Progress of thrombus formation and research on the structure-activity relationship for antithrombotic drugs

Xiaoan Li\textsuperscript{a,b}, Tiantian Guo\textsuperscript{c}, Qian Feng\textsuperscript{a}, Tiantian Bai\textsuperscript{a}, Lei Wu\textsuperscript{a}, Yubo Liu\textsuperscript{a}, Xu Zheng\textsuperscript{d}, Jianzhong Jia\textsuperscript{a}, Jin Pei\textsuperscript{b}, Shaoping Wu\textsuperscript{a*}, Yiming Song\textsuperscript{e*}, Yongmin Zhang\textsuperscript{a,f}

\textsuperscript{a} Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education; Biomedicine Key Laboratory of Shaanxi Province, Northwest University, Xi’an 710069, China
\textsuperscript{b} Pharmacy College, Chengdu University of Traditional Chinese Medicine, Chengdu, 611137, China
\textsuperscript{c} College of Food Science and Technology, Northwest University, Xi’an 710000, China
\textsuperscript{d} Shaanxi Institute for Food and Drug Control, Xi’an 710000, China
\textsuperscript{e} School of Chemical Engineering, Northwest University, 229 Taibai Road, Xi’an, Shaanxi 710069, China
\textsuperscript{f} Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire, UMR 8232, 4 place Jussieu, 75005 Paris, France

* Tel.: +86 029 88304569; Fax: +86 029 88304569. E-mail: wushaoping@nwu.edu.cn

\# These authors contributed equally to this work.

Abstract Many populations suffer from thrombotic disorders such as stroke, myocardial infarction, unstable angina and thromboembolic disease. Thrombus is one of the major threatening factors to human health and the prevalence of cardio-cerebrovascular diseases induced by thrombus is growing worldwide, even some persons got rare and severe blood clots after receiving the AstraZeneca COVID vaccine unexpectedly. In terms of mechanism of thrombosis, antithrombotic drugs have been divided into three categories including anticoagulants, platelet inhibitors and fibrinolytics. Nowadays, a large number of new compounds possessing antithrombotic activities are emerging in an effort to remove the inevitable drawbacks of previously approved drugs such as the high risk of bleeding, a slow onset of action and a narrow therapeutic window. In this review, we describe the causes and mechanisms of thrombus formation firstly, and then summarize these reported active compounds as potential antithrombotic candidates based on their respective mechanism, hoping to promote the development of more effective bioactive molecules for treating thrombotic disorders.

Keywords: Thrombus; Cardio-cerebrovascular diseases; Antithrombotic drugs
Contents

1. Introduction ................................................................................................................................................. 4

2. Three categories of approved antithrombotic drugs .............................................................................. 8

3. New antithrombotic molecules .............................................................................................................. 11
   3.1 Anticoagulants ...................................................................................................................................... 11
   3.2 Platelet inhibitors .................................................................................................................................. 34
   3.3 Fibrinolytics ........................................................................................................................................... 54

4. Conclusion .................................................................................................................................................. 58

Acknowledgements ........................................................................................................................................ 59

References: .................................................................................................................................................... 59
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA: arachidonic acid</td>
<td>LMWHs: low molecular weight heparins</td>
</tr>
<tr>
<td>A3P5P: adenosine 3',5'-diphosphate</td>
<td>MD: molecular dynamics</td>
</tr>
<tr>
<td>APC: activate protein C</td>
<td>NO: nitric oxide</td>
</tr>
<tr>
<td>AIR: aggregation inhibition rate</td>
<td>NSCS: N-succinyl-chitosan</td>
</tr>
<tr>
<td>APTT: activated partial thromboplastin time</td>
<td>NOSCS: N, O-succinyl-chitosan</td>
</tr>
<tr>
<td>ASA: acetylsalicylic acid</td>
<td>PA: platelet aggregation</td>
</tr>
<tr>
<td>AV-SHUNT: arteriovenous shunt</td>
<td>PAF: platelet activating factor</td>
</tr>
<tr>
<td>Ca²⁺: calcium ion</td>
<td>PAI-1: plasminogen activator inhibitor -1</td>
</tr>
<tr>
<td>COX: cyclooxygenase</td>
<td>PAR: protease activated receptor</td>
</tr>
<tr>
<td>CS: Chondroitin sulfate</td>
<td>PDE3: phosphodiesterases 3</td>
</tr>
<tr>
<td>sCS: sulfated chondroitin sulfate</td>
<td>PEG: Polyethylene glycol</td>
</tr>
<tr>
<td>CT: clotting time</td>
<td>PGG₂: prostaglandin G2</td>
</tr>
<tr>
<td>E: fucogalactan</td>
<td>PGH₂: prostaglandin H2</td>
</tr>
<tr>
<td>ECM: extracellular matrix</td>
<td>PGI₂: prostacyclin</td>
</tr>
<tr>
<td>EPS: sulfated esculin:</td>
<td>PRP: platelet-rich plasma</td>
</tr>
<tr>
<td>5-HT: 5-hydroxytryptamine</td>
<td>PT: prothrombin time</td>
</tr>
<tr>
<td>FV: factor V</td>
<td>SBDD: structure-based drug design</td>
</tr>
<tr>
<td>FVII: factor VII</td>
<td>SK: streptokinase</td>
</tr>
<tr>
<td>FXa: factor Xa</td>
<td>TFG: thrombelastography</td>
</tr>
<tr>
<td>FXII: factor XII</td>
<td>TF: tissue factor</td>
</tr>
<tr>
<td>FVIII: factor VIII</td>
<td>TO: trehalose octasulfate</td>
</tr>
<tr>
<td>GpIb: glycoprotein Ib</td>
<td>TT: thrombin time</td>
</tr>
<tr>
<td>GPIIb-IIIa: glycoprotein IIb-IIIa</td>
<td>t-PA: tissues-type plasminogen activator</td>
</tr>
<tr>
<td>HAS: human serum albumin</td>
<td>TXA₂: thromboxane A₂</td>
</tr>
<tr>
<td>HS: heparan sulfate</td>
<td>TXB₂: thromboxane B₂</td>
</tr>
<tr>
<td>sH/HS: shrimp heads/heparan sulfate</td>
<td>UK: urokinase</td>
</tr>
<tr>
<td>EC: endothelial cells</td>
<td>vWF: von Willebrand factor</td>
</tr>
<tr>
<td>WB: wheat bran</td>
<td></td>
</tr>
</tbody>
</table>
1. Introduction

Thrombus, a pathologic product of a solid mass (blood clot), can lead to myocardial infarction, ischemic cerebral infarction and other cardio-cerebrovascular diseases. From the 1990s to the present, prevalent cases suffering cardiovascular diseases increase year by year, nearly doubled from 271 million in 1990 to 523 million in 2019, and the number of deaths from 12.1 million in 1990 to 18.6 million in 2019, which account for global mortality and disability (Fig. 1) [1]. In this grim situation, efficiently suppressing thrombosis and thromboembolism can reduce these incidences [2]. Especially since December 2019, the pandemic COVID-19 has broken out across the globe, causing incalculable harm to human health and the socio-economic development. Medical research institutions around the world have been working to develop vaccines in response to the situation, in which the AstraZeneca COVID vaccine, developed in UK, is one of the world’s first approved vaccines, but was recently suspended in many countries because of the unpredictable emergence of severe thrombus, and has been banned in Denmark entirely in April 2021. Therefore, developing inexpensive, safer and effective antithrombotic drugs has become a top priority for pharmaceutical researchers, and comprehending the conditions and mechanisms of thrombosis is necessary for drug discovery.

![Graph showing prevalence and deaths from 1990 to 2020.](image)

**Fig. 1.** Total numbers of cardiovascular diseases in global from 1990 to 2019.

Blood has coagulation system and the anticoagulation system (fibrinolysis system) at the same time. In a physiological state, the coagulation factors in the blood
are continuously and limited activated, then produce thrombin and form fibrin in trace amounts, which are deposited in the intima of the cardiovascular system, whereas are dissolved by the active fibrinolytic system immediately. The coagulation system and the anticoagulation system (fibrinolysis system) are dynamically balanced, ensuring both the potential coagulability and the fluid property of the blood. However, when some factors induce the clot emerging, the above dynamic balance is upset, thereby triggering the clotting process which includes platelet aggregation, blood coagulation factor activation and sequential cascade to result in the generation of thrombin and a fibrin clot [3]. The coagulation of flowing blood caused by the activation of platelets and the activation of coagulation factors lead to thrombus formation. Endothelial injury, abnormal blood flow status and hypercoagulability are three major factors predisposing to thrombosis, in which cardiovascular intima injury as the most important and dominant condition, frequently occurring in the heart or in the arterial circulation, is responsible for most cardiovascular and cerebrovascular diseases.

Blood vessel has an inner layer (endothelial cells and subcutaneous basement membrane), a middle layer (smooth muscle cells and their stroma) and an outer layer (fibroblasts and their stroma). Endothelial cell is not just a simple lining of blood vessel, many studies have confirmed that it can secret a variety of active substances, and possesses anticoagulant and procoagulant functions, but mainly anticoagulant normally. Intact endothelial cells can secrete prostacyclin (PGI2) and nitric oxide (NO) to inhibit platelet aggregation and dilate blood vessels to resist thrombosis, and thrombin regulate protein on the surface of the endothelial cells could combine with thrombin so as to activate protein C (APC), which would degrade coagulation factor V (FV) and factor VIII (FVIII) to play anticoagulation effect under the assistance of protein S. In addition, tissue plasminogen activator (t-PA) released by endothelial cells can convert plasminogen into plasmin which degrades the water-insoluble fibrin into water-soluble degraded fragments [4]. However, once vascular injury occurs, the underlying basement membrane, termed subendothelial extracellular matrix (ECM) would be exposed, and activate platelets and coagulation factor XII (FXII), initiating the endogenous coagulation process. Simultaneously, injured endothelial cells release
tissue factor (TF) to activate coagulation factor VII (FVII), initiating the exogenous coagulation process. Blood coagulation is initiated by endogenous or exogenous pathways, resulting in coagulation cascades proposed in the early 1960s. In this theory, coagulation is a process that a series of coagulation factors are successively activated by enzymes. Upon the activation of a current coagulation factor, it can cause the activation of the next factor, and then many other coagulation factors would be activated in a certain order, step by step amplification, and finally produce thrombin which can convert fibrinogen into insoluble fibrin to form a large polymerized fibrin net, and then intertwine with platelets and blood cells together to form the thrombus (Fig.2). Moreover, impaired endothelial cells also release plasminogen activator inhibitors (PAI-1) to inhibit fibrinolytic activity and contribute to thrombus stabilization.

**Fig. 2.** The cascade mechanism of blood coagulation and the molecular targets.

Activation of platelets plays a central role in triggering the clotting process, and undergo three sequential reactions in the blood clotting (Fig. 3). (1) Adhesion: Platelets adhere to ECM and are activated by vW factor (vWF) which binds the integrin and glycoprotein Ib (GpIb) on the surface of platelets to the underlying basement. (2) Release reaction: Upon activation, platelets release granular products
including fibronectin, ADP, calcium ion (Ca\textsuperscript{2+}), histamine and 5-hydroxytryptamine (5-HT), in which Ca\textsuperscript{2+} is involved in the process of coagulation cascade, and ADP is a powerful mediator of platelet-platelet adhesion. (3) Aggregation: Under the action of thromboxane A\textsubscript{2} (TXA\textsubscript{2}) produced by Ca\textsuperscript{2+}, ADP and platelets, platelets in the bloodstream continuously adhere to each other and release more ADP and TXA\textsubscript{2} at the same time, which stimulates more and more platelets to form platelet aggregates. The platelet aggregates are reversible in the initial stage of formation, but as the exogenous coagulation process is activated, thrombin is generated and binds to the receptors on platelets to make platelet aggregates further bigger and shrink to become an irreversible platelet mass. In this process, thrombin can convert fibrinogen into insoluble fibrin to form a large polymerized fibrin net, which intertwined with platelets, a few erythrocytes and leukocytes together to be the starting point for thrombus formation. As a crucial component, thrombin is often used as clinical therapeutic target. That is to say, platelet activation, aggregation and fibrin deposition mediated by thrombin lead to thrombosis[5]. Under the synergistic action of continuously produced thrombin, ADP and TXA\textsubscript{2}, platelets in the bloodstream are constantly activated and adhere to the platelet thrombus, causing its increase in size. Blocked by the existing thrombus, blood flow swirls downstream of the clot, forming new mounds of platelets, and so forth, the thrombus gets bigger and bigger, resulting in a series of serious diseases.

Fig. 3. Process of thrombosis formation.
2. Three categories of approved antithrombotic drugs

As described above, endothelial cells can regulate the balance between anti-thrombotic activity (anticoagulant, antiplatelet and fibrinolytic effects), and prothrombotic activity (platelet, procoagulant and antifibrinolytic effects). In view of these properties, approved antithrombotic drugs have been designed and divided into three categories: anticoagulants, platelet inhibitors and fibrinolytics.

By interfering with the body’s physiological clotting process, anticoagulants in clinical application can prevent thrombus formation and limit further development of the pre-existing clots. Thrombin is produced by a series of activated coagulation factors through the initiation of endogenous and exogenous pathways, most factors of which such as FIIa, FVIIa, FIX and FX have serine protein kinase activities. Heparin, a sulfated polysaccharide, is an anticoagulant agent widely used in clinic for treating the patients with venous and arterial thrombotic disorders [6,7], it activate antithrombin III (AT III), leading to the arginine active center of antithrombin combining with serine of many coagulation factors to realize their inactivation (Fig. 4). Heparin or low molecular weight heparin such as enoxaparin, can prolong blood coagulation time with strong and swift action both in vivo and vitro, however they are not orally available and come with the most occurring bleeding side effect owing to no selectivity as well [8]. Fondaparinux (Arixtra®), a synthetic pentasaccharide derivative of heparin approved in 2001 by FDA, can selectively inhibit FXa and thereby has little bleeding risk and more effectivity compared with enoxaparin [9]. Coumarin (2H-chromen-2-one), a natural product from traditional medicine, has a wide range of pharmacological activities and its anticoagulant activity plays a significant role in thrombosis [10]. Warfarin, dicoumarol and acenocoumarol can be used as oral anticoagulants by antagonizing vitamin K, which as an essential cofactor of carboxylase can help carboxylase to carboxylate and activate the precursor of coagulation factors. It is noteworthy in clinic that these coumarins can only prevent the formation of the coagulation factor precursor but have no effect on the formed coagulation factor, which means, they would work only after the formed coagulation
factors are depleted, so they have slow onset of effects and easily lead to bleeding side effects. In addition, thrombin inhibitors like argatroban and hirudin hold important applications in the medical praxis. As a derivative of arginine, argatroban does not affect the process of thrombin formation, but can directly occupy the thrombin active-site to inhibit proteolytic action of thrombin, resulting in the inability of fibrinogen to be converted into fibrin [11]. Hirudin, a polypeptide compound, is the strongest specific inhibitor of thrombin to date. Nowadays, hirudin is often used to be a template to design hirudin variants as potential antithrombotic drugs, whereas, it is restricted in clinical practice owing to the narrow therapeutic window, rapid clearance and risk of bleeding, especially along with other antithrombotic agents [12].

Antiplatelet drugs, just as their name implies, can prevent the thrombus formation by inhibiting platelet adhesion, aggregation and release to treat cardiac or cerebral ischemic diseases and peripheral blood thrombotic diseases. In the light of different mechanism of action, three types of antiplatelet drugs including the agents that inhibit platelet metabolism, block ADP-induced platelet activation and platelet membrane glycoprotein (GP) IIb-IIIa receptor blockers are presented here (Fig. 5). In
the first class, TXA\(_2\) plays an important role in clotting formation, some percentage of cardiovascular morbidity and mortality can be attributed to TXA\(_2\) overdose [13]. Phospholipase A\(_2\) stimulates platelet membrane phospholipids to release arachidonic acid (AA), which can be converted into the prostaglandin G\(_2\) (PGG\(_2\)) and prostaglandin H\(_2\) (PGH\(_2\)) via cyclooxygenase (COX) pathway and the both are further biosynthesized into TXA\(_2\), one of the most potent vasoconstrictors and platelet aggregators, by TXA\(_2\) synthetase [14]. Thus, this class of agents that suppress on PLA\(_2\), COX and TXA\(_2\) will directly or indirectly lead to the reduction of TXA\(_2\) synthesis and inhibition of platelet metabolism, in which COX is the most frequently used as a therapeutic target. As representative drugs in this class, acetyl salicylic acid, named as aspirin, can inactivate COX by acetylating it to perform antithrombotic effect; Ridogrel is a selective TXA\(_2\) synthase inhibitor; Epoprostenol, a PGI\(_2\) congener, has opposing biological effects to that of TXA\(_2\); Dipyridamole, a strong vasodilator, can be used in combination with anticoagulant coumarins to treat thromboembolic diseases. In the second class, Ticlopidine as the representative can selectively interfere with platelet activation mediated by ADP. In the third class, upon activation of platelets, GPIIb-IIIa receptor on platelet exposes new binding site and has a high-affinity binding ability to ligands like fibrinogen, von Willebrand factor (vWF) and endothelium-induced factor and leads to the formation of platelet aggregates [15]. Accordingly, GPIIb-IIIa receptor blockers can prevent platelet from binding to these ligands and inhibit platelet aggregation at maximum extent [16].

![Chemical structures of common antiplatelet drugs.](image-url)
Fibrinolytics is also called thrombolytics because it can convert plasminogen into plasmin which rapidly hydrolyzes fibrin and fibrinogen and causes thrombolysis. The first generation of fibrinolytics was represented by streptokinase (SK) and urokinase (UK). SK, a non-enzymatic single-stranded protein combine with plasminogen to form a complex which facilitates the transformation of plasminogen into plasmin that hydrolyzes fibrin in thrombus to cause thrombolysis. UK, a glycoprotein extracted from urine, can directly transforms plasminogen into plasmin to achieve the procedure of dissolving thrombus. Although SK and UK possess potently thrombolytic activity, they have low specificity and high risk of bleeding. t-PA as a representative in second generation of fibrinolytics has a good selectivity and dispense with the drawbacks of the first generation [17]. Alteplase, a kind of glycoprotein containing 526 amino acids, is the most commonly used thrombolytic drug and the only one approved by the FDA for the treatment of acute ischemic stroke [18].

In recent years, the discovery of novel antithrombotic molecules has attracted considerable attention in medicinal chemistry field. With regard to diverse medicinal mechanism, these new molecules are also divided into anticoagulants, platelet inhibitors and fibrinolytics.

3. New antithrombotic molecules

3.1 Anticoagulants

3.1.1 Coumarin-substituted derivatives

Amin et al. [19] designed and synthesized a battery of 6-heterocycle substituted coumarin derivatives 1, 2a, 2b, 3a-3e for the anticoagulant effects using warfarin as reference substance (Fig. 6). In a mouse thrombus model, most of compounds showed similar clotting time (CT) and prothrombin time (PT) values compared to their precursor 6-aminocoumarin. The most active compound oxadiazole 1 (CT: 17.70 ± 0.88s, PT: 42.30 ± 2.32 s) showed the familiar PT value of warfarin (CT: 23.20 ± 1.25 s, PT: 42.30 ± 1.61 s). All derivatives relied on inhibition for FXa, one of the most attractive targets of oral anticoagulants, resulting in a decrease of thrombin to achieve the anticoagulant effect with less risk of bleeding [20,21].
In 2018, enlightened by the natural coumarin derivative esculin with anticoagulant activity, Ahmad et al. [22] synthesized a sulfated esculin 4 and evaluated its anticoagulant ability (Fig. 7). In vitro clotting times, PT, activated partial thromboplastin time (APTT) and thrombin time (TT) exhibited to be delayed in the presence of 4 compared with esculin. Additionally, the antithrombotic effect of 4 in vivo showed significant decreases in thrombotic rat model, suggesting that 4 can be used as a promising potential agent with dual anticoagulant and antithrombotic effects.

Bang et al. [23] synthesized a series of coumarin conjugates as indirect anticoagulants, the anticoagulant activity of these compounds were evaluated by thrombin progenitor time in mice (Fig. 8). All synthetic compounds except 5c (PT: 8.44 ± 2.35 s) had greater PTs than the reference drug warfarin (PT: 7.97 ± 1.93 s), and compounds 5a (PT: 10.88 ± 0.56 s) and 5b (PT: 13.10 ± 3.56 s) had the best anticoagulant effects.

**Fig. 6.** Chemical structures of 6-heterocycle substituted coumarin derivatives 1-3.

**Fig. 7.** Chemical structures of esculin and sulfated esculin 4.
Kasperkiewicz et al. [24] synthesized coumarin derivatives for the development of new anticoagulant potential drugs with higher efficacy, longer half-life, minor side effects and safer administration in comparison to warfarin (Fig. 9). The experimental data (bioactivity, drug-likeness and blind docking) explored by computational examinations demonstrated that all the compounds had a better safety profile than warfarin, interestingly, compound 6b (n = 1.64, n is the number of ligands binding to the protein) with two methoxy groups in 3- and 4-position in benzene ring was found to have two binding sites with human serum albumin (HSA), while the others (n < 1) were only one binding site, which can be attributed to smaller steric hindrance of 6b than that of other derivatives.

To investigate the activity of quercetin metabolites in cardiovascular disease, Shi et al. [25] synthesized quercetin derivatives (Fig. 10). The thrombus model of male white rabbit showed that in the monomethylated series, the most active compounds were 7a (TT: 19.40 ± 1.15 s, APTT: 7.60 ± 0.64 s, PT: 49.20 ± 5.51 s) and 7b (TT: 23.00 ± 1.17 s, APTT: 6.80 ± 0.38 s, PT: 72.60 ± 5.07 s), and in the dimethylated series, the optimal one was 7c (TT: 21.80 ± 0.85 s, APTT: 6.90 ± 0.48 s, 67.60 ± 6.95 s) whereas 7d (TT: 9.90 ± 1.05 s, APTT: 5.80 ± 0.36 s, 58.70 ± 3.10 s) showed no better anti-thrombic activity than quercetin, although it prolonged APTT.
3.1.2 Dabigatran derivatives

Li et al. [26] synthesized fluorinated dabigatran derivatives 8a-8d, and prodrugs 9, 10 of compounds 8a and 8c. 8a-8d with IC50 values of 3.49, 3.71, 3.23 and 3.39 nM respectively, are comparable to the reference drug dabigatran (IC50: 2.61 ± 0.84 nM) (Fig. 11). Compound 10, the prodrug of compound 8a, had a strong inhibitory effect with an inhibitory rate of 73 ± 6%, similar to dabigatran 76 ± 2% in the rat thrombus model. Moreover, they synthesized a series of fluorinated 2,5-substituted1-ethyl-1H-benzimidazole derivatives [27]. All the 9 derivatives with effective activity can be used as direct thrombin inhibitors (Fig. 12). Among them, the most potent compound 11h (IC50: 3.39 ± 0.10 nM) displayed a good inhibition rate by chromogenic assays comparable to dabigatran (IC50: 2.61 ± 0.84 nM) and most of them showed moderate to good thrombin inhibitory activity. The inhibitory activities of compounds 11a (IC50: 5.63 ± 0.10 nM), 11b (IC50: 7.26 ± 0.91 nM), 11d (IC50: 8.40 ± 0.09 nM) and 11e (IC50: 4.92 ± 0.29 nM) were better than that of argatroban (IC50: 9.46 ± 0.92 nM). According to the molecular docking model, the compound 11h had strong binding affinity with amino acid residues of thrombin active site to play its activity.
The inhibitory effects of compounds 12a-12f on thrombin were stronger than that of argatroban (IC$_{50}$: 9.46 ± 0.92 nM). In combination with the benefits of anti-thrombin drugs and fluorine atoms in the treatment process, Chen et al. [28] designed and synthesized 9 new fluoride anti-thrombin derivatives by introducing fluorine into thrombin inhibitors (Fig. 13). Compound 12a (IC$_{50}$: 3.52 ± 0.32 nM) is the most effective inhibitor of thrombosis in chromogenic assay. Molecular docking studies have found that compound 12a interacts with Asp36, His102, Trp53 and Tyr101 to form hydrogen bonds with thrombin active site.
Based on bioisosteric and scaffold hopping principles, Chen et al. [29] synthesized two dabigatran mimics (13a and 14), and in vitro antithrombotic efficacy study showed that both 13a (IC$_{50}$: 9.20 nM) and 14 (IC$_{50}$: 7.48 nM) had excellent anticoagulant effect (Fig. 14). To extend oral application, a total of 21 ester and carbamate derivatives of the two mimics mentioned above were developed by replacing benzamidine moiety of dabigatran with tricyclic fused scaffold and evaluated for the anticoagulant activity. 13b (IC$_{50}$: 0.64 ± 0.11 μM) had the most excellent property by conducting a series of experiments (including evaluation in vivo antithrombotic efficacy, mice tail bleeding test, rabbit thrombin-induced platelet aggregation assay and molecular modeling study), indicating that 13b was safer than dabigatran (IC$_{50}$: 0.34 ± 0.06 μM) in bleeding risk, with a shorter bleeding time at the same dose in mice-bleeding test.

Ren et al. [30] designed, synthesized and evaluated a class of N-ethyl dabigatran derivatives 15a-15p for inhibition of thrombin activity and most showed similar
inhibitory activity compared to dabigatran (IC_{50}: 1.20 nM) (Fig. 15). Among them, the most potent compound 15p (IC_{50}: 0.96 nM) displayed a good inhibition rate of (85.35 ± 0.72) % at concentration of 1 mg/mL for arteriovenous thrombosis based on blood clotting in rat model. Meanwhile, compounds 15a-15e, 15h and 15l-15n showed the similar inhibition activities as dabigatran, compound 15f showed weaker activity than the others. The SAR revealed that N substituted on the phenyl ring (X position) could improve the activity and introduction of the α-position methyl was more beneficial to the activity compared with p-position methyl. According to molecular docking study, compound 15p could form hydrogen bonds with Asp189 to bind the target thrombin protein strongly.

![Benzimidazole as scaffold is important](image)

Hydrophobic groups are favorable

15a: X= C, R= H; 15b: X= C, R= 2-CH₃; 15c: X= C, R= 3-CH₃; 15d: X= C, R= 4-CH₃; 15e: X= C, R= 2,3-(CH₃)₂; 15f: X= C, R= 2,4-(CH₃)₂; 15h: X= C, R= 2,6-(CH₃)₂; 15i: X= C, R= 4-F-3-CH₃; 15m: X= C, R= 2,4-F₂; 15n: X= N, R= 4-CH₃; 15p: X= N, R= 6-CH₃; Dabigatran: X = N, R = H.

Fig.15. Chemical structures of N-ethyl dabigatran derivatives 15.

### 3.1.3 Biological polysaccharide derivatives

Inspired from heparin, the widely used drug to treat thrombosis as known [31-33], Patey et al. [34] synthesized a series of heparin derivatives 16a-16h by systematic modification at the main sites of O-sulfation and N-sulfation/acetylation (Fig. 16). The anti-FXa activities of these compounds determined by the specific pentasaccharide sequence showed that compound 16a (IC_{50}: 0.03 µg/mL) had the best activity and the interference ability of these derivatives to antithrombin III/FXa complex was greatly affected by N-sulfate substitution with N-acetyl group.
Román et al. [35] extracted fuco-galactan (E) from *agaricus bisporus*, and got sulfated E after further modification to obtain more polysaccharides with anticoagulant effect. As expected, all sulfated E derivatives had different anticoagulant activities and the best one had great response on APTT but showed lesser activity compared with heparin, indicating that sulfated E maybe inhibit the intrinsic pathway rather than extrinsic pathway of blood coagulation.

Marine brown algae, a rich source of sulfated polysaccharide, can be used as a potential anticoagulant. Based on the property of brown algae, Rocha et al. [36] reported that sulfated galactofucan, a new sulfated polysaccharide from brown algae (Fig. 17), had no anticoagulant effect in vitro, whereas showed potent antithrombotic activity with maximum 8 h after administration in animal models of venous thrombosis, much longer than heparin. It was proposed that the sulfated galactofucan can facilitate the synthesis of antithrombotic heparan sulfate by endothelial cell to display the effect indirectly, indicating that it can be used as a potentially antithrombotic agent.
Brito et al. [37] found that a hybrid heparin/heparan sulfate-like compound from *Litopenaeus vannamei* shrimp heads (sH/HS) showed strong anticoagulant and anti-FXa activity with slight bleeding effect. Given strong anticoagulant and anti-FXa activity related to anti-cancer, they further studied the inhibitory potential of sH/HS on thrombin activity and its effect on tumorigenesis. In the thrombin inhibition test, sH/HS showed much greater inhibitory effect than heparin (90.70%, 55.30%, respectively) at 0.50 μg/mL concentration, while from 1.0 μg/mL concentration, both polysaccharides showed similar inhibitory effect, reaching about 95%. Furthermore, the effects of different concentrations of sH/HS or heparin on the synthesis of antithrombotic heparan sulfate in endothelial cells (EC) of rabbit aorta confluence showed that sH/HS compounds were effective as heparin. In addition, sH/HS inhibited the migration of B16F10 cells without affecting normal cellular activity, and showed a 90% decrease in tumor growth. In the case of cancer related thromboembolic diseases, these findings make sH/HS a good focus that inhibits tumor progression and development, as well as prevents coagulopathy.

β-D-xylopyranosides were reported to have antithrombotic activity in previous literature [38], and the SAR indicated that substitution of sulfur for the sugar-ring oxygen and the glycosidic oxygen atoms could lead to an upsurge of activity. 4-cyanophenyl glycoside of 1,5-dithio-β-D-xylopyranose (beciparcil) had fairly well...
activity, however, the severe side effects prevented it from being a potential clinical drug. Inspired by the antithrombotic activity of beciparcil, Bozo et al. [39] synthesized the corresponding 2-deoxy analog \(17\) in order to optimize beciparcil’s structure to reduce the side effects, with beciparcil as a control to evaluate the antithrombotic activity of \(17\) in rat model (Fig. 18). The result displayed that \(17\) (ED\(_{50}\): 7.0 mg/kg) was much safer than beciparcil (ED\(_{50}\): 25.0 mg/kg), suggesting that \(17\) could be a promising antithrombotic agent.

![Fig. 18. Chemical structures of beciparcil and 17.](image)

Xiong et al. [40] selectively synthesized two water-soluble chitosan (WSC) derivatives (Fig. 19), \(N\)-succinyl-chitosan (\(18\)) and \(N,O\)-succinyl-chitosan(\(19\)). A rabbit model of thrombus was employed to study their antithrombotic, hemolytic and anticoagulant properties which were expressed by some pharmacological parameters including APTT, PT and TT to display their anticoagulant performance in normal rabbit plasma. At a concentration of 3 mg/mL, compared to chitosan as control group (APTT = 33.80 s, PT = 12.00 s, TT = 18.10 s), \(18\) (APTT = 48.20 s, PT = 12.00 s, TT = 18.20 s) and \(19\) (APTT = 125.30 s, PT = 12.10 s, TT = 18.60 s) could prolong APTT, while no obvious increase of PT and TT was observed, suggesting that they may inhibit intrinsic rather than extrinsic coagulation pathway.
Bozo´ et al. [41] synthesized thioglycosides 20a, 20β, 21a, 21β and their D-isomers 22a, 22β, 23a, 23β (Fig. 20). The antithrombotic activity of these compounds tested in rats model showed that 20a (ED$_{50}$: 15 mg/kg) had slight more potency than beciparci (ED$_{50}$: 25 mg/kg), and all the other glycosides (ED$_{50}$: 1~5 mg/kg) were much stronger compared to beciparci.

3.1.4 Derivatives from traditional Chinese medicine

Based on good thrombin inhibitory activity of glycyrrhizin and its aglycone glycyrrhetic acid, Paula et al. [42] designed and synthesized 3 glycyrrhizin derivatives 24a-24c (Fig. 21). Compound 24b was a phthalate derivative of glycyrrhizin with better thrombin inhibition (IC$_{50}$: 114.40 ± 1.30 μM) compared to the
saponin (IC₅₀: 235.70 ± 1.40 μM) in human plasma and it can prolong TT (118.30 ± 8.00 s). According to molecular docking, it was speculated that 24b could restrain the hydrolysis of substrate S2238 (H-D-Phe-Pip-Arg-p-nitroanilide), a synthetic peptide specially designed for the catalytic site of thrombin, to exhibit anticoagulant activity.

Isosteviol, a hydrolysate derived from natural stevioside, is a tetracyclic diterpene containing beyerane skeleton, with unique advantages such as low toxicity, low cost and excellent biological characteristics. Chen et al. [43] have discussed that compound 25 contained the tetracyclic diterpene skeleton with significant anticoagulant and antiplatelet activity, and developed a series of isosteviol derivatives (25a-25r) containing effectively bioactive oxime ether (Fig. 22). Compound 25k exhibited excellent selectivity for a panel of serine proteases and the best activity against FXa (Kᵢ = 0.015 mM), as well as showed potent anticoagulant activity (p < 0.01) and antiplatelet activity (p < 0.01) according to the PT and APTT analysis in vitro and the rat ADP-induced platelet aggregation test in vitro (clopidogrel as the positive control). Furthermore, the in vivo and in vitro antithrombotic activity experiments on rats displayed compound 25k had prominent antithrombotic activity (in vitro p < 0.01, in vivo p < 0.0001), with rivaroxaban and clopidogrel as positive controls, indicating that compound 25k can inhibit the factors of various stages of thrombus formation, and could be developed as a promising antithrombotic drug.
Some previous works showed that N-[(7-amidino-2-naphthyl) methyl] aniline derivatives were effective FXa inhibitors [44,45]. YM-60828, one of the derivatives, had a good antithrombotic effect and its L-shaped conformation at the active site of FXa is considered to play an important role in the inhibitory activity. Hirayama et al. [46] synthesized the YM-60828 derivatives by cyclizing on the side chain of amino acetic acid and the central benzene ring, and discussed their activities on FXa (Fig. 23). These compounds showed great anticoagulant activities in vitro. 26a (IC_{50}: 2.10 nM), 26b (IC_{50}: 2.80 nM) and 26c (IC_{50}: 2.50 nM) showed better FXa inhibitory and anticoagulant activity compared to YM-60828 (IC_{50}: 6.00 nM). Furthermore, 26c (PT_1 = 4.00 h, PT_2 = 8.30 h) showed the best anticoagulant activity and effectively prolonged PT in mice and squirrel monkey thrombus model, compared with YM-60828 (PT_1 = 2.60 h, PT_2 = 4.30 h). Later, they obtained several effective oral FXa inhibitors by optimizing the central aniline nitrogen side chain of 7-amino-2-naphthylaniline [47], including a series of 7-amidino-2-naphthoanilide (Fig. 24). Among these compounds, 27a-27j showed favorable efficiency in inhibition for FXa, and 27h (IC_{50}: 3.90 nM) was the most effective, possessing a strongest FXa inhibitory activity in squirrel monkey model at the dose of 3 mg/kg.
Based on the previously reported compound 28 [48], Lu et al. [49] synthesized a series of novel non-amino acid thrombin inhibitors guanidine analogues 29-33 (Fig. 25). The in vitro selectivity towards thrombin and the crystal structures of these compounds were investigated, and it was proved that compound 29 had a remarkable selectivity (>10³ ×) and inhibition for thrombin, whereas even the concentration was reached to 200 μM, it had no inhibitory effect on other enzymes such as urokinase, FXa, plasmin, chymotrypsin and trypsin.
Based on the strategy of combining amidine derivatives and their prodrugs, Ishihara et al. [50] synthesized a series of \( N \)-benzenesulfonylpiperidine derivatives to excavate potent and orally active FXa inhibitors (Fig. 26). Compound 34b exhibited potent inhibitory activity against FXa (\( IC_{50} \): 13 nM) and remarkable selectivity over thrombin (> 7000-fold) within the coagulation cascade. The prodrug 34a from the parent 34b, without activity in vitro, could show an anticoagulant effect via oral administration by biological conversion into its corresponding active ingredients.

Encouraged by the combination of thrombin inhibitors and fibrinogen IIb/IIa antagonist with more benefits in previous work [51,52], Ilic et al. [53] synthesized 1,4-benzodioxine enantiomeric compounds by incorporation of anticoagulant and antiplatelet aggregation activity into the same molecule to be dual antithrombotic drugs, and investigated the effects of chirality and regional heterogeneity on these potential dual antithrombotic compounds (Fig. 27). Compound 35d was the most effective thrombin inhibitor (\( K_{i(thr)} \): 0.078 ± 0.005 \( \mu \)M), and compound 36b had the best inhibitory activity at both targets of thrombin and fibrinogen GPIIb/IIIa \( K_{i(thr)} \):
1.67 ± 0.27 μM, IC$_{50}$(GPIIb/IIIa): 0.66 ± 0.26 μM).

Fig. 27. Chemical structures of 1,4-benzodioxine enantiomeric compounds 35 and 36.

Liu et al. [54] designed and synthesized a series of new 3,4-diaminobenzoyl derivatives with highly selective inhibition on FXa (Fig. 28). The anticoagulant activity of these compounds was determined by PT in rabbit plasma in vitro. Compounds 37a (PT = 10.20 ± 2.00 s), 37b (PT = 8.80 ± 1.70 s) and 37c (PT = 9.50 ± 0.40 s) displayed significant anticoagulant activity, and among them, 37a (PT = 10.20 ± 2.00 s) showed greater inhibition than apixaban (PT = 10.00 ± 1.50 s).

Fig. 28. Chemical structures of 3,4-diaminobenzoyl derivatives 37.

Amide bond (-CONH) was exploited by Tarasov et al. [55] for linking different moiety to form a series of novel FXa inhibitors like P$_1$-(CONH)-P$_2$-(CONH)-P$_3$ (Fig. 29). Compound 38, with doubled prothrombin time (PT × 2) of 0.08 ± 0.02 μM, was the most active amide inhibitor of FXa (IC$_{50}$: 0.50 ± 0.08 nM). The half-life and activity of 38 were superior to other compounds in rabbit and mouse models of thrombus.
In the process of coagulation, prothrombin is converted into active thrombin after combination with thrombin factors, and the generated thrombin can accelerate the combination in turn. One strategy of inhibitor design relies on the powerful action between the positively charged amidinium ions on the thrombin inhibitors and the side chains of Asp189 on the serine protease (FXa is a kind of serine protease). Wiley et al. [56] used the 1,2-dibenzamidobenzene derivative 39 (a competitive inhibitor of FXa with $K_i = 1.00 \, \mu M \, [57]$) as the research object to explore the attachment of amidine substituents of compounds 40 to the inhibitor 39 in order to enhance the interaction with Asp189 (Fig. 30). According to FXa affinity, molecular simulation, evaluation of anticoagulation and antithrombotic activity, FXa affinity and selectivity can be significantly increased by the addition of an amidine to the side chain of dibenzamidobenzene derivatives. These compounds can execute effective anticoagulant and antithrombotic activity in vivo.

The formation of TF/VIIa complex by the combination of tissue factor (TF) with
serine protease factor VIIa (FVIIa) in the blood induces the formation of fibrin clots and leads to thrombus formation, and inhibition for the TF/VIIa complex could not only block the formation of thrombosis but also effectively reduce the side effect of bleeding. Analysis for the crystal structure of tripeptide-α-ketothiazole (effectively inhibiting serine protease) combined with TF/VIIa showed that 3-pyridylphenylalanine played an important role in the selectivity for the TF/VIIa complex [58], so they used benzyl-pyrazinone as the active group to synthesize a series of compounds 41a-41c [59] via structure-based drug design (SBDD) and 5-step PASP library (Fig. 31). These compounds were tested in TF/VIIa enzyme, FXa, thrombin, and trypsin respectively, it was found that 41c had the best effect, with an IC$_{50}$ value of 16 nM and more 6,250 times selectivity for TF/VIIa than FXa and thrombin.

![Fig.31. Chemical structures of benzyl-pyrazinone derivatives 41.](image)

In 2015, Xing et al. [60] synthesized the compounds 42 and 43 as well as their analogues 42a-42g, 43a-43e, respectively (Fig. 32). Betrixaban was used as a positive control to evaluate the inhibitory activity of all compounds on FXa in vitro. Most compounds had significant activity against FXa (IC$_{50}$: 0.023-1.12 µM), in which 43b was the most effective one with IC$_{50}$ value of 23.0 nM, and the selectivity of compound 43b (2 × PT = 8.70 µM) for thrombin was comparable to the reference betrixaban (2 × PT = 2.40 µM). In 2017, using compound 44 with prominent inhibitory activity against FXa as a template, they designed and synthesized a battery of 2,3-dihydroquinazolin -4(1H)-one derivatives 45a-45h for developing novel scaffold of FXa inhibitors [61] (Fig. 33). Compound 45e was the most promising agent with potent FXa inhibitory activity (IC$_{50}$: 21.00 ± 6.00 nM) along with a reduction in thrombus weight, showing similar effectiveness and safety to betrixaban.
in arteriovenous shunt (AV-SHUNT) model of rats. In addition, 45e had a remarkable selectivity against thrombin with the IC$_{50}$ value of 67.0 μM.

Although different FXa inhibitors possess various structures, most of them are constituted of three components consisting of a main scaffold and two hydrophobic moieties. With Betrixaban as a template, a series of anthranilamide derivatives 46a-46j were developed as novel FXa inhibitors [62], in which 46g had the best activity (IC$_{50}$: 3.50 nM) both in vitro and in vivo AV-SHUNT thrombosis model of rats (Fig. 34). In addition, 46g was more effective than betrixaban, with higher thrombus weight reduction and more safety at the same dose of 1 mg/kg and 5 mg/kg.
Based on a dataset containing a total of 38 new tricyclic fused oxazolidinones which have been reported by Yang [63], Xu et al. [64] conducted a series of studies using computational methods including molecular dynamics (MD) simulation, 3D-QSAR, molecular docking and Topomer CoMFA, and studied the conformation or orientation of [6,6,5] tricyclic fused oxazolidinones and offered constructive information for the design of novel FXa inhibitors (Fig. 35). Among all the compounds, compound 47a had the highest bioactivity with actual pIC50 (-logIC50) value of 8.60, and could be employed as the template molecule to illustrate the MD simulations of other compounds.

In view of the catalysis of FXa in thrombin generation, Yang et al. [65] synthesized a series of isoxazolo [5,4-d] pyrimidin-4(5H)-one derivatives, subjected to FXa inhibitors with less bleeding complications [66] (Fig. 36). Compound 48g with acetoxy group exhibited better FXa inhibitory activity (IC50: 0.013 μM) and higher selectivity than other compounds and showed almost the same activity with rivaroxaban’s (98% versus 100%) at the concentration of 0.10 μM. Moreover, 48g also displayed good anticoagulant potency in human plasma (2 × PT = 2.12 μM).
According to benzothiazole guanidine fragment with weak thrombin inhibitory activity, Michael et al. [67] designed and synthesized a series of new type of benzothiazole guanidine compounds 49a-49g with low pKₐ values (Fig. 37). Among them, 49a-49c displayed different degrees of ability to bind to thrombin, while 49d-49g displayed an effect on thrombin. In the thrombin activity assay, 49b was the most active one, 49a showed slight inhibition at high concentrations, and 49c was proved to be more effective than 49a, the unsubstituted one at -N.

Taking into account that the non-amidine inhibitor can undergo a bioconversion into the corresponding conjugate to enhance anticoagulant activity in vivo and in vitro by increasing hydrophilicity, Ishihara et al. [68] exploited antithrombotic compound 50a as a template to synthesize thrombin inhibitors with phenol as the functional group which could bind to the potential substrate to form glucuronic glycoside conjugates with high anticoagulation effect after biological transformation by oral administration (Fig. 38). The in vitro selective activity screening of human thrombin and trypsin showed that compound 50b with phenolic hydroxyl group at the 3-position (IC₅₀: 0.0040 μM) and compound 50c, its corresponding bioconverted glucuronide (IC₅₀: 0.0049 μM) had the similar activity to 50a (IC₅₀: 0.0058 μM) in
inhibition on thrombin factor FXa. The intravenous bolus injection experiment and the anticoagulant activity test in cynomolgus monkeys showed, compared with compounds 50a and 50b, the anticoagulant activity of 50c (the doses of 0.10 mg/kg) was significantly improved, and its inhibitory activity was 10 times higher than that of compound 50a (the doses of 1.00 mg/kg). Taken together, compound 50b was a promising oral anticoagulant inhibitor.

![Chemical structures of antithrombotic compound 50.](image)

FXII as a trypsin-like serine protease can promote coagulation through contact activation, and in thrombus caused by medical-instrument-mediated contact, the inhibition for FXII can effectively increase antithrombotic activity without bleeding side effects [69,70]. Bouckaert et al. [71] designed a series of oxygen-containing compounds (-OH or -COOH), using coumarin derivative (serine protease inhibitor) as the framework (Fig. 39). Moreover, as a result of lack for antithrombotic models in vivo, the FXIIa hybrid model was conducted by combining the advantages of the FXIIa homology model and the FXII protein crystal structure. The activity experiment of single concentration screening at 50 mM on FXIIa showed that 51a-51e had inhibitory activity and compound 51a had the best activity (IC$_{50}$: 5.00 μM). Further, calculations of the binding energy about these compounds to ligands showed that 51a was the most stable compound attributed to the minimum energy. Subsequently, molecular docking displayed that targeting the H1 pocket (unique to FXIIa) was the reason for the selectivity of compound 51a, according to superimpose the crystal structures of proteases (thrombin, FVIIa, and FXa) and the complex of FXIIa mixed model.
Since the C-7 carbamate side chain of vorapaxar is a metabolic site, Chelliah et al. [72] cyclized the carbamate in the form of a spiral ring to obtain a range of C7-spirocyclic analogues of vorapaxar (Fig. 40), 52a-52d (spirocyclic oxazolidinone analogues), then evaluated their activities against PAR-1 receptor by using rat thrombus model. Among those compounds, 2-cyanophenyl analogue 52c (Ki = 5.10 nM) was the most active derivative.

Lucietto et al. [73] designed and synthesized two cyclic dipeptides 53a and 53b (Fig. 41), and found that the both had significant inhibitory effects on thrombin, whereas 53b only showed a 43.30 % reduction at lower concentration (0.025 mM). In addition, 53b did not distinctly inhibit thrombin-induced platelet aggregation, but 53a did (IC50: 0.066 mM).
Based on the antithrombosis drugs reported in the literature mentioned above, we summarized their respective targets and structural characteristics, and found that FXa and thrombin as the attractive targets accounted for majority in drug development, shown in Table 1.

**Table 1. Anticoagulant compounds and their respective targets**

<table>
<thead>
<tr>
<th>NO.</th>
<th>Compound name</th>
<th>Target</th>
<th>Structural characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1~3</td>
<td>FXa</td>
<td>Coumarin derivative</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Thrombin</td>
<td>Coumarin derivative</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>HSA</td>
<td>Coumarin derivative</td>
</tr>
<tr>
<td>4</td>
<td>7~15</td>
<td>Thrombin</td>
<td>Dabigatran derivative</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>FXa</td>
<td>Biological polysaccharide derivatives</td>
</tr>
<tr>
<td>7</td>
<td>25~27</td>
<td>FXa</td>
<td>Isosteviol</td>
</tr>
<tr>
<td>8</td>
<td>28~33</td>
<td>Thrombin</td>
<td>/</td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>FXa</td>
<td>amidine</td>
</tr>
<tr>
<td>10</td>
<td>35~36</td>
<td>Thrombin, fibrinogen GPIIb/IIIa</td>
<td>1,4-benzodioxine enantiomer</td>
</tr>
<tr>
<td>11</td>
<td>37~40</td>
<td>FXa</td>
<td>/</td>
</tr>
<tr>
<td>12</td>
<td>41</td>
<td>TF/VIIa</td>
<td>benzyl-pyrazinone derivative</td>
</tr>
<tr>
<td>13</td>
<td>42~48</td>
<td>FXa</td>
<td>/</td>
</tr>
<tr>
<td>14</td>
<td>49</td>
<td>Thrombin</td>
<td>benzothiazole guanidine fragment</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>FXa</td>
<td>/</td>
</tr>
<tr>
<td>16</td>
<td>51</td>
<td>FXIIa</td>
<td>coumarin derivative</td>
</tr>
<tr>
<td>17</td>
<td>52</td>
<td>PAR-1</td>
<td>spirocyclic oxazolidinone analogue</td>
</tr>
<tr>
<td>18</td>
<td>53</td>
<td>α-glucosidase</td>
<td>cyclic dipeptide</td>
</tr>
</tbody>
</table>

### 3.2 Platelet inhibitors

Using thrombin inhibitor 54 with a new framework of tetrahydrobenzo [4, 5] imidazole and [1, 2-a] pyrazine as the lead compound, Chen et al. [74] designed and synthesized amino formic acid ester derivatives (Fig. 42). Biological activity test (in
vitro) results showed that all the compounds had certain inhibitory activities on the platelet aggregation induced by thrombin. The inhibitory activity of compound 55b (IC$_{50}$: 0.32 ± 0.10 μM), 57b (IC$_{50}$: 0.57 ± 0.11 μM) and 58b (IC$_{50}$: 0.47 ± 0.06 μM) is comparable to that of dabigatran (IC$_{50}$: 0.60 ± 0.05 μM), whereas compound 56b (IC$_{50}$: 0.11 ± 0.08 μM) is better. In vitro inhibitory activity of the four precursors (compounds 55b-58b) was generally better than that of their counterparts in the dual precursors (compounds 55a-58a). In vivo mouse model of thrombus, it is speculated that these derivatives may be hydrolyzed to produce HTMP (ethanol extraction of Chuanxiong) with antiplatelet aggregation activity.

Fig. 42. Chemical structures of amino formic acid ester derivatives 54-58.

Tian et al. [75] synthesized novel 2H-benzo[b][1,4] oxazin-3(4H)-ones (Fig. 43), in which 59a-59g had good activities for antiplatelet aggregation, and compound 59a had the strongest inhibitory effect (IC$_{50}$: 10.14 μM), but the effectiveness is lower than that of the reference material ticlopidine (IC$_{50}$: 3.18 μM) and aspirin (IC$_{50}$: 6.07 μM). Based on the SAR, the inhibition of platelet receptor-binding compounds can be ameliorated by bringing down 4- and 7-position steric hindrance. Moreover, a series of 2H-benzo[b][1,4]-oxazin-3(4H)-ones were synthesized [76] in 2014 based on previous work [75], and aspirin and ticlopidine were used as controls to evaluate the activity of inhibition on ADP-induced platelet-rich plasma in New Zealand rabbits (Fig. 44). As the strongest antiplatelet compound 60 (IC$_{50}$: 9.20 μM) is comparable to aspirin (IC$_{50}$: 7.07 μM).
Taking allylicin as the basic structure, Block et al. [77] synthesized the difluoro allylicin \( 61 \) and its related organosulfur compounds, as well as compared the effects of these fluorinated organosulfur compounds on platelet and coagulation activation by human whole blood thrombelastography (TEG) (Fig. 45). Difluoro allylicin \( 61 \) (51.0 \( \mu \)M) showed similar effect to that of allylicin (62.0 \( \mu \)M, \( p < 0.001 \)) in antiplatelet activity, whereas stronger than that of allylicin and other organic sulfur compounds in anticoagulant activity. Fluorine substitution enhanced the electrophilicity and reactivity of these compounds against the biological mercaptan groups, so the substitution of F atom and the presence of -S=O group made compound \( 61 \) the most active.
Rashid et al. [78] synthesized a novel small sulphated molecule trehalose octasulfate (TO) with anticoagulant and antiplatelet effects (Fig. 46). In vitro and in vivo coagulation tests, TO significantly prolonged the clot time of APTT and PT, as well as showed good anticoagulant property in a dose-dependent manner. When evaluating antiplatelet activity in rat thrombus model, decreased platelet aggregation was observed in blood collected from animals exposed to TO, indicating that TO has dual action mechanism and exhibits antithrombotic property.

Marrakchi et al. [79] isolated a series of lebetin (L1; 11- to 13-mer) from Macrovipera lebetina venom which can inhibit platelet aggregation induced by various agonists (Fig. 47). After purification, 66α, 66β and 66γ were obtained, which contained 13, 12 and 11 amino acid residues respectively. N - Terminal truncation of 66α was used to synthesize 66β and 66γ, and their activities were studied. Based on alanine screening, the substitution of residues and the integrity of polypeptide chains affected antiplatelet activity.

The activation of P2Y₁ receptor could give rise to ADP-mediated platelet aggregation, on the contrary, antagonism of the P2Y₁ receptor can block the aggregation and it is widely used as a validated target for antithrombosis. In 2007, Costanzi et al. [80] employed adenosine 3’,5’-diphosphate (A3P5P), a kind of competitive antagonist of human P2Y₁, as a template to synthesize a bunch of A3P5P.
derivatives (Fig. 48). N-methanocarba derivative 67c showed the best antithrombotic activity in vivo, with the IC\(_{50}\) of 8.40 nM. The R substituent of compound 67e-67g (IC\(_{50}\): 6.90 ± 2.50 µM, 1.75 ± 1.10 µM, 0.87 ± 0.18 µM) with cyano group, polar carboxyl group and carboxyl amide at the position 2 respectively had poor tolerance to P2Y\(_1\) receptor. The antagonistic activity of 2-acetylenyl substituted compound 67k (IC\(_{50}\): 0.093 ± 0.0030 µM) was much higher than that of its corresponding compound 67l (IC\(_{50}\): 2.40 ± 0.60 µM) with a longer hexynyl chain. Compound 67h (IC\(_{50}\): 0.048 ± 0.001 µM) with shorter chain length has higher affinity and potency than that of compound 67i (IC\(_{50}\): 0.45 ± 0.21 µM), 67j (IC\(_{50}\): 1.87 ± 0.59 µM), 67l (IC\(_{50}\): 2.40 ± 0.60 µM) with longer chain length. Similarly, less bulky compound 67k is more liable to bind to the P2Y\(_1\) receptor compared with compound 67m with phenyl-substituted at the position 2. Compound 68 (IC\(_{50}\): 1.56 ± 0.54 µM), a derivative of 67c with the phosphate group removed, would result in disappearance of activity, and compound 69a (IC\(_{50}\): 0.62 ± 0.040 µM) and 69b (IC\(_{50}\): 0.79 ± 1.60 µM) with halogen substitution both had the general activity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>67a</td>
<td>H</td>
</tr>
<tr>
<td>67b</td>
<td>Cl</td>
</tr>
<tr>
<td>67c</td>
<td>I</td>
</tr>
<tr>
<td>67d</td>
<td>SCH(_3)</td>
</tr>
<tr>
<td>67e</td>
<td>CN</td>
</tr>
<tr>
<td>67f</td>
<td>CONH(_2)</td>
</tr>
<tr>
<td>67g</td>
<td>CO(_2)H</td>
</tr>
<tr>
<td>67h</td>
<td>CH(_3)</td>
</tr>
<tr>
<td>67i</td>
<td>(CH(_2)(_2))CH(_3)</td>
</tr>
<tr>
<td>67j</td>
<td>trans-CH=CH(CH(_2)(_2))CH(_3)</td>
</tr>
<tr>
<td>67k</td>
<td>C=CH</td>
</tr>
<tr>
<td>67l</td>
<td>C=CH(_2)CH(_3)</td>
</tr>
<tr>
<td>67m</td>
<td>Ph</td>
</tr>
</tbody>
</table>

Fig. 48. Chemical structures of A3P5P derivatives 67-69.

Compound 70 synthesized by the predecessor is not highly selective for P2Y\(_1\) receptor (K\(_B\) = 100 nM) [81], whereas it shows significant affinity for P2X\(_1\) and P2X\(_3\) receptors, with an IC\(_{50}\) value of 1.15 and 12.90 µM, respectively [82]. To develop novel P2Y\(_1\) antagonists as new antithrombotic agents, Raboisson et al. [83] prepared
**N**\(^6\)-dimethyl-l-2'-deoxyadenosine-3',5'-bisphosphate derivatives 71a-71d, among them, 71d as an antagonist is 4 times more effective than 70 in the platelet aggregation test (Fig. 49).

Based on the SAR profile of a series of non-cyclic fatty chain nucleotide analogues explored as P2Y\(_1\) receptor antagonists, Kim et al. [84] found that compound 72e, a full antagonist for P2Y\(_1\) receptor, was the most potent one with IC\(_{50}\) value of 0.48 μM, while its corresponding 2-H derivative, compound 72d had a weaker effect than 72e (IC\(_{50}\): 1.09 ± 0.51 μM). Compound 72a and its 2-chlorine analogue compound 72b with longer alkyl chain compared to 72e, were moderately potent antagonists with the IC\(_{50}\) values of 1.60 μM and 0.84 μM, respectively (Fig. 50).

Lourenço et al. [85] synthesized a series of \(N, N'\) - disubstituted thioureas derivatives and analyzed their antithrombotic property (Fig. 51). The most active thioureas compounds 73a, 73b, 73c and 73d (IC\(_{50}\): 29.00-84.00 μM) compared to aspirin (IC\(_{50}\): 30.56 ± 0.40 μM) directly affected the formations of PGE\(_2\) and TXA\(_2\), reduced thromboxane B\(_2\) (TXB\(_2\)) production and directly inhibited COX-1 to achieve antiplatelet effect in human thrombus model.
In order to search for new platelet aggregation inhibitors, Xie et al. [86] designed and synthesized two series of 1, 4-benzodioxazine derivatives based on the previous antiplatelet drugs LX2421, which had effective antiplatelet activity and no risk of bleeding [87,88]. By introducing 1,4-bendioxazine to replace the indole structure in LX2421, new 1,4-bendioxazine derivatives were established as the platelet aggregation inhibitors (Fig. 52). Compounds 74 significantly inhibited platelet aggregation induced by ADP and thrombin, with IC₅₀ values of 41.70 μM and 22.20 μM, respectively, stronger than LX2421. Moreover, it had a strong antagonistic activity of GPII/IIIa with an IC₅₀ of 2.30 μM, better than GPIIb/IIIa antagonist RGD.

Zhang et al. [89] designed and synthesized a range of 2-hydroxytetrahydrothienopyridine derivatives using the metabolite of prasugrel as the lead compound (Fig. 53). In the pharmacological experiment of antiplatelet aggregation after oral administration of these compounds in rats, most of the
compounds except compound 75e showed better anti ADP-induced platelet aggregation activity compared with prasugrel. Compounds 75a-75d and 75f showed similar antiplatelet aggregation activity as prasugrel (platelet aggregation (PA): 22.83 ± 4.99%), and compound 75b (PA: 18.50 ± 7.94%) showed slightly stronger activity than prasugrel.

![Chemical structures of 2-hydroxytetrahydrothienopyridine derivatives](image1)

Fig.53. Chemical structures of 2-hydroxytetrahydrothienopyridine derivatives 75.

Jaiswal et al. [90] synthesized a bunch of 2-oxo-2-phenylethylidene linked 2-oxo-benzo [1,4] oxazine analogues 76 (Fig. 54). Compared with the standard drug aspirin (IC50: 21.34 ± 1.09 µg/mL), these compounds exhibited moderate to good inhibition activity on platelet aggregation induced by arachidonic acid. 76a-76f were considered promising platelet aggregation inhibitors, in which 76d (IC50: 17.96 ± 0.18 µg/mL) and 76f (IC50: 16.96 ± 0.18 µg/mL) displayed the best activity. Based on docking analysis, 76d and 76f had a strong binding affinity with anti-platelet target COX-1.

![Chemical structures of 2-oxo-2-phenylethylidene linked 2-oxo-benzo [1,4] oxazine analogues](image2)

Fig.54. Chemical structures of 2-oxo-2-phenylethylidene linked 2-oxo-benzo [1,4] oxazine analogues 76.

Using nicotinic acid as the precursor, He et al. [91] designed and synthesized 10 nicotinic acid derivatives by coupling different junction bridges with acetylsalicylic acid and nicotinic acid. In vitro biological activity study showed that all compounds
had certain anti-platelet aggregation activities. The inhibitory activity of compounds 77a-77e on platelet aggregation is higher than that of niacin (aggregation inhibition rate (AIR): 1.82%), 77a (AIR: 41.13%) and 77b (AIR: 40.73%) were stronger than that of the control drug aspirin (AIR: 32.26%), while compounds 78a-78e are lower than that of aspirin (Fig. 55). These compounds inhibited TXA2 synthesis and promoted PGI2 synthesis or blocked COX-1 activity, thereby playing an anti-platelet aggregation role.

Zhou et al. [92,93] synthesized a series of NO donor aspirin derivatives (79a-79n; 80a-80h) by connecting aspirin with furoxans nitrogen oxide and nitrate via ferulic acid or hydroxycinnamic acid (Fig. 56). In the platelet aggregation test, 79a-79c (AIR: 12.10%, 14.80% and 19.70%), 79i (AIR: 15.70%) and 79l-79n (AIR: 8.00%, 16.00% and 28.70%) reduced the platelet aggregation comparable to aspirin (AIR: 14.70%). In the rat model of arteriovenous bypass thrombosis, 79i (AIR: 16.00%) inhibited arteriovenous bypass thrombosis in rats comparable to aspirin (AIR: 19.20%). The complexes 80b, 80d, 80e, 80f, 80g and 80h decreased platelet aggregation significantly compared with the negative control group (p < 0.05). The activity of 80f was significantly higher than that of aspirin (p < 0.01). 80f inhibited arteriovenous bypass thrombosis in rats with a better inhibition rate (43.90%) than aspirin (28.05%), and significantly increased TXB2 and 6-Keto-PGF1α levels of antithrombotic activity.
Picotamide, a potent platelet inhibitor, can simultaneously inhibit TXA2 synthase and PGH2 receptor of platelet [94]. Wei et al. [95] synthesized a series of picotamide analogs (81a-81j) and assessed their anti-platelet aggregation activity in vitro (picotamide and aspirin as positive controls) by Born Turbidimetry (Fig. 57). Most of them (81a, 81b, 81d, 81f, 81i, 81j) had effective inhibitory ability, in which compound 81b was the most potent one (IC50: 0.35 μM), superior to picotamide (IC50: 0.76 μM) and aspirin (IC50: 0.53 μM).

Liu et al. [96] synthesized 4 series of picotamide derivatives (Fig. 58): series 1 (ortho-substituted phenyl: 82a-82j), series 2 (inter-substituted phenyl: 83a-83k), series 3 (para-substituted phenyl: 84a-84l), and series 4 (aromatic without substituted group and aromatic heterocyclic substituted groups). The anti-platelet aggregation activities in vitro were evaluated by Born method and the results showed that
compounds 82c, 82d, 82i, 82j, 83g, 84a, 84c, 84d, 84f, 84h, 84l, 85b and 85c exhibited better anti-platelet aggregation activity than the control drug picotamide (IC$_{50}$: 0.76 μM). The compound 82i (IC$_{50}$: 0.02 μM), compound 83g (IC$_{50}$: 0.49 μM), compound 84l (IC$_{50}$: 0.49 μM) and compound 85c (IC$_{50}$: 0.24 μM) had the strongest activity in their respective series.

![Chemical structures of compounds 82-85.](image)

A series of 4-methoxy-1, 3-benzenedisulfonamide compounds (86a-86j) were designed [97] based on the SAR of platelet inhibitor picotamide derivatives (Fig. 59).

According to Born turbidimetry, an anti-platelet aggregation activity study was conducted and showed that all compounds except 86g and 86j had various degrees of inhibitory activities against ADP-induced rabbit platelet aggregation in vitro, with aspirin, picotamide and clopidogrel as positive controls. Compound 86b was the most effective inhibitor at 1.30 μM, with IC$_{50}$ value of 0.46 μM, and its inhibition rate of 62.30% was much higher than that of aspirin (16.47%) and picotamide (26.01%). The compounds (86h and 86i) substituted with methyl groups to methylene had no obvious activity in either R or S configurations.
He et al. [98] synthesized a cyclic precursor drug 87a-87d based on RGD (Arg-Gly-Asp) peptidomimetics 88a-88d with different ring sizes ([CH₂]n = 1, 3, 5 and 7) and evaluated the antithrombotic activity of prodrugs 87a-87d and the parent compounds 88a-88d by platelet aggregation test, with RGDF peptide (IC₅₀: 12.00 μM) as a positive control [99] (Fig. 60). The results showed that the antithrombotic activity of 88a-88d increased with the increase of methylene number, and the antithrombotic activity of prodrug 87a-87d was lower than that of the parent compound 88a-88d, indicating that the cyclic prodrug 87a-87d had to be converted into the parent drug by esterase to exert antithrombotic effect. Cyclic prodrug 87d (IC₅₀: 0.40 ± 0.03 μM) was the best antithrombotic one, which is the premise for its transformation into the parent drug 88d (IC₅₀: 0.20 ± 0.01 μM).

The new aspirin derivatives formed by the connection of NO donor (inhibition of platelet aggregation) with the antiplatelet drug aspirin have strong antithrombotic activity and low gastrointestinal side effects. Therefore, Zhou’s group [100] coupled 3-aryl-1, 2, 3, 4-oxatriazole-5-imine (with antiplatelet activity) as a NO donor to aspirin to synthesize a battery of new NO donor-type aspirin derivatives (89a-89l) and
found that 89f, 89k, 89l could not merely inhibit platelet aggregation induced by ADP in vitro (with aspirin as positive control and 0.10% DMSO as negative control) but also effectively inhibit the formation of pulmonary thrombosis in vivo (with CMC-Na as negative control and aspirin as positive control), and 89f had the best inhibition activity in vitro with inhibitory rate of 30.70%. In summary, compounds 89f, 89k and 89l had effective antithrombotic activity whether in vivo or in vitro, and further investigation is needed on their substituents and positions (Fig. 61).

Fig. 61. Chemical structures of NO donor-type aspirin derivatives 89.

Ferulic acid is an effective ingredient in traditional Chinese medicine to promote blood circulation and remove blood stasis. Modern pharmacology has shown that ferulic acid can effectively inhibit the release of serotonin in platelets to display its antithrombotic activity [101]. Li et al. [102] synthesized six ferulic acid derivatives (90a-90f), using ozagrel and dazoxiben (with selective inhibition for TXA2 synthase) as model compounds and ferulic acid as lead compounds. All compounds showed significant inhibitory activities on the ADP-induced platelet aggregation and possessed better activity than ozagrel in vivo. Among them, compounds 90a and 90e were the best, with their respective inhibition rates of 83.10% and 91.40%, which were 3.40 and 3.70 times of ozagrel. Moreover, application of the Energy minizer (MOE software) to calculate the distance between the basic nitrogen atom and the carboxyl group of the ferulic acid derivatives at the lowest energy indicated that the distance of both 90a and 90e were less than 1.0 nm, similarly to ozagrel and dazoxiben.

Compound 91 which was synthesized by the structural characteristics of ferulic acid and TXA2 synthase inhibitor had a pronounced inhibitory effect on ADP-induced
platelet aggregation. Inspired by pyrazine introduced as a pharmacophore with good antiplatelet aggregation activity in previous literature [103], Ma et al. [104] synthesized a series of new [(pyrazine-3-y1) methoxy] aromatic acid derivatives (92a-92f) by replacement of pyridine with pyrazine and different substituted methyl aromatic acid esters in compound 91. The in vitro antiplatelet aggregation activity of 92a-92f was evaluated by Born turbidimetric assay, with ozygrel and ferulic acid as positive control, the data displayed that all compounds had effective suppressions on ADP-induced platelet aggregation in rabbit model. Compounds 92a (IC\textsubscript{50}: 1.14 mM) and 92e (IC\textsubscript{50}: 1.81 mM) had better inhibitory activity than ozagrel (IC\textsubscript{50}: 2.85 mM) and ferulic acid (IC\textsubscript{50}: 19.58 mM), and could be comparable to the compound 91 (IC\textsubscript{50}: 1.76 mM) (Fig. 62).

![Chemical structures of ferulic acid derivatives 90-92.](image.png)

Exposure of GPIIb/IIIa receptors on the surface of platelets can promote the binding of fibrinogen molecules to platelets, causing the aggregate of platelets and leading to thrombosis. Liu et al. [105] synthesized a series of compounds with inhibitory activity of GPIIb/IIIa receptor by different chemical substitutions at 7-position and 4-position of 1,4-benzoxazine-3(4H)-one (Fig. 63). In the anti-platelet aggregation activity test of rabbit arterial blood samples, using aspirin and ticlopidine
as positive controls, the activities of 93c and 93d (IC₅₀: 8.99 µM and 8.94 µM, respectively) with a shorter branched chain or ring structure on C-7 were the best, due to smaller steric hindrance. Molecular docking experiments demonstrated that 93c and 93d could interact with the GPIIb/IIIa receptor and indicated that 1, 4-benzooxazine-3 (4H)-one was a promising inhibitor for platelet aggregation.

Fig. 63. Chemical structures of 1,4-benzooxazine-3(4H)-one substitutions 93.

The dihydropyridazinone compound CCI-17810 has an effective inhibitory effect on platelet aggregation caused by factors such as ADP and platelet activating factor (PAF). In order to develop less toxic and more potent inhibitors, two clusters of dihydropyridazinone derivatives, 94a-94g and 95a-95e, were synthesized by Zhao et al. [106] based on CCI-17810. Born turbidimetry method was used to determine the activity of these derivatives by an anti-platelet aggregation test (with CCI-17810 as positive control) in vitro, which showed that 95a-95e and other two compounds 94f, 94g had inhibitory effects on ADP-induced platelet aggregation in healthy people. Compound 95b exerted the strongest inhibitory activity, with an IC₅₀ value of 0.008 µM, more than 60 times than that of the positive control CCI-17810 (IC₅₀: 0.49 µM). Further analysis of the SAR showed that the anti-platelet aggregation activity of 95 was generally stronger than that of 94.

Taking CCI-17810 as the lead compound, Wu et al. [107] synthesized 18 6-(substituted phenyl)-4,5-dihydro-3(2H)-pyridazinone compounds. Preliminary in vitro pharmacological test results showed that most of the target compounds except 96m and 96q had varying degrees of inhibition for ADP-induced platelet aggregation in New Zealand rabbits, stronger than the lead compound CCI-17810. Compound 96e was the most effective with IC₅₀ value of 2.50 µM, much better than that of the
positive control CCI-17810 (IC_{50}: 12.50 \mu M) (Fig. 64).

In addition, because pyridazinones were effective inhibitors for platelet aggregation, and the pyridazinone derivatives 6- [4'- (4'-pyridylaminobenzene)]-4, 5-dihydro-3 (2H) pyridazinones (MCI-154) had strong cardiotonic and antiplatelet aggregation activity, two types of compounds with similar structure to MCI-154 were designed and synthesized [108], namely 6-(4-substituted chloroacetamido) phenyl-4,5-dihydro-3(2H)-pyridazinone and 6-(4-substituted chloroacetamido) phenyl-5-methyl-4,5-dihydro-3(2H)-pyridazinone, in order to explore the underlying SAR of pyridazinones and find more potent and selective inhibitors (Fig. 65).

Compounds 97a-97h (with MCI-154 as a control) were conducted by an in vitro pharmacological experiment, and most compounds had different degrees of activity against human platelet aggregation in vitro, compound 97a had the strongest activity (IC_{50}: 0.03 \mu M) with more 10 times than that of MCI-154 (IC_{50}: 0.36 \mu M). The activity of compounds 97f-97h (IC_{50}: 0.085, 0.050, 0.060 \mu M, respectively) was also superior to MCI-154, and 97b-97e were comparable to MCI-154.
Based on previous research [109], Nikitina et al. [110] synthesized enantiomerically pure sulfoxide 98 to study its cytotoxicity and ability to inhibit bleeding in vitro (Fig. 66). In addition, the possibility of 98 as a platelet concentration stabilizer was investigated, the results showed that 98 could almost completely inhibit arachidonic acid, collagen, and epinephrine-induced platelet aggregation. Finally, it was confirmed that the anticoagulant property of 98 was related to its ability to stabilize cell membrane by interaction of outer phospholipids and van der Waals forces. In conclusion, compound 98 is expected to be a promising drug for stabilizing platelet blood products, treating and preventing thrombosis.

Giorgio et al. [111] designed and synthesized a series of new compounds 99 and its analogues 100, which were tested in vitro using acetylsalicylic acid (ASA), cilostazol, and milrinone as reference compounds against ADP, collagen, and Ca^{2+} ionophore A23187 in platelet-rich plasma (PRP) inhibitory activity of human platelet aggregation (Fig. 67). The new compounds 99a-99g were proved to be inhibitors of human platelet aggregation in vitro, significantly more active (IC_{50} on ADP: 0.90 ± 0.36 μM-6.10 ± 2.10 μM) than the reference compounds, because they increased the platelet cAMP level by specifically inhibiting phosphodiesterase 3 (PDE3) to display antiplatelet activity, and they were much more active than their analogues 100 (IC_{50}
on ADP: 74.80 ± 10.00 μM and 95.00 ± 15.00 μM, respectively).

In view of the existing drugs such as tirofiban, eptifibatide and abciximab with the disadvantage of thrombocytopenia, Pavel et al. [112] developed and designed two types of αIIbβ3 antagonists which can integrate into closed or open forms of the receptor pocket (Fig. 68). Various theoretical methods such as QSAR, docking studies and pharmacophore models based on structure and ligands have been applied to screen out and provide 4 compounds (101a-101d) and 3 compounds (102a-102c) as ligands for the open and the closed form of the target protein, respectively. The in-vitro assay of inhibition on ADP-induced platelet aggregation indicated that 101c (IC₅₀: 6.20 nM) and 102b (IC₅₀: 11.00 nM) were the most potent in their respective series, as well as more effective than the antithrombotic tirofiban (IC₅₀: 32.00 nM) commonly used in clinical practice.

Compared with other antiplatelet drugs, clopidogrel has the advantages of good efficacy, low cost, and small adverse reactions, but however it also has defects, such as slow onset, large individual variation in platelet inhibition, thrombotic
thrombocytopenic purpura and hemolytic uremic syndrome. To overcome the above weakness, Chen et al. [113] designed and synthesized clopidogrel analogues 103 and 104, and used clopidogrel as a control for determination of anti-platelet aggregation activity (Fig. 69). Preliminary bioactivity test results showed that 103 and 104 had a certain degree of anti-platelet aggregation effects, among them, 103b and 103g were particularly pronounced and their inhibition percentage of platelet aggregation in rats were 46.10% and 38.20%, respectively. Although the activity of these compounds was less active than the reference clopidogrel on anti-thrombosis, it provided important information for the next step of research. What is noteworthy is that inhibition of platelet aggregation is not “the stronger the better”, since excessive inhibition would increase the risk of bleeding.

![Chemical structures of clopidogrel analogs 103 and 104.](image1)

Enlightened by various potency of quinoline, benzimidazole and oxadiazoles derivatives in pharmacology, Bharadwaj et al. [114] designed to combine these three drug scaffolds and synthesized benzimidazole-containing quinolinyl oxadiazoles, as well as explored their anticoagulant and antiplatelet activities by conducting a series of experiments and detecting antiplatelet and toxicity properties in RBC cells (Fig. 70). Among them, compound 105a and 105b were nontoxic and exhibited anticoagulant potency by enhancement in clotting time, further, 105a also inhibited 93.4% of platelet aggregation induced by epinephrine at the concentration of 30 μg.

![Chemical structures of benzimidazole-containing quinolinyl oxadiazoles compound 105.](image2)
Polyethylene glycol (PEG) is not widely used for covalent modification of anti-thrombosis because of its potential steric complications, whereas dendritic scaffolds confer powerful spatial control to overcome the disadvantage of PEG. Given this, Kim et al. [115] used the third generation (G3) PAMAM dendrimer to conjugate nucleoside moieties and PEG chains, in order to study the effect of PEG chains on the anti-thrombosis bioactivity (Fig. 71). Cyclic AMP accumulation assay showed that the dendrimer conjugate 106a with 11 PEG750 chains was 5-fold more active than 106b with 4 PEG2000 chains in the A2A AR-mediated cyclic AMP formation, while ADP-induced platelet aggregation experiment also displayed that the inhibition of 106a (IC50: 0.15 ± 0.053 µM) on platelet aggregation was stronger than that of macromolecule 106b (IC50: 0.49 ± 0.14 µM), suggesting that longer PEG chains were not favorable for the potency due to steric hindrance.

Based on the platelet inhibitors reported in the literature described above, we summarized their respective targets and structural characteristics, and found that the main targets were GPIIb/IIIa, TXA2 and P2Y1, as shown in the Table 2.
Table 2. Platelet inhibitor compounds and their targets

<table>
<thead>
<tr>
<th>NO.</th>
<th>Compound name</th>
<th>Target</th>
<th>Structural characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>α_{IIbβ3}</td>
<td>benzo-oxazin-one</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>GPIIb/IIIa</td>
<td>benzo-oxazin-one</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>PAE</td>
<td>lebetin</td>
</tr>
<tr>
<td>4</td>
<td>67–71</td>
<td>P2Y₁</td>
<td>adenosine 3’,5’-diphosphate</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>P2Y₁</td>
<td>fatty chain nucleotide analogues</td>
</tr>
<tr>
<td>6</td>
<td>73</td>
<td>COX-1</td>
<td>N, N’ - disubstituted thioureas</td>
</tr>
<tr>
<td>7</td>
<td>74</td>
<td>GPIIb/IIIa</td>
<td>benzodioxazine</td>
</tr>
<tr>
<td>8</td>
<td>75</td>
<td>P2Y₁</td>
<td>2-hydroxytetrahydrothienopyridine</td>
</tr>
<tr>
<td>9</td>
<td>76–78</td>
<td>COX-1</td>
<td>phenylethylidene</td>
</tr>
<tr>
<td>10</td>
<td>79–80</td>
<td>TXA₂</td>
<td>hydroxycinnamic acid combine aspirin</td>
</tr>
<tr>
<td>11</td>
<td>82–86</td>
<td>TXA₂</td>
<td>picotamide</td>
</tr>
<tr>
<td>12</td>
<td>87–88</td>
<td>GPIIb/IIIa</td>
<td>cyclic precursor</td>
</tr>
<tr>
<td>13</td>
<td>90–92</td>
<td>TXA₂</td>
<td>ferulic acid</td>
</tr>
<tr>
<td>14</td>
<td>93</td>
<td>GPIIb/IIIa</td>
<td>benzoazine-one</td>
</tr>
<tr>
<td>15</td>
<td>99–100</td>
<td>PDE3A</td>
<td>/</td>
</tr>
<tr>
<td>16</td>
<td>101–102</td>
<td>α_{IIbβ3}</td>
<td>/</td>
</tr>
<tr>
<td>17</td>
<td>106</td>
<td>GPCRs</td>
<td>Polyethylene glycol</td>
</tr>
</tbody>
</table>

### 3.3 Fibrinolytics

Using diosgenin as the lead compound, Fu et al. [116] synthesized 9 compounds and examined their antithrombotic activities by using thrombogenesis model of arteriovenous bypass in rats (Fig. 72). Compounds 107h (quality of thrombosis = 8.90 ± 4.10 mg) and 107i (quality of thrombosis = 7.60 ± 3.20 mg) with salicylic group showed good activities compared to aspirin (quality of thrombosis = 10.50 ± 7.10 mg) in anti-thrombotic activity screening test. Moreover, the C-3 position in the parent structure of diosgenin was modified to obtain a series of derivatives 108 [117], and evaluated their anti-thrombotic activities. 3β- diosgeninyl monosuccinate (quality of
thrombosis = 18.70 ± 9.30 mg) had the strongest inhibitory effect, similar to that of aspirin (quality of thrombosis = 15.40 ± 8.10 mg), indicating that the modification of appropriate hydrophilic groups on the C-3 hydroxyl group could improve the antithrombotic activity of diosgenin.

Chondroitin sulfate (CS), a sulfated polysaccharide, is a class of highly complex biomolecules called glycosaminoglycans, which can be covalently attached to the nuclei of proteins to form proteoglycans. The size and quantity of CS chains, binding site and degree of sulfation make CS a bunch of different molecules that exert various activities. Based on the enormous complexity of the molecular structure and functions of CS in different species, Cavalcante et al. [118] extracted sulfated chondroitin sulfate (sCS: 109a-109c) from the head of the Pacific white shrimp (Fig. 73). In the anticoagulant assay of sCS, the APTT and PT were determined, with pig-heparin (standard anticoagulant) as control, the result showed that sCS could not affect APTT and PT. In the antithrombotic experiment evaluated by rat venous thrombotic model, the control group only accepted saline solution and thrombotic formation rate reached 100%, conversely, administration with sCS and heparin led to a dramatic decrease in the thrombus weight and the inhibition rate of both agents was about 70%.
Fig. 7.3. Proposed structures for the peculiar disaccharide unit found in sCS.

Given involvement of plasminogen activator inhibitor (PAI)-1 in thrombogenesis, Izuhara et al. [119] synthesized a novel PAI-1 inhibitor 110, by the extensive research on the SAR of the lead compound TM5007 and assessed its effectiveness on blood clots by rat model and cynomolgus monkey (a representative of nonhuman primates), aiming to reduce differences between animal trials and practically clinical studies (Fig. 7.4). In rat model, the clot weights after administration of 10 and 50 mg/kg of 110 (60.90 ± 3.00 and 56.80 ± 2.80 mg) were lower than that in no-treated rats (72.50 ± 2.00 mg); while in the monkey thrombosis model, the occlusion time of 110 and clopidogrel group (53.90 ± 19.90 mins and 39.40 ± 25.80 mins, respectively) pronouncedly decreased, compared with no-treated group (119.00 ± 17.40 mins).

Fig. 7.4. Chemical structures of PAI-1 inhibitor TM5007 and 110.

Inflammation can promote the formation of thrombosis, and P-selectin can cause white blood cells and platelets to adhere to the inflammation or injury site, therefore, inhibiting P-selectin and inflammation can effectively treat thrombosis-related diseases. Screening 126 derivatives (with antithrombotic and anti-inflammatory activities) of β-carboline 3-carboxylic acid, tetrahydro-β-carboline-3-carboxylic acid and indoloquinolizine to dock with the active pocket of P-selectin by rational
computer assistant, Wu et al. [120] constructed the optimized compound (2-(3-(hydroxymethyl)-9H-pyrido[3,4-b] indol-1-yl) ethyl)-L-phenylalanine (111) by comparison of the binding energy and analyzing the pharmacophore of these derivatives (Fig. 75). Compound 111 could directly bind to P-selectin and showed dose-dependent behavior, and the thrombus weight of the rats and mice accepted 200 nmol/kg of 111 is equivalent to that of 167 μmol/kg and 240 μmol/kg of aspirin, respectively, suggesting that 111 was favorable for discovering novel, potent antithrombotic leads of P-selectin inhibitors.

![Chemical structure of 111](image)

**Fig. 75.** Chemical structures of (2-(3-(hydroxymethyl)-9H-pyrido[3,4-b] indol-1-yl) ethyl)-L-phenylalanine (111).

Although recombinant t-PA, SK and UK are widely used as thrombolytic agents to inhibit clot formation in clinical practice, the safety of these drugs is low and may bring undesirable results such as severe bleeding complications, therefore, it is necessary to develop a new type of fibrinolytic with high safety. Silver and gold nanoparticles can be introduced into antithrombotic agents to be developed as anticoagulants. Harish et al. [121] isolated xylan from wheat bran (WB) by alkaline treatment [122] which was used to prepare silver nanoparticles with fibrinolytic activity, WB-xylan AgNPs. DPPH free radical scavenging experiment and fibrin plate assay proved that WB-xylan AgNPs not only had favorable free radical scavenging activity but also displayed high fibrinolytic activity (Fig. 76). In addition, in order to further explore the potential of WB-xylan AgNPs to treat thrombosis, blood clot was on a glass plate in vitro (as a negative control), uncoagulated blood was gained by addition of sodium citrate (as a positive control), then WB-xylan AgNPs were added to the blood clot to result in dissolution within 5 minutes, in line with the fibrin plate assay and positive control. However, its specific role in fibrinolysis remains unclear and the safety is needed to further study.
For the present, there is less literature on fibrinolytics than antithrombosis drugs and platelet inhibitors, so the corresponding targets are fewer than those of antithrombosis drugs and platelet inhibitors. We summarized their respective targets and structural characteristics based on the reported literature, as shown in the Table 3.

<table>
<thead>
<tr>
<th>NO.</th>
<th>Compound name</th>
<th>Target</th>
<th>Structural characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>107</td>
<td>TXA$_2$</td>
<td>diosgenin</td>
</tr>
<tr>
<td>2</td>
<td>108</td>
<td>TXA$_2$</td>
<td>diosgenin</td>
</tr>
<tr>
<td>3</td>
<td>110</td>
<td>PAI-1</td>
<td>/</td>
</tr>
<tr>
<td>4</td>
<td>111</td>
<td>P-selectin</td>
<td>pyrido[3,4-b] indol</td>
</tr>
</tbody>
</table>

4. Conclusion

Uncontrolled thrombosis is the main cause for a variety of cardiovascular and cerebrovascular diseases, leading to the cardiovascular morbidity and mortality gradually increasing year by year [123]. Unexpectedly, even some persons occurred rare and severe blood clots after receiving the AstraZeneca COVID vaccine, leading to the suspending in many countries. Antithrombotic medicines are particularly significant due to their potency against thrombotic disorders to ameliorate popular conditions. Up to today, the primary treatment and prevent strategy have been use of the three categories: anticoagulants, platelet inhibitors and fibrinolytics, and the approved drugs like unfractionated heparin, low molecular weight heparins (LMWHs), warfarin, aspirin, ridogrel, dipyridamole, argatroban, dabigatran, streptokinase,
anistreplase and so on, they play important roles in clinical application but have insurmountable disadvantages such as the unexpected high risk of bleeding, a slow onset of action, a narrow therapeutic window, consequently, they require individualized medication and close monitoring. To circumvent these issues, extensive and deep investigations and tremendous endeavors have been made in exploiting novel antithrombotic agents in recent decades. Theoretically, any enzyme or receptor involved in the process of thrombosis can be used as the target of antithrombotic drugs [124], while the major researches focus on thrombin, various coagulation factors, GPIIb-IIIa receptor, ADP receptor and TXA2 receptor [125] as discussed. With further studying on antithrombotic mechanism, discovery of more promising targets will bring along emergence of more anticoagulant agents with efficient, safe and orally bioavailable properties.

Declaration of competing interest

No personal or financial conflicts of interest have been found among all the authors.

Acknowledgements

We sincerely thank the National Key Research and Development Program of Shaanxi Province (No. 2021ZDLSF03-03), Biomedicine Key Laboratory of Shaanxi Province (No. 2018SZS41).

References:


[17] Y. Lu, X. Ning, The mechanism of thrombus and the advances in research of thrombolitics, Food


[34] S.J. Patey, E.A. Edwards, E.A. Yates, J.E. Turnbull, Heparin derivatives as inhibitors of BACE-1,


[73] F.R. Lucietto, P.J. Milne, G. Kilian, C.L. Frost, M. Van De Venter, The biological activity of the histidine-containing diketopiperazines cyclo(His-Ala) and cyclo(His-Gly), Peptides, 27 (2006) 2706-2714.


