ABSTRACT:

Thromboembolic events play a major role in the pathogenesis of global diseases like ischemic heart diseases, stroke and venous thromboembolism (VTE) which constitute a prime cause of morbidity and mortality in developed countries. One of the most conceivable strategies for treating these diseases is to reduce or prevent the formation of thrombus. The traditional antithrombotic therapy has been reported to have limitations like a narrow therapeutic window and interactions with other food and drugs (vitamin K antagonist-warfarin), inconvenience in oral administration, bleeding risks (heparin analogs). Extensive research efforts have been made to develop orally active antithrombotic drugs with a better safety profile.

Factor Xa (FXa), a trypsin-like serine protease, which is located at the common pathway of the coagulation cascade, converts prothrombin to thrombin and plays a crucial role in various thrombotic disorders. Currently, there are four orally active, selective FXa inhibitors, approved by U.S. Food and Drug Administration (FDA): rivaroxaban (**7**), apixaban (**8**), edoxaban (**9**) and betrixaban (**10**).

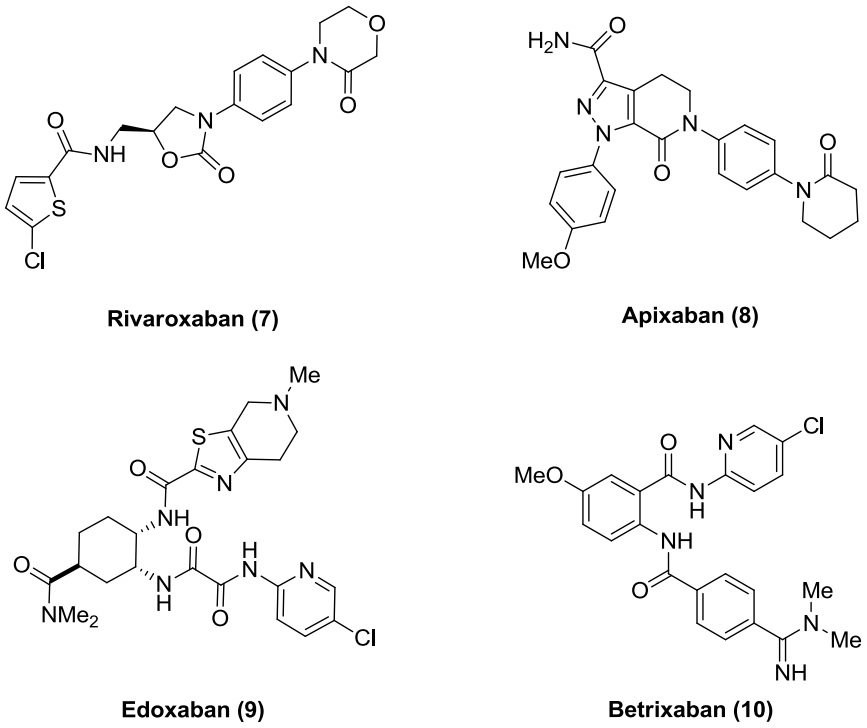


Figure 1. Currently approved FXa inhibitors

These novel FXa inhibitors manifested higher specificity, better oral bioavailability and lesser food and drug interactions compared to the traditional anticoagulant agents. However, they still have many drawbacks like drug-drug interactions, narrow clinical indications and lack of a specific antidote for preventing bleeding. These inhibitors are not recommended to the patients suffering from acute hepatic and renal impairment, and patients with artificial heart valves. So, there is a demand to further develop novel and safer FXa inhibitors to promote their clinical use.

The review of the literature reveals that for selective FXa inhibitory activity, the following structural features are necessary:

- S1 subsite binding ligand
- S4 subsite binding ligand
- A basic scaffold (linker) that connects S1 subsite binding ligand with S4 subsite binding ligand and builds the final U or V shape structure that can be best accommodated in to the active site.

So designing the ligands in such a way that fulfills all the above-mentioned requirements is our primary goal. Synthesis of those designed ligands and biological evaluations are other objectives of this work. It was decided to use three scaffolds like 2-aminobenzamide, 1,3,4-thiadiazole and carbazole to connect it to the two different hydrophobic arms (S1 binding ligand and S4 binding ligand).

Chemical studies:

The work carried out for the fulfillment of the proposed plan has been discussed under three heads:

A. 2-Aminobenzamide-based FXa Inhibitors

B. 1,3,4-Thiadiazole-based FXa inhibitors and

C. Carbazole derivatives as FXa inhibitors

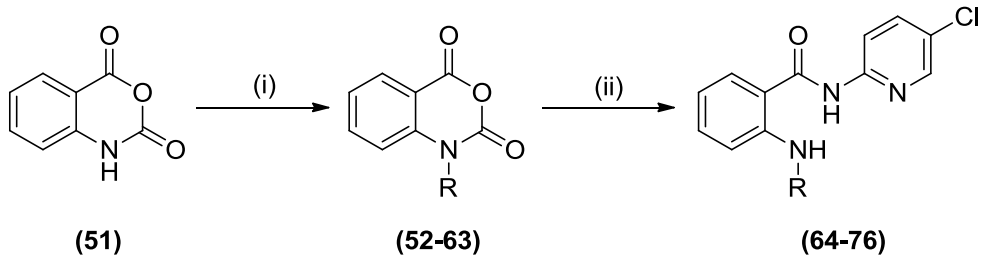
A. 2-Aminobenzamide-based Factor Xa Inhibitors

Inspired by the favorable biological profile of anthranilamide based FXa inhibitors, we selected betrixaban (**10**) as a lead molecule for further chemical modifications. It was contemplated to introduce alkyls, benzyls, biphenyls or substituted piperazines as S4 binding ligands in the

anthranilamide scaffold as the replacements of highly basic amidine group of betrixaban (**10**) and maintain the 5-chloro-2-pyridyl group as such, as the S1 binding ligand.

Synthesis of the designed 2-aminobenzamide derivatives

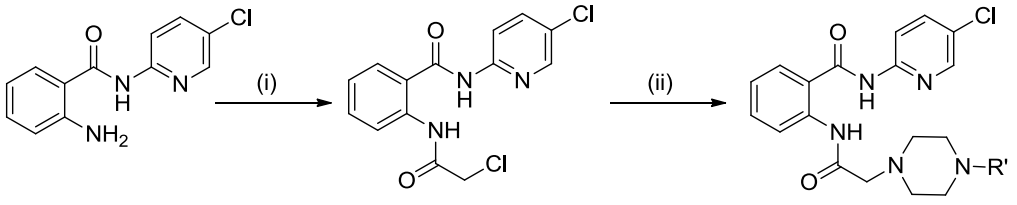
Substituted 2-amino-*N*-(5-chloropyridin-2-yl)benzamides (**64-76**) were synthesized by adopting general **Scheme I**. Commercially available isatoic anhydride (**51**) was treated with alkyl/arylalkyl halides to obtain *N*-substituted isatoic anhydrides (**52-63**)**.** The *N*-substituted isatoic anhydrides (**52-63**) were subjected to ring-opening by reacting with 2-amino-5-chloropyridine to afford the desired substituted 2-amino-*N*-(5-chloropyridin-2-yl)benzamides (**64-76**).



(51) **(52-63)** **(64-76)**

Scheme I. Synthetic route for the preparation of compounds **(64-76)**. Reagents and conditions: (i) Alkyl/arylalkyl halide, DIPEA, DMA, rt; (ii) 2-Amino-5-chloropyridine, pot. *tert*-butoxide, THF, rt.

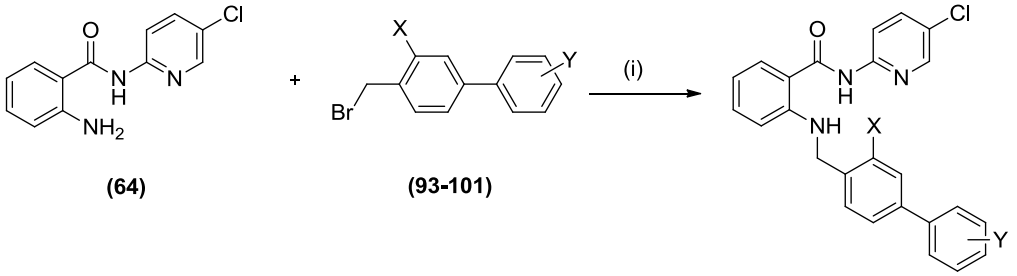
2-(2-(*N*-Substitutedpiperazin-1-yl)acetamido)-*N*-(5-chloropyridin-2-yl) benzamides (**78-83**) were synthesized using the synthetic route as depicted in **Scheme II**. Compound (**64**) was reacted with chloroacetyl chloride to offer the intermediate (**77**). The chloro group of the intermediate (**77**) was displaced by different substituted piperazines to get the desired piperazinyl compounds (**78-83**).



(64) **(77)** **(78-83)**

Scheme II. Synthetic route for the preparation of compounds (**78-83**). Reagents and conditions: (i) Chloroacetyl chloride, K_2CO_3, dry DCM, 0-5 °C; (ii) Substituted piperazines, DMF, 120 °C, 4-6 hrs.

2-(4-(2-/3-/4-substituted phenyl)benzylamino)-*N*-(5-chloropyridin-2-yl)benzamides (**102-110**) were synthesized by adopting **Scheme III**. Compound (**64**) was reacted with different 4-bromomethyl-1,1'-biphenyls (**93-101**) to obtain the targeted compounds (**102-110**).



(64) **(93-101)** **(102-110)**

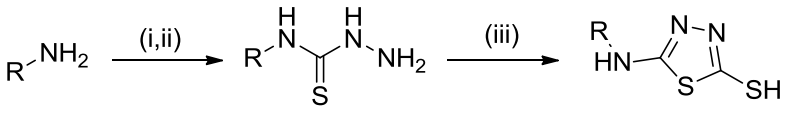
Scheme III. Synthetic route for the preparation of compounds (**102-110**). Reagents and conditions: (i) K_2CO_3, DMF, 120 °C, 4-6 hrs.

B. 1,3,4-Thiadiazole-based FXa inhibitors

A multi-receptor based virtual screening approach was used to identify a novel 1,3,4-thiadiazole scaffold for the development of potential FXa inhibitors. Extensive modifications focusing on the P4 and P1 groups resulted in novel series of FXa inhibitors.

Synthesis of 1,3,4-thiadiazole derivatives

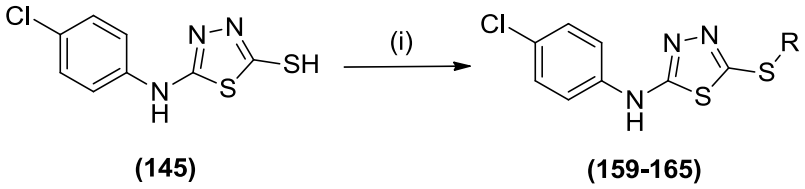
Synthesis of 5-substituted amino-1,3,4-thiadiazole-2-thiols (**141-155**) was carried out as depicted in **Scheme IV**.



(111-125) **(126-140)** **(141-155)**

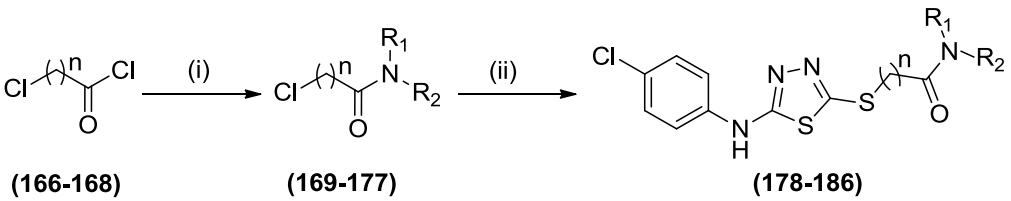
Scheme-IV. Synthetic route for the preparation of thiosemicarbazides (**126-140**) and thiols (**141-155**). Reagents and Conditions: For (**126-134**) (i) Carbon disulphide, sodium hydroxide, DMF, 20-30 °C, 2-3 hrs; (ii) Hydrazine hydrate, 70 °C, 1.5 hrs; For (**135-140**) (i) Carbon disulphide, triethylamine, methanol, 20-30 °C, 2-3 hrs. and iodomethane in methanol at -10 °C (ii) Hydrazine hydrate, methanol, reflux; (iii) Carbon disulphide, potassium hydroxide, methanol, Reflux, 5-6 hrs.

5-(Substituted-benzylthio)-*N*-(4-chlorophenyl)-1,3,4-thiadiazol-2-amines (**159-165**) were synthesized by reacting **(145)** with different benzyl halides as illustrated in **Scheme V**.



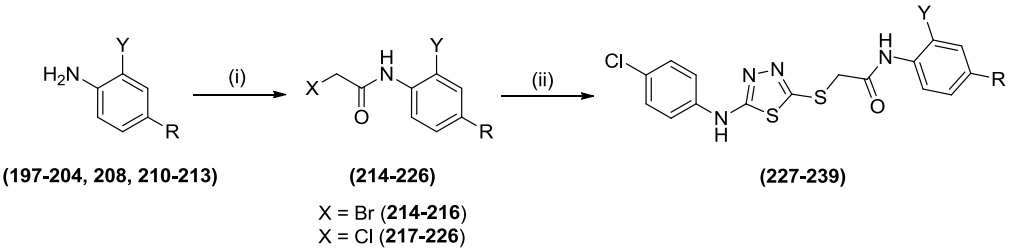
Scheme V. Synthetic route for the preparation of compounds (**159-165**). Reagents and Conditions: (i) Substituted benzyl halides, potassium carbonate, DMF, rt, 2-3 hrs.

Some secondary amines (morpholine, piperidine and pyrrolidine) were reacted with various acid chlorides (**166-168**) to offer the intermediates (**169-177**). These intermediates **(169-177)** without further purification were reacted with compound (**145**) to get the desired 5-thiosubstituted 1,3,4-thiadiazoles (**178-186**) (**Scheme VI**).



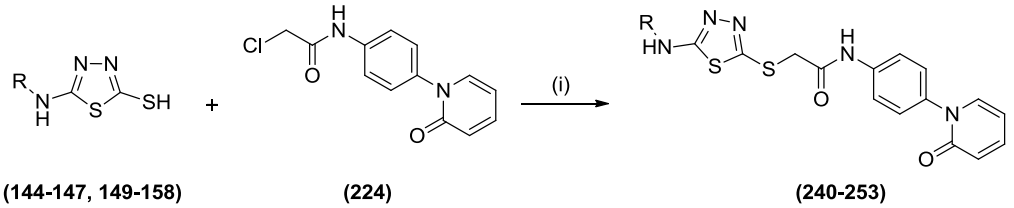
Scheme VI. Synthetic route for the preparation of compounds (**178-186**). Reagents and Conditions: (i) Secondary amines, anhydrous potassium carbonate, dry DCM, rt, 6-7 hrs; (ii) Compound (**145**), potassium carbonate, DMF, rt, 3-4 hrs.

Substituted anilines (**197-204, 208, 210-213**) were reacted with chloroacetyl chloride or bromoacetyl bromide to obtain the intermediates (**214-226**). The acylated compounds (**214-226**) were further reacted with the key intermediate (**145**) to get the desired 2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-phenylacetamides (**227-239**) (**Scheme VII**).



Scheme VII. Synthetic route for the preparation of compounds (**227-239**). Reagents and Conditions: (i) Chloroacetyl chloride, anhydrous potassium carbonate, dry DCM, rt, 2-3 hrs; (ii) Compound (**145**), potassium carbonate, DMF, rt, 3-4 hrs.

As per **scheme VIII**, 5-(substituted amino)-1,3,4-thiadiazole-2-thiols (**141-144, 146-155**) were reacted with 2-chloro-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl)acetamide (**224**) to get desired 2-((5-(substituted phenyl or benzylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-(2-oxopyridin-1(2*H*)-yl)phenyl) acetamides (**240-253**) (**Scheme VIII)**.

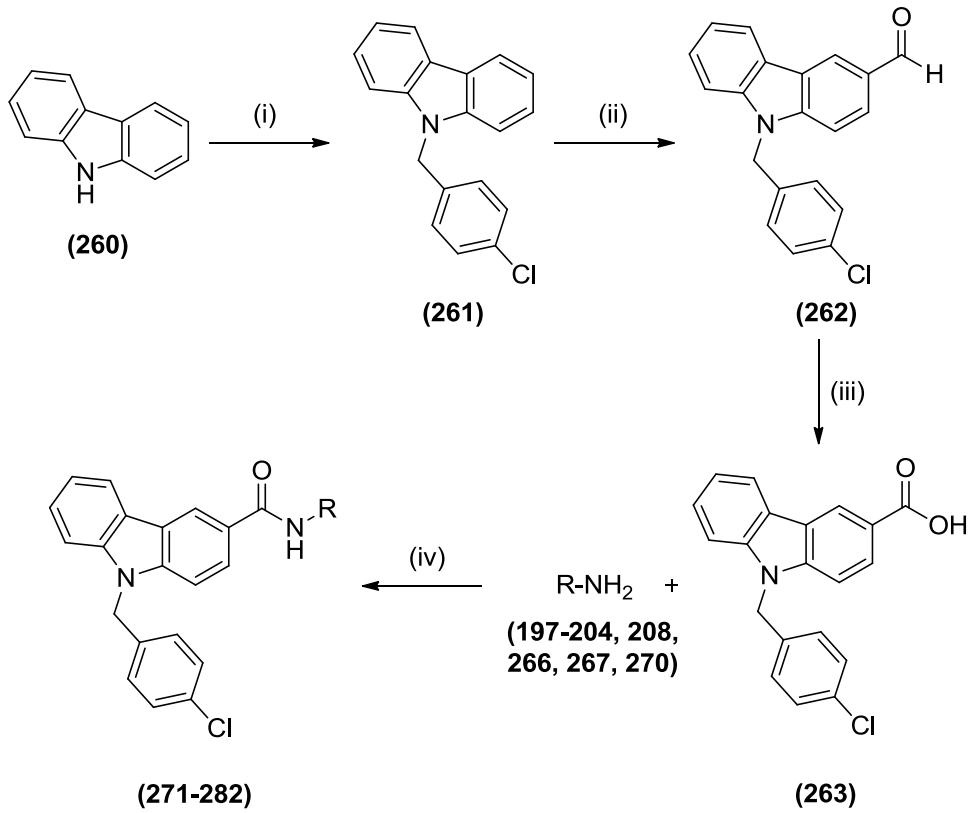


Scheme VIII. Synthetic route for the preparation of compounds (**240-253**). Reagents and Conditions: (i) Potassium carbonate, DMF, rt, 3-4 hrs.

C. Carbazole derivatives as FXa inhibitors

Synthesis of carbazole derivatives (271-282)

As per the **Scheme IX**, the intermediate (**263**) was reacted with different amines (**194-201, 205, 266, 267** and **270**) to obtain the desired carbazole derivatives (**271-282**).



Scheme-IX. Synthetic route for the preparation of compounds (**269-280**). Reagents and Conditions: (i) 4-Chlorobenzylchloride, NaOH, DMSO, 2-3 hrs, RT; (ii) DMF, $POCl_3$, $CHCl_3$,

reflux 12-15 hrs; (iii) TBHP, DMSO, stirred overnight at 110 °C; (iv) Anhydrous HOBt, EDCI, DMAP, DMF, overnight, RT.

Biological studies

All the synthesized compounds were subjected to evaluate for their antithrombotic potential. All the synthesized compounds were tested *in vitro* for FXa inhibitory activity and *In vitro* anticoagulant activity.

A total of twenty-eight 2-aminobenzamide derivatives were evaluated for their effect on antithrombotic activity. Amongst the tested compounds, the benzyl substituted derivative (**71**) and the biphenyl derivatives (**104**, **105** and **107**) showed significant inhibition of the enzyme with IC_{50} values of 11.5 µM, 5.4 µM, 1.3 µM, and 0.7 µM respectively. Compounds showing significant inhibition of the enzyme FXa in the preliminary screening were evaluated further using *ex vivo* measurements of prothrombin time and clotting time. Compound (**107**) (45 sec) exhibited a significant change in clotting time offering the highest value among the selected compounds. Compound (**107**) is the 'best find' of the study offering a high selectivity for FXa over thrombin, with an IC_{50} value in the submicromolar range and causing a significant enhancement in the clotting time.

To check the antithrombotic potential of these 1,3,4-thiadiazole derivatives, all the synthesized compounds were screened against FXa at a concentration of 100 µM. Those compounds with more than 50 % of the FXa inhibition were chosen for the determination of their IC_{50} values. Simple benzyl substituted compounds (**159-165**) and aminoalkyl derivatives (**178-186**) showed <50 % inhibition of the enzyme at a concentration of 100 µM. Compounds (**230-233**) offered good FXa inhibitory activity with IC_{50} values of 16.5 µM, 18.8 µM, 6.5 µM and 4.9 µM, respectively. Compound (**237**) exhibited potent FXa inhibitory activity with an IC_{50} value of 0.22 µM. Moreover, compound (**237**) showed good *in vitro* anticoagulant activity with its 2 × PT value of 25.9 sec and 2 × aPTT value of 27.44 sec. Compound (**237**) displayed good *in vivo* antithrombotic activity in $FeCl_3$ induced arterial thrombosis model (49 % and 32 % inhibition of thrombus formation at 30

mg/kg and 15 mg/kg in rats). These findings suggested that compound (**237**) warrants further evaluation as a potential candidate for the development of antithrombotic agents.

All the synthesized carbazole derivatives (**271-282**) were evaluated for *in vitro* FXa inhibitory and anticoagulant activities. Among these, Compound (**278**) showed the highest FXa inhibitory activity (IC_{50} value of 7.49 µM). Compound (**278**) also exhibited good anticoagulant activity with PT (39.4 sec) and aPTT (69.6 sec) time.

Computational studies:

The promising compounds (**107** and **237**) were subjected to predict their binding interactions with the active site of FXa enzyme. These two compounds exhibited good molecular interactions with the active site of the enzyme. Structure-activity relationship and molecular interaction analysis by docking and dynamics studies within two series (2-aminobenzamide and 1,3,4-thiadizole) of compounds are well discussed in the thesis.