Antiplatelet agents: an overview

Asma Haffouz[a], Ali Gargouri[a], Basma Hadjkacem[a,b]

[a]Laboratory of Molecular Biotechnology of Eukaryotes, Biotechnology Center of Sfax, University of Sfax, Sfax, [b]Department of Life Sciences, Faculty of Sciences of Gafsa, University of Gafsa, Gafsa, Tunisia

Correspondence to Basma Hadjkacem, PhD, Laboratory of Molecular Biotechnology of Eucaryotes (LR15CBS02), Biotechnology Center of Sfax, University of Sfax, Road of Sidi Mansour Km 6, PO Box 1177, Sfax 3018, Tunisia. Tel: +216 9968 3506; fax: 0021674875818; e-mail: hadjkacem_basma@yahoo.fr, basmahadjkacem@gmail.com

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Platelets play a major role in normal hemostatic and thrombotic processes. Aggregation of platelets presents the key pathophysiological step in the development of cardiovascular diseases; hence, antiplatelet agents remain crucial in the treatment of cardiovascular diseases. They may target the three principal phases leading to thrombogenesis, including platelet adhesion, activation, and aggregation. This review presents an overview of the currently available antiplatelet agents, with a particular focus on their targets, pharmacological properties, and limitation of use.

Keywords:
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Introduction

In 2021, the WHO reported that cardiovascular disease (CVD) was the most common cause of death worldwide. The number of deaths from these diseases accounted for 32% of all reported deaths. Of these, 85% were due to stroke and heart attack [1].

The evolution of these diseases involves a combination of the development of atherosclerotic plaque and thrombus formation [2]. The first step of this disease is the accumulation of lipids, coagulation factors, and cells beneath the endothelium, followed by the formation of atheromatous plaques. Biomechanical and physical factors may destabilize these plaques and make them vulnerable to rupture [3]. Upon erosion or rupture, the plaque components are exposed to blood cells, which contribute to tissue factor activation and the subsequent coagulation and, concomitantly, the recruitment of circulating platelets. The interaction between platelet receptors and exposed plaque components leads to platelet activation, aggregation, and the formation of an occlusive thrombus, which leads itself to reduce blood flow and further lesions. Therefore, platelets play a central role in thromboembolic diseases pathophysiology of CVD through their implication in thrombus formation after the rupture of the atherosclerotic plaque [2].

Platelets are anucleated cell fragments that are involved in hemostasis and thrombosis. Under resting conditions, the endothelium prevents platelet adhesion to the vessel walls by secreting inhibitory substances, such as nitric oxide and prostacyclin. Following vascular injury, the subendothelial extracellular matrix becomes exposed to blood circulation [4]. The exposed matrix contains many adhesive molecules that serve as ligands for different platelet surface receptors [5]. First, the von Willebrand factor (VWF) forms a bridge between the platelet glycoprotein GPIb-IX-V and the exposed collagen leading to unstable platelet adhesion. Collagen, released from the endothelium, binds to GPVI and GPIa on the platelet surface allowing consecutive firm adhesion [6]. After these interactions, platelets become activated, change shape, and release their granule contents. Most agonists released by activated platelets such as serotonin and ADP, and plasma mediators, like thrombin and epinephrine, cause further platelet activation [7]. Upon activation, the surface platelet receptor GPIIb/IIIa also becomes activated and able to fix fibrinogen. The activated GPIIb/IIIa mediates the recruitment of neighboring platelets as well as platelet–platelet interactions that trigger platelet aggregation and allows then the formation of a thrombus at the site of the injury [8]. Platelet activation and aggregation play an important role in normal hemostatic and thrombotic processes. However, the plug can cause the arteries to clog, leading to arterial disease [9].

Accordingly, therapies that inhibit platelet activation and aggregation remain the cornerstone of treatment of cardiovascular events. Platelet function inhibition may be achieved either through inhibition of platelet membrane receptors (P2Y12, integrin GPIIb/IIIa, PAR1, and the thromboxane receptor) or by affecting the intracellular signaling pathways (cyclooxygenase 1 and phosphodiesterase inhibitors) [9].

This review focuses on the different types of available antiplatelet drugs and other inhibitors that are being studied. For each agent, the mechanism of action, advantages, and limitations will be discussed.

Antiplatelet agents

Antiplatelet drugs are some of the most prescribed medications in the world [10]. This class of drugs targets the process of platelet aggregation and leads to the inhibition of thrombus genesis. Antiplatelet drugs may inhibit platelet adhesion, activation, or aggregation, as summarized below and shown in Fig. 1.

Inhibitors of platelet adhesion

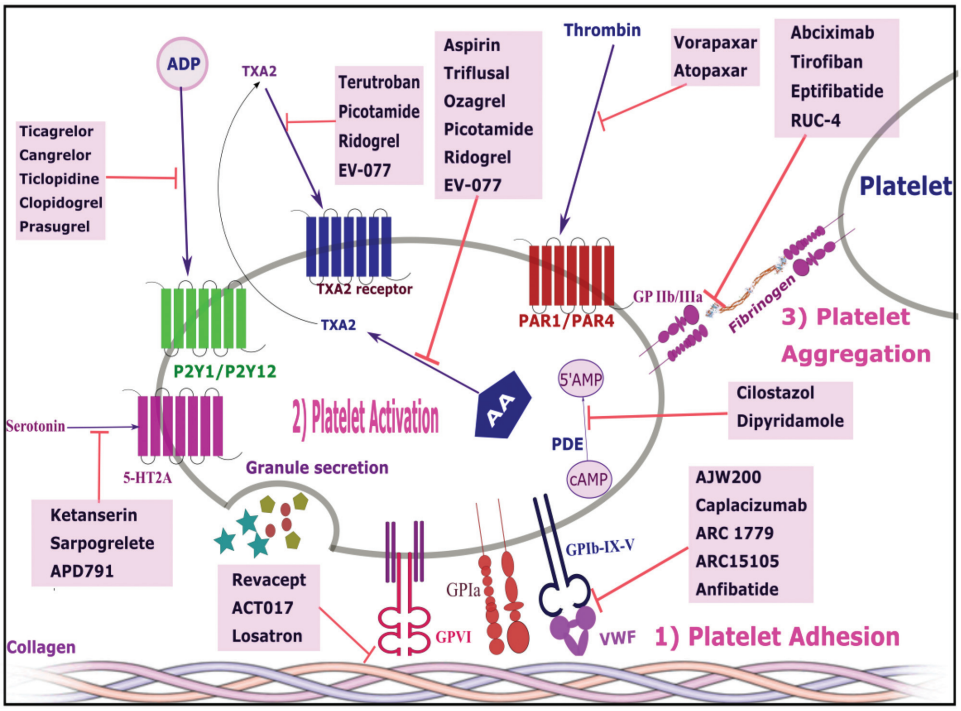
After vascular injury, platelets immediately adhere to the exposed subendothelial matrix through several receptors [5]. The first contact between the platelet and the endothelium occurs by a transient interaction between the GPIb-IX-V platelet receptor and VWF associated with the subendothelial collagen. Following this interaction, collagen binds to its respective platelet receptors GPVI and GPIa leading to firm adhesion [6]. Therefore, several antiplatelet agents target this step through inhibiting VWF-GPIb-IX-V, collagen-GPVI, or collagen–GPIa interactions (Table 1).

Inhibition of the interaction between von Willebrand factor and GPIb-IX-V receptor

VWF is an adhesive glycoprotein playing an essential role in hemostasis specifically, in platelet adhesion to the subendothelial collagen after blood vessel injury. This protein interacts with platelets through the glycoprotein GPIb-IX-V [6]. Before its interaction with platelet, the VWF adheres to the subendothelial collagen and then interacts with platelets through the GPIb-IX-V receptor leading to transient adhesion of platelets to the exposed subendothelium. Following this interaction, platelets will roll on the vascular wall, which allows for more GPIb–VWF interactions [11].

Therefore, the interaction of GPIb-IX-V with VWF plays a pivotal role in platelet adhesion and may trigger thrombotic diseases. Due to these peculiarities, GPIb–VWF interaction is considered as a promising drug target for antiplatelet therapy.

Figure 1



Different targets for antiplatelet drugs.

Table 1 Platelet adhesion inhibitors

Target	Agent	Mechanism	Route of administration	Phase of development	Half-life	Prodrug	Onset of action	Reversibility of platelet inhibition	Side effects and limitations
VWF-GPIb-IX-V interaction	AJW200	Monoclonal antibody against A1 domain of VWF	Intravenous	Phase I	23–27 h	No	Immediately	–	No significant adverse events
	Caplacizumab (ALX-0081/ ALX-0681)	Nanobody specific of VWF A1 domain	ALX-0081: intravenous ALX-0681: subcutaneous	FDA-approved	16–27 h	No	Immediately	–	Mucocutaneous bleeding Headache Shortness of breath
	ARC 1779 and ARC15105	Aptamers that bind to A1 domain of VWF	Intravenous	Phase II/III	2 h for ARC 1779 217 h for ARC15105	No	10 min	Yes	Thrombocytopenia Anemia
	Anfibatide	GPIb receptor antagonist	Intravenous	Phase II	4 h	No	–	Yes	No significant adverse events
GPVI	Revacept	Inhibition of GPVI receptor	Intravenous	Phase II	67–136 h	–	2 h	–	Few bleeding events
	ACT017 (Glenzocimab)	Monoclonal antibody against GPVI	Intravenous	Phase II	9 h	–	6–12 h	Yes	Headache
	Losartan	Inhibition of GPVI receptor	Oral	FDA-approved as an angiotensin receptor blocker	2 h	Yes	6 h	–	Renal insufficiency Hyperkalemia

VWF, von Willebrand factor.

Several antiplatelet agents are used to prevent thrombus formation by blocking the interaction between GPIb-IX-V and VWF. This interaction may be blocked either by targeting the GPIb-IX-V receptor or by targeting the VWF.

Monoclonal antibodies against von Willebrand factor or GPIb-IX-V

Monoclonal antibodies are widely used as inhibitors of these interactions. AJW200 and 82D6A3 are two monoclonal antibodies directed against the A1 domain (binding site for platelet GPIb-IX-V) and the A3 domain of human VWF (binding site of collagen), respectively [12].

In vitro, preclinical and clinical studies have shown that AJW200 inhibited platelet aggregation induced by ristocetin and botrocetin without affecting the bleeding time [13]. However, no clinical studies for 82D6A3 have yet been reported.

Numerous other monoclonal antibodies have been shown to target the GPIb-IX-V receptor such as the 6B4 fab-fragment (and its humanized form h6B4-Fab) and inhibit the binding of VWF to platelets. The h6B4-Fab antibody inhibits ristocetin-induced platelet aggregation *ex vivo* and has also shown potent antithrombotic activity *in vivo*. No thrombocytopenia or prolongation of the bleeding time was observed in preclinical studies of the antiplatelet potential of these antibodies [14].

Nanobodies

Nanobodies are the smallest functional fragments derived from single-chain antibodies developed by the *Camelidae* family and used as a new class of therapeutic proteins [15].

Several nanobodies have been developed to inhibit platelet adhesion by blocking the interaction between GPIb and VWF. ALX-0081 and ALX-0681 are two analogous nanobodies that bind to the A1 domain of VWF and thereby block the binding of VWF to the platelet receptor GPIb-IX-V [16]. These nanobodies showed an antiplatelet effect without bleeding or immunogenic response [15,17]. ALX-0081 and ALX-0681 are named according to their routes of administration intravenously and subcutaneously, respectively [17]. Caplacizumab (formerly ALX-0081 or ALX-0681) was approved by the European Union in August 2018 and by the Food and Drug Administration in February 2019 for use as an antithrombotic drug [18].

Aptamers

Aptamers are chemically synthesized oligonucleotide sequences and represent an interesting new class of antiplatelet agents that can inhibit the interaction of VWF with platelets. ARC 1779 and ARC15105 are two aptamers that have been described to inhibit GPIb–VWF interaction by targeting the A1 domain of VWF [19]. ARC 1779 is a DNA aptamer with a 40-nucleotide stabilized by 20 kDa polyethylene glycol (PEG) conjugation, 3'-capping and backbone modifications [20]. It inhibits VWF-mediated platelet function and thrombogenesis. This aptamer may have an antithrombotic effect without causing significant anticoagulation [19]. ARC 1779 had no significant bleeding complications. Unfortunately, anemia was observed after the administration of ARC 1779 [21]. Therefore, this agent was withdrawn prematurely. ARC15105 is a modified RNA aptamer conjugated with 40 kDa PEG that inhibits shear-induced platelet aggregation. After administration in cynomolgus monkeys, ARC15105 has a high half-life (about 67 h). This long half-life was thought to be the consequence of the incorporation of 40 kDa PEG as compared with ARC 1779 (20 PEG) [22]. No clinical studies have been reported for ARC15105.

Peptides selected by phage-display technology

Several peptides isolated by the phage-display technique are being used for the development of novel antiplatelet agents. In this context, Hagay *et al*. [23] developed a novel single-chain Fv monoclonal antibody (Y1-scFv) toward GPIb that inhibited ristocetin-induced GPIb-dependent platelet aggregation. The cyclic peptide (CTERMALHNLC), isolated from a cysteine-constrained phage display library, also binds to GPIb and blocks the interaction of VWF and its receptor [24].

Recombinant protein

GPG-290 is a chimeric recombinant protein that contains the amino-terminal 290 amino acids of GPIb (specific to the A1 domain of VWF) linked to human immunoglobulin G1 Fc. Its antiplatelet activity has been demonstrated in preclinical studies. This protein binds to the A1 domain of VWF with high affinity and allows inhibition of platelet adhesion without significant prolongation of bleeding time [25].

Others inhibitors of von Willebrand factor–platelet interactions

N-acetylcysteine

N-acetylcysteine is an antioxidant that contains a cysteine group associated with an acetyl group. It

may reduce the size and activity of VWF in human plasma and mice by disrupting the disulfide bond between Cys1272 and Cys1458 in the A1 domain of VWF [12]. As a result, the affinity of VWF to collagen is reduced. This molecule can inhibit platelet aggregation without causing anemia, thrombocytopenia, and organ damage. However, the use of N-acetylcysteine was associated with a significant decrease in plasma levels of vitamin K-dependent factors [26].

Protein purified from snake venom

Snake venom contains several active compounds such as polypeptides and proteins that have significant antiplatelet effects. C-type lectin represents a family of snake venom proteins that potently block platelet function by binding to the GPIb receptor with high affinity [27,28].

Lebecetin, a C-type lectin from *Macrovipera lebetina* venom, has a potent inhibitory effect on thrombin-induced platelet aggregation and showed no effect on platelet aggregation triggered by arachidonic acid or thromboxane A2 (TxA2) mimetic U46619. This protein binds to platelet GPIb and blocks platelet aggregation without interfering with blood coagulation and erythrocyte agglutination [29].

Agkistin purified from *Agkistrodon acutus* venom inhibits ristocetin-induced human platelet aggregation and TxA2 formation [28]. Echicetin, derived from *Echis carinatus* venom, had also inhibitory effects on platelet aggregation induced by thrombin or ristocetin [30]. However, the use of these proteins may cause thrombocytopenia [31]. Anfibatide is another C-type lectin protein purified from *A. acutus* that inhibits ristocetin/botrocetin-induced and thrombin-induced platelet aggregation. In phase I of clinical trials, anfibatide inhibits VWF-mediated platelet aggregation in a competitive manner without affecting the bleeding time or coagulation pathway [32]. Anfibatide is currently in phase II of clinical trials to assess the safety of this protein in patients with acquired thrombotic thrombocytopenic purpura (NCT04021173).

Several snake venom metalloproteinases (SVM) were associated with antiplatelet activity by cleaving the GPIb receptor. Crotalin is an SVM isolated from *Crotalus atrox* venom that inhibits ristocetin, but not platelet aggregation induced by thrombin and collagen. Intravenous administration of this protein did not induce thrombocytopenia in mice [33]. Mocarhagin is another SVM purified from the venom of *Naja mozambique* that cleaves between Glu282 and Asp283 on the GPIb receptor and inhibits VWF-dependent platelet aggregation [34].

GPVI collagen

The second step of adhesion occurs when the exposed collagen binds to the platelet GPVI receptor, allowing firm adhesion. The interaction between GPVI, as well as GPIa and collagen, mediates firm adhesion [6]. Owing to the critical role of GPVI and collagen interaction in thrombosis, anti-GPVI agents are attractive targets in the development of antithrombotic drugs.

GPVI receptor mimics

Revacept (GPVI-Fc) is a recombinant protein consisting of the extracellular collagen-binding domain of GPVI and the constant domain (Fc) of human immunoglobulin G1. This recombinant protein competes with GPVI to bind the subendothelial collagen and prevent collagen-mediated platelet adhesion and activation [35]. Phase I trials have shown that revacept did not increase bleeding time and it is devoid of any signs of toxicity [36]. Phase II clinical trials demonstrated the safety and efficacy of this recombinant protein in symptomatic carotid stenosis and in chronic coronary syndromes [37,38]. In a perfusion chamber, this protein inhibits platelet aggregation at a high shear flow with a shear rate of 1500/s, but its effect is limited to low shear flow (physiological blood flow). GPVI-CD39, a fusion protein formed by the association of GPVI-Fc and the ectonucleotidase CD39, was developed to create dual antiplatelet therapy. This recombinant protein inhibits platelet aggregation without affecting bleeding time *in vivo* [39].

Monoclonal antibodies

Another possibility to interfere with the GPVI pathway is the monoclonal antibodies against the GPVI receptor. This method is prominent owing to the limited copies of GPVI expressed in platelets. In addition, antibodies are characterized by their specificity and high affinity for their targets. Antibodies act either by blocking collagen interaction with GPVI or by depletion of GPVI [40].

The fab fragments of most of these antibodies, including ACT017 (Glenzocimab), SAR264565, JAQ1, OM4, OM2, 1G5, 5C4, and 9O12 have high affinity to GPVI receptor and inhibit platelet adhesion to collagen without depleting platelets [41]. Glenzocimab is in phase II clinical trials for patients with ischemic stroke [42]. A10 and C3 are

two neutralizing antibodies isolated from a combinatorial phage display library that specifically blocked GPVI binding to the collagen. A10 inhibits the interaction between convulxin and GPVI collagen-induced platelet aggregation *in vitro* [43]. 10B12 and 1C3 are also two single-chain antibodies developed by the phage-display method that bind to D1 and D2 domains of GPVI, respectively. The 10B12 antibody blocks the binding of collagen-related peptide (CRP) and collagen to the GPVI receptor [44]. The BLO8-1 antibody has been isolated from phage-display libraries and demonstrated inhibition of the binding of collagen and CRP to the platelet receptor GPVI. Under arterial shear conditions, this antibody inhibits thrombus formation in whole blood [45].

F1201 and F1232 are also used as antibodies that inhibit collagen-induced platelet aggregation. However, their injection into monkeys induces immunodepletion of GPVI. Thrombocytopenia was observed for F1201 but not for F1232 [46].

Others molecules

Small molecules present another way to target the GPVI receptor. Many chemical molecules showed antiplatelet activities mediated by GPVI. Losartan, an angiotensin II receptor antagonist, may block the clustering of GPVI without affecting the binding of collagen [47]. A benzimidazole diamide compound, GSK669, can also block collagen-induced platelet aggregation under flow conditions. This molecule inhibits platelet aggregation induced by collagen and CRP [48]. Artesunate, a semisynthetic derivative of artemisinin extracted from *Artemisia annua*, is a selective and competitive antagonist of collagen receptor GPVI. This molecule inhibits collagen-induced platelet aggregation by inhibiting granule release, intracellular calcium mobilization, and GPIIb-IIIa activation [49].

Collagen–GPIa interactions

GPIa, also known as integrin α2β1, is a second platelet receptor of collagen. This receptor is also a good target to develop new antiplatelet agents. Metalloproteases derived from venoms such as jararhagin, NN-PF3, EMS16, and rhodocetin prevent collagen binding to the GPIa receptor [50–52]. In addition, several antibodies against this receptor have been reported as inhibitors of GPIa–collagen interaction such as 176D7, P1H5, and 6F1 that recognize a small region of the receptor [53].

Inhibitors of platelet activation

The interaction between VWF, subendothelial collagen, and platelets leads to the adhesion of platelets to the site of the injury. After this step, platelets become activated, change shape by the formation of pseudopods, and release their granule contents [7]. During this process, the fibrinogen receptors (GPIIb/IIIa) are activated and become able to form bridges between platelets leading to platelet aggregation. Platelet activation may present a major step in thrombus formation and pathogenesis of various CVDs [54]. Therefore, this step is a potential target to prevent thrombotic events. As shown in Table 2, multiple agents have been developed to block platelet activation by inhibiting ADP pathway, TxA2 pathway, thrombin, and phosphodiesterase.

Inhibitors of the thromboxane A2 pathway

Multiple pathways contribute to platelet activation and aggregation. Among them, TxA2 is a critical pathway that amplifies vasoconstriction and platelet activation [55]. Inhibition of this pathway is a prominent strategy to prevent thrombosis.

TxA2 is synthesized from arachidonic acid, liberated from the plasma membrane by phospholipase A2 through the cyclooxygenase metabolic pathway [56]. Inhibition of the TxA2 pathway may be achieved either by inhibition of the synthesis of TxA2 or by blockade of the TxA2 receptor [55].

At low doses, aspirin (acetyl salicylic acid) is the most famous and prescribed antiplatelet drug that targets TxA2 synthesis by irreversible acetylation of serine529 of COX1, which leads to steric hindrance of arachidonic acid to access the catalytic center (Tyr385). The action of aspirin lasts the lifespan of the platelet (8–10 days) [57]. Clinical use of this drug proved the beneficial effects in the secondary prevention of myocardial infarction, stroke, or peripheral arterial vascular disease [58]. Unfortunately, the use of this molecule was associated with gastrointestinal and/or intracranial bleeding [59]. In some cases, aspirin was unable to prevent thrombotic complications. This phenomenon is known as 'aspirin resistance.' It has been reported that many factors may contribute to this phenomenon including genetic polymorphisms of COX1, drug interactions, and inadequate dose [60]. Triflusal is a second COX inhibitor with a similar structure and action to aspirin. This drug was effective as aspirin but with a more favorable safety profile and reduced bleeding risk [61].

Ozagrel is a drug prescribed for asthma and stroke. It inhibits the synthesis of TxA2. This compound is

Table 2 Inhibitors of platelet activation

Targets	Agent	Mechanism	Route of administration	Phase of development	Half-life	Prodrug	Onset of action	Reversibility of platelet inhibition	Side effects and limitations
TXA2 pathway	Aspirin	TxA2 synthesis inhibitor	Oral	FDA-approved	20 min	No	20 min	No	Bleeding, Gastrointestinal discomfort, Drug resistance
	Triflusal		Oral	FDA-approved	30 min	Yes	10–20 min	No	Hemorrhage, Gastric pain
	Ozagrel		Intravenous	Phase IV	1 h	Yes	3 h	–	Hemorrhage
	Terutroban	Antagonist of TxA2 receptor	Oral	Phase III	6–10 h	No	1 h	Yes	Gastrointestinal bleeding
	Picotamide	Antagonists of TxA2 receptor TXA2 synthase inhibitor	Oral	Drug not approved by the FDA	3 h	–	–	No	Bleeding, Gastrointestinal discomfort
	Ridogrel		Oral	Phase III	6–9 h	Yes	30 min	–	Gastrointestinal hemorrhage
	EV-077		Oral	Phase II	2–6 h	–	30 min	Yes	Headache hematoma
P2Y12	Ticagrelor	Antagonist of P2Y12 receptor	Oral	FDA-approved	6–12 h	No	30 min–4 h	Yes	Dyspnea
	Cangrelor		Intravenous	FDA-approved	5 min	No	5 min	Yes	Dyspnea
	Ticlopidine		Oral	FDA-approved	12 h	Yes	3 days	No	Thrombocytopenia, Neutropenia Bone marrow aplasia
	Clopidogrel		Oral	FDA-approved	6–8 h	Yes	2–8 h	No	Diarrhea bleeding gastrointestinal complaints neutropenia
	Prasugrel		Oral	FDA-approved	8 h	Yes	30 min 4 h	No	Thrombocytopenic purpura anemia
PAR1	Vorapaxar	PAR1 receptor inhibitor	Oral	FDA-approved	5–13 days	No	2–7 days	Yes	Bleeding intracranial hemorrhage
	Atopaxar		Oral	Phase II	22–26 h	No	3–4 h	Yes	Cardiotoxicity liver dysfunction
Phosphodiesterase	Cilostazol	PDE inhibitor	Oral	FDA-approved	11–13 h	No	6 h	Yes	Headache, Diarrhea
	Dipyridamole		Oral	FDA-approved	10–12 h	No	1 h 2 h	Yes	Headache hypotension
5-HT2A	Ketanserin	5-HT2A receptor antagonist	Oral	FDA-approved as an antihypertensive drug	17 h	Yes	1 h	Yes	Hypotension
	Sarpogrelate		Oral	FDA-approved	40–50 min	Yes	30 min	Yes	Liver dysfunction, Allergic reactions
	APD791 (Temanogrel)		Oral	Phase II	1 h	Yes	20 min	–	Dermatitis, Headache
	SL65.0472–00		Oral	Phase II	–	–	1 h	-	Hematoma, Headache

associated with an improvement in neurological impairment [62].

Besides TxA2 synthesis inhibitors, there are antagonists of TxA2 receptor. This strategy is more effective than the inhibition of the ligand synthesis [63]. These antagonists may prolong bleeding time more than TxA2 synthase inhibitors [64].

Terutroban (S18886) is a competitive antagonist of TXA_2 receptor that is used in the secondary prevention of thrombotic events in CVD. Unlike aspirin, terutroban has a reversible effect on platelet activation [55]. This drug showed an antithrombotic effect more important than aspirin [65].

Picotamide, ridogrel, and EV-077 are three antiplatelet agents that have dual activity. They act as TXA2 synthase inhibitors with additional TXA2 receptor antagonism properties [66]. Despite the efficacy of these drugs, clinical trial results were disappointing [67]. Picotamide did not show any difference with aspirin in the primary ischemic endpoints [68]. In patients taking picotamide, the incidence of gastrointestinal bleeding was much lower than in those treated with aspirin [69]. Clinical trial has shown that ridogrel failed to demonstrate any advantage over aspirin in patients with acute myocardial infarction. However, this drug was shown to be more effective in the prevention of new ischemic events [70]. In patients with type-2 diabetes, EV-077 showed strong antiplatelet activity when compared with aspirin [71]. However, clinical trials of this drug were terminated at phase II [72].

P2Y12 antagonists

ADP is another important platelet activator that acts on the purinoreceptors P2Y1 and P2Y12 through the amplification of platelet activation [73]. The ADP pathway inhibitors comprise two classes of drugs: the nucleoside/nucleotide derivatives (ticagrelor and cangrelor) and the thienopyridines family (prasugrel, ticlopidine, and clopidogrel) [74].

The nucleoside/nucleotide derivative drugs act directly on the P2Y12 receptor because they did not need an activation mediated by hepatic cytochrome P450 (CYP450) [75]. Ticagrelor is a reversible noncompetitive antagonist of the P2Y12 receptor that binds in different sites of ADP [76]. Despite this mode of action, the inhibitory effect of this drug lasts 3–5 days after oral administration [77]. Ticagrelor displays a reduction of myocardial infarctions and cardiovascular complications. Cangrelor is an intravenously administered antagonist of P2Y12, which has a short onset and offset of action requiring about 1 h to return to baseline platelet function due to the short half-life (3–5 min) and the reversible binding mode. Like ticagrelor, cangrelor leads to a reversible P2Y12 receptor inhibition but with competitive interaction [78].

In contrast, thienopyridines are a group of orally administered prodrugs that require cytochrome P450 (CYP450)-mediated activation to bind irreversibly to the receptor by forming a disulfide bridge with cysteine 97 residues [79]. Ticlopidine is the first P2Y12 inhibitor effective in the case of cardio-cerebrovascular events and peripheral vascular disease [80]. Although ticlopidine has been shown to have a beneficial role in the prevention of CVD, it is rarely prescribed because it is linked to certain adverse effects, including neutropenia, thrombotic thrombocytopenic, and bone marrow aplasia [81]. Due to these side effects, ticlopidine was replaced by clopidogrel.

Clopidogrel is the best known P2Y12 inhibitor. It binds selectively and irreversibly to the P2Y12 receptor and reduces the interaction of ADP with its receptor [82]. The onset of action of this drug was delayed because of the need for metabolism [83]. Clopidogrel is effective like ticlopidine in preventing CVD but with fewer side effects than ticlopidine [80]. Clopidogrel treatment has also been associated with several side effects such as the risk of bleeding and gastrointestinal disturbances [84]. Several studies have shown that some treated patients still have thrombotic complications [85]. This phenomenon, known as clinical clopidogrel resistance, may be caused by numerous factors such as genetic polymorphism, drug interaction, immature platelets, medication compliance, atherosclerosis, and other factors [86].

Prasugrel is the newest P2Y12 antagonist drug prescribed as an alternative to clopidogrel and ticlopidine. It showed strong inhibition of platelet aggregation and efficient protection from CVD without interindividual variability [87,88]. It is 10 and 100 times more effective than clopidogrel and ticlopidine, respectively [89]. Metabolism of this drug was achieved by a single oxidation step by CYP450 leading to a rapid response [88]. This drug is contraindicated for patients weighing less than 60 kg, patients more than 75 years old, and patients with a history of stroke or ischemic attack, as it may increase the risk of bleeding [90]. Thrombocytopenic purpura and anemia have been reported in patients treated with prasugrel [91].

PAR1 inhibitors

Thrombin is a platelet activator through its interaction with its platelet receptor protease-activated receptor (PAR) [92]. There are four types of PARs (PAR1, PAR2, PAR3, and PAR4) [93]. Thrombin acts on PAR1, PAR3, and PAR4 while PAR2 is activated by trypsin [94]. Human platelets express only PAR1 and PAR4 [95]. These receptors must be cleaved by thrombin to bind the ligand [93]. This interaction leads to platelet activation and aggregation. Thrombin has a high affinity for PAR1 and a low affinity for PAR4 [96]. Blocking PAR1 is a powerful option in antiplatelet therapy. Numerous antagonists of PAR1 display antithrombotic potency. Vorapaxar (SCH 530348), a synthetic analog of natural himbacine, and atopaxar (E5555), a synthetic compound based on the bicyclic amidine motif, are two nonpeptide inhibitors of the thrombin pathway. They are two active oral drugs that produce reversible and competitive PAR1 receptor inhibition. Vorapaxar has a long half-life (126–269 h) while E5555 has a half-life of 22–26 h [97]. Vorapaxar inhibits aggregation induced by thrombin and does not affect the other pathways of platelet aggregation and clotting parameters [98]. However, this drug may lead to severe bleeding and intracranial hemorrhage [99]. Treatment with atopaxar increases cardiotoxicity and liver dysfunction, which led to its use being banned in 2012 [100,101].

RWJ-58259 is a peptide mimetic antagonist of PAR1 receptor that inhibits platelet aggregation triggered by thrombin and does not affect platelet aggregation mediated by collagen or U46619. Unlike vorapaxar and atopaxar, RWJ-58259 is not orally active and needs intravenous administration. Despite its potent activity, clinical trials of this compound did not yield much because of the short half-time (9 min) and the poor oral bioavailability [102].

Phosphodiesterase inhibitors

The cAMP and cGMP secondary messengers inhibit signaling pathways involved in platelet activation. Phosphodiesterase (PDE) is responsible for the hydrolysis of these two messengers [103]. Hence, platelet activation is influenced by PDE activity. Platelets have three isoforms of PDE: PDE2 and PDE3 for hydrolysis of both cAMP cGMP and PDE5 for cGMP only [104]. Inhibitors of these enzymes are potential candidates in the development of antiplatelet agents.

Cilostazol and dipyridamole are the most common PDE inhibitors. Cilostazol, a quinolinone derivative, is a reversible and selective PDE3 inhibitor that inhibits platelet aggregation mediated by ADP, thrombin, collagen, arachidonic acid, and epinephrine without causing any bleeding complications. It is an orally active drug with a half-life of about 11 h. This drug is contraindicated in patients with heart failure and severe renal or hepatic impairment [105].

Dipyridamole, a coronary vasodilator, has also been shown to inhibit PDE activity with a half-life of ~10 h [106]. Contrary to cilostazol, dipyridamole may inhibit both PDE3 and PDE5 [104]. These drugs interfere with platelet activation; nevertheless, they have some adverse effects such as hypotension, tachycardia, and gastrointestinal symptoms [106].

5-HT2A inhibitors

Serotonin (5-hydroxytryptamine) is a platelet activator that is stored in dense granules and binds to the 5-hydroxytryptamine 2A (5-HT2A) platelet receptor. It is a weak and reversible platelet agonist, but it may potentiate the activity of other agonists [107]. Serotonin-mediated platelet activation is a promising target to develop new antiplatelet drugs.

Ketanserin, a 5-HT2A receptor inhibitor, showed antiplatelet activity in response to serotonin. However, the use of this drug was associated with lowering blood pressure [108].

Sarpogrelate is a selective antagonist of 5-HT2A receptor already in clinical use that inhibits vasoconstriction and platelet aggregation induced by serotonin, collagen, and epinephrine [109]. Cyproheptadine and pizotifen (antidepressant drugs) are also clinically used 5-HT2A antagonists that inhibit ADP and TxA2-stimulated platelet aggregation [110]. APD791 and SL65.0472–00 can antagonize the 5-HT2A receptor, but they are only at the end of Phase I clinical trials [111,112].

Inhibitors of platelet aggregation

Platelet aggregation is the last step of primary hemostasis, which leads to the genesis of thrombus. Glycoprotein GPIIb/IIIa is a central player in platelet aggregation that binds fibrinogen to link platelets together and form platelet aggregates [8]. GPIIb/IIIa antagonists are a class of molecules that block the interaction of fibrinogen with platelets and therefore the formation of clots. Three intravenously administered drugs have been successfully developed to inhibit the interaction of fibrinogen and its platelet GPIIb-IIIa receptor (Table 3).

Table 3 Glycoprotein IIb/IIIa antagonists

Targets	Agent	Mechanism	Route of administration	Phase of development	Half-life	Prodrug	Onset of action	Reversibility of platelet inhibition	Side effects and limitations
GP IIb/IIIa	Abciximab		Intravenous	Approved	10–30 min	No	2 h	Yes	Bleeding Thrombocytopenia
	Tirofiban	Glycoprotein GP IIb/IIIa blockade	Intravenous	Approved	2 h	No	20–40 min	Yes	Bleeding Thrombocytopenia
	Eptifibatide		Intravenous	Approved	2.5 h	No	15 min	Yes	Bleeding Thrombocytopenia
	RUC-4		Subcutaneous	Phase II	5–30 min	Yes	15 min	–	–

Abciximab was the first GPIIb/IIIa antagonist to be approved in 1994 and was taken off the market in 2019 after production ceased. It is a humanized monoclonal mouse antibody (Fab fragment) that specifically binds to GPIIb/IIIa receptor with high affinity. The receptor is so unable to interact with fibrinogen and as a consequence platelets do not interact with each other [113]. This drug showed maximal activity within 10 min when at least 80% of the receptors are blocked [114]. Unbound abciximab is rapidly eliminated (half-life ~30 min) [115].

Tirofiban and eptifibatide are two GPIIb/IIIa antagonists that are approved by the FDA in 1998. Tirofiban is a nonpeptide derivative of tyrosine extracted from the snake venom of *E. carinatus*, while eptifibatide is a cyclic heptapeptide derived from the venom of rattlesnakes [116]. They both mimic part of the fibrinogen-binding sequence in GPIIb/IIIa and thus block the binding of fibrinogen to platelets. The half-life was about 2 h for tirofiban and eptifibatide [116].

Furthermore, these GPIIb/IIIa antagonists are associated with significant thrombocytopenia and a higher risk of bleeding particularly abciximab [115]. Alternative molecules were developed to avoid side effects. RUC-2 and RUC-4 are two molecules that display antiplatelet effects by the inhibition of fibrinogen–GPIIb/IIIa interaction in animal models [117]. RUC-4 was used in human patients for the first time in 2020 and was effective and safe following subcutaneous administration [118].

Conclusion

Platelets, although critical in the process of physiological hemostasis, are major mediators in the pathophysiology of CVDs such as stroke and cerebrovascular disease. As such, antiplatelet drugs are widely used for the treatment and prevention of these events. However, these drugs are usually associated with side effects such as the risk of bleeding and variability in individual response, which limits their use. In this way, the research and development of new antiplatelet drugs with increased efficacy and lower risks for patients is highly desired. The current review summarizes the pathways of platelet aggregation in thrombosis and provides an update on the pharmacological properties of current and new antiplatelet agents. This review article also presented guidance for researchers and clinicians to develop new antiplatelet agents with fewer adverse side effects.

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Conflicts of interest

There are no conflicts of interest.

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