JOURNAL OF NATURAL PRODUCTS

ARTICLE

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Cytotoxic and NF-κB Inhibitory Constituents of the Stems of *Cratoxylum cochinchinense* and Their Semisynthetic Analogues

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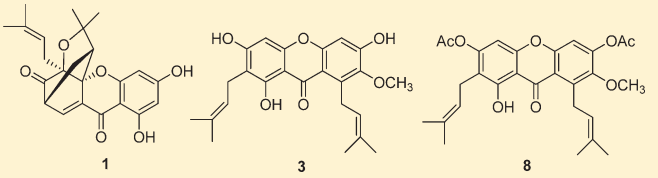
Supporting Information

ABSTRACT: A new caged xanthone (**1**), a new prenylxanthone (**2**), seven known xanthones, and a known sterol glucoside were isolated from the stems of *Cratoxylum cochinchinense*, collected in Vietnam. Compounds **1** and **2** were determined structurally by analysis of their spectroscopic data. In addition, five new (**10** and **16**−**19**) and eight known prenylated xanthone derivatives were synthesized from the known compounds α-mangostin (**3**) and cochinchinone A (**6**). Several of these substances were found to be cytotoxic toward HT-29 human colon cancer cells, with the most potent being 3,6-di-*O*-acetyl-α-mangostin (**8**, ED_{50}, 1.0 μM), which was tested further in an in vivo hollow fiber assay, but found to be inactive at the highest dose used (20 mg/kg; ip). Of the substances evaluated in a NF-κB p65 inhibition assay, 1,3,7-trihydroxy-2,4-diisoprenylxanthone (**5**) exhibited the most potent activity (IC_{50}, 2.9 μM). In a mitochondrial transmembrane potential assay, two new compounds, **1** (IC_{50}, 3.3 μM) and **10** (IC_{50}, 1.4 μM), and two known compounds, **3** (α-mangostin, IC_{50}, 0.2 μM) and **11** (3,6-di-*O*-methyl-α-mangostin, IC_{50}, 0.9 μM), were active. A preliminary analogue development study showed that 3,6-diacetylation and 6-benzoylation both slightly increased the cytotoxicity of α-mangostin (**3**), whereas methylation reduced such activity. In contrast, neither acetylation, benzoylation, nor methylation enhanced the cytotoxicity of cochinchinone A (**6**).



Cratoxylum (Clusiaceae) is a small genus distributed in Southeast Asia, with some of its species used medicinally.[1] Previous phytochemical investigations have demonstrated that xanthones[2,3] are the most characteristic biologically active components of this genus.[4−7] *Cratoxylum cochinchinense* (Lour.) Bl. is a tropical plant used in folk medicine to treat a number of diseases, including cough, diarrhea, fever, and ulcers.[8] Detailed phytochemical studies of *C. cochinchinense* have resulted in the isolation of several xanthones,[3,9−12] including some caged xanthones[10,13,14] and α-mangostin.[9,10,14] The latter compound has shown cytotoxicity toward the NCI-H187 human lung and HT-29 human colon cancer cell lines[3,10] and acts by inducing caspase-3-dependent apoptosis.[4−6]

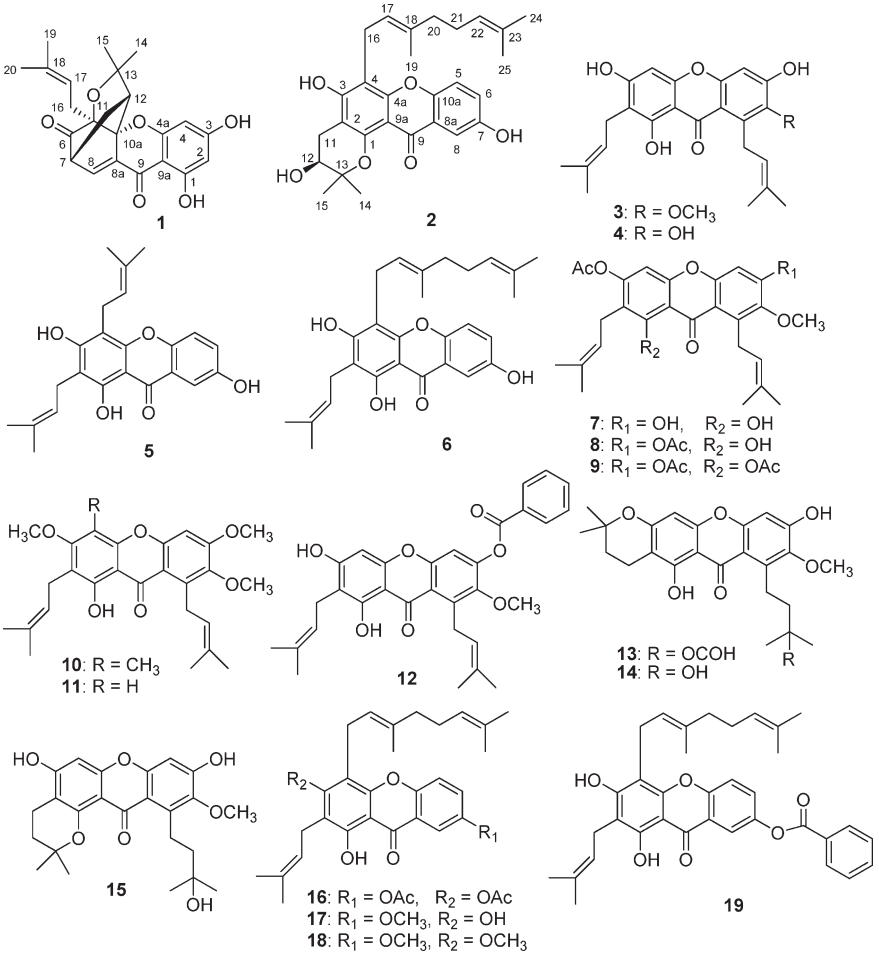
As a part of a research program on the discovery of new natural product anticancer agents from diverse organisms,[15] it was found that a crude methanol extract of the stems of *C. cochinchinense*, collected in Vietnam, exhibited cytotoxicity toward HT-29 human colon cancer cells. Using column chromatography, a new caged xanthone (**1**) and a new prenylxanthone (**2**), together with seven known xanthone compounds and a known sterol glucoside, were isolated from this species. Two of the known compounds were identified as α-mangostin (**3**) and cochinchinone A (**6**), which showed ED_{50} values of 4.1 and 16.1 μM, respectively, when tested for cytotoxicity against the HT-29 human colon cancer cell line. Several semisynthetic derivatives of the lead compounds **3** and **6** were prepared in an attempt to obtain more potent bioactive analogues. All isolated and semisynthetic compounds were evaluated for their cytotoxicity toward HT-29 cells in vitro, with the most active compound obtained (3,6-di-*O*-acetyl-α-mangostin, **8**) being tested in an in vivo hollow fiber assay. Compounds obtained in this investigation were assessed for their NF-κB inhibitory and mitochondrial transmembrane potential (MTP) activities, where the quantities isolated permitted.

RESULTS AND DISCUSSION

A chloroform-soluble extract of the methanol extract of the dried and ground stems of *C. cochinchinense* was separated by column chromatography over silica gel and yielded 15 pooled

Received: January 18, 2011
Published: March 23, 2011

Chart 1



fractions. Combined fractions 3 and 4 were separated by silica gel column chromatography to afford cochinchinone A (**6**)[13] and 3-geranyloxy-1,7-dihydroxyxanthone (cochinchinone G).[11,14] The most active fractions, 5 and 6, were separated by silica gel column chromatography to furnish the new compounds cochinchinoxanthone (**1**) and cochinensoxanthone (**2**), along with the known compounds α-mangostin (**3**),[16] γ-mangostin (**4**),[17] 1,3,7-trihydroxy-2,4-diisoprenylxanthone (**5**),[18] euxanthone,[19] 1,7-dihydroxy-4-methoxyxanthone,[20] and β-sitosterol 3-*O*-β-D-glucopyranoside.[21] Analogue development of α-mangostin (**3**) and cochinchinone A (**6**), which were both obtained in large amounts, yielded five new derivatives, **10** and **16**−**19**. All these new compounds were determined structurally by comparison of their spectroscopic data with those of their parent compounds, **3** and **6**. Also obtained were eight known analogues, 3-*O*-acetyl-α-mangostin (**7**),[22] 3,6-di-*O*-acetyl-α-mangostin (**8**),[23] 3,6,7-tri-*O*-acetyl-α-mangostin (**9**),[24] 3,6-di-*O*-methyl-α-mangostin (**11**),[25] 6-*O*-benzoyl-α-mangostin (**12**),[26] 18-*O*-formyl-3-isomangostin hydrate (**13**),[27] 3-isomangostin hydrate (**14**),[17,27] and 1-isomangostin hydrate (**15**).[17,27] These known semisynthetic compounds were identified by comparison of their spectroscopic data with those of the parent compounds and from literature values.

Compound **1** was isolated as an amorphous, light yellow powder, with a molecular formula of $C_{23}H_{24}O_6$, as determined by HRESIMS (*m*/*z* 419.1468 $[M + Na]^+$, calcd for 419.1471). Both the UV (λ_{max} 226, 327, and 346 nm) and IR [ν_{max} 1744 (unconjugated carbonyl group), 1644 (hydrogen-bonded α,β-conjugated carbonyl group) cm^{-1}] spectra showed absorption bands characteristic of a caged xanthone.[28] This preliminary structural assignment was supported from the four three-proton methyl singlets at δ 1.08, 1.28, 1.37, and 1.67, a one-proton multiplet at δ 3.51 (m), and a one-proton triplet at δ 4.42 (*J* = 7.0 Hz) in the ^{1}H NMR spectrum (Table 1). In turn, a signal at δ 203.1 for a carbonyl group and three signals at δ 84.0, 84.7, and 90.3 for three quaternary carbons, observed in the ^{13}C NMR spectrum of **1** (Table 2), were typical signals for a caged xanthone.[29−32] The NMR spectra of **1** indicated the presence of two prenyl groups, as supported by its elemental formula. In addition, the ^{1}H NMR spectrum also revealed a hydrogen-bonded hydroxy group at C-1 (δ 12.45) and a second hydroxy group at C-3 (δ 6.26), which were confirmed by HMBC correlations between these hydroxy groups and C-1 and C-3, respectively (Figure 1). Furthermore, the HMBC correlations between H-11/C-6, C-8 and H-16/C-5, C-10a suggested that two prenyl groups are connected to C-5 and C-7, respectively. Therefore, **1** was determined as a 1,3-dihydroxy-5,7-diprenyl caged xanthone (Figure 1).

An isomer of **1** has been reported in previous studies.[13,14,29,31] This was derived chemically from bractatin treated with formic acid[29] and later isolated from the roots of *C. cochinchinense*,[13] representing at the time the first discovery of caged xanthones

Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1, 2, 17, and 18

position	1[a]	2[a]	17[b]	18[b]
2	6.01 d (1.5)			
4	6.06 d (1.5)			
5		7.23 m	7.38 d (9.0)	7.40 d (9.0)
6		7.23 m	7.29 dd (3.0, 9.0)	7.32 dd (3.0, 9.0)
7	3.51 m			
8	7.42 d (8.4)	8.35 d (2.4)	7.61 d (3.0)	7.62 d (3.0)
11	2.35 m	2.78 dd (5.1, 17.1)	3.46 d (7.0)	3.41 d (6.8)
	1.31 m	2.95 dd (5.1, 17.1)		
12	2.45 m	3.91 t (5.1)	5.27 m	5.26 t (6.8)
13				
14	1.28 s	1.45 s	1.74 s	1.68 s
15	1.67 s	1.57 s	1.83 s	1.79 s
16	2.61 d (7.0)	3.61 d (7.2)	3.57 d (8.0)	3.53 d (6.7)
17	4.42 t (7.0)	5.28 m	5.27 m	5.21 t (6.7)
19	1.37 s	1.86 s	1.86 s	1.86 s
20	1.08 s	2.10 m	2.08 m	2.09 m
21		2.10 m	2.08 m	2.09 m
22		5.03 brs	5.03 t (8.0)	5.00 t (6.8)
24		1.65 s	1.62 s	1.56 s
25		1.56 s	1.56 s	1.51 s
OH-1	12.45 s		13.21 s	12.97 s
OH-3	6.26 s			
OH-7			6.44 s	
OMe-3				3.80 s
OMe-7			3.89 s	3.90 s

[a] Data (δ) measured in $CDCl_3$ at 300 MHz. [b] Data (δ) measured in $CDCl_3$ at 400 MHz. s = singlet, brs = broad singlet, d = doublet, t = triplet, m = multiplet, dd = double doublet. *J* values presented in Hz and omitted if the signals overlapped as multiplets.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1, 2, 17, and 18

position	1[a]	2[a]	17[b]	18[b]
1	165.5 C	155.1 C	158.4 C	158.7 C
2	97.1 CH	106.4 C	109.1 C	116.9 C
3	165.4 C	159.0 C	160.9 C	163.6 C
4	95.4 CH	105.0 C	105.1 C	113.0 C
4a	161.3 C	153.5 C	153.0 C	153.3 C
5	84.0 C	117.9 CH	119.0 CH	119.2 CH
6	203.1 C	123.5 CH	124.7 CH	124.1 CH
7	47.0 CH	153.5 C	155.9 C	155.9 C
8	133.8 CH	111.8 CH	105.1 CH	105.9 CH
8a	135.6 C	122.9 C	120.6 C	120.6 C
9	179.4 C	176.7 C	181.0 C	181.6 C
9a	101.3 C	103.0 C	103.3 C	105.0 C
10a	90.3 C	149.2 C	150.8 C	151.1 C
11	25.4 CH_2	26.2 CH_2	21.6 CH_2	22.7 CH_2
12	49.0 CH	68.7 CH	121.6 CH	125.2 CH
13	84.7 C	78.3 C	135.1 C	131.4 C
14	29.2 CH_3	24.5 CH_3	25.9 CH_3	25.6 CH_3
15	30.5 CH_3	22.0 CH_3	18.0 CH_3	17.9 CH_3
16	29.2 CH_2	22.2 CH_2	21.8 CH_2	22.5 CH_2
17	118.3 CH	121.3 CH	121.6 CH	122.8 CH
18	134.1 C	140.3 C	137.9 C	135.3 C
19	25.8 CH_3	16.3 CH_3	16.3 CH_3	16.3 CH_3
20	17.1 CH_3	39.7 CH_2	39.7 CH_2	39.7 CH_2
21		26.2 CH_2	26.4 CH_2	26.5 CH_2
22		123.4CH	123.9 CH	122.6 CH
23		132.3 C	131.9 C	131.9 C
24		25.7 CH_3	25.7 CH_3	25.7 CH_3
25		17.7 CH_3	17.7 CH_3	17.7 CH_3
OMe-3				61.9 CH_3
OMe-7			56.0 CH_3	56.0 CH_3

[a] Data (δ) measured in $CDCl_3$ at 75.5 MHz. [b] Data (δ) measured in $CDCl_3$ at 100.6 MHz.

from a plant genus other than *Garcinia*. To further investigate this compound, Li et al. developed a method to synthesize the scaffold of this compound.[31] However, the finalized structural information and detailed spectroscopic data concerning this compound have not been reported.

Several caged xanthones have now been isolated from *C. cochinchinense*, but their absolute configuration was not determined.[10,13,14,29,31] Some caged xanthones isolated from *C. cochinchinense* contain the same structural skeleton but show opposite (positive or negative) specific rotation values.[13] Comparison of the literature data of this earlier caged xanthone[29] with those of **1** revealed that both compounds exhibit similar NMR spectra, except for differences between the methyl group signals, which appeared at δ 1.32 (H-20), 1.42 (H-24, equal to H-14 of **1**), 1.44 (H-19), and 1.70 (H-25, equal to H-15 of **1**) in the substance described in the literature,[29] but were displayed at δ 1.08 (H-20), 1.28 (H-14), 1.37 (H-19), and 1.67 (H-15) in the ^{1}H NMR spectrum of compound **1**. These differences suggested **1** to be an isomer of the literature xanthone, exhibiting one or more different configurations at C-5, -7, -10a, and/or -12.

In a previous collaborative study on the determination of the configuration of caged xanthones using electronic circular dichroism (ECD), the absolute configuration of (−)-morellic acid was determined as 5*R*, 7*S*, 10a*S*, and 27*S*, respectively.[32] The CD spectrum of (−)-morellic acid showed a positive and a negative Cotton effect at 293.5 and at 360.5 nm.[32] However, the CD spectrum of compound **1** exhibited a negative and a positive Cotton effect at 292 and 353 nm. On the basis of a comparison of the CD spectrum of **1** with that of (−)-morellic acid, the absolute configuration of C-5 and C-7 of **1** may be proposed as 5*S* and 7*R*. NOESY correlations between H-7/H-8, -12, H-12/H-8, -15, and H-14, -17/H-19 (Figure 1) indicated the *R* configuration for C-10a and C-12. Accordingly, the absolute configuration of compound **1** could be defined as 5*S*, 7*R*, 10a*R*, and 12*R*. Therefore, **1** was determined as (1*S*,3a*R*,5*R*,12a*R*)-3,3a,4,5-tetrahydro-8,10-dihydroxy-3,3-dimethyl-1-(3-methyl-2-buten-1-yl)-1,5-methano-1*H*,7*H*-furo[3,4-*d*]xanthene-7,13-dione.[33] This compound has been assigned the trivial name cochinchinoxanthone.

Compound **2** was isolated and purified as an amorphous, yellow powder and showed a purple color under UV light at 365 nm. The HRESIMS exhibited a sodiated molecular ion peak at *m*/*z* 487.2131 (calcd 487.2097), consistent with a molecular formula of $C_{28}H_{32}O_6$. The UV (λ_{max} 242, 308 nm) and IR (ν_{max} 3344, 1632, 1616, 1587, 1457 cm^{-1}) spectra showed the absorption characteristics of a prenylxanthone.[34] Analysis of the ^{1}H and ^{13}C NMR data gave evidence of **2** being a C-1, -2, -3, -4,

dx.doi.org/10.1021/np200051j |*J. Nat. Prod.* 2011, 74, 1117–1125

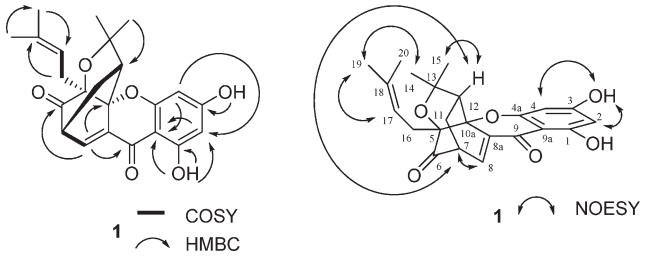


Figure 1. COSY, selected NOESY, and key HMBC correlations of **1**.

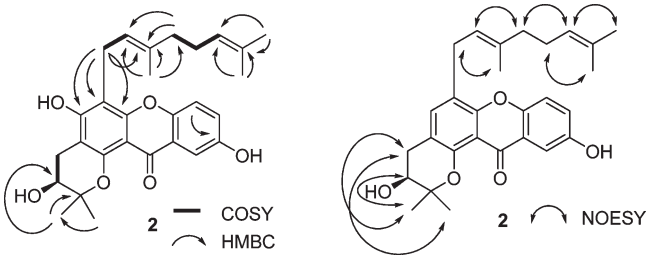


Figure 2. COSY, selected NOESY, and key HMBC correlations of **2**.

and -7 pentasubstituted xanthone. The ^{1}H NMR spectrum (Table 1) showed three aromatic protons at δ 7.23 × 2 and 8.35 (Table 1). The ^{13}C NMR and DEPT spectra contained signals consistent with the presence of a xanthone unit substituted by a prenyl group at C-2 and a geranyl group at C-4. These assignments were supported by HMBC correlations between H-15/C-12 and H-16/C-3, -4a (Figure 2). Comparison of the ^{1}H and ^{13}C NMR data of **2** with those of compound **6**[13] indicated the same partial structures due to their xanthone unit and the geranyl group at C-4, but a difference in regard to the prenyl group at C-2 of these compounds. The signals at δ 121.6 (CH) and 135.0 (C) observed for **6** were replaced in **2** by those at δ 68.7 (CH) and 78.3 (C) (Table 2), due to a 3-hydroxy-2,2-dimethyldihydropyran ring,[34] which was confirmed by the mass spectrum of **2** that showed the molecule to contain an extra oxygen atom when compared with **6**. The HMBC correlation between H-15/C-12, together with the NOESY correlations between H-15/H-11, H-12 indicated that this hydroxy group is linked to C-12 (Figure 2). In the ^{1}H NMR spectrum (Table 1), the lack of a signal at ca. δ 12.8 due to a hydrogen-bonded hydroxy group in **6** suggested that the 3-hydroxy-2,2-dimethyldihydropyran ring is located at C-1 and C-2 rather than at C-2 and C-3. This determination was substantiated by the signals evident for C-12 at δ 69.0/70.4/68.8 (CH) and for C-13 at δ 78.5/79.6/78.2 (C) of some 3-hydroxy-2,2-dimethyldihydropyranoxanthones.[9,35,36]

The absolute configuration at C-3 of a 3-hydroxy-2,2-dimethyldihydropyranoxanthone has not been determined. It has been reported that several natural products containing one stereocenter in a 3(*R*)-hydroxy-2,2-dimethyldihydropyran residue show negative specific rotation values, while their *S* isomers exhibit positive specific rotation values.[37,38] For example, a model compound containing a 3(*R*)-hydroxy-2,2-dimethylchroman system showed a specific rotation value of −102 in pyridine (−11 in $CHCl_3$), while its isomer with a 3(*S*)-hydroxy-2,2-dimethylchroman system exhibited a specific rotation value of +102 in pyridine.[37] Another model compound, (2*R*)-2-hydroxy-1,2-dihydroacronycine, exhibited a specific rotation value of −14.9 in $CHCl_3$, but its isomer, (2*S*)-2-hydroxy-1,2-dihydroacronycine, gave a specific rotation value of +15.2 in $CHCl_3$.[38] The absolute configuration at C-12 of **2** was postulated as being *S* because it displays a positive specific rotation value of +11 in CH_2Cl_2 and is consistent with those of 3(*S*)-hydroxy-2,2-dimethyldihydropyran derivatives.[37,38] Therefore, **2** was proposed as 3,4-dihydro-3(*S*),5,10-trihydroxy-2,2-dimethyl-6-[3,7-dmethylocta-2(*E*),6-dien-1-yl]-2*H*,12*H*-pyrano[2,3-*a*]xanthen-12-one,[39] and it has been assigned the trivial name cochinensoxanthone.

The most potent cytotoxic compound (α-mangostin, **3**), obtained in a relatively large quantity from *C. cochinchinense*, was modified chemically by a series of synthetic methods, including acetylation, methylation, benzoylation, and cyclization (Schemes S1−S4, Supporting Information). All the semisynthetic derivatives obtained were determined structurally by comparison of their spectroscopic data with those of the parent compound (Tables 1−4 and Tables S1−S6, Supporting Information). The new compound **10**, obtained by methyl iodide methylation of **3**, gave a sodiated molecular ion peak at *m*/*z* 475.2052 ($C_{27}H_{32}O_6Na$), indicating the presence of three

Table 3. ^{1}H NMR Spectroscopic Data of Compounds 10, 16, and 19

position	10[a]	16[b]	19[b]
4			
5	6.79 s	7.46 m	8.06 d (2.4)
6		7.46 m	7.58 m
8		7.94 brs	7.58 m
11	3.39 d (6.3)	3.28 brs	3.47 d (7.0)
12	5.26 m	5.14 m	5.25 m
14	1.67 s	1.67 s	1.75 s
15	1.79 s	1.76 s	1.84 s
16	4.12 d (6.3)	3.40 brs	3.58 d (6.9)
17	5.26 m	5.03 t (6.6)	5.25 m
19	1.65 s	1.83 s	1.87 s
20	1.83 s	2.04 m	2.10 m
21		2.00 m	2.10 m
22		5.14 m	5.04 m
24		1.57 s	1.66 s
25		1.53 s	1.56 s
OH-1	13.45 s	12.80 s	13.10 s
OH-3			6.50 s
OMe-3	3.78 s		
OMe-6	3.97 s		
OMe-7	3.77 s		
Me-4	2.30 s		
OAc		2.34 s	
OAc		2.33 s	
3′			8.22 d (7.5)
4′			7.58 m
5′			7.65 t (7.5)
6′			7.58 m
7′			8.22 d (7.5)

[a] Data (δ) measured in $CDCl_3$ at 400 MHz. [b] Data (δ) measured in $CDCl_3$ at 300 MHz. *s* = singlet, brs = broad singlet, *d* = doublet, *t* = triplet, *m* = multiplet. *J* values presented in Hz and omitted if the signals overlapped as multiplets.

Table 4. ^{13}C NMR Spectroscopic Data of Compounds 10, 16, and 19

position	10[a]	16[b]	19[a]
1	158.6 C	158.5 C	158.7 C
2	116.6 C	116.9C	109.7 C
3	162.8 C	154.3 C	161.5 C
4	106.0 C	112.8 C	105.6 C
4a	158.3 C	153.6 C	153.7 C
5	98.3 CH	119.0 CH	118.3 CH
6	152.3 C	129.5 CH	129.1 CH
7	144.0 C	146.6 C	146.9 C
8	137.3 C	118.0 CH	119.0 CH
8a	111.9 C	120.8 C	121.3 C
9	182.8 C	181.4 C	180.7 C
9a	107.6 C	107.0 C	103.5 C
10a	155.7 C	152.5 C	153.1 C
11	22.4 CH_2	22.9 CH_2	21.8 CH_2
12	122.8 CH	121.3 CH	121.6 CH
13	131.9 C	132.3 C	135.5 C
14	25.8 CH_3	25.7 CH_3	25.9 CH_3
15	18.2 CH_3	17.9 CH_3	17.9 CH_3
16	26.2 CH_2	23.1 CH_2	22.1 CH_2
17	123.1 CH	121.3 CH	121.7 CH
18	131.6 C	135.8 C	138.1 C
19	25.9 CH_3	16.3 CH_3	16.5 CH_3
20	17.9 CH_3	39.6 CH_2	39.9 CH_2
21		26.5 CH_2	26.6 CH_2
22		124.0 CH	124.1 CH
23		131.5 C	132.1 C
24		25.6 CH_3	26.1 CH_3
25		17.7 CH_3	18.2 CH_3
OMe-3	61.0 CH_3		
OMe-6	56.1 CH_3		
OMe-7	61.1 CH_3		
Me-4	8.5 CH_3		
OAc-3		168.3 C	
		20.6 CH_3	
OCOH-6			
OAc-7		169.2 C	
		21.0 CH_3	
1′			165.4 C
2′			129.3 C
3′			130.5 CH
4′			128.9 CH
5′			134.1 CH
6′			128.9 CH
7′			130.5 CH

[a] Data (δ) measured in $CDCl_3$ at 100.6 MHz. [b] Data (δ) measured in $CDCl_3$ at 75.5 MHz.

additional methyl groups when compared with **3**. A signal at δ 13.45 in the ^{1}H NMR spectrum suggested a hydrogen-bonded hydroxy group at C-1, and a signal at δ 106.0 in the ^{13}C NMR spectrum was consistent with a methyl group being linked to C-4, because this signal appeared at 93.3 in the ^{13}C NMR spectrum of **3**. Thus, **10** was determined as 4-methyl-3,6-di-*O*-methyl-α-mangostin.

A second compound (cochinchinone A, **6**) isolated in a reasonable quantity from *C. cochinchinense* was derivatized chemically using the same methods as described for compound **3**. The identities of these derivatives were determined by comparison of their spectroscopic data with those of the parent xanthone (Tables 1−4, and Tables S1 and S2, Supporting Information). Compound **16** gave a sodiated molecular ion peak at *m*/*z* 555.2416, corresponding to a molecular formula of $C_{32}H_{36}O_7$, indicating the presence of two additional acetyl groups when compared to **6**. A signal at δ 12.80 in the ^{1}H NMR spectrum was assigned to a hydrogen-bonded hydroxy group at C-1, consistent with the structure of **16** being 3,7-di-*O*-acetylcochinchinone A. Likewise, the pseudomolecular ion peak at *m*/*z* 485.2355 ($C_{29}H_{34}O_5Na$) indicated that **17** contains an additional methyl group when compared with **6**. In the ^{13}C NMR spectrum of **17**, a signal at δ 155.9 indicated a methoxy group linked to C-7, because the signal for this carbon appeared at δ 152.3 in the ^{13}C NMR spectrum of **6**. Therefore, **17** was assigned as 7-*O*-methylcochinchinone A. The HRESIMS at *m*/*z* 499.2484 ($C_{30}H_{36}O_5Na$) showed that **18** contains an additional methyl group when compared with **17**. A signal at δ 12.97 due to a hydrogen-bonded hydroxy group in the ^{1}H NMR spectrum was consistent with the structure of **18** being 3,7-di-*O*-methylcochinchinone A. Compound **19**, also prepared from cochinchinone A (**6**), gave a sodiated ion peak at *m*/*z* 575.2432 ($C_{35}H_{36}O_6Na$), consistent with the presence of a benzoyl group. A signal at δ 146.9 in the ^{13}C NMR spectrum indicated a benzoyl group at C-7, because this carbon signal appeared at δ 152.3 in the ^{13}C NMR spectrum of **6**. Therefore, **19** is 7-*O*-benzoylcochinchinone A.

All isolated and semisynthesized compounds were tested in terms of their cytotoxicity against the HT-29 human colon cancer cell line, using paclitaxel as positive control (Table 5). Compounds **1**, **3**, **4**, **7**−**9**, and **12**−**14** were found to be cytotoxic, of which **8** and **12** were the most potent active substances, with ED_{50} values of 1.0 and 1.9 μM, respectively. Further, compound **8** (3,6-di-*O*-acetyl-α-mangostin) was tested in an in vivo hollow fiber assay, but was found to be inactive at the highest dose used (20 mg/kg; ip).

Comparison of the cytotoxicity of compound **1** with those of the caged xanthones obtained in a previous study[32] indicated that the prenyl groups at C-2 and C-4 and a carboxyl group at C-18 are not necessary for the mediation of cytotoxicity of caged xanthones. Chemical modification of compound **3** (α-mangostin, ED_{50}, 4.1 μM) demonstrated that both 3,6-diacetylation (e.g., **8**, ED_{50}, 1.0 μM) and 6-benzoylation (e.g., **12**, ED_{50}, 1.9 μM) improved the resultant cytotoxicity. Also, cyclization at C-2 and C-3 (e.g., **13**, ED_{50}, 4.4 μM and **14**, ED_{50}, 4.4 μM) retained the initial cytotoxic potency of **3**, but cyclization at C-1 and C-2 (e.g., **15**, ED_{50}, > 20 μM) and 3,6-dimethylation (e.g., **10**, ED_{50}, >20 μM and **11**, ED_{50}, >20 μM) greatly decreased such activity. In turn, neither acetylation, methylation, nor benzoylation enhanced the cytotoxicity of cochinchinone A (**6**, ED_{50}, 16.1 μM).

Some of the compounds with sufficient quantities were tested in a NF-κB p65 inhibition assay, using rocaglamide as positive control (Table 5). Compound **5** (1,3,7-trihydroxy-2,4-diisoprenylxanthone) exhibited the most potent activity (IC_{50} value of 2.9 μM) of the substances tested in this assay. In a mitochondrial transmembrane potential assay (Table 5), the two new compounds **1** (IC_{50}, 3.3 μM) and **10** (IC_{50}, 1.4 μM) were active. The two known compounds **3** (α-mangostin) and **11**

Table 5. Biological Activity of Compounds Isolated from the Stems of *C. cochinchinense* and Their Semisynthetic Analogues

compound	cytotoxicity[a]	NF-κB p65 inhibition[b]	MTP[c]
1	5.8	>10	3.3
2	>10	NT[d]	NT[d]
3	4.1	>10	0.2
4	4.0	NT[d]	>10
5	>10	2.9	NT[d]
6	>10	>10	>10
7	8.8	>10	NT[d]
8	1.0	>10	1.8
9	6.0	>10	NT[d]
10	>10	>10	0.9
11	>10	>10	1.4
12	1.9	>10	>10
13	4.4	NT[d]	>10
14	4.4	NT[d]	>10
15	>10	>10	>10
16	>10	>10	NT[d]
17	>10	>10	>10
18	>10	>10	NT[d]
19	>10	>10	>10

[a] Data presented as ED_{50} values (μM) toward HT-29 cells, with paclitaxel used as a positive control (ED_{50}, 0.10 nM). [b] Data presented as IC_{50} values (μM), with rocaglamide used as positive control (IC_{50}, 0.075 μM). [c] Mitochondrial transmembrane potential, data presented as IC_{50} values (μM), with staurosporine used as positive control (IC_{50}, 2.0 nM). [d] Not tested.

(3,6-di-*O*-methyl-α-mangostin) exhibited promising activity, with IC_{50} values of 0.2 and 0.9 μM, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotation values were measured with a Perkin-Elmer model 343 polarimeter. UV spectra were recorded on a Hitachi U2910 UV spectrophotometer. CD measurements were performed using a JASCO J-810 spectropolarimeter. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer. ^1H and ^{13}C, DEPT, HMQC, HMBC, NOESY, and COSY NMR spectra were recorded at room temperature on a Bruker Avance DPX-300 or DRX-400 NMR spectrometer, with TMS as internal standard. ESIMS and HRESIMS were recorded on a LCT-TOF mass spectrometer. Column chromatography was conducted using silica gel (70−230 mesh, Merck, Darmstadt, Germany). Analytical and preparative thin-layer chromatography (TLC) were performed on precoated silica gel 60 F254 plates (Sorbent Technologies, Atlanta, GA). Sephadex LH-20 was purchased from Amersham Biosciences, Uppsala, Sweden. For visualization of TLC plates, H_2SO_4 reagent was used. Fluorescence was tested under a Spectroline (model ENF-260C) UV light source. HPLC was performed on a Hitachi (Hitachi High Technologies America) HPLC instrument with a model Prep pump, an Elite LaChrom L-2200 autosampler, and an Elite LaChrom L-2400 UV detector. Chromatograms were recorded using EZChrom Elite software (Hitachi). Analytical HPLC was carried out on a YMC (PH12S05-1546 WT) column (150 mm × 4.6 mm i.d., 5 μm) used together with a YMC precolumn, having an injector with a 500 μL loop. Semipreparative HPLC was carried out on a YMC (PH12S05-1520 WT) column (150 mm × 20 mm i.d., 5 μm), again used together with a YMC precolumn. Solvents A and B of the mobile phase were MeOH and H_2O, respectively. A linear gradient was applied from a 50:50 A/B mixture to a 80:20 A/B mixture, over 30 min at room temperature, at a flow rate of 1 mL/min for the analytical column or 5 mL/min for the semipreparative column, and the detection wavelength was at 250 nm.

All other procedures were carried out using anhydrous solvents purchased from commercial sources and employed without further purification. All reagents for chemical synthesis were purchased from Sigma except where indicated. Reactions were monitored by TLC using precoated silica gel plates.

Plant Material. A sample of the stems of *Cratoxylum cochinchinense* was collected at Hon Ba Nature Reserve, Dien Khanh District, Khanh Hoa Province, Vietnam, in November 2004. The voucher herbarium specimen (Soejarto et al. 13598) was identified by D.D.S. as *C. cochinchinense* (Lour.) Bl. (Clusiaceae) and was deposited at the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, under accession number FM 2257409.

Extraction and Isolation. The ground stems of *C. cochinchinens* (450 g) were extracted with MeOH (3 L × 5) at room temperature. The solvent was evaporated in vacuo. The resultant dried MeOH extract (33 g, 7.3%) was suspended in 10% H_2O in MeOH (600 mL) and partitioned with *n*-hexane (300 mL × 3) to yield a hexane-soluble residue (6.0 g, 1.3%). The aqueous-MeOH layer was then partitioned with $CHCl_3$ (500, 400, 300, 200 mL) to afford a chloroform-soluble extract (3.3 g, 0.7%), which was washed with a 1% aqueous solution of NaCl, to partially remove any plant polyphenols present.

The active chloroform-soluble extract (3.0 g, ED_{50} < 20 μg/mL) was subjected to passage over a silica gel column (4.5 × 45 cm), eluted with a gradient mixture of *n*-hexane−acetone (100:1 → 1:1; 500 mL each). Fractions were pooled by TLC analysis to give 15 combined fractions. Of these, fractions 3 and 4 (ED_{50} < 10 μg/mL) were combined and further chromatographed over a silica gel column (2.5 × 20 cm), eluted with a gradient mixture of *n*-hexane−acetone (20:1 → 3:1, 200 mL each), to yield five combined fractions. Fractions 3-1 and 3-2 were chromatographed over silica gel using *n*-hexane−acetone (10:1) as solvent and then purified by separation over a Sephadex LH-20 column (2.5 × 25 cm), eluted with $CHCl_3$−MeOH (1:1), affording cochinchinone A (**6**, 250 mg). Fractions 3-3 to 3-5 were combined and separated by silica gel chromatography, eluted with *n*-hexane−acetone (10:1), and then purified by passage over a Sephadex LH-20 column, eluted with a mixture of $CHCl_3$−MeOH (1:1), to afford 3-geranyloxy-1,7-dihydroxyxanthone (1.0 mg).

Fractions 5 and 6 (ED_{50} < 5 μg/mL) were combined and chromatographed over a silica gel column (2.5 × 20 cm), eluted with a gradient mixture of *n*-hexane−acetone (10:1 → 1:1, 300 mL each), to yield nine combined fractions. Fraction 5-1 was chromatographed over silica gel, using *n*-hexane−acetone (5:1) as solvent, and then purified by passage over a Sephadex LH-20 column, eluted with $CHCl_3$−MeOH (1:1), affording **2** (2.0 mg). Fraction 5-2 was purified by silica gel chromatography, eluted by *n*-hexane−acetone (5:1), and then purified by preparative HPLC, to afford 1,3,7-trihydroxy-2,4-diisoprenylxanthone (**5**) (t_R 13.1 min, 3.5 mg). Fraction 5-3 was chromatographed sequentially over silica gel, eluted by *n*-hexane−acetone (5:1), and over Sephadex LH-20, eluted with a mixture of $CHCl_3$−MeOH (1:1), to afford α-mangostin (**3**, 75 mg). Fractions 5-4 and 5-5 were combined and chromatographed over silica gel, eluted by *n*-hexane−acetone (3:1), and then purified over a Sephadex LH-20 column, with $CHCl_3$−MeOH (1:1) as eluent, affording 1,7-dihydroxy-4-methoxyxanthone (1 mg). Fraction 5-6 was chromatographed over silica gel, eluted by *n*-hexane−acetone (3:1), and then purified over a Sephadex LH-20 column, using $CHCl_3$−MeOH (1:1) for elution, furnishing euxanthone (1.5 mg). Fraction 5-7 was chromatographed over silica gel, eluted by *n*-hexane−acetone (3:1), then separated over Sephadex LH-20, using

$CHCl_3$−MeOH (1:1) for elution, and finally purified by semipreparative HPLC, to yield **1** (t_R 15.5 min, 5 mg). Fraction 5-8 was chromatographed over silica gel, eluted by *n*-hexane−acetone (1:1), and then purified over a Sephadex LH-20 column, using $CHCl_3$−MeOH (1:1) for elution, furnishing γ-mangostin (**4**, 2 mg). Fraction 5-9 was chromatographed over silica gel, eluted by *n*-hexane−acetone (1:1), and then purified over a column containing Sephadex LH-20, using $CHCl_3$−MeOH (1:1) for elution, affording β-sitosterol 3-*O*-β-D-glucopyranoside (7 mg).

Cochinchinoxanthone (1): amorphous, yellow powder (*n*-hexane) showing a purple color under UV light at 365 nm; $[\alpha]^{20}_D$ +10 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 226 (4.67), 327 (4.59), 346 (4.60) nm; CD (MeOH, nm) λ_{max} (Δε) 213 (−26.96), 292 (−2.41), 303.5 (−2.64), 353 (+1.97); IR (dried film) ν_{max} 3230, 1744, 1644, 1597, 1461, 1333, 1274, 1165, 815 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 419.1468, calcd for $C_{23}H_{24}O_6Na$, 419.1471.

Cochinensoxanthone (2): amorphous, yellow powder (*n*-hexane) showing a purple color under UV light at 365 nm; $[\alpha]^{20}_D$ +11 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 242 (4.58), 308 (4.22) nm; CD (MeOH, nm) λ_{max} (Δε) 206.5 (+27.43), 228 (+2.63), 262.5 (+3.90), 283 (−2.83); IR (dried film) ν_{max} 3344, 1632, 1616, 1587, 1457, 1226, 1125 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 487.2131, calcd for $C_{28}H_{32}O_6Na$, 487.2097.

Acetylation of α-Mangostin (3) and Cochinchinone A (6). To a dried 50 mL flask equipped with water condenser and magnetic stirrer, containing 12.3 mg (0.03 mmol) of α-mangostin (**3**), were added acetic anhydride (9.2 mg, 0.09 mmol) and pyridine (5 mL). After the mixture was stirred at 60 °C for 1 h, the mixture was cooled to room temperature. Then, 5 mL of $CHCl_3$ was transferred into the flask, and the solution was extracted with distilled H_2O. The organic layer was washed with distilled H_2O and then evaporated at reduced pressure. The residue was separated by silica gel column chromatography, using *n*-hexane and acetone (5:1 → 1:1), to afford 3-*O*-acetyl-α-mangostin (**7**, 1.5 mg, 0.003 mmol, 11.0%) and 3,6-di-*O*-acetyl-α-mangostin (**8**, 12.0 mg, 0.024 mmol, 81.0%). Using the same procedure, reaction at 70 °C for 2 h yielded 3,6-di-*O*-acetyl-α-mangostin (**8**, 12.5 mg, 0.025 mmol, 84.3%) and 3,6,7-tri-*O*-acetyl-α-mangostin (**9**, 2.5 mg, 0.0047 mmol, 12.4%).

The same reaction as described above with 13.4 mg (0.03 mmol) of cochinchinone A (**6**) at 60 °C for 1 h produced 3,7-di-*O*-acetylcochinchinone A (**16**, 12 mg, 0.022 mmol, 75.2%).

3,7-Di-*O*-acetylcochinchinone A (16): amorphous, white powder (*n*-hexane) showing a purple color under UV light at 365 nm; UV (MeOH) λ_{max} (log ε) 235 (4.57), 260 (4.60), 286 (4.07) nm; IR (dried film) ν_{max} 2968, 1767, 1635, 1616, 1587, 1484, 1463, 1372, 878 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; positive HRESIMS *m/z* 555.2416, calcd for $C_{32}H_{36}O_7Na$, 555.2359.

Methylation of α-Mangostin (3) and Cochinchinone A (6). To a dried flask (50 mL) equipped with a water condenser and a magnetic stirrer, containing 12.3 mg (0.03 mmol) of α-mangostin (**3**), were added 20.8 mg of Ag_2O (0.09 mmol), 12.8 mg of MeI (0.09 mmol), and 5 mL of CH_2Cl_2. After being stirred at 40 °C for 4 h, the mixture was cooled to room temperature and washed with distilled H_2O. The organic layer was evaporated at reduced pressure. The residue was separated by silica gel column chromatography, using *n*-hexane and acetone (5:1 → 1:1) for elution, to afford 4-methyl-3,6-di-*O*-methyl-α-mangostin (**10**, 2.0 mg, 0.0042 mmol, 14.7%) and 3,6-di-*O*-methyl-α-mangostin (**11**, 4.0 mg, 0.009 mmol, 30.4%).

The same reaction performed as described above with 13.4 mg (0.03 mmol) of cochinchinone A (**6**) and 20.8 mg (0.09 mmol) of Ag_2O gave 7-*O*-methylcochinchinone A (**17**, 2.0 mg, 0.0043 mmol, 14.4%) and 3,7-di-*O*-methylcochinchinone A (**18**, 1.5 mg, 0.0032 mmol, 10.5%).

4-Methyl-3,6-di-*O*-methyl-α-mangostin (10): amorphous, yellow powder (*n*-hexane) showing a purple color under UV light at 365 nm; UV (MeOH) λ_{max} (log ε) 238 (4.38), 271 (4.45), 307 (4.25) nm; IR (dried film) ν_{max} 2925, 1645, 1600, 1469, 1373, 1284, 826 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; positive HRESIMS *m/z* 475.2052, calcd for $C_{27}H_{32}O_6Na$, 475.2097.

7-*O*-Methylcochinchinone A (17): amorphous yellow powder (*n*-hexane) showing a purple color under UV light at 365 nm; UV (MeOH) λ_{max} (log ε) 236 (4.75), 266 (4.79), 316 (4.44) nm; IR (dried film) ν_{max} 3390, 1651, 1600, 1455, 1372, 1220, 821 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 485.2355, calcd for $C_{29}H_{34}O_5Na$, 485.2304.

3,7-Di-*O*-methylcochinchinone A (18): amorphous, yellow powder (*n*-hexane) showing a purple color under UV light at 365 nm; UV (MeOH) λ_{max} (log ε) 238 (4.41), 267 (4.53), 297 (3.96) nm; IR (dried film) ν_{max} 2921, 1641, 1608, 1488, 1469, 1281, 821 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m/z* 499.2 $[M + Na]^+$; positive HRESIMS *m/z* 499.2484, calcd for $C_{30}H_{36}O_5Na$, 499.2460.

Benzoylation of α-Mangostin (3) and Cochinchinone A (6). To a dried flask (50 mL) equipped with a water condenser and a magnetic stirrer were added 12.3 mg (0.03 mmol) of α-mangostin (**3**), 11.0 mg of benzoic acid (0.09 mmol), 6.2 mg of DCC (0.03 mmol), and 5 mL of CH_2Cl_2. After the mixture was stirred at 40 °C for 4 h, it was cooled to room temperature and then washed with distilled H_2O. The organic layer was evaporated under reduced pressure, and the residue separated by silica gel column chromatography, using *n*-hexane and acetone (5:1 → 1:1) as eluents, to produce 6-*O*-benzoyl-α-mangostin (**12**, 3.0 mg, 0.0058 mmol, 19.5%).

The same reaction as described above was performed on 13.4 mg (0.03 mmol) of cochinchinone A (**6**) to afford 7-*O*-benzoylcochinchinone A (**19**, 2.0 mg, 0.0036 mmol, 12.1%).

7-*O*-Benzoylcochinchinone A (19): amorphous, yellow powder (*n*-hexane) showing a purple color under UV light at 365 nm; UV (MeOH) λ_{max} (log ε) 239 (4.53), 257 (4.36), 313 (4.05) nm; IR (dried film) ν_{max} 1743, 1645, 1483, 1253, 807 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; positive HRESIMS *m/z* 575.2432, calcd for $C_{35}H_{36}O_6Na$, 575.2410.

Cyclization of α-Mangostin (3). To a dried flask (50 mL) equipped with water condenser and magnetic stirrer, containing 12.3 mg (0.03 mmol) of α-mangostin (**3**), were added 1.44 mg of formic acid (0.09 mmol), 6.18 mg of DCC (0.03 mmol), and 5 mL of CH_2Cl_2. After the mixture was stirred at 40 °C for 4 h and cooled to room temperature, it was washed with distilled H_2O. The organic layer was evaporated at reduced pressure, and the residue was separated by silica gel column chromatography, using *n*-hexane and acetone (5:1 → 1:1) as eluents, to afford 18-*O*-formyl-3-isomangostin hydrate (**13**, 1.5 mg, 0.0033 mmol, 11.0%), 3-isomangostin hydrate (**14**, 3.0 mg, 0.007 mmol, 23.4%), and 1-isomangostin hydrate (**15**, 1.5 mg, 0.0035 mmol, 11.7%).

Cytotoxicity Assay. Cytotoxicity of the samples was evaluated against HT-29 human colon cancer cells by a previously reported procedure.[32] The cells were cultured under standard conditions and trypsinized. Then, the harvested cells were added to 96-well plates and treated by the test samples dissolved in DMSO at different concentrations, the positive control, and the negative control (DMSO). The plates were incubated at 37 °C in 5% CO_2 for three days, and then the cells were fixed, incubated at room temperature for 30 min, washed with tap water, dried at room temperature overnight, and dyed using sulforhodamine B. After the dyed cells were lysed in Tris-base buffer, the plates were read at 515 nm with an ELISA plate reader. Paclitaxel was used as a positive control, and the ED_{50} values of the test samples in serial dilutions were calculated using nonlinear regression analysis (Table Curve2Dv4; AISN Software, Inc., Mapleton, OR). Measurements were performed in triplicate and are representative of two independent experiments in which the values generally agreed within 10%.

Enzyme-Based ELISA NF-κB Inhibition Assay. A NF-κB inhibition assay was carried out using a published procedure, with an

EZ-Detect Transcription Factor Assay System ELISA kit (Pierce Biotechnology, Rockford, IL).[40] Thus, nuclear extracts of HeLa cells (ATCC, American Type Culture Collection) treated with the positive control and the test samples at four different concentrations were used to determine the specific binding ability of the activated p65 subunit of NF-κB to the biotinylated-consensus sequence, which was measured by detecting the chemiluminescent signal in a Fluostar Optima plate reader (BMG Labtech, Inc., Durham, NC). Rocaglamide was used as a positive control,[40] and measurements were performed in duplicate and are representative of two independent experiments, with the values generally agreeing within 10%. The dose−response curve was calculated for IC_{50} determinations using nonlinear regression analysis (Table Curve2DV4; AISN Software Inc., Mapleton, OR).

Mitochondrial Transmembrane Potential (MTP) Assay. Changes in mitochondrial transmembrane potential were determined by a fluorescence cell-based assay, using a previously described protocol.[41] In brief, HT-29 cells at a density of 6×10^4 were incubated overnight at 37 °C in a CO_2 incubator on black 96-well plates. Cells were then treated by the test compounds or the positive control at four different concentrations for 2 h. Soon afterward, cells were incubated with the lipophilic cationic dye 5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzymidazolylcarbocyanide (JC-1, Cayman Chemical Company, Ann Arbor, MI) for 30 min. After incubation, cells were washed to remove unbound staining dye. The black 96-well plates were analyzed by a Fluostar Optima fluorescence plate reader (BMG Labtech, Inc.) with excitation wavelengths of 485 and 560 nm and emission wavelengths of 530 and 595 nm for JC-1 monomers and J-aggregates, respectively. Measurements were performed in triplicate and are representative of at least two independent experiments, with values generally agreeing within 10%. The dose−response curve was determined for IC_{50} determinations using nonlinear regression analysis (Table Curve2DV4; AISN Software Inc., Mapleton, OR).

In Vivo Hollow Fiber Assay. The in vivo hollow fiber assay was conducted as described previously.[32,36] Three human cancer cell lines, designated HT-29 (colon adenocarcinoma), MCF-7 (breast cancer), and MDA-MB-435 (melanoma), were used in this study, and paclitaxel was used as positive control substance. A dose range of 1−20 mg/kg (ip) was used for both the test compounds and the positive control.

■ ASSOCIATED CONTENT

Supporting Information. Mass and ^1H and ^{13}C NMR spectra of compounds **1**, **2**, **10**, **13**, and **16**−**19**; schemes for the synthesis of analogues of α-mangostin (**3**) and cochinchinone A (**6**); ^1H and ^{13}C NMR assignments of known xanthones isolated or derivatized from *C. cochinchinense*; physical data of the known xanthones isolated or derivatized from *C. cochinchinense*; and evaluation of 3,6-di-*O*-acetyl-α-mangostin (**8**) in an in vivo hollow fiber assay. This information is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This investigation was supported by grant P01 CA125066, funded by the National Cancer Institute, NIH, Bethesda, MD. The plant sample *Cratoxylum cochinchinense* (A05984) was collected under a collaborative arrangement between the University of Illinois at Chicago (USA) and the Institute of Ecology and Biological Resources of the Vietnam Academy of Science and Technology, Hanoi (Vietnam). We are grateful to Dr. D. J. Hart, Department of Chemistry, The Ohio State University, for helpful advice concerning the semisystematic names used for compounds **1** and **2**. We thank Dr. K. Green-Church, of the Mass Spectrometry and Proteomics of the Campus Chemical Instrument Center, The Ohio State University, for assistance with the MS measurements. We also thank J. Fowble, College of Pharmacy, The Ohio State University, for access to the NMR instrumentation used in this investigation.

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