2. Literature review

2.1. Development of direct FXa inhibitors

Research programs were started in the early 1980s to develop direct FXa inhibitors. Two naturally occurring FXa inhibitors (TAP and antistasin) were identified in the late 1980s that were used as reference for the development of FXa inhibitors. Antistasin, a polypeptide was extracted from the salivary glands of the Mexican leech *Haementeria officinalis*.[53,54] and found to be a potent FXa inhibitor. After two years, another naturally produced FXa inhibitor, tick anticoagulant peptide (TAP) was isolated from tick *Ornithodoros moubata*.[55] It was stated that TAP and antistasin were used to evaluate FXa as a drug target.[56]

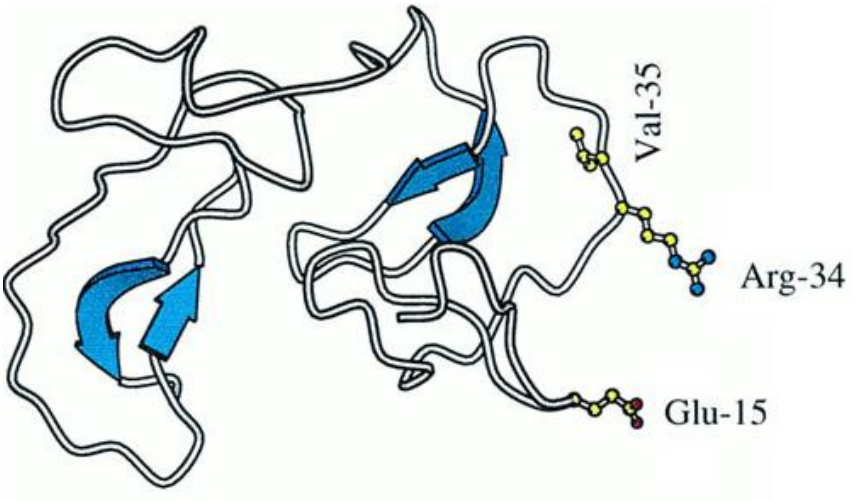
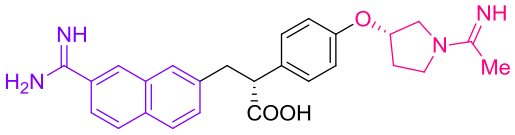


Figure 2.1. Structure of 'Antistasin' first invented FXa inhibitor.[53]

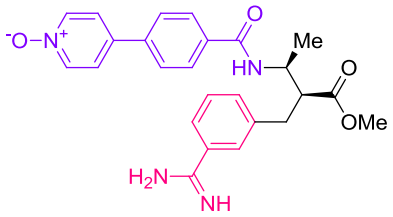
2.2. Early prototype molecules for the development of direct FXa inhibitors

The limitations of the LMWHs such as indirect activity, restricted capacity to inhibit fibrin-bound FXa and administration by intravenous route, motivated researchers to identify orally active small-molecules as direct FXa inhibitors. The first generation of small-molecules were progressed to phase II clinical trials as parenteral agents.[57] DX-9065a (**11**) was discovered as the first potent and selective FXa inhibitor (FXa K_i = 41 nM, thrombin K_i > 2000 μM, trypsin K_i = 620 nM) with good anticoagulant (PT_{2X} = 0.52 μM, $aPTT_{2X}$ =

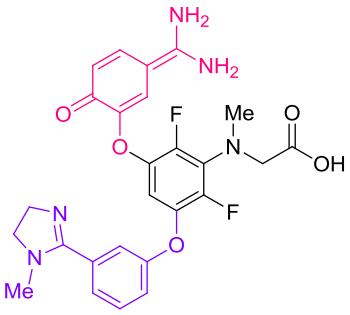
0.97 μM) activity. Due to its very poor human oral bioavailability (F = 2-3%), compound (**11**) was clinically developed as a parenteral agent.[58,59]



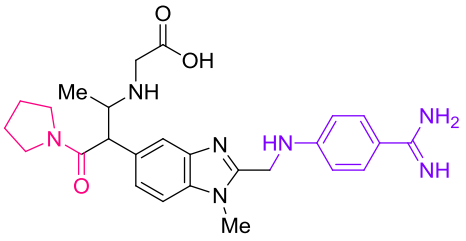
DX9065a (11)



Otamixaban (12)



Fidexaban (13)



Tanogitran (14)

Otamixaban (**12**), a 2,3-disubstituted β-aminoester analog was found to be a potent, reversible FXa inhibitor (K_i = 0.5 nM) with excellent *in vitro* anticoagulant (PT_{2X} = 1.1 μM, $aPTT_{2X}$ = 0.41 μM) activity. Due to its high polarity, benzamidine-based compounds have been reported to have poor oral absorption.[60,61]

Fidexaban (**13**, ZK-807834, Berlex-Pfizer) was identified as a third parenteral agent containing two amidine groups and one carboxylic acid moiety. The dihydrochloride salt of compound (**13**) was identified as a potent FXa inhibitor with the K_i value of 0.10 nM. It also demonstrated greater selectivity over other seine proteases like thrombin (20000-fold) and trypsin (2500-fold).[62]

A dual thrombin/FXa inhibitor, tanogitran (**14**), was also investigated in a phase II clinical trial treating a human model of endotoxin-induced coagulation.[63] Compound (**14**) displayed prolongation of prothrombin time and reduction of *in vivo* thrombin generation in dose-dependant manner.

The discovery programs for the development of orally bioavailable, direct FXa inhibitors leading to clinical studies, along with a brief summary of

the early inhibitors that resulted into some of these agents have been discussed below.

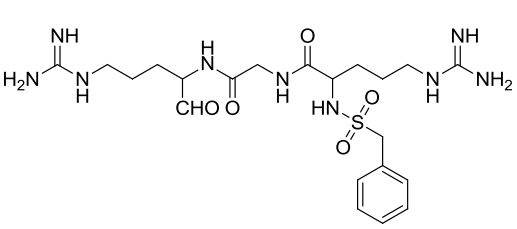
2.3. Approaches for the discovery of oral FXa inhibitors

The first crystal structure of FXa was reported in 1994. As per the inputs obtained from enzymatic studies and the available crystallographic structure of FXa, three pharmacophoric units i.e. P1 moiety, P4 moiety and the central scaffold are required to be present in potential FXa inhibitors. These chemical groups (P1, central scaffold and P4) are considered to form U/V or L shaped molecules. Different P1, P4 and central scaffolds have been identified by various research groups to develop orally active selective FXa inhibitors.

2.3.1. Covalent inhibitors: Peptidomimetics and transition state analogs

Extensive efforts were made to identify FXa inhibitors derived from the previous findings of thrombin inhibitors that incorporated "serine traps" (eg. aldehyde or ketothiazole groups). These moieties can interact covalently with the hydroxyl group of Ser195 to reversibly mimic a tetrahedral transition state. Compounds (**15-17**, IC_{50} values of 15 nM, 0.13 nM and 0.83 nM respectively) were a type of early FXa inhibitors incorporating a "serine trap".[64–66]

Similar to the transition-state thrombin inhibitors, some of the initially developed FXa inhibitors also comprised of an arginine or constrained arginine P1 residue that would closely interact with Asp189 S1 residue, lined by aromatic residues structured to be accommodated into the hydrophobic S1 binding pocket of FXa.[57] It also became clear in the structure of these inhibitors that basic or weakly basic substituents could be maintained in both the S1 and S4 binding pockets, with the basic P4 moiety exhibiting π-cation interaction with the hydrophobic residues of the S4 pocket. Several peptidomimetic inhibitors were ultimately derived by introducing heterocyclic amide-bond substituents, like pyridone (**18**, FXa IC_{50} = 3 nM), while retaining favorable FXa binding affinity. However, oral bioavailability could not be achieved for these compounds.[67] There are no available reports of any transition-state FXa inhibitors progressing into clinical trials.



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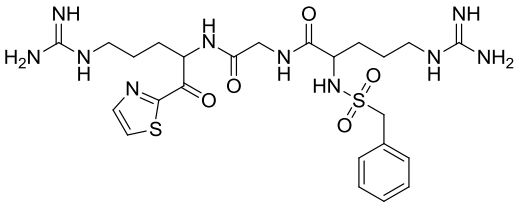

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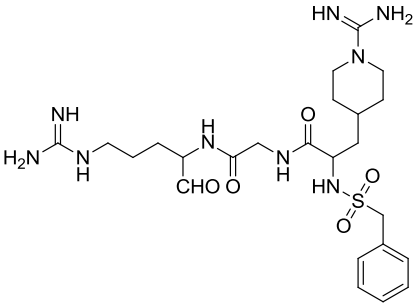

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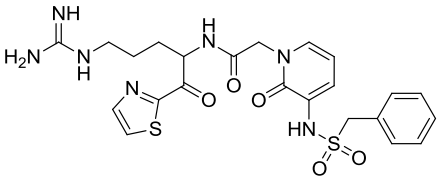
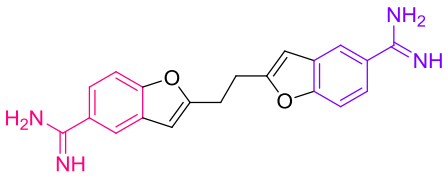


(18)

2.3.2. Early dibasic benzamidine approach

Based on the success of compound (**11**), various research groups tried to further develop amidine-based FXa inhibitors. In most of these reports, amidine groupings were used as P1 and P4 motifs to develop non-peptidic, small-molecule bis-amidine compounds (**19**, bovine FXa K_i = 570 nM) and (**20**, bovine FXa K_i = 610 nM).[68,69]

Initial efforts to improve FXa inhibitory activity and oral bioavailability, researchers at Daiichi Sankyo identified direct analogs of compound (**11**) like indoline compound (**21**, FXa IC_{50} = 7.6 nM) and compound (**22**, FXa IC_{50} = 3.9 nM). These two compounds showed good binding affinity to FXa. But they have some selectivity issues with trypsin (K_i = 24 and 39 nM, respectively).[68] To overcome this issue, modifications were carried out by replacing the naphthyl P1 moiety with substituted benzamidine and inserting a hydroxyl group on the P1 benzamidine moiety to obtain compound (**23**, FXa IC_{50} = 4.4 nM). This compound (**23**) displayed an improved selectivity profile for FXa over trypsin (K_i = 1500 nM). Compound (**23**) was also found to be active in the *in vitro* clotting tests (PT_2 = 0.39 μM, $aPTT_{2X}$ = 0.34 μM); however its oral bioavailability could not be achieved.[70]



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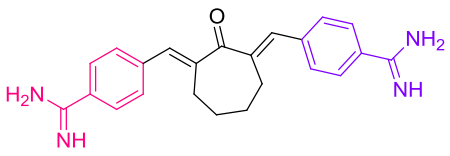

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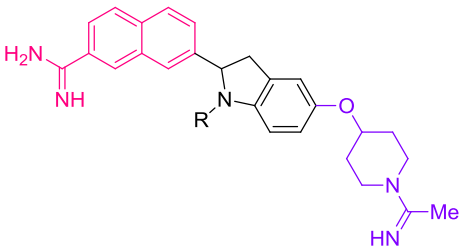
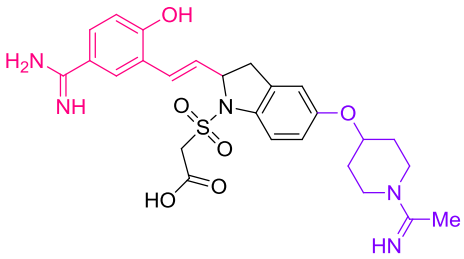


(21) R=SO_2Et
(22) R=SO_2CH_2COOH



(23)

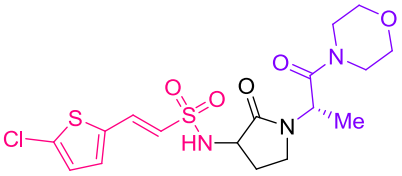
2.4. Transition from benzamidine to oral agents

Due to lack of oral bioavailability and nonselectivity of amidine-based FXa inhibitors, the amidine group was replaced by nonbasic functional groups as P1 motifs. Initially, efforts were made to develop orally active selective FXa inhibitors having halo or other substituted benzenes as P1 motifs.[71] The neutral chlorinated P1 motifs bind effectively to Tyr228 of S1 pocket by C-Cl- -π interaction improving selectivity and oral bioavailability.[72] On the other side, amidine or benzamidine as P4 motifs were also replaced by bi- or mono-aryl motifs.[73] To connect the P1 and P4 moieties, various central scaffolds have been explored to form U/V or L shaped molecules. Different P1, P4 and central scaffolds have been identified by various research groups to develop orally active selective FXa inhibitors. Here, we briefly describe the developments on the basis of their chemical classes.

2.4.1. Pyrrolidine based FXa inhibitors

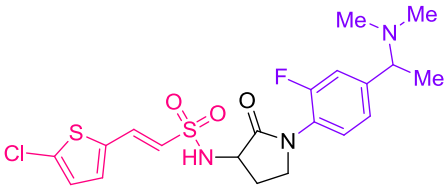
Researchers at Glaxo Smith Kline[74] employed structure and property based drug designing approach to design novel FXa inhibitors having similar size, hydrophobicity and molecular weight as that of compound (**24**), a clinical candidate. Compounds were prepared having different monoaryl motifs as S4 binding ligands. Compound (**25a**, FXa, K_i = 0.8 nM; $PT_{1.5X}$ = 0.9 µM), a mixture of two diastereomers (**25b** and **25c)**, having *N*,*N*-dimethylamino

group as P4 motif and 2-(5-chlorothien-2-yl)ethenyl as P1 motif showed good anticoagulant activity and excellent inhibition of FXa. The individual isomers (**25b**, FXa, K_i = 2 nM; $PT_{1.5X}$ = 0.7 µM, $t_{1/2}$= 1.3h, F = 54 %) and (**25c**, FXa, K_i = 1 nM; $PT_{1.5X}$ = 0.9 µM, $t_{1/2}$ = 1.6 h, F = 23 %) showed similar profiles.



(24)

FXa, K_i = 4 nM
$PT_{1.5X}$ = 1.2 µM

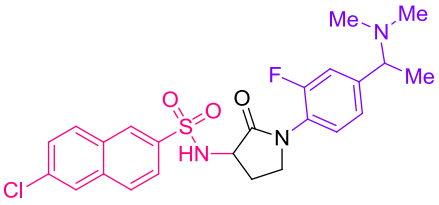


(25a-25c)

	FXa K_i (nM)	$PT_{1.5X}$ (µM)
25a	0.8	0.9
25b (1*R*)	2	0.7
25c (1*S*)	1	0.9

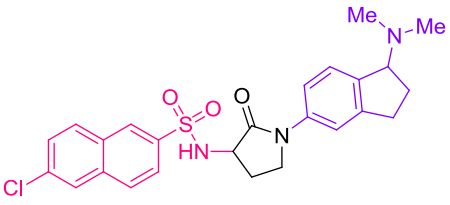
Compound (**26a**, FXa, K_i = 0.8 nM; $PT_{1.5X}$ = 2.9 µM) was a mixture of two diastereomers (**26b** and **26c**) containing the same P4 *N*,*N*-dimethylamino motif as present in (**25a-25c**) with slightly more bulky 6-chloronaphthyl group as the P1 motif. The isomers (**26b**, FXa, K_i = 2 nM) and (**26c**, FXa, K_i = 2 nM) exhibited slightly reduced anticoagulant activity ($PT_{1.5X}$ = 1.9 µM and 2.1 µM respectively) but somewhat improved pharmacokinetic profiles in terms of increased half-lives ($t_{1/2}$ = 2.6 hr and 2.1 hr respectively).

To overcome the time dependent inhibition (TDI) of CYP3A4, α-methylbenzylamine group was converted into cyclic structures like aminoindane (**27**) and phenylpyrrolidine (**28**).[75] Among these, the aminoindane derivatives showed significant time dependent P450 inhibition (TDI = 2.2 fold). Compounds (**28a-28c**) having phenylpyrrolidine as the P4 motif demonstrated comparable biological activity (FXa, K_i = 2-4 nM; $PT_{1.5X}$ = 4.5-8 µM) and pharmacokinetic profiles (plasma clearance 2.6-2.8 ml/min/kg, volumes of distribution 1.7-1.9 L/kg, half-lives 7.5-10.2 h, oral bioavailability 25-38%) to the clinical candidate (**24**, FXa, K_i = 4 nM; $PT_{1.5X}$ = 1.2 µM; Cl_p = 8 ml/min/kg; V_{ss} = 0.29 L/kg; $t_{1/2}$ = 0.7 h and F = 75%).



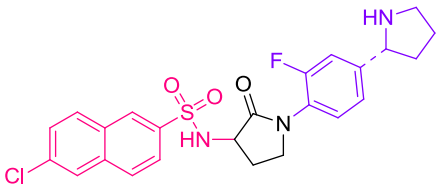
(26a-26c)

	FXa, K_i (nM)	$PT_{1.5X}$ (μM)
26a	0.8	2.9
26b (1*R*)	2	1.9
26c (1*S*)	2	2.1



Aminoindanes (27a-27c)

	FXa, K_i (nM)
27a	3
27b (1*R*)	9
27c (1*S*)	2



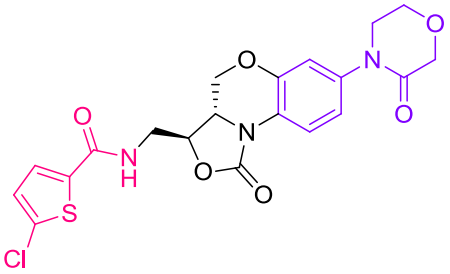
Phenylpyrrolidines (28a-28c)

	FXa, K_i (nM)
28a	2
28b (2*R*)	2
28c (2*S*)	4

2.4.2. Oxazolidinone and isoxazole based FXa inhibitors

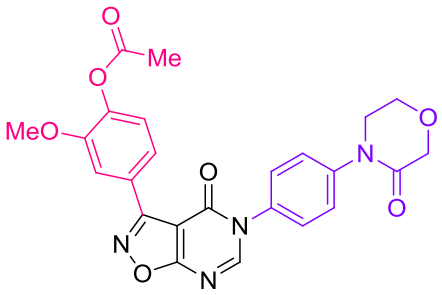
Based on the structure of the approved FXa inhibitor rivaroxaban (**7**), Xue et al. designed a novel series of fused [5,6,6] tricyclic oxazolidinones.[76] An additional linker was introduced to form a bridge between oxazolidine ring and phenyl ring without disturbing the structural planarity of the molecule. Structural modifications of both P1 and P4 groups and the linkers resulted into potent and highly selective FXa inhibitor (**29**, FXa IC_{50} = 3.41 nM; Thrombin IC_{50}> 20 mM) having good oral bioavailability (F = 92% in dogs) and excellent *in vivo* efficacy. Compound (**29**) showed superior *in vivo* efficacy (ED_{50} = 2.97 mg/kg) to rivaroxaban (**7**, ED_{50} = 4.53 mg/kg) in arterial thrombosis model of rats.

Yang et al. employed bicyclic isoxazole scaffold with different P1 and P4 groups to develop novel FXa inhibitors.[77] Optimization of P1 and P4 moieties by attaching various substituents led to the discovery of compound (**30**) having high selectivity (FXa, IC_{50} = 0.013 μM; Thrombin, IC_{50}> 20 mM) and good anticoagulant effect in human plasma (PT_{2x} = 2.12 μM).



(29)

FXa, IC_{50} = 3.41 nM

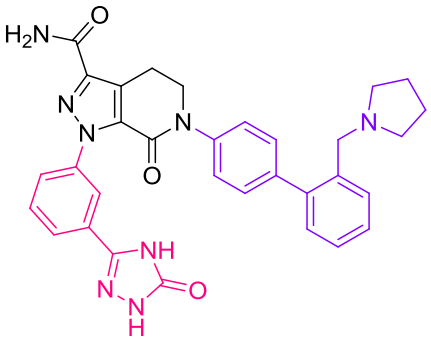


(30)

FXa, IC_{50} = 0.013 µM
PT_{2x} = 2.12 µM

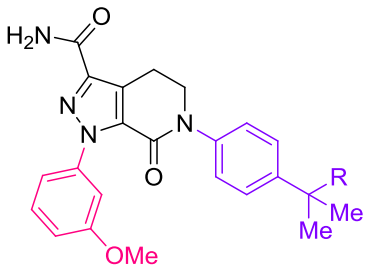
2.4.3. Pyrazole based FXa inhibitors

Due to the success of apixaban (**8**), Bristol-Myers Squibb researchers[78] introduced phenyltriazolinone moiety to the bicyclic pyrazole scaffold of apixaban (**8**) to discover compound (**31**) with good selectivity (FXa, K_i = 0.25 nM; FIIa, K_i > 6000 nM). Compound (**31**) exhibited comparable anticoagulant activity (PT EC_{2x} = 4.4 µM) to apixaban (**8**, FXa, K_i = 0.08 nM; PT EC_{2x} = 3.8 µM). Compound (**32**) bearing a neutral hydroxymethyl group as a P4 motif showed good FXa affinity (FXa, K_i = 0.66 nM; FIIa, K_i = 2578 nM) and moderate anticoagulant activity (PT EC_{2x} = 3.8 µM) along with good pharmacokinetic profile in dogs (clearance value of 0.98 L/kg/h, V_{dss} value of 3.02 L/kg, $t_{1/2}$ value of 4.95 h and F value of 50%). Compound (**33**) with methylsulfone as the P4 moiety exhibited high FXa affinity, good anticoagulant activity (FXa, K_i = 0.48 nM; PT EC_{2x} = 3.90 µM) and a favorable pharmacokinetic profile (clearance = 0.32 L/kg/h, V_{dss} = 0.51 L/kg, $t_{1/2}$ = 1.70 h and F = 79%).[79]



(31)

FXa, K_i = 0.25 nM

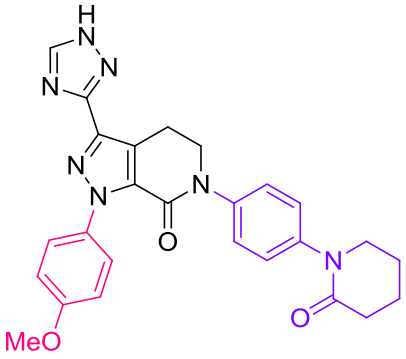


(32,33)

(32) R = CH_2OH; FXa, K_i = 0.66 nM
(33) R = SO_2Me; FXa, K_i = 0.48 nM

Wang et al. designed and synthesized apixaban (**8**) derivatives by retaining the 1,4,5,6-tetrahydro-7*H*-pyrazolo[3,4-*c*]pyridin-7-one scaffold.[80] The carboxamido group at C-3 position of pyrazole ring was replaced by nitrogen containing heterocycles such as 1,2,4-triazole and pyrrole in order to accommodate the S2 binding pocket and strengthen the hydrogen bonding to Glu146. Among the series, compound (**34**, PT = 0.5, aPTT = 0.8, IC_{50} = 0.15 µM) was identified as the most potent FXa inhibitor with superior anticoagulation property to apixaban (PT = 0.8, aPTT = 0.9, IC_{50} = 0.23 µM). Compound (**34**) also displayed excellent *in vivo* efficacy (99 % inhibition rate).

In a similar manner, Sun et al. also used the tetrahydropyrazolopyridone scaffold with 1,3,4-triazole, 1,2,3-triazolomethyl and 1,2,4-triazolomethyl groups as P2 motifs.[81] Introduction of 1,3,4-triazole moiety at C-3 position of pyrazole ring showed pronounced anticoagulant activity. Particularly, compound (**35**) with morpholinyl-methyl group at C-2 position of 1,3,4-triazole demonstrated excellent potency against FXa with an IC_{50} value of 0.23 µM. In addition, compound (**35**) showed 98% inhibition rate in venous thrombosis model.



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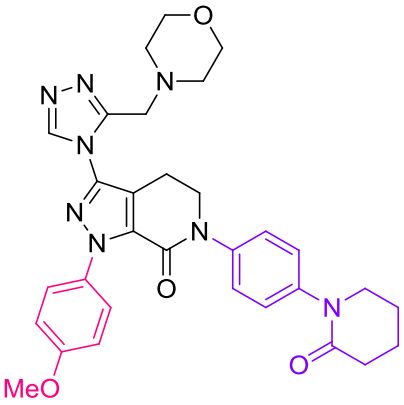
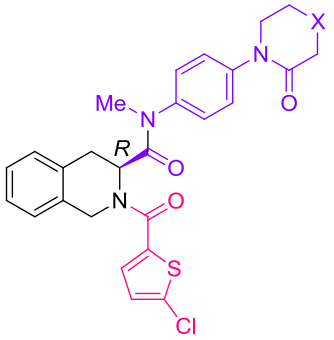


(35)

2.4.4. Tetrahydroisoquinolines as FXa inhibitors

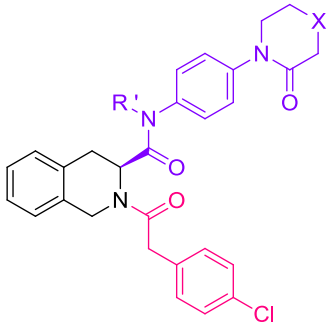
Al Horani et al. discovered tetrahydroisoquinoline as a novel scaffold containing two hydrophobic arms (P1 and P4 motifs).[82] Compound (**36**) was identified as a novel hit from the molecular modeling studies of FXa inhibitors. Replacement of piperidone ring of (**36**, FXa, IC_{50} = 56 µM) with

morpholin-3-one resulted into compound (**37**, FXa, IC_{50} = 36 µM) with somewhat improved activity. The *p*-chlorophenylacetyl group as the P1 moiety in compound (**38**, IC_{50} = 1.3 µM) showed a high affinity for FXa.



(36, 37)

(36) X = CH_2; FXa, IC_{50} = 56 µM
(37) X = O; FXa, IC_{50} = 36 µM



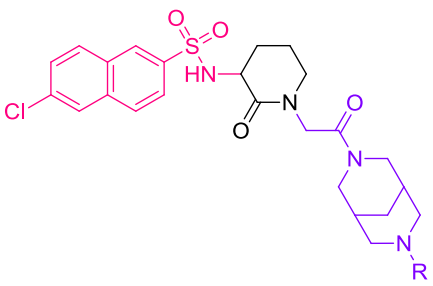
(38, 39)

(38) R' = Me, X = CH_2; FXa, IC_{50} = 1.3 µM
(39) R' = H, X = O; FXa, IC_{50} = 0.27µM; K_i = 135 nM

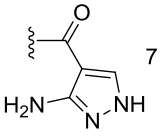
Further optimization of the structure led to the discovery of a dicarboxamide (**39**, IC_{50} = 0.27 µM) as a highly efficacious FXa inhibitor with a K_i value of 135 nM, PT value of 17.1 µM and aPTT value of 20.2 µM in human plasma. Compound (**39**) showed 1852 fold higher selectivity for FXa inhibition over other serine proteases.

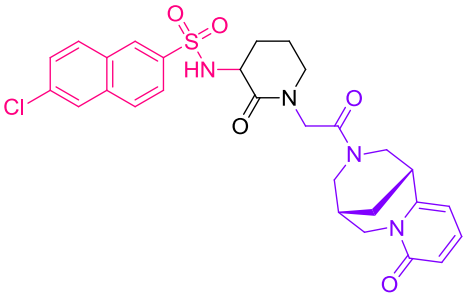
2.4.5. Arylsulfonamidopiperidone derivatives

Researchers at Bristol-Myers Squibb reported a novel series of compounds (**40-42**) having the arylsulfonamide-valerolactam scaffold.[83] Among the series, compounds (**40** and **42**) containing the 3,7-diazabicyclo[3.3.1]nonan-3-yl group and cytosine as P4 motifs showed good FXa inhibitory and anticoagulant activities. X-ray crystal structure of compound (**42**) in the bound form to human FXa revealed that the 6-chloro-2-naphthyl group binds to the S1 pocket, with the acylcytisine group occupying the S4 pocket. Derivatization of -NH group of 3,7-diazabicyclo[3.3.1]nonan-3-yl of compound (**40**) resulted into the formation of the most potent compound (**41**, FXa, IC_{50} 7 nM; PT EC_{2x} = 1.7 µM) of the series.



(40, 41)

	R	FXa, IC_{50} (nM)	PT EC_{2x} (µM)
(40)	H	22	3.5
(41)		7	1.7

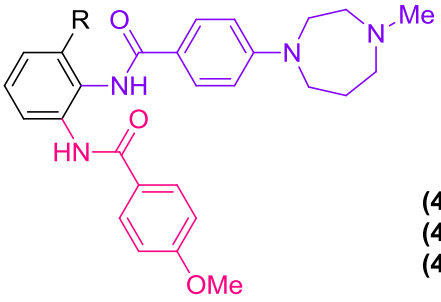


(42)

FXa, IC_{50} = 13 nM
PT EC_{2x} = 4.1 µM

2.4.6. Anthranilamides and disubstituted benzenes as FXa inhibitors

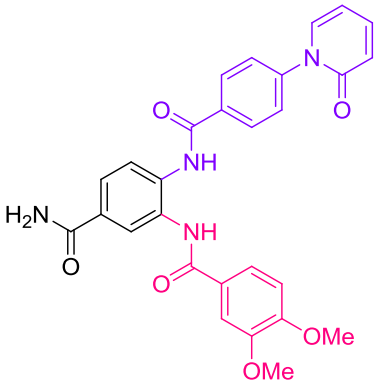
As an output of high-throughput screening (HTS) programme, researchers at Astellas Pharma identified non-amidine group containing FXa inhibitors. Initially, their efforts resulted into compound (**43**) with good FXa inhibitory and anticoagulant activities (FXa, IC_{50} = 103 nM; $PTCT_{2x}$ = 2.8 µM). Introduction of a phenolic hydroxyl group at 3rd position of the central phenyl ring in compound (**43**) led to the discovery of darexaban (**44**) with an improved FXa inhibitory activity (IC_{50} = 54.6 nM) and good anticoagulant property ($PTCT_{2x}$ = 4.1 µM). Subsequent metabolic studies on compound (**44**) demonstrated formation of glucuronide conjugate (**45**, FXa, IC_{50} = 28.6 nM; $PTCT_{2x}$ = 2.5 µM) as an active and stable metabolite of compound (**44**). Compound (**44**, FXa, K_i = 0.031 µM) and its metabolite (**45**, FXa, K_i = 0.020 µM) showed higher selectivity for FXa over other serine proteases (K_i of **44** and **45** >100 µM for FIIa).[84]



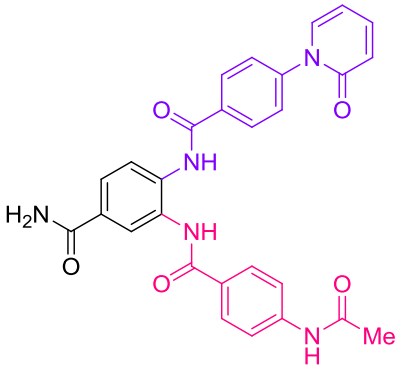
	R	FXa, IC_{50} (nM)	$PTCT_2$ (μM)
(43)	H	103	2.8
(44)	OH	54.6	4.1
(45)	OGlu	28.6	2.5

(43-45)

Yang et al.[85] independently used 3,4-diaminobenzoyl scaffold with different P1 and P4 motifs. Among the reported series of derivatives, compound (**46**) with 3,4-dimethoxyl group and compound (**47**) having 4-acetamido group as S1 binding ligands showed comparable FXa inhibitory activity (FXa, IC_{50} = 17.1 nM and 15.6 nM respectively) to rivaroxaban (**7**, FXa, IC_{50} = 14.4 nM). Compound (**46**) showed higher selectivity for FXa over other serine proteases like thrombin and trypsin (IC_{50} = >100 μM for thrombin). Compound (**46**) also exhibited excellent *in vivo* antithrombotic activity.

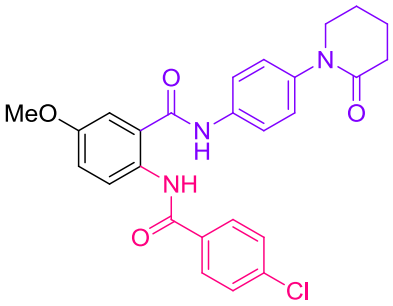


(46)
FXa, IC_{50} = 17.1 nM



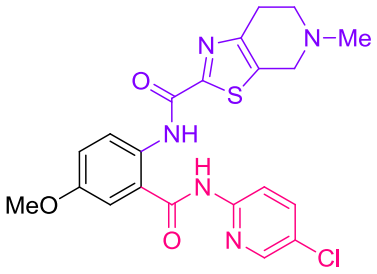
(47)
FXa, IC_{50} = 15.6 nM

Xing et al.[86] successfully applied fragment based drug designing and virtual screening strategies to identify novel compounds possessing FXa inhibitory activity. Compound (**48**) was found as the potent FXa inhibitor (FXa, IC_{50} = 23 nM) having excellent selectivity (FIIa, IC_{50} = 48 μM) and potent anticoagulant activity (PT_{2x} = 8.7 μM).



(48)

FXa, IC_{50} = 23 nM
FIIa, IC_{50} = 48 µM



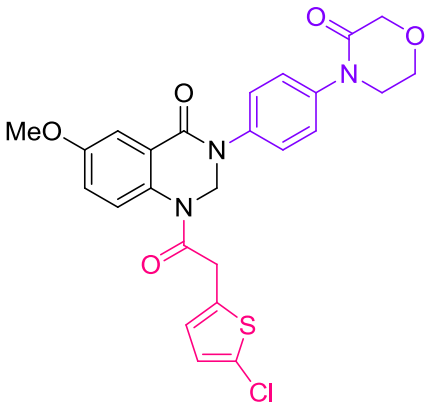
(49)

FXa, IC_{50} = 3.5 nM
FIIa, IC_{50} = 4.2 µM
2 x PT = 0.8 µM
2 x aPTT = 0.5 µM

In continuing efforts, the same research group employed structure-based design strategies to develop safer and effective FXa inhibitors.[87] Modifications at P1 and P4 groups led to compound (**49**) as highly potent and selective FXa inhibitor. Compound (**49**) showed excellent *in vitro* anticoagulant property. It also exhibited good *in vivo* antithrombotic potential in the venous thrombosis (54% at 10 mg/kg) and AV-SHUNT (67% at 10 mg/kg) models. The tail bleeding model indicated that compound (**49**) at 1mg/kg and 5 mg/kg dose was considered to be safer than betrixaban (**10**). Moreover, the pharmacokinetic profile of compound (**49**) was adequate. Furthermore, in the MTT test, Compound (**49**) displayed significant ability to improve the viability of H9C2 cells caused by hypoxia–reoxygenation.

2.4.7. 2,3-Dihydroquinazolin-4(*1H*)-one derivatives

Based on the promising results obtained for compound (**48**), Xing et al. designed a novel series of dihydroquinazolin-4(1*H*)-one derivatives through the cyclization of compound (**48**).[87] Modifications were made to P1 and P4 groups to get excellent potency against FXa. Among the series, Compound (**50**) displayed the strongest potency against FXa with the IC_{50} value of 21 nM and excellent selectivity versus thrombin (IC_{50} = 67 µM). It also exhibited pronounced *in vitro* anticoagulant activity with its 2 x PT value of 1.2 µM and 2 x aPTT value of 0.6 µM. In addition, Compound (**50**) also showed excellent *in vivo* antithrombotic potential in the arteriovenous (AV) shunt model similar to as that of betrixaban (**10**).



(**50**)

Moreover, Compound (**50**) and betrixaban prolonged the bleeding time in a dose-dependent manner to the same extent in a rat tail-bleeding time study which exhibited that they have a similar safety profile in terms of bleeding risks.