Flavonoids with Antithrombotic Activities: a Review

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Thrombus is a severe health problem all over the world, especially in developing countries. However, there is still no satisfactory antithrombotic therapeutic strategy. Currently, the clinical antithrombotic drugs suffer from several drawbacks such as high rate of bleedings and short half-life, causing allergic reactions or complications, etc. In recent years, small-molecule compounds isolated from natural products with antithrombotic activities become a hot issue in the field of drug research. In this review, we will focus on representative naturally occurring small molecule flavonoids with antithrombotic potency and potential for future therapeutic regimens to combat thrombosis disease.

Keywords thrombosis, small-molecule, antithrombotic activities, natural products, flavonoids

Introduction

The rapid development of economy improves the living conditions of human being, increases the average life expectancy, and changes the living habits.[1,2] However, some serious diseases such as obesity and related diseases are threatening the whole society.[2] Among them, thrombotic disease is the main factor of morbidity and mortality in the modern era,[3-5] causing serious damages to human health and heavy economic burden for patients’ family possibly.[6-8] An estimated one in four people dies from causes related to thrombosis worldwide. Therefore, it is urgent to develop antithrombotic drugs with high efficiency, low toxicity, and low cost.[9]

Antithrombotic drugs, including anticoagulant drugs and anti-platelet drugs,[10,11] can dissolve fibrin clot or activate plasminogen into plasmin to cure thrombosis disease.[12] Heparin (ordinary heparin, low molecular weight heparin) and fondaparinux, and oral adjusted-dose vitamin K antagonists[13] aspirin, adenosine monophosphate receptor antagonist and platelet membrane glycoprotein anti-body antagonist[14] have been approved by the FDA as treatments for thrombus. However, thrombotic drugs aren’t satisfactory[15,16] due to several disadvantages[17] such as too long half-life or too short-life, severe anaphylactic reaction, and lack of specificity.[18] Especially, some drugs can cause complications[19] with hemorrhage and limited therapeutic range.[20-22] Only 1.8%-8.9% patients were treated by t-PA-based therapy.[23,24] For example, heparin and vitamin K antagonists suffer from several drawbacks: heparins are involved in parenteral administration, vitamin K antagonists need frequent monitoring and dose adjustments, interactions with several medications, and dietary restrictions.[25] Hence, researchers are searching for small molecules with antithrombotic activities.[26-33]

Natural products are divided into diverse chemical compounds isolated from varied biological sources such as plants, animals, marine organisms, microorganisms, minerals, and organic matters.[36-39] In recent years, the research on natural products becomes a hot issue in drug research. The drug discovery from natural products possesses several advantages such as timeliness, faster and better methods for bioassay screening, and compound isolation.[40] Currently, naturally occurring flavonoids with antithrombotic activities have been identified and appeared in the recent literature or patents. This progress holds a considerable promise to the development of alternative, highly effective and secure therapeutics agents and ultimately the eradication of thrombosis disease. In this paper, we summarized flavonoids from natural products with antithrombotic activities.

Flavonoids

Most flavonoids are isolated from natural materials with various skeletons such as flavonols, flavone aglycon, chalcones, prenylflavones, biflavones, etc. Flavonoids are reported with strong antioxidant activity,[41] reducing blood-sugar effects,[42] and improving blood circulation effects,[43] decreasing cholesterol and benefit cardiovascular disease.[44] Moreover, they exhibit fibrinolytic activities, anticoagulant activities, and antiplatelet activities.

Flavonol

Flavonol kaempferol (1) isolated from fruits of Lagenaria siceraria[45] exhibited fibrinolytic activities on the fibrinolysis assay system (dissolve the plasma clot by 77.37%) in Figure 1, suggesting that kaempferol be a potent aspect for further fibrinolytic activities research on plasma clots in vitro.

Flavone aglycon

Luteolin (2) with flavones-C-glycoside was isolated from Forsomos Gentianaceous plants.[46] Luteolin displayed a potent platelet aggregation effect on platelet aggregation induced by arachidonic acid (AA) and collagen. In addition, it had also a significant effect on platelet aggregation induced by thrombin and platelet-activating factor (PAF) (IC_{50}=43.5 μM). Afitil[47,48] reported luteolin possessed strong inhibition on collagen-induced platelet aggregation (IC_{50}=0.034 mg/mL). Simultaneously, luteolin-7-O-glucoside (3) and vitexin (4) presented strong inhibition of collagen-induced platelet aggregation (IC_{50} of 0.046 and 0.021 mg/mL). Luteolin-7-O-glucoside belongs to flavones-O-glucoside, and vitexin belongs to flavones-C-glycoside.

6-Hydroxykaempferol 3,6,7-tri-O-glucoside (5) and 6-hydroxykaempferol 3,6-di-O-glucoside (6)[49] extracted from the flower of
Carthamus tinctorius L. exhibited significantly inhibitory potency on the collage-induced platelet aggregation by testing the inhibition of platelet aggregation. The inhibition of compounds 5 and 6 were 3.9%~10.0% and 4.3%~19.3% at the concentration of 13–200 μg/mL, respectively. In addition, they also showed weak inhibition on the platelet aggregation induced by adenosine 5 ’-diphosphate (ADP). At the concentration of 200 μg/mL, the inhibition of compounds 5 and 6 were 2.7% and 0.4 %, respectively.

Five flavonoid glycosides, kaempferol 3-O-α-L-(2’E,4’E-di-p-coumaroyl)-rhamnose (7), kaempferol 3-O-α-L-(3’E,4’E-di-p-coumaroyl)-rhamnose (8), kaempferol 3-O-α-L-(4’E-E-p-coumaroyl)-rhamnose (9), afzelin (10) and quercetin (11) were isolated from Trogopterus faeaces[28] and evaluated for antithrombotic activities by the thrombin time (TT) method assay. The Ig(TT) prolongation (%) of compounds 7–11 increased from 1.05 to 2.31 μg/mL linearly with the increase of the dosage from 7 to 70 μg/mL. Among them, compounds 7–9 showed the significant antithrombotic activity. Two flavones diosmetin apiosyl-glucosides 5,7,4’-trihydroxy-falvone (apigenin) (12) (IC50=0.036 mg/mL) and apigenin 7-O-glucoside (cosmosin) (13) (IC50=0.18 mg/mL) with antiplatelet aggregation activity were discovered from the aqueous extract of Petroselinum crispum (Mill) flat leaves specimens.[15–23]

![Figure 1](image-url) The structures of compounds 1–13.

Chalcones

Ju et al.[44] discovered two chalcones, 4-hydroxyderricin (14) and xanthoangelol (15) from Asishatba roots (Angelica keiskei Koidz.) in Figure 2. Compounds 14 and 15 inhibited aggregation induced by collagen and by PAF (IC50=41.9, 46.1 μM; 41.9, 46.1 μM, respectively). Especially for xanthoangelol (15) could also inhibit the platelet aggregation induced by collagen and by PAF (IC50=41.9 and 46.1 μM, respectively). As a result, the two compounds may be potential antithrombotic agents in the future.

Prenylflavones

In 1992, six prenylflavones from Artocarpus communis[55–57] with the antiplatelet aggregation, cyclomorusin (16), artumunoxanthone (17), cyclomulberrin (18), dihydroisocleoartominin (19), cicioocommunoi (20), and cyclocommunin (21) were discovered[38]. Among the compounds, cyclomorusin (16) and artumunoxanthone (17) exhibited strongly inhibitory the platelet aggregation induced by PAF (IC50=8.0 and 6.5 μM). Cyclomulberrin (18), dihydroisocleoartominin (19), cicioocommunoi (20), and cyclocommunin (21) possessed inhibitory the platelet aggregation induced by AA and collagen, of which cyclocommunin (21) was the most potent compound on the antiplatelet activity induced by AA and collagen with IC50 value 12.5 μM and 14.4 μM, respectively.

Two prenylflavones with the antiplatelet aggregation activities, yinanyhuo A (22) and yinanyhuo B (23), were isolated from the aerial part of Epimedium sagittatum[58] exhibiting significant inhibition of antiplatelet aggregation activity induced by AA (IC50=7.14 and 1.67 μM). Cycloaarticarp A (24), cycloaarticarp B (25), broussochalcone A (26), kazinol A (27), broussochalcone A (28), and broussoflavonol F (29) showed inhibitory platelet aggregation induced by AA with IC50 values of 18.5, 10.9, 6.8, 11.4, 15.4 and 16.9 μM, respectively. Meanwhile, compounds 24, 26, 27 could inhibit the platelet aggregation induced by collagen strongly (IC50=23.7, 22.4 and 20.7 μM, respectively). Compound 27 exhibited the inhibition of platelet aggregation induced by PAF (IC50=54.6 μM).

Figure 2 The structures of compounds 14—29.

Biflavones

Two biflavones, hinokiflavone (30) and amentoflavone (31), were isolated from Trogopterus faeaces[49] and tested antithrombotic activity by the TT assay in Figure 3. The results showed that the Ig(TT) prolongation (%) of compounds 30 and 31 were 1.08 and 1.09 with the same dosage of 50 μg/mL.

Others

Except for the above mentioned classifications, more flavonoids have been evaluated for their antithrombotic activity. Five flavonoids 32–36 with inhibition of the PAI-1 activities[43] were discovered by the experiment on t-PA and PAI-1 production in cultured human umbilical vein endothelial cells (HUVECs). The results indicated that baikalein (32) inhibited the decrease of t-PA and the increase of PAI-1 activity efficiently (IC50=3.7 μM). Besides this, wogonin (33), oxorin A (34), skullcapflavone II (35), and 2’,5’,5,7-tetrahydroxy-6,8-dimethoxyflavone (36) had a relatively weaker inhibition for PAI-1 activity (IC50=105, 61, 110, and 88 μM, respectively). The results indicated that baikalein had potentials for the treatment of thrombus.

Tsai et al.[61] reported isobavachalcone (37) and neobavaisoflavo-

![Figure 3](image-url)
Conclusions

This review summarized the small-molecule flavonoids antithrombotic active inhibitors. Flavonoids were characterized in flavonols, flavone aglycon, chalcones, prenylflavones, biflavones. Their structural characteristics and antithrombotic properties (antiplatelet and anticoagulant therapies) were mainly discussed, which will provide valuable information for understanding the progress of antithrombotic activity investigation and developing new antithrombotic therapeutic agents.

Presently, due to drug high rate of bleeding, short half-life, and allergic reactions side-effects of current antithrombotic drugs, the search for novel drugs is mandatory. Therefore, many researchers pay more attention to small-molecule derived from natural resources.

Because of structural specificity, high activity and low toxicity of the natural products, they have been used as lead compounds for antithrombotic agents. Undoubtedly, natural products need to be investigated in more detail to obtain mechanistically and structurally novel and more potent derivatives and to explore their potential as novel adjuncts to established thrombosis disease.

Except these flavonoids as antithrombotic active inhibitors, some newly small molecular thromb fibrinolysis brings into play a similar role about thrombolysis. The mechanism of thrombosis associated with (1) plasminogen regulator pathway; (2) single chain urokinase type plasminogen activator (sc-uPA) regulator pathway; (3) plasminogen activator inhibitor 1 (PAI-1) inhibitor pathway and (4) plasminogen activator regulator pathway based on fibrinolytic features of the newly small molecular. The newly small molecules described act by utilizing or modulating such embedded mechanisms and do not affect the catalytic activity of the mature enzymes. These pathways whether discriminate flavonoids as zymogen modulators from inhibitors or activators that simply act on mature enzymes or enzymogen, particularly those in the coagulation and fibrinolytic systems? Whether any other ways of mechanism? These are important attention in a future.

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