Benzophenones and Xanthones with Isoprenoid Groups from *Cudrania cochinchinensis*[1]

Ai-Jun Hou,[†] Toshio Fukai,[†] Manabu Shimazaki,[†] Hiroshi Sakagami,[‡] Han-Dong Sun,[§] and Taro Nomura*[,†]

Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan, Department of Dental Pharmacology, Meikai University School of Dentistry, Sakado, Saitama 350-0283, Japan, and Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Kunming 650204, Yunnan, the People's Republic of China

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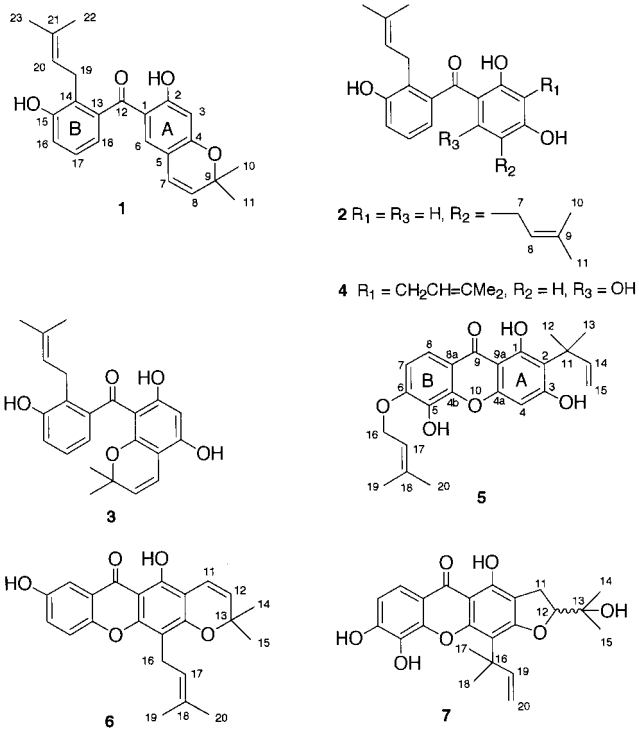
Four new benzophenones with two isoprenoid groups, cudraphenones A−D (**1**−**4**), and three new xanthones also with two isoprenoid units, cudraxanthones P−R (**5**−**7**), were isolated from the roots of *Cudrania cochinchinensis*, together with 19 known phenolic compounds. The structures of the new compounds were elucidated by spectroscopic methods. Some compounds exhibited weak cytotoxicity against human oral squamous carcinoma cells (HSC-2) and normal human gingival fibroblasts (HGF). Among them, benzophenones **1**−**4** showed more potent cytotoxic activities against HSC-2 cells than against HGF cells. On the other hand, xanthones bearing isoprenoid groups showed much lower tumor specificity as compared with the benzophenones, except for geronthxanthone H and isoalvaxanthone. The presence of two sets of hydrophobic and hydrophilic groups in separate domains in each molecule might play a role in the mediation of tumor-specific action.

Members of the family Moraceae are of important economic and medicinal value. We have investigated Moraceous plants in Japan, the People's Republic of China, Indonesia, and Brazil, from which a variety of flavonoids, stilbenes, and xanthones were isolated.[2] Some of these compounds were found to have hypotensive effects, or to act as antagonists of bombesin receptors, or to possess antitumor-promoting activity.[2,3] Recently, we evaluated the cytotoxic potential of flavonoids with an isoprenoid group(s) against human oral tumor cell lines.[4,5] Accordingly, as part of our continuing research on Moraceous plants, further investigations of *Cudrania cochinchinensis* (Lour.) Kudô et Masam. collected in Yunnan Province, People's Republic of China, were carried out. The root of the species is one of the plants used in the Chinese folk medicine "Chuan-po-shi" along with the roots of *C. tricuspidata* (Carr.) Bur.[6−8] This preparation is used for the treatment of gonorrhea, rheumatism, jaundice, boils, scabies, bruising, and dysmenorrhea.[6] Previously, a series of new isoprenoid-substituted xanthones (cudraxanthones A−O) and flavonoids (cudraflavanone A, cudraflavones A−D) from the root bark of Japanese and Chinese *C. tricuspidata* were characterized by our group.[2,9] However, only one new cytotoxic isoflavone and four known compounds were isolated from the roots of *C. cochinchinensis*[10] despite the abundance of xanthones with isoprenoid group(s) in the roots (bark and wood) of Taiwanese *C. cochinchinensis* var. *gerontogea*.[11−13]

The present phytochemical study on the roots of Chinese *C. cochinchinensis* has resulted in the isolation of four new prenylated benzophenones (**1**−**4**), three new isoprenoid-substituted xanthones (**5**−**7**), and 19 known phenolic compounds. We describe here the isolation and structure elucidation of **1**−**7**. The pharmacological properties of xanthones and benzophenones have attracted a great deal of interest.[14] Therefore, we also investigated the cytotoxic activities of some xanthones and benzophenones isolated here against the human oral squamous cell carcinoma cell line HSC-2 and normal human gingival fibroblasts (HGF).



Results and Discussion

The ethanol extract of the roots of *C. cochinchinensis* was suspended in water and partitioned successively with *n*-hexane, C_6H_6, EtOAc, and *n*-BuOH. Repeated column chromatography on Si gel and ODS of the C_6H_6 layer yielded four new benzophenones, cudraphenones A−D (**1**−**4**), three new xanthones, cudraxanthones P−R (**5**−**7**), as well as 19 known compounds (a benzophenone, 11 xanthones with one or more isoprenoid groups, six flavonoids, and a coumarin), namely, cudranone (**8**, 4-methoxy-9-prenyl-2,6,10-trihydroxybenzophenone),[11] 1,3,7-tri-

* To whom correspondence should be addressed. Tel: (+81)-47-472-1780. Fax: (+81)-47-476-6195. E-mail: nomura@phar.toho-u.ac.jp.
[†] Toho University.
[‡] Meikai University School of Dentistry.
[§] Kunming Institute of Botany.

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Table 1. ^{13}C NMR Data for Compounds **1**−**4** in Acetone-d_6 (125 MHz, δ in ppm)

carbon	1	2	3	4
1	115.3	114.4	106.5	105.8
2	166.7	165.2	167.1	164.5
3	104.5	102.9	96.0	107.8
4	161.6	165.2	161.5	163.9
5	114.4	120.8	102.6	95.1
6	132.7	135.9	157.8	161.0
7	121.6	27.8	116.7	21.9
8	129.7	123.0	125.3	124.1
9	78.9	133.3	78.0	130.8
10	28.7	17.7	27.5	17.8
11	28.7	25.6	27.5	25.9
12	203.4	203.1	202.1	201.3
13	140.7	141.0	146.0	145.3
14	126.2	126.2	124.5	124.9
15	156.2	156.1	155.7	155.8
16	117.2	116.8	115.5	116.2
17	127.4	127.1	126.9	127.3
18	119.3	119.3	117.7	118.0
19	26.4	26.3	26.7	26.3
20	123.7	123.7	123.9	123.8
21	132.0	131.8	131.0	131.4
22	17.5	17.5	17.6	17.6
23	25.7	25.7	25.8	25.7

hydroxy-2-(3-methylbut-2-enyl)xanthone (**9**),[15] alvaxanthone (**10**),[16] gerontoxanthones A (**11**), B (**12**),[12a] and G−I (**13**−**15**),[12b] isoalvaxanthone (**16**),[17] macluraxanthone (**17**),[18] 6-deoxyjacareubin (**18**),[19] toxyloxanthone C,[20] naringenin (**19**),[21] sophoraflavanone B (**20**),[22] 3′-*O*-methylorobol (**21**),[23] wighteone (**22**),[24] alpinumisoflavone (**23**),[25] carpachromene,[26] and bergapten (**24**).[27]

Cudraphenone A (**1**), $C_{23}H_{24}O_4$, gave a positive reaction with ferric chloride reagent by TLC, showing its phenolic nature. The IR spectrum indicated the presence of hydroxyl groups (3425 cm^{-1}) and a carbonyl group which conjugated with aromatic rings and also hydrogen-bonded with a hydroxyl group (1625 cm^{-1}). The ^1H NMR spectrum provided signals of most of the functional groups, including two hydroxyl groups [δ 12.76 (1H, s) and δ 8.66 (1H, br s)], a prenyl group [δ 5.09 (1H, m), 3.32 (2H, br d, J = 7 Hz), and 1.39, 1.45 (each 3H, br s)], and a 2,2-dimethyl-2H-pyran ring [δ 6.25 (1H, br d, J = 10 Hz), 5.65 (1H, d, J = 10 Hz), and 1.43 (6H, s)]. It also exhibited evidence for an ABX system (B ring) [δ 7.14 (1H, t, J = 8 Hz), 7.02 (1H, dd, J = 1, 8 Hz), and 6.74 (1H, dd, J = 1, 8 Hz)] and two aromatic protons (A ring) [δ 6.92 (1H, s) and 6.29 (1H, d, J = 1 Hz, coupled with the proton at δ 6.25)]. In the ^{13}C NMR spectrum of **1** (Table 1), the chemical shift of C-19 (C-1 of prenyl group) indicated that one of the *ortho*-positions of the prenyl group was substituted with an oxygenated functional group and the other was a carbon-containing functional (carbonyl) group.[28] The oxygenated carbon signals appeared at δ 156.2−166.7, indicating *meta*-dioxygenation or isolated oxygenation.[29] On the basis of the analysis of its 2D NMR spectra (HMQC and HMBC), **1** was deduced to have a benzophenone skeleton. In the HMBC spectrum (see Supporting Information), the proton of the hydrogen-bonded hydroxyl group at δ 12.76 was correlated with C-1 (δ 115.3), C-2 (δ 166.7), and C-3 (δ 104.5). One *cis*-olefinic proton at δ 5.65 (H-8) coupled with C-5 (δ 114.4), and another at δ 6.25 (H-7) showed cross-peaks with C-6 (δ 132.7) and C-4 (δ 161.6). These results indicated the presence of a 2-hydroxybenzoyl moiety with a 2,2-dimethylpyran ring. On the other hand, the following long-range cross-peaks (^2J and ^3J) were observed for the other ring (B ring): the double doublet at δ 6.74 (H-18) with C-12 (δ 203.4), C-14 (δ 126.2), and C-16 (δ 117.2); the triplet at δ 7.14 (H-17) with C-13 (δ 140.7) and C-15 (δ 156.2); the methylene broad doublet at δ 3.32 (H_2-19) with C-13, C-14, and C-15, which indicated the attachment of the prenyl group at C-14 and the non-hydrogen-bonded hydroxyl group at C-15 on the aromatic ring; and the singlet at δ 6.92 (H-6) with C-12, respectively. Thus, the structure of cudraphenone A was deduced as shown in formula **1**.

Cudraphenone B (**2**) was assigned a molecular formula of $C_{23}H_{26}O_4$. Its NMR spectra showed the presence of two prenyl groups, three hydroxy groups, a carbonyl group, and 1,2,3-trisubstituted and 1,2,4,5-tetrasubstituted benzene rings. By comparison of the NMR spectra of **2** with those of **1**, it could be inferred that the 2,2-dimethylpyran ring in **1** was altered to a prenyl group in **2**. Thus, the chemical shift of the methylene carbon of the prenyl group (δ 27.8, Table 1) indicated that one of the *ortho*-positions of the prenyl group was occupied by an oxygen-containing functional group, and the other was unsubstituted.[28] The chemical shifts of the other prenyl group and B ring carbons resembled those of **1**. The structure was further confirmed by the HMBC spectrum of **2** (Supporting Information). Thus, the structure of cudraphenone B was determined as shown in formula **2**.

Cudraphenone C (**3**), $C_{23}H_{24}O_5$, was also a benzophenone derivative with the same ring B and 2,2-dimethylpyran ring as **1**. The difference between these compounds is that H-6 in **1** is occupied by an oxygenated functional group in **3**, which was confirmed as follows. In the ^1H NMR spectrum of **3**, no signal for H-6 was apparent, while only one singlet signal for H-3 at δ 6.00 was observed. Furthermore, the carbon signal at δ 157.8 in **3** replaced the signal at δ 132.7 (C-6) in **1**, which resulted in upfield ^{13}C NMR chemical shifts for C-1, C-3, and C-5. These observations indicated that **3** is 6-hydroxycudraphenone A or its isomer in which the pyran ring was fused at C-5 and C-6. The methyl protons of the 2,2-dimethylpyran ring (H_3-10, H_3-11) and an olefinic proton of **3** appeared at a higher field position than those of **1** [$\Delta\delta_H$ (**1**−**3**), 0.5 (H_3-10, H_3-11), 0.32 (H-8), −0.23 (H-7)] and normal 2,2-dimethylpyran rings on phenols.[28d] The upfield shifts were caused by the anisotropic effect of the B ring.[30] The H-7 signal was observed as a doublet rather than a doublet of doublets, due to the absence of zigzag coupling between H-7 and an aromatic proton. On the basis of these lines of evidence, the structure of cudraphenone C was assigned as formula **3**. The structure was further confirmed by the NOESY spectrum of **3**. Thus, a cross-peak between H_3-23 and H_3-10 (H_3-11) and a weak cross-peak between H-8 and H-20 were observed in this spectrum.[31] All proton and carbon signals of **3** were fully assigned by the HMBC experiment (Supporting Information).

Cudraphenone D (**4**) was obtained as a yellow oil, the molecular formula of which was shown to be $C_{23}H_{26}O_5$ by HR laser desorption/ionization time-of-flight MS and NMR.[32] This formula was 16 Da (an oxygen atom) larger than **2**. The NMR spectra of **4** indicated the presence of the same B ring as **1**−**3** and a 2,4,6-trihydroxybenzoyl moiety with a *C*-prenyl group. This led to the assumption that the cyclized prenyl group in **3** was transformed into a chain in **4**, which was confirmed by the HMBC spectrum (Supporting Information). Thus, the structure of cudraphenone D was determined as **4**.

Cudraxanthone P (**5**) was obtained as pale yellow prisms and established as $C_{23}H_{24}O_6$ based on HRMS and NMR data.[32] Its UV spectrum (λ_{max} 202, 252, 284 (sh), and 328 nm) resembled those of 1,3,5,6-tetraoxygenated xanthones.[2a,b,12] The ^1H NMR spectrum showed signals for three hydroxyl groups [δ 14.19 (1H, s), 9.51, 8.45 (each 1H,

Table 2. ^{13}C NMR Data for Compounds **5**−**7** in Acetone-d_6 (125 MHz, δ in ppm)

carbon	5	6	7
1	164.0	156.5	157.0
2	115.7[a]	104.8	109.6
3	164.0	158.9	166.3
4	95.3	108.0	109.7
4a	156.7	154.9	156.4
4b	145.7	150.8	146.8
5	135.2	120.0	133.6
6	152.5	125.2	151.4
7	110.4	155.2	113.7
8	116.8	109.2	117.1
8a	115.8[a]	121.6	114.5
9	181.6	181.7	181.6
9a	103.2	103.8	104.3
11	41.7	116.1	26.5
12	29.2	128.6	91.9
13	29.2	79.0	71.4
14	150.8	28.4	26.3
15	108.5	28.4	25.4
16	67.0	22.0	41.5
17	120.4	123.1	29.2
18	138.8	131.8	29.2
19	18.2	18.1	151.2
20	25.8	25.9	108.5

[a] The assignment may be interchanged.

br s)], *ortho*-coupled aromatic protons [δ 7.68 (1H, d, J = 9 Hz) and 7.15 (1H, d, J = 9 Hz)], a singlet aromatic proton [δ 6.47 (1H, s)], a 1,1-dimethylallyl group [δ 6.36 (1H, dd, J = 11, 18 Hz), 4.94 (1H, dd, J = 1, 18 Hz), 4.84 (1H, dd, J = 1, 11 Hz), and 1.62 (6H, s)], and an *O*-prenyl group [δ 5.50 (1H, m), 4.78 (2H, br d, J = 7 Hz), and 1.774, 1.769 (each 3H, br d, J = 1 Hz)]. The chemical shift of the intramolecular hydrogen-bonded hydroxy proton (δ 14.19) indicated that a bulky group was located at C-2.[28b] Analysis of its HMQC and HMBC spectra allowed the unambiguous assignments of all proton and carbon signals (Table 2), which also clarified the position of five substituents. Several HMBC couplings, including correlations of the OH-1 proton with C-1 (δ 164.0), C-2 (δ 115.7), and C-9a (δ 103.2), the two methyl groups at δ 1.62 (H_3-12, H_3-13) with C-2, and the aromatic singlet proton at δ 6.47 (H-4) with C-2, C-3 (δ 164.0), C-4a (δ 156.7), and C-9a, revealed a partial structure of **5** as 1,3-dihydroxy-2-(1,1-dimethyl-2-propenyl)xanthone. The positions of the prenyloxy and hydroxyl groups on B ring were deduced by following HMBC correlation peaks: Cross-peaks between the doublet at δ 7.68 (H-8) with C-4b (δ 145.7), C-6 (δ 152.5), and C-9 (δ 181.6), and the oxygen-bearing methylene at δ 4.78 (H_2-16) with C-6, and the doublet at δ 7.15 (H-7) with C-8a (δ 115.7) and C-5 (δ 135.2) were also observed in the spectrum (Supporting Information). Accordingly, the hydroxyl and the oxygenated prenyl groups were located at C-5 and C-6, respectively. Thus, the structure of cudraxanthone P was concluded to be **5**.

Cudraxanthone Q (**6**), isolated as yellow needles, was assigned a molecular formula of $C_{23}H_{22}O_5$ as inferred from its HRMS and NMR data.[31] UV and IR data suggested that **6** should have a xanthone skeleton. The NMR spectra of **6** indicated the presence of a prenyl group, a 2,2-dimethylpyran ring, a 1,2,4-trisubstituted benzene ring (B ring), and a hexasubstituted benzene ring (A ring). The oxygenated carbon signals on the B ring (Table 2) were observed at δ 155.2 (C-7) and 150.8 (C-4b), which are characteristic chemical shifts for 7-hydroxyxanthones (2-hydroxyxanthones).[33] A comparison of the ^1H and ^{13}C NMR data of **6** with those of osajaxanthone[12a,34] revealed that the proton at C-4 in the latter compound was replaced by a prenyl group in **6**. Thus, cudraxanthone Q (**6**) was assigned as a 4-prenylated osajaxanthone and confirmed by its HMBC spectrum (Supporting Information). Consequently, cudraxanthone Q was elucidated as **6**.

Cudraxanthone R (**7**), yellow prisms, $C_{23}H_{24}O_7$, $[\alpha]_D$ +6.1°, was also regarded as a xanthone based on its UV and IR spectra. The ^1H NMR spectrum of **7** contained one hydrogen-bonded hydroxyl group [δ 13.48 (1H, s)], *ortho*-coupled aromatic signals [δ 7.59 (1H, d, J = 9 Hz) and 6.98 (1H, d, J = 9 Hz)], and a set of signals due to a 1,1-dimethylallyl group [δ 6.53 (1H, dd, J = 11, 18 Hz), 5.09 (1H, dd, J = 1, 18 Hz), 4.92 (1H, dd, J = 1, 11 Hz), and 1.71, 1.72 (each 3H, s)]. Moreover, signals of a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran ring [δ 4.79 (1H, dd, J = 8, 10 Hz), 3.21 (1H, dd, J = 8, 16 Hz), 3.09 (1H, dd, J = 10, 16 Hz), and 1.27, 1.29 (each 3H, s)] were also observed, which were in agreement with the relevant signals [δ 91.9 (C-12), 71.4 (C-13), 26.5 (C-11), and 26.3, 25.4 (C-14, C-15)] observed in the ^{13}C NMR spectrum of **7** (Table 2).[5,35] The chemical shift of OH-1 (δ 13.48) indicated that a 1,1-dimethylallyl group did not occur at the C-2 position.[28c] In the HMBC spectrum of **7** (Supporting Information), the methylene signals at δ 3.21 and 3.09 (H_2-11) showed cross-peaks with C-1 (δ 157.0), C-2 (δ 109.6), and C-3 (δ 166.3), and the methine signal at δ 4.79 (H-12) was correlated with C-3, indicating that a dihydrobenzofuran ring was connected to C-2 and C-3. By a process of elimination, the remaining 1,1-dimethylallyl group could be located at C-4. Therefore, the structure of cudraxanthone R was assigned as **7**. The stereochemistry at C-12 remains to be determined.

The main phenolic constituent of *Cudrania cochinchinensis* obtained in the present investigation is the same as that of *C. cochinchinensis* var. *gerontogea* [i.e., the prenylated isoflavone, wighteone (**22**)],[12b] and not those of *C. tricuspidata* (the 3-prenylated flavones, cudraflavones A−C).[2b] Most of the minor phenols of *C. cochinchinensis* were 1,3,5,6-tetraoxygenated xanthones with two isoprenoid groups (**5**, **7**, **10**−**13**, and **15**−**17**) similar to *C. cochinchinensis* var. *gerontoge* (14 xanthones have been isolated from the plant, among which nine compounds are 1,3,5,6-tetraoxygenated xanthones with one or two isoprenoid groups).[12] On the other hand, the xanthones of *C. tricuspidata* are mostly di-isoprenoid-substituted 1,3,7-trioxygenated xanthones and 1,3,6,7-tetrahydroxyxanthones.[2a,b]

The phenolic compounds isolated here were screened first for cytotoxic activity against the human oral squamous cell carcinoma cell line HSC-2 (Table 3). Among the benzophenones, compounds **1**−**4**, with two isoprenoid groups, showed higher cytotoxic activities against HSC-2 cells [CC_{50} = 0.17, 0.036, 0.092, 0.052 mmol/L (mM)] than the monoprenylated compound cudranone (**8**, CC_{50} = 0.40 mM). For the xanthones, compounds **10** and **16** (1,3,5,6-tetrahydroxyxanthones with a 1,1-dimethylallyl group at the C-2 position and a prenyl group at the B ring) showed the highest cytotoxic activities (CC_{50} = 0.022, 0.035 mM). Previously, we reported that substitution of a hydrophobic group (isoprenoid unit) in polyhydroxylated flavones, flavonols, isoflavones, and a 2-arylbenzofuran modified their cytotoxic activities against HSC-2 cells.[4,5] Furthermore, flavones and flavonols with two sets of hydrophobic and hydrophilic (hydroxyl) groups showed higher cytotoxic activities against HSC-2 cells than monoprenylated flavonoids.[4a] On the other hand, 2-arylbenzofurans with two isoprenoid groups exhibited much lower cytotoxic activities against HSC-2 cells than the monoprenylated 2-arylbenzofuran.[5]

Table 3. Cytotoxic Activities of Phenolic Compounds against the Human Oral Squamous Cell Carcinoma Cell Line HSC-2 and Normal Human Gingival Fibroblasts[a]

compound	cytotoxic activity (CC_{50}) in mM		tumor specificity
	HSC-2 (A)	HGF (B)	B/A
benzophenones			
1	0.17	0.43	2.5
2	0.036	0.090	2.5
3	0.092	0.19	2.1
4	0.052	0.19	3.7
8	0.40	>0.61	>1.5
xanthones			
9	>0.64	>0.64	
10	0.022	0.025	1.1
11	>0.51	>0.51	
12	0.39	>0.51	>1.3
13	>0.51	>0.51	
14	0.12	0.20	1.7
15	0.43	>0.51	>1.1
16	0.035	0.058	1.6
17	>0.51	>0.51	
18	0.48	>0.65	>1.4
flavonoids			
19	0.55	>0.74	>1.3
20	0.13	0.19	1.5
21	0.16	>0.67	>4.2
22[b]	0.12	0.25	2.1
23	0.40	>0.60	>1.5
coumarin			
24	0.72	>0.93	>1.3

[a] The A_{540} values of control HSC-2 and HGF cells (viable cells after incubation for 24 h without test compound) were 1.853 and 0.337, respectively. [b] Positive control (6-prenyl-4′,5,7-trihydroxyisoflavone).

Benzophenones **1**−**4** showed higher cytotoxic activities against HSC-2 cells than against normal human gingival fibroblasts (HGF) (tumor specificity: CC_{50} for HGF (B)/CC_{50} for HSC-2 (A) (B/A ratio) = 2.5, 2.5, 2.1, 3.7, respectively) (Table 3). Previously, we reported high tumor specificity of flavones and flavonols with two sets of hydrophobic and hydrophilic groups at separated domains (B/A = 1.5−2.6) and no specificity of kuwanon C (3,8-diprenyl-2′,4′,5,7-tetrahydroxyflavone, B/A = 1.2).[4a] In addition, we have also reported high tumor specificity of the isoprenoid-substituted sanggenon-type flavanones (3-hydroxy-2-prenylflavanones with a benzofuran ring between B and C rings), B/A = 1.4−3.9.[5] Benzophenones **1**−**4** showed tumor specificity for HSC-2 (B/A = 2.1−3.7). It is likely that polyphenols with two sets of hydrophobic and hydrophilic groups at separated domains have higher tumor specificity. However, further systematic cytotoxicity studies with additional prenylated phenols are necessary to confirm this hypothesis.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanaco MP-500V micro-melting point apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-370 digital polarimeter. IR spectral data were measured on a JASCO FTIR-300E spectrometer with KBr pellets. UV spectra were obtained on a Shimadzu UV-265 spectrophotometer. NMR spectra were run on JEOL JNM ECP-500 and AL-400 FT-NMR instruments. Chemical shifts are reported with respect to acetone-d_6 (δ_H 2.04, δ_C 206.0). EIMS were recorded on a JEOL JMS-AM II 50 spectrometer. HR-LDI-TOF-MS (without matrix) data were obtained on a Voyager-DE STR TOF mass spectrometer. Calibration was performed with two internal standards, cyclomorusin,[2] **9**, or **15** ($[M]^+$). The details of the measurement of HR-MS were reported in our recent paper.[32] The absolute mass error of angiotensin II ($[M + H]^+$) was − 2.1 mDa, and a 95.5% confidence level ($\pm 2\sigma$) of the ion was ±6.6 mDa with this instrument. Wakogel C-200 (Si gel, Wako Pure Chemical Co., Ltd., Osaka, Japan) and Chromatorex octadecylsilyl Si gel (ODS, 100−200 mesh) (Fuji Silysia Chemical, Ltd., Kasugai, Japan) were used for column chromatography.

Plant Material. The roots of *C. cochinchinensis* (Lour.) were collected in Xishuangbanna, Yunnan, People's Republic of China, in July 1998, and air-dried. The identity of plant material was verified by Prof. Zhong-Wen Lin (Kunming Institute of Botany), and a voucher specimen (KIB 98-7-20 Lin) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica.

Extraction and Isolation. The dried and powdered roots (8.7 kg) were extracted with EtOH under reflux three times and filtered. The filtrate was evaporated *in vacuo* to give a residue (1 kg), which was suspended in water and partitioned successively with *n*-hexane, C_6H_6, EtOAc, and *n*-BuOH. The C_6H_6 extract (100 g) was subjected to column chromatography over Si gel eluted with C_6H_6 and C_6H_6−EtOAc (9:1, 4:1, and 1:1) to yield fractions 1−9. Fraction 4 (9 g) was chromatographed on Si gel (*n*-hexane−acetone, 9:1) to give four fractions. The first fraction was subjected to column chromatography on Si gel ($CHCl_3$−acetone, 25:1), followed by ODS (MeOH−H_2O, 9:1) to give **1** (10 mg), **6** (3 mg), **8** (17 mg),[11] **11** (83 mg),[12a] and **18** (11 mg).[19] The second fraction was purified through an ODS column (MeOH−H_2O, 9:1) to afford **24** (15 mg).[27] The third fraction was repeatedly chromatographed over Si gel (*n*-hexane−Et_2O, 3:2) and ODS (MeOH−H_2O, 8:2) to give **5** (3 mg), **14** (54 mg),[12b] **17** (5 mg),[18] and **23** (15 mg).[25] The fourth fraction was eluted with MeOH−H_2O (7:3) over a column of ODS to yield **2** (16 mg) and **24** (34 mg). Fraction 5 (3 g) was repeatedly chromatographed over Si gel (*n*-hexane−acetone, 8:2) and ODS (MeOH−H_2O, 8:2) to give **3** (9 mg), **12** (15 mg),[12a] and **13** (210 mg).[12b] Fraction 6 (4 g) was also purified by column chromatography over Si gel ($CHCl_3$−acetone, 15:1) and ODS (MeOH−H_2O, 8:2) to give **9**[15] (50 mg) and **20** (124 mg).[22] Fraction 7 (9 g) was eluted with C_6H_6−acetone (9:1) over Si gel to afford two fractions. The first fraction was chromatographed on ODS developed with MeOH−H_2O (8:2 and 9:1) to give **7** (3 mg), **15** (100 mg),[12b] **16** (143 mg),[17] **22** (1.14 g),[24] and carpachromene (3 mg).[26] Further separation of the second fraction by passage over on Si gel (C_6H_6−acetone, 9:1, and *n*-hexane−Et_2O, 1:1) yielded **4** (40 mg). Fraction 8 (7 g) was fractionated by column chromatography on Si gel (C_6H_6−acetone, 9:1), followed by passage over ODS (MeOH−H_2O, 6:4 and 8:2) to provide **10** (394 mg),[16] toxyloxanthone C (20 mg),[20] **19** (88 mg),[21] and **21** (4 mg).[23]

Cudraphenone A (1): yellow oil; UV (MeOH) λ_{max} (log ϵ) 204 (4.35), 228 (sh) (4.22), 236 (sh) (4.22), 262 (4.29), 358 (3.71) nm; IR (KBr) ν_{max} 3425, 2974, 2925, 1625, 1578, 1482, 1462, 1370, 1288 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 12.76 (1H, s, OH-2), 8.66 (1H, br s, OH-15), 7.14 (1H, t, J = 8 Hz, H-17), 7.02 (1H, dd, J = 1, 8 Hz, H-16), 6.92 (1H, s, H-6), 6.74 (1H, dd, J = 1, 8 Hz, H-18), 6.29 (1H, d, J = 1 Hz, H-3), 6.25 (1H, br d, J = 10 Hz, H-7), 5.65 (1H, d, J = 10 Hz, H-8), 5.09 (1H, m, H-20), 3.32 (2H, br d, J = 7 Hz, H_2-19), 1.45 (3H, br d, J = 1 Hz, H_3-22), 1.43 (6H, s, H_3-10, H_3-11), 1.39 (3H, br s, H_3-23); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 365 $[M + 1]^+$ (5), 364 $[M]^+$ (10), 346 $[M - H_2O]^+$ (14), 331 (100), 316 (8), 291 (16), 188 (63); HRLDITOFMS *m*/*z* 365.1761 $[M + H]^+$ (calcd for $C_{23}H_{25}O_4$, 365.1753).

Cudraphenone B (2): yellow oil; UV (MeOH) λ_{max} (log ϵ) 206 (4.38), 288 (3.96), 337 (3.84) nm; IR (KBr) ν_{max} 3426, 3122, 2968, 2933, 1631, 1585, 1561, 1481, 1456, 1344, 1295, 1230, 1162 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 12.54 (1H, s, OH-2), 7.13 (1H, t, J = 8 Hz, H-17), 7.00 (1H, dd, J = 1, 8 Hz, H-16), 6.95 (1H, s, H-6), 6.69 (1H, dd, J = 1, 8 Hz, H-18), 6.41 (1H, s, H-3), 5.13 (1H, m, H-8), 5.07 (1H, m, H-20), 3.31 (2H, br d, J = 7 Hz, H_2-19), 3.10 (2H, br d, J = 7 Hz, H_2-7), 1.58 (3H, br s, H_3-11), 1.52 (3H, br s, H_3-10), 1.44 (3H, br s, H_3-22), 1.40 (3H, br s, H_3-23); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 367 $[M + 1]^+$ (2), 366 $[M]^+$ (7), 348 $[M - H_2O]^+$ (33), 333 (32), 308 (5), 293 (12), 279 (17), 267 (6), 253 (6), 241 (10), 205 (10),

188 (100); HRLDITOFMS *m/z* 367.1949 $[M + H]^+$ (calcd for $C_{23}H_{27}O_4$, 367.1909).

Cudraphenone C (3): yellow oil; UV (MeOH) λ_{max} (log ϵ) 203 (4.52), 301 (4.14) nm; IR (KBr) ν_{max} 3397, 2972, 2924, 1642, 1600, 1548, 1460, 1423, 1287 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 13.42 (1H, s, OH-2), 7.05 (1H, t, $J = 8$ Hz, H-17), 6.90 (1H, dd, $J = 1$, 8 Hz, H-16), 6.60 (1H, dd, $J = 1$, 8 Hz, H-18), 6.48 (1H, d, $J = 10$ Hz, H-7), 6.00 (1H, s, H-3), 5.33 (1H, d, $J = 10$ Hz, H-8), 5.19 (1H, m, H-20), 3.30 (2H, br d, $J = 7$ Hz, H_2-19), 1.50 (3H, br d, $J = 1$ Hz, H_3-23), 1.49 (3H, br s, H_3-22), 0.95 (6H, s, H_3-10, H_3-11); ^{13}C NMR data, see Table 1; EIMS *m/z* 381 $[M + 1]^+$ (6), 380 $[M]^+$ (16), 365 (15), 347 (62), 311 (18), 297 (5), 267 (7), 219 (17), 203 (50), 188 (100); HRLDITOFMS *m/z* 381.1741 $[M + H]^+$ (calcd for $C_{23}H_{25}O_5$, 381.1702).

Cudraphenone D (4): yellow oil; UV (MeOH) λ_{max} (log ϵ) 204 (4.53), 304 (4.10) nm; IR (KBr) ν_{max} 3456, 2968, 2921, 1632, 1606, 1565, 1514, 1438, 1316 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 12.98 (1H, s, OH-2), 8.77 (3H, br s, OH-4, 6, 15), 7.04 (1H, t, $J = 8$ Hz, H-17), 6.87 (1H, dd, $J = 1$, 8 Hz, H-16), 6.67 (1H, dd, $J = 1$, 8 Hz, H-18), 5.93 (1H, s, H-5), 5.25 (1H, m, H-8), 5.15 (1H, m, H-20), 3.33 (2H, br d, $J = 7$ Hz, H_2-19), 3.27 (2H, br d, $J = 7$ Hz, H_2-7), 1.75 (3H, br s, H_3-10), 1.64 (3H, br d, $J = 1$ Hz, H_3-11), 1.49 (3H, br d, $J = 1$ Hz, H_3-23), 1.47 (3H, br s, H_3-22); ^{13}C NMR data, see Table 1; EIMS *m/z* 382 $[M]^+$ (2), 364 $[M - H_2O]^+$ (4), 349 (3), 309 (7), 257 (17), 188 (100); HRLDITOFMS *m/z* 383.1827 $[M + H]^+$ (calcd for $C_{23}H_{27}O_5$, 383.1859).

Cudraxanthone P (5): yellow prisms (MeOH); mp 166 °C; UV (MeOH) λ_{max} (log ϵ) 202 (4.55), 252 (4.76), 284 (sh) (4.11), 328 (4.48) nm; IR (KBr) ν_{max} 3409, 2926, 2855, 1623, 1604, 1577, 1515, 1443, 1386, 1281 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 14.19 (1H, s, OH-1), 9.51 (1H, br s, OH), 8.45 (1H, br s, OH), 7.68 (1H, d, $J = 9$ Hz, H-8), 7.15 (1H, d, $J = 9$ Hz, H-7), 6.47 (1H, s, H-4), 6.36 (1H, dd, $J = 11$, 18 Hz, H-14), 5.50 (1H, m, H-17), 4.94 (1H, dd, $J = 1$, 18 Hz, H-15a), 4.84 (1H, dd, $J = 1$, 11 Hz, H-15b), 4.78 (2H, br d, $J = 7$ Hz, H_2-16), 1.774, 1.769 (each 3H, br d, $J = 1$ Hz, H_3-19, H_3-20), 1.62 (6H, s, H_3-12, H_3-13); ^{13}C NMR data, see Table 2; EIMS *m/z* 397 $[M + 1]^+$ (7), 396 $[M]^+$ (25), 381 (3), 363 (5), 328 (68), 313 (100), 299 (49), 287 (52), 273 (59), 257 (8), 244 (8); HRLDITOFMS *m/z* 397.1629 $[M + H]^+$ (calcd for $C_{23}H_{25}O_6$, 397.1651).

Cudraxanthone Q (6): yellow needles (MeOH); mp 205 °C; UV (MeOH) λ_{max} (log ϵ) 233 (4.02), 294 (4.33), 341 (sh) (3.54), 388 (3.34) nm; IR (KBr) ν_{max} 3242, 2970, 2924, 1649, 1609, 1582, 1547, 1480, 1437 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 13.28 (1H, s, OH-1), 7.56 (1H, d, $J = 3$ Hz, H-8), 7.50 (1H, d, $J = 9$ Hz, H-5), 7.36 (1H, dd, $J = 3$, 9 Hz, H-6), 6.69 (1H, d, $J = 10$ Hz, H-11), 5.74 (1H, d, $J = 10$ Hz, H-12), 5.24 (1H, m, H-17), 3.47 (2H, br d, $J = 8$ Hz, H_2-16), 1.88 (3H, br s, H_3-19), 1.65 (3H, br s, H_3-20), 1.49 (6H, s, H_3-14, H_3-15); ^{13}C NMR data, see Table 2; EIMS *m/z* 379 $[M + 1]^+$ (6), 378 $[M]^+$ (25), 363 (100), 335 (10), 323 (6); HRLDITOFMS *m/z* 401.1387 $[M + Na]^+$ (calcd for $C_{23}H_{22}NaO_5$, 401.1356).

Cudraxanthone R (7): yellow prisms (MeOH); mp 237 °C; $[\alpha]^{22}_D + 6.1°$ (*c* 0.10, acetone); UV (MeOH) λ_{max} (log ϵ) 203 (4.16), 255 (4.44), 289 (sh) (3.91), 331 (4.10) nm; IR (KBr) ν_{max} 3428, 2975, 2931, 1660, 1624, 1588, 1470, 1412 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 13.48 (1H, s, OH-1), 7.59 (1H, d, $J =$ 9 Hz, H-8), 6.98 (1H, d, $J = 9$ Hz, H-7), 6.53 (1H, dd, $J = 11$, 18 Hz, H-19), 5.09 (1H, dd, $J = 1$, 18 Hz, H-20a), 4.92 (1H, dd, $J = 1$, 11 Hz, H-20b), 4.79 (1H, dd, $J = 8$, 10 Hz, H-12), 3.21 (1H, dd, $J = 8$, 16 Hz, H-11a), 3.09 (1H, dd, $J = 10$, 16 Hz, H-11b), 1.72, 1.71 (each 3H, s, H_3-17 and H_3-18), 1.29, 1.27 (each 3H, s, CH_3-14 and H_3-15); ^{13}C NMR data, see Table 2; EIMS *m/z* 413 $[M + 1]^+$ (7), 412 $[M]^+$ (29), 397 (15), 379 (25), 353 (26), 339 (40), 325 (100), 311 (13), 297 (11), 285 (17); HRLDITOFMS *m/z* 435.1460 $[M + Na]^+$ (calcd for $C_{23}H_{24}NaO_7$, 435.1420).

Assay for Cytotoxic Activity. All tested samples were dissolved in 50% EtOH at 10 mg/mL. The final concentration of EtOH in the medium was below 1%. Cell culture and the other procedures were the same as those reported previously.[4] All data represent the mean values of duplicate determinations.

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Supporting Information Available: Tables of HMBC NMR data for **1**−**4** (Table 1a) and **5**−**7** (Table 2a) and data for the known compounds **8**−**24**, carpachromene, and toxyloxanthone C. This material is available free of charge via the Internet at http://pubs.acs.org.

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