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Biologically active alkylated coumarins from *Kayea assamica*

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Dedicated to the memory of Professor Jeffrey B. Harborne
Dedicated also to Professor Emeritus Dr. Kyaw Soe, Former Head of the Botany Department, Yangon University, Yangon, Myanmar

Abstract

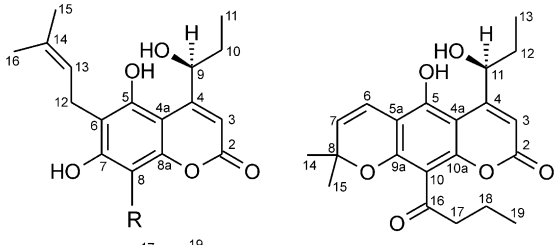
Four coumarin derivatives, theraphins A (**1**), B (**2**), C (**3**), and D (**4**), along with three known xanthones, 2-hydroxyxanthone, 1,7-dihydroxyxanthone, and 5-hydroxy-1-methoxyxanthone, were isolated from the bark of *Kayea assamica* (Clusiaceae) native to Myanmar. Their structures were determined using spectroscopic and chemical techniques. The absolute configuration of **1** was established by the modified Mosher ester method. Theraphins A (**1**), B (**2**), and C (**3**) exhibited good cytotoxicity against Col2, KB, and LNCaP human cancer cell lines. Theraphin D (**4**) showed mild activity only against the KB cell line. The coumarins also exhibited mild antimalarial activities.

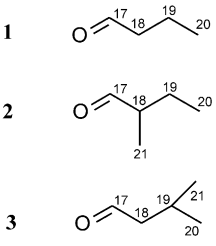
Keywords: *Kayea assamica*; Clusiaceae; Alkylated coumarins; Xanthones; Cytotoxicity; Antimalarial activity

1. Introduction

Kayea assamica King & Prain (Clusiaceae) is a slow growing, tall, handsome evergreen tree and blooms from early in October to May. The bark of this species is light brownish-grey, often exfoliating in large square plates. The inside of the bark is fibrous and reddish with fine, and close whitish veins, soon turning brown (Kanjilal et al., 1934). In India, the fruits of the species are used as a fish poison, and the aqueous extract of the stem bark is used as remedy for treating fevers (Bordoloi et al., 1997). The local name of the species in Myanmar is thera-phi. It is a well-known and highly prized medicinal plant, and was mentioned in a poem by the celebrated Burmese bard Nawadaygi (ca. 1488) (1962) "at the beginning of March,....the heavenly precious blossom thera-phi is opening its bud." The pollen is used for sores, fistulas, fever, and malaria.

As part of a study investigating the active principles of the ethnomedical plants of Myanmar (Tin-Wa et al., 1991), the bark of *Kayea assamica* was examined for cytotoxic activity based on a panel of human cancer cell lines, and for antimalarial activity against the D6 (chloroquine-sensitive) and W2 (chloroquine-resistant) clones of *Plasmodium falciparum*.





Four coumarin derivatives, theraphins A (**1**), B (**2**), C (**3**), and D (**4**), along with three known xanthones, 2-hydroxyxanthone (Fernandes et al., 1998), 1,7-dihydroxy-xanthone

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(Westerman et al., 1977), and 5-hydroxy-1-methoxyxanthone (Poobrasert et al., 1998), were isolated from the EtOAc-soluble extract of the bark of *K. assamica*.

2. Results and discussion

Theraphin A (**1**) was isolated as a yellowish oil, $[\alpha]_D$ −6.2° (*c* 0.1, MeOH). The TOFMS of **1** gave a protonated molecular ion peak at *m*/*z* 375.1810, indicating a molecular formula of $C_{21}H_{26}O_6$. The UV spectrum of **1** showed two absorption maxima bands at 328 (log ε 4.54) and 255 (log ε 4.52) nm, which is characteristic for a coumarin (Chakraborty and Chakraborti, 1961). The IR spectrum of **1** showed characteristic absorptions of carbonyl (ν_{max} 1709 cm^{-1}) and hydroxyl (ν_{max} 3363 cm^{-1}) groups. The ^1H NMR spectrum of **1** displayed four methyl groups, two singlets at δ_H 1.79 and 1.68, and two triplets at δ_H 1.00 and 0.99. Three oxygenated or olefinic protons were observed at δ_H 5.95, 5.15, and 4.59, as well as four methylene groups. Besides these signals, the ^1H NMR spectrum of **1** showed a very downfield singlet at δ_H 14.32, which suggested that a hydroxyl proton was strongly chelated by an adjacent carbonyl oxygen (Cao et al., 1998a). The ^{13}C NMR and DEPT spectra of **1** displayed 21 carbons (Table 1), including four methyls at δ_C 25.8, 17.9, 13.8, and 10.5, supporting the conclusions from the ^1H NMR spectrum, one oxygenated methine at δ_C 78.5, two olefinic methines at δ_C 108.8 and 108.8, four methylene resonances, a ketone carbonyl carbon at δ_C 205.0, and 10 quaternary carbons (Table 1).

Three partial structural units were elucidated using combinations of 1D and 2D NMR spectroscopy. The triplet methyl signals at δ_H 1.00 and 0.99 suggested that the two methyl groups should each be connected to methylene groups. These connections were confirmed by the observation of a correlation from the methyl protons at δ_H 1.00 (H_3-11) to the methylene protons at δ_H 1.74 (H-10a) and 2.00 (H-10b), and from the methyl protons at δ_H 0.99 (H_3-20) to the methylene protons at δ_H 1.70 (H_2-19) in the ^1H–^1H COSY spectrum. In the HMBC spectrum of **1**, the methyl protons at δ_H 1.00 (H_3-11) correlated to the carbon resonance at δ_C 78.5 (C-9), the methyl protons at δ_H 0.99 (H_3-20) correlated to the carbon resonance at δ_C 46.8 (C-18), and the methylene signal at δ_H 1.70 (H_2-19) correlated to the ketone carbonyl carbon at δ_C 205.0 (C-17). These 1D and 2D NMR spectroscopic data suggested the existence of a hydroxypropyl and an oxobutyl substituent in **1**. Additionally, the presence of a prenyl unit in **1** was confirmed by the HMBC correlations from the olefinic triplet at δ_H 5.15 (H-13) to the methyl resonances at δ_C 25.8 (C-15) and 17.9 (C-16), as well as the ^1H-^1H COSY correlations from the H-13 resonance to the methylene protons at δ_H 3.30 (H-12a) and 3.36 (H-12b).

From the determined molecular formula of **1**, $C_{21}H_{26}O_6$, the compound contained nine unsaturation values. Besides the two degrees of unsaturation present in the partial structural units (ketone and double bond), the skeleton of **1** should contain seven unsaturation values. Through a comparison of the chemical shifts of the remaining nine carbons of **1** with the literature data (Cao et al., 1998b; Guilt et al., 1999), compound **1** was considered to be a coumarin derivative. The positional assignments of the three substituents were established based on the observed key HMBC correlations. The methylene protons at δ_H 1.74 and 2.00 (H_2-10) correlated to the C-4 signal at δ_C 157.1, a methine triplet at δ_H 4.59 (H-9) correlated to the resonance of C-4a at δ_C 100.7 and the methine carbon at δ_C 108.8 (C-3), and the olefinic singlet at δ_H 5.95 (H-3) correlated to the C-9 signal at δ_C 78.5 and the quaternary carbon at δ_C 100.7 (C-4a) in the HMBC spectrum. Consequently, the hydroxypropyl unit was placed at position 4. The prenyl unit was placed at position 6 because the olefinic triplet at δ_H 5.15 (H-13) showed a HMBC correlation to the resonance of C-6 at δ_C 115.0, and the oxobutyl unit was placed at position 8 because the phenolic proton at δ 14.32 (H-7) displayed HMBC correlations to the signals of C-8 at δ_C 103.8, C-6 at δ_C 115.0, and C-17 at δ_C 205.0. In addition, H_2-18 correlated with the resonances at δ_C 103.8 (C-8) and 13.8 (C-20). Since the elucidated structure of **1** contained three hydroxyl groups, compound **1** was acetylated to confirm their presence. The ^1H NMR spectrum of the tri-acetate derivative showed a notable shift of the H-9 resonance downfield to δ 6.26 from δ 4.59, in addition to three acetyl methyl singlets at δ 2.43, 2.23, and 2.18. Furthermore, the ESI spectrum of the acetate exhibited a molecular weight of 500 consistent with the molecular formula, $C_{27}H_{32}O_9$, and reflecting the addition of three acetate units. On the basis of the above evidence, the planar structure of theraphin A (**1**) was determined as 5,7-dihydroxy-4-(1-hydroxypropyl)-6-(3-methyl-but-2-enyl)-8-(1-oxobutyl)-2*H*-benzopyran-2-one.

The absolute configuration of **1** was determined by the modified Mosher ester method (Ohtani et al., 1991; Rieser et al., 1992). The (*R*)- and (*S*)-MTPA esters of **1** were obtained by treating **1** with (*S*)- and (*R*)-MTPA chloride, respectively. The proton resonances of the (*R*)- and (*S*)-MTPA esters of **1** were assigned based on the ^1H-^1H correlations, and the differences in the proton chemical shifts of these MTPA ester derivatives, particularly for H-3 ($\Delta\delta = \delta_S - \delta_R + 0.407$) and H_3-11 (Δδ-0.108), indicated that the absolute configuration of C-9 in **1** was *S*. Consequently, the absolute structure of theraphin A is as shown in **1**.

The structures of the isolates **2**, **3**, and **4** were deduced from a comparison of their 1D and 2D NMR spectroscopic data and chiroptical value with those of **1**. Theraphin B (**2**) was isolated as a yellowish oil, $[\alpha]_D$ −12.7° (*c* 0.2, MeOH). The TOFMS of **2** gave a protonated molecular ion peak at *m*/*z* 389.1977, indicating a mole-

Table 1
NMR spectroscopic data for theraphins A (**1**), B (**2**), and C (**3**)[a]

Position	Theraphin A (**1**)		Theraphin B (**2**)		Theraphin C (**3**)	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		161.0 (*s*)		160.2 (*s*)		160.9 (*s*)
3	5.95 (*s*)	108.8 (*d*)	6.07 (*s*)	108.9 (*d*)	5.95 (*s*)	108.8 (*d*)
4		157.1 (*s*)		156.5 (*s*)		157.0 (*s*)
4a		100.7 (*s*)		100.8 (*s*)		100.7 (*s*)
5		157.6 (*s*)		157.7 (*s*)		157.6 (*s*)
6		115.0 (*s*)		114.3 (*s*)		114.9 (*s*)
7	14.32 (OH, *s*)	166.8 (*s*)	14.32 (OH, *s*)	166.4 (*s*)	14.37 (OH, *s*)	166.9 (*s*)
8		103.8 (*s*)		104.0 (*s*)		103.9 (*s*)
8a		156.1 (*s*)		156.1 (*s*)		156.1 (*s*)
9	4.59 (*t*, 7.0)	78.5 (*d*)	4.71 (*t*, 7.7)	77.9 (*d*)	4.60 (*t*, 7.4)	78.4 (*d*)
10	1.74 (*d*)	27.4 (*t*)	1.82 (*d*)	27.8 (*t*)	1.76 (*d*)	27.4 (*t*)
	2.00 (*ddd*, 14.5, 7.5, 3.8)		1.99 (*ddd*, 14.5, 7.1, 3.5)		1.99 (*ddd*, 14.2, 7.4, 3.4)	
11	1.00 (*t*, 7.4)	10.5 (*q*)	1.04 (*t*, 7.4)	10.5 (*q*)	0.96–1.00 (m)	10.5 (*q*)
12	3.30 (*dd*, 14.3, 6.6)	22.0 (*t*)	3.41 (*t*, 7.5)	22.1 (*t*)	3.38 (*dd*, 14.2, 7.3)	22.0 (*t*)
	3.36 (*dd*, 14.3, 6.6)				3.32 (*dd*, 14.2, 7.3)	
13	5.15 (*t*, 6.8)	121.3 (*d*)	5.21 (*t*, 6.9)	121.4 (*d*)	5.17 (*t*, 6.9)	121.3 (*d*)
14		133.3 (*s*)		133.3 (*s*)		133.0 (*s*)
15	1.68 (3H, *s*)	25.8 (*q*)	1.72 (3H, *s*)	25.8 (*q*)	1.72 (3H, *s*)	25.8 (*q*)
16	1.79 (3H, *s*)	17.9 (*q*)	1.82 (3H, *s*)	17.9 (*q*)	1.82 (3H, *s*)	17.9 (*q*)
17		205.0 (*s*)		210.1 (*s*)		204.8 (*s*)
18	2.93 (*ddd*, 14.7, 7.3, 7.3)	46.8 (*t*)	3.69 (*dd*, 6.6, 6.4)	47.0 (*d*)	2.79 (*dd*, 15.8, 6.8)	53.6 (*t*)
	3.01 (*ddd*, 14.7, 7.3, 7.3)				2.97 (*dd*, 15.8, 6.8)	
19	1.70 (2H, *m*)	17.9 (*t*)	1.85 (*d*)	27.0 (*t*)	2.16 (*ddd*, 13.3, 6.7, 3.4)	25.2 (*d*)
			1.43 (*ddd*, 14.0, 6.9, 3.5)			
20	0.99 (3H, *t*, 7.3)	13.8 (*q*)	0.96 (3H, t, 7.3)	11.7 (*q*)	0.96–1.00 (*d*)	22.7[b] (*q*)
			1.21 (3H, d, 6.6)	16.6 (*q*)	0.96–1.00 (*d*)	22.6[b] (*q*)

[a] The data were obtained in $CDCl_3$ at 500 MHz for ^{1}H and 125 MHz for ^{13}C; *J* values are given in Hz; chemical shift values presented in ppm.
[b] These chemical shifts may be interchangeable.

cular formula of $C_{22}H_{28}O_6$. The UV and the IR spectra of **2** were similar to those of **1**, and the ^{1}H NMR spectrum of **2** was almost identical to that of **1** (Table 1). However, the ^{1}H NMR spectrum of **2** showed an additional methyl doublet at δ_H 1.21, and the resonance of H-18 appeared at δ_H 3.69 as a methine doublet of doublets ($J = 6.6$ and 6.4 Hz), which is consistent with the difference of fourteen mass units. Moreover, the ^{13}C NMR and DEPT spectra of **2** showed five methyl signals, one more than **1**, and the methylene at δ_C 47.0 (C-18) was now a methine. The additional methyl group of **2** was placed based on the homonuclear COSY and HMBC spectra. The doublet methyl signal at δ_H 1.21 showed a ^{1}H–^{1}H COSY correlation to the H-18 signal at δ_H 3.69, and HMBC correlations to the resonances of C-17 at δ_C 210.1 and of C-19 at δ_C 27.0, placing the additional methyl group at position 18. The difference between **1** and **2** was therefore the oxobutyl substitutent. Compound **2** possesses two chiral carbons at C-9 and C-18. The absolute configuration of C-18 was not determined because an appropriate technique was not found for such a determination. However, the absolute configuration of C-9 was suggested as *S* because the sign of the optical rotation of compound **2** was also negative ($[\alpha]_D$ −12.7°), like theraphin A (**1**). Thus, theraphin B (**2**) was elucidated as 9(*S*)-(−)-5,7-dihydroxy-4-(1-hydroxypropyl)-6-(3-methyl-but-2-enyl)-8-(2-methyl-1-oxo-butyl)-2*H*-benzopyran-2-one.

Theraphin C (**3**) was isolated as a yellowish oil, $[\alpha]_D$ −7.1° (*c* 0.2, MeOH). The TOFMS of **3** gave a protonated molecular ion peak at *m*/*z* 389.1976, indicating a molecular formula of $C_{22}H_{28}O_6$, the same as **2**. The UV and IR spectra of **3** were also similar to those of **1** and **2**. Further, the ^{1}H NMR spectrum of **3** was almost identical to those of **1** and **2** (Table 1). However, according to the integration of the region δ_H 0.96–1.00 with the ^{1}H NMR spectrum, **3** showed the presence of an additional methyl group overlapped with two other methyl signals. The ^{13}C NMR and DEPT spectra of **3** showed the presence of five methyl groups, and a methylene group instead of a methine carbon in **2**. The location of the additional methyl group was determined based on the homonuclear COSY and HMBC spectra of **3**. The methine signal H-19 at δ_H 2.16 showed ^{1}H–^{1}H COSY correlations to H-21 and to H-20 in the region δ_H 0.96–1.00 and to the H_2-18 methylene signals at δ_H 2.79 and 2.97. The H-21 signal displayed HMBC correlations to C-18 at δ_C 53.6 and to C-20 at δ_C 22.7. Thus, the additional methyl group was placed at position 19, indicating that the difference between **1** and **3** was also in the

oxobutyl substitutent. The absolute configuration of C-9 in **3** was suggested to be *S* because the sign of the optical rotation of **3** was also negative like that of **1**. Thus, theraphin C (**3**) was elucidated as 9(*S*)-(-)-5,7-dihydroxy-4-(1-hydroxypropyl)-6-(3-methyl-but-2-enyl)-8-(3-methyl-1-oxobutyl)-2*H*-benzopyran-2-one.

Theraphin D (**4**) was isolated as a yellowish oil, $[\alpha]_D$ −118.8° (*c* 0.1, MeOH). The TOFMS of **4** gave a protonated molecular ion peak at *m*/*z* 373.1648, indicating a molecular formula of $C_{21}H_{24}O_6$. The IR spectrum of **4** displayed the characteristic absorptions of carbonyl (ν_{max} 1719 cm^{-1}) and hydroxyl (ν_{max} 3422 cm^{-1}) groups. Overall, the ^1H NMR spectrum of **4** was similar to that of **1** (Table 2). The significant differences were the presence of two olefinic doublets at δ_H 5.59 and 6.74 and the absence of the phenolic proton signal in the ^1H NMR spectrum of **4**. The ^{13}C NMR and DEPT spectra of **4** displayed an oxygenated quaternary carbon at δ_C 80.2, suggesting that **4** was a pyranocoumarin derivative resulting from the internal oxidative cyclization of the prenyl substituent at position 6 and the hydroxyl group at either position 5 or 7 on the coumarin nucleus (Sato et al., 2001). The presence of a pyrene ring was apparent based on the COSY and HMBC spectra of **4**. The olefinic signal H-6 at δ_H 6.74 showed a ^1H–^1H COSY correlation to the olefinic signal H-7 at δ_H 5.59. Futhermore, the H-6 signal showed a HMBC correlation to the C-8 resonance at δ_C 80.2, and the H-7 signal showed HMBC correlations to the resonance of C-14 at δ_C 28.1 and C-15 at δ_C 28.3.

Table 2
NMR spectroscopic data for theraphin D (**4**)[a]

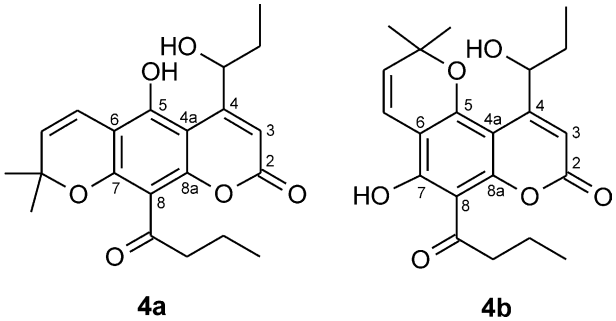
Position	^{13}C	^1H	HMBC correlations
2	161.6 (*s*)		
3	106.9 (*d*)	6.62 (*s*)	C-4, 4a, and 11
4	160.1 (*s*)		
4a	101.4 (*s*)		
5	155.7 (*s*)		
5a	106.2 (*s*)		
6	115.9 (*d*)	6.74 (*d*, 10.0)	C-5, 5a, and 8
7	126.6 (*d*)	5.59 (*d*, 10.0)	C-5a, 8, 14, and 15
8	80.2 (*s*)		
9a	155.7 (*s*)		
10	104.6 (*s*)		
11	71.7 (*d*)	5.43 (*d*, 7.2)	C-12 and 13
12	30.9 (*t*)	1.92 (*d*)	
		1.47 (*ddd*, 15.2, 8.1, 4.1)	C-4, 11, and 13
13	10.3 (*q*)	1.12 (*t*, 7.3)	C-11 and 12
14	28.1 (*q*)	1.52 (*s*)	C-7, 8, and 15
15	28.3 (*q*)	1.57 (*s*)	C-7, 8, and 14
16	206.4 (*s*)		
17	46.9 (*t*)	3.27 (*t*, 7.2)	C-16, 18, and 19
18	18.1 (*t*)	1.78 (*d*)	C-16, 17, and 19
19	13.8 (*q*)	1.04 (*t*, 7.4)	C-17 and 18

[a] The data were obtained in $CDCl_3$ at 500 MHz for ^1H and 125 MHz for ^{13}C; *J* values are given in Hz; chemical shift values presented in ppm.

Since either of the phenolic groups at C-5 and C-7 in **1** could undergo radical promoted oxidative cyclization to form a pyran ring with the double bond in the prenyl group, the structure of **4** could possibly be represented as either **4a** or **4b** (Fig. 1). However, the UV absorption spectrum of **4** showed two zones of absorption maxima (307 and 270 nm), which are considered to be typical of a 5-hydroxycoumarin derivative rather than a 7-hydroxycoumarin derivative, where the two bands are merged to a single absorption around 320 nm (Kalyanmay and Bagchi, 1958). Moreover, the phenolic proton, which had formed a strong hydrogen bond between the phenolic group at position 7 and the carbonyl carbon at position 17 in **1**, was not observed in **4**. This evidence suggested that the structure of **4** was therefore **4a**, rather than **4b**.

Placement of the pyran ring and the substituents of **4** was accomplished using a combination of 1D and 2D NMR spectroscopy, along with comparisons to the assignments of **1**. The olefinic H-7 signal at δ_H 5.59 showed a HMBC correlation to the C-5a resonance at δ_C 106.2, and the olefinic proton H-6 at δ_H 6.74 showed a HMBC correlation to the C-5 at δ_C 155.7. Thus, the position of the new pyran ring was confirmed. The absolute configuration of C-11 in **4** was determined to be *S* because the sign of the optical rotation of **4** was also negative, as in **1**. Thus, theraphin D (**4**) was elucidated as 11(*S*)-(−)-8,8-dimethyl-5-hydroxy-4-(1-hydroxypropyl)-10-(1-oxobutyl)-2*H*,8*H*-benzo(1,2-b:3,4-b′)di-pyran-2-one.

Compounds **1–4** from *K. assamica* were evaluated for their cytotoxic activity in a panel of human cancer cell lines. Table 3 lists the cytotoxicity data for the isolates. Compounds **1–3** exhibited strong cytotoxic activity against Col2, KB, and LNCaP cell lines with IC_{50} values in the range 3.5–13.1 μM. However, the cytotoxic activity of the isolates against the Lu1 cell line varied significantly, with IC_{50} values in the range 7.5–42.8 μM. The results could not be readily explained in terms of the structures of the coumarin derivatives. On the other hand, compound **4**, a pyranocoumarin, exhibited very weak activity with an IC_{50} value of 52.2 μM against only the KB cell line. This preliminary biological assessment suggested that the 7-hydroxyl group must be important for cytotoxic activity of coumarin derivatives,



4a **4b**

Fig. 1. Alternative structures of **4**.

Table 3
Cytotoxic activity of compounds **1–4** from *K. assamica*[a]

Compound	Cell lines[b,c]			
	Lu1	Col2	KB	LNCaP
Theraphin A (**1**)	7.5	7.2	3.5	3.5
Theraphin B (**2**)	16.2	7.2	5.7	3.4
Theraphin C (**3**)	42.8	13.1	6.2	6.4
Theraphin D (**4**)	>53.8	>53.8	52.2	>53.8

[a] Data are given as estimated IC_{50} values in μM.
[b] Lu1 = human lung cancer, Col2 = human colon cancer, KB = epidermoid carcinoma of the nasopharynx, LNCaP = hormone dependent human prostate cancer.
[c] Details of the procedures and the controls are given in Likhitwitayawuid et al. (1993).

Table 4
Antimalarial activity of **1–4** from *Kayea assamica*[a]

Compound	*P. falciparum* clones[b]		Cytotoxicity	SI[c]	
	D6	W2	KB	D6	W2
Theraphin A (**1**)	9.7	7.7	3.5	0.36	0.45
Theraphin B (**2**)	9.8	9.6	5.7	0.58	0.59
Theraphin C (**3**)	9.5	5.1	6.2	0.65	1.22
Theraphin D (**4**)	11.1	10.4	52.2	4.70	5.02
Chloroquine[d]	0.012	0.13	54.5	4542	149

[a] Data are given as estimated IC_{50} values in μM.
[b] D6 (chloroquine-sensitive), W2 (chloroquine-resistant).
[c] SI = KB IC_{50}/*P. falciparum* IC_{50}.
[d] Positive control.

in accord with the report that 7-hydroxycoumarin, the major human metabolite of coumarin, has a growth-inhibitory effect on human malignant cell lines in vitro (Marshall et al., 1994).

The experimental data obtained when compounds **1–4** were evaluated for antimalarial activity are listed Table 4. The coumarin derivatives showed modest activities, with IC_{50} values in the range 9.7–11.1 μM against the D6 clone, and IC_{50} values in the range 5.1–10.4 μM against the W2 clone. However, the Selectivity Indices (SI) (Likhitwitayawuid et al., 1993) of the coumarin derivatives were typically less than 1.0, although the values for **4**, were 4.70 and 5.02 for the D6 and W2 clones, respectively. These observations indicated that the coumarin derivatives possess little potential as antimalarial drugs, although structure modification of **4** might improve the SI level leading to derivatives of greater antimalarial potential.

3. Experimental

3.1. General

HPLC was performed using a Waters 600 E Delivery system equipped with a Waters 966 photodiode detector (Waters Co., Milford, MA). A preparative HPLC CC (Phenomenex, LUNA-C18, 12 μm, 25 × 50 mm) was used as the HPLC column. Optical rotations were measured at room temperature (20–25 °C) at the sodium D line (λ 589 Å) using a Perkin-Elmer 241 automatic polarimeter (Bodenseewerk Perkin Elmer GmbH, Überlingen, Germany). UV absorption spectra were obtained using a Beckman DU-7 spectrometer (Beckman Instruments Inc., Fullerton, CA). IR spectra were obtained using a Jasco 410 FT-IR spectrometer (Jasco Ltd., Great Dunmow, UK). The NMR spectra were obtained using either Bruker Avance DPX-300 or DRX-500 spectrometers (Bruker Instruments Inc., Billerica, MA). HRTOFMS were obtained using a Micromass QTOF spectometer (Micromass Inc., Manchester, UK) and ESIMS were obtained using a Finnigan MAT-90 mass spectrometer (Thermo Finnigan MAT GmbH, Bremen, Germany).

3.2. Plant material

The bark of *Kayea assamica* King & Prain (Clusiaceae) was collected around Pyay in Myanmar in January 1999, and provided by Dr. M. Tin-Wa, PharmChem, Inc., San Francisco, CA. The plant material was identified by Dr. J. C. Regalado, Jr. at the University of Illinois at Chicago. A voucher specimen of the species is deposited at the Field Museum of Natural History, Chicago, Illinois.

3.3. Extraction and isolation

The bark of *Kayea assamica* King & Prain (115 g) was ground, percolated for 24 h, and extracted with MeOH (1 L × 2). The methanolic extracts were concentrated under reduced pressure around 35 °C, dissolved in H_2O (250 ml), and partitioned with EtOAc (250 ml × 3). On the basis of cytotoxic activity results of the extracts against human cancer cell lines, the EtOAc extract (13.2 g) was selected and fractionated over a Si gel column (5.0 × 50 cm; 1.5 kg, 60–200 μm) using mixtures of $CHCl_3$/MeOH (100 ml fractions) ranging from 50:1 to 20:1. After combination, four fractions were obtained, and fraction 1 (4.2 g), representing a major spot in the EtOAc extract, was selected based on the TLC profile. The fraction F1 was further separated with a mixture of $CHCl_3$/acetone (30:1) (50 ml fractions) over a silica gel column (2.5 × 30 cm, 510 g, 60–200 μm) to afford nine combined fractions. Fraction F1.6 (383 mg) was purified over a silica gel column (3.0 × 70 cm, 38 g, 40–63 μm), using a mixture of hexane/acetone (7:3) (10 ml fractions) to afford six combined fractions. The major subfraction (F1.6.2, 235.3 mg) was purified by HPLC on a Waters 600 E delivery system equipped with a Waters 966 photodiode detector, eluting with an isocratic mobile phase of CH_3CN/H_2O (60:40) maintained at 20 ml/min flow rate. Four novel coumarin derivatives, theraphins D (4) (2 mg),

A (1) (30 mg), B (2) (9 mg), and C (3) (18 mg), were isolated as oils from the HPLC separation sequentially.

3.4. Theraphin A (*1*)

Yellowish oil; $[\alpha]_D$ −6.2° (*c* 0.1, MeOH); UV λ_{max} (MeOH) (log ε) 328 (4.54), 255 (4.52), 214 (3.88), 205 (4.35) nm; IR ν_{max} (film) 3363, 2965, 2916, 2850, 2671, 1709, 1594, 1424, 1377, 1280, 1235, 1199, 1149, 1124, 1048, 978 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; TOFMSMS *m/z* (rel. int.) 375 ($[M+H]^+$, 20), 319 (100), 301 (90), 283 (21), 275 (7), 255 (9); EIMS *m/z* (rel. int.) 374 ($[M]^+$, 49), 341 (100), 331 (26), 319 (24), 313 (49), 301 (50), 257 (45), 229 (15), 215 (8), 84 (12), 57 (11), 49 (14), 43 (12), 41 (17), 28 (18); TOFMS *m/z* 375.1810 $[M+H]^+$ (calcd for $C_{21}H_{27}O_6$, 375.1808).

3.5. Acetylation of *1*

Compound **1** (2 mg) was acetylated with acetic anhydride (0.2 ml) and pyridine (0.5 ml) at room temperature for 2 days. The acetate derivative of **1** (**1a**, 1 mg) was purified by preparative TLC using ($CHCl_3$/MeOH, 30:1, R_f=0.4). ^1H NMR data of **1a** ($CDCl_3$, 300 MHz): δ 0.99 (3H, *t*, *J*=7.4 Hz, H_3-20), 1.00 (3H, *t*, *J*=7.4 Hz, H_3-11), 1.54 (3H, *s*, H_3-15), 1.69 (3H, *s*, H_3-16), 1.77 (2H, *m*, H_2-19), 2.18 (3H, *s*, 9-OAc), 2.23 (3H, *s*, 5-OAc), 2.35 (2H, *m*, H_2-10), 2.43 (3H, *s*, 7-OAc), 2.93 (1H, *t*, *J*=7.2 Hz, H_2-18), 3.01 (1H, *t*, *J*=7.2 Hz, H_2-18), 3.10 (1H, *d*, *J*=4.8 Hz, H_2-12), 3.16 (1H, *d*, *J*=5.2 Hz, H_2-12), 4.94 (1H, *t*, *J*=6.0 Hz, H-13), 6.26 (1H, *d*, *J*=5.7 Hz, H-9), 6.55 (1H, *s*, H-3).

3.6. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of *1*

Mosher esters of **1** were prepared following the procedure of Ohtani (Ohtani et al., 1991). In order to prepare the (*R*)- and (*S*)-MTPA ester derivatives of compound **1**, two equal portions of **1** (each 2.1 mg) were dissolved in deuterated pyridine (250 μl), and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride [(*S*)-MTPA-Cl] (5 μl, 25 μmol, Aldrich 65,365) and [(*R*)-MTPA-Cl] (Aldrich 65,363) were added, respectively. The reaction mixtures were retained at room temperature for 12 h. ^1H NMR data of **1s** (pyridine-d_5, 300 MHz): δ 0.96 (H-11), 0.98 (H-20), 1.43 (H-15), 1.53 (H-16), 1.78–1.88 (H_2-19), 3.14 (H-18), 5.26 (H-13), 6.63 (H-3); ^1H NMR data of **1r** (pyridine-d_5, 300 MHz): δ 0.97 (H-20), 1.07 (H-11), 1.43 (H-15), 1.53 (H-16), 1.76–1.84 (H_2-19), 3.12 (H-18), 5.24 (H-13), 6.23 (H-3).

3.7. Theraphin B (*2*)

Yellowish oil; $[\alpha]_D$ −12.7° (*c* 0.2, MeOH); UV λ_{max} (MeOH) (log ε) 328 (3.38), 301 (3.23), 256 (2.90), 217 (3.53), 205 (3.54) nm; IR ν_{max} (film) 3359, 2968, 2933, 2877, 2664, 1708, 1604, 1541, 1419, 1375, 1236, 1202, 1123, 978, 848 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; TOFMSMS *m/z* 389 ($[M+H]^+$, 16), 333 (63), 315 (91), 297 (100), 287 (19), 269 (43), 255 (21), 241 (9), 227 (8); EIMS *m/z* 388 ($[M]^+$, 34), 370 (15), 355 (24), 347 (30), 331 (60), 313 (25), 299 (9), 287 (15), 275 (22), 257 (100), 229 (23), 133 (9), 57 (8), 41 (16), 29 (12); TOFMS *m/z* 389.1977 $[M+H]^+$ (calcd for $C_{22}H_{29}O_6$, 389.1964).

3.8. Theraphin C (*3*)

Yellowish oil; $[\alpha]_D$ −7.1° (*c* 0.2, MeOH); UV λ_{max} (MeOH) (log ε) 329 (4.07), 255 (3.67), 212 (4.39), 205 (4.43) nm; IR ν_{max} (film) 3345, 2963, 2931, 2874, 2670, 1716, 1614, 1592, 1559, 1457, 1420, 1375, 1296, 1237, 1202, 1126, 1101, 979, 848 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; TOFMSMS *m/z* 389 ($[M+H]^+$, 16), 333 (30), 315 (100), 297 (87), 287 (28), 269 (43), 255 (9), 227 (13), 215 (8), 199 (7); EIMS *m/z* 388 ($[M]^+$, 55), 370 (26), 355 (100), 345 (17), 331 (28), 327 (41), 315 (58), 303 (12), 287 (14), 275 (23), 257 (60), 229 (20), 215 (8), 133 (9), 115 (8), 77 (8), 57 (10), 41 (23), 29 (12); TOFMS *m/z* 389.1976 $[M+H]^+$ (calcd for $C_{22}H_{29}O_6$, 389.1964).

3.9. Theraphin D (*4*)

Yellowish oil; $[\alpha]_D$ −118.8° (*c* 0.1, MeOH); UV λ_{max} (MeOH) (log ε) 370 (3.37), 307 (3.99), 270 (3.99), 262 (3.97), 222 (3.88), 205 (3.93) nm; IR ν_{max} (film) 3422, 2968, 2916, 1719, 1607, 1385, 1190, 1151, 1128, 883, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; TOFMSMS *m/z* 373 ($[M+H]^+$, 52), 330 (22), 329 (100), 315 (11), 301 (9); EIMS *m/z* 372 ($[M]^+$, 34), 357 (81), 339 (15), 329 (29), 315 (40), 273 (10), 257 (10), 149 (12), 84 (91), 69 (12), 49 (100), 44 (55), 36 (69), 28 (71); TOFMS *m/z* 373.1648 $[M+H]^+$ (calcd for $C_{21}H_{25}O_6$, 373.1651).

3.10. Bioassays

Cytotoxic activity was evaluated using the standard SRB (sulforhodamine B) assay developed by the National Cancer Institute (Skehan et al., 1990), as modified (Likhitwitayawuid et al., 1993). The results are presented in Table 3.

Antimalarial activity was assessed by measuring [G-^{3}H]-hypoxanthine incorporation of *P. falciparum* as described previously (Desjardins et al., 1979; Likhitwitayawuid et al., 1993). The results are presented in Table 4.

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