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Review Article

COAGULATION FACTOR Xa: THE PROTHROMBINASE COMPLEX AS AN EMERGING THERAPEUTIC TARGET FOR SMALL MOLECULE INHIBITORS

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INTRODUCTION

During the last decade, the pharmaceutical industry has focused numerous drug discovery efforts in the search for novel antithrombotics directed at the coagulation enzyme thrombin,[1] and on platelet antiaggregation mechanisms.[2] While various platelet inhibitors have been quite successful in recent clinical studies and their use is gaining significant momentum, novel, direct antithrombins have not fared as well.[3,4] This seeming lack of success is, in part, because of concerns about their narrow therapeutic index and lack of significantly improved efficacy over traditional regimens which use anticoagulants such as heparin and more recently the low molecular weight

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heparins (LMWHs).[5] Because of these concerns, a new anticoagulation strategy has begun to emerge as an alternative to direct thrombin inhibition and is intended to address both the efficacy and safety concerns of direct thrombin inhibitors. This alternative strategy targets blockade of thrombin generation mediated through the inhibition of the prothrombinase complex which is formed most frequently on the surface of activated platelets. The prothrombinase complex is formed by the association of factor Xa, factor Va, and Ca^{2+} with phospholipids. The prothrombinase complex amplifies clot formation by functioning as the catalytic machinery to convert prothrombin to active thrombin at sites of vascular injury and thrombus growth. The conversion rate is increased 10^7-fold when factor Xa is incorporated into the prothrombinase complex. This novel anticoagulation target is currently being explored by the pharmaceutical industry with increasing intensity.

In recent years, small protein inhibitors of factor Xa have emerged as the initial tools to demonstrate that the prothrombinase complex is a viable alternative target to direct thrombin inhibition.[6] These proteinaceous agents are potent and highly specific inhibitors of factor Xa as well as the prothrombinase complex, and have unambiguously validated the prothrombinase complex as an important *in vivo* target.[7,8] However, the potential development of this class of protein-based agents is complex and their therapeutic potential has been traditionally viewed as less desirable compared to the identification of small molecule inhibitors of factor Xa with similar biological properties. In addition, the identification of orally deliverable anticoagulant therapy continues to be a more attractive target in the development of novel anticoagulants. From these considerations, the last few years have witnessed a growing body of literature describing novel, enzyme-specific, low-molecular weight factor Xa inhibitors that have the potential to be orally effective and to display encouraging therapeutic indices. Because of these recent developments, a summary of the status of these advances is timely and is the purpose of this review.

REVERSIBLE TRANSITION STATE INHIBITORS OF FACTOR Xa

The design of transition-state inhibitors that are both potent and specific for factor Xa based on the known substrate binding sequences for this enzyme has been described.[9–11] Examples of rational design using this approach are the inhibitors (**1**–**3**) (shown in Figure 1) disclosed by COR Therapeutics, which are based on the sequence of one of the better known chromogenic

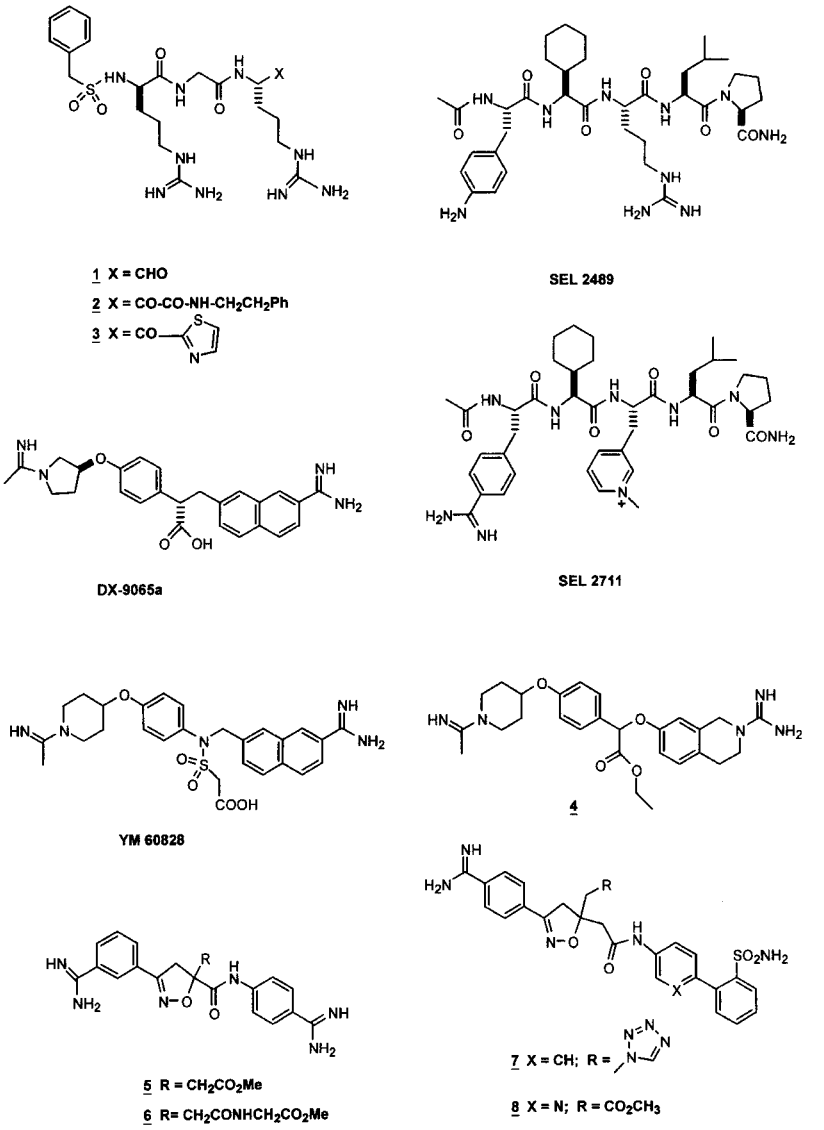

FIGURE 1 Structure of selected inhibitors of coagulation factor Xa/prothrombinase complex.

substrates for factor Xa, Cbz-D-Arg-Gly-Arg-p-nitroanilide, (S-2765). Using the specificity sequence from this substrate (D-Arg-Gly-Arg) and incorporation of polarized carbonyl functionality in the P1 arginine residue, such as an aldehyde, α-ketoamide or ketoheterocycle affords nanomolar

and sub-nanomolar inhibitors of factor Xa as well as the prothrombinase complex.[12] It may be speculated that the high specificity of this inhibitor series is derived from the P3 D-argininyl-residue which binds to the hydrophobic S4 pocket of factor Xa formed by residues Tyr99, Phe174 and Trp215. These residues may afford a surface for π-cation interactions which has been previously proposed.[13] The slow reversibility of this series of inhibitors is exemplified by (**3**) which has a K_i value of 13 pM and a corresponding k_{off} value of $3.4 \times 10^5\,M^{-1}\,s^{-1}$ which translates into a half-time for dissociation of 5.5 h from factor Xa (unpublished observations, U. Sinha and A. Betz).

ACTIVE SITE REVERSIBLE INHIBITORS

A number of active site reversible inhibitors of factor Xa have been recently reported and a subset of the most active structures will be shown in Figures 1–3. Novel peptidomimetic inhibitors reported by Selectide Corporation such as SEL2489 and SEL2711 (Figure 1), have been designed via combinatorial chemistry with the original leads for this series coming from an octapeptide library.[14] SEL2711, with a K_i value of 3 nM for factor Xa and a K_i of 40 μM for thrombin is highly selective versus other coagulation enzymes except tissue plasminogen activator (tPA) but is remarkably inactive with human trypsin ($K_i = 112\,\mu M$). The ability of SEL2489 or SEL2711 to inhibit the prothrombinase complex as effectively as their ability to inhibit uncomplexed factor Xa is unknown.

Nonpeptide bisamidine-containing inhibitors of factor Xa have increasingly been reported following disclosure of DX-9065a (Figure 1) by Daiichi Pharmaceuticals, the first highly specific inhibitor in this class. DX-9065a has a reported K_i for factor Xa of 41 nM and IC_{50} values that range between 80–6300 nM for the prothrombinase complex.[15–18] This inhibitor is quite effective as an anticoagulant in a variety of species when administered intravenously but is fairly ineffective when administered orally. Recently, an X-ray crystal structure of DX-9065a in complex with human des-Gla-factor Xa has been reported which confirms the binding of the naphthamidine group bound in the S1 specificity pocket and the pyrrolidine group bound in the S4 pocket.[19] Researchers at Yamanouchi, using a similar molecular template have reported potent anti-factor Xa activity of YM-60828 (Figure 1).[20,21] This inhibitor, with a K_i value of 1.3 nM for factor Xa and an IC_{50} of 7.7 nM for platelet prothrombinase is greater than 50,000-fold more specific for factor Xa compared to thrombin but is still a fairly effective inhibitor of

trypsin ($K_i = 46$ nM). In rat venous thrombosis models, YM-60828 exerted dose-dependent antithrombotic effects with an ID_{50} value of 8.1 μg/kg. In an arterio-venous shunt model in the rat, YM-60828 also displayed antithrombotic effects in a dose-dependent fashion with an ID_{50} value of 10 μg/kg. Little effect on bleeding time measurements at antithrombotic doses were noted with YM-60828 and suggests that antithrombotic/antihemostatic ratio may be improved over other direct and indirect thrombin inhibitors. Low oral bioavailability of YM-60828 was noted in the rat (4.4%) but was improved in squirrel monkey (20.3%) and guinea pig (33.4%). Only in the squirrel monkey was the half-life of YM-60828 sufficient ($t_{1/2} = 1$ h) to view this agent as having potential for oral administration. Boehringer Mannheim has reported yet another variation of this template in inhibitor (**4**) (Figure 1) with a factor Xa K_i value of 28 nM.[22]

Subsequently, other bisamidine-containing classes of inhibitors have been reported by investigators at Dupont-Merck, which have prepared bisbenzamidine isoxazoline class of inhibitors such as (**5** and **6**) (Figure 1).[23] Inhibitor (**5**) has a reported K_i value for factor Xa of 94 nM and 480 nM for trypsin. Inhibitor (**6**) has a K_i value for factor Xa of 18 nM and 420 nM for trypsin. Prothrombinase inhibitory activity for this class has not been reported. Attempting to remove one of the positive charges from this class of inhibitor, Dupont-Merck has reported the biphenylsulfonamide-containing inhibitors (**7** and **8**) (Figure 1).[24] The tetrazole-containing inhibitor (**7**) has a reported K_i value of 0.5 nM for factor Xa, while the methyl ester-containing analog (**8**) has a K_i value of 0.96 nM.

Rhone-Poulenc Rorer has reported a novel series of highly specific factor Xa inhibitors of the 3-substituted benzamidine class exemplified by structures (**9** and **10**) (Figure 2).[25] The unsubstituted biphenyl-containing inhibitor (**9**) has a reported K_i value of 110 nM for factor Xa. The dimethoxy-substituted biphenyl-containing inhibitor (**10**) has a factor Xa K_i value of 30 nM. No data for prothrombinase inhibition has been reported for this series. Dupont-Merck reported a second class of bis-phenylamidine-containing inhibitors exemplified by structures (**11** and **12**) (Figure 2).[26] The bisbenzamidine inhibitor (**11**) has a K_i of 34 nM for factor Xa, while the guanidine-containing inhibitor (**12**) has a K_i of 9 nM. This series is not absolutely specific for factor Xa. Inhibitor (**12**) has a reported 10-fold selectivity for inhibition of factor Xa versus trypsin. Again, within this series, the ability of inhibitors to inhibit the prothrombinase complex is unknown. Using this same template and replacement of one of the positive charges by the biphenylsulfonamide group has again led to potent inhibitors such as (**13**) (Figure 2) with a reported K_i value of 0.53 nM.[27] A distinct

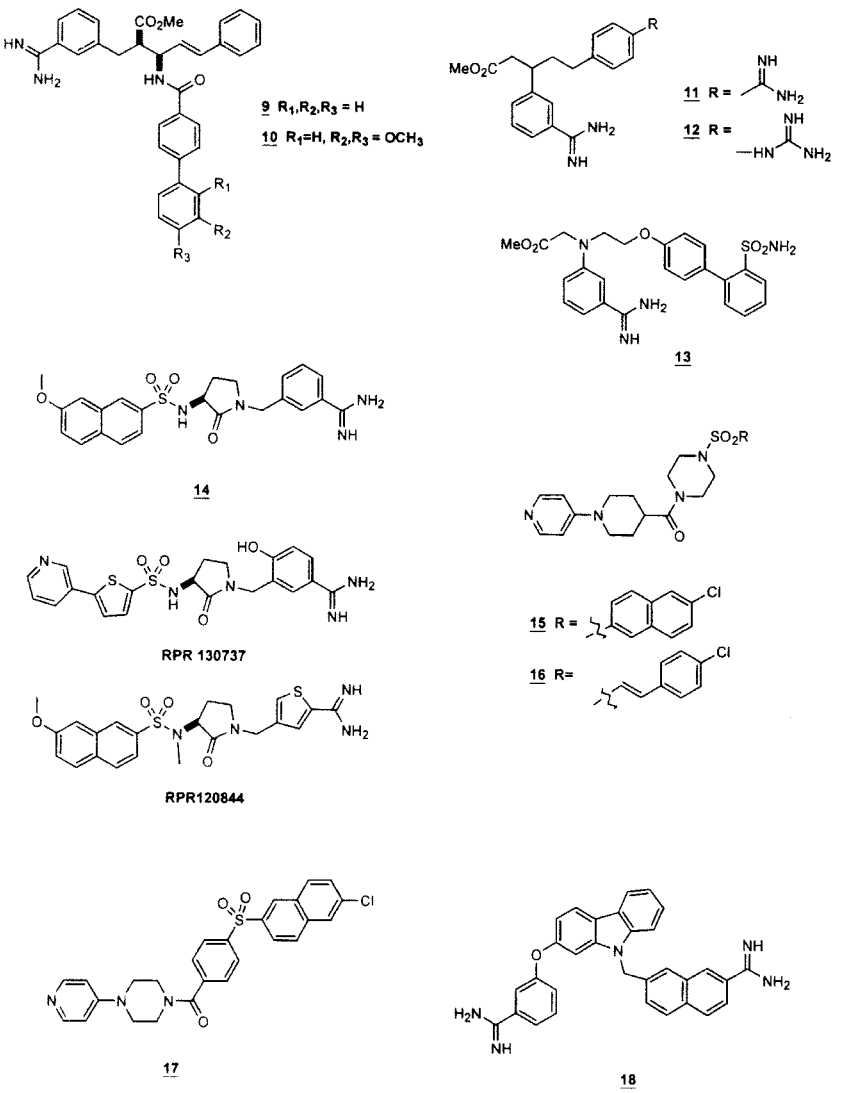

FIGURE 2 Structure of selected inhibitors of coagulation factor Xa/prothrombinase complex.

series of oxopyrrolidines reported by Rhone-Poulenc Rorer, exemplified by structure (**14**) (Figure 2), has a factor Xa K_i value of 35 nM.[28] Other compounds from this series such as RPR130737 and RPR120844 (Figure 2) with a K_i value of 7 nM for factor Xa are effective in arterial models of thrombosis in the rat.[29]

A series of factor Xa inhibitors which do not contain the more usual amidino or guanidino functionality has been reported by Zeneca. Two classes have been described which contain substituted amino pyridines either with sulfonamido groups (**15** and **16**)[30] (Figure 2) or sulfone functionality (**17**) (Figure 2).[31] Both classes of inhibitors are potent against factor Xa with a K_i value of 3 nM for (**15**), 12 nM for (**16**) and 13 nM for (**17**). Berlex has described novel, substituted carbazoles such as (**18**) with a K_i value of 1.6 nM for factor Xa (Figure 2).[32] The poor water solubility of the carbazole series stimulated the search for similar templates with improved properties and afforded the novel benzimidazoles (**19**) and (**20**) (Figure 3),[33] with K_i, values of 0.03 and 0.01 nM respectively and indole (**21**)[34] with a K_i of 0.17 nM as factor Xa inhibitors (Figure 3). The stereodefined (*Z*, *Z*) isomer of BABCH[18] (ZK-805412) (Figure 3), with a K_i value for factor Xa of 660 pM is reportedly 25,000 times more potent than the previously reported (*E*, *E*) isomer of BABCH.[35] This observation has been used to design a series of 2,6-diphenoxypyridine inhibitors of factor Xa such as ZK-805623 with a K_i value of 80 nM,[36] ZK-806530 with a K_i value of 1.2 nM,[37] and (**22**) with a K_i value of 0.19 nM (Figure 3).[38] Since it has been reported that the 4-position of the pyridine ring can be substituted without deleterious effects on potency, in an attempt to increase the oral bioavailability of this series, further analogs have been designed such as ZK-807191,[39] and the bicyclic inhibitor (**23**) (Figure 3).[40] Inhibitor (**23**) has a reported K_i value of 0.42 nM. ZK-807191 (BX807834), has a reported K_i value of 0.1 nM for factor Xa, 2100 nM for thrombin, 320 nM for trypsin and 6.6 nM for prothrombinase, and has been shown to have a half-life value *in vivo* of less than $\sim$30 min when administered intravenously to dogs. The half-life of analogs has not been found to be improved by various substitutions at the 4-position of the pyridine ring. ZK-807191 was undetectable in rat plasma upon oral administration, but showed 20% oral bioavailability in dogs and primates. In a rat venous stasis model, BX-807834 inhibited thrombus formation with an ED_{50} value of 0.69 mg/kg when administered intravenously.[41] In the dog, BX-807834 was rapidly absorbed when administered orally (C_{max} at 1 h) with an elimination half-life of $\sim$70 min. The bioavailability was $\sim$20% which was significantly decreased with prior food administration.[42] In the baboon, p.o. administration (10 mg/kg) of BX-807834 prolonged prothrombin time (PT) greater than 1.5-fold for greater than 12 h following a single oral dose. The bioavailability of this inhibitor was also $\sim$20% in the baboon and displayed a delayed C_{max} at 6–8 h with an elimination half-life of 120 min. These data have led to the choice of ZK-807191(BX-807834) as a parenteral clinical candidate.[43]

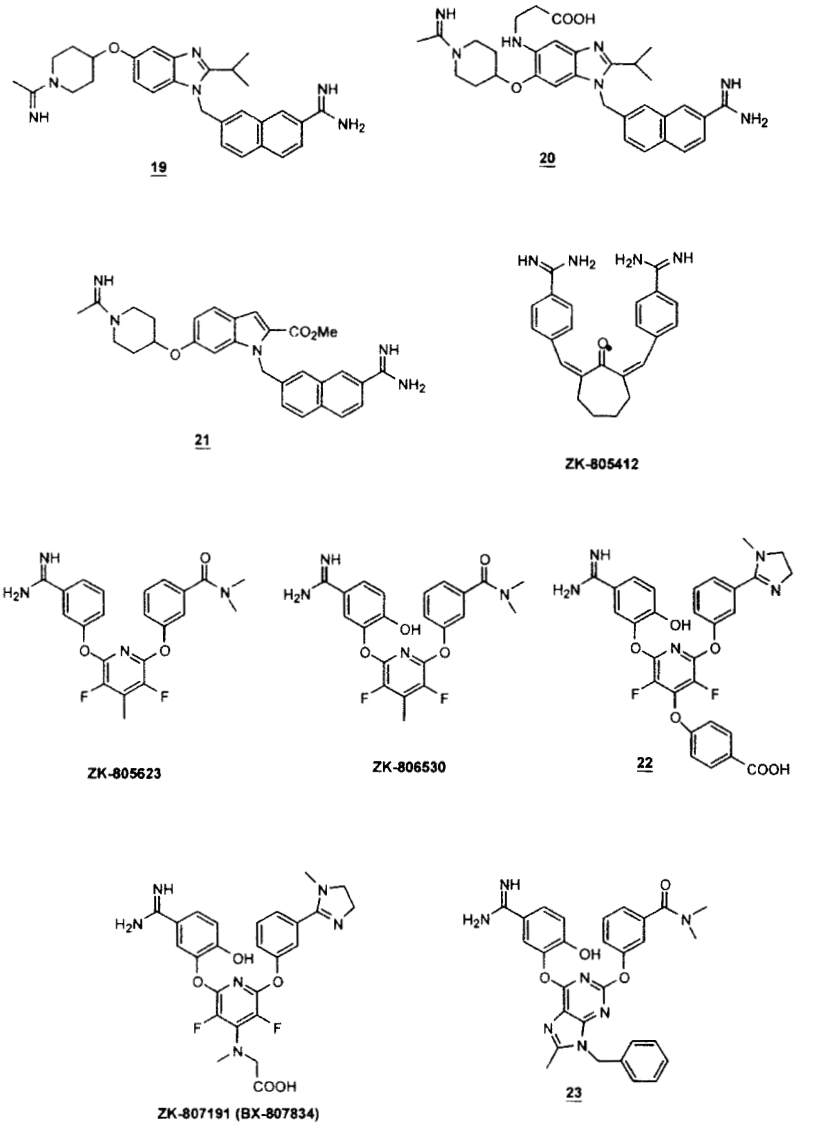

FIGURE 3 Structure of selected inhibitors of coagulation factor Xa/prothrombinase complex.

PERSPECTIVES

The rapid progress in identification of novel and diverse structural families of potent factor Xa inhibitors is evident from the preceeding section and

illustrates the amount of significant effort being devoted to developing clinical candidates to address this novel anticoagulant target. In spite of the impressive progress to date, a number of important unanswered questions concerning the factor Xa target and the approaches being utilized to inhibit this enzyme complex remain. Foremost, from a practical sense is whether factor Xa/prothrombinase inhibition truly affords a greater therapeutic index over currently used antithrombotic regimens. If the answer to this question is indeed yes, then the efforts in this area will prove to be justified. However, animal studies are only suggestive of this potentially improved safety index and clinical trials of agents will be the only definitive way to prove the theory. Secondly, as agents are being synthesized and tested, it is probably quite important to determine and understand the inhibitory constants for both the uncomplexed enzyme (free factor Xa), as well as the corresponding inhibitory constants for the prothrombinase complex. This may be very relevant since the structure-activity relationships for complexed or uncomplexed enzyme may be subtly different and yield distinct correlations with *in vivo* efficacy in animal models. Of additional interest is the observation that certain small molecule antagonists of factor Xa are classically noncompetitive inhibitors of the prothrombinase complex.[3] The practical implications of this observation have not been determined.

Finally, it is apparent from the structures disclosed to date that the benzamidine functionality is a common theme in most classes of inhibitors, with many of the most potent compounds requiring two basic functional groups to afford potent inhibition of the enzyme. Reliance on this structural feature may afford difficulty in obtaining inhibitors with acceptable oral absorption and with sufficient duration of action to qualify as clinical candidates for oral anticoagulant trials. This hurdle is particularly high for therapeutic agents with narrow therapeutic indices. Unless alternate, less basic functionality is discovered which affords potent inhibitors, future progress towards the identification of oral clinical candidates may be a slow and resource-consuming endeavour.

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