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ARTICLE

Design and Synthesis of 3,3'-biscoumarin-based c-Met inhibitors

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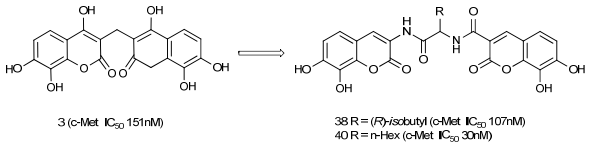
A library of biscoumarin-based c-Met inhibitors was synthesized, based on optimization of 3,3'-biscoumarin hit **3**, which was identified as a non-ATP competitive inhibitor of c-Met from a diverse library of coumarin derivatives. Among these compounds, **38** and **40** not only showed potent enzyme activities with IC_{50} 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.

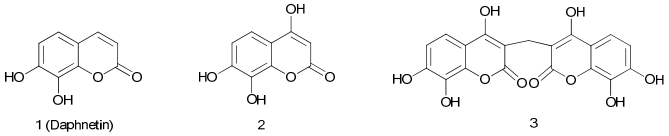


Fig. 1 Daphnetin derivatives

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 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, had 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. 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 IC50 values of **3** remained unchanged with increasing ATP concentration. This suggests

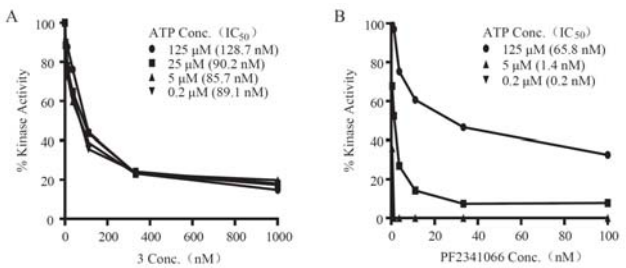


Fig. 2 Compound **3** is an ATP non-competitive inhibitor of Met kinase activity. Inhibition assays with recombinant c-Met protein and different concentrations of **3** (A) or ATP-competitive PF2341066 (B) were performed in the presence of various concentrations of ATP.

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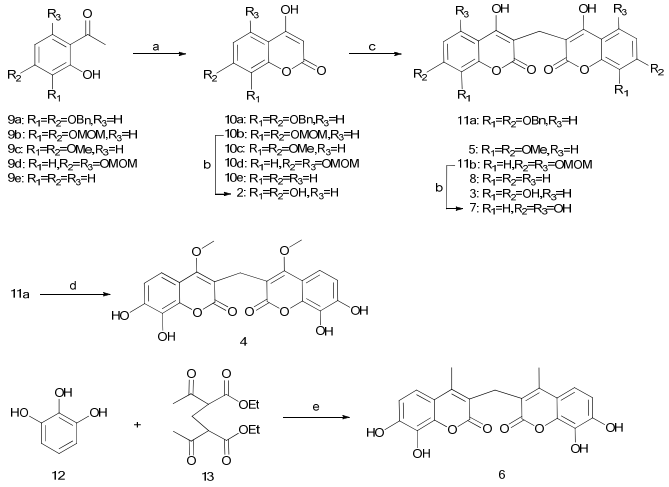
that **3** is an ATP non-competitive inhibitor of c-Met (Figure 2). These initial results encouraged us to pursue a medicinal chemistry program to further optimize **3** as a novel c-Met inhibitor.

Results and discussion



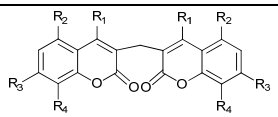
Scheme 1. Synthesis of biscoumarin compounds **3**-**8**. *Reagents and conditions*: (a) $CO(OEt)_2$, NaH, Toluene, 80℃; (b) HCl/AcOEt; (c) Paraformaldehyde, Et_3N, EtOH; (d) i. CH_2N_2, Et_2O; ii. $Pd(OH)_2$, H_2, THF; (e) 70% H_2SO_4.

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

Compound **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 complete loss of potency (**8** and **5**). Moving the phenolic hydroxyl groups from the 8,8'-position to the 5,5'-positon (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 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

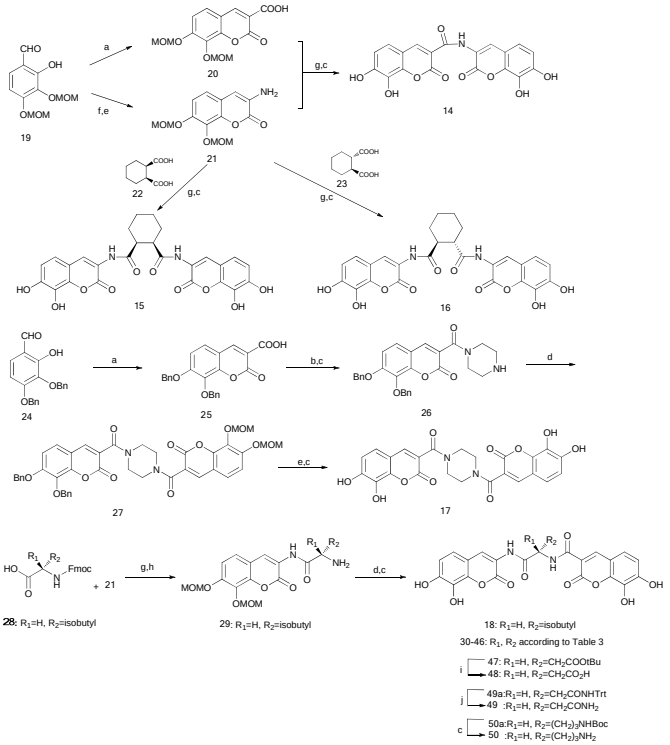
Table 1 c-Met Enzymatic Activity of compounds **3-8**



Compound	R_1	R_2	R_3	R_4	IC_{50} (nM)[a]
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 Logit method from the results of at least two independent tests with eight concentrations each and expressed as means ± SD.

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 conditions, by condensation of **21** with 0.5 equiv of 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



Scheme 2. Synthesis of biscoumarin compounds **14-18** with modified linkers. *Reagents and conditions*: (a) isopropylidene malonate, piperidinium acetate, EtOH, 60℃; (b) 1-Boc-piperazine, EDCI, DMAP, DCM; (c) HCl/AcOEt; (d) 21, 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.

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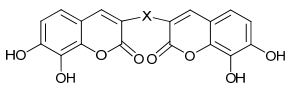
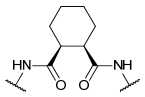
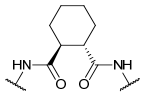
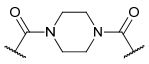
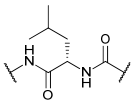
to compound **17** via hydrogenolytic cleavage and acid deprotection. Fmoc-Leu-OH **28** was also condensed with **21** to give an intermediate which was transformed to **29** via piperidine deprotection. The coupling reaction between **29** and **20** was followed by acid deprotection to give compound **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.

Table 2 c-Met Enzymatic Activity of compounds **14-18**



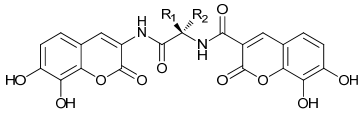
Compound	X	IC_{50} (nM)[a]
14		620.8±70.9
15		168.7±18.6
16		134.1±6.3
17		1370.9±208.5
18		122.0±20.3

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

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.

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),

Table 3 c-Met Enzymatic Activity of compounds **30-50**



Compound	R_1	R_2	IC_{50} (nM)[a]
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 Logit method from the results of at least two independent tests with eight concentrations each and expressed as means ± SD.

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 to **34**. The ester analogue (**47**, IC_{50} = 15 nM) offered potentinhibitory activity, presenting 8-fold higher potency than the corresponding acid (**48**, IC_{50} = 119 nM). For nitrogen-bearing substituents, the amide analogue (**49**) retained

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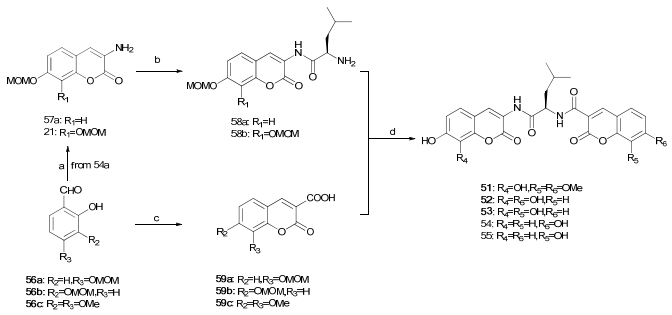
inhibitory potency (IC_{50} = 63 nM) relative to acid **48**. The amine analogue (**50**), however, showed 65-fold lower potency (IC_{50}=4.8 μM) than **34.**

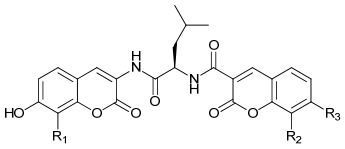
Using D-leucine as a linker, the effect of modifying the hydroxyl groups on the two coumarin rings was explored. Compounds **51-55** were synthesized according to the procedures outlined in Scheme 3. Known compounds **56a-c** were transformed into coumarin acids **59a-c** and aminocoumarins **21** and **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**.



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℃; (d) i. EDCI, DMAP, DCM; ii. HCl/AcOEt.

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.

Table 4 c-Met Enzymatic Activity of compounds **51-55**



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

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

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 Figure 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).

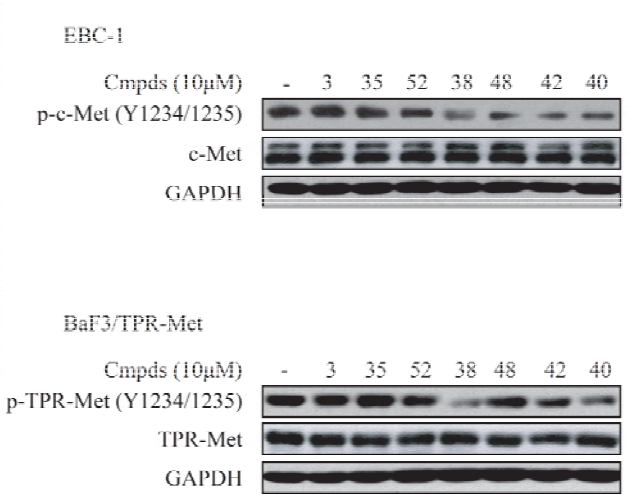


Fig. 3 The effect of selected compounds on c-Met phosphorylation in EBC-1 and BaF3/TPR-Met cells.

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 through 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

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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, ^{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 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 H2O and B = CH3OH.

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 (15.6g, 100%). Magnesium bromide etherate (5.3g, 20.5mmol) was added portionwise to a solution of 1-(2,3,4-tris(benzyloxy)phenyl)ethanone (9.0g, 20.5mmol) 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 (100mL). 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** (5.3g, 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 (2.3g, 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 (2.15g 12.8mmol) and DIPEA in 50mL DCM at 0 °C was added MOMCl (2.14mL, 28.13mmol) dropwise. After stirring for 2 h at 0 °C, the mixture was diluted with 100mL DCM and washed with 10% aqueous citric acid (2×30mL) 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 beige solid (2.1g, 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** (5.30g, 15mmol) and diethyl carbonate (3.54g, 30mmol) in 80mL toluene was added NaH (2.40g, 60% in oil, 60mmol) at 0 °C. The mixture was stirred at 80 °C for 2 h. The solution was then cooled to rt and 30mL 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** (4.6g, 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 (1.0g, 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 (100mg, 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 (2.0g, 7.1mmol) was dissolved in 20mL 3N HCl/EtOAc and 0.5mL MeOH. The reaction mixture was stirred at room temperature for 4 h. The white solid was isolated by filtration to afford **3** (1.3g, 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** (500mg, 2.58mmol) and paraformaldehyde (50mg,0.56mmol) in 10mL EtOH was added Et_3N (0.5mL). 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** (460mg, 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**[28] and **8**[30] were prepared utilizing the same synthetic route as compound **3** starting from **10a,c-e**.

Organic & Biomolecular Chemistry Accepted Manuscript

3, 3'-Methylenebis(7,8-bis(benzyloxy)-4-hydroxy-2*H*-chromen-2-one) (11a). White solid (80mg, 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 (54mg, 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** (50mg, 0.0867mmol) was dissolved in 3mL 2N HCl/EtOAc and stirred at room temperature for 1 h. The reaction mixture was evaporated to afford **7** as brown solid (27mg, 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 (100mg, 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 (400mg, 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, 132.1, 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** (90mg, 0.118mmol) in 6mL THF was added CH_2N_2 in Et_2O (1M, 3.4eq, 0.4mL) at 0 °C. After stirring at 0 °C for 2 h, the reaction was quenched with 0.1mL 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) (45mg, 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) (40mg, 0.0507mmol) and $Pd(OH)_2$ (5mg) in 10mL THF was placed under a hydrogen atmosphere (H_2, 1atm) and stirred for 2h. 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 (12mg, 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 (412μL, 4.0mmol) and CH_2Br_2 (2.1mL, 30.0mmol) was heated to 50 °C for 1.5 h and then cooled to rt. The mixture was added to a solution of ethyl acetoacetate (258mg, 2.0mmol) in 8mL of CH_2Cl_2 and stirred at rt. Upon completion (2 h), the reation mixture was concentrated, and the crude mixture was purified by column chromatography on silica gel (EtOAc/PE) to give the desired product **13** as colorless oil (123mg, 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-*2H*-chromen-2-one) (6). To a mixture of pyrogallol **12** (500mg, 4mmol) and diethyl 2,4-diacetylpentanedioate **13** (544mg, 2mmol) at 0 °C was added 70% H_2SO_4. The reaction mixture was stirred at room temperature for 30 min, then poured into water (50mL). The tan solid was isolated by filtration and dried under vacuum to afford **6** (50mg, 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 (2.4g, 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**[25] and **56a-b**[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 (2.26g, 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 (4.0g, 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 (300mg, 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 (2.1g, 13.6mmol) and K_2CO_3 (6.6g, 47.7mmol) in 50mL acetone was added CH_3I (3.0mL, 47.7mmol). The reaction mixture was stirred at 60 °C for 24 h, and then cooled to rt. The residue was diluted with 100mL EtOAc and 50mL 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 (2.6g, 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 (546mg, 2.78mmol) in 20mL benzene was added anhydrous $AlCl_3$ (408mg, 3.06mmol). After stirring for 5 min at room temperature, the mixture was heated to 80 °C for 6 h, and then cooled to rt. 30mL Ice water and 3mL 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 (398mg, 78.6%). ^{1}H NMR (300 MHz,

Organic & Biomolecular Chemistry Accepted Manuscript

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$CDCl_3$) 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** (2mmol), isopropylidene malonate (2.4mmol) and piperidinium acetate (0.1mmol) in 30mL 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 5mL 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 (780mg, 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 (550mg, 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 (145mg, 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 (237mg, 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 (367mg, 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** (2.26g, 8.80mmol) and ethyl nitroacetate (1.40g, 10.6mmol) in 60mL dry benzene was added piperidine (174μL, 1.76mmol). 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 (1.90g, 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 (350mg, 1.07mmol) and 10% Pd/C (14mg) in 15mL EtOAc was stirred under hydrogen (H_2, 1atm) 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 yellow solid (190mg, 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** (55mg, 0.177mmol), aminocoumarin **21** (50mg, 0.177mmol) and EDCI (52mg, 0.267mmol) 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-2H-chromen-3-yl)-7,8-bis(methoxymethoxy)-2-oxo-2H-chromene-3-carboxamide (17mg, 17%). Mp: >280°C. HPLC: 95.35%, t_R=2.868 min. ^{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 (17mg, 0.0296mmol) was dissolved in 3mL 2M HCl in EtOAc and stirred at rt for 2h. The yellow solid was isolated by filtration to afford **14** (10mg, 85%). Mp: >280°C. ^{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** (26mg, 0.151mmol), aminocoumarin **21** (85mg, 0.302mmol) and EDCI (87mg, 0.453mmol) 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 (25mg, 23.7%). Mp: 284-285°C. HPLC: 95.07%, t_R=2.648 min. ^{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 (25mg, 0.0358mmol) was dissolved in 3mL of 2M HCl in EtOAc and stirred at rt for 2h. The mixture was concentrated and purified by flash chromatography (CH_2Cl_2/ CH_3OH = 20:1) to provide **16** as a yellow solid (14mg, 75%). ^{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 (3mg, 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** (100mg, 0.249mmol), 1-Boc-piperazine (46mg, 0.249mmol), EDCI (72mg, 0.373mmol) and DMAP (6mg, 0.049mmol) in 10mL 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 (130mg, 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 3mL of 2M HCl in EtOAc at 0 °C and then warmed to rt. After stirring at room

Organic & Biomolecular Chemistry Accepted Manuscript

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 (0.228mmol), **24** (85mg, 0.274mmol), EDCI (66mg, 0.342mmol) and DMAP (5mg, 0.041mmol) in 10mL CH_2Cl_2 was added Et_3N (95μL, 0.684mmol). The reaction mixture was stirred at room temperature overnight, then concentrated and purified by flash chromatography column (PE-Acetone) give **27** (70mg, 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(40mg, 0.0524mmol) and 10% Pd/C (5mg) in 2mL CH_3OH and 2mL EtOAc was hydrogenated (H_2, 1atm) 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 (27mg, 90%). This intermediate was dissolved in 2mL DCM and 3 drops of MeOH, then 2mL of 2M 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** (21mg, 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** (227mg, 0.641mmol), aminocoumarin **21** (120mg, 0.427mmol) and EDCI (164mg, 0.854mmol) 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 (190mg, 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 (190mg, 0.308mmol) in 10mL acetonitrile was added piperidine (31μL, 0.308mmol). 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 (85mg, 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** (85mg, 0.216mmol), **24** (80mg, 0.259mmol), EDCI (62mg, 0.324mmol) and DMAP (6mg, 0.049mmol) were dissolved in 10mL 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 (120mg, 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 (140mg, 0.175mmol) was dissolved in 2mL DCM and 2mL of 2M HCl in EtOAc, and stirred at rt for 2 h. The yellow solid was isolated by filtration to afford **18** (96mg, 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 (780mg, 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),

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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 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,

Organic & Biomolecular Chemistry Accepted Manuscript

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** (40mg, 0.0704mmol) was dissolved in 4mL 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 (13mg, 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** (46mg, 0.0704mmol) in 3mL DCM was added 2mL TFA. The mixture was stirred at rt for 2 h and the yellow solid was isolated by filtration to afford **49** (24mg, 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-dihydroxy-2-oxo-2H-chromen-3-yl)amino)-4-(7,8-dihydroxy-2-oxo-2H-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-

Organic & Biomolecular Chemistry Accepted Manuscript

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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** (28mg, 0.0458mmol) in 2mL DCM was added 2mL of 2M HCl in EtOAc. The mixture was stirred at rt for 2 h and the solvent was evaporated to afford **50** as a yellow solid (18mg, 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, 1.2Hz, 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 poly $(Glu,Tyr)_{4:1}$ (Sigma) was pre-coated as a substrate in 96-well plates. 50 μL of 10 μmol/L ATP solution diluted in kinase reaction buffer (50 mmol/L HEPES pH 7.4, 50 mmol/L $MgCl_2$, 0.5 mmol/L $MnCl_2$, 0.2 mmol/L Na_3VO_4, 1mmol/L DTT) was added to each well. 1μL of various concentrations of indicated compounds diluted in 1% DMSO (v/v) (Sigma) were added to each reaction well. 1% DMSO (v/v) was used as 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 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 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 o-phenylenediamine in 0.1 mol/L citrate buffer, pH 5.5, was added. The reaction was terminated by the addition of 50 μL of 2 mol/L H_2SO_4 as 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-(A490/A490 control)] × 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-

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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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1. D. Bottaro, J. Rubin, D. Faletto, A. Chan, T. Kmiecik, G. Vande Woude and S. Aaronson, *Science*, 1991, **251**, 802-804.
2. L. Trusolino, A. Bertotti and P. M. Comoglio, *Nat. Rev. Mol. Cell Biol.*, 2010, **11**, 834-848.
3. P. M. Comoglio, S. Giordano and L. Trusolino, *Nat. Rev. Drug Discov.*, 2008, **7**, 504-516.
4. J. A. Engelman, K. Zejnullahu, T. Mitsudomi, Y. Song, C. Hyland, J. O. Park, N. Lindeman, C.-M. Gale, X. Zhao, J. Christensen, T. Kosaka, A. J. Holmes, A. M. Rogers, F. Cappuzzo, T. Mok, C. Lee, B. E. Johnson, L. C. Cantley and P. A. Jänne, *Science*, 2007, **316**, 1039-1043.
5. R. Straussman, T. Morikawa, K. Shee, M. Barzily-Rokni, Z. R. Qian, J. Du, A. Davis, M. M. Mongare, J. Gould, D. T. Frederick, Z. A. Cooper, P. B. Chapman, D. B. Solit, A. Ribas, R. S. Lo, K. T. Flaherty, S. Ogino, J. A. Wargo and T. R. Golub, *Nature*, 2012, **487**, 500-504.
6. H.-L. Cheng, B. Trink, T.-S. Tzai, H.-S. Liu, S.-H. Chan, C.-L. Ho, D. Sidransky and N.-H. Chow, *J. Clin. Oncol.*, 2002, **20**, 1544-1550.
7. E. Lengyel, D. Prechtel, J. H. Resau, K. Gauger, A. Welk, K. Lindemann, G. Salanti, T. Richter, B. Knudsen, G. F. Vande Woude and N. Harbeck, *Int. J. Cancer*, 2005, **113**, 678-682.
8. L. Lo Muzio, A. Farina, C. Rubini, E. Coccia, M. Capogreco, G. Colella, R. Leonardi, G. Campisi and F. Carinci, *Tumor Biol.*, 2006, **27**, 115-121.
9. K. Sawada, A. R. Radjabi, N. Shinomiya, E. Kistner, H. Kenny, A. R. Becker, M. A. Turkyilmaz, R. Salgia, S. D. Yamada, G. F. Vande Woude, M. S. Tretiakova and E. Lengyel, *Cancer Res.*, 2007, **67**, 1670-1679.
10. K. Matsumoto and T. Nakamura, *Biochem. Biophys. Res. Commun.*, 2005, **333**, 316-327.
11. T. Otsuka, J. Jakubczak, W. Vieira, D. P. Bottaro, D. Breckenridge, W. J. Larochelle and G. Merlino, *Mol. Cell. Biol.*, 2000, **20**, 2055-2065.
12. K. Matsumoto, T. Nakamura, K. Sakai and T. Nakamura, *Proteomics*, 2008, **8**, 3360-3370.
13. T. Burgess, A. Coxon, S. Meyer, J. Sun, K. Rex, T. Tsuruda, Q. Chen, S.-Y. Ho, L. Li, S. Kaufman, K. McDorman, R. C. Cattley, J. Sun, G. Elliott, K. Zhang, X. Feng, X.-C. Jia, L. Green, R. Radinsky and R. Kendall, *Cancer Res.*, 2006, **66**, 1721-1729.
14. H. Jin, R. Yang, Z. Zheng, M. Romero, J. Ross, H. Bou-Reslan, R. A. D. Carano, I. Kasman, E. Mai, J. Young, J. Zha, Z. Zhang, S. Ross, R. Schwall, G. Colbern and M. Merchant, *Cancer Res.*, 2008, **68**, 4360-4368.
15. E. H. van der Horst, L. Chinn, M. Wang, T. Velilla, H. Tran, Y. Madrona, A. Lam, M. Ji, T. C. Hoey and A. K. Sato, *Neoplasia*, 2009, **11**, 355-364.
16. T. L. Underiner, T. Herbertz and S. J. Miknyoczki, *Anticancer Agents Med. Chem.*, 2010, **10**, 7-27.
17. X. Liu, R. C. Newton and P. A. Scherle, *Expert Opin. Investig. Drugs*, 2011, **20**, 1225-1241.
18. J. Porter, *Expert Opin. Ther. Pat.*, 2010, **20**, 159-177.
19. E. B. Yang, Y. N. Zhao, K. Zhang and P. Mack, *Biochem. Biophys. Res. Commun.*, 1999, **260**, 682-685.
20. A. Kumar, B. K. Singh, N. K. Sharma, K. Gyanda, S. K. Jain, Y. K. Tyagi, A. S. Baghel, M. Pandey, S. K. Sharma, A. K. Prasad, S. C. Jain, R. C. Rastogi, H. G. Raj, A. C. Watterson, E. Van der Eycken and V. S. Parmar, *Eur. J. Med. Chem.*, 2007, **42**, 447-455.
21. J. A. Burlison and B. S. J. Blagg, *Org. Lett.*, 2006, **8**, 4855-4858.
22. B. R. Kusuma, L. B. Peterson, H. Zhao, G. Vielhauer, J. Holzbeierlein and B. S. J. Blagg, *J. Med. Chem.*, 2011, **54**, 6234-6253.
23. H. Y. Zou, Q. Li, J. H. Lee, M. E. Arango, S. R. McDonnell, S. Yamazaki, T. B. Koudriakova, G. Alton, J. J. Cui, P.-P. Kung, M. D. Nambu, G. Los, S. L. Bender, B. Mroczkowski and J. G. Christensen, *Cancer Res.*, 2007, **67**, 4408-4417.
24. X. Wan and M. M. Joullié, *J. Am. Chem. Soc.*, 2008, **130**, 17236-17237.
25. S. Saito and J. Kawabata, *Tetrahedron*, 2005, **61**, 8101-8108.
26. Z.-T. Du, Z.-T. Du, J. Lu, J. Lu, H.-R. Yu, H.-R. Yu, Y. Xu, Y. Xu, A.-P. Li and A.-P. Li, *J. Chem. Res.*, 2010, **34**, 222-227.
27. J. D. S. Denis, J. S. Gordon IV, V. M. Carroll and R. Priefer, *Synthesis*, 2010, **2010**, 1590-1592.

Organic & Biomolecular Chemistry Accepted Manuscript

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28. N. J. Desai and S. Sethna, *The Journal of Organic Chemistry*, 1957, **22**, 388-390.
29. J.-C. Jung, Y.-J. Jung and O.-S. Park, *Synth. Commun.*, 2001, **31**, 1195-1200.
30. K. M. Khan, S. Iqbal, M. A. Lodhi, G. M. Maharvi, Z. Ullah, M. I. Choudhary, A.-u. Rahman and S. Perveen, *Bioorg. Med. Chem.*, 2004, **12**, 1963-1968.
31. Y.-S. Hon, T.-R. Hsu, C.-Y. Chen, Y.-H. Lin, F.-J. Chang, C.-H. Hsieh and P.-H. Szu, *Tetrahedron*, 2003, **59**, 1509-1520.
32. K. C. Nicolaou, P. K. Sasmal and H. Xu, *J. Am. Chem. Soc.*, 2004, **126**, 5493-5501.
33. K. Rikimaru, T. Wakabayashi, H. Abe, T. Tawaraishi, H. Imoto, J. Yonemori, H. Hirose, K. Murase, T. Matsuo, M. Matsumoto, C. Nomura, H. Tsuge, N. Arimura, K. Kawakami, J. Sakamoto, M. Funami, C. D. Mol, G. P. Snell, K. A. Bragstad, B.-C. Sang, D. R. Dougan, T. Tanaka, N. Katayama, Y. Horiguchi and Y. Momose, *Bioorg. Med. Chem.*, 2012, **20**, 3332-3358.
34. K. Mori, T. Kawasaki, S. Sueoka and T. Akiyama, *Org. Lett.*, 2010, **12**, 1732-1735.
35. P. Magnus and I. S. Mitchell, *Tetrahedron Lett.*, 1998, **39**, 9131-9134.

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