First Total Synthesis of Protoapigenone and Its Analogues as Potent Cytotoxic Agents

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Protoapigenone (**1**), isolated from *Thelypteris torresiana*, previously showed significant cytotoxic activity against five human cancer cell lines. In a continued structure−activity relationship study, the first total synthesis and modification of **1** were achieved. All synthesized compounds and related intermediates were evaluated for cytotoxic activity against five human cancer cell lines, HepG2, Hep3B, MDA-MB-231, MCF-7, and A549. Among them, **24** showed 2.2−14.2-fold greater cytotoxicity than **1** and naphthyl A-ring analogues remarkably enhanced the activity.

Introduction

Flavonoids are plant pigments that generally display marvelous colors and are ubiquitous to green plants. Their multiple bioactivities (leading to the term bioflavonoid) and medicinal significance have been summarized recently.[1,2] In our previous study, we isolated the unique flavonoid protoapigenone (**1**) from *Thelypteris torresiana* (Gaud.) Alston, which showed potent cytotoxic activity against HepG2 and Hep3B (liver), MDA-MB-231 and MCF-7 (breast), and A549 (lung) human cancer cell lines with IC_{50} values of 0.94−5.59 μM.[3] In our subsequent study, this compound showed 7.5- and 4.6-fold greater cytotoxic activity against two human breast cancer cell lines MCF-7 and MDA-MB-231 compared with the MCF-10A normal human breast epithelial cell line.[4]

Flavonoid **1** has an unusual nonaromatic B-ring with a hydroxy group on the C-1′ position, which might arise from oxidation of a 4′-hydroxyphenyl. Apigenin (**2**), which is the conceivable biosynthetic precursor of **1**, kaempferol (**3**), and quercetin (**4**) are well-known flavonoids bearing a 4′-hydroxyphenyl B-ring (Figure 1). These three compounds showed no antiproliferative activity (IC_{50} > 20 μg/ mL) against six human cancer cell lines,[5] while **1** displayed significant cytotoxic activity. This unique difference spurred our interest in the characteristics of the 1′-hydroxycyclohexa-2′,5′-dien-4′-one B-ring in this flavonoid skeleton. Although the antitumor mechanism of this functional group is still not clear, recent studies on some quinol derivatives showed that the oxidized species are more active than their reduced counterparts.[6−9]

To investigate a new class of anticancer agents based on this novel plant-derived natural flavonoid, the first total synthesis of **1** and the preparation of some analogues were accomplished. All newly synthesized compounds including structurally related intermediates were assayed for *in vitro* cytotoxic activity against five human cancer cell lines, e.g., HepG2, Hep3B, MDA-MB-231, MCF-7, and A549. In this paper, we describe the synthesis, bioactivity data, and preliminary structure−activity relationship (SAR) studies related to **1**.

Chemistry. Our initial attempt to obtain **1** from **2** by oxidation failed because of oxidation of the 5- and 7-hydroxy groups. Because of the difficulty of selectively protecting these two groups on **2** in the presence of the 4′-hydroxyl, diprotected trihydroxyacetophenones (**6** and **7**)[10] (commercially available) were selected as starting materials.

Our successful flavonoid synthesis started with a Claisen−Schmidt condensation carried out individually on **6** and **7** with 4-benzyloxybenzaldehyde in the presence of aqueous potassium hydroxide to provide **8** (87%) and **9** (79%).[11] Chalcone **9** was further cyclized with a catalytic amount of iodine in DMSO to give the corresponding 4′-benzyloxyflavonoid **11** (74%).[11] Similar cyclization of **8** gave only a low yield of the expected product. Instead, the use of pyridine as solvent caused an unexpected cleavage of the MOM[a] (methoxymethyl) ether from the 5-position, along with cyclization, to produce **10** (86%). The benzyl groups of **10** and **11** were removed by treatment with catalytic 10% palladium carbon under H_2 to afford 4′-hydroxyflavonoids **12** (86%) and **13** (83%).[12] Oxidation of **12** and **13** with [bis(trifluoroacetoxy)iodo]benzene (TAIB) in acetonitrile/H_2O at room-temperature gave the novel flavonoids **14** (22%) and **15** (33%).[13,14] The 5-hydroxy group was unaffected under these conditions because of strong intermolecular hydrogen bonding with the carbonyl group on the 4-position. When MeOH rather than acetonitrile/H_2O was used as solvent, trimethoxyprotoapigenone (**16**) (33%) was produced from **13**. Finally, **1** was obtained by cleavage of the MOM group on **14** using 15% HCl in *i*-PrOH (yield 47%),[10,12] while the methoxy groups on **15** were not cleaved using 47% HBr/HOAc at reflux as the demethylation condition (Scheme 1).[15,16]

In addition, five commercially available 4′-hydroxyflavonoids (**17**, **18**, **19**, **25**, and **28**) were oxidized with TAIB using acetonitrile/H_2O or methanol as the solvent to obtain the related quinol derivatives, **20** (36%) and **21** (28%) from **17**, **22** (16%) and **23** (15%) from **18**, **24** (20%) from **19**, **26** (16%) and **27** (15%) from **25**, and **29** (23%) from **28** (Scheme 2).[13,14] Accordingly, the first total synthesis of **1** as well as the synthesis of twelve A-ring and C-1′ analogues, **14**−**16**, **20**−**24**, **26**, **27**, and **29**, were accomplished.

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[a] Abbreviations: TAIB, [bis(trifluoroacetoxy)iodo]benzene; MOM, methoxymethyl.

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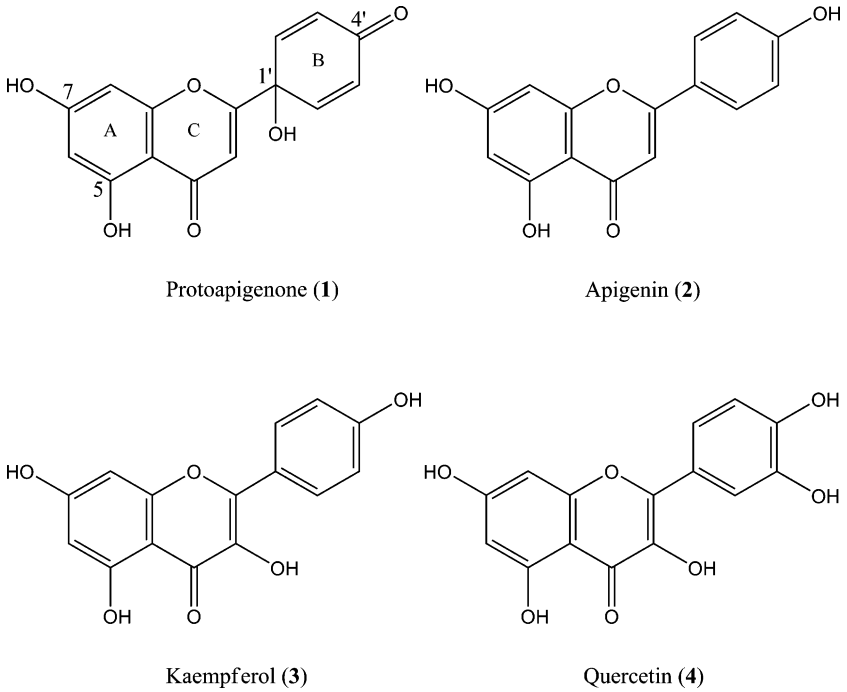


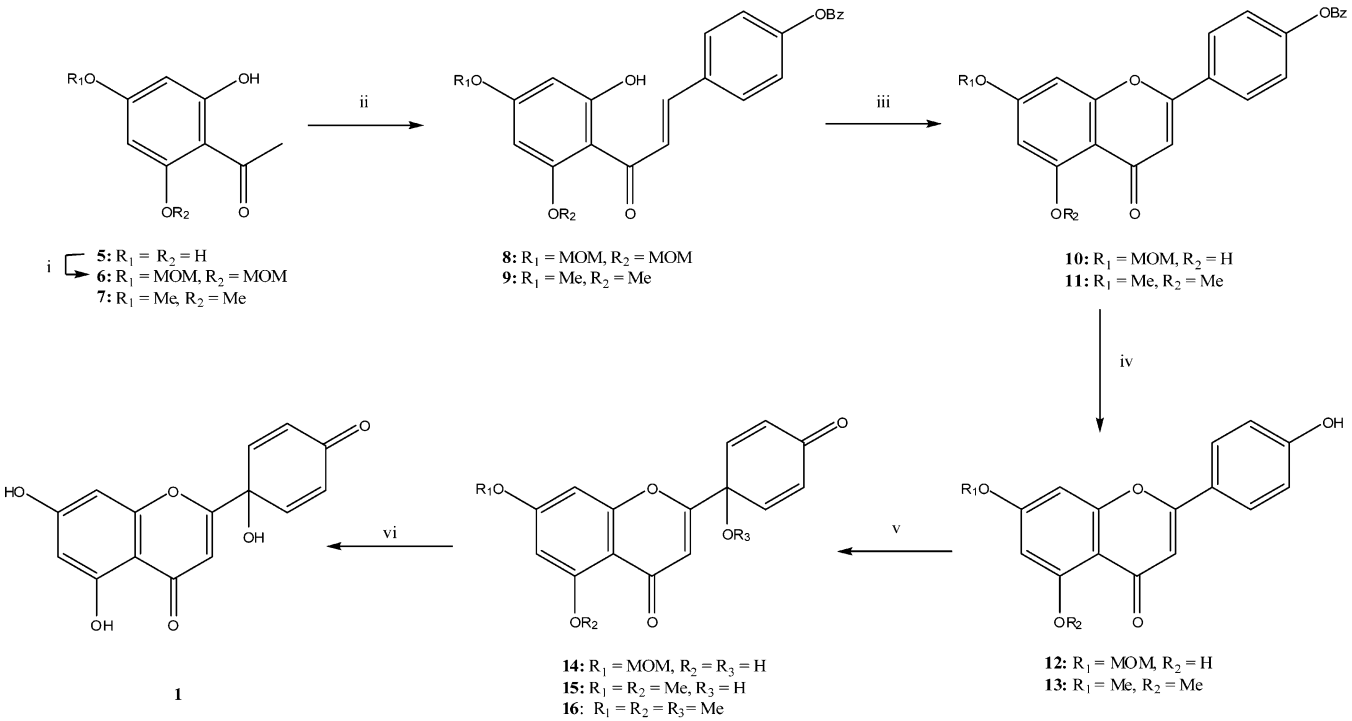
Figure 1. Chemical structures of **1** and related flavonoids.

Results and Discussion

Together with **1,** newly synthesized flavonoids **14**−**16**, **20**−**24**, **26**, **27**, and **29** were examined for *in vitro* cytotoxic activities against several human cancer lines, including HepG2, Hep3B, MDA-MB-231, MCF-7, and A549. Table 1 lists the IC_{50} values obtained with these compounds as well as the related flavonoids protoapigenin (**30**) and 5′,6′-dihydro-6′-methoxyprotoapigenone (**31**) (Figure 2), as well as doxorubicin, included as a positive control.

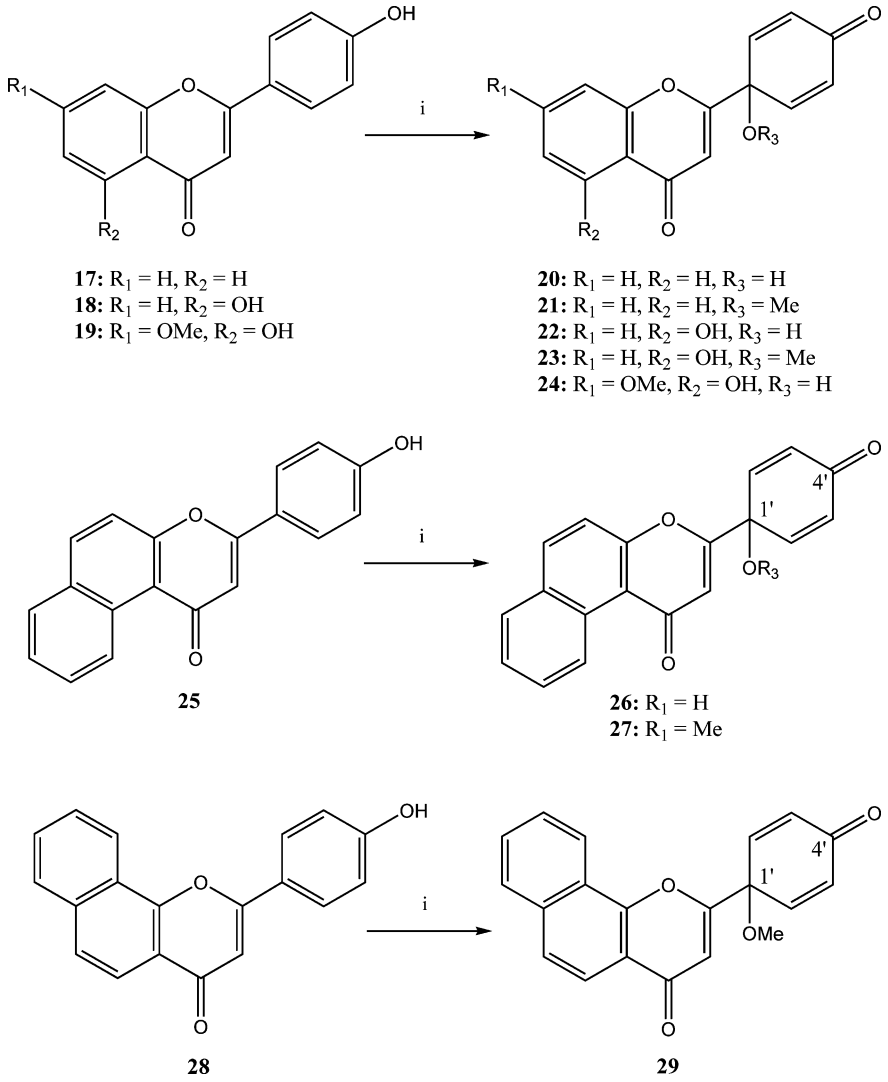
The parent compound **1** showed potent cytotoxic activity against the tested human cancer cell lines. Comparison of **1** to its synthetic analogues **14**−**16** showed that **14**, with a MOM ether at C-7, had comparable or reduced activity compared with **1**, and trimethoxy **16** was uniformly less active. However, 5,7-dimethoxy compound **15** showed enhanced cytotoxic activity with notable IC_{50} values of 0.54−1.27 μM against four cell lines (Hep3B, MDA-MB-231, MCF-7, and A549). Remarkably, 1′-hydroxy analogues **15**, **20**, **22**, and **26** were 1.6 to >48.0 times

Scheme 1. Total Synthesis of **1** and Its Analogues[a]



[a] Reagents: (i) K_2CO_3, MOMCl, acetone, (ii) EtOH, 50% KOH/H_2O, 4-benzyloxybenzaldehyde; (iii) I_2, DMSO or pyridine; (iv) 10% Pd−C/H_2; (v) TAIB (1 equiv), CH_3CN/H_2O 9/1 (**12**→**14**, **13**→**15**) or MeOH (**13**→**16**), 25 °C; (vi) 15% HCl/iPrOH (**14**→**1**).

Scheme 2. Synthesis of Flavonoid and Naphthoflavone Analogues with Quinol B-Ring[a]



[a] Reagent: (i) TAIB (1 equiv), CH_3CN/H_2O 9/1 or MeOH, 25 °C.

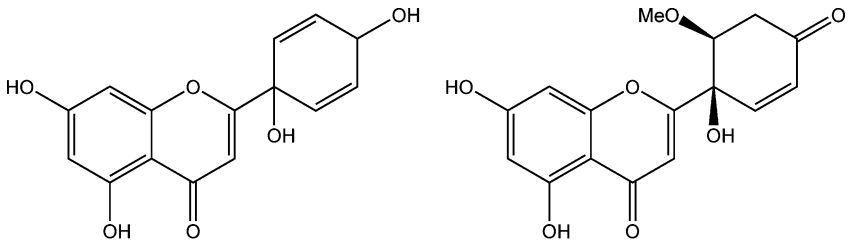
Table 1. Cytotoxic Effects of Synthesized **1** and Analogues

	IC_{50} (μM)[a]				
compound	HepG2	Hep3B	MDA-MB-231	MCF-7	A549
1	8.11 ± 0.01	2.27 ± 0.01	1.43 ± 0.00	3.74 ± 0.02	13.85 ± 0.01
14	15.58 ± 0.08	2.39 ± 0.00	1.73 ± 0.00	5.24 ± 0.01	24.61 ± 0.02
15	5.03 ± 0.01	0.67 ± 0.01	0.54 ± 0.01	1.21 ± 0.01	1.27 ± 0.03
16	27.01 ± 0.01	3.78 ± 0.00	4.70 ± 0.01	4.21 ± 0.04	>60.98
20	6.73 ± 0.01	1.26 ± 0.02	0.71 ± 0.00	1.73 ± 0.01	5.24 ± 0.05
21	40.86 ± 0.08	5.41 ± 0.00	5.49 ± 0.01	7.99 ± 0.03	>74.63
22	4.96 ± 0.02	1.30 ± 0.01	0.67 ± 0.00	3.44 ± 0.04	5.07 ± 0.03
23	26.90 ± 0.01	2.32 ± 0.04	1.90 ± 0.02	5.46 ± 0.06	57.61 ± 0.31
24	0.57 ± 0.02	0.67 ± 0.00	0.43 ± 0.00	1.70 ± 0.06	3.10 ± 0.08
26	3.55 ± 0.04	0.30 ± 0.00	0.39 ± 0.00	0.66 ± 0.00	1.81 ± 0.00
27	9.94 ± 0.10	1.10 ± 0.01	1.16 ± 0.01	2.70 ± 0.01	25.79 ± 0.05
29	14.37 ± 0.03	0.60 ± 0.01	1.07 ± 0.01	2.96 ± 0.08	28.33 ± 0.07
30[b]	5.56 ± 0.33	69.44 ± 0.58	>69.44	>69.44	65.42 ± 0.60
31[b]	18.49 ± 0.47	5.47 ± 0.08	4.09 ± 0.10	18.62 ± 0.29	41.82 ± 0.23
doxorubicin[c]	0.50 ± 0.04	0.62 ± 0.03	0.14 ± 0.01	0.74 ± 0.00	0.36 ± 0.02

[a] Data are expressed as mean ± SD (mean = 2). [b] Data from our previous study. [c] Positive control.

more active than the related 1′-methoxy analogues, **16**, **21**, **23**, and **27**. These findings indicate the importance of a nonsubstituted OH at the C-1′ position.

Among the quinol derivatives, changing the C-5 substituent from hydrogen to methoxy (**20** vs **22**, **21** vs **23**) did not affect potency, while variation in the C-7 functional group did exert an influence. For example, compound **24** with a C-7 methoxy group showed the highest potency against all five cancer cell lines with IC_{50} values of 0.43−3.10 μM. Amazingly, this compound also exhibited significantly enhanced activity against the HepG2 human liver cancer cell line with an IC_{50} value of 0.57 μM and consequently was 14.2 times more active than **1**, while most analogues showed comparatively weak activity against this cell line. Compound **22**, which lacks a C-7 methoxy



30 **31**

Figure 2. Structures of **1**-related cytotoxic flavonoids.

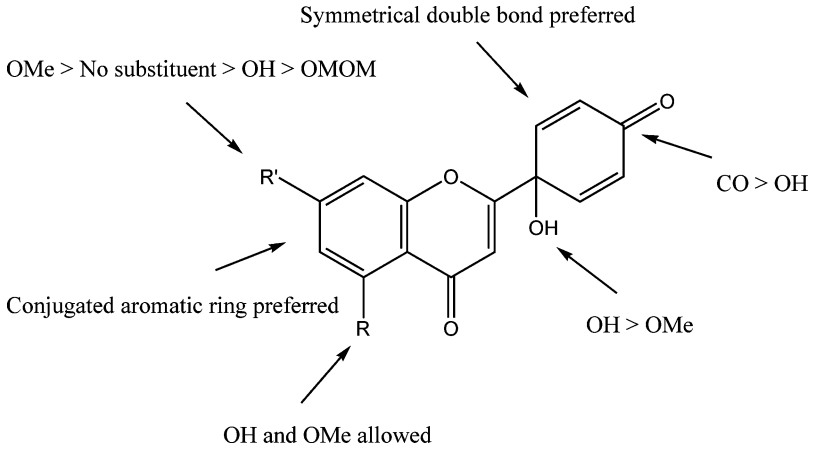


Figure 3. SAR in newly synthesized cytotoxic flavonoids.

group, was generally less active than **24**, but was about 2-fold more active than **1** against all cell lines. In particular, growth inhibition of Hep3B (IC_{50} = 1.30 μM) and MDA-MB-231 (IC_{50} = 0.67 μM) was significantly increased.

Naphthoflavones **26**, **27**, and **29**, which have an additional conjugated aromatic ring attached to the A-ring, showed better activity compared with **1** against the three cell lines Hep3B, MDA-MB-231, and MCF-7. Particularly, analogue **26** was more potent than doxorubicin against the Hep3B cell line with an IC_{50} value of 0.30 μM. In addition, this compound was 7.7 times more potent than **1** against A549 cell growth with an IC_{50} value of 1.81 μM.

Our prior report stated that the 4′-oxocyclohexa-2′,5′-dienyl moiety as found in **1** is critically important for biological activity in flavonoids.[3] Accordingly, we found in this study that the natural product **30**, which instead contains a 4′-hydroxycyclohexa-2′,5′-dienyl moiety, was active against only the HepG2 cell line. However, corresponding synthetic 1′-hydroxyquinol analogues **20**, **22**, and **24**, with different combinations of hydrogen, hydroxy, and methoxy substituents rather than two hydroxy groups on the A-ring, showed significant cytotoxic activity. In addition, we previously found that **31**, which has only a single double bond in the B-ring, exhibited lower cytotoxic activity; thus, a symmetrical double bond structure in the B-ring might also be important.[3] The summary of this preliminary SAR study is shown in Figure 3.

In conclusion, among all screened compounds, the 1′-hydroxycyclohexa-2′,5′-dien-4′-one B-ring is critically important in the bioflavonoid skeleton, but substituent changes on the A-ring can affect selectivity or potency against different kinds of cancer cell lines. A methoxy group on the C-7 position can enhance cytotoxic selectivity toward the human HepG2 liver cancer cell line, and an additional conjugated aromatic ring on the A-ring increases cytotoxicity against all five tested human cancer cell lines. These prototype analogues of **1** have demonstrated valuable growth inhibition of selected human cancer cell lines. In addition, our preliminary data (unpublished) indicate that treatment of MDA-MB-231 with **1** leads to cell cycle arrest at the G2/M phase and apoptosis; the precise mechanisms responsible for these effects are currently under investigation. We are synthesizing additional candidate structures that will address specific cancer cell lines.

Experimental Section

Materials and Methods. Melting points were measured with a Fisher−John melting apparatus without correction. ^{1}H NMR spectra were measured on a 300 MHz Varian Gemini 2000 spectrometer. The solvent used were $CDCl_3$ or pyridine-d_5. Mass spectra were measured on a PECIEX API 3000 instrument with turbo ion spray source, Agilent-1100, LC/MSD-Trap, or Shimadzu LCMS-IT-TOF with ESI interface. Thin-layer chromatography (TLC) and preparative TLC were performed on precoated silica GF plates purchased from Merck, Inc. Biotage Flash+ or Isco Companion systems were used for flash chromatography. Silica gel (200−400 mesh) from Aldrich, Inc. was used for column chromatography. Flavonoids were obtained from Indofine Chemical Company, Inc. All other chemicals were obtained from Aldrich, Inc.

Cell Viability Assays. Human breast (MCF-7 and MDA-MB-231), liver (HepG2 and Hep3B), and lung (A549) cancer cell lines were obtained from American Type Culture Collection. All cell lines were propagated in RPMI-1640 medium supplement with 10% (v/v) FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Cell viability was measured by the MTT colorimetric method. Cells were seeded at densities of 5000−10000 cells/well in 96-well tissue culture plates. On day two, cells were treated with test compounds for another 72 h. After drug treatment, attached cells were incubated with MTT (0.5 mg/mL, 1 h) and subsequently solubilized in DMSO. The absorbency at 550 nm was then measured using a microplate reader. The IC_{50} is the concentration of agent that reduced the cell viability by 50% under the experimental conditions.[3,17]

Protoapigenone (1). Compound **14** (30 mg, 0.09 mmol) was dissolved in 15% HCl/iPrOH (1:5, 5 mL), and the reaction mixture was stirred at the room temperature for 140 min. The solvent was evaporated and the residue chromatographed using silica gel (isocratic elution, 33% MeOH/67% $CHCl_3$) to give **1** (12.0 mg, 47%) as a yellow solid: ESI-MS (*m/z*, %): 285 (M^- − 1, 100); ^{1}H NMR (pyridine-d_5) δ 13.38 (1H, br s, OH-5), 7.24 (2H, d, J = 10.2 Hz, H-2′, H-6′), 7.07 (1H, s, H-3), 6.72 (1H, d, J = 2.4 Hz, H-8), 6.60 (1H, d, J = 2.4 Hz, H-6), 6.54 (2H, d, J = 10.2 Hz, H-3′, H-5′); ^{13}C NMR (pyridine-d_5) δ 185.2 (C-4′), 182.9 (C-4), 167.8 (C-2), 166.3 (C-7), 163.1 (C-5), 158.7 (C-9), 148.4 (C-2′, C-6′), 129.5 (C-3′, C-5′), 107.4 (C-3), 105.1 (C-10), 100.2 (C-6), 95.0 (C-8), 69.7 (C-1′).

1-[2-Hydroxy-4,6-bis(methoxymethoxy)phenyl]ethanone (6). 2′,4′,6′-Trihydroxyacetophenone monohydrate (**5**) (2.0 g, 11.9 mmol) and K_2CO_3 (11.5 g) were dissolved in anhydrous acetone (80 mL). Chloromethyl methyl ether (MOMCl, 2.4 g) was added dropwise to the stirring solution, and then the suspension was refluxed for 90 min. After cooling, the solid was filtered off, and the crude product was evaporated and purified by column chromatography on silica gel (isocratic elution, 90% *n*-hexane/10% EtOAc) to afford **6** (1.5 g, 50%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 13.72 (1H, br s, OH-2), 6.25 (1H, d, J = 2.4 Hz, H-5), 6.23 (1H, d, J = 2.4 Hz, H-3), 5.24, 5.16 (2H each, s, CH_2-MOM), 3.51, 3.46 (3H each, s, OMe-MOM), 2.64 (1H, s, $COCH_3$); ^{13}C NMR (pyridine-d_5) δ 203.2 (CO), 166.8 (C-2), 163.4 (C-4), 160.3(C-6), 106.9 (C-1), 97.1 (C-3), 94.4 (C-5), 93.9 (CH_2-MOM, overlapping), 56.6, 56.4 (OMe-MOM), 33.0 ($COCH_3$).

3-[4-(Benzyloxy)phenyl]-1-[2-hydroxy-4,6-bis(methoxymethoxy)phenyl]prop-2-en-1-one (8). Compound **6** (1.8 g, 7.1 mmol) and 4-benzyloxybenzaldehyde (3.0 g, 14.3 mmol) were dissolved in 50% EtOH, KOH/H_2O solution (20 mL). The reaction was stirred at rt for 30 h, and then the solvent was evaporated under reduced pressure. The mixture was chromatographed on silica gel (isocratic elution, *n*-hexane/EtOAc, 6:1) to afford **8** (2.8 g, 87%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 13.95 (1H, br s, OH-2′), 7.84 (1H, d, J = 15.6 Hz, H-β), 7.78 (1H, d, J = 15.6 Hz, H-α), 7.56 (2H, d, J = 8.7 Hz, H-2, H-6), 7.32−7.46 (5H, m, H-OBz), 7.01 (2H, d, J = 8.7 Hz, H-3, H-5), 6.32 (1H, d, J = 2.4 Hz, H-5′), 6.25 (1H, d, J = 2.4 Hz, H-3′), 5.29, 5.19 (2H each, s, CH_2-MOM), 5.11 (2H, s, CH_2-OBz), 3.54, 3.49 (3H each, s, OMe-MOM); ^{13}C NMR ($CDCl_3$) δ 192.8 (CO), 167.3 (C-4′), 163.3 (C-2′), 160.6 (C-6′), 159.8 (C-4), 142.6 (C-β), 136.4 (C-1-OBz), 130.1 (C-2, C-6), 128.6 (C-3-OBz, C-5-OBz), 128.4 (C-1), 128.1 (C-α), 127.4 (C-2-OBz, C-6-OBz), 125.0 (C-4-OBz), 115.3 (C-3, C-5), 107.5 (C-1′), 97.5 (C-5′), 95.1 (C-3′), 94.7, 94.0 (CH_2-MOM), 70.1 (CH_2-OBz), 56.8, 56.4 (OMe-MOM).

3-[4-(Benzyloxy)phenyl]-1-(2-hydroxy-4,6-dimethoxyphenyl)-prop-2-en-1-one (9). Compound **7** (1.0 g, 5.1 mmol) was reacted with 4-benzyloxybenzaldehyde (2.2 g, 10.2 mmol) as described above for **6** to afford **9** (1.6 g, 79%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 13.87 (1H, br s, OH-2′), 7.82 (1H, d, J = 15.6 Hz, H-β), 7.77 (1H, d, J = 15.6 Hz, H-α), 7.56 (2H, d, J = 9.0 Hz, H-2, H-6), 7.32−7.46 (5H, m, H-OBz), 7.00 (2H, d, J = 9.0 Hz, H-3, H-5), 6.11 (1H, d, J = 2.4 Hz, H-5′), 5.96 (1H, d, J = 2.4 Hz, H-3′), 5.11 (2H, s, CH_2-OBz), 3.92, 3.83 (3H each, s, OMe); ^{13}C NMR ($CDCl_3$) δ 192.6 (CO), 168.4 (C-4′), 166.0 (C-2′), 162.4 (C-6′), 160.5 (C-4), 142.4 (C-β), 136.5 (C-1-OBz), 130.1 (C-2, C-6), 128.7 (C-3-OBz, C-5-OBz), 128.5 (C-1), 128.1 (C-α), 127.5 (C-2-OBz, C-6-OBz), 125.2 (C-4-OBz), 115.2 (C-3, C-5), 106.3 (C-1′), 93.8 (C-5′), 91.2 (C-3′), 70.1 (CH_2-OBz), 55.8, 55.6 (OMe).

2-[4-(Benzyloxy)phenyl]-5-hydroxy-7-(methoxymethoxy)-chromen-4-one (10). Compound **8** (500 mg, 1.1 mmol) was dissolved anhydrous pyridine (5 mL), and a catalytic amount of iodine (28.4 mg, 0.1 mmol) was added. The solution mixture was stirred and refluxed. After 3 h, 10% $Na_2S_2O_3$ (30 mL) was added to remove iodine. The mixture was extracted with EtOAc (50 mL) and then purified by column chromatography on silica gel (isocratic elution, EtOAc only) to give **10** (380 mg, 86%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 12.77 (1H, br s, OH-5), 7.81 (2H, d, J = 8.7 Hz, H-2′, H-6′), 7.32−7.46 (5H, m, H−OBz), 7.06 (2H, d, J = 8.7 Hz, H-3′, H-5′), 6.64 (1H, d, J = 2.1 Hz, H-8), 6.55 (1H, s, H-3), 6.46 (1H, d, J = 2.1 Hz, H-6), 5.23 (2H, s, CH_2-OMOM), 5.13 (2H, s, CH_2-OBz), 3.50 (3H, s, OMe); ^{13}C NMR ($CDCl_3$) δ 182.4 (C-4), 163.9 (C-7), 162.8 (C-2), 161.9 (C-5), 161.7 (C-9), 157.4 (C-4′), 136.1 (C-1-OBz), 128.7 (C-2, C-6), 128.2 (C-4-OBz), 128.0 (C-2-OBz, C-6-OBz), 127.4 (C-3-OBz, C-5-OBz), 123.6 (C-1′), 115.3 (C-3, C-5), 106.1 (C-10), 104.3 (C-3), 100.0 (C-6), 94.2 (C-8), 94.2 (CH_2-MOM), 70.1 (CH_2-OBz), 56.3 (OMe).

2-[4-(Benzyloxy)phenyl]-5,7-dimethoxychromen-4-one (11). Compound **9** (1.6 g, 4.0 mmol) was treated analogously as **8** to give **11** (1.1 g, 74%) as a yellow solid: ^{1}H NMR (pyridine-d_5) δ 7.95 (2H, d, J = 9.0 Hz, H-2′, H-6′), 7.32−7.58 (5H, m, H-OBz), 7.23 (2H, d, J = 9.0 Hz, H-3′, H-5′), 6.96 (1H, s, H-3), 6.83 (1H, d, J = 2.4 Hz, H-8), 6.57 (1H, d, J = 2.4 Hz, H-6), 5.19 (2H, s, CH_2−OBz), 3.85 (6H, s, OMe); ^{13}C NMR ($CDCl_3$) δ 176.6 (C-4), 164.3 (C-7), 161.6 (C-2), 161.3 (C-5), 160.5 (C-4′), 160.2 (C-9), 137.2 (C-1-OBz), 129.0 (C-3-OBz, C-5-OBz), 128.5 (C-4-OBz), 128.1 (C-2-OBz, C-6-OBz), 128.1 (C-2, C-6), 124.5 (C-1′), 115.6 (C-3, C-5), 108.2 (C-10), 109.8 (C-3), 96.7 (C-6), 93.7 (C-8), 70.3 (CH_2-OBz), 56.2, 55.9 (OMe).

General Procedure for Benzyl Group Removal. Synthesis of Compounds 12 and 13. A mixture of **10** (200 mg, 0.5 mmol), dry Pd/C (10%, 106 mg), and EtOAc/ MeOH (1:1, 15 mL) was stirred at 25 °C under an atmosphere of hydrogen for 3 h. The mixture was filtered through a layer of silica gel on cotton, washed with EtOAc, concentrated under vacuum, and purified by column chromatography on silica gel (isocratic elution, EtOAc only) to give **12** (134 mg) as a yellow solid.

5-Hydroxy-2-(4-hydroxyphenyl)-7-(methoxymethoxy)chromen-4-one (12). 86% yield; ^{1}H NMR (pyridine-d_5) 7.93 (2H, d, J = 8.7 Hz, H-2′, H-6′), 7.27 (2H, d, J = 8.7 Hz, H-3′, H-5′), 6.96 (1H, s, H-3), 6.91 (1H, d, J = 2.1 Hz, H-8), 6.78 (1H, d, J = 2.1 Hz, H-6), 5.35 (2H, s, CH_2-OMOM), 3.45 (3H, s, OMe); ^{13}C NMR ($CDCl_3$) δ 182.4 (C-4), 164.4 (C-7), 162.8 (C-2), 163.4 (C-5), 162.1 (C-9), 157.4 (C-4′), 128.5 (C-2, C-6), 121.5 (C-1′), 116.4 (C-3, C-5), 106.0 (C-10), 103.6 (C-3), 99.7 (C-6), 94.3 (C-8), 94.2 (CH_2-MOM), 55.8 (OMe).

2-(4-Hydroxyphenyl)-5,7-dimethoxychromen-4-one (13). 83% yield; ^{1}H NMR (pyridine-d_5) δ 7.93 (2H, d, J = 9.0 Hz, H-2′, H-6′), 7.27 (2H, d, J = 9.0 Hz, H-3′, H-5′), 6.95 (1H, s, H-3), 6.82 (1H, d, J = 2.4 Hz, H-8), 6.56 (1H, d, J = 2.4 Hz, H-6), 3.84 (6H, s, OMe); ^{13}C NMR (pyridine-d_5) δ 176.7 (C-4), 164.2 (C-7), 162.1 (C-2), 161.3 (C-5), 161.1 (C-4′), 160.2 (C-9), 128.4 (C-2′, C-6′), 122.6 (C-1′), 116.8 (C-3′, C-5′), 109.7 (C-10), 107.5 (C-3), 96.7 (C-6), 93.7 (C-8), 56.2, 55.9 (OMe).

General Procedure for Oxidation Reaction with TAIB. Synthesis of Compounds 14−16, 20−24, 26, 27, and 29. The precusor flavonoid [for example, **12** (90 mg)] was dissolved in acetonitrile/H_2O (9:1) or MeOH (5 mL) individually, and a catalytic amount of TEMPO (0.2 eq) was added to the flask, followed by 2 equiv of TAIB. The reaction mixture was stirred vigorously at 25 °C for 90 min. The reaction mixture was then evaporated to dryness under reduced pressure and the residue purified on a silica gel column (isocratic elution, 2.5% MeOH/97.5% CH_2Cl_2) to give **14** in 22% yield. Corresponding oxidations of the remaining flavonoids (**13**, **17**−**19**, **25**, and **28**) gave **15/16**, **20/21**, **22/23**, **24**, **26/27**, and **29**, respectively, with different solvents.

7-Methoxymethoxyprotoapigenone (14). 22% yield from **12**; ^{1}H NMR ($CDCl_3$) δ 12.34 (1H, s, OH-5), 6.87 (2H, d, J = 9.9 Hz, H-2′, H-6′), 6.66 (1H, s, H-3), 6.46 (2H, s, H-6, H-8), 6.41 (2H, d, J = 9.9 Hz, H-3′, H-5′), 5.20 (2H, s, CH_2-OMOM), 3.46 (6H, s, OMe); ^{13}C NMR ($CDCl_3$) δ 184.5 (C-4′), 182.4 (C-4), 165.6 (C-7), 163.4 (C-2), 162.0 (C-5), 157.6 (C-9), 145.5 (C-2′, C-6′), 130.2 (C-3′, C-5′), 107.5 (C-3, C-10), 100.6 (C-6), 94.4 (C-8), 94.2 (CH_2-MOM), 69.5 (C-1′), 56.5 (OMe).

5,7-Dimethoxyprotoapigenone (15). 33% yield from **13**; ESI-MS (*m/z*, %): 313 (M^- − H, 100); ^{1}H NMR (pyridine-d_5) δ 7.26 (2H, d, J = 10.2 Hz, H-2′, H-6′), 7.10 (1H, s, H-3), 6.56 (2H, d, J = 10.2 Hz, H-3′, H-5′), 6.55 (2H, s, H-6, H-8), 3.82, 3.68 (3H each, s, OMe); ^{13}C NMR (pyridine-d_5) δ 185.3 (C-4′), 176.4 (C-4), 164.5 (C-7), 164.3 (C-2), 161.3 (C-5), 160.3 (C-9), 148.8 (C-

2′, C-6′), 129.4 (C-3′, C-5′), 110.9 (C-3), 109.8 (C-10), 96.9 (C-6), 93.4 (C-8), 69.4 (C-1′), 56.2, 55.8 (OMe).

5,7,1′-Trimethoxyprotoapigenone (16). 33% yield from **13**; ESI+MS (*m/z*, %): 328 ($M^+ + H$, 100); ^{1}H NMR (pyridine-d_5) δ 6.95 (2H, d, $J = 10.2$ Hz, H-2′, H-6′), 6.76 (1H, s, H-3), 6.67 (2H, d, $J = 10.2$ Hz, H-3′, H-5′), 6.55 (1H, s, H-8), 6.52 (1H, s, H-6), 3.81, 3.68, 3.25 (3H each, s, OMe); ^{13}C NMR (pyridine-d_5) δ 184.6 (C-4′), 176.0 (C-4), 164.6 (C-7), 161.4 (C-2), 161.3 (C-5), 160.1 (C-9), 146.0 (C-2′, C-6′), 133.2 (C-3′, C-5′), 111.5 (C-3, C-10), 97.0 (C-6), 93.4 (C-8), 74.7 (C-1′), 56.2, 55.8, 52.4 (OMe).

2-(1-Hydroxy-4-oxocyclohexa-2,5-dienyl)-4*H*-chromen-4-one (20). 36% yield from **17**; ESI-MS (*m/z*, %): 254 ($M^- - H$, 100); ^{1}H NMR ($CDCl_3$) δ 8.14 (1H, dd, $J = 7.8$, 1.5 Hz, H-5), 7.66 (1H, ddd, $J = 8.4$, 7.2, 1.5 Hz, H-7), 7.40 (1H, ddd, $J = 7.8$, 7.2, 0.9 Hz, H-6), 7.36 (1H, dd, $J = 8.4$, 0.9 Hz, H-8), 6.95 (2H, d, $J = 10.2$ Hz, H-2′, H-6′), 6.88 (1H, s, H-3), 6.41 (2H, d, $J = 10.2$ Hz, H-3′, H-5′); ^{13}C NMR ($CDCl_3$) δ 185.0 (C-4′), 178.9 (C-4), 166.3 (C-2), 156.2 (C-9), 146.2 (C-2′, C-6′), 134.3 (C-7), 129.9 (C-3′, C-5′), 125.7 (C-5, C-6), 123.5 (C-7), 118.1 (C-8), 108.8 (C-3), 69.8 (C-1′).

2-(1-Methoxy-4-oxocyclohexa-2,5-dienyl)-4*H*-chromen-4-one (21). 28% yield from **17**; ESI+MS (*m/z*, %): 268 (M^+, 100); ^{1}H NMR ($CDCl_3$) δ 8.17 (1H, dd, $J = 7.8$, 1.5 Hz, H-5), 7.65 (1H, ddd, $J = 8.4$, 7.2, 1.5 Hz, H-7), 7.40 (1H, ddd, $J = 7.8$, 7.2, 0.9 Hz, H-6), 7.35 (1H, dd, $J = 8.4$, 0.9 Hz, H-8), 6.82 (2H, d, $J = 10.2$ Hz, H-2′, H-6′), 6.76 (1H, s, H-3), 6.58 (2H, d, $J = 10.2$ Hz, H-3′, H-5′), 3.42 (3H, s, OMe); ^{13}C NMR ($CDCl_3$) δ 185.5 (C-4′), 178.0 (C-4), 163.8 (C-2), 156.0 (C-9), 145.5 (C-2′, C-6′), 134.0 (C-7), 133.1 (C-3′, C-5′), 125.7, 125.5 (C-5, C-6), 123.9 (C-7), 118.0 (C-8), 109.5 (C-8), 74.8 (C-1′), 52.7 (OMe).

5-Hydroxy-2-(1-hydroxy-4-oxocyclohexa-2,5-dienyl)-4*H*-chromen-4-one (22). 16% yield from **18**; ESI-MS (*m/z*, %): 270 ($M^- - H$, 100); ^{1}H NMR ($CDCl_3$) δ 12.24 (1H, s, OH-5), 7.52 (1H, dd, $J = 8.4$, 8.4 Hz, H-7), 6.99 (2H, d, $J = 9.9$ Hz, H-2′, H-6′), 6.81 (1H, d, $J = 8.4$ Hz, H-8), 6.80 (1H, d, $J = 8.4$ Hz, H-6), 6.73 (1H, s, H-3), 6.42 (2H, d, $J = 9.9$ Hz, H-3′, H-5′); ^{13}C NMR ($CDCl_3$) δ 184.4 (C-4′), 183.5 (C-4), 166.4 (C-2), 160.8 (C-5), 156.3 (C-9), 145.3 (C-2′, C-6′), 135.9 (C-7), 130.4 (C-3′, C-5′), 112.0 (C-6), 110.7 (C-10), 107.7 (C-3), 107.0 (C-8), 69.6 (C-1′).

5-Hydroxy-2-(1-methoxy-4-oxocyclohexa-2,5-dienyl)-4*H*-chromen-4-one (23). 15% yield from **18**; ESI-MS (*m/z*, %): 283 ($M^- - H$, 25), 268 ($M^- - CH_3$, 8), 253 ($M^- - CH_3 - OH$, 100); ^{1}H NMR ($CDCl_3$) δ 12.29 (1H, s, OH-5), 7.50 (1H, dd, $J = 8.4$, 8.4 Hz, H-7), 6.79 (2H, m, H-6, H-8), 6.78 (2H, d, $J = 9.9$ Hz, H-2′, H-6′), 6.70 (1H, s, H-3), 6.58 (2H, d, $J = 9.9$ Hz, H-3′, H-5′), 3.41 (3H, s, OMe); ^{13}C NMR ($CDCl_3$) δ 184.3 (C-4′), 183.5 (C-4), 165.1 (C-2), 160.7 (C-5), 156.2 (C-9), 145.1 (C-2′, C-6′), 135.7 (C-7), 133.4 (C-3′, C-5′), 111.8 (C-6), 110.8 (C-10), 108.2 (C-3), 107.0 (C-8), 74.8 (C-1′), 52.8 (OMe).

5-Hydroxy-2-(1-hydroxy-4-oxocyclohexa-2,5-dienyl)-7-methoxy-4*H*-chromen-4-one (24). 20% yield from **19**; ESI-MS (*m/z*, %): 299 ($M^- - H$, 100); ^{1}H NMR (pyridine-d_5) δ 13.22 (1H, s, OH-5), 7.27 (2H, d, $J = 10.2$ Hz, H-2′, H-6′), 7.11 (1H, s, H-3), 6.61 (1H, d, $J = 2.7$ Hz, H-8), 6.59 (2H, d, $J = 10.2$ Hz, H-3′, H-5′), 6.49 (1H, d, $J = 2.7$ Hz, H-6), 3.67 (3H, s, OMe); ^{13}C NMR (pyridine-d_5) δ 185.2 (C-4′), 183.0 (C-4), 168.3 (C-7), 166.2 (C-2), 162.6 (C-5), 158.3 (C-9), 148.4 (C-2′, C-6′), 129.6 (C-3′, C-5′), 107.7 (C-3), 106.1 (C-10), 99.0 (C-6), 92.9 (C-8), 69.7 (C-1′), 56.0 (OMe).

3-(1-Hydroxy-4-oxocyclohexa-2,5-dienyl)-1*H*-benzo[*f*]chromen-1-one (26). 16% yield from **25**; ESI-MS (*m/z*, %): 303 ($M^- - H$, 100); ^{1}H NMR ($CDCl_3$) δ 9.92 (1H, dd, $J = 9.0$, 1.0 Hz, H-5), 8.04 (1H, d, $J = 9.0$ Hz, H-9), 7.88 (1H, dd, $J = 8.4$, 1.2 Hz, H-8), 7.76 (1H, ddd, $J = 8.4$, 8.4, 1.0 Hz, H-7), 7.63 (1H, ddd, $J = 9.0$, 8.4, 1.2 Hz, H-6), 7.36 (1H, d, $J = 9.0$ Hz, H-10), 7.02 (1H, s, H-3), 7.00 (2H, d, $J = 10.2$ Hz, H-2′, H-6′), 6.43 (2H, d, $J = 10.2$ Hz, H-3′, H-5′); ^{13}C NMR ($CDCl_3$) δ 184.9 (C-4′), 180.4 (C-4), 163.3 (C-2), 157.6 (C-10a), 146.1 (C-2′, C-6′), 130.0 (C-3′, C-5′), 136.1, 130.7, 130.1, 129.6, 128.3, 127.0, 126.9 (C-4b, C-5, C-6, C-7, C-8, C-8a, C-9), 117.2 (C-10), 117.1 (C-4a), 111.8 (C-3), 69.6 (C-1′).

3-(1-Methoxy-4-oxocyclohexa-2,5-dienyl)-1*H*-benzo[*f*]chromen-1-one (27). 15% yield from **25**; ESI-MS (*m/z*, %): 288 ($M^+ + H - OCH_3$, 100), 319 ($M^+ + H$, 95); ^{1}H NMR ($CDCl_3$) δ 9.98 (1H, dd, $J = 9.0$, 1.2 Hz, H-5), 8.07 (1H, d, $J = 9.0$ Hz, H-9), 7.94 (1H, dd, $J = 8.4$, 1.5 Hz, H-8), 7.76 (1H, ddd, $J = 8.4$, 8.4, 1.2 Hz, H-7), 7.62 (1H, ddd, $J = 9.0$, 8.4, 1.2 Hz, H-6), 7.40 (1H, d, $J = 9.0$ Hz, H-10), 6.88 (1H, s, H-3), 6.88 (2H, d, $J = 9.9$ Hz, H-2′, H-6′), 6.60 (2H, d, $J = 9.9$ Hz, H-3′, H-5′), 3.45 (3H, s, OMe); ^{13}C NMR ($CDCl_3$) δ 184.5 (C-4′), 179.8 (C-4), 161.1 (C-2), 157.4 (C-10a), 145.9 (C-2′, C-6′), 133.1 (C-3′, C-5′), 135.8, 130.6, 130.3, 129.4, 128.2, 127.0, 126.8 (C-4b, C-5, C-6, C-7, C-8, C-8a, C-9), 117.3 (C-10), 117.3 (C-4a), 112.6 (C-3), 74.7 (C-1′), 52.8 (OMe).

2-(1-Methoxy-4-oxocyclohexa-2,5-dienyl)-4*H*-benzo[*h*]chromen-4-one (29). 23% yield from **28**; ESI-MS (*m/z*, %): 319 ($M^+ + H$, 100), 288 ($M^+ + H - OCH_3$, 17); ^{1}H NMR ($CDCl_3$) δ 8.12 (1H, dd, $J = 7.8$, 1.2 Hz, H-10), 8.10 (1H, d, $J = 8.7$ Hz, H-5), 7.91 (1H, dd, $J = 7.2$, 1.5 Hz, H-7), 7.76 (1H, d $J = 8.7$ Hz, H-6), 7.69 (1H, ddd, $J = 8.4$, 7.8, 1.5 Hz, H-9), 7.62 (1H, ddd, $J = 8.4$, 7.2, 1.2 Hz, H-8), 6.94 (1H, s, H-3), 6.93 (2H, d, $J = 10.2$ Hz, H-2′, H-6′), 6.67 (2H, d, $J = 10.2$ Hz, H-3′, H-5′), 3.48 (3H, s, OMe); ^{13}C NMR ($CDCl_3$) δ 184.6 (C-4′), 177.9 (C-4), 162.9 (C-2), 160.6 (C-10b), 146.2 (C-2′, C-6′), 133.1 (C-3′, C-5′), 136.0, 129.5, 128.1, 127.5, 125.7, 123.7, 122.0, 120.5, 120.3 (C-4a, C-5, C-6, C-6a, C-7, C-8, C-9, C-10, C-10a), 110.8 (C-3), 75.1 (C-1′), 52.9 (OMe).

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Supporting Information Available: Additional information on compound purity, high-resolution mass spectral data, and HPLC analysis results of the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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