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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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Abstract

1 R = OMe, R' = OH

2 R = OH, R' = OMe

3 R = H, R' = OH

The synthesis of two novel 2-methylchromone-7-O-rutinosides, whose synthetic precursors and themselves *in vitro* biological activities have been investigated based on the cytotoxicity against several human tumor cell lines, was reported. The synthesis features early-stage assembly of the acidic labile glycosidic bond between sugar and 2-methylchromone aglycon under a phase transfer catalyzed glycosidation condition, 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)CCl₃, Br, OH groups) failed to furnish any detectable products.

Flavonoids, a class of polyphenolic compounds found 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, anti-carcinogenic to modulation of enzymatic activities.¹ Most prominent of these are their potential roles serving as promising anti-tumor agents, and much of recent research efforts have been directed towards flavonoid based anti-

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Supporting Information **Available**. Experimental procedure detail and copies of ¹H NMR and ¹³C NMR of all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

cancer 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 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, HT-29 tumor cell lines at micromol concentration level ($GI_{50} = 0.4\text{--}6\text{ }\mu\text{M}$), while, interestingly, no activity was observed with their non-glycosylated aglycone counterpart.³ Intrigued with these bioactivities, we decided to set out to develop a straightforward and concise synthetic strategy to access these relative simple yet biologically remarkable agents, meanwhile 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, *in vitro* anti-proliferative 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 aglycon **4** was synthesized based on literature precedures⁴ commencing from commercially available Visnagin, which was first oxidized with chromic acid to destruct 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-glucosylbromide **5** in a bi-phase reaction media ($\text{CHCl}_3/\text{H}_2\text{O}$) in presence of phase transfer catalyst Bu_4NBr and K_2CO_3 as a base at $60\text{ }^\circ\text{C}$ for 20 hours afforded the compound **6** in 56% yield along with small amount of di-glycosylated by-product. The regioselectivity of the glycosylation was confirmed by the nOe relationship between 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 $\rightarrow$ 6)-glucopyranosyl diasaccharides) as coupling partners which bears different standard anomeric leaving groups such as Br, OH, SPh, and $\text{OC}(\text{NH})\text{CCl}_3$ to couple with 6-hexonyl protected **4** (for the purpose of increasing its solubility⁶) were not successful under the corresponding standard glycosylation conditions (data not shown). Such difficulties in glycosylation resembles Linhardt's recent work⁷ for their synthesis of quercetin 3-sophorotrioside, in which the convergent assembly of target molecule via glycosylation of sophorotriosidic donor with protected quercetin was not successful either, highlighting a common challenge in quick entry into flavonoid glycosides of such type 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 tritylation⁸ of the resultant primary OH group; 3) re-capping of the remaining hydroxyl groups as acetates. It should be noted that in the tritylation step extremely low conversion (< 20%) was observed under conventional condition (trityl chloride/pyridine) even after long reaction time (72 h). Removal of trityl protecting group was proved not to be trivial either. We found that the glycosidic bond of **7** was susceptible to cleavage even under mild acidic detritylation condition like using 1% I_2 in methanol at elevated temperature ($60\text{ }^\circ\text{C}$).⁹ Extensive experimentation disclosed that sabitha's recently developed protocol¹⁰ 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 hours 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** underwent uneventfully in dichloromethane with the promotion of TMSOTf, affording **10a** in 60% yield as a single isomer. The reaction yield was subsequently optimized to increase to 92% simply by protecting groups swap of the trichloroacetimidate **9a** with the reaction

executed under the otherwise identical condition. Final global removal of 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 natural 2-methylchromone rutoside **3** was conducted in a similar route as that of **2**, commencing with 5,7-dihydroxy-2-methylchromone **11** which was readily prepared in gram scale using the Kostanecki-Robinson synthesis from 2,4,6-trihydroxyacetophenone based on the literature protocol.¹¹ Next, NBu₄Br catalyzed glycosylation of 2,3,4,6-tetra-O-acetyl- α -D-glucosyl bromide **5** with **11** have 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 while with concomitant formation of significant amount of hemiacetal arose 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 commercially available PTC catalyst Aliquat 336 could dramatically accelerate the reaction to completion within 18 hours, affording **12** cleanly with remarkably improved 74% yield without the need of extra addition of bromide **5** during the course of reaction. The exclusive regioselective glycosylation at 7-OH position could be explained by the strong hydrogen bond formation between 5-OH and adjacent carbonyl group (Scheme 3).

Analogous to the preparation of **7**, compound **13** was synthesized in 86% yield based on a one pot three-step sequence (zemplén deacetylation $\rightarrow$ selective tritylation of primary OH group $\rightarrow$ acetylation of remaining OH groups). Interestingly, 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 CDCl₃ 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 CH₂Cl₂ in the presence of activated molecular sieves at -30 °C, followed by slowly warming of the reaction temperature within 3 hours, afforded glycosylated product **15** in 68% yield as a single isomer. Global removal of acetyl protecting groups of **15** using 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 to that of natural **3** as reported by McCloud and co-workers³ (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**, **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, and K562, a leukemia cancer cell line with the well established MTS assay, wherein the anti-cancer drug Doxorubicin was used as a positive control. Specifically, all the cancer cell lines were treated by the compounds at various concentrations (0.01 μ M, 0.1 μ M, 1.0 μ M, 10 μ M, 100 μ M) 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 non-treated cell control. Much to our surprise, although compound **10b** and **15** in MCF-7 cell line, compound **12** and **15** in K562 cell line showed some inhibition at 100 μ M concentration, none of the rest tested compounds showed any significant inhibitory effect against these cancer cells even at high concentration (see Figure 1-3 in SI). Most strikingly, the result obtained for compound **3** in our assays is in sharp contrast with that by McCloud and coworkers, where they have demonstrated that **3** has 0.8 μ M of GI₅₀ against MCF-7 cell line.³ Such bioassay discrepancy underlines 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), 5,7-dihydroxy-2-methylchromone **11** (0.5 g, 2.60 mmol) in a mixed solution of $\text{CHCl}_3/\text{H}_2\text{O}$ (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 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) were added and the stirring was kept for 24 h at 50 °C. The reaction mixture was cooled down to rt, and diluted with CHCl_3 (100 mL), washed with cold 1N HCl solution, 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 isolated 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), 5,7-dihydroxy-2-methylchromone **11** (0.20 g, 1.05 mmol) in a mixed solution of $\text{CHCl}_3/\text{H}_2\text{O}$ (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 down to rt, and diluted with CHCl_3 (50 mL). The organic solution was washed with cold 1N HCl solution, brine, dried over anhydrous Na_2SO_4 , and concentrated. Column chromatographic purification using the condition same as the method 1 afforded the title product **12** (0.40 g, 0.78 mmol) in 74% yield. ^1H 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. ESIHRMS Calcd for $\text{C}_{24}\text{H}_{26}\text{O}_{13}\text{Na}$ $[\text{M}+\text{Na}]^+$: 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 additional 12 h before it was quenched with aq satd 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 desire product **14** (67 mg, 0.14 mmol) in 75% yield, meanwhile recovering the unreacted starting material (11 mg, 0.015 mmol). ^1H 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. ESIHRMS Calcd for $\text{C}_{22}\text{H}_{24}\text{O}_{12}\text{Na}$ $[\text{M}+\text{Na}]^+$: 503.1165, found 503.1183.

Representative rhamnosylation of 2-methylchromone glucosides

7-O-[2,3,4-tri-O-acetyl- α -L-rhamnosyl-(1 $\rightarrow$ 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 4Å molecular sieves (100 mg) in anhydrous CH₂Cl₂ (4.0 mL) was stirred at rt for 1 h before it was cooled down 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 allowed to warm up to 0 °C. The reaction mixture was then quenched with aq NaHCO₃ solution, diluted with CH₂Cl₂ (50 mL) and filtered through Celite. The filtrate was washed with brine, dried over anhydrous Na₂SO₄ 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. ¹H NMR (CDCl₃, 500 MHz) δ 12.69 (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); ¹³C NMR (CDCl₃, 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. ESIHRMS Calcd for C₃₄H₄₀O₁₉Na [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 1M 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 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 chromatography (CH₂Cl₂/MeOH, 4:1) to isolate the final product **3** (12 mg, 0.027 mmol) in quantitative yield. $[\alpha]_{24}^D$ -50.0 (*c* 0.1, MeOH); ¹H 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, 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); ¹³C NMR (CDCl₃, 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. ESIHRMS Calcd for C₂₂H₂₈O₁₃Na [M+Na]⁺: 523.1422, found 523.1468.

MTS Assay—The cytotoxicity of synthesized compounds was evaluated by MTS assay using three cancer cell lines, MCF-7, K562 and LS174T. The cancer cells were cultured in DMEM (Gibco, USA) with 10% FBS (Gibco, USA) (MCF-7, K562) or 20% FBS (LS174T) at 37 °C with 5% CO₂. 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 interested compounds or Doxorubicin were added to each well at varied concentrations (0.01 μ M, 0.1 μ M, 1 μ M, 10 μ M, 100 μ M). After 4 days, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, 0.2 mg/mL) and PMS (phenazine methosulfate, 2.5 μ M) were added to the cell culture and incubated at 37 °C for 2-3 hours. 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 the non-treated cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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5. The alternative approach to constructing 6,7-dimethylated **4** using the method recently developed by Fillion and coworkers^a via 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 based on 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 coumarin one moves upfield at 155-165 ppm.^{b,c} What we observed in the ¹³C-NMR spectrum of the product have the carbonyl carbon's chemical shift at 160.9 ppm. Additionally, both ¹H and ¹³C-NMR of the product was in full agreement with that of the literature reported 5,6,7-trimethoxy-4-methylcoumarine which was synthesized via methylation of 5,7-dimethoxy-6-hydroxy-4-methylcoumarine.^d For related references, see: Agrawal PK. *Carbon-13 NMR of Flavonoids*. Elsevier Science Publishers B. V. Amsterdam 1989 Breitmaier E, Voelter W. *Carbon-13 NMR spectroscopy*. VCH Verlagsgesellschaft Weinheim 1987 Parmar VS, Sharma NK, Husain M, Watterson AC, Kumar J, Samuelson LA, Cholli AL, Prasad AK, Kumar A, Malhotra S, Kumar N, Jha A, Singh A, Singh I, Himanshu, Vats A, Shakil NA, Trikha S, Mukherjee S, Sharma SK, Singh SK, Kumar A, Jha HN, Olsen CE, Stove CP, Bracke ME, Mareel MM. *Bioorg Med Chem*. 2003; 11:913. [PubMed: 12614877]
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Scheme 1. Synthesis of 2-methylchromone-7-*O*-glucopyranoside 8

Scheme 2. Synthesis of 5-methoxy-6-hydroxy-2-methylchromon-7-O-rutinoside **2**

Scheme 3. Synthesis of 5-hydroxy-2-methylchromone-7-O-rutinoside 3