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Research Article

Design, Synthesis, and *In Vitro* Antiplatelet Aggregation Activities of Ferulic Acid Derivatives

Peng-Xuan Zhang, Hang Lin, Cheng Qu, Yu-Ping Tang, Nian-Guang Li, Jun Kai, Guanxiong Shang, Baoquan Li, Li Zhang, Hui Yan, Pei Liu, and Jin-Ao Duan

Jiangsu Collaborative Innovation Center of Chinese Medicine Resources Industrialization, Jiangsu Key Laboratory for High Technology Research of TCM Formulae, and National and Local Collaborative Engineering Center of Chinese Medicine Resources, Nanjing University of Chinese Medicine, Nanjing 210023, China

Correspondence should be addressed to Yu-Ping Tang; yupingtang@njucm.edu.cn
and Nian-Guang Li; linianguang@njutcm.edu.cn

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In order to discover new compounds with antiplatelet aggregation activities, some ferulic acid (FA) derivatives were designed and synthesized. The *in vitro* antiplatelet aggregation activities of these compounds were assessed by turbidimetric test. The results showed that the target compound **7f** had potent antiplatelet aggregation activity with its IC_{50} 27.6 μmol/L, and **7f** can be regarded as a novel potent antiplatelet aggregation candidate.

1. Introduction

Thrombosis is mainly distributed over the visible part of the circulation of the blood of human or animal body, including the abnormal agglutination of blood clot and the condensed sediment of the inner wall of the heart [1, 2]. Thrombotic diseases can be divided into arterial thrombosis, venous thrombosis, and capillary thrombosis [3, 4].

With the rapid development of social life and the raising level of people's daily life, the prevalence rate of thrombotic disease has been rising, which has caused widespread concern in society [5]. Up to two million people lost their precious lives because of the cardiac and cerebral vascular diseases per year [6, 7].

The first choice for the treatment of thrombotic disease is antithrombotic drug. It can be divided into three categories, which are anticoagulant drugs, antiplatelet drugs, and thrombolytic drugs. Anticoagulant drugs and antiplatelet aggregation drugs can be used for the prevention and treatment of thrombosis, and thrombolytic drugs can be mainly used to cure thrombus dissolution [8]. According to statistics, antiplatelet aggregation drugs have 64.49% market share, and they are the first choice medicine and the mainstream first-line drugs for prevention and cure of arterial thrombus diseases [9], so the research on antiplatelet aggregation drugs is very important [10].

Antiplatelet drugs (Figure 1) are commonly divided into cyclooxygenase (COX-1) inhibitors, adenosine diphosphate (ADP) antagonists, and platelet membrane glycoprotein IIb/IIIa receptor antagonists [11]. The COX-1 inhibitors include aspirin (**1**). Although they have good activities on thromboembolic diseases induced by platelet hyperfunction, acute myocardial infarction, and unstable angina [12], the negative side effects are that they can injure gastric mucosa, decrease the number of platelets and white cells, irritate gastrointestinal and induce asthma [13–15]. Ticlopidine (**2**) and Clopidogrel (**3**) are ADP inhibitors to treat multiple thromboses [16, 17], including peripheral vascular disease, microvascular disease, venous thrombosis caused by diabetes [18], myocardial ischemia [19], and bypass thrombosis [20]. However, they also have adverse effects on digestive system and so on [21]. Tirofiban (**4**), which is a platelet membrane glycoprotein IIb/IIIa receptor antagonist on the treatment of atherectomy and coronary artery bypass grafting (CABG) [22], has exhibited some side effects with bleeding and platelet purpura [23].

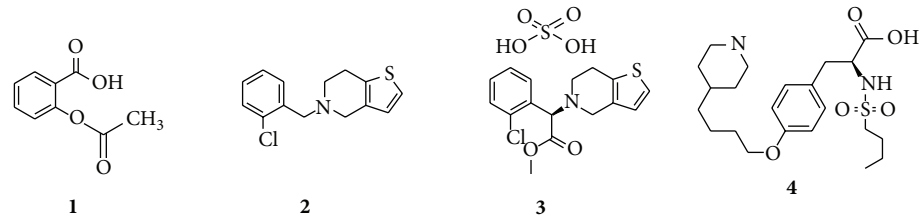
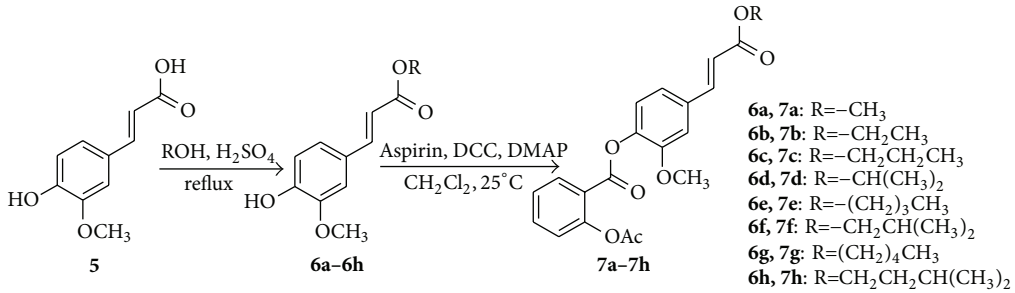


Figure 1: Structures of antiplatelet drugs.



Scheme 1: Synthetic route for the construction of **6a**–**6h** and **7a**–**7h**.

Therefore, developing antithrombotic drugs with little side effects is still an arduous task for the current pharmacy workers. Traditional Chinese herbs can be regarded as rich source for drug lead compound discovery because they have been used clinically for many years. Ferulic acid (FA) (**5**) (Scheme 1) is a kind of phenolic acids and present as glycoside, connecting with some arabinose residues on the side chain of the arabinoxylan in the cell wall [24, 25] and it is a very common secondary metabolite, present in many plants and food including legumes, cocoa, fruits, oils, herbs, spices, nuts, vegetables, and cereals. It is also present in beverages such as coffee, beer, and wine [26–28]. FA has been used to treat thrombosis, because it can activate blood circulation to dissipate blood stasis [29–31]. However, FA can be metabolized by the gut microflora undergoing hydrogenation of α,β-unsaturated bond, demethylation, and selective dehydroxylation at C4 to form a plethora of related phenolic metabolites [32]. Besides, the disadvantage of FA is that it is difficult to pass through the biological membrane lipid bilayer because of its strong hydrophilicity, which can weaken its activities [33].

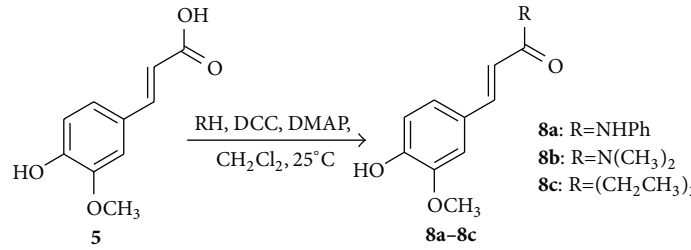
In order to improve the lipophilicity of FA, some FA esters (**6a**–**6h**) were synthesized through the reaction of FA and alcohols. In addition, some diesters (**7a**–**7h**) were obtained after **6a**–**6h** condensed with aspirin. Furthermore, some FA amides (**8a**–**8c**) and FA salts (**9a**–**9c**) were also synthesized in order to study the structure-activity relationships of FA in antiplatelet aggregation.

2. Materials and Methods

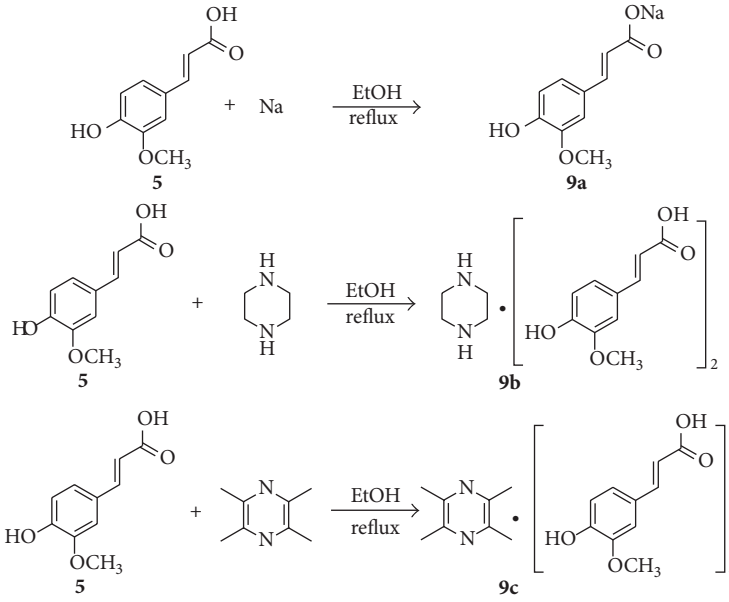
2.1. Materials. Reagents and solvents were purchased from commercial sources and used without further purification unless otherwise specified. Air- and moisture-sensitive liquids and solutions were transferred *via* syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation below 45°C at approximately 20 mm Hg. All nonaqueous reactions were carried out under anhydrous conditions using flame-dried glassware within an argon atmosphere in dry and freshly distilled solvents, unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.15~0.20 mm Yantai silica gel plates (RSGF 254) using UV light as the visualizing agent. Chromatography was performed on Qingdao silica gel (160~200 mesh) using petroleum ether (60~90) and ethyl acetate as the eluting solvent. The melting points (mp) were measured on a WRS-1B apparatus and were not corrected. ^{1}H NMR spectra were obtained using Bruker AV-300 (300 MHz) and AV-500 (500 MHz). Chemical shifts were recorded in ppm downfield from tetramethylsilane. J values were given in Hz. Abbreviations used were s (singlet), d (doublet), t (triplet), q (quartet), b (broad), and m (multiplet). ESI-MS spectra were recorded on a Waters Synapt HDMS spectrometer.

2.2. Chemistry. In general, FA esters were synthesized as described in Scheme 1 [34]. FA (**5**) was firstly esterified and converted to its esters **6a**–**6h** by refluxing it with series of alcohols using the concentrated sulfuric acid as a catalyst. Subsequently, the diesters **7a**–**7h** were obtained after **6a**–**6h** reacted with aspirin under the catalysts of dicyclohexylcarbodiimide (DCC) and 4-N,N-dimethylaminopyridine (DMAP) in dichloromethane at room temperature.

Secondly, FA amides **8a**–**8c** were produced after FA (**5**) was amidated with phenylamine, dimethylamine, and diethylamine, respectively, and catalyzed by DCC and DMAP at room temperature in dichloromethane (Scheme 2) [35].



Scheme 2: Synthetic route for the construction of **8a**–**8c**.



Scheme 3: Synthetic route for the construction of **9a**–**9c**.

At last, FA salts **9a**–**9c** were synthesized by refluxing FA (**5**) with sodium, piperazine, and ligustrazine in ethanol, respectively (Scheme 3) [36].

2.3. Antiplatelet Aggregation Assays. The *in vitro* activity studies on antiplatelet aggregation of the target compounds have been done by using turbidimetric test: carotid arterial blood gathering from the rabbit was collected into tubes which was containing 3.8% sodium citrate (1 : 9, v/v). Platelet aggregation was assessed in platelet-rich plasma (PRP), obtained by centrifugation of citrated whole blood at room temperature for 10 min (800 g). The aggregation rate was measured by platelet aggregation analyzer after stimulation with ADP (5 μM) using platelet-poor plasma (PPP) to set zero. The PRP was obtained by centrifugation of PRP at room temperature for 15 min (3000 g). The solution of the compounds dissolved in DMSO or Chloroform (5 μL) was added into PRP (200 μL), and the same volume of DMSO or Chloroform with no test compound was added as a reference sample (according to the preexperiment, 5 μL DMSO or Chloroform shows no significant effect on the platelet aggregation). After 2 min of incubating, we assessed the platelet aggregation activities and calculated the percentage inhibition of platelet aggregation using the corresponding ADP.

3. Results and Discussion

*3.1. General Procedure for the Preparation of **6a**–**6h**.* To a stirring solution of **5** (2.0 g, 10.31 mmoL) in concentrated sulfuric acid (1.8 mmoL) series of alcohols (30 mL) was added, respectively; the reaction mixture was refluxed for 2.5 h, neutralized by 10% Na_2CO_3 solutions, and extracted with EtOAc subsequently. The ethyl acetate layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated, and then the crude materials were purified by column chromatography (ethyl acetate-petroleum ether).

*(E)-3-(3-Methoxyl-4-hydroxy phenyl) Methyl Acrylate (**6a**).* Yellow oil, 79% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.80 (s, 3H, OCH_3), 3.91 (s, 3H, $COOCH_3$), 6.29 (d, J = 15.9 Hz, 1H, C=CH), 6.88 (d, J = 8.0 Hz, 1H, Ar-H), 7.04 (m, 2H, Ar-H), 7.62 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 209 $[M+H]^+$.

*(E)-3-(3-Methoxyl-4-hydroxy phenyl) Ethyl Acrylate (**6b**).* Yellow oil, 81% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.31 (t, J = 7.1 Hz, 3H, CH_3), 3.92 (s, 3H, OCH_3), 4.25 (q, J = 7.1 Hz, 2H, $COOCH_2$), 6.29 (d, J = 15.9 Hz, 1H, C=CH), 6.91 (d, J = 8.0 Hz, 1H, Ar-H), 7.06 (m, 2H, Ar-H), 7.61 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 223 $[M+H]^+$.

*(E)-3-(3-Methoxyl-4-hydroxy phenyl) Propyl Acrylate (**6c**)*. Yellow oil, 77% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.01 (t, J = 6.8 Hz, 3H, CH_3), 1.75 (m, 2H,CH_2), 3.94 (s, 3H, OCH_3), 4.15 (t, J = 7.4 Hz, 2H, $COOCH_2$), 6.31 (d, J = 15.9 Hz, 1H, C=CH), 6.93 (d, J = 8.0 Hz, 1H, Ar-H), 7.05 (m, 2H, Ar-H), 7.63 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 237 $[M+H]^+$.

*(E)-3-(3-Methoxyl-4-hydroxy phenyl) Isopropyl Acrylate (**6d**)*. Red solid, 69% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.32 (d, J = 6.2 Hz, 6H, $2CH_3$), 3.92 (s, 3H, OCH_3), 5.14 (m, 1H, COOCH), 6.27 (d, J = 15.9 Hz, 1H, C=CH), 6.91 (d, J = 8.0 Hz, 1H, Ar-H), 7.03 (m, 2H, Ar-H), 7.60 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 237 $[M+H]^+$.

*(E)-3-(3-Methoxyl-4-hydroxy phenyl) Butyl Acrylate (**6e**)*. Red oil, 73% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.97 (t, J = 7.3 Hz, 3H, CH_3), 1.45 (m, 2H, CH_2), 1.69 (m, 2H, CH_2), 3.93 (s, 3H, OCH_3), 4.20 (t, J = 6.6 Hz, 2H, $COOCH_2$), 6.29 (d, J = 15.9 Hz, 1H, C=CH), 6.92 (d, J = 8.0 Hz, 1H, Ar-H), 7.04 (m, 2H, Ar-H), 7.61 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 251 $[M+H]^+$.

*(E)-3-(3-Methoxyl-4-hydroxy phenyl) Isobutyl Acrylate (**6f**)*. Yellow oil, 63% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.98 (d, J = 6.8 Hz, 6H, $2CH_3$), 2.03 (m, 1H, CH), 3.92 (s, 3H, OCH_3), 3.98 (d, J = 6.6 Hz, 2H, $COOCH_2$), 6.30 (d, J = 15.9 Hz, 1H, C=CH), 6.91 (d, J = 8.0 Hz, 1H, Ar-H), 7.05 (m, 2H, Ar-H), 7.62 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 251 $[M+H]^+$.

*(E)-3-(3-Methoxyl-4-hydroxy phenyl) Amyl Acrylate (**6g**)*. Yellow oil, 58% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.88 (m, 3H, CH_3), 1.36 (m, 4H, $2CH_2$), 1.70 (m, 2H, CH_2), 3.91 (s, 3H, OCH_3), 4.20 (t, J = 6.7 Hz, 2H, $COOCH_2$), 6.28 (d, J = 15.9 Hz, 1H, C=CH), 6.90 (d, J = 8.0 Hz, 1H, Ar-H), 7.04 (m, 2H, Ar-H), 7.60 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 265 $[M+H]^+$.

*(E)-3-(3-Methoxyl-4-hydroxy phenyl) Isoamyl Acrylate (**6h**)*. Yellow oil, 46% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.95 (d, J = 6.6 Hz, 6H, $2CH_3$), 1.56 (m, 2H, CH_2), 1.81 (m, 1H, CH), 3.91 (s, 3H, OCH_3), 4.22 (t, 2H, J = 6.8 Hz, $COOCH_2$), 6.29 (d, J = 15.9 Hz, 1H, C=CH), 6.91 (d, J = 8.0 Hz, 1H, Ar-H), 7.07 (m, 2H, Ar-H), 7.61 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 265 $[M+H]^+$.

*3.2. General Procedure for the Preparation of **7a–7h**.* To a stirring solution of **6** (1.0 mmoL), DCC (618 mg, 3 mmoL), and DMAP (73.2 mg, 0.60 mmoL) in dichloromethane (50 mL) aspirin (540 mg, 3 mmoL) was added at room temperature. Then the mixture was stirred at 25°C for 3-4 h. After being filtered and concentrated, the target compounds were obtained by column chromatography (ethyl acetate-petroleum ether).

*(E)-2-Acetoxyl Benzoic Acid-[4-(3-methoxyl-3-oxo-1-propenyl)-2-methoxyl] Phenyl Ester (**7a**)*. Yellow solid, 42% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.33 (s, 3H, $COCH_3$), 3.86 (s, 3H, OCH_3), 3.89 (s, 3H, $COOCH_3$), 6.44 (d, J = 15.9 Hz, 1H, C=CH), 7.20 (m, 4H, Ar-H), 7.44 (m, 1H, Ar-H), 7.70 (m, 2H, Ar-H), 8.28 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 371 $[M+H]^+$.

*(E)-2-Acetoxyl Benzoic Acid-[4-(3-oxethyl-3-oxo-1-propenyl)-2-methoxyl] Phenyl Ester (**7b**)*. Yellow solid, 48% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.37 (t, J = 7.1 Hz, 3H, CH_3), 2.32 (s, 3H, $COCH_3$), 3.88 (s, 3H, OCH_3), 4.28 (q, J = 7.1 Hz, 2H, $COOCH_2$), 6.42 (d, J = 15.9 Hz, 1H, C=CH), 7.18 (m, 4H, Ar-H), 7.39 (m, 1H, Ar-H), 7.68 (m, 2H, Ar-H), 8.26 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 385 $[M+H]^+$.

*(E)-2-Acetoxyl Benzoic Acid-[4-(3-propoxy-3-oxo-1-propenyl)-2-methoxyl] Phenyl Ester (**7c**)*. Yellow solid, 43% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.00 (t, J = 7.4 Hz, 3H, CH_3), 1.74 (m, 2H, CH_2), 2.30 (s, 3H, $COCH_3$), 3.86 (s, 3H, OCH_3), 4.18 (t, J = 6.8 Hz, 2H, $COOCH_2$), 6.42 (d, J = 15.9 Hz, 1H, C=CH), 7.8 (m, 4H, Ar-H), 7.39 (m, 1H, Ar-H), 7.68 (m, 2H, Ar-H), 8.26 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 399 $[M+H]^+$.

*(E)-2-Acetoxyl Benzoic Acid-[4-(3-isopropoxy-3-oxo-1-propenyl)-2-methoxyl] Phenyl Ester (**7d**)*. Yellow oil, 46% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.32 (d, J = 6.2 Hz, 6H, $2CH_3$), 2.30 (s, 3H, $COCH_3$), 3.84 (s, 3H, OCH_3), 5.15 (m, 1H, COOCH), 6.39 (d, J = 15.9 Hz, 1H, C=CH), 7.14 (m, 4H, Ar-H), 7.36 (m, 1H, Ar-H), 7.66 (m, 2H, Ar-H), 8.24 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 399 $[M+H]^+$.

*(E)-2-Acetoxyl Benzoic Acid-[4-(3-butoxy-3-oxo-1-propenyl)-2-methoxyl] Phenyl Ester (**7e**)*. Yellow solid, 41% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.97 (t, J = 7.4 Hz, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.69 (m, 2H, CH_2), 2.28 (s, 3H, $COCH_3$), 3.82 (s, 3H, OCH_3), 4.21 (t, J = 6.6 Hz, 2H, $COOCH_2$), 6.40 (d, J = 15.9 Hz, 1H, C=CH), 7.16 (m, 4H, Ar-H), 7.37 (m, 1H, Ar-H), 7.64 (m, 2H, Ar-H), 8.22 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 413 $[M+H]^+$.

*(E)-2-Acetoxyl Benzoic Acid-[4-(3-isobutoxy-3-oxo-1-propenyl)-2-methoxyl] Phenyl Ester (**7f**)*. Yellow solid, 45% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.99 (d, J = 6.8 Hz, 6H, $2CH_3$), 2.01 (m, 1H, CH), 2.30 (s, 3H, $COCH_3$), 3.84 (s, 3H, OCH_3), 4.00 (d, J = 6.6 Hz, 2H, $COOCH_2$), 6.42 (d, J = 15.9 Hz, 1H, C=CH), 7.16 (m, 4H, Ar-H), 7.37 (m, 1H, Ar-H), 7.66 (m, 2H, Ar-H), 8.24 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 413 $[M+H]^+$.

*(E)-2-Acetoxyl Benzoic Acid-[4-(3-pentyloxy-3-oxo-1-propenyl)-2-methoxyl] Phenyl Ester (**2g**)*. Yellow solid, 42% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.95 (t, J = 6.8 Hz, 3H, CH_3), 1.39 (m, 2H, CH_2), 1.55 (m, 2H, CH_2), 1.72 (m, 2H, CH_2), 2.30 (s, 3H, $COCH_3$), 3.86 (s, 3H, OCH_3), 4.21 (t, J = 6.8 Hz, 2H, $COOCH_2$), 6.41 (d, J = 15.9 Hz, 1H, C=CH), 7.16 (m, 4H, Ar-H), 7.39 (m, 1H, Ar-H), 7.66 (m, 2H, Ar-H), 8.25 (d, J = 15.9 Hz, 1H, CH=C); MS ESI-MS: m/z 427 $[M+H]^+$.

*(E)-2-Acetoxyl Benzoic Acid-[4-(3-isopentylvoxy-3-oxo-1-propenyl)-2-methoxyl] Phenyl Ester (**7h**)*. Yellow solid, 42% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.97 (brs, 6H, $2CH_3$), 1.60 (m, 2H, CH_2), 1.81 (m, 1H, CH), 2.30 (s, 3H, $COCH_3$), 3.84 (s, 3H, OCH_3), 4.25 (d, J = 6.8 Hz, 2H, $COOCH_2$), 6.41 (d, J = 15.9 Hz, 1H, C=CH), 7.12 (m, 4H, Ar-H), 7.37 (m, 1H, Ar-H), 7.64 (m, 2H, Ar-H), 8.25 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 427 $[M+H]^+$.

Table 1: Effects of target compounds on the platelet aggregations induced by adenosine diphosphate (ADP) *in vitro*.

Compd.	IC_{50} (μM)	Compd.	IC_{50} (μM)	Compd.	IC_{50} (μM)
Aspirin	90.0	**6g**	>200	**7g**	79.9
FA	66.3	**6h**	>200	**7h**	35.8
6a	80.2	**7a**	87.4	**8a**	>200
6b	>200	**7b**	44.7	**8b**	>200
6c	93.0	**7c**	107.4	**8c**	82.2
6d	81.3	**7d**	>200	**9a**	>200
6e	>200	**7e**	43.1	**9b**	>200
6f	>200	**7f**	27.6	**9c**	64.3

*3.3. General Procedure for the Preparation of **8a–8c**.* To a stirring mixture of **5** (2.0 g, 10.31 mmoL), DCC (433 mg, 2.1 mmoL), and DMAP (25.5 mg, 0.21 mmoL) dissolved in dichloromethane (40 mL) amide compounds (2.1 mmoL) were added; then the mixture was stirred at room temperature for 6–8 h. After being filtered and concentrated, the crude materials were purified by column chromatography (ethyl acetate-petroleum ether).

*(E)-3-(4-Hydroxy-3-methoxy phenyl)-N-phenyl Acrylamide (**8a**).* Yellow solid, 33% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.89 (s, 3H, OCH_3), 4.13 (m, 1H, NH), 6.30 (d, J = 15.9 Hz, 1H, C=CH), 6.88–7.64 (m, 8H, Ar-H), 7.65 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 270 $[M+H]^+$.

*(E)-3-(4-Hydroxy-3-methoxy phenyl)-N,N-dimethylacrylamide (**8b**).* Yellow solid, 19.3% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.99 (s, 6H, NCH_3), 3.93 (s, 3H, OCH_3), 6.66 (d, J = 15.9 Hz, 1H, C=CH), 6.84–7.10 (m, 3H, Ar-H), 7.59 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 222 $[M+H]^+$.

*(E)-3-(4-Hydroxy-3-methoxy phenyl)-N,N-diethylacrylamide (**8c**).* Yellow solid, 23% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.63–1.94 (m, 6H, CH_3), 3.48 (d, 4H, NCH_2), 3.93 (s, 3H, OCH_3), 6.67 (d, J = 15.9 Hz, 1H, C=CH), 6.90–7.13 (m, 3H, Ar-H), 7.63 (d, J = 15.9 Hz, 1H, CH=C); ESI-MS: m/z 250 $[M+H]^+$.

*3.4. General Procedure for the Preparation of **9a–9c**.* To a stirring solution of **5** (3.9 g, 0.02 moL) dissolved in ethanol (30 mL) sodium (0.01 moL), piperazine (0.01 moL), and ligustrazine (0.01 moL) were added, respectively. The reaction mixture was refluxed for 6–8 h. After being poured into ethanol, the reaction mixture was filtered to get crude materials, which was washed with ethanol and recrystallized with water to give the target compounds.

*Sodium Ferulate (**9a**).* Light yellow needle crystal, 99% yield. ESI-MS: m/z 239 $[M+Na]^+$.

*Piperazine Ferulate (**9b**).* White needle crystal, 90% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.78 (s, 4H, NH_2), 3.80 (s, 3H, OCH_3), 6.34 (d, J = 15.9 Hz, 1H, C=CH), 6.77–7.22 (m, 3H, Ar-H), 7.38 (d, J = 15.9 Hz, 1H, CH=C), 8.50 (s, 1H, COOH), 8.55 (s, 1H, NH). ESI-MS: m/z 513 $[M+K]^+$.

*Ligustrazine Ferulate (**9c**).* Yellow solid, 23% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.37 (s, 6H, CH_3), 3.81 (s, 3H, OCH_3), 6.36 (d, J = 15.9 Hz, 1H, C=CH), 6.78 (d, 1H, Ar-H), 7.08 (d, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 7.48 (d, J = 15.9 Hz, 1H, CH=C), 9.52 (s, 1H, OH), 12.10 (s, 1H, COOH). ESI-MS: m/z 563 $[M+K]^+$.

3.5. Antiplatelet Aggregation Activity. The *in vitro* antiplatelet aggregation activities of these compounds were assessed by turbidimetric test [37, 38], and the results were shown in Table 1. In the series of monoesters **6a–6h**, the most potent compound was **6a** with its IC_{50} which was 80.2 μM. Furthermore, the carbon chain length of the alcohol played an important role in biological activities. The antiplatelet aggregation activity became weaker as the number of carbons in the side chain increased, such as in the case of **6b**, **6c**, and **6d**, where the IC_{50}s were >200, 93.0, and 81.3 μM, respectively. In the series of diesters **7a–7h** in which the aspirin was introduced, the antiplatelet aggregation activity of some diesters became weaker, such as in the case of **7a**, **7c**, and **7d**, with their IC_{50}s which were 87.4, 107.4, and >200 μM, respectively, and higher than **6a**, **6c**, and **6d**. To our delight, the antiplatelet aggregation activity of some diesters became stronger, such as in the case of **7b**, **7e**, **7f**, **7g**, and **7h**; they showed lower IC_{50}s compared to **6b**, **6e**, **6f**, **6g**, and **6h**. The most potent compound was **7f**, with its IC_{50} which was 27.6 μM; the antiplatelet aggregation activity of this compound was stronger than aspirin and FA, whose IC_{50}s were only 90.0 μM and 66.3 μM, respectively.

In the series of **8a–8c** and **9a–9c**, no compound exhibited more potent antiplatelet aggregation activity than aspirin and FA. Although compound **8c** showed the most activity in **8a–8c**, it was much weaker than FA, with its IC_{50} which was 82.2 μM. Furthermore, compound **9c** showed no more potent activity than FA with its IC_{50} which was 64.3 μM which was almost the same as that of FA.

4. Conclusion

In summary, some FA derivatives were designed and synthesized and the *in vitro* antiplatelet aggregation activities of these compounds were assessed by turbidimetric test. The target compound **7f** had a higher antiplatelet aggregation activity than **5**. Further study of **7f** on some activities [39] such as aggregatory stimulus, toxicity, and metabonomics will

be carried out, and the results will be reported in the near future.

Conflict of Interests

The authors have reported no conflict of interests.

Authors' Contribution

Peng-Xuan Zhang, Hang Lin, Cheng Qu, Yu-Ping Tang, Nian-Guang Li, Jun Kai, Guanxiong Shang, Baoquan Li, Li Zhang, Hui Yan, Pei Liu, and Jin-Ao Duan revised the paper.

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