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Factor Xa Inhibitors: Next-Generation Antithrombotic Agents

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1. Introduction

Thrombosis is the underlying cause of a host of common, debilitating, and often fatal cardiovascular disorders. Formation of thrombi in the arterial circulation can lead to acute myocardial infarction (MI[a]) or ischemic stroke. In the venous circulation, deep vein thrombosis (DVT) may result in chronic leg pain, swelling, and ulceration and can, if partially or fully dislodged, be followed by life-threatening pulmonary embolism (PE). The public health consequences of thromboembolic disease are vast. For example, approximately 2.5 million people in the United States are affected by atrial fibrillation (AF), a cardiac arrhythmia associated with a 4- to 5-fold increase in the risk of stroke of primarily cardioembolic origin.[1] Another group at elevated risk of ischemic stroke, as well as recurrent acute MI, is the large and growing population of patients with acute coronary syndrome (ACS).[2] Furthermore, it has been estimated that DVT and PE, which together comprise venous thromboembolism (VTE), afflict up to 600 000 individuals in the United States each year and are implicated in at least 100 000 deaths.[3]

Despite the continued morbidity and mortality caused by thromboembolic disease, recent advances in drug development provide cause for optimism that we are about to enter a new era in antithrombotic therapy. One of the most important advances has been the development, and recent introduction into clinical practice, of a new class of anticoagulants, the direct factor Xa (FXa) inhibitors.[4,5] In this Perspective, we provide a detailed insight into the development of these important new agents, describe how structure-based design played a pivotal role in this process, and review the wealth of preclinical and clinical data that have emerged to date. Finally, we consider the issues that will determine the future impact of the direct FXa inhibitors on clinical practice. We begin by briefly highlighting the limitations of the current standards of care in antithrombotic therapy, reviewing some key concepts in hemostasis and thrombosis, and explaining the rationale for targeting FXa.

1.1. Current Antithrombotic Therapy. Numerous clinical trials have confirmed the efficacy of traditional anticoagulants, including vitamin K antagonists (VKAs), unfractionated heparin (UFH), and low-molecular-weight heparins (LMWHs, fractionated heparin with reduced activity toward thrombin compared to UFH), in the prevention and treatment of a range of arterial and venous thromboembolic diseases.[1,6−8] Despite the fact that these drugs are the current standard of care and despite their proven efficacy, these anticoagulants all possess significant limitations that restrict their usefulness in the clinic and have created the need for new therapies. Use of warfarin and other VKAs is especially problematic, even though these anticoagulants offer the convenience of oral administration.[9] For example, warfarin is associated with numerous drug and food interactions, an unpredictable pharmacokinetic (PK) and pharmacodynamic (PD) profile, and considerable intra- and interpatient variability in drug response.[9] As a result, the appropriate therapeutic dose varies, necessitating monitoring and frequent dose adjustment. Monitoring of warfarin therapy is critical because of this variability and relatively narrow therapeutic index, which often leads to subtherapeutic anticoagulation and a higher risk of thromboembolism or to excessive anticoagulation and an increased risk of bleeding.[9] Furthermore, the delayed onset of action of warfarin means that in critical situations therapy must be initiated with a rapid-acting, parenteral anticoagulant. Urgent surgical or invasive procedures may also be complicated by the fact that the anticoagulant effects of warfarin are retained for several days after discontinuation of treatment.

Agents for short-term anticoagulation include UFH, LMWHs, the indirect FXa inhibitor fondaparinux, and the direct thrombin inhibitors (DTIs) argatroban, bivalirudin, and hirudin. These anticoagulants all require parenteral administration, which makes their use outside the hospital problematic and which can also be associated with injection-site hematomas. UFH and, to a lesser extent, LMWHs carry the risk of thrombocytopenia, and since they are produced from animal tissue, they are sometimes associated with serious side effects.[10] In addition, UFH has an unpredictable PK profile and anticoagulant response that necessitate monitoring.[5] The limitations of parenteral anticoagulants, particularly the need for injection, mean that warfarin and other VKAs, which in many countries are still the only orally administered anticoagulants approved for

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[a]Abbreviations: ACS, acute coronary syndrome; AF, atrial fibrillation; aPTT, activated partial thromboplastin time; ATIII, antithrombin III; AV, arteriovenous; DTI, direct thrombin inhibitor; DVT, deep vein thrombosis; ECAT, electric-current-induced arterial thrombosis; FVa, factor Va; FVIIa, factor VIIa; FVIII, factor FVIII; FVIIIa, factor FVIIIa; FIX, factor IX; FX, factor X; FXa, factor Xa ; FXIa, factor XIa; GPIIb/IIIa, glycoprotein IIb/IIIa; HLM, human liver microsomes; hERG, human ether-a-go-go related gene; HTS, high-throughput screen; LMWH, low-molecular-weight heparin; MI, myocardial infarction; PD, pharmacodynamic; PDB, Protein Data Bank; PE, pulmonary embolism; PK, pharmacokinetic; PT, prothrombin time; SAR, structure−activity relationship; TAFI, thrombin-activatable fibrinolysis inhibitor; TAP, tick anticoagulant peptide; TF, tissue factor; TG, thrombin generation; THR, total hip replacement; TKR, total knee replacement; UFH, unfractionated heparin; VKA, vitamin K antagonist; VTE, venous thromboembolism; vWF, von Willebrand factor.

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use, represent the sole viable option for long-term anticoagulation therapy.

As discussed below, hemostasis and thrombosis are interrelated processes, and it is not surprising that anticoagulant and antiplatelet agents may have hemorrhagic complications. Dose selection, therefore, is primarily influenced by the maximum dose that can be safely administered rather than the dose providing greatest efficacy. Although this will remain true for new antithrombotic agents on the horizon, benefit-to-risk profiles do vary.[11] These limitations result in the underutilization of existing therapies in the very patients who are at highest risk of serious thrombotic events. For example, use of antithrombotic drugs is often avoided in the very elderly, despite the fact that advanced age is a major risk factor for both arterial and venous thromboembolism.[12,13] Concerns over bleeding have also restricted long-term use of warfarin and other anticoagulants in combination with antiplatelet drugs in patients with ACS and AF.[4]

All of these factors have been the driving force for the development of new anticoagulants, including the direct FXa inhibitors. For any new anticoagulant to be considered ideal, it would need to possess a predictable PK profile that allows fixed oral dosing without routine monitoring and to possess a relatively wide therapeutic index with low peak-to-trough plasma concentrations to provide high levels of efficacy and low rates of bleeding. Since the target is in the central compartment, the ideal PK profile also features a low volume of distribution (to reduce the potential for off-target liabilities) and low systemic clearance (to minimize drug−drug interactions). Other key features of the "ideal" new anticoagulant include a rapid onset of action and an ability to bind clot-bound coagulation factors.[14]

1.2. Hemostasis and Thrombosis. Hemostasis is the physiologic process during which bleeding is antagonized, and possibly stopped, in order to minimize blood loss.[15] Unlike hemostasis, which is a necessary physiologic response to bleeding, thrombosis is a pathological process involving an exaggerated hemostatic response and is often ascribed to the combined influence of a triad of causative factors, namely, prothrombotic vascular endothelial changes or injury, stasis of blood flow, and/or other causes of hypercoagulability.[16]

Primary hemostasis begins immediately after endothelial damage and involves localized vasoconstriction and the activation and adhesion of platelets to form a soft aggregate plug. In contrast, secondary hemostasis comprises a complex series of reactions during which several trypsin-like serine proteases, including FXa, are formed from their respective proenzymes. Figure 1A depicts how the "coagulation cascade" has classically been viewed, with two parallel and largely independent pathways, the extrinsic and intrinsic pathways, converging at the point of factor X (FX) activation.[4] Upon the formation of FXa, the common pathway is initiated, leading to the activation of prothrombin to thrombin and the subsequent conversion of fibrinogen to fibrin (described in more detail in section 1.3). Polymerized fibrin strands, together with activated platelets, then form stable clots that seal the breach at the site of injury. While this classic view of the coagulation cascade aids understanding of some of the key processes involved in secondary hemostasis, recent advances have led to the development of a cell-based model that may describe the time course and physiology of thrombogenesis more accurately, including the roles of tissue-factor (TF)-bearing cells and platelets. In this model, shown schematically in Figure 1B, coagulation occurs in three overlapping phases (initiation, amplification, and propagation) and is critically dependent on the contribution of cell surface proteins such as TF.[17,18] In the initiation

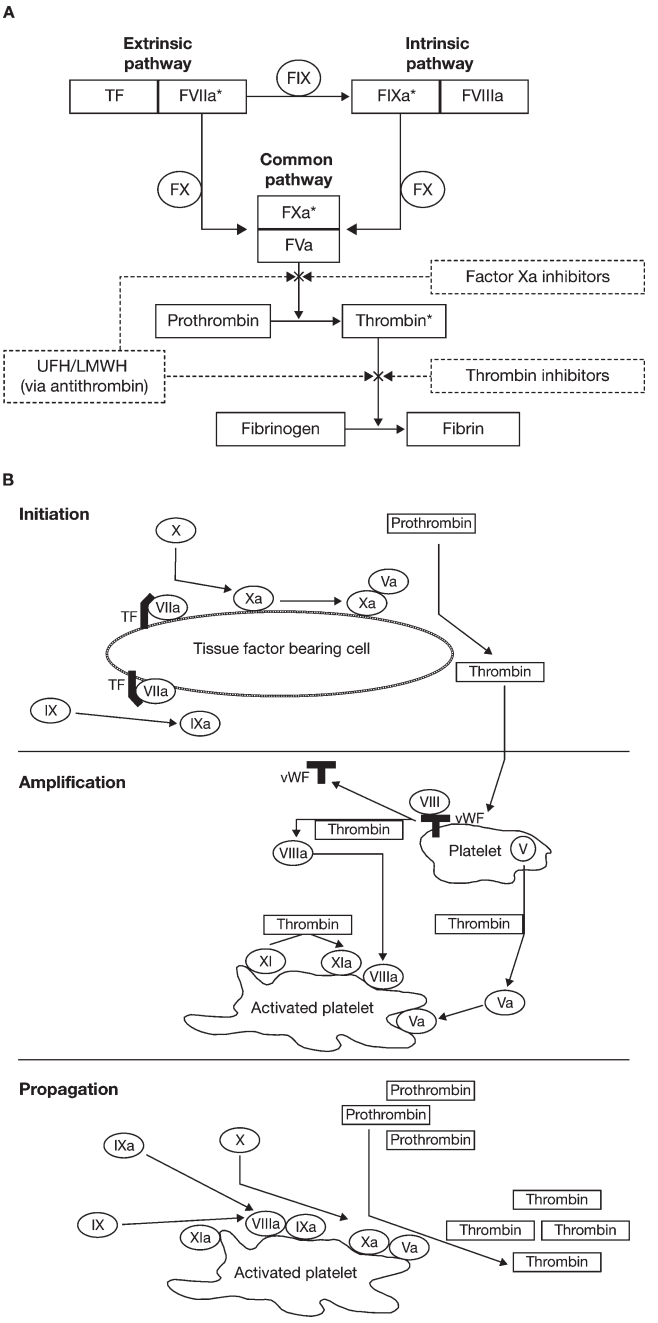


Figure 1. (A) Classic view of the coagulation cascade. Sites of action of traditional and new anticoagulants are also depicted (coagulation factors affected by VKAs are indicated by an asterisk). Adapted by permission from Macmillan Publishers Ltd.: *Clinical Pharmacology & Therapeutics* (http://www.nature.com/clpt/index.html) (Gross, P. L.; Weitz, J. I. New antithrombotic drugs. *Clin. Pharmacol.Ther.* **2009,** *86*, 139−146),[4] Copyright 2009. (B) Cell-based model of coagulation. Adapted by permission from Wolters Kluwer Health/Lippincott, Williams & Wilkins: *Journal of Cardiovascular Pharmacology* (Hammwöhner, M.; Goette, A. Will warfarin soon be passé? New approaches to stroke prevention in atrial fibrillation. *Journal of Cardiovascular Pharmacology* **2008,** *52*, 18−27),[17] Copyright 2008, and *Arteriosclerosis, Thrombosis and Vascular Biology* (Monroe, D. M.; Hoffman, M.; Roberts, H. R. Platelets and thrombin generation. *Arterioscler., Thromb., Vasc. Biol.* **2002,** *22*, 1381−1389),[18] Copyright 2002.

phase, TF−factor VIIa (FVIIa) complexes activate factor IX (FIX) and FX; subsequently formed FXa−factor Va (FVa) complexes then generate small amounts of thrombin. During the amplification phase, thrombin generated in the initiation phase activates platelets, leading to release of factor VIIIa (FVIIIa) from factor VIII (FVIII)−von Willebrand factor (vWF) complexes on the platelet surfaces and also generation of FVa and factor XIa (FXIa). In the final propagation phase, factor XIa (FXIa) generates more FIXa; this FIXa, together with that generated in the amplification phase, activates FX, leading to the formation of numerous FXa−FVa complexes and a burst of thrombin generation.

The inhibitory activity of anticoagulants can be assessed by a number of methods. Assays that employ purified coagulation proteases and synthetic substrates are most commonly used for the initial characterization of the affinity of synthetic inhibitors toward their target enzyme. Modifications of these methods can also be used to measure the activity of inhibitors in blood samples as, for example, the anti-Xa assay that can be used with the LMWHs, fondaparinux, or synthetic inhibitors. Anticoagulants can be further characterized using clotting assays (including prothrombin time (PT) and activated partial thromboplastin time (aPTT)) that measure the time for formation of a fibrin clot after addition of an activator of coagulation to whole blood or plasma.[19] Clotting assays can be used for either in vitro characterization of the potency of compounds or for ex vivo assessments in laboratory animals or in humans. In vitro potency is conventionally reported as the concentration of inhibitor required to produce a doubling of the uninhibited clotting time ($PT_{2\times}$ or $aPTT_{2\times}$). Other useful laboratory methods include the thrombin generation (TG) assay, which measures time-dependent changes in thrombin concentration.[20]

1.3. Targeting FXa. 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. Regulation of thrombin generation is the primary physiologic function of FXa, and few other roles have been identified.[21] FXa is therefore an attractive and potentially specific target for new anticoagulant agents. As will be discussed, experience with indirect inhibitors of FXa has helped to validate FXa inhibition as an effective and safe anticoagulant strategy. However, indirect FXa inhibitors possess two significant limitations. First, these agents require parenteral administration; second, they rely on the activity of antithrombin and are therefore not able to inhibit FXa bound within the prothrombinase complex.[22] Development of orally administered direct inhibitors of FXa that can effectively inhibit prothrombinase-associated and clot-bound FXa, and thereby offer potentially greater anticoagulant activity, is therefore a highly significant advance.

Once formed via the actions of FXa, thrombin plays key roles in both coagulation and platelet activation. Within the coagulation cascade, thrombin directly cleaves fibrinopeptides from fibrinogen, participates in positive feedback reactions via activation of FV and FVIII, promotes the cross-linking of fibrin through activation of factor XIII (FXIII), and renders fibrin resistant to fibrinolysis through activation of thrombin-activatable fibrinolysis inhibitor (TAFI).[23] Oral anticoagulant drug discovery efforts initially focused on the development of small-molecule anticoagulants that target thrombin directly, the oral DTIs. Although the rationale for targeting thrombin is clear, there is some evidence to suggest that inhibition earlier in the coagulation cascade at the level of FXa may have greater antithrombotic potential.[21] Furthermore, preclinical studies suggest that FXa inhibitors may possess a wider therapeutic index than DTIs.[24] Therefore, it is not surprising that the oral anticoagulant drug discovery efforts of many pharmaceutical companies ultimately focused aggressively on small-molecule, direct FXa inhibitors. Differences between the direct FXa inhibitors and DTIs, and the potential consequences of these differences for clinical practice, are discussed in more detail in section 5.

Before we begin our review of the development of the direct FXa inhibitors, it is worth briefly considering some important molecular features of the target protein. FXa belongs to the family of trypsin-like serine proteases, the catalytic domain of which consists of two similar antiparallel β-barrel folds that together form the catalytic triad and substrate binding site.[25] Schechter and Berger have developed a useful nomenclature to describe the prototypical binding site of a serine protease that has been widely adopted and that we will use herein.[26] Accordingly, each protein subsite, labeled S_i, binds the corresponding substrate amino acid labeled P_i, with "i" increasing toward the substrate N-terminus. Similarly, the corresponding subsites and substrate amino acids to the left of the scissile amide bond in Figure 2 are designated as S_i' and P_i', respectively, increasing toward the substrate C-terminus. Substrate cleavage occurs at the P1′−P1 amide bond. As the discovery of small-molecule protease inhibitors has advanced, this convention has been extended to denote drug substructures that bind in a manner similar to substrate amino acids.[27] Figure 2A depicts the serine protease subsites primarily responsible for the recognition and binding of substrate and druglike molecules. It is noteworthy that all reported small-molecule serine protease inhibitors for which structural data exist bind in the S1 and one or more of the remaining subsites.

The FXa binding site is defined by the S1 and S4 subsites and surrounding residues (Figure 2B and Figure 2C). S1 is a deep, largely hydrophobic cleft at the bottom of which lies the Asp189 and Tyr228 side chains. S4 is a strongly hydrophobic pocket defined principally by the side chains of Tyr99, Phe174, and Trp215. The most potent ligands reported in the literature invariably engage both sites. Other features include the catalytic triad consisting of His57, Asp102, and Ser195 and the β-strand region defined by the 214−217 backbone.

Selectivity is a significant issue in the development of factor Xa inhibitors, since, as discussed above, several trypsin-like serine proteases play key regulatory roles in the coagulation cascade, among them FVIIa, FIXa, FXa, FXIa, and thrombin. Trypsin itself is an important enzyme for digestion of proteins in the gastrointestinal tract. Although the consequences of trypsin inhibition in humans have not been well-studied, results in laboratory animals could be problematic in preclinical toxicity testing that is required of any new drug.[30] Orally administered drugs can reach concentrations many-fold higher within the GI tract compared to concentrations within blood; therefore, a high degree of selectivity for the target coagulation enzyme over trypsin is important. In addition, selectivity over trypsin may be considered as a surrogate determination for achieving selectivity over other members of the trypsin-like protease family.

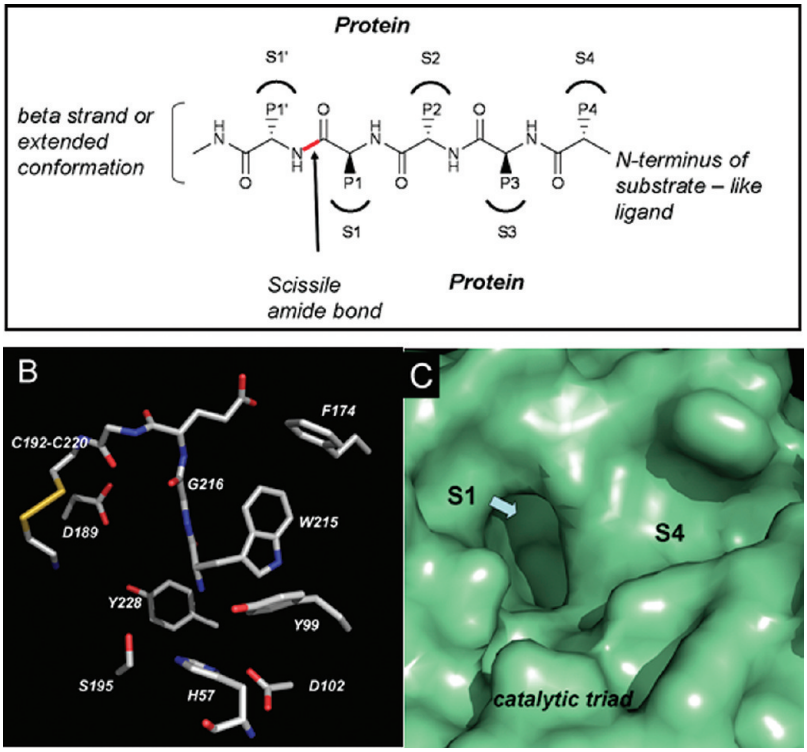


Figure 2. Serine protease and factor Xa structure and nomenclature. (A) Depiction of substrate/protease nomenclature based on the convention of Schechter and Berger.[26] This figure is based on that of Leung et al.[28] (B, C) Overview of the factor Xa binding site,[29] with subsites and several residues important for ligand binding labeled.

As will be discussed in section 3 in more detail, a differentiating feature that can be exploited for selectivity among serine proteases is the nature of the S1 pocket, being smaller and lipophilic in some such as trypsin, or larger and more hydrophobic such as in FXa.

2. Development of Direct FXa Inhibitors

2.1. Precedence for FXa Inhibition as an Effective and Safe Anticoagulant Therapy. Proof of principle for the effectiveness of direct FXa inhibition was established in preclinical animal models of thrombosis with naturally occurring FXa inhibitors of the prothrombinase complex such as tick anticoagulant peptide (TAP)[31] and antistasin.[32,33] Both are highly potent inhibitors of FXa (K_i = 0.59 and 0.3−0.6 nM, respectively), with > 50 000-fold selectivity for FXa over other related serine proteases.[34,35] Compound **1** (Figure 3), a synthetic pentasaccharide, is selective for FXa but acts indirectly via binding to antithrombin and has demonstrated improved or similar clinical benefit over LMWHs in venous thrombotic indications.[36] Superiority in ACS patients with unstable angina/non-ST-segment elevation MI for reducing risk of death or recurrent heart attack was also demonstrated.[37,38] The safety and efficacy of **1** provided the first clinical proof of principle that targeting FXa would be an important advancement in the area of anticoagulation therapy.[36]

More recently, Sanofi-Aventis advanced a hypermethylated derivative of **1** with a high affinity for antithrombin III (ATIII), in which the amino functional groups were replaced with hydroxyl or methoxy groups.[39] This compound, idraparinux (**2a**, K_d = 1 nM, Figure 3), interacts more strongly with ATIII than **1** (K_d = 50 nM) and in patients has a longer half-life ($t_{1/2} \approx$ 80 h), allowing for once-weekly dosing. The results of phase II/III trials with **2a** were mixed, however, and

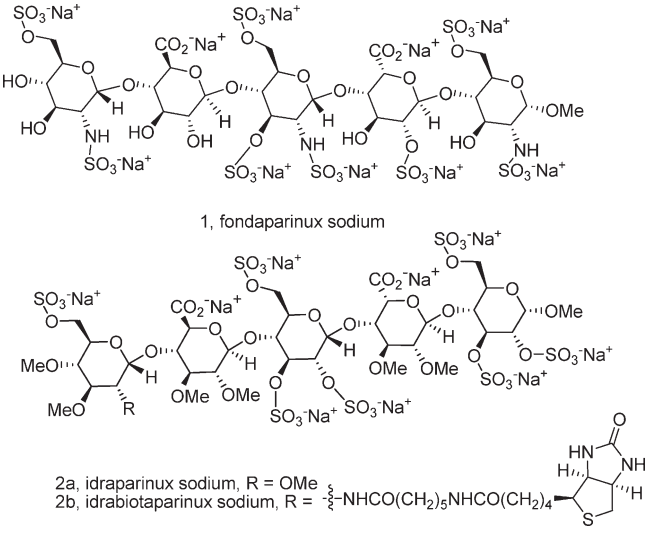


Figure 3. Structures of the pentasaccharide indirect FXa inhibitors.

did not demonstrate a clear advantage over **1**.[40] In a phase III trial, long-term treatment with **2a** (once weekly) for the prevention of stroke and systemic embolism in patients with AF was noninferior to warfarin but caused more bleeding.[41] Development of **2a** has since been discontinued. A second-generation synthetic pentasaccharide, idrabiotaparinux (**2b**, SSR126517E), is in late-stage clinical trials for treatment of VTE and for stroke prevention in patients with AF,[4,42] although the company recently announced discontinuation of development of the drug for the latter indication.[43] Compound **2b** incorporates a biotin moiety that enables the selective reversal of anticoagulant activity by intravenous (iv) administration of avidin, which binds the biotin group.

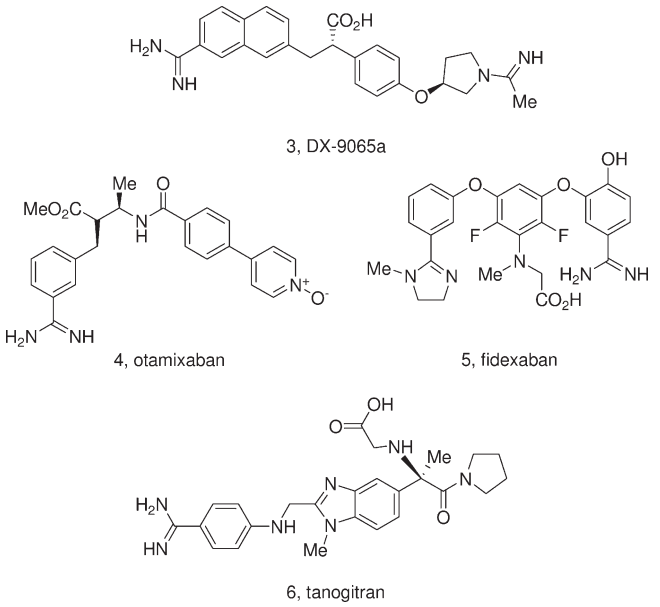


Figure 4. Parenteral direct FXa inhibitors **3**, **4**, and **5** and the dual FXa/thrombin inhibitor **6**.

2.2. Early Prototype Direct FXa Inhibitors: Parenteral Agents. The shortcomings of the LMWHs (e.g., indirect activity, limited ability to inhibit fibrin-bound FXa, parenteral use only) provided great impetus for the discovery of synthetic, small-molecule direct FXa inhibitors. The first generation of these were iv agents, and several small-molecule, nonpeptidic, direct inhibitors of FXa were advanced to phase II clinical trials as parenteral agents.[44,45] DX-9065a (**3**, Daiichi Sankyo, Figure 4, FXa K_i = 41 nM, thrombin K_i > 2000 μM, trypsin K_i = 620 nM) was clearly one of the first potent and selective FXa inhibitors identified, with good clotting activity (activated partial thromboplastin time twice the control [$aPTT_{2\times}$] = 0.97 μM, prothrombin time twice the control [$PT_{2\times}$] = 0.52 μM).[46,47] The compound was studied extensively in preclinical models; found to be efficacious in animal models of thrombosis after iv, subcutaneous, and oral administration; and did not show prolongation of bleeding time.[48] Because of its very low human oral bioavailability (F = 2−3%), **3** was advanced clinically as a parenteral agent.[49] The compound was well tolerated, with no increase in bleeding over the dose range. Human PK for **3** showed a long half-life ($t_{1/2}$ > 20 h), low clearance (120 mL/min), and low plasma protein binding (60% bound). Plasma concentrations correlated well with PD markers. In a phase II study in patients with non-ST-elevation ACS, dose-related trends toward reductions in ischemic events with high-dose **3** compared with heparin were observed.[50] Safety parameters such as bleeding increased dose proportionally.

Otamixaban (**4**, FXV-673, Sanofi-Aventis, Figure 4), a 2,3-disubstituted β-aminoester derivative, is a potent, reversible FXa inhibitor (K_i = 0.5 nM) with good in vitro anticoagulant activity ($aPTT_{2\times}$ = 0.41 μM, $PT_{2\times}$ = 1.1 μM).[51,52] Like **3**, compound **4** belongs to the benzamidine class of molecules, and its polarity precludes significant oral absorption. In vivo, **4** was efficacious in canine models of thrombosis and demonstrated minimal effect on bleeding at effective doses.[53,54] In phase I/II studies, **4** was administered intravenously and was found to be well tolerated in healthy volunteers and patients with coronary artery disease and was rapidly distributed in the plasma and rapidly eliminated with a half-life of 1.5−2 h.[55] In a phase II trial setting, **4** significantly reduced prothrombin fragment 1 + 2, a marker of thrombin generation, when compared with UFH and a glycoprotein (GPIIb/IIIa) antagonist, eptifibatide.[56] Two phase II clinical trials for the management of ACS and patients with ACS undergoing percutaneous coronary intervention have also been completed and showed the potential for reduced ischemic events with bleeding rates similar to those of UFH plus eptifibatide.[56,57] A third parenteral agent that was advanced to human clinical trials was fidexaban (**5**, ZK-807834, Berlex-Pfizer; Figure 4),[58] a compound that contains two amidine groups and a polar carboxylic acid moiety.[59] The dihydrochloride salt of **5** (ZK-807191) is a potent inhibitor of FXa (K_i = 0.10 nM) and exhibits nearly 20 000-fold selectivity over thrombin and 2500-fold selectivity over trypsin; it has been shown to be efficacious in several in vivo animal models of thrombosis.[60−62] In human clinical trials, infusion of **5** was found to be well tolerated.[63] Phase II trials were underway in unstable angina in 2001; however, no results from these studies were published.[64] In addition to the above direct FXa parenteral compounds, a dual thrombin/Xa inhibitor, tanogitran (**6**, BIBT 986, Boehringer Ingelheim, FXa K_i = 26 nM, thrombin K_i = 2.7 nM, Figure 4), was evaluated more recently in a phase II clinical trial involving a human model of endotoxin-induced coagulation.[65] In this study, **6** prolonged plasma aPTT, reduced in vivo thrombin generation in a dose-dependent manner, and was safe and well tolerated. No further development has been reported.

Clinical success of indirect FXa inhibitors such as **1** and the improved therapeutic index with the early direct FXa inhibitors such as the parenteral inhibitors described above fueled an intense effort to discover and develop safer and more effective oral FXa inhibitors. The discovery and advancement of orally bioavailable, direct-acting FXa inhibitors that progressed to clinical studies, including a brief survey of some of the early inhibitors that led up to these agents, are now discussed.

2.3. Approach to Oral FXa Inhibitors. 2.3.1. Transition State and Peptidomimetic Approach: Covalent Inhibitors. Early efforts to identify inhibitors of FXa stemmed from the prior discoveries of thrombin inhibitors that contained "serine traps", such as aldehyde or ketothiazole moieties, which are capable of interacting covalently with the catalytic Ser195 hydroxyl group to mimic a tetrahedral transition state in a reversible manner. Compounds **7** (FXa IC_{50} = 15 nM),[66] **8** (FXa K_i = 0.13 nM),[67] and **9** (FXa IC_{50} = 0.83 nM)[68] are examples of early FXa inhibitors that contain a "serine trap" (Figure 5). As with the thrombin inhibitors in this class, several of the first transition-state FXa inhibitors also contained arginine or constrained arginine P1 residues that would interact strongly with the acidic Asp189 S1 residue, flanked by aromatic residues designed to fit into the hydrophobic S1 pocket of FXa.[44] In the design of these inhibitors, it also became apparent that basic substituents could be tolerated in both the S1 and S4 regions, with a basic P4 moiety interacting in a π−cation manner with the hydrophobic residues in the S4 subsite. These peptide-like inhibitors eventually evolved to incorporate heterocyclic amide-bond replacements,[69] such as pyridone (**10**, FXa IC_{50} = 3 nM), ketopiperazine (**11**, FXa IC_{50} = 2 nM), and caprolactam (**12**, FXa IC_{50} = 3 nM), while maintaining good FXa binding affinity; however, oral bioavailability was

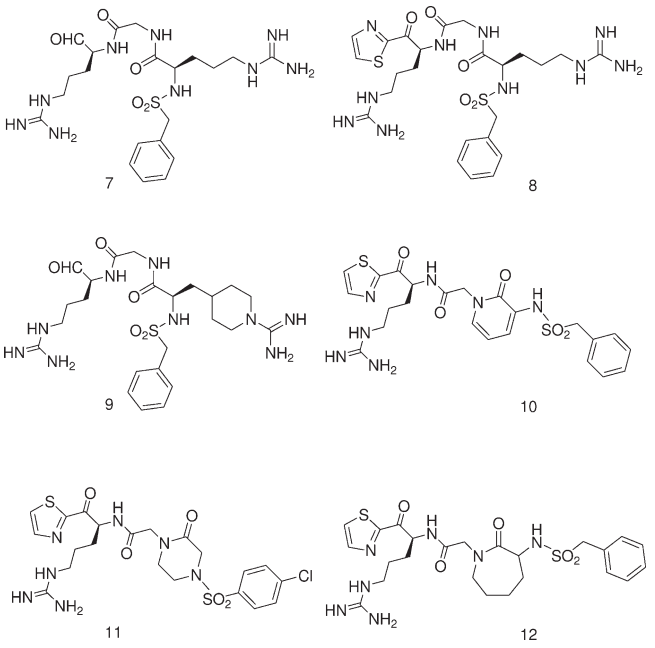


Figure 5. Examples of transition-state inhibitors of FXa.

not achieved. There are no published reports of any transition-state FXa inhibitors advancing into clinical trials.

2.3.2. Early Dibasic Benzamidine Approach. Reports of nonpeptidic, small-molecule inhibitors of FXa such as bisamidine compounds **13** (DABE, bovine FXa K_i = 570 nM)[70] and **14** (BABCH, bovine FXa K_i = 610 nM)[71] set the stage for the evolution of additional dibasic inhibitors (Figure 6), of which **3** is an early example. The success of **3** prompted multiple research groups to further optimize compounds in this class. Initial attempts at Daiichi Sankyo to improve FXa potency and oral bioavailability in direct analogues of **3** resulted in constrained indoline compounds **15** (FXa IC_{50} = 7.6 nM)[72] and **16** (FXa IC_{50} = 3.9 nM).[73] Although these analogues demonstrated potent binding affinity against FXa, selectivity over trypsin remained an issue (trypsin K_i = 24 and 39 nM, respectively). This was addressed by modification of the naphthyl P1 moiety to a substituted benzamidine and introduction of a hydroxyl moiety on the P1 benzamidine group to afford **17** (FXa IC_{50} = 4.4 nM),[74] which demonstrated an improved selectivity profile for FXa relative to trypsin (K_i = 1500 nM). Compound **17** was potent in the in vitro clotting assays ($PT_{2\times}$ = 0.39 μM, $aPTT_{2\times}$ = 0.34 μM); however, oral exposure was not achieved.

Several other laboratories have explored variants of **3** to arrive at potent inhibitors of FXa. For example, researchers at Astellas Pharma prepared the structurally related compound **18** (YM-60828, FXa K_i = 1.3 nM, $PT_{2\times}$ = 0.21 μM),[75,76] which was highly selective over thrombin (K_i > 10 000 nM) but showed less selectivity for FXa compared with trypsin (K_i = 46 nM). This compound showed oral activity in squirrel monkeys (F=20%), was efficacious in several animal models of thrombosis, and did not show bleeding increases at effective doses. YM-75466, the methanesulfonate salt of **18**, was reported to have entered clinical development,[75] although development of this compound appears to have ceased.

In a parallel effort, investigators at Portola Pharmaceuticals also prepared compounds (e.g., **19**, FXa IC_{50} = 6 nM and **20**, FXa IC_{50} = 7 nM) that bear structural similarities to **15**.[77] Constraining the central aniline moiety in the form of a benzoxazinone ring resulted in analogues, such as **21**, that retained potent binding affinity against FXa (K_i = 6 nM). Kissei Laboratories reported the discovery of **22** (KFA-1411, FXa K_i = 1.7 nM), which is highly selective over a broad range of serine proteases, including trypsin, and has good in vitro anticoagulant potency ($PT_{2\times}$ = 0.28 μM, $aPTT_{2\times}$ = 0.87 μM).[78] The compound was efficacious when compared with dalteparin and LMWH in a hemodialysis model in monkeys.[79] Additional early bisamidine-containing analogues have been covered extensively in previous review articles.[80−82]

2.4. Transition From Benzamidine to Oral Agents. 2.4.1. Isoxazoline, Isoxazole, and Pyrazole-Based Inhibitors. In the quest for potent inhibitors of FXa, Bristol-Myers Squibb researchers recognized the similarity between the platelet GPIIb/IIIa peptide sequence Arg-Gly-Asp and the prothrombin substrate FXa sequence Glu-Gly-Arg.[83] Isoxazoline derivative **23** (FXa $K_i \approx$ 39 000 nM, Figure 7) was identified from a high-throughput screen (HTS) of a library of proprietary GPIIb/IIIa antagonists. Modifications to incorporate the bisbenzamidine motif of the known FXa inhibitors, along with further optimization, provided **24** with greatly enhanced affinity (FXa K_i = 94 nM).[83] Guided by structure-based design, these researchers replaced the 4-amidino moiety of **24** with a neutral *o*-phenylsulfonamide group to reduce the basicity of this dibasic lead and thus improve permeability. This afforded the first known monobasic FXa inhibitor **25**, which also exhibited enhanced potency (FXa K_i = 6.3 nM).[84] The biaryl moiety of **25** was designed to interact with the hydrophobic S4 aryl binding domain of the FXa active site (see Figure 2B) and, from modeling experiments, was shown to be neatly stacked between the residues Tyr99, Phe174, and Trp215, with the terminal *o*-phenylsulfonamide ring designed to make an edge-to-face interaction with Trp215. Further scaffold optimization led to isoxazoline compound **26** (FXa K_i = 0.55 nM) with the benzamidine P1 and the biarylsulfonamide vicinally substituted on the five-member ring.[85] Replacement of the isoxazoline ring with a planar aromatic isoxazole ring in **27** provided further improvement in FXa potency (K_i = 0.15 nM).[85] The lack of chirality of this template, and the potency, made it an attractive starting point for further optimization. Rapid evaluation of a variety of vicinally substituted five- and six-member ring scaffolds resulted in the determination that the five-member nitrogen-based, N-linked templates were most potent.[86] This effort led to the discovery of a very potent pyrazole analogue **28** (SN429, FXa K_i = 0.013 nM).[87] An X-ray crystal structure of **28** in bovine trypsin showed the following interactions: S1-Asp189 with the benzamidine P1, the pyrazole N2 group with the backbone of Gln192, a lipophilic interaction of the C3 methyl at the outer ridge of the enzyme, the linker carboxamide carbonyl oxygen with the NH of Gly216, and the P4 biarylsulfonamide neatly stacked in the S4 hydrophobic box formed by Tyr99, Trp215, and Phe174. In vivo, in the rabbit arteriovenous (AV) shunt model, administration of **28** by iv infusion resulted in reduction of thrombus weight by 50% at a dose of 0.02 μmol/kg/h (ID_{50}).[87] Poor oral bioavailability (F < 4% in dogs), combined with a short half-life ($t_{1/2}$ = 0.82 h) and lack of selectivity over related trypsin-like serine proteases, precluded further development of **28** as an oral anticoagulant.

The high affinity of **28** for FXa afforded a unique opportunity to give up potency in an effort to improve the oral

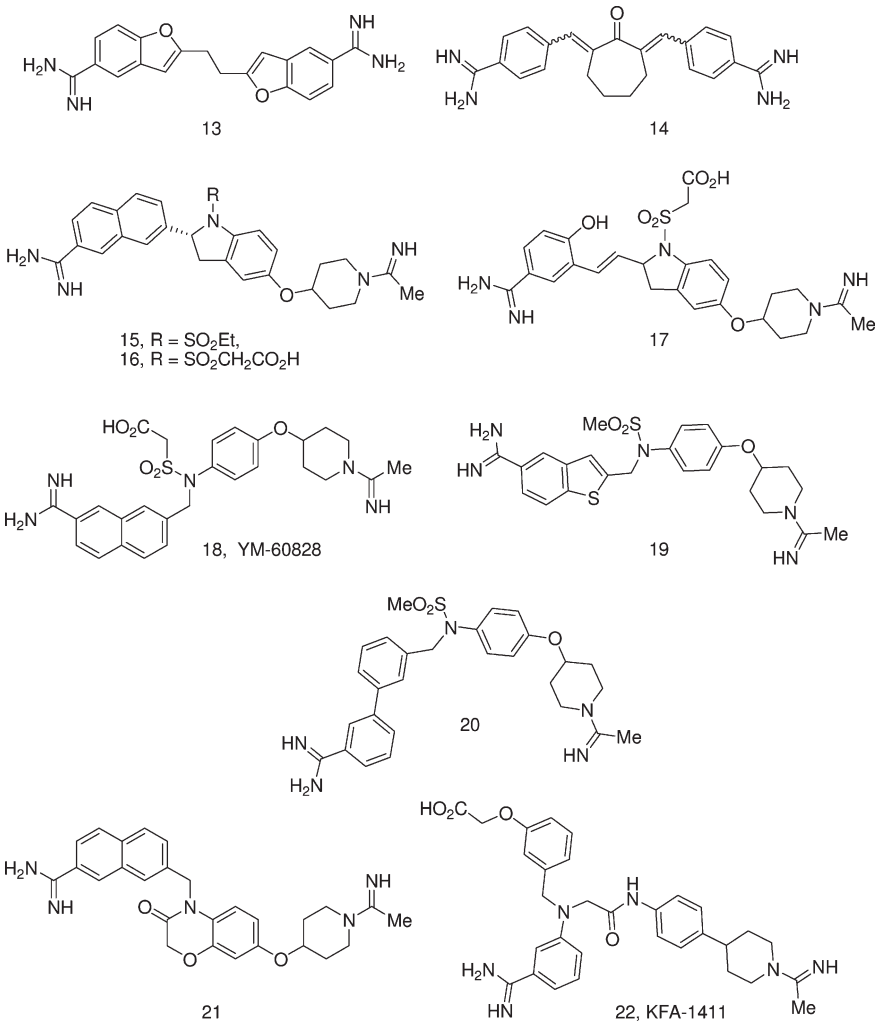


Figure 6. Examples of bis-amidine FXa inhibitors.

bioavailability and selectivity of the pyrazole class of compounds. The initial strategy centered on replacing the highly basic amidine P1 moiety ($pK_a \approx 11.5$) with a less basic P1 group such as a benzylamine ($pK_a \approx 8.8$). This culminated in the discovery of the first oral clinical candidate from the pyrazole series, **29** (DPC423, Figure 8), which demonstrated excellent affinity for FXa ($K_i = 0.15$ nM), good selectivity (thrombin $K_i = 6000$ nM, trypsin $K_i = 60$ nM),[87] and potency in clotting assays ($aPTT_{2\times} = 4.86\ \mu M$). The compound showed good oral bioavailability in dogs ($F = 57\%$), low clearance (Cl = 0.24 L/h/kg), moderate volume of distribution at steady state ($V_{dss} = 0.90$ L/kg), and a relatively long half-life ($t_{1/2} = 7.5$ h). In the rabbit AV shunt and the rabbit electrically induced carotid artery (ECAT) thrombosis models, **29** administered as a continuous iv infusion was efficacious, with 50% reduction in thrombus weight at a plasma concentration (IC_{50}) of 150 nM and restoration of integrated blood flow to 50% of pre-ECAT injury values at an effective plasma concentration (EC_{50}) of 137 nM, respectively.[88,89] In phase I clinical trials, high oral exposure was observed, with a half-life of approximately 30 h.[90]

In a continuing effort to find pyrazole FXa inhibitors with improved selectivity and oral bioavailability profiles, a systematic and comprehensive search for novel benzamidine mimics with reduced basicity was explored.[91] To optimize **28**, the strategy focused on lowering the pK_a of the P1 ligand by replacing the amidine with less basic and neutral benzamidine mimics, some of which is summarized in Table 1. This effort proved successful and resulted in a number of analogues that maintained nanomolar potency and good selectivity while demonstrating improved PK profiles and oral absorption.

Further modifications led to a diverse set of pyrazole compounds, including **30** (DPC602)[92] and **31** (razaxaban, BMS-561389, Figure 8).[93] Compound **30** (FXa $K_i = 0.91$ nM) showed excellent potency against FXa and improved selectivity for FXa relative to trypsin ($K_i = 3500$ nM) and thrombin ($K_i = 3600$ nM). The compound was permeable in the Caco-2 assay and efficacious in the iv rabbit AV shunt model ($ID_{50} = 4.2\ \mu$mol/kg/h) and demonstrated excellent oral bioavailability ($F = 100\%$ in dogs).[92] Compound **31** was also highly potent against FXa ($K_i = 0.19$ nM) and very selective (> 5000-fold) against other serine proteases.[93] As described in more detail in section 3, the excellent selectivity of **31** was in part due to the presence of the bulky aminobenzisoxazole P1 moiety, which is in close contact with the side chain of Ala190 in FXa and thus forms an unfavorable interaction with the larger serine residue at this position in the trypsin S1 pocket. This compound is potent in the in vitro clotting assays ($aPTT_{2\times} = 6.1\ \mu M$ and $PT_{2\times} = 2.1\ \mu M$) and shows antithrombotic efficacy in the iv rabbit AV shunt

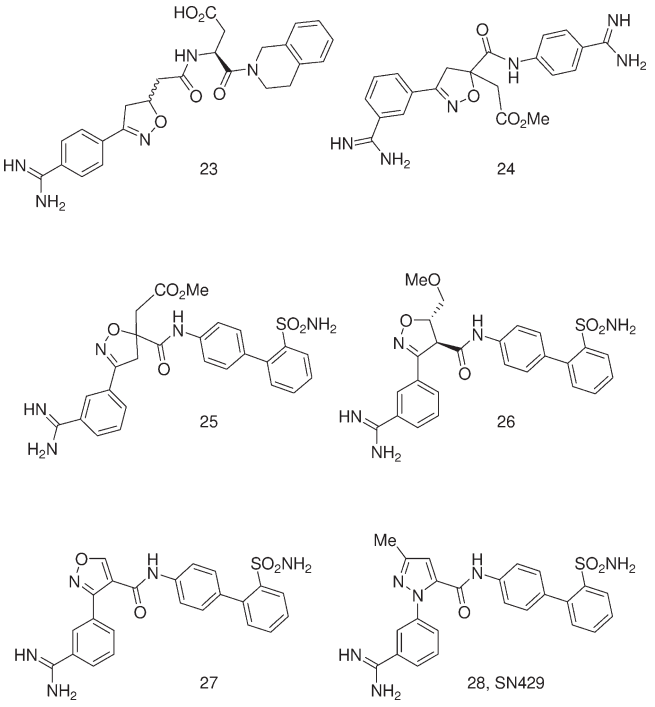


Figure 7. Evolution of the first pyrazole-based FXa inhibitors.

with ID_{50} = 1.6 μmol/kg/h [93] and in the ECAT model with ED_{50} = 0.22 mg/kg/h.[94] Additionally, **31** was highly permeable in a Caco-2 assay and orally bioavailable in dogs (F = 84%), with a moderate clearance (Cl = 1.1 L/h/kg) and a moderate to high volume of distribution (V_{dss} = 5.3 L/kg) and half-life of 3.4 h. The HCl salt of **31** was selected for clinical development and was the first orally bioavailable pyrazole FXa inhibitor to demonstrate efficacy in a phase II proof-of-principle clinical trial.[95]

An early concern in the pyrazole series was the presence of the 5-amido linkage to biarylamine P4 moieties, creating the possibility of liberation of an aniline-containing metabolite in vivo that has the potential to be mutagenic. The cleavage of the amide linker of **31** was not observed in vivo, and the imidazoaniline P4 moiety was not mutagenic in either in vitro or in vivo experiments. However, during lead optimization, screening each new aniline P4 fragment for mutagenicity was cumbersome and no clear structure−activity relationship (SAR) emerged. As a result, several strategies were developed to remove the cleavable amide in subsequent follow-on compounds. Replacement of the amide linkage with a ketone linker led to compound **32** (FXa K_i = 0.97 nM), which demonstrated a high affinity for FXa and good clotting activity ($PT_{2\times}$ = 2.0 μM).[96] In dogs, **32** exhibited high clearance (Cl = 2.5 L/h/kg), a large volume of distribution (V_{dss} = 4.5 L/kg), a short half-life ($t_{1/2}$ = 1.6 h), and low oral bioavailability (F = 16%). Another approach involved the rigidification of the pyrazole ring by cyclization of the amide NH to the C4 position of the pyrazole ring to form a bicyclic scaffold (Figure 9), as in the pyrazolopyrimidinones **33** (FXa K_i = 1.1 nM, $PT_{2\times}$ = 2.2 μM)[97] and **34** (FXa K_i = 0.17 nM; $PT_{2\times}$ = 4.1 μM).[98] Further optimization resulted in several potential follow-on compounds to **31**.[99,100] For example, tetrahydropyrazolopyridinone **35** (BMS-740808, FXa K_i = 0.030 nM, $PT_{2\times}$ = 3.6 μM), containing the aminobenzisoxazole P1 moiety, was shown to be a highly potent FXa inhibitor, with good antithrombotic

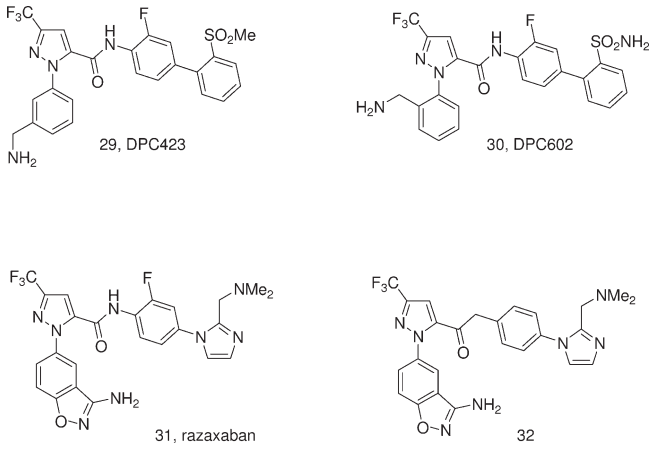


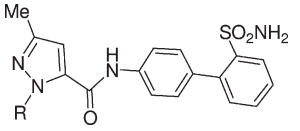
Figure 8. Optimization of pyrazole-based FXa inhibitors.

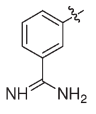
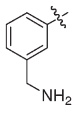
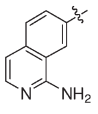
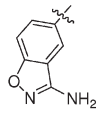
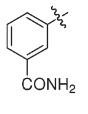
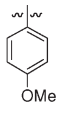
efficacy in the iv rabbit AV shunt model and a PK profile similar to that of **31**.[99] Evaluation of other P1 groups with this bicyclic template resulted in the *m*-carboxamidophenyl analogue **36**, which retained potent FXa affinity (K_i = 0.18 nM), good in vivo efficacy (iv rabbit AV shunt IC_{50} = 500 nM), and similar PK profile to **31**.[100] In identifying a suitable backup for **31**, a compound with both low clearance and low volume of distribution was targeted to keep the drug in the central compartment. A significant breakthrough in achieving this ideal PK profile was ultimately realized by the incorporation of a P4 lactam group in combination with a *p*-methoxyphenyl P1 group. To further increase the free fraction and decrease protein binding, the trifluoromethyl substituent was replaced by a carbamoyl group. This led to the discovery of **37** (apixaban, BMS-562247, FXa K_i = 0.08 nM, Figure 9), a highly potent and selective FXa inhibitor with good in vitro anticoagulant activity ($PT_{2\times}$ = 3.8 μM, $aPTT_{2\times}$ = 5.1 μM).[101] Compound **37** was permeable in the Caco-2 assay and demonstrated an ultralow clearance (Cl = 0.02 L/h/kg), low volume of distribution (V_{dss} = 0.2 L/kg), and high oral bioavailability (F > 50%) in dogs.[101] In rabbits, **37** was highly efficacious in three iv thrombosis models, AV shunt (ED_{50} = 0.27 mg/kg/h), ECAT (ED_{50} = 0.07 mg/kg/h), and DVT (ED_{50} = 0.11 mg/kg/h).[102] The overall preclinical profile demonstrated by **37** was considered superior to its predecessor compounds. Compound **37** is currently in late-stage (phase III) clinical trials for multiple indications as described in section 4.

The pyrazole scaffold was subsequently also employed by other research groups, including researchers at Portola who identified pyrazole compounds **38** (FXa K_i = 1.5 nM)[103] and **39** (FXa K_i = 0.7 nM),[104] in which the P1 moiety is a halogenated naphthyl group (Figure 10). These compounds were orally bioavailable in rats (F = 35% and 47%, respectively) with long half-lives ($t_{1/2}$ = 7.2 and 8.8 h, respectively) and inhibited thrombus formation by iv infusion in a rabbit DVT model. Researchers at Johnson & Johnson also reported a series of benzofused pyrazole FXa inhibitors.[105] An example is compound **40** (FXa K_i = 4.4 nM, $aPTT_{2\times}$ = 4.4 μM),[105] in which the bicyclic pyrazole scaffold of **37** has been replaced with a 7-fluoroindazole core, and the aminobenzisoxazole P1 moiety of **31** was employed. The fluoro substituent on the indazole ring mimics the carbonyl−Gly216 binding interaction seen with the amide carbonyl group present in **37**.

2.4.2. Disubstituted Heterocycles. In addition to the 3,5-isoxazoline cores described above,[84] other 1,3-disubstituted

Table 1. Benzamidine Mimics: Effect of pK_a on Potency and Oral Bioavailability in Analogues of **28**[91]



R =						
pKa	10.7	8.8	6.7	<2.3	<0	<0
FXa K_i (nM)	0.13	2.7	0.3	1.4	19	11
F% (dogs)	4	13	13	26	46	48

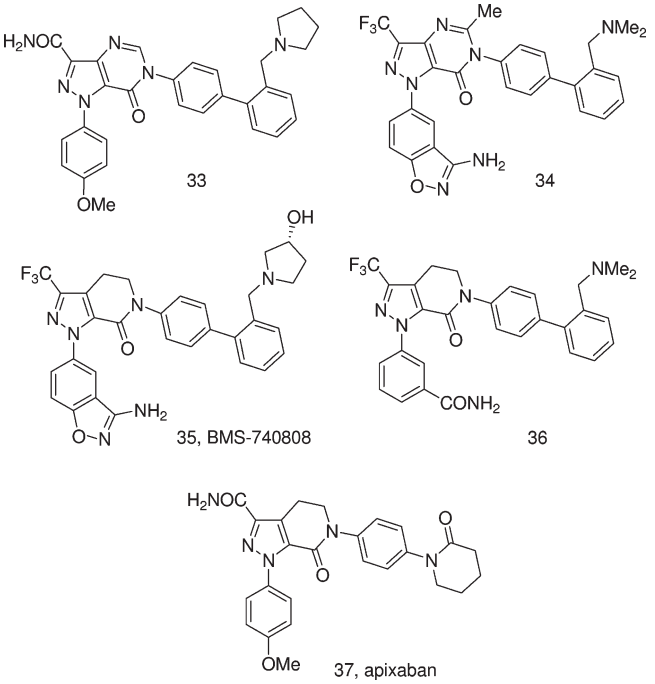


Figure 9. Bicyclic pyrazole FXa inhibitors leading to **37**.

five-membered heterocyclic cores were investigated by various groups. The Sanofi-Aventis group identified a 1,3-disubstituted pyrrolidinone analogue **41** (FXa K_i = 230 nM), which combined a (2-naphthylsulfonamido) P4 moiety attached at the C3 position of a pyrrolidinone core with a 3-amidinobenzyl P1 group appended at N1 (Figure 11).[106] The introduction of a methoxy group at the 7-position on the naphthyl P4 group and methylation of the sulfonamide nitrogen led to further potency enhancements in **42** (FXa K_i = 47 nM) and **43** (FXa K_i = 22 nM). Both **42** and **43** had moderate selectivity for FXa compared with thrombin (30- to 50-fold) and trypsin (18- to 32-fold). Affinity for FXa and selectivity with respect to thrombin and trypsin (140- and 76-fold, respectively) was further improved by replacing the benzamidine P1 group of **42** with an amidinothiophene P1 moiety in **44** (RPR120844, FXa

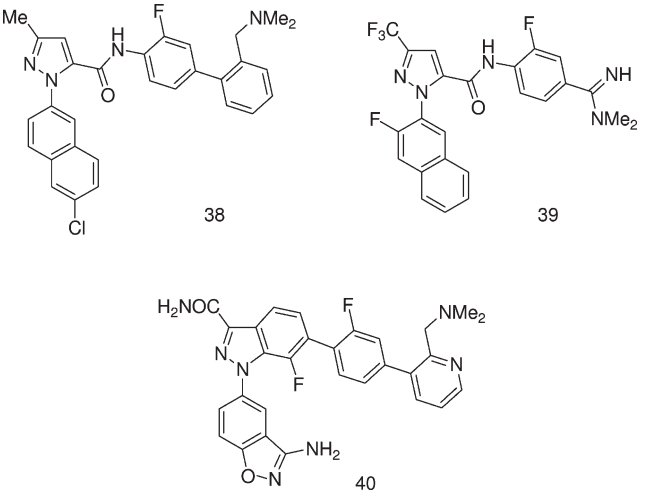


Figure 10. Additional examples of pyrazole and bicyclic pyrazole FXa inhibitors.

K_i = 7 nM). Modest plasma exposure was observed in rats with this thiophene analogue after an oral dose of 50 mg/kg.[106] To overcome the historical problem of poor oral bioavailability associated with amidine-containing compounds, the highly basic P1 moiety of **42** was replaced with 2-aminoisoquinoline.[107] The resulting compound, **45**, maintained moderate binding affinity for FXa (K_i = 180 nM) and showed improved permeability in the Caco-2 assay. Replacement of the methoxy naphthyl P4 substituent with a thienopyridine group afforded compound **46** (FXa K_i = 22 nM), which demonstrated good exposure in dogs with a maximum plasma concentration of 2.7 μM at 2 h after a 10 mg/kg oral dose, oral bioavailability (F = 33%), and good ex vivo anti-FXa activity up to 4 h postdose. The introduction of a second amino group on the isoquinoline P1 moiety led to improved FXa binding affinity in **47** (FXa K_i = 6 nM) along with improved selectivity. Interestingly, the 6-azaindol-2-yl group also served as a good benzamidine P1 replacement in this scaffold, as shown by compounds **48** (FXa K_i = 43 nM)[108] and **49** (RPR208707, FXa K_i = 18 nM).[109] X-ray structures of **46** and **48** show that the aminoisoquinoline and

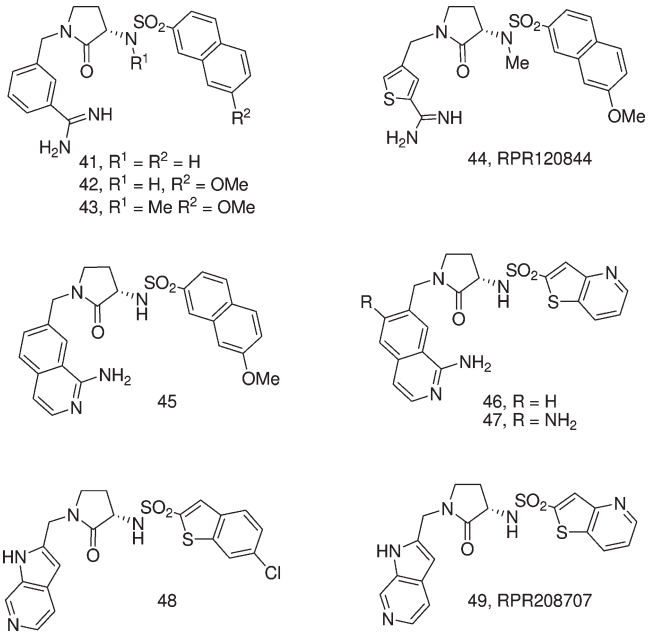


Figure 11. 3-Aminopyrrolidinone FXa inhibitors.

azaindole moieties do not form the typical direct salt-bridge interaction to Asp189 seen with the more basic benzamidine compounds. Instead, these P1 groups sit higher in the S1 pocket and interact with Asp189 via a water molecule.[109]

A 3-aminopyrrolidinone scaffold was also used by GlaxoSmithKline researchers to prepare FXa inhibitors wherein a 6-chloronaphthalene-2-sulfonamide as the P1 group and the biaryl P4 group of **29** were employed to afford compound **50** (Figure 12), which was potent in the binding assay (FXa K_i < 0.1 nM) but showed weak clotting activity (aPTT$_{1.5\times}$ = 52 μM).[110] Replacement of the naphthylsulfonamide P1 moiety with (*E*)-2-(5-chlorothiophen-2-yl)ethenesulfonamide led to compound **51** (FXa K_i = 0.2 nM, aPTT$_{1.5\times}$ = 21.3 μM).[110] The low potency in the clotting assay for these compounds was attributed to high plasma protein binding, since **51** was 97.6% bound. Introduction of polar substitution in the P4 region was tolerated but did not provide the desired increased potency in the aPTT assay in **52** (FXa K_i < 0.3 nM, aPTT$_{1.5\times}$ = 38.9 μM), **53** (FXa K_i < 0.4 nM, aPTT$_{1.5\times}$ = 17 μM), or **54** (FXa K_i = 0.6 nM, aPTT$_{1.5\times}$ = 19.2 μM).[111] The combination of the basic dimethylaminomethylimidazole P4 moiety with the (*E*)-2-(5-chlorothiophen-2-yl)ethenesulfonamide P1 group in compound **55** (FXa K_i = 0.2 nM), however, did result in a 4- to 5-fold improvement in clotting activity (aPTT$_{1.5\times}$ = 4.2 μM).[111] In rats, **55** showed low clearance (Cl = 13 mL/min/kg), low volume of distribution (V_{dss} = 0.6 L/kg), and good oral bioavailability (F = 52%) but a short half-life ($t_{1/2}$ = 1 h). Introduction of a novel morpholino amide P4 group provided **56** (FXa K_i = 6 nM, aPTT$_{1.5\times}$ = 5.4 μM) and **57** (GW813893, FXa K_i = 4 nM, aPTT$_{1.5\times}$ = 1.2 μM),[112] which had similar in vitro anticoagulant potency but reduced FXa binding affinity compared with **55**. Compound **57** demonstrated good oral bioavailability in rats and dogs (F = 75% and 53%, respectively), with low clearance and low volume of distribution in both species but with a short half-life (rat $t_{1/2}$ = 0.7 h; dog $t_{1/2}$ = 1.2 h). This compound was efficacious in animal models of thrombosis[113] and was selected for further development but has since been terminated.[114] Additional structurally diverse, pyrrolidine-based FXa inhibitors were also reported by researchers at Roche

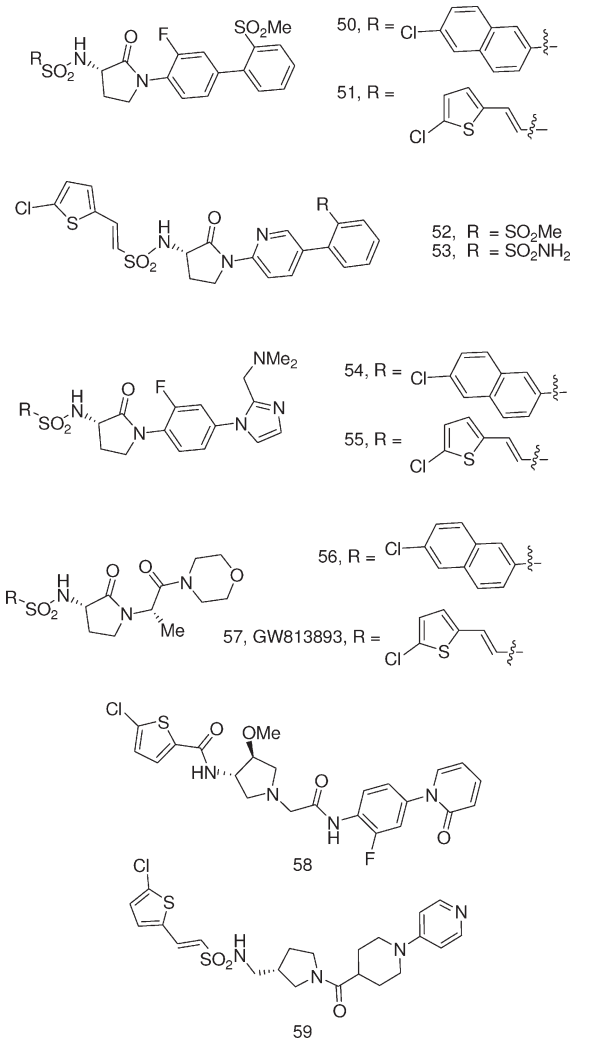


Figure 12. Additional examples of pyrrolidinone and pyrrolidine FXa inhibitors.

(**58**, FXa K_i = 3.0 nM, PT$_{2\times}$ = 1.7 μM)[115] and Bristol-Myers Squibb (**59**, FXa IC$_{50}$ = 5.5 nM).[116] No further development was reported with either of these analogues.

The most successful approach employing a 1,3-substituted heterocycle core was the discovery of the oxazolidinone class of potent FXa inhibitors at Bayer HealthCare AG (Figure 13). Beginning with compound **60** (FXa IC$_{50}$ = 120 nM), identified from HTS,[117] an early lead compound **61** was obtained by introduction of an isoindoline core and replacement of the highly basic amidinomethoxy moiety with a 4-pyridylaminomethyl group. While **61** had excellent FXa affinity (IC$_{50}$ = 8 nM), it lacked oral bioavailability. Incorporation of the chlorothiophene P1 moiety from **61** into another weakly active oxazolidinone-containing HTS hit provided compound **62** (FXa IC$_{50}$ = 90 nM), which was further improved by replacement of the pendent P4 thiomorpholine moiety with a morpholine group to provide **63** (FXa IC$_{50}$ = 32 nM). Various other heterocyclic P4 modalities were also investigated. Lactam analogue **64** (FXa IC$_{50}$ = 4.0 nM), which lacks the fluorine substitution on the phenyl ring, showed significant improvement in binding affinity. The introduction of the carbonyl moiety into the morpholine P4 group provided compound **65** (rivaroxaban, BAY 59-7939, FXa IC$_{50}$ = 0.7 nM, PT$_{2\times}$ = 0.23 μM). In a rabbit AV shunt model, **65** reduced thrombus formation in a

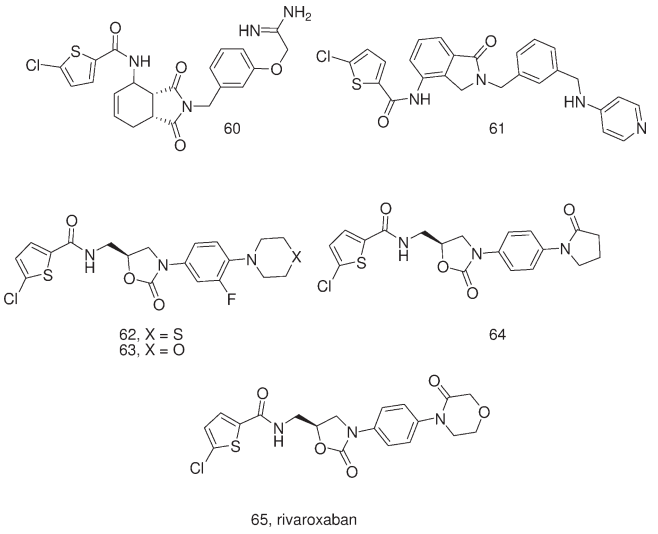


Figure 13. Oxazolidinone-based FXa inhibitors leading to **65**.

dose-dependent manner with an ED_{50} value of 0.6 mg/kg (po), with no significant increase in bleeding time.[118] The oral bioavailability was high (F = 60−86% and 57−66% in dogs and rats, respectively), with low clearance (Cl = 0.3 and 0.4 L/h/kg, respectively) and short half-life ($t_{1/2}$ = 0.9 and 1.2−2.3 h, respectively).[119] An X-ray structure of **65** bound in human FXa (2.08 Å) shows a Cl−Tyr228 interaction in the S1 region, with the morpholinone substituent occupying the S4 hydrophobic box and the oxazolidinone carbonyl forming a hydrogen bond to the backbone NH of Gly219. The *S*-oxazolidinone enantiomer provided optimal binding to FXa. Compound **65** was advanced into clinical development.[117] Currently, supported by four phase III trials showing superior efficacy versus enoxaparin, **65** is approved in Europe, Canada, and several other countries outside the United States for the prevention of VTE in patients undergoing total hip replacement (THR) or total knee replacement (TKR) surgery.[120]

2.4.3. Vicinal Diamide Inhibitors. Pioneering efforts in this area include the contributions from the Berlex and the Lilly groups. Researchers at Berlex identified anthranilamide compound **66** (FXa K_i = 11 nM, Figure 14) through library screening.[121] Replacement of the fluoroanilide with a chloroanilide moiety in **67** led to a 34-fold improvement in binding affinity toward FXa (K_i = 0.32 nM). Further structural modifications to the central phenyl core and to both the P1 and P4 moieties of **67** provided additional potent compounds such as **68** (FXa K_i = 0.005 nM, $PT_{2\times}$ = 1.2 μM).[122] Compound **68** demonstrated excellent oral bioavailability in dogs (F = 98%) and showed good efficacy in the rat stasis thrombosis model.

Researchers at Lilly separately disclosed anthranilamide and diaminobenzene compounds as inhibitors of FXa (Figure 14). Compound **69** (FXa K_{ass} ($\sim 1/K_i$) = 57.9 × 10^6 L/mol, K_i = 11.5 nM) was the most potent analogue disclosed in the anthranilamide series.[123] In a related diaminobenzene series, benzamidine **70** was highly active against FXa (K_{ass} = 250 × 10^6 L/mol), demonstrated good clotting activity ($aPTT_{2\times}$ = 0.67 μM, $PT_{2\times}$ = 0.96 μM), and was efficacious in rabbit and rat models of thrombosis.[124] Other diaminobenzene analogues devoid of the amidine moiety, such as **71** (FXa K_{ass} = 2.3 × 10^6 L/mol) and **72** (FXa K_{ass} = 100 × 10^6 L/mol), were also reported to have moderate FXa

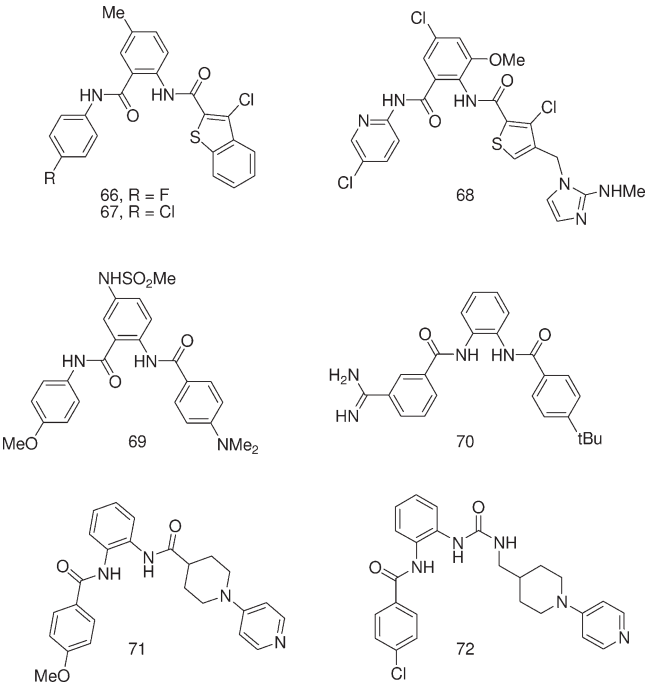


Figure 14. Anthranilamide and diaminobenzene FXa inhibitors.

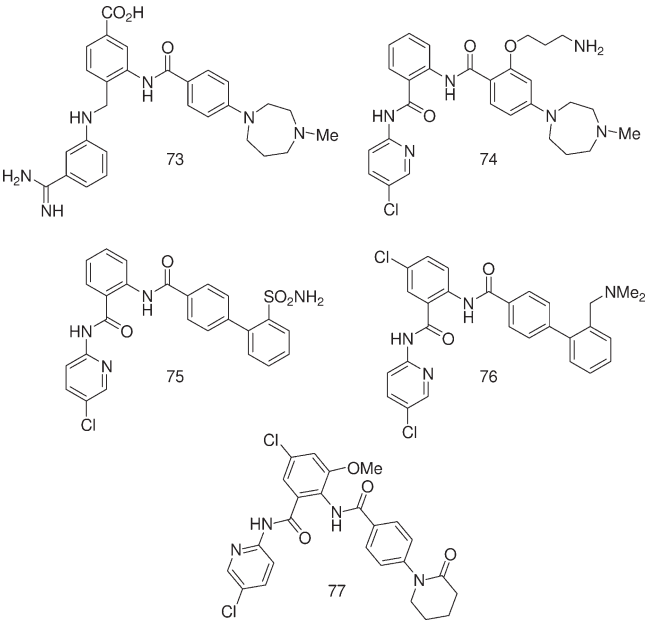


Figure 15. Additional anthranilamide-like FXa inhibitors.

activity.[125] A structurally related analogue **73** (FXa IC_{50} = 3.5 nM, $PT_{2\times}$ = 0.09 μM, Figure 15) with a 4-methyl-1,4-diazepane P4 moiety was reported by Astellas.[126] In a separate effort, the Lilly group disclosed **74** (K_{ass} = 8970 × 10^6 L/mol, $aPTT_{2\times}$ = 0.1 μM, $PT_{2\times}$ = 0.14 μM), which also contained the diazepane P4 group.[127]

Investigators at Portola successfully incorporated several biaryl P4 groups onto the anthranilamide scaffold, e.g., **75** and **76** (Figure 15).[128] Compound **76** (FXa K_i = 0.1 nM) showed excellent oral bioavailability in rats (F = 100%). These compounds had poor potency in the TG assay ($TG_{2\times}$ > 5 μM), owing to their high lipophilicity (cLogD > 4). Bristol-Myers Squibb scientists also reported on related anthranilamide analogues bearing the P4 lactam group of **37**, such as

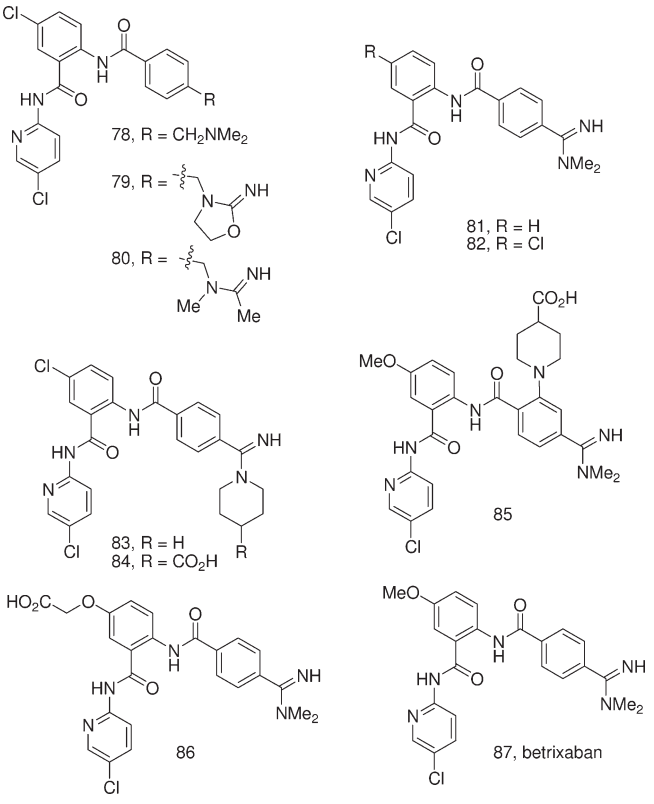


Figure 16. Anthranilamide FXa inhibitors related to **87**.

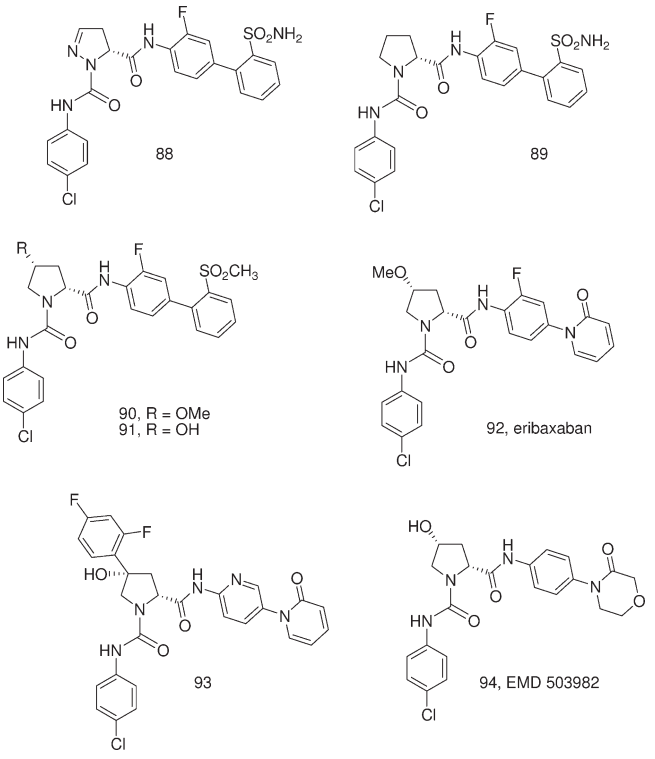


Figure 17. Proline-derived FXa inhibitors.

77 (FXa $K_i = 0.057$ nM), which displayed comparable efficacy to **37** in the rabbit AV shunt model and was orally bioavailable in dogs.[129]

To reduce the lipophilicity and to lower protein binding in **75** and **76**, the Portola group incorporated a polar aminoalkyl P4 moiety (Figure 16) to provide **78** (FXa $K_i = 1.3$ nM), which retained good FXa binding affinity with good activity in the TG assay ($TG_{2\times} = 3.2$ μM).[130] In rats, **78** had moderate clearance (Cl = 20.6 mL/min/kg), very high volume of distribution ($V_{dss} = 33$ L/kg), long half-life ($t_{1/2} = 7.5$ h), and high oral bioavailability ($F = 100\%$). Replacement of the dimethylamine moiety of **78** with a 2-iminooxazolidine group afforded **79** (FXa $K_i = 1.5$ nM, $TG_{2\times} = 0.56$ μM), which was also orally bioavailable ($F = 44\%$). The FXa affinity of these compounds was also retained in acyclic amidine compound **80** (FXa $K_i = 0.6$ nM, $TG_{2\times} = 0.5$ μM); however, oral exposure was greatly reduced ($F < 1\%$ in rats). The SAR of the anthranilamide series was next extended to include *N*,*N*-dialkylbenzamidine P4 groups (Figure 16).[131] Compound **81** (FXa $IC_{50} = 3$ nM, $TG_{2\times} = 0.54$ μM) demonstrated good binding affinity for FXa, as well as good potency in the TG assay. In rats and dogs, **81** demonstrated good oral bioavailability ($F = 31\%$ and 69%, respectively) and a moderate PK profile with a large volume of distribution. Other dialkylamidines and imidazolines in this series show similar properties.

While interest in these series was peaking, it was found that many of these benzamidine-containing compounds were potent hERG inhibitors.[132,133] Compounds **81** and **82** (FXa $K_i = 0.044$ nM), for example, both inhibited hERG with K_i values of 100 nM. Similarly, the piperidinylamidine analogue **83** was also a potent hERG inhibitor (hERG $K_i = 200$ nM). To circumvent the hERG issue, acidic groups were introduced as in **84**, **85**, and **86** to provide compounds with hERG $K_i > 10\,000$ nM; however, these zwitterionic species were no longer orally bioavailable. Ultimately, methoxy analogue **87** (betrixaban, PRT054021, Figure 16) was identified as having the best overall profile from this series and was chosen for advancement into clinical trials. Compound **87** has good binding affinity for FXa ($K_i = 0.12$ nM) and potency in a TG assay ($TG_{2\times} = 0.33$ μM) and has a lower affinity for the hERG channel (patch clamp hERG $IC_{50} = 8.9$ μM) compared with other lead compounds in this class.[133] Oral bioavailability was observed in rat, dog, and monkey ($F = 23.8\%$, 51.6%, and 58.7%, respectively), and **87** was efficacious in iv animal models of thrombosis.[134]

In a slightly different manner, the Pfizer group also applied a vicinal diamide strategy using five-membered nitrogen heterocyclic scaffolds and employing a chloroaniline P1 moiety (Figure 17). For example, the pyrazoline derivative **88** (FXa $IC_{50} = 8$ nM) incorporated a biarylsulfonamide P4 group, which provided good FXa-binding affinity.[135] Replacement of the pyrazoline with pyrrolidine resulted in a 2-fold drop in FXa binding affinity in **89** (FXa $IC_{50} = 18$ nM).[136] Incorporation of a cis-3-methoxy group on the proline moiety (analogous to the comparable substitution in the anthranilamide series described above) led to a substantial improvement in the FXa affinity in **90** (FXa $IC_{50} = 0.16$ nM) and good in vitro clotting activity ($PT_{2\times} = 1.7$ μM).[136] Compound **90** was permeable in the Caco-2 assay and was orally bioavailable in rats ($F = 28\%$) with a half-life of 2.4 h.[136,137] The desmethyl analogue **91** (FXa $IC_{50} = 0.38$ nM, $PT_{2\times} = 1.9$ μM) had modest oral bioavailability in rats ($F = 17\%$) and was active in a canine electrolytic injury model of thrombosis. Replacement of the pendent P4 sulfonylphenyl group with pyridone afforded **92** (eribaxaban, PD-0348292), a potent and selective inhibitor of FXa ($IC_{50} = 0.32$ nM) with excellent in vitro clotting activity ($PT_{2\times} = 0.58$ μM).[137] In dogs, **92** had low clearance (Cl = 2.5 mL/min/kg), low volume of distribution ($V_{dss} = 0.87$ L/kg), and moderate half-life ($t_{1/2} = 4.9$ h) and was orally bioavailable ($F = 41\%$). A similar

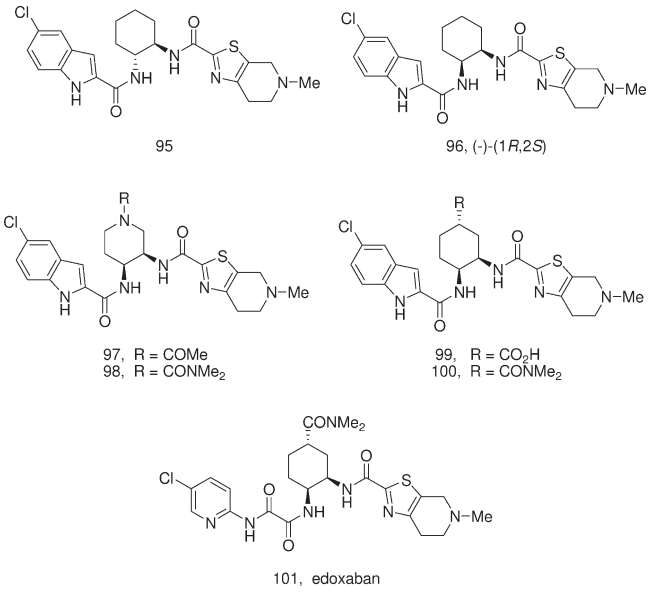


Figure 18. Evolution of **101**.

profile was also observed in rats, with improved oral exposure ($F = 82\%$). A dose-dependent inhibition of thrombus weight in the rabbit AV shunt model was observed with **92**, with an estimated oral EC_{50} value of 55 ng/mL. On the basis of the overall favorable profile exhibited, **92** was selected for clinical development[137] and has completed a phase II trial. According to dose−response modeling from this adaptive-design trial, the dose of **92** equivalent to enoxaparin 30 mg twice daily for the prevention of VTE in TKR patients was estimated to be 1.16 mg once daily.[138] Clinical development of **92** has since been discontinued.[139] The Pfizer group further explored the 3-substituted proline SAR with the intention of identifying compounds with a longer duration of action ($t_{1/2}$).[140] This effort yielded compound **93** (FXa $IC_{50} = 0.10$ nM), which displayed good in vivo activity, excellent PK in rats and dogs, including both low clearance and volume of distribution, a moderate to long half-life ($t_{1/2} = 12.6$ h in dogs), and modest oral bioavailability (F of 19% and 24% in rats and dogs, respectively). No further development has been reported for these analogues. Compound **94** (EMD-503982), a close analogue of **92**, in which the morpholinone P4 moiety of **65** was incorporated, was selected for development at Merck KGaA.[141] FXa affinity data have not been disclosed for **94**, and it is not clear whether this compound was advanced to clinical trials.

On a separate front, researchers at Daiichi Sankyo investigated vicinal substituted cycloalkyl scaffolds as potential mimics of the previously reported 1,2-diaminobenzene series (Figure 18).[142] Both cis- and trans-1,2-diaminocyclohexyl derivatives were explored using 5-chloroindole at the P1 position and a novel 5-methyl-4,5,6,7-tetrahydro-thiazolo-[5,4-*c*]pyridine group as the P4 moiety. Initial examples demonstrated comparable FXa binding affinity for both the trans-isomer **95** (FXa $IC_{50} = 13$ nM, $PT_{2\times} = 6.2$ μM) and the corresponding (−)-(1*R*,2*S*)-cis-isomer **96** (FXa $IC_{50} = 16$ nM, $PT_{2\times} = 2.9$ μM).[142] Good oral exposure was demonstrated in rats with **96**, as measured by ex vivo anti-FXa and anticoagulant activity up to 6 h after a 30 mg/kg oral dose. Compound **96** showed modest oral bioavailability in monkeys ($F = 6.1\%$) and poor stability in human liver microsomes (HLM; 46% remaining after 5 min incubation).

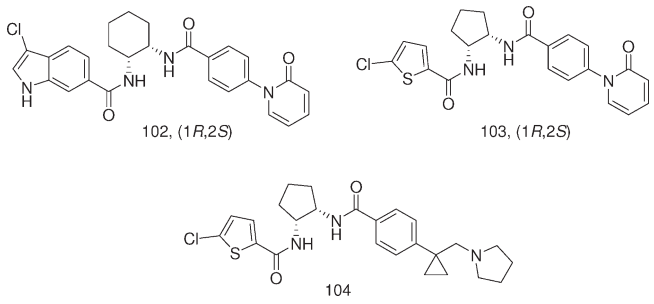


Figure 19. FXa inhibitors with vicinal cycloalkyl cores.

To address the microsomal instability, racemic 3,4-diaminopiperidine analogues were explored.[143] Good FXa binding affinity and potent clotting activity were observed with the *N*-acetyl compound **97** (FXa $IC_{50} = 8.6$ nM, $PT_{2\times} = 0.67$ μM). A similar in vitro profile was observed with the *N*,*N*-dimethylurea analogue **98** (FXa $IC_{50} = 8.4$ nM, $PT_{2\times} = 0.79$ μM). In the HLM assay, however, both compounds had a high turnover rate, similar to **96**. Introduction of a carboxylic group in the cyclohexyl scaffold led to **99** (FXa $IC_{50} = 7.5$ nM, $PT_{2\times} = 2.8$ μM), which was stable in HLM but not orally bioavailable, presumably because of poor intrinsic permeability.[144,145] Conversion of the acid to the corresponding *N*,*N*-dimethylamide provided **100** (FXa $IC_{50} = 2.8$ nM, $PT_{2\times} = 0.34$ μM),[145] which had slightly better affinity for FXa and significantly improved potency in the PT assay compared with **99**. In monkeys, **100** demonstrated moderate clearance (Cl = 12.4 mL/min/kg), moderate volume of distribution ($V_{dss} = 1.49$ L/kg), short half-life ($t_{1/2} = 1.5$ h), and good oral bioavailability ($F = 68\%$), the last being an indication of better liver microsome stability. An X-ray structure of **100** bound in FXa shows the chloroindole and the methyltetrahydrothiazolo[5,4-*c*]pyridine moieties in the S1 and the S4 pocket of the FXa enzyme, respectively. The compound was highly selective against most serine proteases and was active in animal models of thrombosis. Replacing the chloroindole P1 moiety of **100** with a 5-chloropyridin-2-yloxalamide group provided **101** (edoxaban, DU-176b).[146] Compound **101** is a potent inhibitor of human FXa in vitro (FXa $K_i = 0.56$ nM), with > 10 000-fold selectivity against relevant serine proteases, and demonstrated good anticoagulant activity ($PT_{2\times} = 0.26$ μM) and activity in various animal models of thrombosis, with minimal bleeding.[144,146] Significant FXa inhibition was observed in rat plasma 0.5 h after oral administration of **101** and was sustained up to 4 h. In cynomolgus monkeys, a rapid onset of FXa inhibition that peaked at 4 h after oral dosing and persisted at gradually decreasing levels out to 24 h was observed. Compound **101** is currently in phase II/III clinical trials (see section 4.2.3).

Researchers at Bristol-Myers Squibb also employed 1,2-diaminocycloalkyl scaffolds but incorporated a phenylpyridone P4 moiety in combination with various P1 groups (Figure 19). The cis-cyclohexyl compound **102** (FXa $K_i = 0.67$ nM, $PT_{2\times} = 3.2$ μM), with a chloroindole P1 moiety, and the cis-cyclopentyl analogue **103** (FXa $K_i = 0.43$ nM, $PT_{2\times} = 1.7$ μM), with the 5-chlorothiophene of **65** at P1, both displayed good affinity for FXa and were potent in the in vitro clotting assays.[147] An analogue of **103** with a basic substituent at the P4 position, **104** (FXa $K_i = 0.51$ nM, $PT_{2\times} = 2.1$ μM), also showed good binding affinity and in vitro anticoagulant activity.

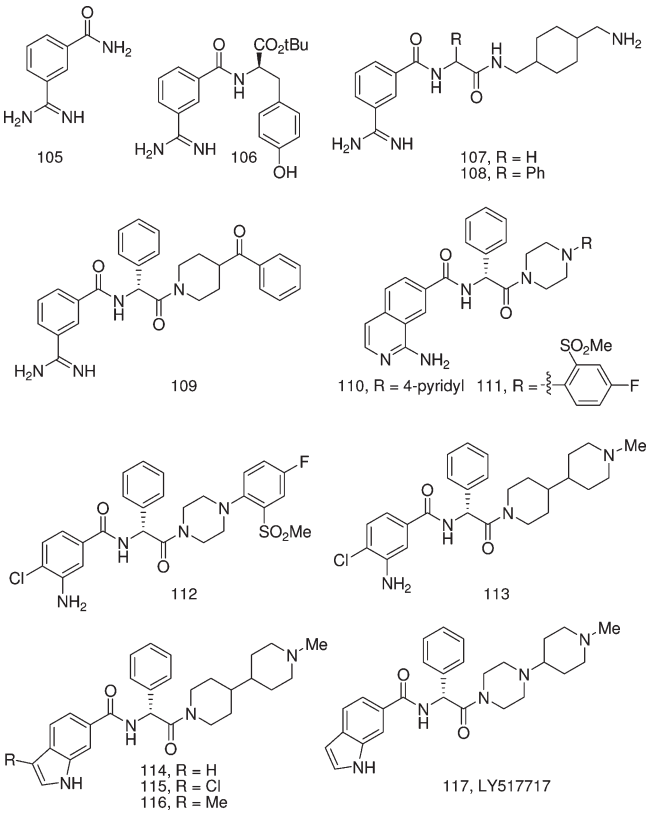


Figure 20. Design and optimization of phenylglycine FXa inhibitors leading to **117**.

2.4.4. D-Amino Acid Based Inhibitors. Investigators at Lilly used iterative structure-based screening of virtual chemical libraries coupled with a structure-based design approach in their search for FXa lead molecules.[148] To simplify the library design, the initial effort focused on retaining the benzamidine P1 moiety because of its strong binding affinity with Asp189 in the S1 region. Libraries generated off the 3- and 4-position of benzamidine were evaluated, and it was quickly realized that the meta-substituted compounds provided the best fit. A set of hydrophobic and polar S4 substituents was selected and attached to the benzamidine via an appropriate linker (Figure 20). Compared with 3-carbamoylbenzamidine, **105** (FXa K_i = 250 000 nM), the tyrosine derivative **106** had a FXa K_i = 16 000 nM. The derivatized glycine analogue **107** (FXa K_i = 11 000 nM) also had weak FXa binding affinity. A virtual screen of other amino acid derivatives suggested that D-phenylglycine analogues, wherein the phenyl ring of the amino acid was in proximity to the Cys190−Cys220 disulfide bridge, were preferred. This was confirmed by evaluation of the enantiomers of structure **108** where the D-isomer had 5- to 6-fold better affinity for FXa (K_i = 210 nM) compared with the corresponding L-isomer (FXa K_i = 1100 nM). A 15-fold improvement in FXa-binding affinity was achieved with 4-benzoylpiperidine analogue **109** (FXa K_i = 16 nM). Further optimization included replacing the benzamidine of **109** with an aminoisoquinoline in **110** (FXa K_i = 100 nM), which showed reduced FXa binding affinity. Various P4 replacements were then explored while maintaining the aminoisoquinoline at the P1 position. The 4-fluoro-2-methylsulfonylphenylpiperazine analogue, **111** (FXa K_i = 3 nM), showed improvement in FXa binding affinity, with greatly improved selectivity over trypsin compared with earlier benzamidine compounds (trypsin K_i = 11 000 nM). This compound was orally bioavailable in rats (F = 27%) and demonstrated oral antithrombotic activity in a rat thrombosis model. The aminoisoquinoline in the P1 position of **111** was successfully replaced by an *o*-chloroaniline substituent in **112** (FXa K_i = 2 nM). This P1 substitution was also compatible with a 4-*N*-methylpiperidinylpiperidine P4 moiety in compound **113** (FXa K_i = 4 nM). By use of this basic P4 moiety, a neutral 6-indolyl P1 group could be installed without a significant drop in FXa affinity (**114**, FXa K_i = 4 nM). Substitution at the C3 position of the indole with either chlorine or methyl provided enhanced binding affinity in compounds **115** (FXa K_i = 0.7 nM) and **116** (FXa K_i = 0.9 nM). The piperazine analogue of **114**, compound **117** (LY517717, FXa K_i = 4.6−6.6 nM, $aPTT_{1.5\times}$ = 0.46 μM),[149] was advanced into clinical development. Compound **117** is 1000-fold more selective for FXa over other serine proteases and displays good aqueous solubility. Oral bioavailability was observed in rats and dogs (F = 25−82%),[150] with plasma half-life of 7−10 h. In a rat AV shunt model, **117** had an ED_{50} value of 5−10 mg/kg po.[149] Clinical development of **117** has since been discontinued.[151]

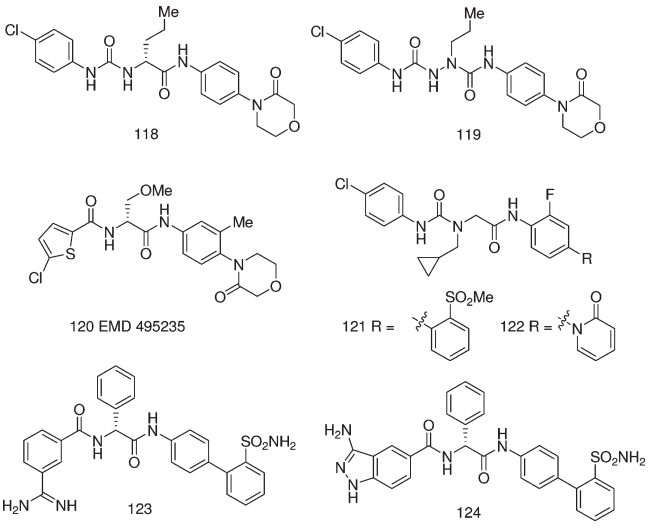


Figure 21. Amino acid derived FXa inhibitors.

Using D-norvaline as a scaffold, researchers at Merck KGaA incorporated a urea-linked *p*-chlorophenyl P1 moiety and the phenylmorpholinone P4 group (Figure 21) to arrive at **118** (FXa IC_{50} = 5.6 nM).[152] The corresponding aza analogue **119** (FXa IC_{50} = 85 nM), which lacks the chiral center, showed reduced FXa affinity.[153] Amide-linked P1 analogues were also investigated in combination with a variety of aliphatic D-amino acid templates and substituted phenylmorpholinone P4 groups.[152] This effort provided a potent FXa inhibitor derived from (D)-*O*-methylserine, compound **120** (EMD-495235, FXa IC_{50} = 5.5 nM, $aPTT_{2\times}$ = 1 μM, and $PT_{2\times}$ = 1 μM), which was orally active in rats, dogs, and monkeys (F = 60%, 60%, and 80%, respectively).[152] Clearance of **120** in rats and dogs was moderate (0.94 and 1.3 L/h/kg, respectively), but low in monkeys (0.25 L/h/kg).

In a similar manner, investigators from Pfizer identified compounds **121** and **122**,[154] containing biarylsulfone and phenylpyridone as the P4 moieties, respectively (Figure 21). Similar FXa binding affinity (IC_{50} = 4−5 nM) was seen with these entities; however, **122** ($PT_{2\times}$ = 1.7 μM) was more

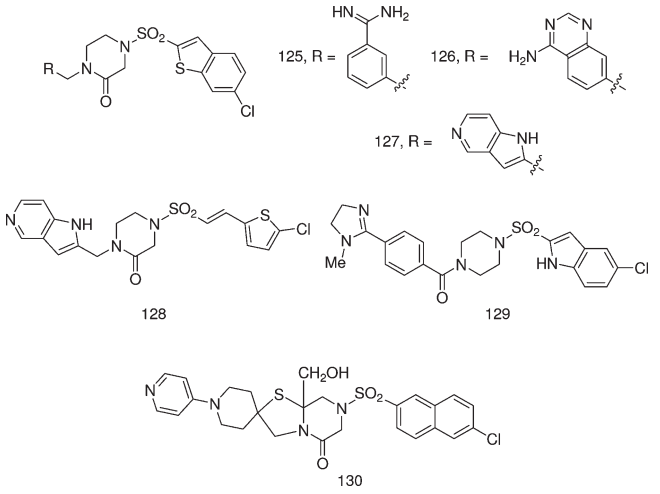


Figure 22. Ketopiperazine FXa inhibitors.

potent in the clotting assay compared with **121** ($PT_{2\times}$ = 7.6 μM). In a rat PK/PD model, **122** exhibited good oral exposure with short apparent half-life as measured by ex vivo FXa inhibition and plasma concentration 4−6 h after oral gavage. The Portola group investigated both amidine and amidine replacements at the P1 position, including 2-aminoisoquinoline, 3-aminobenzisoxazole, and 3-aminoindazole moieties.[155] The amidine analogue **123** (FXa IC_{50} = 35 nM) was 100-fold more active than the aminoindazole analogue, **124** (FXa IC_{50} = 3450 nM). No further advancement of these templates was reported by either research group.

2.4.5. Other Scaffolds. An extensive effort at Sanofi-Aventis to find an alternative to the aminopyrrolidinone scaffold resulted in the identification of ketopiperazine as a suitable replacement (Figure 22).[44] Compound **125** from this series, with an amidine as the P1 moiety, demonstrated potent affinity for FXa (K_i = 4 nM), with good selectivity relative to other serine proteases (thrombin K_i > 4000 nM, trypsin K_i = 1200 nM). The corresponding 4-aminoquinazoline analogue, **126** (FXa K_i = 0.8 nM, $aPTT_{2\times}$ = 12 μM), showed improved FXa activity but was less potent in the in vitro clotting assay. Incorporation of the 5-azaindole P1 group in **127** (FXa K_i = 4 nM, $aPTT_{2\times}$ = 2 μM) provided improved potency in the in vitro clotting assay while maintaining good FXa binding affinity. In dogs, **127** showed good oral bioavailability (F = 46%); however, the half-life was short ($t_{1/2}$ = 0.6 h). Incorporation of the (*E*)-2-(5-chlorothiophen-2-yl)ethenesulfonamide substituent to afford **128** (FXa K_i = 1.1 nM, $aPTT_{2\times}$ = 0.6 μM) further increased binding affinity and potency in the aPTT assay. The compound was efficacious in various animal models of thrombosis and, when dosed in dogs at 5 mg/kg, showed good oral bioavailability (F = 97%) but a short half-life ($t_{1/2}$ = 52 min). Separately, Portola investigators installed an *N*-2-imdazolinylphenyl P4 group in combination with 5-chloroindole at the P1 position to arrive at **129** (FXa K_i = 1.9 nM, Figure 22), which also possessed good activity in a TG assay ($TG_{2\times}$ = 1.3 μM) and efficacy in a rabbit DVT model.[156] The Mochida group reported novel fused spirocyclic piperazinone compound **130** (FXa IC_{50} = 1.2 nM, Figure 22), which was efficacious in a rat thrombosis model.[157]

Other structurally diverse compounds (Figure 23) include cyanoguanidine **131** (BMS-269223, FXa K_i = 6.5 nM, $PT_{2\times}$ = 30 μM)[158] and aroylguanidine **132** (BMS-344577,

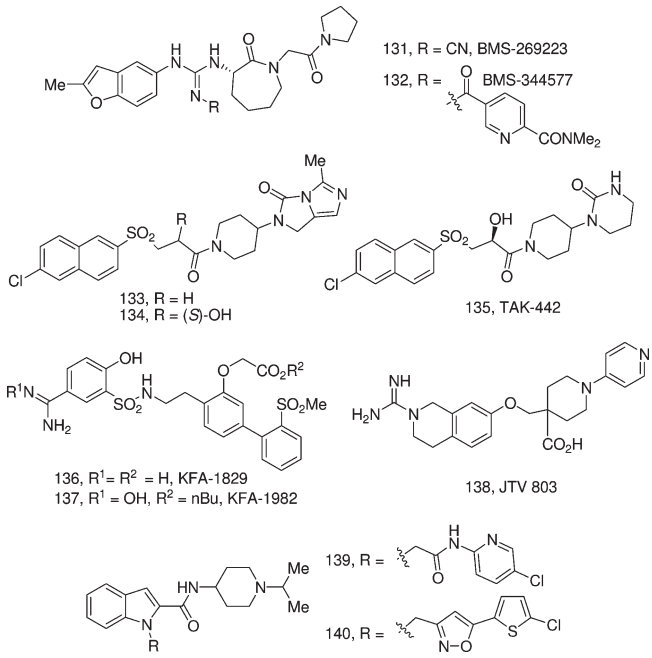


Figure 23. Other structurally diverse FXa inhibitors.

FXa IC_{50} = 9 nM, $PT_{2\times}$ = 2.5 μM) reported by researchers at Bristol-Myers Squibb.[159] Compound **131** showed good oral bioavailability in rats, dogs, and monkeys (F = 50%, 77%, and 49%, respectively). Similar oral bioavailability was demonstrated for **132** (F = 55%, 77%, and 28% in rats, dogs, and monkeys, respectively). Both compounds were efficacious in iv rat models of venous and arterial thrombosis. Ethylsulfone **133** (FXa K_i = 2 nM, $PT_{2\times}$ = 1.0 μM) from Takeda also showed good oral bioavailability in rats (F = 24%) and monkeys (F = 46%).[160] The hydroxy analogue **134** (FXa K_i = 0.5 nM, $PT_{2\times}$ = 0.92 μM) was identified as an active metabolite of **133**.[161] This hydroxyl substitution was also employed in **135** (TAK-442, FXa IC_{50} = 3.5 nM, $PT_{2\times}$ = 0.58 μM), wherein the 5-methyl-1,2-dihydro-3*H*-imidazo-[1,5-*c*]imidazol-3-one P4 moiety was replaced with a tetrahydropyrimidin-2(1*H*)-one.[162] Compound **135** is orally bioavailable in monkeys (F = 52.5%) with low clearance (Cl = 708 mL/h/kg) and volume of distribution (V_{dss} = 579 mL/kg) and a mean residence time of 6.96 h. The compound was efficacious in an iv rabbit venous thrombosis model and did not prolong bleeding times.[163] Compound **135** is currently in phase II clinical trials. Sulfonamide **136** (KFA-1829, FXa K_i = 5 nM, $PT_{2\times}$ = 1.0 μM), an earlier compound from Kissei, was advanced into clinical trials as a double prodrug, KFA-1982 (**137**).[164] The prodrug had modest oral bioavailability in multiple species (F = 4.9%, 11%, 23%, and 15% in dogs, monkeys, rabbits, and mice, respectively). Compound **138** (JTV-803, FXa K_i = 19 nM)[165] from Japan Tobacco showed dose-dependent efficacy in an iv venous thrombosis model in rats, as well as oral exposure in monkeys as measured by ex vivo inhibition of FXa, and was selected as a candidate for further studies. Sanofi-Aventis has described indole carboxamides **139** (FXa K_i = 1 nM)[166] and **140** (FXa K_i = 3 nM),[167] with good binding affinity for FXa. It was recently reported that a compound from this class, AVE-3247, was advanced into clinical development.[168] YM150 (Astellas, structure undisclosed) is currently in mid- to late-stage clinical trials in multiple indications. In preclinical studies, YM150 inhibited FXa

Table 2. Preclinical Characterization of Oral Direct FXa Inhibitors[a]

	rivaroxaban (**65**)	apixaban (**37**)	edoxaban (**101**)	betrixaban (**87**)	YM150 (structure undisclosed)	TAK-442 (**135**)
company	Bayer, Johnson & Johnson	Bristol-Myers Squibb, Pfizer	Daiichi Sanyko	Portola Pharmaceuticals, Merck & Co.	Astellas Pharma	Takeda
current clinical trial status	III[b]	III	III	II	III	II
MW (Da)	436	460	NR	452	NR	NR
in vitro activity						
FXa K_i (nM)	0.4[118]	0.08[101]	0.56[146]	0.12[170]	31[169]	1.8[163]
clotting assays (μM)	$PT_{2\times}$ = 0.23; $aPTT_{2\times}$ = 0.69[118]	$PT_{2\times}$ = 3.8; $aPTT_{2\times}$ = 5.1[101]	$PT_{2\times}$ = 0.26; $aPTT_{2\times}$ = 0.51[146]	$PT_{2\times}$ = 0.4[134]	$PT_{2\times}$ = 1.2[169]	$aPTT_{2\times}$ = 0.59[163]
preclinical efficacy/ bleeding studies	rabbit and rat models of thrombosis and bleeding[118,171]	rabbit and rat models of thrombosis and bleeding[102,172,173]	rabbit and rat models of thrombosis and bleeding[146]	baboon, rabbit, and rat models of thrombosis and bleeding[134,170]	monkey, rabbit, and rat models of thrombosis and bleeding[169,174]	rabbit and rat models of thrombosis and bleeding[163]
			Preclinical Pharmacokinetics			
oral bioavailability	57−66% (rat), 60−86% (dog)[119]	51% (chimpanzee), 88% (dog), 34% (rat)[175]	50% (monkeys)[176]	23.8% (rat), 51.6% (dog), 58.7% (monkey)[133]	NR	NR
volume of distribution	0.3 L/kg (rat), 0.4 L/kg (dog)[119]	0.17 L/kg (chimpanzee), 0.29 L/kg (dog), 0.31 L/kg (rat)[175]	NR	32.9 L/kg (rat), 48.8 L/kg (dog), 13.4 L/kg (monkey)[133]	NR	NR
protein binding	99% (rat), 90% (dog)[119]	95% (chimpanzee), 92% (dog), 96% (rat)[175]	NR	NR	NR	NR
systemic clearance	0.4 L/h/kg (rat), 0.3 L/h/kg (dog)[119]	0.018 L/h/kg (chimpanzee), 0.052 L/h/kg(dog), 0.26 L/h/kg (rat)[175]	NR	43.6 mL/min/kg (rat), 26.5 mL/min/kg (dog), 18.7 mL/min/kg (monkey)[133]	NR	NR
elimination (% of dose)	fecal 67%, renal 25% (rat) fecal 43%, renal 52% (dog)[177]	fecal > 54%, renal < 15% (mouse, rat, rabbit, dog)[178]	NR	NR	NR	NR

[a] aPTT, activated partial thromboplastin time; $aPTT_{2\times}$ and $PT_{2\times}$, concentration required to prolong clotting time 2-fold; K_i, inhibition constant; NR, not reported; PT, prothrombin time. [b] Rivaroxaban is approved in Canada, Europe, and other countries outside the United States for the prevention of VTE in adults after hip or knee replacement surgery; phase III trials of rivaroxaban in other indications are ongoing.

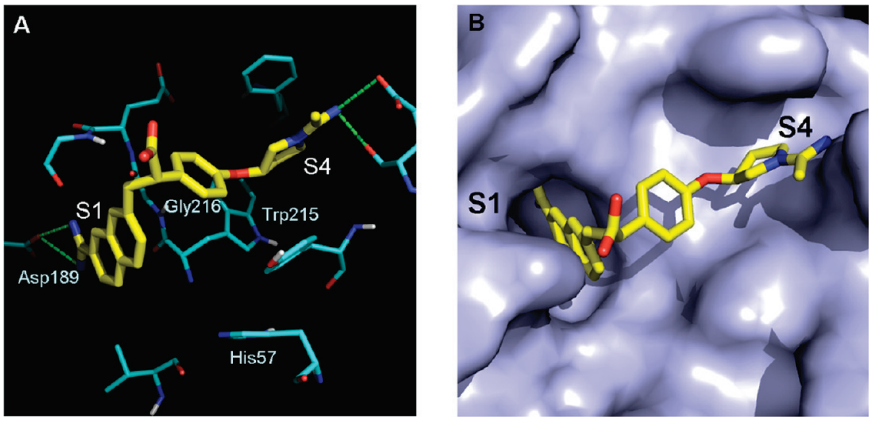


Figure 24. (A) Stick representation of **3** bound to FXa.[180,181] (B) Same structure in which the protein is rendered with a solvent-accessible surface.

Table 3. Homology of S1 Pockets across Serine Proteases Involved in the Coagulation Cascade[a]

enzyme	189	190	191	192	193	194	195	213	218	220
factor Xa	Asp	**Ala**	Cys	**Gln**	Gly	Asp	Ser	Val	Gly	Cys
factor XIa	Asp	**Ala**	Cys	**Lys**	Gly	Asp	Ser	Thr	Gly	Cys
thrombin	Asp	**Ala**	Cys	**Glu**	Gly	Asp	Ser	Val	Gly	Cys
factor VIIa	Asp	**Ser**	Cys	**Lys**	Gly	Asp	Ser	Val	Gly	Cys
factor IXa	Asp	**Ser**	Cys	**Gln**	Gly	Asp	Ser	Ile	Glu	Cys
trypsin	Asp	**Ser**	Cys	**Gln**	Gly	Asp	Ser	Val	Gly	Cys

[a] The S1 pocket is well conserved among trypsin-like serine proteases, with the notable exceptions of residues at the 190 and 192 positions (in boldface).

with a K_i value of 31 nM and prolonged PT and aPTT in human plasma. The compound was efficacious in animal models without affecting bleeding times.[169]

Preclinical data for the oral FXa inhibitors that have advanced into clinical trials and are still being actively developed are summarized in Table 2.

3. Structure and Molecular Modeling

To date, over 90 crystallographic structures involving FXa are known to have been solved, reflecting the integral role that structure-based modeling and design have played in the discovery of FXa inhibitors. In this section, we describe how molecular modeling was employed to guide development of oral FXa inhibitors. Figure 24A and Figure 24B depict the first crystallographic structure of a small molecule, **3**, bound to FXa.[179,180] This early structure exemplifies many key features involved in FXa−drug-molecule binding. The naphthylamidine P1 is positioned in the S1 subsite, a deep, largely hydrophobic recess formed by the backbone atoms of Ala190−Gly192, Ser214−Gly218, the side chains of Asp189, Val213, and Tyr228, and the Cys191−Cys220 disulfide bridge. The basic amidine forms a salt bridge interaction with the Asp189 side chain in a manner similar to the peptide substrate.

The S1 pocket is well conserved among trypsin-like serine proteases, with the notable exceptions of residues at the 190 and 192 positions (Table 3), which can influence ligand selectivity.[182] In FXa, as with thrombin and FXIa, alanine occupies position 190, creating a slightly larger and more lipophilic pocket than the so-called "serine 190" serine proteases such as FVIIa and FIXa. Position 192 is more variable across the coagulation serine proteases. Compound **3** provides a striking example of 192-dependent selectivity, where a

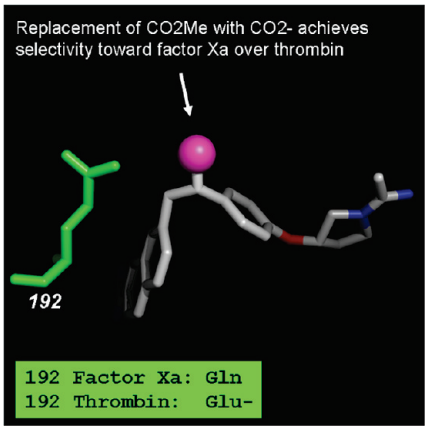


Figure 25. Residue at position 192 of serine proteases can be an important determinant of selectivity, as was demonstrated by the SAR of an early Daiichi Sankyo inhibitor series.[180,181]

high degree of selectivity for FXa over thrombin is achieved, presumably arising from electrostatic repulsion of the ligand CO_2^- with the Glu192 side chain. The corresponding methyl ester is nonselective (Figure 25).

The phenoxy moiety of **3** lies over the Trp215−Gly216 backbone and is partially inserted into the S4 pocket. Gly216 NH appears to be desolvated, a phenomenon that is commonly observed in other FXa−small-molecule complexes. The expected desolvation penalty is possibly offset by attractive electrostatic interactions resulting from the complementary alignment of the Gly216 NH and Gly216 CO dipoles.

The basic P4 group is engaged in complex interactions in S4, which is defined by the aromatic side chains of Tyr99, Trp215, and Phe174 and the backbone atoms of Thr98−Tyr99. Friesner et al. have described subsites such as the FXa S4 as "hydrophobic enclosures", that is, deep clefts in the protein surface where water molecules are particularly unfavored enthalpically and entropically.[183,184] Displacement of such waters can lead to very significant gains in the free energy of ligand binding. Hydrophobic groups avidly bind in the S4 pocket, but organocationic groups bind as well, some examples being quaternary nitrogen, 4-aminopyridine, amidines, and imidazoles.[185] Cheney and Mason used quantum chemical methods to elucidate the relative electrostatic potentials of the S4 subsites of FXa, trypsin, and thrombin and showed a relatively large negative potential that is unique to FXa[186] but absent in thrombin, which is not known to bind organocations (Figure 26).

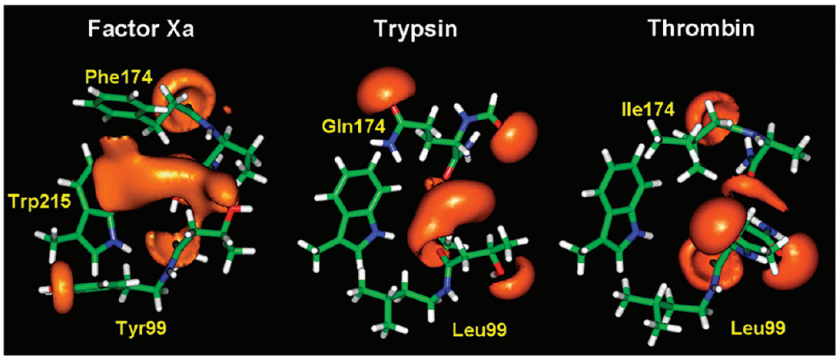


Figure 26. Quantum chemically derived electrostatic potentials of S4 subsites of FXa, trypsin, and thrombin.[186−188]

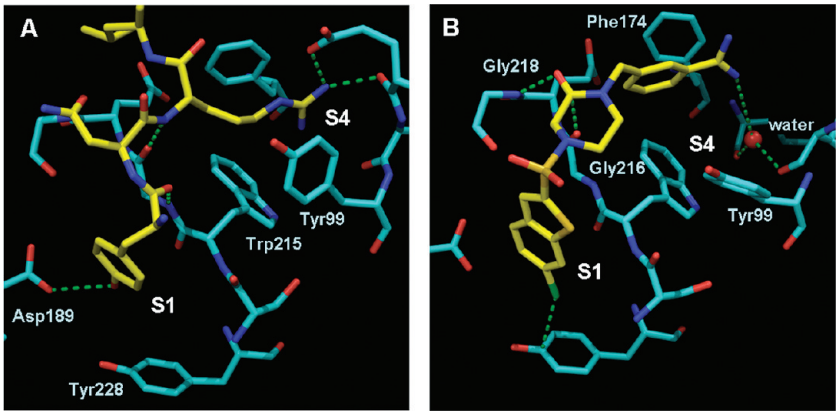


Figure 27. (A) TAP−FXa complex (3.0 Å).[190] (B) Sanofi-Aventis inhibitor (**125**) bound to FXa (2.1 Å).[191]

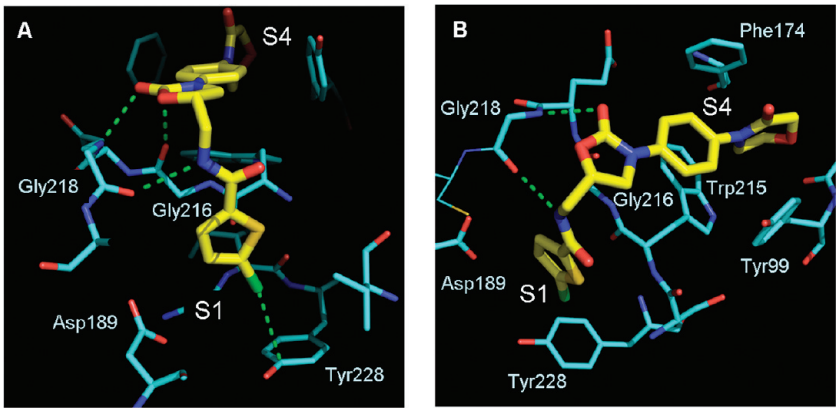


Figure 28. Crystallographic structure of **65** bound to FXa:[181,194] (A) view detailing key intermolecular interactions in the S1 pocket of FXa (2.08 Å); (B) overview of **65** bound in FXa.

The basic P4 moiety of **3** appears to engage in cation−π interactions with the aryl side chains of Phe174 and Tyr99 and forms a hydrogen bond with the backbone carbonyl of Glu97.

The salt bridge interaction between Asp189 and the P1 amidine of **3** characterizes first-generation thrombin and FXa inhibitors and was rationalized on the basis of mimicking the substrate P1 arginine side chain. However, in 1998, Lumma and colleagues reported the discovery of potent thrombin inhibitors that incorporated nonbasic P1 groups.[189] In the same year, the crystal structure of TAP bound to FXa was reported, in which the N-terminal tyrosine side chain unexpectedly bound in S1 and an arginine side chain bound in S4 (Figure 27A).[190] Together, these findings challenged the convention whereby a basic P1 was required for strong binding affinity, and enabled the discovery of second-generation trypsin-like serine protease inhibitors containing neutral P1s with improved PK properties, for thrombin and FXa, respectively. The unexpected manner of binding observed in the TAP−FXa crystal structure (i.e., a neutral group in S1 and basic group in S4) was mirrored in a subsequent report[191] by scientists at Sanofi-Aventis of a crystallographic structure (Figure 27B) in which the intended P1 benzamidine was bound in the S4 pocket and the P4 aryl halogen was bound in S1, displacing a water above Tyr228 in a manner very similar to the thrombin inhibitors described earlier by Merck.[189] The amidine group of **125** is positioned at the solvent edge of S4 and forms a hydrogen bond to a structural water to the rear of the pocket. The ketopiperazine carbonyl forms a pair of contacts involving a strong hydrogen bond with Gly218 NH and a T-shaped carbonyl−carbonyl

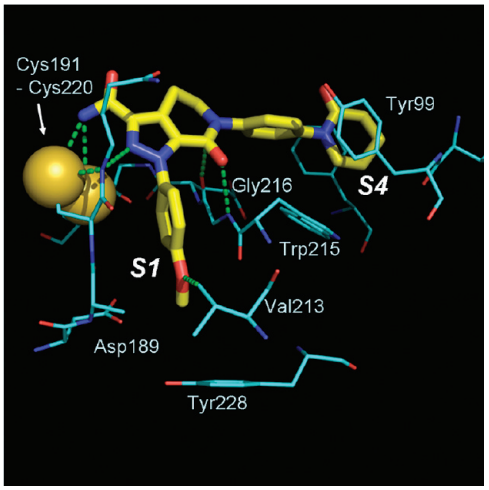


Figure 29. Crystallographic structure of **37** bound to FXa (2.3 Å).[101,181,196]

interaction with Gly216. This latter interaction has been observed in several FXa−small-molecule complexes involving diverse chemotypes and its geometry likened to the trajectory of nucleophilic attack on carbonyl groups.[192,193]

Figure 28 depicts the crystallographic structure of **65** bound to FXa.[117,118,194] Arylhalogen binding[189] is observed in the S1 pocket, while the central core engages in interactions very similar to the ketopiperazine ring of **125** in Figure 27B. The phenylmorpholinone group engages in extensive hydrophobic interactions in the S4 pocket. Crystal structures of **65** and other second-generation FXa inhibitors suggest that effective FXa−drug molecule binding is largely a hydrophobically driven process. A detailed statistical mechanical analysis of the hydration of the FXa binding site using lengthy molecular dynamic simulations showed that enthalpically and entropically disfavored waters coincide closely with known high-affinity subsites, such as S4 and much of S1, particularly the water site above Tyr228.[195]

Figure 29 depicts the crystallographic structure of **37** in FXa at 2.3 Å resolution in which the anisole ring is bound in the S1 subsite, displacing the water characteristically located above Tyr228.[101,196] The methoxy group forms contacts with the Val213 side chain. High-level quantum chemical calculations suggest a weak hydrogen bonding potential of the oxygen of anisole, suggesting this oxygen to be relatively lipophilic.[197,198] The identities of the two heteroatoms of the amide are not discernible at the level of resolution (2.3 Å) but in this case can be deduced from the nature of the attached pyrazole ring. Thus, the amide N−H is estimated to be about 2.5 and 2.8 Å away from the Cys191Sγ and Cys220Sγ, respectively. Given the relatively large van der Waal radius for sulfur (1.8 Å vs 1.52 Å for oxygen and 1.55 Å for nitrogen), these can be regarded as weak to moderate contacts. There is some hesitancy in characterizing these as hydrogen bonds. A recent review of high-resolution protein structures in the Protein Data Bank (PDB) concluded that such contacts with disulfide bridges and methionines occur infrequently, suggesting that the interaction is not strong.[199] Although the pyrazole nitrogen atom is positioned in proximity to Gln192 backbone NH, the geometry is suboptimal for hydrogen bonding. However, a contact between this nitrogen and Cys220Sγ is observed (3.5 Å). The Merck group observed a very similar contact between a tetrazole nitrogen and the thrombin Cys191−Cys220 disulfide and postulated a

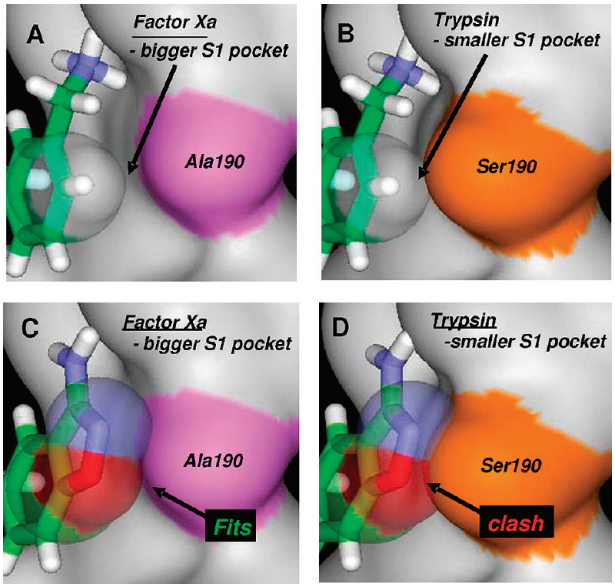


Figure 30. Structure-based design of selective FXa inhibitors. Parts A and B depict the S1 region of the crystallographic structures of **29** bound in factor Xa and trypsin, respectively.[92,204,205] Part C depicts the crystallographic structure of **31** in factor Xa.[29,92] Part D is a model portraying the steric clash **31** encounters in the trypsin S1 pocket.[93,206]

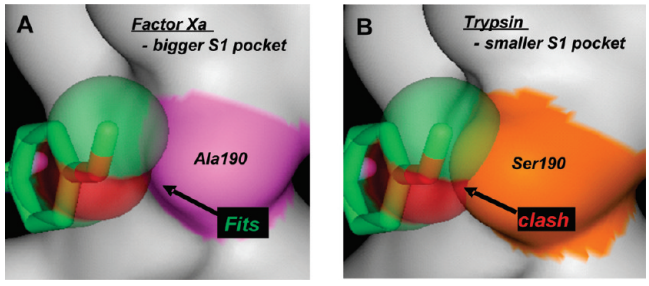


Figure 31. Comparison of **37** in a crystallographic structure bound to FXa and in a docked model involving trypsin.[206]

polar interaction, citing computational work by Rotello to support their theory.[200,201] Diederich and Iwaoka performed a detailed statistical analysis of experimental structural data and showed that this contact likely involves favorable interactions.[193,202]

The lactam oxygen of the central bicyclic core of **37** forms a hydrogen bond to Gly216 NH. As with the ketopiperazine **125** (Figure 27B) and oxazolidinone **65** (Figure 28), a T-shaped carbonyl−carbonyl interaction is observed between the ligand and Gly216.[193] The P4 *N*-phenyl lactam moiety binds deep in the S4 pocket, with the plane of the lactam amide stacking with the aryl rings of Phe174 and Tyr99, the edge of the lactone ring forming an edge-to-face interaction with Trp215, and the carbonyl solvent exposed. The very high binding affinity to FXa of **37** and related compounds (subpicomolar in some cases) is likely due in part to the low internal entropy of binding resulting from the rigidity of these compounds. Conformational analysis using the parameters of Boström et al.[203] shows no more than eight distinct and energetically viable conformations of **37**, one of which is closely related to the bound conformation.

The evolution of **37** exemplifies how insights into the comparative structures of serine protease S1 pockets were utilized to design highly selective FXa inhibitors. Figure 30A

and Figure 30B depict crystallographic structures of **29** bound to S1 pockets of trypsin and FXa[92,204,205] and provide a clear rationale for the poor selectivity of **29** for FXa over trypsin: the benzylamine P1 of **29** is easily accommodated in both S1 pockets. Molecular modeling,[206] on the other hand, suggests that the aminobenzisoxazole ring of **31** fits poorly into the S1 pocket of trypsin, clashing with the side chain of Ser190 and resulting in improved selectivity (40 000-fold) for FXa (Figure 30C and Figure 30D). Similarly, the putative clash of the anisole P1 of **37** in trypsin S1[206] results in a 20 000-fold increase in selectivity for FXa (Figure 31).

4. Clinical Investigations of Oral FXa Inhibitors

Numerous clinical studies of the oral FXa inhibitors have been performed or are ongoing. In this section, we review key published clinical data for those agents that are under active development in phase II or III trials or have already entered clinical practice in some countries. For details of individual ongoing trials see www.clinicaltrials.gov.

4.1. Clinical Pharmacology. Early phase clinical trials have provided detailed information on the PK and PD characteristics of rivaroxaban and apixaban in humans; relatively limited data are also available for other oral FXa inhibitors (Table 4).

4.1.1. Early Phase Clinical Trials with Rivaroxaban (65). In healthy subjects, **65** dose dependently inhibited FXa, with maximum effects occurring 1−4 h after administration.[207,208] Compound **65** also inhibited thrombin generation induced by collagen or tissue factor in a single-dose phase I study.[209] Clinical PK studies have shown that **65** is rapidly absorbed, with C_{max} occurring 2−4 h after oral administration.[207,208] The terminal half-life of **65** in healthy young subjects is 5−9 h.[207,208] During multiple-dosing studies, a dose-proportional PK profile was observed and steady-state conditions were achieved without significant drug accumulation.[208] The population PK profiles of **65** in healthy subjects and in patients undergoing THR are predictable and dose-dependent and are well described by two- and one-compartment models, respectively.[210,211]

In humans, approximately 66% of the total dose of **65** is excreted through the kidneys, either as the parent compound or as various metabolites, and the remainder is excreted in the feces as unchanged drug.[177] Compound **65** should be used with caution in patients with severe renal impairment and in some patients with moderate renal impairment; use of **65** is not recommended in patients with creatinine clearance of <15 mL/min.[212] This agent is also contraindicated in patients with hepatic disease associated with coagulopathy and clinically relevant bleeding risk.[212] Administration of **65** with food delays the time at which C_{max} is reached (T_{max}) by 1.3 h and increases drug exposure by 30−40%.[213] Studies to date suggest that **65** has a low propensity for drug interactions;[120] use of **65**, however, is contraindicated in patients receiving strong inhibitors of both CYP3A4 and P-glycoprotein (e.g., ketoconazole).[120]

4.1.2. Early Phase Clinical Trials with Apixaban (37). Single- and multiple-dose studies in healthy male subjects have shown that the PK and PD profiles of **37** in humans are consistent and predictable.[214,215] In these studies, clotting times increased in proportion to the dose of **37**. Compound **37** is rapidly absorbed after oral administration, with C_{max} reached 1.5−3.5 h after dosing and a reported half-life of 8−15 h.[214,215] In a multiple-dose study, dose-proportional increases in drug exposure were observed and steady-state plasma concentrations of **37** were achieved after 3 days, with only mild drug accumulation.[215] As expected, lower peak-to-trough concentration ratios were observed with twice-daily versus once-daily dosing.

Compound **37** is excreted by multiple elimination pathways in humans. Renal and fecal elimination account for 25−29% and 47−56% of excretion of **37**, respectively.[216] Unchanged **37** is the major circulating component, although several metabolites are produced.[216] It is postulated that multiple elimination pathways may allow a drug to be used without dose reduction in patients with renal impairment; a PK study of **37** in patients with renal impairment is ongoing. Systemic clearance of **37** is low, as predicted by preclinical PK studies.[175] Compound **37** has a low volume of distribution, suggesting that it is mainly distributed at the site of therapeutic action, the blood.[5,175] The PK of compound **37** in patients undergoing knee replacement surgery has been described by a one-component linear model with first-order absorption.[217]

Exposure to **37** is not affected by food (although time to C_{max} is delayed), and the potential for drug interactions with this agent is low.[120,175,218−220] For example, **37** has no effect on the PK of digoxin, a drug frequently administered to patients with AF.[219] Similarly, the histamine H_2-receptor antagonist famotidine does not influence the PK of **37**.[221] Exposure to **37** is affected by strong inhibitors or inducers of CYP3A4 and P-glycoprotein, although available data suggest it is not a sensitive CYP3A4 substrate.[222,223]

4.1.3. Early Phase Clinical Trials with Other Oral FXa Inhibitors in Development. Of the oral FXa inhibitors in the earlier stages of clinical development, most data are available for **101**. In healthy subjects, single doses of **101** inhibited thrombin generation and ex vivo thrombus formation[224] and were associated with rapid and sustained inhibition of coagulation.[225] The effects of food on the PK and PD of **101** are minimal.[226] Published data for other compounds are scarce, although **87** is reported to possess good oral bioavailability (47%), a long elimination half-life (19 h), minimal renal excretion, and a low potential for drug−drug interactions.[120,227] Reports state that YM150 displays predictable PK and PD in humans,[228] but detailed data have not been published.

4.2. Phase II and Phase III Clinical Trials. Phase II and phase III clinical development of new anticoagulants generally begins with assessment of effectiveness for VTE prevention in patients undergoing THR or TKR. Rates of VTE after these orthopedic procedures are high, and therapy is usually short (∼2−5 weeks); as a result, fewer patients and a relatively short duration of drug exposure are required to assess anticoagulant efficacy. In addition, patients remain in the controlled environment of the hospital for the first few days after surgery, where potential adverse events such as bleeding can be effectively managed.[229] Once efficacy and safety have been established in this setting or while these studies are ongoing, phase II and III trials are initiated in other areas of high unmet need, including the treatment of patients with acute VTE, the secondary prevention of VTE, the prevention of systemic embolism and stroke in patients with AF, and the prevention of cardiovascular events in patients with ACS. Of all the oral FXa inhibitors, **65** and **37** are the most advanced (Table 4).

4.2.1. Phase II/III Clinical Trials with 65. The efficacy and safety of **65** as VTE prophylaxis in patients undergoing TKR or THR were first assessed in four phase II dose-ranging trials

Table 4. Clinical Studies of Oral FXa Inhibitors[a]

	rivaroxaban (**65**)	apixaban (**37**)	edoxaban (**101**)	betrixaban (**87**)	YM150 (structure undisclosed)
PD	dose-dependent inhibition of FXa in healthy subjects[207,208]	dose-dependent increases in clotting time in healthy subjects[214,215]	inhibition of thrombin generation and ex vivo thrombus formation in healthy subjects[224]	dose- and concentration-dependent inhibition of thrombin generation and FXa activity in TKR patients[251]	NR
	inhibition of thrombin generation after single dosing in healthy subjects[209]	inhibition of thrombin generation after single dosing in healthy subjects[264]	inhibition of coagulation in shed blood model in healthy subjects[225]		
T_{max} (h)	2−4[207,208]	1.5−3.5[214,215]	1−2[224]	NR	NR
half-life (h)	5−9[207,208]	8−15[215]	9−11[224]	19[120]	NR
excretion	renal (66%), fecal (28%)[177]	renal (25−29%), fecal (47−56%)[216]	reported to be predominantly renally cleared[224]	renal excretion reported to be minimal[227]	NR
food interactions	food delays drug absorption and increases exposure[213]	no effect of high-calorie, high-fat meal on drug exposure[220]	no or minimal effects of standard high-fat breakfast on PD and PK[226]	NR	NR
drug interactions	avoid potent CYP3A4 and P-gp inhibitors[212]	< 2-fold increase in exposure in presence of ketoconazole suggests apixaban is not a sensitive CYP3A4 substrate[223]	NR	potential for drug interactions reported to be low[227]	NR
phase II/III trials published to date	VTE prevention (phase II): ODIXa-HIP,[232] ODIXa-HIP2,[230] ODIXa-QD-HIP,[231] ODIXa-KNEE[234]	VTE prevention (phase II): APROPOS[243]	VTE prevention (phase II): Raskob et al.,[250] Fuji et al.[249]	VTE prevention (phase II): EXPERT[251]	VTE prevention (phase II): ONYX,[228] Eriksson et al.[248]
	VTE prevention (phase III): RECORD 1,[235] RECORD 2,[236] RECORD 3,[237] RECORD 4[238]	VTE prevention (phase III): ADVANCE-1,[245] ADVANCE-2[244]	AF (phase II): Weitz et al., 2008;[252] Yasaka et al., 2009[253]		
	VTE treatment (phase II): ODIXa-DVT,[240] EINSTEIN-DVT dose-ranging study[241]	VTE treatment (phase II): BOTTICELLI[247]			
	ACS (phase II): ATLAS ACS-TIMI 46[242]	ACS (phase II): APPRAISE-1[246]			
ongoing phase III trials (by study population)	treatment and secondary prevention of VTE	VTE prophylaxis in THR	prevention of systemic embolism and stroke in AF		VTE prophylaxis in THR and TKR
	prevention of VTE in hospitalized patients	treatment and secondary prevention of VTE			VTE prophylaxis in major abdominal surgery and hip fracture-related or other lower-extremity surgery
	prevention of cardiovascular events in ACS	prevention of VTE in hospitalized patients			prevention of recurrent VTE
	prevention of systemic embolism and stroke in AF	prevention of cardiovascular events in ACS			
		prevention of systemic embolism and stroke in AF			

[a] No data for TAK-442 reported to date. ACS, acute coronary syndromes; AF, atrial fibrillation; BID, twice daily; DVT, deep vein thrombosis; CYP3A4, cytochrome P450 3A4; FXa, factor Xa; LMWH, low-molecular-weight heparin; NR, not reported; PD, pharmacodynamics; P-gp, P-glycoprotein; PK, pharmacokinetics; QD, once daily; THR, total hip replacement; TKR, total knee replacement; T_{max}, time to maximum plasma concentration; VTE, venous thromboembolism.

(Table 4).[230−234] Subsequently, a once-daily 10 mg dose of **65** was selected for evaluation in four phase III trials in TKR or THR patients (RECORD1−4).[235−238] These trials compared the efficacy and safety of **65** with those of the current standard of care for VTE prevention in major orthopedic surgery, the LMWH enoxaparin. In all RECORD trials, **65** was associated with a significant reduction in the risk of the primary outcome (the composite of any DVT, nonfatal PE, and all-cause mortality) compared with enoxaparin. Event rates in the TKR studies were 9.6% and 18.9% with **65** and enoxaparin, respectively, in RECORD3[237] and 6.9% and 10.1% in RECORD4.[238] In the THR study with both agents given for up to 35 days (RECORD1),[235] the incidence of the primary outcomes was 1.1% in the **65** group and 3.7% in the enoxaparin group. In pooled analyses of all four RECORD trials,[239] rates of major bleeding at day 12 were increased with **65** (0.34% vs 0.21%), although they did not reach statistical significance ($p = 0.175$). The RECORD trials did not include bleeding at the site of surgery in the definition of major bleeding. Surgical site bleeding is of particular concern to orthopedic surgeons and, as a consequence of its exclusion, reported rates of major bleeding in the RECORD trials are lower than for other anticoagulant trials.[11] To date, **65** has been approved in a number of other countries outside the United States for VTE prevention in adults undergoing THR or TKR.

Proof of concept for **65** as a treatment for acute VTE has been provided by two dose-ranging phase II trials.[240,241] Ongoing phase III trials are investigating **65** in the treatment of acute DVT and PE and for the long-term prevention of recurrent VTE. Phase II trial data have also been reported for **65** in ACS,[242] and a phase III trial in this indication is underway. Other ongoing phase III trials will assess **65** for prevention of systemic embolism and stroke in patients with AF and for VTE prophylaxis in hospitalized patients.

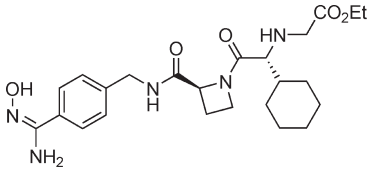
4.2.2. Phase II/III Clinical Trials with 37. Phase II investigations of **37** for the prevention of VTE in patients undergoing TKR helped identify the optimal dosing regimen for further evaluation in this setting.[243] Two phase III, double-blind trials have since compared the efficacy and safety of **37** (2.5 mg twice daily) with that of two different doses of enoxaparin.[244,245] In ADVANCE-1, the proportion of TKR patients experiencing DVT, nonfatal PE, or death by any cause was numerically similar in patients receiving **37** (9.0%) and enoxaparin (30 mg twice daily, 8.8%).[245] Statistical criteria for noninferiority of **37** versus enoxaparin were not met. However, achievement of noninferiority was made difficult because the observed rate of events in the enoxaparin arm (8.8%) was lower than the predicted rate (16%) upon which the study sample size was calculated. Compound **37** was significantly more effective than enoxaparin (40 mg once daily) in ADVANCE-2, a second phase III trial of **37** in TKR patients (rates of DVT, PE, or death: 15.1% vs 24.4%, respectively; $p < 0.001$).[244] In both trials, rates of clinically relevant bleeding (a composite of major bleeding and clinically relevant nonmajor bleeding, including bleeding at the surgical site) were lower with **37** than with enoxaparin (for ADVANCE-1, 2.9% vs 4.3%, $p = 0.03$; for ADVANCE-2, 3.5% vs 4.8%, $p = 0.09$). A phase III trial of **37** in THR patients is ongoing.

Phase II trials of **37** for the treatment of symptomatic VTE and the prevention of cardiovascular events in patients with ACS have also been reported,[246,247] with phase III trials in these indications underway. Two ongoing phase III trials are investigating **37** for the prevention of systemic embolism and stroke in patients with AF, one comparing **37** to warfarin in a broad AF population (CHADS2 risk score of ≥ 1) and a second comparing **37** to aspirin in patients intolerant of or unable to take warfarin. Another phase 3 trial is ongoing for VTE prophylaxis in hospitalized medical patients.

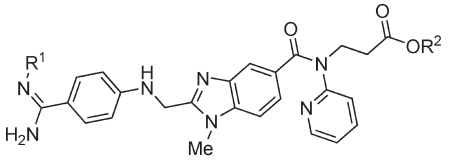
4.2.3. Other Oral FXa Inhibitors in Phase II/III Clinical Trials. Phase II studies of **87**, **101**, and YM150 for VTE prevention following THR or TKR have been reported (Table 4).[228,248−251] All of these agents displayed dose-dependent antithrombotic activity in this setting. The results of two phase II trials of **101** in AF have also been presented,[252,253] and a phase III trial in this indication is underway. Phase III trials of YM150 are currently investigating this agent in the prevention of recurrent VTE and for VTE prevention in patients undergoing THR, TKR, major abdominal surgery, or lower-extremity surgery. A phase II trial of **87** in AF is also ongoing. A phase II trial of **135** in TKR patients has reportedly been completed, and another phase II trial in ACS is underway.

5. Oral FXa Inhibitors and Oral DTIs: Differences in Clinical Practice?

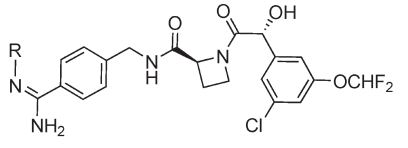
The first oral DTI, ximelagatran (**141**, AstraZeneca; Figure 32), was launched in Europe in 2004 for the prevention of VTE following orthopedic surgery. However, marketing and development of **141** were halted in 2006 because of potential hepatotoxicity.[254] Despite its withdrawal from the market, **141** provided proof of concept for the efficacy of oral, direct thrombin inhibition and several new oral DTIs have since been developed. At the time of writing, the most advanced oral DTI, dabigatran etexilate (**142**, Boehringer Ingelheim), had been approved in several regions outside the United States for the prevention of VTE in adults undergoing THR or TKR surgery. In the phase III RE-NOVATE trial, **142** (150 or 220 mg once daily) was noninferior to enoxaparin (40 mg once daily) for VTE prevention in THR patients.[255]



141, ximelagatran



142 $R^1 = CO_2$-n-hex, R^2 = Et, dabigatran etexilate
143 $R^1 = R^2$ = H, dabigatran



144, R = OMe, AZD0837
145, R = H, AR-H067637

Figure 32. Structures of direct thrombin inhibitors.

Noninferiority of **142** in TKR was also demonstrated versus enoxaparin (40 mg once daily) in the RE-MODEL trial.[256] In the RE-MOBILIZE trial, both doses of **142** were statistically inferior to enoxaparin (30 mg twice daily; $p < 0.001$ for 150 mg **142** and $p = 0.02$ for 220 mg **142**).[257] In a meta-analysis of RE-NOVATE, RE-MODEL, and RE-MOBILIZE, no significant differences between **142** (220 mg once daily) and all doses of enoxaparin were detected for the primary end-point of the trials (rates of total VTE and all-cause mortality) or major bleeding rates.[258]

The phase III trial RE-LY compared **142** with warfarin for the prevention of stroke and systemic embolism in patients with AF.[259] Compound **142** (110 mg once daily) and adjusted-dose warfarin were associated with similar rates of stroke and systemic embolism (1.53% vs 1.69% per year, respectively; $p < 0.0001$ for noninferiority) and lower rates of major bleeding (3.36% vs 2.71% per year, respectively; $p = 0.003$). A higher dose of **142** (150 mg once daily) was associated with lower rates of stroke and systemic embolism (1.11%, $p < 0.0001$ for superiority vs warfarin) and similar rates of bleeding (3.11%, $p = 0.31$). Another oral DTI in development is AZD0837 (**144**, AstraZeneca). This compound is currently in phase II development, and preparations for phase III trials of **144** are underway.[260]

Both **142** and **144** are prodrugs that are metabolized to their active forms (**143** and **145** (AR-H067637), respectively) following oral administration. Compound **143** is a nonpeptidic molecule that was synthesized as a derivative of the peptide-like thrombin inhibitor, *N*-α-naphthylsulfonylglycyl-4-amidinophenylamine piperidine.[120,261] Subsequent addition of a hydrophobic side chain created orally absorbed **142**. Compounds **143** and **145** are specific and reversible inhibitors of both free and clot-bound thrombin.[120]

The comparative efficacy and safety of oral FXa inhibitors and DTIs have not been assessed in head-to-head clinical trials. Assessment of relative therapeutic indices is also hampered by the wide variation in bleeding definitions employed within different VTE prophylaxis clinical trial programs.[11] Preclinical comparator studies have, however, highlighted differences between FXa inhibitors and DTIs. In rabbit models of venous thrombosis, **37**, **65**, and **143** were equally efficacious.[24] In the same study, however, the bleeding potential of equivalent doses of these agents differed. Cuticle bleeding times increased 4.4-fold with **143**, compared with 1.1- and 1.9-fold increases for **37** and **65**, respectively. Overall, these results suggest that in rabbits the FXa inhibitors **37** and **65** preserve hemostasis more effectively and possess a favorable efficacy−bleeding profile compared with the oral DTI **142**. Earlier animal studies also showed that inhibition of FXa was associated with reduced bleeding times compared with thrombin inhibition.[88,262]

Differences in bleeding profiles observed in preclinical studies may be due to the fact that, unlike DTIs, FXa inhibitors do not affect existing levels of thrombin. Therefore, the small amounts of thrombin remaining after FXa inhibition may be sufficient to activate platelet thrombin receptors and preserve hemostatic function.[24] Whether these differences will afford FXa inhibitors a wider therapeutic index in clinical practice remains to be determined. Interestingly, different LMWH preparations have varying anti-Xa and antithrombin activity, and evidence from clinical trials suggests that preparations with the highest ratio of anti-Xa/antithrombin activity are more effective and safer.[21] In addition to its prothrombotic role in platelet activation, thrombin has other important functions outside the coagulation cascade that, in theory, may be inhibited when thrombin is blocked. These include an antithrombotic role in protein C activation and functions related to cellular inflammation and proliferation.[218] Further studies are required to determine whether loss of these additional functions of thrombin during long-term therapy with oral DTIs will prove important. Unlike thrombin, FXa has relatively few functions outside its role in the coagulation cascade; FXa inhibition may therefore represent a method of anticoagulation associated with a reduced potential for "off-target" effects.

6. Future Perspectives

Direct FXa inhibitors and other new small-molecule, orally available anticoagulants have the potential to revolutionize the management of thromboembolic disease. Large clinical trial programs have already established the effectiveness of the FXa inhibitors **37** and **65** and the oral DTI **142** for VTE prevention in patients undergoing THR or TKR. In phase III trials in this setting, these agents provided efficacy comparable or superior to that of the current standard of care, enoxaparin. Of particular interest is whether differences in benefit−risk profiles (risk of clinically relevant bleeding or serious hemorrhagic complications) of one or more of the new oral anticoagulants will be borne out in clinical practice. Such a step forward may help combat the perhaps disproportionate fear of bleeding that leads some orthopedic surgeons to withhold anticoagulant therapy despite the high risk of VTE following THR and TKR surgery and the established efficacy of traditional agents such as enoxaparin.[11]

The potential introduction of new oral anticoagulants into the orthopedic surgeon's armamentarium is a welcome advance. It is in other settings, however, that these agents are likely to have the biggest impact. Because it is administered orally, the VKA warfarin has, for many decades, been the agent of choice when long-term anticoagulation is required to prevent recurrent VTE or reduce the risk of arterial thromboembolism in patients with ACS or AF. Unfortunately, the numerous limitations of warfarin (in particular, its unpredictable PK and anticoagulant effects) have led to substantial underutilization of anticoagulant therapy in these patients. Even when warfarin is administered, treatment remains suboptimal; indeed, it has been estimated that therapeutic levels of anticoagulation are maintained only about 50% of the time.[4] Issues with warfarin therapy have been the driving force for the development of oral FXa inhibitors and DTIs, agents with predictable pharmacologic profiles that offer the convenience of fixed, once- or twice-daily dosing and can be administered without anticoagulation monitoring or frequent dose adjustment. These characteristics offer the hope that many patients currently considered unsuitable for warfarin therapy will be eligible for treatment with these new agents and that levels of compliance with oral anticoagulant therapy will be increased. If approved for longer-term indications currently being investigated in phase III trials, **37**, **65**, and/or **142** will be the first new oral anticoagulants to rival warfarin since its introduction in 1954. A new era of antithrombotic therapy beckons.

In this new era, will differences between new oral anticoagulants emerge? As we have discussed, theoretical and observed differences between and within the new classes of oral anticoagulants do exist. For example, preclinical evidence suggests that FXa inhibitors may possess a wider therapeutic

index than DTIs.[24] PK differences between agents may also prove important in the clinic. Agents that are eliminated via multiple routes may, for instance, be more suitable for use in patients with renal impairment than drugs that are predominately renally excreted. Because renal function declines with age, such differences may be important in the future management of age-related conditions such as AF. While published and ongoing clinical trials can provide useful information on potential clinical differences between agents, variations in study design limit the validity of cross-trial comparisons. For example, the broad range of definitions of "bleeding" used in studies involving patients undergoing THR or TKR makes it difficult to compare the relative risk of bleeding associated with new oral agents.[11] In the absence of head-to-head trials, experiences in clinical practice are likely to play an important role in determining the relative roles of these new agents.

Widespread clinical use of a new drug sometimes reveals previously undetected safety signals, such as the hepatotoxicity detected following the brief introduction into clinical practice of **141**. Evidence to date suggests that oral FXa inhibitors do not affect liver function and, in general, possess favorable safety profiles. These findings are perhaps not surprising given the fact that, from the earliest stages of development, the selection and refinement of candidate compounds with optimal PK characteristics have been a primary objective. Minimization of peak-to-trough plasma concentration ratios, for example, may be expected to contribute to a reduced risk of hemorrhagic complications. Similarly, drugs with low volumes of distribution are, by definition, primarily maintained in the central compartment and may therefore have a reduced potential for off-target toxicities. Despite such observations, ongoing vigilance for potential long-term non-hematologic side effects remains of critical importance. Furthermore, additional clinical experience is needed to further characterize the safety of oral FXa inhibitors in patients receiving concomitant medications, including other antithrombotic agents such as clopidogrel, aspirin, warfarin, and enoxaparin.

Structure-based drug design has played an important role in the discovery and development of oral FXa inhibitors particularly in terms of achieving selectivity and oral bioavailability. The increased throughput of crystallography and increasingly sophisticated molecular modeling techniques have been critical in understanding how lead molecules bind to FXa and related serine proteases and integral to the process of evolving these molecules into highly optimized orally bioavailable FXa inhibitors.[263]

We are about to enter a new frontier in the management of thromboembolic disease, with some new agents offering the promise of fixed-oral dosing that does not require time- and resource-intensive monitoring, efficacy that is at least as impressive as current standards-of-care, and uniquely favorable safety profiles. Phase II and phase III clinical trials have clearly shown that direct oral FXa inhibitors are efficacious in patients experiencing, or at risk of, arterial or venous thromboembolism. Data from these trials, together with evidence from preclinical studies, also suggest that these agents may possess wider therapeutic indices than traditional agents or other new oral anticoagulants. It is therefore anticipated that orally administered, direct inhibitors of FXa will afford a significant advance in the next generation of anticoagulants and have the potential to provide a long-awaited alternative to the current standard of care.

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Joanne M. Smallheer received her B.A. in Chemistry from Douglass College, NJ, and her M.S. in Organic Chemistry from Rutgers University, NJ. She began her career at Hoffmann-La Roche in 1975 and subsequently moved to Smith-Kline & French in 1986. She joined E. I. du Pont de Nemours and Company in 1988, and in 2001 she went with Bristol-Myers Squibb where she is currently a Senior Principal Scientist. She has coauthored 48 publications and patents, including previous reviews in the fields of coagulation serine protease inhibitors and antiplatelet agents.

Daniel L. Cheney graduated with a Ph.D. degree in Organic Chemistry from the Ohio State University where he worked with Dr. Leo Paquette on the total synthesis of natural products. After serving as Visiting Professor in Organic Chemistry at the University of Pretoria, South Africa, he joined Rhone-Poulenc Rorer, initially as a medicinal chemist before joining the Department of Computer-Aided Drug Design (CADD). In 1998 he joined the CADD group at Bristol-Myers Squibb, where he has been actively involved in several cardiovascular and metabolic disease programs. He has made numerous contributions to the field of CADD, including successful applications of structure- and ligand-based drug design and the development of methods involving molecular docking and scoring, quantum chemistry, molecular recognition, and receptor-based similarity.

Robert M. Knabb received a B.S. degree from the Pennsylvania State University in 1976 and a Ph.D. in Physiology from the University of Virginia in 1982. After postdoctoral training in the Cardiology Division, Department of Internal Medicine at Washington University, MO, he began his pharmaceutical career with the E. I. du Pont de Nemours and Company in 1985 and worked in drug discovery programs in reperfusion injury, thrombolysis, and coagulation factor inhibitors. He co-led the factor Xa inhibitor team that advanced two orally active factor Xa inhibitors to clinical studies with DuPont and three additional compounds with Bristol-Myers Squibb. He is currently a Group Director in Bristol-Myers Squibb Global Clinical Research.

Ruth R. Wexler received a B.A. in Chemistry from Boston University, MA, and a Ph.D. in Organic Chemistry from the University of Pennsylvania. She joined E. I. du Pont de Nemours and Company in 1982 as a Research Chemist and advanced to Executive Director. In 2001, she moved to Bristol-Myers Squibb, as Executive Director, Discovery Chemistry, where she currently heads the medicinal chemistry efforts directed at cardiovascular diseases. During her career, her group has focused on targets in cardiovascular/metabolic diseases (hypertension, atherosclerosis, thrombosis, and obesity), inflammatory disease (arthritis), and Alzheimer's disease. These

research efforts resulted in Cozaar, a marketed angiotension II receptor antagonist; apixaban, a factor Xa inhibitor, currently in phase III; and several additional compounds selected for clinical evaluation. She has coauthored over 170 scientific publications and patents.

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