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Synthesis and Biological Evaluation of Novel Homoisoflavonoids for Retinal Neovascularization

Halesha D. Basavarajappa^{#,†,‡,§}, Bit Lee^{##}, Hyungjun Lee[#], Rania S. Sulaiman^{†,‡,||,T}, Hongchan An[∞], Carlos Magaña[⊥], Mehdi Shadmand^{†,‡}, Alexandra Vayl^{†,‡}, Gangaraju Rajashekhar^{†,‡,∇}, Eun-Yeong Kim[×], Young-Ger Suh[∞], Kiho Lee[×], Seung-Yong Seo^{##,*}, and Timothy W. Corson^{#,†,‡,§,||,⊥,*}

[†]Eugene and Marilyn Glick Eye Institute, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States

[‡]Department of Ophthalmology, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States

[§]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States

^{||}Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States

[⊥]Melvin and Bren Simon Cancer Center, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States

[#]College of Pharmacy and Gachon Institute of Pharmaceutical Sciences, Gachon University, Incheon 406-840, South Korea

^TDepartment of Biochemistry, College of Pharmacy, Cairo University, Cairo, Egypt

[∞]College of Pharmacy, Seoul National University, Seoul, South Korea

[×]College of Pharmacy, Korea University, Sejong, South Korea.

[#] These authors contributed equally to this work.

Abstract

Eye diseases characterized by excessive angiogenesis such as wet age-related macular degeneration, proliferative diabetic retinopathy, and retinopathy of prematurity are major causes of blindness. Cremastranone is an anti-angiogenic, naturally occurring homoisoflavanone with efficacy in retinal and choroidal neovascularization models and antiproliferative selectivity for endothelial cells over other cell types. We undertook a cell-based structure-activity relationship

*Corresponding Authors syseo@gachon.ac.kr. Phone: (+82) 32-899-6050. Fax: (+82) 32-820-4829. tcorson@iupui.edu. Phone: (+1) 317-274-3305. Fax: (+1) 317-274-2277.

[∇]Present Addresses Department of Ophthalmology, University of Tennessee Health Science Center.

ASSOCIATED CONTENT

Supporting Information. Purity analysis, supplementary schemes, figures and tables, and biological methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

HDB, BL, SYS, and TWC are named on a patent application disclosing the novel compounds described here. The authors declare no other competing financial interest.

study to develop more potent cremastranone analogs, with improved antiproliferative selectivity for retinal endothelial cells. Phenylalanine-incorporated homoisoflavonoids showed improved activity and remarkable selectivity for retinal microvascular endothelial cells. A lead compound inhibited angiogenesis in vitro without inducing apoptosis, and had efficacy in the oxygen-induced retinopathy model in vivo.

Keywords

Homoisoflavanone; Chroman-4-one; Anti-angiogenic; Angiogenesis; Ocular neovascularization

INTRODUCTION

Abnormal formation of new blood vessels in the eye is a pathological event in the development of common, blinding ocular diseases such as retinopathy of prematurity (ROP), proliferative diabetic retinopathy (PDR) and wet age-related macular degeneration (wet AMD).¹ Treatment of neovascularization in the eye is the standard therapy. The present therapies include photocoagulation, photodynamic therapy, and administration of antiangiogenic biologics such as ranibizumab, bevacizumab, and pegaptanib.² However, an innate drawback of surgical and laser treatments is the accrual of retinal tissue damage and partial vision loss. On the other hand, the use of biologics has shown promising results in halting progression of these diseases and even improving vision. But these drugs have limitations: they all only target the Vascular Endothelial Growth Factor (VEGF) pathway at the VEGF ligand-receptor level and nearly 35% of AMD patients are resistant to these biologics.^{2a, 3} As VEGF is required for the survival of normal blood vessel endothelial cells, treatment with anti-VEGF biologics can also result in several adverse effects. At present there are no FDA approved small molecule drugs targeting pathological angiogenesis in the eye, although there are some promising small molecules such as squalamine, OC-10X, vatalanib, and pazopanib being developed as antiangiogenic drugs for wet AMD and PDR (www.clinicaltrials.gov).⁴ To supplement these and existing drugs, novel, specific antiangiogenic small molecules need to be developed to strengthen the drug pipeline for wet AMD, PDR and ROP.

We⁵ and others⁶ have shown previously that a natural product homoisoflavanone, cremastranone (**1**; Figure 1), and a regioisomer (**2**)⁷ inhibit angiogenesis in vitro, with some selectivity for blocking proliferation of endothelial cells over other ocular cell types. Selectivity for antiproliferative effects on microvascular endothelial cells (such as those in the eye) over macrovascular endothelial cells and non-endothelial cell lines is suggestive of a desirable lack of off-target cytotoxicity in vivo.⁸ Indeed, cremastranone inhibits ocular angiogenesis, without obvious toxicity, in mouse models of choroidal neovascularization⁹ and oxygen-induced retinopathy (OIR),¹⁰ animal models recapitulating some of the features of wet AMD and ROP respectively.

Even though cremastranone's antiangiogenic activity is well established, other homoisoflavonoids have not been extensively explored for their effects on endothelial cells. One compound, (*E*)-3-(3-hydroxy-4-methoxybenzylidene)-6-methyl-4-chromanone was

shown to have submicromolar antiangiogenic effects in vitro and efficacy in a laser-induced choroidal neovascularization model,¹¹ while a related compound (*E*)-3-(2-methoxybenzylidene)-4-chromanone was shown to have antiproliferative effects on human umbilical vein endothelial cells (HUVECs).¹² Of natural-source homoisoflavanones, methylophiopogonanone B blocked HUVEC angiogenesis and the hypoxic response in vitro,¹³ while very recently methylophiopogonanone A was shown to block brain endothelial cell activation.¹⁴ In addition, considerable work has documented antiangiogenic effects of the related chalcones, flavones and flavanones.^{8, 15} However, to our knowledge no systematic exploration of this class of compounds as antiangiogenic agents has been reported.

Multiple signaling pathways are modulated by cremastranone. It induces expression of p21^{WAF1} (*CDKN1A*), an inhibitor of the cyclin-dependent kinase Cdc2 (*CDK1*).¹⁰ It also blocks prostaglandin synthesis from arachidonic acid;^{16a, 17} and decreases phosphorylation of the mitogen activated protein kinases. Finally, it blocks nuclear translocation of NF- κ B and production of inflammatory cytokines.^{7, 16} The search for direct molecular targets is underway. However, since its exact mechanism of action remains unknown, a cell-based analysis of efficacy is the most appropriate route to developing novel derivatives. Here, we describe development of a structure-activity relationship of homoisoflavonoids for inhibiting proliferation of endothelial cells, which resulted in identification of potent, microvascular endothelial-cell specific antiangiogenic molecules for lead optimization. Using **1** as our primary scaffold, we envisioned homoisoflavonoid analog design by which several substituents on the A and B rings could be varied as shown in Figure 1. We planned to synthesize homoisoflavonoids with highly oxygenated substitutions such as methoxy and hydroxy on the A ring,¹⁸ whereas those with a wide range of substitution patterns on the B ring would be anticipated to exhibit more efficient and selective antiproliferative activity than natural product homoisoflavonoids.

RESULTS AND DISCUSSION

Synthesis of Cremastranone and A ring Modified Homoisoflavanones

We previously reported the total synthesis of cremastranone (**1**) for the first time with a six-step reaction sequence.⁵ However, to improve the reaction efficiency and develop a modular synthesis toward cremastranone analogs, three chroman-4-ones, 7-hydroxy-5,6-dimethoxychroman-4-one (**6a**), 5,6,7-trimethoxychroman-4-one (**6b**) and 5,7-dimethoxychroman-4-one (**6c**) were considered as key intermediates (Scheme 1). Thus three ethanones (**3**–**5**) were treated with *N,N*-dimethylformamide dimethyl acetal, followed by catalytic hydrogenation of the resulting 4-chromenes to afford chroman-4-ones (**6a**–**6c**), respectively. Among them, **6a** was treated with isovanillin and *p*-TsOH, followed by catalytic hydrogenation to afford the resulting homoisoflavanone **8**. Finally the treatment of **8** with TMSI gave cremastranone (**1**) with improved yield and reaction steps. In a similar manner, **2** as well as homoisoflavanone **9** were prepared from **6b** which was converted into homoisoflavanone **10** before TMSI demethylation. Also, homoisoflavanone **11** was synthesized from **6c** in 2 steps.

Synthesis of B-ring Modified Homoisoflavanones

To prepare homoisoflavanones modified on the B ring, 3-benzylidene-4-chromanones **7d** and **7e** were prepared from **7b** by treatment with benzyl bromide and allyl bromide. Aldol condensation of 5,6,7-trimethoxy-4-chromanone (**6b**) with the appropriate arylaldehydes under acidic conditions gave the other 3-benzylidene-4-chromanones (**7f~7r**) (Scheme 2 and Supplementary Scheme 1). As previously described, 3-benzyl-5,6,7-trimethoxychroman-4-one (**12a**) was prepared from 3,4,5-trimethoxyphenol (Supplementary Scheme 2).^{7,19} Catalytic hydrogenation of 3-benzylidene-4-chromanones **7f** and **7g** gave **12b** and **12c** which were hydrolyzed to **12d** and **10** was transformed to the benzylated and acylated 3-benzyl-4-chromanones **12f~12i**.

As shown in Scheme 3, to improve the biological activity along with druglike properties, several amino acids functionalized on the NH₂ group such as carbamate, urea and sulfonamide were introduced on the C3' position of **10** via EDCI-mediated coupling to afford aryl ester analogs (**14a~14j**). Similar to **14a~14c**, amine **12b** was coupled with the *N*-substituted amino acids to afford the *N*-arylamide analogs (**15a~15c**).

Biological Evaluation of A-ring Modified Homoisoflavanones

In the homoisoflavanone series modified on the A ring, the majority of synthetic compounds except cremastranone exhibited weak inhibitory activity of cell proliferation and poor selectivity for human microvascular retinal endothelial cells (HRECs) compared to HUVECs and human ocular tumor cell lines 9-21 (uveal melanoma) and Y79 (retinoblastoma) (Table 1). Cremastranone **1** showed potent inhibitory activity of HREC and HUVEC proliferation, as reported already,⁵ whereas regioisomers with the different site-combinations of hydroxy and methoxy groups on the A ring had lower activity than the natural compound. Compounds **8** and **9** lost the inhibitory activity on HREC cell proliferation, while homoisoflavanones **10** and **11** functionalized only with methoxy groups had good activity. Although compound **10** with trimethoxy on C5, C6 and C7 did not show stronger activity than cremastranone, it did show good selectivity for HRECs over other cell types including HUVECs. Thus, it was chosen as the starting point for further analogs in order to discover potent, microvascular endothelial-cell specific, antiangiogenic agents and to expand chemical space.

Biological Evaluation of B-ring Modified Homoisoflavanones

In a series of homoisoflavanones modified on the B ring, 3-benzylidene-4-chromanones with mono-substituents on C2' and C4', di-substituents on C2'/C3' and C3'/C4', and trimethoxy groups on C2'/C3'/C4' and C3'/C4'/C5' of the B ring were evaluated (Table 2 and Supplementary Table 1). Compared to the 3-benzyl-4-chromanones **10** and **11**, 3-benzylidene-4-chromanones **7b** and **7c** did not exhibit satisfactory activity against HRECs nor selectivity. On the other hand, 3-(3',4'-disubstituted-benzylidene)-4-chromanones (**7d~7h**) with a methoxy group at the C4' position had moderate anti-proliferative activity (GI₅₀ 3~6 μM for HRECs), although still lacked selectivity. 3-Benzylidene-4-chromanones substituted on either C2' or C4' and those with a trimethoxy substituent on C2'/C3'/C4' and C3'/C4'/C5' had substantially decreased inhibitory activity on HREC proliferation.

(Supplementary Table 1). Additionally, the bulkier benzyl group at the C4 position led to lower activity than the hydroxyl or methoxy group (**7i** vs **7j**, **7k** vs **7l**).

In contrast to the 3-benzylidene-4-chromanones with a planar conformation, the freedom of rotation of 3-benzyl-4-chromanones might affect the selectivity for HRECs over human ocular tumor cell lines (Table 3). Mainly 3-benzyl-4-chromanones bearing methoxy on C4' of the B ring were evaluated along with **12a**, which shows little antiproliferative activity. Aniline **12b** showed excellent antiproliferative activity, but ester **12c** and acid **12d** showed little or no antiproliferative activity. Benzyl (**12e**) and carbamoyl (**12i**) compounds were found to be weak growth inhibitors. Interestingly, introduction of acyl groups such as benzoyl (**12f**), cinnamoyl (**12g**) and dihydrocinnamoyl (**12h**) strongly increased activity with GI₅₀ values of 0.14~0.65 μ M for HRECs. Moreover **12f**~**12h** were selective for HREC inhibition over HUVECs, Y79, and 9-21 cells. The antiproliferative activities were obviously dependent on the substitution pattern of the B ring.

Biological Evaluation of Homoisoflavanones Coupled with Amino Acids on the C3' position

Encouraged by the potent activity of aryl esters (**12f**~**12h**), phenol **10** and aniline **12b** were coupled with some *N*-protected amino acids to obtain the ester (**14a**~**14j**) and amide (**15a**~**15c**) analogs, respectively. Interestingly, **14a** which was given by coupling **10** with Boc-Phe-OH showed the most potent activity with GI₅₀ = 55 nM against HRECs (Table 4; Figure 2A). Moreover **14a** selectively inhibited HREC proliferation about 14-fold over HUVECs, 218-fold over Y79, and >1000-fold over 92-1, suggestive of cytostatic effects on HRECs rather than general cytotoxicity. The analogs (**14b** and **14c**) for which **10** was coupled with Boc-Tyr(Bn)-OH and Boc-Tyr(Allyl)-OH had similar activity to **14a**, potentially indicating that a bulkier (or longer) chemical spacer to the phenyl ring of the phenylalaninyl moiety is not detrimental to potency. An isoleucinyl analog (**14d**) had lower antiproliferative activity, whereas analogs (**14e**, **14f** and **14g**) generated with Boc-Leu-OH, Boc-homophe-OH and Cbz-Phe-OH have more preferable activity and selectivity to cremastranone. The antiproliferative activity of ethylurea (**14h**), butylurea (**14i**) and sulfonamide (**14j**) analogs was not improved. Noteworthy, the inhibition of HREC proliferation with *N*-arylamide analogs (**15a**~**15c**) decreased substantially, compared with the corresponding phenyl ester analogs (**14a**~**14c**). Conversely, *N*-arylamide analogs were considered to be moderate inhibitors against 92-1 and/or Y79 cells rather than antiangiogenic compounds.

Validation of a Potent Cremastranone Derivative In Vitro

In alamarBlue proliferation assays, **14a** had the highest potency of any compound tested. In addition, it was more potent and endothelial-cell specific than previously described antiangiogenic homoisoflavonoids.^{5, 11-14} Given this, we tested it in a secondary cell proliferation assay, which monitors the incorporation of a thymidine analogue 5-ethynyl-2'-deoxyuridine into DNA of proliferating HRECs. Here, we confirmed the dose-dependent inhibition of HREC proliferation by **14a**, without any signs of apoptotic nuclear morphology (Supplementary Figure 1).

After establishing the antiproliferative activity of this promising lead, we further tested its antiangiogenic activities in vitro. First we monitored the migration of HRECs, testing this important property of endothelial cells during blood vessel formation in the presence of **14a** in a scratch wound assay (Figure 2B). **14a** blocked the ability of HRECs to migrate in a dose dependent manner. Then we tested the ability of HRECs to form tubes in the presence of **14a** in a Matrigel tube formation assay, an in vitro assay that recapitulates most of the events of physiological angiogenesis such as migration, proliferation, and cell-cell adhesion. **14a** inhibited the tube formation ability of HRECs in the Matrigel assay at sub micromolar concentrations (Figure 2C).

Although **14a** did not induce changes in cell morphology in these assays, since the compound was so potent in inhibiting tube formation, we tested if **14a** induces apoptosis in HRECs. We employed both activated caspase (Figure 2D) and TUNEL (Supplementary Figure 2) assays to monitor the apoptosis of HRECs in the presence of different concentrations of **14a**. We observed less than 10% HREC cells undergoing apoptosis treated with up to 2 μ M **14a**, indicating that the compound may not be cytotoxic at effective concentrations. Furthermore, a trypan blue exclusion assay confirmed that treated HRECs retained viability (Supplementary Figure 3), further implicating a cytostatic rather than cytotoxic mechanism for this compound. This finding was further supported by analysis of the cell cycle profile in HRECs treated with **14a**, which revealed a dose-dependent G₂/M phase blockade with few sub-G₀ cells (Supplementary Figure 4), as documented previously for cremastranone.¹⁰ These results established that **14a** is a potent inhibitor of angiogenesis in vitro.

In vivo efficacy of a Potent Cremastranone Derivative

After establishing antiangiogenic activity of **14a** in vitro, we next explored the in vivo efficacy of this compound in preventing neovascularization in the OIR mouse model. Intravitreal injection of **14a** to a final concentration of 1 μ M in each eye significantly inhibited retinal neovascularization in OIR mice as compared to vehicle. Moreover, efficacy of the compound in vivo was similar to that observed with standard-of-care anti-VEGF antibody treatment (Figure 3). We did not observe any overt systemic or ocular toxicity in mice treated with **14a**, or gross morphological changes in the retinal vasculature. However, more extensive toxicological assessment of **14a** remains to be done.

The in vivo antiangiogenic activity of **14a** observed here provides evidence that novel synthetic homoisoflavonoids that show potent and selective antiangiogenic activity in vitro can be used as lead molecules to develop drugs for treatment of ocular diseases arising from pathological angiogenesis.

CONCLUSION

We synthesized a series of homoisoflavonoids from chroman-4-ones, including successfully synthesizing the natural product cremastranone. Antiproliferative compounds with endothelial cell specificity, with a homoisoflavonoid-based scaffold, were developed as potent inhibitors of angiogenesis. The scaffold is sensitive to changes on the substituents on both the A and B rings. Exploring modification at the C3' position revealed that addition of

N-carbamate amino acids improved inhibitory activity on HREC proliferation. The most potent phenylalanyl-incorporated **14a** showed improved activity and remarkable selectivity for retinal microvascular endothelial cells, with antiangiogenic efficacy in vitro and in the oxygen-induced retinopathy model in vivo.

EXPERIMENTAL SECTION

Chemistry

All starting materials and reagents were obtained from commercial suppliers and were used without further purification. Air and moisture sensitive reactions were performed under an argon atmosphere. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). ¹H and ¹³C NMR spectra were recorded on a Bruker 600 MHz spectrometer as solutions in deuteriochloroform (CDCl₃) or methanol-d₄. ¹H NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet and/or multiple resonances), number of protons, and coupling constant (*J*) in hertz (Hz). High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-700 (FAB and EI) and an Agilent 6530 Q-TOF LC/MS/MS system (ESI). All assayed compounds had purity 95% as determined by HPLC (Supplementary Table 2).

7-Hydroxy-5,6-dimethoxychroman-4-one (6a)

To a solution of 1-(4-(benzyloxy)-6-hydroxy-2,3-dimethoxyphenyl)ethanone (100 mg, 0.33 mmol) in toluene (2.0 mL) was added *N,N*-dimethylformamide dimethyl acetal (0.052 mL, 0.39 mmol). After stirring for 18 h at 80 °C, the mixture was cooled to 0 °C and *c*-HCl (0.2 mL) was added. After stirring for 1 h at 50 °C, The reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure and purified by flash column chromatograph on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford 72(benzyloxy)-5,6-dimethoxy-4*H*-chromen-4-one (101 mg, 97%). ¹H-NMR (CDCl₃, 600 MHz) δ 7.63 (d, 1H, *J* = 6.0 Hz), 7.46-7.40 (m, 4H), 7.37 (t, 1H, *J* = 6.6 Hz), 6.7 (s, 1H), 6.1 (d, 1H, *J* = 6.0 Hz), 5.20 (s, 2H), 3.97 (s, 3H), 3.92 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 176.2, 156.8, 154.6, 152.9, 140.7, 135.5, 128.8, 128.4, 127.2, 114.2, 113.8, 97.6, 70.9, 62.1, 61.5, 30.9. An anhydrous MeOH solution of the above 4-chromenone (47 mg, 0.15 mmol) and 10% Pd/C (16 mg) was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the chroman-4-one (**6a**) (33 mg, 99%). ¹H-NMR (CDCl₃, 600 MHz) δ 6.31 (s, 2H), 4.43 (t, 2H, *J* = 6.6 Hz), 3.90 (s, 3H), 3.90 (s, 3H), 2.72 (d, 2H, *J* = 6.6 Hz). ¹³C-NMR (150 MHz, CDCl₃) δ 189.3, 160.1, 155.5, 153.3, 135.1, 109.6, 98.9, 66.6, 61.5, 61.43, 38.7; HRMS (ESI): mass calcd for C₁₁H₁₂O₅ [M + H⁺], 224.0685; found, 224.0677.

5,6,7-Trimethoxychroman-4-one (6b)

Chromen-4-one formation of 1-(6-hydroxy-2,3,4-trimethoxyphenyl)ethan-1-one with *N,N*-dimethylformamide dimethyl acetal followed by the catalytic hydrogenation, performed according to the procedure described above, gave the chroman-4-one (**6b**) in 94% yield. ¹H-NMR (CDCl₃, 600 MHz) δ 6.22 (s, 1H), 4.42 (t, 2H, *J* = 6.6 Hz), 3.88 (s, 3H), 3.84 (s, 3H), 3.77 (s, 3H), 2.69 (t, 2H, *J* = 6.6 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 189.1, 160.0, 159.3, 154.3, 137.3, 109.6, 96.0, 66.8, 61.5, 61.3, 56.0, 38.7.

5,7-Dimethoxychroman-4-one (6c)

Chromen-4-one formation of 1-(2-hydroxy-4,6-dimethoxyphenyl)ethan-1-one with *N,N*-dimethylformamide dimethyl acetal followed by the catalytic hydrogenation, performed according to the procedure described above, gave the chroman-4-one (**6c**) in 95% yield. ¹H-NMR (CDCl₃, 600 MHz) δ 6.06 (s, 2H), 4.45 (t, 2H, *J* = 6.6 Hz), 3.87 (s, 3H), 3.82 (s, 3H), 2.73 (d, 2H, *J* = 6.6 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 189.1, 165.7, 165.2, 162.3, 106.4, 93.3, 92.9, 66.8, 56.1, 55.5, 38.8.

(E)-7-Hydroxy-3-(3'-hydroxy-4'-methoxybenzylidene)-5,6-dimethoxychroman-4-one (7a)

To a solution of the chroman-4-one (**6a**) (32 mg, 0.14 mmol) in benzene (3 mL) was added isovanillin (26 mg, 1.7 mmol) and *p*-toluenesulfonic acid (3 mg) at 0 °C. The reaction mixture was refluxed for 10 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure to afford the title product. The residue was used in subsequent reactions without further purification. HRMS (ESI): mass calcd for C₁₉H₁₈O₇ [M + H⁺], 358.1053; found, 358.1073.

(E)-3-(3'-Hydroxy-4'-methoxybenzylidene)-5,6,7-trimethoxychroman-4-one (7b)

To a solution of the chroman-4-one (**6b**) (238 mg, 1.0 mmol) in benzene (25 mL) was added isovanillin (170 mg, 1.1 mmol) and *p*-toluenesulfonic acid (20 mg, 0.1 mmol) at 0 °C. The reaction mixture was refluxed for 12 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate / *n*-hexane = 1 : 1) to afford the 3-benzylidene-chroman-4-one (**7b**) (215 mg, 58%). ¹H-NMR (600 MHz, CDCl₃) δ 7.74 (s, 1H), 6.91-6.84 (m, 3H), 6.26 (s, 1H), 5.67 (s, 1H), 5.24 (d, 2H, *J* = 1.8 Hz); 3.98 (s, 3H), 3.94 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 179.5, 159.3, 159.1, 154.7, 147.5, 145.5, 137.8, 136.2, 130.1, 128.1, 123.2, 115.7, 110.5, 96.1, 67.6, 61.6, 61.3, 60.3, 60.3, 56.0, 55.9; HRMS (EI): mass calcd for C₂₀H₂₀O₇ [M⁺], 372.1209; found, 372.1208.

(E)-3-(3'-Hydroxy-4'-methoxybenzylidene)-5,7-dimethoxychroman-4-one (7c)

To a solution of the chroman-4-one (**6c**) (71 mg, 0.34 mmol) in benzene (2 mL) was added isovanillin (62 mg, 0.41 mmol) and *p*-toluenesulfonic acid (7 mg, 0.03 mmol) at 0 °C. The reaction mixture was refluxed for 12 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzylidene-chroman-4-one (**7c**) (72 mg, 62%). ¹H-NMR (600 MHz, CDCl₃) δ 7.72 (s, 1H), 6.89-6.87 (m, 2H), 6.83 (d, 1H, *J* = 8.4 Hz), 6.11 (s, 1H), 6.06 (s, 1H), 5.23 (s, 2H), 3.93 (s, 3H), 3.90

(s, 3H), 3.82 (s, 3H); ^{13}C -NMR (150 MHz, CDCl_3) δ 179.5, 165.6, 164.6, 162.7, 147.4, 145.5, 135.7, 130.5, 128.3, 123.0, 115.8, 110.5, 107.3, 93.05, 93.5, 67.6, 56.1, 56.0, 55.5; HRMS (EI): mass calcd for $\text{C}_{19}\text{H}_{18}\text{O}_6$ [M^+], 342.1103; found, 342.1101.

7-Hydroxy-3-(3'-hydroxy-4'-methoxybenzyl)-5,6-dimethoxychroman-4-one (8)

A solution of the 3-benzylidene-chroman-4-one (**7a**) (35 mg, 0.07 mmol) and 10% Pd/C (10 mg) in MeOH was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzyl-chroman-4-one (**8**) (22 mg, 87%). ^1H -NMR (600 MHz, CD_3OD) δ 6.82 (d, 1H, J = 14.4 Hz), 6.67 (d, 1H, J = 1.8 Hz), 6.63 (dd, 1H, J = 8.4 and 2.4 Hz), 6.16 (s, 1H), 4.21 (dd, 1H, J = 11.4 and 4.2 Hz), 4.04 (dd, 1H, J = 11.4 and 7.2 Hz), 3.82 (s, 3H), 3.79 (s, 3H), 3.75 (s, 3H), 3.00 (dd, 1H, J = 13.2 and 4.2 Hz), 2.66 (m, 1H), 2.58 (dd, 1H, J = 13.8 and 10.8 Hz); ^{13}C -NMR (150 MHz, CD_3OD) δ 192.4, 160.0, 158.5, 154.4, 146.3, 146.2, 136.4, 131.2, 119.9, 115.6, 111.5, 107.3, 99.1, 68.6, 60.4, 60.1, 55.0, 48.2, 32.0; HRMS (ESI): mass calcd for $\text{C}_{19}\text{H}_{20}\text{O}_7$ [$\text{M} + \text{H}^+$], 361.1287; found, 361.1270. Compound **8** was reported. See ref 7.

3-(3'-Hydroxy-4'-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (10)

An anhydrous MeOH solution of the 3-benzylidene-chroman-4-one (**7b**) (415 mg, 1.2 mmol) and 5% Pd/C (59 mg) was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzyl-chroman-4-one (**10**) (327 mg, 78%). ^1H -NMR (400 MHz, CDCl_3) δ 7.24 (s, 1H), 6.83 (d, 1H, J = 7.8 Hz); 6.71 (d, 2H, J = 1.9 Hz); 6.23 (s, 1H), 5.53 (s, 1H), 4.23 (m, 1H), 4.10 (m, 1H), 3.91 (s, 3H), 3.85 (d, 6H, J = 1.9 Hz); 3.79 (s, 3H), 3.16 (m, 1H), 2.70 (m, 1H), 2.63 (m, 1H); ^{13}C -NMR (100 MHz, CDCl_3) δ 191.3, 159.6, 159.2, 154.4, 146.5, 144.2, 137.4, 130.2, 121.8, 114.3, 111.4, 108.6, 95.9, 69.0, 61.5, 61.2, 56.0, 55.9, 48.5, 32.5. HRMS (ESI): mass calcd for $\text{C}_{20}\text{H}_{22}\text{O}_7$ [$\text{M} + \text{H}^+$], 375.1444; found, 375.1432. Compound **10** was reported. See ref 5.

5-Hydroxy-3-(3'-hydroxy-4'-methoxybenzyl)-6,7-dimethoxychroman-4-one (9)

To a CHCl_3 solution (2 mL) of the 3-benzyl-chroman-4-one (**10**) (60 mg, 0.16 mmol) was added TMSI (50 μL , 0.4 mmol) at 0 $^\circ\text{C}$ and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the demethylated 3-benzyl-chroman-4-one (**9**) (23 mg, 42%). ^1H -NMR (400 MHz, CDCl_3) δ 11.96 (s, 1H), 6.86 (d, 1H, J = 7.8 Hz), 6.72-6.70 (m, 2H), 6.02 (s, 1H), 5.60 (s, 1H), 4.30 (dd, 1H, J = 11 and 4.3 Hz), 4.14 (dd, 1H, J = 6.8 and 11 Hz), 3.87 (s, 6H), 3.82 (s, 3H), 3.17 (dd, 1H, J = 13 and 3.9 Hz), 2.83-2.77 (m, 1H), 2.72 (dd, 1H, J = 13 and 10 Hz); ^{13}C -NMR (100 MHz, CDCl_3) δ 198.4, 160.7, 158.7, 155.2, 146.6, 144.1, 130.4, 129.5, 121.8, 114.4, 111.3, 102.6, 91.2, 69.1, 60.8, 56.1, 55.8, 46.8, 32.4; HRMS

(ESI): mass calcd for $C_{20}H_{22}O_7$ [$M + H^+$], 375.1439; found, 375.1459. Compound **9** was reported. See ref 7.

3-(3'-Hydroxy-4'-methoxybenzyl)-5,7-dimethoxychroman-4-one (**11**)

An anhydrous MeOH solution of the 3-benzylidene-chroman-4-one (**7c**) (12 mg, 0.04 mmol) and 10% Pd/C (4 mg) was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzyl-chroman-4-one (**11**) (10 mg, 73%). 1H -NMR (600 MHz, $CDCl_3$) δ 6.81-6.77 (m, 2H), 6.71 (d, 1H, J = 8.4 Hz), 6.06 (d, 1H, J = 8.4 Hz), 5.58 (s, 1H), 4.27 (dd, 1H, J = 11.4 and 4.2 Hz), 4.10 (dd, 1H, J = 10.8 and 7.2 Hz), 3.88 (s, 3H), 3.87 (s, 3H), 3.82 (s, 3H), 3.19 (dd, 1H, J = 13.8 and 4.2 Hz), 2.75-2.72 (m, 1H), 2.58 (t, 1H, J = 12.6 Hz); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 191.3, 165.7, 164.9, 162.5, 145.6, 145.2, 131.9, 120.6, 115.2, 110.7, 105.4, 93.1, 92.9, 68.9, 56.1, 56.0, 55.5, 48.4, 32.2; HRMS (EI): mass calcd for $C_{19}H_{20}O_6$ [M^+], 344.1260; found, 344.1267.

(*E*)-3-(3'-(Benzyloxy)-4'-methoxybenzylidene)-5,6,7-trimethoxychroman-4-one (**7d**)

To an acetone (5 mL) solution of the 3-benzylidene-chroman-4-one (**7b**) (124 mg, 0.33 mmol) benzyl bromide (70 mg, 0.4 mmol) and K_2CO_3 (144 mg, 0.80 mmol) were added. After stirring for 3 h at room temperature, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the benzylated 3-benzylidene-chroman-4-one (**7d**) (120 mg, 79%). 1H -NMR (400 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.42-7.24 (m, 5H), 6.93 (d, 1H, J = 8.3 Hz); 6.87 (dd, 1H, J = 8.3 and 2.0 Hz); 6.75 (d, 1H, J = 2.0 Hz); 6.22 (s, 1H), 5.17 (s, 2H), 5.03 (d, 2H, J = 1.5 Hz); 3.95 (s, 3H), 3.92 (s, 3H), 3.86 (s, 3H), 3.81 (s, 3H); ^{13}C -NMR (100 MHz, $CDCl_3$) δ 179.4, 159.3, 159.1, 154.7, 150.8, 147.8, 137.8, 136.7, 136.2, 129.9, 128.6, 128.0, 127.3, 127.1, 124.0, 115.9, 111.5, 110.5, 96.1, 71.1, 67.4, 61.6, 61.3, 56.1, 56.0; HRMS (EI): mass calcd for $C_{27}H_{26}O_7$ [M^+], 462.1679; found, 462.1679.

(*E*)-3-(3'-(Allyloxy)-4'-methoxybenzylidene)-5,6,7-trimethoxychroman-4-one (**7e**)

To an acetone (2 mL) solution of the 3-benzylidene-chroman-4-one (**7b**) (9.9 mg, 0.02 mmol) allylbromide (2.5 μ L, 0.02 mmol) and K_2CO_3 (18 mg, 0.10 mmol) were added. After stirring for 3 h at room temperature, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate : *n*-hexane = 1 : 1) to afford the allylated 3-benzylidene-chroman-4-one (**7e**) (6.8 mg, 83%). 1H -NMR (600 MHz, $CDCl_3$) δ 7.75 (s, 1H), 6.93 (d, 1H, J = 8.4 Hz), 6.88 (dd, 1H, J = 8.4 and 1.8 Hz), 6.83 (d, 1H, J = 1.8 Hz), 6.25 (s, 1H), 6.11 - 6.04 (m, 1H), 5.43 - 5.40 (m, 1H), 5.33 - 5.31 (m, 1H), 5.23 (d, 1H, J = 1.8 Hz), 4.64 (m, 2H), 3.97 (s, 3H), 3.91 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 179.51, 159.32, 159.19, 154.78, 150.57, 147.82, 137.88, 136.36, 133.05, 130.01, 127.41,

123.76, 118.32, 115.26, 111.36, 110.64, 96.15, 69.99, 67.70, 61.66, 61.35, 56.13, 56.01; HRMS (EI): mass calcd for $C_{23}H_{24}O_7$ [M^+], 412.1522; found, 412.1519.

(E)-5,6,7-Trimethoxy-3-(4'-methoxy-3'-nitrobenzylidene)chroman-4-one (7f)

To a benzene solution (2 mL) of the chroman-4-one (**6b**) (17 mg, 0.071 mmol) were added 4-methoxy-3-nitrobenzaldehyde (13 mg, 0.071 mmol) and *p*-toluenesulfonic acid (2 mg, 0.1 mmol) at 0 °C. After refluxing for 12 h with a Dean-Stark apparatus, the reaction mixture was cooled and quenched with saturated $NaHCO_3$. The reaction mixture was diluted with ethyl acetate (5 mL $\times$ 3) and washed with water and the combined organic phases were dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzylidene-chroman-4-one (**7f**) (17 mg, 60%). 1H -NMR (600 MHz, $CDCl_3$) δ 7.76 (d, 1H, J = 2.4 Hz), 7.72 (s, 1H), 7.51 (dd, 1H, J = 9.0 and 2.4 Hz), 7.17 (d, 1H, J = 8.4 Hz), 6.26 (s, 1H), 5.20 (d, 2H, J = 1.8 Hz), 4.02 (s, 3H), 3.98 (s, 3H), 3.89 (s, 3H), 3.83 (s, 3H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 178.8, 159.5, 159.4, 154.8, 153.3, 139.5, 138.0, 135.7, 133.0, 132.4, 127.1, 126.5, 113.8, 110.3, 96.2, 67.1, 61.6, 61.3, 56.7, 56.2; HRMS (EI): mass calcd for $C_{20}H_{19}NO_8$ [M^+], 401.1111; found, 401.1113.

Methyl (E)-2-methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-ylidene) methyl)benzoate (7g)

To a benzene solution (2 mL) of the chroman-4-one (**6b**) (103 mg, 0.43 mmol) were added methyl 5-formylsalicylate (78 mg, 0.43 mmol) and *p*-toluenesulfonic acid (17 mg, 0.04 mmol) at 0 °C. After refluxing for 12 h with a Dean-Stark apparatus, the reaction mixture was cooled and quenched with saturated $NaHCO_3$. The reaction mixture was diluted with ethyl acetate (5 mL $\times$ 3) and washed with water and the combined organic phases were dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford methyl (*E*)-2-hydroxy-5-((5,6,7-trimethoxy-4-oxochroman-3-ylidene)methyl) benzoate (115 mg, 66%). 1H -NMR (600 MHz, $CDCl_3$) δ 10.87 (s, 1H), 7.66 (s, 1H), 7.61 (s, 1H), 7.30 (d, 1H, J = 8.4 Hz), 6.93 (dd, 1H, J = 8.4 and 1.8 Hz), 6.15 (s, 1H), 5.11 (s, 2H), 3.88 (s, 6H), 3.78 (s, 3H), 3.74 (s, 3H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 179.1, 169.9, 162.1, 159.2, 159.2, 154.7, 137.8, 137.0, 134.8, 131.7, 130.6, 125.9, 118.1, 112.4, 110.4, 96.1, 67.3, 61.5, 61.2, 56.1, 52.6. To an acetone solution (3 mL) of methyl (*E*)-2-hydroxy-5-((5,6,7-trimethoxy-4-oxochroman-3-ylidene)methyl)benzoate (100 mg, 0.24 mmol) were added dimethyl sulfate (0.23 mL, 2.4 mmol) and K_2CO_3 (138 mg, 0.72 mmol). After refluxing for 4h, the reaction mixture was diluted with ethyl acetate (10 mL $\times$ 3) and washed with water and the combined organic phases were dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzylidene-chroman-4-one (**7g**) (100 mg, 97%). 1H -NMR (600 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.71 (d, 1H, J = 2.4 Hz), 7.41 (dd, 1H, J = 9 and 2.4 Hz), 7.03 (d, 1H, J = 8.4 Hz), 6.23 (s, 1H), 5.20 (d, 2H, J = 1.2 Hz), 3.96 (s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.81 (s, 3H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 179.2, 166.0, 159.6, 159.3, 159.3, 154.8, 137.9, 135.3, 134.8, 133.2, 130.9, 126.7, 120.2, 112.2, 110.5, 96.1, 67.4, 61.6, 61.3, 56.2, 56.1, 52.3; HRMS (EI): mass calcd for $C_{22}H_{22}O_8$ [M^+], 414.1315; found, 414.1317.

(E)-3-(3'-Fluoro-4'-methoxybenzylidene)-5,6,7-trimethoxychroman-4-one (7h)

To a benzene solution (5 mL) of the chroman-4-one (**6b**) (101 mg, 0.42 mmol) were added 3-fluoro-4-hydroxybenzaldehyde (59 mg, 0.42 mmol) and *p*-toluenesulfonic acid (17 mg, 0.08 mmol) at 0 °C. After refluxing for 12 h with a Dean-Stark apparatus, the reaction mixture was cooled and quenched with saturated NaHCO₃. The reaction mixture was diluted with ethyl acetate (30 mL × 3) and washed with water and the combined organic phases were dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzylidene-chroman-4-one (**7h**) (62 mg, 40%). ¹H-NMR (600 MHz, CDCl₃) δ 7.67 (s, 1H), 7.01-6.95 (m, 3H), 6.22 (s, 1H), 5.18 (s, 2H), 3.94 (s, 3H), 3.90 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 179.2, 159.3, 154.7, 152.8, 151.1, 148.6, 148.5, 137.9, 134.8, 130.8, 126.8, 117.4, 117.3, 113.2, 110.5, 96.1, 67.4, 61.6, 61.3, 56.2; HRMS (EI): mass calcd for C₂₀H₁₉FO₆ [M⁺], 374.1166; found, 374.1166.

(E)-3-(4'-hydroxy-3'-methoxybenzylidene)-5,6,7-trimethoxychroman-4-one (7i) and (E)-3-(4'-(benzyloxy)-3'-methoxybenzylidene)-5,6,7-trimethoxychroman-4-one (7j)

To a benzene solution (25 mL) of 5,6,7-trimethoxychroman-4-one (**6b**) (238 mg, 1.0 mmol) were added 4-benzyloxy-3-methoxybenzaldehyde (265 mg, 1.1 mmol) and *p*-toluenesulfonic acid (20 mg, 0.1 mmol) at 0 °C. After refluxing for 12 h with a Dean-Stark apparatus, the reaction mixture was cooled and quenched with saturated NaHCO₃. The reaction mixture was diluted with ethyl acetate (15 mL × 3) and washed with water and the combined organic phases were dried over anhydrous MgSO₄, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzylidene-chroman-4-ones (**7j**) (48 mg, 11%) and (**7i**) (62 mg, 17%). For **7i**, ¹H-NMR (600 MHz, CDCl₃) δ 7.75 (s, 1H), 6.96 (s, 1H), 6.96 (d, 1H, *J* = 8.4 Hz), 6.82 (s, 1H), 6.79 (d, 1H, *J* = 8.4 Hz), 5.24 (s, 2H), 3.97 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 179.5, 159.3, 159.1, 154.7, 147.0, 146.5, 137.8, 136.5, 129.8, 127.0, 123.7, 114.6, 112.7, 110.6, 96.1, 67.6, 61.6, 61.3, 56.1, 55.9; ; HRMS (EI): mass calcd for C₂₀H₂₂O₇ [M⁺], 372.1209; found, 372.1210. For **7j**, ¹H-NMR (600 MHz, CDCl₃) δ 7.75 (s, 1H), 7.44–7.30 (m, 5H), 6.92 (d, 1H, *J* = 7.8 Hz), 6.85 (s, 1H), 7.78 (d, 1H, *J* = 7.8 Hz), 6.25 (s, 1H), 5.23 (s, 2H), 5.20 (s, 2H), 3.97 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 179.4, 159.3, 159.1, 154.7, 149.4, 149.2, 137.8, 136.5, 136.3, 130.1, 128.6, 128.0, 127.9, 127.2, 123.1, 113.6, 113.3, 110.6, 96.1, 70.8, 67.6, 61.6, 61.3, 56.1, 56.1; HRMS (EI): mass calcd for C₂₇H₂₆O₇ [M⁺], 462.1679; found, 462.1674.

(E)-3-(3',4'-Dihydroxybenzylidene)-5,6,7-trimethoxychroman-4-one-(7k)

To a benzene solution (3 mL) of the chroman-4-one (**6b**) (83 mg, 0.34 mmol) were added 3,4-dihydroxybenzaldehyde (47 mg, 0.34 mmol) and *p*-toluenesulfonic acid (14 mg, 0.07 mmol) at 0 °C. After refluxing for 12 h with a Dean-Stark apparatus, the reaction mixture was cooled and quenched with saturated NaHCO₃. The reaction mixture was diluted with ethyl acetate (10 mL × 3) and washed with water and the combined organic phases were dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-

benzylidene-chroman-4-one (**7k**) (13 mg, 10%). ¹H-NMR (600 MHz, DMSO-d₆) δ 7.52 (s, 1H), 6.85-6.84 (m, 2H), 6.78 (dd, 1H, *J* = 8.4 and 1.8 Hz), 6.49 (s, 1H), 5.27 (s, 2H), 3.86 (s, 3H), 3.81 (s, 3H), 3.69 (s, 3H); ¹³C-NMR (150 MHz, DMSO-d₆) δ 178.8, 159.2, 159.2, 154.4, 147.9, 154.7, 137.6, 136.3, 129.1, 125.8, 123.4, 117.9, 116.2, 110.5, 97.1, 67.8, 61.7, 61.2, 56.7; HRMS (EI): mass calcd for C₁₉H₁₈O₇ [M⁺], 358.1053; found, 358.1057.

(*E*)-3-(3',4'-Bis(benzyloxy)benzylidene)-5,6,7-trimethoxychroman-4-one (**7l**)

To a benzene solution (5 mL) of the chroman-4-one (**6b**) (108 mg, 0.45 mmol) were added 3,4-dibenzyloxybenzaldehyde (102 mg, 0.45 mmol) and *p*-toluenesulfonic acid (9 mg, 0.04 mmol) at 0 °C. After refluxing for 12 h with a Dean-Stark apparatus, the reaction mixture was cooled and quenched with saturated NaHCO₃. The reaction mixture was diluted with ethyl acetate (10 mL × 3) and washed with water and the combined organic phases were dried over anhydrous MgSO₄, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the 3-benzylidene-chroman-4-one (**7l**) (110 mg, 45%). ¹H-NMR (600 MHz, CDCl₃) δ 7.67 (s, 1H), 7.44 (t, 4H, *J* = 7.8 Hz), 7.37 (t, 4H, *J* = 7.8 Hz), 7.32 (t, 2H, *J* = 7.2 Hz), 6.94 (d, 1H, *J* = 8.4 Hz), 6.80 (m, 2H), 6.22 (s, 1H), 5.20 (s, 2H), 5.18 (s, 2H), 5.04 (d, 2H, *J* = 1.2 Hz), 3.95 (s, 3H), 3.86 (s, 3H), 3.81 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 179.4, 159.3, 159.1, 154.7, 150.0, 148.4, 137.8, 136.8, 136.7, 136.1, 128.6, 128.6, 128.0, 127.2, 124.1, 116.9, 114.2, 110.6, 96.1, 71.4, 71.0, 67.5, 61.6, 61.3, 56.1; HRMS (EI): mass calcd for C₃₃H₃₀O₇ [M⁺], 538.1992; found, 538.1992.

3-(3'-Amino-4'-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**12b**)

An anhydrous MeOH solution of **7f** (12 mg, 0.05 mmol) and 10% Pd/C (4 mg) was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with Ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzyl-chroman-4-one (**12b**) (13 mg, 73%). ¹H-NMR (600 MHz, CDCl₃) δ 6.70 (d, 1H, *J* = 8.4 Hz), 6.58 (d, 1H, *J* = 1.8 Hz), 6.56 (dd, 1H, *J* = 8.4 and 2.4 Hz), 4.26 (dd, 1H, *J* = 10.8 and 4.2 Hz), 4.11 (m, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.12 (dd, 1H, *J* = 14.4 and 4.2 Hz), 2.71 (m, 1H), 2.54 (dd, 1H, *J* = 13.8 and 10.8 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 191.7, 166.4, 159.8, 159.2, 136.1, 133.5, 131.2, 125.7, 118.9, 115.6, 110.4, 108.9, 95.9, 69.1, 61.6, 61.3, 56.0, 55.5, 48.4, 32.1; HRMS (EI): mass calcd for C₂₀H₂₃NO₆ [M⁺], 373.1525; found, 373.1519.

Methyl 2-methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl) benzoate (**12c**)

To an anhydrous MeOH (3 mL) solution of **7g** (102 mg, 0.24 mmol) and 10% Pd/C (26 mg) was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the 3-benzyl-chroman-4-one (**12c**) (72 mg, 74%). ¹H-NMR (600 MHz, CDCl₃) δ 7.58 (d, 1H, *J* = 2.4 Hz), 7.29 (dd, 1H, *J* = 8.4 and 2.4 Hz), 6.88 (d, 1H, *J* = 9 Hz), 6.19 (s, 1H), 4.23 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.03 (dd, 1H, *J* = 9 and 5.4 Hz), 3.86 (s, 3H), 3.82 (s, 9H), 3.74 (s, 3H), 3.15 (dd, 1H, *J* = 13.8 and 4.2 Hz), 2.72 (m,

1H), 2.63 (dd, 1H, J = 14.4 and 10.8 Hz); ^{13}C -NMR (150 MHz, CDCl_3) δ 190.9, 166.5, 159.6, 159.3, 157.8, 154.4, 137.5, 134.1, 132.0, 130.1, 120.0, 112.3, 108.6, 95.9, 69.0, 61.5, 61.2, 60.3, 56.0, 52.0, 58.1, 20.9; HRMS (EI): mass calcd for $\text{C}_{22}\text{H}_{24}\text{O}_8$ [M^+], 416.1471; found, 416.1470.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)benzoic acid (**12d**)

The methyl ester **12c** (29 mg, 0.07 mmol) was suspended in 0.4 mL of THF. In a separate flask, 2.5 mg of lithium hydroxide was dissolved in 0.4 mL of deionized water. Both mixtures were chilled to 4 °C and combined to form a turbid white mixture. After 1 h of stirring, the mixture had become homogeneous. After 24 h, 0.5 mL of 3 M HCl was added, and the mixture was allowed to warm to room temperature. The reaction mixture was diluted with ethyl acetate (3 mL $\times$ 3) and washed with water and the combined organic phases were dried over MgSO_4 , and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzyl-chroman-4-one (**12d**) (16 mg, 56%). ^1H -NMR (600 MHz, CD_3OD) δ 7.31 (d, 1H J = 1.8 Hz), 7.16 (dd, 1H, J = 8.4 and 2.4 Hz), 6.93 (d, 1H, J = 8.4 Hz), 6.39 (s, 1H), 4.30 (dd, 1H, J = 11.4 and 4.2 Hz), 4.13 (dd, 1H, J = 11.4 and 7.8 Hz), 3.87 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 3.74 (s, 3H), 3.11 (dd, 1H, J = 13.8 and 4.2 Hz), 2.77 (m, 1H), 2.66 (dd, 1H, J = 14.4 and 10.8 Hz); ^{13}C -NMR (150 MHz, CD_3OD) δ 192.3, 160.2, 159.8, 155.2, 153.9, 137.1, 129.7, 129.7, 129.0, 111.3, 108.0, 96.0, 68.8, 60.7, 60.6, 60.2, 59.9, 55.3, 54.6, 31.5, 22.5; HRMS (EI): mass calcd for $\text{C}_{21}\text{H}_{22}\text{O}_8$ [M^+], 402.1315; found, 402.1317.

3-(3'-(Benzyloxy)-4'-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**12e**)

To an acetone (5 mL) solution of **10** (33 mg, 0.08 mmol), benzyl bromide (16 μL , 0.12 mmol) and K_2CO_3 (37 mg, 0.25 mmol) were added. After stirring for 3 h at room temperature, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the 3-benzyl-chroman-4-one (**12e**) (34 mg, 84%). ^1H -NMR (600 MHz, CDCl_3) δ 7.58 (d, 1H, J = 2.4 Hz), 7.29 (dd, 1H, J = 8.4 and 2.4 Hz), 6.88 (d, 1H, J = 9 Hz), 6.19 (s, 1H), 4.23 (dd, 1H, J = 11.4 and 4.2 Hz), 4.03 (dd, 1H, J = 9 and 5.4 Hz), 3.86 (s, 3H), 3.82 (s, 9H), 3.74 (s, 3H), 3.15 (dd, 1H, J = 13.8 and 4.2 Hz), 2.72 (m, 1H), 2.63 (dd, 1H, J = 14.4 and 10.8 Hz); ^{13}C -NMR (150 MHz, CDCl_3) δ 190.9, 166.5, 159.6, 159.3, 157.8, 154.4, 137.5, 134.1, 132.0, 130.1, 120.0, 112.3, 108.6, 95.9, 69.0, 61.5, 61.2, 60.3, 56.0, 52.0, 58.1, 20.9; HRMS (EI): mass calcd for $\text{C}_{27}\text{H}_{28}\text{O}_7$ [M^+], 464.1835; found, 464.1837.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl benzoate (**12f**)

To an acetone (5 mL) solution of **10** (36 mg, 0.09 mmol), benzoyl chloride (17 μL , 0.11 mmol) and K_2CO_3 (41 mg, 0.29 mmol) were added. After stirring for 17 h at room temperature, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the 3-benzyl-chroman-4-one (**12f**) (38 mg, 82%). ^1H -NMR (600

MHz, CDCl₃) δ 8.19 (dd, 1H, J = 8.4 and 1.2 Hz), 7.62 (t, 1H, J = 7.2 Hz), 7.50 (t, 2H, J = 7.8 Hz), 7.10 (dd, 1H, J = 8.4 and 1.8 Hz), 7.02 (d, 1H, J = 2.4 Hz), 6.94 (d, 1H, J = 8.4 Hz), 6.23 (s, 1H), 4.29 (dd, 1H, J = 11.4 and 4.2 Hz), 4.13 (dd, 1H, J = 11.4 and 7.8 Hz), 3.90 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H), 3.78 (s, 3H), 3.21 (dd, 1H, J = 13.8 and 4.2 Hz), 2.76-2.72 (m, 1H), 2.67 (dd, 1H, J = 13.8 and 4.8 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 191.1, 164.7, 159.7, 159.3, 154.5, 150.0, 139.9, 137.5, 133.5, 131.0, 130.3, 129.3, 128.5, 127.4, 123.6, 112.7, 108.7, 96.0, 69.0, 61.6, 61.3, 56.1, 56.0, 48.3, 31.9; HRMS (EI): mass calcd for C₂₇H₂₆O₈[M⁺], 478.1628; found, 478.1628.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl cinnamate (12g)

To an acetone (5 mL) solution of **10** (77 mg, 0.2 mmol), cinnamoyl chloride (41 mg, 0.24 mmol) and K₂CO₃ (86 mg, 0.6 mmol) were added. After stirring for 17 h at room temperature, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (Ethyl acetate / *n*-hexane = 1 : 2) to afford the 3-benzyl-chroman-4-one (**12g**) (80 mg, 77%). ¹H-NMR (600 MHz, CDCl₃) δ 7.86 (d, 1H, J = 16.2 Hz), 7.56 (d, 2H, J = 3.0 Hz), 7.40 (t, 3H, J = 2.4 Hz), 7.08 (d, 1H, J = 8.4 Hz), 6.98 (d, 1H, J = 1.8 Hz), 6.93 (d, 1H, J = 8.4 Hz), 6.65 (d, 1H, J = 15.6 Hz), 6.23 (s, 1H), 4.30 (dd, 1H, J = 11.4 and 4.2 Hz), 4.12 (dd, 1H, J = 12 and 7.8 Hz), 3.91 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.20 (dd, 1H, J = 13.8 and 4.2 Hz), 2.75-2.71 (m, 1H), 2.66 (dd, 1H, J = 14.4 and 10.8 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 191.1, 164.9, 159.7, 159.3, 154.5, 149.9, 146.6, 139.7, 137.5, 134.2, 131.0, 130.6, 129.0, 128.3, 127.3, 123.6, 116.9, 112.6, 108.7, 96.0, 69.0, 61.6, 61.3, 56.1, 56.0, 48.3, 31.8; HRMS (EI): mass calcd for C₂₉H₂₈O₈ [M⁺], 504.1784; found, 504.1779.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl 3-phenylpropanoate (12h)

An anhydrous MeOH solution of **12g** (28 mg, 0.05 mmol) and 10% Pd/C (6 mg) was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with Ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzyl-chroman-4-one (**12h**) (26 mg, 92 %). ¹H-NMR (600 MHz, CDCl₃) δ 7.3327.22 (m, 5H), 7.06 (d, 1H, J = 8.4 Hz), 6.90 (d, 1H, J = 7.8 Hz), 6.84 (s, 1H), 6.25 (s, 1H), 4.28 (dd, 1H, J = 11.4 and 4.2 Hz), 4.10 (dd, 1H, J = 10.8 and 4.2 Hz), 3.93 (s, 3H), 3.88 (s, 3H), 3.81 (s, 3H), 3.77 (s, 3H), 3.19 (dd, 1H, J = 14.4 and 4.2 Hz), 3.10 (t, 2H, J = 7.8 Hz), 2.92 (t, 2H, J = 7.8 Hz), 2.74-2.71 (m, 1H), 2.64 (dd, 1H, J = 13.8 and 10.8 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 191.1, 170.9, 159.7, 159.3, 154.5, 149.8, 140.3, 139.7, 137.5, 131.0, 128.5, 128.4, 127.3, 126.3, 123.4, 112.5, 108.7, 96.0, 69.0, 61.6, 61.3, 56.1, 55.9, 48.2, 35.5, 31.8, 30.9; HRMS (EI): mass calcd for C₂₉H₃₀O₈ [M⁺], 506.1941; found, 506.1944.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl diethylcarbamate (12i)

To a toluene (1 mL) solution of **10** (16 mg, 0.04 mmol), diethyl carbamoyl chloride (6 μ L, 0.05 mmol) and Et₃N (17 μ L, 0.12 mmol) were added. After refluxing for 17 h, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the 3-benzyl-chroman-4-one (**12i**) (8 mg, 40%). ¹H-NMR (600 MHz, CDCl₃) δ 7.0 (d, 1H, *J* = 8.4 Hz), 6.95 (s, 1H), 6.86 (d, 1H, *J* = 8.4 Hz), 6.22 (s, 1H), 4.28 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.10 (dd, 1H, *J* = 11.4 and 7.8 Hz), 3.90 (s, 3H), 3.85 (s, 3H), 3.79 (s, 6H), 3.43 (d, 2H, *J* = 6.6 Hz), 3.36 (d, 2H, *J* = 6.6 Hz), 3.18 (dd, 1H, *J* = 13.8 and 3.6 Hz), 2.74-2.70 (m, 1H), 2.62 (dd, 1H, *J* = 13.8 and 10.8 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 191.2, 159.7, 159.2, 154.4, 154.0, 150.4, 140.6, 137.4, 130.8, 126.6, 124.0, 112.5, 108.7, 96.0, 69.0, 61.6, 61.3, 56.1, 56.0, 48.3, 42.2, 42.0, 31.8, 14.0, 13.4; HRMS (EI): mass calcd for C₂₅H₃₁NO₈ [M⁺], 473.2050; found, 473.2040.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl (tert-butoxycarbonyl)-L-phenylalaninate (14a)

To a CH₂Cl₂ solution (6 mL) of **10** (169 mg, 0.45 mmol) were added Boc-Phe-OH (155 mg, 0.54 mmol), EDCI (84 mg, 0.54 mmol) and DMAP (11 mg, 0.09 mmol). After stirring for 17 h, the reaction mixture was diluted with CH₂Cl₂ and washed with water and brine, dried over anhydrous MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**14a**) (247 mg, 87%). ¹H-NMR (600 MHz, CDCl₃) δ 7.34 (m, 2H), 7.29 (m, 3H), 7.08 (dd, 1H, *J* = 8.4 and 1.8 Hz), 6.92 (d, 1H, *J* = 8.4 Hz), 6.83 (bs, 1H), 6.25 (s, 1H), 4.88 (m, 1H), 4.29 (dd, 1H, *J* = 14 and 4.2 Hz), 4.10 (m, 1H), 4.13-4.07 (m, 2H), 3.93 (s, 3H), 3.88 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.35 (m, 1H), 3.23 (m, 1H), 3.19 (m, 1H), 2.72 (m, 1H), 2.64 (m, 1H), 1.42 (s, 9H); ¹³C-NMR (150 MHz, CDCl₃) δ 191.0, 170.2, 159.7, 159.4, 154.4, 150.9, 137.5, 136.0, 131.0, 129.6, 128.5, 127.0, 122.6, 121.3, 113.1, 112.6, 108.6, 96.0, 79.9, 69.0, 61.6, 61.3, 56.1, 55.8, 54.3, 48.2, 38.2, 32.7, 31.8, 28.3; HRMS (EI): mass calcd for C₃₄H₃₉NO₁₀ [M⁺], 621.2574; found, 621.2573.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl (2S)-3-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (14b)

To a CH₂Cl₂ solution (2 mL) of **10** (63 mg, 0.16 mmol) were added Boc-Tyr(Bzl)-OH (75 mg, 0.19 mmol), EDCI (40 mg, 0.24 mmol) and DMAP (4 mg, 0.03 mmol). After stirring for 17 h, the reaction mixture was diluted with CH₂Cl₂ and washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**14b**) (115 mg, 94%). ¹H-NMR (600 MHz, CDCl₃) δ 7.39 (d, 2H, *J* = 7.2 Hz), 7.33 (t, 3H, *J* = 7.2 Hz), 7.28 (t, 1H, *J* = 7.2 Hz), 7.19 (d, 2H, *J* = 8.4 Hz), 7.05 (dd, 1H, *J* = 8.4 and 1.8 Hz), 6.92 (m, 3H), 6.22 (d, 1H, *J* = 2.4 Hz), 5.01 (s, 2H), 4.80 (m, 1H), 4.26 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.06 (dd, 1H, *J* = 7.8 and 3.6 Hz), 3.89 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H), 3.26 (dd, 1H, *J* = 14.4 and 6.0 Hz), 3.16 (dd,

1H, J = 13.8 and 4.2 Hz), 2.72 (m, 1H), 2.62 (m, 1H), 1.40 (s, 9H); ^{13}C -NMR (150 MHz, CDCl_3) δ 191.0, 170.1, 159.6, 159.3, 157.9, 155.0, 154.4, 149.6, 139.2, 137.5, 136.9, 131.0, 130.6, 128.5, 128.2, 127.9, 127.6, 127.4, 123.4, 114.8, 112.6, 108.6, 96.0, 79.9, 70.0, 68.9, 61.6, 61.3, 56.0, 55.8, 54.4, 48.2, 37.3, 31.8, 28.3; HRMS (ESI): mass calcd for $\text{C}_{41}\text{H}_{45}\text{NO}_{11}$, $[\text{M} + \text{H}^+]$, 727.2993; found, 727.302.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl (2S)-3-(4-allyloxy)phenyl-2-((tert-butoxycarbonyl)amino)propanoate (14c)

To a CH_2Cl_2 solution (3 mL) of **10** (56 mg, 0.15 mmol) were added Boc-Tyr(All)-OH (58 mg, 0.18 mmol), EDCI (35 mg, 0.22 mmol) and DMAP (4 mg, 0.03 mmol). After stirring for 17 h, the reaction mixture was diluted with CH_2Cl_2 and washed with water and brine, dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (Ethyl acetate / *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**14c**) (85 mg, 84%). ^1H -NMR (600 MHz, CDCl_3) δ 7.16 (d, 1H, J = 8.4 Hz), 7.04 (dd, 1H, J = 8.4 and 1.8 Hz), 6.88 (d, 1H, J = 8.4 Hz), 6.85 (d, 3H J = 12 Hz), 6.22 (d, 1H, J = 2.4 Hz), 6.03-5.96 (m, 1H), 5.37 (dd, 1H, J = 16.8 and 1.2 Hz), 5.24 (dd, 1H, J = 10.8 and 1.2 Hz), 5.02 (d, 1H, J = 7.8 Hz), 4.79 (q, 1H, J = 6.0 Hz), 4.48 (dd, 2H, J = 5.4 and 1.2 Hz), 4.25 (dd, 1H, J = 11.4 and 4.2 Hz), 4.04 (m, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 3.77 (s, 3H), 3.76 (s, 3H), 3.24 (dd, 1H, J = 14.4 and 6.0 Hz), 3.15 (dd, 1H, J = 13.8 and 7.2 Hz), 2.71-2.66 (m, 1H), 2.61 (dd, 1H, J = 13.8 and 10.2 Hz), 1.39 (s, 9H); ^{13}C -NMR (150 MHz, CDCl_3) δ 191.0, 170.1, 159.6, 159.3, 157.7, 155.0, 154.4, 149.6, 139.2, 137.4, 133.2, 131.0, 130.5, 128.1, 127.5, 123.4, 117.5, 114.7, 112.6, 108.6, 96.0, 79.9, 68.9, 68.7, 61.5, 61.2, 56.0, 55.8, 54.4, 48.1, 37.3, 31.8, 28.2; HRMS (ESI): mass calcd for $\text{C}_{37}\text{H}_{43}\text{NO}_{11}$ $[\text{M} + \text{H}^+]$, 677.2836; found, 677.2847.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl (tert-butoxycarbonyl)-L-isoleucinate (14d)

To a CH_2Cl_2 solution (3 mL) of **10** (22 mg, 0.05 mmol) were added Boc-Ile-OH (15 mg, 0.06 mmol), DCC (13 mg, 0.6 mmol) and DMAP (1 mg, 0.01 mmol). After stirring for 17 h, the reaction mixture was diluted with CH_2Cl_2 and washed with water and brine, dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**14d**) (33 mg, 95%). ^1H -NMR (600 MHz, CDCl_3) δ 7.18 (d, 2H, J = 7.8 Hz), 7.09 (dd, 1H, J = 8.4 and 2.4 Hz), 6.95 (m, 4H), 6.26 (s, 1H), 5.01 (m, 1H), 4.85 (dd, 1H, J = 8.4 and 6.0 Hz), 4.29 (dd, 1H, J = 10.8 and 4.2 Hz), 4.10 (m, 1H), 3.93 (s, 3H), 3.88 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.48 (m, 1H), 3.32 (dd, 1H, J = 14.4 and 6.0 Hz), 3.20 (dd, 1H, J = 13.8 and 4.2 Hz), 2.75 (m, 1H), 2.64 (m, 1H), 1.42 (s, 9H), 1.32 (s, 9H); HRMS (FAB): mass calcd for $\text{C}_{31}\text{H}_{41}\text{NO}_{10}$ $[\text{M} + \text{H}^+]$, 588.2730; found, 588.2807.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl (tert-butoxycarbonyl)-L-leucinate (14e)

To a CH_2Cl_2 solution (3 mL) of **10** (20 mg, 0.084 mmol) were added Boc-Leu-OH (16 mg, 0.06 mmol), DCC (16 mg, 0.06 mmol) and DMAP (1 mg, 0.01 mmol). After stirring for 17

h, the reaction mixture was diluted with CH₂Cl₂ and washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**14e**) (30 mg, 61 %). ¹H-NMR (600 MHz, CDCl₃) δ 7.07 (dd, 1H, *J* = 8.4 and 1.8 Hz), 6.93 (s, 1H), 6.90 (d, 1H, *J* = 8.4 Hz), 6.24 (s, 1H), 4.98 (d, 1H, *J* = 7.2 Hz), 4.57 (m, 1H), 4.28 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.11 (m, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 3.19 (dd, 1H, *J* = 13.8 and 4.2 Hz), 2.74 (m, 1H), 2.65 (m, 1H), 1.87 (m, 2H), 1.65 (m, 1H), 1.46 (s, 9H), 1.01 (s, 3H), 1.00 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 191.0, 171.5, 159.7, 159.3, 155.3, 154.4, 149.6, 139.4, 137.5, 131.0, 127.5, 123.4, 112.6, 108.6, 96.0, 79.9, 68.9, 61.6, 61.3, 56.1, 55.9, 52.2, 48.2, 41.9, 31.8, 28.3, 24.8, 22.9; HRMS (FAB): mass calcd for C₃₁H₄₁NO₁₀ [M + H⁺], 588.2730; found, 588.2810.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl (2S)-2-((tert-butoxycarbonyl)amino)-4-phenylbutanoate (14f)

To a CH₂Cl₂ solution (3 mL) of **10** (27 mg, 0.07 mmol) were added *N*2-(tert-butoxycarbonyl)-l-homophenylalanine (25 mg, 0.08 mmol), EDCI (14 mg, 0.08 mmol) and DMAP (2 mg, 0.01 mmol). After stirring for 17 h, the reaction mixture was diluted with CH₂Cl₂ and washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**14f**) (31 mg, 67 %). ¹H-NMR (600 MHz, CDCl₃) δ 7.28 (m, 2H), 7.21 (m, 3H), 7.06 (d, 1H, *J* = 8.4 Hz), 6.89 (d, 1H, *J* = 8.4 Hz), 4.63 (m, 1H), 4.26 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.09 (m, 1H), 3.89 (s, 3H), 3.84 (s, 3H), 3.78 (s, 3H), 3.75 (s, 3H), 3.17 (dd, 1H, *J* = 13.8 and 4.2 Hz), 2.81 (t, 2H, *J* = 8.4 Hz), 2.72 (m, 1H), 2.63 (dd, 1H, *J* = 13.8 and 10.8 Hz), 2.32 (m, 1H), 2.15 (m, 1H), 1.45 (s, 9H); ¹³C-NMR (150 MHz, CDCl₃) δ 191.0, 159.7, 159.3, 154.4, 149.6, 139.3, 137.5, 131.1, 128.5, 128.4, 127.6, 126.1, 123.4, 120.5, 112.6, 108.6, 96.0, 69.0, 61.6, 61.3, 56.1, 56.0, 55.9, 55.8, 53.4, 48.2, 34.5, 31.8, 31.8, 31.4, 28.3; HRMS (EI): mass calcd for C₃₅H₄₁NO₁₀ [M⁺], 635.2730; found, 635.2733.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl ((benzyloxy)carbonyl)-L-phenylalaninate (14g)

To a CH₂Cl₂ solution (3 mL) of **10** (36 mg, 0.09 mmol) were added *N*-carbobenzoyl-l-phenylalanine (35 mg, 0.11 mmol), EDCI (18 mg, 0.11 mmol) and DMAP (3 mg, 0.01 mmol). After stirring for 17 h, the reaction mixture was diluted with CH₂Cl₂ and washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**14g**) (28 mg, 44 %). ¹H-NMR (600 MHz, CDCl₃) δ 7.32-7.24 (m, 10H), 7.06 (d, 1H, *J* = 7.8 Hz), 6.89 (d, 1H, *J* = 8.4 Hz), 6.82 (bs, 1H), 6.23 (s, 1H), 5.28 (dd, 1H, *J* = 8.4 and 3.0 Hz), 5.09 (dd, 2H, *J* = 6.0 and 3.0 Hz), 4.94 (dd, 1H, *J* = 13.8 and 6.0 Hz), 4.26 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.06 (m, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.34 (dd, 1H, *J* = 13.8 and 5.4 Hz), 3.25 (dd, 1H, *J* = 13.8 and 6.6 Hz), 3.17 (dd, 1H, *J* = 13.8 and 4.2 Hz), 2.71-2.68 (m, 1H), 2.62 (t, 1H, *J* = 12 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 191.0, 159.7, 159.3, 154.4, 149.6, 139.3, 137.5, 131.1, 128.5, 128.4, 127.6, 126.1, 123.4, 120.5, 112.6, 108.6, 96.0, 69.0, 61.6, 61.3, 56.1,

56.0, 55.9, 55.8, 53.4, 48.2, 34.5, 31.8, 31.8, 31.4, 28.3; HRMS (EI): mass calcd for $C_{37}H_{37}NO_{10}$ [M^+], 655.2417; found, 655.2419.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl (ethylcarbamoyl)-L-phenylalaninate (14h)

To a CH_2Cl_2 solution (2 mL) of **10** (16 mg, 0.04 mmol) were added (2*S*)-2-[(ethylcarbamoyl)amino]-3-phenylpropanoic acid (10 mg, 0.04 mmol), EDCI (10 mg, 0.06 mmol) and DMAP (1 mg, 0.004 mmol). After stirring for 24h, the reaction mixture was diluted with CH_2Cl_2 and washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the acylated 3-benzyl-chroman-4-one (**14h**) (15 mg, 56 %). 1H -NMR (600 MHz, $CDCl_3$) δ 7.28(bs, 5H), 7.05(d, 1H, J = 8.4Hz), 6.89(d, 1H, J = 8.4Hz), 6.82(d, 1H, J = 1.8Hz), 6.23(s, 1H), 5.06(dd, 1H, J = 13.2 and 6Hz), 4.25(dd, 1H, J = 10.2 and 3.6Hz), 4.05(m, 1H), 3.90(s, 3H), 3.85(s, 3H), 3.78(s, 3H), 3.77(s, 3H), 3.05-3.21(m, 2H), 3.14(d, 3H, J = 9Hz), 2.69(m, 1H), 2.61(dd 1H, J = 13.8 and 10.8Hz), 1.06(t, 3H, J = 3Hz); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 191.1, 159.7, 159.3, 157.0, 154.4, 149.6, 149.6, 139.2, 137.5, 136.2, 131.0, 129.7, 128.5, 127.6, 127.0, 123.5, 112.6, 108.6, 96.0, 69.0, 61.6, 61.3, 56.1, 55.8, 53.7, 48.2, 38.4, 35.3, 31.8, 15.3; HRMS (FAB): mass calcd for $C_{32}H_{36}N_2O_9$ [$M + H^+$], 593.2421; found, 593.2506.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl (butylcarbamoyl)-L-phenylalaninate (14i)

To a CH_2Cl_2 solution (2 mL) of **10** (23 mg, 0.06 mmol) were added *N*-[(butylamino)carbonyl]-L-phenylalanine (16 mg, 0.06 mmol), EDCI (13 mg, 0.09 mmol) and DMAP (2 mg, 0.01 mmol). After stirring for 15 h, the reaction mixture was diluted with CH_2Cl_2 and washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the acylated 3-benzyl-chroman-4-one (**14i**) (18 mg, 49 %). 1H -NMR (600 MHz, $CDCl_3$) δ 7.29-2.22 (m, 5H), 7.05 (d, 1H, J = 8.4 Hz), 6.88 (d, 1H, J = 9 Hz), 6.81 (s, 1H), 6.23 (s, 1H), 5.06-5.02 (m, 1H), 4.25 (dd, 1H, J = 11.4 and 4.2 Hz), 4.06 (dd, 1H, J = 11.4 and 7.2 Hz), 3.89 (s, 3H), 3.85 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H), 3.27 (m, 1H), 3.23 (dd, 1H, J = 13.8 and 5.4 Hz), 3.15 (d, 1H, J = 13.8 Hz), 3.07 (s, 2H), 2.7022.67 (m, 1H), 2.61 (dd, 1H, J = 13.8 and 10.2 Hz), 1.39 (m, 2H), 1.27 (m, 2H), 0.86 (t, 3H, J = 4.8 Hz); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 191.1, 159.7, 159.3, 157.2, 124.4, 151.8, 149.6, 139.2, 137.5, 136.3, 131.0, 129.7, 128.4, 127.5, 126.9, 123.5, 112.6, 108.6, 96.0, 69.0, 61.6, 61.2, 56.1, 55.8, 53.8, 48.2, 40.2, 38.4, 32.1, 31.8, 19.9, 13.7; HRMS (FAB): mass calcd for $C_{34}H_{40}N_2O_9$ [$M + H^+$], 621.2734; found, 621.2830.

2-Methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl tosyl-L-phenylalaninate (14j)

To a CH_2Cl_2 solution (3 mL) of **10** (26 mg, 0.07 mmol) were added *N*-(*p*-toluenesulfonyl)-L-phenylalanine (27 mg, 0.08 mmol), EDCI (13 mg, 0.08 mmol) and DMAP (2 mg, 0.01 mmol). After stirring for 17 h, the reaction mixture was diluted with CH_2Cl_2 and washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The

residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**14j**) (24 mg, 51%). ¹H-NMR (600 MHz, CDCl₃) 7.66 (d, 2H, *J* = 7.8 Hz), 7.26-7.20 (m, 7H), 7.04 (dt, 1H, *J* = 8.4 and 2.4 Hz), 6.55 (d, 1H, *J* = 1.8 Hz), 6.24 (s, 1H), 5.08 (dd, 1H, *J* = 9 and 5.4 Hz), 4.48 (m, 1H), 4.25 (dd, 1H, *J* = 11.4 and 4.2 Hz), 4.05 (m, 1H), 3.91 (s, 3H), 3.86 (s, 3H), 3.79 (s, 3H), 3.70 (s, 3H), 3.26 (dd, 1H, *J* = 7.8 and 5.4 Hz), 3.17 (m, 2H), 2.69 (m, 1H), 2.60 (m, 1H), 2.38 (d, 3H, *J* = 7.2 Hz); ¹³C-NMR (150 MHz, CDCl₃) δ 191.1, 159.7, 159.3, 157.0, 154.4, 149.6, 149.6, 139.2, 137.5, 136.2, 131.0, 129.7, 128.5, 127.6, 127.0, 123.5, 112.6, 108.6, 96.0, 69.0, 61.6, 61.3, 56.1, 55.8, 53.7, 48.2, 38.4, 35.3, 31.8, 15.3; HRMS (EI): mass calcd for C₃₆H₃₇NO₁₀ [M + H⁺], 675.2138; found, 675.2136.

***tert*-Butyl ((2*S*-1-((2-methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**15a**)**

To a CH₂Cl₂ solution (3 mL) of **12b** (35 mg, 0.09 mmol) were added Boc-Phe-OH (27 mg, 0.09 mmol), EDCI (17 mg, 0.1 mmol) and DMAP (3 mg, 0.01 mmol). After stirring for 17 h, the reaction mixture was diluted with CH₂Cl₂ and washed with water and brine, dried over anhydrous MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**15a**) (24 mg, 42%). ¹H-NMR (600 MHz, CDCl₃) δ 8.20 (s, 1H), 8.08 (bs, 1H), 7.28 (d, 2H, *J* = 7.2 Hz), 7.22 (d, 3H, *J* = 7.2 Hz), 6.88 (d, 1H, *J* = 8.4 Hz), 6.74 (d, 1H, *J* = 7.8 Hz), 6.23 (d, 1H, *J* = 1.2 Hz), 5.11 (bs, 1H), 4.48 (bs, 1H), 4.28 (dd, 1H, *J* = 10.8 and 3.6 Hz), 4.11 (q, 1H, *J* = 7.2 Hz), 3.90 (s, 3H), 3.85 (s, 3H), 3.78 (s, 1H), 3.71 (s, 3H), 3.21 (dd, 1H, *J* = 13.8 and 3.6 Hz), 3.12 (bs, 2H), 2.79 (m, 1H), 2.61 (t, 1H, *J* = 12 Hz), 1.4 (s, 9H); ¹³C-NMR (150 MHz, CDCl₃) δ 191.2, 169.2, 159.7, 159.2, 155.3, 154.4, 146.7, 137.4, 136.5, 131.0, 129.2, 128.7, 127.0, 126.9, 124.4, 120.3, 110.0, 108.7, 96.0, 69.1, 61.6, 61.3, 56.6, 56.0, 55.6, 48.3, 48.3, 38.5, 32.2, 28.2; HRMS (ESI): mass calcd for C₃₄H₄₀N₂O₁₀ [M + H⁺], 621.2807; found, 621.2804.

***tert*-Butyl ((2*S*-3-(4''-(benzyloxy)phenyl)-1-((2-methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl)amino)-1-oxopropan-2-yl)carbamate (**15b**)**

To a CH₂Cl₂ solution (6 mL) of **12b** (35 mg, 0.09 mmol) were added Boc-Tyr(Bzl)-OH (35 mg, 0.09 mmol), EDCI (18 mg, 0.1 mmol) and DMAP (2 mg, 0.01 mmol). After stirring for 17 h, the reaction mixture was diluted with CH₂Cl₂ and washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**15b**) (50 mg, 72%). ¹H-NMR (600 MHz, CDCl₃) δ 8.22 (s, 1H), 8.10 (bs, 1H), 7.41 (d, 2H, *J* = 7.8 Hz), 7.37 (t, 2H, *J* = 7.2 Hz), 7.31 (t, 1H, *J* = 7.2 Hz), 7.16 (d, 2H, *J* = 8.4 Hz), 6.90 (d, 3H, *J* = 8.4 Hz), 6.75 (d, 1H, *J* = 7.8 Hz), 6.24 (s, 1H), 5.01 (s, 2H), 4.46 (bs, 1H), 4.29 (dd, 1H, *J* = 10.8 and 3.6 Hz), 4.12 (m, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H), 3.73 (s, 3H), 3.23 (dd, 1H, *J* = 14.4 and 3.6 Hz), 3.10 (m, 2H), 2.81 (m, 1H), 2.63 (m, 1H), 1.42 (s, 9H); ¹³C-NMR (150 MHz, CDCl₃) δ 191.3, 169.4, 159.7, 159.2, 127.8, 154.4, 146.7, 137.4, 136.9, 131.0, 130.3, 128.5, 127.9, 127.4, 127.0, 124.4, 120.3, 115.0, 110.0, 108.7, 96.0, 70.0, 69.2, 69.2, 61.6, 61.3, 56.0, 55.6, 48.3, 48.3, 37.7, 32.2, 30.9, 29.7, 28.2; HRMS (FAB): mass calcd for C₄₁H₄₆N₂O₁₀ [M + H⁺], 727.3152; found, 727.3241.

***tert*-Butyl ((2*S*-3-(4''-(allyloxy)phenyl)-1-((2-methoxy-5-((5',6',7'-trimethoxy-4'-oxochroman-3'-yl)methyl)phenyl)amino)-1-oxopropan-2-yl)carbamate (15c)**

To a CH₂Cl₂ solution (3 mL) of **12b** (35 mg, 0.09 mmol) were added Boc-Tyr(Bzl)-OH (27 mg, 0.10 mmol), EDCI (17 mg, 0.10 mmol) and DMAP (3 mg, 0.01 mmol). After stirring for 17 h, the reaction mixture was diluted with CH₂Cl₂ and washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the acylated 3-benzyl-chroman-4-one (**15c**) (42 mg, 71%). ¹H-NMR (600 MHz, CDCl₃) δ 8.22 (d, 1H, *J* = 2.4 Hz), 8.07 (bs, 1H), 7.15 (d, 2H, *J* = 8.4 Hz), 6.90 (d, 1H, *J* = 8.4 Hz), 6.84 (d, 2H, *J* = 7.2 Hz), 6.76 (d, 1H, *J* = 8.4 Hz), 6.25 (d, 1H, *J* = 1.2 Hz), 6.06-5.99 (m, 1H), 5.40 (dd, 1H, *J* = 17.4 and 1.8 Hz), 5.27 (dd, 1H, *J* = 10.8 and 1.2 Hz), 5.12 (bs, 1H), 4.49 (dd, 2H, *J* = 5.4 and 1.2 Hz), 4.30 (dd, 1H, *J* = 11.4 and 3.6 Hz), 4.13 (m, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 3.74 (s, 3H), 3.24 (dd, 1H, *J* = 14.4 and 4.2 Hz), 3.10 (m, 1H), 3.06 (bs, 1H), 2.81-2.75 (m, 1H), 2.63 (m, 1H), 1.43 (s, 9H); HRMS (FAB): mass calcd for C₃₇H₄₄N₂O₁₀ [M+H⁺], 677.2996; found, 677.3074.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AMD	age-related macular degeneration
homophe	homophenylalanine
HREC	retinal human microvascular endothelial cell
HUVEC	human umbilical vein endothelial cell
OIR	oxygen-induced retinopathy
PDR	proliferative diabetic retinopathy
ROP	retinopathy of prematurity
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
VEGF	vascular endothelial growth factor

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Figure 1.
Homoisoflavonoid analog design.

Reagents and conditions: (i) $(\text{CH}_3)_2\text{NCH}(\text{OCH}_3)_2$, toluene, reflux (82~97%); (ii) H_2 , Pd/C, MeOH, rt (87~99%); (iii) isovanillin, *p*-TsOH, benzene, reflux (60~77%); (iv) TMSI, CHCl_3 , 60 °C (45~83%).

Scheme 1.

Synthesis of Cremastranone and Its A-ring Modified Analogs via Chroman-4-ones.

Reagents and conditions: (i) benzyl bromide or allyl bromide, K₂CO₃, acetone (79% for **7d**, 83% for **7e** and 84% for **12e**); (ii) arylaldehydes, *p*-TsOH, benzene, reflux (59~87%); (iii) cinnamoyl chloride, BF₃·Et₂O, reflux (98%); (iv) H₂, Pd/C, MeOH, rt (96%); (v) aq. HCHO, NaOH, 60 °C (43%); (vi) LiOH, H₂O, THF (56%); (vii), benzoyl chloride or cinnamoyl chloride, acetone (82% for **12f** and 80% for **12g**); (viii) ClCONEt₂, Et₃N, toluene, reflux (40%).

Scheme 2.

Synthesis of B Ring-Modified Homoisoflavonoids (**7d**~**7l** and **12a**~**12i**).

Reagents and conditions: (i) *N*-substituted amino acids, EDCI (or DCC), DMAP, CH₂Cl₂, rt (42~92%).

Scheme 3.

Synthesis of Homoisoflavonoids (**14a~14j** and **15a~15c**) Comprising Amino Acids on the C3' Position of the B-Ring.

Figure 2.

Compound **14a** inhibits angiogenic behavior of HRECs in vitro. (A) Dose-response of the effects of **14a** on HREC proliferation as measured by alamarBlue fluorescence. (B) **14a** dose-dependently inhibits migration of HRECs in a scratch-wound assay. (C) **14a** dose-dependently inhibits tube formation of HRECs on Matrigel. (D) **14a** caused negligible apoptosis as assayed by activated caspase 3 (pink) immunofluorescence. 1 μ M staurosporine (SP) is positive control. DAPI (blue) indicates normal nuclear morphology. Error bars indicate SEM, n=3, representative results from at least triplicate experiments. *P<0.05, **P<0.01 and ***P<0.001. Scale bars = 200 μ m.

Figure 3.

Homoisoflavonoid **14a** inhibits retinal neovascularization in the OIR mouse model. Retinal whole mounts from treated mice were stained for blood vessels using Alexa-488-conjugated isolectin and imaged by confocal microscopy; neovascular area was measured using Adobe Photoshop. Error bars indicate SEM, n=6. **P<0.01.

Table 1

Growth Inhibitory Activity (GI₅₀, μ M) of A-ring Modified Homoisoflavanones on the Proliferation of Microvascular (HREC), Macrovascular (HUVEC) and Ocular Tumor (92-1 and Y79) Cells. 95% Confidence Interval Shown in Parentheses.

Cpd	A ring	HREC	HUVEC	92-1	Y79
1		0.22 (0.12 – 0.39)	0.38 (0.24 – 0.59)	48 (17 – 132)	9.8 (2.1 – 45)
2		45 (26 – 75)	18 (16 – 21)	10 (4.4 – 23)	>100
8		>100	>100	>100	>100
9		>100	44 (34 – 58)	>100	>100
10		2 (0.81 – 5.1)	12 (2.7 – 55)	>100	>100
11		1.6 (1.0 – 2.6)	2.5 (0.87 – 7.1)	>100	4.2 (2.0 – 9.1)

Table 2

Growth Inhibitory Activity (GI₅₀, μ M) of B-ring Modified 3-Benzylidene-4-chromanone Analogs. 95% Confidence Interval Shown in Parentheses.

Cpd	B ring	HREC	HUVEC	92-1	Y79
7b		46 (17 – 122)	5.6 (3.2 – 9.8)	0.22 (0.061 – 0.81)	44 (22 – 89)
7c ^a		42 (12 – 146)	15 (4.4 – 51)	>100	14 (7.2 – 25)
7d		4.3 (1.9 – 9.7)	16 (5.2 – 49)	3.5 (1.4 – 9.0)	33 (6.1 – 180)
7e		3.9 (1.9 – 8.1)	12 (7.6 – 18)	1.1 (0.34 – 3.6)	2.8 (1.5 – 5.1)
7f		4.8 (2.2 – 11)	15 (7.2 – 31)	12 (6.5 – 24)	2.2 (0.72 – 6.4)
7g		5.6 (2.6 – 12)	3.2 (1.4 – 7.3)	>100	6.1 (3.4 – 11)
7h		2.8 (1.1 – 7.1)	7.6 (3.2 – 18)	7.0 (1.9 – 26)	9.8 (4.1 – 23)
7i		3.3 (1.7 – 6.4)	6.2 (5.1 – 7.7)	26 (8.6 – 77)	5.2 (3.2 – 8.2)

Cpd	B ring	HREC	HUVEC	92-1	Y79
7j		35 (14 – 83)	52 (27 – 100)	68 (25 – 186)	14 (8.7 – 24)
7k		7.6 (2.7 – 22)	5.0 (2.2 – 11)	9.5 (4.8 – 19)	4.0 (0.86 – 19)
7l		72 (18 – 277)	43 (9.1 – 204)	>100	32 (12 – 88)

^a3-(3'-hydroxy-4'-methoxybenzylidene)-5,7-dimethoxychroman-4-one

Table 3

Growth Inhibitory Activity (GI₅₀, μ M) of B-ring Modified 3-Benzyl-4-chromanone Analogs. 95% Confidence Interval Shown in Parentheses.

Cpd	B ring	HREC	HUVEC	92-1	Y79
12a		>100	>100	>100	>100
12b		1.1 (0.29 – 4.4)	0.51 (0.11 – 25)	>100	>100
12c		18 (6.1 – 50)	17 (5.1 – 57)	>100	40 (24 – 66)
12d		>100	92 (27 – 317)	>100	>100
12e		22 (13 – 40)	>100	61 (14 – 278)	20 (8.1 – 51)
12f		0.65 (0.26 – 1.6)	>100	>100	>100
12g		0.17 (0.030 – 0.97)	>100	>100	>100
12h		0.22 (0.064 – 0.77)	38 (13 – 109)	>100	>100

Cpd	B ring	HREC	HUVEC	92-1	Y79
12i	 <chem>CCOC(=O)c1ccccc1C(=O)NCC</chem>	49 (17 – 141)	>100	>100	24 (6.1 – 97)

Table 4

Growth Inhibitory Activity (GI₅₀, μ M) of Homoisoflavonoids Comprising Amino Acids on the C3' Position of the B-Ring. 95% Confidence Interval Shown in Parentheses.

Cpd	B ring	HREC	HUVEC	92-1	Y79
14a		0.055 (0.032 – 0.094)	0.75 (0.37 – 1.5)	>100	12 (5.7 – 25)
14b		0.51 (0.26 – 1.0)	>100	>100	>100
14c		0.16 (0.020 – 1.3)	0.091 (0.013) – 0.63)	>100	52 17 – 166
14d		3.1 (1.3 – 7.4)	>100	>100	24 (7.1 – 79)
14e		0.13 (0.026 – 0.69)	>100	>100	>100
14f		0.17 (0.035 – 0.82)	>100	>100	>100

X = O for 14a~14j X = NH for 15a~15c					
Cpd	B ring	HREC	HUVEC	92-1	Y79
14g		0.14 (0.027 – 0.73)	>100	>100	>100
14h		1.0 (0.031 – 3.6)	34 (7.2 – 165)	98 (37 – 265)	48 (33 – 69)
14i		1.4 (0.73 – 2.4)	>100	>100	64 (26 – 158)
14j		1.5 (0.41 – 5.4)	22 (11 – 43)	>100	>100
15a		22 (16 – 32)	8.6 (1.2 – 6.1)	3.1 (0.93 – 10)	4.7 (1.2 – 18)
15b		>100	>100	>100	0.39 (0.12 – 1.3)
15c		13 (9.7 – 17)	4.5 (1.2 – 17)	1.9 (0.65 – 5.3)	2.5 (1.4 – 4.6)