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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].
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].

![Thrombus production on blood vessels.](http://dx.doi.org/10.5772/intechopen.76518)
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.
Several clinical trials have confirmed the efficacy of classic anticoagulants, including vitamin K antagonists (VKA), unfractionated heparin (UFH), and low molecular weight heparins (LMWH) 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 ratio.
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.

<table>
<thead>
<tr>
<th>Antithrombotic agent</th>
<th>Commercial agents disadvantages and side effects</th>
</tr>
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<tr>
<td><strong>Antiplatelet drugs</strong></td>
<td></td>
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</table>
Aspirin: gastrointestinal complaints, allergy, hepatic and renal pathologies, aspirin resistance  
Thienopyridines: gastrointestinal complaints, hematologic side effects  
Dipyridamole: gastrointestinal complaints, headache, facial flushing, dizziness, hypotension, caution in patients with coronary artery diseases  
GP IIb/IIIa receptor antagonists: bleeding, thrombocytopenia. |
| **Anticoagulants** |  
Heparin, UFH, and LMWH: parenteral administration, bleeding, thrombocytopenia, increasing level of bilirubin, osteoporosis.  
Fondaparinux: bleeding, there is no antidote  
Leprudin, bivalirudin, argatroban: parenteral administration, serious bleeding, hepatic insufficiency  
Dabigatran etexilate: bleeding, renal excretion  
Warfarin or acenocoumarol: 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.  
Rivaroxaban: expensive, renal excretion  
Apixaban: expensive, only for major orthopedic surgery, anemia, hemorrhage, nausea  
Betrixaban: expensive, anemia, skin rash, drug-drug interactions |
| **Fibrinolytics** |  
Streptokinase: expensive, hypotension, rash, fever, chills and rigors, blurred vision, confusion, dizziness, faintness, unusual tiredness or weakness, drug-drug interactions.  
Urokinase: bleeding gums, difficulty with breathing or swallowing, headache, increased menstrual flow or vaginal bleeding, nosebleeds, paralysis, prolonged bleeding from cuts  
Anistreplase, alteplase, tenecteplase, reteplase: 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].
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 anti-platelet 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 Si, binds its related amino acid substrate, labeled Pi [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...
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 antistasin—was extracted from the salivary glands of the Mexican leech *Haementeria officinalis*. Antistasin 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].](image-url)
novel synthetic route was designed 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 [Ki = 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 10. Razaxaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.](image1)

![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].](image2)
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 Ki = 0.08 nM with 50% oral bioavailability [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].
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
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].
This new DOAC is a competitive and reversible FXa inhibitor and it has a Ki $0.117 \text{ pM}$ and $\text{IC}_{50} 1.5 \text{ nM}$ [119]. Betrixaban may therefore have several potential advantages over the other FXa inhibitors (Figure 20).
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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