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FXa Direct Synthetic Inhibitors

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Abstract

Factor Xa (FXa) is an enzyme belonging to the serine protease family which plays a vital role in hemostasis, being an essential part in the blood-clotting cascade by catalyzing the thrombin and clot production, and wound closure. Moreover, the improvement of new anticoagulants drugs is essential to prevent cardiovascular thrombotic and pathologies. FXa has been a main target for the design of new drugs with important antithrombotic action; nevertheless direct FXa inhibitors that are available still have side effects and drawbacks. This chapter describes the FXa function in the blood-clotting cascade, the molecular and structural characteristics of this essential enzyme, and the novel FXa synthetic drug characteristics. This chapter highlights the importance of continuing the efforts towards searching and designing novel and safer anticoagulant drugs.

Keywords: FXa, coagulation cascade, anticoagulants, synthetic inhibitors, direct FXa inhibitors

1. Introduction

1.1. Primary and secondary hemostasis

Hemostasis is the human body's physiological response to blood vessel injury and subsequent prevention of hemorrhage [1–3]. This significant three-step biological process involves a concerted coordination between blood clotting proteins and platelets with the consequent formation of a clot (repair of a damaged vascular tissue) or thrombus (clot in a healthy blood vessel). According to the cell-based coagulation model (**Figure 1**), this process involves: primary and secondary hemostasis, and fibrinolysis [4].

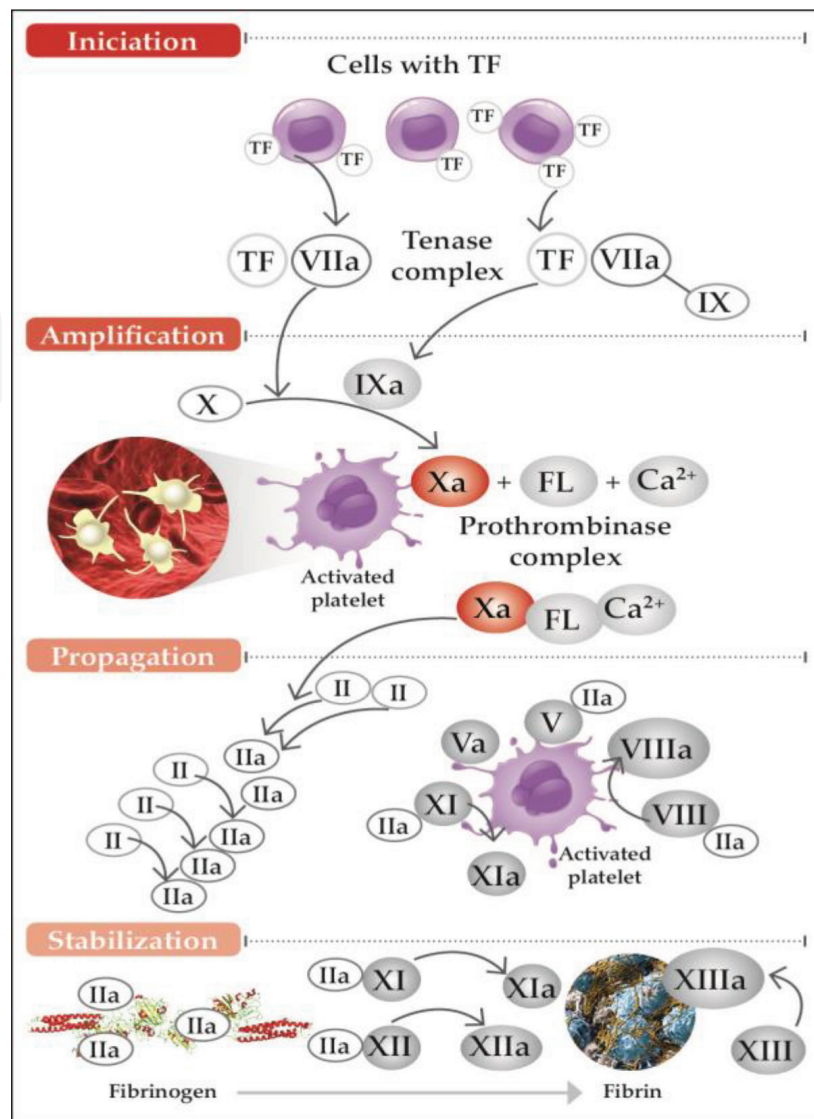


Figure 1. Cell-based coagulation model. Figure adapted from Vojacek [8].

Primary hemostasis causes local vasoconstriction which diminishes blood flow at the injury site and platelet plug formation. *Secondary hemostasis* implicates a series of enzymatic reactions between coagulation factors and cellular activity. These enzymatic reactions convert fibrinogen to fibrin, an insoluble strand, which together with platelets forms a thrombus.

Lastly, *fibrinolysis* is the biological mechanism which disperses the clot after the blood vessel has healed.

The cell-based model includes the interactions between cells, platelets, and coagulation factors. This model postulates a three-phase process:

- *Initiation:* occurs after vascular injury and leads to the production of a small amount of thrombin. Tissue factor (TF) localized to the cell membrane is activated by non-coagulation and coagulation proteases (blood clotting proteins or factors). The produced FVIIa/TF

complex activates **Factor X to FXa**, and FXa combines with FVa to produce small amounts of thrombin, which subsequently activate platelets during the amplification phase.

- *Amplification*: produces small amounts of thrombin activated platelets increasing platelet adhesion and promoting **FXa and cofactors to catalyze the production of more than 1000 thrombin molecules from each FXa unit**.
- *Propagation*: the protein complexes are assembled on the platelet surface resulting in large-scale thrombin generation. After that, fibrin production starts with the clot formation. Finally, stabilization contributes to the formation of a thrombus, thus producing the worldwide pathology called thrombosis [5–7].

1.2. Thrombosis

Over the past few decades, the fact that cardiovascular syndromes are a leading cause of heart problems and rising death rates in the US and Europe has been gradually accepted. With more than 24,000 deaths annually, cerebrovascular accidents (CVA) represent almost a third of all deaths [8–12].



Figure 2. Thrombus production on blood vessels.

In 2012, the World Health Assembly (WHA) set a global target to reduce premature deaths from non-infectious disease, including cardiovascular disease, by 25% by 2025. Later, in May 2015, the International Society on Thrombosis and Hemostasis (ISTH) and the World Thrombosis Day (WTD) committee appealed for increased attention to thrombosis in a message to the Assembly of the World Health Organization (WHO) [13].

A thrombus formation, which obstructs arterial circulation, can end in acute myocardial infarction (AMI) or ischemic stroke. In venous circulation, deep vein thrombosis (DVT) can cause chronic leg pain, edema, and ulcers [14–16].

A thrombus can partly or completely block blood vessels, which may deprive tissues of a supply of oxygen and nutrients. An embolus (stroke) is a dislodged thrombus that moves through the bloodstream and obstructs another vessel (**Figure 2**). The thrombus is formed by aggregations of activated platelets, red blood cells, and cross-linked fibrin protein.

Thrombosis is a common causal pathology for three prevalent cardiovascular disorders: stroke, acute coronary syndrome (ACS), and venous thromboembolism (VTE) [17, 18]. Additionally, the latest statistical study from the Global Burden of Diseases, Injuries, and Risk Factors (GBD) shows that 25% of the people around the world die from thrombosis-related events. As an example of this statistic, a recent study carried out in Chile found the incidence risk rate for thromboembolic diseases among patients under general surgery is 55%, and the main cause of death in Chile is cardiovascular disease [9, 10].

Besides, it is important to point out that these diseases have a harsh effect on these people's quality of life and health care costs [19]. Clearly, the high prevalence of thrombosis and its serious implications create an urgent need for safe and reliable prophylaxis and treatment.

2. Nowadays antithrombotic therapy

Antithrombotic drugs are used for prevention and treatment of thrombosis. Targeting the thrombi components and the pathology, these agents include *antiplatelet* drugs, *anticoagulants*, and *fibrinolytic* agents [20]. The two first agents act to prevent the thrombus formation during the primary and secondary hemostasis process, while the fibrinolytic agents act when the thrombus is already formed (**Figure 3**) [21].

There is a great variety of commercial drugs for the treatment of antithrombotic pathologies with a wide range of disadvantages and side effects as summarized in **Table 1**.

Various clinical trials have verified the value of standard antiplatelet and anticoagulant agents, which include aspirin (antiplatelet), vitamin K antagonists (VKA) (warfarin), FXa indirect inhibitors (fondaparinux sodium), DTI (argatroban), UFH, LMWH, and TII for wide-ranging prevention and treatment of arterial and venous thromboembolic diseases and cardiovascular pathologies [21–30].

It is evident that, given the prevalence and implications of serious thrombosis, there exists a strong necessity for effective prophylaxis and treatment, and the use of oral anticoagulants is widespread.

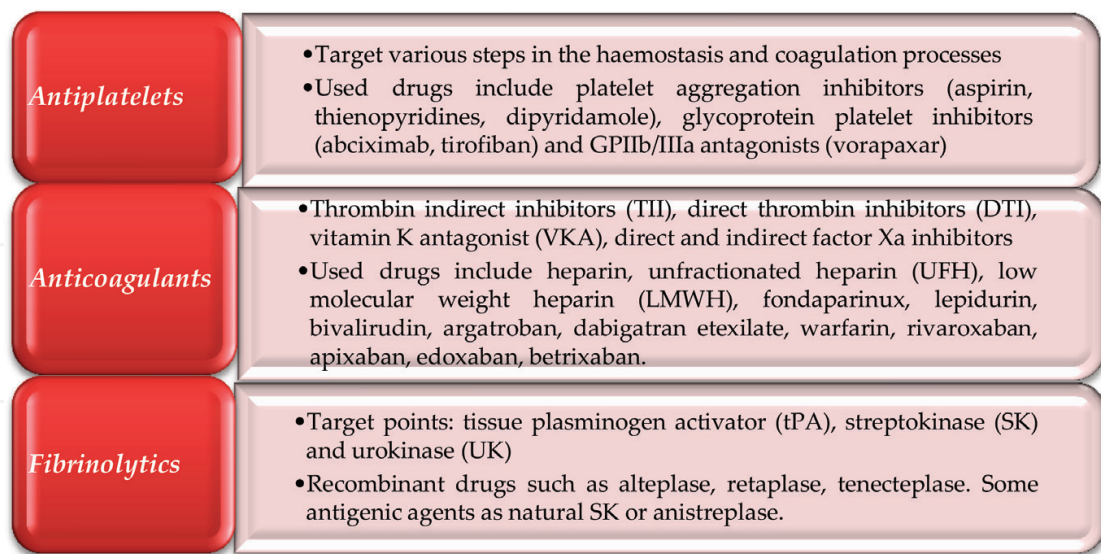


Figure 3. Antithrombotic agents' classification, target points, and commercial drugs [20–27].

Several clinical trials have confirmed the efficacy of classic anticoagulants, including vitamin K antagonists (VKA), unfractionated heparin (UFH), and low molecular weight heparins (LMWH weight heparin with reduced activity towards thrombin versus UFH) in prevention and treatment of a wide range of arterial and venous thromboembolic disease prevention [21]. Many approaches have been explored in the development of antithrombotic agents which inhibit enzymes in the coagulation pathways [29, 30, 32].

Unfractionated heparin (UFH) was discovered in 1916 and it targets multiple factors in the coagulation cascade, but it has a number of disadvantages, including a parenteral route of administration, frequent laboratory monitoring of coagulation activity, and the risk for patients to develop mortal heparin-induced thrombocytopenia. Low-molecular-weight heparins (LMWHs), developed in the 1980s, promote the inactivation of both thrombin (factor IIa) and factor Xa. LMWHs have largely replaced UFH due to its lower risk of causing bleeding, lower levels of plasma protein binding, good bioavailability and superior pharmacokinetic properties in comparison with UFH.

However, its use remains limited owing to the need for parenteral administration to patients who will eventually need either to be trained to self-inject or to find assistance from a trained nurse. These anticoagulants all have the limitations mentioned above that restrict their use in the clinic and have created the need for new treatments (**Figure 4**) [33–35].

Warfarin, which was discovered in 1941, is the prototype vitamin K antagonist (VKA, **Figure 5a** and **b**). Its use and other VKAs' uses are especially problematic, albeit these anticoagulants offer the convenience of oral administration. Until recently, the VKAs were the only available oral anticoagulants and the most commonly prescribed. However, VKAs have a number of well-documented drawbacks, including a slow onset and offset of action, unpredictable pharmacokinetics and pharmacodynamics, variability in response to the dosage, and multiple food-drug and drug–drug interactions. Furthermore, regular monitoring of coagulation and dose adjustments are required to maintain patients in the target international normalized

Antithrombotic agent	Commercial agents disadvantages and side effects
Antiplatelet drugs	<ul style="list-style-type: none"> • <i>Aspirin</i>: gastrointestinal complaints, allergy, hepatic and renal pathologies, aspirin resistance • <i>Thienopyridines</i>: gastrointestinal complaints, hematologic side effects • <i>Dipyridamole</i>: gastrointestinal complaints, headache, facial flushing, dizziness, hypotension, caution in patients with coronary artery diseases • <i>GPIIb/IIIa receptor antagonists</i>: bleeding, thrombocytopenia.
Anticoagulants	<ul style="list-style-type: none"> • <i>Heparin, UFH, and LMWH</i>: parenteral administration, bleeding, thrombocytopenia, increasing level of bilirubin, osteoporosis. • <i>Fondaparinux</i>: bleeding, there is no antidote • <i>Lepirudin, bivalirudin, argatroban</i>: parenteral administration, serious bleeding, hepatic insufficiency • <i>Dabigatran etexilate</i>: bleeding, renal excretion • <i>Warfarin or acenocoumarol</i>: only 3% of administered warfarin is biologically active due to its binding to albumin, narrow therapeutic window, frequent monitoring, drug-food and drug-drug interactions, interference with the synthesis of vitamin K-dependent clotting proteins (FII, FVII, FIX and FX), bleeding, skin necrosis, and fetal abnormalities. • <i>Rivaroxaban</i>: expensive, renal excretion • <i>Apixaban</i>: expensive, only for major orthopedic surgery, anemia, hemorrhage, nausea • <i>Betrixaban</i>: expensive, anemia, skin rash, drug-drug interactions
Fibrinolytics	<ul style="list-style-type: none"> • <i>Streptokinase</i>: expensive, hypotension, rash, fever, chills and rigors, blurred vision, confusion, dizziness, faintness, unusual tiredness or weakness, drug-drug interactions. • <i>Urokinase</i>: bleeding gums, difficulty with breathing or swallowing, headache, increased menstrual flow or vaginal bleeding, nosebleeds, paralysis, prolonged bleeding from cuts • <i>Anistreplase, alteplase, tenecteplase, reteplase</i>: hemorrhage or hematoma formation at the site of venipuncture, gastrointestinal and genitourinary tract hemorrhage, blood in urine

Table 1. Disadvantages and side effects of commercial antithrombotic agents [31].

ratio (INR) range. Monitoring of warfarin therapy is critical due to the variability and relatively narrow therapeutic index, which frequently leads to a higher risk of thromboembolism or excessive anticoagulation with subsequent increased risk of bleeding [36, 37].

Other drugs available for short-term anticoagulation include UFH, LMWHs, fondaparinux (**Figure 5c**) as an indirect FXa inhibitor, and direct thrombin inhibitors (DTIs) such as argatroban, bivalirudin, and hirudin. All these anticoagulants require parenteral administration with their consequent disadvantage. However, LMWH and VKA are the basis for contemporary thromboprophylaxis and treatment in Chile as it is all around the world. The difficulties and inadequacies around the practical and medical aspects of these anticoagulants have encouraged the development of novel drugs that are less expensive for the patient and the health care system [38–41].

Despite the accumulated understanding of the clotting system, its complexity has provided a considerable number of obstacles to the discovery and development of potent anticoagulants that are simultaneously effective and safe.

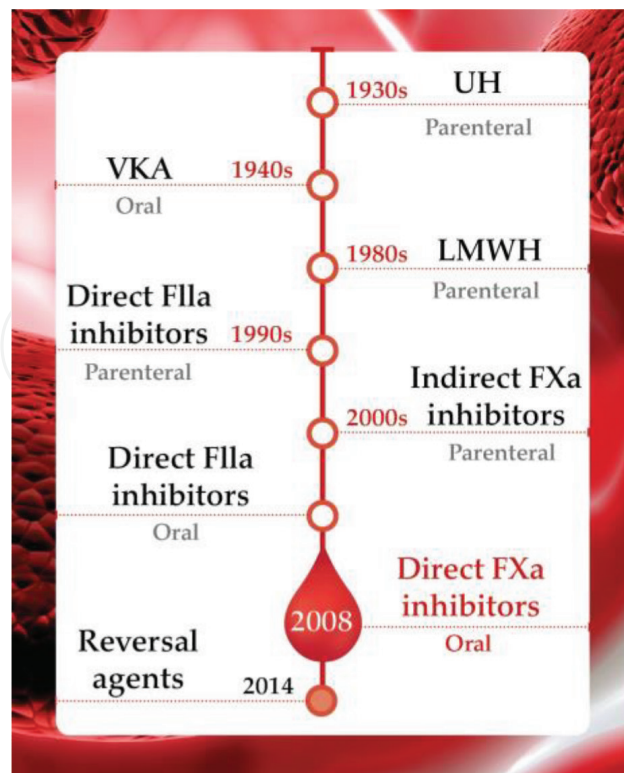


Figure 4. Chronological development of anticoagulants.

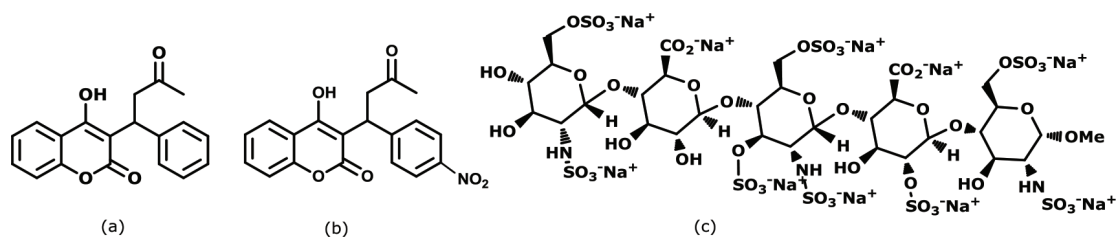


Figure 5. (a-b) VKA. (c) Indirect FXa inhibitor.

In recent years, investigation has been focused on novel classes of anticoagulants (small molecules) which target a specific enzyme or coagulation step in the coagulation cascade, including complex inhibitor of factor VIIa-tissue factor, factor IXa inhibitors, and factor XIa, direct thrombin inhibitors, and synthetic direct and indirect inhibitors of Factor Xa (activated Factor X). All of the features mentioned have led to the development of new anticoagulants, including direct FXa inhibitors [42–44].

3. Clotting cascade: factor Xa function

Factor X or Stuart-Prower Factor named after the male patient named Stuart in 1957 and a female patient named Prower with FX deficiency [45, 46]. Moreover, Factor X has long been known to have a key role in hemostasis and plays a central part in the blood-clotting cascade by catalyzing the production of thrombin, which leads to clot formation and wound closure [33, 47].

An ideal anticoagulant would prevent thrombosis without inducing systemic hypocoagulation, and would thereby prevent undesired bleeding complications. Thus, a factor Xa inhibitor could potentially have the properties of a desirable anticoagulant. In the search for new drugs, anticoagulant serine protease activated factor Xa is a particularly promising target and has attracted a strong interest in the last 5 years.

FXa plays an important role in first and secondary hemostasis. It produces the core catalyzing reaction that results in thrombin enzyme formation by means of the blood coagulation cascade which results in clot formation and wound closure [33, 47]. Moreover, FXa was found to play a central role in the coagulation process leading to hemostasis in the original extrinsic/intrinsic model [33] as well as in the newly proposed cell-based model. Factor X can be activated through either the intrinsic or extrinsic pathway. Initiation of both pathways activates the inactive precursor FX to FXa. Considering that one molecule of FXa catalyzes the formation of 1000 thrombin molecules, this amplification step can be substantial. Moreover, both pathways lead to the propagation and amplification of coagulation through the activation of FX.

The perfect antithrombotic agent would not induce systemic hypocoagulation and thus provides equilibrium between clot formation and secondary problems such as bleeding. The investigation into finding new anticoagulant agents reveals that serine protease FXa is an important validated pharmaceutical achievement whose use has grown remarkably since the beginning of the twenty-first century [42–44]. Thus, an FXa inhibitor combined with an antiplatelet moiety could possibly provide the features of an effective drug, thus preventing the platelet aggregation during the hemostasis process, avoiding the thrombus formation and inhibiting the catalyzing FXa reaction [33, 48–53].

As explained above, FXa performs a crucial function in the coagulation process. Thus, FXa provides a specific target for novel anticoagulant agents. The synthesis of direct FXa inhibitors that are able to effectively inhibit prothrombinase-associated and clot-bound FXa, and therefore provide greater potential anticoagulant activity, is therefore a significantly important advance. There is enough evidence to imply that inhibition earlier during primary hemostasis in the coagulation cascade at the FXa level could provide higher antithrombotic potential by using inhibition of platelet adhesion drugs. Furthermore, preclinical studies indicate that FXa inhibitors possibly possess a broader therapeutic index. Therefore, there is a significant number of pharmaceutical companies, which are working to discover new anticoagulant drugs, and have finally decided to focus on small molecules such as direct FXa inhibitors [54–59].

FXa is a serine protease which catalyzes the production of 1000 thrombin molecules involving the interaction on the platelets surface, Ca^{2+} ions, and FVa called the prothrombinase complex. The prothrombinase complex acts on the natural substrate producing the catalytic coagulation process.

Structurally FXa, like trypsin, belongs among the family of serine proteases within the catalytic domain, which is formed by two antiparallel β -barrel folds that act in tandem to produce the catalytic triad and the substrate binding site. Schechter and Berger (**Figure 6**) have provided a nomenclature adopted by scientists, which describes the prototypical binding site of a serine protease. Consequently, each protein subsite, labeled S_i , binds its related amino acid substrate, labeled P_i [60].

As the discovery of small-molecule protease inhibitors has progressed, this convention has been amplified to denote drug substructures that similarly bind to substrate amino acids (**Figure 6**) [54].

In the 1980s, early attempts to identify FXa inhibitors were prompted by prior thrombin inhibitor discoveries such as the compounds illustrated in **Figure 7 (a and b)**, which are examples of early [61] and most recent direct FXa inhibitors rivaroxaban (**Figure 7c**) and apixaban (**Figure 7d**) chemical structures.

Development of rivaroxaban was a major breakthrough in anticoagulation drug discovery and was the first approved orally active direct FXa inhibitor. However, recently studies have shown that rivaroxaban and apixaban discontinuation could result in thromboembolic events, and the use of rivaroxaban associated with warfarin increases the risk of major bleeding in non-valvular atrial fibrillation patients [62–64]. Through the study of the chemical structures of these inhibitors illustrated below, it also became evident that various substitutes could be accepted in both the S1 and S4 regions [45, 65–73].

In spite of extensive knowledge about the clotting mechanism, its complexity poses a considerable challenge to the research and development of powerful anticoagulants that are both safe and effective.

3.1. FXa structural target points

As it was exposed before, FXa plays a critical role in coagulation. Together with FVa and calcium ions on a phospholipid surface, FXa forms the prothrombinase complex, which is responsible for the conversion of prothrombin to thrombin, the final effector of coagulation (**Figure 1**).

Oral anticoagulant drug discovery efforts initially focused on the development of small-molecule anticoagulants that target thrombin directly—the oral DTIs. But, there is some evidence to suggest that inhibition earlier in the coagulation cascade at the level of FXa may have greater antithrombotic potential. In addition preclinical studies suggest that FXa inhibitors may possess a wider therapeutic index than DTIs. Thus, it is understandable that a great number of pharmaceutical companies dedicated to the discovery of this oral anticoagulant drug have finally and determinedly concentrated on small-molecule, direct FXa inhibitors [33, 56, 57, 74–78].

It is worth considering briefly some of the significant molecular characteristics of the target protein. FXa belongs to the family of serine proteases such as trypsin; the catalytic domain consists of two antiparallel β -barrel folds that together form the catalytic triad and the substrate binding site. Accordingly to the Schetcher and Berger nomenclature each protein subsite (Si) binds the amino acid (Pi) residue [79]. Specifically, FXa is composed by four principal subsites S1, S2, S3, and S4.

S1 is an anionic pocket—hydrophobic and deep cleft—formed by Tyr228, Ser195, and Asp189; and S4 subsite has three domains to link with the ligand: one hydrophobic pocket defined by Tyr99, Trp215, and Phe174, one cationic hole formed by Glu97 and Lys96, and a water pocket where the natural substrate is trapped under the following amino acids: Thr98, Ile175, and

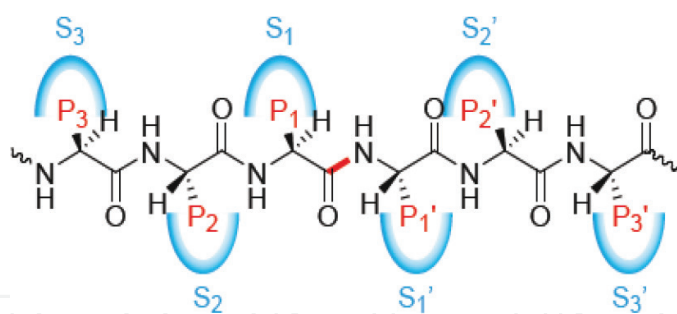


Figure 6. Nomenclature based on the Schechter and Berger convention. Figure adapted from Berg et al [67].

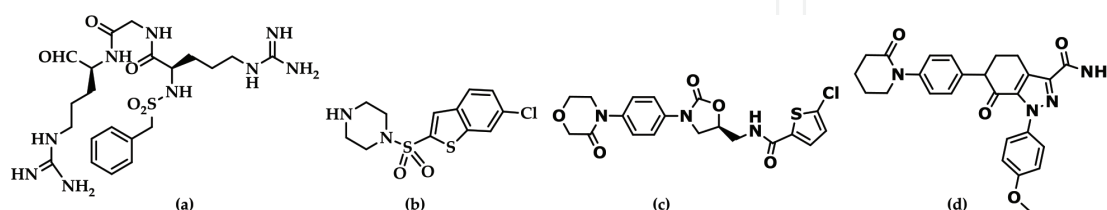


Figure 7. Early and more recent direct FXa inhibitors.

Tyr175. Besides, the S2 subsite is not well defined, has a slightly profound pocket, and is fused with the S4 subsite (Figure 8). Finally, the S3 pocket is exposed to the solvent, and it is situated at the borderline of the S1 subsite region [78, 80].

All reported data indicates that small-molecule serine protease inhibitors bind one or more of the subsites. Figure 5 shows the most important serine protease subsites responsible for the design molecules recognition and binding characteristics.

In view of the small size of these synthetic inhibitors, they allow inhibiting both bound prothrombinase and free FXa. In addition, these drugs are able to penetrate the blood clot and inhibit FXa [61, 81, 82].

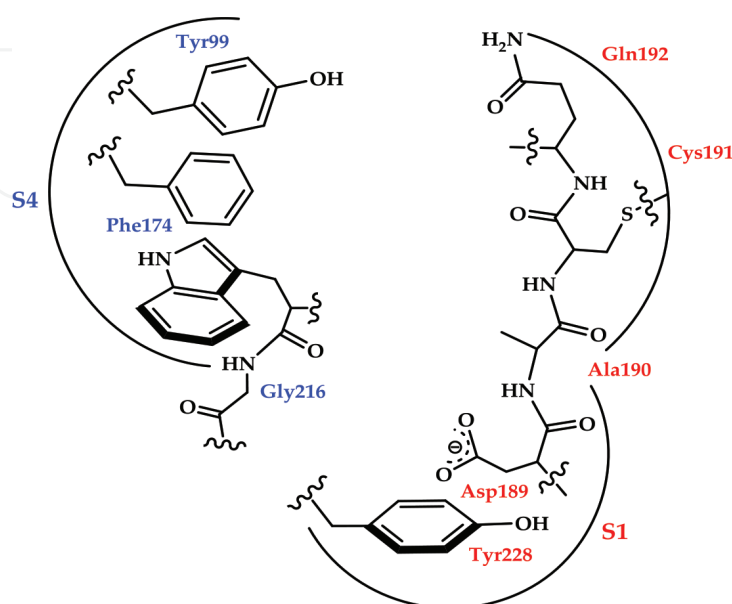


Figure 8. Most important amino acid residues in S1 and S4 pockets.

4. Direct FXa inhibitors development

In spite of the fact that as early as the beginnings of the 1980s, the factor Xa had already been recognized as an auspicious target for the development of new anticoagulants, its viability inhibition was not tested until the late 1980s. It was in 1987 that the first factor Xa inhibitor—naturally occurring compound antistatin—was extracted from the salivary glands of the Mexican leech *Haementeria officinalis*. Antistatin is a 119 amino-acid polypeptide; kinetic studies revealed that it is a slow, tight-binding, potent factor Xa inhibitor [83–85]. Similar properties show another factor Xa inhibitor—the tick anticoagulant peptide (tAP) [86]. This anticoagulant peptide is a single-chain, amino-acid peptide which was isolated in 1990 from extracts of the soft tick *Ornithodoros moubata*. The antithrombotic effects of these compounds were compared with those of direct thrombin inhibitors, and of indirect thrombin and factor Xa inhibitors in animal models of thrombosis.

The discovery of FXa's role in the clotting cascade produced an increasing interest in this enzyme due to its pharmacological target for the treatment of a diverse number of hemostatic pathologies.

The first FXa inhibitory studies were carried by using natural anticoagulants obtained from ticks (antistatin), leeches (Yagin), and bats (Draculin). These natural anticoagulant proteins had indirect activity on FXa. Antistatin produce a slow-release FXa-complex which reduces the cascade amplification. Besides, Draculin directly inhibits FXa without activity on thrombin [87].

Several investigation groups started designing and synthesizing novel small molecules for the treatment of thrombotic-related pathologies such as deep vein thrombus (DVT), acute coronary syndrome (ACS), stroke, as well as for the prevention of clot production during surgeries. Moreover, pharmaceuticals companies have been financing strongly in the research and development of new synthetic oral FXa inhibitors.

For example, fondaparinux (**Figure 5c**), is selective for FXa but acts indirectly via binding to antithrombin and has demonstrated similar clinical benefit over LMWHs in venous thrombotic indications. The safety and efficacy of one provided the first clinical proof of the principle that targeting FXa would be an important advancement in the area of anticoagulation therapy [79, 88–91].

A second generation of synthetic derivatives, idrabiotaparinux, is in late-stage clinical trials for treatment of VTE and for stroke prevention in patients with AF [92]. Early efforts to identify inhibitors of FXa stemmed from the prior discoveries of thrombin inhibitors such as compounds showing in **Figure 6** are examples of early FXa inhibitors. Because of the success of indirect dual factor Xa and thrombin inhibitors, such as LMWHs, indirect inhibitors of factor Xa with greater selectivity, such as fondaparinux, were developed in parallel with oral direct factor Xa inhibitors, such as rivaroxaban [93] (**Figure 7c**) and apixaban [94] (**Figure 7d**).

These last two small molecules have been demonstrated to have the best pharmacokinetics characteristics, and they have a fully complete preclinical characterization as an oral direct FXa inhibitor. Furthermore, rivaroxaban was approved in Canada, Europe, and other countries for the prevention of VTE in adults undergoing hip and knee surgery, and it has a predictable anticoagulant response avoiding the need for monitoring. Moreover, apixaban was

approved by the Food and Drug Administration (FDA) in December 2012 with an indication of reducing the risk of stroke and dangerous blood clots (systemic embolism) in patients with atrial fibrillation (AF) [95–98].

Based on these discoveries, in the mid-1990s, it was assumed that small-molecule, direct factor Xa inhibitors could most likely become a better option than the antithrombotic therapies used in those days [99].

4.1. Direct synthetic FXa inhibitors

Indirect FXa inhibitor development led to the advance of direct oral FXa inhibitors, such as rivaroxaban [100] (**Figure 7c**) and apixaban [98] (**Figure 7d**). Interestingly, the “Xa” suffix comes from FXa and “ban” indicating inhibition [101].

These latest FXa direct oral inhibitors comprised a group of small molecules. Taking into account the chronological order, these new direct oral anticoagulants (DOACs) are razaxaban, rivaroxaban, apixaban, darexaban, edoxaban, and betrixaban; however, some of them did not obtain the approval during clinical trials and they are not commercialized [48].

4.1.1. Razaxaban

This novel FXa synthetic and orally active compound was developed by Bristol Myers Squibb in 2004. The production of razaxaban was carried out through a seven synthetic pathway (**Figure 9**) [56]. It acts as a selective and reversible direct FXa inhibitor which was the first synthetic direct FXa inhibitor developed. Moreover, razaxaban has demonstrated FXa selectivity in venous thrombosis in human beings and arterial thrombosis prevention in animal models [94].

The razaxaban structural L shape allowed it to fit within the FXa S1 pocket where the nitrogen atom of the benzoisoxazole moiety interacts with Ala190 and Asp189 (**Figure 10**) [48, 100]. Moreover, the development was discontinued in Phase II at the end of 2004.

4.1.2. Rivaroxaban

Rivaroxaban (Xarelto®), was the first direct oral FXa inhibitor developed by Bayer Schering Pharma AG and it obtained the clinical approved in 2008 [102]. At the beginning, the production of rivaroxaban was carried out through a nine synthetic pathway [68]. In recent years, a

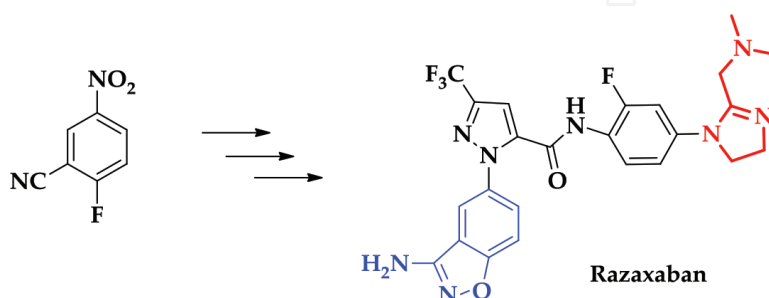


Figure 9. Chemical structures: Commercial starting material and Razaxaban. The moiety that interacts with S1, is shown in blue, and the portion involved in the S4 interaction is shown in red [50].

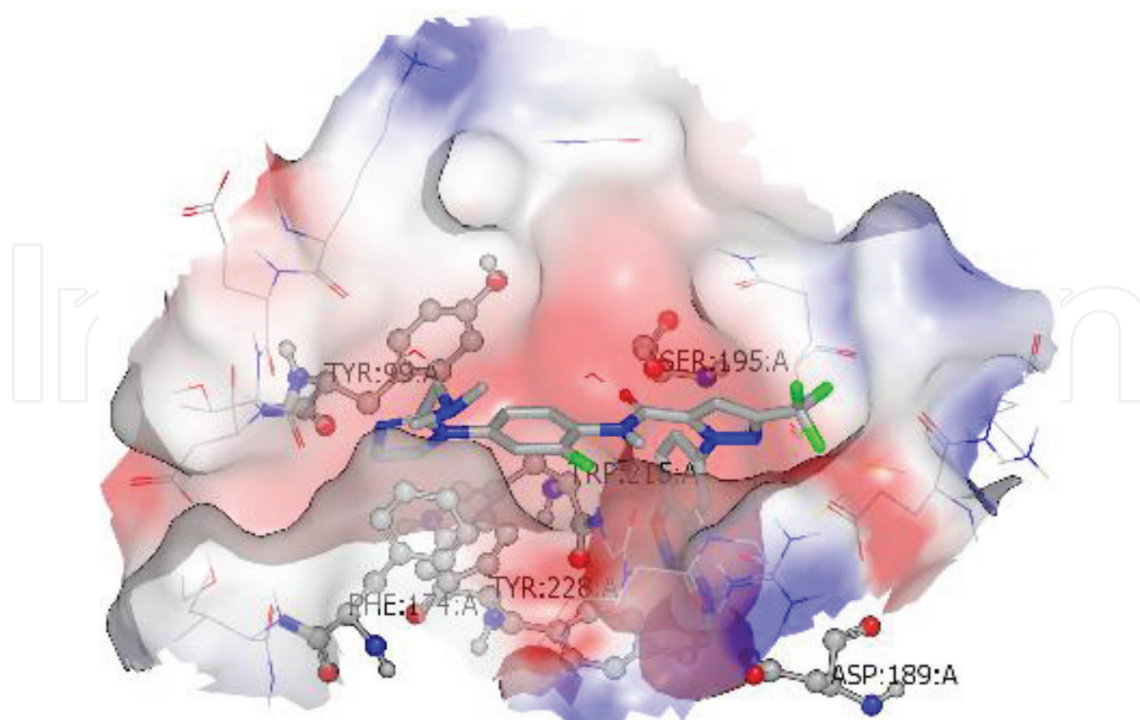


Figure 10. Razaxaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.

novel synthetic route was design by using only a seven-step procedure diminishing the environmental impact and increasing the reaction yields (**Figure 11**) [103]. This novel DOAC is a selective and reversible FXa inhibitor which shows 100-fold greater selectivity for FXa over any other serine protease. Rivaroxaban inhibits the complex between FXa and prothrombinase with an IC_{50} 2.1 nM; moreover, it shows nanomolar inhibitory constant [$K_i = 0.4$ nM] [33, 93, 104].

As it is shown in **Figure 12**, the two-ringed moiety, including the morpholinone and benzenic moieties, produced S4 hydrophobic interactions with Phe174 and Tyr99 residues. Moreover, the oxazolidone ring interacts with Gly219 through hydrogen bonds and the chlorothiophene moiety produces necessary interactions with Asp189, Ala190 and Tyr228 in the profound S1 site (**Figure 12**) [33, 68, 82].

This first commercially available DOAC doesn't show food interactions and it is prescribed as one dose-per-day drug after heart attack or stroke [105, 106]. Furthermore, rivaroxaban was approved for prophylaxis after knee or hip surgery [96].

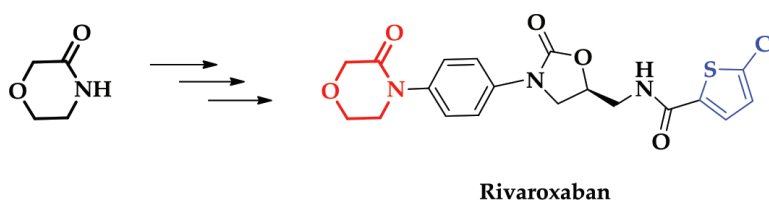


Figure 11. Chemical structures: Commercial starting material and rivaroxaban. The moiety that interacts with S1 is shown in blue and the portion involved in the S4 interaction is shown in red [594].

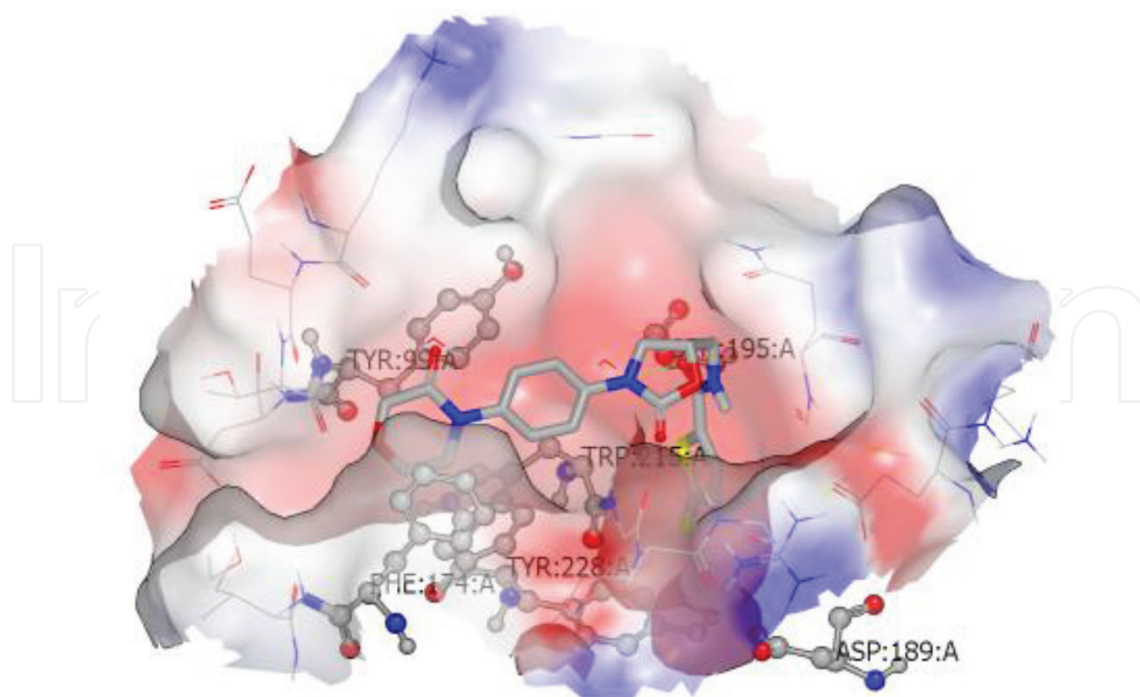


Figure 12. Rivaroxaban bound to FXa (PDB ID 2w26) [68]. The binding site is shown in surface mode. Specificity sites and important residues are labeled.

4.1.3. Apixaban

Apixaban (Eliquis®) was the second FXa oral inhibitor approved by the European Medicines Agency (EMA) in 2011 and by the Food and Drug Administration (FDA) in 2012 [98]. This novel molecule is a design evolution of razaxaban, and it was developed by Bristol Myers Squibb [53]. Moreover, apixaban is a reversible and selective FXa inhibitor which is prescribed in thromboembolic prophylaxis events such as preventing thrombus production and strokes in persons with atrial fibrillation. Furthermore, apixaban is prescribed to prevent blood clots in deep vein thrombosis (DVT) and pulmonary embolus formation according to the United States regulations [52, 107, 108].

Currently, apixaban has FXa inhibitory activity showing a $K_i = 0.08$ nM with 50% oral bio-availability [104, 107]. In addition, its action showed selectivity for clot-bound (IC_{50} 1.3 nM) vs. free FXa (IC_{50} 7.6 nM) [107]. Besides, this DOAC induces hepatotoxicity as its adverse effect (**Figure 13**) [109].

The characteristic FXa inhibitors L shape is produced by the peptide bond present between the two ring pyrazole linked to a phenyl piperidinone (**Figure 14**). Apixaban shows the same interactions than Rivaroxaban in the S1 pocket by using the methoxyphenyl portion at the bottom of the S1 pocket [100].

4.1.4. Darexaban

Darexaban was designed by Astellas Pharma in 2007 for venous and arterial thromboembolic disease prophylaxis such as venous thrombosis, myocardial infarction, and ischemic stroke [110].

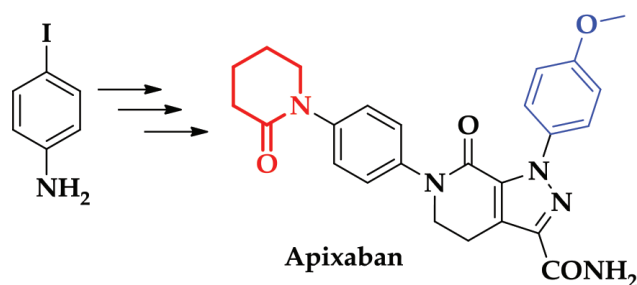


Figure 13. Chemical structures: Commercial starting material and apixaban. The moiety that interacts with S1 is shown in blue, and the portion involved in the S4 interaction is shown in red [53].

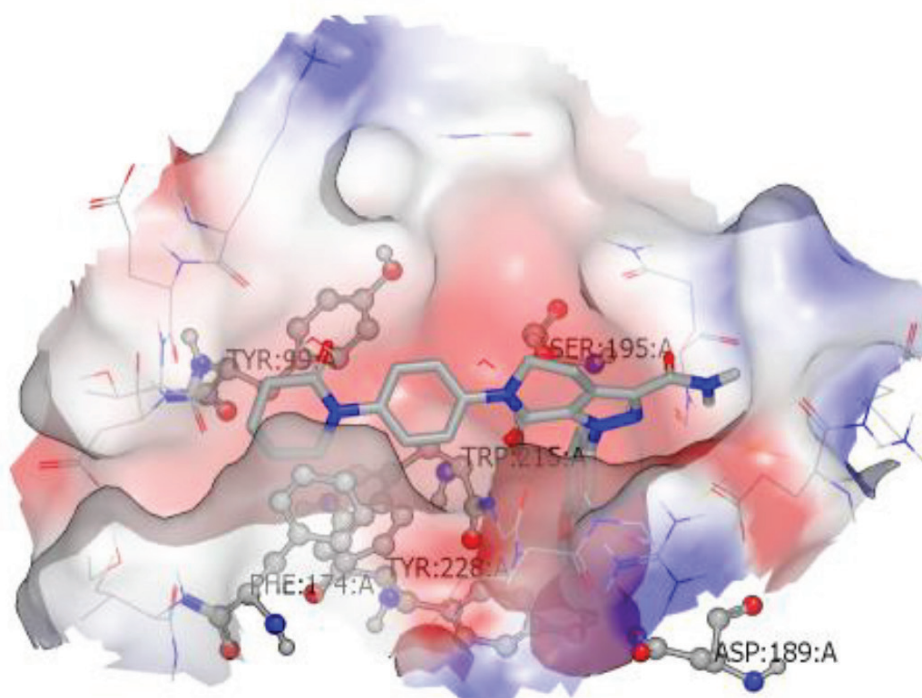


Figure 14. Apixaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.

It showed an inhibition constant K_i 0.031 μM and IC_{50} 40 nM for free FXa and IC_{50} 80 nM for blood clots [111]. However, darexaban development was discontinued in September 2011, after a phase II in Australia, Canada, and the European Union (EU) because the clinical trial showed that the combination of darexaban with an antiplatelet agent such as acetyl salicylic acid (ASA) caused a fourfold increase in bleeding rates and had no effect on acute coronary syndrome (ACS) (**Figure 15**) [112].

Darexaban establishes the same interactions with Asp189, Ala190, and Tyr228 in the S1 pocket as other DOACs (**Figure 16**) [100].

4.1.5. Edoxaban

Edoxaban (Savaysa[®] in USA and Lixiana[®] in Canada and outside the USA) was developed by Daiichi Sankyo and it was approved in Japan (2011) and by the FDA (2015) [71, 113]. This

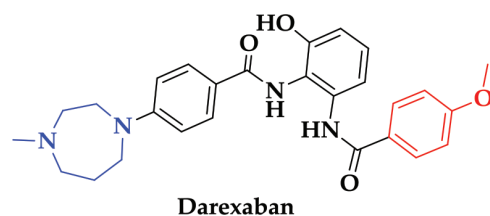


Figure 15. Darexaban chemical structure. The moiety that interacts with S1 is shown in blue, and the portion involved in the S4 interaction is shown in red.

novel DOAC is used for stroke and VTE prophylaxis in patients with atrial fibrillation [114]. Edoxaban was synthesized through a twelve-step procedure (**Figure 17**) [113].

Edoxaban bioavailability is 62%, and it is prescribed at 15–150 mg daily. It has been shown a nanomolar value for its K_i (0.56 nM) and IC_{50} (3 nM) [115–117]. Currently, Daiichi Sankyo is developing a phase III trial for cardiovascular disorders during February 2018.

This DOAC interacts with the same amino acidic residues in the S1 serine enzyme pocket as the other FXa direct inhibitors (**Figure 18**). The chloropyridine moiety is responsible for the S1 pocket interaction meanwhile the tetrahydrothiazolo-pyridine moiety interacts with the S4 pocket [100].

4.1.6. Betrixaban

Betrixaban (Bevyxxa[®]) is the newest DOAC developed by Portola Pharmaceuticals and it was designed through structure activity relationship (SAR) studies [80, 118]. It was approved by the FDA in June 2017 for prevention of venous thromboembolism in acute hospitalized medical adult patients by using an initial single dose of 80 mg (**Figure 19**) [119].

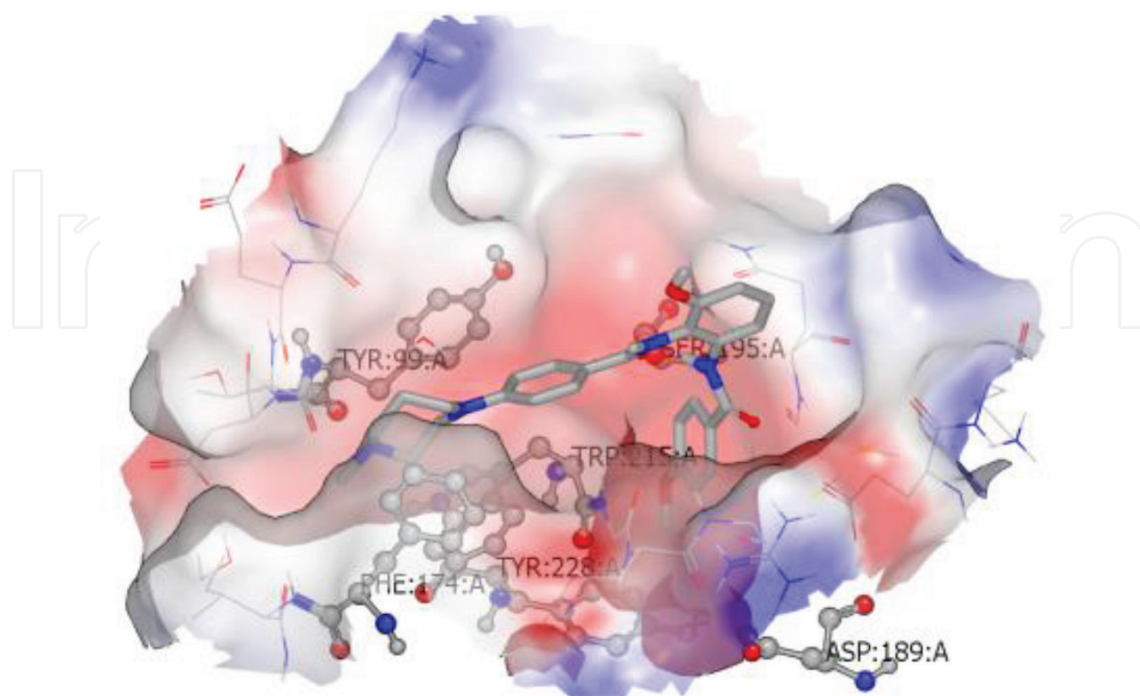


Figure 16. Darexaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.

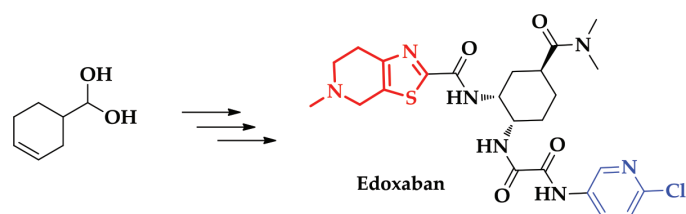


Figure 17. Chemical structures: commercial starting material and Edoxaban. The moiety that interacts with S1 is shown in blue, and the portion involved in the S4 interaction is shown in red [113].

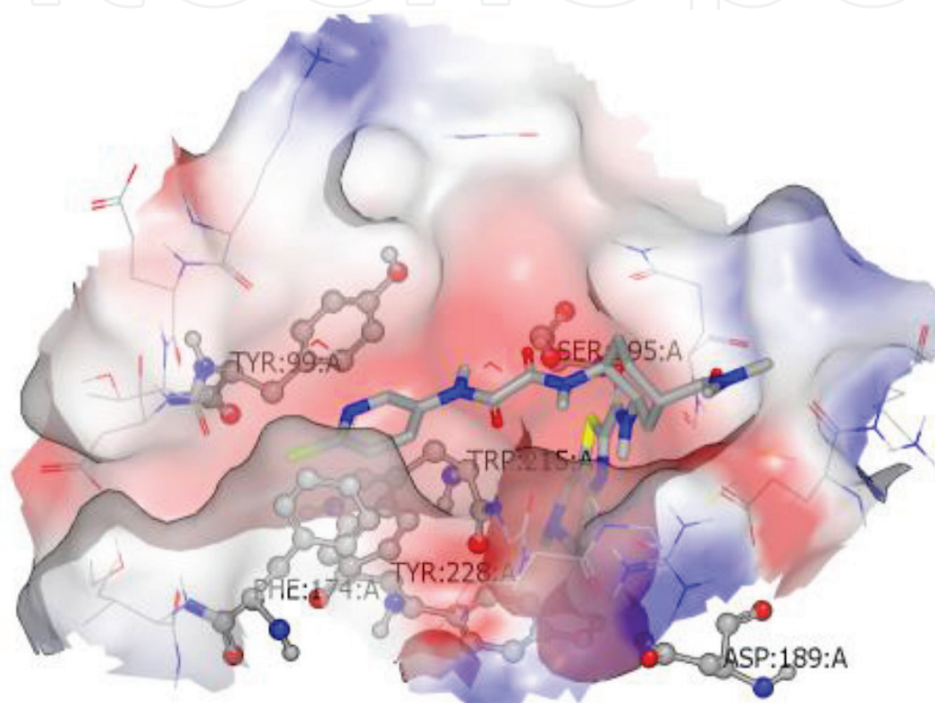


Figure 18. Edoxaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.

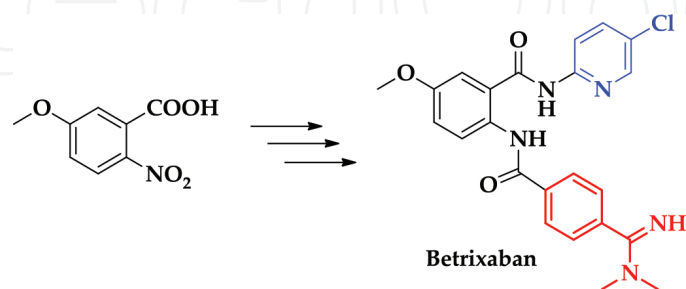


Figure 19. Chemical structures: commercial starting material and Betrixaban. The moiety that interacts with S1 is shown in blue, and the portion involved in the S4 interaction is shown in red.

This new DOAC is a competitive and reversible FXa inhibitor and it has a K_i 0.117 pM and IC_{50} 1.5 nM [119]. Betrixaban may therefore have several potential advantages over the other FXa inhibitors (**Figure 20**).

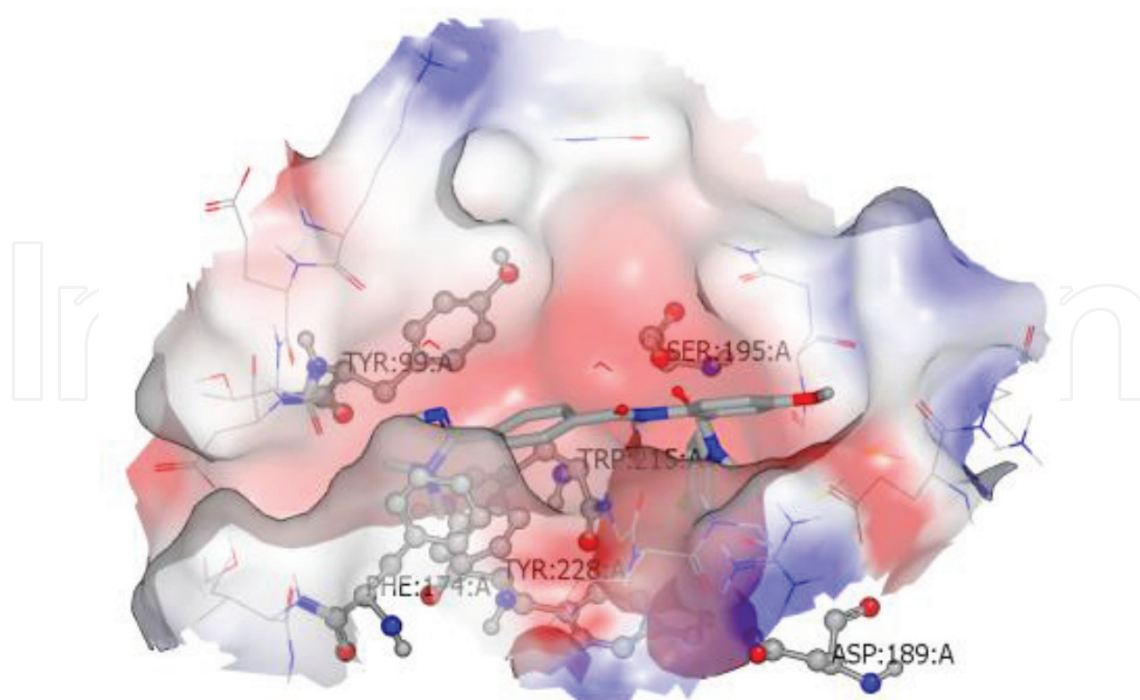


Figure 20. Betrixaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.

5. Conclusions

A perfect anticoagulant would prevent thrombosis without inducing systemic hypocoagulation, and would prevent undesired bleeding complications. Thus, an FXa inhibitor could potentially have the properties of a desirable anticoagulant. In the search for new anticoagulant drugs, the serine protease FXa is a particularly promising target and has attracted a strong interest over the last 15 years.

Development of DOACs such as direct FXa inhibitors is an important innovation in the anticoagulation drug discovery field. In spite of widespread knowledge about the clotting mechanism, its complexity generates significant challenge for the investigation and production of innovative anticoagulants that are both efficient and safe. DOACs availability embraces a vast field of the clinical health system for prevention of diverse pathologies. Currently, with the development and approval of DOACs such as FXa inhibitors, clinical health professionals can use these novel therapeutics approaches.

The continuous and future development of an innovative oral anticoagulant drug that is designed to prevent several thrombotic disorders and related pathologies would be of remarkable wide-reaching health value.

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Conflict of interest

The author declares no conflict of interest.

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References

- [1] Austin S. Haemostasis. *Medicine*. 2013;**41**:208-211
- [2] Pérez-Gómez F, Bover R. La nueva cascada de la coagulación y su posible influencia en el difícil equilibrio entre trombosis y hemorragia. *Revista Española de Cardiología* 2007;**60**:1217-1219
- [3] Riddel J, Aouizerat B, Miaskowski C, Lillicrap D. Theories of blood coagulation. *Journal of Pediatric Oncology Nursing*. 2007;**24**:123-131
- [4] Smith S. The cell-based model of coagulation. *Journal of Veterinary Emergency and Critical Care*. 2009;**19**:3-10
- [5] Hoffman M, Monroe D III. A cell-based model of hemostasis. *Thrombosis and Haemostasis*. 2001;**85**:958-965
- [6] Hoffman M, Monroe D. Rethinking the coagulation cascade. *Current Hematology Reports*. 2005;**4**:391-396
- [7] Palta S, Saroa R. Overview of the coagulation system. *Indian Journal of Anaesthesia*. 2014;**58**:515-523
- [8] Vojacek J. Should we replace the terms intrinsic and extrinsic coagulation pathways with tissue factor pathway? *Clinical and Applied Thrombosis/Hemostasis*. 2016;**1**:1-6

- [9] Principales causas de muerte en Chile por regiones 1997-2003. Instituto Nacional de Estadísticas; 2006
- [10] Feigin V, Lawes C, Bennett D, Anderson C. Stroke epidemiology: a review of population based studies of incidence, prevalence and case fatality in the late 20th century. *The Lancet Neurology*. 2003;**2**:43-53
- [11] Estrategia Nacional de Salud. 2011-2020. Ministerio de Salud. Gobierno de Chile
- [12] Protocolo prevención enfermedad tromboembólica en pacientes quirúrgicos. Dirección Servicio de Salud Metropolitano Oriente. Ministerio de Salud de Chile; 2010
- [13] Raskob G, Angchaisuksiri P, Blanco A, Buller H, Gallus A, Hunt B, Hylek E, Kakkar A, Konstantinides S, McCumber M, Ozaki Y, Wendelboe A, Weitz J. A major contributor to global disease burden. ISTH steering Committee for World Thrombosis day. *Journal of Thrombosis and Haemostasis*. 2014;**12**:1580-1590
- [14] Gustafsson D, Bylund R, Antonsson T, Nilsson I, Nyström J-E, Eriksson U, Bredberg U, Teger-Nilsson A-C. A new oral anticoagulant: the 50-year challenge. *Nature Reviews Drug Discovery*. 2004;**3**:549-659
- [15] Wolf P, Abbott R, Kannel W. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. *Stroke*. 1991;**22**:983-988
- [16] Flisfisch H, Aguiló J, Lillo Cuevas D. Trombosis venosa profunda. *Revista Medicina y Humanidades*. 2014;**VI**:46-50
- [17] Bombin F, Kotlik A, Díaz G, Vera O, Contreras T, Vásquez Z. Secuelas de la trombosis venosa profunda de las extremidades inferiores luego de un tratamiento anticoagulante controlado. *Revista Chilena de Cirugía*. 2005;**57**:311-319
- [18] Palomo G, Pereira G, Alarcón L, Pinochet P, Vélez S, Hidalgo P, Skagerberg K, Poblete CF. Factor V Leiden y mutación de la protrombina G20210A en pacientes con trombosis venosa y arterial. *Revista Médica De Chile*. 2005;**133**:1425-1433
- [19] Wolf P, Mitchell J, Baker C, Kannel W, D'Agostino R. Impact of atrial fibrillation on mortality, stroke, and medical costs. *Archives of Internal Medicine*. 1998;**158**:229-234
- [20] Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. *Harrison's Principles of Internal Medicine*, 1 and 2. 18th ed. New York: McGraw-Hill Professional; 2011
- [21] Abraham D, editor. *Burger's Medicinal Chemistry and Drug Discovery. Cardiovascular Agents and Endocrines*. 6th ed. Vol. 3. New Jersey: Wiley-Interscience; 2003
- [22] Lee M, Smith S, Galor A, Hoffman G. Antiplatelet and anticoagulant therapy in patients with giant cell arteritis. *Arthritis and Rheumatism*. 2006;**54**:3306-3309
- [23] Rao P, Burkart T. Advances in oral anticoagulation therapy – What's in the pipeline? *Blood Reviews*. 2017;**31**:205-211
- [24] Oh J, Smiddy W, Kim S. Antiplatelet and anticoagulation therapy in vitreoretinal surgery. *The American Journal of Ophthalmology*. 2011;**151**:934-939

- [25] Cohen A, Imfeld S, Markham J, Granziera S. The use of aspirin for primary and secondary prevention in venous thromboembolism and other cardiovascular disorders *Thrombosis Research*. 2015;**135**:217-225
- [26] Koenig-Oberhuber V, Filipovic M. New antiplatelet drugs and new oral anticoagulants. *British Journal of Anaesthesia*. 2016;**117**:ii74-ii84
- [27] Aizman A, Abbott E, Rojas L. Profilaxis de enfermedad tromboembólica en pacientes hospitalizados con patología médica, estrechando la brecha entre las guías y la práctica clínica. *Revista Médica de Chile*. 2011;**139**:1210-1217
- [28] Sobieraj-Teague M, O'Donnell M, Eikelboom J. New anticoagulants for atrial fibrillation. *Seminars in Thrombosis and Hemostasis*. 2009;**35**:515-524
- [29] Gómez-Outes A, Suárez-Gea M, Lecumberri R, Rocha E, Pozo-Hernández C, Vargas-Castrillón E. Nuevos anticoagulantes parenterales en desarrollo clínico. *Actualidad en Farmacología y Terapéutica*. 2011;**9**:167-181
- [30] Weits JI. Emerging anticoagulants for the treatment of venous thromboembolism. *Thrombosis and Haemostasis*. 2006;**96**:274-284
- [31] Side effects of commercial antithrombotic agents. <https://www.drugs.com/sfx/> [Accessed: July 12, 2017]
- [32] Dimitropoulos G, Rahim S, Moss A, Lip G. New anticoagulants for venous thromboembolism and atrial fibrillation: what the future holds. 2018;**27**:71-86
- [33] Perzborn E, Roehrig S, Straub A, Kubitza D, Misselwitz F. The discovery and development of rivaroxaban, an oral, direct factor Xa inhibitor. *Nature Reviews Drug Discovery*. 2011;**10**:61-75
- [34] Eriksson BI, Dahl OE, Lassen MR, Ward DP, Rothlein R, Davis G, Turpie AGG. Partial factor IXa inhibition with TTP889 for prevention of venous thromboembolism: An exploratory study. *Journal of Thrombosis and Haemostasis*. 2008;**6**:457-463
- [35] Goto S. Factor XIa as a possible new target of antithrombotic therapy. *Journal of Thrombosis and Haemostasis*. 2006;**4**:1494-1495
- [36] Schumacher WE. Antithrombotic and hemostatic effects of a small molecule factor XIa inhibitor in rats. *European Journal of Pharmacology*. 2007;**570**:167-174
- [37] Kearon C. Dose-response study of recombinant human soluble thrombomodulin (ART-123) in the prevention of venous thromboembolism after total hip replacement. *Journal of Thrombosis and Haemostasis*. 2005;**3**:962-968
- [38] Koller F. History of factor X. *Thrombosis Et Diathesis Haemorrhagica*. 1960;**4**:58-65
- [39] Brown DL, Kouides PA. Diagnosis and treatment of inherited factor X deficiency. *Haemophilia*. 2008;**14**:1176-1182
- [40] Telfer TP, Denson KW, Wright DR. A "new" coagulation defect. *British Journal of Haematology*. 1956;**2**:308-316

- [41] Butenas S, van't Veer C, Mann KG. "Normal" thrombin generation. *Blood*. 1999;**94**:2169-2178
- [42] Mann G. Thrombin formation. *Chest*. 2003;**124**:S4-S10
- [43] Di Cera E. Thrombin interactions. *Chest*. 2003;**124**:S11-S17
- [44] Mann KG, Brummel K, Butenas S. What is all that thrombin for? *Journal of Thrombosis and Haemostasis*. 2003;**1**:1504-1514
- [45] Komoriya S, Kobayashi S, Osanai K, Yoshino T, Nagata T, Haginoya N, Nakamoto Y, Mochizuki A, Nagahara T, Suzuki M, Shimada T, Watanabe K, Isobe Y, Furugoori T. *Bioorganic & Medicinal Chemistry*. 2006;**14**:1309-1330
- [46] Medscape. Factor X deficiency. <https://emedicine.medscape.com/article/209867-overview#a4> [Accessed: January 25, 2018]
- [47] Ansell J. Factor Xa or thrombin: Is factor Xa a better target? *Journal of Thrombosis and Haemostasis*. 2007;**5**:60-64
- [48] Perzborn E. Factor Xa inhibitors-new anticoagulants for secondary haemostasis. *Hämostaseologie*. 2009;**29**:260-267
- [49] Beynon R, Bond J, editors. *Proteolytic Enzymes*. 2nd ed. New York: Oxford University Press; 2001
- [50] Quan M, Lam P, Han Q, Pinto D, He M, Li R, Ellis C, Clark C, Teleha C, Sun J, Alexander R, Bai S, Luetgen J, Knabb R, Wong P, Wexler R. Discovery of 1-(3'-aminobenzisoxazol-5'-yl)-3-trifluoromethyl-N-[2-fluoro-4-[(2'-dimethylaminomethyl)imidazol-1-yl]phenyl]-1H-pyrazole-5-carboxamide hydrochloride (razaxaban), a highly potent, selective, and orally bioavailable factor Xa inhibitor. *Journal of Medicinal Chemistry*. 2005;**48**:1729-1744
- [51] Nagar S, Argikar U, Tweedie D, editors. *Enzyme Kinetics in Drug Metabolism. Fundamentals and Applications*. New York: Humana Press/Springer; 2014
- [52] Hanna M, Mohan P, Knabb R, Gupta E, Frost C, Lawrence J. Development of Apixaban: A novel anticoagulant for prevention of stroke in patients with atrial fibrillation. *Annals of the New York Academy of Sciences*. 2014;**1329**:93-106
- [53] Jiang J, Ji Y. Alternate synthesis of Apixaban (BMS-562247), an inhibitor of blood coagulation factor Xa. *Synthetic Communications*. 2013;**43**:72-79
- [54] Meyer S, Vanhoorelbeke K, Ulrichs H, Staelens S, Feys H, Salles I, Fontayne A, Deckmyn H. Development of Monoclonal Antibodies that Inhibit Platelet Adhesion or Aggregation as Potential Anti-Thrombotic Drugs *Cardiovascular & Hematological Disorders - Drug Targets*. 2006;**6**:191-207
- [55] Vanhoorelbeke K, Ulrichs H, Van de Walle G, Fontayne A, Deckmyn H. Inhibition of Platelet Glycoprotein Ib and Its Antithrombotic Potential. *Current Pharmaceutical Design*. 2007;**13**:2684-2697

- [56] Rezaie R. Prothrombin protects factor Xa in the prothrombinase complex from inhibition by the heparin-antithrombin complex. *Blood*. 2001;**97**:2308-2313
- [57] Desai U. New antithrombin-based anticoagulants. *Medicinal Research Reviews*. 2004;**24**:151-181
- [58] Undas A, Brummel-Ziedins K, Mann K. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. *Blood*. 2007;**109**:2285-2292
- [59] Assessing and treating disorders of primary hemostasis. *Clinical Advisor*. <https://www.clinicaladvisor.com/features/treating-disorders-primary-hemostasis/article/706749/> [Accessed March 07, 2018]
- [60] Berg J, Tymoczko J, Stryer L. *Biochemistry*. 5th Edition: International Version. New York: W. H. Freeman; 2002
- [61] Núñez-Navarro N, Santana F, Parra L, Zacconi F. Surfing the blood coagulation Cascade: Insight into the vital factor Xa. *Current Medicinal Chemistry*. DOI: 10.2174/0929867325666180125165340
- [62] Lip G, Pan X, Kamble S, Kawabata H, Mardekian J, Masseria C, Bruno A, Phatak H. Major bleeding risk among non-valvular atrial fibrillation patients initiated on apixaban, dabigatran, rivaroxaban or warfarin: a “real- world” observational study in the United States. *International Journal of Clinical Practice*. 2016;**70**:752-763
- [63] Vene N, Mavri A, Gubensek M, Tratar G, Cuderman T, Perme M, Blinc A. Risk of Thromboembolic Events in Patients with Non-Valvular Atrial Fibrillation After Dabigatran or Rivaroxaban Discontinuation - Data from the Ljubljana Registry. *PLoS One*. 2016;**11**:e0156943
- [64] Acanfora D, Acanfora C, Scicchitano P, Longobardi M, Furgi G, Casucci G, Lanzillo B, Dentamaro I, Zito A, Incalzi R, Ciccone M. Evidence Gaps in the Era of Non-Vitamin K Oral Anticoagulants. *Clinical Drug Investigation*. 2016;**36**:857-862
- [65] Pruitt J, Pinto D, Galembo R, Alexander R, Rossi K, Wells B, Drummond S, Bostrom L, Burdick D, Bruckner R, Chen H, Smallwood A, Wong P, Wright M, Bai S, Luetgen J, Knabb R, Lam P, Wexler R. Discovery of 1-(2-aminomethylphenyl)-3-trifluoromethyl-N-[3-fluoro-2'-(aminosulfonyl)[1,1'-biphenyl]-4-yl]-1H-pyrazole-5-carboxamide (DPC602), a potent, selective, and orally bioavailable factor Xa inhibitor(1). *Journal of Medicinal Chemistry*. 2003;**46**:5298-5315
- [66] Mederski W, Cezanne B, van Amsterdam C, Buhning K-U, Dorsch D, Gleitz J, Marz J, Tsaklakidis C. Chlorothiophenecarboxamides as P1 surrogates of inhibitors of blood coagulation factor Xa. *Bioorganic & Medicinal Chemistry Letters*. 2004;**14**:5817-5822
- [67] Wang W, Yuan J, Fu X, Meng F, Zhang S, Xu W, Xu Y, Huang C. Novel Anthranilamide-Based FXa Inhibitors: Drug Design, Synthesis and Biological Evaluation. *Molecules*. 2016;**21**:491-507

- [68] Roehrig S, Straub A, Pohlmann J, Lampe T, Pernerstorfer J, Schlemmer K-H, Reinemer P, Perzborn E. Discovery of the novel antithrombotic agent 5-chloro-*N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methyl}thiophene-2-carboxamide (BAY 59-7939): An oral, direct factor Xa inhibitor. *Journal of Medicinal Chemistry*. 2005;**48**:5900-5908
- [69] Anselm L, Banner D, Benz J, Zbinden K, Himber J, Hilpert H, Huber W, Kuhn B, Mary J-L, Otteneder M, Panday N, Ricklin F, Stahl M, Thomi S, Haap W. Discovery of 1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-*c*]pyridine-3-carboxamide (Apixaban, BMS-562247), a Highly Potent, Selective, Efficacious, and Orally Bioavailable Inhibitor of Blood Coagulation Factor Xa. *Bioorganic & Medicinal Chemistry Letters*. 2010;**20**:5313-5319
- [70] Song H, Cho Y, Lee D, Park H, Baek S, Chae S, Jo S, Kim Y, Lee H, Park J, Park T, Woo S, Kim Y. 2011. US 2011/0112083 A1
- [71] Samama MM, Mendell J, Guinet C, Le Flem L, Kunitada S. In vitro study of the anticoagulant effects of edoxaban and its effect on thrombin generation in comparison to fondaparinux. *Thrombosis Research*. 2012;**129**:E77-E82
- [72] Douxfils J, Ageno W, Samama C, Lessire S, Ten Cate H, Verhamme P, Dogné J, Mullier F. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. *Journal of Thrombosis and Haemostasis*. 2018;**16**:209-219
- [73] Myerson J, He L, Lanza G, Tollefsen D, Wickline S. Thrombin-inhibiting perfluorocarbon nanoparticles provide a novel strategy for the treatment and magnetic resonance imaging of acute thrombosis. *Thrombosis and Haemostasis*. 2011;**9**:1292-1300
- [74] Merlini P, Ardissino D, Bauer K, Oltrona L, Pezzano A, Bottasso B, Rosenberg R, Mannucci P. Persistent thrombin generation during heparin therapy in patients with acute coronary syndromes. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 1977;**17**:1325-1330
- [75] Hermans C, Claeys D. Review of the rebound phenomenon in new anticoagulant treatments. *Current Medical Research and Opinion*. 2006;**22**:471-481
- [76] Weitz J, Hudoba M, Massel D, Maraganore J, Hirsh J. Clot-bound thrombin is protected from inhibition by heparin-antithrombin III independent inhibitors. *The Journal of Clinical Investigation*. 1990;**86**:385-391
- [77] Pinto D, Smallheer J, Cheney D, Knabb R, Wexler R. Factor Xa inhibitors: Next-generation antithrombotic agents. *Journal of Medicinal Chemistry*. 2010;**53**:6243-6274
- [78] Young R. The successful quest for oral factor Xa inhibitors; learnings for all of medicinal chemistry? *Bioorganic & Medicinal Chemistry Letters*. 2011;**21**:6228-6235
- [79] Bauer K, Hawkins D, Peters P, Petitou M, Herbert J-M, van Boeckel C, Meuleman D. Fondaparinux, a synthetic Pentasaccharide: The first in a new class of antithrombotic agents—The selective factor Xa inhibitors. *Cardiovascular Drug Reviews*. 2002;**20**:37-52
- [80] Zhang P, Huang W, Wang L, Bao L, Jia Z, Bauer S, Goldman E, Probst G, Song Y, Su T, Fan J, Wu Y, Li W, Woolfrey J, Sinha U, Wong P, Edwards S, Arfsten A, Clizbe L,

- Kanter J, Pandey A, Park G, Hutchaleelaha A, Lambing J, Hollenbach S, Scarborough R, Zhu B. Discovery of betrixaban (PRT054021), *N*-(5-chloropyridin-2-yl)-2-(4-(*N,N*-dimethylcarbamimidoyl)benzamido)-5-methoxybenzamide, a highly potent, selective, and orally efficacious factor Xa inhibitor. *Bioorganic & Medicinal Chemistry Letters*. 2009;**19**:2179-2185
- [81] Cabral K, Ansell J. The role of factor Xa inhibitors in venous thromboembolism treatment. *Vascular Health and Risk Management*. 2015;**11**:117-123
- [82] Samama M. The mechanism of action of rivaroxaban—an oral, direct factor Xa inhibitor—compared with other anticoagulants. *Thrombosis Research*. 2011;**127**:497-504
- [83] Nutt E, Gasic T, Rodkey J, Gasic GJ, Jacobs JW, Friedman PA, Simpson E. The amino acid sequence of antistasin. A potent inhibitor of factor Xa reveals a repeated internal structure. *The Journal of Biological Chemistry*. 1988;**263**:10162-10167
- [84] Tuszynski GP, Gasic TB, Gasic GJ. Isolation and characterization of antistasin. An inhibitor of metastasis and coagulation. *The Journal of Biological Chemistry*. 1987;**262**:9718-9723
- [85] Dunwiddie C, Thornberry NA, Bull HG, Sardana M, Friedman PA, Jacobs JW, Simpson E. Antistasin, a leech-derived inhibitor of factor Xa. Kinetic analysis of enzyme inhibition and identification of the reactive site. *The Journal of Biological Chemistry*. 1989;**264**:16694-16699
- [86] Waxman L, Smith DE, Arcuri KE, Vlasuk GP. Tick anticoagulant peptide (TAP) is a novel inhibitor of blood coagulation factor Xa. *Science*. 1990;**248**:593-596
- [87] Al-Obeidi F, Ostrem J. Factor Xa inhibitors. *Expert Opinion on Therapeutic Patents*. 1999;**9**:931-953
- [88] Nagler M, Haslauer M, Wuillemin W. Fondaparinux—Data on efficacy and safety in special situations. *Thrombosis Research*. 2012;**129**:407-417
- [89] Bauer K. Fondaparinux sodium: A selective inhibitor of factor Xa. *American Journal of Health-System Pharmacy*. 2001;**58**:14-17
- [90] Savi P, Chong B, Greinacher A. Effect of fondaparinux on platelet activation in the presence of heparin-dependent antibodies: A blinded comparative multicenter study with unfractionated heparin. *Blood*. 2005;**105**:139-144
- [91] Warkentin T, Pai M, Sheppard J. Fondaparinux treatment of acute heparin-induced thrombocytopenia confirmed by the serotonin-release assay: A 30-month, 16-patient case series. *Journal of Thrombosis and Haemostasis*. 2011;**9**:2389-2396
- [92] Harenberg J. Development of idraparinux and idrabiotaparinux for anticoagulant therapy. *Thrombosis and Haemostasis*. 2009;**102**:811-815
- [93] Kubitza D, Becka M, Mueck W, Zuehlsdorf M. Rivaroxaban (BAY 59-7939) – An oral, direct factor Xa inhibitor – Has no clinically relevant interaction with naproxen. *British Journal of Clinical Pharmacology*. 2007;**63**:469-476

- [94] Wong P, Crain E, Xin B, Wexler R, Lam P, Pinto D, Luetzgen J, Knabb R. Apixaban, an oral, direct and highly selective factor Xa inhibitor: In vitro, antithrombotic and antihe-mostatic studies. *Journal of Thrombosis and Haemostasis*. 2008;**6**:820-829
- [95] Kubitza D, Becka M, Mueck W, Zuehlsdorf M. Effects of renal impairment on the phar-macokinetics, pharmacodynamics and safety of rivaroxaban – An oral, direct factor Xa inhibitor. *British Journal of Clinical Pharmacology*. 2010;**70**:703-712
- [96] Eriksson B, Quinlan D, Weitz J. Comparative pharmacodynamics and pharmacokinet-ics of oral direct thrombin and factor Xa inhibitors in development. *Clinical Pharma-cokinetics*. 2009;**48**:1-22
- [97] Pinto D, Orwat M, Koch S, Rossi K, Alexander R, Smallwood A, Wong P, Rendina A, Luetzgen J, Knabb R, He K, Xin B, Wexler R, Lam P. Discovery of 1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyri-dine-3-carboxamide (Apixaban, BMS-562247), a highly potent, selective, efficacious, and orally bioavailable inhibitor of blood coagulation factor Xa. *Journal of Medicinal Chemistry*. 2007;**50**:5339-5356
- [98] Wong P, Pinto D, Zhang D. Preclinical discovery of apixaban, a direct and orally bio-available factor Xa inhibitor. *Journal of Thrombosis and Thrombolysis*. 2011;**31**:478-492
- [99] Collins B, Hollidge C. Antithrombotic drug market. *Nature Reviews Drug Discovery*. 2002;**2**:11-12
- [100] De Candia M, Lopopolo G, Altomare C. Novel factor Xa inhibitors: A patent review. *Expert Opinion on Therapeutic Patents*. 2009;**19**:1535-1580
- [101] Masotti L, Campanini M. Pharmacology of new oral anticoagulants: Mechanism of action, pharmacokinetics, pharmacodynamics. *The Italian Journal of Medicine*. 2013;**7**:1-7
- [102] Perzborn E, Roehrig S, Straub A, Kubitza D, Mueck W, Laux V. Rivaroxaban: A new oral factor Xa inhibitor. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2010;**30**:376-381
- [103] Yuan J, Liu K, Li L, Yuan Y, Liu X, Li Y. A novel synthesis of the oxazolidinone anti-thrombotic agent rivaroxaban. *Molecules*. 2014;**19**:14999-15004
- [104] Ufer M. Comparative efficacy and safety of the novel oral anticoagulants dabigatran, rivaroxaban and Apixaban in preclinical and clinical development. *Thrombosis and Haemostasis*. 2010;**103**:572-585
- [105] Wong K, Hu D, Oomman A, Tan R, Patel M, Singer D, Breithardt G, Mahaffey K, Becker R, Califf R, Fox K, Berkowitz S, Hacke W, Hankey G. Rivaroxaban for stroke prevention in east Asian patients from the ROCKET AF trial. *Stroke*. 2014;**45**:1739-1747
- [106] Dempfle C. Direct oral anticoagulants-pharmacology, drug interactions, and side effects. *Seminars in Hematology*. 2014;**51**:89-97
- [107] Frost C, Wang J, Nepal S, Schuster A, Barrett Y, Mosqueda-Garcia R, Reeves R, Lacreata F. Apixaban, an oral, direct factor Xa inhibitor: Single dose safety, pharmacokinetics, pharmacodynamics and food effect in healthy subjects. *British Journal of Clinical Pharmacology*. 2013;**75**:476-487

- [108] Pinto DJP, Orwat MJ, Quan ML, Han Q, Galembo RA, Amparo E, Wells B, Ellis C, He MY, Alexander RS, Rossi KA, Smallwood A, Wong PC, Luettgen JM, Rendina AR, Knabb RM, Mersinger L, Kettner C, Bai S, He K, Wexler RR, Lam PYS. 1-[3-Aminobenzisoxazol-5-yl]-3-Trifluoromethyl-6-[2-(3-(*R*)-Hydroxy-*N*-Pyrrolidinyl)methyl-[1,1]-Biphen-4-yl]-1,4,5,6-Tetrahydropyrazolo-[3,4-*C*]-Pyridin-7-one (BMS-740808) a highly potent, selective, efficacious, and orally bioavailable inhibitor O. *Bioorganic Medicinal Chemistry Letters*. 2006;**16**:4141-4147
- [109] Cordeanu M, Lambert A, Gaertner S, Nouri S, Mirea C, Alt-Tebacher M, Stephan D. Apixaban-induced hepatotoxicity. *The International Journal of Cardiology*. 2016;**204**:4-5
- [110] Hashimoto T, Suzuki K, Kihara Y, Iwatsubo T, Miyashita A, Heeringa M, Onkels H, Groenendaal D, Verheggen F, Van Marle S, Usui T. Absorption, metabolism and excretion of darexaban (YM150), a new direct factor Xa inhibitor in humans. *Xenobiotica*. 2013;**43**:534-547
- [111] Iwatsuki Y, Sato T, Moritani Y, Shigenaga T, Suzuki M, Kawasaki T, Funatsu T, Kaku S. Biochemical and pharmacological profile of darexaban, an oral direct factor Xa inhibitor. *European Journal of Pharmacology*. 2011;**673**:49-55
- [112] Steg PG, Mehta SR, Jukema JW, Lip GYH, Gibson CM, Kovar F, Kala P, Garcia-Hernandez A, Renfurm RW, Granger CB. RUBY-1: A randomized, double-blind, placebo-controlled trial of the safety and tolerability of the novel oral factor Xa inhibitor darexaban (YM150) following acute coronary syndrome. *European Heart Journal*. 2011;**32**:2541-2554
- [113] Nakamura Y, Mukesh KM, Inamdar MI. Process for the preparation of (1*S*,4*S*,5*S*)-4-bromo-6-oxabicyclo[3.2.1] Octan-7-one, U.S. patent 20150239909, August 27, 2015
- [114] Minguet J, Sims HM, Smith KH, Bramlage P. The factor Xa inhibitor edoxaban for the prevention of stroke and systemic embolism in patients with atrial fibrillation. *Expert Review of Clinical Pharmacology*. 2016;**10**:1-11
- [115] Parasrampur DA, Truitt KE. Pharmacokinetics and pharmacodynamics of edoxaban, a non-vitamin K antagonist oral anticoagulant that inhibits clotting factor Xa. *Clinical Pharmacokinetics*. 2016;**55**:641-655
- [116] Poulakos M, Walker JN, Baig U, David T. Edoxaban: A direct oral anticoagulant. *The American Journal of Health-System Pharmacy*. 2017;**74**:117-129
- [117] Lip GYH, Agnelli G. Edoxaban: A focused review of its clinical pharmacology. *European Heart Journal*. 2014;**35**:1844-1855
- [118] Thoenes M, Minguet J, Bramlage K, Bramlage P, Ferrero C. Betrixaban – The next direct factor Xa inhibitor? *Expert Review of Hematology*. 2016;**9**:1111-1117
- [119] Administration, F. & D. FDA Approved Betrixaban (BEVYXXA, Portola) for the Prophylaxis of Venous Thromboembolism (VTE) in Adult Patients. <https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm564422.htm> [Accessed September 21, 2017]

