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Design, synthesis, and characterization of novel apigenin analogues that suppress pancreatic stellate cell proliferation in vitro and associated pancreatic fibrosis in vivo

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ABSTRACT

Accumulating evidence suggests that activated pancreatic stellate cells (PSC) play an important role in chronic pancreatitis (CP), and inhibition of the activated PSC is considered as a potential strategy for the treatment and prevention of CP. Herein, we disclose our findings that apigenin and its novel analogues suppress the proliferation and induce apoptosis in PSC, which reduce the PSC-mediated fibrosis in CP. Chemical modifications of apigenin have been directed to build a focused library of *O*-alkylamino-tethered apigenin derivatives at 4′-*O* position of the ring C with the attempt to enhance the potency and drug-like properties including aqueous solubility. A number of compounds such as **14**, **16**, and **24** exhibited potent antiproliferative effects as well as improved aqueous solubility. Intriguingly, apigenin, new analogues **23** and **24** displayed significant efficacy to reduce pancreatic fibrosis even at a low dose of 0.5 mg/kg in our proof-of-concept study using a preclinical in vivo mouse model of CP.

1. Introduction

Chronic pancreatitis (CP) is a progressive, non-curable disorder of the pancreas.[1] Pathologically, both the endocrine and exocrine pancreas undergo progressive and often irreversible morphological changes, including glandular fibrosis.[2–5] In the United States, disorders of the exocrine pancreas affect over 1 million patients and result in a cost over $3.7 billion annually.[6,7] Current treatment options for CP are limited to supportive and palliative care; patients with advanced disease can be managed with endoscopic and/or surgical pancreatic decompression, denervation, resection, bypass or transplantation.[8,9] Overall, patients have a poor quality of life and are burdened by chronic abdominal pain, increased hospitalizations, impaired digestion, diarrhea, weight loss, diabetes, complications like pseudocysts and an increased risk of pancreatic cancer.[9] Therefore, the development of effective, safe and affordable therapeutic agents remains a critical need.

Within the last two decades, it has been well-established that pancreatic stellate cells (PSC) are responsible for the fibrotic component of CP, and suppressing PSC is therefore a potential therapeutic target for the disease.[10–12] In the normal pancreas, PSC are inactive/quiescent, whereas during tissue injury, PSC display an activated, myofibroblastic phenotype, with increased proliferation, motility, and secretion of extracellular matrix proteins.[10–12] CP favors the perpetual activation of PSC.[13] Consequently, a promising strategy for the prevention and treatment of CP involves limiting the proliferation and inducing apoptosis of activated PSC.[13–16]

The sentinel acute pancreatitis event (SAPE) hypothesis provides a unified model for the pathogenesis of CP.[17] After studying cases of hereditary pancreatitis, Whitcomb et al. found that 50% of patients with gain-of-function trypsinogen mutations experienced repeated episodes of acute pancreatitis (AP) that later developed into CP.[1,17] Regardless of the inciting etiology(s) of the sentinel event of AP, recurrent episodes of AP cause CP. AP is initiated with acinar cell injury, characterized by premature acinar zymogen activation, recruitment of inflammatory cells, autodigestion and necrosis of acinar and ductal cells, subsequent anti-inflammatory response, and PSC-dependent scarring.[1,5,17–19] Recurrent pancreatic injury overwhelms normal repair mechanisms, favoring progressive irreversible fibrosis.[1,5,17] We have directed our efforts to develop novel compounds that would limit

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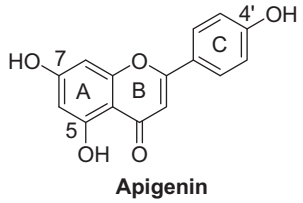


Figure 1. Chemical structure of apigenin.

repeated pancreatic injury, and thus minimize the progression of CP.

Natural products, especially common dietaries consumed on a daily basis, continue to serve as a valuable source in developing drug-like candidates for chemoprevention and chemotherapy.[20–22] Flavonoids, which are ubiquitously distributed in many dietary plant materials, have received a great deal of attention because some of them have been shown to exert various beneficial effects on human health.[23] Apigenin (Fig. 1) is abundantly present in common fruits and vegetables, and has gained particular interest in recent years as a beneficial and health-promoting agent because of its low intrinsic toxicity.[24] Apigenin has been demonstrated to possess various clinically relevant properties such as anti-inflammatory, anti-oxidant, antiproliferative and pro-apoptotic activities likely through multiple mechanisms.[25] Our research efforts on this natural product with low intrinsic toxicity have led to the discovery that apigenin can ameliorate the stromal fibrosis characteristic of CP. Despite its promising anti-fibrotic property, apigenin suffers from poor aqueous solubility and low metabolic stability like most flavonoids, limiting its clinical potential. While many research groups focused on developing novel compounds based on the apigenin structure as anticancer agents, most of these apigenin derivatives suffered from unfavorable physicochemical properties, including limited aqueous solubility, and none of them has been approved for clinical investigation.[26,27] Herein, we report our design and synthesis of novel apigenin analogues that suppress the proliferation and promote apoptosis in activated PSC with improved potency and favorable physicochemical properties. More importantly, we also disclose that at a low dose, new analogues **23** and **24** are as efficacious as apigenin in reducing pancreatic fibrosis in a preclinical animal model, providing the proof-of-concept as potential therapeutics for CP.

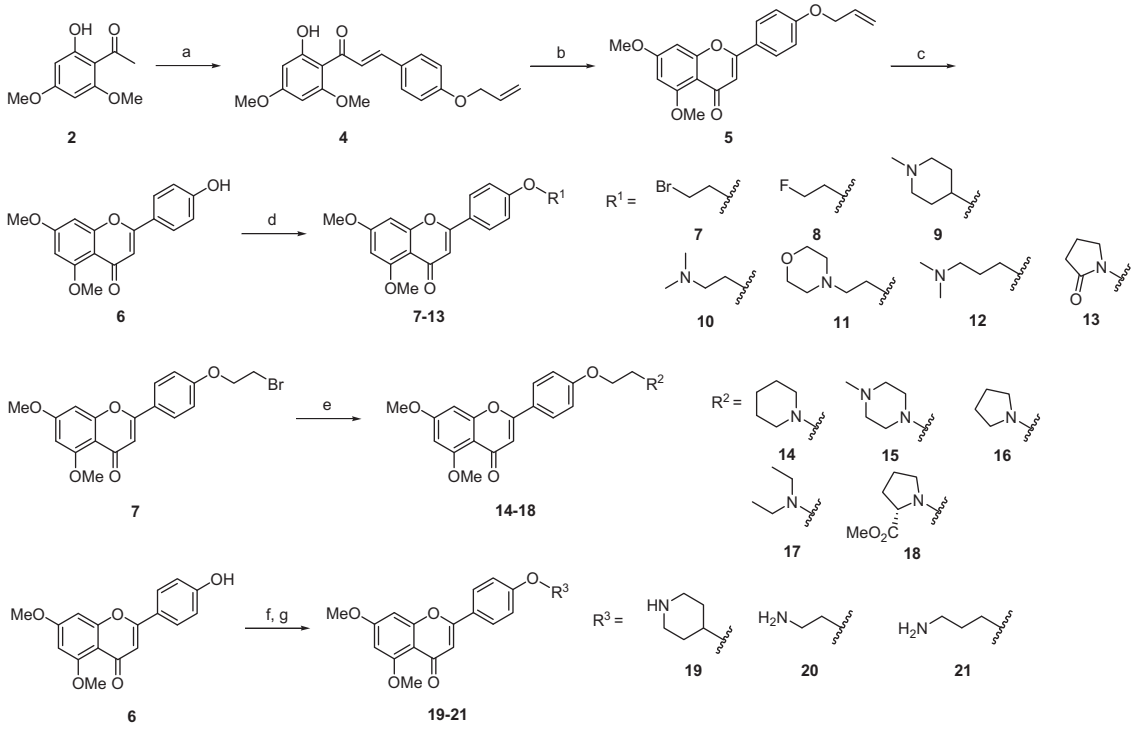
2. Results and discussion

2.1. Design

Considerable efforts on the modifications of apigenin as the lead compound in anticancer drug design have been made. Reported structural-activity relationship (SAR) studies indicate that the A ring of apigenin as well as its C ring are suitable for diverse modifications.[28–31] In addition, increasing studies have demonstrated that the polymethoxylated flavones or flavone analogues with nonpolar and hydrophobic substituents on A ring generally exhibit more potent antiproliferative activities against various human cancer cell lines.[28,32] Moreover, methylated flavones have dramatically higher intestinal permeability and higher metabolic stability.[33–35] Combined with the structural features of the SAR trends, we directed our initial optimization effort to discover novel apigenin derivatives by introducing aqueous solubility-enhancing moieties at 4′-*O* position of apigenin with 5,7-dimethoxy groups on the A ring.

2.2. Chemistry

The synthesis of new apigenin derivatives with chemical optimizations on 4′-hydroxyl group is outlined in Scheme 1. The key intermediate **5** was prepared in a three-step synthesis starting



Scheme 1. Reagents and conditions: (a) 4-allyloxybenzaldehyde (**3**), 50% NaOH/H_2O, EtOH, rt, 16 h, 76%; (b) cat. I_2, DMSO, 140 °C, 4 h, 91%; (c) cat. $Pd(PPh_3)_4$, K_2CO_3, MeOH, 90 °C, 4 h, 95%; (d) R^1OH, Ph_3P, DIAD, THF, rt, 16 h, 81–94%; (e) R^2H, KI, K_2CO_3, acetone, reflux, 18 h, 66–99%; (f) Boc-R^3OH, Ph_3P, DIAD, THF, rt, 2 h; (g) TFA, CH_2Cl_2, 0 °C to rt, 3 h, 59–90% (two steps).

with 1-(2-hydroxy-4,6-dimethoxyphenyl)ethanone (**2**) and 4-allyloxybenzaldehyde (**3**) according to a literature procedure.[36] As shown in Scheme 1, base-catalyzed aldol condensation of **2** with **3** afforded the chalcone **4** in a yield of 76% with a simple purification. The chalcone was cyclized in the presence of catalytic iodine in dimethyl sulfoxide at 140 °C to provide the flavone **5** in high yield. The allyl protecting group of flavones **5** was cleaved with a catalytic amount of $Pd(PPh_3)_4$ in the presence of K_2CO_3 in MeOH at reflux for 4 h to obtain the key intermediate 4′-hydroxyflavone **6** for direct use without further purification.

New analogues **7**–**13** were conveniently synthesized by Mitsunobu reaction of the key intermediate **6** with appropriate substituted alcohols in high yields (81–94%). Alkylation of the bromide intermediate **7** with the corresponding amine in the presence of K_2CO_3 and KI in acetone introduced basic functionalities providing final compounds **14**–**18** in 66–99% yields. Mitsunobu coupling of **6** with *N*-Boc-protected amino alcohols followed by the Boc-deprotection with the treatment of TFA in CH_2Cl_2 afforded analogues **19**–**21** with diversified *O*-alkylamino side chains in 59–90% yields (two steps).

The synthetic route to 3-amino-2-hydroxypropoxy-flavonesis outlined in Scheme 2. The reaction of the key intermediate **6** with excessive epichlorohydrin (**22**) and successive treatment of the intermediate epoxide (**23**) with appropriate amines under reflux afforded the desired derivatives **24**–**26** in yields of 71–94%. The Mitsunobu coupling of **6** with (2,2-dimethyl-1,3-dioxolan-4-yl)methanol (solketal, **27**) generated an intermediate which was subjected to cleavage conditions using 0.5% HCl (aq) in EtOH to produce the 1,2-diol derivative **28**. For the synthesis of diversified apigenin analogues on side chain of the ring C at *para*-position as depicted in Scheme 3, the starting material **2** was condensed with 4-bromobenzaldehyde (**29**) to give chalcone **30**, which was then cyclized to give the key intermediate **31**. Compound **33** was obtained by Suzuki coupling reaction of **31** with 2-fluoropyridine-5-boronic acid (**32**) in the presence of $Pd(dppf)Cl_2$ catalyst in a yield of 85%. Palladium-catalyzed Buchwald–Hartwig amination reaction of **31** with *N*,*N*-dimethylethylenediamine (**34**) or 2-(pyrrolidin-1-yl)ethanamine (**35**) afforded the targeted compounds **36** and **37** in yield of 64% and 57%, respectively.

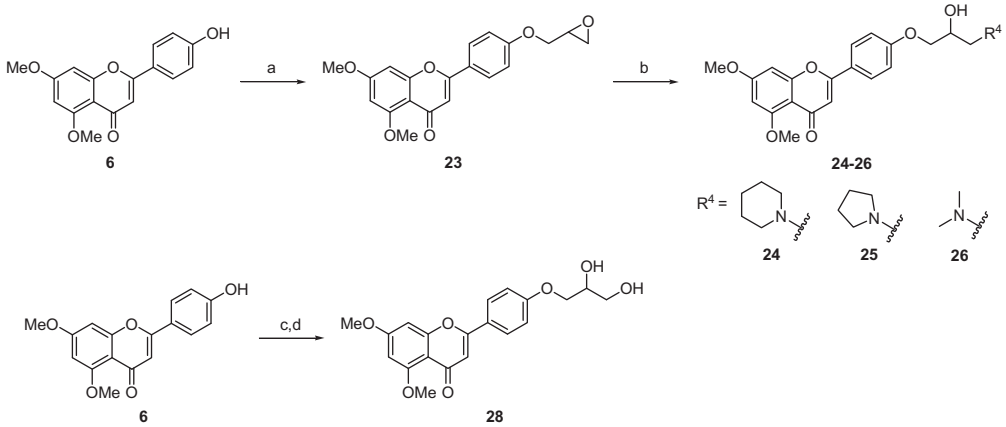
Scheme 4 outlines the synthesis of demethylated derivatives **38** and **39**. Generation of **38** was achieved in 79% yield by a monodemethylation of **16** using 2 equiv of boron tribromide at room temperate for 2 h. Both methyl groups on A-ring of **16** were successfully removed by treatment with 3 equiv of boron tribromide for 24 h, leading to the demethylated analogue **39** in a yield of 73%.

2.3. Biology

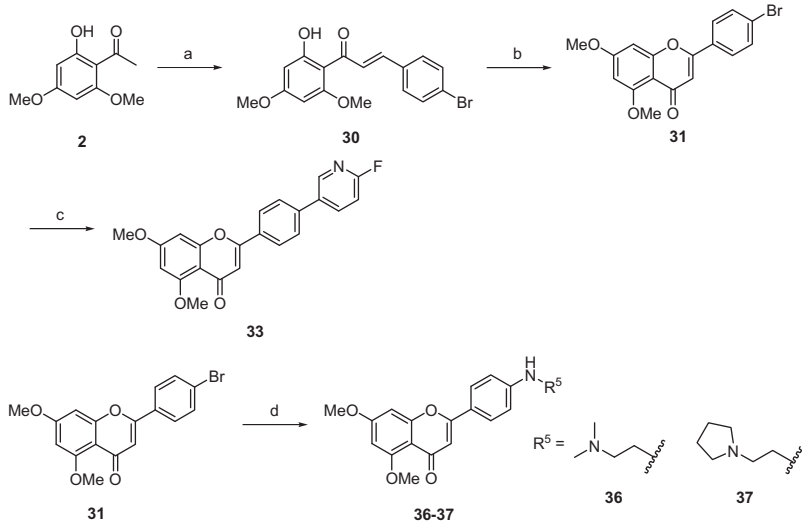
The calculated lipophilicity (*c*Log*P*) and topological polar surface area (tPSA) values of all newly synthesized analogues are listed in Table 1. The results indicate that all these new compounds meet the criteria of Lipinski's 'Rule of Five' and may have favorable physicochemical properties. To explore a meaningful SAR and examine how the substitutions on the key moieties affect biological activities of new apigenin derivatives, we first evaluated the in vitro antiproliferative effects of these analogues on transformed PSC using AlamarBlue Cell Viability Assay (Life Technologies) as described in the Section 4. AlamarBlue is a non-toxic reagent that is converted to a highly fluorescent end product by viable cells. The capabilities of these new analogues to inhibit the proliferation of transformed PSC are summarized in Table 1.

The key intermediate **6** was found to display no significant antiproliferative effect even at 20 μM. After introduction of an *O*-bromoalkyl moiety or an *O*-fluoroalkyl moiety into the derivative **6** at 4′-*O* position, compounds **7** and **8** showed a slightly increased antiproliferative effects in comparison with **6**, indicating that appropriate modifications on 4′-*O* position may regain the antiproliferative activity. *O*-Alkylamino-tethered derivatives **9**, **10** and **12** exhibited a moderate antiproliferative activity at 10 μM with inhibitory effects of 49%, 51% and 59%, respectively. This finding suggests that optimizations with a nitrogen-containing hydrophilic moiety at 4′-*O* position appears to be a viable strategy to yield more potent compounds with a better aqueous solubility. To this end, compounds **14** with a piperidinyl moiety and **16** with a pyrrolidinyl group displayed potent antiproliferative activity at 10 μM with inhibitory effects of 64% and 66%, respectively. Meanwhile, we found that the tertiary amines with alkylated amino groups appeared to be more favorable than secondary or primary amines with free amino groups at the terminal of the side chains. For instance, in comparison with compounds **14** and **16**, analogues **19**–**21** only exhibited a moderate to low inhibitory effects. The similar trend of SAR was also observed for derivatives **24**–**26** and **28**. Compound **28** with a terminal OH group at the tail resulted in a dramatic loss of activities compared with its according analogues **24**–**26** with a terminal amino moiety. Interestingly, compound **23** with an epoxide was identified as a highly potent inhibitor suppressing PSC proliferation.

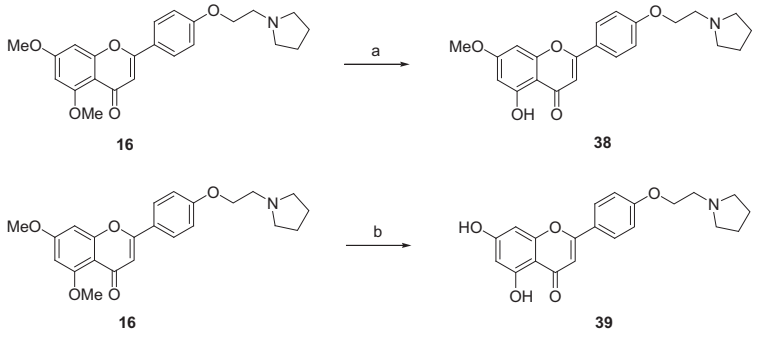
Structural modifications of *para*-position on C-ring with diversified substitutions were also investigated. Compound **33** with a pyridinyl group on the *para*-position resulted in a substantial loss of the activity. In contrast, introduction of



Scheme 2. Reagents and conditions: (a) epichlorohydrin (**22**), K_2CO_3, acetone, reflux, 24 h, 67%; (b) R^4H, K_2CO_3, EtOH, reflux, 3 h, 71–94%; (c) Ph_3P, DIAD, THF, (2,2-dimethyl-1,3-dioxolan-4-yl)methanol (**27**), rt, 4 h; (d) 0.5 N HCl (aq), EtOH, reflux, 2 h, 71% (two steps).



Scheme 3. Reagents and conditions: (a) 4-bromobenzaldehyde (**29**), 50% NaOH/H_2O, EtOH, rt, 16 h, 65%; (b) cat. I_2, DMSO, 140 °C, 4 h, 92%; (c) 2-fluoropyridine-5-boronic acid (**32**), Pd(dppf)Cl_2, KOAc, THF/EtOH/H_2O, 80 °C, 18 h, 85%; (d) NH_2R^5 [*N*,*N*-dimethylethylenediamine (**34**) for **36** or 2-(pyrrolidin-1-yl)ethanamine (**35**) for **37**], $Pd_2(dba)_3$, NaO^tBu, (±)-BINAP, toluene, 80 °C, 48 h, 57–64%.



Scheme 4. Reagents and conditions: (a) 1 N BBr_3 (in CH_2Cl_2), CH_2Cl_2, rt, 2 h, 79%; (b) 1 N BBr_3 (in CH_2Cl_2), CH_2Cl_2, rt, 24 h, 73%.

2-dimethylamino-ethylamino moiety or 2-pyrrolidin-1-yl-ethylamino group displayed significantly better antiproliferative activities at 10 μM with inhibitory effects of 60% and 51%, respectively. Demethylation of compound **16** resulted in generation of two *O*-demethylated compounds **38** and **39**. We found that neither mono-demethylation nor full demethylation of the methoxy groups on the A-ring is favorable for the enhancement of activity, and thus more extensive SAR study on the A-ring was not pursued.

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Structural modifications of *para*-position on C-ring with diversified substitutions were also investigated. Compound **33** with a pyridinyl group on the *para*-position resulted in a substantial loss of the activity. In contrast, introduction of 2-dimethylamino-ethylamino moiety or 2-pyrrolidin-1-yl-ethylamino group displayed significantly better antiproliferative activities at 10 μM with inhibitory effects of 60% and 51%, respectively. Demethylation of compound **16** resulted in generation of two *O*-demethylated compounds **38** and **39**. We found that neither mono-demethylation nor full demethylation of the methoxy groups on the A-ring is favorable for the enhancement of activity, and thus more extensive SAR study on the A-ring was not pursued.

Since one goal of our drug discovery effort was to identify new apigenin derivatives with improved water-solubility and oral bioavailability, aqueous solubility of several selected analogues with enhanced antiproliferative effects was determined by an HPLC analysis method. Since one goal of our drug discovery effort was to identify new apigenin derivatives with improved water-solubility and

Table 1
Effects of apigenin and newly synthesized apigenin analogues on PSC proliferation

Entry	*c*Log*P*[a]	tPSA[b]	Inhibitory effect[c] (%)		
			5 μM	10 μM	20 μM
Apigenin (1)	2.33	90.9	11	34	64
6	2.73	68.9	NE[d]	NE	NE
7	3.86	57.9	NE	NE	20
8	3.35	57.9	NE	NE	21
9	3.29	61.2	28	49	ND[e]
10	2.73	61.2	19	51	65
11	2.45	70.4	NE	NE	NE
12	3.06	61.2	36	59	86
13	2.69	78.2	7	14	33
14	3.84	61.2	45	64	97
15	2.66	64.4	22	42	67
16	3.48	61.2	48	66	98
17	3.54	61.2	33	60	72
18	3.22	87.5	22	37	67
19	3.08	69.9	NE	21	ND
20	1.93	83.9	6	35	70
21	2.43	83.9	22	39	ND
23	2.50	70.4	69	89	93
24	3.03	81.4	34	65	95
25	2.66	81.4	21	37	57
26	2.13	81.4	42	60	71
28	1.77	98.4	NE	11	16
31	3.80	48.7	12	10	22
33	4.05	61.6	NE	6	19
36	2.72	63.9	30	60	84
37	3.13	63.9	21	51	80
38	3.28	72.1	NE	39	99
39	2.98	83.1	2	71	90

[a] *c*Log*P*: http://146.107.217.178/lab/alogps/start.html.
[b] tPSA: http://www.molinspiration.com/cgi-bin/properties.
[c] Values are mean of at least three independent experiments.
[d] NE: no effect.
[e] ND: not determined.

oral bioavailability, aqueous solubility of several selected analogues with enhanced antiproliferative effects was determined by an HPLC analysis method.[37] As depicted in Figure 2, compounds **14**, **16** and **24** (in the form of HCl salt) have demonstrated to possess more favorable aqueous solubility, with a saturated concentration of 17.4, 13.7 and 84.1 mg/mL, respectively, while that of apigenin is only 2.16 μg/mL.[26]

To investigate more detailed information about the antiproliferative effects of this series of apigenin derivatives against human PSC, compounds **23** (**HJC0561**) and **24** (**HJC05100**) were selected based on their potency or aqueous solubility for further evaluations using cell proliferation and cell death assays, as well as an in vivo model of CP. As shown in Figure 3A, both analogues **23** and **24** inhibited PSC proliferation at lower doses than apigenin, indicating their enhanced potency in vitro. With logarithmic transformation and nonlinear regression, a best-fit curve was generated, allowing determination of their IC_{50}, representing the concentration at which the compound causes 50% inhibition of PSC proliferation (Fig. 3B). Compound **23** was identified as a highly potent analogue with an IC_{50} value of 2.5 ± 0.6 μM. Compound **24** was slightly less potent with an IC_{50} value of 8.0 ± 1.8 μM, and that of apigenin was 18.6 ± 1.6 μM. The irreversible fibrosis that defines CP is mediated by activated PSC, which produce and remodel the extracellular matrix (ECM).[10] Therefore, the potent antiproliferative effect of these new analogues on PSC supports their further development as anti-fibrotic agents for CP.

These two representative analogues were also investigated to determine whether the growth inhibition induced by compounds **23** and **24** in PSC was attributed to apoptosis. Apoptosis was determined using the Cell Death Detection ELISA^{PLUS} assay. This sandwich-enzyme-immunoassay involved antibodies binding to cytoplasmic nucleosomes, which were specific to the process of apoptosis rather than necrosis. Analogue **23** was found to be quite potent, inducing greater PSC cell death at lower concentrations than apigenin (Fig. 4A). At low concentrations, compound **24** failed to induce apoptosis; however, between the concentrations of 25–35 μM, it produced a steep dose–response, and concentrations beyond 35 μM induced significant cell death. Next, the dose–response curve was generated (Fig. 4B). The EC_{50} represented the concentration at which the compound yielded half of the maximal amount of cell death. The most potent analogue **23** exhibited the lowest EC_{50} value of 9.6 ± 1.8 μM, while the EC_{50} value of apigenin was 24.5 ± 2.5 μM, and that of **24** was 35.2 ± 5.5 μM. The capacity of the compounds to induce cell death in activated PSC would most likely translate into reduced fibrosis in CP. Analogue **23** more potently induced PSC apoptosis than apigenin, while **24** appeared to act through a different cellular mechanism, inducing stellate cell toxicity at concentrations greater than 25 μM.

These encouraging results prompted us to further test **23** and **24** as lead compounds for the development of a new series of anti-fibrotic agents which may be useful for treating CP.[38] In order to evaluate the anti-fibrotic effect of these new compounds as a proof-of-concept, we tested the compounds in a preclinical animal model of CP. CP was induced in mice using the well-established model of repeated cerulean (CR) injections.[39,40] Treatment with vehicle, apigenin or analogues (0.5 mg/kg daily gavage) was started the second week of the experiment and continued with CP

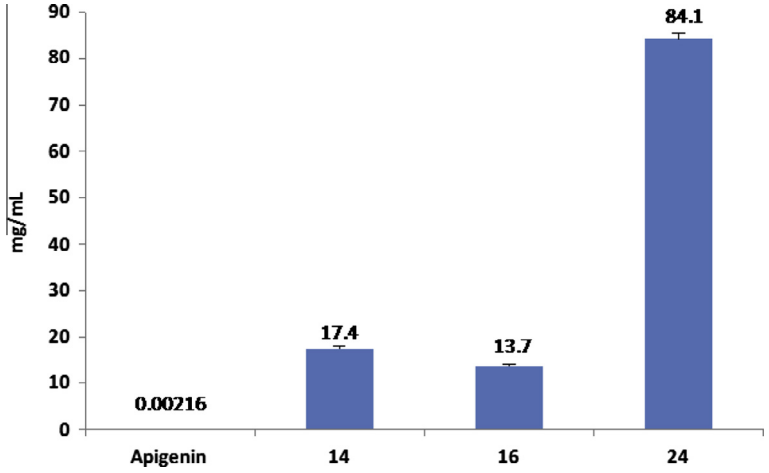

Figure 2. Aqueous solubility of apigenin and selected apigenin derivatives. Compounds **14**, **16** and **24** (in the form of HCl salt) showed significantly improved solubility as compared with apigenin.

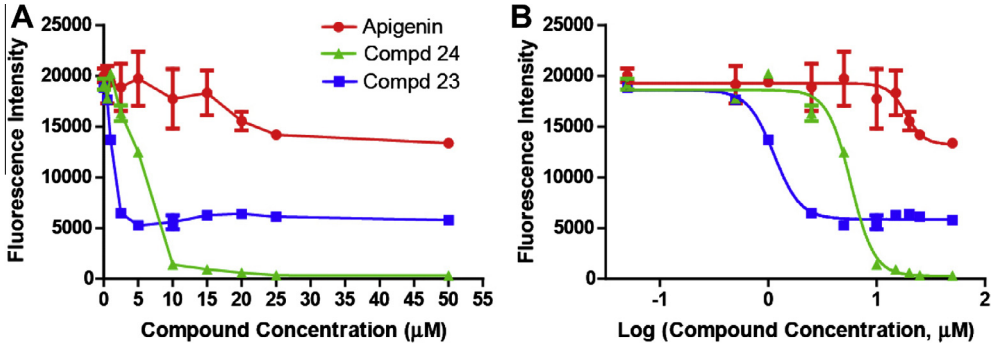

Figure 3. Effect of apigenin and its analogues on PSC proliferation. Human PSC were treated with apigenin or analogues at various doses for 24 h. (A) Representative data from one experiment: cell viability was measured using the AlamarBlue colorimetric assay. (B) The IC_{50} values of apigenin, **23**, and **24** are 18.6 ± 1.6 μM, 2.5 ± 0.6 μM, and 8.0 ± 1.8 μM, respectively. The calculated IC_{50} values are derived from the mean ± SEM of at least three independent experiments.

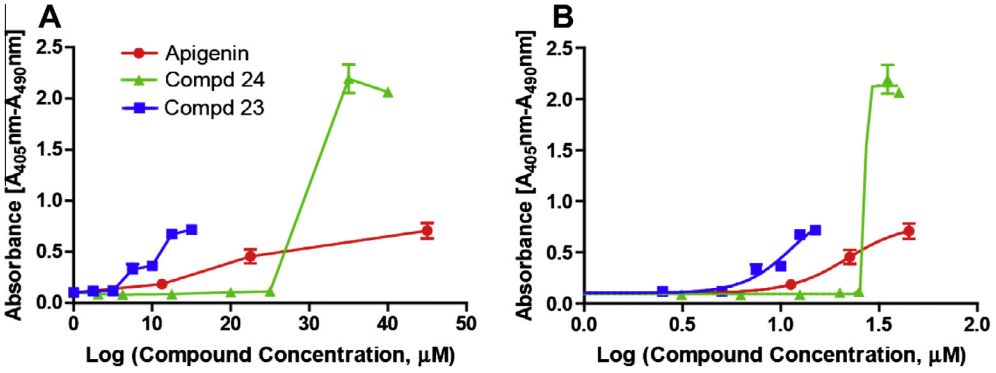

Figure 4. Effect of apigenin and its analogues on PSC apoptosis. Human PSC were treated with apigenin or analogues at various doses for 14 h. (A) Representative data from one experiment: Apoptosis was measured using the Cell Death Detection ELISA^{PLUS} assay. (B) The EC_{50} values of apigenin, **23**, and **24** are 24.5 ± 2.5 μM, 9.6 ± 1.8 μM, and 35.2 ± 5.5 μM, respectively. The calculated EC_{50} values are derived from the mean ± SEM of at least three independent experiments.

induction. After four weeks, the pancreata were processed and stained for fibronectin, which is a major component of the ECM. The 400× microscope image in Figure 5A showed a large amount of periacinar and perilobular fibrosis (brown color), edema (the space between lobules), and atrophic, irregularly shaped acini.[41] Treatment with apigenin and analogues **23** and **24** significantly decreased the stromal fibrosis of CP, reduced tissue edema, and limited acinar cell damage (Fig. 5B–D). ImageJ analysis of the slides quantified the significant decrease in fibrosis (p <0.001) (Fig. 5E). Despite no statistical difference between apigenin and compounds **23** or **24** at the low dose of 0.5 mg/kg, these data demonstrated that apigenin and new analogues significantly reduced fibrosis in the pre-clinical animal model of CP. The present investigation has provided a proof-of-concept study that apigenin and analogues have the potential to function as anti-fibrotic agents in CP. The relevant mechanistic studies and further dose–response investigation of in vivo efficacy are currently ongoing.

3. Conclusion

Accumulating evidence suggests that activated PSC play an important role in CP. We have proposed a potential strategy for the prevention and treatment of CP by decreasing the proliferation and inducing apoptosis of PSC. While a few flavone-based compounds have previously been reported to possess antiproliferative effects for cancer, we are the first to show that apigenin and its novel analogues suppress the proliferation of PSC and reduce the associated fibrosis. Chemical modifications of apigenin have been directed to build a focused library of *O*-alkylamino-tethered apigenin derivatives at 4′-*O* position of the ring C with the attempt to enhance the potency and drug-like properties including aqueous solubility. A series of novel apigenin analogues have been synthesized from the key intermediate **6**, and a number of compounds such as **14**, **16**, and **24** have been identified to exhibit potent antiproliferative effects as well as improved aqueous solubility. Even at a low dose of 0.5 mg/kg, apigenin and new analogues **23** and **24** significantly reduced the fibrotic response in a preclinical animal model of CP, providing a proof-of-concept study that supports their development as promising therapeutics for CP.

4. Experimental section

4.1. Chemistry

4.1.1. General

All commercially available starting materials and solvents were reagent grade, and used without further purification. Reactions were performed under a nitrogen atmosphere in dry glassware with magnetic stirring. Preparative column chromatography was performed using silica gel 60, particle size 0.063–0.200 mm (70–230 mesh, flash). Analytical TLC was carried out employing silica gel 60 F254 plates (Merck, Darmstadt). Visualization of the developed chromatograms was performed with detection by UV (254 nm). NMR spectra were recorded on a Bruker-600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with TMS as an internal reference. Chemical shifts were expressed in ppm, and J values were given in Hz. High-resolution mass spectra (HRMS) were obtained from Thermo Fisher LTQ Orbitrap Elite mass spectrometer. Parameters include the following: Nano ESI spray voltage was 1.8 kV; Capillary temperature was 275 °C and the resolution was 60,000; Ionization was achieved by positive mode. Melting points were measured on a Thermo

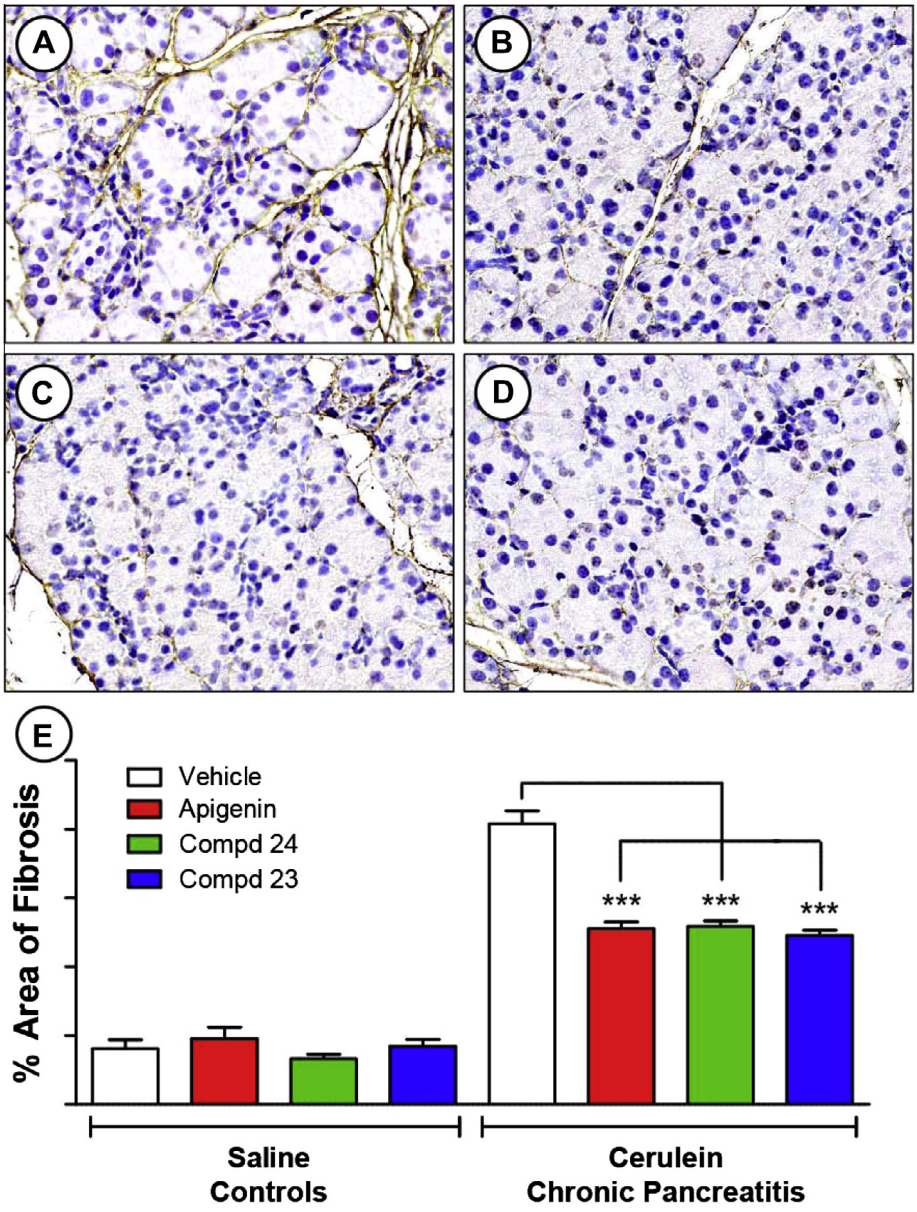

Figure 5. Effect of apigenin and its analogues in preclinical animal model of CP. CP was induced using repeated cerulein (CR) injections as outlined in the methods. Control mice received PBS ($n = 5$), and mice injected with CR developed CP ($n = 10$–11). Treatment with vehicle (0.5% methylcellulose + 0.025% Tween 20 in ddH_2O), apigenin or analogues (0.5 mg/kg, daily gavage) started the second week and was continued 3 additional weeks while continuing CP induction. Pancreata were stained for fibronectin by IHC, and counter-stained with hematoxylin. Representative 400× images of each group are shown: (A) CR + Vehicle; (B) CR + Apigenin; (C) CR + **24**; and (D) CR + **23**. ImageJ analysis of the slides quantified the percent area of brown fibronectin stain in (E). *** Represents p <0.001, comparing to the effect from apigenin at the same dosage.

Scientific Electrothermal Digital Melting Point Apparatus and uncorrected. Purity of final compounds was determined by analytical HPLC, which was carried out on a Shimadzu HPLC system (model: CBM-20A LC-20AD SPD-20A UV/VIS). HPLC analysis conditions: Waters μBondapak C18 (300 × 3.9 mm); flow rate 0.5 mL/min; UV detection at 270 and 254 nm; linear gradient from 30% acetonitrile in water [0.1%, trifluoroacetic acid (TFA)] to 100% acetonitrile (0.1% TFA) in 20 min followed by 30 min of the last-named solvent. All biologically evaluated compounds are >95% pure.

4.1.2. 2-(4-Hydroxy-phenyl)-5,7-dimethoxy-chromen-4-one (6)

Starting from the commercially available 4-allyloxybenzaldehyde (**3**) and 1-(2-hydroxy-4,6-dimethoxy-phenyl)-ethanone (**2**), the key intermediate **6** was prepared in three steps according to literature procedures.[36] HPLC purity 97.0% (t_R = 16.94 min). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88 (d, 2H, J = 9.0 Hz), 6.90 (d, 2H, J = 9.0 Hz), 6.83 (d, 1H, J = 2.4 Hz), 6.58 (s, 1H), 6.49 (d, 1H, J = 2.4 Hz), 3.89 (s, 3H), 3.82 (s, 3H). HRMS (ESI) calcd for $C_{17}H_{15}O_5$ 299.0914 $(M + H)^+$, found 299.0917.

4.1.3. 2-(4-(2-Bromoethoxy)phenyl)-5,7-dimethoxy-chromen-4-one (7)

To a solution of **6** (200 mg, 0.67 mmol) and Ph_3P (351 mg, 1.34 mmol) in THF (10 mL) was added 2-bromoethanol (168 mg, 1.34 mmol) and diisopropylazodicarboxylate (DIAD, 271 mg, 1.34 mmol). The mixture was stirred at rt for 16 h. The reaction mixture was diluted with ethyl acetate (EtOAc, 200 mL) and extracted with H_2O (40 mL). The organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated to give the crude product. This residue was purified with silica gel column (EtOAc) to provide **7** (240 mg, 88%) as a white solid (mp 202–203 °C). HPLC purity 99.6% (t_R = 21.17 min). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.00 (d, 2H, J = 8.4 Hz), 7.12 (d, 2H, J = 9.0 Hz), 6.86 (d, 1H, J = 2.4 Hz), 6.68 (s, 1H), 6.50 (d, 1H,

J = 1.8 Hz), 4.43 (t, 2H, J = 5.4 Hz), 3.90 (s, 3H), 3.85(t, 2H, J = 5.4 Hz), 3.83 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.6, 163.6, 160.4, 160.2, 159.5, 159.1, 127.8, 123.5, 115.1, 108.3, 106.9, 96.2, 93.4, 68.0, 56.1, 56.0, 31.2. HRMS (ESI) calcd for $C_{19}H_{18}BrO_5$ 405.0332 $(M+H)^+$, found 405.0334.

4.1.4. 2-(4-(2-Fluoroethoxy)phenyl)-5,7-dimethoxy-chromen-4-one (8)

Compound **8** was prepared in 90% yield by a procedure similar to that used to prepare **7**. The title compound was obtained as a white solid (mp 187–188 °C). HPLC purity 97.3% (t_R = 19.56 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 (d, 2H, J = 10.2 Hz), 7.03 (d, 2H, J = 10.2 Hz), 6.59 (s, 1H), 6.55 (d, 1H, J = 2.4 Hz), 6.37 (d, 1H, J = 1.8 Hz), 4.82–4.84 (m, 1H), 4.74–4.76 (m, 1H), 4.30–4.32 (m, 1H), 4.26–4.27 (m, 1H), 3.95 (s, 3H), 3.91 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.1, 161.0, 160.9, 160.6, 160.0, 127.8, 124.6, 115.1, 109.4, 108.0, 96.2, 93.0, 81.9 (d, J = 171.5 Hz), 67.4 (d, J = 20.3 Hz), 56.6, 55.9. HRMS (ESI) calcd for $C_{19}H_{18}FO_5$ 345.1133 $(M+H)^+$, found 345.1135.

4.1.5. 5,7-Dimethoxy-2-(4-(1-methylpiperidin-4-yloxy)phenyl)-chromen-4-one (9)

Compound **9** was prepared in 94% yield by a procedure similar to that used to prepare **7**. The title compound was obtained as a white solid (mp 143–144 °C). HPLC purity 99.3% (t_R = 15.86 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, 2H, J = 9.0 Hz), 6.99 (d, 2H, J = 9.0 Hz), 6.58 (s, 1H), 6.55 (d, 1H, J = 2.4 Hz), 6.37 (d, 1H, J = 3.0 Hz), 4.44 (s, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 2.72 (s, 2H), 2.38 (s, 2H), 2.34 (s, 3H), 2.04–2.08 (m, 2H), 1.88–1.92 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.0, 161.0, 160.8, 160.1, 160.0, 127.8, 123.9, 116.2, 109.4, 107.8, 96.2, 93.0, 56.6, 55.9, 52.4, 46.1, 30.6. HRMS (ESI) calcd for $C_{23}H_{26}NO_5$ 396.1806 $(M+H)^+$, found 396.1808.

4.1.6. 2-(4-(2-Dimethylaminoethoxy)phenyl)-5,7-dimethoxy-chromen-4-one (10)

Compound **10** was prepared in 81% yield by a procedure similar to that used to prepare **7**. The title compound was obtained as a pale yellow solid (mp 153–154 °C). HPLC purity 98.8% (t_R = 15.25 - min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, 2H, J = 9.0 Hz), 7.02 (d, 2H, J = 9.0 Hz), 6.59 (s, 1H), 6.55 (d, 1H, J = 1.8 Hz), 6.37 (d, 1H, J = 1.8 Hz), 4.14 (t, 2H, J = 6.0 Hz), 3.95 (s, 3H), 3.91 (s, 3H), 2.78 (t, 2H, J = 6.0 Hz), 2.36 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.0, 161.4, 161.0, 160.8, 160.0, 127.7, 124.1, 115.1, 109.4, 107.9, 96.2, 92.9, 66.3, 58.2, 56.6, 55.9, 46.0. HRMS (ESI) calcd for $C_{21}H_{24}NO_5$ 370.1649 $(M+H)^+$, found 370.1652.

4.1.7. 5,7-Dimethoxy-2-(4-(2-morpholin-4-yl-ethoxy)phenyl)-chromen-4-one (11)

Compound **11** was prepared in 82% yield by a procedure similar to that used to prepare **7**. The title compound was obtained as a white solid (mp 149–150 °C). HPLC purity 99.8% (t_R = 15.26 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, 2H, J = 9.0 Hz), 6.99 (d, 2H, J = 9.0 Hz), 6.58 (s, 1H), 6.55 (s, 1H), 6.37 (s, 1H), 4.18 (t, 2H, J = 6.0 Hz), 3.95 (s, 3H), 3.90 (s, 3H), 3.74 (t, 4H, J = 3.0 Hz), 2.83 (t, 2H, J = 6.0 Hz), 2.59 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.1, 161.3, 161.0, 160.7, 160.0, 127.7, 124.2, 115.1, 109.4, 107.9, 96.2, 93.0, 67.0, 66.2, 57.6, 56.6, 55.9, 54.3. HRMS (ESI) calcd for $C_{23}H_{26}NO_6$ 412.1755 $(M+H)^+$, found 412.1757.

4.1.8. 2-(4-(3-Dimethylaminopropoxy)phenyl)-5,7-dimethoxy-chromen-4-one (12)

Compound **12** was prepared in 91% yield by a procedure similar to that used to prepare **7**. The title compound was obtained as a white solid (mp 105–106 °C). HPLC purity 98.2% (t_R = 15.76 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, 2H, J = 9.0 Hz), 6.99 (d, 2H, J = 9.0 Hz), 6.59 (s, 1H), 6.56 (d, 1H, J = 2.4 Hz), 6.37 (d, 1H, J = 2.4 Hz), 4.09 (t, 2H, J = 9.0 Hz), 3.95 (s, 3H), 3.91 (s, 3H), 2.48 (t, 2H, J = 7.2 Hz), 2.27 (s, 6H), 1.98–2.00 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.0, 161.7, 161.1, 160.9, 160.0, 127.7, 123.9, 115.0, 109.4, 108.0, 96.2, 93.0, 66.6, 56.6, 56.4, 55.9, 45.6, 27.6. HRMS (ESI) calcd for $C_{22}H_{26}NO_5$ 384.1806 $(M+H)^+$, found 384.1808.

4.1.9. 1-(2-(4-(5,7-Dimethoxy-4-oxo-4*H*-chromen-2-yl)phenoxy)ethyl)-pyrrolidin-2-one (13)

Compound **13** was prepared in 85% yield by a procedure similar to that used to prepare **7**. The title compound was obtained as a white solid (mp 115–116 °C). HPLC purity 99.3% (t_R = 18.44 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, 2H, J = 8.4 Hz), 6.97 (d, 2H, J = 8.4 Hz), 6.58 (s, 1H), 6.55 (d, 1H, J = 2.4 Hz), 6.37 (d, 1H, J = 1.8 Hz), 4.18 (t, 2H, J = 5.4 Hz), 3.95 (s, 3H), 3.91 (s, 3H), 3.71 (t, 2H, J = 5.4 Hz), 3.58 (t, 2H, J = 7.2 Hz), 2.39 (t, 2H, J = 8.4 Hz), 2.01–2.08 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 175.6, 164.1, 161.0, 160.9, 160.7, 160.0, 127.8, 124.4, 114.9, 109.3, 107.9, 96.2, 92.9, 66.8, 56.6, 55.9, 49.1, 42.4, 30.9, 18.3. HRMS (ESI) calcd for $C_{23}H_{24}NO_6$ 410.1598 $(M+H)^+$, found 410.1601.

4.1.10. 5,7-Dimethoxy-2-(4-(2-piperidin-1-yl-ethoxy)phenyl)-chromen-4-one (14)

To a solution of **7** (30 mg, 0.074 mmol), KI (25 mg, 0.15 mmol) and K_2CO_3 (102 mg, 0.74 mmol) in acetone (5 mL) was added piperidine (31 mg, 0.37 mmol) at 0 °C. The mixture was stirred at 75 °C for 18 h. The solution was diluted with EtOAc (100 mL), washed with 0.1 N HCl (aq) (10 mL) and brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc) to give the desired product **14** (30 mg, 99%) as a white solid (mp 79–80 °C). HPLC purity 95.1% (t_R = 16.24 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (d, 2H, J = 6.6 Hz), 7.02 (d, 2H, J = 6.6 Hz), 6.62 (s, 1H), 6.58 (d, 1H, J = 2.4 Hz), 6.39 (d, 1H, J = 2.4 Hz), 4.22 (t, 2H, J = 6.0 Hz), 3.98 (s, 3H), 3.94 (s, 3H), 2.85 (t, 2H, J = 6.0 Hz), 2.57–2.59 (m, 4H), 1.65–1.67 (m, 4H), 1.50–1.52 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.1, 161.4, 161.0, 160.8, 160.0, 127.7, 124.0, 115.1, 109.4, 107.8, 96.2, 93.0, 66.3, 57.8, 56.6, 55.9, 55.2, 25.9, 24.2. HRMS (ESI) calcd for $C_{24}H_{28}NO_5$ 410.1962 $(M+H)^+$, found 410.1964.

4.1.11. 5,7-Dimethoxy-2-(4-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-chromen-4-one(15)

Compound **15** was prepared in 80% yield by a procedure similar to that used to prepare **14**. The title compound was obtained as a white solid (mp 144–145 °C). HPLC purity 98.7% (t_R = 16.43 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79–7.81 (m, 2H), 6.98–7.00 (m, 2H), 6.58 (s, 1H), 6.55 (d, 1H, J = 2.4 Hz), 6.36 (d, 1H, J = 2.4 Hz), 4.17 (t, 2H, J = 6.0 Hz), 3.95 (s, 3H), 3.90 (s, 3H), 2.85 (t, 2H, J = 6.0 Hz), 2.50–2.65 (m, 8H), 2.30 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.0, 161.4, 161.0, 160.8, 160.0, 127.7, 124.1, 115.1, 109.4, 107.8, 96.2, 92.9, 66.3, 57.1, 56.6, 55.9, 55.1, 53.7, 46.1. HRMS (ESI) calcd for $C_{24}H_{29}N_2O_5$ 425.2071 $(M+H)^+$, found 425.2070.

4.1.12. 5,7-Dimethoxy-2-(4-(2-pyrrolidin-1-yl-ethoxy)phenyl)-chromen-4-one (16)

Compound **16** was prepared in 85% yield by a procedure similar to that used to prepare **14**. The title compound was obtained as a pale brown solid (mp 114–115 °C). HPLC purity 99.2% (t_R = 15.91 - min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78(d, 2H, J = 6.6 Hz), 6.99 (d, 2H, J = 6.6 Hz), 6.55 (d, 1H, J = 3.6 Hz), 6.52 (d, 1H, J = 2.4 Hz), 6.34 (d, 1H, J = 2.4 Hz), 4.17 (t, 2H, J = 9.0 Hz), 3.92 (s, 3H), 3.88 (s, 3H), 2.93 (t, 2H, J = 9.0 Hz), 2.65–2.66 (m, 4H), 1.81–1.82 (m,

4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.0, 161.4, 161.0, 160.8, 160.0, 127.7, 123.9, 115.0, 109.3, 107.7, 96.2, 93.0, 67.3, 56.5, 55.8, 54.9, 54.8, 23.6. HRMS (ESI) calcd for $C_{23}H_{26}NO_5$ 396.1806 $(M+H)^+$, found 396.1809.

4.1.13. 2-(4-(2-Diethylaminoethoxy)-phenyl)-5,7-dimethoxy-chromen-4-one (17)

Compound **17** was prepared in 85% yield by a procedure similar to that used to prepare **14**. The title compound was obtained as a pale yellow solid (mp 116–117 °C). HPLC purity 99.5% (t_R = 16.09 - min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, 2H, J = 9.0 Hz), 7.00 (d, 2H, J = 9.0 Hz), 6.59 (d, 1H, J = 2.4 Hz), 6.56 (d, 1H, J = 1.8 Hz), 6.37 (d, 1H, J = 1.8 Hz), 4.12 (t, 2H, J = 6.0 Hz), 3.95 (s, 3H), 3.91 (s, 3H), 2.91 (t, 2H, J = 6.0 Hz), 2.65–2.68 (m, 4H), 1.09 (t, 6H, J = 7.2 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.0, 161.5, 161.0, 160.8, 160.0, 127.7, 124.0, 115.1, 109.4, 107.8, 96.2, 93.0, 67.0, 56.6, 55.9, 51.8, 48.0, 12.0. HRMS (ESI) calcd for $C_{23}H_{28}NO_5$ 398.1962 $(M+H)^+$, found 398.1964.

4.1.14. 1-(2-(4-(5,7-Dimethoxy-4-oxo-4*H*-chromen-2-yl)phenoxy)ethyl)-pyrrolidine-2-carboxylic acid methyl ester (18)

Compound **18** was prepared in 66% yield by a procedure similar to that used to prepare **14**. The title compound was obtained as a white solid (mp 121–122 °C). HPLC purity 98.4% (t_R = 16.15 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (d, 2H, J = 9.0 Hz), 7.00 (d, 2H, J = 9.0 Hz), 6.61 (s, 1H), 6.58 (d, 1H, J = 2.4 Hz), 6.40 (d, 1H, J = 1.8 Hz), 4.18–4.24 (m, 2H), 3.98 (s, 3H), 3.94 (s, 3H), 3.66 (s, 3H), 3.39–3.41 (m, 1H), 3.32–3.35 (m, 1H), 3.11–3.14 (m, 1H), 3.04–3.07 (m, 1H), 2.59–2.62 (m, 1H), 2.20–2.22 (m, 1H), 1.96–2.00 (m, 2H), 1.86–1.88 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 174.9, 164.0, 161.2, 161.0, 160.8, 160.0, 127.7, 124.1, 115.0, 109.4, 107.9, 96.2, 93.0, 67.5, 66.2, 56.6, 55.9, 55.1, 53.6, 52.0, 29.9, 23.5. HRMS (ESI) calcd for $C_{25}H_{28}NO_7$ 454.1860 $(M+H)^+$, found 454.1865.

4.1.15. 5,7-Dimethoxy-2-(4-(piperidin-4-yloxy)-phenyl)-chromen-4-one (19)

To a solution of **6** (60 mg, 0.2 mmol) and Ph_3P (210 mg, 0.8 mmol) in THF (5 mL) was added 4-hydroxy-piperidine-1-carboxylic acid *tert*-butyl ester (121 mg, 0.6 mmol) in THF (5 mL) and DIAD (121 mg, 0.6 mmol). The mixture was stirred at rt for 2 h, and was then concentrated to give the crude product. This residue was purified with silica gel column (EtOAc) to afford 80 mg of the intermediate as a white solid. To the solution of the intermediate (80 mg) in CH_2Cl_2 (3 mL) was added TFA (1 mL) at 0 °C. The mixture was stirred at rt for 3 h, and was then concentrated. The residue was partitioned between EtOAc (250 mL) and 1 N $NaHCO_3$ (aq, 10 mL). The organic layer was washed with H_2O (10 mL) and dried over Na_2SO_4. The organic layer was concentrated. The residue was purified with silica gel column (EtOAc) to afford **19** (60 mg, 78%, two steps) as a pale yellow solid (mp 205–206 °C). HPLC purity 98.6% (t_R = 15.43 min). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.98–8.00 (m, 2H), 7.15–7.17 (m, 2H), 6.85 (d, 1H, J = 1.8 Hz), 6.68 (s, 1H), 6.51 (d, 1H, J = 2.4 Hz), 4.77 (t, 1H, J = 4.2 Hz), 3.90 (s, 3H), 3.83 (s, 3H), 3.21–3.25 (m, 4H), 3.03–3.07 (m, 2H), 2.09–2.12 (m, 2H), 1.78–1.81 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 178.6, 164.5, 161.3, 160.9, 160.0, 159.4, 128.1, 124.4, 116.1, 108.9, 107.4, 96.4, 93.0, 69.2, 56.3, 55.9, 40.6, 28.0. HRMS (ESI) calcd for $C_{22}H_{24}NO_5$ 382.1649 $(M+H)^+$, found 382.1651.

4.1.16. 2-(4-(2-Aminoethoxy)-phenyl)-5,7-dimethoxy-chromen-4-one (20)

Compound **20** was prepared in 59% yield (two steps) by a procedure similar to that used to prepare **19**. The title compound was obtained as a white solid (mp 165–166 °C). HPLC purity 98.4% (t_R = 14.50 min). ^{1}H NMR (600 MHz, CD_3OD) δ 7.87 (d, 2H, J = 9.0 Hz), 7.05 (d, 2H, J = 8.4 Hz), 6.61 (d, 2H, J = 9.0 Hz), 6.42 (s, 1H), 4.12 (t, 2H, J = 4.8 Hz), 3.94 (d, 6H, J = 2.4 Hz), 3.14 (s, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ 178.9, 164.7, 161.7, 161.6, 161.0, 160.1, 128.1, 124.0, 115.1, 108.8, 107.2, 96.5, 93.1, 69.1, 56.2, 56.0, 40.7. HRMS (ESI) calcd for $C_{19}H_{20}NO_5$ 342.1336 $(M+H)^+$, found 342.1337.

4.1.17. 2-(4-(3-Aminopropoxy)-phenyl)-5,7-dimethoxy-chromen-4-one (21)

Compound **21** was prepared in 90% yield (two steps) by a procedure similar to that used to prepare **19**. The title compound was obtained as a white solid (mp 170–171 °C). HPLC purity 98.4% (t_R = 15.07 min). ^{1}H NMR (600 MHz, CD_3OD) δ 7.88 (d, 2H, J = 9.0 Hz), 7.05 (d, 2H, J = 9.0 Hz), 6.66 (d, 1H, J = 1.8 Hz), 6.60 (s, 1H), 6.45 (d, 1H, J = 1.8 Hz), 4.18 (t, 2H, J = 6.0 Hz), 3.95 (d, 6H, J = 3.0 Hz), 3.35 (s, 2H), 3.09 (t, 2H, J = 7.2 Hz), 2.14 (t, 2H, J = 6.0 Hz). ^{13}C NMR (150 MHz, CD_3OD) δ 179.3, 165.2, 162.2, 161.9, 161.1, 160.4, 128.3, 124.1, 115.3, 108.9, 107.1, 96.8, 93.4, 65.9, 56.3, 56.2, 38.2, 29.4. HRMS (ESI) calcd for $C_{20}H_{22}NO_5$ 356.1493 $(M+H)^+$, found 356.1496.

4.1.18. 5,7-Dimethoxy-2-(4-oxiranylmethoxyphenyl)-chromen-4-one (23)

To a solution of **6** (200 mg, 0.67 mmol) and epichlorohydrin (**22**) (617 mg, 6.7 mmol) in acetone (10 mL) was added K_2CO_3 (462 mg, 3.35 mmol). The mixture was stirred at 80 °C for 24 h, and was then concentrated to give the crude product. The residue was diluted with EtOAc (100 mL), and washed with 0.1 N HCl (aq) (10 mL) followed by brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20/1) to give the desired product **23** (160 mg, 67%) as a pale yellow solid (mp 191–192 °C). HPLC purity 98.9% (t_R = 18.99 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80–7.82 (m, 2H), 7.00–7.03 (m, 2H), 6.58 (s, 1H), 6.55 (d, 1H, J = 2.4 Hz), 6.36 (d, 1H, J = 2.4 Hz), 4.31–4.33 (m, 1H), 3.99–4.02 (m, 1H), 3.95 (s, 3H), 3.90 (s, 3H), 3.37–3.39 (m, 1H), 2.93–2.94 (m, 1H), 2.78–2.79 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.7, 164.1, 161.0, 161.0, 160.6, 160.0, 127.8, 124.6, 115.1, 109.4, 108.0, 96.2, 93.0, 69.1, 56.6, 55.9, 50.1, 44.7. HRMS (ESI) calcd for $C_{20}H_{19}O_6$ 355.1176 $(M+H)^+$, found 355.1180.

4.1.19. 2-(4-(2-Hydroxy-3-piperidin-1-yl-propoxy)phenyl)-5,7-dimethoxy-chromen-4-one (24)

To a solution of **23** (30 mg, 0.085 mmol) and piperidine (72 mg, 0.85 mmol) in EtOH (5 mL) was added K_2CO_3 (117 mg, 0.85 mmol). The mixture was stirred at 100 °C for 3 h, and was then concentrated to give the crude product. The residue was diluted with EtOAc (100 mL), and washed with 0.1 N HCl (aq) (10 mL) followed by brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10/1) to give the desired product **24** (35 mg, 94%) as a white solid (mp 109–110 °C). HPLC purity 98.5% (t_R = 15.78 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79–7.81 (m, 2H), 7.00–7.03 (m, 2H), 6.58 (s, 1H), 6.54 (d, 1H, J = 3.0 Hz), 6.36 (d, 1H, J = 1.8 Hz), 4.11–4.15 (m, 1H), 4.02–4.06 (m, 2H), 3.95 (s, 3H), 3.90 (s, 3H), 2.66 (br s, 1H), 2.54–2.56 (m, 2H), 2.50–2.55 (m, 2H), 2.41–2.43 (m, 2H), 1.58–1.65 (m, 4H), 1.46–1.48 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.0, 161.4, 161.0, 160.8, 160.0, 127.7, 124.2, 115.1, 109.3, 107.8, 96.2, 92.9, 70.7, 65.3, 61.1, 56.6, 55.9, 54.9, 26.0, 24.2. HRMS (ESI) calcd for $C_{25}H_{30}NO_6$ 440.2068 $(M+H)^+$, found 440.2071.

4.1.20. 2-(4-(2-Hydroxy-3-pyrrolidin-1-yl-propoxy)phenyl)-5,7-dimethoxy-chromen-4-one (25)

Compound **25** was prepared in 94% yield by a procedure similar to that used to prepare **24**. The title compound was obtained as a pale yellow solid (mp 137–138 °C). HPLC purity 99.9% (t_R = 15.42 - min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 (d, 2H, J = 9.0 Hz), 7.03 (d, 2H, J = 9.0 Hz), 6.59 (s, 1H), 6.55 (d, 1H, J = 2.4 Hz), 6.36 (d, 1H, J = 2.4 Hz), 4.12–4.15 (m, 1H), 4.06 (d, 2H, J = 5.4 Hz), 3.95 (s, 3H), 3.91 (s, 3H), 3.10–3.30 (br s, 1H), 2.86–2.89 (m, 1H), 2.76–2.79 (m, 2H), 2.58–2.62 (m, 2H), 1.82–1.85 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.1, 161.3, 161.0, 160.7, 160.0, 127.7, 124.3, 115.1, 109.4, 107.9, 96.2, 93.0, 70.7, 67.2, 58.6, 56.6, 55.9, 54.4. HRMS (ESI) calcd for $C_{24}H_{28}NO_6$ 426.1911 $(M+H)^+$, found 426.1915.

4.1.21. 2-(4-(3-Dimethylamino-2-hydroxypropoxy)-phenyl)-5,7-dimethoxy-chromen-4-one (26)

Compound **26** was prepared in 71% yield by a procedure similar to that used to prepare **24**. The title compound was obtained as a white solid (mp 92–93 °C). HPLC purity 99.6% (t_R = 10.65 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (d, 2H, J = 9.0 Hz), 7.04 (d, 2H, J = 9.0 Hz), 6.61 (s, 1H), 6.58 (d, 1H, J = 2.4 Hz), 6.39 (d, 1H, J = 2.4 Hz), 4.12–4.15 (m, 1H), 4.11–4.12 (m, 2H), 3.98 (s, 3H), 3.93 (s, 3H), 2.61–2.65 (m, 1H), 2.42–2.48 (m, 2H), 2.39 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 164.1, 161.3, 161.0, 160.8, 160.0, 127.7, 124.2, 115.1, 109.3, 107.9, 96.2, 93.0, 70.7, 66.1, 61.8, 56.6, 55.9, 45.6. HRMS (ESI) calcd for $C_{22}H_{26}NO_6$ 400.1755 $(M+H)^+$, found 400.1756.

4.1.22. 2-(4-(2,3-Dihydroxypropoxy)phenyl)-5,7-dimethoxy-chromen-4-one (28)

To a solution of **6** (50 mg, 0.17 mmol) and Ph_3P (88 mg, 0.34 mmol) in THF (5 mL) was added (2,2-dimethyl-1,3-dioxolan-4-yl)methanol (**27**) (44 mg, 0.34 mmol) in THF (5 mL) and DIAD (68 mg, 0.34 mmol). The mixture was stirred at rt for 4 h, and was then concentrated to give the crude product. This residue was purified with silica gel column (CH_2Cl_2/MeOH = 20/1 to 10/1) to afford 70 mg of the intermediate as a white solid. To the solution of the intermediate (70 mg) in EtOH (5 mL) was added 0.5 N HCl (aq, 0.5 mL) at 0 °C. The mixture was stirred at 100 °C for 2 h, and was then concentrated. The residue was partitioned between EtOAc (50 mL) and 1 N $NaHCO_3$ (aq, 10 mL). The organic layer was washed with H_2O (10 mL) and dried over Na_2SO_4. The organic layer was concentrated. The residue was purified with silica gel column (CH_2Cl_2/MeOH = 10/1) to afford **28** (45 mg, 71%, two steps) as a white solid (mp 203–204 °C). HPLC purity 97.6% (t_R = 15.51 min). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.97–8.00 (m, 2H), 7.08–7.11 (m, 2H), 6.86 (d, 1H, J = 2.4 Hz), 6.66 (s, 1H), 6.50 (d, 1H, J = 2.4 Hz), 5.00 (d, 1H, J = 5.4 Hz), 4.70 (t, 1H, J = 6.0 Hz), 4.09–4.11 (m, 1H), 3.95–3.98 (m, 1H), 3.90 (s, 3H), 3.83 (s, 3H), 3.80–3.83 (m, 1H), 3.46 (t, 2H, J = 6.0 Hz). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.6, 163.6, 161.4, 160.2, 159.7, 159.1, 127.7, 122.9, 115.0, 108.3, 106.7, 96.2, 93.4, 69.9, 69.8, 62.6, 56.1, 56.0. HRMS (ESI) calcd for $C_{20}H_{21}O_7$ 373.1282 $(M+H)^+$, found 373.1284.

4.1.23. 2-(4-Bromophenyl)-5,7-dimethoxy-chromen-4-one (31)

Starting from the commercially available 4-bromobenzaldehyde (**29**) and 1-(2-hydroxy-4,6-dimethoxy-phenyl)ethanone (**2**), **31** was prepared in two steps according to literature procedures.[36] HPLC purity 96.5% (t_R = 21.53 min). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.99 (d, 2H, J = 8.4 Hz), 7.75 (d, 2H, J = 9.0 Hz), 6.87 (d, 1H, J = 2.4 Hz), 6.82 (s, 1H), 6.51 (d, 1H, J = 1.8 Hz), 3.90 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.6, 163.9, 160.3, 159.1, 158.5, 132.0, 130.2, 127.9, 125.1, 108.6, 108.4, 96.4, 93.4, 56.1, 56.0. HRMS (ESI) calcd for $C_{17}H_{14}BrO_4$ 361.0070 $(M+H)^+$, found 361.0070.

4.1.24. 2-(4-(6-Fluoropyridin-3-yl)phenyl)-5,7-dimethoxy-chromen-4-one (33)

To a solution of **31** (90 mg, 0.25 mmol) and 2-fluoropyridine-5-boronic acid (**32**) (42 mg, 0.3 mmol) in THF/EtOH/H_2O (2 mL/2 mL/2 mL) was added KOAc (94 mg, 0.75 mmol) and then Pd(dppf)Cl_2 (20 mg, 0.025 mmol). The resulting mixture was deoxygenated via five vacuum/N_2-refill cycles. The mixture was stirred at 80 °C for 18 h, and was then concentrated under vacuum. The residue was partitioned between EtOAc (100 mL) and H_2O (20 mL). The organic layer was separated and washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtrated and concentrated to give an oily residue. This residue was purified with silica gel column (CH_2Cl_2/MeOH = 10/1) to obtain **33** (80 mg, 85%) as a pale red solid (mp 209–210 °C). HPLC purity 98.4% (t_R = 20.91 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.48 (d, 1H, J = 1.8 Hz), 8.01–8.04 (m, 1H), 7.98 (s, 1H), 7.97 (s, 1H), 7.04–7.06 (m, 1H), 6.72 (s, 1H), 6.59 (d, 1H, J = 2.4 Hz), 6.40 (d, 1H, J = 2.4 Hz), 3.97 (s, 3H), 3.93 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.5, 164.5, 164.4, 162.9, 161.2, 160.1, 160.0, 146.2, 146.1, 139.8, 139.5, 133.8, 131.6, 127.6, 126.9, 110.0, 109.8, 109.6, 96.5, 93.1, 56.6, 55.9. HRMS (ESI) calcd for $C_{22}H_{17}FNO_4$ 378.1136 $(M+H)^+$, found 378.1138.

4.1.25. 2-(4-(2-Dimethylaminoethylamino)phenyl)-5,7-dimethoxy-chromen-4-one (36)

NaO^tBu (43 mg, 0.45 mmol), $Pd_2(dba)_3$ (28 mg, 0.03 mmol) and (±)-BINAP (19 mg, 0.03 mmol) were placed into a flask and dissolved into distilled toluene (10 mL). To this solution was added **31** (108 mg, 0.3 mmol) and *N*,*N*-dimethylethylenediamine (**34**) (40 mg, 0.45 mmol) dropwise with stirring at room temperature and the mixture was refluxed at 80 °C for 48 h. After the mixture was cooled, 20 mL of H_2O was added and extracted with EtOAc (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10/1) to give the desired product (65 mg, 64%) as a pale yellow solid (mp 159–160 °C). HPLC purity 98.8% (t_R = 15.10 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (d, 2H, J = 9.0 Hz), 6.66 (d, 2H, J = 9.0 Hz), 6.54 (d, 1H, J = 1.8 Hz), 6.53 (s, 1H), 6.35 (d, 1H, J = 1.8 Hz), 4.84 (s, 1H), 3.95 (s, 3H), 3.90 (s, 3H), 3.22 (s, 2H), 2.60 (t, 1H, J = 6.0 Hz), 2.28 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.9, 163.8, 161.7, 161.0, 160.0, 151.1, 127.7, 119.4, 112.5, 109.4, 106.2, 96.0, 93.0, 57.7, 56.6, 55.8, 45.2, 40.6, 31.1. HRMS (ESI) calcd for $C_{21}H_{25}N_2O_4$ 369.1809 $(M+H)^+$, found 369.1811.

4.1.26. 5,7-Dimethoxy-2-(4-(2-pyrrolidin-1-yl-ethylamino)phenyl)-chromen-4-one (37)

Compound **37** was prepared in 57% yield by a procedure similar to that used to prepare **36**. The title compound was obtained as a pale yellow solid (mp 148–149 °C). HPLC purity 98.2% (t_R = 15.73 - min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.69 (d, 2H, J = 9.0 Hz), 6.66 (d, 2H, J = 8.4 Hz), 6.53 (d, 1H, J = 2.4 Hz), 6.52 (s, 1H), 6.34 (d, 1H, J = 2.4 Hz), 4.91 (s, 1H), 3.94 (s, 3H), 3.89 (s, 3H), 3.27 (d, 2H, J = 2.4 Hz), 2.78 (t, 1H, J = 6.0 Hz), 2.59 (s, 4H), 1.81 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.9, 163.8, 161.7, 161.0, 160.0, 151.1, 127.6, 119.3, 112.5, 109.4, 106.1, 96.0, 93.0, 56.5, 55.8, 54.6, 54.0, 41.8, 23.6. HRMS (ESI) calcd for $C_{23}H_{27}N_2O_4$ 395.1965 $(M+H)^+$, found 395.1968.

4.1.27. 5-Hydroxy-7-methoxy-2-(4-(2-pyrrolidin-1-yl-ethoxyphenyl)-chromen-4-one (38)

To a solution of **16** (30 mg, 0.076 mmol) in 5 mL of CH_2Cl_2 was added 1 N BBr_3 in CH_2Cl_2 (0.15 mL, 0.15 mmol) at 0 °C. The resulting mixture was stirred at rt for 2 h. The solution was diluted with CH_2Cl_2/MeOH (10/1, 50 mL), washed with H_2O (15 mL) and brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The residue was

purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10/1) to give the desired product (23 mg, 79%) as a pale yellow solid (mp 127–129 °C). HPLC purity 99.7% (t_R = 18.02 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.81 (s, 1H), 7.83 (d, 2H, J = 9.0 Hz), 7.03 (d, 2H, J = 9.0 Hz), 6.60 (s, 1H), 6.48 (d, 1H, J = 2.4 Hz), 6.36 (d, 1H, J = 2.4 Hz), 4.21 (t, 2H, J = 9.0 Hz), 3.88 (s, 3H), 2.97 (t, 2H, J = 9.0 Hz), 2.67–2.69 (m, 4H), 1.83–1.85 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 182.6, 165.6, 164.2, 162.3, 162.0, 157.9, 128.2, 123.8, 115.2, 105.7, 104.5, 98.2, 92.8, 67.4, 55.9, 54.9, 54.8, 23.6. HRMS (ESI) calcd for $C_{22}H_{24}NO_5$ 382.1649 $(M+H)^+$, found 386.1652.

4.1.28. 5,7-Dihydroxy-2-(4-(2-pyrrolidin-1-yl-ethoxy)phenyl)-chromen-4-one (39)

To a solution of **16** (22 mg, 0.056 mmol) in 5 mL of CH_2Cl_2 was added 1 N BBr_3 in CH_2Cl_2 (0.17 mL, 0.17 mmol) at 0 °C. The resulting mixture was stirred at rt for 24 h. The solution was diluted with CH_2Cl_2/MeOH (10/1, 50 mL), washed with H_2O (5 mL) and brine (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10/1) to give the desired product (15 mg, 73%) as a pale yellow solid (mp 228–229 °C). HPLC purity 97.5% (t_R = 16.04 min). ^{1}H NMR (600 MHz, $CDCl_3$/CD_3OD 2:1) δ 7.80 (d, 2H, J = 9.0 Hz), 6.98 (d, 2H, J = 9.0 Hz), 6.50 (s, 1H), 6.39 (d, 1H, J = 2.4 Hz), 6.22 (d, 1H, J = 2.4 Hz), 4.16 (t, 2H, J = 6.0 Hz), 2.94 (t, 2H, J = 6.0 Hz), 2.65–2.67 (m, 4H), 1.80–1.83 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$/CD_3OD 2:1) δ 182.6, 164.4, 164.3, 161.8, 161.7, 158.1, 128.2, 123.8, 115.1, 104.7, 103.9, 99.4, 94.5, 66.8, 54.8, 54.7, 23.4. HRMS (ESI) calcd for $C_{21}H_{22}NO_5$ 368.1493 $(M+H)^+$, found 368.1495.

4.2. Biology

4.2.1. Human pancreatic stellate cell culture and reagents

Under an IRB-approved tissue protocol, discarded human resected pancreatic tissue (500 mm^3) was attained fresh from the operating room at the University of Texas Medical Branch. The tissue was minced and plated on a collagen-coated flask (15 μg/mL; Invitrogen, Carlsbad, CA) with DMEM (VWR, Radnor, PA) supplemented with penicillin 200 U/mL, streptomycin 200 μg/mL, amphotericin B 0.25 μg/mL and gentamicin 50 μg/mL (Invitrogen, Carlsbad CA), 10% fetal bovine serum (Lonza, Walkersville, MD), 1% insulin-transferrin, selenium-ethanolamine (Gibco, Grand Island, NY), and 1% non-essential amino acids (Sigma–Aldrich, St. Louis, MO). The human PSC were isolated by the outgrowth method.[41–43] The purity of PSC culture was confirmed by immunohistochemical staining for vimentin, α-smooth muscle actin, glial fibrillar acidic protein, or Oil red O staining. Primary PSC were transformed and immortalized for use in experiments using lentiviral vectors containing SV40 Large T antigen and human telomerase (plasmid # 12245 and 12246; Addgene, Cambridge, MA). Cultures were maintained in DMEM with 10% FBS, at 37 °C in a humidified 95% O_2/5% CO_2 atmosphere.

4.2.2. Cell proliferation assay

Transformed PSC (3×10^3) were plated in 96-well plates, in sextuplicate. The next day, the media was changed to 1% FBS with apigenin or analogues for 24–48 h. AlamarBlue reagent (10% sample volume, DAL1025) was added to each well per Invitrogen's protocol. Fluorescence was recorded using excitation/emission wavelengths of 544/590 nm and the SpectraMax M2 Microplate Reader (Molecular Devices, Sunnyvale CA).

4.2.3. Cell death assay

Transformed PSC (8×10^3) were plated in 96-well plates in triplicate. The next day, the media was changed to 1% FBS with apigenin or analogues for a 14 h incubation. The Cell Death Detection ELISA^{PLUS} assay (Roche Applied Science, Indianapolis, IN) was used and protocol followed (Version 11.0). Absorbance was measured at 405 nm, using the ELx800 Automated Microplate Reader (Bio-TEK Instruments, Inc., Winooski, VT).

4.2.4. Chronic pancreatitis (CP) animal model

Animal experiments were conducted under an Institutional Animal Care and Use Committee-approved protocol. CP was induced in C57BL/6 mice using supraoptimal pancreatic stimulation with cerulein (CR), a cholecystokinin analogue (Bachem, Torrance, CA). CP (50 μg CR/kg mouse weight) was administered via intraperitoneal route hourly for 5 h for 3 d/week for a total of 4 weeks. Control mice received phosphate buffered saline (PBS) injections following the same schedule. Starting Week 2 of the experiment, apigenin, analogue, or vehicle (0.5% methylcellulose + 0.025% Tween 20 in ddH_20) were administered (0.5 mg/kg/d, oral gavage, once daily, 6 d/week, for 3 week) while continuing CR injections. At the end of Week 4, the mice were anesthetized with isoflurane and sacrificed per protocol. The pancreata were quickly harvested and processed.

4.2.5. Immunohistochemistry (IHC) and image analysis

Pancreata were formalin-fixed and paraffin-embedded. Prior to staining, sections (5 μM) were deparaffinized with xylene, rehydrated with ethanol, and subjected to heat-mediated antigen retrieval (DAKO, Carpinteria, CA) to optimize antigen immunoreactivity. Fibronectin antibody (1:600; Santa Cruz Biotechnology, Dallas, TX), and biotinylated anti-goat IgG (1:400; Vector Laboratories Inc., Burlingame, CA) were used. IHC staining was completed with the VECTASTAIN Elite ABC kit (Vector Lab), color development with DAB (DAKO), and counterstaining with hematoxylin 7211 (Thermo Scientific, Kalamazoo, MI). Five non-overlapping images of each pancreas (400×) were taken using an Olympus BX51 microscope connected to a DP71 Olympus digital camera. The percent area of brown fibronectin staining was quantified using the Image Processing and Analysis in Java (ImageJ) 1.46r software (NIH, Bethesda, MD) and a color deconvolution plug-in.[44,45]

4.2.6. Statistical analysis

Dose–response curves were generated by plotting fluorescence or absorbance versus log (compound concentration). A best-fit curve was created using nonlinear regression, and the IC_{50} or EC_{50} determined from the graph (GraphPad Prism 5; GraphPad Software Inc., La Jolla, CA). SPSS (IBM, Armonk, NY) was used to conduct statistical analysis, which included SEM, two-way ANOVA, and one-way ANOVA with post-hoc Tukey-Kramer multiple comparisons test. Significance was set at p <0.05.

5. Notes

The authors declare no competing financial interest.

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References and notes

1. Schneider, A.; Whitcomb, D. C. *Best Pract. Res. Clin. Gastroenterol.* **2002**, *16*, 347.
2. Bordalo, O.; Goncalves, D.; Noronha, M.; Cristina, M. L.; Salgadinho, A.; Dreiling, D. A. *Am. J. Gastroenterol.* **1977**, *68*, 278.
3. Shimizu, K. *J. Gastroenterol.* **2008**, *43*, 823.
4. Sahel, J.; Sarles, H. *Dig. Dis. Sci.* **1979**, *24*, 897.
5. Witt, H.; Apte, M. V.; Keim, V.; Wilson, J. S. *Gastroenterology* **2007**, *132*, 1557.
6. Yang, A. L.; Vadhavkar, S.; Singh, G.; Omary, M. B. *Arch. Intern. Med.* **2008**, *168*, 649.
7. Everhart, J. E.; Ruhl, C. E. *Gastroenterology* **2009**, *136*, 376.
8. Trikudanathan, G.; Navaneethan, U.; Vege, S. S. *Gastroenterol. Clin. North Am.* **2012**, *41*, 63.
9. Forsmark, C. E. *Gastroenterology* **2013**, *144*, 1282.
10. Erkan, M.; Adler, G.; Apte, M. V.; Bachem, M. G.; Buchholz, M.; Detlefsen, S.; Esposito, I.; Friess, H.; Gress, T. M.; Habisch, H. J.; Hwang, R. F.; Jaster, R.; Kleeff, J.; Kloppel, G.; Kordes, C.; Logsdon, C. D.; Masamune, A.; Michalski, C. W.; Oh, J.; Phillips, P. A.; Pinzani, M.; Reiser-Erkan, C.; Tsukamoto, H.; Wilson, J. *Gut* **2012**, *61*, 172.
11. Jaster, R.; Emmrich, J. *Best Pract. Res. Clin. Gastroenterol.* **2008**, *22*, 17.
12. Masamune, A.; Watanabe, T.; Kikuta, K.; Shimosegawa, T. *Clin. Gastroenterol. Hepatol.* **2009**, *7*, S48.
13. Madro, A.; Slomka, M.; Celinski, K. *Adv. Med. Sci.* **2011**, *56*, 132.
14. Gao, X.; Cao, Y.; Yang, W.; Duan, C.; Aronson, J. F.; Rastellini, C.; Chao, C.; Hellmich, M. R.; Ko, T. C. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2013**, *304*, G804.
15. Spector, I.; Honig, H.; Kawada, N.; Nagler, A.; Genin, O.; Pines, M. *Pancreas* **2010**, *39*, 1008.
16. He, J.; Sun, X.; Qian, K. Q.; Liu, X.; Wang, Z.; Chen, Y. *Biochim. Biophys. Acta* **2009**, *1792*, 56.
17. Whitcomb, D. C. *Gut* **1999**, *45*, 317.
18. Kloppel, G.; Maillet, B. *Pancreas* **1993**, *8*, 659.
19. Comfort, M. W.; Gambill, E. E.; Baggenstoss, A. H. *Gastroenterology* **1946**, *6*, 376.
20. Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Disc.* **2005**, *4*, 206.
21. Harvey, A. L. *Drug Discovery Today* **2008**, *13*, 894.
22. Li, J. W.; Vederas, J. C. *Science* **2009**, *325*, 161.
23. Srinivas, N. R. *Curr. Clin. Pharmacol.* **2009**, *4*, 67.
24. Shukla, S.; Gupta, S. *Pharm. Res.* **2010**, *27*, 962.
25. Patel, D.; Shukla, S.; Gupta, S. *Int. J. Oncol.* **2007**, *30*, 233.
26. Keller, T. H.; Pichota, A.; Yin, Z. *Curr. Opin. Chem. Biol.* **2006**, *10*, 357.
27. Di, L.; Kerns, E. H.; Carter, G. T. *Curr. Pharm. Des.* **2009**, *15*, 2184.
28. Manthey, J. A.; Guthrie, N. *J. Agric. Food Chem.* **2002**, *50*, 5837.
29. Chan, K. F.; Zhao, Y.; Burkett, B. A.; Wong, I. L.; Chow, L. M.; Chan, T. H. *J. Med. Chem.* **2006**, *49*, 6742.
30. Lin, A. S.; Nakagawa-Goto, K.; Chang, F. R.; Yu, D.; Morris-Natschke, S. L.; Wu, C. C.; Chen, S. L.; Wu, Y. C.; Lee, K. H. *J. Med. Chem.* **2007**, *50*, 3921.
31. Wong, I. L.; Chan, K. F.; Tsang, K. H.; Lam, C. Y.; Zhao, Y.; Chan, T. H.; Chow, L. M. *J. Med. Chem.* **2009**, *52*, 5311.
32. Walle, T. *Mol. Pharm.* **2007**, *4*, 826.
33. Gradolatto, A.; Canivenc-Lavier, M. C.; Basly, J. P.; Siess, M. H.; Teyssier, C. *Drug Metab. Dispos.* **2004**, *32*, 58.
34. Gradolatto, A.; Basly, J. P.; Berges, R.; Teyssier, C.; Chagnon, M. C.; Siess, M. H.; Canivenc-Lavier, M. C. *Drug Metab. Dispos.* **2005**, *33*, 49.
35. Wen, X.; Walle, T. *Drug Metab. Dispos.* **2006**, *34*, 1786.
36. Chan, K. F.; Zhao, Y.; Chow, T. W.; Yan, C. S.; Ma, D. L.; Burkett, B. A.; Wong, I. L.; Chow, L. M.; Chan, T. H. *ChemMedChem* **2009**, *4*, 594.
37. Chen, H.; Yang, Z.; Ding, C.; Chu, L.; Zhang, Y.; Terry, K.; Liu, H.; Shen, Q.; Zhou, J. *ACS Med. Chem. Lett.* **2013**, *4*, 180.
38. Apte, M.; Pirola, R.; Wilson, J. *Antioxid. Redox. Signal.* **2011**, *15*, 2711.
39. Lerch, M. M.; Gorelick, F. S. *Gastroenterology* **2013**, *144*, 1180.
40. Aghdassi, A. A.; Mayerle, J.; Christochowitz, S.; Weiss, F. U.; Sendler, M.; Lerch, M. M. *Fibrogenesis Tissue Repair* **2011**, *4*, 26.
41. Chao, C.; Tallman, M. L.; Ives, K. L.; Townsend, C. M., Jr.; Hellmich, M. R. *J. Surg. Res.* **2005**, *129*, 313.
42. Apte, M. V.; Haber, P. S.; Applegate, T. L.; Norton, I. D.; McCaughan, G. W.; Korsten, M. A.; Pirola, R. C.; Wilson, J. S. *Gut* **1998**, *43*, 128.
43. Bachem, M. G.; Schneider, E.; Gross, H.; Weidenbach, H.; Schmid, R. M.; Menke, A.; Siech, M.; Beger, H.; Grunert, A.; Adler, G. *Gastroenterology* **1998**, *115*, 421.
44. Ruifrok, A. C.; Johnston, D. A. *Anal. Quant. Cytol. Histol.* **2001**, *23*, 291.
45. Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. *Nat. Methods* **2012**, *9*, 671.

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