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 homoisoflavane 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
study to develop more potent cremastranone analogs, with improved antiproliferative selectivity for retinal endothelial cells. Phenylalanyl-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.

We\(^5\) and others\(^6\) 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 neovascularization\(^9\) 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\text{-}hydroxy\text{-}4\text{-}methoxybenzylidene)\text{-}6\text{-}methyl\text{-}4\text{-}chromanone was
shown to have submicromolar antiangiogenic effects in vitro and efficacy in a laser-induced choroidal neovascularization model, while a related compound (E)-3-(-2methoxybenzylidene)-4-chromanone was shown to have antiproliferative effects on human umbilical vein endothelial cells (HUVECs). Of natural-source homoisoflavanones, methylophiopogonanone B blocked HUVEC angiogenesis and the hypoxic response in vitro, while very recently methylophiopogonanone A was shown to block brain endothelial cell activation. In addition, considerable work has documented antiangiogenic effects of the related chalcones, flavones and flavanones. 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). It also blocks prostaglandin synthesis from arachidonic acid, and decreases phosphorylation of the mitogen activated protein kinases. Finally, it blocks nuclear translocation of NF-κB and production of inflammatory cytokines.

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 homoisoflavanoids 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 homoisoflavanoid analog design by which several substituents on the A and B rings could be varied as shown in Figure 1. We planned to synthesize homoisoflavanoids with highly oxygenated substitutions such as methoxy and hydroxy on the A ring, 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 homoisoflavanoids.

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. 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.

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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 arylden aldehydes 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).

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 NH$_2$ 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, 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 32benzyl-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 (GI$_{50}$ 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.
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 μM 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. 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 μM 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 μM 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-4H-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), 6.20 (s, 1H), 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. $^1$H-NMR (CDCl$_3$, 600 MHz) $\delta$ 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); $^{13}$C-NMR (150 MHz, CDCl$_3$) $\delta$ 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. $^1$H-NMR (CDCl$_3$, 600 MHz) $\delta$ 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); $^{13}$C-NMR (150 MHz, CDCl$_3$) $\delta$ 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 C$_{19}$H$_{18}$O$_7$ [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%). $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 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); $^{13}$C-NMR (150 MHz, CDCl$_3$) $\delta$ 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 C$_{20}$H$_{20}$O$_7$ [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%). $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 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 C$_{19}$H$_{18}$O$_{6}$ [M$^+$], 342.1103; found, 342.1101.

7-Hydroxy-3-(3′-hydroxy-4′-methoxybenzyl)-5,6-dimethoxycroman-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%).

$^1$H-NMR (600 MHz, CD$_3$OD) 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$_3$OD) 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 C$_{19}$H$_{20}$O$_{7}$ [M + H$^+$], 361.1287; found, 361.1270. Compound 8 was reported. See ref 7.

3-(3′-Hydroxy-4′-methoxybenzyl)-5,6,7-trimethoxycroman-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%).

$^1$H-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, 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 C$_{20}$H$_{22}$O$_{7}$ [M + H$^+$], 375.1444; found, 375.1432. Compound 10 was reported. See ref 5.

5-Hydroxy-3-(3′-hydroxy-4′-methoxybenzyl)-6,7-dimethoxycroman-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 °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 afforded the demethylated 3-benzyl-chroman-4-one (9) (23 mg, 42%).

$^1$H-NMR (400 MHz, CDCl$_3$) δ 11.96 (s, 1H), 6.86 (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 198.4, 160.7, 158.6, 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 C$_{20}$H$_{22}$O$_{7}$ [M + H$^+$], 375.1444; found, 375.1432. Compound 9 was reported. See ref 5.
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, CDCl3) 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 = 11.4 and 4.2 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); 13C-NMR (150 MHz, CDCl3) 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 C19H20O6 [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 K2CO3 (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 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 benzylated 3-benzylidene-chroman-4-one (7d) (120 mg, 79%).

1H-NMR (400 MHz, CDCl3) 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); 13C-NMR (100 MHz, CDCl3) 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 C27H26O7 [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 μL, 0.02 mmol) and K2CO3 (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 MgSO4 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, CDCl3) 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), 3.97 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H); 13C-NMR (150 MHz, CDCl3) δ 179.51, 159.32, 159.19, 154.78, 150.57, 147.82, 137.88, 136.36, 133.05, 130.01, 127.41, 127.31, 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 C27H26O7 [M+], 462.1679; found, 462.1679.
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 × 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%). $^1$H-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 × 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%). $^1$H-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$_2$CO$_3$ (138 mg, 0.72 mmol). After refluxing for 4h, the reaction mixture was diluted with ethyl acetate (10 mL × 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 methyl (E)-2-hydroxy-5-((5,6,7-trimethoxy-4-oxochroman-3-ylidene)methyl)benzoate (100 mg, 24 mmol) and K$_2$CO$_3$ (138 mg, 0.72 mmol). After refluxing for 4h, the reaction mixture was diluted with ethyl acetate (10 mL × 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%). $^1$H-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.

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(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), 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, 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 87.58 (d, 1H, J = 2.4 Hz), 7.29 (dd, 1H, J = 8.4 and 2.4 Hz), 6.88 (d, 1H, J = 9Hz), 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,
2-Methoxy-5-(5′,6′,7′-trimethoxy-4′-oxochroman-3′-yl)methylbenzoic 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 × 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\(_3\)OD) \(\delta\) 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); 13C-NMR (150 MHz, CD\(_3\)OD) \(\delta\) 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, 50.2, 55.3, 54.6, 31.5, 22.5; HRMS (EI): mass calcd for C\(_{21}\)H\(_{22}\)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\(_2\)CO\(_3\) (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\(_3\)) \(\delta\) 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); 13C-NMR (150 MHz, CDCl\(_3\)) \(\delta\) 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 C\(_{27}\)H\(_{28}\)O\(_7\) [M\(^+\)], 464.1835; found, 464.1837.

2-Methoxy-5-(5′,6′,7′-trimethoxy-4′-oxochroman-3′-yl)methylphenyl benzoate (12f)

To an acetone (5 mL) solution of 10 (36 mg, 0.09 mmol), benzoyl chloride (17 μL, 0.11 mmol) and K\(_2\)CO\(_3\) (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, CDCl\(_3\)) \(\delta\) 7.63 (dd, 1H, J = 14.4 and 10.8 Hz); 13C-NMR (150 MHz, CDCl\(_3\)) \(\delta\) 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 C\(_{27}\)H\(_{28}\)O\(_7\) [M\(^+\)], 464.1835; found, 464.1837.
MHz, CDCl$_3$ $\delta$ 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); $^{13}$C-NMR (150 MHz, CDCl$_3$) $\delta$ 191.1, 164.7, 159.7, 159.3, 154.5, 150.0, 139.9, 137.5, 133.5, 131.0, 129.3, 128.5, 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 C$_{27}$H$_{26}$O$_8$ [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 K$_2$CO$_3$ (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 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 (12g) (80 mg, 77%). $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 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); $^{13}$C-NMR (150 MHz, CDCl$_3$) $\delta$ 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 C$_{29}$H$_{28}$O$_8$ [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 %). $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 7.33-7.22 (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); $^{13}$C-NMR (150 MHz, CDCl$_3$) $\delta$ 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 C$_{29}$H$_{30}$O$_8$ [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 μL, 0.05 mmol) and Et₃N (17 μL, 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 C₂₅H₃₁NO₸ [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, 154.0, 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 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 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 (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); 13C-NMR (150 MHz, CDCl3) δ 191.0, 170.1, 159.6, 159.3, 157.9, 155.0, 149.6, 139.2, 137.5, 136.9, 131.0, 130.6, 128.5, 128.2, 127.9, 127.6, 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 C41H45NO11, [M + 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 CH2Cl2 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 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 (14c) (85 mg, 84%). 1H-NMR (600 MHz, CDCl3) 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), 3.24 (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); 13C-NMR (150 MHz, CDCl3) δ 191.0, 170.1, 159.6, 159.3, 157.7, 157.6, 150.4, 149.6, 139.2, 137.4, 133.2, 131.0, 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 C37H43NO11 [M + 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 CH2Cl2 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 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 (14d) (33 mg, 95%). 1H-NMR (600 MHz, CDCl3) 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 4.2Hz), 2.75 (m, 1H), 2.64 (m, 1H), 1.42 (s, 9H), 1.32 (s, 9H); HRMS (FAB): mass calcd for C31H41NO10 [M+ 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 CH2Cl2 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,
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, 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 C₃₅H₄₁NO₁₀ [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 N₂(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, 31.4, 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-carbobenzyo-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) 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, 31.4, 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 (ethylcarbamoyl)-L-phenylalaninate (14h)

To a CH\textsubscript{2}Cl\textsubscript{2} solution (2 mL) of 10 (16 mg, 0.04 mmol) were added (2S)-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\textsubscript{2}Cl\textsubscript{2} and washed with water and brine, dried over MgSO\textsubscript{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 %).

\textsuperscript{1}H-NMR (600 MHz, CDCl\textsubscript{3}) \(\delta\) 7.28(bs, 5H), 7.05(d, 1H, \(J = 8.4\)Hz), 6.89(d, 1H, \(J = 8.4\)Hz), 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, \(J = 9\)Hz), 2.69(m, 1H), 2.61(dd 1H, \(J = 13.8\) and 10.8Hz), 1.06(t, 3H, \(J = 3\)Hz); \textsuperscript{13}C-NMR (150 MHz, CDCl\textsubscript{3}) \(\delta\) 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\textsubscript{32}H\textsubscript{36}N\textsubscript{2}O\textsubscript{9} [M + H\textsuperscript{+}], 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\textsubscript{2}Cl\textsubscript{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\textsubscript{2}Cl\textsubscript{2} and washed with water and brine, dried over MgSO\textsubscript{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 %).

\textsuperscript{1}H-NMR (600 MHz, CDCl\textsubscript{3}) \(\delta\) 7.30-2.22 (m, 5H), 7.05 (d, 1H, \(J = 8.4\) Hz), 6.88(d, 1H, \(J = 8.4\)Hz), 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); \textsuperscript{13}C-NMR (150 MHz, CDCl\textsubscript{3}) \(\delta\) 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\textsubscript{34}H\textsubscript{40}N\textsubscript{2}O\textsubscript{9} [M + H\textsuperscript{+}], 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\textsubscript{2}Cl\textsubscript{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\textsubscript{2}Cl\textsubscript{2} and washed with water and brine, dried over MgSO\textsubscript{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\%). \(^1\)H-NMR (600 MHz, CDCl\(_3\)) \(\delta\) 7.66 (d, 2H, \(J = 7.8\) Hz), 7.26-7.20 (m, 7H), 7.04 (dt, 1H, \(J = 9\) and 5.4 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); \(^1\)C-NMR (150 MHz, CDCl\(_3\)) \(\delta\) 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 C\(_{36}\)H\(_{37}\)NO\(_{10}\) [M + H\(^+\)], 675.2138; found, 675.2136.

tert-Butyl ((2S-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 \(\text{CH}_2\text{Cl}_2\) 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 \(\text{CH}_2\text{Cl}_2\) 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 \((15a)\) (24 mg, 42\%). \(^1\)H-NMR (600 MHz, CDCl\(_3\)) \(\delta\) 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); \(^1\)C-NMR (150 MHz, CDCl\(_3\)) \(\delta\) 191.2, 169.2, 159.7, 159.2, 155.3, 154.4, 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, 56.1, 56.0, 55.6, 48.3, 48.3, 38.5, 32.2, 28.2; HRMS (ESI): mass calcd for C\(_{34}\)H\(_{40}\)N\(_2\)O\(_{10}\) [M + H\(^+\)], 621.2807; found, 621.2804.

tert-Butyl ((2S-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 \(\text{CH}_2\text{Cl}_2\) 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 \(\text{CH}_2\text{Cl}_2\) 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 \((15b)\) (50 mg, 72\%). \(^1\)H-NMR (600 MHz, CDCl\(_3\)) \(\delta\) 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); \(^1\)C-NMR (150 MHz, CDCl\(_3\)) \(\delta\) 191.3, 169.4, 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, 56.1, 56.0, 55.6, 48.3, 48.3, 38.5, 32.2, 28.2; HRMS (FAB): mass calcd for C\(_{41}\)H\(_{46}\)N\(_2\)O\(_{10}\) [M + H\(^+\)], 727.3152; found, 727.3241.

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**tert-Butyl (2S-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 CH$_2$Cl$_2$ 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 CH$_2$Cl$_2$ 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 (15c) (42 mg, 71%). $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 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 C$_{37}$H$_{44}$N$_2$O$_{10}$ [M+H$^+$], 677.2996; found, 677.3074.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**ACKNOWLEDGMENT**

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**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMD</td>
<td>age-related macular degeneration</td>
</tr>
<tr>
<td>homophe</td>
<td>homophenylalanine</td>
</tr>
<tr>
<td>HREC</td>
<td>retinal human microvascular endothelial cell</td>
</tr>
<tr>
<td>HUVEC</td>
<td>human umbilical vein endothelial cell</td>
</tr>
<tr>
<td>OIR</td>
<td>oxygen-induced retinopathy</td>
</tr>
<tr>
<td>PDR</td>
<td>proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>ROP</td>
<td>retinopathy of prematurity</td>
</tr>
<tr>
<td>TUNEL</td>
<td>Terminal deoxynucleotidyl transferase dUTP nick end labeling</td>
</tr>
<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
</tr>
</tbody>
</table>

_J Med Chem._ Author manuscript; available in PMC 2016 July 14.
REFERENCES


15. a Fotis T, Pepper MS, Aktas E, Breit S, Rasku S, Adlercreutz H, Wahala K, Montesano R, Schweigerer L. Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro


Figure 1.
Homoisoflavonoid analog design.
Reagents and conditions: (i) (CH$_3$)$_2$NCH(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, K$_2$CO$_3$, acetone (79% for 7d, 83% for 7e and 84% for 12e); (ii) arylaldehydes, p-TsOH, benzene, reflux (59–87%); (iii) cinnamoyl chloride, BF$_3$·Et$_2$O, reflux (98%); (iv) H$_2$, Pd/C, MeOH, rt (96%); (v) aq. HCHO, NaOH, 60 ºC (43%); (vi) LiOH, H$_2$O, THF (56%); (vii), benzoyl chloride or cinnamoyl chloride, acetone (82% for 12f and 80% for 12g); (viii) ClCONEt$_2$, Et$_3$N, 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, CH$_2$Cl$_2$, rt (42~92%).

Scheme 3.
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 μM 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 μm.
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 (GI_{50}, μM) 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.

<table>
<thead>
<tr>
<th>Cpd</th>
<th>A ring</th>
<th>HREC</th>
<th>HUVEC</th>
<th>92-1</th>
<th>Y79</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.22 (0.12 – 0.39)</td>
<td>0.38 (0.24 – 0.59)</td>
<td>48 (17 – 132)</td>
<td>9.8 (2.1 – 45)</td>
</tr>
<tr>
<td>2</td>
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<td>45 (26 – 75)</td>
<td>18 (16 – 21)</td>
<td>10 (4.4 – 23)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>&gt;100</td>
<td>44 (34 – 58)</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>2 (0.81 – 5.1)</td>
<td>12 (2.7 – 55)</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>1.6 (1.0 – 2.6)</td>
<td>2.5 (0.87 – 7.1)</td>
<td>&gt;100</td>
<td>4.2 (2.0 – 9.1)</td>
</tr>
</tbody>
</table>
Table 2
Growth Inhibitory Activity (GI_{50}, \mu M) of B-ring Modified 3-Benzylidene-4-chromanone Analogs. 95% Confidence Interval Shown in Parentheses.

<table>
<thead>
<tr>
<th>Cpd</th>
<th>B ring</th>
<th>HREC</th>
<th>HUVEC</th>
<th>92-1</th>
<th>Y79</th>
</tr>
</thead>
<tbody>
<tr>
<td>7b</td>
<td></td>
<td>46 (17 – 122)</td>
<td>5.6 (3.2 – 9.8)</td>
<td>0.22 (0.061 – 0.81)</td>
<td>44 (22 – 89)</td>
</tr>
<tr>
<td>7c</td>
<td></td>
<td>42 (12 – 146)</td>
<td>15 (4.4 – 51)</td>
<td>&gt;100</td>
<td>14 (7.2 – 25)</td>
</tr>
<tr>
<td>7d</td>
<td></td>
<td>4.3 (1.9 – 9.7)</td>
<td>16 (5.2 – 49)</td>
<td>3.5 (1.4 – 9.0)</td>
<td>33 (6.1 – 180)</td>
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<tr>
<td>7e</td>
<td></td>
<td>3.9 (1.9 – 8.1)</td>
<td>12 (7.6 – 18)</td>
<td>1.1 (0.34 – 3.6)</td>
<td>2.8 (1.5 – 5.1)</td>
</tr>
<tr>
<td>7f</td>
<td></td>
<td>4.8 (2.2 – 11)</td>
<td>15 (7.2 – 31)</td>
<td>12 (6.5 – 24)</td>
<td>2.2 (0.72 – 6.4)</td>
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<tr>
<td>7g</td>
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<td>5.6 (2.6 – 12)</td>
<td>3.2 (1.4 – 7.3)</td>
<td>&gt;100</td>
<td>6.1 (3.4 – 11)</td>
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<td>7h</td>
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<td>2.8 (1.1 – 7.1)</td>
<td>7.6 (3.2 – 18)</td>
<td>7.0 (1.9 – 26)</td>
<td>9.8 (4.1 – 23)</td>
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<tr>
<td>7i</td>
<td></td>
<td>3.3 (1.7 – 6.4)</td>
<td>6.2 (5.1 – 7.7)</td>
<td>26 (8.6 – 77)</td>
<td>5.2 (3.2 – 8.2)</td>
</tr>
<tr>
<td>Cpd</td>
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<td>HREC</td>
<td>HUVEC</td>
<td>92-1</td>
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<tr>
<td>7j</td>
<td><img src="7j.png" alt="Image" /></td>
<td>35 (14 – 83)</td>
<td>52 (27 – 100)</td>
<td>68 (25 – 186)</td>
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</tr>
<tr>
<td>7k</td>
<td><img src="7k.png" alt="Image" /></td>
<td>7.6 (2.7 – 22)</td>
<td>5.0 (2.2 – 11)</td>
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<tr>
<td>7l</td>
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<td>72 (18 – 277)</td>
<td>43 (9.1 – 204)</td>
<td>&gt;100</td>
<td>32 (12 – 88)</td>
</tr>
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</table>

\(^a\)3-(3′-hydroxy-4′-methoxybenzylidene)-5,7-dimethoxychroman-4-one
Table 3
Growth Inhibitory Activity (GI_{50}, μM) of B-ring Modified 3-Benzyl-4-chromanone Analogs. 95% Confidence Interval Shown in Parentheses.

<table>
<thead>
<tr>
<th>Cpd</th>
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<th>HREC</th>
<th>HUVEC</th>
<th>92-1</th>
<th>Y79</th>
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</thead>
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<td>&gt;100</td>
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<td>&gt;100</td>
</tr>
<tr>
<td>12b</td>
<td><img src="https://example.com/image2.png" alt="image" /></td>
<td>1.1 (0.29 – 4.4)</td>
<td>0.51 (0.11 – 25)</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>12c</td>
<td><img src="https://example.com/image3.png" alt="image" /></td>
<td>18 (6.1 – 50)</td>
<td>17 (5.1 – 57)</td>
<td>&gt;100</td>
<td>40 (24 – 66)</td>
</tr>
<tr>
<td>12d</td>
<td><img src="https://example.com/image4.png" alt="image" /></td>
<td>&gt;100</td>
<td>92 (27 – 317)</td>
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<tr>
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<td><img src="https://example.com/image5.png" alt="image" /></td>
<td>22 (13 – 40)</td>
<td>&gt;100</td>
<td>61 (14 – 278)</td>
<td>20 (8.1 – 51)</td>
</tr>
<tr>
<td>12f</td>
<td><img src="https://example.com/image6.png" alt="image" /></td>
<td>0.65 (0.26 – 1.6)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>12g</td>
<td><img src="https://example.com/image7.png" alt="image" /></td>
<td>0.17 (0.030 – 0.97)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>12h</td>
<td><img src="https://example.com/image8.png" alt="image" /></td>
<td>0.22 (0.064 – 0.77)</td>
<td>38 (13 – 109)</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Cpd</td>
<td>B ring</td>
<td>HREC</td>
<td>HUVEC</td>
<td>92-1</td>
<td>Y79</td>
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<td>-------</td>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>12i</td>
<td><img src="image" alt="Structure" /></td>
<td>49 (17 – 141)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>24 (6.1 – 97)</td>
</tr>
</tbody>
</table>
**Table 4**

Growth Inhibitory Activity (GI\textsubscript{50}, μM) of Homoisoflavonoids Comprising Amino Acids on the C3' Position of the B-Ring. 95% Confidence Interval Shown in Parentheses.

<table>
<thead>
<tr>
<th>Cpd</th>
<th>B ring</th>
<th>HREC</th>
<th>HUVEC</th>
<th>92-1</th>
<th>Y79</th>
</tr>
</thead>
<tbody>
<tr>
<td>14a</td>
<td><img src="structure1.png" alt="Structure" /></td>
<td>0.055 (0.032 – 0.094)</td>
<td>0.75 (0.37 – 1.5)</td>
<td>&gt;100</td>
<td>12 (5.7 – 25)</td>
</tr>
<tr>
<td>14b</td>
<td><img src="structure2.png" alt="Structure" /></td>
<td>0.51 (0.26 – 1.0)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>14c</td>
<td><img src="structure3.png" alt="Structure" /></td>
<td>0.16 (0.020 – 1.3)</td>
<td>0.091 (0.013) – 0.63</td>
<td>&gt;100</td>
<td>52 17 – 166</td>
</tr>
<tr>
<td>14d</td>
<td><img src="structure4.png" alt="Structure" /></td>
<td>3.1 (1.3 – 7.4)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>24 (7.1 – 79)</td>
</tr>
<tr>
<td>14e</td>
<td><img src="structure5.png" alt="Structure" /></td>
<td>0.13 (0.026 – 0.69)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>14f</td>
<td><img src="structure6.png" alt="Structure" /></td>
<td>0.17 (0.035 – 0.82)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Cpd</td>
<td>B ring</td>
<td>HREC</td>
<td>HUVEC</td>
<td>92-1</td>
<td>Y79</td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
<td>------------</td>
<td>-----------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>14g</td>
<td></td>
<td>0.14 (0.027 – 0.73)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>14h</td>
<td></td>
<td>1.0 (0.031 – 3.6)</td>
<td>34 (7.2 – 165)</td>
<td>98 (37 – 265)</td>
<td>48 (33 – 69)</td>
</tr>
<tr>
<td>14i</td>
<td></td>
<td>1.4 (0.73 – 2.4)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>64 (26 – 158)</td>
</tr>
<tr>
<td>14j</td>
<td></td>
<td>1.5 (0.41 – 5.4)</td>
<td>22 (11 – 43)</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>15a</td>
<td></td>
<td>22 (16 – 32)</td>
<td>8.6 (1.2 – 6.1)</td>
<td>3.1 (0.93 – 10)</td>
<td>4.7 (1.2 – 18)</td>
</tr>
<tr>
<td>15b</td>
<td></td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.39 (0.12 – 1.3)</td>
</tr>
<tr>
<td>15c</td>
<td></td>
<td>13 (9.7 – 17)</td>
<td>4.5 (1.2 – 17)</td>
<td>1.9 (0.65 – 5.3)</td>
<td>2.5 (1.4 – 4.6)</td>
</tr>
</tbody>
</table>

$X = O$ for 14a–14j
$X = NH$ for 15a–15c

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