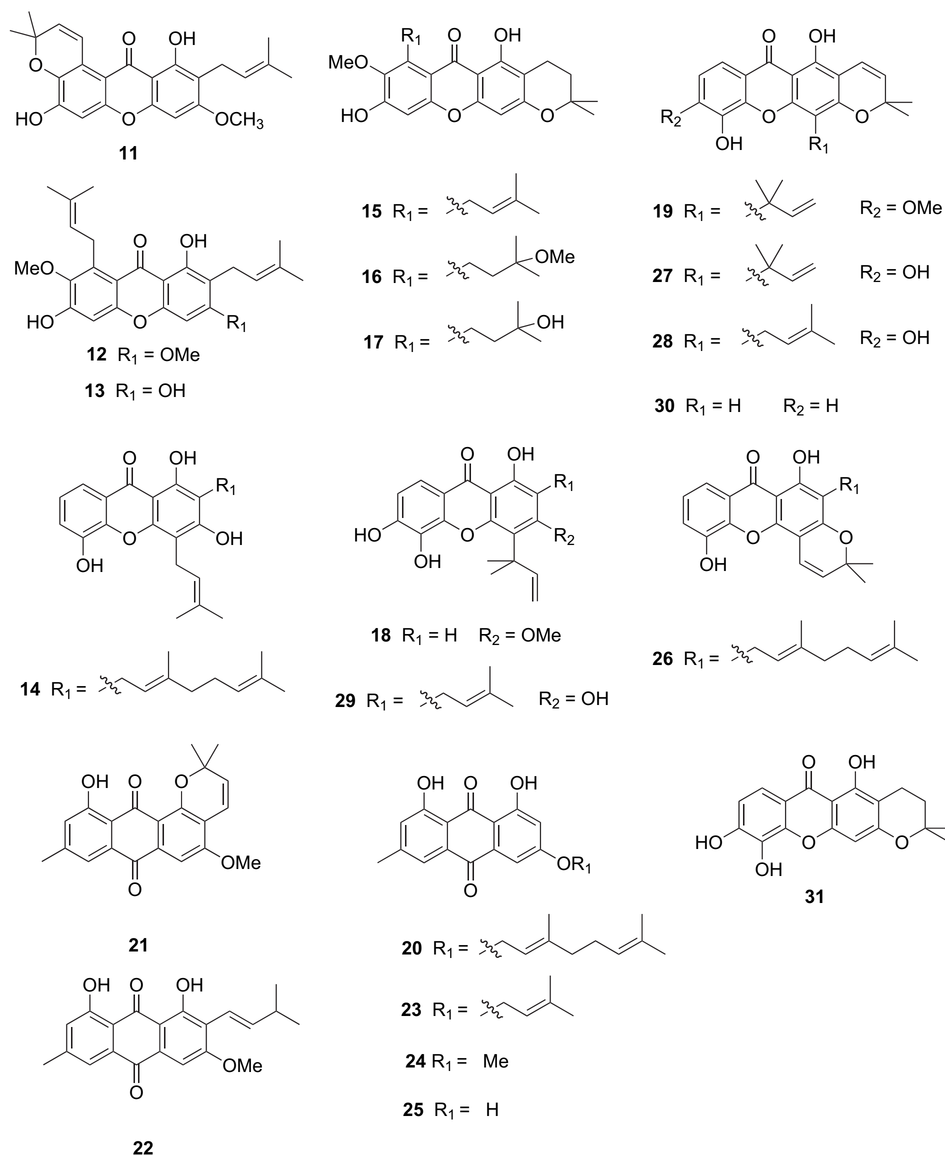
[a] Recorded at 300 MHz.
[b] Recorded at 75 MHz.
[c] Reduced from HMQC experiment.

Only stable compounds of sufficient quantity were evaluated for their antibacterial activity against both Gram-positive (*Bacillus substilis* and *Staphylococcus aureus*) and Gram-negative (*Streptococcus faecalis*, *Salmonella typhi*, *Shigella sonei* and *Pseudomonas aeruginosa*) bacteria. Cytotoxicity against MCF-7 (breast adenocarcinoma), HeLa (Human cervical cancer), HT-29 (colon cancer) and KB (human oral cancer) cell lines was also evaluated. The results of antibacterial activity of the tested compounds are given in Table 9. Pruniflorone E (**5**) and **13** showed potent antibacterial activity against *B. substilis*, *S. aureus* and *S. faecalis*, whereas pruniflorone C (**3**) and **31** exhibited strong activity against *B. substilis* and *S. aureus*. Compounds **28** and **29** showed strong and broad spectrum of antibacterial activity compared to vancomycin. For this investigation, only **29** showed inhibition against *S. sonei* and *P. aeruginosa*. It is interesting to note that, compounds **12**, **17** and **30** were highly active specifically against *S. aureus* therefore it might be worthwhile to further investigate the structure–activity relationships (SAR) of these compounds against *S. aureus*. Compounds **20–25** exhibited no antibacterial activity. According to the MIC values shown in Table 9, it seems that the isoprenyl or 3-hydroxyl-3-methylbutyl moiety at C-2 and C-8, and the catechol unit are both important for antibacterial activity, whereas isoprenyl unit at C-8, which was cyclized to 3,3-dimethylchromene or 3,3-dimethylchromane rings might decrease the antibacterial activity as shown in compounds **6** and **11**. In addition to antibacterial activity, compound **29** strongly inhibited all cancer cell lines used in this investigation compared to camptothecin, whereas compounds **12**, **13**, **28** and **31** showed less inhibitory activity. Compounds **1**, **5**, **6**, **11** and **20–25** were found to be inactive for cytotoxic activity (Table 10).

3. Experimental

3.1. General experimental procedures

Melting points were determined on the Fisher-John melting point apparatus. Optical rotations were measured on



a JASCO P-1020 digital polarimeter. UV and IR spectra were recorded on SPECORD S 100 (Analytikjena) and Perkin–Elmer FTS FTIR spectrophotometer, respectively. The ^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz Varian UNITY INOVA and/or 300 MHz Bruker FTNMR Ultra Shield™ spectrometers in $CDCl_3$ or CD_3OD with TMS as

Table 6. HMBC and NOESY (300 MHz) spectral data of **7** and **8** in $CDCl_3$

Position	7		8	
	HMBC	NOESY	HMBC	NOESY
7		H-8	C-5, C-6, C-8a	H-8, 6-OMe
8	C-6, C-9	H-7	C-6, C-9	H-7
1′	C-1, C-2, C-3, C-3′	H-2′	C-1, C-2, C-3, C-3′	H-2′
2′	C-2, C-3, C-3′ C-4′, C-9′	H-1′, H-4′	C-2, C-3′	H-1′
4′	C-3′, C-5′	H-2′, H-6′	C-3′	
5′	C-3′, C-4′, C-6′, C-7′	H-6′	C-4′	
6′	C-5′, C-8′, C-10′	H-4′, H-5′	C-4′	
8′	C-6′, C-7′		C-6′, C-7′	
9′	C-2′, C-3′, C-4′		C-3′, C-4′	
10′	C-6′, C-7′		C-6′, C-7′	
2″	C-4, C-1″	H-3″	C-4, C-1″	H-3″
3″	C-1″, C-2″	H-2″	C-1″, C-2″, C-4″, C-5″	H-2″, H-4″, H-5″
4″	C-4, C-1″		C-4, C-1″, C-2″	H-3″
5″	C-4, C-1″		C-4, C-1″, C-2″	H-3″
1-OH	C-1, C-2, C-9a		C-1, C-2, C-9a	
6-OMe			C-6	H-7

Table 7. NMR (300 MHz) spectral data of **9** in $CDCl_3$

Position	9			
	^{1}H (*J* in Hz)	^{13}C (δ)	HMBC	NOESY
1		160.7		
2		108.5		
3		162.1		
4	6.19 s	93.2	C-2, C-3, C-9, C-4a, C-9a	
5	7.17 s	116.7	C-6, C-9, C-4b, C-8a	
6		152.0		
7	7.18 s	123.7	C-6, C-8	
8		127.1		
9		183.4		
4a		155.3		
4b		151.3		
8a		118.4		
9a		104.1		
1′	3.35 d (6.9)	21.5	C-1, C-2, C-3, C-2′, C-3′	H-2′, H-4′
2′	5.19 br t (6.9)	121.4	C-4′	H-1′, H-5′
3′		135.5		
4′	1.66 s	25.8	C-2′, C-3′	H-1′
5′	1.74 s	17.9	C-2′, C-3′	H-2′
1″	4.20 d (6.6)	25.6	C-7, C-8, C-4a, C-8a, C-2′, C-3′	H-2″, H-9″
2″	5.16 br t (6.6)	121.5	C-8, C-4′, C-9′	H-1″, H-4″
3″		138.6		
4″	1.98 m	39.7	C-3′, C-9′	H-2″
5″	1.98 m	26.4	C-4′, C-6′, C-7′	H-6″
6″	4.94 m	123.8	C-4′, C-5′, C-8′	H-5″, H-8″
7″		132.0		
8″	1.55 s	25.8	C-6′, C-7′	H-6″
9″	1.77 s	16.4	C-2′, C-3′	H-1″
10″	1.48 s	17.7	C-6′, C-7′	
1-OH	13.54 s		C-1, C-2, C-9a	

Table 8. NMR (500 MHz) spectral data of **10** in $CDCl_3$

Position	10			
	^{1}H (*J* in Hz)	^{13}C (δ)	HMBC	NOESY
1-OH	12.30 s	165.1	C-1, C-2, C-9a	
2	6.68 d (2.5)	107.6	C-1, C-4	H-1′
3		165.8		
4	7.37 d (2.5)	108.7	C-3, C-10, C-9a	H-1′
5	7.62 br s	121.3	C-7, C-10, C-8a, C-6(Me)	6-Me
6		148.4		
7	7.08 br s	124.5	C-5, C-8, C-8a, C-6(Me)	6-Me
8-OH	12.13 s	163.0	C-7, C-8, C-8a	
9		190.8		
10		182.0		
4a		135.2		
4b		133.2		
8a		113.7		
9a		110.1		
1′	4.68 d (6.5)	65.8	C-3, C-2′, C-3′	
2′	5.50 br t (6.5)	119.0	C-4′	
3′		141.5		
4′	2.79 d (6.5)	42.1	C-2′, C-3′, C-5′, C-6′, C-9′	
5′	5.62 dd (6.5, 15.5)	123.9	C-4′, C-7′	
6′	5.69 d (15.5)	140.5	C-4′, C-7′, C-8′, C-10′	
7′		70.8		
8′	1.33 s	29.8	C-6′, C-7′	
9′	1.77 s	16.8	C-2′, C-3′, C-4′	
10′	1.33 s	29.8	C-6′, C-7′	
6-Me	2.45 s	22.2	C-5, C-6, C-7	H-5, H-7

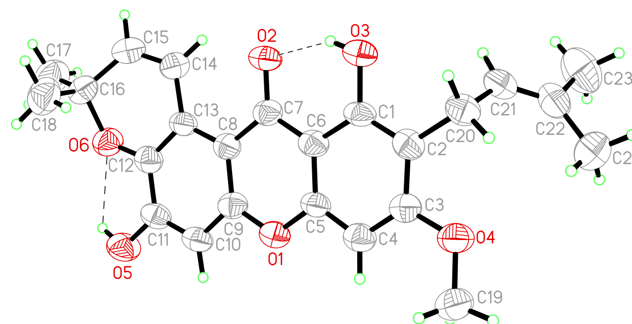


Figure 2. The ORTEP plot of **11**.

the internal standard. Chemical shifts are reported in δ (ppm) and coupling constants (*J*) are expressed in Hertz. EI and HREIMS were measured on a Kratos MS 25 RFA spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively.

3.2. Plant material

Barks and roots of *C. formosum* ssp. *pruniflorum* were collected in May 2004 from Nong Khai Province, northeastern part of Thailand. Identification was made by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and a specimen (No. 0012677) was deposited at Prince of Songkla University Herbarium.

3.3. Isolation and extraction

Air-dried roots (5.30 kg) were extracted with CH_2Cl_2 (2×20 L, for 5 days) at room temperature. The crude CH_2Cl_2 extracts were evaporated under reduced pressure to afford a brownish crude (30.04 g) extract. The crude extract was subjected to QCC on silica gel using hexane as the first eluent and then increasing polarity with EtOAc and acetone, respectively, to give eight fractions (FR1–FR8). Fraction FR2 was separated by CC eluting with CH_2Cl_2–hexane (4:1, v/v) to afford four subfractions (FR2A–FR2D). Subfraction FR2A was further purified by CC with EtOAc–hexane (3:7, v/v) to give **12** (45.0 mg). Subfraction FR2B was further purified by CC eluting with acetone–hexane (1:9, v/v) to give **13** (15.0 mg). Subfraction FR2C was further purified by CC on reversed-phase silica gel C-18 with MeOH to give **7** (8.0 mg) and **18** (2.5 mg). Fraction FR3 (2.56 g) was separated by CC with acetone–hexane (3:17, v/v) to give **14** (15.0 mg), **6** (5.8 mg) and **11** (10.2 mg), which was further recrystallized in $CHCl_3$–MeOH (4:1, v/v) to yield yellow needle single crystals. Fraction FR4 was subjected to CC with acetone–hexane (1:4, v/v) to afford five subfractions (FR4A–FR4E). Subfraction FR4B was separated by CC with acetone–hexane to give three fractions (FR4BA–FR4BC). Subfraction FR4BC was further purified by CC on reversed-phase silica gel C-18 with MeOH to give **9** (15.0 mg). Subfraction FR4D was further purified by CC on reversed-phase silica gel C-18 with MeOH to give **8** (3.0 mg) and **19** (3.0 mg). Fraction FR6 was purified by CC with acetone–hexane (1:4, v/v) to give **2** (3.3 mg)**,** **15** (5.0 mg) and **16** (5.0 mg). Fraction FR7 was further purified by CC with EtOAc–hexane (2:3, v/v) to give **3** (8.2 mg),

Table 9. Antibacterial activity of compounds isolated from *C. formosum* ssp. *pruniflorum*

Compound	Minimum inhibitive concentration (μg/mL)					
	B. substilis	*S. aureus*	*S. faecalis*	*S. typhi*	*S. sonei*	*P. aeruginosa*
1	300	18.7	—	—	300	—
3	<1.1	<1.1	150	—	300	—
5	<1.1	<1.1	<1.1	—	300	18.7
6	300	9.3	4.6	—	300	37.5
10	300	75	150	—	300	—
11	75	18.7	—	—	300	—
12	18.7	<1.1	75	—	—	—
13	<1.1	<1.1	<1.1	—	18.7	18.7
14	18.7	37.5	—	—	—	—
17	9.3	<1.1	—	—	—	—
27	4.6	4.6	2.3	9.6	—	—
28	<1.1	<1.1	<1.1	<1.1	—	9.3
29	<1.1	<1.1	4.6	37.5	<1.1	<1.1
30	4.6	<1.1	75	—	150	150
31	<1.1	<1.1	37.5	—	—	37.5

— = Inactive at >50 μg/mL.

Table 10. In vitro cytotoxic activity of compounds isolated from *C. formosum* ssp. *pruniflorum*

Compound	Cell line			
	MCF-7	HeLa	HT-29	KB
	IC_{50} (μg/mL)			
12	3.6	4.9	4.8	4.6
13	3.7	3.2	4.5	3.2
28	>25.0	4.7	6.0	2.7
29	0.6	0.7	0.7	0.6
31	>5.0	3.4	>5.0	>5.0

4 (1.5 mg) and **5** (2.0 mg). Fraction FR8 was separated by CC with a gradient of acetone–hexane to give four fractions (FR8A–FR8D). Subfraction FR8C was further purified by CC with a gradient of acetone–hexane to give **17** (2.1 mg) and **1** (32.2 mg), which was further recrystallized from $CHCl_3$–MeOH (4:1, v/v) to yield pale yellow single crystals.

Ground-dried barks (4.00 kg) were extracted with CH_2Cl_2 and acetone (each 2×20 L, for 5 days) at room temperature, successively. The crude extracts were evaporated under reduced pressure to afford brownish crude CH_2Cl_2 (76.28 g) and acetone (21.74 g) extracts. The crude CH_2Cl_2 extract was subjected to QCC eluting with increasing polarities of EtOAc and acetone in hexane to afford 10 fractions (F1–F10). Fraction F1 (2.01 g) was separated by CC with acetone–hexane (1:19, v/v) to afford three subfractions (F1A–F1C). Subfraction F1B was further purified by CC with EtOAc–hexane (1:9, v/v) to give **22** (3.3 mg) and **23** (5.6 mg). Fraction F2 (58.06 g) was further separated by CC using a gradient of hexane with EtOAc to afford eight subfractions (F2A–F2H) and **27** (150.0 mg). Subfraction F2C (120.02 g) was further purified by CC with EtOAc–hexane (1:4, v/v) to give **10** (5.2 mg) and **20** (68.2 mg). Subfraction F2D was purified by CC with CH_2Cl_2–hexane (3:2, v/v) to give three fractions (F2DA–F2DC). Subfraction F2DB was further purified by prep TLC with CH_2Cl_2–hexane (3:7, v/v) to give **26** (1.5 mg). Subfraction F2G was subjected to CC with acetone–hexane (1:9, v/v) to give **24** (5.0 mg). Fraction F3 was separated by CC with acetone–hexane (1:9, v/v) to afford five fractions (F3A–F3E). Subfraction F3D was purified by CC with acetone–hexane (3:17, v/v) to give **29** (25.0 mg). Fraction F6 was separated by CC with acetone–hexane (3:17, v/v) to afford seven subfractions (F6A–F6G). Subfraction F6B was further purified by CC with EtOAc–hexane (3:7, v/v) to give **28** (8.0 mg). The crude acetone was subjected to QCC eluting with a gradient of hexane–acetone to afford 12 fractions (FA1–FA12). Fraction FA2 (1.98 g) was further separated by CC with acetone–hexane (3:97, v/v) to give six subfractions (FA2A–FA2F). Subfraction FA2B (422.0 mg) was further purified by CC with acetone–hexane (1:19, v/v) to give **21** (3.0 mg). Fraction FA3 was further purified by CC with EtOAc–hexane (1:9, v/v) to give **30** (4.0 mg). Fraction FA7 was separated by CC with acetone–hexane (1:4, v/v) to give **25** (3.1 mg) and **31** (5.0 mg).

3.3.1. Pruniflorone A (1). Pale yellow needle crystals, mp 259-260 °C, $[\alpha]_D^{26}$ −5.1 (*c* 0.430, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 247 (4.29), 261 (4.34), 314 (4.17), 355 (3.55) nm; IR (KBr) ν_{max} 3414, 1642, 1614 cm^{-1}; HREIMS *m/z* $[M]^+$ 442.1994 (calcd for $C_{25}H_{30}O_7$, 442.1992); ^1H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR (CD_3OD/$CDCl_3$, 75 MHz), see Table 2.

3.3.2. Pruniflorone B (2). Yellow powder, mp 215–217 °C, $[\alpha]_D^{26}$ −4.0 (*c* 0.165, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 246 (4.01), 299 (3.79), 334 (3.40) nm; IR (neat) ν_{max} 3177, 1639, 1611 cm^{-1}; HREIMS *m/z* $[M]^+$ 456.2116 (calcd for $C_{26}H_{32}O_7$, 456.2148); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD/$CDCl_3$, 125 MHz), see Table 2.

3.3.3. Pruniflorone C (3). Yellow solid, mp 134–136 °C, $[\alpha]_D^{27}$ −5.5 (*c* 0.145, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245 (3.89), 259 (3.86), 313 (3.75), 353 (3.25) nm; IR (KBr) ν_{max} 3414, 1632, 1614 cm^{-1}; HREIMS *m/z* $[M]^+$ 442.1995 (calcd for $C_{25}H_{30}O_7$, 442.1992); ^1H NMR (CD_3OD/$CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$/CD_3OD, 125 MHz), see Table 2.

3.3.4. Pruniflorone D (4). Yellow viscous oil, $[\alpha]_D^{26}$ 17.5 (*c* 0.075, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 249 (5.00), 259 (4.98), 312 (4.92), 352 (4.35) nm; IR (neat) ν_{max} 3170, 1646, 1597 cm^{-1}; HREIMS *m/z* $[M]^+$ 456.2198 (calcd for

$C_{26}H_{32}O_7$, 456.2148); ^{1}H NMR ($CD_3OD/CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CD_3OD/CDCl_3$, 125 MHz), see Table 2.

3.3.5. Pruniflorone E (5). Yellow gum, $[\alpha]_D^{27}$ −4.4 (*c* 0.130, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245 (3.91), 260 (3.88), 312 (3.78), 353 (3.25) nm; IR (KBr) ν_{max} 3414, 1635, 1614 cm^{-1}; HREIMS *m/z* $[M]^+$ 442.2000 (calcd for $C_{25}H_{30}O_7$, 442.1992); ^{1}H NMR ($CD_3OD/CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CD_3OD/CDCl_3$, 125 MHz), see Table 2.

3.3.6. Pruniflorone F (6). Pale yellow powder, mp 235–236 °C, $[\alpha]_D^{26}$ −9.2 (*c* 0.290, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 255 (3.94), 258 (3.99), 302 (3.77), 349 (3.40) nm; IR (KBr) ν_{max} 3177, 1614 cm^{-1}; HREIMS *m/z* $[M]^+$ 410.1728 (calcd for $C_{24}H_{26}O_6$, 410.1729); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2.

3.3.7. Pruniflorone G (7). Brown powder, mp 143–145 °C, $[\alpha]_D^{27}$ −7.4 (*c* 0.425, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243 (4.56), 288 (4.81), 335 (4.53) nm; IR (KBr) ν_{max} 3414, 1646, 1628, 1580 cm^{-1}; EIMS *m/z* 462 (11) $[M]^+$, 447 (5), 379 (100); HREIMS *m/z* $[M]^+$ 462.2063 (calcd for $C_{28}H_{30}O_6$, 462.2042); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.8. Pruniflorone H (8). Yellow powder, mp 175–177 °C, $[\alpha]_D^{27}$ −56.5 (*c* 0.050, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 252 (4.06), 289 (4.22), 336 (4.01) nm; IR (KBr) ν_{max} 3400, 1632, 1597, 1573 cm^{-1}; EIMS *m/z* 476 (31) $[M]^+$, 461 (15), 393 (100), 279 (15), 167 (39), 149 (94), 97 (21), 85 (22), 83 (29); HREIMS *m/z* $[M]^+$ 476.2215 (calcd for $C_{29}H_{32}O_6$, 476.2199); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.9. Pruniflorone I (9). Brown viscous oil, $[\alpha]_D^{27}$ −11.3 (*c* 1.150, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 264 (4.70), 310 (4.45), 380 (3.94) nm; IR (neat) ν_{max} 3400, 1642, 1608 cm^{-1}; HREIMS *m/z* $[M]^+$ 448.2277 (calcd for $C_{28}H_{32}O_5$, 448.2250); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 4.

3.3.10. Pruniflorone J (10). Orange viscous oil, $[\alpha]_D^{27}$ −98.4 (*c* 0.250, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 269 (4.33), 283 (4.32), 366 (3.37), 440 (3.86) nm; IR (neat) ν_{max} 3414, 1673, 1625 cm^{-1}; HREIMS *m/z* $[M]^+$ 422.1737 (calcd for $C_{25}H_{26}O_6$, 422.1729); ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 5.

3.4. X-ray crystallographic studies of 1 and 11

Crystallographic data were collected at 100.0(1) K with the Oxford Cyrosystem Cobra low-temperature attachment. The data were collected using a Bruker Apex2 CCD diffractometer with a graphite monochromated Mo Kα radiation at a detector distance of 5 cm and with APEX2 software.[29] The collected data were reduced using *SAINT* program,[29] and the empirical absorption corrections were performed using *SADABS* program.[29] The structures were solved by direct methods and refined by least-squares using the *SHELXTL* software package.[30] All non-hydrogen atoms were refined anisotropically, whereas all H atoms were placed in calculated positions with an O–H distance of 0.82 Å and C–H distances in the range 0.93–0.98 Å after checking their positions in the difference map. The U_{iso} values were constrained to be $1.5U_{eq}$ of the carrier atoms for methyl H atoms and $1.2U_{eq}$ for hydroxyl and the other H atoms. The final refinement converged well. Materials for publication were prepared using *SHELXTL*[30] and *PLATON*.[31]

Crystal data for **1**: $C_{25}H_{30}O_7$, M=442.49, 0.52×0.19×0.05 mm^3, monoclinic, $P2_1/n$, a=11.9303(4) Å, b=19.3361(7) Å, c=19.6631(7) Å, β=96.64(2), V=4505.1(3) $Å^3$, Z=8, D_x=1.305 Mg m^{-3}, μ(Mo Kα)=0.097 mm^{-1}, 79,107 reflection measured, 7928 unique reflections, R=0.0759, R_w=0.1699.

Crystal data for **11**: $C_{24}H_{24}O_6$, M=408.43, 0.54×0.22×0.08 mm^3, triclinic, P-1, a=8.1342(6) Å, b=8.9103(6) Å, c=14.2437(9) Å, α=82.229(4)°, β=80.494(4)°, γ=83.065(4)°, V=1003.70(12) $Å^3$, Z=2, D_x=1.351 Mg m^{-3}, μ(Mo Kα)=0.097 mm^{-1}, 25,932 reflection measured, 3926 unique reflections, R=0.1064, R_w=0.2883

The crystallographic-information files for **1** and **11** have been deposited in the Cambridge Crystallographic Data Center as CCDC293266 and CCDC293267, respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

3.5. Bioassays

3.5.1. Antibacterial assay. The isolated compounds from roots and barks of *C. formosum* ssp. *pruniflorum* were tested against the microorganisms, *B. substilis* (obtained from Department of Industrial Biotechnology, PSU), *S. aureus* (TISTR517) (obtained from Microbial Resources Center (MIRCEN), Bangkok, Thailand), *S. faecalis*, *S. typhi*, *S. sonei* and *P. aeruginosa*. The last four microorganisms were obtained from Department of Pharmacognosy and Botany, PSU. The antibacterial assay employed was the same as described in Boonsri et al.[8] Vancomycin, which was used as a standard, showed antibacterial activity of 75 µg/mL.

3.5.2. Cytotoxic assay. The procedure for cytotoxic assay was performed by the sulphorhodamine B (SRB) assay as described by Skehan et al.[32] In this study, four cancer cell lines obtained from National Cancer Institute, Bangkok, Thailand, were used: MCF-7 (breast adenocarcinoma), KB (human oral cancer), HeLa (Human cervical cancer) and HT-29 (colon cancer). Camptothecin, which was used as a standard, showed cytotoxic activity in the range of 0.2–2.0 µg/mL.

Acknowledgements

We are greatly indebted to the Directed Basic Research in Medicinal Chemistry (Thailand Research Fund), the Development and Promotion of Science and Technology Talents Project, the Higher Education Development Project: Postgraduate Education and Research and Training Program in

Chemistry, the Graduate School and the Natural Products from Mangrove Plants and Synthetic Material Research Unit, Prince of Songkla University for financial supports. The authors would also like to thank the Malaysian Government and Universiti Sains Malaysia for Scientific Advancement Grant Allocation (SAGA) grant no. 304/PFIZIK/653003/A118.

References and notes

1. Smitinand, T. *Thai Plant Names*; Prachachon: Bangkok, 2001; p 152.
2. Veesommai, U.; Kavduengtain, P. *Wild Trees in Thailand*; HN Group: Bangkok, 2004; pp 249–252.
3. Kitanov, G. M.; Assenov, I.; The Van, D. *Pharmazie* **1988**, *43*, H12–H13.
4. Aderson, E. F. *Econ. Bot.* **1986**, *40*, 442–450.
5. Grosvenor, P. W.; Gothard, P. K.; William, M. C.; Supriono, A.; Gray, D. O. *J. Ethnopharmacol.* **1995**, *45*, 75–95.
6. (a) Kijjoa, A.; Jose, M.; Gonzales, T. G.; Pinto, M. M. M.; Damas, A. M.; Mondranondra, I. O.; Silva, A. M.; Herz, W. *Phytochemistry* **1998**, *49*, 2159–2162; (b) Nguyen, L. H. D.; Harrison, L. J. *Phytochemistry* **1998**, *50*, 471–476; (c) Sia, G. L.; Bennett, G. J.; Harrison, L. J.; Sim, K. Y. *Phytochemistry* **1995**, *38*, 1521–1528.
7. Bennett, G. J.; Harrison, L. J.; Sia, G. L.; Sim, K. Y. *Phytochemistry* **1993**, *32*, 1245–1251.
8. Boonsri, S.; Karalai, C.; Ponglimanont, C.; Kanjana-opas, A.; Chantrapromma, K. *Phytochemistry* **2006**, *67*, 723–727.
9. Rukachaisirikul, V.; Kamkaew, M.; Sukavisit, D.; Phongpaichit, S.; Sawangchote, P.; Taylor, W. C. *J. Nat. Prod.* **2003**, *66*, 1531–1535.
10. Seo, E.-K.; Kim, N.-C.; Wani, M. C.; Wall, M. E.; Navarro, H. A.; Burgess, J. P.; Kawanishi, K.; Kardono, L. B. S.; Riswan, S.; Rose, W. C.; Fairchild, C. R.; Farnsworth, N. R.; Kinghorn, A. D. *J. Nat. Prod.* **2002**, *65*, 299–305.
11. Chang, C.-H.; Lin, C.-C.; Kawata, Y.; Hattori, M.; Namba, T. *Phytochemistry* **1989**, *28*, 2823–2826.
12. Pattalung, P. N.; Thongtheeraparp, W.; Wiriyachitra, P.; Taylor, W. C. *Planta Med.* **1994**, *60*, 365–368.
13. Wu, T.-S.; Lin, D.-M.; Shi, L.-S.; Damu, A. G.; Kuo, P.-C.; Kuo, Y.-H. *Chem. Pharm. Bull.* **2003**, *51*, 948–950.
14. Botta, B.; Delle Monache, F.; Delle Monache, G.; Marini Bettolo, G. B.; Oguakwa, J. U. *Phytochemistry* **1983**, *22*, 539–542.
15. Deachathai, S.; Mahabusarakam, W.; Phongpaichit, S.; Taylor, W. C.; Zhang, Y. J.; Yang, C. R. *Phytochemistry* **2006**, *67*, 464–469.
16. Mahabusarakam, W.; Wiriyachitra, P.; Taylor, W. C. *J. Nat. Prod.* **1987**, *50*, 474–478.
17. Dutta, P. K.; Sen, A. K.; Sarkar, K. K.; Banerji, N. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **1987**, *26B*, 281–282.
18. Kobayashi, M.; Mahmud, T.; Yoshioka, N.; Shibuya, H.; Kitagawa, I. *Chem. Pharm. Bull.* **1997**, *45*, 1615–1619.
19. Gunasekera, S. P.; Selliah, S.; Sultanbawa, M. U. S. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1539–1544.
20. Delle Monache, F.; Ferrari, F.; Marini-Bettolo, G. B.; Maxfield, P.; Cerrini, S.; Fedeli, W.; Gavuzzo, E.; Vaciago, A. *Gazz. Chim. Ital.* **1979**, *109*, 301–310.
21. Goncalves, M. L. S.; Mors, W. B. *Phytochemistry* **1981**, *20*, 1947–1950.
22. Ritchie, E.; Taylor, W. C. *Tetrahedron Lett.* **1964**, *23*, 1431–1436.
23. Kalidhar, S. B. *Phytochemistry* **1989**, *28*, 2455–2458.
24. Cohen, P. A.; Towers, G. H. N. *Phytochemistry* **1995**, *40*, 911–915.
25. Delle Monache, F.; Botta, B.; Nicoletti, M.; De Barros Coelho, J. S.; Lyra, F. D.; De Andrade Lyra, F. D. *J. Chem. Soc., Perkin Trans. 1* **1981**, 484–488.
26. Botta, B.; Delle Monache, G.; Delle Monache, F.; Marini Bettolo, G. B.; Menichini, F. *Phytochemistry* **1986**, *25*, 1217–1219.
27. Rocha, L.; Marston, A.; Kaplan, M. A. C.; Stoeckli-Evans, H.; Thull, U.; Testa, B.; Hostettmann, K. *Phytochemistry* **1994**, *36*, 1381–1385.
28. King, F. E.; King, T. J.; Manning, L. C. *J. Chem. Soc.* **1953**, 3932–3937.
29. Bruker. *APEX2 Version 1.27, SAINT and SADABS*; Bruker AXS: Madison, WI, USA, 2005.
30. Sheldrick, G. M. *SHELXTL Version 5.1*; Bruker AXS: Madison, WI, USA, 1998.
31. Spek, A. L. *J. Appl. Crystallogr.* **2003**, *36*, 7–13.
32. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.