CHARLES UNIVERSITY IN PRAGUE
FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
AND PHARMACEUTICAL ANALYSIS

Literature review in field of blood clotting influencing drugs

Diploma Thesis

Hradec Králové, 2015 Konstantinos Kolotsios

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Hradec Králové 15. 5. 2015 Konstantinos Kolotsios

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ABSTRACT
Charles University in Prague
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Title of Thesis: Literature review in field of blood clotting influencing drugs

From 1919, when the heparin and 1920's, when hydroxycoumarin, were isolated, many important steps in agents affecting blood clotting have been done. All these years until now, several agents were tested and used for prevention and treatment of thromboembolism and other conditions like deep vein thrombosis, acute coronary syndrome and others.
This thesis is a review of agents that influence blood clotting. The first two chapters, are to understand the pathways that are activated after an injured blood vessel wall and all these enzymes, factors and receptors that play a crucial role in formation of clots.
On third chapter, all agents, old, current, discontinued, or under development are analyzed according to their mechanism of action.
Many agents are used in our days with great efficacy and safety, but also with important side effects. A lot of agents are under investigation for better safety, quality and efficacy. It is important to develop the ideal agent and many scientists try to overcome this challenge nowadays.

ABSTRAKT
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Název diplomové práce: Rešerše literatury v oblasti látek, ovlivňujících krevní srážení.

Od roku 1919, kdy byl poprvé izolován heparin a dvacátých let minulého století, kdy byly objeveny účinky hydroxykumarinu, bylo učiněno mnoho významných kroků ve vývoji látek, ovlivňujících krevní srážení. Za toto období byla testována řada sloučenin pro prevenci a léčbu tromboembolických a podobných onemocnění, jako jsou hluboká žilní trombóza, akutní koronární syndrom aj.

Tato diplomová práce je zpracována jako přehled látek ovlivňujících krevní srážení. První dvě kapitoly vysvětlují hlavní procesy, které jsou aktivovány po porušení cévní stěny a enzymy, faktory a receptory, které hrají klíčové role v procesu tvorby krevní sraženiny.
Třetí kapitola zahrnuje všechny typy látek – používané v minulosti i současnosti včetně již z terapie vyřazených, i látky, které jsou ještě ve stadiu vývoje. Jsou řazeny podle mechanismů účinku.
Řada aktuálně používaných léčiv vykazuje vysokou účinnost a bezpečnost, nicméně i významné vedlejší účinky. Mnohé další sloučeniny jsou zkoumány za účelem dosažení vyšší bezpečnosti. Je důležité usilovat o získání ideální látky o což se snaží mnoho vědeckých týmů.

CONTENTS

Abbreviations

AA: amino acids
ADP: adenosine diphosphate
ATIII: antithrombin III
ATP: adenosine triphosphate
cAMP: cyclic adenosine monophosphate
cGMP: cyclic guanosine monophosphate
COX: cyclooxygenase
Da: Daltons
DNA: deoxyribonucleic acid
DVT: deep vein thrombosis
EDTA: ethylenediaminetetraacetic acid
EMA: European Medicine Agency
FDA: Food and Drug Administration
FI-FXIII: factor I-XIII
GP: glycoprotein
INR: international normalized ratio
LMWH: low molecular weight heparin
MI: myocardial infarction
NSAID: non-steroidal anti-inflammatory drug
Par: proteinase activated receptor
PCI: percutaneous coronary intervention
PDE: phosphodiesterase
RNA: ribonucleic acid
t-PA: tissue plasminogen activator
u-PA: urokinase plasminogen activator
vWF: von Willebrand factor

1 Introduction

Hemostasis is a mechanism of our organism, in order to arrest blood loss and protect us from an injured blood vessel wall. Blood clot is the final product of hemostasis, that can be lifesaving but also life threating. In order to form a clot, two factors are playing a crucial role, activated platelets and formation of fibrin. It is a process divided in three phases:

- Primary, which resulted in platelets aggregation
- Secondary, a two pathway process that lead to formation of fibrin
- Tertiary, the formation of solid thrombus by platelets and fibrin[1,2].

Blood clot can be lifesaving, by prevent bleeding from an injured blood vessel wall, but sometimes, imbalance of factors can be harmful. Imbalance of coagulation factors or platelets disorders can lead to bleeding and thrombotic disorders. These disorders can be hereditary or acquired.
Bleeding disorders can be a result of platelet dysfunction or thrombocytopenia, but also by lack of coagulation factors, like inherited hemophilia (deficiency of FVIII, FIX). Thrombotic disorders can lead to thromboembolism, and inherited thrombotic disorders can be caused by deficiency of natural anticoagulants, like ATIII, protein C, etc. Acquired thrombotic disorders can be affected by risk factors like pregnancy, age, surgery and smoking[3,4].

Agents affecting blood clotting are used and have great importance in pharmacotherapy of thrombotic disorders and diseases. Prevention and treatment of several diseases caused by excitation of factors like ischemic disorders, stroke, atrial fibrillation DVT and others is why these agents are crucial for better quality of life and decreasing mortality rate. There are several drugs with different mechanism and site of actions and can be categorized as antiplatelets, anticoagulants, fibrinolytic and antifibrinolytic agents. History of these agents started at 1916 by isolation of heparin and new agents are discovered and manufactured with better safety, efficacy and quality until today.

2 Aim of the work

For almost one hundred years, several aspects, enzymes, mechanisms and drugs have been found that are affecting blood clotting. The aim of this diploma thesis is:

- to perform a literature review of agents affecting blood clotting
- to summarize and analyze the pathways that lead to the formation thrombus
- to review all factors, enzymes, receptors and proteins that play important role in blood clot formation
- to categorize all drugs according to mechanism of actions,
- to review old, current and new drugs and technologies in this field.

3 Overview of blood clotting scheme

3.1 Pathway of platelets activation and aggregation

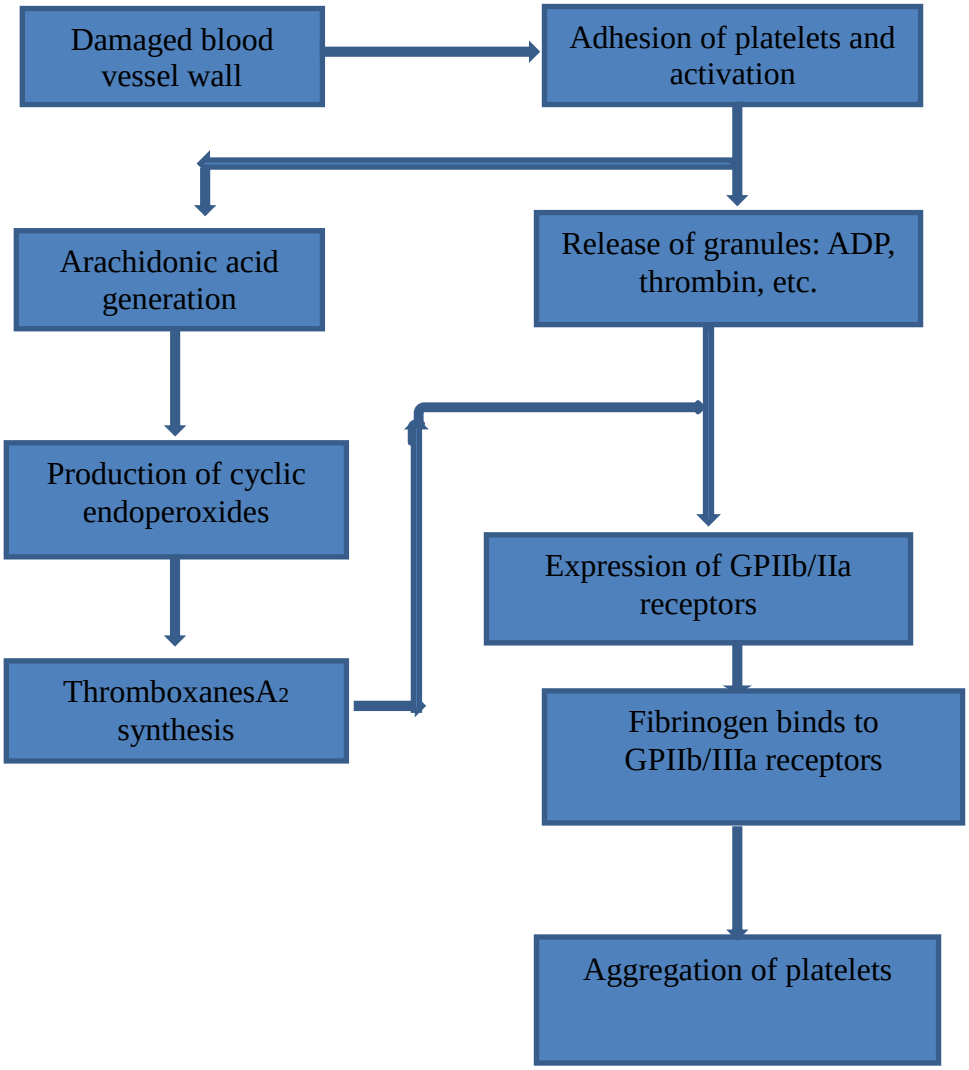


Figure 1: Platelets pathway

Activation and aggregation of platelets is a very complicated pathway that involves many receptors, protein and factors. Platelets mainly are in resting phase but after a damaged wall of blood vessels they are activated. After an injury, vasoconstriction and exposure of collagen by endothelium is happened. Adhesion undergoing platelets, through GPVI receptors, are bound to collagen receptors, mainly integrin $\alpha_2\beta_1$ directly but also with using von Willebrand factor as a bridge for collagen and platelets GPIb-IX-V receptors. Formation of these complexes is the first step for activation of platelets by activation of phospholipase C[5].

Activation of phospholipase C is followed by increasing of Ca^{++}, which leads to secretion of granules like releasing ADP, coagulation factors like thrombin and

arachidonic acid generation, which can lead to formation of thromboxane A_2. These agonists can further form a complex with G-proteins coupled receptors, ADP with $P2Y_{12}$ receptors and thromboxane A_2 with thromboxane A_2 receptors. Expression of the G-proteins coupled receptor will lead to changes in platelets shape but also it will be followed by the expression of GPIIb/IIIa receptors. GPIIb/IIIa receptor is the receptor which binds fibrinogen, in order to happen aggregation of platelets, using fibrinogen as bridges[6].

3.2 Coagulation pathway

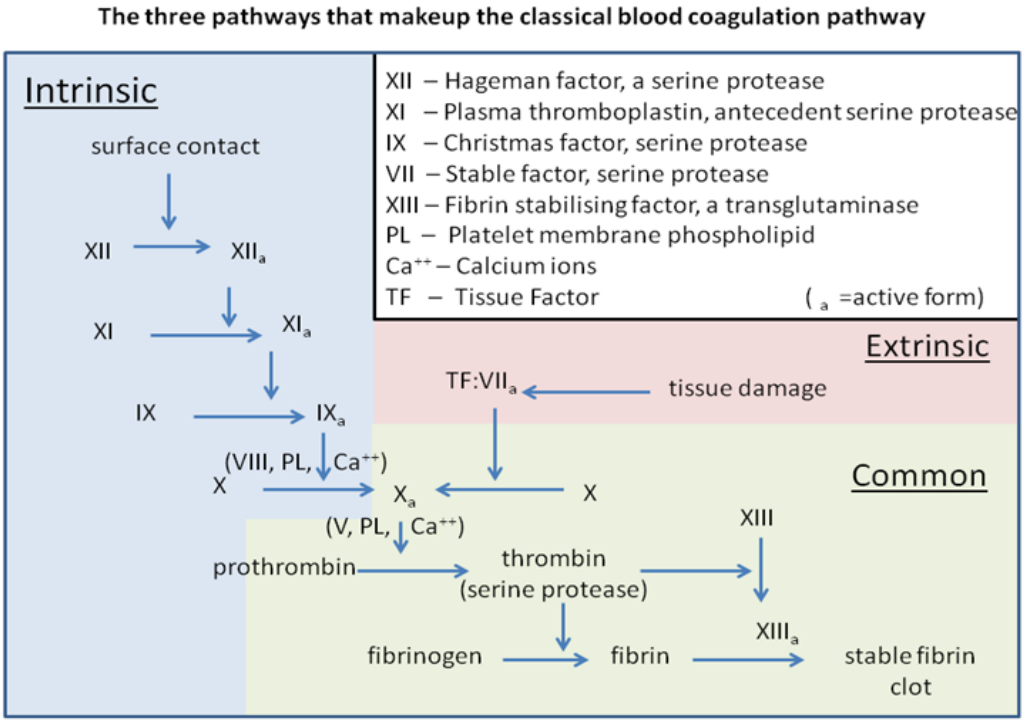


Figure 2: The three pathways that makeup the classical blood coagulation pathway (Image adapted from Wikipedia[7])

Coagulation cascade is a pathway that involves several coagulation factors, tissue factor, acidic phospholipids and Ca^{++} ions. It is divided in two pathways, extrinsic and intrinsic, that both result to activation of FX to FXa and continue together to a common pathway for the formation of fibrin, which is responsible for the formation of clot.

In order to understand correctly this pathway, it is preferable to analyze it step by step. First of all, initiation of extrinsic pathway is characterized by tissue factor (TF), a protein that is bound to membrane after an injury of a blood vessel wall and it forms a bond with the FVII. The complex of TF-FVIIa is important for the activation of FX and FIX[8].

The intrinsic pathway is responsible for the activation of coagulation factors. Activation happened by a numerous proteolytic reactions of serine proteases with

cofactors. It starts with a process called contact activation, the formation of a complex between collagen and FXII and continues with activation of FXI and FIX[9].
Results of both pathways are responsible for the activation of FX to FXa. FXa, accompanied with FV, phospholipids and calcium ions are responsible for the cleavage of prothrombin into thrombin. Thrombin's action is not only for further activation of FV, FVIII and FIX, or for the important role that has in platelets aggregation, but the main role is the conversion of fibrinogen to fibrin and through the activation of FVIII to form more stable fibrin[8].

3.3 Fibrinolysis pathway

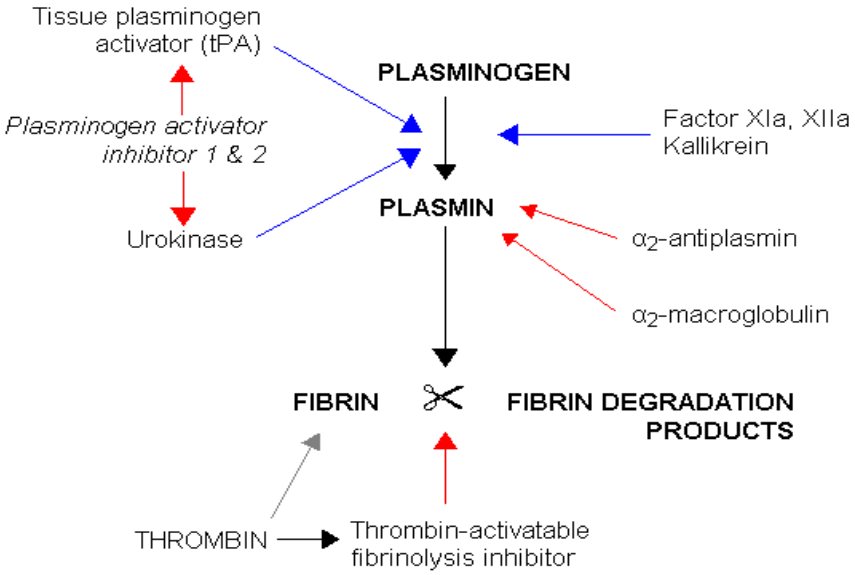


Figure 3: Fibrinolysis

(Image adapted from Wikipedia[10])

At the same time that coagulation pathway is activated, the fibrinolytic pathway is starting. Fibrinolysis involves plasminogen activators, plasminogen, plasmin, fibrin and serine protease inhibitors which are responsible for the excessive plasmin regulation[11].

Plasmin is responsible for degradation of fibrin. Plasminogen is activated by proteases like tissue plasminogen activators or urokinase plasminogen activators, resulting in plasmin. Plasmin degrades fibrin and fibrin degradation products like fibrinopeptide B and other monomers and dimers products. In order to regulate the degradation of fibrin by an excessive amount of plasmin, serine proteases (serpins) inhibitors, like plasminogen activator inhibitors 1, 2 and α2-antiplasmin, are involved in the pathway[11].

4 Mechanisms (enzymes, receptors, other molecules) employed to affect the blood clotting process.

In this chapter, most important enzymes, proteins, receptors, factors, cofactors and other substances involved in the mechanism of pathways are analyzed.

4.1 Platelet pathway

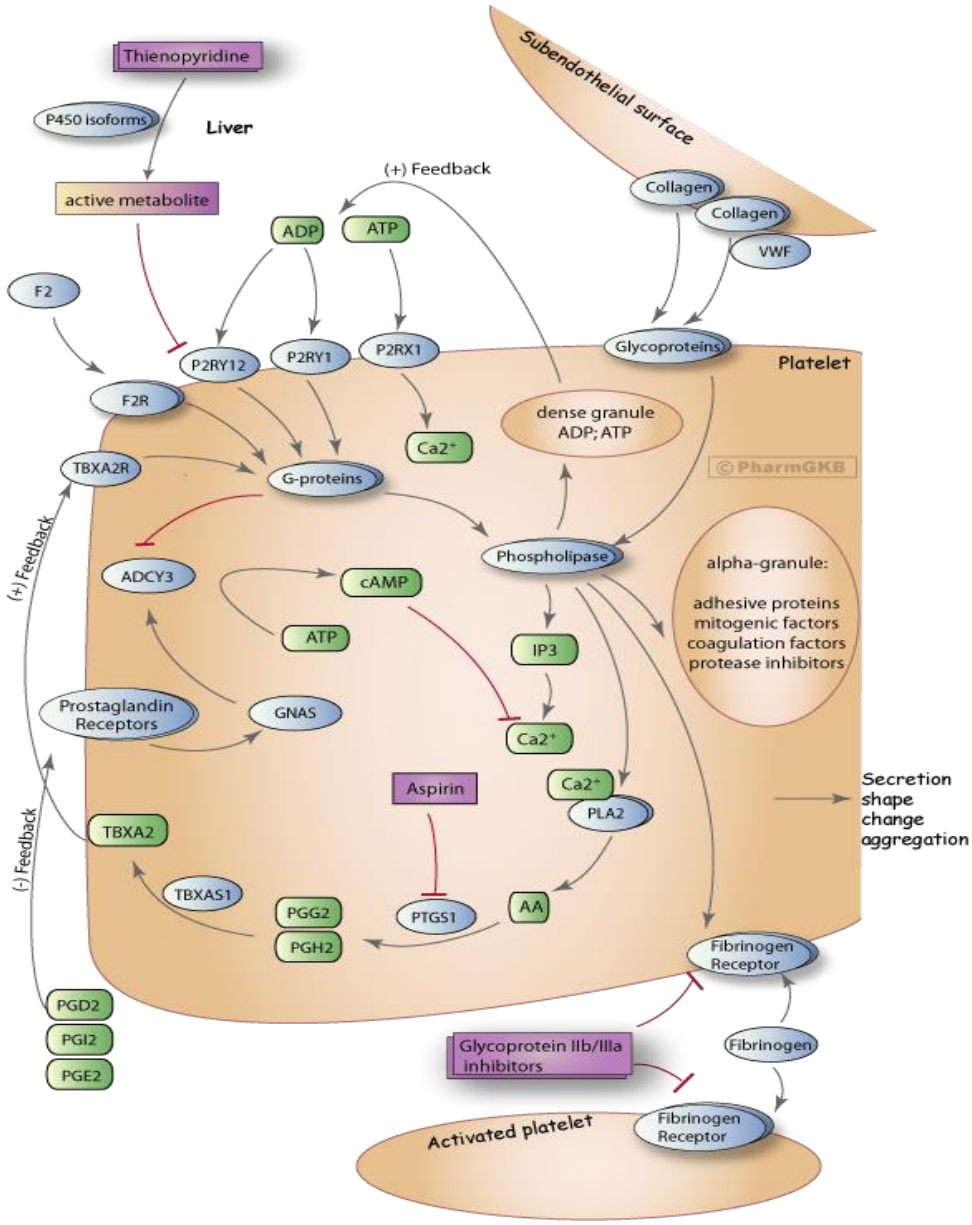


Figure 4: Platelet Aggregation Inhibitor Pathway, Pharmacodynamics

(Image adapted from Sangkuhl, K.[6])

Glycoprotein (GP) receptors are part of integrin's complex receptors. There are different GP receptors in the membrane of platelets, for binding different substances but mainly fibrinogen, vWF, collagen and prothrombin. They are activated after injury of blood vessel walls by ADP and thromboxane A_2. Important tool of therapy, affecting glycoprotein receptors, are GPIIb/IIIa receptors inhibitors[12].

P2Y receptors are G protein-coupled receptor and in humans are divided into $P2Y_1$ and $P2Y_{12}$ receptors. It has an important role in platelets aggregation by being ADP binding site and they are the targets of thienopyridine drugs like clopidogrel[13].

Protease-activated receptor (Par) is also a G-protein-coupled receptor that can be found in endothelial cells and also on the surface of platelets. There are four types of Par receptors and the binding site of thrombin and FXa in platelets. Binding of thrombin in platelets through Par-1 receptor, will lead in platelets aggregation, thromboxane A_2 production, degranulation and exposure of phospholipids. Drugs for inhibition of Par-1 are under development[14].

Von Willebrand factor is a glycoprotein, located between endothelium collagen and platelets and bind with them by GPIb receptor. It can be considered as coagulation factor, by binding on platelets and it is essential for the first step of platelets adhesion and activation[3].

Phosphodiesterase (PDE) is an enzyme that breaks phosphodiester bond of cAMP and cGMP. There are three phosphodiesterase enzymes found in platelets, PDE_2, PDE_3 and PDE_5 which works as regulators of platelets aggregation. Drug for inhibition of PDE_3 is cilostazol[15].

ADP is released after the first activation of platelets. ADP after releasing, form a complex with $P2Y_{12}$ receptors and lead to morphological changes in platelets, but most importantly, with thromboxane A_2 can help for the expression of GPIIb/IIIa receptors[6] ADP-dependent platelets aggregation inhibitors are used to prevent the action of ADP but not directly. They inhibit $P2Y_{12}$ receptors.

Thromboxane A_2 is an eicosanoid and a result of arachidonic acid metabolism in platelets and together with ADP it contributes to the expression of GPIIb/IIIa receptor and further aggregation of platelets. Drugs against thromboxane A_2 action are used widely, like aspirin, and block COX, an important enzyme for the production of thromboxane A_2[3,16].

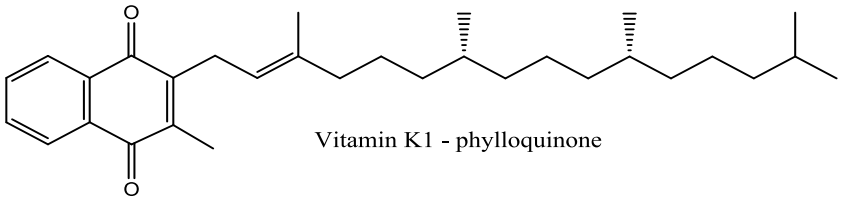
4.2 Coagulation pathway

This pathway is important, as it is written above, for the conversion of fibrinogen to fibrin by thrombin. Coagulation factors are responsible for the activation of this pathway and for the formation of fibrin.

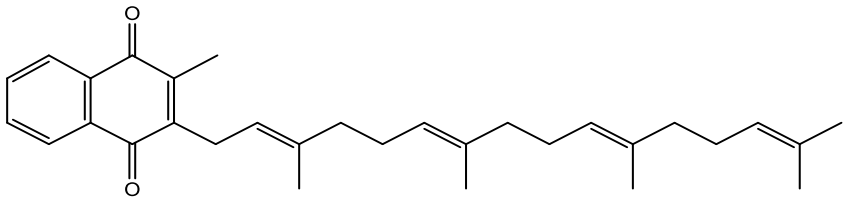
4.2.1 Vitamin K

Vitamin K is a fat soluble vitamin, a 2-methyl-1,4-naphthoquinone derivative according to its chemical structure, and it can be categorized as vitamin K1 and Vitamin K2. Vitamin K1 is also known as phylloquinone that is obtained from green vegetables and vitamin K2 is also called menaquinone, found in microbes. Main difference is the side chain in position-3, which in vitamin K2 is characterized by isoprenoid residues.

Vitamin K plays an important role in several metabolic functions of organism especially in blood coagulation, but also in bone metabolism, vascular calcification and cells growth. Finding of the presence of γ-carboxyglutamic acid, a product of vitamin K in prothrombin, shows us the relation of vitamin K with coagulation. Vitamin K influences important enzymes like vitamin K epoxide reductase and γ-glutamate carboxylase, and so helps proteins to form γ-carboxyglutamate residues and activate them in order to bind with Ca^{++} ions. The proteins that are activated by vitamin K on coagulation pathway are prothrombin (FII), FVII, FIX, FX and proteins C, S and Z[17].



Vitamin K1 - phylloquinone



Vitamin K2 - menaquinone

4.2.2 Coagulation factors

Coagulation factors are serine proteases, except FIII, FIV (calcium), FVIII, that are produced in liver. They are in circulation in an inactive form. Damage of blood vessel wall will lead to the activated form of factors through proteolytic reaction. Each of them, have a special action in the coagulation cascade, starting by FVII that is activated by TF, when they bind each other[3].

Thrombin is the central protease in the blood coagulation cascade. It is the result of cleavage of prothrombin by FXa, phospholipids and calcium ions. It is essential for further activation of FV, FVIII, FXI in order to increase its own production, can also activate platelets by binding to Par receptors (protease-activated receptors), but more importantly, it cleaves fibrinogen to form fibrin[3,18].Thrombin can be inhibited directly by thrombin inhibitors and indirectly by activation of ATIII.

Fibrinogen plays a crucial role in coagulation pathway. It is a glycoprotein with three polypeptide chains with disulphide bonds. It is a precursor of fibrin, but also it is important for the last step of platelet activation pathway by binding to GPIIb/IIIa receptors[19].

Fibrin is the result of the cleavage of fibrinogen by thrombin and the main product of coagulation pathway. It is responsible, together with activated platelets, for the formation of a blood clot.

Factor Xa (FXa), is the activated form of FX, also known as Stuart-Prower factor. It is a serine protease that is produced in liver as FX. The action of FXa is to cleave prothrombin to active thrombin. FXa can be inhibited by activation of AT III but can be also inhibited directly by a category that is known as xabans or direct inhibitor of FXa.

Tissue factor (TF) is a glycoprotein in subendothelial tissue. It is activated after an injury of a blood vessel and is the initial step of extrinsic pathway that activates FVII by binding. The complex of TF-FVIIa leads to the activation of FIX and FX[3, 20].

4.2.3 Natural anticoagulants

In coagulation pathway, except of coagulation factors, regulators also exist. Regulators act as natural anticoagulants. Some of them are thrombomodulin, tissue factor pathway inhibitors, antithrombin and proteins C, S.

Thrombomodulin is a protein receptor in endothelial cells. It binds thrombin in order to prevent the cleavage of fibrinogen. Also, it activates protein C[3, 21].
Protein C is a serine protease that is activated by thrombomodulin and is responsible for inhibition of FV and FVIII[3,20].

Tissue factor plasminogen inhibitor is a polypeptide of endothelial cells that acts by blocking FX to bind on complex of TF-FVIIa[3,20].

Antithrombin III (ATIII) is an important regulator of coagulation pathway and a member of the serine protease inhibitor family (serpin). ATIII is composed of 432 AA and it is produced mainly in the liver. ATIII is an important protease inhibitor of thrombin, FIXa and FXa. It also can inhibit FXIa and FXIIa, plasmin and kallikrein[22]. So, ATIII is a major physiological inhibitor of blood coagulation, and its hereditary deficiency is associated with venous thrombotic diseases[23].
The category of thrombin/FXa inhibitors includes three different subcategories of drugs, heparin, LMWH and synthetic pentasaccharides. They have the same mechanism of action, they activate of ATIII, but with small differences.

4.3 Fibrinolytic pathway

Plasmin is an important and potent serine protease that is produced after the activation of plasminogen by their activators like tPA or uPA. It is responsible for the degradation of fibrin. Action of plasmin can be regulated either from antiplasmin, a glycoprotein that inhibits plasmin action or TAFI (thrombin activate fibrinolysis inhibitor), which is blood proenzyme responsible for decreasing affinity of plasminogen[3, 24].

5 Groups of drugs

5.1 Antiplatelet drugs

This category of drugs is used mainly for arterial thrombosis, acute MI or in high risk for MI, after angioplasty and stent placement or after heart bypass surgery and in atrial fibrillation (only if oral anticoagulants are contraindicated)[2].

5.1.1 Inhibitors of adhesion

AJW200 is an immunoglobulin G4 humanized monoclonal antibody that inhibit platelets adhesion by having direct action on vWF and not allow the formation of complex with GPIba. It can be administrated alone or with tPA and it was tested in monkeys, rabbits and humans. First studies shown efficacy but can cause hematomas on the site of administration[25,26,27].

ALX-0081 is an anti-vWF agent that is tested as inhibitor of platelet adhesion. It has two identical humanized nanobodies and it is divalent, so it can interact with vWF but it is specialized on targeting GPIb site. It is in trials in animals like baboons and cynomolgus monkeys and in humans it is under phase II in percutaneous coronary intervention[25,26,28].

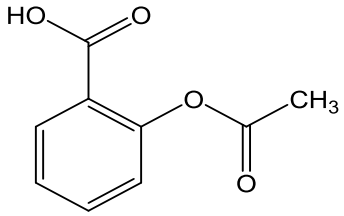
82D6A3, is a monoclonal antibody that acts against vWF- collagen I and III complex. A3 in the name is because it acts directly in vWF A3-domain on Arg-963, Pro-901, Asp-1009, Arg-1016, Ser-1020, Met-1022 and His-1023. It was tested in baboons with not incidence of bleeding, but there is not documentation until today for clinical trials in humans[25,29].

GPG-290, is a recombinant chimeric protein, linked to Fc fragment of human immunoglobulin G, that contain the N-terminal 290 AA of GPIba[25]. It is tested in mice as inhibitor of vWF- GPIba complex[30].

5.1.2 COX1-Inhibitors

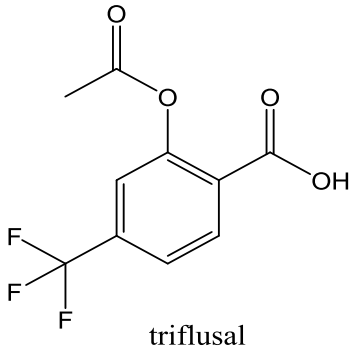
Drugs that inhibit COX1 can be considered having an anti-inflammatory action. Some of these drugs can have antithrombotic action like acetylsalicylic acid in low dose (75-100mg). Inhibiting COX1 can affect synthesis of thromboxane A_2. These agents can be prescribed for heart attack or stroke or as prevention for a second stroke and after angioplasty or bypass surgery[31].

Acetylsalicylic acid was the first drug that was found to have antiplatelet action. In 1960 to 1980's, scientists found that acetylsalicylic acid can affect platelets and help in prevention of heart attack and stroke in low doses (75-100mg)[31]. Acetylsalicylic acid is a NSAID, which belongs to the family of salicylates and inhibit COX. Through inhibition of COX by acetyl group that bind to a serine of COX (Serine530 of COX-1, Serine516 of COX-2)[32], acetylsalicylic acid can affect synthesis of thromboxane A_2. It can be used alone or on dual therapy.



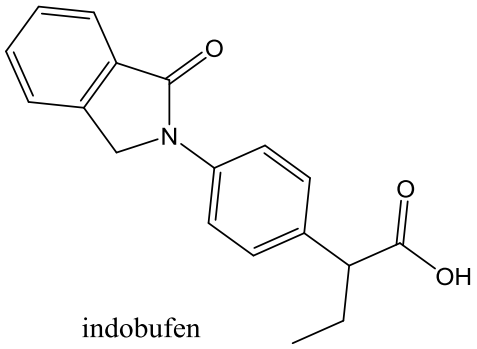
acetylsalicylic acid

Triflusal, is an agent similar to acetylsalicylic acid in structure but with a trifluoromethyl group at position-4. Triflusal not only inhibits thromboxane A_2 biosynthesis due to inhibition of COX1 but also helps the production of nitric oxide to increase the potential of vasodilatation and also inhibits phosphodiesterase. It has both antithrombotic and neuroprotective action. Main metabolite 2-OH-4-trifluoromethyl benzoic acid is formed, after triflusal pass the liver[33]. It is a drug produced on late 1970's but now it is considered as a good alternative of acetylsalicylic acid due to better safety after lot of testing[34].



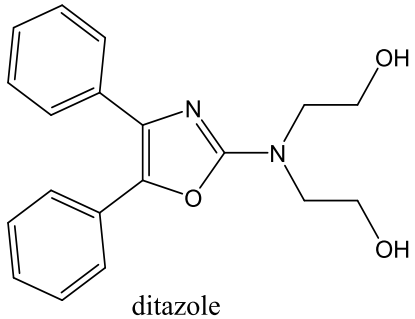
triflusal

Indobufen is a reversible inhibitor of COX which leads to inhibition of platelets aggregation. It is an old drug that is used today in some countries (e.g. Czech Republic) and it is considered as a good alternative to warfarin and acetylsalicylic acid on prevention of thromboembolism[35].



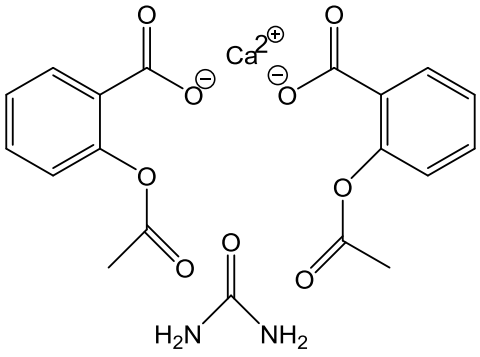
indobufen

Ditazole is a NSAID that is used as an antiplatelet agent only in Spain and Portugal for thromboembolism prophylaxis[36].



ditazole

Carbasalate calcium is also known as salt of acetylsalicylic acid that is formulated by two molecules of acetylsalicylic acid and urea. As salt of acetylsalicylic acid, carbasalate calcium is considered as NSAID and inhibits platelet aggregation. Can be used as alternative of acetylsalicylic acid in patients dealing with gastrointestinal problems (e.g. ulcers formation)[37].

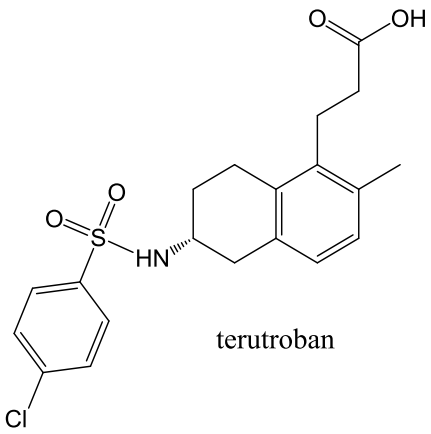


carbasalate calcium

5.1.3 Inhibitors of thromboxansynthase

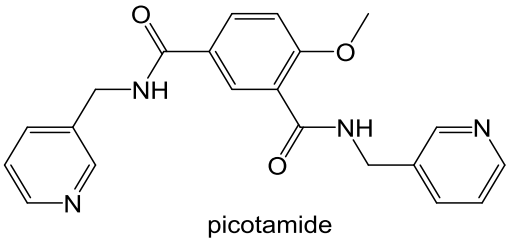
Terutroban is a thromboxane receptor of platelets antagonist, and also an inhibitor of thromboxane synthase. Both these mechanisms result in an anti-thrombotic effect[38]. But the results of clinical trials-phase II, which was terutroban versus acetylsalicylic

acid for patients with cerebral ischemic events, were not positive and the testing of drug was stopped[39].



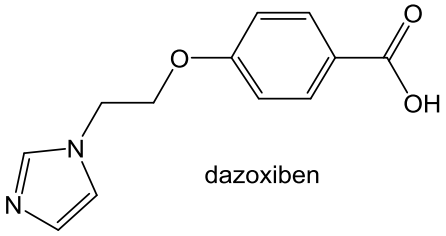
terutroban

Picotamide, is a double action antiplatelet drug. It can inhibit both thromboxane A2 receptors and thromboxane A2 synthase. It has a methoxy isophtalic acid derivative structure and it is tested to be used in patients with peripheral arterial disease. After some clinical testing, the result was that there is no clinical benefit in patient with peripheral arteries disease but it was beneficial in peripheral arterial disease patient with diabetes. This data from testing can be helpful in further investigation of drug[40].



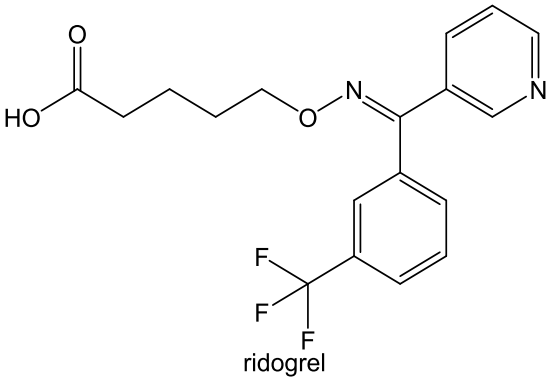
picotamide

Dazoxiben, is an oral drug that inhibits thromboxane synthase but at the same time it increases the production of prostacyclin. It is a drug that was tested from early 1980's, for prevention of ischemia and also in patients with Raynaud's disease and shown smaller activity than aspirin. There is no further documentation the last 10 years[41,42].



dazoxiben

Ridogrel is an agent under development the last 20 years. It inhibits thromboxane synthase, but also thromboxane receptors. It was tested versus aspirin for prevention of thromboembolism and for acute MI. It was also tested for inflammatory bowel disease, because of pro-inflammatory activity of platelets. This agent still has no significant indication versus aspirin and the last 5 years, there is not further documentation about the drug[43,44,45].

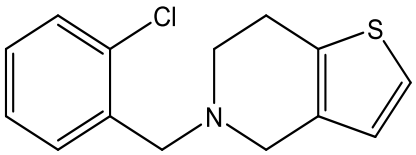


ridogrel

5.1.4 Blockers of membrane ADP receptors

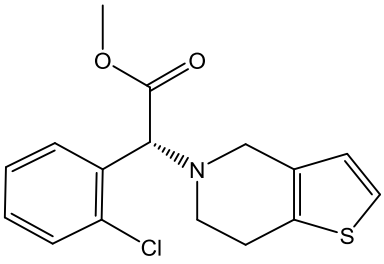
This category of action includes two kinds of drugs, prodrugs that metabolized in liver by cytochrome P450 to active metabolites and drugs that do not require metabolism. Thienopyridines are the first category, they bind irreversibly in $P2Y_{12}$ receptors by disulfide bond and do not allow ADP bind to platelet. As the second group nucleoside analogues are considered with similar action to thienopyridines but binding reversibly at $P2Y_{12}$ receptors [46].

Ticlopidine is the first thienopyridine produced to inhibit platelets aggregation by the mechanism of ADP-dependent platelet aggregation. Ticlopidine has a tetrahydrothieno[3,2-c]pyridine structure and it is used twice per day, with side effects like neutropenia and aplastic anemia. It is used prophylactically or for treatment of stroke and as an alternative in patients that are allergic to acetylsalicylic acid[47,48].



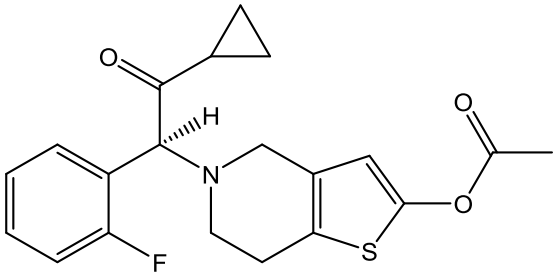
ticlopidine

Clopidogrel is a second generation thienopyridine and it acts as inhibitor of ADP-dependent platelets aggregation by binding irreversibly in $P2Y_{12}$ receptors of platelets[49]. It is administered once per day, orally, alone or as a part of dual therapy with acetylsalicylic acid for PCI, prevention of peripheral vascular and coronary artery disease. In some cases it can cause neutropenia and bleeding[50].



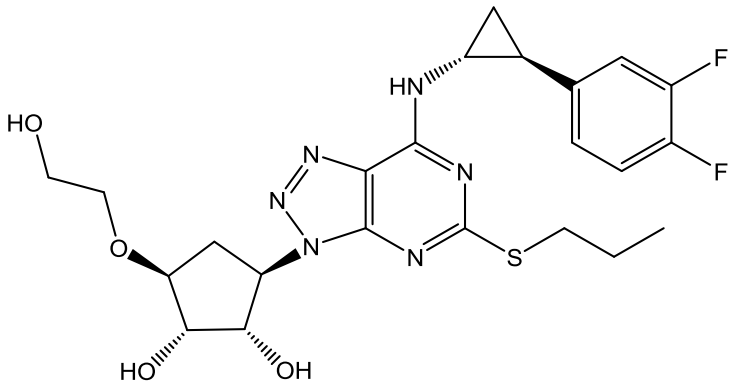
clopidogrel

Prasugrel, is the most recent thienopyridine that is approved by FDA since 2009. It has the same mechanism of action as the two previous drugs. Unlike of clopidogrel it contains a cyclopropyl substituent[51]. One more difference is that prasugrel is more lipophilic prodrug metabolized by esterases, so it has better oral bioavailability, rapid onset of action and smaller loading dose (60mg) than clopidogrel (300mg)[49].



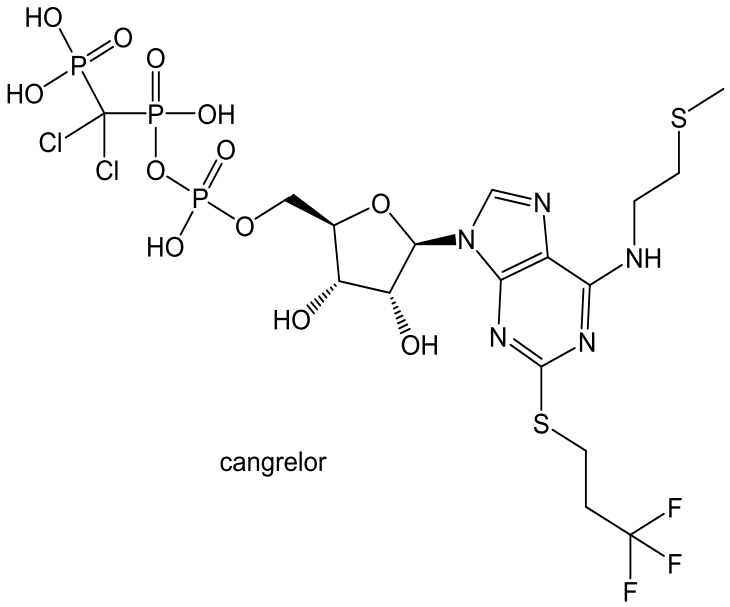
prasugrel

Ticagrelor is a nucleoside analogue type oral inhibitor of ADP, it binds in P2Y12 but reversibly. It is a triazolopyrimidine nucleoside derivative, which means that it not needs cytochrome to metabolize in order to act. It has faster action than clopidogrel and after clinical trials against clopidogrel, it reduces mortality caused by vascular diseases[52,53].



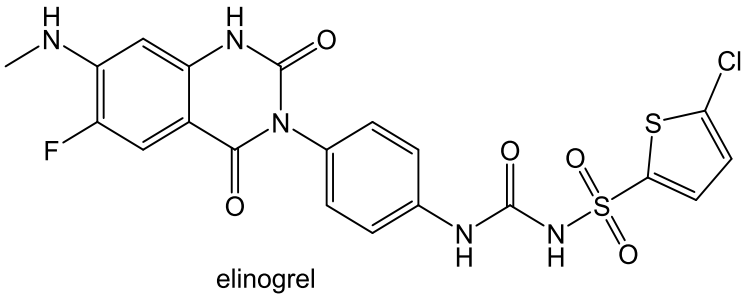
ticagrelor

Cangrelor is an intravenous ATP analogue structure that is in clinical trials tested as ADP-dependent platelets aggregation inhibitor for reducing thrombotic cardiovascular events. It is faster and has more inhibitory action than clopidogrel[54]. FDA in 2014 did not approve the drug due to poor performance in clinical trials for PCI[55]. In January of 2015, EMA gave market authorization on cangrelor[56].



cangrelor

Elinogrel was another ATP-dependent platelets aggregation inhibitor of non-nucleoside type that binds reversibly in P2Y12 receptors. It was the only inhibitor that was tested for both oral and intravenous administration and had quinazoline-2,4-dione structure. The development stopped after clinical trial phase II[54].

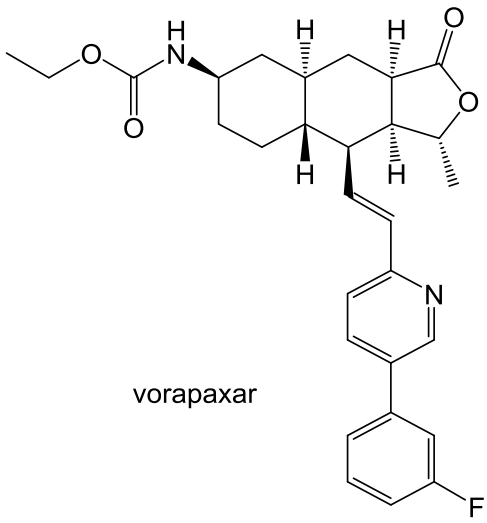


elinogrel

5.1.5 Blockers of membrane PAR-1 thrombin receptors

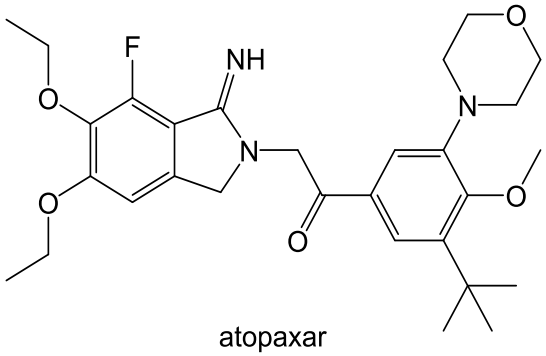
Protease activated receptors (Par) are responsible for activating platelets through thrombin, and drugs inhibiting Par-1 receptors represent one more way for prevention and treatment of thrombotic events.

Vorapaxar, is an agent structurally similar to alkaloid himbacine with a tricyclic structure substituted with 3-phenylpyridine. It is a new synthetic, reversible Par-1 inhibitor. It is an agent with a high bioavailability, long half-life (120-262 hours)[57]. There were problems in clinical trials due to high incidence of bleeding in Phase III for treatment of acute coronary syndrome and in patients with a history of stroke but the trials continued and the drug is waiting for the FDA's approval [58].



vorapaxar

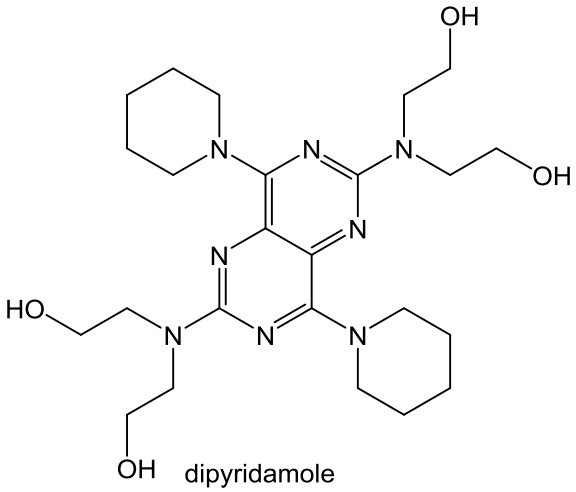
Atopaxar, another Par-1 inhibitor with quite different structure, is also potent and reversible agent, but with more rapid elimination (half-life is 22-26 hours) and it has also a primary gastrointestinal metabolism[57]. Clinical trials phase II (LANCELOT) results were not very positive, due to bleeding incidence. Phase III trials were supposed to clear the effect of atopaxar, but after 2012 there is no further information about clinical trials phase III[58].



atopaxar

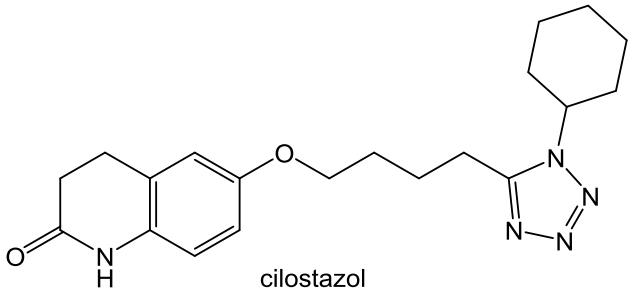
5.1.6 Phosphodiesterase inhibitors

Dipyridamole is an oral inhibitor of adenosine reuptake by red blood cells, that causes an increase in cAMP and the result is to reduce thromboxane A_2 synthesis, but also inhibits PDE. Structure characteristic is the diaminopyrimidine and it is used for secondary prevention of cerebrovascular events and for 50 years now is used also as vasodilator and this is the reason that main side effect is hypotension[59,60].



dipyridamole

Cilostazol is an inhibitor of phosphodiesterase and a quinolinone derivative that is used in peripheral artery disease. It is a new drug, similar drugs (ex. milrinone) can affect mortality in patients with congestive heart failure but a trial after approval of the drug shown, that cilostazol does not affect mortality[61].



cilostazol

5.1.7 Glycoprotein IIb/IIIa blockers

Drugs in this category are considered to inhibit more pathways of platelets activation. This group includes a monoclonal antibody (abciximab) and some synthetic molecules like tirofiban. The use of GPIIb/IIIa antagonists will be associated with greatest clinical benefit in the following clinical scenarios:

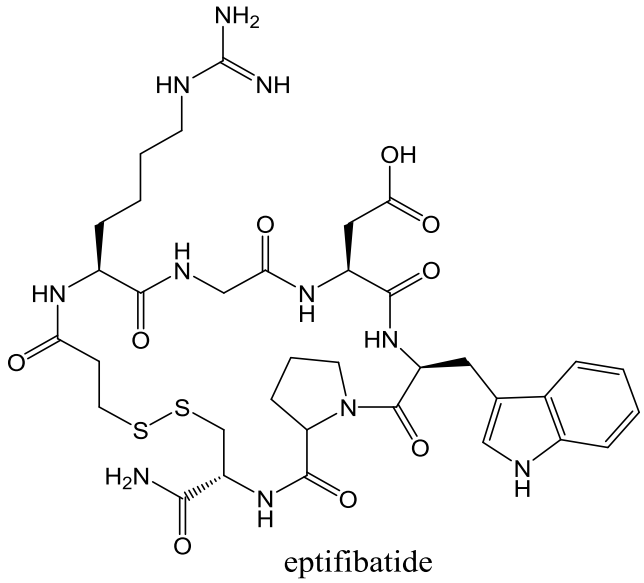
- Conditions associated with a high likelihood of intracoronary thrombosis.
- When the anticoagulant is heparin rather than bivalirudin.
- When the patient has not been treated before PCI with a $P2Y_{12}$ antagonist.
- In unstable ACS patients who require transfer to a PCI center.
- To reduce the risk of stent thrombosis in a patient with ST-elevation myocardial infarction (particularly when there is not adequate pretreatment with a $P2Y_{12}$ antagonist)[62].

Main disadvantage of this group is that it increases the risk of bleeding.

Abciximab, is a large size (48kDa) chimeric monoclonal antibody, developed on mice in 1985 and it is approved as intravenous agent from 1994 as GPIIb/IIIa inhibitor. It is a nonselective antagonists of GPIIb/IIIa receptor. It is used for treatment and prevention of unstable angina, acute coronary syndrome and for

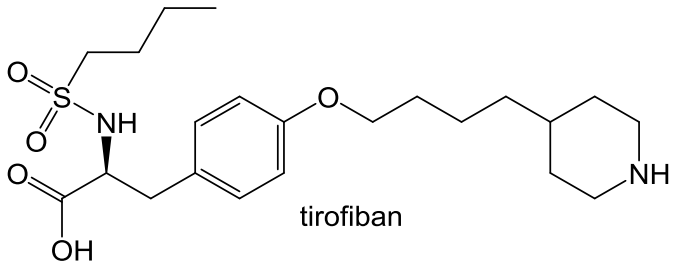
patients under PCI. Because of originally murine antibody and in order to prevent immunogenicity, the constant part of the murine γ-globulin was replaced by constant region of human γ-immunoglobulin to get the less immunogenic chimeric human-murine protein. Main side effect is severe bleeding[63,64.]

Eptifibatide, is a cyclic heptapeptide, with six AA (lysine-glycine-aspartic acid-tryptophane-proline-cysteinamide), and one mercaptopropionyl residue and disulfide bridges discovered in rattlesnake's venom. It is an intravenous antagonist of GPIIb/IIIa receptors which also antagonize fibrinogen and vWF. It acts faster than abciximab and is used for acute coronary syndrome and ischemic episodes after percutaneous coronary intervation[62].



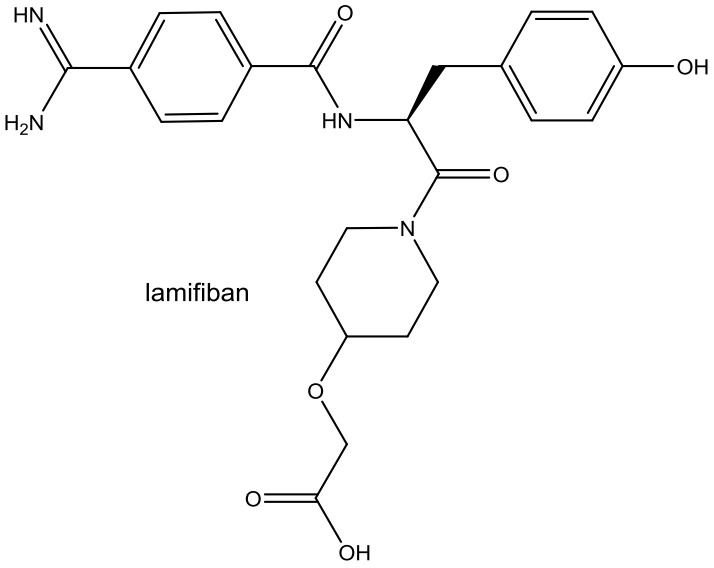
eptifibatide

Tirofiban, is a non-peptide tyrosine like peptidomimetic recognizing Arg-Gly-Asp sequence in order to act in similar ways to Arg-Gly-Asp integrins. It is a reversible inhibitor of GPIIb/IIIa receptors with high specificity. It is also an intravenous agent with half-life 2 hours. Monitoring is necessary for all drugs of this category[62].



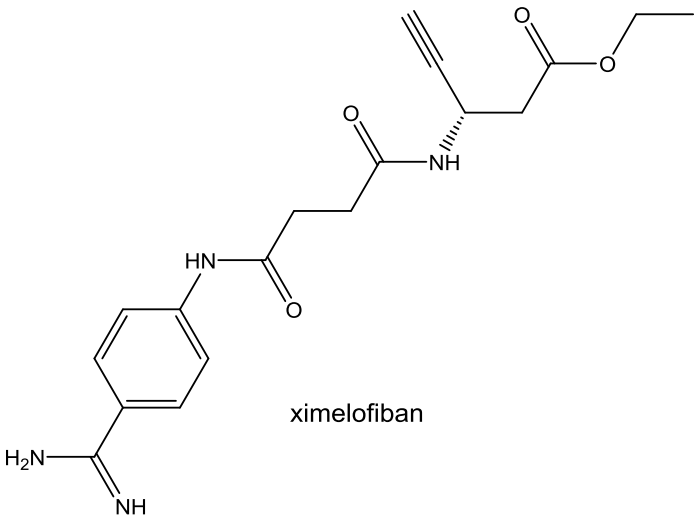
tirofiban

Lamifiban, is a synthetic and reversible nonpeptide that prevent fibrinogen to bind in platelets by inhibiting of GPIIb/IIIa receptors. It was tested in patients with non-Q wave myocardial infarction (MI) or unstable angina pectoris and also for percutaneous coronary interventions, but also the main side effect was incidence of bleeding[65,66].



lamifiban

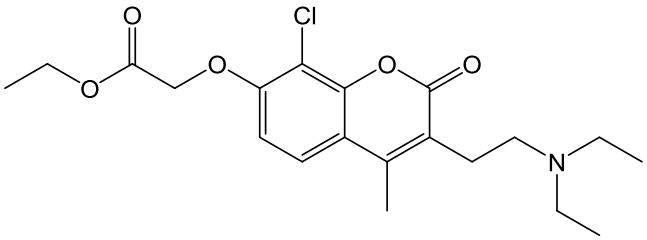
Ximelofiban is an orally prodrug that is metabolized to its active drug. It works as a selective inhibitor of GPIIb/IIIa receptor. Drug was tested for thrombosis in patients with unstable angina pectoris and acute myocardial infarction in phase III but the results did not shown advantages in therapy and its manufacture discontinued. From 2003, VDDI Pharmaceuticals started developing the drug again for percutaneous coronary interventions. No further documentation is known until now [67].



ximelofiban

5.1.8 Another antiplatelet drugs

Cloricromen, a coumarin derivative of carbonyl-methoxy-coumarin structure is an agent with anti-ischemic effect. It is an inhibitor of PLA2, which is an enzyme of the synthesis pathway of Platelet Activation Factor. PLA2 activation leads to the release of arachidonic acid followed by leukotriene synthesis. So, cloricromen is a drug with antiplatelet and antileukotriene properties[68].



cloricromen

Defibrotide is an agent discovered in 1968 from animal tissue and since then, it was found that the drug has a multifunctional action, as fibrinolytic, antithrombotic, anti-ischemic, anti-shock and anti-atherosclerotic action. It is obtained by depolymerization of porcine DNA and consists of 90% single-stranded and 10% double-stranded phosphodiester oligonucleotides. It increases levels of prostaglandins I_2 and E_2, which leads to prevention of platelets adhesion and aggregation but also enhance the activity of tPA and decreases its inhibition. Currently, it is used in Europe for sinusoidal obstruction syndrome and in USA only with an expanded-access protocol[69,70].

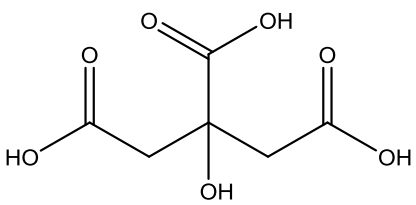
5.2 Anticoagulant drugs

In this category, we can found four different mechanisms of actions. It is a complicated pathway that is affected by activation of coagulation factors.

5.2.1 Calcium binding agents

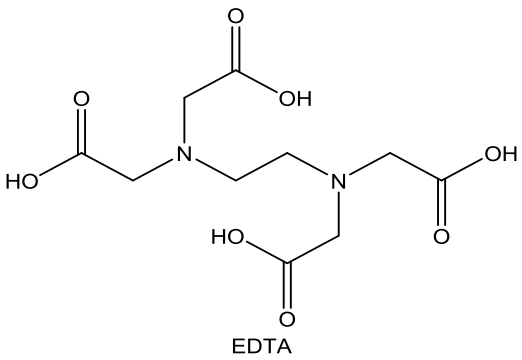
Calcium binding agents are used in laboratories for examination purpose and for blood transfusion as agents that *in vitro* prevent blood to form clots. Citric acid and EDTA are used most frequently as the calcium binding agents, but they also have some other functions. On preventing blood clots, they act mainly as inhibitors of thrombin formation, by chelating calcium ions that is required for coagulation pathway, by binding to them strongly. Their use should be limited in blood transfusion, considering their toxic effects[71,72,73].

Citric acid contains three carboxylic groups and can be used as sodium citrate, citrate dextrose and citrate phosphate dextrose[72].



citric acid

EDTA contains four carboxylic groups. It works also as chelating agent of calcium and also heavy metals[73].



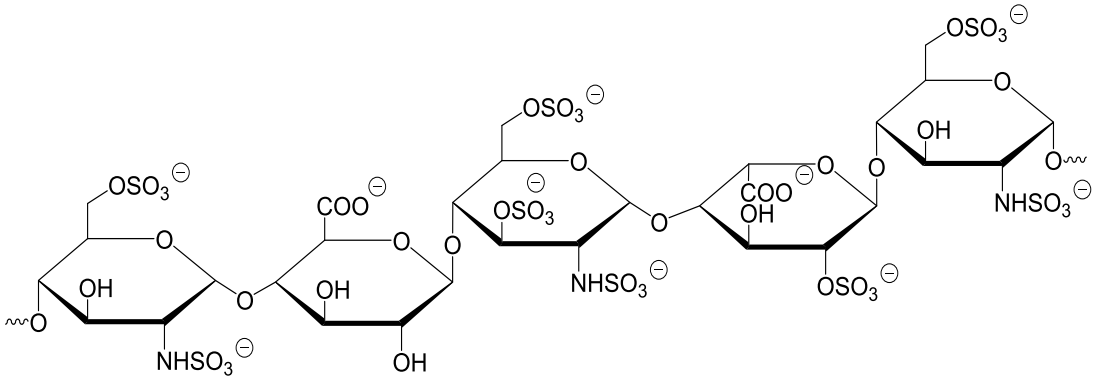
5.2.2 Antithrombins (thrombin inhibitors)

5.2.2.1 Heparin and its analogues

ATIII is one of the main inhibitors of blood coagulation. Deficiency of ATIII is related with venous thrombotic disease [74]. This group includes three different categories of drugs; heparin, LMWH and synthetic pentasaccharides. Their mechanism of action is to activate ATIII with small but therapeutically significant differences in their action.

Heparin binds to ATIII and forms a complex, heparin-ATIII complex that is able to inhibit both FXa and thrombin in the same degree, as shown in Table 1 (FXa: FIIa is 1) On the contrary, LMWHs bind to ATIII, but due to their smaller size, they have greater affinity for FXa than thrombin. As shown in Table 1, the anti-FXa action: anti-FIIa ratio is ranged between 1.9 to 3.8. Synthetic pentasaccharides, are chemical molecules that bind selectively and reversible to ATIII, inducing a conformational change in the ATIII molecule that increases the affinity to FXa more than 300-folds[75]. This complex has higher specific anti-Xa activity than that of LMWH, 800 IU anti-FXa/mg vs. 100 IU/mg[78].

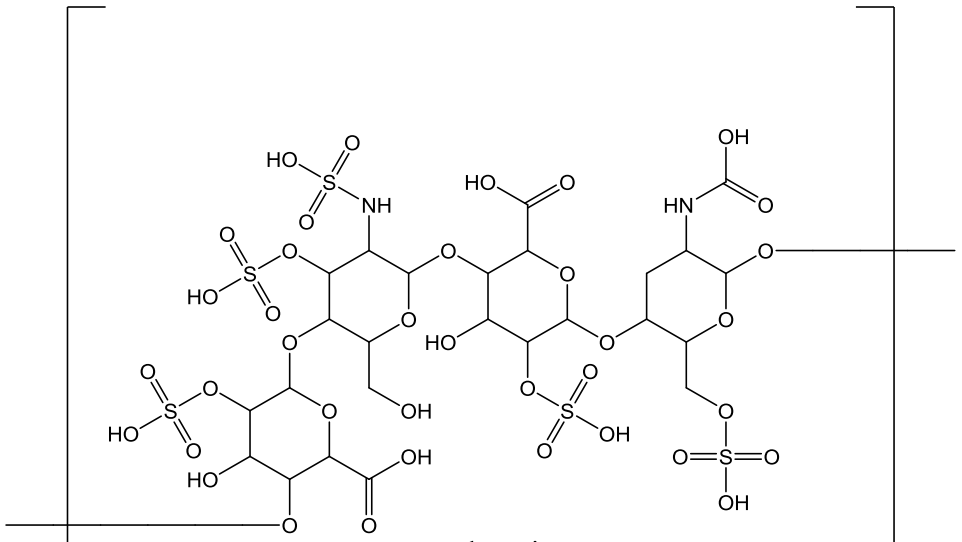
Heparin was discovered in 1916 and named because it was extracted from liver[2]. It can be isolated from liver of mammalian animals like cattle and pig. It is a strongly acidic polysaccharide that consists of many disaccharides (α-L-iduronic acid 2-sulfate, β-D-glucoronic acid). Heparin mechanism of action is to activate ATIII, binding by pentasaccharide's part consisting of 1-D-glucoronic acid and D-glucosamine-sulfate, in order to inhibit FXa and thrombin, but also affects platelets formation. As antidote for heparin, protamine can be used. Main disadvantages of heparin are bleeding and heparin induced thrombocytopenia[76,77].



glucosamine 2-N-sulphated and 6-O sulphated | glucuronic acid | glucosamine 2-N sulphated, 3-O sulphated and 6-O sulphated | iduronic acid 2-O sulphated | glucosamine 2-N sulphated and 6-O sulphated

Pentasaccharide sequence of heparin that binds to ATIII

LMWHs were discovered at late 1970s. They are obtained by enzymatic depolymerisation of heparin, but for every LMWH, this procedure is different. Their weight is between 4,500-5,000 Da (***Table-1***)[77]. LMWHs have still the pentasaccharide part that is needed for binding on ATIII and activate it, but their small size is responsible for preferable inhibition of FXa than thrombin. Advantages are their higher bioavailability and better predictable half-life. They can cause bleeding, hypersensitivity and thrombocytopenia but the risks are lower than at heparin. Drugs of this category are ardeparin, certoparin, dalteparin, enoxaparin, nadroparin, parnaparin, reviparin and tinzaparin[78].



ardeparin

Table 1: Characteristics of heparin, LMWHs, ultra-LMWHs and synthetic pentasaccharides
(Table adapted from Cosmi B, Palareti G[78])

Drugs	Method of preparation	Mean molecular weight (Daltons)	Ratio of anti-factor Xa to anti-factor IIa
heparin	porcine intestinal mucosa extraction	12000-15000	1.0
LMWHs			
Ardeparin	peroxidative depolymerization	6000	1.9
Dalteparin	nitrous acid depolymerization	6000	2.7
Certoparin	deaminative cleavage with isoamyl nitrate degradation	6000	2.4
Nandroparin	nitrous acid depolymerization	4500	3.6
Tinzaparin	heparinase digestion	4500	1.5
Parnaparin	hydrogen peroxide and cupric salt depolymerization	4500	3.0
Enoxaparin	benzylation and alkaline depolymerization	4200	3.8
Reviparin	nitrous acid depolymerization, chromatographic purification	4200	3.5
Ultra-LMWHs			
Bemiparin	quaternary ammonium fractionation	3600	8.1
Semuloparin	partial and controlled chemiselectivedepolymerization	2400	8.0
Pentasaccharides			
Fondaparinux	chemical synthesis	1728	~850 IU anti-Xa/mg
Idraparinux	chemical synthesis	1728	~1600 IU anti-Xa/mg
Idrabiotaparinux	chemical synthesis	2052	~1600 IU anti-Xa/mg

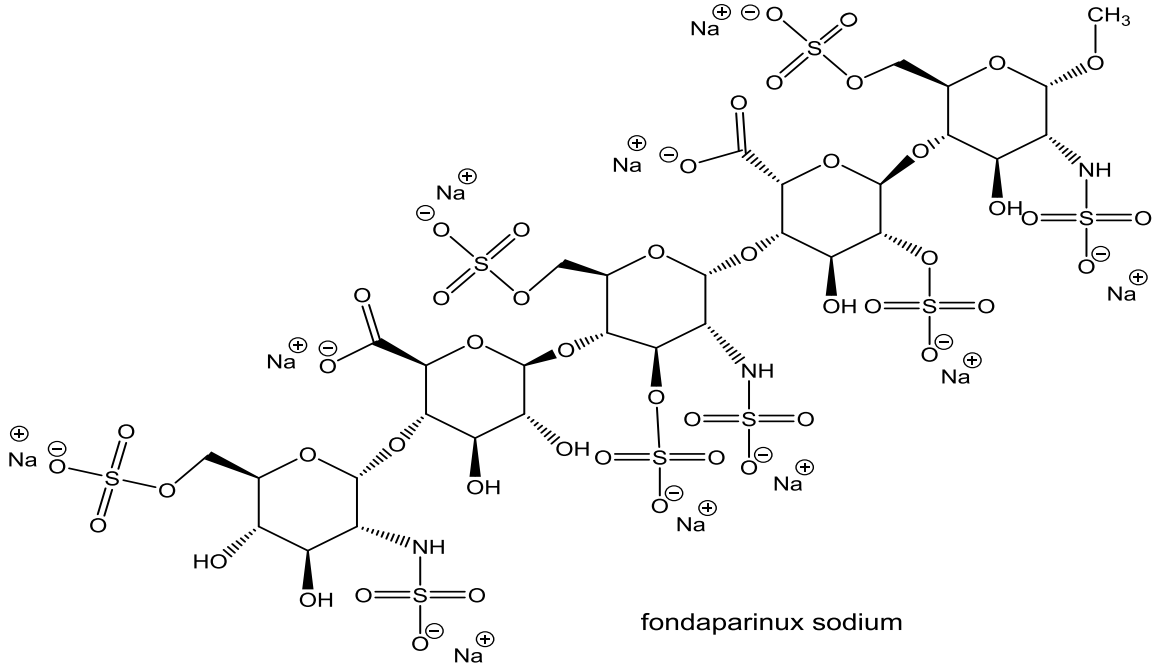
Second generation or Ultra-LMWHs

Bemiparin is considered as a second generation of LMWH and it is product of alkaline depolymerization of porcine heparin with high proportion of saccharides[79]. Molecular weight is 3600 Da and has long half-life of 5,6 hours[80]. It works as activator of ATIII, but because of its molecular weight, it has higher affinity for inhibition of FXa than heparin and it is used for DVT and in thromboembolism[79]. Risk of bleeding is lower but it still remains the main side effect of bemiparin[81]. It is on European market since 1998.

Semuloparin is a hemi-synthetic ultra-LMWH that is prepared by selective chemical depolymerization of heparin. It is used for the prevention of thromboembolism and molecular weight of this drug is 2400Da with an exclusive oligosaccharide sequence for binding to ATIII. It is still under clinical trials[82].

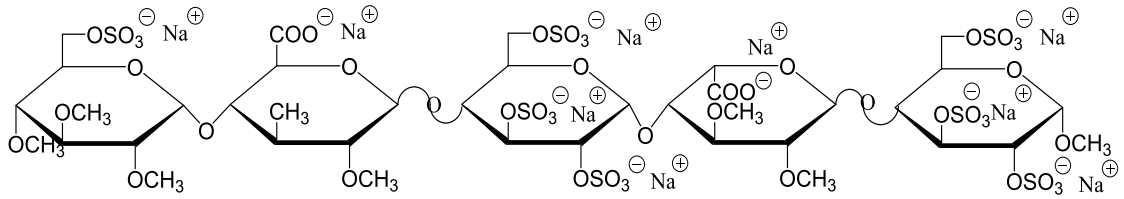
Synthetic pentasaccharides

Fondaparinux was the first synthetic pentasaccharide anticoagulant agent. It works as an inhibitor of FXa, it has a relatively small molecule with structure corresponding to part of heparin and LMWHs[31] sequence[31]. Due to pentasaccharide structure, fondaparinux is in low risk to heparin induced thrombocytopenia[83]. It is administered subcutaneously, half-life is 17-21 hours and it should be avoided in patients with renal insufficiency[84].



fondaparinux sodium

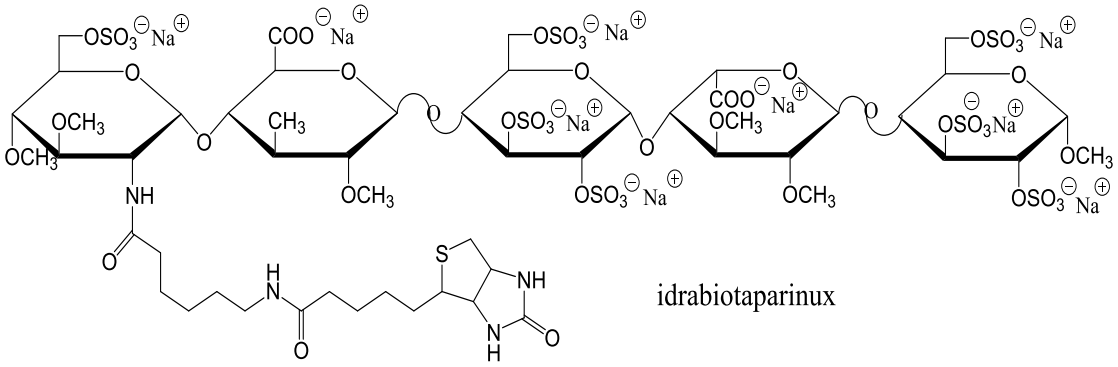
Idraparinux is an O-sulfated, O-methylated analogue of fondaparinux. It is a synthetic pentasaccharide which inhibits FXa and characteristic was the long half-life of 120 hours, which mean that it was considered to administer once a week. But it failed to pass clinical trials-phase III, because of the high bleeding incidence in some patient and its development has been terminated[85,86].



idraparinux

Idrabiotaparinux is developed following idraparinux structure. It is a hyper-methylated derivative of fondaparinux but the difference was the biotin attached on

first glucose, which enables deactivation of the drug by avidin, a protein highly specifically binding to biotin. It had the same results like idraparinux but with better safety results[85, 87]. Last clinical trial about comparison of idrabiotaparinux with warfarin was terminated by sponsors without any results[88].



5.2.2.2 Direct thrombin inhibitors

In order to inhibit the action of thrombin, direct thrombin inhibitors are used. Thrombin has three binding sites, that drugs can attach them and inhibiting. These are the active part, the exo-site 1 which is the fibrin binding site and the exo-site 2, the binding site of heparin. Drugs that directly inhibit thrombin are divided in two groups, divalent direct inhibitors like hirudin and its analogues, which bind to active site and exo-site and univalent direct inhibitors, which bind only in active site of thrombin. Direct thrombin inhibitors (DTIs) have been developed and investigated for their utility in prophylaxis and treatment of venous thromboembolism, heparin-induced thrombocytopenia, acute coronary syndromes (ACS), secondary prevention of coronary events after ACS, and non-valvular atrial fibrillation[89]. Drug of this category can inhibit both thrombin and also fibrin-bound-thrombin. Other key advantages include a more predictable anticoagulant effect compared with heparins because of their lack of binding to other plasma proteins, an anti-platelet effect and the absence of immune-mediated thrombocytopenia[83].

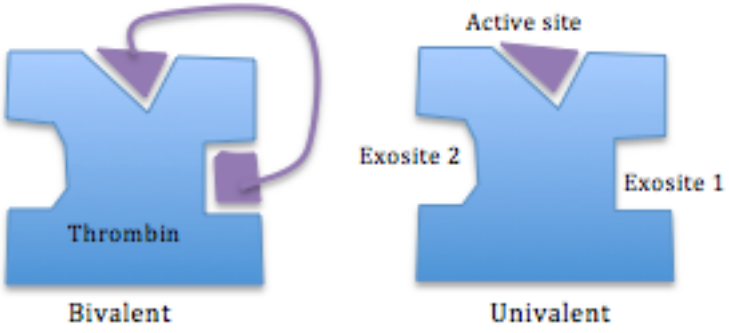
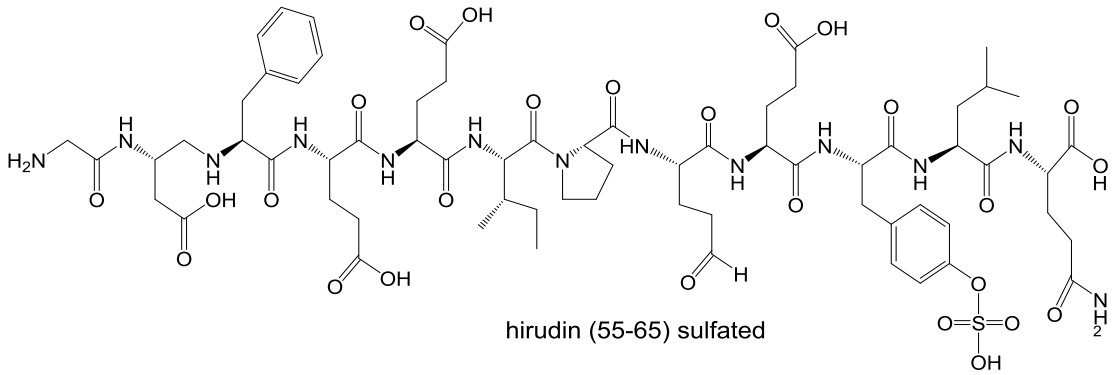


Figure 5: Thrombin's binding sites

(Image adapted from Wikipedia[90])

Hirudin was isolated by Markwardt in 1950s from leech saliva. It has antithrombotic properties[91]. Its mechanism of action is to inhibit directly thrombin. It is a protein

consisting of 65-AA, chemical structure has NH_2 and COOH terminals that are responsible for binding to thrombin directly (NH_2) and at exo-site (COOH)[85,92]. Last decades, technology helped us to form recombinant hirudins (r-hirudin) that are discussed further in the text.



in biological environment as salts

Bivalirudin, is a recombinant hirudin analogue that consists of 20 AA and inhibits thrombin reversibly. As Theodore E. Warkentin, Andreas Greinacher, Andreas Koster (2008, p.1)[93]
Put it:

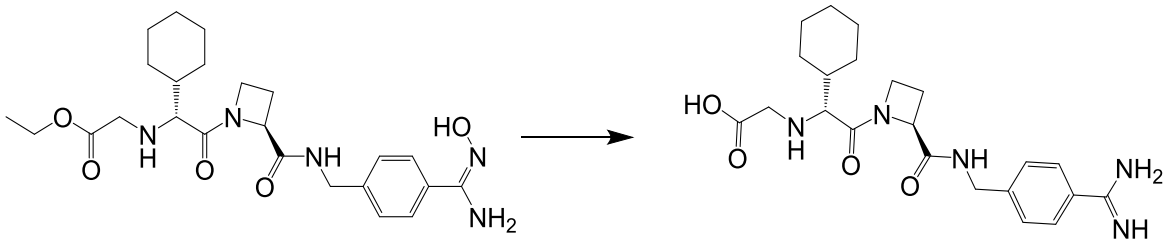
"It combines a carboxy-terminal segment of 12 amino acids (dodecapeptide) derived from native hirudin (residues 53–64, the fibrin[ogen] binding site, also known as exosite1), to an active site-binding tetrapeptide sequence (d-Phe-Pro-Arg-Pro) at its amino-terminus; four glycine residues bridge these two segments together."

It has the shortest half-life and monitoring is necessary (INR, blood pressure). It is administrated intravenously and it is used for PCI and in heparin induced thrombocytopenia[94].

Lepirudin is an irreversible inhibitor of thrombin. It is similar with hirudin structurally, but without a sulfate group on tyrosine position-63 and with isoleucine taking place of the leucine[95]. It was considered to be used as alternative anticoagulant, in case of heparin induced thrombocytopenia, but its development stopped because of bleeding and development of anti-hirudin antibodies[96].

Ximelagatran was the first oral direct inhibitor of thrombin. It is a prodrug that after absorption was rapidly converted into **melagatran**. It had rapid action and it was administrated twice per day. It was approved in some countries for DVT and prevention of thromboembolism as an alternative of warfarin but manufacturer AstraZeneca, stopped its production and withdrew the drug from the markets due to hepatotoxicity[97,98].

Conversion of ximelagatran to melagatran

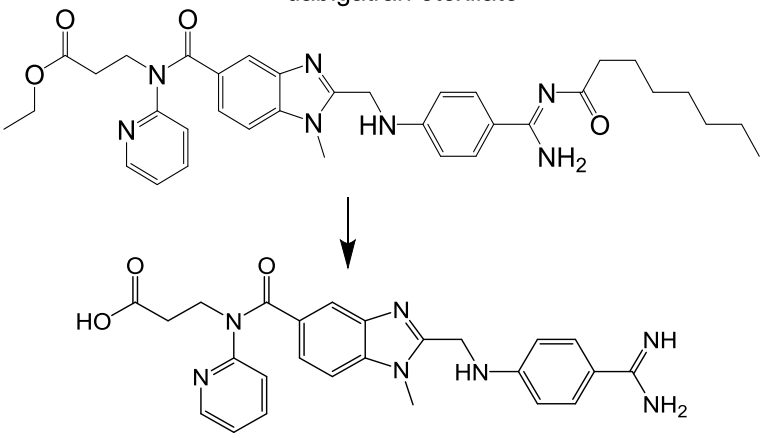


ximelagatran melagatran

Dabigatran etexilate is the prodrug affording active dabigatran after metabolism. **Dabigatran** etexilate is an oral direct thrombin inhibitor (benzimidazole class). It binds reversibly to thrombin and is used for stroke in patients with atrial fibrillation, also used to prevent venous thromboembolism and in knee-hip replacement surgery[99]. Main declared advantage except oral administration was needless of monitoring. However, FDA after post market surveys and evaluation again stated the necessity of monitoring because of the bleeding which is the main side effect of the drug[100].

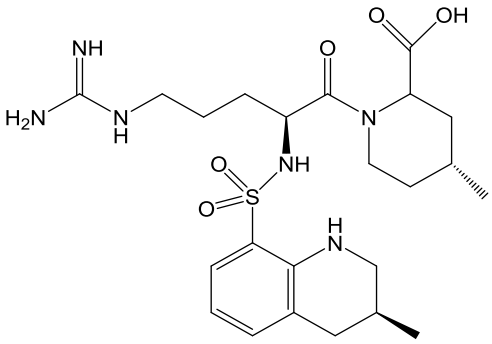
Conversion of dabigatran etexilate to dabigatran

dabigatran etexilate



dabigatran

Argatroban is a synthetic molecule, that is derived from arginine and it is used as inhibitor of thrombin and it is used in heparin induced thrombocytopenia parenterally[101]. Piperidine and guanidine groups are the two parts of the drug that binds to thrombin and can also affect platelets aggregation and thromboxanes A_2 synthesis[102].



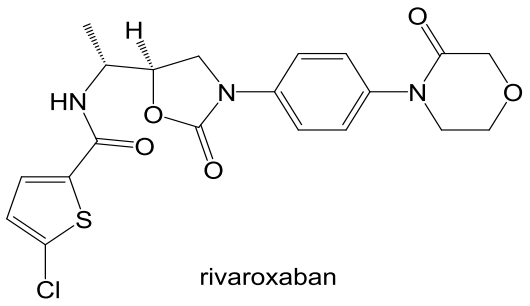
argatroban

5.2.2.3 Direct factor Xa (FXa) inhibitors

FXa can be inhibited by activation of ATIII, but can be also inhibited directly by a category of drugs also known as xabans, the direct inhibitors of FXa. It is a new category with the benefits of oral administration, rapid onset of action and monitoring is not obligatory. On the other hand, patients with renal dysfunction cannot use these drugs. On the contrary no monitoring can be also a problem, due to bleeding as side effect. Direct FXa inhibitors are used for prevention and treatment of stroke, embolism in patients with atrial fibrillation, DVT and for prevention of venous thromboembolism in patients undergoing hip and knee replacement surgery[103].

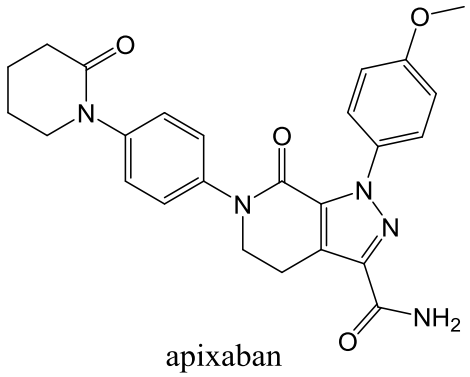
Xabans as direct inhibitors of FXa were developed as an equal alternative of warfarin, with milder side effects and presumption that monitoring of INR will not be compulsory. Most of these agents are administrated orally and have higher bioavailability and half-life than warfarin. Some of the drugs below are on market or under clinical trials.

Rivaroxaban is a selective inhibitor of FXa that approved on 2011 with the trade name Xarelto and it is an oxazolidine derivative with a chlorothiophene non-basic moiety[103]. It prevents and treats DVT, stroke, embolism in patients with atrial fibrillation and after hip or knee surgeries[104]. Advantages are the oral administration and that monitoring is not compulsory. In USA, Xarelto Development Company was suited by various law firms for the excessive bleeding and deaths of people that was taking rivaroxaban without monitoring[105].



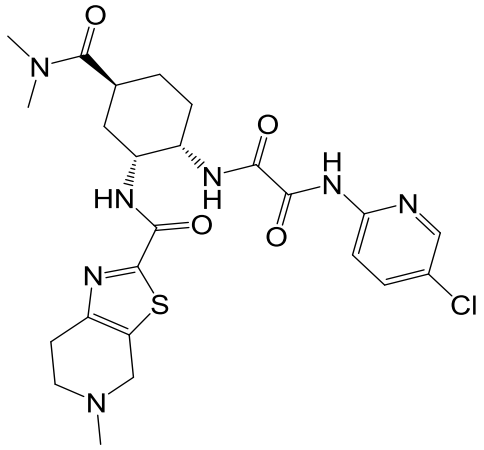
rivaroxaban

Apixaban is a direct and reversible inhibitor of FXa with half-life of 12 hours. In position 1, it has a methoxy phenyl group as a non-basic moiety[103]. It is on markets in Europe from May of 2012 but in USA is still under clinical trial, Phase III[106].



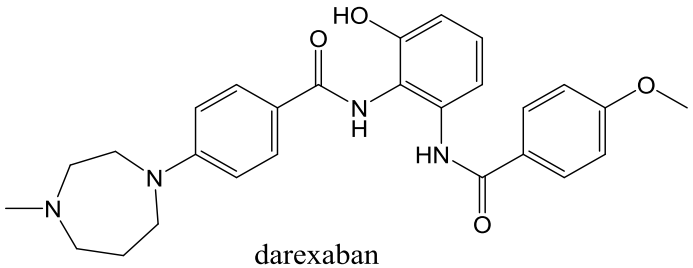
apixaban

Edoxaban is another oral inhibitor of FXa, which is on market on Japan and just recently approved by FDA for the prevention and treatment of stroke and non-Central Nervous System- embolism in patient with atrial fibrillation[103,106].



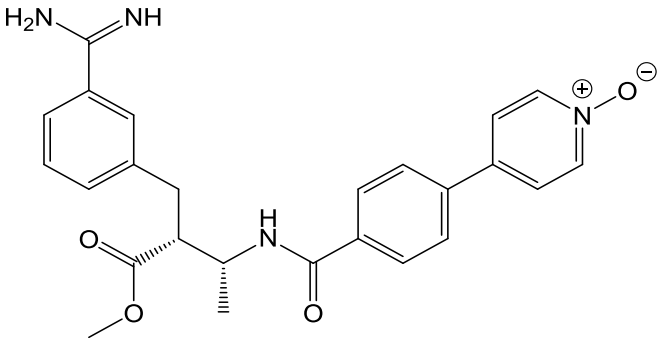
edoxaban

Darexaban and its major metabolite, **darexaban glucoronide** is also an inhibitor of FXa and was developed and tested for stroke in patients with atrial fibrillation, for prevention of thromboembolism and also for patients with Acute Coronary Syndrome[106,107]. After clinical trials-phase II (RUBY-10), it was found that doses higher than 10mg can cause serious bleeding and the development stopped on September of 2011[108].



darexaban

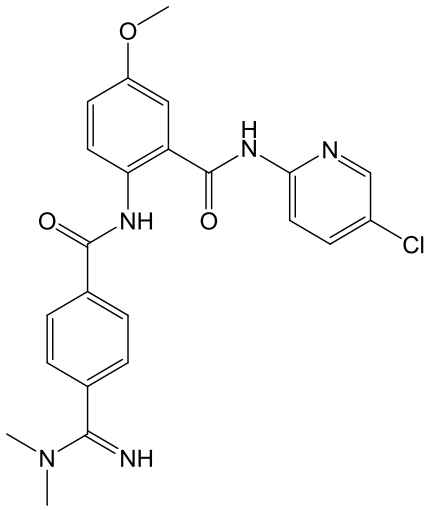
Otamixaban, is another inhibitor of FXa but with a great difference from others xabans. Otamixaban is the only parenteral inhibitor of FXa, which means, short half-life and rapid action. It was developed as alternative of heparin for Acute Coronary Syndrome[109], but results of clinical trial-Phase III was poor and its development has been stopped[106].



otamixaban

Betrixaban, a highly selective inhibitor of FXa is still on clinical trials, phase III. It is a unique small molecule that has half-life of 12 hours and a different elimination (biliary) than other oral inhibitors of FXa[109]. It is tested for prevention of embolism after knee-hip surgery but also for stroke in patients with atrial fibrillation[106]. The same manufacturing company, Portola Pharmaceuticals are also testing Andexanet Alfa as an antidote of oral inhibitors of FXa[110,111].

Andexanet Alfa is a recombinant protein that is under development as an antidote of direct inhibitor of FXa but also for partial inhibition of LMWHs. It has not a procoagulatory action and the lack of γ-carboxyglutamic acid domain is responsible for not binding to FVIIa, TF and phospholipids. Phase III of clinical trials started on March of 2015[112].



betrixaban

Eribaxaban is an inhibitor of FXa that is under clinical trial phase III for venous thromboembolism after a knee replacement surgery. Phase II shown that increasing dose will increase bleeding but results was strong enough to continue at Phase III[113,114].

5.2.3 Vitamin K antagonists

As mentioned above, vitamin K is one of the fat soluble vitamins and it is important for the formation of clotting factors like FII, FVII, FIX and FX in liver. Vitamin K cycle is starting by reduction of vitamin K by quinone reductase and converted to vitamin K epoxide by transformation of glutamate into γ-carboxyglutamate. Last step is the conversion of vitamin K epoxide to vitamin K by vitamin K epoxide reductase[115,116].

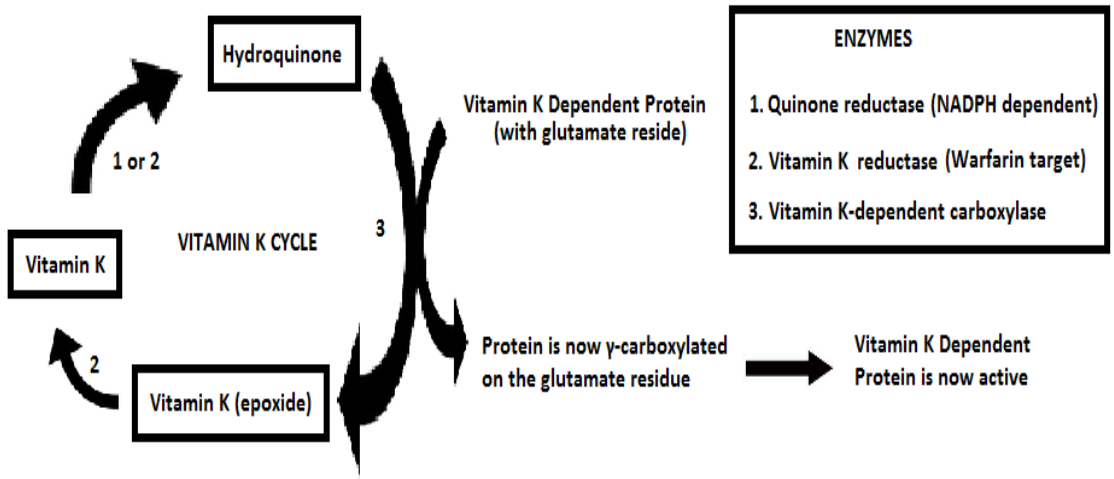


Figure 6: Vitamin K cycle

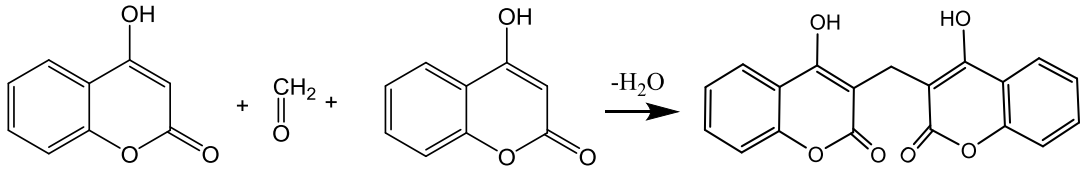
(Image adapted from Examine[117])

Vitamin K antagonists are one of the most significant groups of drugs that affect blood clotting. This category includes drugs with coumarin or indandione structure. Both of them work by antagonizing vitamin K epoxide reductase that takes part in vitamin K cycle, which is responsible of the synthesis of coagulation factors. Vitamin K antagonists are used for the prevention or treatment of thrombotic events, DVT, atrial fibrillation, for patients that have prosthetic valves[118].

Coumarins are the most important oral anticoagulants, especially warfarin. Coumarins are derivatives of 4-OH-coumarin. Dicoumarol was the first drug of this group but it was replaced by warfarin in 1950s. Vitamin K antagonists are used for the primary and secondary prevention of venous thromboembolism, for the prevention of systemic embolism in patients with prosthetic heart valves or atrial fibrillation, for the primary prevention of acute MI in high-risk men, and for the prevention of stroke, recurrent infarction, or death in patients with acute MI[119]. The main advantage of vitamin K antagonists is the oral administration and that their long half-life, which are important for long term use. But vitamin K antagonists are challenging to use because:

- their narrow therapeutic index
- of many drug-drug and food-drug interactions
- frequently monitoring by measuring INR
- of major side effects including life-threatening bleeding, skin necrosis
- they can be teratogenic when used during pregnancy.

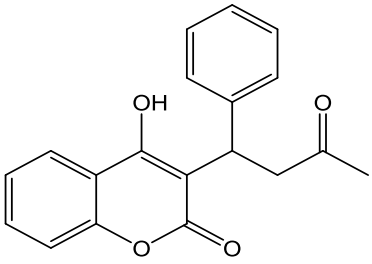
Coumarins were discovered by mold of sweet clover, on the late 1930's, in Wisconsin University. Dicoumarol is formed as the result of the reaction of two molecules of 4-hydroxy-coumarin with formaldehyde.



4-hydroxy-coumarin dicoumarol

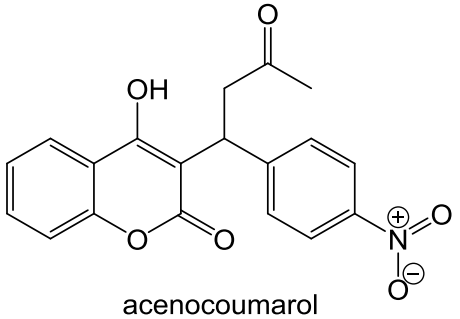
Dicoumarol was firstly used as rat poison and after as anticoagulant. Mechanism of action is to inhibit hepatic synthesis of vitamin K-dependent coagulation factors. Half-life is 10-30 hours according to dose[120,121]. Bleeding is the main side effect that can lead also to anemia[122]. On 1950's, dicoumarol was replaced by warfarin.

Warfarin inhibits vitamin-K epoxide reductase, which means that is stop vitamin K cycle. Warfarin is 4-hydroxycoumarin which is substituted at position 3 by a 1-phenyl-3-oxo-1-butyl group[123]. Enolic group make the drug acidic and warfarin is used a racemic mixture of R- and S-enantiomers[124]. Half-life is 40-70 hours, it has narrow therapeutic index, so monitoring and measuring INR is important but it also has many drug-drug or food-drug interactions, it can cause bleeding and many other side effects[125].



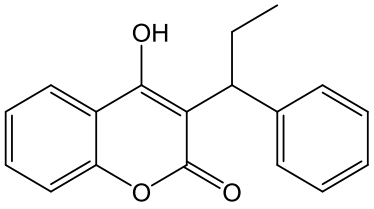
warfarin

Acenocoumarol is also a vitamin K antagonist and has the same mechanism with warfarin. In Greece, acenocoumarol is the most used drug of this category. It has the same properties, uses, interactions and concerns with warfarin. The phenolic group of warfarin is substituent by p-nitrophenyl group. Due to half-life, which is 3-10 hours, it can be taken twice per day[126].



acenocoumarol

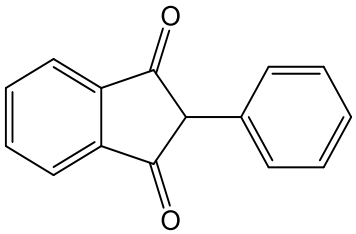
Phenprocoumon is a long-acting antagonist of vitamin K, with half-life of 5,5 days. It has R- and S- enantiomer also but is metabolized by different cytochromes than warfarin (cytochrome P2C9)[118]. Because of the long action of phenprocoumon, monitoring is not so frequent than in other agents of this group because INR is in therapeutic ranges[127].



phenprocoumon

Another category of vitamin K antagonists is drugs with parent structure of indandione.

Phenindione, is a vitamin K antagonist that inhibits vitamin K reductase. It is an oral drug that is considered as prophylactic anticoagulant[128]. "Phenindione has actions similar to warfarin, but it is now rarely employed because of its higher incidence of severe adverse effects. (From Martindale, The Extra Pharmacopoeia, 30th ed, p234)".



phenindione

5.2.4 Defibrinating agents

Defibrinating agents are responsible for conversion of fibrinogen to fibrin degradation products that eliminate, in order to decrease levels of blood fibrinogen.

Ancrod is a protease that is obtained by venom of Malayan snake, *Agkistrodon rhodostoma*. Ancrod acts by breaking down fibrinogen to smaller polymers, which are then removed by phagocytosis or fibrinolysis. It was used in some countries until 1980's, but then withdrawn from the market. On 2005, started a new clinical trial for its use in patients with acute ischemic stroke, without success. Further investigation should be done on this drug. [129,130].

Batroxobin is obtained from *Bothrops atrox* and *moojeni*. It is a thrombin like protease, consisting of 231 AA and after analysis; it was shown high contents of

proline and carbohydrates. Mechanism of batroxobin action is to cleave fibrinopeptide A of fibrinogen and convert it to fibrin degradation products in order to eliminate them. Batroxobin is given parenterally for prevention of thrombosis and used in laboratories for determination of fibrinogen sufficiency[131,132].

5.3 Fibrinolytic agents

As mentioned above, blood fibrinolytic system contains plasminogen, that can be converted to the active enzyme, plasmin. Plasmin degrades fibrin into soluble fibrin degradation products, and this reaction can be catalyzed by two physiological plasminogen activators (PA), the tissue PA (t-PA) and the urokinase PA (u-PA)[133]. Drugs that promote conversion of plasminogen to plasmin are called fibrinolytics and it can be used for MI, acute ischemic stroke, pulmonary embolism, DVT and peripheral thromboembolism[1]. Studies shown that fibrinolytic agents can reduce mortality if given 12 hours of the onset symptoms of MI or acute thrombotic stroke within 3 hours[2]. Main disadvantage is the hemorrhagic complication that can be occurred by administration.

Streptokinase is a protein derived from Streptococci and consists of 440 AA, which after secretion are broke down to 414AA. These AA of streptokinase, are derived into 3 groups (1-150, 151–287 and 288–414).Each of these groups binds to plasminogen and activate (tPA) it in order to convert plasminogen to plasmin[134]. Streptokinase is used on acute MI, embolism, DVT and arterial thrombosis but is contraindicated in severe hypertension and in active bleeding[135].

Urokinase (u-PA) is a two chain enzyme that is isolated from urine and can be found in several locations in human cells. It consists of 411 AA and it is characterized by three domains; N-terminal growth factor domain (1-46 AA), kringle domain (47-137 AA) and serine protease domain (138-411 AA). Urokinase is synthesized by prourokinase, an inactive one chain enzyme precursor. Prourokinase is cleaved by plasmin, in the serine protease domain, result in the formation of two chains. Disulfide bonds maintain the two chain urokinase structure[136]. The N-terminal growth factor and the kringle domains are responsible for binding to urokinase plasminogen activation receptors[137]. U-PA binds to plasminogen and by cleaving arginine-valine bond converts plasminogen to plasmin in order to degrade fibrin clots[138]. Both urokinase and streptokinase are intravenously administered.

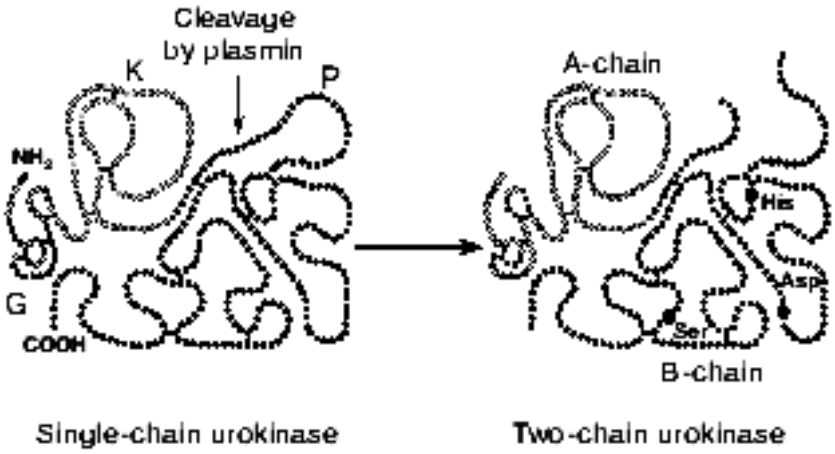


Figure 7: Structure of single-chain and two-chain urokinase forms. G, growth factor-like domain; K, kringle domain; P, protease domain.

(Image adapted from Stepanova, V. V.[136])

Anistreplase, consists of bacterial streptokinase and human plasminogen, with the active site of plasminogen acylated for its protection[139]. After administration, acyl group is hydrolyzed releasing the streptokinase-proactivator complex that converts plasminogen to plasmin, with greater selectivity on clots than free plasminogen and greater activity. Main difference unlike sole streptokinase is the longer half-life (40-90 min.)[1].

Staphylokinase is obtained by Staphylococcus aureus and can be used as an alternative plasminogen activator[139]. It consists of 136 AA, with a structure that is separated in three parts, an α-helix with residues of Lys57-Thr71, a five strand β-sheet and connecting loops. In order to activate, it should form a complex 1:1 with plasminogen[140].

Alteplase is a second generation fibrinolytic, which is a result of recombinant DNA technology. It is a serine protease, that consists of 527 AAs and it binds and activates the fibrin-bound plasminogen only, via breaking arginine-valine bond and plasminogen is converted to plasmin[140].

Reteplase, another second generation agent, it consists of 355 AA (4-175 AA from 527AA of tPA is not in this protease). It still has domains of Kringle-2 (help to bind to fibrin) and serine protease (important for breaking Arg-Val bond in plasminogen to form plasmin). It is not so specific to fibrin, but smaller molecule it is cheaper to produce. It has longer half-life than alteplase[140,141].

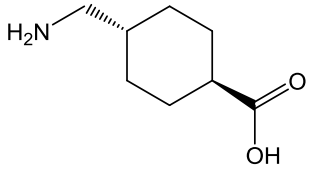
Tenecteplase has three basic differences from tPA. It is produced by modifications of complementary DNA, changing Thr-103 with Asp-103, Asp-117 with Glu-117 and by removal of 296-299 AA and substitute by 4 Ala[140]. It also acts like alteplase and reteplase, but it is more specific for tissue plasminogen activator and has longer half-life than the other two drugs[142].

Desmoteplase is isolated from saliva of vampire bat (*Desmodus rotundus*) and it is structurally similar to tPA. It consists of 477AA without the domains of Kringle-2 and plasmin cleavage site. It has longer half-life than anyone other fibrinolytic agent and does not affect blood-brain barriers (no neurotoxicity)[143]. It is under clinical trials phase III and first results were disappointing, but Lundbeck, the manufacturing company will continue trials[144].

5.4 Antifibrinolytic agents

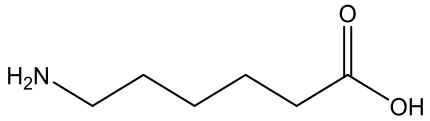
Antifibrinolytic agents are used in order to inhibit plasminogen activation and prevent fibrinolysis.

Tranexamic acid, chemically *trans*-4-(aminomethyl) cyclohexane-1-carboxylic acid, inhibits the activation of plasminogen to plasmin. It is used in hemophilia for short-term treatment but mainly as antidote in overdose of fibrinolytic drugs[145].



tranexamic acid

Aminocaproic acid is a derivative of lysine missing α-amino moiety. It is a short fatty acid compound with aliphatic tail and it binds to kringle domain of plasminogen and inhibits tPA. In other words it inhibits fibrin breakdown. It is used for excessive postoperative bleeding treatment or in case of fibrinolytic drugs overdose[146].



aminocaproic acid

Aprotinin, a bovine pancreatic trypsin inhibitor, is a protein that can inhibit proteases like trypsin, plasmin, kallikrein (lead to formation of FXIIa). It is used in surgeries as antifibrinolytic to stop bleeding. It was in market from early 1990s but on 2007 it was withdrawn. After more trials, EMA gave authorization and aprotinin is now on market again[147].

5.5 New technologies

Aptamers are small oligonucleotide molecules that bind with high specificity and affinity to a target that can be a protein, nucleic acids, and tissues. It is a new technology that is used in order to produce safer, more effective and more specific

drugs. They were discovered at early 1990's and testing for various targets and one of them is coagulation factors. Aptamers are similar to monoclonal antibodies in principle of their action, but their advantages are easier production and controlling of the agent and that they are of decreased toxicity and immunogenicity[148].

Pegnivacogin (RB006) is a 2-fluoropyrimidine modified 31-nucleotide-RNA aptamer bearing 40 kDA PEG unit at 5´-end for better pharmacokinetic profile. It is administrated subcutaneously and inhibits FIX-FIXa in order not to allow generation of thrombin. It is under clinical trials phase III for acute coronary syndrome and PCI. Phase II results shown less bleeding complications than in case of heparin[148].

Anivamersen (RB007) is the controlling agent of pegnivacogin. Is a complementary antisense 17-mer oligonucleotide to pegnivacogin chemically 2-O-methyl modified RNA[148]. It is administrated intravenously and binds tightly to pegnivacogin, changing its conformation and neutralizing the effect on FIX rapidly[149].

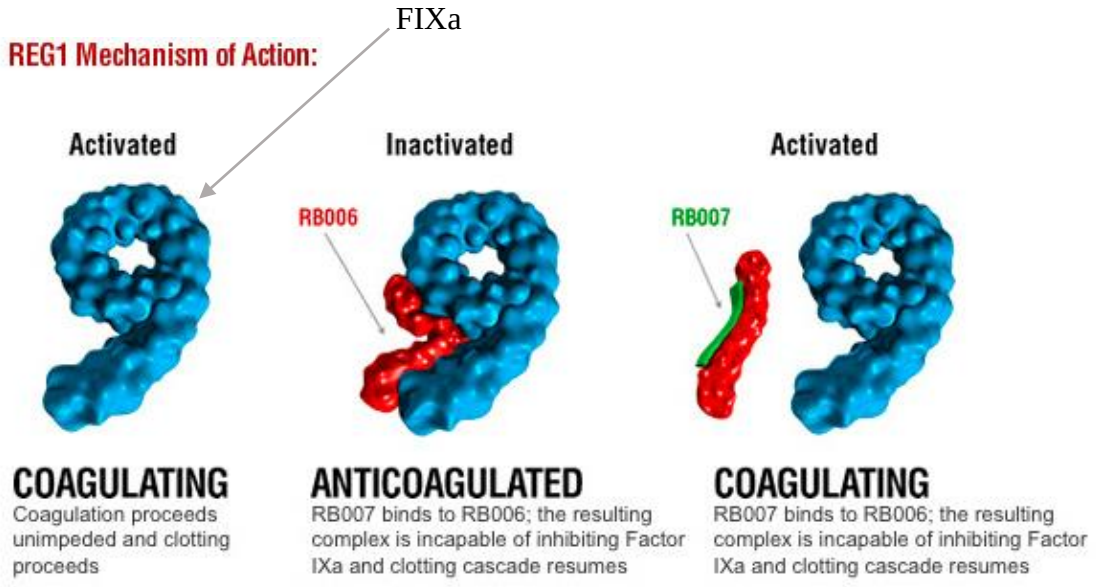


Figure 8: REG1 Mechanism of Action

(Image adapted from Regado Biosciences[150])

ARC1779, is a drug on clinical trials phase II for acute coronary syndrome, von Willebrand disease and for carotic arteries disease. It is an inhibitor of vWF on A1 domain, which mean that block GPIb receptor and stop further aggregation of platelets. It is a 40 nucleotide DNA aptamer, consists of 13 unmodified 2'-deoxy nucleotides, 26 modified 2'-O-methyl and 1 deoxythymidine nucleotide with a 20 kDa polyethylene glycol conjugation[151].

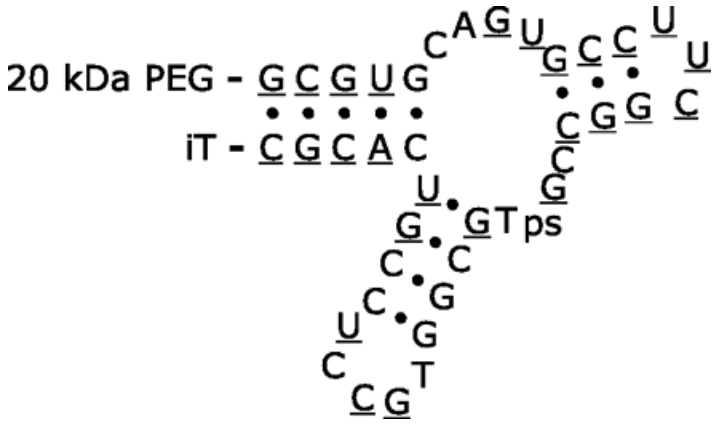


Figure 9: Proposed secondary structure of ARC1779

(Image adapted from Gilbert, J. C.[151])

NU172 is a 26 unmodified nucleotide DNA aptamer, which inhibits thrombin and is under clinical trials-Phase II as anticoagulant[148].

6 Conclusion

Since the isolation of heparin, on 1916, and discovery of coumarin at early 1920's, several new categories of drugs with different mechanisms of actions were found for prevention and therapy of thromboembolism. But also, platelets and coagulation pathways were understood completely and new technologies like aptamers were discovered.

An ideal anticoagulant should have oral rapid action, with greater safety than old agents, larger therapeutic index and less drug-drug interactions. This can be our guide in our days, for developing newer agents with greater benefit- risk ratio, larger safety than older ones, but still there are some steps to do in order to reach our aims and develop the ideal agent that affect blood clotting.

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