SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL COUMARIN DERIVATIVES AS ANTIPLATELET AGENTS

Peng Huang, Lei-Lei Gao, Zhuo Zhang, Jing-Ru Liu, and Li-Qin He*

School of Pharmacy, Anhui University of Chinese Medicine, Hefei 230012, China; E-mail: hlq661125@126.com; ‡These Authors contributed equally to the work.

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 worldwide. Platelet aggregation plays an important role in thrombotic processes. 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. 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, antiplatelet, anticoagulant, antihyperglycemic and antitumor etc. Moreover, some coumarins possess low cytotoxicity on and excellent cell permeability, and have a relatively low molecular weight suitable for modification. 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.\textsuperscript{11,12} Compound A (Figure 1)\textsuperscript{13} amide-substituent at the C3-position of coumarin, had better antithrombotic activity and less bleeding 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.\textsuperscript{14} Among them, Compound B (Figure 1) showed the highest inhibitory effect. These results suggest that the presence of a substituted group at C-3 of coumarin is beneficial for antiplatelet activity.

![Figure 1. Chemical structures of carbochromene, compound A and compound B](image)

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.\textsuperscript{15-17} The antiplatelet properties of 2-aminochromones have been investigated by many researchers.\textsuperscript{18-21} 2-Morpholinochromones have been suggested to be the most potent analogue.\textsuperscript{16} In view of this, we designed and synthesized a series of coumarin derivatives containing various substituted amines, such as diethylamine, piperidin, \textit{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 \textmu M. 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 \textbf{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 1H 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, H2O, EtOH, reflux, 15 min; 2) HCl; (iii)Br-(CH2)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 IC50 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 (IC50 = 0.89 mM).
Table 1. Effect of the target compounds 5a-m (0.1mmol/L) on the ADP-induced platelet aggregation in vitro

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

*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

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>———</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0.89</td>
</tr>
<tr>
<td>5c</td>
<td>0.45</td>
</tr>
<tr>
<td>5e</td>
<td>0.38</td>
</tr>
</tbody>
</table>

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
Chemistry. Melting points were measured using a WRS-1B apparatus without any correction. 1H 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 CDCl3 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-2H-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 Na2SO4, 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 C19H37N2O 296.9684); IR 3051, 2943, 1762, 1715, 1609, 1567, 1454 cm⁻¹; 1H NMR (400 MHz, CDCl3) 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-2H-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_{2}O_{3} 310.9841); IR 3065, 2943, 1752, 1716, 1610, 1590, 1456 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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-2H-chromene-3-carboxylate (5a). 2-Bromoethyl 2-oxo-2H-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, CDCl₃) 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-2H-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, 1456, 1245, 1212 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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-2H-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, 2970, 2930, 1757, 1702, 1615, 1566, 1456, 1245, 1212 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (CDCl₃) 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-2H-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, CDCl₃) 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-2H-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,
3-Morpholinopropyl 2-oxo-2H-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 C17H19NO5 318.1263); IR 3056, 2975, 2936, 1770, 1606, 1560, 1449, 1255, 1242 cm⁻¹; 1H NMR (400 MHz, CDCl₃) 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), 8.57 (s, 1H).

2-(4-Methylpiperazin-1-yl)ethyl 2-oxo-2H-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 C17H20N2O4 317.1423); IR 3050, 2924, 2854, 1769, 1716, 1610, 1568, 1456, 1246, 1218 cm⁻¹; 1H NMR (400 MHz, CDCl₃) 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-2H-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 solid in 49% yield, mp 55.7-56.3 °C; ESI-MS m/z: 331.3398 [M+H]+ (Calcd for C18H22N2O4 331.1580); IR 3045, 2940, 2836, 1766, 1706, 1609, 1568, 1455, 1251, 1214 cm⁻¹; 1H NMR (400 MHz, CDCl₃) 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-2H-chromene-3-carboxylate (5i). 2-Bromoethyl 2-oxo-2H-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 C21H16O8 397.0845); IR 2955, 2854(CH₃), 1747(C=O), 1609, 1563, 1484 cm⁻¹; 1H NMR (400 MHz, CDCl₃) 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-2H-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$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 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-2H-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$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 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-2H-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$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 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-2H-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$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 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 platelet-poor 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.

ACKNOWLEDGEMENTS
This study was supported by a grant from the Nature and Science Foundation of Department of Education, Anhui province in China (No. KJ2010A204; No. KJ2013A168).

REFERENCES