2. LITERATURE REVIEW

Two different categories of compounds have been described in this thesis. Hence the literature on both of these categories of compounds has been described under separate heads on Factor Xa inhibitors and Thrombin inhibitors.

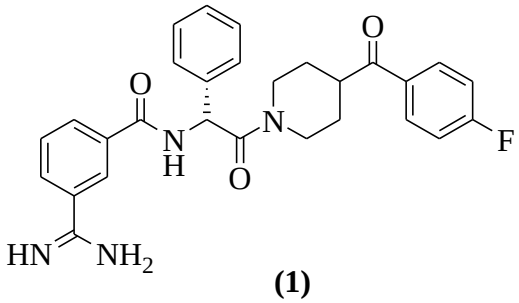
2.1 Factor Xa inhibitors

Factor Xa inhibitors falling under various chemical categories are described as given below.

2.1.1. Piperazinyl amides

Jones *et.al* have designed and synthesized benzamidinecarboxamide derivatives with phenylglycine moiety as selective anti-FXa agents using PRO_SELECT software programme where they reported interaction of *D*-amino acids with lipophilic disulphide pocket nearby to S1 subsite made up of Gln192, Cys191, Cys220 and Gly218 residues.[1] Exploration of S4 pocket using primary and secondary amines like benzyl and cyclohexylamines and also piperazines and piperidines resulted into a number of selective FXa inhibitors. (e.g. **1**, FXa, K_i = 0.01 µM; **2**, FXa, K_i = 0.015 µM and **3**, FXa, K_i = 0.013 µM).

From their research on orally active FXa inhibitors, Imaeda *et.al* have discovered novel piperazinylimidazo[1,2-a]pyridine derivatives as potent and selective FXa inhibitors.[2] The detailed SAR studies and rigorous modifications for S4 binding region resulted in active compound **(4)** with FXa IC_{50} value of 0.021 µM and *ex-vivo* PT prolongation at 1.4 µM concentration. To improve the potency, further changes in the imidazopyridine moiety successfully led to the discovery of 2-hydroxymethyl derivative, **(5)** (FXa IC_{50} = 0.009 µM and PT_{2x} = 1.4 µM), a selective, potent and orally bioavailable FXa inhibitor (F = 31% in cynomolgus monkeys) having diminished CYP3A4 inhibition properties.



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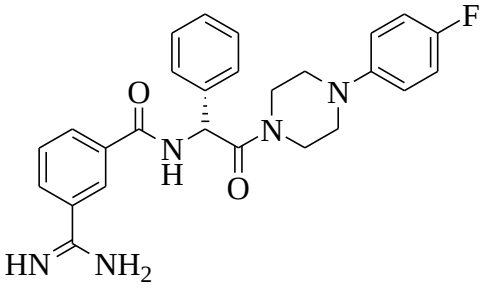

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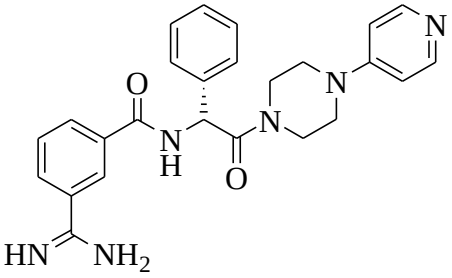

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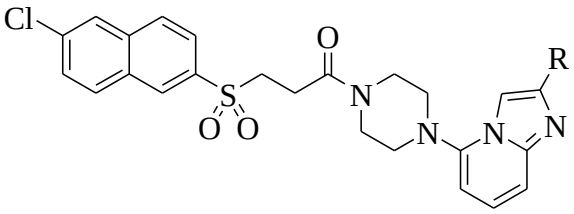


(4) R = H,
(5) R = CH_2OH

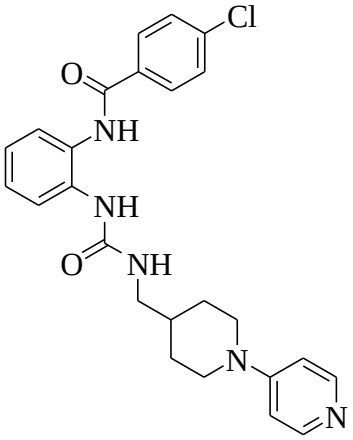
2.1.2. Anthranilamides

A major contribution in this research area was made by research groups at Berlex, Lilly, Portola, Millennium, Bristol-Myers-Squibb, Daiichi-Sankyo and Astellas Pharmaceuticals. A decade of research at these companies led to successful development of many novel active molecules like betrixaban, edoxaban, darexaban and some other compounds that are under clinical trials.

To get improved oral bioavailability, Masters *et.al* at Lilly Laboratories disclosed non-amidine group comprising of 1,2-diamidobenzene derivatives as potent antithrombotic agents.[3] The most active compound of the series, **(6)** (FXa, K_{ass} = 100×10^{-6} L/mol and PT_{2x} = 0.58 µM) exhibited high selectivity (>500 fold) towards FXa and good antithrombotic efficacy at different doses in rabbit arterio-venous shunt model.

Zhang *et. al* at Millennium Pharmaceuticals Inc**.** developed anthranilamide class of selective potent FXa inhibitors. Compounds **(7** and **8)** exhibited potent anti-FXa activities (IC_{50} values of 3.4 nM and 6.7 nM respectively) but poor 2xTG (>5 µM) which was due to high lipophilicity.[4] Hence, in order to lower lipophilicity of these compounds various structural

optimizations in S4 binding biphenyl moiety and central ring conferred enhanced *in vitro* potency and improved thrombin generation activity to compounds **(9**, FXa, IC_{50} = 0.2 nM and 2xTG = 1.4 µM) and **(10**, FXa, IC_{50} = 9.5 nM and 2xTG = 2.2 µM) but these compounds showed poor bioavailability.



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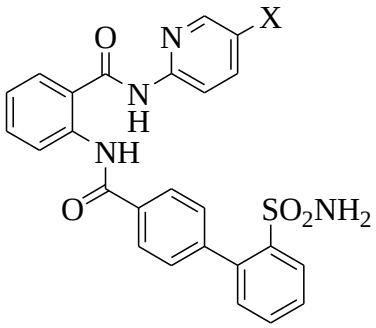
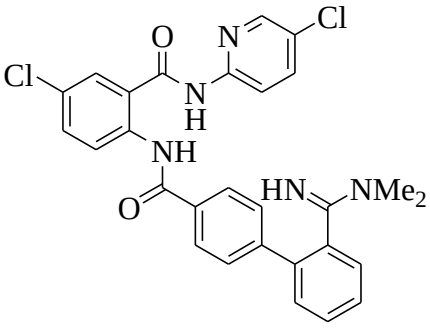


(7) X = -Br
(8) X = -Cl



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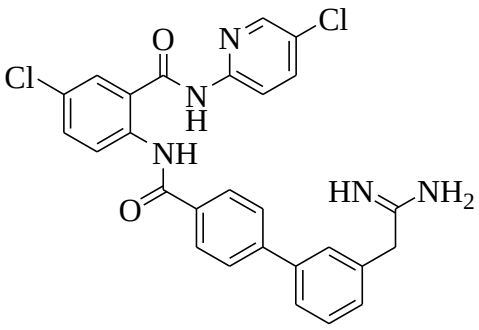
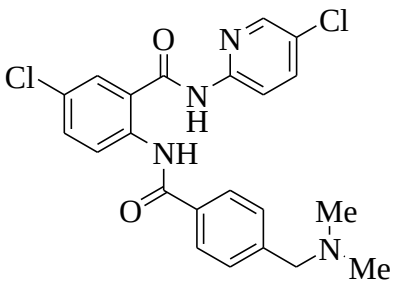


(10)

From their previously reported biphenyl anthranilamides (**7, 8,** 2XTG value > 5 µM) with reduced anticoagulant activity in TG assay, Zhang *et.al* modified lipophilicity of the anthranilamide compounds and synthesized potent FXa active analogs **(11** and **12)** with substituted aminomethylbenzoyl groups as P4 moieties and these derivatives exhibited higher potency in human thrombin generation assay.[5] The derivative **(12)** (FXa, K_i = 1.5 nM and 2xTG = 0.56 µM) displayed excellent antithrombotic potency in *in vivo* deep vein thrombosis model (40 % inhibition at 0.83 µM) along with good pharmacokinetic profile (F = 44 %; $t_{1/2}$ = 8.5 hr).



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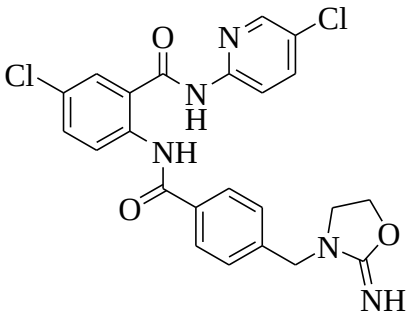
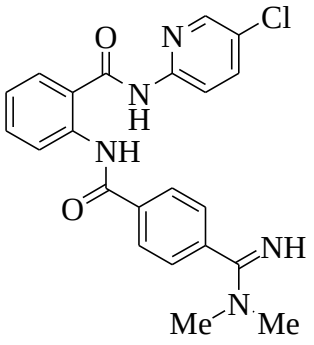


(12)

A series of anthranilamide derivatives containing substituted benzamidines as S4 binding motifs was reported by researchers at Millennium Pharma. Inc. where thorough exploration led to the discovery of compounds **(13-15)** (FXa IC_{50} values of 3.0 nM, 3.0 nM and 20 nM respectively) as potent FXa inhibitors with *N*,*N*-dialkylbenzamidine moieties.[6] These compounds displayed good activity in human TG assay (at 0.54 µM, 0.36 µM and 1.1 µM concentrations) and oral bioavailability in beagle dogs (F = 69 % and 50 % for **13** and **15** respectively). Due to its balanced activity profile compound **(13)** was chosen for further advanced development.



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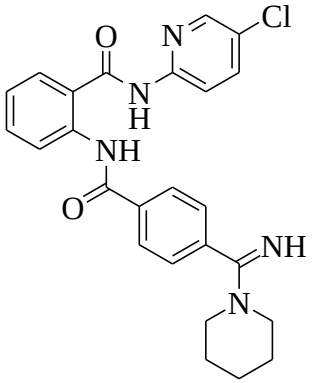

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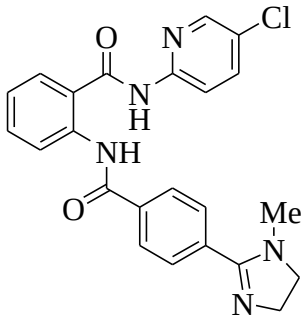
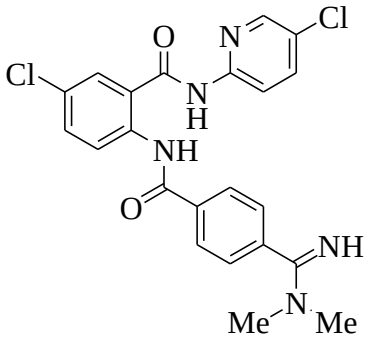


(15)

A detailed investigation on SAR studies of active compound (**13)** by Zhang *et.al* resulted into a series of compounds with high potency, efficacy and selectivity for direct FXa inhibition activity.[7] Derivatives **(16**, FXa, K_i = 0.04 nM, PT_{2x} = 0.83 µM); **17**, FXa, K_i = 0.11 nM, PT_{2x} = 1.7 µM) and **18**, FXa, K_i = 0.06 nM, PT_{2x} = 1.1 µM) were found to possess superior *in vitro* anti-FXa potency and antithrombotic activity along with good oral bioavailability in dogs (**16**; 64.9 % and **18**; 68.8%) and monkeys (**16**; 73% and **18**; 86%). However further evaluation of these

compounds for safety studies revealed their hERG channel inhibition at 0.11 µM and 0.42 µM respectively while compound **(17)** showed IC_{50} of 8.9 µM in hERG patch clamp assay. Thus, due to better overall biological activity profile, favorable PK data, safety concerns and low production cost, compound (**17**) was selected for clinical studies.



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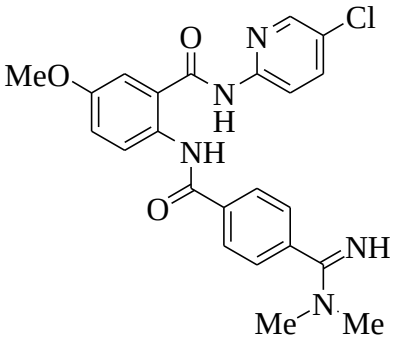
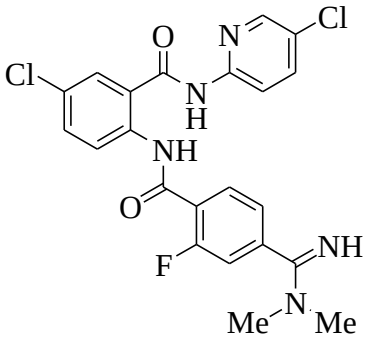
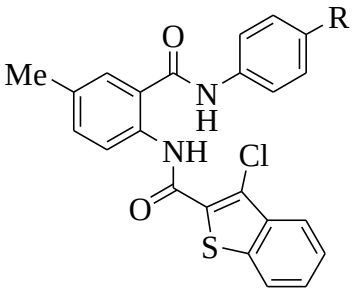


(**17**)

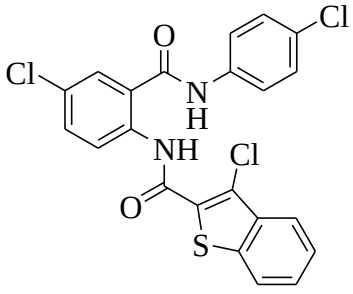


(**18**)

From high throughput screening program at Berlex, Chou *et.al* first invented compound (**19)** (FXa, $K_{i,app}$ = 11 nM) as selective and potent FXa inhibitor.[8] A detailed investigation by optimizing all three rings of **19** resulted into a series of non-amidine anti-FXa agents e.g **(20**, FXa $K_{i,app}$ = 0.32 nM and **21**, FXa $K_{i,app}$ = 0.60 nM), with subnanomolar activity but poor *in vitro* PT prolongation properties (PT_{2X} ~ 500 µM) which was likely to be due to their high lipophilicity and poor solubility. SAR studies disclosed that small groups like methyl or chloro at the C-5 position of the central phenyl ring were essential for FXa potency and 4-chloro or 4-bromo substituents at the aniline ring resulted in high activity.

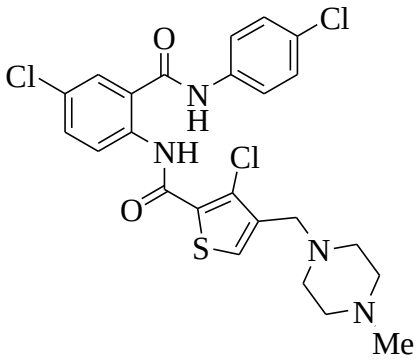


(**19**) R= 4-F
(**20**) R= 4-Cl



(21)

Further modifications for the improvement of the physicochemical properties of analog **(20)**, Kochanny *et.al* reported thiophene derivatives containing basic or hydrophilic substituents displaying more potent *in vitro* antithrombotic activity.[9] Introduction of aliphatic diamines or piperazines at C-4 of thiophene ring afforded compounds with better FXa inhibitory potency (e.g. **22**, FXa $K_{i,app}$ = 1.0 nM, PT_{2X} = 12µM; **23**, FXa $K_{i,app}$ = 0.3 nM, PT_{2X} = 5 µM). Compound **(23)** showed 41% oral bioavailability in dogs at 10 mg/kg dose and the crystal structure of **22** revealed that it binds with the enzyme in characteristic L-shape conformation with chlorobenzene occupying the S1 pocket and piperazine in the S4 site.



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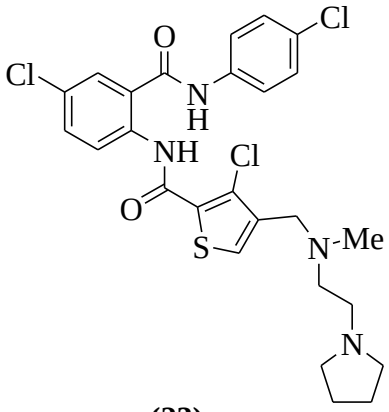

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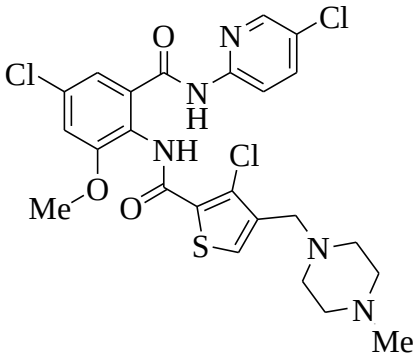
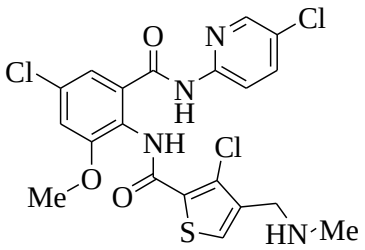


(24)

A detailed SAR of compound **(22)**, a poor anticoagulant (PT_{2X} = 12µM) obtained from the previous studies, was explored by Ye *et.al* to get more potent derivatives.[10] Initial modifications like introduction of 5-chloropyridine ring in place of 4-chlorobenzene and attachment of C-3 methoxy group to the phenyl ring of anthranilamide yielded compound **(24)** (FXa $K_{i,app}$ = 0.16 nM, PT_{2X} = 1.6 µM) with enhanced anti-FXa potency and improved antithrombotic activity. Substitution on C-4 of the thiophene moiety by different aliphatic, alicyclic and heterocyclic amines led to a series of active FXa inhibitors having picomolar potency and good *in vitro* activity. e.g. **(25**, FXa $K_{i,app}$ = 0.21 nM, PT_{2X} = 1.64 µM**)**, **(26**, FXa $K_{i,app}$ = 0.007 nM, PT_{2X} = 0.36 µM**)** and **(27**, FXa $K_{i,app}$ = 0.005 nM, PT_{2X} = 1.25 µM**)**. All these compounds displayed good oral bioavailability in dogs (F = 75 % for **25**; 56 % for **26** and 98 % for **27**) at 10 mg/kg p.o. dose and also high selectivity (>10,000 fold) towards FXa compared to other serine proteases. They also showed high efficacy in rat venous thrombosis model (ED_{50} = 4.4 mg/kg i.v., 0.4 mg/kg i.v. and 0.36 mg/kg i.v. for **25**, **26** and **27** respectively).



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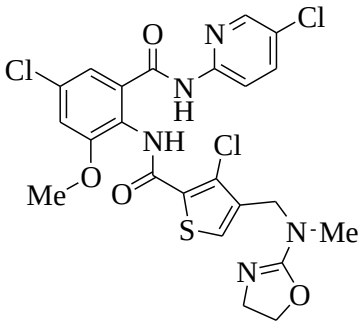

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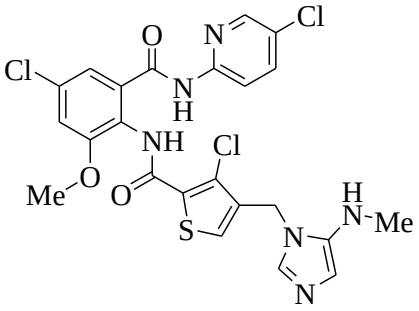
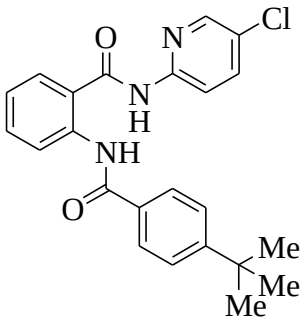


(27)

Mendel *et.al* reported N2-aroyl anthranilamide derivatives as potent FXa inhibitors through SAR optimizations.[11] Compound **(28)** (FXa K_{ass} = 42×10^6 L/mol) with 5-chloropyridine as S1 binding moiety was chosen as prototype for SAR studies. Substitution of 3-aminopropyl ether at C-2 of benzoyl group led to compound **(29)** (FXa K_{ass} = 1440 ×10^6 L/mol, 2_XPT = 0.79 µM) with 34-fold improved potency while modification at C-4 by introduction of *N*-methylhomopiperazine moiety resulted in compound **(30)** (FXa K_{ass} = 8970 ×10^6 L/mol, 2_XPT = 0.14 µM) with picomolar FXa inhibition potency and nanomolar anticoagulant activity.



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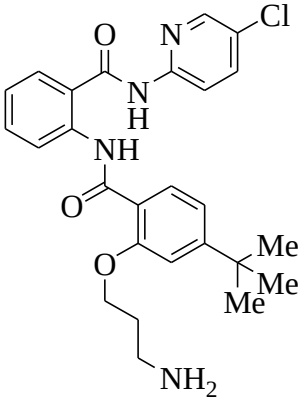

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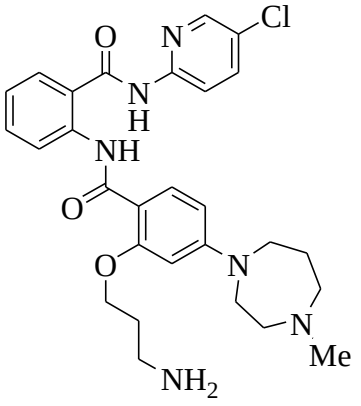
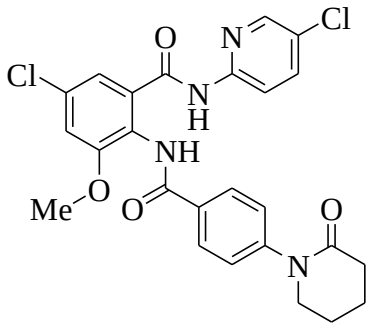


(30)

As a back-up plan to the discovery of a lead molecule apixaban at Bristol-Myers Squibb, Corte *et.al* developed potent FXa active antithrombotic agents by incorporation of phenyl piperidinone and phenylpyridinone as P4 motifs in anthranilamide scaffold.[12] Initial SAR modifications on P1 and P4 groups and then on central phenyl ring led to the very potent compounds of the series (**31**, FXa, K_i = 0.005 nM, EC_{2x} PT = 1.6 µM and **32**, FXa, K_i = 0.057

nM, EC_{2x} PT = 1.5 µM). On further detailed PK and *in-vivo* studies, compound **(32)** exhibited (F = 44 %) good oral bioavailability and good efficacy in rabbit arteriovenous-shunt model with 34 %, 70 % and 80 % inhibition at 0.3, 1.0 and 3.0 mg/kg/hr doses respectively.



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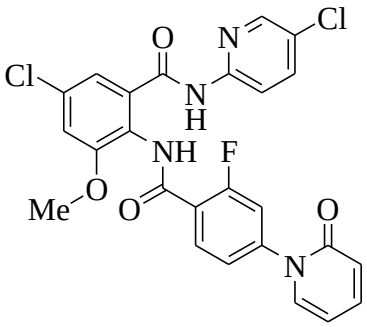
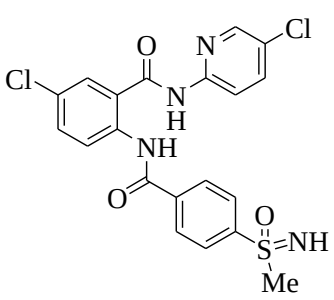


(32)

Pandya *et.al* at Zydus Research Centre have designed and developed novel anthranilamide derivatives as FXa inhibitors bearing sulfoximine moiety as S4 binding motif in place of basic amidine functionality of betrixaban while 2-amino-5-chloropyridine as S1 ligand was retained.[13] The simple sulfoximine lead molecule, **(33)** exhibited 76% inhibition at 0.1 µM. Further modifications by various substitutions on both S and N atoms afforded compound **(34)** (FXa, IC_{50} = 2.1 nM; human $PTCT_2$ = 0.68 µM) which displayed potent anti-FXa and PT prolongation activities. Detailed PK studies of compound **(34)** revealed that its metabolite **(35)** (FXa, IC_{50} = 2.7 nM; human $PTCT_2$ = 0.77 µM) was equally potent. Compound (**34**) also showed high efficacy in animal arterial (32% & 71% reduction at 10 mg/kg and 30 mg/kg doses respectively) and venous (45% & 81% reduction at 10 mg/kg and 30 mg/kg doses respectively) thrombosis models and demonstrated higher selectivity over other enzymes and negligible effect on CYP3A4 inhibition.

Yang *et.al* reported a series of active FXa inhibitors containing 3,4-diaminobenzoyl linker moiety.[14] These new derivatives through intensive SAR modifications were found to show moderate to high *in vitro* anticoagulant potency. Two compounds **(36)** (FXa, IC_{50} = 17.1 nM; 39 % wt. reduction) and **(37)** (FXa, IC_{50} = 15.6 nM; 41% wt. reduction) displayed better *in vivo* activity than the standard rivaroxaban (FXa, IC_{50} = 14.4 nM; 32 % wt. reduction) in venous

models of thrombosis, and high selectivity for compound **(36)** towards FXa compared to thrombin and trypsin.



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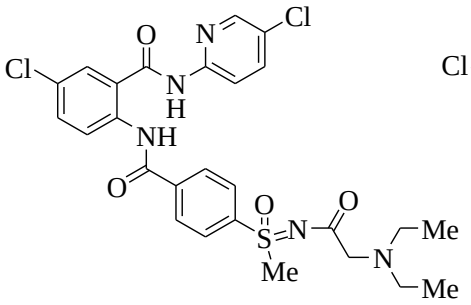

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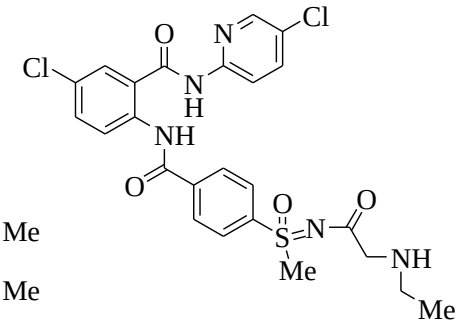

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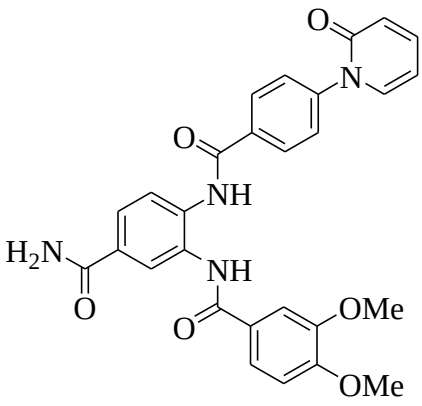

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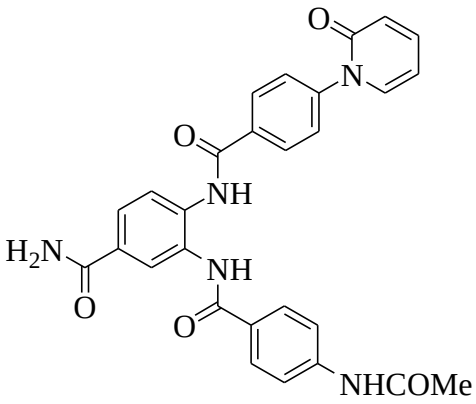
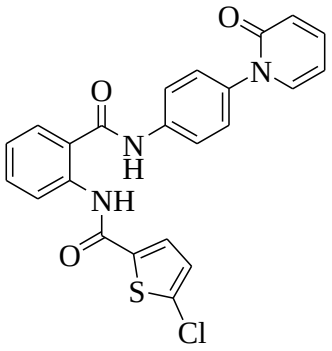


(37)

Wang *et.al* designed a new series of anthranilamides where the connection order of diamides in standard drugs like rivaroxaban was reversed to get compounds with different safety profile or pharmacokinetic properties.[15] Three compounds, **(38)** (FXa IC_{50} = 30 nM), **(39)** (FXa IC_{50} = 25 nM; human PT_{2x} = 12.8 µM) and **(40)** (FXa IC_{50} = 71 nM; human PT_{2x} = 10.4 µM) were identified as potent FXa inhibitors with promising *in vitro* antithrombotic activity and good selectivity against thrombin. Further optimizations of S1 binding moieties from 5-chlorothiophene to 5-bromothiophene or 2,4-dicholrophenyl groups resulted in active derivatives **(41)** (FXa, K_i = 20 nM, human PT_{2X} = 8.4 µM), **(42)** (FXa, K_i = 13 nM, human PT_{2X} = 4.2 µM)

and **(43)** (FXa, K_i = 17 nM, PT_{2X} = 16.4 µM) which displayed pronounced anti-FXa activity and selectivity.[16]



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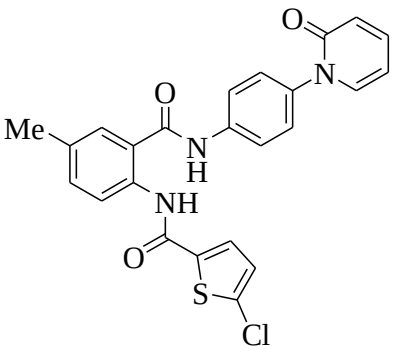

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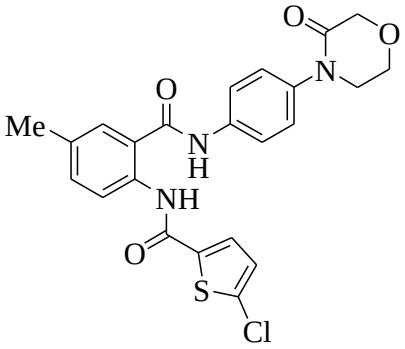

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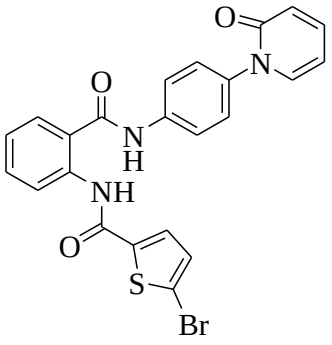

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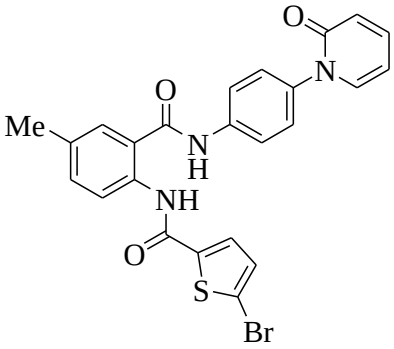

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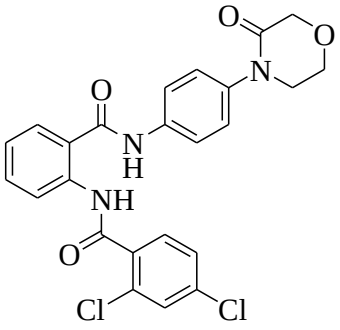
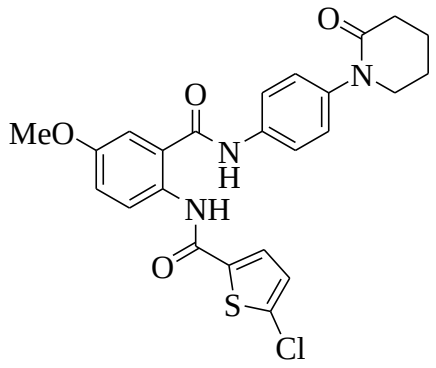


(43)

In pursuit of novel factor Xa inhibitors, Xing *et. al* have designed and synthesized anthranilamide derivatives with potential anti-FXa activity using 3D fragment-based drug design (FBDD) and structure-based pharmacophore modeling approaches.[17] Multi-stage virtual screening further led to the identification of highly persistent two hit compounds **(44** and **45)**, whose derivatives were synthesized and tested for *in-vitro* FXa inhibition. From this series of active compounds, the most potent compound **(46)** has exhibited IC_{50} value of 23 nM and high selectivity against thrombin (IC_{50} = 40 µM). Also compound **(46)** displayed doubling of prothrombin time (PT) at a concentration of 8.7 µM.



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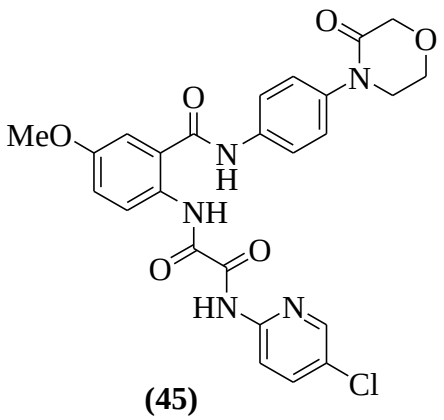

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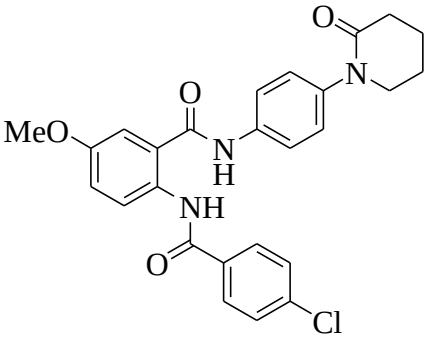

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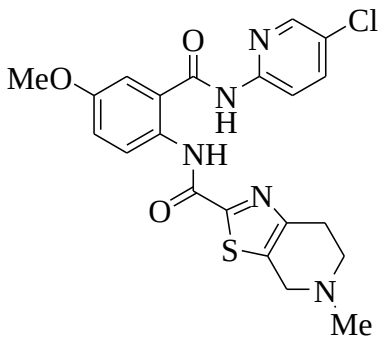
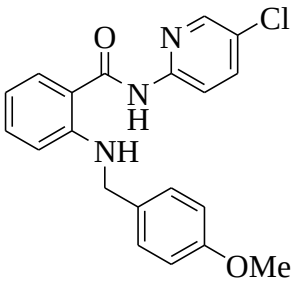


(47)

Continuing their research on development of antithrombotic agents, Xing *et. al* reported novel anthranilamide analogs as direct FXa inhibitors from structure-based design technique wherein optimization of P1 and P4 moieties led to the development of promising derivative **(47)** (IC_{50} = 3.5 nM) exhibiting superior *in vivo* antithrombotic activity in AV-SHUNT model and $FeCl_3$-induced venous thrombosis model in rats.[18] Compound **(47)** displayed safety profile comparable to the standard drug, betrixaban in terms of bleeding at 1 mg/kg and 5 mg/kg doses, along with moderate pharmacokinetic profile. Further investigation using MTT assay showed alleviation of hypoxia-induced H9C2 cell toxicity by the compound (**47**).

Patel *et.al* developed 2-aminobenzamide derivatives as potential FXa inhibitors where a series of aryl, biaryl and piperazinyl analogs were used as S4 binding elements and tested them for antithrombotic activities.[19] Compounds **(48)** (IC_{50} = 11.5 µM), **(49)** (IC_{50} = 5.4 µM) and **(50)** (IC_{50} = 1.3 µM) showed moderate FXa inhibitory activities. The most potent derivative **(51)** displayed an IC_{50} value of 0.7 µM with good selectivity against thrombin and 46 % inhibition

from *in vivo* arterial thrombosis model as well as enhanced *in vitro* clotting time. Molecular dynamics and docking studies supported binding interactions of compound **(51)** with the enzyme.



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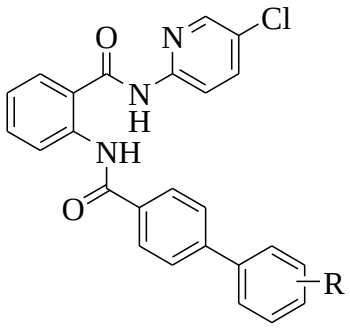
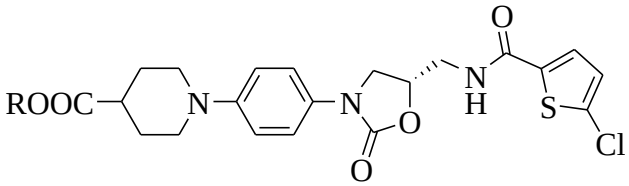


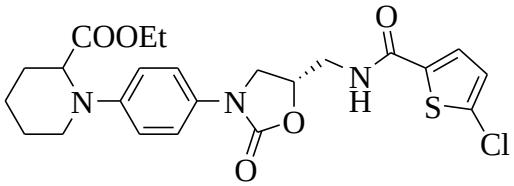
(**49**) R = 2-CN
(**50**) R = 3,5-diF
(**51**) R = 2-F

2.1.3. Oxazolidinone analogs

In their ongoing research on antithrombotic agents, Trstennjak *et. al* designed and synthesized novel dual factor Xa and thrombin inhibitory agents by modification of the morpholin-3-one ring of rivaroxaban to piperidine-4-carboxylate moiety using "designing in" approach.[20] Compound **(52)** with ethyl ester group exhibited K_i value of 0.76 µM for FXa without thrombin activity while its acid derivative, **(53)** displayed dual inhibitory potencies towards FXa (K_i = 0.08 µM) and thrombin (K_i = 16.6 µM). The most active dual inhibitory compound **(54)** (FXa K_i = 0.06 µM) possessing 2-substituted piperidine moiety showed 12-fold increase in FXa inhibition potency than 4-substituted derivative (**53**), along with good antithrombin potency (FIIa, K_i = 0.35 µM)).



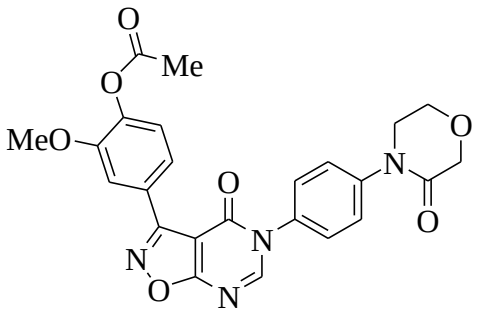
(**52**) R = Et
(**53**) R = H



(54)

2.1.4. Pyrimidinone analogs

Yang *et.al* synthesized and evaluated novel isoxazolopyrimidinone derivatives for their potential antithrombotic activity. Compounds **(55;** IC_{50}= 0.013 µM and **56;** IC_{50}= 0.018µM**)** exhibited strong FXa inhibitory activities. They also displayed good anticoagulant activity in human plasma (2xPT, **55** = 2.12 µM and **56** = 3.37 µM) and high selectivity (> 1000 fold) over other serine proteases. Molecular docking of compound **(55)** with the FXa enzyme demonstrated the formation of π-π interaction of phenyl ring of Tyr99 with the pyrimidinone moiety and multiple hydrogen bonding of carbonyl P1 motif to Ser214 and Trp215.



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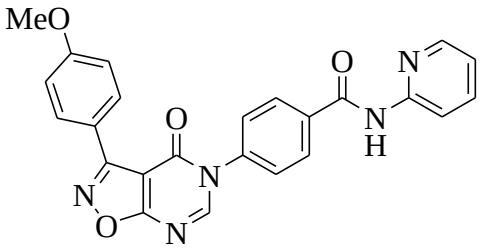


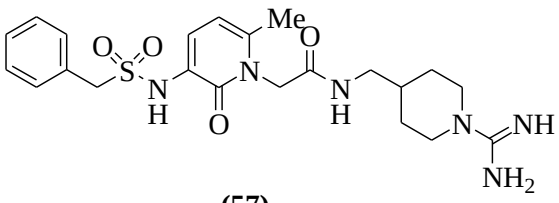
(56)

2.2 Thrombin Inhibitors

2.2.1. Pyrazinone derivatives

Researchers at Merck Laboratories in pursuit of highly efficacious thrombin inhibitors, had designed and developed pyridinone acetamide derivatives for potential antithrombotic activity where compound **(57)** (thrombin, K_i = 0.5 nM) was found to be highly potent but exhibited poor oral bioavailability due to high hydrophilicity of amidinopiperidine moiety.[22] Therefore further studies aimed at improvement in pharmacokinetic properties, led to replacement of P1 amidinopiperidine moiety of compound **(57)** by less basic 2-amino-6-methylpyridine group resulting in derivative (**58**) (thrombin, K_i = 0.5 nM) with similar potency. Methyl substituent in pyridine provided higher selectivity for thrombin against trypsin and improved the potency too. Compound (**58**) also displayed good efficacy in *in vivo* model of thrombosis and better oral bioavailability (F % = 44) in beagle dogs at 1 mg/kg i.v. dose.[23] But

due to short half-lives in monkeys and dogs and also poor pharmacokinetics, its further development was halted. Then, the chemically stable bioisosteres of 3-sulphonylaminopyridinone scaffold viz. 3-alkylaminopyrazinones were designed to circumvent the drawbacks of compound (**58**). These analogs [e.g. compounds (**59)**, FIIa K_i = 0.8 nM and **(60)**, FIIa K_i = 0.35 nM] conferred better pharmacokinetics in rats, monkeys and dogs (compound (**59)**, F = 42%, 60% and 91% respectively) than the previous pyridinone compounds and showed good efficacy *in vivo* and consistent 2x aPTT values.[24]



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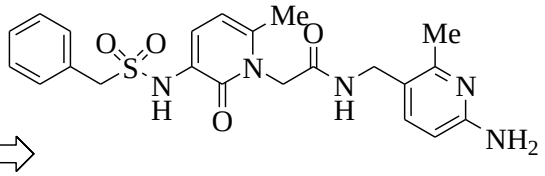

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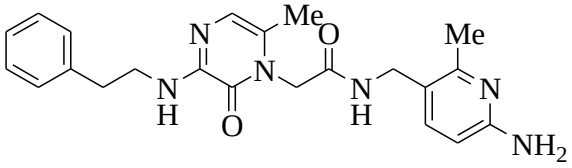

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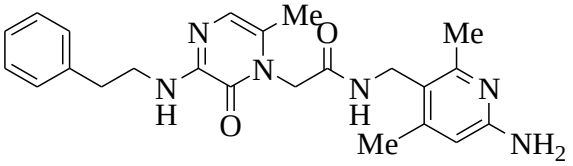
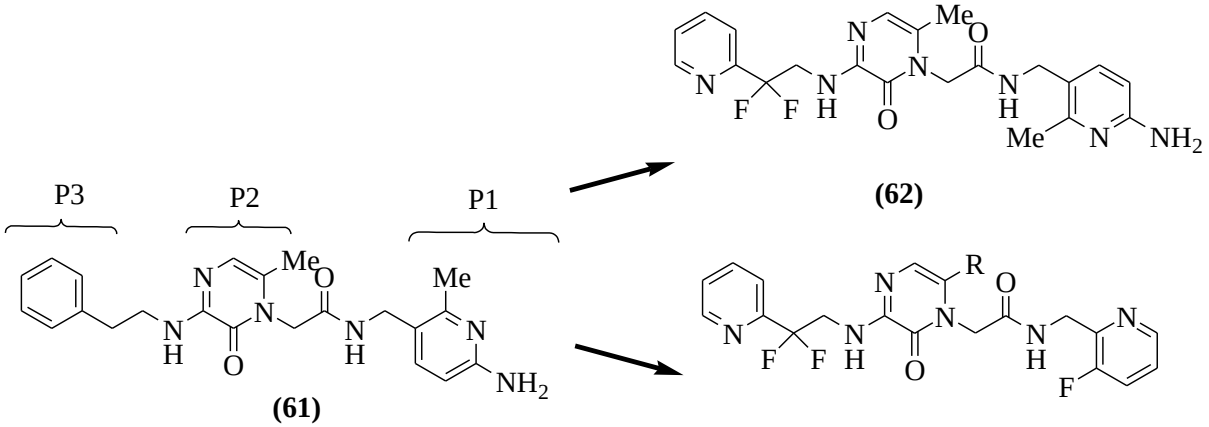


(60)

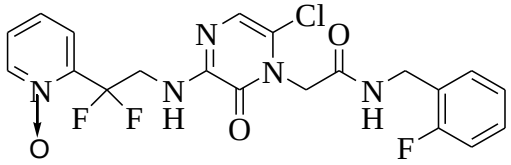
The same research team at Merck developed selective and orally efficacious potent thrombin inhibitors through rigorous modifications at three major sites in 3-aminopyrazinone acetamide analog **(61)** viz. P1, P2 and P3, which were thought to be susceptible to metabolism exhibiting altered pharmacokinetics.[25] Initially, optimization of P3 site via introduction of difluoro group and 2-pyridyl moiety in place of phenyl ring provided the most potent compound of the series, **(62)** (FIIa, K_i = 0.042 nM, 2xaPTT = 0.20 µM). Later, P1 and P2 modifications led to the discovery of new potent derivatives **(63)** (FIIa, K_i = 4.2 nM; 2xaPTT = 0.67 µM), **(64)** (FIIa, K_i = 5.2 nM, 2xaPTT = 0.78 µM) and **(65)** (FIIa, K_i = 2.3 nM, 2xaPTT = 0.41 µM) which exhibited longer half-lives (**(63)**, $t_{1/2}$ = 3.5 hr.; **(64)**, $t_{1/2}$ = 6.6 hr. and **(65)**, $t_{1/2}$ = 9.7 hr.) and improved pharmacokinetic properties. Compounds **(63** and **64)** demonstrated good oral bioavailability in dogs (F = 87% and 66%) and rats (F = 55% and 23%) and good *in vivo* efficacy in $FeCl_3$ assay.



(61)

(62)

(**63**) R = Me
(**64**) R = Cl
(**65**) R = CN



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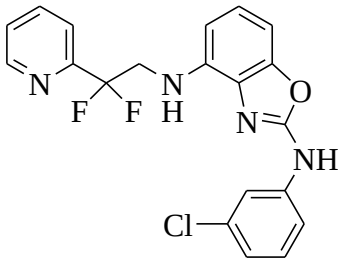

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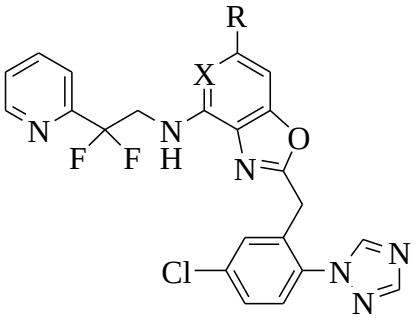
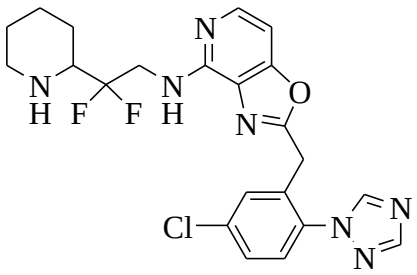


(**68**) X= CH, R= H
(**69**) X = N, R = Me



(70)

In an attempt to improve low solubility of the previous lead derivative **(64)**, Merck scientists invented a novel series of thrombin inhibitory compounds bearing pyridine N-oxides as the P3 element along with P1 benzylamides.[26] A detailed investigation on SAR of P1 site in terms of pharmacokinetics, potency and human microsomal stability, afforded compound **(66)** as

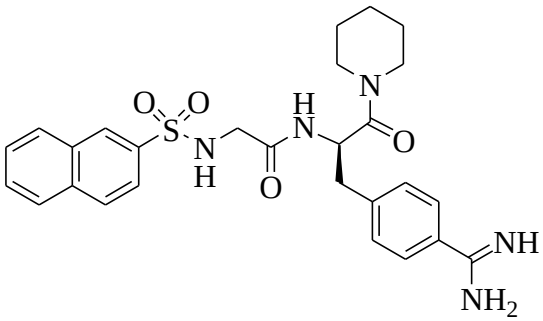
the 'best fit' analog from the series. It displayed K_i value of 2.3 nM, 2xaPTT at 0.40 µM and longer $t_{1/2}$ of 4.9 hr as well as excellent selectivity for thrombin (>49 µM). Compound **(66)** also showed good bioavailability in rats (F = 52%) and dogs (F = 40%) and dose-dependent inhibition in arterial thrombosis model.

Structure-based lead optimization of weakly active triazolopyrimidine core by Deng *et.al* led to a series of oxazolopyridine analogs as potent dual thrombin and FXa inhibitors.[27] Extensive modifications in the lead derivative **(67)** provided compounds with excellent potencies. Substitution of triazole ring at the P1 binding motif resulted in compound **(68)** with thrombin K_i value of 0.08 nM. Methyl group substituent in the central pyridine ring imparted better potency and selectivity over other proteases (e.g. **69**, FIIa, K_i = 0.04 nM; 2xaPTT = 0.41 µM). In order to decrease plasma protein binding, converting pyridine to piperidine moiety (e.g. **70**, FIIa, K_i = 0.04 nM; FXa, K_i = 3.9 nM; 2xaPTT = 0.07 µM) improved antithrombin potency and *in vitro* anticoagulant activity and additionally furnished significant FXa inhibitory potency to the compound. Compound **(70)** displayed promising *in vivo* efficacy in rat thrombosis model and long half-life of 4.2 hr but showed poor oral bioavailability.

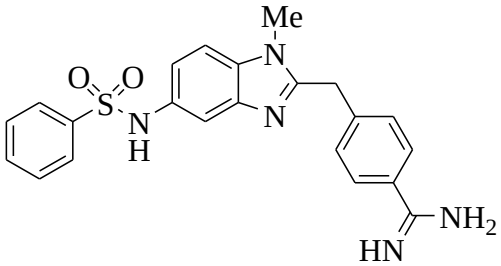
2.2.2. Dabigatran mimics

From the X-ray crystal structure of thrombin inhibitor (**71**), NAPAP (IC_{50} = 0.2 µM) complexed with bovine thrombin, a new class of antithrombotic nonpeptide trisubstituted benzimidazole derivatives was designed by Hauel *et.al*.[28] The first designed compound (**72)** (IC_{50} = 1.5 µM) showed cardiovascular side effects at a low dose of 1 mg/kg but it acted as a lead for optimization and development of new derivatives with improved potency, tolerability and pharmacokinetics. To increase overall hydrophilicity, structural modifications like introduction of carboxylate group in the amide side chain resulted into the most potent compound of the series, **(73)** (FIIa, K_i = 4.5 nM) which also displayed excellent thrombin selectivity and *in vivo* tolerability. Unfortunately it exhibited insufficient oral bioavailability. Its double prodrug **(74)** was developed which possessed promising oral activity in different animals and it was selected for further clinical development. The binding interactions of **73** in the thrombin-inhibitor complex revealed that amidine group formed a salt bridge with Asp189 and the central

benzimidazole ring interacted with P-pocket while the pyridine ring was placed between Leu99 and Ile174 in D-pocket of the enzyme.



(**71**) NAPAP



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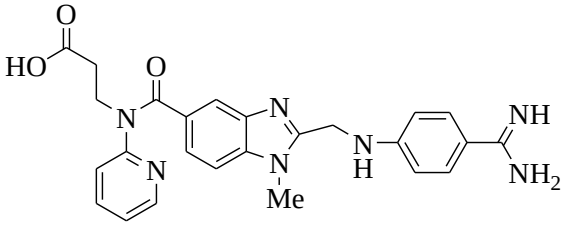

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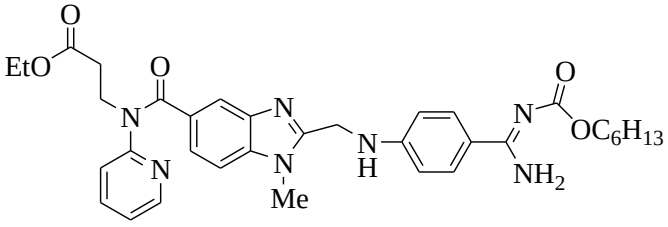
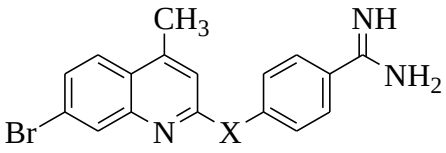
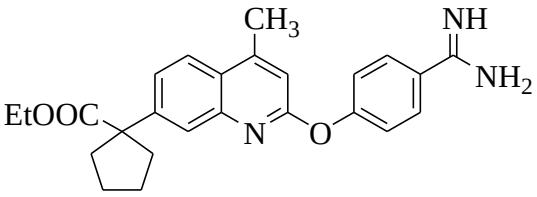


(74)



(**75**) X = -O
(**76**) X = -NH

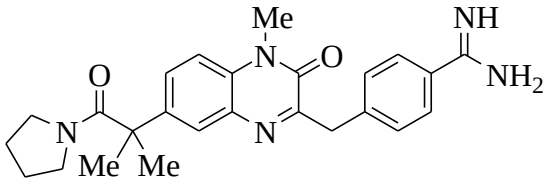


(77)

In search of orally active antithrombotic agents, Ries *et. al* disclosed novel quinoline based low molecular weight derivatives as selective thrombin inhibitors.[29] First lead derivative **(75)** (IC_{50} = 0.066 µM) and its amino analog **(76)** (IC_{50} = 0.087 µM), displayed good antithrombin potency. Different modifications were carried out at 7-position of the quinoline ring to get compounds with better inhibition properties along with good solubility and binding properties with the enzyme. Ethyl cyclopentylacetate derivative (**77),** was found to show the

most potent antithrombin activity with IC_{50} value of 0.003 µM and caused weak FXa inhibition (IC_{50} = 1.5 µM).

Ries *et. al* further designed and invented new quinoxalinone derivatives as dual inhibitors of thrombin and FXa enzymes, from their previous studies on active amidinophenoxy quinoline derivatives which were not suitable for therapeutic use because of high lipophilicity.[30] Low lipophilicity of quinoxalinone derivatives improved the *in vitro* antithrombotic properties and thrombin and FXa inhibitory activities up to nanomolar level. From the series, compound **(78)** (IC_{50} = 5 nM) exhibited the most potent antithrombin activity while the cyclopropyl analog **(79)** was reported as the most active dual inhibitor of thrombin (IC_{50} = 8 nM) and FXa (IC_{50} = 84 nM), and showed 2xaPTT value at 0.095 µM concentration. Unfortunately these derivatives were not tested for their *in vivo* activity because of cardiovascular adverse effects. Therefore further modification of the amide side chain resulted into zwitterionic compounds (e.g. **80** with better tolerability in rats but unfavorable pharmacodynamic properties requiring still more efforts to obtain compounds with favourable biological profile.



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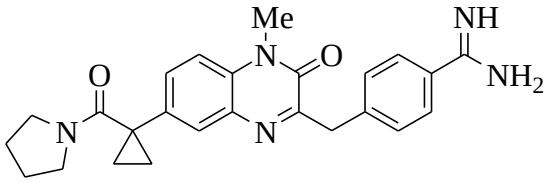

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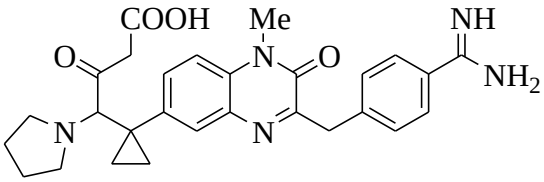
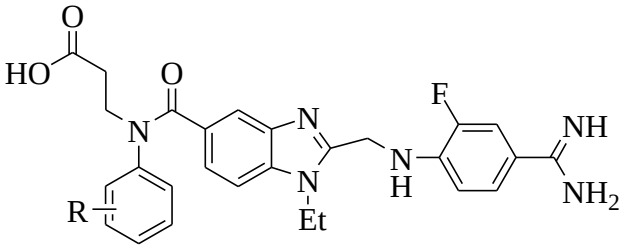


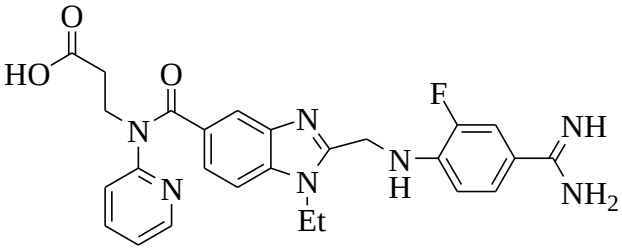
(80)

Li *et.al* reported a series of novel fluorinated benzimidazole derivatives possessing potential antithrombotic activity.[31] Compounds **(81** FIIa, IC_{50} = 5.63 nM; **82** FIIa, IC_{50} = 7.26 nM; **83,** FIIa, IC_{50} = 8.40 nM and **84** FIIa, IC_{50} = 4.92 nM) displayed potent *in vitro* thrombin inhibitory activity when compared to argatroban (FIIa, IC_{50} = 9.6 nM) while compound **(85** FIIa,

IC_{50} = 3.39 nM) containing pyridine substituent was found to be the most active derivative of the series. Structure-activity relationship studies have revealed that aryl or heteroaryl substituents binding to the S4 pocket were essential for activity. Electron-withdrawing groups like fluoro especially at meta-position resulted into potent compounds e.g (**84**) while electron-donating moieties had negligible effect on the activity. The pyridine ring was found to be superior for *in vitro* activity.

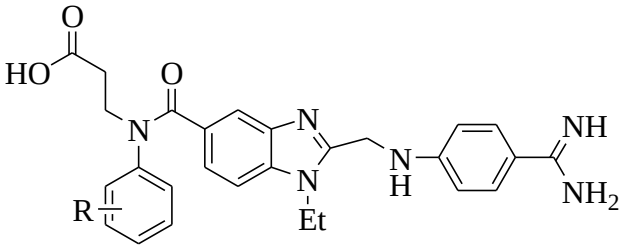


Compound	R
(81)	H
(82)	2-Me
(83)	3-OMe
(84)	3-F



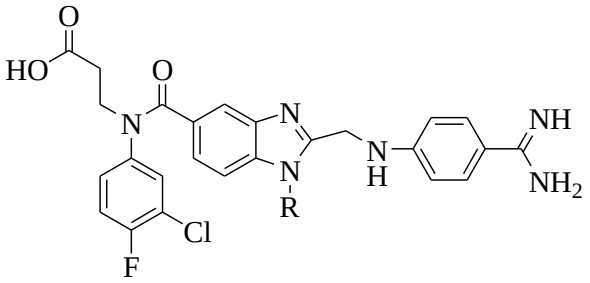
(85)

Fluorine-substituted benzimidazole derivatives as direct thrombin inhibitors were discovered from computer-aided simulation studies by Chen *et.al*.[32] All the derivatives exhibited nano- to subnanomolar *in vitro* potency. Compounds **(86-89)** displayed excellent activity with IC_{50} values of 3.52, 4.26, 9.55 and 6.21 nM/L respectively which was comparable to the reference, argatroban (IC_{50} = 9.46 nM). It was observed that fluorine substitution at 2- and 4-positions of the phenyl ring yielded more active compounds.

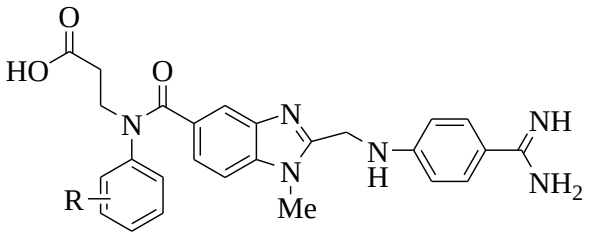


Compound	R
(86)	2-F
(87)	4-F
(88)	3,4-diF
(89)	2-CF3

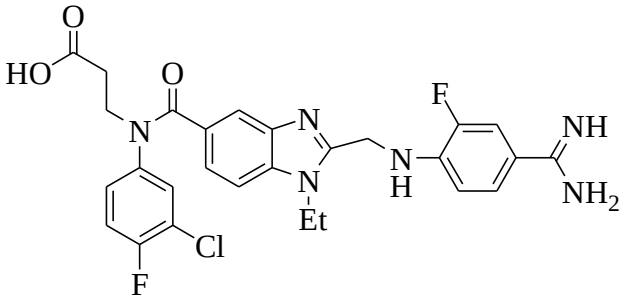
Li *et. al* discovered new dabigatran-mimic compounds by substitution of different alkyl side chains at N-1 of central benzimidazole moiety.[33] Among the derivatives, *N*-methyl (**90** FIIa, IC_{50} = 8.29 nM) and *N*-ethyl (**91** FIIa, IC_{50} = 8.86 nM) analogs disclosed potent *in vitro* antithrombin activity which was better than the standard drug, argatroban (FIIa, IC_{50} = 9.46 nM) and other alkylated compounds. Same research team also developed new derivatives like dabigatran as antithrombotic agents from docking simulations.[34] After synthesis and biological activity studies, compounds **(92** and **93)** were found to be the most active derivatives with IC_{50} values of 2.74 nM and 2.99 nM respectively which were comparable to the standard dabigatran (IC_{50} = 1.20 nM) in potency.



(90) R = Me
(91) R = Et



(92) R = 2-Me
(93) R = 2,3-diMe

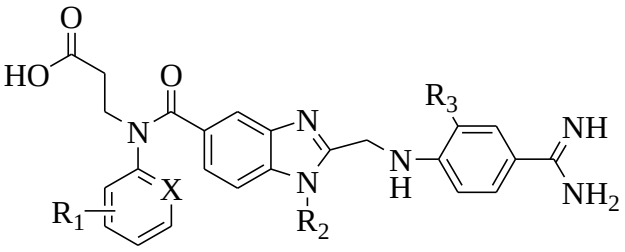


(94)

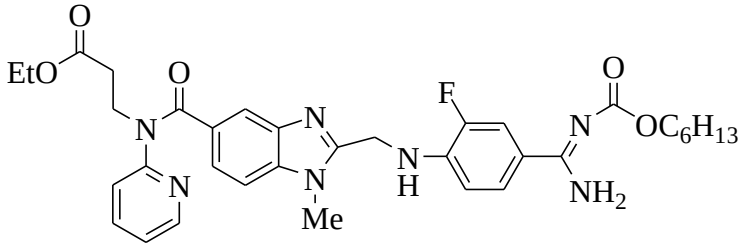
Li *et.al* prepared and evaluated novel dabigatran type compounds for potential antithrombotic activities.[35] Alkyl chain substitution at N-1 of benzimidazole moiety affected biological activities of the resulting compounds due to steric factors. As the length of the chain increased, the biological activity concurrently decreased. Fluorine substitution at C-2 of the side chain phenyl ring improved thrombin potency. 3-Chloro-4-fluoro substituted terminal phenyl moiety was retained for optimum potency. Ethyl substituent at N-1 displayed superior

antithrombotic activity because of its hydrophobic binding with the nearby amino acid residues and its ability to occupy the active pocket. Thus, compound **(94)** demonstrated the most potent antithrombin activity *in vitro* with IC_{50} value of 2.04 nM which was slightly better than dabigatran (IC_{50} = 2.61 nM).

By using bioisosteric strategies, Ren *et.al* have designed and synthesized 21 novel dabigatran like derivatives for antithrombotic activity.[36] Among the compounds, **(95**, IC_{50} = 3.49 nM); **96**, IC_{50} = 3.71 nM; **97**, IC_{50} = 3.23 nM and **98**, IC_{50} = 3.39 nM) were found to possess potent *in vitro* activity against thrombin which was better than argatroban (IC_{50} = 9.46 nM) and close to the standard dabigatran (IC_{50} = 2.61 nM). The prodrug of compound **(97)** i.e. derivative **(99)** displayed strong inhibition of thrombin-associated platelet aggregation, and 73% inhibition at 3 mg/ml dose in *in vivo* arteriovenous model of thrombosis. The SAR data revealed that electron-donating groups on phenyl ring diminished the anti-thrombin potency while reverse was the case for electron-withdrawing substituents.



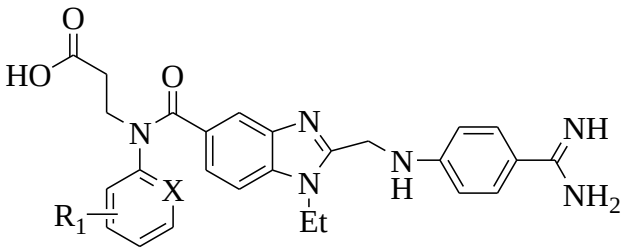
Compound	X	R_1	R_2	R_3
(95)	N	H	Me	F
(96)	CH	2-F	Et	F
(97)	CH	2-F	Me	F
(98)	N	H	Et	F



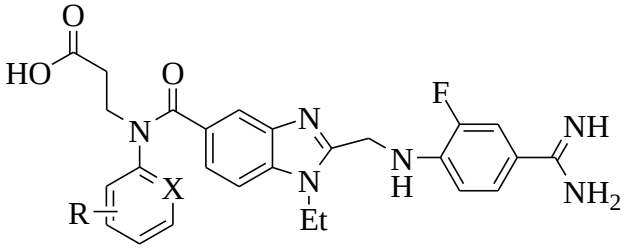
(99)

From earlier research on dabigatran derivatives, a novel series of *N*-ethyl substituted dabigatran analogs were designed wherein the biological activity prediction by CoMFA modeling exhibited comparable activity to dabigatran.[37] After synthesis and biological evaluation, compounds **(100**, IC_{50} = 2.13 nM; **101**, IC_{50} = 3.10 nM; **102**, IC_{50} = 1.62 nM and **103**,

IC_{50} = 0.92 nM) displayed strong *in vitro* inhibitory activity close to the reference dabigatran (IC_{50} = 1.20 nM). Compound **(103)** was found to demonstrate better *in vitro* antithrombin properties than dabigatran and excellent *in vivo* antithrombotic activity with 85.35% inhibition comparable to the standard (dabigatran, 85.07 % inhibition) at 0.5 mg/ml concentration. The SAR studies demonstrated that methyl substituent at any position of the aryl ring was beneficial for the activity.



Compound	X	R_1
(100)	CH	3-Me
(101)	CH	2,6-diMe
(102)	N	4-Me
(103)	N	6-Me

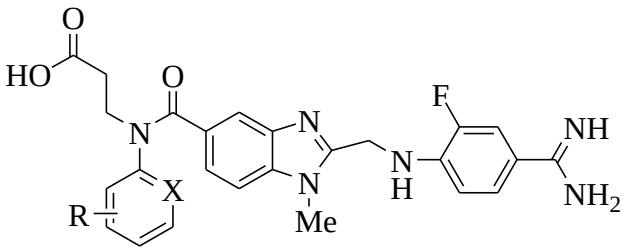


Compound	X	R
(104)	CH	2,6-diMe
(105)	CH	2,4-diF
(106)	N	6-Me

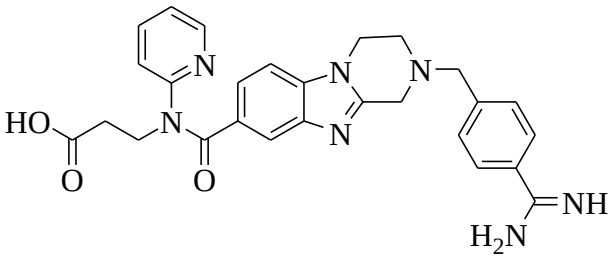
From the theoretical biological activity predictions using CADD approach, Wang *et.al* developed and reported fifteen fluorinated dabigatran-mimics as potential antithrombotic agents.[38] All the analogs showed fair inhibition towards thrombin close to 90%. The derivatives **(104**, IC_{50} = 1.81 nM; **105**, IC_{50} = 3.21 nM and **106**, IC_{50} = 2.16 nM) displayed promising *in vitro* anticoagulant activities close to the reference (IC_{50} = 1.23 nM). Compounds **(105** and **106)** presented 76.23 % and 84.66 % inhibition respectively from *in vivo* thrombosis evaluation. Molecular docking studies further supported inhibitory mechanisms and binding mode of the active compounds. Either methyl or fluorine moiety at ortho-position of the phenyl ring enhanced antithrombin activity.

Pharmacophore modeling and QSAR studies were employed and fifteen new fluorinated derivatives similar to dabigatran were designed, synthesized and tested for their inhibitory potentials against thrombin.[39] Initial screening where compounds exhibiting >80% inhibition,

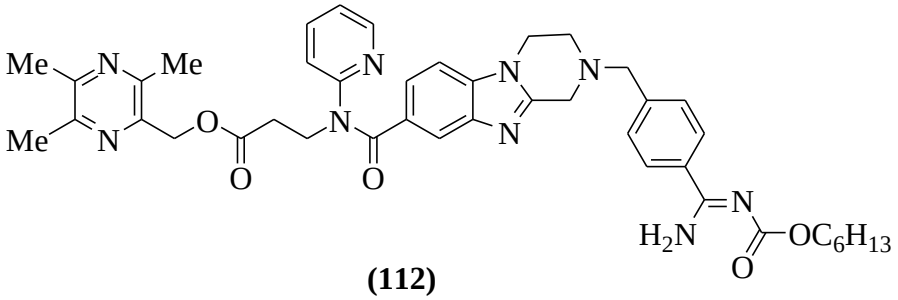
were subjected to *in vitro* studies yielding potent compounds, **(107**, IC_{50} = 1.54 nM; **108**, IC_{50} = 1.42 nM) and the most potent derivatives were compounds **(109**, IC_{50} = 0.84 nM and **110**, IC_{50} = 1.18 nM) with better inhibition than dabigatran (IC_{50} = 1.20 nM). Further evaluation of compounds **(109** and **110)** showed remarkable antithrombotic efficacy in *in vivo* arteriovenous thrombosis model with 84.24% and 84.57% inhibition respectively. Incorporation of two fluorine atoms in the aryl ring improved activity while methyl substitution at para-position lowered the activity.



Compound	X	R
(107)	CH	2,3-diMe
(108)	N	6-Me
(109)	CH	2,4-diF
(110)	CH	3,5-diF



(111)



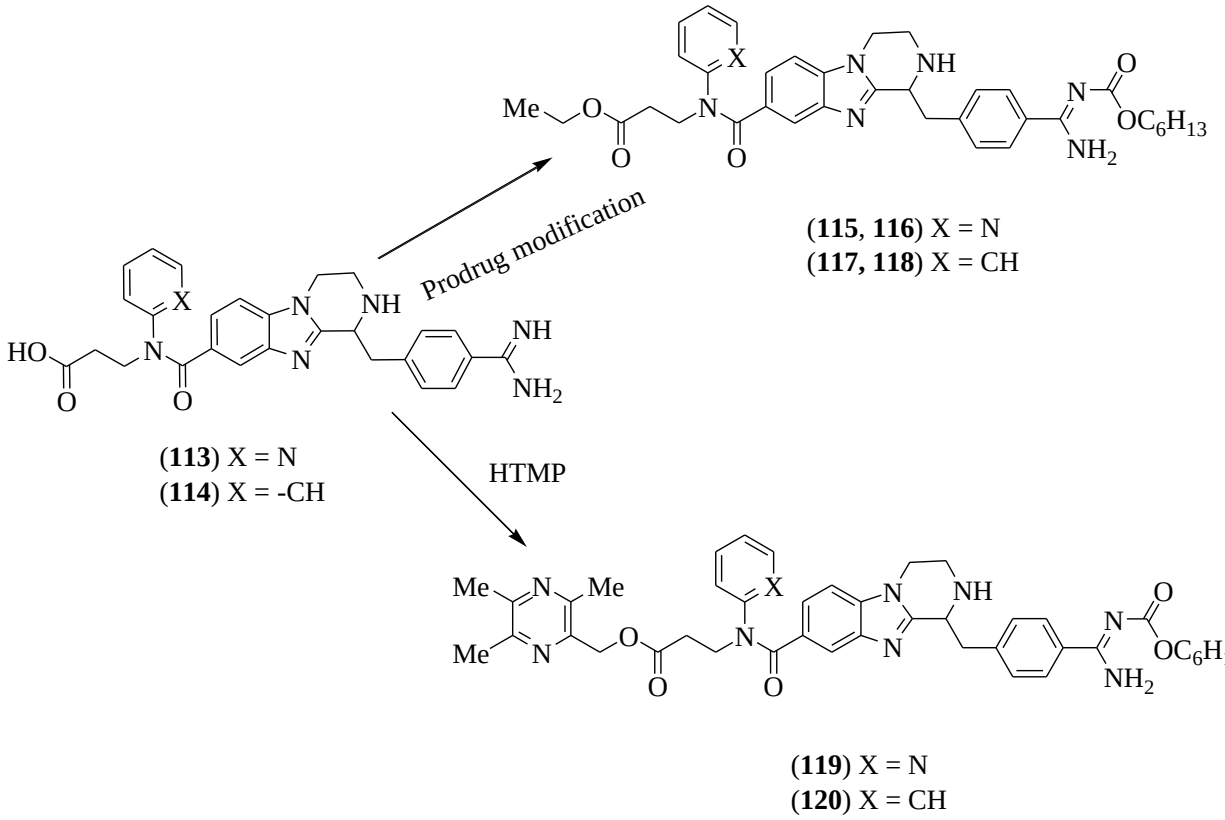
(112)

Wang *et.al* reported a series of dabigatran etexilate mimics with novel tricyclic fused scaffold.[40] All the derivatives from the series displayed moderate *in vitro* thrombin-induced antiplatelet aggregation activity. The bifunctional prodrug of compound **(111)**, (thrombin, IC_{50} =

9.09 µM**)**, viz. **(112)**, (thrombin, IC_{50} = 9.74 µM) containing 2-hydroxymethyl-3,5,6-trimethylpyrazine (HTMP), a moiety possessing anti-platelet pharmacophore exhibited the most potent *in vivo* anticoagulant activity in venous thrombosis model (50% and 80% inhibition at 5 mg/kg and 20 mg/kg doses respectively) which was weaker than dabigatran (70% and 90 % inhibition at 5 mg/kg and 20 mg/kg doses respectively) but showed better safety profile with lower bleeding (462 sec.) tendency compared to dabigatran etexilate (722 sec.)

From the bioisosteric principle and scaffold hopping strategy, Chen *et.al* synthesized and evaluated tricyclic fused novel dabigatran type compounds for their antithrombotic activities.[41] Compounds **(113,** FIIa IC_{50} = 9.20 nM and **114,** FIIa IC_{50} = 7.48 nM) exhibited excellent *in vitro* antithrombin potency close to dabigatran. From these results, a series of ester and carbamate prodrugs of **113** and **114** were synthesized and tested for anticoagulant potential wherein compounds **(115**, IC_{50} = 0.73 µM; **116**, IC_{50} = 0.75 µM; **117**, IC_{50} = 1.44 µM and **118**, IC_{50} = 0.91µM) showed promising inhibitory properties in thrombin-associated platelet aggregation. The prodrugs coupled with antiplatelet aggregatory functionality, 2-hydroxymethyl-3,5,6-trimethylpyrazine (HTMP) viz. **119** and **120** exhibited better antithrombotic efficacy in rat venous thrombosis model**.** Finally, compound **(119)** demonstrated better safety in terms of haemorrhagic risk compared to dabigatran.

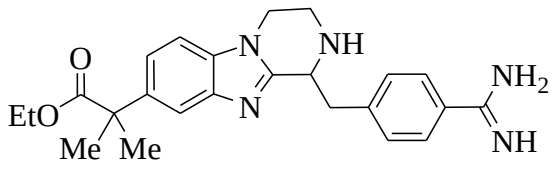
Encouraged with the results from previous findings, Chen *et.al* synthesized and reported benzimidamide derivatives for human antithrombotic properties.[42] Initial structural optimizations led to compounds **(121**, IC_{50} = 1440 nM and **122**, IC_{50} = 82.8 nM) with moderate to good *in vitro* antithrombotic activity. From next modifications in order to get oral bioavailability, ten new amide compounds were synthesized wherein most of them showed inhibition of platelet aggregation associated with thrombin function. Compounds **(123**, IC_{50} = 8.16 µM and **124**, IC_{50} = 1.95 µM) displayed dose-dependent inhibition in *in vivo* thrombosis studies. The potency of prodrug, **(124)** was attributed to the synergistic effect of HTMP and (**122**) which were produced after *in vivo* hydrolysis.



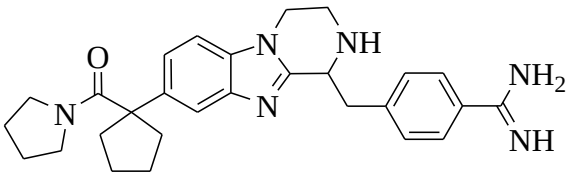
(**115**, **116**) X = N
(**117, 118**) X = CH

(**113**) X = N
(**114**) X = -CH

(**119**) X = N
(**120**) X = CH



(121)



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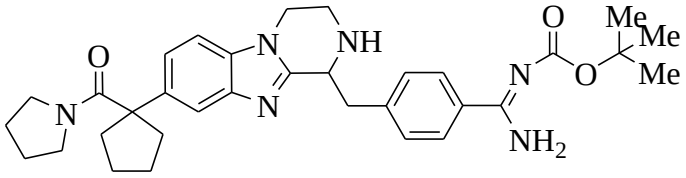
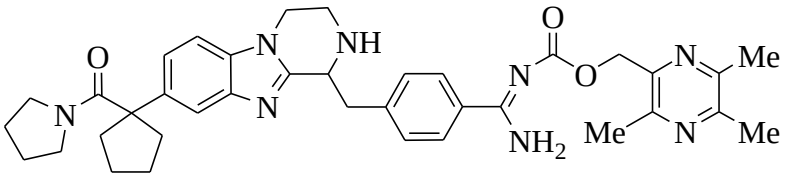


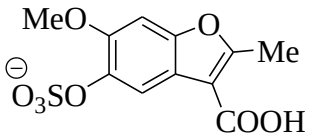
(123)



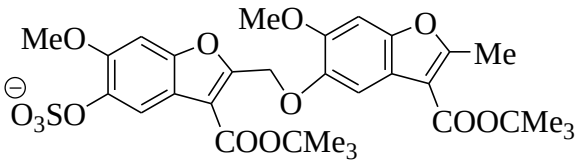
(124)

2.2.3. Allosteric thrombin inhibitiors

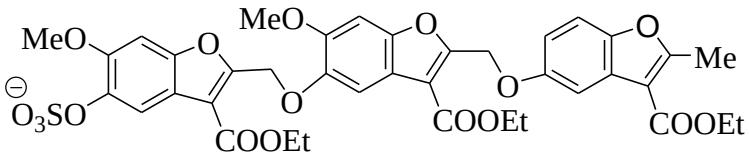
Allosteric inhibition as a new strategy to design novel thrombin inhibitors with low bleeding tendencies was used by Desai and his group. In the initial work, they designed low molecular weight sulphated lignins as heparin mimetics and studied their binding on exosite-II like sites of thrombin and other enzymes where these lignins displayed nanomolar potency against thrombin.[43] In 2009, they synthesized small aromatic monomeric benzofuran derivatives and tested them for activity against factor Xa and thrombin. The compounds like **125** showed weak inhibition of both the enzymes with different selectivity of recognition and allosteric mechanism.[44] Later, they synthesized a library of 28 sulphated benzofuran molecules as potent allosteric antithrombin agents. The Michaelis-Menten kinetic data supported allosteric inhibition and most of the derivatives also exhibited potent anticoagulant activity. tert-Butyl derivative (**126**, IC_{50} = 7.3 µM) was found to be the most potent analog of the series.[45]



(125)



(126)



(127)

Motivated by earlier results, the same research group developed monosulphated trimeric and tetrameric benzofuran compounds derived from their previous studies.[46] The trimer (**127**) was found to show 9-fold more potency than the corresponding dimer and was the most active antithrombotic agent with IC_{50} of 0.67 µM and nearly 80% efficacy.

2.3 References

1. Jones, S. D.; Liebeschuetz, J. W.; Morgan, P. J.; Murray, C. W.; Rimmer, A. D.; Roscoe, J.M.E.; Waszkowycz, B.; Welsh, P.M.; Wylie, W.A.; Young, S.C.; Martin, H.; Mahler, J.; Brady, L.; Wilkinson, K. The design of phenylglycine containing benzamidine carboxamides as potential and selective inhibitors of Factor Xa. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 733-736.
2. Imaeda, Y.; Kawamoto, T.; Tobisu, M.; Konishi, N.; Hiroe, K.; Kawamura, M.; Tanaka, T.; Kubo, K. Discovery of piperazinylimidazo[1,2-a]pyridines as novel S4 binding elements for orally active factor Xa inhibitors. *Bioorg. Med. Chem.*, **2008**, *16*, 3125–3140.
3. Masters, J. J.; Franciskovich, J. B.; Tinsley, J. M.; Campbell, C.; Craft, T. J.; Froelich, L. L.; Gifford- Moore, D. S.; Hay, L. A.; Herron, D. K.; Klimkowski, V. J.; Kurz, K. D.; Metz, J. T.; Ratz, A. M.; Shuman, R. T.; Smith, G. F.; Smith, T.; Towner, R. D.; Wiley, M. R.; Wilson, A.; Yee, Y. K. Nonamidine-containing 1,2-dibenzamidobenzene inhibitors of human factor Xa with potent anticoagulant and antithrombotic activity. *J. Med. Chem.* **2000**, *43*, 2087-2092.
4. Zhang, P.; Bao, L.; Zuckett, J.F.; Goldman, E.A.; Jia, Z.J.; Arfsten, A.; Edwards, S.; Sinha, U.; Hutchaleelaha, A.; Park, G.; Lambing, J.L.; Hollenbach, S.J.; Scarborough, R.M.; Zhu, B. Y. Design, synthesis, and SAR of anthranilamide-based factor Xa inhibitors incorporating substituted biphenyl P4 motifs. *Bioorg. Med. Chem. Lett*., **2004**, *14*, 983-987.
5. Zhang, P.; Bao, L.; Zuckett, J. F.; Jia, Z.I.; Woolfrey, J.; Arfsten, A.; Edwards, S.; Sinha, U.; Hutchaleelaha, A.; Lambing, J. L.; Hollenbach, S.J.; Scarborough, R.M.; Zhu, B.Y. Design, synthesis, and SAR of anthranilamide-based factor Xa inhibitors with improved functional activity. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 989-993.
6. Zhang, P.; Bao, L.; Fan, J.; Jia, Z.J.; Sinha, U.; Wong, P.W.; Park, G.; Hutchaleelaha, A.; Scarborough, R. M.; Zhu, B.Y. Anthranilamide-based N,N-dialkylbenzamidines as potent and orally bioavailable factor Xa inhibitors: P4 SAR. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 2186-2189.

7. Zhang, P.; Huang, W.; Wang, L.; Bao, L.; Jia, Z.J.; Bauer, S.M.; Goldman, E.A.; Probst, G.D.; Song, Y.; Su, T.; Fan, J.; Wu, Y.; Li, W.; Woolfrey, J.; Sinha, U.; Wong, P.W.; Edwards, S.T.; Arfsten, A.E.; Clizbe, L.A.; Kanter, J.; Pandey, A.; Park, G.; Hutchaleelaha, A.; Lambing, J. L.; Hollenbach, S. J.; Scarborough, R. M.; Zhu, B.Y. Discovery of betrixaban (PRT054021), *N*-(5-chloropyridin-2-yl)-2-(4-(*N*,*N*-dimethylcarbamimidoyl)benzamido)-5-methoxybenzamide, a highly potent, selective and orally efficacious factor Xa inhibitor. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 2179-2185.
8. Chou, Y. L.; Davey, D. D.; Eagen, K. A.; Griedel, B. D.; Karanjawala, R.; Phillips, G. B.; Sacchi, K. L.; Shaw, K. J.; Wu, S. C.; Lentz, D.; Liang, A. M.; Trinh, L.; Morrissey, M. M.; Kochanny, M. J. Structure-activity relationships of substituted benzothiophene anthranilamide factor Xa inhibitors. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 507–511.
9. Kochanny, M.J.; Adler, M.; Ewing, J.; Griedel, B.D.; Ho, E.; Karanjawala, R.; Lee, W.; Lentz, D.; Liang, A.M.; Morrissey, M.M.; Phillips, G.B.; Post, J.; Sacchi, K.L.; Sakata, S.T.; Subramanyam, B.; Vergona, R.; Walters, J.; White, K.A.; Whitlow, M.; Ye, B.; Zhao, Z.; Shaw, K.J. Substituted thiopheneanthranilamides as potent inhibitors of human factor Xa. *Bioorg. Med. Chem.* **2007**, *15*, 2127–2146.
10. Ye, B.; Arnaiz, D.O.; Chou, Y.L.; Griedel, B.D.; Karanjawala, R.; Lee, W.; Morrissey, M.M.; Sacchi, K.L.; Sakata, S.T.; Shaw, K.J.; Wu, S.C.; Zhao, Z.; Adler, M.; Cheeseman, S.; Dole, W.P.; Ewing, J.; Fitch, R.; Lentz, D.; Liang, A.; Light, D.; Morser, J.; Post, J.; Rumennik, G.; Subramanyam, B.; Sullivan, M.E.; Vergona, R.; Walters, J.; Wang, Y.X.; White, K.A.; Whitlow, M.; Kochanny, M.J. Thiopheneanthranilamides as highly potent and orally available factor Xa inhibitors. *J. Med. Chem.* **2007,** *50*, 2967-2980.
11. Mendel, D.; Marquart, A.L.; Joseph, S.; Waid, P.; Yee, Y.K.; Tebbe, A.L.; Ratz, A.M.; Herron, D.K.; Goodson, T.; Masters, J.J.; Franciskovich, J.B.; Tinsley, J.M.; Wiley, M.R.; Weir, L.C.; Kyle, J.A.; Klimkowski, V.J.; Smith, G.F.; Towner, R.D.; Froelich, L.L.; Buben, J.; Craft, T.J. Anthranilamide inhibitors of factor Xa. *Bioorg. Med. Chem. Lett*., **2007**, *17*, 4832–4836.
12. Corte, J.R.; Fang, T.; Pinto, D.J.; Han, W.Z.; Hu, X.J.; Jiang, Y.L.; Li, J.F.; Gauuan, M.; Hadden, D.; Orton, A.R.; Rendina, J.M.; Luettgen, P.C.; Wong, K.; He, P.E.; Morin, C.H.; Chang, D.L.; Cheney, Knabb, R.M.; Wexler, R.R.; Lam, P.Y. Structure-activity

relationships of anthranilamide-based factor Xa inhibitors containing piperidinone and pyridinone P4 moieties, *Bioorg. Med. Chem. Lett*., **2008**, *18*, 2845–2849.

13. Pandya, V.; Jain, M.; Chakrabarti, G.; Soni, H.; Parmar, B.; Chaugule, B.; Patel, J.; Jarag, T.; Joshi, J.; Joshi, N.;, Unadkat, V.; Sharma, B.; Ajani, H.; Kumar, J.; Sairam, K.V.M.; Patel, H.; Patel P. Synthesis and structure-activity relationship of potent, selective and orally active anthranilamide-based factor Xa inhibitors: Application of weakly basic sulfoximine group as novel S4 binding element. *Eur. J. Med. Chem*., **2012**, *58*, 136-152.
14. Yang, J.; Su, G.; Ren, Y.; Chen, Y. Synthesis of 3,4-diaminobenzoyl derivatives as factor Xa inhibitors. *Eur. J. Med. Chem.*, **2015**, *101*, 41-51.
15. Wang, W.; Yuan, J.; Fu, X.; Meng, F.; Zhang, S.; Xu, W.; Xu, Y.; Huang, C. Novel Anthranilamide-based FXa Inhibitors: Drug Design, Synthesis and Biological Evaluation. *Molecules*, **2016**, *21*, 491.
16. Huang, C.; Wang, W.; Li, Y.; Zhang, S.; Meng, F.; Xu, W.; Yuan, J.; Chen, L. Synthesis and evaluation of anthranilamide-based derivatives as FXa inhibitors. *Oncotarget*, **2017**, *8(23)*, 37186-37199.
17. Xing, J.; Yang, L.; Li, H.; Li, Q.; Zhao, L.; Wang, X.; Zhang, Y.; Zhou, M.; Zhou, J.; Zhang, H. Identification of anthranilamide derivatives as potential factor Xa inhibitors: Drug design, synthesis and biological evaluation. *Eur. J. Med. Chem*., **2015**, *95*, 388-399.
18. Xing, J.; Yang, L.; Zhou, J.; Zhang, H. Design, synthesis and biological evaluation of anthranilamide derivatives as potential factor Xa (fXa) inhibitors. *Bioorg. Med. Chem.*, **2018**, *26*, 5987–5999.
19. Patel, N.R.; Patel, D.V.; Kanhed, A.M.; Patel, S.P.; Patel, K.V.; Afosah, D.K.; Desai, U.R.; Karpoormath, R.; Yadav, M.R. 2-Aminobenzamide-based factor Xa inhibitors with novel mono-and bi-aryls as S4 binding elements. *Chemistry Select*, **2019**, *4*, 802-809.
20. Trstenjak, U.; Ilas J.; Kikelj, D. Transformation of a selective factor Xa inhibitor rivaroxaban into a dual factor Xa/thrombin inhibitor by modification of the morpholin-3-one moiety. *Med. Chem. Comm*., **2014**, *5*, 197-202.
21. Yang, J.; Su, G.; Ren Y.; Chen Y. Design, synthesis and evaluation of isoxazolo[5,4-d]pyrimidin-4(5H)-one derivatives as antithrombotic agents. *Bioorg. Med. Chem. Lett*., **2015**, *25*, 492-495.

22 Sanderson, P.E.J., Dyer, D.L. Naylor-Olsen, A.M., Vacca, J.P., Gardell, S.J., Lewis, S.D., Lucas, Jr. B.J., Lyle, E.A., Lynch Jr., J.J., Mulichak, A.M. L-373,890, an achiral, noncovalent, subnanomolar thrombin inhibitor. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1497-1500.

23. Sanderson, P.E.J.; Cutrona, K.J.; Dorsey, B.D.; Dyer, D.L.; McDonough, C.M.; Naylor-Olsen, A.M.; Chen, I-W.; Chen, Z.; Cook, J.J.; Gardell, S.J.; Krueger, J.A.; Lewis, S.D.; Lin, J.H.; Lucas, Jr. B.J.; Lyle, E.A.; Lynch Jr., J.J.; Stranieri, M.T.; Vastag, K.; Shafer, J.A.; Vacca, J.P. L-374,087, an efficacious, orally bioavailable, pyridinone acetamide thrombin inhibitor. *Bioorg. Med. Chem. Lett*., **1998**, *8*, 817-822.

24. Sanderson, P.E.J.; Lyle, T.A.; Cutrona, K.J.; Dyer, D.L.; Dorsey, B.D.; McDonough, C.M.; Naylor-Olsen, A.M.; Chen, I-W.; Chen, Z.; Cook, J.J.; Cooper, C.M.; Gardell, S.J.; Hare, T.R.; Krueger, J.A.; Lewis, S.D.; Lin, J.H.; Lucas, Jr. B.J.; Lyle, E.A.; Lynch, Jr. J.J.; Stranieri, M.T.; Vastag, K.; Yan, Y.; Shafer, J.A.; Vacca, J.P. Efficacious, orally bioavailable thrombin inhibitors based on 3-aminopyridinone or 3-aminopyrazinone acetamide peptidomimetic templates. *J. Med. Chem.* **1998**, *41*, 4466-4474.

25. Burgey, C. S.; Robinson, K. A.; Lyle, T. A.; Sanderson, P. E. J.; Lewis, S. D.; Lucas, B. J.; Krueger, J. A.; Singh, R.; Miller-Stein, C.; White, R. B.; Wong, B.; Lyle, E. A.; Williams, P. D.; Coburn, C. A.; Dorsey, B. D.; Barrow, J. C.; Stranieri, M. T.; Holahan, M. A.; Sitko, G. R.; Cook, J. J.; McMasters, D. R.; McDonough, C. M.; Sanders, W.M.; Wallace, A. A.; Clayton, F. C.; Bohn, D.; Leonard, Y. M., Jr.; Detwiler, T. J., Jr.; Lynch, J. J., Jr.; Yan, Y.; Chen, Z.; Kuo, L.; Gardell, S. J.; Shafer, J. A.; Vacca, J. P. Metabolism-directed optimization of 3-aminopyrazinone acetamide thrombin inhibitors. Development of an orally bioavailable series containing P1 and P3 pyridines. *J. Med. Chem.* **2003**, 46, 461-473.

26. Burgey, C.S.; Robinson, K. A.; Lyle, T. A.; Nantermet, P. G.; Selnick, H. G.; Isaacs, R. C. A.; Lewis, S. D.; Lucas, B. J.; Krueger, J. A.; Singh, R.; Miller-Stein, C.; White, R. B.; Wong, B.; Lyle, E. A.; Stranieri, M. T.; Cook, J. J.; McMasters, D. R.; Pellicore, J. M.; Pal, S.; Wallace, A. A.; Clayton, F. C.; Bohn, D.; Welsh, D. C.; Lynch, Jr., J. J.; Yan, Y.; Chen, Z.; Kuo, L.; Gardell, S. S. J.; Shafer, J. A.; Vacca, J. P. Pharmacokinetic optimization of 3-amino-6-chloropyrazinone acetamide thrombin inhibitors.

Implementation of P3 pyridine N-Oxides to deliver an orally bioavailable series containing P1 N-benzylamides. *Bioorg. Med. Chem. Lett*., **2003**, 13, 1353–1357.

27. Deng, J.Z.; McMasters, D.R.; Rabbat, P.M.A.; Williams, P.D.; Coburn, C.A.; Yan, Y.; Kuo, L.C.; Lewis, D.; Lucas, B.J.; Krueger, J.A.; Strulovici, B.; Vacca, J.P.; Lyle T.A.; Burgey, C.S. Development of an oxazolopyridine series of dual thrombin/factor Xa inhibitors via structure-guided lead optimization. *Bioorg. Med. Chem. Lett*., **2005**, 15, 4411–4416.
28. Hauel, N.H.; Nar, H.; Priepke, H.; Ries, U.; Stassen, J-M.; Wienen, W. Structure-based design of novel potent nonpeptide thrombin inhibitors. *J. Med. Chem.* **2002**, *45*, 1757-1766.
29. Ries, U.J.; Priepke, H. W.M.; Hauel, N.H.; Haaksma, E.J.; Stassen, J.M.; Wienen, W.; Nar, H. Heterocyclic thrombin inhibitors. Part 1: Design and synthesis of amidino-phenoxy quinoline derivatives. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 2291–2295.
30. Ries, U.J.; Priepke, H.W.M.; Hauel, N.H.; Handschuh, S.; Mihm, G.; Stassen, J.M.; Wienen, W.; Nar, H. Heterocyclic thrombin inhibitors. Part 2: Quinoxalinone derivatives as novel, potent antithrombotic agents. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 2297–2302.
31. Li, M.; Ren, Y-J. Synthesis and Biological Evaluation of Some New 2,5-Substituted 1-Ethyl-1H-benzoimidazole Fluorinated Derivatives as Direct Thrombin Inhibitors. *Arch. Pharm. Chem. Life Sci*., **2015**, *348*, 353–365.
32. Chen, H.; Ren, Y-J. Design, synthesis and anti-thrombotic evaluation of some novel fluorinated thrombin inhibitor derivatives. *Arch. Pharm. Chem. Life Sci.*, **2015**, *348*, 408–420.
33. Li, C-L.; Ren, Y-J. Synthesis and biological evaluation of novel dabigatran derivatives as thrombin inhibitors. *Res. Chem. Intermed*., **2015**.
34. Liu, Q.; Ren, Y-J.; Gao, X. Design, Synthesis and Bioactivity Evaluation of Novel Dabigatran Derivatives as Potential Thrombin Inhibitors. *Lett. Drug Des. Discov.*, **2016**, *13(8)*, 809-817.
35. Li, C-H.; Dong, M-H.; Ren, Y-J.; Li, L-H. Design, synthesis, biological evaluation and molecular docking of novel dabigatran derivatives as potential thrombin inhibitors. *RSC Adv.*, **2015**, *5*, 23737.

36. Li, M-L.; Ren, Y-J.; Dong, M-H.; Ren, W-X. Design, synthesis and structural exploration of novel fluorinated dabigatran derivatives as direct thrombin inhibitors. *Eur. J. Med. Chem*., **2015**, *96*, 122-138.
37. Ren, W.; Ren, Y-J.; Wang, S. Design, synthesis, anticoagulant activity evaluation and molecular docking studies of a class of N-ethyl dabigatran derivatives. *Eur. J. Med. Chem*., **2016**, *120*, 148-159.
38. Wang, F.; Ren, Y-J.; Dong, M-H. Molecular design, synthesis and anticoagulant activity evaluation of fluorinated dabigatran analogues. *Bioorg. Med. Chem.*, **2016**, *24*, 2739–2753.
39. Yang, H.; Liu, Q.; Gao, X.; Ren, Y-J.; Gao, Y. Novel dabigatran derivatives with a fluorine atom at the C-2 position of the terminal benzene ring: Design, synthesis and anticoagulant activity evaluation. *Eur. J. Med. Chem*., **2017**, *126*, 799-809.
40. Wang, S.; Dai, P.; Xu, Y.; Chen, Q.; Zhu, Q.; Gong, G. Design, synthesis and biological evaluation of dabigatran etexilate mimics, a novel class of thrombin inhibitors. *Arch. Pharm. Chem. Life Sci*., **2015**, *348*, 1–11.
41. Chen, D.; Wang, S.; Diao, X.; Zhu, Q.; Shen, H.; Han, X.; Wang, Y.; Gong, G.; Xu, Y. Design, synthesis and antithrombotic evaluation of novel dabigatran etexilate analogs, a new series of non-peptides thrombin inhibitors. *Bioorg. Med. Chem.*, **2015,** *23*, 7405–7416.
42. Chen, D.; Shi, J.; Liu, J.; Zhang, X.; Deng, X.; Yang, Y.; Cui, S.; Zhu, Q.; Gong, G.; Xu, Y. Design, synthesis and antithrombotic evaluation of novel non-peptide thrombin inhibitors. *Bioorg. Med. Chem.*, **2017**, *25*, 458–470.
43. Henry, B. L.; Monien, B. H.; Bock, P. E.; Desai, U. R. A novel allosteric pathway of thrombin inhibition. Exosite II mediated potent inhibition of thrombin by chemo-enzymatic, sulfated dehydropolymers of 4-hydroxycinnamic acids. *J. Biol. Chem.*, **2007**, *282*, 31891-31899.
44. Verghese, J.; Liang, A.; Sidhu, P. S.; Hindle, M.; Zhou, Q.; Desai, U. R. First steps in the direction of synthetic, allosteric, direct inhibitors of thrombin and factor Xa. *Bioorg. Med. Chem. Lett*., **2009**, *19*, 4126−4129.

45. Sidhu, P.S.; Liang, A.; Mehta, A.Y.; Abdel Aziz, M.H.; Zhou, Q.; Desai, U.R. Rational design of potent, small, synthetic allosteric inhibitors of thrombin. *J. Med. Chem.* **2011**, *54*, 5522–5531.
46. Sidhu, P.S; Abdel Aziz, M.H.; Sarkar, A.; Mehta, A.Y.; Zhou, Q.; Desai, U.R. Designing allosteric regulators of thrombin. Exosite 2 features multiple subsites that can be targeted by sulfated small molecules for inducing inhibition. *J. Med. Chem.* **2013**, *56*, 5059–5070.