Chapter-1a

Platelets in atherothrombosis:
Triggers, targets and treatments for thrombosis
1.1 Introduction

Thrombotic disorders are the major cause of morbidity and mortality in the developed world, while their incidents are rapidly taking the upper hand in developing countries. According to the WHO, 17.1 million people died worldwide of cardiovascular diseases (CVD), per year, accounting for one-third of all deaths globally. By 2020 heart disease and stroke will become the leading cause of both death and disability in the world. On the basis of heart disease and stroke statistics—2010 update, nearly 2300 Americans die of CVD each day, an average of 1 death every 38 seconds.\(^1\) Over the past decade, CVD has exploded in India, emerging as a critical threat not only to the country's health, but also to its economy. Within 10 years, experts say, cardiovascular deaths in India will almost double. WHO estimates that by 2010, India will have 60% of the world's heart patients.\(^1\)

![Pie graph of status report on noncommunicable diseases in India](image)

**Figure 1:** Pie graph of status report on noncommunicable diseases in India

Primary cause of CVD or atherosclerosis – now referred to as atherothrombosis – is the hardening and narrowing of the arteries. It is caused by a slow and progressive build-up of plaque under the lining of the arterial wall which may gradually narrow the artery and restrict blood flow to the target organ. Plaque consists of fat, cholesterol, and calcium found in the blood. Atherothrombosis is characterized by a sudden, unpredictable, atherosclerotic plaque disruption, leading to platelet activation and thrombus (blood clot) formation. The blood clot will most likely enter the bloodstream, partially or totally blocking the blood flow in the artery and causing a local decrease in oxygen supply (ischemia) to the target organ. Plaque rupture is generally accompanied by an acute thrombotic phenomenon leading to the abrupt closure of the vessel, and subsequently leads to ischemic events in the arteries of the brain, heart, kidneys, legs, etc.
and arms. Therefore, atherothrombosis is the underlying condition that results in serious disease and complications such as coronary artery disease, cerebrovascular disease or peripheral arterial disease. Many if not most episodes of thrombosis can be prevented by use of an appropriate primary antithrombotic therapy and almost all instances of recurrence can be prevented by use of an appropriate secondary therapy.

1.2 Haemostasis

Maintenance of blood fluidity within the vascular system is an important human physiological process. The term ‘haemostasis’ refers to the normal response of the vessel to injury by forming a clot that serves to limit haemorrhage\textsuperscript{2,3} and is defined as a complex process that defends against uncontrolled hemorrhage in the event of damage to blood vessel. It can be activated either by vessel injury, tissue injury or the presence of foreign bodies in blood stream. The sol-gel transformation that blood can undergo is popularly considered one of the important ways in which blood loss can be limited. Additionally it is important for wound healing and plays a role in numerous pathological conditions.\textsuperscript{4,6} The haemostatic mechanism of action involves:

1. \textit{Vasospasm of injured vessel}: The initial phase of the process is vascular constriction. This limits the flow of blood to the area of injury.

2. \textit{Formation of a short term platelet plug}: Platelets become activated by thrombin and aggregate at the site of injury, forming a temporary, loose platelet plug. Upon activation, platelets change their shape to accommodate the formation of the plug.

3. \textit{Formation of a strong fibrin clot, thrombus}: To insure stability of the initially loose platelet plug, a fibrin mesh forms and entraps the plug called thrombus.

4. \textit{Dissolution of the clot (fibrinolysis)}: Finally, the clot must be dissolved in order for normal blood flow to resume following tissue repair. The dissolution of the clot occurs through the action of plasmin.

Various factors are involved\textsuperscript{7,8} in the haemostatic process; the established ones include:

1. the extravascular tissues;
2. the vasculature itself, the size and type of vessel being important;
3. the number of functioning platelets, and
4. the plasma coagulation system.
1.3 The Clotting Mechanism

The plasma clotting system, like platelet physiology, has been an area of intense research, and much has been done in the past few years to elucidate this complex and vital link in the haemostatic mechanism. Schmidt (1892) and later Morawitz (1905) first postulated the so-called classical theory of blood coagulation. Since that time many more factors (Table 1) have been postulated, and some identified but this has not made the understanding any simpler or easier.

<table>
<thead>
<tr>
<th>Standard Nomenclature</th>
<th>Traditional Name</th>
<th>Molecular Weight (Da)</th>
<th>Plasma concentration (μg / ml)</th>
<th>Half life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor I</td>
<td>Fibrinogen</td>
<td>340000</td>
<td>2000-4000</td>
<td>90</td>
</tr>
<tr>
<td>Factor II</td>
<td>Prothrombin</td>
<td>72000</td>
<td>120</td>
<td>65</td>
</tr>
<tr>
<td>Factor III</td>
<td>Tissue factor</td>
<td>45000</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Factor IV</td>
<td>Calcium</td>
<td>40</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Factor V</td>
<td>Proaccelerin</td>
<td>330000</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Factor VII</td>
<td>Proconvertin</td>
<td>48000</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Anti haemophilic factor</td>
<td>360000</td>
<td>0.05</td>
<td>10</td>
</tr>
<tr>
<td>Factor IX</td>
<td>Christmas Factor</td>
<td>57500</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Factor X</td>
<td>Stuart-Prower factor</td>
<td>55000</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>Factor XI</td>
<td>Plasma thromboplastin</td>
<td>160000</td>
<td>6</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>antecedent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor XII</td>
<td>Hageman Factor</td>
<td>85000</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>Fibrin stabilising factor</td>
<td>320000</td>
<td>20</td>
<td>200</td>
</tr>
</tbody>
</table>

Table 1: Factors involved in blood coagulation

Blood clotting can be regarded as the end result of a series of enzymatic reactions leading to the conversion of soluble fibrinogen into insoluble fibrin under the influence of thrombin. It is also universally accepted that thrombin comes from a precursor prothrombin, which circulates in the inactive form. Under physiologic conditions, the conversion of prothrombin to thrombin is an enzymatic process. While a specific enzyme has never been identified, the majority of investigators accept thromboplastin as the triggering substance. There are two principal mechanisms of the coagulation process in higher organisms. They are intrinsic system (all factors present in blood) and an extrinsic system (which depends on the release of thromboplastin from the
Chapter 1

The extrinsic pathway requires tissue factor, which is located in the tissue adventitia and comes in contact with blood only after vascular injury. When vascular injury occurs, tissue factor apoprotein in the presence of calcium ions and phospholipids facilitates the conversion of factor VII to factor VIIa by minor proteolysis. The factor VIIa-tissue factor complex then converts factor X to factor Xa. This activated factor Xa converges with the intrinsic pathway to generate thrombin thereby leading to the formation of fibrin. Introduction of a foreign object into the blood vessel endothelium leads to the intrinsic pathway in which factor XIIa serves as activator of factor XI to factor Xla and also activates prekallikrein and high molecular kininogen (HMWK). Factor Xla converts factor IX to factor IXa in presence of calcium ions. This factor IXa in presence of factor VIIIa, calcium ions and phospholipids convert factor X to factor Xa. This newly generated factor Xa forms a one to one complex with factor Va, calcium ions and phospholipids leading to prothrombinase, which converts prothrombin to thrombin. Thrombin in turn, cleaves soluble fibrinogen to form soluble fibrin. This soluble fibrin is converted to stable fibrin polymer by cross-linking fibrin monomers in presence of factor XIIa and calcium ions.

1.4 Fibrinolysis

Fibrinolysis is a process that prevents blood clots from growing and becoming problematic. This process has two types: primary fibrinolysis and secondary fibrinolysis. The primary type is a normal body process, whereas secondary fibrinolysis is the breakdown of clots due to a medicine, a medical disorder, or some other cause. The clot that is formed after a vessel ruptures represents only a temporary haemostatic barrier, which disappears when new tissue or capillaries form. This disappearance of the fibrin clot is brought about by a proteolytic enzymatic system opposed to the coagulation system, and referred to in general terms as fibrinolysis.

1.5 Thrombin

Thrombin is a pivotal enzyme for all major thrombotic processes, including physiologic hemostasis and pathologic thrombosis. Despite its clear relevance in the pathogenesis of arterial thrombotic disorders and well-founded reputation as the “gatekeeper” for a complex series of biochemical events that culminate in intravascular clot formation, there is mounting evidence that the inhibition of coagulation proteins more proximally
injured cells and tissue). Initially when an injury to the subendothelial cells of a blood vessel or a tissue occurs there is an immediate vasoconstrictive reflex which reduces the volume of blood flow. The platelets adhere to the site of injury and plasma proteins forms a bridge between the activated platelet and the subendothelium. Figure 2 represents the schematic coagulation cascade.

![Coagulation Cascade Diagram](image)

**Figure 2: The Coagulation Cascade**

Fibrinogen forms a bridge between activated platelets by binding to surface receptors on adjacent activated platelet, thus leading to the formation of a platelet plug. The coagulation factors interact through a series of reactions to form fibrin, which intertwines, strengthens and reinforces the platelet plug. The coagulation factors except for calcium and phospholipids are all proenzymes.
The extrinsic pathway requires tissue factor, which is located in the tissue adventitia and comes in contact with blood only after vascular injury. When vascular injury occurs, tissue factor apoprotein in the presence of calcium ions and phospholipids facilitates the conversion of factor VII to factor VIIa by minor proteolysis. The factor VIIa-tissue factor complex then converts factor X to factor Xa. This activated factor Xa converges with the intrinsic pathway to generate thrombin thereby leading to the formation of fibrin. Introduction of a foreign object into the blood vessel endothelium leads to the intrinsic pathway in which factor XIIa serves as activator of factor XI to factor Xla and also activates prekallikrein and high molecular kininogen (HMWK). Factor Xla converts factor IX to factor IXa in presence of calcium ions. This factor IXa in presence of factor VIIIa, calcium ions and phospholipids convert factor X to factor Xa. This newly generated factor Xa forms a one to one complex with factor Va, calcium ions and phospholipids leading to prothrombinase, which converts prothrombin to thrombin. Thrombin in turn, cleaves soluble fibrinogen to form soluble fibrin. This soluble fibrin is converted to stable fibrin polymer by cross-linking fibrin monomers in presence of factor XIIa and calcium ions.

1.4 Fibrinolysis

Fibrinolysis is a process that prevents blood clots from growing and becoming problematic. This process has two types: primary fibrinolysis and secondary fibrinolysis. The primary type is a normal body process, whereas secondary fibrinolysis is the breakdown of clots due to a medicine, a medical disorder, or some other cause. The clot that is formed after a vessel ruptures represents only a temporary haemostatic barrier, which disappears when new tissue or capillaries form. This disappearance of the fibrin clot is brought about by a proteolytic enzymatic system opposed to the coagulation system, and referred to in general terms as fibrinolysis.

1.5 Thrombin

Thrombin is a pivotal enzyme for all major thrombotic processes, including physiologic hemostasis and pathologic thrombosis. Despite its clear relevance in the pathogenesis of arterial thrombotic disorders and well-founded reputation as the “gatekeeper” for a complex series of biochemical events that culminate in intravascular clot formation, there is mounting evidence that the inhibition of coagulation proteins more proximally
positioned in the coagulation cascade is paramount to treatment success in unstable angina and myocardial infarction, collectively referred to as acute coronary syndromes.  

1.5.1 Structure and Biochemistry

Thrombin is a serine protease and, like trypsin, cleaves peptide bonds involving arginine residues. In contrast to trypsin, it possesses exquisite selectivity and cleaves peptide bonds in only a limited number of macromolecular substrates. Thrombin is a 308 amino acid peptide and, after cleavage by factor Xa at residue 49, becomes a two-chain active enzyme composed of an A-chain of 49 residues (36 residues and is non-essential for proteolytic activities) and a B-chain composed of 259 amino acids and is derived from the carboxyl terminal sequence of prothrombin. The B chain contains the three active site amino acids, His57, Asp102, and Ser195. The disulfide loops in the serine protease family are characteristic structures, and the four disulfide bridges of thrombin are entirely homologous to those found in chymotrypsins. Serine254 has been identified as the active-site in human thrombin, and the sequence surrounding this residue is entirely homologous with the equivalent peptide sequence for other serine proteases.

In 1989, Bode et al. published the X-ray structure of thrombin. The same group reported a refined crystal in 1992. X-ray crystal structure of a number of thrombin inhibitor complexes provides invaluable information about the interaction of the inhibitor with the active site of the enzyme. The numbering of human α-thrombin residues is based on the chymotrypsin sequence. The crystallographic structure of thrombin reveals that it is organized around two β-barrels with an overall folding pattern to adopt the geometric arrangement required for enzyme inhibitor interaction. Various subsites and possible interactions are:

(a) **Recognition site or Active site, S1**: The S1 pocket of thrombin has Asp189 in the bottom to recognize and develop ionic interactions with inhibitors having a basic group.

(b) **Active site, S2**: Tyr60A and Trp60D of the thrombin specific Tyr-Pro-Pro-Trp insertion loop primarily creates S2 pocket. It forms the proximal hydrophobic pocket for interaction with the lipophilic groups.
(c) Active site S3: The distal S3 pocket which is larger than S2 pocket is created by the side chains of Leu_99, Ile_174 and Trp_215, also has a hydrophobic character for edge to face of Vander Walls interactions with a hydrophobic group.

(d) Active sites, S1’, S2’ and S3’: Active sites S1’, S2’ and S3’ utilize bulky amino acid, hydrophobic and hydrophobic amino acid residues. Ser_{214}, Trp_{235}, Gly_{236} which forms a segment of β-sheet structure bind to the inhibitor via at least one hydrogen bond to straddle the active site.

In addition to its active sites, thrombin possesses two exosites, which are positively charged domains located at opposite poles of the enzyme. Thrombin utilizes exosite-1 to dock on its substrates, thereby orienting the appropriate peptide bonds into its active site cleft. Exosite-2 serves as the heparin-binding domain.

1.5.2 Biological Function(s)

Thrombin is the last enzyme in the clotting cascade functioning to cleave fibrinogen to fibrin which forms the fibrin gel of a hemostatic plug or a pathologic thrombus. In addition, thrombin potentiates the procoagulant process by activating Factors V, VIII, XI and XIII. Thrombin is also involved with other activities including inflammation and wound healing. In response to injury, thrombin is chemotactic for monocytes, fibroblasts and smooth muscle cells. Thrombin can activate neutrophils and platelets which release a myriad of mediators including cytokines, chemotactic factors and growth factors, all of which influence inflammation and lead to resolution of an injury. Thrombin has been shown to be mitogenic for chick embryo fibroblasts and smooth muscle cells. Apart from fibrinogen cleavage it also plays a key role in the initiation of the inhibitory pathways to down regulate the coagulation process, and also activates the fibrinolytic system. Thus the development of thrombin inhibitors as therapeutic agents has become a major focus for the current investigators.

1.6 Platelets

Platelets (thrombocytes) have a crucial role in haemostasis and thrombosis. Platelets are small subcellular fragments (2–5 μm diameter, 0.5 μm thickness and 6–10 fl volume) that circulate in the blood for 7–10 days at a concentration of 150–400 x 10^9 per litre. Structural features of platelets include granules (dense lysosomal and α-granules), mitochondria, a cytoskeleton, a surface-connected canalicular system and a
dense tubular system, but no nucleus. Platelets are derived from the cytoplasm of megakaryocytes, the only polyploid haematopoietic cells.\textsuperscript{41} Polyploid megakaryocytes and their platelet progeny are found only in mammals. In all other animal species, cells involved in haemostasis and blood coagulation are nucleated. The evolutionary events that produced mammalian megakaryocytes and platelets, as well as the biological advantage of this system, remain elusive.\textsuperscript{42-45} The membranes associated with each fragment then quickly close to form anucleate, disk-shaped platelets, which have a lifespan of approximately 8 to 10 days.\textsuperscript{46-48}

The main function of the platelet is to scan the vascular system and respond to endothelial damage by tethering to the site of injury or lesion. It circulates to 'survey' the integrity of the vascular system, where they discriminate between normal endothelial cell lining and areas with lesions. Thus, it is an essential component of the clotting process. To this end, platelets contain several elements crucial to coagulation. Negatively charged elements within its phospholipid membrane provide a surface on which coagulation enzymes can function efficiently, whereas glycoprotein (GP) receptors on the platelet's surface act as points of attachment for both other platelets and various plasma components that are necessary for the clot mass to grow.\textsuperscript{49}

There are many theories advanced in an attempt to explain the functions of the platelets. Their principal role has been described as the ability to form an efficient haemostatic plug at the site of injury of a vessel, with the following sequence of reactions:\textsuperscript{50}

- About one to three seconds after injury to a small vessel, the platelets adhere to the damaged endothelial cells at the edge of the ruptured vessel, and to tissue fibres, especially collagen, which are exposed on the wound surface.
- New platelets adhere to those already fixed and to each other, thus forming loose platelet aggregates which eventually cover the rent in the vessel. These aggregates form a temporary haemostatic plug which is permeable to the outflowing blood.
- After a few minutes the plug becomes impermeable and the bleeding stops. At this stage, electron microscope shows that structural changes do occur in the platelets with loss of granules and mitochondria, but the membrane of most
platelets is well preserved. These steps result in changes in the platelets, which take the form of swelling, pseudopodial movements, agglutination, fusion, and contraction of the platelet mass. These morphological changes have been collectively referred to as viscous metamorphosis.

- The last step is the formation of visible fibrin by coagulation of plasma; this occurs somewhat later. The fibrin reinforces and consolidates the haemostatic plug, but seems of secondary importance only and not essential for provisional primary haemostasis.

The following statements regarding the interaction of thrombin and blood platelets are generally accepted as fact:

- Thrombin can cause the aggregation of blood platelets and the subsequent release of adenosine diphosphate in process requiring the presence of calcium ions.
- Thrombin has been demonstrated to bind to blood platelets in a reversible and saturable manner. This process does not require the presence of calcium ions.
- A small amount of thrombin is irreversibly bound to blood platelets.
- Platelets have been demonstrated to have antithrombin activity.
- Thrombin has been demonstrated to physically interact with two chemically different receptors on the platelet surface.
- Thrombin cleaves platelet membrane proteins.

A schematic representation for the mechanism of haemostasis involving the platelets is diagrammatically represented in Figure 3. More recently, a cell-based model (Figure 4) was proposed to elucidate the key role of the activated platelet in the coagulation process, results in the generation of thrombin.

The participation of platelets in atherogenesis and the subsequent formation of occlusive thrombi (clot) depend on platelets' adhesive properties and the inability to respond to stimuli with rapid activation. By understanding the multifaceted mechanisms involved in platelet interactions with vascular surfaces and aggregation, new approaches can be tailored to selectively inhibit the pathways most relevant to the pathological aspects of atherothrombosis.
Figure 3: Schematic representation of haemostasis involving the platelets.

Figure 4: The cell-based model of coagulation involving platelets
The role of platelets in pathophysiology of intravascular thrombosis is well established and collagen is one of the strong inducer of platelets. Glycoprotein, GPIa/IIa (Integrin α2β1) and GPVI are the two well established collagen receptors present on platelets. It is thus appropriate to develop novel drugs to prevent collagen mediated thrombosis. It has been accepted fact that GPVI is essential for platelet activation by collagen but that GPIa/IIa is required for adhesion.

1.6.1.1.1 GPIa/IIa (Integrin α2β1): The most important collagen-binding sites have been identified on the platelet surface is α2β1 integrin that mediates platelet adhesion to vessel wall collagen and was the first collagen receptor to be identified on platelets.60, 61 It also required for normal platelet development and function and plays a significant role in vascular pathology. Fibril-forming collagens, type-I and III, are the best ligands for GPIa/IIa receptor. Patient platelets devoid of GPIa membrane glycoprotein did not react with collagen type-I. Moreover, antibodies raised against GPIa/IIa blocked the aggregation in response to soluble type-I collagen and adhesion to immobilized collagen. Recently, the α2-I domain of GPIa/IIa has been implicated in the adhesion and aggregation induced by type I collagen. GPIa/IIa was shown to recognize different types of collagen via specific sequences, and thus appears to be an important receptor for collagen.62

1.6.1.1.2 GPVI: GPVI, a 60-65 kDa glycoprotein belongs to the immunoglobulin super family consisting of about 319 amino acids residue and signal sequence of about 20 amino acids.63-66 Platelet GPVI is related to members of the T-cell receptor family. It has two extracellular immunoglobulin-like domains, a mucin-like domain, a transmembrane domain and a cytoplasmic tail.55 GPVI is constitutively expressed on platelets, but in vivo, it is only engaged when collagen is exposed in the subendothelial matrix after disruption of the endothelium. Exposure of collagen to the bloodstream occurs at the site of plaque rupture and initiates platelet activation and platelet-mediated thrombus formation.67, 68 Previously, several platelet collagen receptors have been identified, and it has become clear by now that collagen binding to GPVI is the initial trigger of platelet activation and aggregation at the site of vascular injury where extracellular matrix components, including collagen, are exposed. GPVI-mediated platelet activation leads to firm platelet adhesion and secretion of procoagulative and proinflammatory
1.6.1 Primary Platelet Adhesion Receptors

The most widely studied platelet receptors involved in activation and aggregation phenomenon are:

1) Collagen receptors
2) ADP receptors
3) Thrombin receptors (Protease activated receptors, PARs)
4) Fibrinogen receptors
5) Thromboxane receptors

Over the last 20 years, immense effort has been spent on the identification of these receptors and their individual contribution to the complex processes of platelet tethering, adhesion, secretion, aggregation, and coagulant activity. However, the multiplicity of candidate collagen receptors and lack of detailed knowledge of the molecular events that underlie these responses have severely hampered developments in this field.

1.6.1.1 Platelet collagen receptors

Collagen, a matrix protein has various structural characteristics with diverse function. It is a family of proteins consisting of about 28 different types of proteins and synthesised by a wide variety of cells, such as macrophages, smooth muscle cells, endothelial cells keratinocytes, epithelial cells and fibroblasts. In blood vessels it constitutes about 40% of the total protein which help to maintain vessel wall integrity and elasticity. All types of collagens in the appropriate polymeric form can induce platelet aggregation in vitro. Among various collagens, type-I, III (fibrinellar collagen) and IV (non-fibrinellar collagen) are the most platelet reactive and can induce both adhesions as well as aggregation and are present in the vessel wall. Collagen type-I and III are exposed to platelets mainly when the injury extends to the deeper layer of the vessel wall, the media and adventitia, but in some vessel matrices these collagens are also found in the subendothelium. Fibrillar collagens, types-I, II and III, which occur in skin, bone, cartilage, tendon and in the vitreous humour of the eye. More subtle roles are played by the more complex nonfibrillar collagens, such as types IV and VI, which form two and three-dimensional networks, supporting the interstitial tissues of the body and being the fundamental component of the basement membranes to which epithelial and endothelial cell layers can attach.
The role of platelets in pathophysiology of intravascular thrombosis is well established and collagen is one of the strong inducer of platelets. Glycoprotein, GPIa/IIa (Integrin α2β1) and GPVI are the two well established collagen receptors present on platelets. It is thus appropriate to develop novel drugs to prevent collagen mediated thrombosis. It has been accepted fact that GPVI is essential for platelet activation by collagen but that GPIa/IIa is required for adhesion.

1.6.1.1.1 GPIa/IIa (Integrin α2β1): The most important collagen-binding sites have been identified on the platelet surface is α2β1 integrin that mediates platelet adhesion to vessel wall collagen and was the first collagen receptor to be identified on platelets.60, 61 It also required for normal platelet development and function and plays a significant role in vascular pathology. Fibril-forming collagens, type-I and III, are the best ligands for GPIa/IIa receptor. Patient platelets devoid of GPIa membrane glycoprotein did not react with collagen type-I. Moreover, antibodies raised against GPIa/IIa blocked the aggregation in response to soluble type-I collagen and adhesion to immobilized collagen. Recently, the α2-I domain of GPIa/IIa has been implicated in the adhesion and aggregation induced by type I collagen. GPIa/IIa was shown to recognize different types of collagen via specific sequences, and thus appears to be an important receptor for collagen.62

1.6.1.1.2 GPVI: GPVI, a 60-65 kDa glycoprotein belongs to the immunoglobulin super family consisting of about 319 amino acids residue and signal sequence of about 20 amino acids.63-66 Platelet GPVI is related to members of the T-cell receptor family. It has two extracellular immunoglobulin-like domains, a mucin-like domain, a transmembrane domain and a cytoplasmic tail.55 GPVI is constitutively expressed on platelets, but in vivo, it is only engaged when collagen is exposed in the subendothelial matrix after disruption of the endothelium. Exposure of collagen to the bloodstream occurs at the site of plaque rupture and initiates platelet activation and platelet-mediated thrombus formation.67, 68 Previously, several platelet collagen receptors have been identified, and it has become clear by now that collagen binding to GPVI is the initial trigger of platelet activation and aggregation at the site of vascular injury where extracellular matrix components, including collagen, are exposed. GPVI-mediated platelet activation leads to firm platelet adhesion and secretion of procoagulative and proinflammatory
compounds, which, in turn, aggravate and consolidate thrombus formation. Inhibition of GPVI results in the substantial attenuation of platelet adhesion to atherosclerotic tissue. Although GPVI is constitutively surface-expressed, activation of platelets leads to further release and enhanced plasma membrane expression activation of collagen induced platelet GPVI. Preliminary data suggest that platelet collagen receptor GPVI density may be related to myocardial infarction. Thus, an altered GPVI expression level on circulating platelets may indicate a prothrombotic disease state such as imminent myocardial infarction. 69

1.6.1.1.3 GPIb-IX-V: This binding site can be considered as an indirect collagen receptor acting via von Willebrand (vWb) factor as bridging molecule and is essential for platelet interactions with collagen at high shear rates. GPIb-IX-V is a complex of GPIbα (-135 kDa) disulfide-linked to GPIbβ (-25 kDa) and noncovalently associated with GPIIX (-20 kDa) and GPV (-85 kDa) in the ratio 2:2:2:1. 70, 71 There are approximately 30,000-40,000 copies of GPIb-IX per platelet and half as many copies of GPV. GPIbα, GPIbβ, GPIIX and GPV are type I membrane-spanning glycoproteins, and are all members of the leucine-rich repeat family, with one or more -24-residue leucine rich repeats, plus N- and C-terminal disulfide-looped flanking sequences, in their extracellular domains. 72 The first step in the hemostatic process involves the binding of the platelet adhesion receptor, GPIb/V/IX, to the vascular adhesive protein, vWf. 73, 74

1.6.1.1.4 Other Collagen Receptors: In addition to GPVI and GPIa/IIa, a number of collagen binding proteins such as GPIIb, GP61, GPIV and GPV have been identified. However they are not well established and their role in collagen induced platelet activation is controversial.

1.6.1.2 Platelet ADP Receptors

ADP was described about 40 years ago as a factor derived from red blood cells, affecting platelet adhesion and inducing platelet aggregation. 75-77 It has been well documented that ADP is one of the most important mediators of both physiological haemostasis and thrombosis. 78, 79 Although ADP is regarded as a weak agonist of circulating blood platelets, it is an important mediator of platelet activation induced by other activators (thrombin, collagen), which promote ADP release from intraplatelet storage pools, like dense granules, where it is present in high concentrations. This results in a positive
feedback that enhances platelet aggregation and proliferation of platelet plug. Additionally, ADP acts synergistically to all other platelet agonists, even the weak ones, such as serotonin, adrenaline or chemokines.\textsuperscript{80} Currently, it is known that ADP initiates two signalling pathways in blood platelets;

(i) Phospholipase-C mediated increase in cytosolic concentration of \( \text{Ca}^{2+} \), and
(ii) Inhibition of the formation of cyclic adenosine monophosphate (cAMP).

Adenine nucleotides act on cells via purinoreceptors (P2 receptors), which are present in various cell types: endothelial cells, smooth muscle cells, mastocytes, neuronal cells and blood cells. P2 receptors are divided into two major classes: the superfamily of G-protein coupled receptors (P2Y receptors), and the superfamily of ion channels-coupled receptors (P2X receptors). Blood platelets express three types of P2 purinoreceptors: P2X\textsubscript{1}, P2Y\textsubscript{1} and P2Y\textsubscript{12}.\textsuperscript{81-83}

1.6.1.2.1 \textbf{P2X\textsubscript{1}} receptor: Human P2X\textsubscript{1} is a protein composed of 399 amino acids and consists of two transmembrane domains and a large extracellular domain with 10 cysteine residues.\textsuperscript{84} The N- and C-terminal regions are located inside the cell. P2X\textsubscript{1} is a receptor for ATP but not ADP. Several years ago it was believed that P2X\textsubscript{1} is mainly a receptor for adenosine triphosphate and its potential role in platelet response to ADP could be minor. Interestingly, however, some authors reported that P2X\textsubscript{1} was responsible for a rapid ADP-induced entry of calcium ions into platelet cytoplasm. Recently, it was pointed out that commercially available ADP preparations are contaminated by ATP. Using HPLC and hexokinase in order to remove the ATP contamination, it was found that such a treatment abolishes P2X\textsubscript{1} activation. Altogether, most data indicate that P2X\textsubscript{1} is a receptor for ATP but not for ADP.

1.6.1.2.2 \textbf{P2Y\textsubscript{1}} receptor: P2Y\textsubscript{1} receptor is composed of 373 amino acids and has a structure typical for G protein-coupled receptors. P2Y\textsubscript{1} displays rather low tissue specificity being found in heart, blood vessels, smooth muscle cells, connective and neural tissues, testis, prostate, ovary and blood platelets.\textsuperscript{82} Initially, on the basis of pharmacological studies, it was proposed that in platelets P2Y\textsubscript{1} is responsible for ADP induced aggregation, \( \text{Ca}^{2+} \) mobilisation and inhibition of adenylyl cyclase. Currently, it is known that this receptor plays a key role in platelet shape change, as well as in the initiation of platelet response to ADP.\textsuperscript{85} It mediates the first, reversible phase of platelet
aggregation, whilst the amplification of platelet aggregation and the enhancement of platelet secretion are caused by the other receptor for ADP-P2Y\textsubscript{12}, coupled to and responsible for adenylate cyclase inhibition.

1.6.1.2.3 **P2Y\textsubscript{12} receptor**: P2Y\textsubscript{12} receptor inhibits adenylate cyclase, thus leading to a drop in platelet cAMP concentration. According to the present knowledge, ADP-dependent platelet aggregation is initiated by the P2Y\textsubscript{1} receptor, whereas P2Y\textsubscript{12} receptor enhances the activating signal. Stimulation of P2Y\textsubscript{12} is also essential for complete activation of glycoprotein IIb/IIIa by ADP or the stabilization of platelet aggregates. In blood platelets it is mainly P2Y\textsubscript{12} that underlies the ADP-induced generation of thromboxane A\textsubscript{2}, and it also promotes a release reaction from intraplatelet granules. Overall, P2Y\textsubscript{12} plays a crucial role in activation of circulating platelets and their recruitment to the site of vascular injury, as well as in the enhancement of platelet activation evoked by other platelet agonists. It has been demonstrated that P2Y\textsubscript{12}, unlike the other purinoreceptors described above, is present mainly on blood platelets and, to a much lesser extent, on neuronal cells in the brain, and it does not occur in other tissues. It seems apparent, therefore, that P2Y\textsubscript{12} is an ideal candidate for pharmacological approaches aimed at anti-platelet effects.\textsuperscript{81}

1.6.1.3 **Thrombin receptors (Protease activated receptors, PARs)**

Thrombin mediates its cellular effects primarily through PARs.\textsuperscript{86-89} These receptors are activated by a unique mechanism in which the protease exposes tethered ligands at the extracellular N-terminus by specific minor proteolysis, resulting in intramolecular activation.\textsuperscript{90-91} Three of the four known protease activated receptors, PAR1, PAR3, and PAR4, are activated by thrombin. PAR-2 is activated by other proteases, such as trypsin and tryptase, and coagulation factors VIIa and Xa. Current evidence suggests, however, that PAR1 and PAR4 are the major human platelet thrombin receptors. Specific agonist peptides have been designed for the thrombin-independent activation of PAR1 and PAR4. No agonist peptide for PAR3 has been described. Activation of platelet PAR1 by the PAR1-specific agonist peptide, SFLRN, results in platelet aggregation and degranulation. Human platelets express dual thrombin receptors, specifically protease-activated receptor PAR1 and PAR4,\textsuperscript{92} but there are still no therapeutic strategies that effectively target both receptor subtypes. PAR1 and PAR4 appear to form a stable heterodimer that enables thrombin to act as a bivalent functional agonist. Recent
studies have identified that simultaneous antagonism of PAR1 and PAR4 is synergistic and provides more effective inhibition of thrombin-induced platelet activation than either PAR1 or PAR4 antagonism alone.\textsuperscript{93 (b)}

1.6.1.4 Fibrinogen receptor, GP IIb/IIIa

Platelet glycoprotein (GP) IIb/IIIa complex (also called integrin $\alpha_{IIb}\beta_3$) is the major integrin present on the platelet surface and plays a central role in haemostasis by providing binding sites for fibrinogen, vWF, fibronectin and vitronectin.\textsuperscript{94,95} The glycoprotein GP IIb/IIIa belongs to the integrin superfamily and is the most abundant surface protein. A normal platelet contains approximately 50,000 receptor complexes which bind like several other integrins to an Arg-Gly-Asp-Ser (RGDS) tetrapeptide recognition sequence. Fibrinogen binding to activated GP IIb/IIIa is absolutely required for platelet aggregation. The deficiency of integrin $\alpha_{IIb}\beta_3$ results in the severe bleeding disorder Glanzmann thrombasthenia characterized by a lack of platelet aggregation.\textsuperscript{96} The final common pathway of platelet aggregation involves the activation of GP IIb/IIIa receptors and the subsequent crosslinking of neighboring platelets through fibrinogen and vWF, by the arginine–glycine–aspartic acid (RGD) sequence. A unique feature of integrin $\alpha_{IIb}\beta_3$ is that it is activated upon platelet activation.\textsuperscript{97} In resting platelets, the integrin $\alpha_{IIb}\beta_3$ does not bind soluble fibrinogen; whereas, following platelet activation, there are conformational changes in the GPIIib/IIIa receptor enabling it to bind soluble fibrinogen. Recent biophysical studies are continuing to investigate the molecular basis for the transformation from the inactive to an active configuration.\textsuperscript{98} Recent studies on the biochemical mechanisms of platelet activation indicate that the final obligatory step in platelet aggregation is the cross-linking of plasma protein fibrinogen and platelet membrane GPIIib/IIIa exposed on activated platelets. The inhibition of GPIIib/IIIa receptor binding therefore has been thought of as an attractive target in the development of more effective antithrombotic agents.\textsuperscript{99–103}

1.6.1.5 Thromboxane receptors (TP)

Thromboxane is generated within platelets through the stimulation of phospholipase A2 (PLA2), which itself generated in response to stimulation by other agonists. PLA2 releases arachidonic acid (AA) from membrane phospholipids, which is rapidly transformed in platelets by cyclooxygenase-1 (COX-1), first to the unstable endoperoxides prostaglandin-G2 and prostaglandin-H2, and then converted to
thromboxane A2 \((\text{TxA}2)\) by thromboxane synthase. TxA2 released from the platelet can then act at close range on thromboxane receptors on platelets and other vascular cells.\textsuperscript{104-106} The released thromboxane A2 acts as a positive feedback mediator in the activation and recruitment of more platelets to the primary hemostatic plug. Thromboxane A2 exerts its actions via specific G protein-coupled receptors and has been described as either a potent platelet agonist\textsuperscript{107} or as a weak agonist with an important role in amplifying the response of platelets to more potent agonists.\textsuperscript{108, 109}

### 1.6.2 Epinephrine

Epinephrine (also known as adrenaline) is a hormone and a neurotransmitter. The receptor for epinephrine on the platelets has been identified as \(\alpha_2\)-adrenergic receptor (A2AR) encoded by chromosome-10 and is associated with hypertension and an increase in epinephrine-mediated platelet aggregation in humans. The mechanism by which epinephrine induces platelets aggregation is unknown. Warren \textit{et al} reasoned that epinephrine-induced platelet aggregation is mediated by A2AR-dependent activation of sodium and chloride transport.\textsuperscript{110}

### 1.6.3 Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is synthesized in brain neuronal cells and in the enterochromaffin cells of the gastrointestinal tract and is, apart from acting as a neurotransmitter, important for a variety of physiological functions, including platelet aggregation and smooth muscle contraction. After platelets have fragmented from the parent megakaryocyte, serotonin is acquired through two serotonin transport systems, one situated at the plasma membrane and the other on the dense granule membrane. As a result, all of the serotonin present in blood is, in fact, localized in the platelet dense granules.\textsuperscript{111}

### 1.6.4 von Willebrand factor (vWF)

von Willebrand factor the largest human plasma protein, is an adhesive multimeric protein present in platelets, endothelial cells, and the subendothelium.\textsuperscript{112} It mediates the initiation and progression of thrombus formation at sites of vascular injury by means of specific interactions with extracellular matrix components and platelet receptors. Platelet adhesion to exposed subendothelium at sites of vascular injury is mediated, in part, by interaction of the platelet plasma membrane GP Ib/V/IX complex
with vonWillebrand factor presented on collagen-exposed surfaces.\textsuperscript{113-115} vWF also associates with the procoagulant factor-VIII (FVIII), forming a complex that regulates FVIII secretion inside cells and prevents rapid clearance of the factor from the circulation. vWF has two major functions in hemostasis. First, it is essential for platelet-subendothelium adhesion and platelet-to-platelet interactions and also causes platelet aggregation in vessels. The second major function of vWF in haemostasis is that of being the specific carrier of FVIII in plasma.\textsuperscript{116, 117}

### 1.7 Platelets in atherothrombosis

The platelet is intimately involved in the successive phases of atherothrombosis that are triggered by damage to the endothelial lining of the arterial wall.\textsuperscript{118, 119} The following is a simplified summary of these complex processes, and conveniently divided into 3 stages; platelet adhesion, activation and aggregation. (Figure 5)

![Figure 5. Stages of platelet adhesion, activation and aggregation](image)

#### 1.7.1 Platelet Adhesion

Under normal (nonhemostatic) conditions, platelets are at rest and flow through blood vessels without interacting with any other cells.\textsuperscript{120} However, once a site of vessel damage is detected, passing platelets will adhere to the endothelium within seconds of the injury occurring.\textsuperscript{121} The catalysts for this interaction are the constituents of the exposed subendothelium, including collagen, von Willebrand factor (vWF), fibronectin, laminin, and thrombospondin. Platelets have a range of surface membrane receptors that interact with these constituents, helping the platelet to overcome the high shear forces generated by blood flow and so attach itself to the target site. Collagen is the
most thrombogenic component of the subendothelial matrix, but it requires vWF to stabilize its interaction with the platelet's GP Ia/IIa receptor. Once the platelet has bound to both the exposed collagen and vWF (via the GP-Ib/IX/V receptor complex), it changes shape from a disk to a ball.\textsuperscript{122} This conformational change triggers an internal signaling network that encourages further morphing of the cell into a hemispherical shape, increasing the surface area in contact with the artery wall. Thus, the platelet becomes more firmly anchored, but its adhesion remains reversible. Irreversible adhesion is achieved as a result of another signalling cascade, which causes extensive flattening of the platelet over the injured site.

1.7.2 Platelet Activation

The process by which a resting platelet changes shape and becomes an integral part of clot formation is known as activation.\textsuperscript{123} The binding of collagen and vWF to platelet receptors triggers intracellular signals within the platelet mediated by calcium. This in turn causes degranulation of storage vesicles which contain the platelet-activating substances [ADP] and serotonin, synthesis of thromboxane-A\textsubscript{2} (also a platelet activator and a vasoconstrictor), and an increase in the surface population of the GP IIb/IIIa receptor. At the same time, circulating thromboxane-A\textsubscript{2} and ADP, in concert with other soluble agonists (eg, \textalpha{-thrombin, epinephrine}, bind to adherent platelets and synergistically induce their activation. Thus, platelet activation is amplified by several secondary feedback pathways. Another important step in platelet activation is the exposure of phospholipid membrane components on the platelet's surface. This triggers the well-known coagulation cascade eventually resulting in the generation of insoluble fibrin, which provides stabilizing cross-links between adjacent platelets. However, this traditional model reflects only the interactions of the proteins involved in blood coagulation. The cell-based model proposed (Figure 4) that the interactions among activated platelets, coagulation cofactors and their associated enzymes result in the generation of large amounts of thrombin, which is itself a potent stimulant of platelet activation.\textsuperscript{124}

1.7.3 Platelet Aggregation

Aggregation is primarily mediated by GP IIb/IIIa receptors on the platelet surface that bind various adhesion proteins. These receptors have low affinity for their ligands under resting conditions but are up-regulated during platelet activation and become
operational. One of the most important adhesion molecules bound by GP IIb/IIIa receptors is fibrinogen. Because platelet activation increases the surface density of these receptors, more fibrinogen can be bound, making higher concentrations accessible for thrombin-mediated conversion into fibrin. Concurrently, the GP IIb/IIIa receptors on the surface of adherent platelets immobilize other soluble adhesion proteins such as vWF, fibronectin, and vitronectin. Altogether, these processes attract more platelets to the site of injury, and the binding of fibrinogen encourages fibrin cross-linking with these newcomers. Fibrin then strengthens the structure, allowing continued platelet aggregation, thrombus formation, and growth.\textsuperscript{123, 125, 126}

<table>
<thead>
<tr>
<th>Phase of response</th>
<th>Substrates, Agonists, Ligands</th>
<th>Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initiation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tethering and Adhesion</td>
<td>vWF</td>
<td>GPIb-IX-V</td>
</tr>
<tr>
<td></td>
<td>Collagen</td>
<td>$\alpha_2\beta_1$, GPVI-FcR_1</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen, fibrin</td>
<td>$\alpha_{IIb}\beta_3$</td>
</tr>
<tr>
<td></td>
<td>Fibronectin</td>
<td>$\alpha_4\beta_1$</td>
</tr>
<tr>
<td></td>
<td>Laminin</td>
<td>$\beta_6\beta_1$</td>
</tr>
<tr>
<td><strong>Propagation</strong></td>
<td>Activation</td>
<td>$\alpha$-Thrombin</td>
</tr>
<tr>
<td></td>
<td>ADP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thromboxane A_2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\alpha_{2A}$</td>
</tr>
<tr>
<td><strong>Aggregation</strong></td>
<td></td>
<td>Fibrinogen, vWF, fibronectin</td>
</tr>
<tr>
<td><strong>Stabilization</strong></td>
<td>P-selectin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ephrin B1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD40 ligand</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GAS6</td>
<td></td>
</tr>
<tr>
<td><strong>Inhibition</strong></td>
<td>PECAM-1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PGI2</td>
<td></td>
</tr>
</tbody>
</table>

*Table 2: Receptors, ligands and signalling pathways in platelet thrombus formation*

1.8 **Platelet Aggregation Inhibitors and Antiplatelet Therapy**

Medical therapies targeting various pathways in this cascade of events have been or are currently being developed for use as antiplatelet agents. These include therapies aimed at inhibiting TxA\_2, ADP, GPIIb/IIIa, thrombin, collagen, and vWF. Complex interactions between these various factors and the mechanisms involved (Figure 6) in this process
ensure redundancy in the pathways responsible for platelet activation and thrombus formation.

Figure 6: Platelet function and molecular targets of antiplatelet agents

1.8.1 ADP-Receptor Antagonists

The use of inhibitors blocking the interaction of ADP with the P2Y₁ and P2Y₁₂ receptors seems promising as antiplatelet agents. The outcomes of large clinical trials and smaller studies on the effects of purinoreceptor antagonists indicate that new generation drugs antagonizing ADP receptors may be more effective than the most commonly used antiplatelet drug, aspirin, in reducing the combined risk of myocardial infarction, ischaemic stroke, vascular disease or cardiovascular fatal events. Also, the overall clinical safety profile of thienopyridine-derived blockers, at least as good as that of medium-dose aspirin, seems encouraging. Despite these advantageous characteristics, the cost-effectiveness ratio is much worse for thienopyridines, the only widely used purinoreceptor blockers in today’s clinics, than for aspirin. Therefore, the use of currently available purinoreceptor antagonists is considered rather in a combination therapy with other antiplatelet agents than as a single agent treatment in
long-term antiplatelet therapy. Hence, the invention of low-cost new generation blocking agents of ADP receptors, preferably suitable for oral administration, is challenging. The fact that ADP is a rather small and relatively simple molecule makes designing and testing novel competitive blockers with a desirable pharmacokinetic profile fairly straightforward.

1.8.1.1 **P2Y\textsubscript{12} antagonists:** The first P2Y\textsubscript{12} antagonists were thienopyridine derivatives. They irreversibly block P2Y\textsubscript{12} as a result of a covalent modification of cysteine residues located in the extracellular portion of the receptor.\textsuperscript{128, 129}

**Ticlopidine:** Ticlopidine (trade name Ticlid) (1) was the first major thienopyridine used in the treatment of patients with vascular disease that is metabolized by cytochrome P450 in the liver. Chemically it is 5-[(2-chlorophenyl)methyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridine.\textsuperscript{127, 130-132a}

![Chemical structure of ticlopidine](image)

Ticlopidine inhibits platelet aggregation by altering the function of platelet membranes by blocking ADP receptor, P2Y\textsubscript{12}. This prevents platelet expression of GP\textsubscript{IIb/IIIa} which prevents platelet binding to fibrinogen. In a study of 652 patients, those receiving ticlopidine versus conventional therapy had a 46.8% relative reduction in the combined rate of vascular death or nonfatal MI at 6 months.\textsuperscript{132b}

**Clopidogrel:** Clopidogrel (2) is the present choice owing to its more favourable side-effect profile and rapid onset of action.\textsuperscript{133-136} Clopidogrel is absorbed from the intestine and extensively metabolized by hepatic cytochrome-P450 (CYP-3A4) to an active thiol metabolite. This short-lived metabolite irreversibly binds to the P2Y\textsubscript{12} receptor for the lifetime of the platelet. In addition, clopidogrel may attenuate thrombin generation by inhibiting platelet activation. The first large randomized clinical trial to evaluate the effectiveness and safety of clopidogrel was the CAPRIE (Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events) study, which was a secondary prevention study comparing clopidogrel (75 mg/d) versus aspirin (325 mg/d) therapy in patients with recent MI, ischemic stroke, or symptomatic peripheral arterial disease.
Clopidogrel use was associated with an 8.7% relative risk reduction for the composite outcome of vascular death, MI or stroke. Clopidogrel also reduced rehospitalisation for ischemic events to a greater extent than aspirin.

**Prasugrel: (CS-747; LY-640315):** Prasugrel \(^{137-139}\) (3) is a third-generation oral thienopyridine that is chemically distinct from clopidogrel. Like clopidogrel, prasugrel is a specific, irreversible antagonist of the platelet adenosine 5'-diphosphate (ADP) P2Y\(_{12}\) receptor. Prasugrel is a prodrug that must be converted to an active metabolite (R-138727) by the hepatic cytochrome-P450 (CYP) system to exert antiplatelet activity. It is rapidly absorbed and metabolized, with a median time for achieving the maximal concentration of its active metabolite in the circulation of about 30 minutes.

The mean elimination half-life of the prasugrel active metabolite is 3.7 hours, and renal excretion (approximately 70%) is the major route for elimination of prasugrel metabolites in humans. An important difference between the metabolism of prasugrel and that of clopidogrel is that a significant portion of the administered dose of clopidogrel is deactivated in the early stages of its metabolism, resulting in less availability of the active metabolite. The active metabolites of both prasugrel and clopidogrel covalently bind to the active site of the P2Y12 receptor in a similar manner through a disulfide linkage.\(^{140-146}\)

**Cangrelor (ARC-669931MX):** Cangrelor (4) is a member of an investigational novel chemical class of ATP analogs undergoing late-stage clinical development for intravenous use during coronary procedures.\(^{127, 131}\) This compound is a high-affinity, reversible antagonist for the P2Y\(_{12}\) receptor that causes a pronounced, virtually total inhibition of ADP-induced platelet aggregation.
Cangrelor, like Ticagrelor, is a chemically modified ATP derivative that is stable to enzymatic degradation. It does not require metabolic conversion to an active metabolite and hence is immediately active after infusion\textsuperscript{147} The human pharmacokinetics of cangrelor is favourable and the results of the preclinical studies imply that cangrelor has the potential to prevent thrombus formation and reduce emboli formation as well as restore arterial patency and improve blood flow in combination with fibrinolytic agents\textsuperscript{148-150}.

**Ticagrelor (AZD6140):** Ticagrelor (5) is the first of a new class of orally active antiplatelet agents, the cyclopentyltriazolopyrimidines. Similar to the thienopyridines, AZD6140 inhibits the prothrombotic effects of ADP by blocking the platelet P2Y\textsubscript{12} receptor.

This adenosine 5'-triphosphate (ATP) analog evolved through the chemical modification of ATP to yield cangrelor, an intravenously administered P2Y\textsubscript{12} receptor antagonist, followed by β,γ-methylene substitutions of the ester linkages in the triphosphate group\textsuperscript{151} Unlike the thienopyridines, AZD6140 binds to the P2Y\textsubscript{12} receptor in a reversible fashion. AZD6140 confers nearly complete inhibition of ADP-induced platelet aggregation and is highly specific for the P2Y\textsubscript{12} receptor with insignificant affinity for other P2 receptors. Furthermore, AZD6140 does not require metabolic activation for activity. AR-C124910XX, an active metabolite of AZD6140, has been identified in the
circulation; it is about as potent as the parent molecule with respect to blocking the P2Y\textsubscript{12} receptor and is thought to contribute to the antiplatelet effect of the parent molecule. Peak plasma levels of AZD6140 are reached between 1.5 and 3 hours after treatment, with steady state reached after 2 to 3 days. The mean elimination half-life is 6-12 hours, independent of dose.\textsuperscript{152-155}

**Elinogrel**: Elinogrel (6) is an investigational, direct-acting, reversible P2Y\textsubscript{12} antagonist with a novel structure. It can be administered orally or intravenously and is currently undergoing Phase II trials.\textsuperscript{156}

\[ \text{6} \]

1.8.1.2 **P2Y\textsubscript{1} antagonists**: The action of ADP on P2Y\textsubscript{1} receptor can be blocked by specific antagonists, including adenosine-2',5'-bisphosphate (A2P5P), adenosine-3',5'-bisphosphate (A3P5P) and adenosine-3'-phosphate,5'-phosphosulphate (A3P5PS). They competitively block the receptor, however, only at concentrations as high as micromolar. Another group of efficient agents includes N6-methyl-2'-deoxyadenosine-3',5'-bisphosphate (MRS2179)\textsuperscript{157} and (N)-methanocarba-N6-methyl-2-chloro-2'-deoxyadenosine-3',5'-bisphosphate (MRS2279)\textsuperscript{158}

1.8.2 **GPIIb/IIIa Antagonists**

The GP IIb/IIIa receptor antagonists act by blocking the binding of fibrinogen to the platelet GP IIb/IIIa receptor, the final common pathway of platelet to platelet aggregation.\textsuperscript{159-163} The potent in vivo antiplatelet activity of this group of drugs has led to the development of both intravenous and oral formulations of GP IIb/IIIa receptor antagonists.\textsuperscript{164, 165}

**Abciximab**: Abciximab (Figure 7)\textsuperscript{166} is a recombinant monoclonal antibody that not only binds to the platelet GP IIb/IIIa receptor with high affinity, but also to the vitronectin receptor present on platelets, smooth muscle cells, monocytes and leukocytes and to
the macrophage (MAC)-1 receptor present on leukocytes. Abciximab has also been reported to inhibit tissue factor-induced thrombin generation.

**Figure 7:** Protein structure of Abciximab

Following intravenous administration, free plasma concentrations of abciximab decrease rapidly, with an initial half-life of less than 10 minutes and a second phase half-life of 30 minutes, probably related to rapid binding to the platelet GPIIb/IIIa receptor. Platelet function generally recovers over the course of 48 hours; however, abciximab remains in the circulation up to 10 days in the platelet bound state. Upon completion of a constant infusion, free plasma concentrations fall rapidly over the next 6 hours, then decline at a slower rate. Intravenous administration of abciximab in doses ranging from 0.15 mg/kg to 0.3 mg/kg produces a rapid dose-dependent inhibition of platelet functions as measured by ex vivo platelet aggregation in response to ADP. It has been proposed that the multiple binding possibilities of abciximab for receptors characterized with a similar binding motif may provide additional antithrombotic effects.

**Lamifiban:** Lamifiban (7) is part of a new class of GPIIb/IIIa antagonists that has been developed without peptide bonds. The potential advantage of the “peptidomimetic” inhibitors are their specificity and increased circulating half-life. Following intravenous administration, lamifiban binds minimally to plasma proteins and has a relatively short terminal half-life of 84 minutes. Saturation of the platelet GPIIb/IIIa receptor takes place at relatively low concentrations of lamifiban, and ADP-induced aggregation declines to 10% of baseline within 60 minutes of infusion. A return to 50% of baseline occurs within 5–6 hours of discontinuation.\textsuperscript{167}
Fradasiban: Fradasiban (8) is a nonpeptide mimetic of the arginine-glycine-aspartic acid recognition sequence. Single doses of 1–15 mg fradasiban administered intravenously to human volunteers produced significant occupancy of the platelet GPIIb/IIIa receptor within 30 minutes. At doses 3 mg, ADP (20 nM) induced ex vivo platelet aggregation was inhibited completely. The platelet inhibition effect persisted for several hours.

Lefradasiban: Lefradasiban (9), a prodrug of fradasiban (8), has been developed as an oral platelet GPIIb/IIIa antagonist. Fradasiban has limited oral activity because of its high polarity and recotting, and thus poor absorption. After oral ingestion, esterification of the carboxyl group and acylation of the amino fradasiban has yield a far less polar prodrug, lefradasiban. However, for platelet GPIIb/IIIa receptor inhibition to occur, lefradasiban must be converted metabolically to fradasiban by esterases (non-cytochrome P450–dependent enzymes). Varying doses of lefradasiban, ranging from 10-150 mg, have been administered to healthy human subjects.

Although platelet inhibition was not observed with low doses, a 50 mg oral dose inhibited ADP-mediated platelet aggregation by 90.5% at 2 hours, 59.14% at 8 hours, and, 10% at 24 hours following administration. Platelet aggregation was completely
inhibited by 75 mg and all higher doses of lefradafiban. Complete suppression of ADP-induced aggregation was observed in both the 50 mg and 75 mg dose groups on all days.

**Tirofiban**: Tirofiban (10) (Aggrastat), a tyrosine derivative (N-(butylsulfonyl)-O-(4-[4-piperidinyl]butyl)-L-tyrosine monohydrochloride monohydrate) with a molecular weight of 495 kD, is a nonpeptide inhibitor of the platelet GPIIb/IIIa receptor. Tirofiban, like other nonpeptides, mimics the geometric, stereotactic, and change characteristics of the RGD sequence and thus interferes with platelet aggregation.

![Chemical Structure](image)

Three doses of tirofiban were studied in a Phase I study of patients undergoing coronary angioplasty. Patient received one of three graduated regimens of tirofiban intravenously with a bolus dose of 5, 10, and 10 and 10 mg/kg and a continuous infusion of 0.05, 0.10, and 0.15 lg/kg/min. A dose-dependent inhibition of ex-vivo platelet aggregation was observed within minutes of bolus administration and was sustained during the continuous infusion.

**Eptifibatide (Integrilin)**: (N⁶-(aminomimonomethyl)-N²-(3-mercaptop-1-oxopropyl-L-lysylglycyl-L-α-aspartyl-L-tryptophyl-L-prolyl-L-cysteinamide, cyclic disulfide) (11) is a nonimmunogenic cyclic heptapeptide with an active pharmacophore that is derived from the structure of barbourin, a platelet GPIIb/IIIa inhibitor from the venom of the Southeastern pigmy rattlesnake. The plasma half-life of eptifibatide is 10–15 minutes, and clearance is predominantly renal (75%) and hepatic (25%). The antiplatelet effect has a rapid onset of action and is rapidly reversible. In all dosing groups, platelet-function returned to >50% of baseline within 4 hours of terminating the infusion. It is important to mention that the use of citrate tubes for sample collection may have overestimated the antiplatelet effects of the doses of eptifibatide studied given the requirement for calcium displacement at RGD binding sites (on the GPIIb/IIIa receptor) prior to adhesive protein binding.
**Roxifiban** (DMP-754): DMP-754 (Dupont-Merck Pharmaceuticals) (roxifiban), (12) is an ester prodrug that requires in vivo hydrolysis (blood, liver) to the active compound XV459, a selective and potent antagonist of the platelet GPIIb/IIIa receptor.

The rate of gastrointestinal absorption of roxifiban has been investigated in animals and healthy human volunteers, with peak plasma concentrations being obtained within 4 hours of oral administration. The initial disposition half-life is under 24 hours. Plasma concentrations continue to decline in the terminal disposition phase with a half-life of approximately 5 days. In phase I clinical trials, platelet inhibition increased with daily dosing and peaked on day 4. The average platelet inhibition after the fifth daily dose was 41.9% (1 mg dose), increasing to 64.8% (with 2 mg dose).\(^{171}\)

**Xemilofiban** (SC-54684A): Xemilofiban (13) is one of a class of potent and orally active agents for the prevention of thrombotic complications. It is the prodrug of an active nonpeptide mimetic of the RGD-containing peptide sequence that is recognized by the platelet GPIIb/IIIa receptor.\(^{172}\) Following oral administration, xemilofiban is converted to the active moiety in plasma by ester metabolism and hydrolysis. It has excellent bioavailability and a long duration of action.
The lowest dose yielded 30% inhibition, while the highest dose produced 70% inhibition within 4 hours. After prolonged daily administration (16 days), platelet function was fully restored within 4 days of duration.\textsuperscript{173}

\textbf{Sibrafiban:} Sibrafiban (14) is a double prodrug of Ro44-3888 that is a potent and selective antagonist of platelet GPIIb/IIIa. After oral administration, the double prodrug is absorbed and metabolized to Ro48-3656 (prodrug) and Ro44-3888 (active drug), both of which can be found in plasma and urine.

The active drug exhibits a terminal half-life of 10–12 hours in dogs and 95% is cleared by renal excretion within 4 days. Oral administration of sibrafiban causes a dose-dependent inhibition of platelet aggregation. High levels of platelet inhibition were achieved, and mean peak values of 47–97% were seen on day 28. Twice-daily dosing provided more sustained platelet inhibition than daily dosing. In most cases, the antiplatelet effect was observed within 6 hours of administration and dissipated by 24 hours.\textsuperscript{174,175}

\textbf{Orbofiban:} Orbofiban (15) is an oral ethyl-ester pro-drug that specifically blocks the binding of fibrinogen to the platelet GP IIb/IIIa receptor, thereby interfering with the platelet aggregation induced by various agonists. The active molecule is excreted renally and has a terminal elimination half-life of 16 to 18 hours. Oral GP IIb/IIIa inhibition with orbofiban appears not to be beneficial in the long-term treatment of acute coronary
symptoms. Moreover, the Orbofiban in Patients with Unstable Coronary Syndromes (OPUS) trial had to be terminated prematurely, due to an unexpected increase in mortality among patients treated with orbofiban\textsuperscript{176}.

\begin{center}
\includegraphics[width=0.3\textwidth]{image1}
\end{center}

\textbf{Lotrafiban (SB-214587):} Lotrafiban (16) is a selective, nonpeptide antagonist of the human platelet fibrinogen receptor (glycoprotein IIb/IIIa) for treatment of coronary or cerebral atherosclerotic disease.

\begin{center}
\includegraphics[width=0.3\textwidth]{image2}
\end{center}

Chemically it is (S)-2,3,4,5-tetrahydro-4-methyl-3-oxo-7-((4-(4-piperidyl)piperidino) carbonyl)- 1H-1,4-benzodiazepine-2-acetic acid. Lotrafiban entered phase III trials; the BRAVO (Blockade of the IIb/IIIa Receptor to Avoid Vascular Occlusion) trial assessed the efficacy and regimen of the drug candidate. The drug was showing a higher mortality rate than placebo, and was also associated with an increased incidence of serious thrombocytopenia and major bleeding. As a result of these findings the company has discontinued development of lotrafiban.\textsuperscript{177,178}

\textbf{Elarofiban (RWJ-3308),\textsuperscript{179-180} (17)} which recently completed phase II trials, resulted from the optimization of RWJ-50042, an early prototype compound which was designed to mimic the gamma chain of fibrinogen.
In phase I trials, a 1mg/Kg dose had a $t_{1/2}$ of 25.5h and inhibited ADP-induced platelet aggregation by 51% after 2 h. Another compound with a long half-life reaching advanced clinical trials is gantofiban $^{181}$ (EMD-122347) (18) after getting rapidly absorbed on oral administration and converted into its active metabolite, EMD-132338.

**Other GP IIb/IIIa antagonists in pipeline:** SR121787 $^{182, 183}$, (19) is a diester, N-ethylcarbamoyl prodrug of SR-121566 $^{184, 185}$ ($IC_{50} = 46$ nM). A 2mg/Kg oral dose in baboon's resulted in rapid inhibition of ex vivo platelet aggregation (15 min) and significant inhibition (43%) was still detected 24 h post dose. No bleeding events or drop in platelet counts were observed at doses which gave maximal inhibition of platelet aggregation.

![Chemical Structures](image)

Other molecules in pipeline are MS-180 $^{189}$ (23) its active metabolite (24) ($IC_{50} = 35$ nM), AR0510, $^{190}$ the ethyl ester prodrug of (25) ($IC_{50} = 18$nM). Hydantoin, S1197 $^{191}$ (26) is orally active when administered as its ethyl ester prodrug S-5740 $^{191}$ (27).
NSL-96173\(^{192}\) identified from an SAR study of a series of compounds that incorporate a tri-substituted β-amino acid as a conformational restriction, had limited bioavailability even as its ethyl ester prodrug (28). Introducing thiazolidine moiety at one of the amidine nitrogens the so obtained analog NSL-96184 \(^{192}\) (29) had shown improved and prolonged half-life after oral administration. Thienothiophene \(^{193}\) (30) (IC\(_{50}\)=17nM) and benzodiazepinedione \(^{193}\) derivatives (31) (IC\(_{50}\)= 54 nM) are also mentioned in the literature having good selectivity profile.

![Chemical Structures]

A sudden halt in the drug discovery paradigm for GPIIb/IIIa inhibitors evoked scientific community to understand the precise underlying molecular mechanism of inhibition and the consequences thereof; thus the competition of these inhibitors with fibrinogen for the target receptor GPIIb/IIIa results in the inhibition of platelet aggregation. All the RGD or fibrinogen γ-chain dodecapeptide-based inhibitors possessing specificity and high affinity for the receptor bind to resting and activated forms of GPIIb/IIIa. It is well documented that besides the receptor conformational alteration upon agonist-induced platelet activation, ligand binding to GPIIb/IIIa induces further conformational changes of the receptor extracellular domain, resulting in clustering and the exposure of neoantigenic sites termed ligand-induced binding sites (LIBS).\(^{194}\) LIBS modulate secondary function by shifting the conformational equilibrium in the presence of ligand. Some of the affected functions include the secondary wave of platelet aggregation, platelet adhesion to collagen, clot retraction and platelet secretion.
One leading explanation for the failure of these inhibitors is their suboptimal pharmacokinetic properties which has been very recently addressed and surmounted by Mehrotra et al through an elegant approach of appending the acidic and basic residues onto a constrained template of diazaspirodecane scaffold. The distance between these acidic and basic terminals was synchronized through the use of different linkers so as to mimic the RGD motif of the fibrinogen and thus Mehrotra et al successfully came up with the spirolactam and spirohydantoin prototypes, based potent inhibitors with selective and more favorable pharmacokinetic properties (in vitro assays).

The intrinsic activating effect of GPIIb/IIIa blockers is widely discussed as one potential contribution factor for the disappointing outcome of trials with GPIIb/IIIa inhibitors. Although the existence of an activating property of GPIIb/IIIa blockers leading to platelet aggregation under physiological conditions is questioned, the ligand-induced conformational alteration, activation, clustering and signaling of the receptor have been extensively documented. Now strategy undertaken for the development of newer mechanism based inhibitors should involve the exploitation of the accumulated knowledge of GPIIb/IIIa ligand binding sites. To achieve this target the design of the fibrinogen ligands could be based the sequences derived from GPIIb/IIIa ligand binding sites and sequences complementary to RGD and/or to fibrinogen γ-chain.

1.8.3 Thromboxane Pathway Inhibitors

**Aspirin:** Aspirin (Acetyl salicylic acid) (35) has been used clinically for more than 50 years and is the most commonly used antiplatelet drug. Aspirin not only significantly reduces the incidence of a first myocardial infarction in men at risk of
cardiovascular disease (primary prevention) but also reduces the risk in patients who have had a myocardial infarction (secondary prevention).

Aspirin therapy, however, is not without risk and can cause stomach ulcers and bleeding. It inhibits platelet cyclooxygenase1 (COX-1), which is required for the synthesis of thromboxane A₂ (TXA₂), a potent activator of platelets.²⁰¹,²⁰² It irreversibly acetylates Ser529 of COX-1, rendering the catalytic site of COX-1 inaccessible to arachidonic acid and therefore inhibiting the generation of prostaglandin H₂ and, subsequently, thromboxane A₂.²⁰³ Although a repeated dose of 30 mg/day is sufficient to fully inhibit COX-1 in platelets, a 75–150 mg daily dose for long-term prevention and a 150–325 mg daily dose for rapid and complete inhibition of platelets in high-risk CV patients are recommended.²⁰⁴ Since aspirin is approximately 150-fold more potent in inhibiting platelet COX-1 (antithrombotic effect) than COX-2 present in endothelial and other cells (anti-inflammatory effect), it is more effective as an antithrombotic agent at a 75–325 mg/day dose.

Sulfinpyrazone (36)²⁰⁵ and indobufen (37)²⁰⁶ are also used as COX-1 inhibitors.

Recent studies of the selective cyclooxygenase-2 inhibitors rofecoxib and valdecoxib have shown that inhibiting the ‘wrong’ cyclooxygenase can lead to a significant increase
in the incidence of myocardial infarction and stroke, resulting in the withdrawal of these drugs from the market. It has been proposed that these inhibitors reduce COX-2-dependent synthesis of prostacyclin (also known as PGI2), an inhibitor of platelet activation.

**Ridogrel**: Ridogrel (38) is a TxA2 inhibitor with additional prostaglandin endoperoxide receptor antagonist properties that further enhances its antiaggregatory effects by diverting endoperoxide intermediates into the prostacyclin production pathway.

![Chemical Structure of Ridogrel](image)

Ridogrel has been studied primarily as an adjunctive agent to thrombolytic therapy in acute MI. Despite positive results from initial pilot studies, the largest clinical study, the Ridogrel versus Aspirin Patency Trial (RAPT) failed to demonstrate any advantage with this agent over aspirin.46 In the study of 907 patients with AMI, there was no difference in the primary end point of infarct vessel patency rate between those randomized to ridogrel (72.2%) or aspirin (75.5%). Various mechanisms are likely responsible for the results seen with ridogrel in clinical trials, including potentially ineffective thromboxane receptor inhibition with the concentrations of ridogrel used in human studies. As such, there currently are no clinical indications for preferential use of ridogrel over aspirin.

**Other Thromboxane Inhibitors**: Additional thromboxane inhibitors currently under investigation include a NO-releasing aspirin, NCX-4016 (39) and a thromboxane receptor antagonist, S18886 (40). The potential advantages of NO-releasing aspirin include all the benefits of aspirin therapy, combined with the multiple properties of NO including its gastroprotective, antithrombotic, antiatherogenic, and vasodilatory effects. Unlike aspirin or its derivatives, S18886 acts directly on the thromboxane receptor. As a reversible inhibitor of the thromboxane receptor, S18886, has been shown to prevent atherogenesis and cause plaque regression in animal studies, properties likely independent of any TxA2 effects. In a small study of aspirin-treated patients with CAD, a
single dose of S18886 (10 mg) resulted in improved endothelial function as assessed by vasodilatory response to acetylcholine.\textsuperscript{212} Dazoxiben (41)\textsuperscript{213, 214} is used as thromboxane synthase inhibitor and sulotroban (42)\textsuperscript{215}, while, GR32191 (43)\textsuperscript{216} is used as thromboxane receptor antagonist. Another drug picotamide (44)\textsuperscript{217, 218} is also used as combined thromboxane synthase/thromboxane receptor inhibitor.

1.8.4 Protease Activated Receptor Antagonists

There have been several peptide and nonpeptide antagonists of PAR1 developed, but only two PAR4 antagonists (YD-3 and YC-1). YD-3, as the first nonpeptide PAR4 antagonist, had little or no effect on thrombin-induced platelet aggregation alone and significantly enhanced the anti-aggregatory activity of PAR1 antagonists.\textsuperscript{93} In human platelets, the inhibitory effect of YD-3 was significant only when function of PAR1 was blocked or attenuated.\textsuperscript{219} This may be one reason why YD-3 has not made it into clinical use. However, the results indicate that thrombin-induced platelet activation cannot be inhibited effectively by blocking just one of the thrombin receptor pathways and suggest a rationale for potential combination therapy in arterial thrombosis.\textsuperscript{93} Although
the two PAR4 antagonists are rabbit models that terutroban sodium inhibits the still under preclinical investigation, two of the orally administered PAR1 antagonists, namely Atopaxar (E5555), (45) and Vorapaxar (SCH530348), (46), are currently undergoing evaluation in phase II studies. The efficacy and safety of the former is being evaluated in patients with coronary artery disease, whereas the safety of various doses of the latter is being investigated in PCI.

As a selective antagonist of PAR1, RWJ-58259 (47) has been shown to interfere with thrombotic activity in nonhuman primates, even in the presence of an active PAR4 receptor.

Preclinical results suggest potential clinical usefulness of RWJ-58259 in the treatment of thrombotic disorders and vascular injury associated with acute coronary interventions and atherosclerosis.

1.8.5 Collagen Receptor Antagonists

Various molecules both from synthetic and natural sources have been found to inhibit platelet collagen interactions and the detailed mechanisms of their modulating effects are yet to be discovered. JAQ1, a monoclonal antibody against GPIV receptor, induces internalization and proteolytic degradation of GPIV in circulating mice platelets.
Single chain antibodies with variable domains, 10B12 and 6F1 against GPIV, have been used to study their effects on platelet activation and found to inhibit intracellular calcium mobilization. IgG autoantibody against GPIV also inhibits collagen induced platelet aggregation. DGEA, a synthetic peptide, antagonists of α2β1 collagen receptor, which inhibits collagen mediated adhesion, aggregation. A peptide sequence KOGEOGPK and a recombinant peptide rMIII, in type-III collagen inhibits interaction of type-III collagen with platelets. Quercetin, a flavanoid found in onion, apple, tea and wine inhibit collagen induced platelet tyrosine phosphorylation and intracellular calcium mobilization. Snake venom peptides obtained from various varieties of snakes act as platelet inhibitors. Natural substances such as Frangulin-B (from the plant Rhamnus formosana) inhibits collagen induced insitol trisphosphate formation. D-003, a higher fatty acid from sugar cane wax inhibits collagen induced platelet aggregation in rats. Caffeic acid phenethyl ester derived from honey bee hives also inhibits collagen induced platelet aggregation. GPIb antagonists, hindering platelet adhesion, are various snake venoms, such as the C-type lectin like proteins echicetin, agkistin and dabocetin, all of which have high binding affinity for the GPIb receptor. On the other hand, the metalloproteinase-disintegrin crotalin cleaves and inactivates the GPIb receptor with concomitant inhibition of the platelet adhesion.

1.8.6 Phosphodiesterase (PDEs) Inhibitors

PDEs are a family of nine enzymes catalyzing the hydrolysis of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Dipyridamole and Cilostazol act as PDE inhibitors, raising platelet levels of cyclic adenosine monophosphate and thereby inhibiting platelet function. Dipyridamole is a pyrimidopyrimidine derivative with antiplatelet and vasodilator properties. The antiplatelet effects of dipyridamole have been reported to be due to several mechanisms of action, including inhibition of cyclic nucleotide phosphodiesterase and blockade of adenosine uptake, both of which result in increased intraplatelet cAMP levels, which inhibits signal transduction. Dipyridamole also enhances the release and prevents the degradation of endothelial prostaglandin PGi2, a potent inhibitor of platelet aggregation. Cilostazol is an oral selective cyclic nucleotide phosphodiesterase-3 (PDE3) inhibitor with antiplatelet, vasodilatory and antimitogenic effects. Cilostazol, in addition to aspirin and clopidogrel, seems to be effective and safe.
in reducing the risk of restenosis and repeat revascularization after PCI, but available evidence is limited by the small size of the observed effects.\textsuperscript{236-238}

\begin{center}
\includegraphics[width=0.5\textwidth]{images/58.png}
\end{center}

1.8.7 \textbf{Serotonin receptor antagonists}

Release of serotonin from platelet dense granules with subsequent activation of $5\mathrm{HT}_2\mathrm{A}$ receptors on the platelet surface is a potent augmentative stimulus for platelet aggregation.

\begin{center}
\includegraphics[width=0.5\textwidth]{images/59.png}
\end{center}

However, serotonin receptor antagonists have not been successfully exploited as antiplatelet agents, possibly owing to their lack of specificity for the $5\mathrm{HT}_2\mathrm{A}$ receptor subtype. Recently, APD791 (50) has been developed as a potent and highly selective inverse agonist of the $5\mathrm{HT}_2\mathrm{A}$ receptor that displays no functional activity at the $5\mathrm{HT}_2\mathrm{B}$ or $5\mathrm{HT}_2\mathrm{C}$ subtypes.\textsuperscript{239}

1.8.8 \textbf{New Drug Targets: von Willebrand factor}

The activation of vWF is crucial in initiating platelet adhesion to the injured vessel, especially under high-shear-rate conditions, such as in arterial stenosis. The A1 domain exposed on vWF by high shear rate binds the GPIb platelet receptor.\textsuperscript{240} Aptamers are a novel class of oligonucleotides antagonizing the A1 domain of vWF. An A1 antagonist would potentially display arterial selectivity and specifically target the initial phase of thrombus formation (adhesion) while sparing other activating targets. ARC1779, a
parenteral aptamer, did not cause serious adverse events or spontaneous bleedings in phase I studies.\textsuperscript{240} It has a half-life of \(~2\) h and caused >95\% inhibition of vWF activity at the higher dose, which returned to baseline by 12–16 h after the infusion.\textsuperscript{240} A recently terminated phase II trial randomized patients with myocardial infarction undergoing PCI to three different doses of ARC1779 versus the active comparator abciximab.\textsuperscript{241} Another agent under development is the C1qTNF-related protein-1 (CTRP-1), which prevents collagen-induced platelet aggregation by specific blockade of vWF binding to collagen\textsuperscript{242}, thereby hampering platelet adhesion. In studies of carotid thrombosis in nonhuman primates, CTRP-1 effectively prevented thrombus formation.\textsuperscript{242, 243}

1.9 Future Directions for Antiplatelet Therapy

Platelet activation is an integral component of the pathophysiology that leads to thrombotic and ischaemic diseases such as MI, cerebral stroke and peripheral arterial disease. Platelet inhibition is a major strategy to prevent arterial thrombosis, but is frequently associated with increased bleeding because of impaired primary haemostasis. Therefore, ideal antiplatelet agent should specifically block thrombogenic platelet-dependent mechanisms in vascular diseases without interfering with normal functions that are required in haemostasis and wound healing. Although several antiplatelet strategies have been developed or are under preclinical or clinical investigation, none of the currently available antiplatelet drugs meets all these criteria. The current most widely used antiplatelet drugs are relatively well tolerated by patients being treated for ischaemic diseases. However, the limited efficacy of these drugs in the setting of arterial thrombosis, their unfavourable adverse effect profiles, cost-to-benefit issues and the ‘resistance’ phenomenon substantiate the need for the development of newer and more efficacious antiplatelet and antithrombotic drugs. Because of the problems associated with the use of current antiplatelet drugs, such as resistance, optimal dosage and safety, future strategies for the development of new antiplatelet drugs and new treatment regimens may include consideration of the following:

- a shift from single targets within the signaling cascade to multiple targets;
- a shift from therapy with a single drug to combination therapy; and
- investigating drugs in current clinical use for novel antiplatelet properties.
  (discovery of new antiplatelet drugs in other widely used drugs)
1.10 REFERENCES


7. Perkins H A. Anesthesiology, 1966; 27: 456


33. Verstraete, M.; Zoldhely, P. Drugs 1995, **49**, 856.


77. Mills DCB. Thromb Haemost., 1996; 76: 835–856


93. 93(a) Leger AJ, Jacques SL, Badar J. *Circulation*, 2006; 113(9): 1244-1254; (b) Wu CC, Teng CM. *Eur J Pharmacol* 2006; 546 (1-3): 142-147
100. Coller BS. *J Clin Invest.*, 1997; 100: 57
101. Tscheng JE. Am J Cardiol., 1996; 78: 35
102. Coller BS. *Thromb Haemost.*, 1997; 78: 730
104. Alison H G. *The British Journal Of Cardiology*(Supplement), 2002; 9(8); S2-S7


(b) Todd L. K, Richard C B. Circulation, 2009; 120: 2488-2495

120. Samara WM, Gurbel PA. Coron Artery Dis., 2003; 14: 65-79

121. Ferguson JJ, Quinn M, Moake JL. Antiplatelet Therapy in Clinical Practice (London) 2000: 15-34


146. Michelson A D. Eur Heart J. 2009; 30, 1753–1763
147. Nassim MA, Sanderson JB, Clarke C. J Am Coll Cardiol., 1999; 33: 255A
175. Merlos M, Graul A, Castaner J. Drugs Fut., 1998; 23(12): 1297


213. The Lancet. 1983; 321: 627-628


240. Gilbert JC, DeFeo-Fraulini T, Hutabarat RM. *Circulation* 2007; **116**: 2678–2686
Chapter-1b

Basis of the Work
1b.1 Design and synthesis of N-heterocyclic carboxamides with a basic group as antiplatelets: Literature review

The curiosity in designing various amino acid derived lactam carboxamides with differentially substituted diamine like 3-aminomethyl piperidine/bispidine hoisted from the literature survey where we found that the substitution of N-heterocycles like purines 1, indazoles 2, triazoles 3, oxadiazoles 4, imidazoles 5, pyrimidocinnolines 6, phthalazines 7, or thiazoles 8 with a carboxamide partial structure in addition to a hydrophobic moiety and basic groups leads to a wide variety of compounds with antiplatelet activities in micromolar concentrations.

![Chemical structure](image)

Rehse et al prepared twenty five new triazolecarboxamides [2(a-s)] and tested for their antiplatelet (in vitro) and antithrombotic (in vivo) activities 3.

![Chemical structure](image)

Five of them inhibited the aggregation of blood platelets (Born test, inducer collagen) with IC\text{50} values between 90 and 130 μM. Nine compounds exhibited significant antithrombotic properties with an inhibition of thrombus formation between 7-11%. They inhibit platelet aggregation with IC\text{50} = 90 and 95 μM. In vivo, 2k showed the strongest inhibition of thrombus formation with 11% in arterioles (5% in venules) after a single oral dose of 60 mg/kg. With serotonin as inducer both, 2r and 2s, showed lower IC\text{50} values namely 25 or 30 μM, respectively. Additional antiplatelet activities were found for 2r against adrenaline (IC\text{50} = 25 μM) and for 2s against platelet activating factor (PAF) (IC\text{50} = 15 μM) as inducer. (Table 1)
Table 1. In vitro (Born test, inducer collagen) antiplatelet and in vivo antithrombotic properties of triazolecarboxamides; n. s., not significant.

K Rehse et al prepared imidazole-4-carboxylic acid esters and their carboxamides with an additional secondary amino group (diamines), and identified as antiplatelet agents in a low micromolar range (Born-test, collagen as inducer, Table 2). The carboxamide 3a shows ADP antagonistic properties (IC$_{50}$ = 2 μM). Compound 3b is as well PAF antagonistic (IC$_{50}$ = 4 μM) and a COX-1 inhibitor (IC$_{50}$ = 1 μM). The derivative 3c shows a strong antiadrenergic (IC$_{50}$ = 0.15 μM) and PAF antagonistic (IC$_{50}$ = 0.66 μM) effect.

![Diagram](image)

<table>
<thead>
<tr>
<th>Cpd.</th>
<th>R</th>
<th>X</th>
<th>Born test IC$_{50}$ (μmol/L)</th>
<th>venules % ± sx (α)</th>
<th>arterioles % ± sx (α)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>cyclohexyl</td>
<td>OH</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>2b</td>
<td>benzyl</td>
<td>OH</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>4 ± 1 (0.05)</td>
</tr>
<tr>
<td>2c</td>
<td>4-methylbenzyl</td>
<td>OH</td>
<td>130</td>
<td>n.s.</td>
<td>7 ± 1 (0.05)</td>
</tr>
<tr>
<td>2d</td>
<td>benzyl</td>
<td>OCH$_3$</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>7 ± 3 (0.01)</td>
</tr>
<tr>
<td>2e</td>
<td>heptyl</td>
<td>O-CH$_2$-CH$_2$-OH</td>
<td>&gt; 300</td>
<td>not tested</td>
<td></td>
</tr>
<tr>
<td>2f</td>
<td>cyclohexyl</td>
<td>O-CH$_2$-CH$_2$-OH</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>2g</td>
<td>benzyl</td>
<td>O-CH$_2$-CH$_2$-OH</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>2h</td>
<td>4-methylbenzyl</td>
<td>O-CH$_2$-CH$_2$-OH</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>10 ± 1 (0.002)</td>
</tr>
<tr>
<td>2i</td>
<td>4-fluorbenzyl</td>
<td>O-CH$_2$-CH$_2$-OH</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>9 ± 1 (0.02)</td>
</tr>
<tr>
<td>2j</td>
<td>biphenylmethyl</td>
<td>O-CH$_2$-CH$_2$-OH</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>2k</td>
<td>heptyl</td>
<td>NH-CH$_2$-CH$_2$-OH</td>
<td>&gt; 300</td>
<td>5 ± 2 (0.05)</td>
<td>11 ± 2 (0.002)</td>
</tr>
<tr>
<td>2l</td>
<td>benzyl</td>
<td>NH-CH$_2$-CH$_2$-OH</td>
<td>&gt; 300</td>
<td>4 ± 1 (0.05)</td>
<td>7 ± 1 (0.01)</td>
</tr>
<tr>
<td>2m</td>
<td>4-methylbenzyl</td>
<td>NH-CH$_2$-CH$_2$-OH</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>2n</td>
<td>biphenylmethyl</td>
<td>NH-CH$_2$-CH$_2$-OH</td>
<td>100</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>2o</td>
<td>phenyl</td>
<td>CH$_2$-N(CH$_3$)$_2$</td>
<td>&gt; 300</td>
<td>3 ± 1 (0,1)</td>
<td>10 ± 1 (0,002)</td>
</tr>
<tr>
<td>2p</td>
<td>cyclohexyl</td>
<td>CH$_2$-N(CH$_3$)$_2$</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>not tested</td>
</tr>
<tr>
<td>2q</td>
<td>benzyl</td>
<td>CH$_2$-N(CH$_3$)$_2$</td>
<td>&gt; 300</td>
<td>n.s.</td>
<td>9 ± 2 (0,01)</td>
</tr>
<tr>
<td>2r</td>
<td>biphenylmethyl</td>
<td>CH$_2$-N(CH$_3$)$_2$</td>
<td>90</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>2s</td>
<td>cyclohexyl</td>
<td>CH$_2$-N(CH$_3$)$_2$</td>
<td>95</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

**Table 2.** Inhibition of platelet aggregation (Born test) induced by collagen after an incubation time of 4 and 20 min respectively. The IC$_{50}$ of acetylsalicylic acid is 175 ± 20 μM (n = 10).
Rehse et al. synthesized indazole carboxamide derivatives (4) and they inhibited the blood platelet aggregation induced by collagen with an IC$_{50}$ ranging 85-90 μM.

New 1,2,4-oxadiazole carboxamides, 5(a-c) and 1,3,4-oxadiazole-carboxamides, 6(a-b) containing different lipophilic moieties (i.e. 4-biphenyl, 1-naphthyl, phenylpropyl and n-hexyl substituents) and additional basic groups which are mainly alkyl and dialkylaminoalkyl residues have been synthesized by Rehse et al. and tested for collagen induced platelet aggregation and the inhibitory effects (IC$_{50}$) were between 58 μM and 300 μM.

![Chemical Structure](image)

**Table 3.** IC$_{50}$ values for 1,2,4- and 1,3,4-oxadiazole carboxamides using different inducers of aggregation in the Born test. The relative standard deviation is < 10%.

<table>
<thead>
<tr>
<th>Inducer of aggregation</th>
<th>5a</th>
<th>5b</th>
<th>5c</th>
<th>6a</th>
<th>6b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>65</td>
<td>160</td>
<td>84</td>
<td>93</td>
<td>58</td>
</tr>
<tr>
<td>ADP</td>
<td>118</td>
<td>60</td>
<td>53</td>
<td>105</td>
<td>63</td>
</tr>
<tr>
<td>5-HT</td>
<td>1.0</td>
<td>110</td>
<td>75</td>
<td>6.7</td>
<td>27</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>110</td>
<td>215</td>
<td>&gt;300</td>
<td>217</td>
<td>160</td>
</tr>
<tr>
<td>PAF</td>
<td>28</td>
<td>52</td>
<td>120</td>
<td>28</td>
<td>150</td>
</tr>
</tbody>
</table>

K Rehse et al. synthesized a series of pyrazole-4-carboxamides, 7(a-i) and investigated in the Born test for their antiplatelet activities against collagen, ADP, adrenaline and platelet activating factor (PAF) as inducers of the aggregation. Using collagen three compounds with IC$_{50}$ values below 100 μM were found. Activities in nanomolar concentrations were observed against ADP (IC$_{50}$ = 9.4 nM), adrenaline (IC$_{50}$ = 5.8 nM), and platelet activating factor (IC$_{50}$ = 0.45 nM).
Table 4. Inhibition of platelet aggregation induced by collagen, adrenaline, ADP, or PAF by pyrazole-4-carboxamides.

K Rehse et al\textsuperscript{11} prepared 2-amino-thiazole-4-acetamides in which a 4-fluorophenyl sulfonamido amino rest in 2-position of the thiazoles combined with a cyclohexyl aminopropyl acetamide in 4-position (8) exhibits the strongest antiplatelet activity with an IC\textsubscript{50} = 1 µM against collagen induced platelet aggregation.

Marschenz et al\textsuperscript{1} prepared N-(purin-2-yl)benzenecarboxamides and N-(purin-2-yl)-2-furanecarboxamide and tested for their inhibition of blood platelet aggregation and exhibited platelet aggregation induced by collagen with IC\textsubscript{50} values between 3 and 10 µM/L in the Born test. ADP, PAF, and adrenaline were used as specific aggregation inducers to examine the mechanism of the anti-aggregating activity. An astonishing pattern of activities in the nanomolar, with 9\textsubscript{a}, 9\textsubscript{b} and even subnanomolar range, with 9\textsubscript{c}, was observed. Compound 9\textsubscript{c} inhibited the platelet aggregation induced by ADP with an IC\textsubscript{50} = 0.45 nM (9\textsubscript{a}: 3.5 nM). Compound 9\textsubscript{b} showed an antagonism against the
inducer adrenaline with an IC$_{50}$ = 1.8 nM (9d: 20 nM). The strongest antagonism against PAF was observed with 9b showing an IC$_{50}$ = 1 nM (9c: 35 nM).

![Chemical structures](image)

9 (a-d)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R/R'</th>
<th>IC$_{50}$ [μmol/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>9a</td>
<td>4-SO$_2$-NH-(CH$_2$)$_2$-OCH$_3$</td>
<td>5</td>
</tr>
<tr>
<td>9b</td>
<td>3-CN</td>
<td>18</td>
</tr>
<tr>
<td>9c</td>
<td>3-CN</td>
<td>3</td>
</tr>
<tr>
<td>9d</td>
<td>4-SO$_2$-NH-(CH$_2$)$_2$-OCH$_3$</td>
<td>3</td>
</tr>
<tr>
<td>Asa</td>
<td>–</td>
<td>175</td>
</tr>
</tbody>
</table>

**Table 5:** In vitro antiplatelet activities (Born test) of N-(purin-2-yl) enzenecarboxamides and N-(purin-2-yl)-2-furanecarboxamides

Twenty one new pyrimido[5,4-c]cinnolines containing different lipophilic moieties (10) (viz. phenyl, 4-methoxyphenyl, 2-furanyl, 2-thienyl) in position 2 and additional basic groups (e.g., alkylaminopropyl, dialkylaminopropyl and cyclohexylaminopropyl) in position 4 of the title ring system have been prepared by Rehse et al. and investigated for antiplatelet effects (Born test). Ten of them inhibited the platelet aggregation induced by collagen with an IC$_{50}$ < 10 μmol/L

![Chemical structure](image)

10

1b.2 Design and synthesis of nipecotamide derivatives as human platelet aggregation inhibitors: Literature review

The interest in the designing of cyclic diamine scaffold stems from the finding of 3-carbamoylpiperidine (nipecotamide, piperidine-3-carboxamide) derivatives have been reported to possess structural features that can inhibit platelet aggregation.
Compounds in this class inhibit human platelet aggregation induced by ADP,\textsuperscript{13} collagen,\textsuperscript{14} thrombin,\textsuperscript{15} epinephrine,\textsuperscript{16} and the stable TxA2-mimetic U46619 \textit{in vitro}.\textsuperscript{17} They inhibited collagen induced platelet adhesion and thrombus growth, under simulated physiological conditions. These compounds also inhibited polymer surface-induced clustering of platelets in human whole blood.\textsuperscript{18} Nipecotamides inhibited platelet aggregation in Beagle dogs \textit{ex vivo}.\textsuperscript{19} These compounds reduced the deposition of platelets and fibrin on Dacron grafts, which were surgically implanted in exteriorized femoral arteriovenous shunts, in normal male baboons.\textsuperscript{20} Nipecotamides also protected mice from thromboembolic death caused by the intravenous injection of collagen plus epinephrine.\textsuperscript{21} Nipecotamides have been shown to interact with phospholipids of the platelet membrane, thus stabilizing it against agonist-induced activation.\textsuperscript{22-25}

Gollamudi et al\textsuperscript{24} designed, synthesized a series of 3-carbamoylpiperidines, 1-alkyl (aralkyl) nipecotamides, 11, bis-nipecotamidoalkanes and aralkanes, 12(a-f) are and tested for their inhibitory action against ADP-induced aggregation of human platelets. Nipecotamides are shown here to inhibit the basal and collagen-induced rise in platelet inositol trisphosphate (IP3) levels, as well as phosphoinositide turnover.\textsuperscript{26-38}

\begin{align*}
11, \quad R = \text{alkyl or aralkyl} & \quad 12 (a-f) \\
& \quad 12a; R = \text{NH(CH}_3\text{)}_2\text{CH}_3 \\
& \quad 12b; R = \text{NH(CH}_3\text{)}_2\text{CH}_3 \\
& \quad 12c; R = \text{NH}_2 \\
& \quad 12d; R = \text{NH}_2 \ (4\text{-substituted}) \\
& \quad 12e; R = \text{O} \\
& \quad 12f; R = \text{NO}_2 
\end{align*}

1b.2.1 \textbf{Influence of the 3-functional group}

The presence of a piperidine-3 substituent reported to be essential for the antiplatelet activity of carbamoyl piperidines, since compounds with unsubstituted piperidine ring were inactive.\textsuperscript{27} Moreover, when the 3-substituent of compound was replaced with a substituent on the piperidine-4 position resulting in a precipitous decline in the antiplatelet potency was noticed. It has been hypothesized that the capability of nipecotamides to stabilize the platelet membrane may be a consequence of their lipophilic interaction with the 3-OH group of the inositol portion of phosphoinositides.
rendering them resistant to hydrolysis catalyzed by phospholipase-C to the second messengers inositol 1,4,5-triphosphate (IP3) and 1,2-diacylglycerol (DAG). The 3-amide oxygen of these compounds is capable of forming a H-bond with the 3-OH group of the inositol portion of phosphoinositides. It then follows that the stronger this H-bond, the greater would be the potential for stabilization. The strength of this H-bond can be related to the electronegativity of the 3-amide oxygen of the compounds. Thus, the binding affinity would increase with increasing electronegativity. A structure-activity analysis of the bis(nipecotamido)aralkane type showed that a substituent on the piperidine ring should preferably be an amide and that the electronegativity of the carbonyl oxygen and the orientation of the amide group affected activities.

1.2.1.2 Importance of the chiral centre at the 3-position

In order to examine the functional importance of the chiral centre at the 3-position of the piperidine ring, compound 13 was synthesized. Chirality in the 3-position of compound 13 is eliminated due to the introduction of a double bond at the 3,4-positions of the piperidine ring. It had much lower activity (IC₅₀ = 44.5 mM) than 14 (IC₅₀ = 1.412 mM). Because of the conjugation of the amide group with the 3,4-double bond, the electronegativity of the 3-carbonyl oxygen is higher in 13 (-0.373) than in 14 (-0.368).

A possible explanation is that, because of the double bond, the hybridization state of the piperidine 3-carbon is changed from sp³ to sp². This changes the orientation of the 3-amide group. Since the 3-amide group is important for forming hydrogen bonding with membrane phosphoinositides, and also because this binding exhibits stereoselectivity, the relatively rigid amide group in 13 may be unfavourably oriented for interacting with the 3-OH group of the inositol portion of phosphoinositides. In fact, when the 3-D structures of 13 and 14 were superimposed, these two amide groups showed different orientations.
Altomare et al. synthesized a series of anilides and phenyl esters of piperidine-3-carboxylic acid (nipecotic acid) and tested for the ability to inhibit aggregation of human platelet rich-plasma triggered by ADP and adrenaline. Among the tested compounds, 4-hexyloxyanilide of nipecotic acid (15) was found to be the most active one, its IC\textsubscript{50} value (45.1 mM) being close to that of the most active bis-3-carbamoylpiperidines reported in literature (ca. 40 mM) and aspirin (ca. 60 mM) in ADP and adrenaline-induced aggregation, respectively.

Altomare et al. synthesized a series of benzylxoy anilides of nipecotic (16, 17) and isonipecotic (18, 19) acids and assayed in vitro as inhibitors of ADP-induced platelet aggregation and the blood coagulation enzymes factor Xa (FXa) and thrombin (FIIa). Addition of m-F and p-CF\textsubscript{3} on the distal phenyl ring resulted in a 6-18 fold enhancement of the FXa potency and in 2-4 fold increase of the antiplatelet potency. 20a and 20b proved to be potent FXa-selective inhibitors (130 and 57 nM, respectively) and antiplatelet agents and were identified as leads for developing new dual function antithrombotic drugs.
Hoekstra et al\textsuperscript{41} designed and synthesized potent, orally active GPIIb/IIIa antagonists containing a nipecotamide subunit. The studies culminated in the discovery of RWJ-53308 (22), a potent, orally active GPIIb/IIIa antagonist. To progress from RWJ-50042 (21) to a suitable candidate for clinical development, a series of optimization cycles was conducted that employed solid-phase parallel synthesis for the rapid, efficient preparation of nearly 250 analogues, which were assayed for fibrinogen receptor affinity and inhibition of platelet aggregation induced by four different activators.\textsuperscript{42, 43}

\[
\text{HN}
\]
\[
\begin{array}{c}
\text{HO} \\
\text{R} \\
\text{COOH}
\end{array}
\]
\[
\text{\text{21; R = H, RWJ-50042}} \\
\text{\text{22; R = 3-pyridyl, RWJ-53308}} \\
\text{\text{23; R = 3,4-methylenedioxyphenyl}}
\]

Nipecotamide is an orally active antagonist of the platelet fibrinogen receptor (IC\textsubscript{50} = 6 nM) which exhibits inhibition of collagen-induced platelet aggregation (ex vivo) after oral administration of 10 mg/kg to dogs.\textsuperscript{44} This series was discovered by using the solution structure of the C-terminal γ-chain of fibrinogen for drug design.\textsuperscript{45-47} Furthermore, substituted nipecotamide unit modelled the β-turn structure contained within the KQAGD sequence of the γ-chain of fibrinogen.\textsuperscript{48, 49}

Moreover, the present work apprehends the benefit of very recently appeared patent\textsuperscript{50}, describing the design and synthesis of constrained cyclic amino acids based amides where proline, pyroglutamic acid [24(a-i)], morpholine, thiomorpholine [25(a-b)], and piperazine based carboxylic acids have been utilized which were made to couple either with amino acids or some specific diamines, based on QSAR studies, as (VLA-4) inhibitors.

The integrin α\textsubscript{4}β\textsubscript{1} is expressed primarily on monocytes, lymphocytes, eosinophils, basophils, and macrophages but not on neutrophils. The primary ligands for α\textsubscript{4}β\textsubscript{1} are the endothelial surface protein vascular cell adhesion molecule-1 (VCAM-1) and the extracellular matrix protein fibronectin. The binding of the α\textsubscript{4}β\textsubscript{1} receptor to cytokine induced VCAM-1 expressed on high-endothelial venules (HEVs) at sites of inflammation results in firm adhesion of the leukocyte to the vascular endothelium followed by
extravasations into the inflamed tissue. Thus following are some representative compounds described in these patent series:

24a; $X = 4\text{-CH}_3, R_1 = \text{CO}_2\text{Et}, R_2 = \text{NO}_2$

24b; $X = H, R_1 = \text{CO}_2\text{Et}, R_2 = \text{NH}_2$

24c; $X = 4\text{-NO}_2, R_1 = \text{CO}_2\text{CH}_3, R_2 = \text{OCON(CH}_3)_2$

24d; $X = H, R_1 = \text{CO}_2\text{CH}_3, R_2 = \text{OCON(CH}_3)_2$

24e; $X = H, R_1 = \text{COOH, R}_2 = H$

24f; $X = 3,4\text{-diCl, R}_1 = \text{CO}_2\text{CH}_3, R_2 = \text{NHCOPh}$

24g; $X = 4\text{-CH}_3, R_1 = \text{CO}_2\text{CH}_3, R_2 = \text{NHCOPh}$

24h; $X = 4\text{-F, R}_1 = \text{CO}_2\text{CH}_3, R_2 = \text{OCON(CH}_3)_2$

24i; $X = 4\text{-NO}_2, R_1 = \text{CO}_2\text{CH}_3, R_2 = \text{OCON(CH}_3)_2$

25a; $X = H, Y = O, R_1 = \text{COOH}$

25b; $X = 4\text{-NO}_2, Y = S, R_1 = \text{COOH}$

These molecules thus offer promising leads for the development of drugs inhibiting platelet aggregation. Since they possess several sites with potential for structural manipulation, systematic modification of their structure may yield superior antiplatelet compounds with improved specificity and intensity of action.

Given the highly competitive environment surrounding this area of antithrombotic therapy, we decided to pursue a synthetic protocol to design and synthesise selectively substituted cyclic chiral diamines (in the form of 3-aminomethylpiperidine and bispidine) derivatives of constrained cyclic amino acids based amides as potential antithrombotics.
1b.3 REFERENCES


44. Andrade-Gordon P., unpublished results; details will be published separately.


