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Chromen-based TNF-α converting enzyme (TACE) inhibitors: Design, synthesis, and biological evaluation

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Abstract—A series of coumarin types MMP inhibitors were designed based on gelastatin hydroxamates (**1**) and evaluated for TACE, cellular TNF-α, and NO inhibitory activities. Among them, compounds **9b** had potent inhibitory activities in enzymatic and cellular assays and good selectivity to MMP-2 and MMP-9. Further investigation of **9b** will be carried out for its efficacy in RA animal model system.

1. Introduction

Tumor necrosis factor α (TNF-α) is known to be an important cytokine in rheumatoid arthritis (RA) pathological progress.[1] Clinical success of anti-TNF-α biological drugs has driven more attention to targeting TNF-α in RA therapy.[2] One of the promising approaches to discovery of inhibitors of TNF-α is the inhibition of zinc containing metalloproteinase, TNF-α converting enzyme (TACE).[3,4] TACE is a member of the reprolysin family of the metzincin superfamily and converts membrane bound pro TNF-α to mature and soluble TNF-α.[4]

Recently, we reported design, synthesis, and biological evaluation of gelastatin hydroxamates (**1**), dual functional inhibitors for TACE and MMPs.[5] As a part of our ongoing discovery program of potent TACE inhibitors, a series of coumarin-base analogues were intended to be prepared because of their structural similarity based on the idea of conformational restriction through connection between C5d and C6 position of gelastatin hydroxamates (**1**) leading chromen analogues (**2** in Fig. 1). Furthermore, chromen analogues showed the similar binding pattern as **1** in docking model using the crystal structures of TACE (Fig. 2).

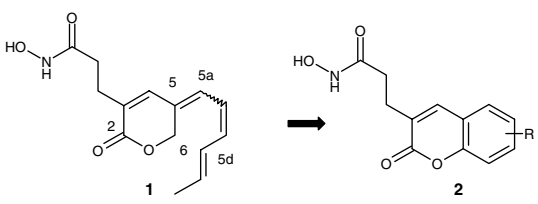

Figure 1. Design of chromene based TACE inhibitors based on gelastatin hydroxates (**1**).

Keywords: TNF-α converting enzyme (TACE); Metalloproteinase-2 (MMP-2); Metalloproteinase-9 (MMP-9); Rheumatoid arthritis (RA); Gelastatin hydroxamates; Coumarin derivatives.
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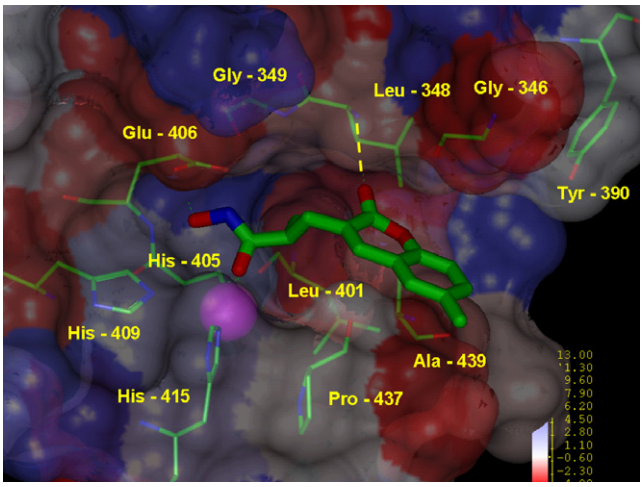


Figure 2. Docking model for the (*S*)-methyl chromen analogue (**9b**) binding to the binding pocket of TACE. Proposed hydrogen bonds between **9b** and the protein are shown as yellow lines.

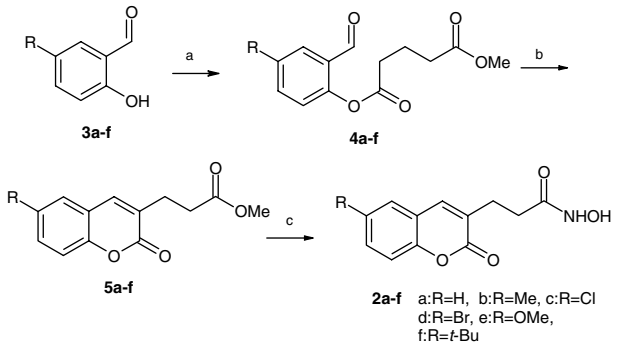
2. Chemistry

The first series of chromen-based analogues (**2a**–**f**) were prepared from the commercially available 2-hydroxybenzaldehydes **3a**–**f** (Scheme 1). Acylation of phenol **3a**–**f** with acid chloride yielded esters **4a**–**f** and the following intramolecular aldol reaction gave chromen moieties (**5a**–**f**). Conversion to hydroxamic acid using potassium hydroxylamide gave the chromen-based analogues **2a**–**f**.

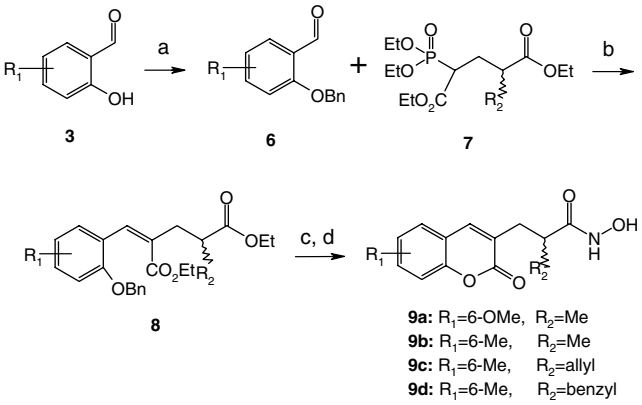
Next, α-alkylated analogues (**9a**–**d**) were designed and intended to increase an activity and selectivity to MMPs by occupying P1′ pocket of TACE enzyme (Fig. 1). Hydroxybenzaldehydes **3** were benzylated and the subsequent Wittig reaction with phosphonates **7** gave *Z*-isomers **8** (Scheme 2). Debenzylation with boron tribromide and the subsequent cyclization gave the chromene core, and the treatment with potassium hydroxylamide gave the racemic α-alkylated hydroxamate analogues **9a**–**d**.

3. Results and discussion

The first series of newly prepared analogues (**2a**–**f**) were tested in vitro for inhibition of TACE, MMP-2 and



Scheme 1. Reagents: (a) $MeO_2C(CH_2)_3COCl$, Et_3N, CH_2Cl_2; (b) DBU, toluene, reflux; (c) NH_2OK, MeOH.

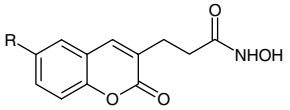


Scheme 2. Reagents: (a) BnBr, K_2CO_3, DMF; (b) NaH, THF; (c) BBr_3, CH_2Cl_2; (d) NH_2OK, MeOH.

MMP-9 activities, and cellular TNF-α and NO production. The results are summarized in Table 1. Among these seven analogues, simple chromen analogue (**2a**), methyl substituted analogue (**2b**), and methoxy substituted analogue (**2e**) had comparable activities and were more active than other analogues for in vitro TACE enzyme inhibition. Larger substituent on the chromene core decreased the inhibitory activities; methyl analogue (**2b**) and methoxy analogue (**2e**) were more potent than *tert*-butyl substituted analogue (**2f**). For the inhibitory activities to MMP-2 and MMP-9, these were very parallel to TACE activity but most of analogues were more potent to TACE and MMP-2 compared to MMP-9. Most of these analogues had dual functional inhibitory activities to TACE and MMP-2 although some analogues had some selectivity (for **2b**–**c** and **2e**, 3- to 5-fold selectivity to TACE over MMP-2). Thus, obtaining more selective TACE inhibitor was the primary goal for TNF-α modulation.[6] All of the prepared analogues were examined for in vitro cellular inhibition of TNF-α and NO on RAW264.7 cells. As shown in Table 1, **2b** and **2e** showed most potent inhibitory activities. All analogues did not inhibit the production of NO.

The alkylated analogues (**9a**–**d**) showed potent in vitro inhibitory activity profiles although the configuration

Table 1. In vitro inhibitory activities of compounds **2a**–**f**



Compound	R	IC_{50}[a] (μM)			IC_{50}[a,b] (μM)	
		TACE	MMP-2	MMP-9	TNF-α	NO
2a	H	0.15	0.20	1.41	5.64	NA
2b	Me	0.06	0.23	3.05	1.30	NA
2c	Cl	0.55	1.47	16.4	25.6	NA
2d	Br	0.84	0.86	24.5	13.0	NA
2e	OMe	0.11	0.59	5.66	2.21	NA
2f	*t*-Bu	12.4	NT	NT	NA	NA
1		0.028	0.006	0.023	8.8	NA

[a] Values are means of three experiments.
[b] RAW264.7 cells were used, NT, not tested; NA, no inhibition at 10 μM.

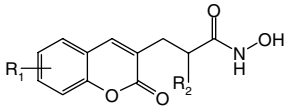
of α-position of hydroxamic acid was racemic; α-methylated analogues (**9a**, **b**) showed IC_{50} at 36 and 3 nM, and 0.22 and 0.32 μM for TACE enzyme inhibition and TNF-α inhibition, respectively, and allyl (**9c**) and benzylated (**9d**) analogues showed the same range of the inhibitory activities at around 30 and 5 nM in the same assay (Table 2). Furthermore, **9b** showed 347-fold selectivity to MMP-2 and even higher than MMP-9 (Table 3). The structural difference of P1′ pocket between TACE and MMP-2[8] may explain this selectivity.

Based on the crystal structure of human TACE (1bkc.pdb),[8] (*S*)-isomer of **9b** was docked into the catalytic domains of the enzymes using the program Discover/InsightII[9] (Fig. 2). The docking data suggests that the hydroxamic acid moiety of **9b** binds the zinc metal in the active sites of TACE enzyme. The carbonyl group in chromen ring interacts with the amide nitrogen proton of Leu348 in TACE through hydrogen bonding and it could afford an extra stabilization. The C6-methyl substituent of **9b** is not located in the extended S1′–S3′ channel and exposed to the outer hydrophilic environment. This offers an explanation why larger and hydrophobic substituent had less potent activities (*t*-Bu group in **2g**). It should also be pointed out that the only (*S*)-configuration of α-position of hydroxamic acid would be allowed to fit into the empty P1′ pocket and would account for improving the TACE inhibitory activity and selectivity to MMPs.[7]

4. Conclusion

A series of chromen-2-one and quinoloin-2-one derivatives have been rationally designed from gelastatin hydroxamates (**1**) and synthesized. Among the initial series of the chromen analogues, **2a**, **b**, **e** showed promising in vitro activity profiles in TACE, MMP-2, and -9 enzyme inhibition and TNF-α inhibition. Compound **9a–d** were designed from TACE docking study and SAR results and found out to be the most potent TACE inhibitor. Based on these results, further evaluations were conducted to investigate the abilities of inhibition TNF-α production in vivo and the efficacy for RA in animal model. The further study of **9a** and other selected compound of these series will be the subject of future publications.

Table 2. In vitro inhibitory activity profiles of compounds **9a–d**



Compound	R_1	R_2	IC_{50}[a] (μM)		
			TACE	TNF-α[b]	NO[b]
9a	6-OMe	Me	0.036	0.22	NA
9b	6-Me	Me	0.003	0.32	NA
9c	6-Me	Allyl	0.030	0.52	NA
9d	6-Me	Benzyl	0.005	0.54	NA

[a] Values are means of three experiments.
[b] RAW264.7 cells were used, NT, not tested; NA, no inhibition at 10 μM.

Table 3. In vitro inhibitory activities of compound **9b**

Compound	IC_{50}[a] (μM)				
	TACE	MMP-2	Selectivity (MMP-2)	MMP-9	Selectivity (MMP-9)
9b	0.003	1.04	347	>10	NA

NA, no inhibition at 10 μM.
[a] Values are means of three experiments.

5. Experimental

All chemicals were obtained from commercial suppliers and used without further purification. Flash column chromatography was performed with silica (Merck EM9385, 230–400 mesh). ^1H and ^{13}C NMR spectra were recorded at 300 and at 75 MHz, respectively. Proton and carbon chemical shifts are expressed in ppm relative to internal tetramethylsilane, coupling constants (*J*) are expressed in Hertz.

5.1. General procedures for 4a–g

2-Hydroxybenzaldehyde (**3**, 1 equiv) was dissolved in CH_2Cl_2 and DIPEA (2 equiv) and methyl-5-chloro-5-oxo-valerate was added at 0 °C under argon. The mixture was stirred for 2 h at room temperature. $NaHCO_3$ solution was added to the reaction mixture and then the mixture was diluted with ethyl acetate and washed with brine. The combined organic layer was dried over anhydrous $MgSO_4$. Compound **4a–g** was obtained by flash chromatography (ethyl acetate/hexanes 1:3) in 60–90% yield.

5.1.1. 2-Formylphenylmethylglutarate (4a). ^1H NMR ($CDCl_3$, 300 MHz) δ 10.02 (s, 1H), 7.81 (d, *J* = 7.2 Hz, 1H), 7.57 (t, *J* = 7.7 Hz, 1H), 7.34 (t, *J* = 7.4 Hz, 1H), 7.12 (d, *J* = 8.1 Hz, 1H), 3.64 (s, 3H), 2.70 (t, *J* = 8.1 Hz, 1H), 2.45 (t, *J* = 7.3 Hz, 1H), 2.10–2.00 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 188.7, 173.2, 171.1, 151.2, 135.2, 131.4, 127.9, 126.3, 123.3, 51.5, 32.9, 32.7, 19.7; ESI (*m*/*z*) 250.8 (MNa^+).

5.1.2. 2-Formyl-4-methylphenylmethylglutarate (4b). ^1H NMR ($CDCl_3$, 300 MHz) δ 9.99 (s, 1H), 7.60 (d, *J* = 1.8 Hz, 1H), 7.37 (dd, *J* = 2.1, 1.8 Hz, 1H), 7.01 (d, *J* = 7.8 Hz, 1H), 3.65 (s, 3H), 2.69 (t, *J* = 7.3 Hz, 2H), 2.45 (t, *J* = 7.1 Hz, 2H), 2.35 (s, 3H), 2.10 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 188.8, 173.2, 171.3, 149.1, 136.3, 135.8, 131.5, 127.5, 123.1, 51.5, 32.9, 32.8, 20.5, 19.7; ESI (*m*/*z*) 263.4 (MNa^+).

5.1.3. 4-Bromo-2-formylphenylmethylglutarate (4d). ^1H NMR ($CDCl_3$, 300 MHz) δ 10.2 (s, 1H), 7.53 (q, *J* = 3.0 Hz, 1H), 7.33–7.27 (m, 1H), 7.16 (q, *J* = 4.2 Hz, 1H), 3.676 (s, 3H), 2.73 (t, *J* = 7.3 Hz, 2H), 2.47 (t, *J* = 7.3 Hz, 2H), 2.13–2.03 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 187.1, 173.1, 170.8, 150.4, 137.9, 133.6, 129.3, 125.3, 119.6, 51.7, 32.9, 32.7, 19.7.

5.1.4. 2-Formyl-4-methoxyphenylmethylglutarate (4e). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.98 (s, 1H), 7.27 (d, $J = 2.1$ Hz, 1H), 7.11–7.01 (m, 2H), 3.77 (s, 3H), 3.63 (s, 3H), 2.67 (t, $J = 7.2$ Hz, 2H), 2.42 (t, $J = 7.1$ Hz, 2H), 2.05–1.97 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 188.2, 173.0, 171.5, 157.4, 145.3, 128.3, 124.3, 121.7, 113.1, 55.6, 51.5, 32.8, 32.7, 19.7; ESI (*m*/*z*) 281.3 (MNa^+).

5.1.5. 4-*tert*-Butyl-2-formylphenylmethylglutarate (4f). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.05 (s, 1H), 7.84 (d, $J = 3.3$ Hz, 1H), 7.63 (dd, $J = 2.6$, 2.7 Hz, 1H), 7.08 (d, $J = 8.4$ Hz, 1H), 3.68 (s, 3H), 2.72 (t, $J = 7.3$ Hz, 2H), 2.48 (t, $J = 7.0$ Hz, 2H), 2.09 (t, $J = 7.4$ Hz, 2H), 1.33–1.29 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 189.1, 173.3, 171.4, 149.5, 149.1, 132.5, 128.0, 127.3, 122.9, 51.6, 34.6, 33.0, 32.9, 32.8, 31.1, 31.1, 19.8; ESI (*m*/*z*) 306.7 (MNa^+).

5.2. General procedures for 5a–f

2-Formylphenylmethylglutarate (**4**, 1 equiv) was dissolved in toluene and DBU (1,8-iazabicyclo[5.4.0]undec-7-ene) (2 equiv) was added at room temperature. The mixture was heated by reflux (150 °C) for 1 day. Then the solvent was removed by rotary evaporator. Compound was obtained by flash chromatography (chloroform) in 35–45% yield.

5.2.1. Methyl-3-(2-oxo-2*H*-chromen-3-yl)propionate (5a). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.53 (s, 1H), 7.42–7.37 (m, 2H), 7.25–7.16 (m, 2H), 3.60 (s, 3H), 2.82 (t, $J = 6.9$ Hz, 2H), 2.65 (t, $J = 7.1$ Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 173.2, 171.1, 151.2, 135.2, 2.2, 131.4, 127.9, 126.3, 126.0, 123.7, 123.3, 51.5, 32.8, 19.7; ESI (*m*/*z*) 230.5 (MNa^+).

5.2.2. Methyl 3-(6-methyl-2-oxo-2*H*-chromen-3-yl)propionate (5b). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.51 (s, 1H), 7.27–7.16 (m, 3H), 3.64 (s, 3H), 2.86 (t, $J = 7.3$ Hz, 2H), 2.69 (t, $J = 7.1$ Hz, 2H), 2.37 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.9, 161.6, 151.3, 139.9, 133.9, 131.8, 127.4, 127. 2, 116.0, 51.6, 32.0, 26.5, 20.7; ESI (*m*/*z*) 264.2 (MNa^+).

5.2.3. Methyl 3-(6-bromo-2-oxo-2*H*-chromen-3-yl)propionate (5d). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (t, $J = 9.4$ Hz, 3H), 7.16 (d, $J = 8.5$ Hz, 1H), 3.64 (s, 3H), 2.86 (t, $J = 7.1$ Hz, 2H), 2.68 (d, $J = 7.1$ Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.8, 161.0, 151.0, 138.6, 133.6, 129.7, 129.1, 120.9, 118.2, 166.9, 50.6, 31.8, 26.6.

5.2.4. Methyl 3-(6-methoxy-2-oxo-2*H*-chromen-3-yl)propionate (5e). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.53 (s, 1H), 7.20 (s, 1H), 7.03 (dd, $J = 2.7$, 3.0 Hz, 1H), 6.86 (d, $J = 3.0$ Hz, 1H), 3.81 (s, 3H), 3.81 (s, 3H), 2.86 (t, $J = 7.1$ Hz, 2H), 2.69 (t, $J = 7.1$ Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.9, 161.5, 155.9, 147.6, 139.7, 127.9, 119.5, 118.5, 117.3, 109.4, 55.7, 51.5, 31.9, 26.5; ESI (*m*/*z*) 263.2 (MNa^+).

5.2.5. Methyl-3-(6-*tert*-butyl-2-oxo-2*H*-chromen-3-yl)propionate (5f). ^{1}H NMR ($CDCl_3$, 300 MHz) 7.58 (s, 1H), 7.50 (dd, $J = 2.1$, 2.4 Hz, 1H), 7.39 (d, $J = 2.4$ Hz, 1H), 7.21 (s, 1H), 3.65 (s, 3H), 2.87 (t, $J = 7.1$ Hz, 2H), 2.70 (t, $J = 7.4$ Hz, 2H), 1.32 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 173.3, 162.0, 151.5, 147.7, 140.8, 128.8, 127.5, 123.9, 119.0, 116.2, 51.9, 34.7, 32.3, 31.6, 26.8; ESI (*m*/*z*) 289.2 (MNa^+).

5.3. General procedures for 2a–f

Methyl-3-(2-oxo-2*H*-chromen-3-yl)propionate (**5**, 1 equiv) was dissolved in MeOH and NH_2OK in MeOH was added at 0 °C. The mixture was stirred for 1–2 h at room temperature. Then the mixture was filtered through on a pad of Celite with eluting MeOH and concentrated in vacuo. Compound was obtained by flash chromatography (10% methanol in chloroform) in 18–32% yields.

5.3.1. *N*-Hydroxy-3-(2-oxo-2*H*-chromen-3-yl)propanamide (2a). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (s, 1H), 7.59–7.51 (m, 2H), 7.31–7.28 (m, 2H), 2.85 (t, $J = 7.2$ Hz, 2H), 2.45 (t, $J = 7.3$ Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 170.2, 162.7, 153.6, 141.4, 131.5, 128.1, 127.6, 125.0, 119.8, 116.6, 27.4, 23.8; ESI (*m*/*z*) 232.6 (MNa^+).

5.3.2. *N*-Hydroxy-3-(6-methyl-2-oxo-2*H*-chromen-3-yl)propanamide (2b). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.61 (s, 1H), 7.25 (d, $J = 6.3$ Hz, 2H), 7.16 (d, $J = 8.1$ Hz, 1H), 2.82 (t, $J = 7.0$ Hz, 2H), 2.43–2.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 170.4, 163.0, 151.8, 141.5, 134.9, 132.6, 127.9, 127.5, 119.7, 116.4, 31.4, 27.5, 20.8; ESI (*m*/*z*) 248.2 (MNa^+).

5.3.3. 3-(6-Chloro-2-oxo-2*H*-chromen-3-yl)-*N*-hydroxy-propanamide (2c). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.64–7.24 (m, 4H), 2.85 (t, $J = 7.1$ Hz, 2H), 2.42 (t, $J = 7.3$ Hz, 2H); ESI (*m*/*z*) 290 (MNa^+).

5.3.4. 3-(6-Bromo-2-oxo-2*H*-chromen-3-yl)-*N*-hydroxy-propanamide (2d). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.66–7.55 (m, 3H), 7.21–7.16 (d, $J = 8.7$ Hz, 1H), 2.85 (t, $J = 6.3$ Hz, 2H), 2.43 (t, $J = 6.8$ Hz, 2H); ESI (*m*/*z*) 335 (MNa^+).

5.3.5. *N*-Hydroxy-3-(6-methoxy-2-oxo-2*H*-chromen-3-yl) propanamide (2e). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (s, 1H), 7.19 (d, $J = 8.7$ Hz, 1H), 7.04 (dd, $J = 2.6$, 2.7 Hz, 2H), 6.94 (t, $J = 7.3$ Hz, 1H), 3.80 (s, 3H), 2.83 (t, $J = 7.0$ Hz, 2H), 2.39 (q, $J = 7.2$ Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 169.4, 162.3, 156.1, 147.5, 140.9, 127.3, 119.7, 118.9, 117.2, 109.6, 30.9, 27.2; ESI (*m*/*z*) 264.2 (MNa^+).

5.3.6. 3-(6-*tert*-Butyl-2-oxo-2*H*-chromen-3-yl)-*N*-hydroxy-propanamide (2f). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (s, 1H), 7.63–7.57 (m, 2H), 7.24 (d, $J = 8.4$ Hz, 1H), 2.84 (t, $J = 7.3$ Hz, 2H), 2.44 (t, $J = 7.3$ Hz, 2H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.5, 163.6, 152.6, 149.0, 142.4, 129.9, 128.2, 125.3, 120.3, 116.7, 35.4, 32.0, 31.7, 28.1; ESI (*m*/*z*) 290.2 (MNa^+).

5.4. Procedures for compounds 9a–d

5.4.1. 2-(Benzyloxy)-5-methoxybenzaldehyde (6). 2-Hydroxy-5-methoxybenzaldehyde (500 mg, 3.29 mmol,

1 equiv) was dissolved in DMF and K_2CO_3 (1.82 g, 4 equiv) was added at room temperature. The mixture was stirred for 30 min at 0 °C under Ar atmosphere. Then benzyl bromide (722 mg, 1.3 equiv) was added and the mixture was stirred for 1 day at room temperature. The mixture was poured to water and diluted with ethyl acetate and washed with brine. And the organic was dried over anhydrous $MgSO_4$ and then concentrated. 2-(Benzyloy)-5-methoxybenzaldehyde (**16**, 650 mg, 81.5%) was obtained by flash chromatography (ethyl acetate/hexanes 1:5); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.49 (s, 1H), 7.40–7.37 (m, 3H), 7.35–7.32 (m, 3H), 7.10 (dd, $J = 3.6$ Hz, 1H), 6.98 (d, $J = 9.6$ Hz, 1H), 5.13 (s, 2H), 3.78 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 189.5, 155.8, 153.9, 136.2, 128.7, 128.5, 128.2, 127.3, 126.9, 125.5, 123.4, 115.1, 110.2, 71.3, 55.8; ESI (*m*/*z*) 242.8 (MNa^+).

5.4.2. (*Z*)-Diethyl-2-(2-(benzyloxy)-5-methoxybenzylidene)-4-ethylpentane dioate (8). Phosphonate **7** (1.2 equiv) was dissolved in THF and NaH (2.5 equiv) was added at 0 °C. And the mixture was stirred for 30 min at −20 °C under Ar atmosphere. Then 2-(benzyloxy)-5-methoxy benzaldehyde (300 mg, 1.24 mmol, 1 equiv) was added and the mixture was stirred for 1 day at room temperature. Water was added to the mixture and diluted with ethyl acetate and washed with brine. And the organic was dried over anhydrous $MgSO_4$ and concentrated. (*Z*)-Diethyl-2-(2-(benzyloxy)-5-methoxybenzylidene)-4-methylpentanedioate (**8**, 433 mg, 68%) was obtained by flash chromatography (ethyl acetate/hexanes 1:5). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.92 (s, 1H), 7.40–7.25 (m, 5H), 7.91–6.75 (m, 3H), 5.04 (s, 2H), 4.27 (q, $J = 7.5$ Hz, 1H), 4.12–3.99 (m, 2H), 3.78 (s, 3H), 2.94–2.80 (m, 1H), 2.77–2.63 (m, 2H), 1.34 (t, $J = 7.4$ Hz, 3H), 1.23–1.14 (m, 6H); ^{13} NMR ($CDCl_3$) δ 176.0, 167.9, 153.5, 150.8, 137.1, 136.7, 131.1, 128.4, 128.4, 127.7, 127.2, 126.9, 126.0, 115.0, 114.9, 114.1, 71.1, 60.7, 60.2, 55.7, 38.4, 31.3, 16.6, 14.2, 14.1; ESI (*m*/*z*) 427.0 (MNa^+).

5.4.3. Ethyl-2-((6-methoxy-2*H*-chromen-3-yl)methyl)propanoate. (*Z*)-Diethyl-2-(2-(benzyloxy)-5-methoxybenzylidene)-4-methylpentanedioaten (**8**, 200 mg, 0.47 mmol, 1 equiv) was dissolved in methylene chloride and BBr_3 (0.47 mL, 1 M solution) was added at −20 °C and the mixture was stirred for 1 h at −20 °C under argon. The mixture was stirred for overnight at room temperature. The water was added to the mixture, and the mixture was diluted with ethyl acetate and washed with brine. And the organic was dried over anhydrous $MgSO_4$ and concentrated. Ethyl-2-((6-methoxy-2*H*-chromen-3-yl)methyl)propionate (30 mg, 22%) was obtained by flash chromatography (ethyl acetate/hexanes 1:3). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.69 (s, 1H), 6.82–6.73 (m, 3H), 6.68 (d, $J = 2.4$ Hz, 1H), 4.25 (q, $J = 7.5$ Hz, 2H), 4.01 (t, $J = 6.9$ Hz, 2H), 3.74 (s, 3H), 2.83–2.74 (m, 1H), 2.57 (t, $J = 6.5$ Hz, 2H), 1.32 (t, $J = 7.1$ Hz, 3H), 1.15 (t, $J = 7.1$ Hz, 3H), 1.07 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 175.8, 161.7, 156.1, 147.8, 140.3, 127.4, 119.7, 118.5, 117.4, 109.6, 60.4, 55.8, 38.1, 35.2, 17.5, 14.1; ESI (*m*/*z*) 291.0 (MNa^+).

5.4.4. *N*-Hydroxy-2-((6-methoxy-2*H*-chromen-3-yl)methyl)propanamide (9a). Ethyl-2-((6-methoxy-2-chromen-3-yl)methyl)propionate (30 mg, 0.1 mmol, 1 equiv) was dissolved in methanol and NH_2OK was added at 0 °C. The mixture was stirred for 1–2 h at room temperature. Then the mixture was filtered on a pad of Celite with eluting 10% MeOH in CH_2Cl_2. After removal of the solvent in vacuo, the residue was purified by flash chromatography (10% methanol in chloroform) to give *N*-hydroxy-2-((6-methoxy-2*H*-chromen-3-yl)methyl)propanamide (**9a**, 4 mg, 14.2%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.68 (s, 1H), 7.24 (d, $J = 8.4$ Hz, 1H), 7.14–7.08 (m, 2H), 3.83 (s, 3H), 2.76 (d, $J = 3.3$ Hz, 2H), 2.66 (q, $J = 4.2$ Hz, 3H), 1.19 (d, $J = 6.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.9, 162.5, 156.1, 141.7, 139.9, 128.6, 126.1, 119.6, 118.9, 117.1, 110.3, 109.4, 55.6, 35.8, 35.4, 17.2; ESI (*m*/*z*) 277.9 (MNa^+).

5.4.5. *N*-Hydroxy-2-methyl-3-(6-methyl-2-oxo-2*H*-chromen-3-yl)-propionamide (9b). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.65 (s, 1H), 7.36–7.34 (m, 2H), 7.20–7.19 (m, 1H), 3.83 (s, 3H), 2.77–2.63 (m, 3H), 2.38 (s, 3H), 1.19 (d, $J = 6.2$ Hz, 3H); ^{13}C NMR (CD_3OD) δ 174.8, 163.6, 152.8, 142.7, 135.7, 132.2, 128.8, 127.4, 120.6, 116.9, 37.6, 20.7, 18.3; ESI (*m*/*z*) 284 (MNa^+).

5.4.6. 2-(6-Methyl-2-oxo-2*H*-chromen-3-ylmethyl)-pent-4-enoic acid hydroxyamide (9c). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.64 (s, 1H), 7.36–7.19 (m, 3H), 5.82–5.75 (m, 1H), 5.10 (d, $J = 16.9$ Hz, 1H), 5.03 (d, $J = 10.0$ Hz, 1H), 2.77–2.24 (m, 5H), 2.38 (s, 3H); ^{13}C NMR (CD_3OD) δ 173.2, 163.6, 152.8, 142.8, 136.3, 135.7, 133.2, 128.8, 127.2, 120.6, 117.6, 116.9, 43.3, 38.0, 34.6, 20.7; ESI (*m*/*z*) 310 (MNa^+).

5.4.7. 2-Benzyl-*N*-hydroxy-3-(6-methyl-2-oxo-2*H*-chromen-3-yl)-propionamide (9d). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.62 (s, 1H), 7.33–7.15 (m, 8H), 2.99–2.76 (m, 5H), 2.36 (s, 3H); ^{13}C NMR (CD_3OD) δ 172.9, 163.5, 152.8, 140.2, 135.6, 133.2, 130.1, 129.4, 128.8, 127.4, 127.1, 120.5, 116.8, 45.4, 39.8, 35.1, 20.7; ESI (*m*/*z*) 360 (MNa^+).

5.5. Biology

5.5.1. TACE, MMP-2, and MMP-9 enzyme inhibition assay. The enzymatic activity of human TACE (R&D Systems, Minneapolis, MN, USA) was determined at 25 °C with a synthetic fluorogenic substrate, Mca-Pro-Leu-Ala-Gln-Ala-Val-Dpa-Arg-Ser-Ser-Ser-Agr-NH_2 (Mca: [7-methoxycoumarin-4-yl] acetyl; Dpa: *N*-3-[2,4-dinitrophenyl]-L-2,3-diaminopropionyl, R&D Systems, Minneapolis, MN, USA). The enzymatic activities of MMP-2 and MMP-9 were determined with recombinant human version catalytic domains (Roche Applied Science, D-68298 Manheim, Germany) and a fluorogenic peptide substrate, Mca – Pro – Leu – Gly – Leu – Dpa – Ala – Arg – NH_2 (Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178, USA). Fluorescence measurements were performed in a Perkin-Elmer Luminescence Spectrometer LS50B. Cleavage of internal quenched substrate liberates emission-active Mca

product, causing an increase in fluorescence emission at 420 nM (the excitation wavelength is 324 nM). The rate of emission change is proportional to enzyme activity.

5.5.2. Cellular NO assay and TNF-α immunoassay. RAW264.7 cells were cultured with inhibitors for 24 h. NO_2^- accumulation was used as an indicator of NO production in the medium. The isolated supernatants were mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, and 2% phosphoric acid) and incubated at room temperature for 10 min. Nitrite production was determined by measuring absorbance at 540 nm versus a $NaNO_2$ standard curve. The concentration of TNF-α secreted in the culture supernatant of RAW264.7 cells was determined by ELISA, according to the manufacture's instruction (R&D Systems, Minneapolis, MN, USA).

5.6. Docking study

The computational complex model was solvated using a solvent sphere of water extending 20.0 Å around the hydrogen of amide backbone aspartic acid 486, only residues within 5.0 Å of compound **9a** were allowed to move during the geometry optimizations using 500 steps of steepest decent and 3000 steps of conjugated gradient.

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