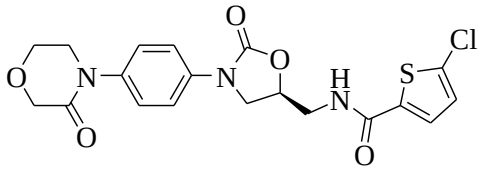
Abstract

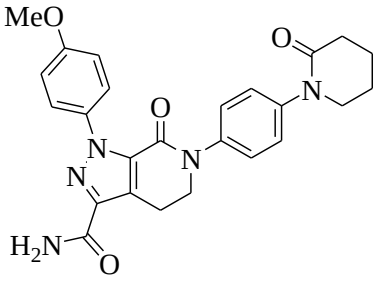
Thrombotic disorders like deep vein thrombosis, pulmonary embolism, myocardial infarction and stroke, all are a major concern and one of the primary causes of mortality today. Anticoagulant therapy comprising of heparins and vitamin K antagonists has been used over decades in the prevention and treatment of thromboembolic diseases. Though effective, these agents are associated with many clinical limitations like constant monitoring, drug-drug interactions and serious bleeding events. Hence, it is imperative need to develop new antithrombotic agents with better efficacy and safety profile.

The discovery program for oral anticoagulants largely targeted two serine proteases, factor Xa (FXa) and thrombin (FIIa). Due to the characteristic location of FXa at the junction of coagulation cascade, direct inhibition of FXa has emerged as an effective strategy to achieve anticoagulation maintaining normal hemostasis. FXa catalyzes conversion of prothrombin to thrombin generating more than 1000 thrombin molecules in one activation cycle. Selective inhibition of FXa blocks the thrombin burst without affecting the normal thrombin levels and the associated platelet functions, thus exhibiting minimal bleeding risks and maintaining normal hemostasis.

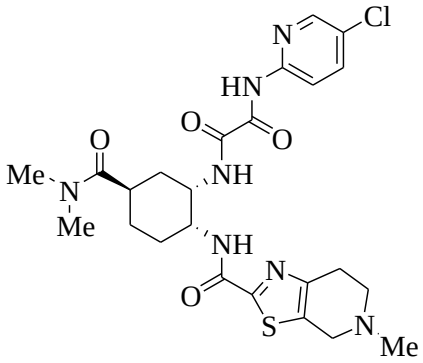
Currently for oral antithrombotic therapy, four clinical candidates as factor Xa inhibitors viz. rivaroxaban (**15**), apixaban (**16**), edoxaban (**17**) and betrixaban (**18**) are available. These agents have exhibited good oral bioavailability and pharmacokinetic properties. However, they have offered narrow clinical utility as they cannot be used in patients with renal and hepatic diseases and patients having mechanical heart valves, and they lack suitable antidotes in severe bleeding events. Recently it has been reported that discontinuation of rivaroxaban and apixaban therapy may result in rebound thrombosis. Therefore the search for new FXa inhibitors with positive attributes to overcome these issues is the need of the time.



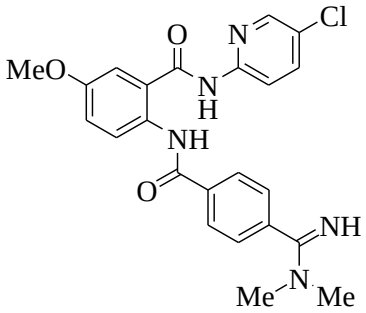
Rivaroxaban (**15**)



Apixaban (**16**)



Edoxaban (**17**)



Betrixaban (**18**)

Thrombin or factor IIa, a serine protease enzyme plays a pivotal role in the regulation of coagulation and fibrinolysis processes and maintenance of hemostasis. It possesses both procoagulant and anticoagulant properties and catalyzes the final step of coagulation cascade viz. conversion of insoluble fibrinogen to soluble fibrin and activates factor XIII forming cross-linked fibrin strands and stable clot. It also facilitates activation of clotting factors FXI, FIX, FX and cofactors FV and FVIII. Direct thrombin inhibitors exhibit either direct or indirect mechanism of action. Currently one drug direct thrombin inhibitor viz. dabigatran is available for oral use.

Thus based on the favorable therapeutic potential of factor Xa inhibitors in the antithrombotic therapy, we have taken rivaroxaban (**15**) and anthranilamide based molecule, betrixaban (**18**) for structural modifications where amidine moiety of betrixaban, responsible for its poor bioavailability was replaced with less basic piperazinyl moiety and different substituted aromatic P1 and P4 ligands were incorporated to get new derivatives with better selectivity and

efficacy. Different haloaromatic groups like 5-chlorothiophen-2-yl (rivaroxaban), 6-chloropyridin-3-yl (betrixaban isostere) and 4-chlorophenyl (eribaxaban) groups present in the reported FXa inhibitors have been employed to improve selectivity for the enzyme FXa. Taking these findings into consideration, a novel series of anthranilamide derivatives was designed, synthesized and biologically evaluated.

Taking into consideration the antithrombotic role of direct thrombin inhibitors, we planned and synthesized new series of furanopyrimidinone compounds. From the fragment-based design strategy we introduced amino and phenoxy linked aromatic fragments as S1 binding moieties and S3 occupying ethylene linked aromatic moieties on central furanopyrimidinone scaffold. Structural optimization in pyrimidine ring by methyl substitution was aimed for better thrombin selectivity.

Chemical Studies:

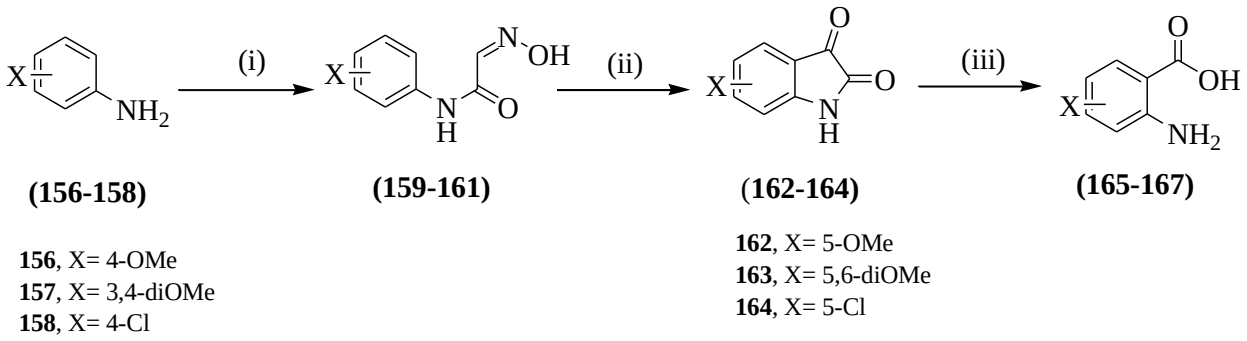
The research work carried out as per planned scheme has been discussed as following:

1. Anthranilamide-based Factor Xa inhibitors
2. Furanopyrimidinone-based Thrombin inhibitors

1. Anthranilamide-based factor Xa inhibitors

Synthesis of designed targeted anthranilamide derivatives

Target anthranilamide derivatives were synthesized by adopting **Scheme I-II**. As depicted in **Scheme-I,** first commercially available anilines (**156-158**) were treated with chloral hydrate and hydroxylamine hydrochloride to get substituted 2-(hydroxyimino)-*N*-phenylacetamide derivatives (**159-161**) which were further reacted with concentrated sulphuric acid giving cyclized products, substituted indoline-2,3-diones (**162-164**). Compounds (**162-164**) were hydrolyzed into substituted 2-aminobenzoic acids (**165-167**) using alkaline hydrogen peroxide.



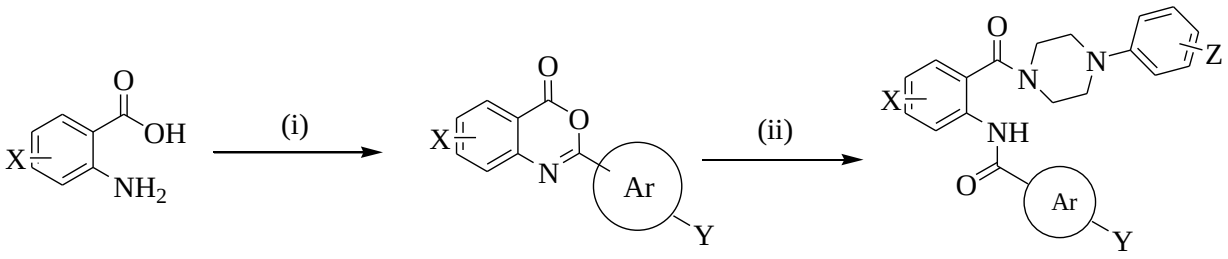
(156-158) **(159-161)** **(162-164)** **(165-167)**

156, X= 4-OMe
157, X= 3,4-diOMe
158, X= 4-Cl

162, X= 5-OMe
163, X= 5,6-diOMe
164, X= 5-Cl

Scheme I: Synthesis of compounds (**165-167**). Reagents and conditions: (i) $Cl_3CCHO.H_2O$, $NH_2OH.HCl$, 130 ^oC; (ii) Conc. H_2SO4, 85 ^oC, 20 min.; (iii) 5% NaOH, 30% H_2O_2, conc. HCl.

Substituted 2-aminobenzoic acids (**165-167**) with different aromatic acid chlorides in presence of dry pyridine afforded different 1,3-benzoxazin-4-one analogs (**168-175**). The benzoxazinone intermediates (**168-175**) were finally heated with different piperazine bases offering the target compounds (**177-235**). The nitro derivatives were further reduced to amino analogs by iron powder and ammonium chloride in methanol. All the targeted compounds were purified by column chromatography.



(165-167) **(168-175)** **(177-235)**

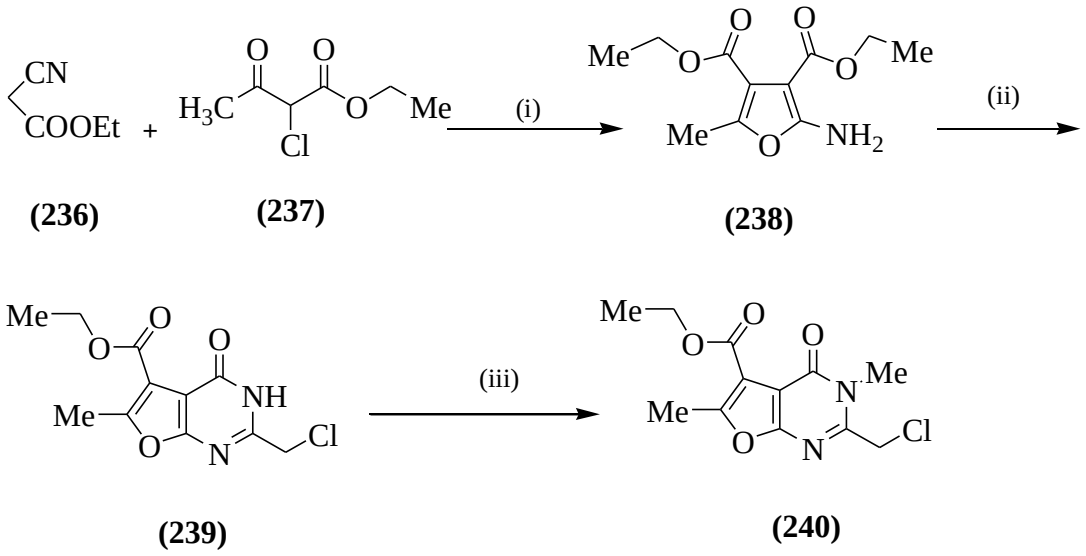
Scheme II: Synthesis of target anthranilamide compounds (**177-235**). Reagents and conditions: (i) Acid chlorides, 0-5 ^oC, Dry pyridine; (ii) Substituted piperazine free base or substituted piperazine HCl, Dry DMF, 6-8 hr, 100 ^oC.

2. Furanopyrimidinone-based Thrombin inhibitors

Synthesis of Furanopyrimidinone-amide derivatives

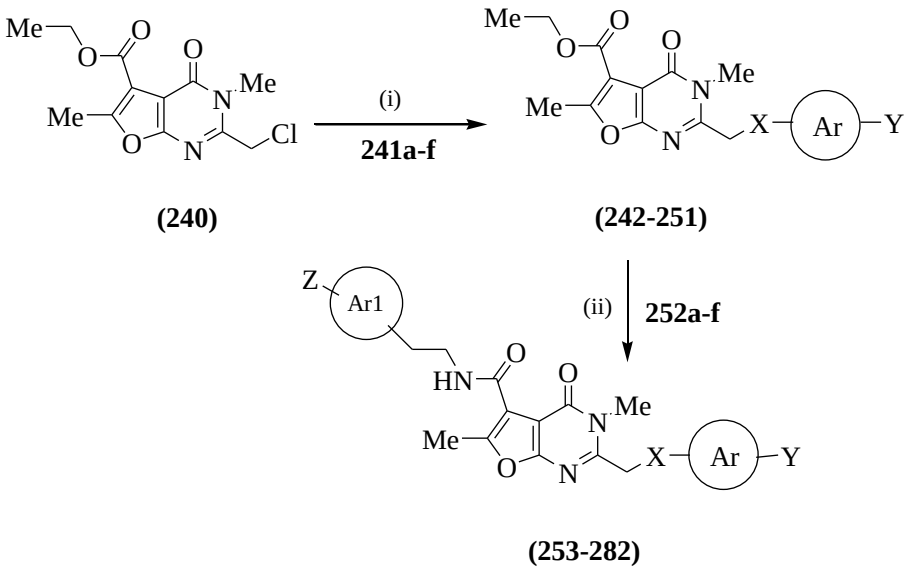
Synthesis of furanopyrimidinone-amides was carried out by employing **Scheme III-IV.** Reaction between ethyl cyanoacetate (**236**) and ethyl 2-chlroacetoacetate (**237**) with triethylamine at RT afforded 2-amino-5-methylfuran diethyl dicarboxylate (**238**) which on

treatment with chloroacetonitrile and dry HCl in dry dioxane resulted in the cyclized product, ethyl-2-(chloromethyl)-3,4-dihydro-6-methyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**239**). Compound (**239**) after methylation using dimethyl sulphate furnished ethyl-2-((4-chloromethyl)-3,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate (**240**).



Scheme III: Synthesis of compounds (**238-240**). Reagents and conditions: (i) TEA/ iPrOH, RT (ii) Dry dioxane in dry HCl, 0-5^{o}C, (iii) Dimethyl sulphate/ acetone, $NaHCO_3$, reflux.

Compound (**240**) after nucleophilic substitution with anilines or phenols (**241a-f**) yielded ethyl-2-((arylamino)methyl)-3,4-dihydro-3,6-dimethyl-4-oxofuro[2,3-*d*]pyrimidine-5-carboxylate derivatives (**242-251**). Finally, the target compounds, 4-oxo-*N*-phenethylfuro[2,3-*d*]pyrimidine-5-carboxamides (**253-282**) were prepared from the reaction of compounds (**242-251)** with substituted phenethylamines (**252a-f**) by Weinreb transamidation method using trimethyl aluminium as the coupling reagent.



Scheme IV: Synthesis of compounds (**252-282**). Reagents and conditions: (i) Dry dioxane, reflux (ii) $AlMe_3$, EDC, RT

Biological Studies:

All the novel synthesized compounds were evaluated for antithrombotic activity. *In vitro* FXa and thrombin inhibitory activities and *ex-vivo* anticoagulant and bleeding time activity were performed.

Initial biological screening of anthranilamide series of derivatives for FXa enzyme inhibition activity has resulted into some good to moderate active compounds showing > 60% activity. During further evaluation for IC_{50} determination of these compounds, two compounds viz. (**201**) and (**208**) both containing 4-fluorophenyl piperazinyl S4-binding and 5-chlorothienyl as S1-binding moieties displayed potent *in vitro* antithrombotic activity with IC_{50} values of 0.61 and 0.74 μM respectively. Compounds (**187**; IC_{50} = 6.3 μM) and (**224;** IC_{50} = 1.8 μM) also showed good to moderate biological activities. Derivatives containing *para* halo-substitution on both P1 and P4 ligands were found to show better antithombotic potency than those having electron donating methyl or methoxy groups. Further evaluation of these potent compounds for *ex-vivo* anticoagulant activity revealed compounds (**201,** PT= 19.9, aPTT= 40.8 sec.) and (**224,** PT= 13.6, aPTT= 40.0 sec.) exhibiting prolongation in prothrombin and activated partial thromboplastin time than standard drug rivaroxaban (PT= 11.4, aPTT= 37.9 sec.). At the same

time compound (**201**) was found to show comparable bleeding tendency in rats suggesting its antithrombotic potential for further development.

For the assessment of antithrombotic activity, series of furanopyrimidinone compounds were subjected to *in-vitro* thrombin inhibition assay where twenty-two compounds showed > 60% inhibition results. From these compounds, compounds (**255**; IC_{50} = 0.96 µM) and **(259**; IC_{50} = 1.36 µM) having 4-chloro and 4-fluorophenyl substituents respectively as P1 fragments were found to be the most potent compounds of the series. Other compounds like (**263,** IC_{50} = 3.01 µM) and (**264,** IC_{50} = 4.55 µM) containing 3-chloro-4-fluorophenyl P1 fragments also afforded moderate inhibitory activity. Removal or replacement of halogen substituents by methyl or methoxy on S1-binding ligands resulted in compounds (**261**; IC_{50} = 18.8 µM and **282**; IC_{50} = 21.4 µM) with diminished activity. In *ex-vivo* anticoagulant evaluation of some potent compounds, two compounds (**255**; PT= 18.7, aPTT= 33.4 sec.**)** and (**264;** PT= 11.7, aPTT= 35.3 sec.**)** showed prolongation in prothrombin time compared to control (PT = 8.7 and aPTT = 25.3 sec.). Compound (**255**; BT= 91 sec.) displayed low bleeding time than the standard drug dabigatran (BT= 102 sec.) in haemorrahgic tendency analysis in rats.

Computational Studies:

The most potent compounds (**201** and **255**) were selected to assess their binding interactions with the active site of FXa and thrombin enzymes respectively. Both the compounds displayed good molecular interactions with proper conformations. In addition, these compounds also exhibited favorable ADMET properties in prediction of physicochemical and pharmacokinetic properties calculations. Molecular simulation studies of anthranilamide compounds are well described in the thesis.