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# SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL COUMARIN DERIVATIVES AS ANTIPLATELET AGENTS

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**Abstract** – In order to develop anti-thrombotic agents with higher potency, a series of novel coumarin derivatives (**5a-m**) were designed, synthesized and biologically evaluated. Compound **5e** displayed the strongest activity in inhibiting the adenosine diphosphate (ADP)-induced platelet aggregation in vitro, with 2.3-fold more effectiveness than clinically used antiplatelet drug aspirin (ASP). Thus, Compound **5e** could deserve further investigations as antiplatelet agents.

Thrombosis is a major cause of human morbidity and mortality worldwild.[1] Platelet aggregation plays an important role in thrombotic processes.[2] Traditional antiplatelet drugs, such as aspirin (ASP) and ticlopidine, are used to protect against thrombosis and reduce the risk of myocardial infarction, ischaemic stroke, vascular disease and cardiovascular fatal events. However, current antiplatelet drugs have certain disadvantages such as notable side effects and inefficient therapy. For example, ASP may cause stomach ulcers and bleeding while ticlopidine lead to indigestion and diarrhoea.[3] Therefore, development of new antiplatelet drugs with more effectivity and fewer side effects will be of great significance for the treatment of thrombotic disease.

Coumarins (benzopyran-2-one) are a large family of compounds of both natural and synthetic origin and displayed extensive biological activities, such as antioxidant,[4] antiplatelet,[5] anticoagulant,[6] antihyperglycemic[7] and antitumor,[8] etc. Moreover, some coumarins possess low cytotoxicity on and excellent cell permeability, and have a relatively low molecular weight suitable for modification.[9,10] Hence coumarins have attracted much attention in drug research. It has been reported that appropriate substituents at the C3-position of coumarin might contribute to the antiplatelet aggregation activity of these compounds. For instance, carbochromen (Figure 1), diethylaminoethyl-substituent at the C3-position of coumarin, was a potent specific coronary vasodilator that had been used for many years in

the treatment of angina pectoris.[11,12] Compound A (Figure 1)[13] amide-substituent at the C3-position of coumarin, had better antithrombotic activity and less blooding time than ASP and warfarin. Our previously studies showed that the presence of ester-substituent at the C-3 positions of the coumarin could enhance the antiplatelet activity against ADP-induced aggregation.[14] Among them, Compound B (Figure 1) showed the highest inhibitory effect. These results suggest that the presence of a substituent group at C-3 of coumarin is beneficial for antiplatelet activity.

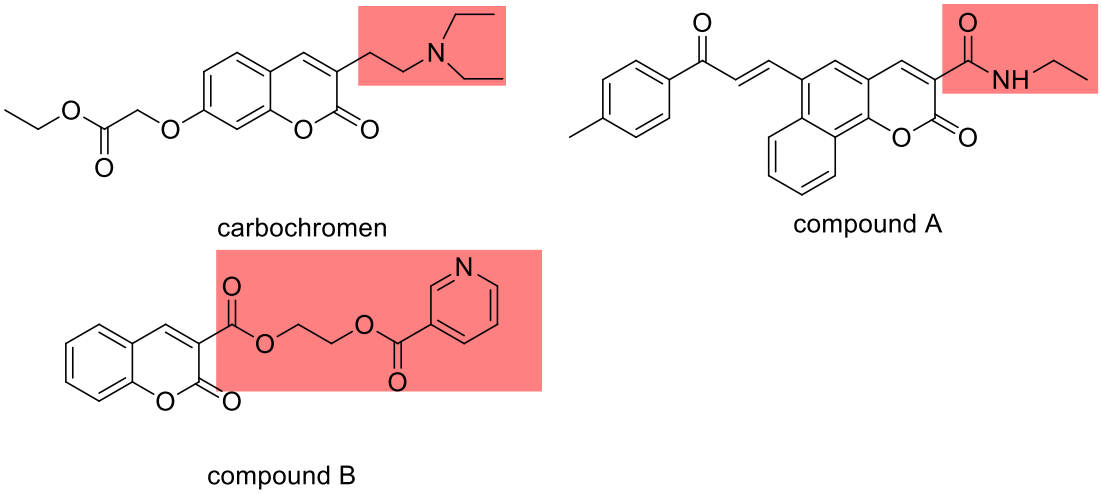


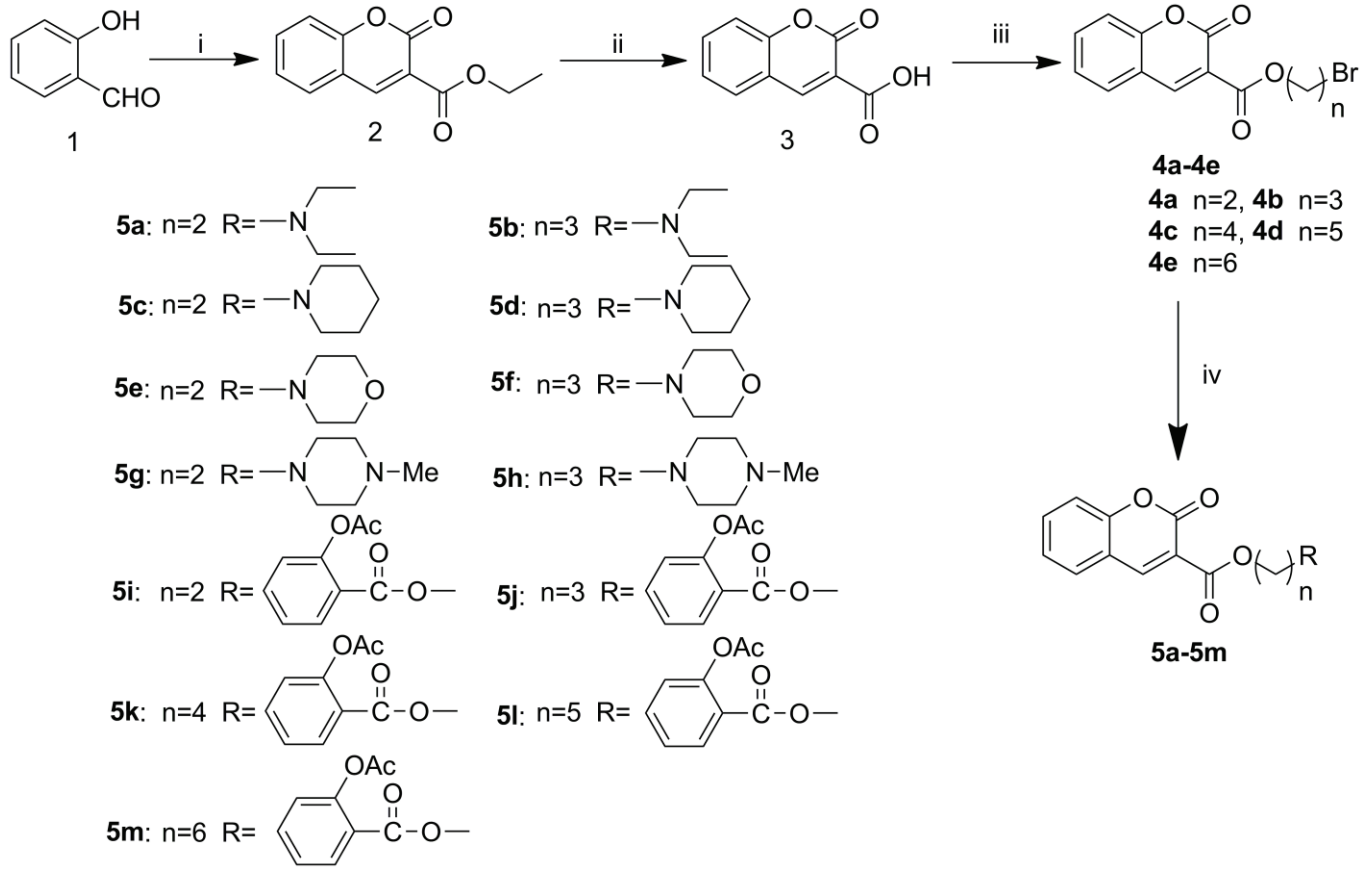

**Figure 1.** Chemical structures of carbochromene, compound A and compound B

Modification of a molecule with amino moiety usually improves its aqueous solubility, and promotes the interaction of both H-bond donor and acceptor with the intended biological targets.[15-17] The antiplatelet properties of 2-aminochromones have been investigated by many researchers.[18-21] 2-Morpholino-chromones have been suggested to be the most potent analogue.[16] In view of this, we designed and synthesized a series of coumarin derivatives containing various substituted amines, such as diethylamine, piperidin, *N*-methylpiperazine, and morpholine at C-3 position of coumarin and evaluated the inhibitory effects of these derivatives on aggregation of washed rabbit platelets induced by ADP at concentrations of 10 μM. Preliminary structure-activity relationship (SAR) correlations are also discussed.

Besides, in order to further study on the relation both the antiplatelet activity and the modification of coumarin derivatives at C-3 position, a series of coumarin derivatives containing aspirin unit by different carbon chain have been designed and synthesized.

As shown in Scheme 1, the target compounds **5a-m** were synthesized from salicylaldehyde **(1)**. Coumarin-3-carboxylic acid ethyl ester **2** was obtained by Knoevenagel cyclization between **1** and diethyl malonate with piperidine as catalyst. Subsequently, compound **2** underwent alkali hydrolysis and

acidification to form coumarin-3-carboxylic acid **3**. Treatment of **3** with dibromoalkanes in the presence of triethylamine generated brominated compounds **4a-e**, which was reacted with corresponding amines and aspirin moiety to gain the target compounds **5a-m**. All of new compounds were purified by column chromatography and characterized by IR, ESI-MS and $^1$H NMR.




**Scheme 1.** Synthesis of novel coumarin derivatives **5a-m**. Reagents and conditions: (i) diethyl malonate, piperidine (cat.), EtOH, reflux, 2 h; (ii) 1) NaOH, $H_2O$, EtOH, reflux, 15 min; 2) HCl; (iii)Br-($CH_2$)n-Br (n=2-6), DMF, TEA, rt, 6 h; (iv) 1) For compounds **5a-h**: diethylamine, piperidine, morpholine, or *N*-methylpiperazidine, MeCN, 50 °C, 7 h; 2) For compounds **5i-m**: aspirin, TEA, MeCN, 60-70 °C, 8 h.

The novel coumarin derivatives **5a-m** were evaluated for inhibition of platelet aggregation in rabbit platelet rich plasma (PRP) in response to ADP (10 μM) using Born's turbidimetric method. Each assay was performed three times, taking ASP as positive controls. As shown in Table 1, for the ADP-induced platelet aggregation, ten out of thirteen target compounds displayed significantly inhibitory effects. All target compounds, with exception of **5b**, **5l** and **5m**, presented better inhibitory effects than ASP against the ADP-induced platelet aggregation. Notably, **5c** (32.83%) and **5e** (38.20%) displayed the most potent inhibitory effects, significantly superior to ASP (15.40%). As shown in Table 2, the $IC_{50}$ values of **5c** and **5e** on the ADP-induced platelet aggregation were 0.45 mM and 0.38 mM respectively, which were 2-fold and 2.34-fold stronger than that of ASP ($IC_{50}$ = 0.89 mM).

**Table 1.** Effect of the target compounds **5a-m** (0.1mmol/L) on the ADP-induced platelet aggregation in vitro

| Compound | Max aggregation at 5 min (%) | Inhibition rate (%) |
|---|---|---|
| Control | 33.96 ± 1.61 | -- |
| Aspirin | 28.73 ± 2.71 | 15.40 |
| **5a** | 27.29 ± 2.96 | 19.65 |
| **5b** | 30.69 ± 2.55 | 9.64 |
| **5c** | 22.81 ± 3.84### | 32.83 |
| **5d** | 27.29 ± 3.14 | 19.65 |
| **5e** | 20.99 ± 2.67###* | 38.20 |
| **5f** | 23.97 ± 2.21## | 29.40 |
| **5g** | 25.52 ± 4.65# | 24.86 |
| **5h** | 25.44 ± 3.41# | 25.10 |
| **5i** | 26.77 ± 3.94 | 21.18 |
| **5j** | 27.61 ± 3.92 | 18.69 |
| **5k** | 26.91 ± 1.69 | 20.75 |
| **5l** | 30.51 ± 2.90 | 10.15 |
| **5m** | 29.67 ± 4.62 | 12.63 |

*P < 0.05 versus aspirin group, #P < 0.05, ##P < 0.01, ###P < 0.001 versus control group.

**Table 2.** Antiplatelet activity ($IC_{50}$) for tested compounds

| **Compound** | **$IC_{50}$ (mmol/L)** |
|---|---|
| Control | —— |
| Aspirin | 0.89 |
| **5c** | 0.45 |
| **5e** | 0.38 |

Analysis of SAR revealed that the antiplatelet aggregation activity of the target compounds depended on both the length of carbon chain of ester and the different moiety (such as amino and aspirin). Firstly, variation in the length of carbon chain affected significantly the antiplatelet aggregation activity of these derivatives. For example, compounds **5a**, **5c** and **5e** with two-carbon chain displayed stronger antiplatelet aggregation activity than those with three-carbon linker. Secondly, the morpholine derivatives **5e** and **5f** exhibited much stronger antiplatelet aggregation activity than **5a** and **5b** which containing the

diethylamine moiety. Indeed, compound **5e** with a two-carbon linker and a morpholine moiety displayed the strongest antiplatelet aggregation activity among the all tested derivatives. In addition, the compounds **5i-m** with a aspirin moiety displayed relatively weak antiplatelet aggregation activity than these compounds with amino moiety *in vitro*. It may be that because these compounds couldn't decompose coumarin and aspirin to strengthen antiplatelet aggregation activity together *in vitro*. However, the precise SAR of these derivatives remains to be further investigated.

## EXPERIMENTAL

**Chemstry.** Melting points were measured using a WRS-1B apparatus without any correction. $^1$H NMR spectra were recorded on 400 MHz Bruker Avance DPX spectrometers and referenced with TMS as an internal standard. All NMR spectra were recorded in $CDCl_3$ at room temperature. IR spectra were collected on Nicolet Avatar 6700 spectrometer using KBr film. ESI mass spectra were acquired using a Thermo Fisher LTQ Orbitrap XL Liquid chromatography-mass spectrometry instrument.

**Preparation of coumarin-3-carboxylic acid (3).** The mixture of salicylaldehyde **1** (4.2 mL), diethyl malonate (6.8 mL), anhydrous EtOH (20 mL), piperidine (0.5 mL), and AcOH (2 drops) was refluxed for 2 h. Subsequence, the resulting suspension was placed in ice bath after added 30 mL water. The raw product was gained by filtration, washed with 50% EtOH, and dried. Finally, the raw product was purified by recrystallized from 25% EtOH to give compound **2**.

The mixture of coumarin-3-carboxylic acid ethyl ester **2** (4 g), NaOH (3 g), EtOH (15 mL) and water (10 mL) was refluxed for 15 min. After cooling, the reaction mixture was added to a solution of 10 mL concentrated hydrochloric acid in 50 mL water under stirring. Then the raw product was obtained by filtration and washed with ice water. Finally, the raw product was purified by recrystallized from 50% EtOH to give compound **3** as a white crystals.

**2-Bromoethyl 2-oxo-2*H*-chromene-3-carboxylate (4a).** Coumarin-3-carboxylic acid (2 g, 10.5 mmol) was added to the mixture of 1,2-dibromoethane (3.6 mL, 42 mmol) and triethylamine (2.9 mL, 21 mmol) in DMF, and reacted at room temperature for 6 h. Then water (100 mL) was poured into the reaction mixture and extracted with EtOAc (3 × 50 mL). The organic layer was then washed with saturated aq. NaCl solution, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The obtained residue was purified by silica gel chromatography with petroleum ether/EtOAc (10:1) as eluent to give compound **4a** as white solid (2.2 g, 71%); mp 126.5-127.4 °C; ESI-MS *m/z*: 296.9756 $[M+H]^+$ (Calcd for $C_{19}H_{37}N_2O$ 296.9684); IR 3051, 2943, 1762, 1715, 1609, 1567, 1454 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 3.68 (t, $J$ = 6.1 Hz, 2H), 4.68 (t, $J$ = 6.1 Hz, 2H), 7.36-7.41 (m, 2H), 7.66-7.72 (m, 2H), 8.61 (s, 1H).

**3-Bromopropyl 2-oxo-2*H*-chromene-3-carboxylate (4b).** Compound **4b** was synthesized according to

the procedure of synthesizing compound **4a**. Compound **4b** was afforded as white solid (2.5 g, 76%); 94.8-95.2 °C; ESI-MS *m/z*: 310.9912 $[M+H]^+$ (Calcd for $C_{19}H_{37}N_2O$ 310.9841); IR 3065, 2943, 1752, 1716, 1610, 1590, 1456 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 2.31-2.41 (m, 2H), 3.62 (t, *J* = 5.7 Hz, 2H), 4.53 (t, *J* = 5.4 Hz, 2H), 7.34-7.43 (m, 2H), 7.62-7.77 (m, 2H), 8.57 (s, 1H).

**2-(Diethylamino)ethyl 2-oxo-2*H*-chromene-3-carboxylate (5a).** 2-Bromoethyl 2-oxo-2*H*-chromene-3-carboxylate **4a** (500 mg, 1.68 mmol) was solved in MeCN, and diethylamine (368 mg, 5.04 mmol) was then added. The reaction mixture was heated to 50 °C for 7 h. The raw product was purified by column chromatography over silica gel to give 250 mg compound **5a** as yellow solid in 51% yield, mp 151.0-151.9 °C; ESI-MS *m/z*: 290.1388 $[M+H]^+$ (Calcd for $C_{16}H_{19}NO_4$ 290.1314); IR 3050, 2968, 2927, 1770, 1707, 1609, 1568, 1456, 1242, 1209 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 1.45 (t, *J* = 7.2 Hz, 6H), 3.02-3.21 (m, 6H), 4.68 (t, *J* = 6.1 Hz, 2H), 7.36-7.41 (m, 2H), 7.66-7.72 (m, 2H), 8.61 (s, 1H).

**3-(Diethylamino)propyl 2-oxo-2*H*-chromene-3-carboxylate (5b).** Compound **5b** was synthesized according to the procedure of synthesizing compound **5a**. Compound **5b** was obtained as yellow solid in 55% yield, mp 165.8-166.8 °C; ESI-MS *m/z*: 304.1635 $[M+H]^+$ (Calcd for $C_{17}H_{21}NO_4$ 304.1504); IR 3025, 2970, 2930, 1757, 1702, 1615, 1566, 1456, 1245, 1212 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 1.22 (t, *J* = 6.9 Hz, 6H), 1.82-1.85 (m, 2H), 2.84-3.00 (m, 6H), 4.41 (t, *J* = 5.5 Hz, 2H), 7.40-7.37 (m, 2H), 7.75-7.68 (m, 2H), 8.59 (s, 1H).

**2-(Piperidin-1-yl)ethyl 2-oxo-2*H*-chromene-3-carboxylate (5c).** Piperidine (429 mg, 5.04 mmol) and **4a** (500 mg, 1.68 mmol) were used as reactants, and column chromatography gave 230 mg of compound **5c** as yellow solid, yield 45%, mp 97.3-98.1 °C; ESI-MS *m/z*: 302.3051 $[M+H]^+$ (Calcd for $C_{17}H_{19}NO_4$ 302.1314); IR 3100, 2936, 2898, 1770, 1716, 1601, 1543, 1456, 1229, 1218 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 1.75-1.81 (m, 6H), 2.82-2.73 (m, 6H), 4.40 (t, *J* = 5.7 Hz, 2H), 7.32-7.35 (m, 2H), 7.63-7.66 (m, 2H), 8.58 (s, 1H); $^{13}C$ NMR ($CDCl_3$) δ 24.38, 25.39, 26.22, 43.01, 48.37, 116.74, 118.39, 124.87, 125.82, 128.47, 132.62, 142.20, 153.97, 158.06, 163.26.

**3-(Piperidin-1-yl)propyl 2-oxo-2*H*-chromene-3-carboxylate (5d).** Piperidine (411 mg, 4.83 mmol) and **4b** (500 mg, 1.61 mmol) were used as reactants. Compound **5d** (240 mg) was obtained as yellow solid in 47% yield, mp 89.7-91.4 °C; ESI-MS *m/z*: 316.1532 $[M+H]^+$ (Calcd for $C_{18}H_{21}NO_4$ 316.1471); IR 3036, 2947, 2930, 1774, 1751, 1608, 1566, 1450, 1251, 1219, 1209 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 1.72-1.75 (m, 6H), 1.81-1.84 (m, 2H), 2.48-2.55 (m, 6H), 4.45 (t, *J* = 5.6 Hz, 2H), 7.35-7.38 (m, 2H), 7.68 (t, *J* = 7.9 Hz, 2H), 8.77 (s, 1H).

**2-Morpholinoethyl 2-oxo-2*H*-chromene-3-carboxylate (5e).** Morpholine (439 mg, 5.04 mmol) and **4a** (500 mg, 1.68 mmol) were used as reactants. The raw product was purified by column chromatography (petroleum ether/EtOAc 12:1) to give 288 mg of compound **5e** as yellow solid, yield 56%, mp 108.5-109.8 °C; ESI-MS *m/z*: 304.2997 $[M+H]^+$ (Calcd for $C_{16}H_{17}NO_5$ 304.1107); IR 3054, 2976, 2930,

1771, 1711, 1608, 1567, 1456, 1247, 1211 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 2.40-2.47 (m, 6H), 3.72 (t, *J* = 8.0 Hz, 4H), 4.38 (t, *J* = 6.4 Hz, 2H), 7.33-7.38 (m, 2H), 7.61-7.67 (m, 2H), 8.52 (s, 1H); $^{13}C$ NMR ($CDCl_3$) δ 53.86, 56.91, 63.03, 68.88, 116.85, 117.88, 118.15, 124.91, 134.50, 148.95, 155.24, 156.64, 163.14.

**3-Morpholinopropyl 2-oxo-2*H*-chromene-3-carboxylate (5f).** Morpholine (420 mg, 4.83 mmol) and **4b** (500 mg, 1.61 mmol) were used as reactants. Compound **5f** (338 mg)was obtained as yellow solid in 66% yield, mp 91.6-93.3 °C; ESI-MS *m/z*: 318.1334 $[M+H]^+$ (Calcd for $C_{17}H_{19}NO_5$ 318.1263); IR 3056, 2975, 2936, 1770, 1606, 1560, 1449, 1255, 1242 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 1.97-2.00 (m, 2H), 2.51-2.57 (m, 6H), 3.72 (t, *J* = 7.8 Hz, 4H), 4.41 (t, *J* = 6.3 Hz, 2H), 7.32-7.40 (m, 2H), 7.61-7.67 (m, 2H), 8.52 (s, 1H).

**2-(4-Methylpiperazin-1-yl)ethyl 2-oxo-2*H*-chromene-3-carboxylate (5g).** *N*-Methylpiperazidine (505 mg, 5.04 mmol) and **4a** (500 mg, 1.68 mmol) were used as reactants. Compound **5g** (236 mg) was obtained as yellow solid in 44% yield, mp 62.0-62.8 °C; ESI-MS *m/z*: 317.1493 $[M+H]^+$ (Calcd for $C_{17}H_{20}N_2O_4$ 317.1423); IR 3050, 2924, 2854, 1769, 1716, 1610, 1568, 1456, 1246, 1218 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 2.74 (s, 3H), 2.85-2.91 (m, 10H), 4.51 (t, *J* = 6.0 Hz, 2H), 7.36-7.44 (m, 2H), 7.68-7.70 (m, 2H), 8.57 (s, 1H).

**3-(4-Methylpiperazin-1-yl)propyl 2-oxo-2*H*-chromene-3-carboxylate (5h).** *N*-Methylpiperazidine (484 mg, 4.83 mmol) and **4b** (500 mg, 1.61 mmol) were used as reactants. Compound **5h** (262 mg) was obtained as yellow slid in 49% yield, mp 55.7-56.3 °C; ESI-MS *m/z*: 331.3398 $[M+H]^+$ (Calcd for $C_{18}H_{22}N_2O_4$ 331.1580); IR 3045, 2940, 2836, 1766, 1706, 1609, 1568, 1455, 1251, 1214 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 1.83-1.90 (m, 2H), 2.37 (s, 3H), 2.51-2.61 (m, 10H), 3.83 (t, *J* = 6.2 Hz, 2H), 7.31-7.36 (m, 2H), 7.54-7.61 (m, 2H), 7.93 (s, 1H).

**2-((2-Acetoxybenzoyl)oxy)ethyl 2-oxo-2*H*-chromene-3-carboxylate (5i).** 2-Bromoethyl 2-oxo-2*H*-chromene-3-carboxylate **4a** (500 mg, 1.68 mmol) and aspirin (900 mg, 5.04 mmol) was solved in MeCN, and subsequently, diethylamine (2.1 mL, 10.08 mmol) was added. The reaction mixture was stirred at 70 °C for 8 h. At the end of reaction, the mixture filtered and concentrated under reduced pressure. Compound **5i** (500 mg) was obtained as white solid by silica gel chromatography (petroleum ether/EtOAc, 15:1), yield 76.8%; mp 103.6-105.0 °C; ESI-MS *m/z*: 397.0913 $[M+H]^+$ (Calcd for $C_{21}H_{16}O_8$ 397.0845); IR 2955, 2854($CH_2$), 1747(C=O), 1609, 1563, 1484 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.03 (d, *J* = 7.8 Hz, 1H), 7.72-7.57 (m, 3H), 7.43-7.32 (m, 3H), 7.12 (d, *J* = 8.1 Hz, 1H), 4.40 (t, *J* = 6.6 Hz, 2H), 4.32 (t, *J* = 6.6 Hz, 2H), 2.37 (s, 3H), 1.85-1.79 (m, 5H), 1.60-1.49 (m, 4H).

**3-((2-Acetoxybenzoyl)oxy)propyl 2-oxo-2*H*-chromene-3-carboxylate (5j).** Reference to the synthetic method of compound **5i**, reaction of **4b** (500 mg, 1.61mmol) with aspirin (869 mg, 4.83 mmol) gave compound **5j** (532 mg, 80.8%) as white solid, mp 99.2-99.9 °C; ESI-MS *m/z*: 411.1071 $[M+H]^+$ (Calcd

for $C_{22}H_{18}O_8$ 411.1002); IR 2997, 2911($CH_2$), 1762(C=O), 1608, 1566, 1486 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.04 (d, *J* = 7.8 Hz, 1H), 7.69-7.55 (m, 3H), 7.39-7.31 (m, 3H), 7.11 (d, *J* = 8.1 Hz, 1H), 4.45 (t, *J* = 6.5 Hz, 2H), 4.37 (t, *J* = 6.5 Hz, 2H), 2.37 (s, 3H), 1.93-1.85 (m, 4H), 1.74-1.56 (m, 2H).

**4-((2-Acetoxybenzoyl)oxy)butyl 2-oxo-2*H*-chromene-3-carboxylate (5k).** Compound **5k** was synthesized according to the procedure of synthesizing compound **5i**. Aspirin (831 mg, 4.62 mmol) and **4c** (500 mg, 1.54 mmol) were used as reactants. Compound **5k** was obtained as white solid (519 mg, 79.6%), mp 91.7-92.4 °C; ESI-MS *m/z*: 425.1227 $[M+H]^+$ (Calcd for $C_{23}H_{20}O_8$ 425.1158); IR 2969, 2897($CH_2$), 1754(C=O), 1610, 1570, 1485 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.02 (d, *J* = 8.8 Hz, 1H), 7.64 (m, 2H), 7.56 (t, *J* = 7.7 Hz, 1H), 7.33 (m, 3H), 7.10 (d, *J* = 8.1 Hz, 1H), 4.39 (m, 4H), 2.35 (s, 3H), 1.94 (m, 4H).

**5-((2-Acetoxybenzoyl)oxy)pentyl 2-oxo-2*H*-chromene-3-carboxylate (5l).** Compound **5l** was synthesized according to the procedure of synthesizing compound **5i**. Aspirin (793 mg, 4.41 mmol) and **4d** (500 mg, 1.47 mmol) were used as reactants and compound **5l** was obtained as white solid (517 mg, 80.1%), mp 80.5-81.1 °C; ESI-MS *m/z*: 439.1375 $[M+H]^+$ (Calcd for $C_{24}H_{22}O_8$ 439.1315); IR 2958, 2894($CH_2$), 1739(C=O), 1611, 1582, 1485 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.04 (d, *J* = 7.8 Hz, 1H), 7.73-7.51 (m, 3H), 7.42-7.31 (m, 3H), 7.11 (d, *J* = 8.1 Hz, 1H), 4.41 (t, *J* = 6.5 Hz, 2H), 4.33 (t, *J* = 6.5 Hz, 2H), 2.37 (s, 3H), 1.91-1.82 (m, 4H), 1.72-1.55 (m, 2H).

**6-((2-Acetoxybenzoyl)oxy)hexyl 2-oxo-2*H*-chromene-3-carboxylate (5m).** Compound **5m** was synthesized according to the procedure of synthesizing compound **5i**. Aspirin (766 mg, 4.26 mmol) and **4e** (500 mg, 1.42 mmol) were used as reactants. Compound **5m** was obtained as white solid (508 mg, 79.4%); mp 65.3-66.0 °C; ESI-MS *m/z*: 453.1540 $[M+H]^+$ (Calcd for $C_{25}H_{24}O_8$ 453.1471); IR 2926, 2855($CH_2$), 1723(C=O), 1609, 1568, 1486 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.03 (d, *J* = 7.8 Hz, 1H), 7.71-7.61 (m, 2H), 7.57 (t, *J* = 7.7 Hz, 1H), 7.42-7.30 (m, 3H), 7.11 (d, *J* = 8.1 Hz, 1H), 4.38 (t, *J* = 6.6 Hz, 2H), 4.31 (t, *J* = 6.6 Hz, 2H), 2.37 (s, 3H), 1.82 (dt, *J* = 13.1, 6.6 Hz, 4H), 1.63-1.47 (m, 4H).

**Biological activity test**

Rabbit blood was obtained by cardiac puncture and transferred to a test tube containing 3.8% sodium citrate aqueous solution 1 part citrate : 9 part blood. Platelet-rich plasma (PRP) was obtained following blood sample centrifugation at 500 rpm for 10 min. The PRP samples were again centrifuged at 3000 rpm for 10 min to obtain plateletpoor plasma (PPP). Then platelet aggregation was performed, following Born's turbidimetric method. All platelet preparations were conducted at room temperature. The 260 μL PRP and 30 μL sample solution were added into the test tube and incubated on 37 °C for 5 min. 10 μM ADP was the inducer. Maximal platelet aggregation was observed and recorded within 5 min. The effects of test compounds were assessed as percent inhibition compared with the control sample. In blank tests,

DMSO 1% was as the control.

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