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Concise Synthesis of 5-Methoxy-6-hydroxy-2-methylchromone-7-*O*- and 5-Hydroxy-2-methylchromone-7-*O*-rutinosides. Investigation of Their Cytotoxic Activities against Several Human Tumor Cell Lines

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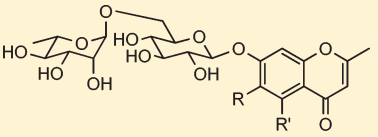
Supporting Information

ABSTRACT: The synthesis of two novel 2-methylchromone-7-*O*-rutinosides is reported, and the in vitro biological activities of these compounds and their synthetic precursors have been investigated on the basis of their cytotoxicity against several human tumor cell lines. The synthesis features early stage assembly of the acidic labile glycosidic bond between sugar and 2-methylchromone aglycon under phase transfer catalyzed glycosidation conditions, whereas all the other standard glycosylation conditions specific to a wide array of rutinosyl donors bearing different anomeric leaving groups (e.g., SPh, $OC(NH)CCl_3$, Br, OH groups) failed to furnish any detectable products.



1 R = OMe, R' = OH
2 R = OH, R' = OMe
3 R = H, R' = OH

Flavonoids, a class of polyphenolic compounds ubiquitous in plants, are emerging as a potentially important new class of pharmaceutical lead substrates which have been shown to possess a wide spectrum of biological activities spanning from antiallergic, anti-inflammatory, antioxidant, antimutagenic, and anticarcinogenic to modulation of enzymatic activities.[1] Most prominent of these are their potential roles serving as promising antitumor agents, and much of the recent research efforts have been directed toward flavonoid-based anticancer drug development and elucidation of their mechanism of action against tumor cell growth, due to their attractively low side effects on normal cells even at high dosage.[2] In 2009, McCloud and co-workers isolated the two new 2-methylchromone-7-*O*-rutinosides **1** and **3** from *Crossosoma bigelovii*, which they reported exhibit potent in vitro cytotoxicity against A-547, MCF-7, and HT-29 tumor cell lines at a micromolar concentration level ($GI_{50} = 0.4-6\ \mu M$), while, interestingly, no activity was observed with their nonglycosylated aglycone counterpart.[3] Intrigued by these bioactivities, we decided to set out to develop a straightforward and concise synthetic strategy to access these relatively simple yet biologically remarkable agents, with the purpose of gleaning insight into the structure−activity relationship. Herein we disclose our synthetic results for 5-methoxy-6-hydroxy-2-methylchromone-7-*O*-rutinoside (**2**), an isomeric form of **1**, and natural 5-hydroxy-2-methylchromone 7-*O*-rutinoside (**3**). Additionally, the in vitro antiproliferative activities of the synthetic Intermediates **2** and **3** against MCF-7, LS174T, and K562 human cancer cell lines have been assayed.

As depicted in Scheme 1, 5-methoxy-6,7-dihydroxy-2-methylchromone aglycone (**4**) was synthesized on the basis of literature precedures[4] commencing from the commercially available Visnagin, which was first oxidized with chromic acid to destroy the furan ring, furnishing the intermediate formylchromone, followed by subsequent hydrogen peroxide induced rearrangement in alkaline solution to afford chromone **4**.[5] Subsequent glycosylation of **4** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (**5**) in a biphasic reaction media ($CHCl_3/H_2O$) in presence of the phase transfer catalyst Bu_4NBr and K_2CO_3 as a base at 60 °C for 20 h afforded compound **6** in 56% yield along with a small amount of diglycosylated byproduct. The regioselectivity of the glycosylation was confirmed by the NOE relationship between the anomeric proton and H8 phenyl proton (Scheme 1). Of note in this transformation is that our original attempts for convergent assembly of this type of glycosidic linkage using per-*O*-benzoylated rutinosyl donors (e.g., rhamnopyranosyl-α-(1→6)-glucopyranosyl disaccharides) as coupling partners which bear different standard anomeric leaving groups such as Br, OH, SPh, and $OC(NH)CCl_3$ to couple with 6-hexonyl-protected **4** (for the purpose of increasing its solubility[6]) were not successful under the corresponding standard glycosylation conditions (data not shown). Such difficulties in glycosylation resembles Linhardt's recent work[7] for their synthesis of quercetin 3-sophorotrioside, in which the convergent assembly of the target molecule via glycosylation of the sophorotriosidic donor with protected

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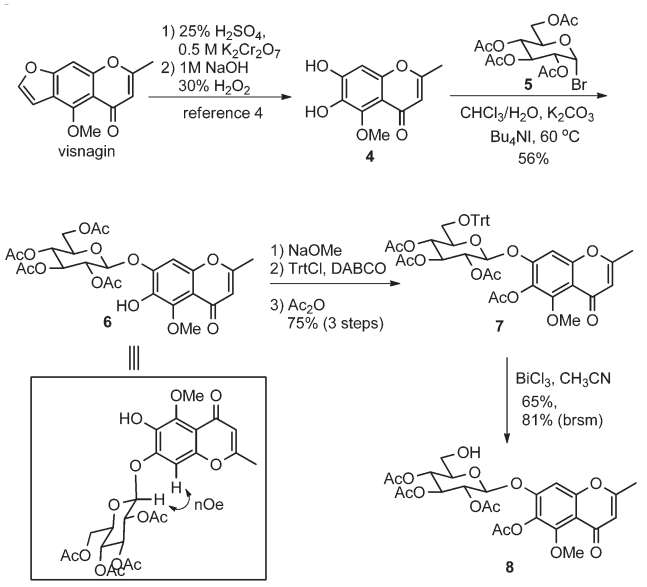
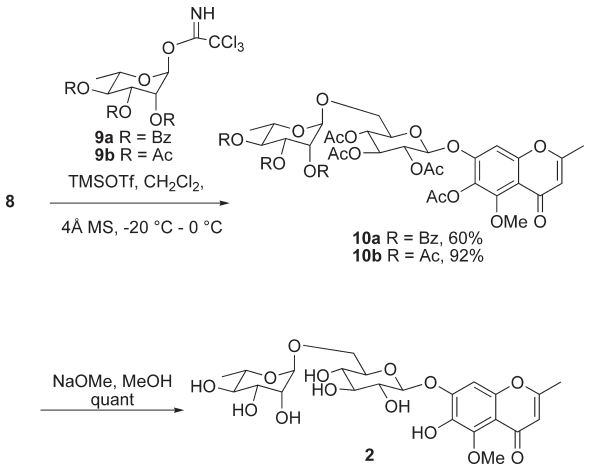
Scheme 1. Synthesis of 2-Methylchromone-7-*O*-glucopyranoside (8)



Scheme 2. Synthesis of 5-Methoxy-6-hydroxy-2-methylchromone-7-*O*-rutinoside (2)



quercetin was also not successful, highlighting a common challenge in a quick entry into flavonoid glycosides of such types via a convergent strategy. Next, subsequent conversion of compound **6** to compound **7** was carried out in a one-pot three-step sequence: (1) Zemplén deacetylation using catalytic amount of NaOMe in methanol, (2) 1,4-diazabicyclo-[2.2.2]octane (DABCO) promoted tritylation[8] of the resultant primary OH group, and (3) recapping of the remaining hydroxyl groups as acetates. It should be noted that in the tritylation step extremely low conversion (<20%) was observed under conventional conditions (trityl chloride/pyridine) even after long reaction times (72 h). Removal of the trityl protecting group also proved not to be trivial. We found that the glycosidic bond of **7** was susceptible to cleavage even under mild acidic detritylation conditions such as using 1% I_2 in methanol at elevated temperature (60 °C).[9] Extensive experimentation disclosed that Sabitha's recently developed protocol[10] using bismuth trichloride as a detritylation agent was the method of choice by which the desired product **8** could be obtained cleanly in 65% yield after 48 h of stirring at room temperature in dry CH_3CN, albeit with the recovery of some unreacted starting material (Scheme 1).

Glycosylation of per-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**9a**) with **8** took place uneventfully in dichloromethane with the promotion of TMSOTf, affording **10a** in 60% yield as a single isomer. The reaction yield was subsequently optimized to 92% simply by swapping protecting groups of the trichloroacetimidate **9a**, with the reaction executed under otherwise identical conditions. Final global removal of the protecting groups using catalytic NaOMe in anhydrous methanol at room temperature for several hours afforded the target compound **2** in quantitative yield (Scheme 2).

Synthesis of the natural 2-methylchromone rutinoside **3** was conducted by a route similar to that for **2**, commencing with 5,7-dihydroxy-2-methylchromone (**11**), which was readily prepared on a gram scale using the Kostanecki−Robinson synthesis from 2,4,6-trihydroxyacetonphenone on the basis of the literature protocol.[11] Next, NBu_4Br-catalyzed glycosylation of 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (**5**) with **11** has proven to be extremely sluggish, requiring addition of another aliquot of bromide donor after 24 h of stirring at 60 °C to drive the reaction to completion. The glycosylated product **12** was obtained in 46% yield after 2 days, with concomitant formation of a significant amount of hemiacetal arising from the hydrolysis of glycosyl bromide **5**. In order to circumvent this problem, we have screened various PTC catalysts and finally were delighted to find that the commercially available PTC catalyst Aliquat 336 could dramatically accelerate the reaction to completion within 18 h, affording **12** cleanly with remarkably improved 74% yield without the need of extra addition of bromide **5** during the course of the reaction. The exclusive regioselective glycosylation at the 7-OH position could be explained by the strong hydrogen bond formation between 5-OH and the adjacent carbonyl group (Scheme 3).

Analogous to the preparation of **7**, compound **13** was synthesized in 86% yield on the basis of a one-pot three-step sequence (Zemplén deacetylation → selective tritylation of primary OH group → acetylation of remaining OH groups). Interestingly, the 5-OH group was resistant to the acetylation reaction, as evidenced by a downfield proton signal resonating at 12.67 ppm in ^1H NMR spectrum of **13** in $CDCl_3$ at 500 MHz, obviously due to the hydrogen bond formation between 5-OH and 4-carbonyl oxygen. Bismuth trichloride catalyzed detritylation of **13** in anhydrous acetonitrile at room temperature for 48 h smoothly delivered the desired product **14** in 75% yield. Next, addition of catalytic TMSOTf into a mixture of trichloroacetimidate **9b** and **14** in anhydrous CH_2Cl_2 in the presence of activated molecular sieves at −20 °C, followed by a slow increase of the reaction temperature over 3 h, afforded glycosylated product **15** in 68% yield as a single isomer. Global removal of the acetyl protecting groups of **15** using a catalytic amount of NaOMe in dry methanol at room temperature furnished **3** in quantitative yield. All spectroscopic data of **3** (NMR, optical rotation, HRMS) were identical with those of natural **3**, as reported by McCloud and coworkers[3] (Scheme 3).

Driven by the purpose of gleaning insight into the structure and activity relationship (SAR) of these synthetic compounds, we have performed a systematic bioassay to evaluate the cell toxicity of the target glycosides **2** and **3** and their respective synthetic precursors against three tumor cell lines, including MCF-7, a breast cancer cell line, LS174T, a colon cancer cell line,

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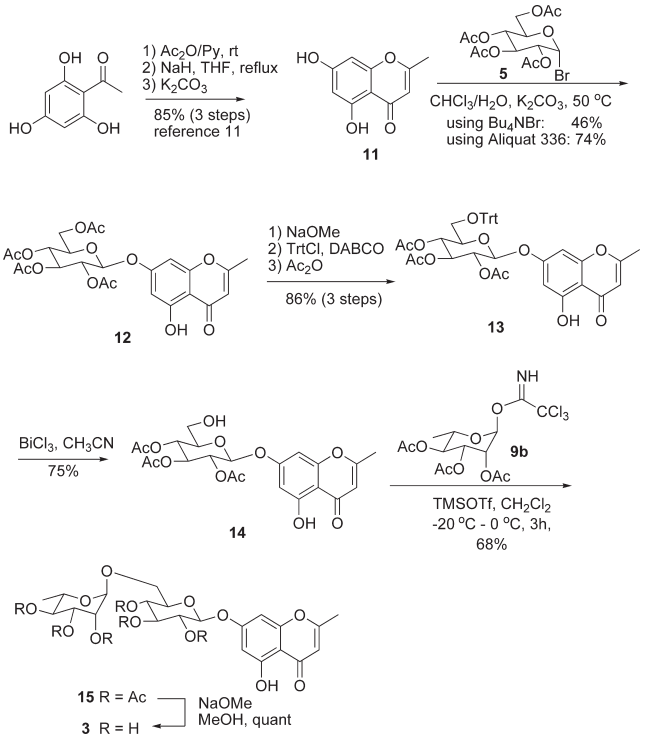
Scheme 3. Synthesis of 5-Hydroxy-2-methylchromone-7-*O*-rutinoside (3)



and K562, a leukemia cancer cell line with the well established MTS assay, wherein the anticancer drug doxorubicin was used as a positive control. Specifically, all the cancer cell lines were treated by the compounds at various concentrations (0.01, 0.1, 1.0, 10, 100 μM) for 4 days, followed by monitoring of inhibition of the cell growth by MTS assay, in which the growth ratio of treated cells was calculated by comparison with the nontreated cell control. Much to our surprise, although compounds **10b** and **15** in the MCF-7 cell line and compounds **12** and **15** in the K562 cell line showed some inhibition at 100 μM concentration, none of the other tested compounds showed any significant inhibitory effect against these cancer cells, even at high concentration (see Figures 1−3 in the Supporting Information). Most strikingly, the result obtained for compound **3** in our assays is in sharp contrast with that by McCloud and co-workers, where they have demonstrated that **3** has a 0.8 μM value of GI_{50} against the MCF-7 cell line.[3] Such a bioassay discrepancy underlines the notion that the cytotoxicity of this type of compounds may be cell-type dependent and/or the compounds themselves are inherently inactive.

EXPERIMENTAL SECTION

Representative Procedure for Phase Transfer Catalyzed Glycosylation of 2-Methylchromone Aglycon with Glycosyl Bromide using either TBAB or Aliquat 336. 7-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-hydroxy-2-methylchromone (12). *Method 1 (using TBAB).* To a stirred solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (**5**; 1.28 g, 3.10 mmol) and 5,7-dihydroxy-2-methylchromone (**11**; 0.5 g, 2.60 mmol) in a mixed solution of $CHCl_3$ and H_2O (20 mL/20 mL) was added K_2CO_3 (0.36 g, 2.59 mmol), followed by Bu_4NBr (0.84 g, 2.59 mmol). The resulting mixture was stirred at 50 °C for 12 h under an argon atmosphere; then one portion of 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (0.53 g, 1.29 mmol) was added. The stirring was continued for another 12 h, after which another portion of 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (0.53 g, 1.29 mmol) was added and the stirring was continued for 24 h at 50 °C. The reaction mixture was cooled to room temperature, diluted with $CHCl_3$ (100 mL), washed with cold 1 N HCl solution and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (toluene/ethyl acetate, 3/1) to isolate the desired product **12** (0.63 g, 1.2 mmol) in 46% yield.

Method 2 (using Aliquat 336). To a stirred solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (**5**; 0.86 g, 2.09 mmol) and 5,7-dihydroxy-2-methylchromone (**11**; 0.20 g, 1.05 mmol) in a mixed solution of $CHCl_3$ and H_2O (10 mL/10 mL) was added K_2CO_3 (0.29 g, 2.09 mmol), followed by Aliquat 336 (0.21 g, 0.50 mmol). The resulting reaction mixture was stirred at 50 °C for 18 h before it was cooled to room temperature and diluted with $CHCl_3$ (50 mL). The organic solution was washed with cold 1 N HCl solution and brine, dried over anhydrous Na_2SO_4, and concentrated. Column chromatographic purification using the same conditions as for method 1 afforded the title product **12** (0.40 g, 0.78 mmol) in 74% yield. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 12.65 (s, 1H), 6.42 (d, J = 2.0 Hz, 1H), 6.36 (d, J = 2.0 Hz, 1H), 6.02 (s, 1H), 5.24−5.32 (m, 2H), 5.11−5.15 (m, 2H), 4.25 (dd, J = 6.0, 12.5 Hz, 1H), 4.18 (dd, J = 3.0, 12.5 Hz, 1H), 3.93 (m, 1H), 2.32 (s, 3H), 2.10 (s, 3H), 2.044 (s, 3H), 2.041 (s, 3H), 2.02 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 182.4, 170.5, 170.1, 169.4, 169.2, 167.3, 162.2, 161.8, 157.6, 108.9, 106.5, 99.7, 98.1, 95.3, 77.3, 72.6, 72.4, 70.9, 68.2, 61.9, 20.6, 20.5, 20.4. ESI HRMS: m/z calcd for $C_{24}H_{26}O_{13}Na$ $[M + Na]^+$ 545.1271, found 545.1259.

Representative Detritylation Procedure. 7-*O*-(2,3,4-Tri-*O*-acetyl-β-D-glucopyranosyl)-5-hydroxy-2-methylchromone (14). To a stirred solution of compound **13** (0.14 g, 0.19 mmol) in dry CH_3CN (10.0 mL) was added $BiCl_3$ (0.12 g, 0.38 mmol) at room temperature. After 6 h of stirring at room temperature, another aliquot of $BiCl_3$ (0.12 g, 0.38 mmol) was added and the stirring was continued for an additional 12 h before the reaction was quenched with aqueous saturated $NaHCO_3$ solution. The mixture was filtered through Celite and washed with ethyl acetate. The filtrate was concentrated under reduced pressure, and the residue was partitioned between ethyl acetate (50.0 mL) and water (50.0 mL). The organic solution was washed with brine, dried, and concentrated. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate, 1/1) to isolate the desired product **14** (67 mg, 0.14 mmol) in 75% yield, while recovering the unreacted starting material (11 mg, 0.015 mmol). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 12.67 (s, 1H), 6.41 (d, J = 2.0 Hz, 1H), 6.35 (d, J = 2.0 Hz, 1H), 6.04 (s, 1H), 5.36 (t, J = 9.5 Hz, 1H), 5.14−5.28 (m, 3H), 3.67−3.85 (m, 3H), 2.69 (t, J = 6.5 Hz, 1H), 2.37 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 182.5, 170.2, 170.0, 169.2, 167.4, 162.2, 161.8, 157.7, 109.0, 106.4, 99.5, 98.0, 95.0, 77.2, 74.8, 72.5, 71.0, 68.3, 61.1, 20.63, 20.60, 20.58, 20.5. ESI HRMS: m/z calcd for $C_{22}H_{24}O_{12}Na$ $[M + Na]^+$ 503.1165, found 503.1183.

Representative Rhamnosylation of 2-Methylchromone Glucosides. 7-*O*-[2,3,4-Tri-*O*-acetyl-α-L-rhamnosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl]-5-hydroxy-2-methylchromone (15). A mixture of compound **14** (25.0 mg, 0.054 mmol), 2,3,4-tri-*O*-acetyl-α-L-rhamnosyl trichloroacetimidate (**9b**; 56.0 mg, 0.11 mmol), and activated 4 Å molecular sieves (100 mg) in anhydrous CH_2Cl_2 (4.0 mL) was stirred at room temperature for 1 h before it was cooled to −20 °C. One equivalent of TMSOTf was then added, and the resulting reaction mixture was stirred for 3 h, during which time the reaction temperature was raised to 0 °C. The reaction mixture was then quenched with aqueous $NaHCO_3$ solution, diluted with CH_2Cl_2 (50 mL), and filtered through Celite. The filtrate was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate, 1/1.2) to isolate the title compound **15** (27 mg, 0.037 mmol) in 68% yield. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 12.69

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(s, 1H), 6.45 (d, J = 2.0 Hz, 1H), 6.39 (d, J = 2.0 Hz, 1H), 6.06 (s, 1H), 5.25−5.34 (m, 4H), 5.19 (d, J = 8.0 Hz, 1H), 5.13 (t, J = 9.5 Hz, 1H), 5.03 (t, J = 9.5 Hz, 1H), 3.90−3.94 (m, 1H), 3.84 (dd, J = 6.5, 9.5 Hz, 1H), 3.79 (dd, J = 3.0, 12.0 Hz, 1H), 3.67 (dd, J = 6.0, 12.0 Hz, 1H), 2.38 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.17 (d, J = 6.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 182.6, 170.2, 170.0, 169.8, 169.7, 169.4, 169.2, 167.5, 162.3, 161.8, 157.8, 108.9, 106.7, 100.1, 98.1, 98.0, 94.7, 77.2, 73.6, 72.5, 71.0, 70.9, 69.4, 69.0, 68.8, 66.7, 66.2, 20.8, 20.73, 20.68, 20.61, 20.58, 20.4, 17.3. ESI HRMS: m/z calcd for $C_{34}H_{40}O_{19}Na$ $[M + Na]^+$ 775.2061, found 775.2052.

Representative Global Removal of Protecting Groups of 2-Methylchromone Rutinosides. 5-Hydroxy-7-*O*-β-rutinosyl-2-methylchromone (3). To a stirred solution of compound **15** (20 mg, 0.027 mmol) in anhydrous methanol (4.0 mL) was added several drops of freshly prepared 1 M NaOMe solution in methanol at room temperature. The reaction mixture was stirred at room temperature for 1 h before it was neutralized with Amberlyst-15 ion-exchange resin until the pH value was around 7. The resin was filtered off and washed with methanol several times. The collected filtrate was concentrated under reduced pressure, affording a residue which was purified by a short silica gel column chromatograph (CH_2Cl_2/MeOH, 4/1) to isolate the final product **3** (12 mg, 0.027 mmol) in quantitative yield. $[\alpha]^D_{24} = -50.0°$ (c 0.1, MeOH). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.78 (s, 1H), 6.64 (d, J = 2.0 Hz, 1H), 6.38 (d, J = 2.0 Hz, 1H), 6.24 (s, 1H), 5.44 (d, J = 4.5 Hz, 1H), 5.23 (t, J = 5.5 Hz, 1H), 5.00 (d, J = 7.0 Hz, 1H), 4.71 (d, J = 5.0 Hz, 1H), 4.60 (d, J = 4.5 Hz, 1H), 4.52 (s, 1H), 4.48 (d, J = 5.5 Hz, 1H), 3.87 (d, J = 10.0 Hz, 1H), 3.67 (br s, 1H), 3.59 (t, J = 8.0 Hz, 1H), 3.25−3.49 (m, 4H), 3.09−3.19 (m, 2H), 2.42 (s, 3H), 1.10 (d, J = 6.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 182.0, 168.7, 162.7, 161.0, 157.5, 108.2, 105.1, 100.7, 99.8, 99.6, 94.4, 76.4, 75.6, 73.0, 72.1, 70.7, 70.2, 69.9, 68.3, 66.4, 20.0, 17.8. ESI HRMS: m/z calcd for $C_{22}H_{28}O_{13}Na$ $[M + Na]^+$ 523.1422, found 523.1468.

MTS Assay. The cytotoxicity of synthesized compounds was evaluated by MTS assays using three cancer cell lines: MCF-7, K562, and LS174T. The cancer cells were cultured in DMEM (Gibco) with 10% FBS (Gibco) (MCF-7, K562) or 20% FBS (LS174T) at 37 °C with 5% CO_2. Briefly, a total of 2000 (LS174T) or 4000 (MCF-7, K562) cells for each well were cultured overnight in a 96-well plate. Then, the compounds of interest or Doxorubicin was added to each well at varying concentrations (0.01, 0.1, 1, 10, 100 μM). After 4 days, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, 0.2 mg/mL) and PMS (phenazine methosulfate, 2.5 μM) were added to the cell culture and the cells were incubated at 37 °C for 2−3 h. The absorbance of formazan (the metabolite of MTS by viable cells) was measured at 490 nm to quantify the viable cells. The growth ratio of treated cells was calculated by comparing the absorbance to that of the nontreated cells.

■ ASSOCIATED CONTENT

Supporting Information. Text and figures giving details of the experimental procedures and ^{1}H NMR and ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) The alternative approach to constructing 6,7-dimethylated **4** using the method recently developed by Fillion and co-workers[5a] via a trifluoroacetic acid promoted annulation reaction between 3,4,5-trimethoxyphenol and 5-(1-methoxyethylidene) Meldrum's acid afforded only 5,6,7-trimethoxy-4-methylcoumarin instead of the desired 5,6,7-trimethoxy-2-methylchromone on the basis of our careful NMR analysis of the product. These two types of compounds could be unequivocally differentiated by their diagnostic conjugated carbonyl carbon's chemical shift, where the chromone carbonyl carbon usually resonates around 175−185 ppm while that of coumarin moves upfield at 155−165 ppm.[5b,c] We observed in the ^{13}C NMR spectrum of the product that the carbonyl carbon's chemical shift was 160.9 ppm. Additionally, both ^{1}H and ^{13}C NMR spectra of the product were in full agreement with that of the reported 5,6,7-trimethoxy-4-methylcoumarin, which was synthesized via methylation of 5,7-dimethoxy-6-hydroxy-4-methylcoumarin. For related references, see: (a) Agrawal, P. K. *Carbon-13 NMR of Flavonoids*; Elsevier Science: Amsterdam, 1989. (b) Breitmaier, E.; Voelter, W. *Carbon-13 NMR spectroscopy*; VCH: Weinheim, Germany, 1987. (c) Parmar, V. S.; Sharma, N. K.; Husain, M.; Watterson, A. C.; Kumar, J.; Samuelson, L. A.; Cholli, A. L.; Prasad, A. K.; Kumar, A.; Malhotra, S.; Kumar, N.; Jha, A.; Singh, A.; Singh, I.; Himanshu; Vats, A.; Shakil, N. A.; Trikha, S.; Mukherjee, S.; Sharma, S. K.; Singh, S. K.; Kumar, A.; Jha, H. N.; Olsen, C. E.; Stove, C. P.; Bracke, M. E.; Mareel, M. M. *Bioorg. Med. Chem.* **2003**, *11*, 913.

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