Bioactive Benzophenones from *Garcinia xanthochymus* Fruits

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A MeOH extract of *Garcinia xanthochymus* fruits was subjected to activity-guided fractionation, yielding two new benzophenones, guttiferone H (**1**) and gambogenone (**2**). Compound **1** contains a seven-membered ring attached to the bicyclo[3.3.1]nonane system at positions 7 and 8 and displayed cytotoxicity in the SW-480 colon cancer cell line (IC_{50} = 12 μM). Compound **2** has a novel benzophenone bicyclo[3.3.2]decane system and displayed cytotoxicity in the SW-480 colon cancer cell line (IC_{50} = 188 μM). Both **1** and **2** induced apoptosis in SW-480 colon cancer cells and displayed antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (IC_{50} = 64 and 38.7 μM, respectively). The structures of **1** and **2** were established by 1D and 2D NMR data analysis. Eleven known compounds, aristophenone A, alloathyriol, amentoflavone, 3,8″-biapigenin, cycloxanthochymol, (±)-fukugetin, (±)-fukugiside, guttiferone E, isoxanthochymol, (±)-volkensiflavone, and xanthochymol, were also obtained. The 11 known compounds were also tested against SW-480 colon cancer cells and in the DPPH assay.

Garcinia xanthochymus Hook.f. (Clusiaceae), commonly known as gamboge, is a tree endemic to India growing 8−10 m in height. The trees have dark green leaves and a gummy yellow sap and bear yellow fruits 6−7 cm in diameter with juicy, acidic, yellow pulp containing two seeds. The acidic fruits are used in jams, preserves, and vinegar.[1] Gamboge is used in watercolors and as a yellow fabric dye.[2] Gamboge fruits are used in traditional medicine for treating diarrhea and dysentery.[3] Previous phytochemical studies of the leaves, seeds, fruits, and heartwood of *G. xanthochymus* have shown the presence of two benzophenones,[4−6] xanthochymol (**4**) and isoxanthochymol (**7**), 10 biflavonoids,[4,5,7,8] including fukugetin (**11**), fukugiside (**12**), and volkensiflavone (**13**), the flavonoid vitexin,[7] and a number of triterpenes,[9] xanthones,[10−12] and lipids.[13] Biological activities, including analgesic,[14] antibacterial,[15] antioxidant,[16] antiviral,[17−19] and cytotoxic,[20] have been reported for previously isolated benzophenones and biflavonoids.

As part of a program to isolate novel antioxidant and cytotoxic compounds from plants,[21−23] the $CHCl_3$ and EtOAc partitions from *G. xanthochymus* fruits were analyzed for their cytotoxic and DPPH activities. Both partitions displayed activity in the DPPH assay (IC_{50} = 32 and 105 μg/mL, respectively) and cytotoxicity against the SW-480 colon cancer cell line (IC_{50} = 15 and 50 μg/mL, respectively) and were selected for further analysis. In the present paper we describe the activity-guided isolation of guttiferone H (**1**) and gambogenone (**2**), two new benzophenones, and 11 known compounds (Figure 1): aristophenone A (**3**), xanthochymol (**4**), guttiferone E (**5**), cycloxanthochymol (**6**), isoxanthochymol (**7**), alloathyriol (**8**), amentoflavone (**9**), 3,8″-biapigenin (**10**), (±)-fukugetin (**11**), (±)-fukugiside (**12**), and (±)-volkensiflavone (**13**). This is the first reported isolation of compounds **3**, **5**, **6**, and **8**−**10** from *G. xanthochymus* fruits. The antioxidant activity and cytotoxicity of **1**−**13** are also reported.

Results and Discussion

The MeOH extract of *G. xanthochymus* fruits was sequentially partitioned with $CHCl_3$ and EtOAc. Chromatography of the $CHCl_3$ partition over Sephadex LH-20, followed by repeated gradient reversed-phase column chromatography using mixtures of MeOH or MeCN in H_2O, led to the isolation of two new benzophenones, **1** and **2**.

Guttiferone H (**1**) was isolated as a yellow oil, and the molecular formula was established as $C_{38}H_{50}O_6$ (corresponding to 14 units of unsaturation) from HRESIMS and ^{13}C and DEPT NMR data (Table 1). The UV spectrum of **1** showed maxima at λ_{max} (log ϵ) 229 (1.87) and 278 nm (2.40), similar to previously isolated polyisoprenylated benzophenones.[18,24] COSY and HMBC experiments (Figure 2) established the presence of the following: (i) two pairs of *gem*-dimethyl groups, the protons at δ 1.20 (Me-22) and 0.97 (Me-23) comprising one group and those at δ 1.21 (Me-37) and 0.95 (Me-38) the other; (ii) an isopropenyl moiety composed of alkenic methylene protons at δ 4.81 (35A) and 4.67 (35B) and the methyl group at δ 1.68 (Me-36); (iii) two isopent-2-enyl groups, the olefinic proton at δ 5.0 (H-18), methylene protons at δ 2.58 (17A) and 2.52 (17B), and two methyls at δ 1.68 (Me-20) and 1.67 (Me-21) comprising one group and the olefinic proton at δ 5.0 (H-25), methylene protons at δ 2.45 (24A) and 2.28 (24B), and two methyls at δ 1.67 (Me-27) and 1.62 (Me-28) the other; (iv) the distinctive, aromatic three-spin system consisting of proton signals 12 (δ 7.36 d), 15 (δ 6.70 d), and 16 (δ 7.21 dd) and carbons 10−16; and (v) an enolized β-diketone group (carbons 1 and 3) at δ 190.9 and 190.6.

Strong HMBC cross-peaks (Figure 2) from the protons of geminal Me-22 and Me-23 to the carbons at δ 67.1, 48.0, and 47.6 established the latter as C-4, C-5, and C-6, while HMBC cross-peaks from the geminal Me-37 and Me-38 to the carbons at δ 40.2, 40.9, and 49.4 identified them as

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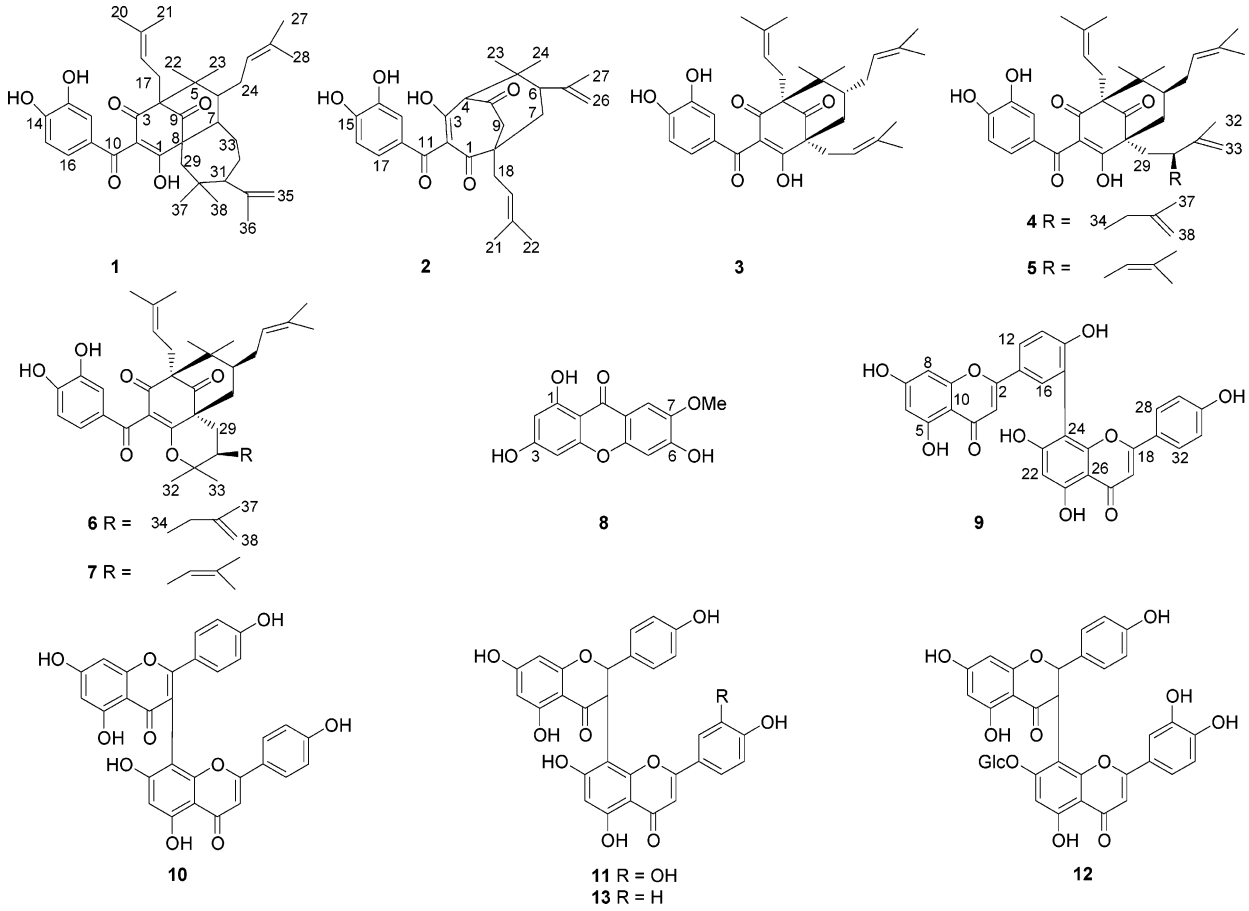

Figure 1. Compounds isolated from *G. xanthochymus* fruits.

C-29, C-30, and C-31. HMBC and COSY connectivities were then sought that would extend the ring system beyond C-6.

H-6 exhibited both a COSY cross-peak to a proton at δ 1.92, suggesting that it was H-7, and HMBC correlations to C-4 and the carbon at δ 60.2. The proton at δ 1.92 also displayed an HMBC connectivity to the carbon at δ 60.2 and, additionally, one to C-6 and the carbon at δ 190.9. These data indicated that C-7 (δ 38.6) is methine in **1**, not methylene as in other guttiferone-type benzophenones, and that the carbons at δ 60.2 and 190.9 are C-8 and C-1, respectively. In addition, H-7 showed an HMBC cross-peak to the carbon at δ 212.7, which suggested that it is C-9 and that guttiferone H might possess a bicyclo[3.3.1]nonane system similar to those of **4** and other guttiferones.[18,24,25]

The next concern was the location of the isopropenyl and the two isopent-2-enyl side chains. HMBC connectivities from H-17A and H-17B to C-4 and the carbon at δ 190.6 identified the latter as C-3 and placed an isopent-2-enyl group including C-17 at C-4. In addition, H-29A exhibited an HMBC cross-peak to the carbon at δ 212.7, lending support to its identity as C-9. Similarly, HMBC correlations between the protons at δ 2.18 (29A) and 1.92 (29B) and the carbons at δ 38.6 and 60.2 confirmed the identities of C-7 and C-8, respectively. Additional cross-peaks between these protons and the carbons at δ 190.9 and 212.7 confirmed the assignments of C-1 and C-9, respectively, and required that the three-carbon fragment that includes C-29 (carbons 29, 30, and 31) be positioned at C-8. Moreover, HMBC connectivities between H-6 and H-7 and the carbon at δ 29.7 (C-24) and COSY cross-peaks between H-6 and the protons at δ 2.45 (24A) and 2.28 (24B) necessitated that the isopent-2-enyl group that includes C-24 be located at C-6.

At this point only the methylene carbons at δ 28.8 and 32.9 and the isopropenyl group remained unassigned. H-31, at δ 2.34, displayed a multitude of HMBC cross-peaks, most notably to the isopropenyl methyl carbon at δ 23.5 (C-36) and olefinic carbons at δ 146.7 (C-34) and 108.3 (C-35). In addition, the protons at δ 1.68 (Me-36) and 4.67 (H-35B) exhibited HMBC connectivities back to the carbon at δ 49.4 (C-31). These correlations all required that the isopropenyl group be placed at C-31. The previously isolated benzophenones sampsoniones D and I are examples of isopentenyl side chains being effectively replaced by an isopropenyl moiety.[26]

In addition, H-31 showed a COSY cross-peak to the methylene proton at δ 1.82 (32B), while it, in turn, exhibited HMBC connectivities to both C-31 and the carbon at δ 32.9 (C-33). These correlations suggested that C-32 is attached to both C-31 and C-33. Moreover, the methylene proton at δ 1.76 (33B) displayed HMBC cross-peaks to both C-7 and C-8 and the carbon at δ 28.8 (C-32), while its geminal partner (33A) exhibited an HMBC correlation to C-6. These HMBC connectivities together with a COSY correlation between H-32B and H-33A complete the novel seven-membered D-ring by requiring that C-33 be bonded to C-7.

There are no HMBC correlations to C-2 (δ 119.1); however, it is reasonable to depict C-2, together with C-1 and C-3, as an enolized 1,3-diketone comprising part of the B-ring of a bicyclo[3.3.1]nonane system. Note that only one of two possible equilibrating, endocyclic, enolized 1,3-diketone structures is depicted in **1**. The nearly identical ^{13}C chemical shifts of carbons 1 and 3 indicate that an equally probable isomer exists in which C-3 is a carbonyl

Table 1. NMR Data for Guttiferone H (**1**) in CD_3OD

position	$^{13}C^a$ (δ)	$^1H^b$ (δ, mult., J in Hz)	HMBC connectivities[b,c]
1	190.9		7, 29A, 29B
2	119.1		
3	190.6		17A, 17B
4	67.1		6, 17A, 17B, 22, 23
5	48.0		22, 23
6	47.6	1.40 m	22, 23, δ 1.92
7	38.6	1.92 dd (14, 6.7)	32B, δ 1.92
8	60.2		6, 7, 29A, 29B, 33B
9	212.7		7, 17A, 29A, 29B
10	198.0		12, 16
11	131.6		15
12	115.5	7.36 d (1.8)	16
13	145.1		15
14	150.4		12, 16
15	114.1	6.70 d (8.2)	
16	124.1	7.21 dd (8.2, 1.8)	12
17	25.8	2.58 dd (14, 6.5)	
		2.52 dd (14, 6)	
18	121.8	5.0, dd (6.5, 6)	17A, 20, 21
19	131.9		20, 21
20	25.4	1.68 s	21
21	17.4	1.67 s	20
22	22.6	1.20 s	23
23	26.6	0.97 s	22
24	29.7	2.45 ddd (14, 6, 5)	6, 7
		2.28 ddd (14, 6, 1)	
25	126.0	5.0 t (6)	27, 28
26	132.5		27, 28
27	24.5	1.67 s	28
28	17.4	1.62 s	25, 27
29	40.2	2.18 d (14)1.90 d (14)	37, 38
30	40.9		29B, 31, 32A, 37, 38
31	49.4	2.34 d (3.6)	29A, 32B, 35A(w), 35B, 36, 37, 38
32	28.8	1.93 m1.82 dd (12, 3.6)	33B, δ 1.92
33	32.9	1.92 m1.76 dd (14, 8.7)	32B
34	146.7		31, 32A, 36
35	108.3	4.81 brs	31, 36
		4.67 brs	
36	23.5	1.68 s	31, 35A, 35B(w)
37	25.1	1.21 s	31, 38
38	23.6	0.95 s	31, 37

[a] Recorded at 75 MHz. [b] Recorded at 500 MHz. [c] Protons correlating with carbon resonance; (w) = weak.

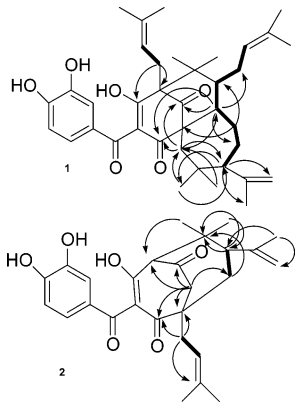

Figure 2. Arrows denote key HMBC cross-peaks and bold lines indicate COSY correlations for **1** and **2**.

carbon and an endocyclic double bond occurs between carbons 1 and 2.

Additional evidence for the structure of **1** was obtained from 2D-TOCSY experiments. TOCSY cross-peaks were observed from H-31 (δ 2.34) to H-32B (δ 1.82) and H-33B (δ 1.76) and to the signals at δ 1.92 (H-7, H-32A, and/or H-33A). This established that C-31, C-32, and C-33 are contiguous. In addition, a coupled spin system was observed consisting of protons H-25 (δ 5.0), H-24A and B (δ 2.45 and 2.28), H-6 (δ 1.4), and H-7 (δ 1.92). The optical rotation of **1**, $[\alpha]_D$ +94°, suggests that its absolute stereochemistry is more like that of the majority of the guttiferones, e.g., **4** and **5**, rather than that of garcinol (the enantiomer of **5**) and guttiferone F. The large vicinal coupling between protons 6 and 7 ($J_{6,7}$ = 14 Hz) indicates that they exist in an *anti* orientation.

Table 2. NMR Data for Gambogenone (**2**) in CD_3OD

position	$^{13}C^a$ (δ)	$^1H^b$ (δ, mult., J in Hz)	HMBC connectivities[b,c]
1	201.5		9, 18
2	130.2		
3	173.0		
4	38.2	1.62 s	9, 23, 24
5	40.4		4, 6, 7B, 23, 24
6	48.7	2.42 dd (10, 7)	23, 24, 26B, 27
7	26.7	2.05 dd (12, 7)	9
		1.67 dd (12, 10)	
8	54.9		7B, 9, 18
9	35.6	1.85 d (10.5)	
		1.82 d (10.5)	
10	207.6		9
11	191.2		13, 17
12	129.9		16
13	115.6	7.38 d (1.7)	17
14	145.6		13, 16
15	151.8		13, 16
16	114.5	6.79 d (8.3)	
17	124.3	7.25 dd (8.3, 1.7)	13
18	34.1	2.41 d (8)	9
19	118.2	4.99 t (8)	21, 22
20	135.9		18, 21, 22
21	16.8	1.60 s	22
22	25.2	1.70 s	21
23	23.4	1.08 s	24
24	24.0	0.83 s	23
25	145.8		6, 7A, 27
26	108.8	4.81 brs	27
		4.64 brs	
27	23.0	1.64 s	26A

[a] Recorded at 75 MHz. [b] Recorded at 500 MHz. [c] Protons correlating with carbon resonance.

Gambogenone (**2**) was isolated as a yellow oil whose molecular formula was established as $C_{27}H_{32}O_6$ (corresponding to 12 units of unsaturation) from HRESIMS and ^{13}C and DEPT NMR data, Table 2. The UV spectrum of **2** showed maxima at λ_{max} (log ϵ) 281 (2.88) and 322 nm (2.58), suggesting that it was also a member of the polyisoprenylated benzophenone family.[18,24] Additional similarities to known benzophenones, such as **4**, included (i) two high-field methyl groups at δ 1.08 and 0.83, characteristic of a *gem*-dimethyl group (Me-23 and Me-24); (ii) a triplet at δ 4.99 (J = 7.8 Hz), a methylene group at δ 2.41, and two methyl groups at δ 1.60 and 1.70, indicative of an isopent-2-enyl group (protons 18−22); and (iii) a 3,4-dihydroxybenzophenone moiety composed of protons at δ 6.79 d (H-16), 7.25 dd (H-17), and 7.38 d (H-13). COSY and HMBC experiments (Figure 2) confirmed the presence of the first and second above-mentioned systems and the 3,4-dihydroxybenzophenone moiety (carbons 11−17). In addition, they revealed that the olefinic methylene protons at δ 4.81 (26A) and 4.64 (26B) and the methyl group at δ 1.64 (Me-27) are part of an isopropenyl group (carbons 25−27).

Benzophenones, such as xanthochymol (**4**), typically possess side chains at the 4-, 6-, and 8-positions.[6,18,24,25] Strong HMBC cross-peaks from the protons of geminal Me-23 and Me-24 to the carbons at δ 38.2, 40.4, and 48.7 established the latter as C-4, C-5, and C-6, respectively. Surprisingly, C-4 was methine and, therefore, appeared to lack a side chain. HMBC connectivities between (i) the

methine proton at δ 2.42 (H-4 or H-6) and C-25 and (ii) both H-26B and Me-27 and the methine carbon at δ 48.7 suggested the latter carbon was C-6 and that the isopropenyl group was attached at C-6. In addition, COSY correlations (Figure 2) between the proton at δ 2.42 and the diastereotopic methylene protons at δ 2.05 (7A) and 1.67 (7B) not only necessitated that the methylene carbon at δ 26.7 (C-7) be also bonded to the methine carbon at δ 48.7 but also confirmed that this carbon was C-6. This, in turn, confirms that C-4 is methine and lacks its usual side chain.

HMBC cross-peaks between H-7A and C-25 confirmed the isopropenyl group was attached to C-6, while those between H-7B and the quaternary carbons at δ 40.4 (C-5) and 54.9 established the latter as C-8. HMBC connectivities between the methylene protons at δ 2.41 of the isopent-2-enyl group (H-18) and both the quaternary carbon at δ 54.9 (C-8) and carbonyl carbon at δ 201.5 (C-1) required that the isopent-2-enyl moiety be placed at C-8.

At this point only carbons at δ 207.6, 173.0, 130.2, and 35.6 remained unassigned. The methylene group (δ_C = 35.6; $\delta_{H(A)} = 1.85$, $\delta_{H(B)} = 1.82$) is unusual in that its protons exhibit more combined HMBC correlations than any other protons, namely, to C-1, C-4, C-7, C-8, C-10, and C-18. In addition, H-9A and H-9B display weak COSY cross-peaks to H-4. Due to the extent of its HMBC connectivities, the 9-methylene group had to be located between C-8 and C-10, creating a novel seven-membered B/C-ring system. Dreiding models demonstrate that the COSY cross-peaks between H-4 and the methylene protons are due to *W*-coupling. *W*-arrangements exist between H-4 and H-9A in one $CH_2C(O)$ bridge conformation and between H-4 and H-9B in the other. While there are no HMBC connectivities to either C-2 (δ 130.2) or C-3 (δ 173.0), it is reasonable to depict them as an enolized 1,3-diketone comprising part of the B-ring of the bicyclo[3.3.2]decane system of **2**.

The NMR data of certain carbons of **2** suggest that the nature of the α,β-unsaturated ketone in its B-ring is substantially different from those of the other isoprenylated benzophenones. Specifically, the chemical shift of C-3 (δ 173.0) is nearly identical to that (δ 173.4) reported for the 3-*O*-methyl derivative of xanthochymol (**4**).[25] In addition, the chemical shift of C-11 (δ 191.2) is ca. 5 ppm more shielded, while that of C-2 (δ 130.2) is ca. 12 ppm more deshielded, than those of analogous benzophenones.[18,24] These data suggest that structure **2** is a fairly accurate representation of gambogenone with carbons 1 and 11 being essentially carbonyl carbons with an endocyclic 2,3-double bond largely localized at these two carbons. The optical rotation of **2**, $[\alpha]_D$ −5°, suggests that its absolute stereochemistry might be also more like that of the majority of the guttiferones, e.g., **1**, **4**, and **5**. However, the new bicyclo[3.3.2]decane structure of **2** makes such a conclusion uncertain. A large vicinal coupling between protons 6 and 7B ($J_{6,7B}$ = 10 Hz) indicates that these two protons exist in an *anti* orientation. H-6 and H-7A, therefore, have α-orientations, while H-7B and the isopropenyl group at C-6 have β-orientations.

In addition, the previously isolated benzophenone **3** and the xanthone **8** were also obtained. After repeated column and preparative chromatography compounds **4**−**5** and **6**−**7** were isolated as isomeric mixtures; their separation was achieved using normal-phase TLC impregnated with Ag^+ ions. The EtOAc partition was chromatographed over Sephadex LH-20, followed by preparative HPLC, affording the biflavonoids **9**−**13**. The structures of the 11 known compounds (**3**−**13**), Figure 1, were determined by comparing their spectroscopic data with reported literature values. The biflavonoids **11**−**13** were isolated as enantiomeric pairs, i.e., the 2*R*/3*S* and 2*S*/3*R* isomers.

Table 3. DPPH and SW-480 Cytotoxicity IC_{50} Values for Isolated Compounds

compound	DPPH IC_{50} μM (± SD)	SW-480 IC_{50} μM (95% CI)
1	64 (2.1)	12.4 (10.5−12.0)
2	38.7 (2.3)	188 (172−214)
3	125 (4.1)	33.3 (24−33)
4	53 (1.0)	8.3 (7.0−8.2)
5	68 (0.33)	7.5 (6.1−7.8)
6, **7**[a]	73 (1.5)	16.6 (15−17.3)
8	na[b]	117 (101−123)
9	184 (5.0)	111 (102−122)
10	≫400	185 (170−203)
11	62 (5.1)	89 (82−98)
12	116 (9.0)	>200
13	298 (13.8)	185 (170−203)

[a] Mixture. [b] na = not active.

Compounds **1**−**13** were screened for their cytotoxicity in the SW-480 colon cancer cells and antioxidant activity in the DPPH free radical assay (Table 3). The new benzophenone **1**, as well as its analogues, **3**−**7**, displayed potent cytotoxicity in the SW-480 colon cancer cells. The effects of **1** on apoptosis and cell cycle distribution in SW-480 colon cancer cells were studied by flow cytometry. Compound **1** exhibited a potent dose-dependent increase in sub-diploid cells at 24 h from 10.2% in untreated controls to 11.8% at 8 μM, 15.6% at 16 μM, and 19.8% at 25 μM. After 48 h of treatment with **1** at 16 μM, 89% of cells were sub-diploid compared to 12% in untreated controls. Additionally, SW-480 cells treated with compound **1** at 16 μM for 3 h exhibited rapid loss of mitochondrial potential in virtually all cells, while controls exhibited active mitochondrial transport indicative of healthy cells. Moreover, SW-480 cells treated with compound **1** at 16 μM for 12 h also exhibited positive Annexin V staining in 80% of the treated cells but not in controls. Annexin-positive cells were virtually negative for propidium iodide staining, which is an indication of early apoptosis. Previous researchers have reported potent cytotoxicity and apoptosis induction for **4** and related benzophenones in leukemia cell lines.[20]

Garcinol induces apoptosis in human leukemia cells[20,27] and has chemopreventive activity in a rodent model of colorectal carcinogenesis.[28] In addition, Balasubramanyam et al.[29] recently identified garcinol as a potent inhibitor of histone acetyltransferases p300, a key regulatory step in gene expression and cell cycle. Therefore, benzophenones from *G. xanthochymus* (**1**, **3**−**7**) may exhibit similar effects.

Compound **2** was the least cytotoxic (IC_{50} = 188 μM) of the isolated benzophenones and is structurally different from compounds **1** and **3**−**7**. Compound **2** has a bicyclo-[3.3.2]decane system with an isopropenyl group at C-6 and only one prenyl side chain. Changes in the attached prenyl side chains and/or the bicyclo[3.3.2]decane system likely cause this significant reduction in cytotoxicity.

In addition, the isolated biflavonoids displayed cytotoxicity against SW-480 colon cancer cells, in the range IC_{50} = 89−185 μM. The biflavonoid glycoside, fukugiside (**12**), displayed weak activity in a SW-480 colon cancer assay.

The new benzophenones **1** and **2** displayed antioxidant activity in the DPPH free radical assay with IC_{50} = 64 and 38.7 μM, respectively. The previously isolated benzophenones, **3**−**7**, also displayed antioxidant activity in the DPPH assay in the range IC_{50} = 73−125 μM. The biflavonoid fukugetin (**11**) displayed the highest antioxidant activity in the DPPH assay (IC_{50} = 62 μM), and fukugiside (**12**), the glycoside of **11**, was half as active, IC_{50} = 116 μM.

The remaining biflavonoids (**9**, **10**, and **13**) displayed different levels of activity (IC_{50} = 184, >400, and 298 μM, respectively), despite having the same number of phenolic functional groups.

Experimental Section

General Experimental Procedures. ^1H, ^{13}C, DEPT, COSY, HSQC, and HMBC NMR spectra were measured using a JEOL GX 400 MHz, a Bruker DMX 500 MHz, or a Bruker DRX 300 MHz spectrometer. The 2D-TOCSY experiments were recorded at 300 MHz using standard Bruker pulse sequences with mixing times of 80, 160, 200, and 300 ms. A Waters 2695 separation system, equipped with a 996 photodiode array detector, using a Phenomenex (Torrance, CA) Luna C_{18} column (250 × 4.6 mm, 5 μm) or a Nucleosil C_{18} column (250 × 4.6 mm, 5 μm) was used for analytical HPLC. Preparative HPLC separations used a Waters Delta 600 pump, equipped with a Waters 486 single-wavelength detector at 254 nm, and a Phenomenex Nucleosil C_{18} column (250 × 21.1 mm, 10 μm). Molecular weights were determined using a ThermoFinnigan electrospray LCQ mass spectrometer in the positive and negative modes. HRESIMS was performed on a Micromass Q-TOF Ultima mass spectrometer. Optical rotations were measured on a JASCO DIP-140 polarimeter. UV spectra were measured on a Lambda 2 UV/vis spectrophotometer. Microplates were read using a Molecular Devices $Versa_{Max}$ plate reader at 515 nm. Microplates for MTT assay were read using a Biokinetics plate reader. The MTT assay was purchased from Boehringer-Mannheim (Indianapolis, IN). The SW-480 cell cultures were maintained in Dulbecco's modified Eagle medium (Gibco-BRL, Grand Island, NY). Apoptosis and cell cycle distribution were quantified on a FACScan flow cytometer (Becton-Dickinson, San Jose, CA).

Reversed-phase TLC analysis was performed on RP-18 F_{254} (Merck, Darmstadt, Germany) plates. Normal-phase TLC was performed on silica gel 60 (20 × 20 cm) F_{254}, 250 μm (Merck, Darmstadt, Germany) plates. Compounds were visualized by spraying with a mixture of 1 g of vanillin, 10 mL of H_2SO_4 (concentrated), and 90 mL of ethanol and heated. ACS grade silver nitrate was purchased from Carolina Biological Supply Company (Burlington, NC). Sephadex LH-20 (25−100 μm; Pharmacia Fine Chemicals, Piscataway, NJ) and reversed-phase C_{18} silica gel (40 μm; J. T. Baker, Phillipsburg, NJ) were used for column chromatography. Amentoflavone was purchased from Indofine Chemical Company (Hillsborough, NJ). Propidium iodide, RNAse A, and PBS were purched from Sigma Chemical Company (St. Louis, MO).

Plant Material. *G. xanthochymus* fruits were collected from the Fruit and Spice Park (Homestead, FL) in February 2002. Fresh frozen fruits were shipped to New York City by overnight courier and stored at −20 °C until extracted. A voucher specimen of *G. xanthochymus* was prepared and deposited at The William and Lynda Steere Herbarium, New York Botanical Garden (Bronx, NY).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Assay. The DPPH assay was performed on samples as previously described.[30] In this assay, 50 μL of plant extract was dissolved in DMSO and mixed in a 96-well microtiter plate with 150 μL of 50 mM ethanolic DPPH. The microtiter plate was incubated at 37 °C for 30 min and absorbance read at 515 nm. Antioxidant activity was determined as a percent inhibition of DPPH by sample and comparison with DMSO-treated controls. IC_{50} values signify the concentration of sample necessary to scavenge 50% of DPPH free radicals.

Cell Culture. SW-480 human colon cancer cells were maintained in Dulbecco's modified Eagle medium (Gibco-BRL, Grand Island, NY) with 10% fetal bovine serum in a normal atmosphere with 5% CO_2 at 37 °C. Cells were grown to 40% confluence prior to treatment with test compounds. All cultures were passaged weekly, and the medium was changed three times a week. No antibiotics were added at any time during the experiment. In all experiments, study compounds were dissolved in DMSO and added to the medium at the start of the incubation.

Microtetrazolium (MTT) Assay. The MTT assay was carried out according to the manufacturer's instructions. Briefly, approximately 30 000 cells were plated in 96-well flat-bottom plates with 100 μL of medium. When cells reached 40% confluence, the medium was changed and cells were exposed to pure compounds or extracts. After 72 h cells were washed 3× with PBS, and then 100 μL of Dulbecco's modified Eagle medium containing 10 μL of 5 mg/mL MTT solution in PBS was added for 4 h. Finally, 100 μL of MTT solubilization solution was added to each well to dissolve the formazan crystals. Absorbance was read at 570 nm. Octuplet wells were assayed for all tested compounds or extracts. Linear regression analysis was used to determine IC_{50} values.

Flow Cytometry. The flow cytometry procedure is described in detail by Darzynkiewicz et al.[31] Briefly, culture media containing the floating cells were harvested. Remaining adherent cells were trypsinized and harvested as well. The cells were then washed twice with PBS and fixed with 70% EtOH chilled at −20 °C and kept in 70% EtOH overnight at −20 °C. Before analysis, cells were washed a third time with PBS, resuspended, and incubated for 30 min in a staining solution containing 0.05 mg/mL propidium iodide and 1 mg/mL RNAse A in PBS. The cells in the suspensions were then analyzed on a flow cytometer. At least 10 000 cells were gated for analysis by flow cytometry. Data were plotted on FL2-A histograms, and sub-diploid cells were considered apoptotic. Cell cycle analysis was performed using the FlowJo software version 6.0. Apoptosis is expressed as percentage of sub-diploid cells out of all analyzed cells. Cell cycle distribution is expressed as percentage of analyzed cells in G1, S, or G2/M phase of the cell cycle.

Argentation TLC. Silica gel plates were soaked in a 10% ethanolic solution of $AgNO_3$ for 5 min and dried overnight in a 50 °C oven protected from light. The benzophenone mixture (ca. 0.8 mg) was applied to the TLC plate and developed using a solvent system of hexane−EtOAc−95% EtOH−TFA (40:10:1.25:0.2). Compounds were scraped from the silica gel plates and eluted with MeOH. To remove the Ag^+ ions, the MeOH/compound mix was concentrated in vacuo to ca. 5 mL and diluted with 95 mL of water. This mixture was partitioned twice with EtOAc. The EtOAc layers were combined and extracted three times with 10% NaCl and three times with H_2O. This procedure was adapted from Lima et al.[32] and Momchilova et al.[33]

Extraction and Isolation. *G. xanthochymus* fruit pulp (13 kg) was extracted exhaustively with MeOH and concentrated in vacuo to yield 310 g of dark green residue. This residue was resuspended in H_2O and partitioned sequentially with $CHCl_3$ and EtOAc. The $CHCl_3$ and EtOAc partitions were concentrated in vacuo to give 4.91 and 4.21 g of residue, respectively.

The $CHCl_3$ partition (4.91 g) was chromatographed over Sephadex LH-20 (200 × 2.5 cm) and eluted with MeOH. Seven combined fractions, A−G, were obtained by RP_{18} TLC analysis (1:1 or 15:85 10 mM ammonium acetate−MeCN).

Fraction C (2.5 g) was separated by reversed-phase column chromatography (CC) (200 g; 4:6−0:1, H_2O−MeCN, 5% steps), and fractions were combined by RP_{18} TLC analysis (1:1 or 15:85 10 mM ammonium acetate−MeCN) to afford six combined subfractions, A2−F2.

Subfraction C2 (56 mg) was repeatedly chromatographed over Sephadex LH-20 (65 × 1.0 cm) and eluted with MeOH to yield **3** (2.1 mg). Subfraction E2 (130 mg) was rechromatographed over reversed-phase CC (100 g; 3:7−0:1, H_2O−MeCN, 5% steps) and recombined into six subfractions by reversed-phase HPLC to yield subfraction E3 (63 mg), a mixture of **6** and **7**. Similarly, subfraction F2 (1.12 g) was repeatedly chromatographed over reversed-phase CC (100 g; 15:85−0:1, H_2O−MeCN, 5% steps) to yield **2** (13.5 mg) and subfraction F3 (900 mg), a mixture of **4** and **5**.

Despite repeated attempts to separate **4** from **5** and **6** from **7** by normal- and reversed-phase preparative TLC; Sephadex LH-20, reversed- and normal-phase, polyamide, and cyano column chromatography; plus HPLC using C_{18}, C_8, cyano,

phenyl, and silica columns, **4** and **5** plus **6** and **7** remained mixtures. Other researchers have encountered difficulties in separating benzophenone double-bond mixtures.[18,25,34] After a protracted method development using various types of argentation chromatography, compounds **4**−**7** were isolated by argentation TLC. Ag-TLC yielded **6** (2.3 mg) and **7** (2.2 mg) from subfraction E3 and **4** (21.2 mg) and **5** (22.4 mg) from subfraction F3.

Fraction D (340 mg) was purified over C_{18} CC (100 g; 6:4−0:1, H_2O−MeOH, 10% steps) to yield **1** (38 mg). Fraction G (70 mg) was separated over C_{18} CC (20 g; 8:2−0:1, H_2O−MeOH, 5% steps), and collected fractions were recombined based on RP_{18} TLC (1:1 10 mM ammonium acetate−MeCN) into two subfractions, A1 and B1. Subfraction A1 (17 mg) was purified by preparative HPLC (45:55 H_2O−MeOH, 5 mL/min) to yield **8** (3.0 mg).

The EtOAc partition (4.21 g) was chromatographed over Sephadex LH-20 (200 × 2.5 cm) and eluted with MeOH. Fractions were combined using RP_{18} TLC (1:1 10 mM ammonium acetate−MeCN) analysis to give 13 fractions, A−M. Fraction G (200 mg) was chromatographed over Sephadex LH-20 (200 × 2.5 cm) and eluted with MeOH to yield **12** (42.3 mg). Fraction H (40.4 mg) was separated by preparative HPLC (65:35 H_2O−MeCN, 10 mL/min) to yield **10** (17.5 mg). Fraction J (340 mg) was chromatographed over Sephadex LH-20 (200 × 2.5 cm) and eluted with MeOH, yielding **11** (86.3 mg) and subfraction J1 (155 mg), a mixture of **11** and **13**. Subfraction J1 (137 mg) was repeatedly separated by preparative HPLC (65:35 H_2O−MeCN, 10 mL/min) to yield **13** (9.8 mg). Fraction M (50 mg) was chromatographed over Sephadex LH-20 (30 × 1.5 cm) to yield **9** (23.0 mg).

Guttiferone H (1): yellow oil; $[\alpha]_D$ +94° (*c* 0.0061, $CHCl_3$); $[\alpha]_D$ +57° (*c* 0.0061, MeOH); UV (MeOH) λ_{max} (log ϵ) 278 (2.40) and 229 (1.87) nm; ^1H and ^{13}C NMR data, see Table 1; negative ESIMS *m/z* 601 $[M - H]^-$; positive HRESIMS *m/z* $[M + H]^+$ 603.3672 (calcd for $C_{38}H_{50}O_6$ + H, 603.3686).

Gambogenone (2): yellow oil; $[\alpha]_D$ −5° (*c* 0.0034, MeOH); UV (MeOH) λ_{max} (log ϵ) 281 (2.88) 322 (2.58) nm; ^1H and ^{13}C NMR data, see Table 2; negative ESIMS *m/z* 451 $[M - H]^-$; positive HRESIMS *m/z* $[M + H]^+$ 453.2294 (calcd for $C_{27}H_{32}O_6$ + H, 453.2277).

Aristophenone A (3): light yellow oil; negative ESIMS *m/z* 533 $[M - H]^-$; ^1H and ^{13}C NMR data are consistent with published data.[35]

Xanthochymol (4): yellow oil; negative ESIMS *m/z* 601 $[M - H]^-$; ^1H and ^{13}C NMR data and UV data are consistent with published data.[6,25] The identification was further supported by HSQC and HMBC experiments.

Guttiferone E (5): yellow oil; negative ESIMS *m/z* 601 $[M - H]^-$; $[\alpha]_D$ +106° (*c* 0.0065, $CHCl_3$); $[\alpha]_D$ +120° (*c* 0.0065, MeOH); ^1H and ^{13}C NMR data and UV data are consistent with published data.[18]

Cycloxanthochymol (6): yellow oil; negative ESIMS *m/z* 601 $[M - H]^-$; ^1H and ^{13}C NMR data are consistent with published data.[34]

Isoxanthochymol (7): yellow oil; negative ESIMS *m/z* 601 $[M - H]^-$; ^1H and ^{13}C NMR data are consistent with published data.[18]

Alloathyriol (8): light yellow powder; negative ESIMS *m/z* 273 $[M - H]^-$; ^1H and NOE NMR data and UV spectrum are consistent with published data.[36]

Amentoflavone (9): light yellow powder; negative ESIMS *m/z* 537 $[M - H]^-$; ^1H and ^{13}C NMR data are consistent with published data.[37−39] The identification was further supported by comparison of ^1H NMR data and HPLC analysis (65:35 10 mM ammonium acetate−MeCN) with purchased standard (Indofine Chemical Company, Hillsborough, NJ).

3,8″-Biapigenin (10): light brown powder; negative ESIMS *m/z* 537 $[M - H]^-$; ^1H and ^{13}C NMR data and UV spectrum are consistent with published data.[40]

(±)-Fukugetin (11): light yellow powder; negative ESIMS *m/z* 555 $[M - H]^-$; ^1H and ^{13}C NMR data are consistent with published data.[41,42]

(±)-Fukugiside (12): red-brown powder; negative ESIMS *m/z* 717 $[M - H]^-$; ^1H and ^{13}C NMR data are consistent with published data.[43]

(±)-Volkensiflavone (13): light brown powder; negative ESIMS *m/z* 539 $[M - H]^-$; ^1H and ^{13}C NMR data and UV spectrum are consistent with published data.[8,37,44]

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