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Design and synthesis of 3,3′-biscoumarin-based c-Met inhibitors†

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Jimin Xu,‡[a] Jing Ai,‡[b] Sheng Liu,[a] Xia Peng,[b] Linqian Yu,[a] Meiyu Geng*[b] and Fajun Nan*[a]

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A library of biscoumarin-based c-Met inhibitors was synthesized, based on optimization of 3,3′-biscoumarin hit **3**, which was identified as a non-ATP competitive inhibitor of c-Met from a diverse library of coumarin derivatives. Among these compounds, **38** and **40** not only showed potent enzyme activities with IC_{50} values of 107 nM and 30 nM, respectively, but also inhibited c-Met phosphorylation in BaF3/TPR-Met and EBC-1 cells.

Introduction

c-Met is a receptor tyrosine kinase that is normally activated by its natural ligand hepatocyte growth factor/scatter factor (HGF/SF).[1] The HGF/c-Met axis plays an important role in normal embryonic development and organ regeneration. However, aberrant c-Met activation has been frequently found in many human solid tumours and hematologic malignancies. Overactivation of c-Met is known to initiate tumorigenesis and promote metastasis, and also cause therapeutic resistance.[2–5] Importantly, both c-Met and HGF elevation have been associated with poor clinical outcome or metastatic progression in many major human cancers.[6–9] As a result, c-Met is considered to be a potential target for cancer treatment.

A variety of approaches have previously been used to target Met signaling. These include HGF antagonists,[10–12] anti-HGF humanized antibodies,[13] and MET extracellular domain monoclonal antibodies.[14,15] Additionally, a large number of small-molecule kinase inhibitors targeting c-Met are now in clinical trials; most of them target the ATP binding site in an ATP-competitive manner.[3,16–18] Here, we report our efforts toward the development of 3,3′-biscoumarin analogues as novel, potent and non-ATP-competitive kinase inhibitors.

Daphnetin **1**, a derivative of coumarin, is a protein kinase inhibitor which inhibits tyrosine-specific protein kinase, EGFR (IC_{50} = 7.67 μM), and serine/threonine-specific protein kinases, including PKA (IC_{50} = 9.33 μM) and PKC (IC_{50} = 25.01 μM).[19] During our initial efforts to synthesize a diverse library of coumarin derivatives, we found that the simple dimeric analogue **3** displayed potent c-Met inhibitory activity with an IC_{50} of 151 nM. Daphnetin **1** and 4-hydroxyl daphnetin **2**, in contrast, showed weak inhibitory activity (IC_{50} = 100 μM). Compound **3**, which has a novel structure type compared to other reported c-Met inhibitors, is a dimer of **2** through a one-carbon linker (Fig. 1). Its acetoxy derivative has been reported as a tool to study protein transacetylase,[20] and similar coumarin dimers with different linkers have been reported as Hsp90 inhibitors by Blagg *et al.*[21,22] The c-Met inhibitory activities of these compounds, however, have not previously been reported. As most kinase inhibitors to date are ATP competitive, we examined whether compound **3** functions in a similar manner. PF2341066, a typical ATP-competitive inhibitor, was used as a reference control.[23] In contrast to PF2341066, the IC_{50} values of **3** remained unchanged with increasing ATP concentration. This suggests that **3** is an ATP non-competitive inhibitor of c-Met (Fig. 2). These initial results encouraged us to pursue a medicinal chemistry program to further optimize **3** as a novel c-Met inhibitor.

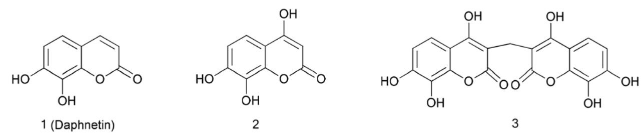

Fig. 1 Daphnetin derivatives.

Results and discussion

To explore the SAR of **3**, simple modifications were made to its structure. These changes, as shown in Scheme 1, yielded

[a]*Chinese National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Science, 189 Guoshoujing Road, Zhangjiang Hi-Tech Park, Shanghai 201203, Republic of China. E-mail: fjnan@simm.ac.cn*
[b]*Division of Antitumor Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Science, 555 Zuchongzhi Road, Shanghai 201203, Republic of China. E-mail: mygeng@simm.ac.cn*

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‡ These two authors contributed equally to this work.

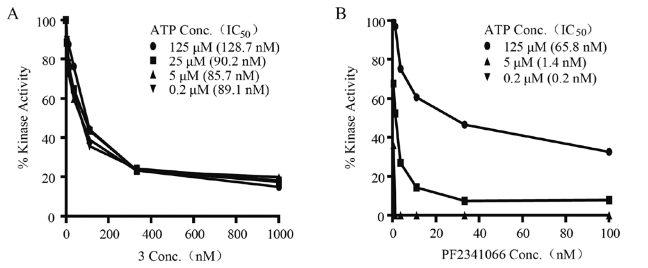
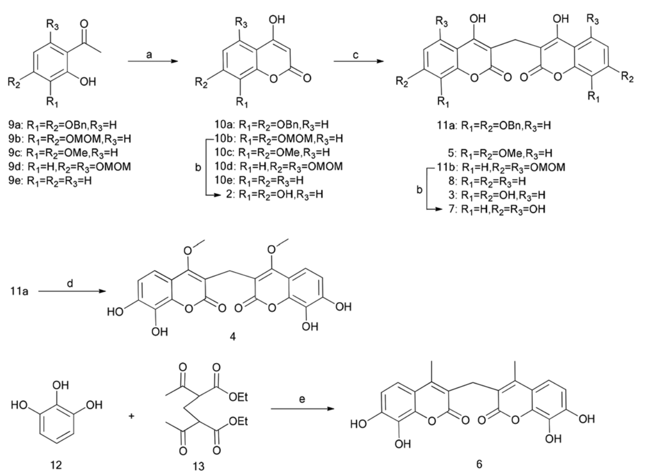


Fig. 2 Compound **3** is an ATP non-competitive inhibitor of Met kinase activity. Inhibition assays with recombinant c-Met protein and different concentrations of **3** (A) or ATP-competitive PF2341066 (B) were performed in the presence of various concentrations of ATP.



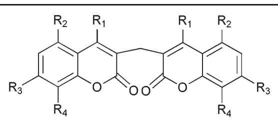
Scheme 1 Synthesis of biscoumarin compounds **3–8**. *Reagents and conditions*: (a) $CO(OEt)_2$, NaH, toluene, 80 °C; (b) HCl–AcOEt; (c) paraformaldehyde, Et_3N, EtOH; (d) i. CH_2N_2, Et_2O; ii. $Pd(OH)_2$, H_2, THF; (e) 70% H_2SO_4.

compounds **3–8** (Table 1). To this end, condensation of differently substituted starting materials **9a–e** with diethyl carbonate in the presence of sodium hydride formed coumarin monomers **10a–e**. Subsequent deprotection of **10b** under acidic conditions gave monomer **2**. The monomers (**2**, **10a**, **10c–e**) were then treated with formaldehyde in ethanol to provide the corresponding dimers (**3**, **11a**, **5**, **11b**, **8**, respectively). **11a** was converted into compound **4** *via* methylation with diazomethane and subsequent debenzylation using $H_2/Pd(OH)_2$. Deprotection of **11b** under acidic conditions afforded compound **7**. The Pechmann reaction of pyrogallol **12** with compound **13** in 70% sulfuric acid provided compound **6** directly.

Compounds **4** and **6** showed potent inhibitory activities, with IC_{50} of 112 nM and 63 nM respectively. Modifying the bis-(7,8-dihydroxyl) moiety (R_3 and R_4, Table 1) with hydrogen or methoxy groups led to a complete loss of potency (**8** and **5**). Moving the phenolic hydroxyl groups from the 8,8′-position to the 5,5′-position (R_2), as in compound **7**, also led to a large loss of activity (IC_{50} = 3.5 μM). These results indicate that retaining the bis-(7,8-dihydroxy) moiety of **3** is important for maintaining its inhibitory activity, and that methoxy and methyl groups are well-tolerated at the C-4,4′ position (R_1).

Next, we explored c-Met's inhibitory activity as it relates to the linker between the two coumarin moieties (Table 2). Compounds **14–18** were prepared as outlined in Scheme 2. Coumarin acids **20** and **25** were formed by reaction of compounds **19** and **24**, respectively, with isopropylidene malonate and piperidinium acetate in ethanol. Aminocoumarin **21** was similarly accessed from **19** *via* a nitrocoumarin intermediate, which was converted into aminocoumarin **21** by hydrogenation. With intermediates **20**, **21**, and **25** in hand, the desired compounds were readily synthesized by a series of condensations and deprotections. To this end, condensation of aminocoumarin **21** with acid **20** using EDCI in 30% pyridine/CH_2Cl_2 afforded compound **14** upon acidic deprotection.[24] Compounds **15** and **16** were accessed under the same coupling

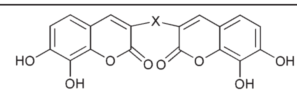
Table 1 c-Met enzymatic activity of compounds **3–8**

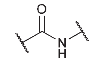
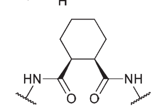
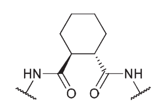
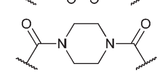
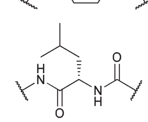


Compound	R_1	R_2	R_3	R_4	IC_{50} [a] (nM)
3	OH	H	OH	OH	150.9 ± 5.8
4	OCH_3	H	OH	OH	112.2 ± 23.6
5	OH	H	OCH_3	OCH_3	0%@10 μM
6	Me	H	OH	OH	62.5 ± 6.9
7	OH	OH	OH	H	3545.7 ± 159.7
8	OH	H	H	H	0%@10 μM

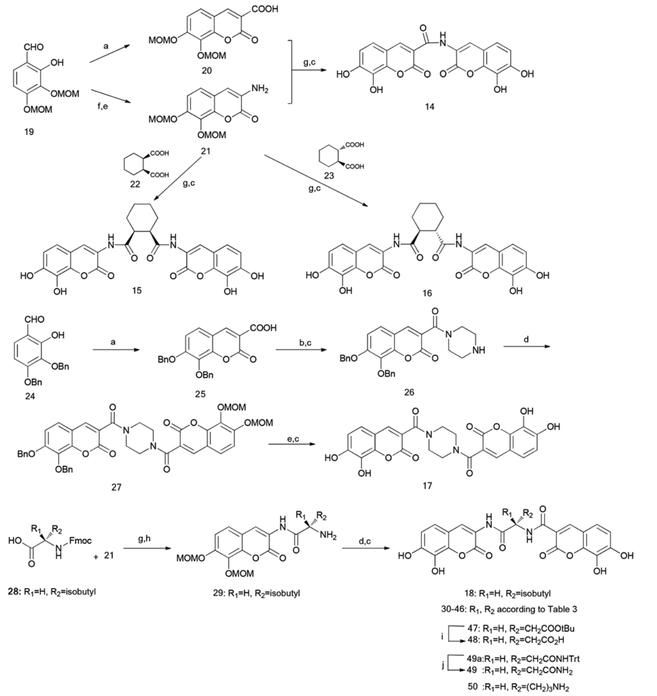
[a] IC_{50}s were calculated by the logit method from the results of at least two independent tests with eight concentrations each and expressed as means ± SD.

Table 2 c-Met enzymatic activity of compounds **14–18**



Compound	X	IC_{50} [a] (nM)
14		620.8 ± 70.9
15		168.7 ± 18.6
16		134.1 ± 6.3
17		1370.9 ± 208.5
18		122.0 ± 20.3

[a] IC_{50}s were calculated by the logit method from the results of at least two independent tests with eight concentrations each and expressed as means ± SD.



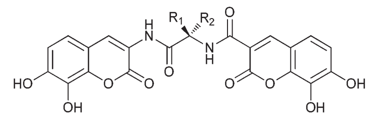
Scheme 2 Synthesis of biscoumarin compounds **14–18** with modified linkers. *Reagents and conditions*: (a) isopropylidene malonate, piperidinium acetate, EtOH, 60 °C; (b) 1-Boc-piperazine, EDCI, DMAP, DCM; (c) HCl–AcOEt; (d) **20**, EDCI, DMAP, DCM; (e) Pd/C, H_2, AcOEt; (f) ethyl nitroacetate, piperidine, benzene, Dean–Stark trap, reflux; (g) EDCI, 30% pyridine, DCM; (h) piperidine, CH_3CN; (i) HCOOH; (j) TFA, DCM.

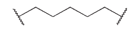
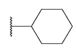
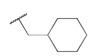
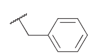
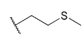
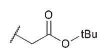
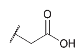
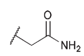
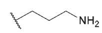
conditions, by condensation of **21** with 0.5 equiv. of the corresponding di-acids **22** and **23**, respectively, followed by the removal of the MOM groups. Condensation of **25** with 1-Boc-piperazine was followed by Boc deprotection under acidic conditions to provide intermediate **26**. Subsequent condensation of **26** with coumarin acid **20** afforded **27**, which was converted to compound **17** *via* hydrogenolytic cleavage and acid deprotection. Fmoc-Leu-OH **28** was also condensed with **21** to give an intermediate which was transformed to **29** *via* piperidine deprotection. The coupling reaction between **29** and **20** was followed by acid deprotection to give compound **18**.

Biological testing revealed that compounds with cyclohexane-1,2-dicarboxamide linkers (**15**, **16**) had potent inhibitory activities, with IC_{50} of 169 nM and 134 nM respectively. Compounds with piperazine-1,4-diyl (**17**) and amide linkers (**14**) displayed reduced potency, with IC_{50} of 1.40 μM and 0.62 μM respectively. Use of L-leucine as a linker (**18**) retained potency against c-Met (IC_{50} = 122 nM). These results indicate that the length of the linker can be adjusted and that substitution on the linker has a great impact on inhibitory activity.

Considering the inhibitory activity of **18**, we further explored the effect of the substitution on the linker moiety using various α-amino acids. Compounds **30–50** were synthesized by a method analogous to that used to access **18**, starting from different Fmoc-protected amino acids (Scheme 2). Their biological activities are shown in Table 3.

Table 3 c-Met enzymatic activity of compounds **30–50**



Compound	R_1	R_2	IC_{50} [a] (nM)
30	H	H	3620.7 ± 444.1
31	H	Me	62.5 ± 5.4
32	H	*n*-Pr	40.8 ± 3.7
33	H	i-Pr	72.2 ± 1.2
34	H	*n*-Bu	70.9 ± 13.6
35	Et	H	21.9 ± 1.2
36	*n*-Pr	H	36.6 ± 2.6
37	*n*-Bu	H	168.8 ± 7.0
38	i-Bu	H	107.0 ± 1.3
39	*n*-Pen, H		48.3 ± 13.1
40	*n*-Hex, H		30.2 ± 0.7
41	Me	Me	38.8 ± 6.3
42			115.4 ± 8.4
43	H		129.0 ± 14.0
44	H		21.7 ± 0.7
45	H		24.5 ± 0.8
46	H		121.8 ± 10.2
47	H		14.9 ± 4.2
48	H		90.6 ± 1.7
49	H		62.5 ± 5.4
50	H		4805.8 ± 1300.9

[a] IC_{50}s were calculated by the logit method from the results of at least two independent tests with eight concentrations each and expressed as means ± SD.

Compounds **30–45** were evaluated to determine the effect of size and chirality of linker substitution on c-Met inhibitory potency. Use of an unsubstituted glycine linker (**30**, $R_1 = R_2$ = H) resulted in weak inhibitory activity (IC_{50} = 3.6 μM). Alkyl substitution significantly increased potency compared to **30**; linkers with (*S*)-methyl, dimethyl, (*R*)-ethyl, and (*S*)-isopropyl substitution (**31**, **41**, **35**, **33**) displayed potent c-Met inhibition with IC_{50} of 63 nM, 39 nM, 22 nM and 72 nM, respectively. Compounds **42–45** with cycloalkyl or benzyl substitution also showed potent inhibition (IC_{50} = 22–130 nM). While the size of the alkyl group is of significant importance to the enzymatic inhibition potency, its configuration proved unimportant. Compound **38** ((*R*)-isobutyl, IC_{50} = 107 nM) was as potent as its epimer **18** ((*S*)-isobutyl, IC_{50} = 122 nM); (*R*)- and (*S*)-*n*-propyl substituted compounds **32** and **36** also showed similar inhibitory potencies (IC_{50} = 41 nM and 37 nM, respectively), while **37** ((*R*)-*n*-butyl, IC_{50} = 169 nM) was slightly less potent than **34** ((*S*)-*n*-butyl, IC_{50} = 71 nM). Racemic compounds **39** and **40** with *n*-Pen and *n*-Hex substituents displayed potent inhibitory activities with IC_{50} of 48 nM and 30 nM respectively.

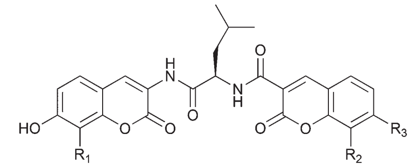
Analogues **46–50** investigated the effect of heteroatom introduction on the side chain. Compound **46**, which bears a thioether, retained potency (IC_{50} = 122 nM) in comparison with **34**. The ester analogue (**47**, IC_{50} = 15 nM) offered potent inhibitory activity, presenting 8-fold higher potency than the corresponding acid (**48**, IC_{50} = 119 nM). For nitrogen-bearing substituents, the amide analogue (**49**) retained inhibitory potency (IC_{50} = 63 nM) relative to acid **48**. The amine analogue (**50**), however, showed 65-fold lower potency (IC_{50} = 4.8 μM) than **34**.

Using D-leucine as a linker, the effect of modifying the hydroxyl groups on the two coumarin rings was explored. Compounds **51–55** were synthesized according to the procedures outlined in Scheme 3. Known compounds **56a–c** were transformed into coumarin acids **59a–c** and aminocoumarins **21** and **57a** by condensation. Coupling of Fmoc-D-Leu-OH with **57a** and **21**, followed by piperidine deprotection, provided **58a–b** respectively. Condensation of **58a–b** with **59a–c** followed by deprotection afforded compounds **51–55**.

As shown in Table 4, methylation of two hydroxyl groups on one coumarin ring (**51**) led to a significant loss of potency. Removal of a hydroxyl group (**52**, **53**) decreased the inhibitory effects on c-Met, with IC_{50} of 1.37 μM and 0.93 μM, respectively. Upon removal of one hydroxyl group on each coumarin ring (**54**, **55**), no inhibition of the enzyme expressing the c-Met receptor was observed. These results are consistent with our initial modification results (Table 1), which showed that the existence of four hydroxyl groups is important to retain good inhibitory activity.

Subsequently, compounds with different structures were selected to evaluate their effect on c-Met phosphorylation in BaF3/TPR-Met and EBC-1 NSCLC cell lines. BaF3/TPR-Met cells stably express a constitutively active, ligand-independent, oncogenic form of c-Met derived from chromosomal rearrangement, whereas EBC-1 NSCLC cells harbor amplified *MET* genes. As shown in Fig. 3, at the concentration of 10 μM, **38** and **40** markedly inhibited c-Met phosphorylation in both cell lines; **42** and **48** only effectively inhibited c-Met phosphorylation in EBC-1 NSCLC cells. Other compounds (**3**, **35** and **52**) failed to inhibit c-Met phosphorylation in either cell line. The poor cell potencies of these compounds can be attributed to their poor permeability, as they have many hydrophilic groups. The relatively good cell potencies of **38** and **40**, in contrast, can be ascribed to improved liposolubility due to their large alkyl substituents (isobutyl and *n*-Hex, respectively).

Table 4 c-Met enzymatic activity of compounds **51–55**



Compound	R_1	R_2	R_3	IC_{50} [a] (nM)
51	OH	OCH_3	OCH_3	51.8%@10 μM
52	OH	H	OH	1370.9 ± 208.5
53	OH	OH	H	927.9 ± 98.7
54	H	H	OH	0%@10 μM
55	H	OH	H	0%@10 μM

[a] IC_{50}s were calculated by the logit method from the results of at least two independent tests with eight concentrations each and expressed as means ± SD.

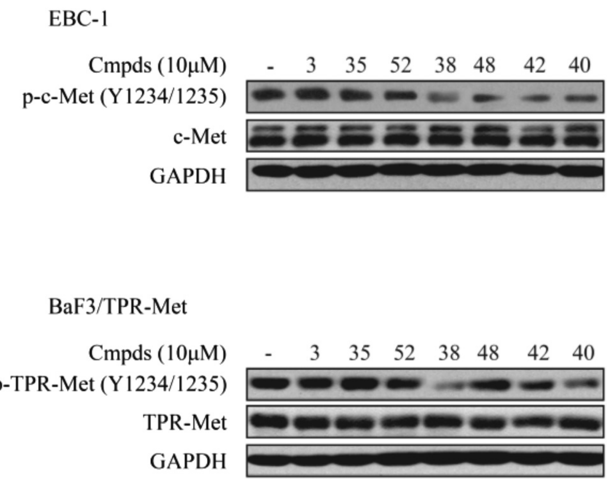
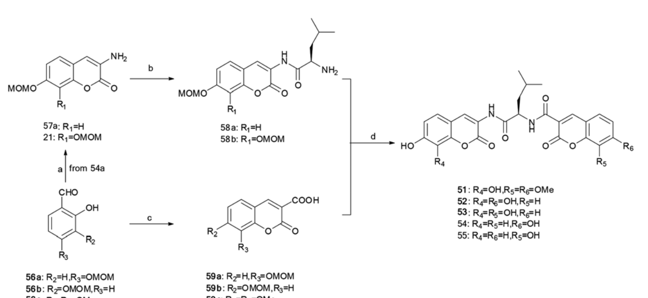


Fig. 3 The effect of selected compounds on c-Met phosphorylation in EBC-1 and BaF3/TPR-Met cells.



Scheme 3 Synthesis of compounds **51–55** with D-leucine linker. *Reagents and conditions*: (a) (i) ethyl nitroacetate, piperidine, benzene, Dean–Stark trap, reflux; (ii) Pd/C, H_2, AcOEt; (b) (i) Fmoc-D-Leu-OH, EDCI, 30% pyridine, DCM; (ii) piperidine, CH_3CN. (c) isopropylidene malonate, piperidinium acetate, EtOH, 60 °C; (d) (i) EDCI, DMAP, DCM; (ii) HCl–AcOEt.

Conclusions

In summary, we have developed a series of 3,3′-biscoumarin-based, non-ATP competitive c-Met inhibitors initiated from 3,3′-methylenebis(4,7,8-trihydroxy-coumarin). Among these compounds, **38** and **40** showed potent enzyme activities with IC_{50} of 107 nM and 30 nM respectively. Significantly, they inhibit c-Met phosphorylation in BaF3/TPR-Met and EBC-1 NSCLC cell lines. These compounds represent a novel structural type for non-ATP competitive c-Met inhibitors, and are worth developing further thorough investigation of the SAR. Such efforts are currently underway, and will be reported in due course.

Experimental section

Chemistry

Starting materials, reagents, and solvents were purchased from commercial suppliers and used without further purification, unless otherwise stated. Anhydrous THF, benzene, diethyl ether and CH_2Cl_2 were obtained by distillation over sodium wire or CaH_2. All non-aqueous reactions were run under an argon atmosphere with exclusion of moisture from reagents, and all reaction vessels were oven-dried. The progress of reactions was monitored by TLC on SiO_2. Spots were visualized by UV or by dipping into $KMnO_4$ solution followed by heating. SiO_2 for flash chromatography was of 230–400 mesh particle size. Petroleum ether refers to the fraction with boiling range 60–90 °C. ^{1}H NMR spectra were recorded on a Varian Mercury-Vx 300M Fourier transform spectrometer at a frequency of 300 MHz, and ^{13}C NMR spectra at 75 MHz. ^{1}H chemical shifts are reported in δ (ppm) using the δ 7.26 signal of $CDCl_3$, the δ 3.31 signal of CD_3OD or the δ 2.50 signal of DMSO-d_6 as an internal standard. ^{13}C chemical shifts are reported in δ (ppm) using the δ 77.23 signal of $CDCl_3$, the δ 49.15 signal of CD_3OD, or the δ 39.51 signal of DMSO-d_6 as an internal standard. The purity of final compounds was assessed by the analytical HPLC method and found to be >95%. An Agilent 1200 series HPLC with a Zorbax SB-C18 (4.6 × 50 mm, 5 μm particle sizes) reversed-phase column was used for analytical HPLC analyses. The elution buffer was an A/B gradient, where A = 0.1% HCOOH in H_2O and B = CH_3OH.

1-(3,4-Bis(benzyloxy)-2-hydroxyphenyl)ethanone (9a).[25] 1-(2,3,4-Trihydroxyphenyl)ethanone (6.0 g, 35.7 mmol) was added to a suspension of benzyl bromide (6.0 g, 35.0 mmol), K_2CO_3 (8.0 g, 58.0 mmol) and KI (0.3 g, 1.5 mmol) in DMF (100 mL). The reaction mixture was heated to 60 °C for 4 h. Upon completion, 200 mL H_2O and 300 mL EtOAc were added. The aqueous phase was extracted with EtOAc and the combined organic phase was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified through column chromatography (eluent, PE–EtOAc = 4 : 1) to afford 1-(2,3,4-tris(benzyloxy)phenyl)ethanone (15.6 g, 100%). Magnesium bromide etherate (5.3 g, 20.5 mmol) was added portionwise to a solution of 1-(2,3,4-tris(benzyloxy)phenyl)ethanone (9.0 g, 20.5 mmol) in ether (50 mL). The mixture was stirred at room temperature for 14 h, and then cooled to 0 °C and quenched with 1 M aqueous HCl (100 mL). The aqueous phase was extracted with EtOAc and the combined organic phase was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash chromatography (PE–EtOAc) to give compound **9a** (5.3 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.62 (s, H), 7.46–7.28 (m, 11H), 6.49 (d, J = 8.7 Hz, 1H), 5.16 (s, 2H), 5.11(s, 2H), 2.56 (s, 3H).

1-(2-Hydroxy-3,4-bis(methoxymethoxy)phenyl)ethanone (9b). Compound **9b** was prepared utilizing the same synthetic route as compound **9d** starting from 1-(2,3,4-trihydroxyphenyl)ethanone. Yellow oil (2.3 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.63 (s, 1H), 7.48 (d, J = 9.0 Hz, 1H), 6.71 (d, J = 8.7 Hz, 1H), 5.27 (s, 2H), 5.19 (s, 2H), 3.63 (s, 3H), 3.50 (s, 3H), 2.57 (s, 3H).

1-(2-Hydroxy-3,4-dimethoxyphenyl)ethanone (9c).[26] Compound **9c** was prepared utilizing the same synthetic route as compound **56c** starting from 1-(2,3,4-trihydroxyphenyl)ethanone. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.97 (s, 1H), 7.49 (d, J = 9.0 Hz, 1H), 6.50 (d, J = 9.0 Hz, 1H), 3.98 (s, 3H), 3.92 (s, 3H), 2.57 (s, 3H).

1-(2-Hydroxy-4,6-bis(methoxymethoxy)phenyl)ethanone (9d).[27] To a mixture of 1-(2,4,6-trihydroxyphenyl)ethanone (2.15 g, 12.8 mmol) and DIPEA in 50 mL DCM at 0 °C was added MOMCl (2.14 mL, 28.13 mmol) dropwise. After stirring for 2 h at 0 °C, the mixture was diluted with 100 mL DCM and washed with 10% aqueous citric acid (2 × 30 mL) and brine. The organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified through column chromatography (eluent, PE–EtOAc = 10 : 1) to afford **9d** as a beige solid (2.1 g, 64.1%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.71 (s, 1H), 6.26 (d, J = 2.2 Hz, 1H), 6.24 (d, J = 2.2 Hz, 1H), 5.25 (s, 2H), 5.17 (s, 2H), 3.52 (s, 3H), 3.47 (s, 3H), 2.65 (s, 3H).

7,8-Bis(benzyloxy)-4-hydroxy-2*H*-chromen-2-one (10a). To a solution of 1-(3,4-bis(benzyloxy)-2-hydroxyphenyl)ethanone **9a** (5.30 g, 15 mmol) and diethyl carbonate (3.54 g, 30 mmol) in 80 mL toluene was added NaH (2.40 g, 60% in oil, 60 mmol) at 0 °C. The mixture was stirred at 80 °C for 2 h. The solution was then cooled to rt and 30 mL 5% aqueous NaOH was added. After stirring for 10 min, the solution was acidified with 10% aqueous citric acid. The pale precipitate was collected and dried under reduced pressure to give **10a** (4.6 g, 80%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.61 (d, J = 9.0 Hz, 1H), 7.47–7.26 (m, 10H), 7.14 (d, J = 9.0 Hz, 1H), 5.24 (s, 2H), 5.19 (s, 1H), 5.13 (s, 2H).

Compounds **10b–e** were prepared utilizing the same synthetic route as compound **10a** starting from **9b–e**, respectively.

4-Hydroxy-7,8-bis(methoxymethoxy)-2*H*-chromen-2-one (10b). White solid (1.0 g, 91%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.60 (br, 1H), 7.47 (d, J = 8.7 Hz, 1H), 7.00 (d, J = 8.7 Hz, 1H), 5.53 (s, 1H), 5.20 (s, 2H), 5.13 (s, 2H), 3.58 (s, 3H), 3.43 (s, 3H).

4-Hydroxy-7,8-dimethoxy-2*H*-chromen-2-one (10c).[28] Pale solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.41 (s, 1H), 7.54 (d, J = 9.0 Hz, 1H), 7.09 (d, J = 9.0 Hz, 1H), 5.45 (s, 1H), 3.90 (s, 3H), 3.80 (s, 3H).

4-Hydroxy-5,7-bis(methoxymethoxy)-2*H*-chromen-2-one (10d). Pale solid (100 mg, 30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.36 (s, 1H), 6.74 (d, J = 2.1 Hz, 1H), 6.71 (d, J = 2.1 Hz, 1H), 5.57 (s, 1H), 5.39 (s, 2H), 5.20 (s, 2H), 3.57 (s, 3H), 3.48 (s, 3H).

4-Hydroxy-2*H*-chromen-2-one (10e).[29] Pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.48 (s, 1H), 7.82 (d, J = 7.2 Hz, 1H), 7.67–7.58 (m, 1H), 7.38–7.30 (m, 2H), 5.88 (s, 1H).

4,7,8-Trihydroxy-2*H*-chromen-2-one (2). **10b** (2.0 g, 7.1 mmol) was dissolved in 20 mL 3 N HCl–EtOAc and 0.5 mL MeOH.

The reaction mixture was stirred at room temperature for 4 h. The white solid was isolated by filtration to afford **2** (1.3 g, 94.5%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.10 (s, 1H), 9.97 (br s, 1H), 9.20 (br s, 1H), 7.15 (d, J = 8.4 Hz, 1H), 6.77 (d, J = 8.4 Hz, 1H), 5.39 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.5, 162.3, 149.7, 143.9, 132.0, 113.3, 111.8, 108.4, 87.8.

3,3′-Methylenebis(4,7,8-trihydroxy-2*H*-chromen-2-one) (3). To a solution of **2** (500 mg, 2.58 mmol) and paraformaldehyde (50 mg, 0.56 mmol) in 10 mL EtOH was added Et_3N (0.5 mL). The reaction mixture was stirred at room temperature for 24 h. The resulting pale solid was isolated by filtration, washed with 10% aqueous citric acid, and dried under vacuum to afford **3** (460 mg, 89%). Mp: >280 °C. HPLC: 99.37%, t_R = 5.408 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.01 (br s, 6H), 7.27 (d, J = 8.7 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 3.71 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.8, 163.3, 149.4, 142.3, 132.0, 113.5, 112.5, 109.0, 99.5, 18.9. HRMS (ESI): calcd for $C_{19}H_{12}O_{10}Na$ $[M + Na]^+$, 423.0328. Found: $[M + Na]^+$, 423.0321.

Compounds **11a–b**, **5** (ref. 28) and **8** (ref. 30) were prepared utilizing the same synthetic route as compound **3** starting from **10a,c–e**.

3,3′-Methylenebis(7,8-bis(benzyloxy)-4-hydroxy-2*H*-chromen-2-one) (11a). White solid (80 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 9.0 Hz, 2H), 7.47–7.26 (m, 20H), 6.89 (d, J = 8.7 Hz, 2H), 5.15 (s, 4H), 5.12 (s, 4H), 3.83 (s, 2H).

3,3′-Methylenebis(4-hydroxy-5,7-bis(methoxymethoxy)-2*H*-chromen-2-one) (11b). White solid (54 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.55 (s, 2H), 6.72 (d, J = 2.1 Hz, 2H), 6.71 (d, J = 2.1 Hz, 2H), 5.31 (s, 4H), 5.19 (s, 4H), 3.79 (s, 2H), 3.55 (s, 6H), 3.47 (s, 6H).

3,3′-Methylenebis(4,5,7-trihydroxy-2*H*-chromen-2-one) (7). Compound **11b** (50 mg, 0.0867 mmol) was dissolved in 3 mL 2 N HCl–EtOAc and stirred at room temperature for 1 h. The reaction mixture was evaporated to afford **7** as a brown solid (27 mg, 80%). Mp: >280 °C. HPLC: 95.51%, t_R = 3.911 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.03 (s, 2H), 6.05 (d, J = 2.1 Hz, 2H), 5.99 (d, J = 1.5 Hz, 2H), 3.45 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.1, 163.1, 160.7, 158.0, 154.6, 98.3, 98.2, 97.6, 93.4, 17.7. HRMS (ESI): calcd for $C_{19}H_{12}O_{10}Na$ $[M + Na]^+$, 423.0328. Found: $[M + Na]^+$, 423.0316.

3,3′-Methylenebis(4-hydroxy-7,8-dimethoxy-2*H*-chromen-2-one) (5). Pale solid (100 mg, 86%). Mp: 286–287 °C. HPLC: 95.58%, t_R = 7.945 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.63 (d, J = 8.7 Hz, 2H), 7.09 (d, J = 9.0 Hz, 2H), 3.89 (s, 6H), 3.78 (s, 6H), 3.71 (s, 2H). HRMS (ESI): calcd for $C_{23}H_{21}O_{10}$ $[M + H]^+$, 457.1135. Found: $[M + H]^+$, 457.1119.

3,3′-Methylenebis(4-hydroxy-2*H*-chromen-2-one) (8). White solid (400 mg, 90%). Mp: 264–265 °C. HPLC: 97.52%, t_R = 11.516 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (d, J = 7.8 Hz, 2H), 7.58 (t, J = 7.5 Hz, 2H), 7.41–7.27 (m, 4H), 3.79 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.7, 162.7, 151.9, 131.7, 123.9, 123.4, 116.9, 116.1, 102.3, 19.4. HRMS (ESI): calcd for $C_{19}H_{12}O_6Na$ $[M + Na]^+$, 459.0532. Found: $[M + Na]^+$, 459.0521.

3,3′-Methylenebis(7,8-dihydroxy-4-methoxy-2*H*-chromen-2-one) (4). To a solution of **11a** (90 mg, 0.118 mmol) in 6 mL THF was added CH_2N_2 in Et_2O (1 M, 3.4 eq., 0.4 mL) at 0 °C. After stirring at 0 °C for 2 h, the reaction was quenched with 0.1 mL AcOH and concentrated under reduced pressure. The residue was purified through column chromatography (eluent, PE–EtOAc = 2 : 1) to afford 3,3′-methylenebis(7,8-bis(benzyloxy)-4-methoxy-2*H*-chromen-2-one) (45 mg, 43%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52–7.29 (m, 22H), 6.90 (d, J = 8.7 Hz, 2H), 5.18 (s, 8H), 4.06 (s, 6H), 3.93 (s, 2H). 3,3′-methylenebis(7,8-bis(benzyloxy)-4-methoxy-2*H*-chromen-2-one) (40 mg, 0.0507 mmol) and $Pd(OH)_2$ (5 mg) in 10 mL THF were placed under a hydrogen atmosphere (H_2, 1 atm.) and stirred for 2 h. The mixture was then filtered through a Celite pad. The Celite pad was washed with 4 × 10 mL of MeOH. The filtrate was concentrated and purified through column chromatography on reverse phase C-18 silica gel (eluent, H_2O–CH_3CN = 3 : 2 to 1 : 1). After lyophilization, **4** was obtained as a yellowish brown solid (12 mg, 55.3%). Mp: 262–264 °C. HPLC: 98.28%, t_R = 4.005 min. ^{1}H NMR (300 MHz, CD_3OD) δ 7.13 (d, J = 8.7 Hz, 2H), 6.82 (d, J = 8.4 Hz, 2H), 4.00 (s, 6H), 3.87 (s, 2H). ^{13}C NMR (75 MHz, CD_3OD + $CDCl_3$) δ 167.2, 165.7, 150.4, 143.8, 133.6, 115.2, 113.5, 112.3, 111.3, 62.7, 21.2. HRMS (ESI): calcd for $C_{21}H_{16}O_{10}Na$ $[M + Na]^+$, 451.0641. Found: $[M + Na]^+$, 451.0637.

Diethyl 2,4-diacetylpentanedioate (13).[31] A mixture of Et_2NH (412 μL, 4.0 mmol) and CH_2Br_2 (2.1 mL, 30.0 mmol) was heated to 50 °C for 1.5 h and then cooled to rt. The mixture was added to a solution of ethyl acetoacetate (258 mg, 2.0 mmol) in 8 mL of CH_2Cl_2 and stirred at rt. Upon completion (2 h), the reaction mixture was concentrated, and the crude mixture was purified by column chromatography on silica gel (EtOAc–PE) to give the desired product **13** as a colorless oil (123 mg, 45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.27–4.13 (m, 4H), 3.53 (t, J = 7.2 Hz, 1H), 2.46–2.29 (m, 2H), 2.26 (s, 6H), 1.34–1.22 (m, 6H).

3,3′-Methylenebis(7,8-dihydroxy-4-methyl-2*H*-chromen-2-one) (6). To a mixture of pyrogallol **12** (500 mg, 4 mmol) and diethyl 2,4-diacetylpentanedioate **13** (544 mg, 2 mmol) at 0 °C was added 70% H_2SO_4. The reaction mixture was stirred at room temperature for 30 min, and then poured into water (50 mL). The tan solid was isolated by filtration and dried under vacuum to afford **6** (50 mg, 5%). Mp: >280 °C. HPLC: 96.23%, t_R = 2.799 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.12 (d, J = 7.2 Hz, 1H), 6.79 (d, J = 7.2 Hz, 1H), 3.87 (s, 2H), 2.41 (s, 6H). HRMS (ESI): calcd for $C_{21}H_{16}O_8Na$ $[M + Na]^+$, 419.0743. Found: $[M + Na]^+$, 419.0735.

3,4-Bis(benzyloxy)-2-hydroxybenzaldehyde (24).[32] Compound **24** was prepared utilizing the same synthetic route as compound **9a** starting from 2,3,4-trihydroxybenzaldehyde. White solid (2.4 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.24 (s, 1H), 9.74 (s, 1H), 7.50–7.29 (m, 10H), 7.24 (d, J = 8.7 Hz, 1H), 6.62 (d, J = 8.4 Hz, 1H), 5.18 (s, 2H), 5.13 (s, 2H).

Compounds **19** (ref. 25) and **56a–b** (ref. 33,34) were prepared utilizing the same synthetic route as compound **9d** starting from different salicylaldehydes.

2-Hydroxy-3,4-bis(methoxymethoxy)benzaldehyde (19). White solid (2.26 g, 29%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.29 (s, 1H), 9.76 (s, 1H), 7.28 (d, *J* = 8.7 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 1H), 5.30 (s, 2H), 5.20 (s, 2H), 3.64 (s, 3H), 3.51 (s, 3H).

2-Hydroxy-4-(methoxymethoxy)benzaldehyde (56a). White solid (4.0 g, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.36 (s, 1H), 9.74 (s, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 6.65 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.60 (d, *J* = 2.4 Hz, 1H), 5.22 (s, 2H), 3.48 (s, 3H).

2-Hydroxy-3-(methoxymethoxy)benzaldehyde (56b). White solid (300 mg, 15%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.12 (s, 1H), 9.91 (s, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.26 (dd, *J* = 7.8, 1.8 Hz, 1H), 6.96 (t, *J* = 7.8 Hz, 1H), 5.26 (s, 3H), 3.53 (s, 3H).

2-Hydroxy-3,4-dimethoxybenzaldehyde (56c).[35] To a solution of 2,3,4-trihydroxybenzaldehyde (2.1 g, 13.6 mmol) and K_2CO_3 (6.6 g, 47.7 mmol) in 50 mL acetone was added CH_3I (3.0 mL, 47.7 mmol). The reaction mixture was stirred at 60 °C for 24 h, and then cooled to rt. The residue was diluted with 100 mL EtOAc and 50 mL H_2O. The aqueous phase was extracted with EtOAc. The combined organic phase was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give 2,3,4-trimethoxybenzaldehyde (2.6 g, 97.2%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.23 (s, 1H), 7.59 (d, *J* = 9.0 Hz, 1H), 6.74 (d, *J* = 9.0 Hz, 1H), 4.02 (s, 3H), 3.92 (s, 3H), 3.87 (s, 3H). To a solution of 2,3,4-trimethoxybenzaldehyde (546 mg, 2.78 mmol) in 20 mL benzene was added anhydrous $AlCl_3$ (408 mg, 3.06 mmol). After stirring for 5 min at room temperature, the mixture was heated to 80 °C for 6 h, and then cooled to rt. 30 mL ice water and 3 mL concentrated HCl were added with stirring. The aqueous phase was extracted with EtOAc. The combined organic phase was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified through column chromatography (eluent, PE–EtOAc = 10 : 1 to 8 : 1) to give **56c** as a white solid (398 mg, 78.6%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.21 (s, 1H), 9.75 (s, 1H), 7.29 (d, *J* = 8.7 Hz, 1H), 6.61 (d, *J* = 8.7 Hz, 1H), 3.95 (s, 3H), 3.90 (s, 3H).

General procedures for the preparation of acidcoumarins (20, 25, 59a–c). A mixture of the corresponding salicylaldehydes **19**, **24** or **56a–c** (2 mmol), isopropylidene malonate (2.4 mmol) and piperidinium acetate (0.1 mmol) in 30 mL anhydrous ethanol was heated to 60 °C for 24 h. Then the mixture was cooled to 0 °C and the precipitate was collected by filtration, washed with 5 mL cold ethanol and dried *in vacuo* to afford the corresponding acidcoumarins (**20**, **25**, **59a–c**).

7,8-Bis(methoxymethoxy)-2-oxo-2*H*-chromene-3-carboxylic acid (20). White solid (780 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.25 (br, 1H), 8.85 (s, 1H), 7.46 (d, *J* = 8.7 Hz, 1H), 7.29 (d, *J* = 8.7 Hz, 1H), 5.36 (s, 2H), 5.26 (s, 2H), 3.71 (s, 3H), 3.53 (s, 3H).

7,8-Bis(benzyloxy)-2-oxo-2*H*-chromene-3-carboxylic acid (25). White solid (550 mg, 46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.23 (br, 1H), 8.82 (s, 1H), 7.47–7.38 (m, 8H), 7.36–7.28 (m, 3H), 7.08 (d, *J* = 8.7 Hz, 1H), 5.27 (s, 2H), 5.21 (s, 2H).

8-(Methoxymethoxy)-2-oxo-2*H*-chromene-3-carboxylic acid (59a). Beige solid (145 mg, 35%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (s, 1H), 7.59 (t, *J* = 4.8 Hz, 2H), 7.38 (d, *J* = 4.5 Hz, 2H), 5.35 (s, 2H), 3.56 (s, 3H).

7-(Methoxymethoxy)-2-oxo-2*H*-chromene-3-carboxylic acid (59b). White solid (237 mg, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.76 (s, 1H), 7.63 (d, *J* = 8.7 Hz, 1H), 7.13–7.06 (m, 2H), 5.30 (s, 2H), 3.51 (s, 3H).

7-(Methoxymethoxy)-2-oxo-2*H*-chromene-3-carboxylic acid (59c). Yellowish-white solid (367 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.85 (s, 1H), 7.47 (d, *J* = 8.7 Hz, 1H), 7.06 (d, *J* = 8.7 Hz, 1H), 4.04 (s, 3H), 4.02 (s, 3H).

3-Amino-7,8-bis(methoxymethoxy)-2*H*-chromen-2-one (21). To a mixture of 2-hydroxy-3,4-bis(methoxymethoxy)benzaldehyde **19** (2.26 g, 8.80 mmol) and ethyl nitroacetate (1.40 g, 10.6 mmol) in 60 mL dry benzene was added piperidine (174 μL, 1.76 mmol). The reaction mixture was heated to reflux for 6 h with a Dean–Stark trap to collect the water. The reaction was then cooled to rt and purified by flash chromatography (eluent, CH_2Cl_2) to give 7,8-bis(methoxymethoxy)-3-nitro-2*H*-chromen-2-one as a yellow solid (1.90 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74 (s, 1H), 7.46 (d, *J* = 8.7 Hz, 1H), 7.28 (d, *J* = 8.7 Hz, 1H), 5.37 (s, 2H), 5.25 (s, 2H), 3.71 (s, 3H), 3.55 (s, 3H). 7,8-Bis(methoxymethoxy)-3-nitro-2*H*-chromen-2-one (350 mg, 1.07 mmol) and 10% Pd/C (14 mg) in 15 mL EtOAc was stirred under hydrogen (H_2, 1 atm.) for 2 h. The mixture was collected through a Celite pad. The Celite pad was washed with 4 × 20 mL of EtOAc. The filtrate was concentrated and purified by flash chromatography column (PE–EtOAc) to provide aminocoumarin **21** as a yellow solid (190 mg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (d, *J* = 8.7 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 1H), 6.65 (s, 1H), 5.24 (s, 2H), 5.22 (s, 2H), 4.13 (br, 2H), 3.70 (s, 3H), 3.51 (s, 3H).

***N*-(7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (14).** Acid **20** (55 mg, 0.177 mmol), aminocoumarin **21** (50 mg, 0.177 mmol) and EDCI (52 mg, 0.267 mmol) were dissolved in 3 mL of 30% pyridine/CH_2Cl_2 and stirred at rt for 2 h. The solvent was evaporated and the residue was purified by column chromatography (CH_2Cl_2–acetone = 50 : 1 to 40 : 1) to give *N*-(7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromen-3-yl)-7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromene-3-carboxamide (17 mg, 17%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.46 (s, 1H), 8.86 (s, 1H), 8.79 (s, 1H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.26–7.21 (m, 2H), 7.14 (d, *J* = 8.7 Hz, 1H), 5.34 (s, 2H), 5.28 (s, 2H), 5.26 (s, 2H), 5.26 (s, 2H), 3.74 (s, 3H), 3.72 (s, 3H), 3.53 (s, 3H), 3.52 (s, 3H). This intermediate (17 mg, 0.0296 mmol) was dissolved in 3 mL 2 M HCl in EtOAc and stirred at rt for 2 h. The yellow solid was isolated by filtration to afford **14** (10 mg, 85%). Mp: >280 °C. HPLC: 95.35%, t_R = 2.868 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.22 (s, 1H), 10.80 (br s, 1H), 9.98 (br s, 1H), 9.69 (br s, 1H), 9.42 (br s, 1H), 8.89 (s, 1H), 8.73 (s, 1H), 7.41 (d, *J* = 8.7 Hz, 1H), 7.06 (d, *J* = 8.7 Hz, 1H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.3, 160.6, 158.0, 152.9, 149.6, 148.1, 144.3, 139.9, 132.2, 132.0, 125.1, 122.0, 120.7, 118.2, 113.8, 113.2, 112.3, 112.2, 112.0. HRMS (ESI): calcd for $C_{19}H_{11}NO_9Na$ $[M + Na]^+$, 420.0332. Found: $[M + Na]^+$, 420.0312.

***trans*-N^1,N^2-Bis(7,8-dihydroxy-2-oxo-2*H*-chromen-3-yl)cyclohexane-1,2-dicarboxamide (16).** *trans*-1,2-Cyclohexanedicarboxylic acid **23** (26 mg, 0.151 mmol), aminocoumarin **21**

(85 mg, 0.302 mmol) and EDCI (87 mg, 0.453 mmol) were dissolved in 3 mL of 30% pyridine/CH_2Cl_2. The mixture was heated to 50 °C for 40 h. The solvent was evaporated and the residue was purified by column chromatography (PE–EtOAc) to give *trans*-N^1,N^2-bis(7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromen-3-yl)cyclohexane-1,2-dicarboxamide (25 mg, 23.7%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (s, 2H), 8.12 (s, 2H), 7.14 (d, *J* = 8.9 Hz, 2H), 7.09 (d, *J* = 8.1 Hz, 2H), 5.24 (s, 4H), 5.22 (s, 4H), 3.69 (s, 6H), 3.49 (s, 6H), 2.81–2.69 (m, 2H), 2.14–2.02 (m, 2H), 1.95–1.85 (m, 2H), 1.65–1.50 (m, 2H), 1.45–1.35 (m, 2H). LS-MS: *m*/*z*: 721.2 [M + Na]$^+$. This intermediate (25 mg, 0.0358 mmol) was dissolved in 3 mL of 2 M HCl in EtOAc and stirred at rt for 2 h. The mixture was concentrated and purified by flash chromatography (CH_2Cl_2–CH_3OH = 20 : 1) to provide **16** as a yellow solid (14 mg, 75%). Mp: 284–285 °C. HPLC: 95.07%, t_R = 2.648 min. ^{1}H NMR (300 MHz, CD_3OD) δ 8.45 (s, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.77 (d, *J* = 8.4 Hz, 2H), 2.94–2.86 (m, 2H), 2.14–2.04 (m, 2H), 1.94–1.80 (m, 2H), 1.62–1.38 (m, 4H). LS-MS: *m*/*z*: 523.1 [M + H]$^+$, 545.1 [M + Na]$^+$.

***cis*-N^1,N^2-Bis(7,8-dihydroxy-2-oxo-2*H*-chromen-3-yl)cyclohexane-1,2-dicarboxamide (15).** This compound was prepared utilizing the same synthetic route as compound **16** starting from *cis*-1,2-cyclohexanedicarboxylic acid **22** and aminocoumarin **21**. Yellow solid (3 mg, 3%). Mp = 216–218 °C. HPLC: 95.33%, t_R = 2.851 min. ^{1}H NMR (300 MHz, CD_3OD) δ 8.47 (s, 2H), 6.90 (d, *J* = 8.4 Hz, 2H), 6.79 (d, *J* = 8.4 Hz, 2H), 3.04 (s, 2H), 2.32–2.20 (m, 2H), 1.92–1.76 (m, 4H), 1.62–1.46 (m, 2H). LS-MS: *m*/*z*: 523.2 [M + H]$^+$, 545.2 [M + Na]$^+$.

7,8-Bis(benzyloxy)-3-(piperazine-1-carbonyl)-2*H*-chromen-2-one (26). A mixture of **25** (100 mg, 0.249 mmol), 1-Boc-piperazine (46 mg, 0.249 mmol), EDCI (72 mg, 0.373 mmol) and DMAP (6 mg, 0.049 mmol) in 10 mL CH_2Cl_2 was stirred at room temperature for 4 h and then concentrated. The residue was purified by flash chromatography column (PE–EtOAc) to give *tert*-butyl 4-(7,8-bis(benzyloxy)-2-oxo-2*H*-chromene-3-carbonyl)piperazine-1-carboxylate (130 mg, 91.7%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.51–7.27 (m, 10H), 7.19 (d, *J* = 8.7 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 1H), 5.21 (s, 2H), 5.20 (s, 2H), 3.73 (s, 2H), 3.57–3.47 (m, 4H), 3.34 (s, 2H), 1.47 (s, 9H). This intermediate was dissolved in 3 mL of 2 M HCl in EtOAc at 0 °C and then warmed to rt. After stirring at room temperature for 1 h, the mixture was concentrated to give **26** as its hydrochloride salt.

7,8-Bis(benzyloxy)-3-(4-(7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromene-3-carbonyl)piperazine-1-carbonyl)-2*H*-chromen-2-one (27). To a mixture of **26** hydrochloride (0.228 mmol), **20** (85 mg, 0.274 mmol), EDCI (66 mg, 0.342 mmol) and DMAP (5 mg, 0.041 mmol) in 10 mL CH_2Cl_2 was added Et_3N (95 μL, 0.684 mmol). The reaction mixture was stirred at room temperature overnight, and then concentrated and purified by flash chromatography column (PE–acetone) give **27** (70 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.89 (s, 1H), 7.54–7.07 (m, 13H), 6.95 (d, *J* = 8.4 Hz, 1H), 5.32–5.15 (m, 8H), 3.87 (s, 4H), 3.69 (s, 3H), 3.51 (s, 4H), 3.48 (s, 3H).

3,3′-(Piperazine-1,4-dicarbonyl)bis(7,8-dihydroxy-2*H*-chromen-2-one) (17). **27** (40 mg, 0.0524 mmol) and 10% Pd/C (5 mg) in 2 mL CH_3OH and 2 mL EtOAc were hydrogenated (H_2, 1 atm.) for 4 h. The mixture was filtered through a Celite pad. The Celite pad was washed with 4 × 10 mL of MeOH. The filtrate was concentrated and purified by flash chromatography column (eluent, CH_2Cl_2–CH_3OH = 20 : 1) to give 3-(4-(7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromene-3-carbonyl)piperazine-1-carbonyl)-7,8-dihydroxy-2*H*-chromen-2-one (27 mg, 90%). This intermediate was dissolved in 2 mL DCM and 3 drops of MeOH, then 2 mL of 2 M HCl in EtOAc was added. The mixture was stirred at room temperature for 2 h, then the yellow solid was isolated by filtration to afford **17** (21 mg, 91%). Mp: >280 °C. HPLC: 97.06%, t_R = 2.825 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.31 (s, 2H), 9.45 (br s, 2H), 8.08 (s, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 3.66–3.38 (m, 8H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.8, 158.0, 150.6, 144.0, 143.7, 132.0, 119.7, 119.2, 113.0, 111.5, 46.7, 46.1, 41.7, 41.2. HRMS (ESI): calcd for $C_{24}H_{18}N_2O_{10}Na$ [M + Na]$^+$, 517.0859. Found: [M + Na]$^+$, 517.3680.

(*S*)-2-Amino-*N*-(7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromen-3-yl)-4-methylpentanamide (29). Fmoc-Leu-OH **28** (227 mg, 0.641 mmol), aminocoumarin **21** (120 mg, 0.427 mmol) and EDCI (164 mg, 0.854 mmol) were dissolved in 5 mL of 30% pyridine/CH_2Cl_2. The mixture was stirred at rt for 36 h. The solvent was evaporated and the residue was purified by column chromatography (PE–EtOAc) to give (*R*)-(9*H*-fluoren-9-yl)methyl (1-((7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromen-3-yl)-amino)-4-methyl-1-oxopentan-2-yl)carbamate as a white solid (190 mg, 72.1%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.57 (br, 1H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.62–7.56 (m 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.30 (t, *J* = 7.2 Hz, 2H), 7.18 (d, *J* = 8.7 Hz, 1H), 7.13 (d, *J* = 8.7 Hz, 1H), 5.32–5.18 (m, 5H), 4.48 (d, *J* = 6.0 Hz, 2H), 4.38 (s, 1H), 4.23 (t, *J* = 6.6 Hz, 1H), 3.70 (s, 3H), 3.52 (s, 3H), 1.78–1.54 (m, 3H), 0.96 (s, 6H). To a solution of this intermediate (190 mg, 0.308 mmol) in 10 mL acetonitrile was added piperidine (31 μL, 0.308 mmol). The reaction mixture was stirred at rt for 6 h. The solvent was evaporated and the residue was purified by column chromatography (PE–EtOAc = 2 : 1 to 1 : 1) to give **29** as a white solid (85 mg, 70.1%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.10 (s, 1H), 8.65 (s, 1H), 7.18 (d, *J* = 8.7 Hz, 1H), 7.11 (d, *J* = 8.7 Hz, 1H), 5.26 (s, 2H), 5.24 (s, 2H), 3.70 (s, 3H), 3.60–3.53 (m, 1H), 3.51 (s, 3H), 1.92–1.63 (m, 5H), 1.06–0.88 (m, 6H).

(*S*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (18). Compound **29** (85 mg, 0.216 mmol), **20** (80 mg, 0.259 mmol), EDCI (62 mg, 0.324 mmol) and DMAP (6 mg, 0.049 mmol) were dissolved in 10 mL CH_2Cl_2 and stirred at rt for 2 h. The solvent was evaporated and the residue was purified by column chromatography (PE–EtOAc = 2 : 1 to 1 : 1) to give (*R*)-*N*-(1-((7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromen-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromene-3-carboxamide (120 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.15 (d, *J* = 6.9 Hz, 1H), 8.88 (s, 1H), 8.87 (s, 1H), 8.63 (s, 1H), 7.40 (d, *J* = 9.0 Hz, 1H), 7.21 (d, *J* = 9.0 Hz, 1H), 7.17 (d, *J* = 8.7 Hz, 1H), 7.11 (d, *J* = 8.7 Hz, 1H), 5.33 (s, 2H), 5.25 (s, 4H), 5.22 (s, 2H), 4.78–4.70 (m, 1H), 3.71 (s, 3H), 3.68 (s, 3H), 3.52 (s, 3H), 3.50 (s, 3H), 1.95–1.75 (m, 3H), 1.02 (d, J = 6.0 Hz, 3H), 0.98 (d, J = 6.3 Hz, 3H). This intermediate (140 mg, 0.175 mmol) was dissolved in 2 mL DCM and 2 mL of 2 M HCl in EtOAc, and stirred at rt for 2 h. The yellow solid was isolated by filtration to afford **18** (96 mg, 92%). Mp: 180–184 °C. HPLC: 97.97%, t_R = 3.511 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.66 (br s, 1H), 9.92 (s, 2H), 9.59 (br s, 1H), 9.32 (br s, 1H), 9.04 (d, J = 8.1 Hz, 1H), 8.77 (s, 1H), 8.46 (s, 1H), 7.33 (d, J = 8.7 Hz, 1H), 7.00 (d, J = 8.7 Hz, 1H), 6.91 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H), 5.04–4.96 (m, 1H), 1.68–1.66 (m, 3H), 0.94 (d, J = 5.1 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.8, 161.4, 161.1, 157.7, 152.3, 149.1, 148.1, 144.2, 140.3, 132.0, 131.9, 127.8, 121.6, 120.2, 118.1, 113.6, 113.1, 112.7, 112.1, 111.8, 52.0, 41.4, 24.6, 23.1, 21.8. HRMS (ESI): calcd for $C_{25}H_{22}N_2O_{10}Na$ [M + Na]$^+$, 533.1172. Found: [M + Na]$^+$, 533.1167.

3-Amino-7-(methoxymethoxy)-2*H*-chromen-2-one (57a). This compound was prepared utilizing the same synthetic route as compound **21**, starting from 2-hydroxy-4-(methoxymethoxy) benzaldehyde **56a**. Yellow solid (780 mg, 36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (d, J = 8.7 Hz, 1H), 6.99 (d, J = 2.4 Hz, 1H), 6.92 (dd, J = 8.4, 2.4 Hz, 1H), 6.68 (s, 1H), 5.18 (s, 2H), 3.48 (s, 3H).

Compounds **30–47**, **49a**, **50a** and **51–55** were prepared utilizing the same synthetic route as compound **18** starting from the appropriate Fmoc-amino acids, **57a** (or **21**), and **20** (or **59a–c**).

***N*-(2-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-2-oxoethyl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (30).** Yellow solid. Mp: >280 °C. HPLC: 95.91%, t_R = 2.772 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.65 (s, 1H), 9.94 (s, 1H), 9.78 (s, 1H), 9.60 (s, 1H), 9.32 (s, 1H), 9.15 (t, J = 5.4 Hz, 1H), 8.78 (s, 1H), 8.50 (s, 1H), 7.35 (d, J = 8.7 Hz, 1H), 6.99 (d, J = 8.7 Hz, 1H), 6.91 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 4.28 (d, J = 5.1 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.6, 161.9, 160.8, 157.7, 152.3, 149.0, 148.0, 144.3, 140.1, 132.1, 131.9, 126.5, 121.6, 120.4, 118.0, 113.6, 113.1, 112.8, 112.1, 111.8, 43.6. HRMS (ESI): calcd for $C_{21}H_{14}N_2O_{10}Na$ [M + Na]$^+$, 477.0546. Found: [M + Na]$^+$, 477.0543.

(*S*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxopropan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (31). Yellow solid. Mp: >280 °C. HPLC: 97.36%, t_R = 2.043 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.66 (s, 1H), 9.96 (s, 1H), 9.86 (s, 1H), 9.61 (s, 1H), 9.33 (s, 1H), 9.16 (d, J = 7.2 Hz, 1H), 8.77 (s, 1H), 8.49 (s, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 8.7 Hz, 1H), 5.03–4.86 (m, 1H), 1.42 (d, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.0, 161.1, 161.0, 157.7, 152.3, 149.0, 148.1, 144.3, 140.2, 132.1, 131.9, 127.5, 121.6, 120.2, 118.1, 113.6, 113.1, 112.7, 112.1, 111.8, 49.1, 18.9. HRMS (ESI): calcd for $C_{22}H_{16}N_2O_{10}Na$ [M + Na]$^+$, 497.0703. Found: [M + Na]$^+$, 497.0695.

(*S*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxopentan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (32). Yellow solid. Mp: >280 °C. HPLC: 98.37%, t_R = 2.807 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.65 (br s, 1H), 10.10–9.75 (br s, 1H), 9.89 (s, 1H), 9.70–9.44 (br s, 1H), 9.43–9.20 (br s, 1H), 9.10 (d, J = 7.8 Hz, 1H), 8.77 (s, 1H), 8.47 (s, 1H), 7.34 (d, J = 8.7 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 8.7 Hz, 1H), 5.02–4.93 (m, 1H), 1.90–1.65 (m, 2H), 1.45–1.30 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.5, 161.3, 161.1, 157.7, 152.3, 149.0, 148.1, 144.2, 140.3, 132.0, 131.9, 127.7, 121.6, 120.2, 118.1, 113.6, 113.1, 112.8, 112.1, 111.8, 53.0, 34.8, 18.3, 13.7. HRMS (ESI): calcd for $C_{21}H_{14}N_2O_{10}Na$ [M + Na]$^+$, 519.1016. Found: [M + Na]$^+$, 519.1012.

(*S*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-3-methyl-1-oxobutan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (33). Yellow solid. Mp: 179–183 °C. HPLC: 96.66%, t_R = 2.653 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.65 (s, 1H), 9.94 (s, 1H), 9.90 (s, 1H), 9.60 (s, 1H), 9.31 (s, 1H), 9.13 (d, J = 8.7 Hz, 1H), 8.78 (s, 1H), 8.46 (s, 1H), 7.34 (d, J = 8.7 Hz, 1H), 7.00 (d, J = 8.7 Hz, 1H), 6.91 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 4.97–4.90 (m, 1H), 2.27–2.08 (m, 1H), 1.05–0.85 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.89, 161.5, 161.2, 157.7, 152.3, 149.1, 148.1, 144.2, 140.3, 132.0, 131.9, 127.9, 121.5, 120.1, 118.1, 113.6, 113.0, 112.8, 112.1, 111.9, 57.7, 31.3, 19.2, 17.4. HRMS (ESI): calcd for $C_{21}H_{14}N_2O_{10}Na$ [M + Na]$^+$, 519.1016. Found: [M + Na]$^+$, 519.1003.

(*S*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxohexan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (34). Yellow solid. Mp: 246–250 °C. HPLC: 97.35%, t_R = 3.667 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.11 (d, J = 7.5 Hz, 1H), 8.77 (s, 1H), 8.47 (s, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 5.00–4.91 (m, 1H), 1.90–1.65 (m, 2H), 1.40–1.20 (s, 4H), 0.86 (t, J = 6.3 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.5, 161.4, 161.1, 157.7, 152.3, 149.0, 148.2, 144.3, 140.3, 132.1, 131.9, 127.7, 121.6, 120.2, 118.1, 113.6, 113.1, 112.8, 112.1, 111.9, 53.2, 40.3, 40.1, 39.8, 39.5, 39.2, 39.0, 38.7, 32.4, 27.1, 21.9, 13.8. HRMS (ESI): calcd for $C_{25}H_{22}N_2O_{10}Na$ [M + Na]$^+$, 533.1172. Found: [M + Na]$^+$, 533.1166.

(*R*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxobutan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (35). Yellow solid. Mp: >280 °C. HPLC: 98.61%, t_R = 2.294 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.66 (br s, 1H), 9.93 (br s, 1H), 9.88 (s, 1H), 9.61 (br s, 1H), 9.33 (br s, 1H), 9.13 (d, J = 7.5 Hz, 1H), 8.77 (s, 1H), 8.48 (s, 1H), 7.34 (d, J = 8.7 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 5.03–4.83 (m, 1H), 1.94–1.70 (m, 2H), 0.93 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.3, 161.4, 161.1, 157.7, 152.3, 149.0, 148.1, 144.2, 140.3, 132.0, 131.9, 127.7, 121.6, 120.1, 118.1, 113.6, 113.1, 112.8, 112.1, 111.8, 54.2, 25.9, 9.6. HRMS (ESI): calcd for $C_{23}H_{18}N_2O_{10}Na$ [M + Na]$^+$, 505.0859. Found: [M + Na]$^+$, 505.0850.

(*R*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxopentan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (36). Yellow solid. Mp: >280 °C. HPLC: 95.90%, t_R = 2.843 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.89 (s, 1H), 9.11 (d, J = 8.1 Hz, 1H), 8.77 (s, 1H), 8.47 (s, 1H), 7.33 (d, J = 8.7 Hz, 1H), 7.00 (d, J = 8.7 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H), 5.08–4.88 (m, 1H), 1.89–1.62 (m, 2H), 1.47–1.27 (m, 2H), 0.91 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6)

δ 171.5, 161.4, 161.1, 157.7, 152.3, 149.0, 148.1, 144.2, 140.3, 132.1, 131.9, 127.7, 121.6, 120.2, 118.1, 113.6, 113.1, 112.8, 112.1, 111.9, 53.0, 34.8, 18.3, 13.7. HRMS (ESI): calcd for $C_{21}H_{14}N_2O_{10}Na$ [M + Na]$^+$, 519.1016. Found: [M + Na]$^+$, 519.1011.

(*R*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxohexan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (37). Yellow solid. Mp: 252–255 °C. HPLC: 96.79%, t_R = 3.660 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.66 (br s, 1H), 10.30–9.25 (br s, 3H), 9.89 (s, 1H), 9.11 (d, *J* = 7.8 Hz, 1H), 8.77 (s, 1H), 8.47 (s, 1H), 7.34 (d, *J* = 8.7 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 4.99–4.93 (m, 1H), 1.90–1.65 (m, 2H), 1.33 (s, 4H), 0.86 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.5, 161.3, 161.1, 157.7, 152.3, 149.0, 148.1, 144.2, 140.3, 132.0, 131.9, 127.7, 121.5, 120.2, 118.1, 113.6, 113.1, 112.8, 112.1, 111.8, 53.2, 32.4, 27.1, 21.9, 13.8. HRMS (ESI): calcd for $C_{25}H_{22}N_2O_{10}Na$ [M + Na]$^+$, 533.1172. Found: [M + Na]$^+$, 533.1166.

(*R*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (38). Yellow solid. Mp: 174–178 °C. HPLC: 98.99%, t_R = 3.519 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.65 (s, 1H), 9.90 (s, 2H), 9.58 (br s, 1H), 9.30 (br s, 1H), 9.04 (d, *J* = 7.8 Hz, 1H), 8.77 (s, 1H), 8.46 (s, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.00 (d, *J* = 8.7 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 5.04–4.94 (m, 1H), 1.74–1.60 (m, 3H), 0.94 (d, *J* = 5.1 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.8, 161.4, 161.0, 157.7, 152.3, 149.1, 148.1, 144.2, 140.3, 132.0, 131.9, 127.7, 121.6, 120.2, 118.1, 113.6, 113.0, 112.7, 112.1, 111.8, 52.0, 41.4, 24.5, 23.1, 21.8. HRMS (ESI): calcd for $C_{25}H_{22}N_2O_{10}Na$ [M + Na]$^+$, 533.1172. Found: [M + Na]$^+$, 533.1165.

***N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxoheptan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (39).** Grey solid. Mp: 266–267 °C. HPLC: 98.06%, t_R = 5.512 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.64 (br s, 1H), 9.93 (br s, 1H), 9.87 (s, 1H), 9.59 (br s, 1H), 9.30 (br s, 1H), 9.11 (d, *J* = 7.8 Hz, 1H), 8.77 (s, 1H), 8.47 (s, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 4.99–4.90 (m, 1H), 1.90–1.65 (m, 2H), 1.40–1.20 (m, 6H), 0.85 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.6, 163.1, 162.2, 159.0, 153.2, 150.3, 149.0, 145.0, 141.2, 132.8, 132.7, 129.8, 122.9, 120.8, 119.4, 114.7, 114.1, 113.3, 113.0, 112.7, 54.6, 33.0, 31.6, 25.3, 22.7, 14.6. HRMS (ESI): calcd for $C_{26}H_{24}N_2O_{10}Na$ [M + Na]$^+$, 547.1329. Found: [M + Na]$^+$, 547.1323.

***N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxooctan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (40).** Grey solid. Mp: 252–254 °C. HPLC: 98.68%, t_R = 7.652 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.64 (s, 1H), 9.94 (s, 1H), 9.88 (s, 1H), 9.59 (s, 1H), 9.32 (s, 1H), 9.11 (d, *J* = 8.1 Hz, 1H), 8.77 (s, 1H), 8.47 (s, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.00 (d, *J* = 8.1 Hz, 1H), 6.91 (d, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 4.98–4.90 (m, 1H), 1.90–1.65 (m, 2H), 1.40–1.15 (m, 8H), 0.84 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.5, 161.3, 161.1, 157.7, 152.3, 149.0, 148.1, 144.2, 140.3, 132.0, 131.9, 127.6, 121.6, 120.1, 118.1, 113.6, 113.0, 112.8, 112.1, 111.8, 53.2, 32.7, 31.0, 28.3, 24.8, 21.9, 13.8. HRMS (ESI): calcd for $C_{27}H_{26}N_2O_{10}Na$ [M + Na]$^+$, 561.1485. Found: [M + Na]$^+$, 561.1480.

***N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-2-methyl-1-oxopropan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (41).** Yellow solid. Mp: 273–276 °C. HPLC: 95.24%, t_R = 2.228 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.70 (br s, 1H), 9.97 (br s, 1H), 9.61 (br s, 1H), 9.33 (br s, 1H), 9.12 (s, 1H), 9.00 (s, 1H), 8.71 (s, 1H), 8.34 (s, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 1.60 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.0, 162.4, 161.7, 158.9, 152.9, 149.5, 148.7, 144.7, 140.8, 132.6, 132.5, 128.5, 122.3, 120.7, 118.9, 114.3, 113.8, 113.7, 112.7, 112.4, 57.8, 25.3. HRMS (ESI): calcd for $C_{23}H_{18}N_2O_{10}Na$ [M + Na]$^+$, 505.0859. Found: [M + Na]$^+$, 505.0855.

***N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)carbamoyl)cyclohexyl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (42).** Yellow solid. Mp: 170–172 °C. HPLC: 95.13%, t_R = 3.883 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.73 (br s, 1H), 9.94 (br s, 1H), 9.61 (br s, 1H), 9.31 (br s, 1H), 9.09 (s, 1H), 9.03 (s, 1H), 8.72 (s, 1H), 8.38 (s, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 9.0 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 2.28–2.18 (m, 2H), 1.88–1.74 (m, 2H), 1.72–1.56 (m, 3H), 1.54–1.36 (m, 2H), 1.35–1.20 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.0, 161.5, 161.4, 158.0, 152.4, 148.9, 148.0, 144.2, 140.0, 132.1, 131.9, 126.4, 121.6, 120.3, 118.0, 113.8, 113.2 (two carbons), 112.1, 111.8, 59.9, 31.28 (two carbons), 24.71, 20.92 (two carbons). HRMS (ESI): calcd for $C_{26}H_{22}N_2O_{10}Na$ [M + Na]$^+$, 545.1172. Found: [M + Na]$^+$, 545.1166.

(*S*)-*N*-(1-Cyclohexyl-2-((7,8-dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-2-oxoethyl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (43). Yellow solid. Mp: 190–203 °C. HPLC: 97.83%, t_R = 2.992 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.91 (s, 1H), 9.13 (d, *J* = 8.4 Hz, 1H), 8.77 (s, 1H), 8.47 (s, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.00 (d, *J* = 8.7 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 5.02–4.85 (m, 1H), 1.89–1.55 (m, 6H), 1.26–1.00 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.8, 161.4, 161.2, 157.7, 152.4, 149.1, 148.1, 144.2, 140.3, 132.0, 131.9, 127.8, 121.6, 120.1, 118.1, 113.6, 113.0, 112.7, 112.1, 111.8, 57.3, 41.0, 29.2, 27.8, 25.7, 25.6 (two carbons). HRMS (ESI): calcd for $C_{27}H_{24}N_2O_{10}Na$ [M + Na]$^+$, 559.1329. Found: [M + Na]$^+$, 559.1325.

(*S*)-*N*-(3-Cyclohexyl-1-((7,8-dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxopropan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (44). Yellow solid. Mp: 151–154 °C. HPLC: 97.17%, t_R = 7.583 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.89 (s, 1H), 9.05 (d, *J* = 7.5 Hz, 1H), 8.77 (s, 1H), 8.45 (s, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.00 (d, *J* = 9.0 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 5.05–4.95 (m, 1H), 1.85–1.55 (m, 6H), 1.45–1.35 (m, 1H), 1.24–1.04 (m, 4H), 1.02–0.84 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.9, 161.4, 161.1, 157.7, 152.3, 149.1, 148.1, 144.2, 140.3, 132.0, 131.9, 127.7, 121.6, 120.2, 118.1, 113.6, 113.0, 112.7, 112.1, 111.8, 51.5, 33.8, 33.2, 32.0, 25.9, 25.7, 25.6. HRMS (ESI): calcd for $C_{28}H_{26}N_2O_{10}Na$ [M + Na]$^+$, 573.1485. Found: [M + Na]$^+$, 573.1476.

(*S*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (45). Orange solid. Mp: 225–228 °C. HPLC: 95.21%, t_R = 3.219 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.65 (br s, 1H), 9.99 (s, 1H), 9.09 (d, *J* = 7.8 Hz, 1H), 8.71 (s, 1H), 8.46 (s, 1H), 7.35–7.15 (m, 6H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 5.25–5.16 (m, 1H), 3.22 (dd, *J* = 13.8, 4.8 Hz, 1H), 3.03 (dd, *J* = 13.5, 8.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.6, 161.3, 160.9, 157.7, 152.4, 149.1, 148.1, 144.2, 140.2, 136.8, 132.1, 131.9, 129.3 (two carbons), 128.1 (two carbons), 127.3, 126.5, 121.6, 120.2, 118.1, 113.7, 113.1, 112.4, 112.1, 111.8, 54.6, 38.0. HRMS (ESI): calcd for $C_{28}H_{20}N_2O_{10}Na$ [M + Na]$^+$, 567.1016. Found: [M + Na]$^+$, 567.1005.

(*S*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-4-(methylthio)-1-oxobutan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (46). Yellow solid. Mp: 174–176 °C. HPLC: 95.60%, t_R = 2.580 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.60 (br s, 1H), 10.30–9.25 (br s, 3H), 9.88 (s, 1H), 9.18 (d, *J* = 7.8 Hz, 1H), 8.76 (s, 1H), 8.47 (s, 1H), 7.34 (d, *J* = 9.0 Hz, 1H), 7.01 (d, *J* = 9.0 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 5.05–4.95 (m, 1H), 2.58–2.50 (m, 2H), 2.21–2.10 (m, 1H), 2.06 (s, 3H), 2.04–1.93 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7, 161.6, 161.0, 157.7, 152.3, 149.0, 148.2, 144.2, 140.3, 132.1, 131.9, 127.8, 121.6, 120.1, 118.1, 113.6, 113.1, 112.8, 112.1, 111.8, 52.8, 32.6, 29.3, 14.7. HRMS (ESI): calcd for $C_{24}H_{20}N_2O_{10}SNa$ [M + Na]$^+$, 551.0736. Found: [M + Na]$^+$, 551.0732.

(*S*)-*tert*-Butyl 4-((7,8-dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-3-(7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamido)-4-oxobutanoate (47). Yellow solid. Mp: 255–258 °C. HPLC: 95.80%, t_R = 3.192 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.63 (s, 1H), 9.38 (d, *J* = 7.5 Hz, 1H), 8.81 (s, 1H), 8.46 (s, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 1H), 6.81 (d, *J* = 8.9 Hz, 1H), 5.15–5.06 (m, 2H), 2.84 (d, *J* = 5.7 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.6, 169.2, 161.8, 160.9, 157.7, 152.6, 149.4, 148.2, 144.3, 140.2, 132.1, 131.9, 126.8, 121.8, 120.2, 118.2, 113.7, 113.2, 112.4, 112.1, 111.8, 80.7, 50.5, 37.5, 27.6. HRMS (ESI): calcd for $C_{27}H_{24}N_2O_{10}Na$ [M + Na]$^+$, 591.1227. Found: [M + Na]$^+$, 591.1222.

(*S*)-4-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-3-(7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamido)-4-oxobutanoic acid (48). Compound **47** (40 mg, 0.0704 mmol) was dissolved in 4 mL formic acid and stirred at rt for 6 h. The solvent was evaporated and the residue was purified by column chromatography (DCM–MeOH–AcOH = 10 : 1 : 1) to give **48** as a yellow solid (13 mg, 36%). Mp: >280 °C. HPLC: 95.57%, t_R = 2.364 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.70 (s, 1H), 9.40 (d, *J* = 7.5 Hz, 1H), 8.72 (s, 1H), 8.47 (s, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 2H), 5.08–5.02 (m, 1H), 2.84 (d, *J* = 6.0 Hz, 2H). HRMS (ESI): calcd for $C_{23}H_{16}N_2O_{12}Na$ [M + Na]$^+$, 535.0601. Found: [M + Na]$^+$, 535.0598.

(*S*)-*N*^{1}-(7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)-2-(7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamido)-*N*^{4}-tritylsuccinamide (49a). White solid. ^{1}H NMR (300 MHz, CD_3OD + $CDCl_3$) δ 8.72 (s, 1H), 8.48 (s, 1H), 7.27–7.05 (m, 16H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 5.13 (t, *J* = 6.3 Hz, 1H), 3.08–3.00 (m, 2H).

(*S*)-*N*^{1}-(7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)-2-(7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamido)succinamide (49). To a solution of compound **49a** (46 mg, 0.0704 mmol) in 3 mL DCM was added 2 mL TFA. The mixture was stirred at rt for 2 h and the yellow solid was isolated by filtration to afford **49** (24 mg, 77%). Mp: 273–274 °C. HPLC: 95.92%, t_R = 2.004 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.67 (br s, 1H), 9.92 (br s, 1H), 9.64 (s, 2H), 9.43 (d, *J* = 7.5 Hz, 1H), 9.32 (br s, 1H), 8.80 (s, 1H), 8.48 (s, 1H), 7.52 (s, 1H), 7.35 (d, *J* = 8.7 Hz, 1H), 7.04 (s, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.81 (d, *J* = 8.7 Hz, 1H), 5.08–4.99 (m, 1H), 2.73 (d, *J* = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.6, 170.1, 161.7, 160.8, 157.7, 152.4, 149.2, 148.0, 144.3, 140.0, 132.1, 131.9, 126.0, 121.7, 120.3, 118.0, 113.6, 113.1, 112.5, 112.1, 111.8, 50.6, 37.0. HRMS (ESI): calcd for $C_{23}H_{17}N_3O_{11}Na$ [M + Na]$^+$, 534.0761. Found: [M + Na]$^+$, 534.0765.

(*S*)-*tert*-Butyl (5-((7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromen-3-yl)amino)-4-(7,8-bis(methoxymethoxy)-2-oxo-2*H*-chromene-3-carboxamido)-5-oxopentyl)carbamate (50a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.24 (d, *J* = 7.2 Hz, 1H), 8.91 (s, 1H), 8.87 (s, 1H), 8.63 (s, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 7.21 (d, *J* = 8.7 Hz, 1H), 7.16 (d, *J* = 8.7 Hz, 1H), 7.11 (d, *J* = 8.7 Hz, 1H), 5.32 (s, 2H), 5.25 (s, 4H), 5.22 (s, 2H), 4.85–4.75 (m, 1H), 4.65 (br s, 1H), 3.71 (s, 3H), 3.67 (s, 3H), 3.52 (s, 3H), 3.50 (s, 3H), 3.28–3.13 (m, 2H), 2.20–2.05 (m, 1H), 1.97–1.82 (m, 1H), 1.68 (d, *J* = 7.2 Hz, 2H), 1.43 (s, 9H).

(*S*)-*N*-(5-Amino-1-((7,8-dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-1-oxopentan-2-yl)-7,8-dihydroxy-2-oxo-2*H*-chromene-3-carboxamide (50). To a solution of compound **50a** (28 mg, 0.0458 mmol) in 2 mL DCM was added 2 mL of 2 M HCl in EtOAc. The mixture was stirred at rt for 2 h and the solvent was evaporated to afford **50** as a yellow solid (18 mg, 72%). Mp: 233–235 °C. HPLC: 95.81%, t_R = 1.520 min. ^{1}H NMR (300 MHz, CD_3OD) δ 9.54 (d, *J* = 7.5 Hz, 1H), 8.78 (s, 1H), 8.51 (s, 1H), 7.23 (d, *J* = 9.0 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 4.96–4.89 (m, 1H), 3.03 (t, *J* = 6.9 Hz, 2H), 2.18–2.06 (m, 1H), 2.04–1.91 (m, 1H), 1.90–1.78 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 172.4, 164.8, 163.2, 160.0, 154.1, 151.1, 149.8, 145.8, 141.8, 133.6, 133.5, 129.6, 123.1, 121.5, 119.8, 115.0, 114.4, 113.9, 113.7, 113.6, 54.9, 40.6, 30.7, 25.0. HRMS (ESI): calcd for $C_{24}H_{22}N_3O_{10}$ [M + H]$^+$, 512.1305. Found: [M + H]$^+$, 512.1293.

(*R*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-7,8-dimethoxy-2-oxo-2*H*-chromene-3-carboxamide (51). Pale solid. Mp: 199–200 °C. HPLC: 95.70%, t_R = 6.199 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.95 (s, 1H), 9.93 (s, 1H), 9.32 (s, 1H), 9.01 (d, *J* = 8.1 Hz, 1H), 8.84 (s, 1H), 8.46 (s, 1H), 7.75 (d, *J* = 9.3 Hz, 1H), 7.24 (d, *J* = 8.7 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 5.05–4.96 (m, 1H), 3.96 (s, 3H), 3.86 (s, 3H), 1.75–1.60 (s, 3H), 0.94 (d, *J* = 4.8 Hz, 6H). ^{13}C NMR (75 MHz, DMSO) δ 171.8, 161.1, 160.6, 157.7, 157.2, 148.5, 148.1, 147.8, 140.3, 134.8, 132.0, 127.8, 126.1, 120.2, 118.1, 114.6, 113.1, 113.0, 112.1, 110.3, 60.8, 56.6, 52.0, 41.3, 24.5, 23.2, 21.8. HRMS (ESI): calcd for $C_{27}H_{26}N_2O_{10}Na$ $[M + Na]^+$, 561.1485. Found: $[M + Na]^+$, 561.1476.

(*R*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-7-hydroxy-2-oxo-2*H*-chromene-3-carboxamide (52). Pale solid. Mp: 192–193 °C. HPLC: 97.67%, t_R = 5.108 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.14 (s, 1H), 10.00 (s, 1H), 9.90 (s, 1H), 9.33 (s, 1H), 9.01 (d, J = 7.8 Hz, 1H), 8.80 (s, 1H), 8.44 (s, 1H), 7.82 (d, J = 8.4 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.82–6.79 (m, 2H), 5.02–4.92 (m, 1H), 1.75–1.62 (m, 3H), 0.93 (d, J = 4.8 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.9, 163.9, 161.4, 161.2, 157.8, 156.4, 148.6, 148.2, 140.3, 132.2, 132.1, 127.9, 120.2, 118.2, 114.5, 113.1 (two carbons), 112.1, 111.2, 101.9, 52.0, 41.4, 24.6, 23.2, 21.8. HRMS (ESI): calcd for $C_{25}H_{22}N_2O_9Na$ $[M + Na]^+$, 517.1223. Found: $[M + Na]^+$, 517.1215.

(*R*)-*N*-(1-((7,8-Dihydroxy-2-oxo-2*H*-chromen-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-8-hydroxy-2-oxo-2*H*-chromene-3-carboxamide (53). Pale solid. Mp: 179–182 °C. HPLC: 97.89%, t_R = 4.291 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.45 (s, 1H), 9.95 (s, 2H), 9.33 (s, 1H), 9.09 (d, J = 7.8 Hz, 1H), 8.84 (s, 1H), 8.46 (s, 1H), 7.43–7.36 (m, 1H), 7.25–7.21 (m, 2H), 7.00 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 5.06–4.96 (m, 1H), 1.73–1.62 (m, 3H), 0.95 (d, J = 4.2 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.7, 160.9, 160.4, 157.7, 148.4, 148.2, 144.4, 142.6, 140.3, 132.0, 127.8, 125.1, 120.3, 120.2 (two carbons), 118.1, 113.0, 112.1, 52.0, 41.4, 24.52, 23.1, 21.8. HRMS (ESI): calcd for $C_{25}H_{22}N_2O_9Na$ $[M + Na]^+$, 517.1223. Found: $[M + Na]^+$, 517.1217.

(*R*)-7-Hydroxy-*N*-(1-((7-hydroxy-2-oxo-2*H*-chromen-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-2-oxo-2*H*-chromene-3-carboxamide (54). Beige solid. Mp: 175–176 °C. HPLC: 96.22%, t_R = 6.802 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.10 (s, 1H), 10.39 (s, 1H), 9.92 (s, 1H), 9.01 (d, J = 7.9 Hz, 1H), 8.81 (s, 1H), 8.50 (s, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 6.89 (dd, J = 8.4 Hz, 1.2 Hz, 1H), 6.85–6.70 (m, 3H), 5.03–4.98 (m, 1H), 1.68–1.66 (m, 3H), 0.94 (d, J = 5.1 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.8, 163.8, 161.3, 161.1, 159.8, 157.8, 156.4, 151.7, 148.5, 132.1, 129.1, 127.2, 120.4, 114.4, 113.6, 113.0, 111.2, 111.1, 101.9, 101.8, 52.0, 41.4, 24.5, 23.1, 21.8. HRMS (ESI): calcd for $C_{25}H_{22}N_2O_8Na$ $[M + Na]^+$, 501.1274. Found: $[M + Na]^+$, 501.1264.

(*R*)-8-Hydroxy-*N*-(1-((7-hydroxy-2-oxo-2*H*-chromen-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-2-oxo-2*H*-chromene-3-carboxamide (55). Pale solid. Mp: 158–160 °C. HPLC: 98.90%, t_R = 5.604 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.45 (s, 1H), 10.38 (s, 1H), 9.96 (s, 1H), 9.09 (d, J = 8.1 Hz, 1H), 8.84 (s, 1H), 8.50 (s, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.44–7.35 (m, 1H), 7.26–7.22 (m, 2H), 6.79 (d, J = 8.4 Hz, 1H), 6.74 (s, 1H), 5.06–4.96 (m, 1H), 1.74–1.62 (m, 3H), 0.94 (d, J = 4.5 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.7, 160.9, 160.4, 159.8, 157.8, 151.8, 148.4, 144.4, 142.6, 129.1, 127.3, 125.1, 120.4, 120.3, 120.1, 119.3, 118.1, 113.6, 111.2, 101.9, 52.0, 41.4, 24.5, 23.1, 21.8. HRMS (ESI): calcd for $C_{25}H_{22}N_2O_8Na$ $[M + Na]^+$, 501.1274. Found: $[M + Na]^+$, 501.1270.

ELISA kinase assay

Met tyrosine kinase activity was evaluated according to the following procedure: briefly, in enzyme-linked-immunosorbent assay (ELISA), 20 μg mL^{-1} poly (Glu,Tyr)$_{4:1}$ (Sigma) was precoated as a substrate in 96-well plates. 50 μL of 10 μmol L^{-1} ATP solution diluted in kinase reaction buffer (50 mmol L^{-1} HEPES pH 7.4, 50 mmol L^{-1} $MgCl_2$, 0.5 mmol L^{-1} $MnCl_2$, 0.2 mmol L^{-1} Na_3VO_4, 1 mmol L^{-1} DTT) was added to each well. 1 μL of various concentrations of indicated compounds diluted in 1% DMSO (v/v) (Sigma) was added to each reaction well. 1% DMSO (v/v) was used as a negative control. The kinase reaction initiated after the addition of purified tyrosine kinase proteins diluted in 49 μL of kinase reaction buffer solution. After incubation for 60 min at 37 °C, the plate was washed three times with phosphate buffered saline (PBS) containing 0.1% Tween 20 (T-PBS). 100 μL anti-phosphotyrosine (PY99) antibody (1 : 500 diluted in 5 mg mL^{-1} BSA T-PBS) was then added. After 30 min incubation at 37 °C, the plate was washed three times. 100 μL horseradish peroxidase-conjugated goat anti-mouse IgG (1 : 2000 diluted in 5 mg mL^{-1} BSA T-PBS) was added. The plate was then incubated at 37 °C for 30 min, and washed 3 times. 100 μL of a solution containing 0.03% H_2O_2 and 2 mg ml^{-1} *o*-phenylenediamine in 0.1 mol L^{-1} citrate buffer, pH 5.5, was added. The reaction was terminated by the addition of 50 μL of 2 mol L^{-1} H_2SO_4 as the color changed, and the plate was read using a multi-well spectrophotometer (SpectraMAX 190, Molecular Devices) at 490 nm. The inhibition rate (%) was calculated using the following equation: $[1 - (A_{490}/A_{490}\ \text{control})] \times 100\%$. IC_{50} values were calculated from the inhibition curves from two separate experiments. For ATP competition assay, various concentrations of ATP were diluted for the kinase reaction.

Western blot analysis

EBC-1 and BaF3/TPR-Met cells were treated with indicated compounds for 4 h at 37 °C and then lysed in 1× SDS sample buffer. The cell lysates were subsequently resolved on 10% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were probed with appropriate primary antibodies (c-Met [Santa Cruz] and phospho-c-Met [Cell Signaling Technology], and GAPDH [KangChen Biotech] antibody), and then subsequently with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG. Immunoreactive proteins were detected using enhanced chemiluminescence detection reagent (Thermo Fisher).

Abbreviations

NSCLC	Non-small cell lung cancer
EGFR	Epidermal growth factor receptor
PKA	Protein kinase A
PKC	Protein kinase C
SAR	Structure–activity relationship
Leu	Leucine
DMF	*N*,*N*-Dimethylformamide

DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
BOC	*t*-Butyloxycarbonyl
EDCI	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
Fmoc	Fluorenylmethoxycarbonyl
MOM	Methoxymethyl

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