Synthesis and Biological Evaluation of Novel Homoisoflavonoids for Retinal Neovascularization

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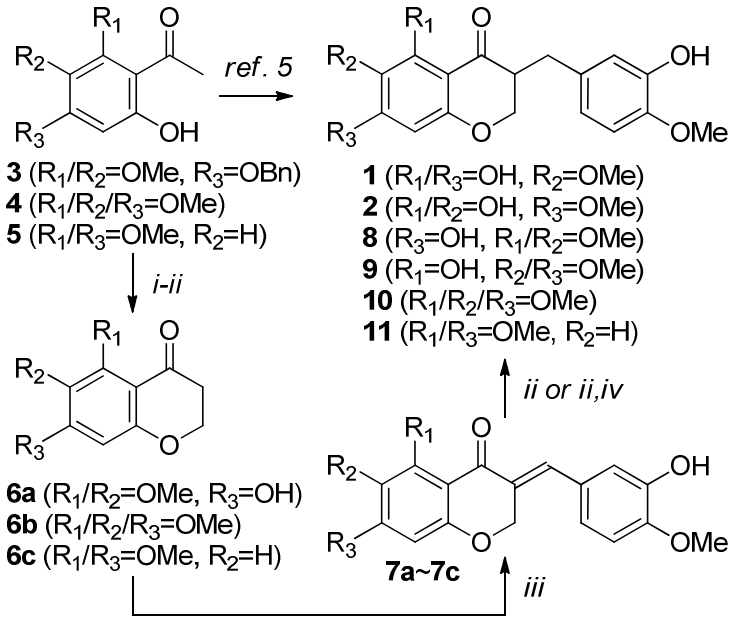
Basavarajappa, H. D., Lee, B., Lee, H., Sulaiman, R. S., An, H., Magaña, C., ... & Corson, T. W. (2015). Synthesis and Biological Evaluation of Novel Homoisoflavonoids for Retinal Neovascularization. Journal of medicinal chemistry. http://dx.doi.org/10.1021/acs.jmedchem.5b00449

ABSTRACT

Eye diseases characterized by excessive angiogenesis such as wet age2related macular degeneration, proliferative diabetic retinopathy, and retinopathy of prematurity are major causes of blindness. Cremastranone is an anti2angiogenic, 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 structure2activity relationship study to develop more potent cremastranone analogs, with improved antiproliferative selectivity for retinal endothelial cells. Phenylalanyl2incorporated 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 oxygen2induced retinopathy model in vivo.

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.

Scheme 1. Synthesis of Cremastranone and Its A2ring Modified Analogs via Chroman242ones.

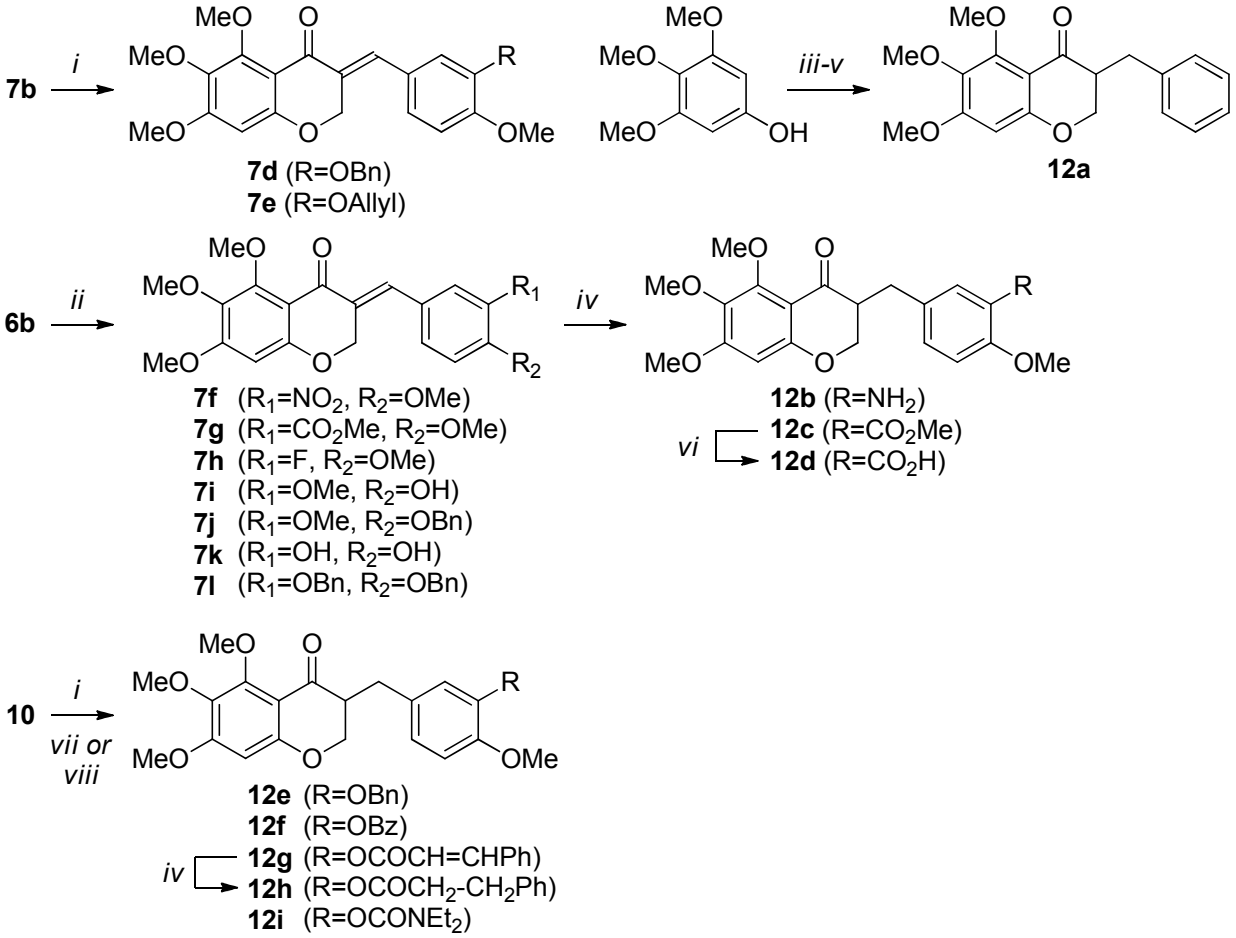


Reagents and conditions: (i) $(CH_3)_2NCH(OCH_3)_2$, toluene, reflux (82~97%); (ii) H_2, Pd/C, MeOH, rt (87~99%); (iii) isovanillin, *p*2TsOH, benzene, reflux (60~77%); (iv) TMSI, $CHCl_3$, 60 °C (45~83%).

Synthesis of B-ring Modified Homoisoflavanones. To prepare homoisoflavanones modified on the B ring, 32benzylidene242chromanones **7d** and **7e** were prepared from **7b** by treatment with benzyl bromide and allyl bromide. Aldol condensation of 5,6,72trimethoxy242chromanone (**6b**) with the appropriate arylaldehydes under acidic conditions gave the other 32benzylidene242 chromanones (**7f**~**7r**) (Scheme 2 and Supplementary Scheme 1). As previously described, 32benzyl25,6,72trimethoxychroman242one (**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**.

Scheme 2. Synthesis of B Ring-Modified Homoisoflavonoids (**7d**~**7l** and **12a**~**12i**).

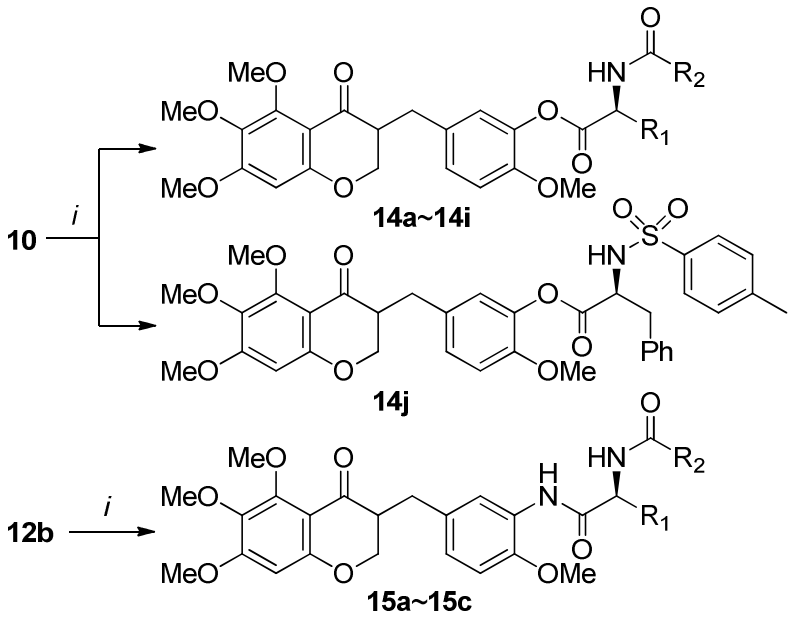


Reagents and conditions: (i) benzyl bromide or allyl bromide, K_2CO_3, acetone (79% for **7d**, 83% for **7e** and 84% for **12e**); (ii) arylaldehydes, *p*-TsOH, benzene, reflux (59~87%); (iii) cinnamoyl chloride, $BF_3 \cdot Et_2O$, reflux (98%); (iv) H_2, Pd/C, MeOH,

rt (96%); (v) aq. HCHO, NaOH, 60 °C (43%); (vi) LiOH, H_2O, THF (56%); (vii), benzoyl chloride or cinnamoyl chloride, acetone (82% for **12f** and 80% for **12g**); (viii) $ClCONEt_2$, Et_3N, toluene, reflux (40%).

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 EDCI2mediated coupling to afford aryl ester analogs (**14a**~**14j**). Similar to **14a**~**14c**, amine **12b** was coupled with the *N*2substituted amino acids to afford the *N*2arylamide analogs (**15a**~**15c**).

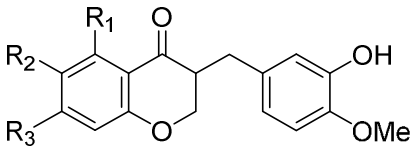
Scheme 3. Synthesis of Homoisoflavonoids (**14a**~**14j** and **15a**~**15c**) Comprising Amino Acids on the C3′ Position of the B2Ring.

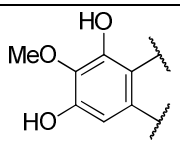
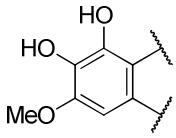
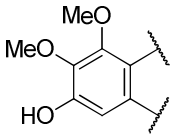
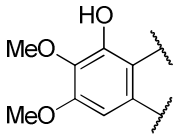
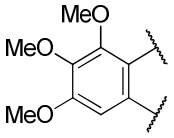
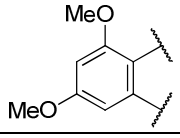


Reagents and conditions: (i) *N*-substituted amino acids, EDCI (or DCC), DMAP, CH_2Cl_2, rt (42~92%).

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 92.1 (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.

Table 1. Growth Inhibitory Activity (GI_{50}, SM) of A2ring Modified Homoisoflavanones on the Proliferation of Microvascular (HREC), Macrovascular (HUVEC) and Ocular Tumor (9221 and Y79) Cells. 95% Confidence Interval Shown in Parentheses.

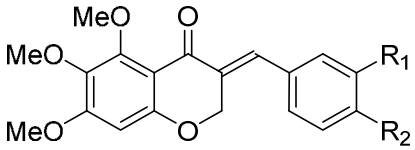


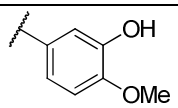
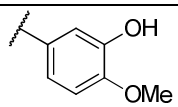
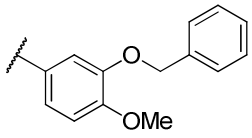
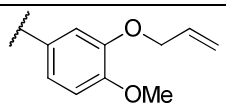
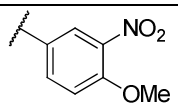
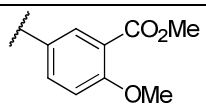
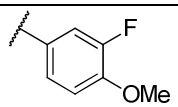
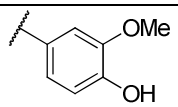
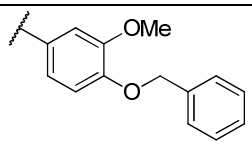
Cpd	A ring	HREC	HUVEC	9221	Y79
1		0.22 (0.12 – 0.39)	0.38 (0.24 – 0.59)	48 (17 – 132)	9.8 (2.1 – 45)
2		45 (26 – 75)	18 (16 – 21)	10 (4.4 – 23)	>100
8		>100	>100	>100	>100
9		>100	44 (34 – 58)	>100	>100
10		2 (0.81 – 5.1)	12 (2.7 – 55)	>100	>100
11		1.6 (1.0 – 2.6)	2.5 (0.87 – 7.1)	>100	4.2 (2.0 – 9.1)

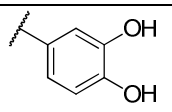
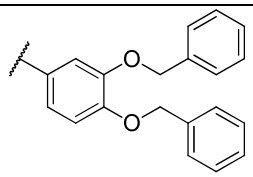
Biological Evaluation of B-ring Modified Homoisoflavanones. In a series of homoisoflavanones modified on the B ring, 32benzylidene242chromanones with

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 (GI_{50} 3~6 SM 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**).

Table 2. Growth Inhibitory Activity (GI_{50}, SM) of B-ring Modified 3-Benzylidene-4-chromanone Analogs. 95% Confidence Interval Shown in Parentheses.



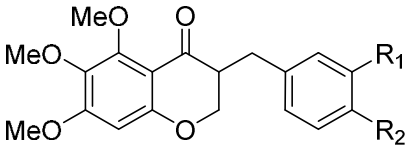
Cpd	B ring	HREC	HUVEC	9221	Y79
7b		46 (17 – 122)	5.6 (3.2 – 9.8)	0.22 (0.061 – 0.81)	44 (22 – 89)
7c[a]		42 (12 – 146)	15 (4.4 – 51)	>100	14 (7.2 – 25)
7d		4.3 (1.9 – 9.7)	16 (5.2 – 49)	3.5 (1.4 – 9.0)	33 (6.1 – 180)
7e		3.9 (1.9 – 8.1)	12 (7.6 – 18)	1.1 (0.34 – 3.6)	2.8 (1.5 – 5.1)
7f		4.8 (2.2 – 11)	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)
7j		35 (14 – 83)	52 (27 – 100)	68 (25 – 186)	14 (8.7 – 24)

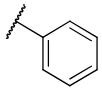
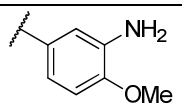
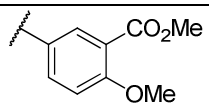
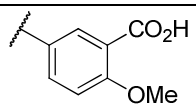
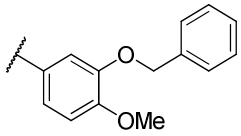
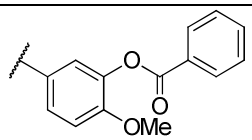
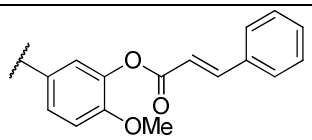
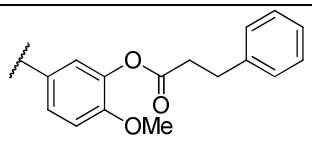
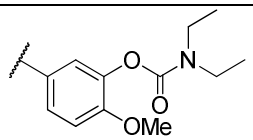
7k		7.6 (2.7 – 22)	5.0 (2.2 – 11)	9.5 (4.8 – 19)	4.0 (0.86 – 19
7l		72 (18 – 277	43 (9.1 – 204	>100	32 (12 – 88)

a. 32(3′2hydroxy24′2methoxybenzylidene)25,72dimethoxychroman242one

In contrast to the 32benzylidene242chromanones with a planar conformation, the freedom of rotation of 32benzyl242chromanones might affect the selectivity for HRECs over human ocular tumor cell lines (Table 3). Mainly 32benzyl242 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 GI_{50} values of 0.14~0.65 SM for HRECs. Moreover **12f**~**12h** were selective for HREC inhibition over HUVECs, Y79, and 9221 cells. The antiproliferative activities were obviously dependent on the substitution pattern of the B ring.

Table 3. Growth Inhibitory Activity (GI_{50}, SM) of B2ring Modified 32Benzyl242 chromanone Analogs. 95% Confidence Interval Shown in Parentheses.

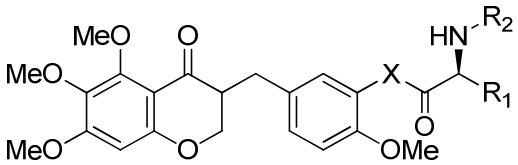


Cpd	B ring	HREC	HUVEC	922l	Y79
12a		>100	>100	>100	>100
12b		1.1 (0.29 – 4.4)	0.51 (0.11 – 25)	>100	>100
12c		18 (6.1 – 50)	17 (5.1 – 57)	>100	40 (24 – 66)
12d		>100	92 (27 – 317)	>100	>100
12e		22 (13 – 40)	>100	61 (14 – 278)	20 (8.1 – 51)
12f		0.65 (0.26 – 1.6)	>100	>100	>100
12g		0.17 (0.030 – 0.97)	>100	>100	>100
12h		0.22 (0.064 – 0.77)	38 (13 – 109)	>100	>100
12i		49 (17 – 141)	>100	>100	24 (6.1 – 97)

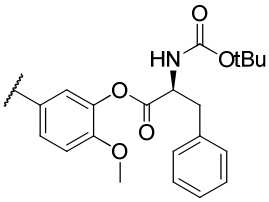
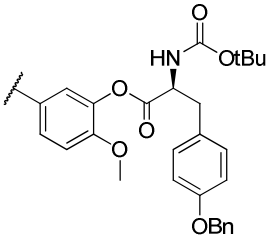
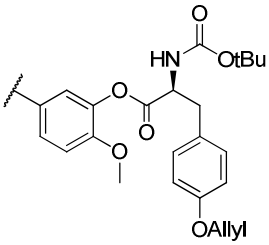
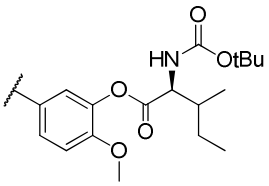
Biological Evaluation of Homoisoflavanones Coupled with Amino Acids on the C3′ position. Encouraged by the potent activity of aryl esters (**12f**~**12h**), phenol **10** and aniline **12b** were coupled with some *N*2protected amino acids to obtain the ester (**14a**~**14j**) and amide (**15a-15c**) analogs, respectively. Interestingly, **14a** which was given by coupling **10** with Boc2Phe2OH showed the most potent activity with GI_{50} = 55 nM against HRECs (Table 4; Figure 2A). Moreover **14a** selectively inhibited HREC proliferation about 142fold over HUVECs, 2182fold over Y79, and >10002fold over 9221, suggestive of cytostatic effects on HRECs rather than general cytotoxicity. The analogs (**14b** and **14c**) for which **10** was coupled with Boc2Tyr(Bn)2OH and Boc2Tyr(Allyl)2OH 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 Boc2Leu2OH, Boc2homophe2OH and Cbz2Phe2OH 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*2arylamide analogs (**15a**~**15c**) decreased substantially, compared with the corresponding phenyl ester analogs (**14a**~**14c**). Conversely, *N*2arylaminde analogs were

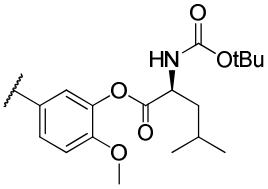
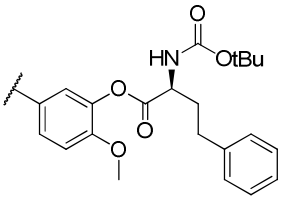
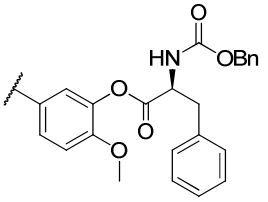
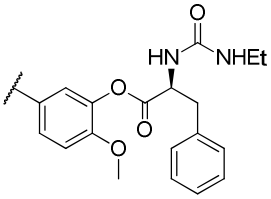
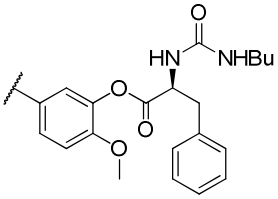
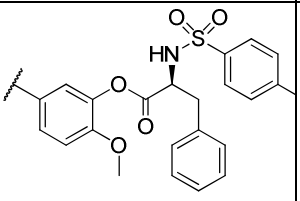
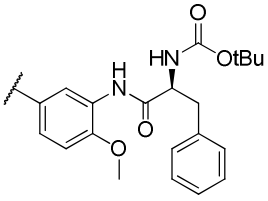
considered to be moderate inhibitors against 9221 and/or Y79 cells rather than antiangiogenic compounds.

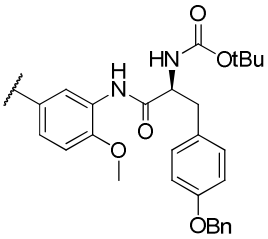
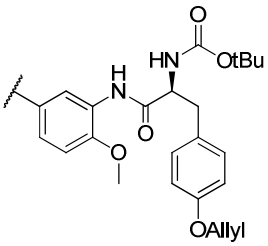
Table 4. Growth Inhibitory Activity (GI_{50}, SM) of Homoisoflavonoids Comprising Amino Acids on the C3′ Position of the B2Ring. 95% Confidence Interval Shown in Parentheses.



X = O for **14a~14j**
X = NH for **15a~15c**

Cpd	B ring	HREC	HUVEC	9221	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
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)

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 endothelial2cell specific than previously described antiangiogenic homoisoflavonoids.[5, 11214] Given this, we tested it in a secondary cell proliferation assay, which monitors the incorporation of a thymidine analogue 52 ethynyl22′2deoxyuridine into DNA of proliferating HRECs. Here, we confirmed the dose2dependent 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 cell2cell 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 dose2dependent G_2/M phase blockade with few sub2G_0 cells (Supplementary Figure 4), as documented previously for cremastranone.[10] These results established that **14a** is a potent inhibitor of angiogenesis in vitro.

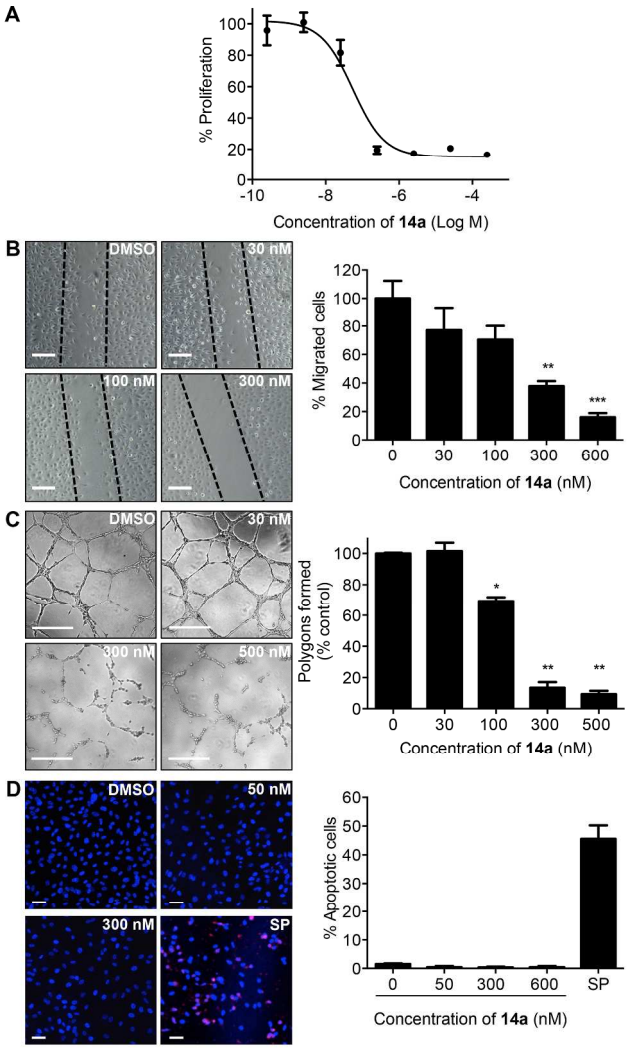


Figure 2. Compound **14a** inhibits angiogenic behavior of HRECs in vitro. (A) Dose2response of the effects of **14a** on HREC proliferation as measured by alamarBlue fluorescence. (B) **14a** dose2dependently inhibits migration of HRECs in a scratch2wound assay. (C) **14a** dose2dependently inhibits 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.

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 standard2of2care anti2 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.

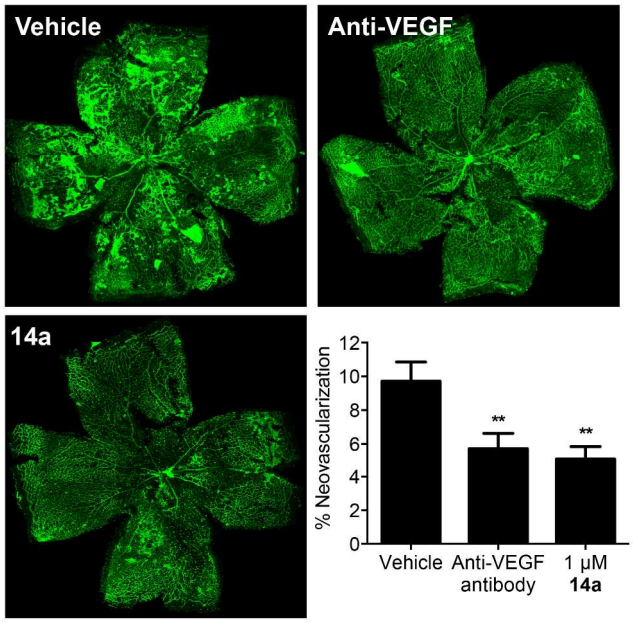


Figure 3. Homoisoflavonoid **14a** inhibits retinal neovascularization in the OIR mouse model. Retinal whole mounts from treated mice were stained for blood vessels using Alexa2488 2conjugated isolectin and imaged by confocal microscopy; neovascular area was measured using Adobe Photoshop. Error bars indicate SEM, n=6. **P<0.01.

CONCLUSION

We synthesized a series of homoisoflavonoids from chroman242ones, including successfully synthesizing the natural product cremastranone. Antiproliferative compounds with endothelial cell specificity, with a homoisoflavonoid2based 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*2carbamate amino acids

improved inhibitory activity on HREC proliferation. The most potent phenylalanyl2incorporated **14a** showed improved activity and remarkable selectivity for retinal microvascular endothelial cells, with antiangiogenic efficacy in vitro and in the oxygen2induced 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 (2302400 mesh, Merck) with the indicated solvents. Thin2layer chromatography was performed using 0.25 mm silica gel plates (Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 600 MHz spectrometer as solutions in deuteriochloroform ($CDCl_3$) or methanol2d4. ^{1}H 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). High2resolution mass spectra (HRMS) were recorded on a JEOL JMS2700 (FAB and EI) and an Agilent 6530 Q2TOF 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 12(42 (benzyloxy)262hydroxy22,32dimethoxyphenyl)ethanone (100 mg, 0.33 mmol) in

toluene (2.0 mL) was added *N*,*N*2dimethylformamide 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*2 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 $MgSO_4$. The solvent was removed under reduced pressure and purified by flash column chromatograph on silica gel (ethyl acetate : *n*2hexane = 1 : 2) to afford 72(benzyloxy)25,62dimethoxy24*H*2chromen242one (101 mg, 97%). ^{1}H2NMR ($CDCl_3$, 600 MHz) δ 7.63 (d, 1H, *J* = 6.0 Hz), 7.4627.40 (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); ^{13}C2NMR (150 MHz, $CDCl_3$) 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 42chromenone (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*2hexane = 1 : 2) to afford the chroman242one (**6a**) (33 mg, 99%). ^{1}H2NMR ($CDCl_3$, 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). ^{13}C2 NMR (150 MHz, $CDCl_3$) 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 $C_{11}H_{12}O_5$ [M + H^+], 224.0685; found, 224.0677.

5,6,7-Trimethoxychroman-4-one (6b). Chromen242one formation of 12(62 hydroxy22,3,42trimethoxyphenyl)ethan212one with *N*,*N*2dimethylformamide dimethyl acetal followed by the catalytic hydrogenation, performed according to the procedure described above, gave the chroman242one (**6b**) in 94% yield. ^{1}H2 NMR ($CDCl_3$, 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); ^{13}C2NMR (150 MHz, $CDCl_3$) 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). Chromen242one formation of 12(22hydroxy2 4,62dimethoxyphenyl)ethan212one with *N*,*N*2dimethylformamide dimethyl acetal followed by the catalytic hydrogenation, performed according to the procedure described above, gave the chroman242one (**6c**) in 95% yield. ^{1}H2NMR ($CDCl_3$, 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); ^{13}C2NMR (150 MHz, $CDCl_3$) 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 chroman242one (**6a**) (32 mg, 0.14 mmol) in benzene (3 mL) was added isovanillin (26 mg, 1.7 mmol) and *p*2

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, $CDCl_3$) 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); ^{13}C-NMR (150 MHz, $CDCl_3$) 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 $C_{20}H_{20}O_7$ [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, $CDCl_3$) δ 7.72 (s, 1H), 6.89-6.87 (m, 2H), 6.83 (d, 1H, *J* = 8.4Hz), 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, 9305, 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-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%). ^{1}H-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 10.8Hz); ^{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 $C_{19}H_{20}O_7$ [M + H^+], 361.1287; found, 361.1270. Compound **8** was reported. See ref 7.

3-(3′-Hydroxy-4′-methoxybenzyl)-5,6,7-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%). ^{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, 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 **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 SL, 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), 6.72-6.70 (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%). ^{1}H-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%). ^{1}H-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}C2NMR (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 32benzylidene2chroman242one (**7b**) (9.9 mg, 0.02 mmol) allylbromide (2.5 SL, 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*2hexane = 1 : 1) to afford the allylated 32benzylidene2chroman242one (**7e**) (6.8 mg, 83%). ^{1}H2NMR (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 26.04 (m, 1H), 5.43 25.40 (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}C2NMR (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 chroman242one (**6b**) (17 mg, 0.071 mmol) were added 42methoxy232nitrobenzaldehyde (13 mg, 0.071 mmol) and *p*2toluenesulfonic acid (2 mg, 0.1 mmol) at 0 °C. After refluxing for 12 h with a Dean2Stark apparatus, the reaction mixture was cooled and quenched with saturated $NaHCO_3$. The reaction mixture was diluted with ethyl acetate (5 mL x 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*2hexane = 1 : 1) to afford the 32benzylidene2chroman242one (**7f**) (17 mg, 60%). ^{1}H2NMR (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}C2NMR (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 x 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_2CO_3 (138 mg, 0.72 mmol). After refluxing for 4h, the reaction mixture was diluted with ethyl acetate (10 mL x 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.

(*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-hydroxybenzaldhyde (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 $NaHCO_3$. The reaction mixture was diluted with ethyl acetate (30 mL x 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 (**7h**) (62 mg, 40%). ^{1}H-NMR (600 MHz, $CDCl_3$) 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); ^{13}C2NMR (150 MHz, $CDCl_3$) 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 $C_{20}H_{19}FO_6$ [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 5,6,72 trimethoxychroman242one (**6b**) (238 mg, 1.0 mmol) were added 42benzyloxy232 methoxybenzaldehyde (265 mg, 1.1 mmol) and *p*2toluenesulfonic acid (20 mg, 0.1 mmol) at 0 °C. After refluxing for 12 h with a Dean2Stark apparatus, the reaction mixture was cooled and quenched with saturated $NaHCO_3$. The reaction mixture was diluted with ethyl acetate (15 mL x 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*2hexane = 1 : 1) to afford the 32benzylidene2chroman2 42ones (**7j**) (48 mg, 11%) and (**7i**) (62 mg, 17%). For **7i**, ^{1}H2NMR (600 MHz, $CDCl_3$) 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); ^{13}C2NMR (150 MHz, $CDCl_3$) 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 $C_{20}H_{22}O_7$ [M^+], 372.1209; found, 372.1210. For **7j**, ^{1}H NMR (600 MHz, $CDCl_3$) 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); ^{13}C NMR (150 MHz, $CDCl_3$) 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 $C_{27}H_{26}O_7$ [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 $NaHCO_3$. The reaction mixture was diluted with ethyl acetate (10 mL x 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 (**7k**) (13 mg, 10%).^{1}H 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); ^{13}C NMR (150 MHz, DMSO-d_6) 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 $C_{19}H_{18}O_7$ [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 chroman242one (**6b**) (108 mg, 0.45 mmol) were added 3,42dibenzyloxybenzaldhyde (102 mg, 0.45 mmol) and *p*2toluenesulfonic acid (9 mg, 0.04 mmol) at 0 °C. After refluxing for 12 h with a Dean2Stark apparatus, the reaction mixture was cooled and quenched with saturated $NaHCO_3$. The reaction mixture was diluted with ethyl acetate (10 mL x 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*2hexane = 1 : 2) to afford the 32benzylidene2chroman242one (**7l**) (110 mg, 45%). ^{1}H2NMR (600 MHz, $CDCl_3$) 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); ^{13}C2NMR (150 MHz, $CDCl_3$) 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 $C_{33}H_{30}O_7$ [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 32 benzyl2chroman242one (**12b**) (13 mg, 73%). ^{1}H2NMR (600 MHz, $CDCl_3$) 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); ^{13}C2NMR (150 MHz, $CDCl_3$) 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 $C_{20}H_{23}NO_6$ [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*2hexane = 1 : 2) to

afford the 3-benzyl-chroman-4-one (**12c**) (72 mg, 74%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ7.58 (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, 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 $C_{22}H_{24}O_8$ [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 x 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%). ^{1}H-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 $C_{21}H_{22}O_8$ [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 SL, 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 32 benzyl-chroman-4-one (**12e**) (34 mg, 84%). ^{1}H 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 $C_{27}H_{28}O_7$ [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 SL, 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*2hexane = 1 : 2) to afford the 32benzyl2chroman242one (**12f**) (38 mg, 82%). ^{1}H2NMR (600 MHz, $CDCl_3$) 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), 2.7622.72 (m 1H), 2.67 (dd, 1H, *J* = 13.8 and 4.8 Hz); ^{13}C2NMR (150 MHz, $CDCl_3$) 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 $C_{27}H_{26}O_8$ [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_2CO_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, $CDCl_3$) 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); ^{13}C-NMR (150 MHz, $CDCl_3$) 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 $C_{29}H_{28}O_8$ [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 32benzyl2chroman242one (**12h**) (26 mg, 92 %). ^{1}H2NMR (600 MHz, $CDCl_3$) 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), 2.742 2.71 (m, 1H), 2.64 (dd, 1H, *J* = 13.8 and 10.8 Hz); ^{13}C2NMR (150 MHz, $CDCl_3$) 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 $C_{29}H_{30}O_8$ [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 Et_3N (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 $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*2hexane = 1 : 1) to afford the 32 benzyl2chroman242one (**12i**) (8 mg, 40%). ^{1}H2NMR (600 MHz, $CDCl_3$) 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), 2.7422.70 (m, 1H), 2.62 (dd, 1H, *J* = 13.8 and 10.8 Hz); ^{13}C2 NMR (150 MHz, $CDCl_3$) 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 $C_{25}H_{31}NO_8$ [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 CH_2Cl_2 solution (6 mL) of **10** (169 mg, 0.45 mmol) were added Boc2Phe2OH (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 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*2hexane = 1 : 2) to afford the acylated 32benzyl2chroman242one (**14a**) (247 mg, 87%). ^{1}H2NMR (600 MHz, $CDCl_3$) 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), 4.1324.07 (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); ^{13}C NMR (150 MHz, $CDCl_3$) 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 $C_{34}H_{39}NO_{10}$ [M^+], 621.2574; found, 621.2573.

2-Methoxy-5-((5′,6′,7′-trimethoxy-4′-oxochroman-3′-yl)methyl)phenyl (2*S*)-3-(4-(benzyloxy)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate (14b). To a CH_2Cl_2 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 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 (**14b**) (115 mg, 94%). ^{1}H NMR (600 MHz, $CDCl_3$) 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 $C_{41}H_{45}NO_{11}$ [M + H^+], 727.2993; found, 727.302.

2-Methoxy-5-((5′,6′,7′-trimethoxy-4′-oxochroman-3′-yl)methyl)phenyl (2*S*)-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 Boc2Tyr(All)2OH (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*2hexane = 1 : 2) to afford the acylated 32benzyl2chroman242one (**14c**) (85 mg, 84%). ^{1}H2NMR (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), 6.0325.96 (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), 2.7122.66 (m, 1H), 2.61 (dd, 1H, *J* = 13.8 and 10.2 Hz), 1.39 (s, 9H); ^{13}C2NMR (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 $C_{37}H_{43}NO_{11}$ [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 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%). ^{1}H-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 4.2Hz), 2.75 (m, 1H), 2.64 (m, 1H), 1.42 (s, 9H), 1.32 (s, 9H); HRMS (FAB): mass calcd for $C_{31}H_{41}NO_{10}$ [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 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 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 (**14e**) (30 mg, 61 %). ^{1}H-NMR (600 MHz, $CDCl_3$) 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); ^{13}C-NMR (150 MHz, $CDCl_3$) 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 $C_{31}H_{41}NO_{10}$ [M + H^+], 588.2730; found, 588.2810.

2-Methoxy-5-((5′,6′,7′-trimethoxy-4′-oxochroman-3′-yl)methyl)phenyl (2*S*)-2-((*tert*-butoxycarbonyl)amino)-4-phenylbutanoate (14f). To a CH_2Cl_2 solution (3 mL) of **10** (27 mg, 0.07 mmol) were added *N*-(*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

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 (**14f**) (31 mg, 67 %). ^{1}H-NMR (600 MHz, $CDCl_3$) 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) ; ^{13}C-NMR (150 MHz, $CDCl_3$) 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 $C_{35}H_{41}NO_{10}$ [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 CH_2Cl_2 solution (3 mL) of **10** (36 mg, 0.09 mmol) were added *N*-carbobenzoxy-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 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 (**14g**) (28 mg, 44 %). ^{1}H-NMR

(600 MHz, $CDCl_3$) 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); ^{13}C-NMR (150 MHz, $CDCl_3$) 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-2-chroman-4-one (**14h**) (15 mg, 56 %). ^{1}H-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), 3.0523.21(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}C2NMR (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*2[(butylamino)carbonyl]212phenylalanine (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*2hexane = 1 : 1) to afford the acylated 32benzyl2chroman242one (**14i**) (18 mg, 49 %). ^{1}H2NMR (600 MHz, $CDCl_3$) δ 7.2922.22 (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), 5.0625.02 (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}C2NMR (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*2(*p*2toluenesulfonyl)212phenylalanine (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*2hexane = 1 : 2) to afford the acylated 32benzyl2chroman242one (**14j**) (24 mg, 51%). ^{1}H2NMR (600 MHz, $CDCl_3$) 7.66 (d, 2H, J = 7.8 Hz), 7.2627.20 (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) ; ^{13}C2NMR (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 (EI): mass calcd for $C_{36}H_{37}NO_{10}S$ [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 CH_2Cl_2 solution (3 mL) of **12b** (35 mg, 0.09 mmol) were added Boc2Phe2OH (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 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*2hexane = 1 : 2) to afford the acylated 32benzyl2chroman242one (**15a**) (24 mg, 42%). ^{1}H2NMR (600 MHz, $CDCl_3$) 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); ^{13}C2NMR (150 MHz, $CDCl_3$) 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 $C_{34}H_{40}N_2O_{10}$ [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 CH_2Cl_2 solution (6 mL) of **12b** (35 mg, 0.09 mmol) were added Boc2Tyr(Bzl)2OH,(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 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*2hexane = 1 : 2) to afford the acylated 32benzyl2 chroman242one (**15b**) (50 mg, 72%). ^{1}H2NMR (600 MHz, $CDCl_3$) 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); ^{13}C2NMR (150 MHz, $CDCl_3$) 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, 110.0108.7, 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 $C_{41}H_{46}N_2O_{10}$ [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 CH_2Cl_2 solution (3 mL) of **12b** (35 mg, 0.09 mmol) were added Boc2Tyr(Bzl)2OH (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_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*2hexane = 1 : 2) to afford the acylated 32benzyl2 chroman242one (**15c**) (42 mg, 71%). ^{1}H2NMR (600 MHz, $CDCl_3$) 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), 6.0625.99 (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), 2.8122.75 (m, 1H), 2.63 (m, 1H), 1.43 (s, 9H); HRMS (FAB): mass calcd for $C_{37}H_{44}N_2O_{10}$ [M+H$^+$], 677.2996; found, 677.3074.

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.

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Notes

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.

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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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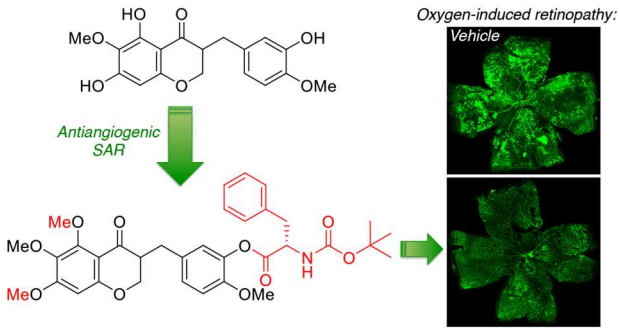

Supporting Information

Synthesis and Biological Evaluation of Novel Homoisoflavonoids for Retinal Neovascularization

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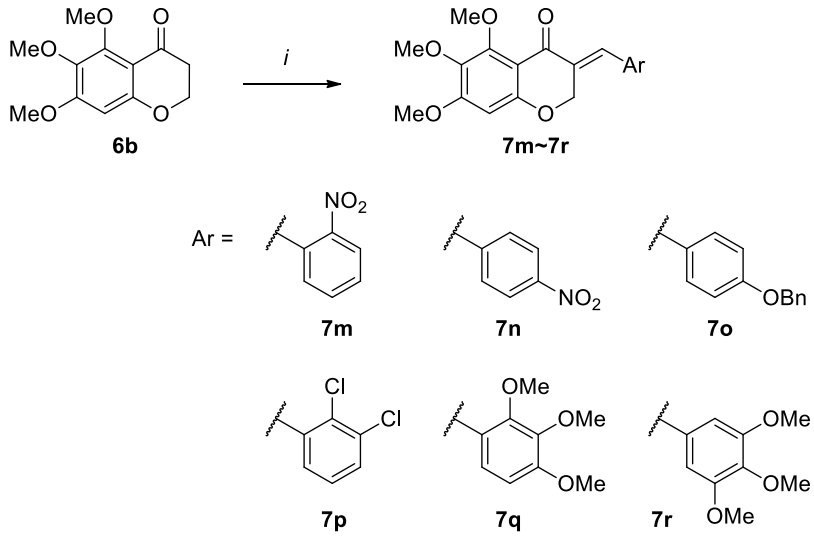
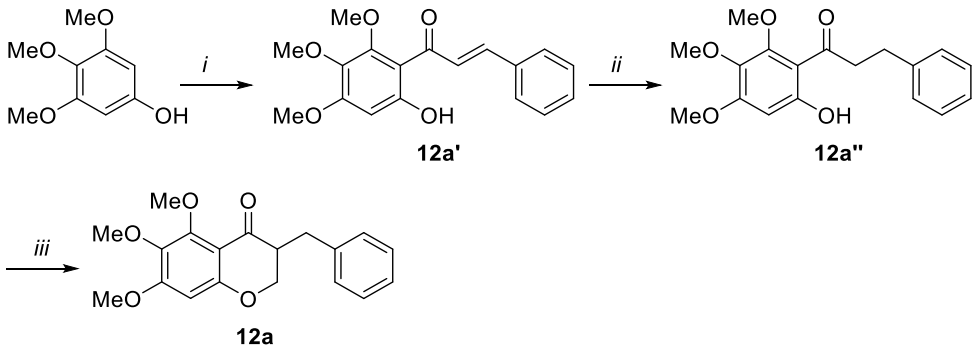
Supporting Information Scheme 1. Synthesis of B-ring Modified 3-Benzylidene-5,6,7-trimethoxychroman-4-ones (**7m–7r**).



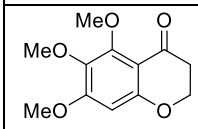
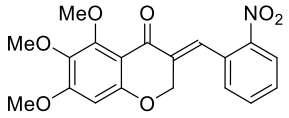
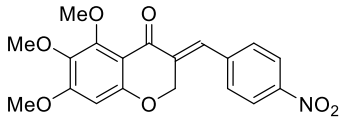
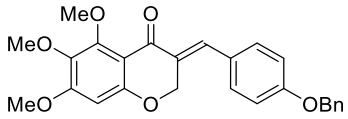
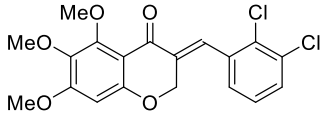
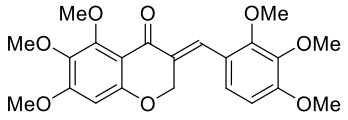
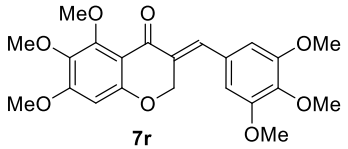
Reagents and conditions: (i) arylaldehydes (Ar-CHO), *p*-TsOH, benzene, reflux (47–78%).

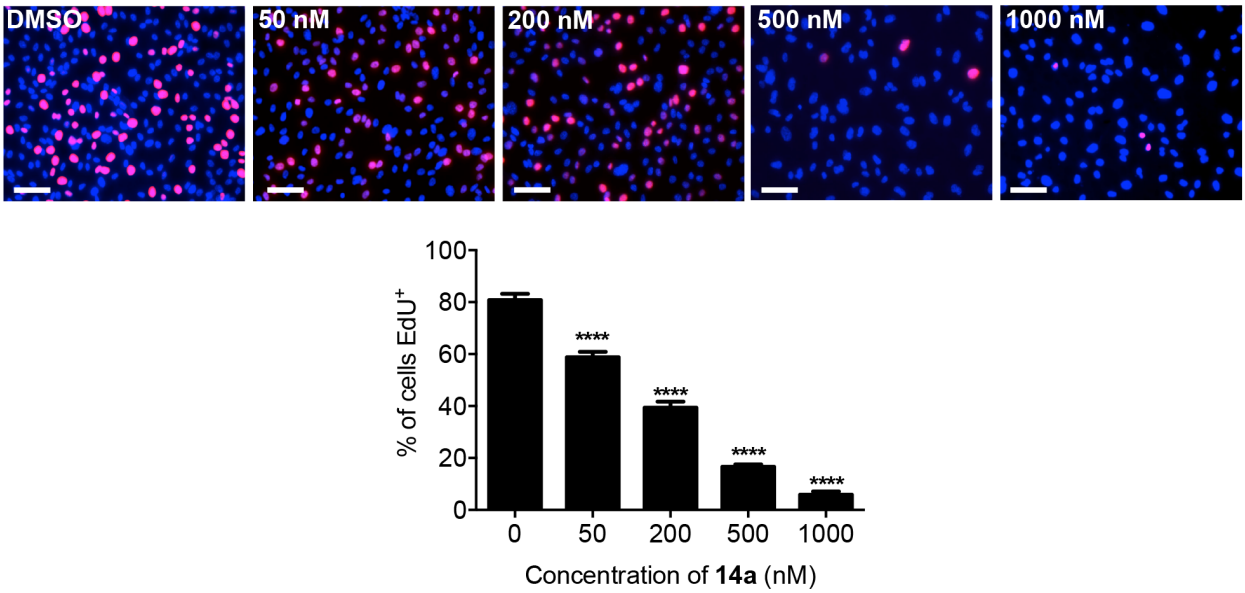
Supporting Information Scheme 2. Synthesis of 3-Benzyl-5,6,7-trimethoxychroman-4-one (**12a**).



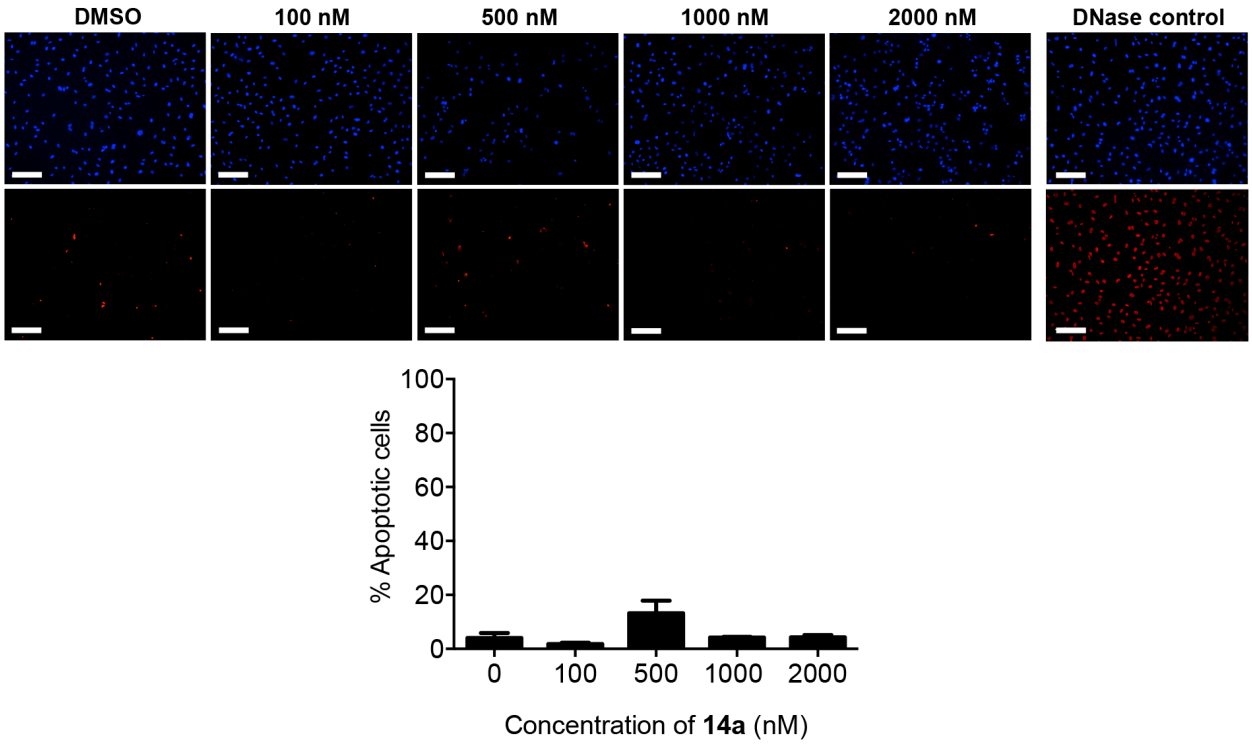
Reagents and conditions: (i) cinnamoyl chloride, $BF_3 \cdot Et_2O$, 98%; (ii) H_2, Pd/C, MeOH, rt, 96%; (iii) aq HCHO, NaOH, 60 °C, 43%.

Supporting Information Table 1. Growth Inhibitory Activity (GI_{50}, μM) of 5,6,7-Trimethoxychroman-4-one (**6b**) and B-ring Modified 3-Benzylidene-4-chromanone Analogues (**7m–7r**). 95% Confidence Interval Shown in Parentheses.

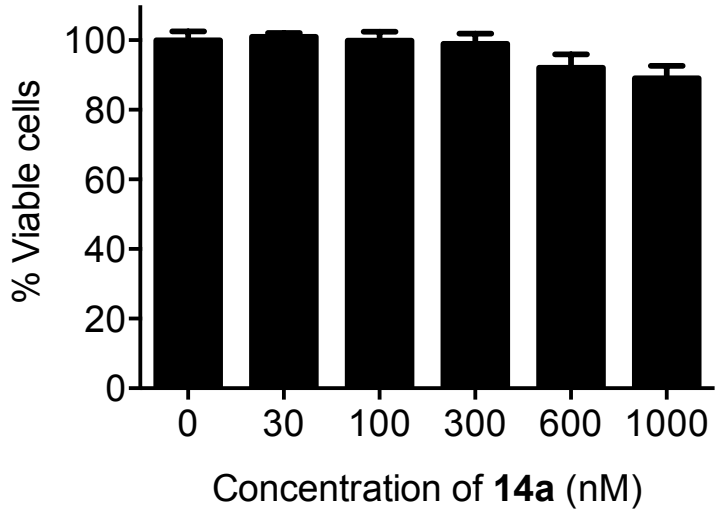
Cpd	HREC	HUVEC	92-1	Y79
 **6b**	>100	>100	>100	>100
 **7m**	8.6 (2.7 – 27)	9.1 (3.6 – 23)	30 (5.5 – 160)	8.2 (2.2 – 30)
 **7n**	17 (9.2 – 33)	5.8 (3.1 – 11)	8.5 (3.4 – 21)	4.2 (2.3 – 7.5)
 **7o**	>100	>100	>100	>100
 **7p**	26 (9.9 – 67)	10 (3.2 – 31)	31 (5.7 – 167)	18 (7.3 – 44)
 **7q**	91 (45 –187)	45 (21 – 96)	>100	>100
 **7r**	17 (4.7 – 59)	9.1 (2.9 – 28)	>100	9.1 (4.5 – 18)



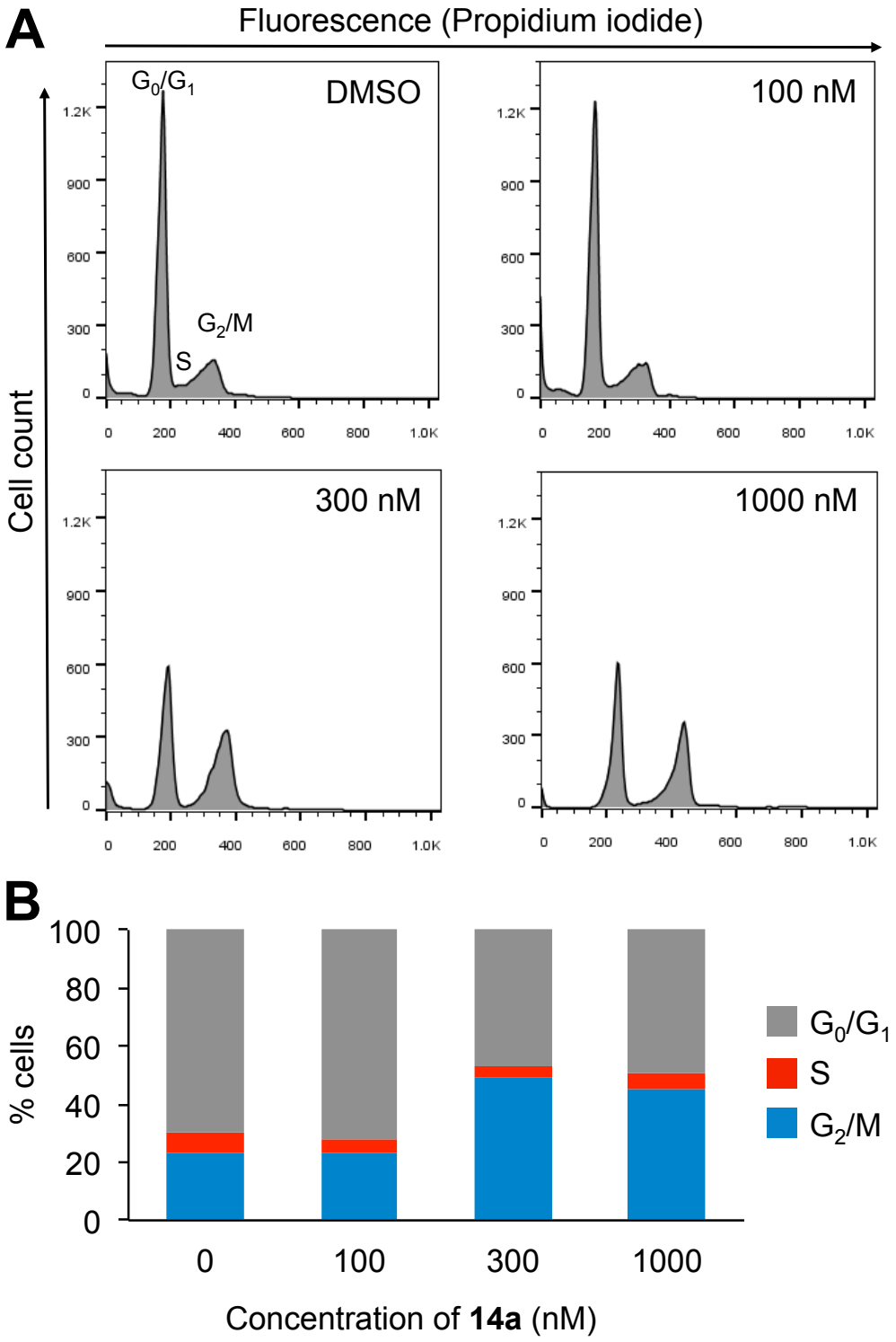
Supporting Information Figure 1. Compound **14a** inhibits proliferation of HRECs as measured by EdU incorporation assay. The nuclei of all cells are labeled with DAPI (blue) and actively dividing cells are labeled with EdU (pink). Error bars indicate SEM, n = 3, representative results from at least triplicate experiments. **** P<0.0001. Scale bars = 200 μm.



Supporting Information Figure 2. Compound **14a** does not induce apoptosis significantly, as evidenced by TUNEL assay. The nuclei of all cells are shown in blue (DAPI staining) and TUNEL$^+$ cells undergoing apoptosis are shown in red. Error bars indicate SEM, n = 3, representative results from at least triplicate experiments. Scale bars = 200 μm.



Supporting Information Figure 3. Viability of HRECs is not significantly altered by compound **14a** as monitored in Trypan blue exclusion assays. Error bars indicate SEM, $n = 3$, representative results from triplicate experiments.



Supporting Information Figure 4. (A) Compound **14a** arrests HRECs at the G_2/M phase of the cell cycle. HRECs were treated with the indicated concentrations of **14a** for 48 hours and then stained with propidium iodide before flow cytometry analysis. (B) Quantitative analysis of the cell cycle progression data. Representative results from duplicate experiments.

Supporting Information Experimental Procedures

*(E)-5,6,7-Trimethoxy-3-(2'-nitrobenzylidene)chroman-4-one (**7m**).* To a benzene solution (3 mL) of 5,6,7-trimethoxychroman-4-one (**6b**) (50 mg, 0.21 mmol) were added 2-nitrobenzaldehyde (31 mg, 0.21 mmol) and *p*-toluenesulfonic acid (4 mg, 0.02 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 (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 = 1:1.5) to afford 3-benzylidene-5,6,7-trimethoxychromanone (**7m**) (49 mg, 63%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.19 (d, 1H, J = 14.4 Hz), 8.01 (s, 1H), 7.68 (t, 1H, J = 7.2 Hz), 7.56 (t, 1H, J = 7.2 Hz), 7.21 (d, 1H, J = 7.8 Hz), 6.22 (s, 1H), 4.90 (d, 2H, J = 1.8 Hz), 3.98 (s, 3H), 3.86 (s, 3H), 3.81 (s, 3H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 178.8, 159.8, 159.6, 155.0, 148.0, 138.0, 133.5, 133.1, 132.7, 131.0, 130.7, 129.7, 125.3, 110.4, 96.2, 67.0, 61.6, 61.3, 56.1. HRMS (EI): mass calcd for $C_{19}H_{17}NO_7$ [M^+], 371.1005; found, 371.1005.

*(E)-5,6,7-Trimethoxy-3-(4'-nitrobenzylidene)chroman-4-one (**7n**).* To a benzene solution (2 mL) of 5,6,7-trimethoxychroman-4-one (**6b**) (24 mg, 0.1 mmol) were added 4-nitrobenzaldehyde (17 mg, 0.11 mmol) and *p*-toluenesulfonic acid (2 mg, 0.01 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 (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 4-benzylidene-5,6,7-trimethoxychromanone (**7n**) (19 mg, 52%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.30 (d, 2H, J = 9.0 Hz), 7.82 (s, 1H), 7.44 (d, 2H, J = 8.4 Hz), 6.27 (s, 1H), 5.17 (d, 2H, J = 1.8 Hz), 3.99 (s, 3H), 3.90 (s, 3H), 3.84 (s, 3H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 178.6, 159.8, 154.8, 147.6, 141.1, 134.6, 133.3, 130.3, 123.9, 96.2, 67.1, 61.6, 61.3, 56.2, 31.6, 14.2, 14.1. HRMS (EI): mass calcd for $C_{19}H_{17}NO_7$ [M^+], 371.1005; found, 371.1002.

*(E)-3-(4'-Benzyloxybenzylidene)-5,6,7-trimethoxychroman-4-one (**7o**).* To a benzene solution (2 mL) of 5,6,7-trimethoxychroman-4-one (**6b**) (24 mg, 0.10 mmol) were added 4-benzyloxybenzaldehyde (23 mg, 0.11 mmol) and *p*-toluenesulfonic acid (5 mg, 0.025 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 (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 4-benzylidene-5,6,7-trimethoxychromanone (**7o**) (24 mg, 56%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.44 (m, 5H), 7.25 (d, 2H, J = 9 Hz), 7.03 (d, 2H, J = 9 Hz), 6.26 (s, 1H), 5.23 (d, 2H, J = 1.8 Hz), 5.11 (s, 2H), 3.98 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 179.6, 160.0, 159.6, 159.3, 159.1, 154.7, 137.8, 136.4, 136.1, 131.8, 129.8, 128.7, 128.2, 127.5, 115.0, 110.6, 96.1, 70.1, 67.6, 61.6, 61.3, 56.1. HRMS (EI): mass calcd for $C_{26}H_{24}O_6$ [M^+], 432.1573; found, 432.1572.

*(E)-3-(2',3'-Dichlorobenzylidene)-5,6,7-trimethoxychroman-4-one (**7p**)*. To a benzene solution (3 mL) of 5,6,7-trimethoxychroman-4-one (**6b**) (50 mg, 0.21 mmol) were added 2,3-dichlorobenzaldehyde (37 mg, 0.21 mmol) and *p*-toluenesulfonic acid (5 mg, 0.02 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 (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 4-benzylidene-5,6,7-trimethoxychromanone (**7p**) (39 mg, 47%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.46 (dd, 1H, J = 8.4 and 1.2 Hz), 7.23 (dd, 1H, J = 12.6 and 4.8 Hz), 6.98 (d, 1H, J = 8.4 Hz), 6.23 (s, 1H), 4.97 (d, 2H, J = 1.2 Hz), 3.96 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 178.9, 159.7, 159.6, 154.9, 138.0, 135.4, 133.9, 133.6, 133.0 ,132.9, 130.8, 128.2, 127.0, 110.3, 96.2, 67.2, 61.6, 61.3, 56.1. HRMS (EI): mass calcd for $C_{19}H_{16}Cl_2O_5$ [M^+], 394.0375; found, 394.0378.

*(E)-5,6,7-Trimethoxy-3-(2',3',4'-trimethoxybenzylidene)chroman-4-one (**7q**)*. To a benzene solution (3 mL) of 5,6,7-trimethoxychroman-4-one (**6b**) (50 mg, 0.21 mmol) were added 2,3,4-trimethoxybenzaldehyde (41 mg, 0.21 mmol) and *p*-toluenesulfonic acid (5 mg, 0.02 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 (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 = 1:5) to afford 4-benzylidene-5,6,7-trimethoxychromanone (**7q**) (68 mg, 78%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.83 (s, 1H), 6.74 (d, 1H, J = 8.4 Hz), 6.59 (d, 1H, J = 8.4 Hz), 6.19 (s, 1H), 5.04 (d, 2H, J = 1.8 Hz), 3.94 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 179.7, 159.5, 159.1, 155.0, 154.7, 153.0, 142.4, 137.7, 132.2, 130.8, 125.1, 121.7, 110.6, 106.9, 96.1, 68.1, 61.6, 61.4, 61.2, 60.9, 56.1, 56.0. HRMS (EI): mass calcd for $C_{22}H_{24}O_8$ [M^+], 416.1471; found, 416.1467.

*(E)-5,6,7-Trimethoxy-3-(3′,4′,5′-trimethoxybenzylidene)chroman-4-one (**7r**).* To a benzene solution (3 mL) of 5,6,7-trimethoxychroman-4-one (**6b**) (100 mg, 0.42 mmol) were added 3,4,5-trimethoxybenzaldehyde (82 mg, 0.42 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 $NaHCO_3$. 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 = 1:5) to afford 4-benzylidene-5,6,7-trimethoxychromanone (**7r**) (130 mg, 75%). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.65 (s, 1H), 6.41 (s, 1H), 6.16 (s, 1H), 5.16 (s, 2H), 3.89 (s, 3H), 3.80 (s, 12H), 3.75 (s, 3H). ^{13}C-NMR (150 MHz, CDCl3) δ 179.2, 159.3, 159.2, 154.7, 153.1, 139.1, 137.8, 136.3, 131.1, 130.1, 110.4, 107.2, 96.1, 67.5, 61.5, 61.2, 60.9, 56.1, 56.1. HRMS (EI): mass calcd for $C_{22}H_{24}O_8$ [M^+], 416.1471; found, 416.1465.

*3-Benzyl-5,6,7-trimethoxychroman-4-one (**12a**).* To 3,4,5-trimethoxyphenol (184 mg, 1 mmol) was added cinnamoyl chloride (199 mg, 1.2 mmol). To the reaction mixture was added $BF_3 \cdot Et_2O$ (1 mL) at 0 °C, then refluxed at 120 °C for 4 h, followed by concentration in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate:*n*-hexane = 1:2) to afford the chalcone **12a′** (309 mg, 98%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 13.67 (1H, d, J = 6.36), 7.96 (1H, s), 7.83 (1H, s), 7.61 (2H, s), 7.39 (3H, m), 6.28 (1H, s), 3.91 (3H, s), 3.88 (3H, s), 3.81 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 192.9, 162.7, 160.1, 155.0, 143.2, 135.3, 130.2, 128.9, 128.4, 126.5, 108.7, 96.5, 62.9, 61.2, 56.1. A solution of chalcone **12a′** (102 mg, 0.32 mmol) and 10% Pd/C (16 mg) in absolute EtOH (3 mL) was placed under an atmosphere of hydrogen. After stirring for 4 h, the reaction mixture was diluted with ethyl acetate, filtered through a short pad of silica gel and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (ethyl acetate:*n*-hexane = 1:1) to

afford the dihydrochalcone (**12a″**) (115 mg, 96%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 13.33 (s, 1H), 7.21 (m, 4H), 7.18 (s, 1H), 6.17 (s, 1H), 3.88 (s, 3H), 3.81 (s, 3H), 3.69 (s, 3H), 3.31 (m, 2H), 2.97 (d, 2H, J = 7.8 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 204.7, 161.8, 159.9, 155.0, 141.4, 134.7, 128.4, 125.9, 108.2, 96.1, 61.0, 60.9, 56.0, 44.7, 30.4. IR (neat) ν_{max} 2960, 2924, 2852 cm^{-1}. LRMS (ESI) *m/z* 317 [M+H$^+$]. The dihydrochalcone (**12a″**) (100 mg, 0.31 mmol) was dissolved in 50 % aqueous NaOH (2 mL), H_2O (6 mL) and stirred with formalin (0.04 mL, 1.61 mmol) at 60 °C for 3 h. After stirring for 3 h, the reaction mixture was diluted with ethyl acetate and washed with sat. NH_4Cl 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 = 1:4) to afford **12a** (44 mg, 43%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29 (m, 2H), 7.22 (m, 3H), 6.23 (s, 1H), 4.24 (d, 1H, J = 4.4 Hz), 4.09 (d, 1H, J = 8.0 Hz), 3.91 (s, 3H), 3.86 (s, 3H), 3.79 (s, 3H), 3.27 (dd, 1H, J = 14.4 and 4.0 Hz), 2.76 (m, 1H), 2.68 (dd, 1H, J = 10.2 and 4.8 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.1, 159.6, 159.2, 154.4, 138.5, 137.4, 129.1, 128.6, 126.5, 108.6, 95.9, 69.0, 61.5, 61.3, 56.0, 48.2, 32.7. HRMS (EI): mass calcd for $C_{19}H_{20}O_5$ [M$^+$], 328.1311; found, 328.1312.

Supporting Information Table 2. Purity and Peak Attributions of the Homoisoflavonoid Analogues.

Compounds	Retention time (min)	Purity (area %)	Compounds	Retention time (min)	Purity (area %)
6b	0.58	98.8	**12b**	0.85	95.4
7b	0.79	95.3	**12c**	0.92	98.0
7c	0.73	96.6	**12d**	0.81	99.7
7d	2.37	95.2	**12e**	2.11	98.6
7e	1.53	95.1	**12f**	2.15	97.7
7f	1.19	96.7	**12g**	3.25	96.9
7g	1.02	97.5	**12h**	2.62	96.7
7h	0.79	96.5	**12i**	1.88	95.9
7i	0.79	95.1	**14a**	3.27	96.8
7j	2.54	97.7	**14b**	7.18	96.7
7k	0.62	98.5	**14c**	4.36	97.6
7l	5.84	97.8	**14d**	3.55	99.1
7m	1.09	97.3	**14e**	3.41	96.3
7n	1.36	95.4	**14f**	4.12	95.6
7o	3.24	96.0	**14g**	3.20	97.2
7p	2.63	95.8	**14h**	1.14	96.6
7q	1.26	95.8	**14i**	1.71	98.5
7r	1.13	96.8	**14j**	2.83	97.9
10	1.11	97.9	**15a**	2.42	98.5
11	0.68	95.4	**15b**	0.55	98.1
12a	1.40	99.3	**15c**	3.22	96.4

HPLC conditions:

System: Agilent 1260 infinity binary LC

Detector: Agilent 1260 infinity UV detector, 256 nm

Column: Waters Sunfire 5 μM 4.6 × 50 mm

Sample diluent: 99.6 % methanol

Mobile phase: 60% MeCN/Water

Mode: Isocratic system

Flow rate: 1.5 mL/min

Injection volume: 20 μL

Biological Methods

Materials

EBM-2 and IMDM growth media were purchased from Lonza (Walkersville, MD, USA). RPMI and Ham's/F-10 media were purchased from Thermo Scientific (Waltham, MA, USA). HRECs and Attachment Factor were purchased from Cell Systems (Kirkland, WA, USA). Clonetics® HUVECs were purchased from Lonza. All endothelial cells were used between passages 5 and 8. Endothelial Growth Medium (EGM-2) was prepared by mixing the contents of an EGM-2 "Bullet Kit" (Cat. no. CC-4176) with Endothelial Basal Medium (EBM) (Lonza). The EGM-2 "Bullet Kit" contains hydrocortisone, human fibroblast growth factor (hFGF), VEGF, R3-insulin like growth factor (R3-IGF-1), ascorbic acid, human epidermal growth factor (hEGF), gentamycin and heparin along with 2% fetal bovine serum (FBS). 92-1 cells[1] (a kind gift from Dr. Martine Jager) were grown in RPMI medium containing 10% FBS and 1% penicillin-streptomycin (pen-strep). Y79 cells[2] (a kind gift from Dr. Brenda Gallie) were grown in RB medium (IMDM + 10% FBS + 55 μM β-mercaptoethanol + 10 µg/mL Insulin + 1% pen-strep). Identity of 92-1 and Y79 cell lines was confirmed by short tandem repeat profiling. Click-iT TUNEL Alexa Fluor-594 imaging assay kit (Cat. no. C10246) was purchased from Molecular Probes (Eugene, OR, USA).

Cell Proliferation Assay

The proliferation of cells was monitored by an alamarBlue based fluorescence assay as described previously.[3] Four cell types were used: HRECs, HUVECs, 92-1, and Y79. Briefly, 2,500 cells in 100 μL growth medium were incubated in 96-well clear bottom black plates for 24 hours followed by 48 hours' incubation with different concentrations of each test compound (range: 0.5 nM to 500 μM). At the end of the incubation, 11.1 μL of alamarBlue reagent was added and 4 hours after, fluorescence readings were taken with excitation and emission wavelengths of 560 nm and 590 nm respectively. Data were analyzed and dose response curves generated using GraphPad Prism software (v. 6.0). Only those compounds that reduced cell number by 50% or more at the highest concentration tested (relative to DMSO control) were reported as having a $GI_{50} < 100$ μM.

EdU Incorporation Assay

The assay was carried out as described before.[3] HRECs (25,000) were seeded onto coverslips coated with attachment factor and grown for 24 hours before starving in serum-free EBM-2 medium. After starvation for 12 hours, the cells were incubated with 10 μM EdU in the

presence of various concentrations of **14a** for 8 hours. Then the cells were processed according to the manufacturer's instructions for the click-iT EdU assay kit (Life Technologies, Grand Island, NY, USA). The images were taken using an EVOS-fl digital microscope (AMG, Mill Creek, WA, USA) and data were analyzed using ImageJ software v. 1.48V (http://imagej.nih.gov/ij/).

Cell Viability Assay

HRECs were grown in complete EGM-2 medium in a 6-well plate to 70 % confluency and then incubated with different concentrations of **14a** for 48 hours. After the incubation and trypsinization, the number of live and dead cells were counted in each well (including floating cells in the medium) using Trypan blue dye and a hemocytometer. The percentage of viable cells was calculated as the ratio of the number of live cells to the total number of cells (sum of live and dead cells). The resulting data were analyzed in GraphPad Prism software (v. 6.0).

Cell Cycle Analysis

HRECs were grown in complete EGM-2 medium in a 10 cm plate until 50 % confluency was reached and then the cells were serum starved overnight. The medium was replaced with fresh EGM-2 medium containing different concentrations of **14a**. After 48 hours of incubation, cells were washed twice in ice-cold PBS, trypsinized and collected in 1.5 mL tubes. Cells were fixed in 66 % EtOH solution for 2 hours at 4 °C. After the fixation, the cells were washed twice in ice-cold PBS. Fixed cells were resuspended in 300 μL of propidium iodide staining solution (20 μg/mL propidium iodide solution prepared in 0.1 % (v/v) Triton X-100 in PBS and 1× RNase [Qiagen]). Cells were incubated at 37 °C for 30 min before analysis by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA). Cell cycle profiles were then generated using FlowJo software (v. 10).

Apoptosis Assays

Caspase-3 Immunofluorescence Assay: The assay was carried out as described previously.[3] Briefly, cells were plated on coated coverslips and incubated in EGM-2 medium overnight before treating with indicated concentrations of **14a**. After 4 hours of compound treatment the cells were fixed in 4% paraformaldehyde and permeabilized using 0.5 % Triton X-100 solutions prepared in PBS. Then cells were immunostained using an antibody against

activated caspase 3 (9661S, Cell Signaling, Beverly, MA, USA) and imaged using an LSM 700 confocal microscope (Zeiss, Thornwood, NY, USA).

TUNEL Assay: Cells (25,000 per coverslip) were seeded on each coverslip coated with attachment factor in a 6-well plate and grown overnight in EGM-2 medium. Next day, fresh medium with the indicated concentrations of **14a** was added to cells and they were incubated for 4 hours. Cells were then fixed in 4 % paraformaldehyde prepared in PBS for 20 min at room temperature. The coverslips were washed twice in PBS and incubated further for 20 min in 0.25 % Triton X-100 in PBS. Then coverslips were washed in PBS twice and apoptotic cells were visualized using the Click-iT TUNEL assay kit as per the manufacturer's instructions, with DAPI counter-stain. The percentage of apoptotic cells was counted on three low-power fields per coverslip using Image J software and analyzed using GraphPad Prism software (v. 6.0).

In Vitro Scratch-Wound Assay

HRECs (10^5) were seeded in each well of a 6-well plate coated with attachment factor. The cells were incubated in EGM-2 medium until confluent (~24 hours). The cells were then starved for 12 hours in serum free EBM-2 medium. After starvation, a straight scratch was introduced in the well with a sterile fine 10 µL micropipette tip. The well was rinsed twice with EBM-2 medium to remove unbound cells and debris and the well was imaged at time = 0. Then cells were incubated in EGM-2 medium in the presence of the indicated concentrations of **14a** at 37°C and 5% CO_2. After 8 hours, images were taken using the EVOS microscope and the number of migrated cells into the scratched area was counted.

In Vitro Angiogenesis Assay

A Matrigel based assay was performed to monitor the tube formation ability of HRECs in the presence of **14a** as described previously.[3] Briefly, 7,500 cells in 100 μL EGM-2 medium were incubated in the presence or absence of **14a** in 96-well clear plates coated with 50 μL of Matrigel basement membrane. After 8 hours, the images were recorded using the EVOS microscope and the tube length was measured using angiogenesis analyzer macros in ImageJ (http://image.bio.methods.free.fr/ImageJ/?Angiogenesis-Analyzer-for-ImageJ).

Oxygen Induced Retinopathy (OIR) Mouse Model

All animal experiments were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee and adhered to all standards set forth in the

Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. OIR in mice was induced as described.[4] Briefly, newborn C57BL/6 mice pups along with nursing mother were incubated in a hyperoxia chamber (75% O_2) from postnatal day (P)7 to P12. On day P12, pups were anesthetized using isoflurane and vehicle/anti-VEGF/**14a** was intravitreously injected into each eye under a dissecting microscope. The clogP of **14a** is 4.64. It dissolved readily in DMSO up to 100 mM and was then diluted into phosphate-buffered saline solution up to 50 μM (final [DMSO] = 0.02%). By injecting 0.5 μL of a 10 μM aqueous solution of **14a**, we estimate a final intravitreal concentration of 1 μM, based on the average vitreous volume of young mice of 4.6 μL.[5] For control experiments, a total of 5 ng of anti-VEGF antibody in 0.5 μL vehicle, or 0.5 μL vehicle alone, was delivered to the vitreous. The experimenters (HDB, GR, and AV) were masked to the identity of the treatments throughout experimentation and data analysis.

After the injections, pups along with the nursing mother were returned to normoxia (room air) conditions from P12 to P17. On day P17, pups were killed and the eyes were enucleated and fixed in 4 % paraformaldehyde for 4 hours. Then retinas were isolated under a dissecting microscope. Retinas were washed twice in PBS and then permeabilized for 2 hours in 0.1 % Triton X-100 in 10 % goat serum prepared in PBS. Then 1:200 diluted isolectin B4-Alexa 488 in 10% goat serum was added to each retina and incubated overnight at room temperature protected from light. After the incubation, retinas were washed 4 times in PBS with each wash lasting for 15 minutes. Retinas were incised into four sections on a glass slide and mounted using Vectashield mounting medium. Using the LSM700 confocal microscope, approximately 20 images were taken for each retina with a 10× objective and all of these images were stitched using Adobe Photoshop CS5. Retinal neovascularization was quantified as described[6] using Adobe Photoshop CS5 software.

Statistical Analysis

The data obtained from all experiments were analyzed by one-way ANOVA with Dunnett's post hoc tests for comparisons between compound treatments and control. All analyses were performed using GraphPad Prism software (v. 6.0). A P value of < 0.05 was considered statistically significant in all tests.

Supporting Information References

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