A SYNOPSIS ON

"DESIGN AND SYNTHESIS OF SOME NOVEL HETEROCYCLIC COMPOUNDS FOR ANTITHROMBOTIC ACTIVITY"

For submission of Ph.D. thesis

By

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INTRODUCTION

Thrombosis is a leading contributor to global diseases like ischemic heart diseases, stroke and venous thromboembolism (VTE).[1] As per the impact report published by the International Society on Thrombosis and Hemostasis (ISTH), 1 in 4 deaths worldwide is due to thrombosis, and up to 60 % of VTE are reported during hospitalization.[2] Thrombosis is obstruction of blood in the arterial and venous circulation. Depending upon the site of formation of thrombus, thrombosis is classified as arterial or venous thrombosis. Different types of antithrombotic drugs are used to treat both arterial and venous thrombosis depending on their pathological differences.[3] Arterial thrombosis is generally treated by antiplatelet agents because the condition is associated with platelet aggregation and activation induced by ruptured atherosclerotic plaque, leading to MI and ACS.[4] Obstruction of veins is in the form of deep vein thrombosis (DVT) or pulmonary embolism (PE) which can be treated by anticoagulant agents.[5]

The current antithrombotic therapy includes vitamin K antagonists, coagulation enzymes' inhibitors and heparins such as unfractionated heparins (UFHs), low molecular weight heparins (LMWHs) or fractionated heparins.[3b, 6] Although these drugs have proved their efficacy in clinical practice but they have been found to be associated with several problems. Warfarin, a vitamin K antagonist is the most widely used antithrombotic drug. Unfortunately warfarin has a narrow therapeutic window, causes undesirable interactions with food and drugs and possesses risk of bleeding.[7] Debigatran etexilate, an oral thrombin inhibitor was found to have uncontrolled bleeding which could prove fatal.[8] Other anticoagulants like UFH, LMWHs, direct thrombin inhibitors (Argatroban, Hirudin derivatives) and indirect factor Xa (FXa) inhibitor (Fondaparinux) require parenteral administration which is responsible for clot formation at the site of injection limiting their use in clinical practice. Heparin analogs are associated with thrombocytopenia, immunological reactions and certain other serious side effects.[9] These shortcomings in the existing drugs motivate researchers to discover new orally bioavailable antithrombotic drugs with better safety profile.

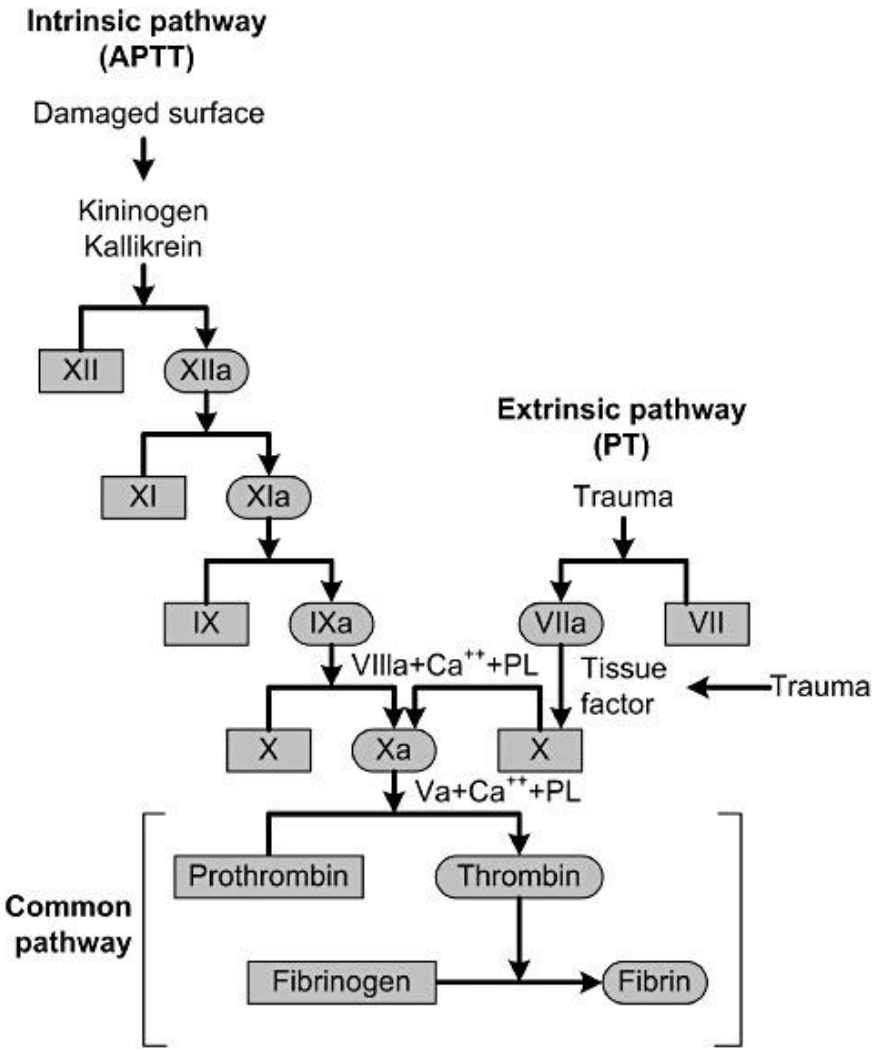


Figure 1. The cascade model of coagulation

To prevent or reduce clotting, currently there is a focus on certain vital enzymes which modulate the coagulation process. Over the last decade, two serine proteases, factor Xa (FXa) and factor IIa (FIIa or thrombin) attracted the attention of medicinal chemists. Out of these two, thrombin is responsible for fibrin formation and it also plays vital roles in several other physiological processes like platelet activation, platelet aggregation and development of new blood vessels. Factor X, a vitamin K-dependent serine protease is secreted in blood as an inactive zymogen. This inactive form is converted into the active form FXa by the factor TF-VIIa complex through the extrinsic pathway or by factor IXa-VIIIa complex through the intrinsic pathway (Figure 1). FXa is the key enzyme in the blood clotting pathway which converts prothrombin to thrombin. On the surface of the phospholipid membrane of activated platelets,

FXa forms a complex with prothrombinase and factor Va in the presence of calcium ions.[8] Thus, FXa plays a central role in the enzymatic activation of blood coagulation cascade and controls hemostasis by regulating thrombin generation and formation of fibrin subsequently in both the models i.e. cascade model (Figure 1) as well as the cell-based model (Figure 2). Compared to thrombin, inhibition of FXa is more specific and involves lower bleeding risks as it does not affect the existing levels of thrombin.[9] Several preclinical studies suggested that FXa could be a better target for anticoagulation therapy as compared to thrombin.[12] One molecule of FXa is responsible for the generation of more than 1000 thrombin molecules.[13] Due to its upstream position in amplification cascade, inhibiting FXa could prove to be a better strategy than direct inhibition of thrombin. The latest results indicated that the use of indirect FXa inhibitors and recently approved direct FXa inhibitors had been associated with lower bleeding risks. Thus, FXa is considered to be an attractive target for the development of antithrombotic drugs.

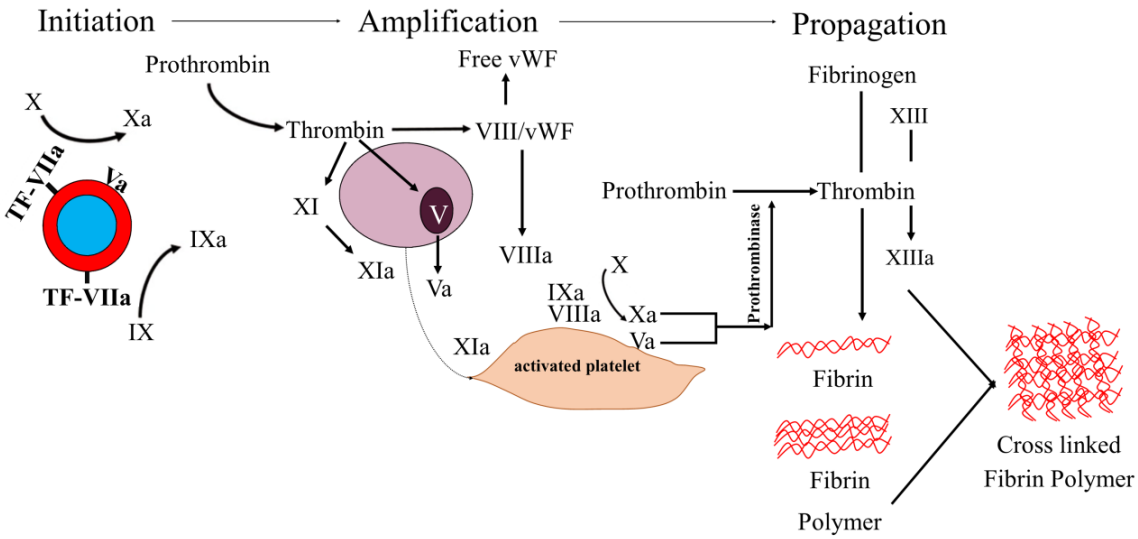
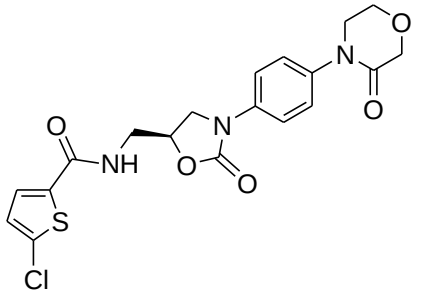


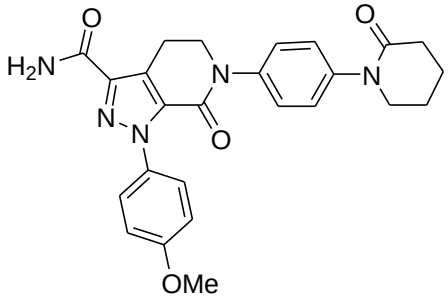
Figure 2. Cell-based model of coagulation

Currently U.S. Food and Drug Administration (FDA) has approved four orally active, selective FXa inhibitors, Rivaroxaban[14] **1**, Apixaban[15] **2**, Edoxaban[16] **3** and Betrixaban[17] **4** (Figure 3). These novel FXa inhibitors displayed higher specificity, better oral bioavailability and lesser food and drug interactions compared to the conventional anticoagulant agents. However, they still have many drawbacks like drug-drug interactions, narrow clinical indications and lack of specific antidote for preventing bleeding.[18] These inhibitors are not recommended to

the patients suffering from acute hepatic and renal impairment[19], and patients with artificial heart valves.[20] So, there is a need to further develop novel and safer FXa inhibitors to advance their clinical use.



1
Rivaroxaban



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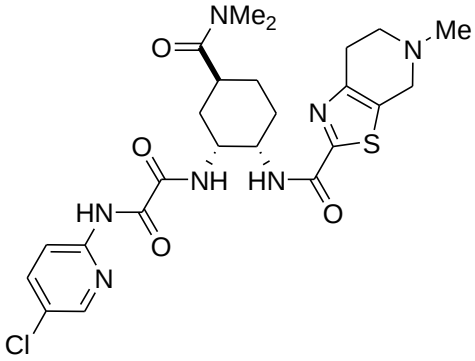
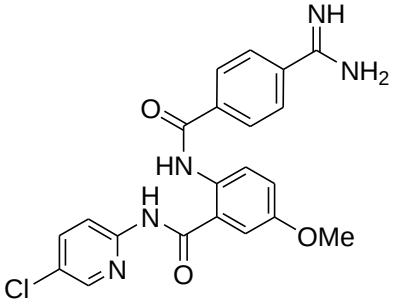
Apixaban



3
Edoxaban



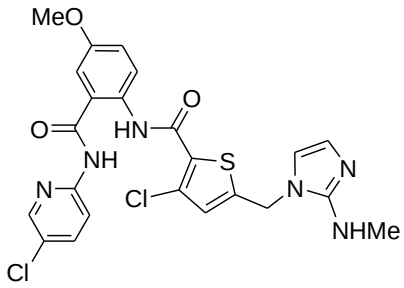
4
Betrixaban

Figure 3. Some well-known FXa inhibitors.

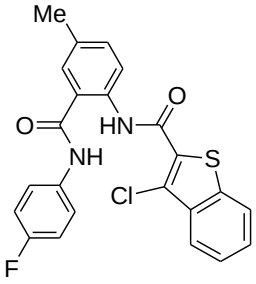
Literature Review

As per the inputs obtained from the enzymatic studies and the available crystallographic structure of FXa, three pharmacophore units i.e. P1 moiety, P4 moiety and a central scaffold are required to be present in potential FXa inhibitors. A large number of FXa inhibitors containing various scaffolds such as anthranilamide[21], diamidobenzene, pyrrolidine[22], pyrazole[23], oxazolidinone, isoxazole[24], piperazine, indole[25], indazole, dihydropyrazolopyridinone, tetrahydroisoquinoline[26], coumarin[27], arylsulfonamidopiperidone[28] and amino acids (eg. glycine, proline)[29] have been reported by various research groups.

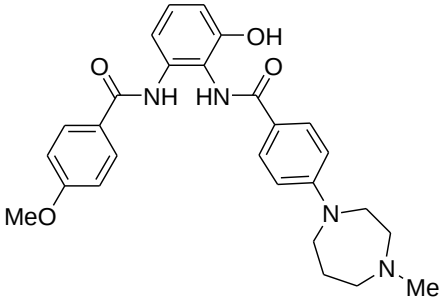
Among these, anthranilamide (**5, 6**) and diamidobenzene (7, **8a, 8b**) based FXa inhibitors have been explored extensively for developing orally active antithrombotic drugs. These vicinal diamide-based FXa inhibitors with U or V shape of the molecule demonstrated good binding to FXa. The thiophene/benzothiophene substituted anthranilamides (**5)** and (**6)** have been reported to inhibit human FXa with potent oral anticoagulant activity. Darexaban, a 1,4-diazepanylbenzamide (**7)** has been reported to be a potent orally active FXa inhibitor. Favourable pharmacokinetic profile and metabolic stability with high potency promoted darexaban as a clinical candidate.



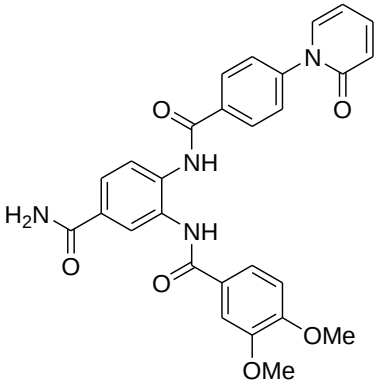
5



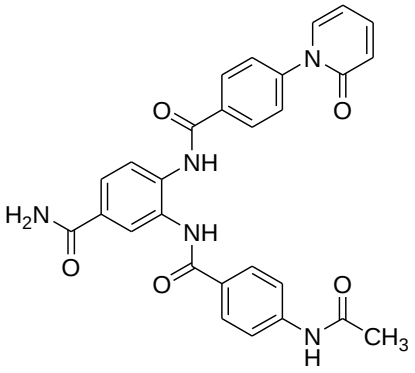
6



7
Darexaban



8a
FXa, IC_{50} = 17.1 nM



8b
FXa, IC_{50} = 15.6 nM

Aims and objectives

The review of 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 fulfill all above mentioned requirement is our primary goal. Synthesis of those designed ligands and biological evaluations are other objectives of this work. The work has been discussed into two parts.

Part-1: 2-Aminobenzamide-based Factor Xa Inhibitors

Inspired by the favourable biological profile of anthranilamide based FXa inhibitors, we selected betrixaban **4** as a lead molecule for further chemical modifications. It was planned to use anthranilamide as the central scaffold to connect it to the two different hydrophobic arms (S1 binding ligand and S4 binding ligand). The neutral haloaromatics, particularly the chlorinated ones have proven their worth as successful S1 binding ligands as they bind effectively to Tyr228 of S1 pocket by Cl-π interaction, improving selectivity and oral bioavailability.[30] The highly basic amidine group, initially used as successful S1 and S4 binding ligand, has been reported to be the culprit for poor oral bioavailabilty.[31]

To develop orally active antithrombotic agents, 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 **4** and maintain the 5-chloro-2-pyridyl group as such, as the S1 binding ligand as represented in Figure 4.

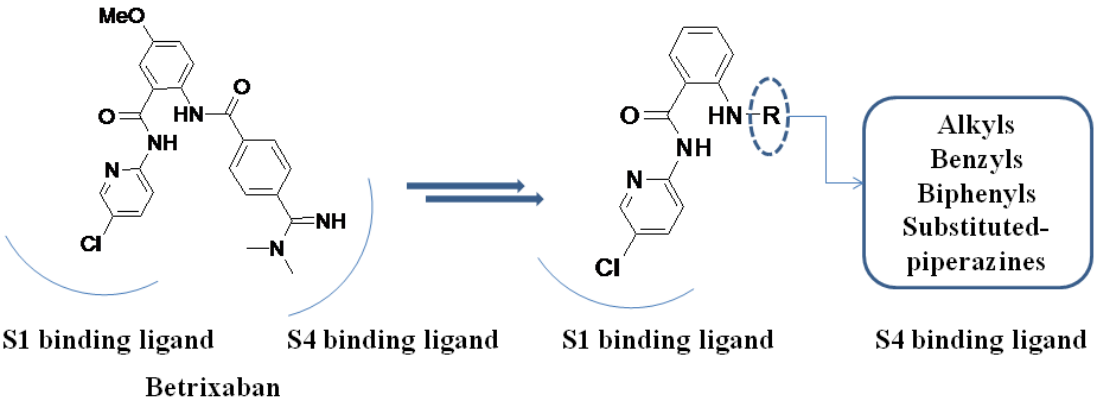


Figure 4. Anthranilamide derivatives possessing novel S4 binding ligands.

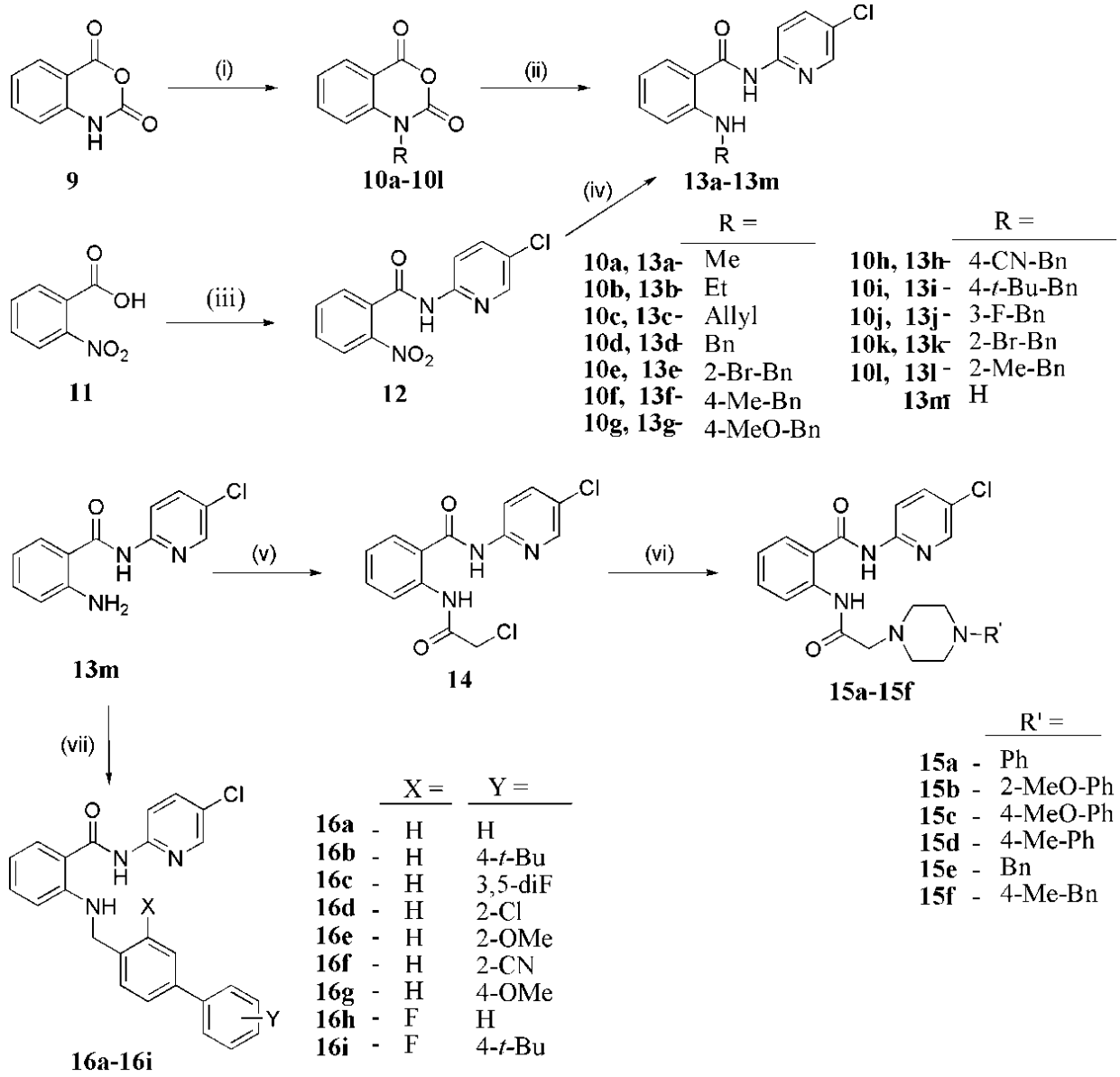
Results and discussion

Chemistry

The designed anthranilamide derivatives were synthesized by adopting Schemes **I** and **II**. The compounds **13a-13m** were prepared from commercially available isatoic anhydride in two steps. In the first step, isatoic anhydride **9** was reacted with alkyl/arylalkyl halides in presence of some organic base to obtain *N*-substituted isatoic anhydrides **10a-10l.**[32] The *N*-substituted isatoic anhydrides **10a-10l** 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 **13a-13l**.

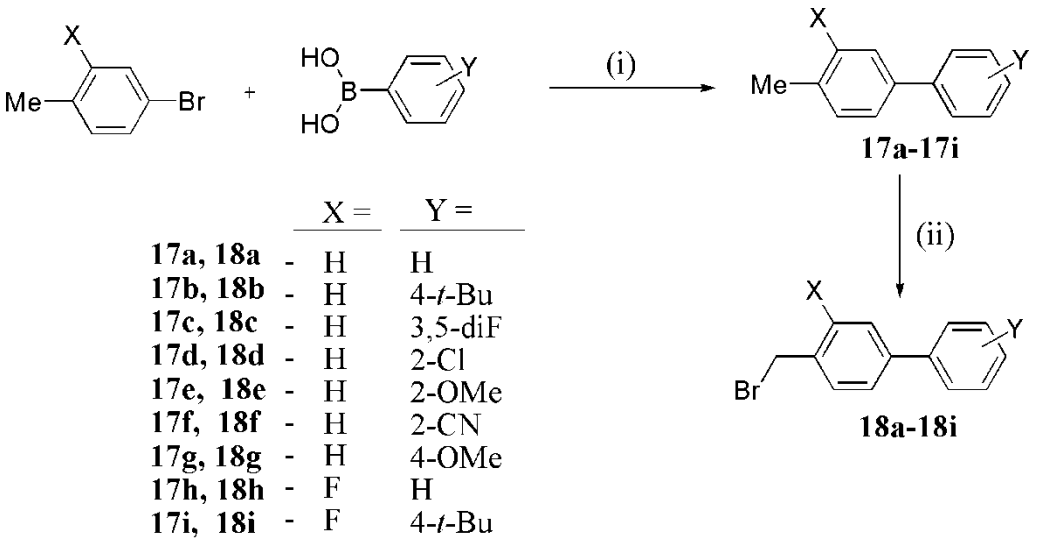
The synthetic routes for the preparation of compounds **15a-15f** and **16a-16i** are also illustrated in **Scheme I**. Commercially available 2-nitrobenzoic acid **11** was used as the starting material to obtain the targeted 2-aminobenzamide **13m**. Controlled condensation of 2-nitrobenzoic acid **11** with 2-amino-5-chloropyridine gave intermediate **12** which was reduced with $SnCl_2$ to get the aniline derivative **13m**. The 4-bromomethyl-1,1'-biphenyls **18a-18i**, prepared from **17a-17i** as per **Scheme II**, were used to obtain compounds **16a-16i**. Suzuki reaction was used to get biphenyls **17a-17i** from aryl halides and phenylboronic acids.[33] The key intermediate **13m** was reacted with chloroacetyl chloride to offer the intermediate **14**. The chloro group of the intermediate **14** was displaced by different substituted piperazines to get the desired piperazinyl compounds **15a-15f**.

Scheme I



Scheme I: Synthetic route for the preparation of compounds **15a-15f** and **16a-16i**. Reagents and conditions: (i) Alkyl/arylalkyl halide, DIPEA, DMA, rt; (ii) 2-Amino-5-chloropyridine, pot. *tert*-butoxide, THF, rt (Method A); (iii) $POCl_3$, dry pyridine, 0-5 °C, 3 hrs (iv) $SnCl_2.2H_2O$, EtOAc, reflux; (v) Chloroacetyl chloride, K_2CO_3, dry DCM, 0-5 °C; (vi) Substituted piperazines, DMF, 120 °C, 4-6 hrs (Method B);; (vii) K_2CO_3, DMF, **18a-18i**, 120 °C, 4-6 hrs (Method C).

Scheme II



	X =	Y =
17a, 18a	H	H
17b, 18b	H	4-*t*-Bu
17c, 18c	H	3,5-diF
17d, 18d	H	2-Cl
17e, 18e	H	2-OMe
17f, 18f	H	2-CN
17g, 18g	H	4-OMe
17h, 18h	F	H
17i, 18i	F	4-*t*-Bu

Scheme II: Synthetic route for the preparation of compounds **18a-18i**. Reagents and conditions: (i) K_2CO_3, $Pd(OAc)_2$, PEG 4000, H_2O, 50 °C, 30 mins (ii) *N*-Bromosuccinimide, AIBN, CCl_4, reflux, 20 h.

Biology

In vitro FXa and thrombin inhibition assays

In vitro enzyme inhibition assays for FXa and thrombin were performed for the synthesized compounds at a concentration of 100 µM by using a chromogenic substrate (Spectrozyme TH for thrombin and S-2222 for FXa) as per the previously reported procedure. The residual enzyme activity was determined from the change in the absorbance at 405 nm with hydrolysis of the substrate by the enzyme. Those compounds with less than 60 % of the residual FXa activity were chosen for determination of their IC_{50} values. Compounds **13e-13g**, **13i-13k**, **15e** and **16a**, **16c-16f**, **16h** and **16i** showed inhibition of FXa in the preliminary screening (Figure 5). All the synthesized compounds showed more than 50 % thrombin residual activity (Figure 6). All the selected compounds demonstrated good selectivity for FXa over thrombin (IC_{50} values of >100 µM). **Table 1** represents IC_{50} of compounds **13e-13g**, **13i-13k**, **15e** and **16a**, **16c-16f**, **16h** and **16i** against both the enzymes.

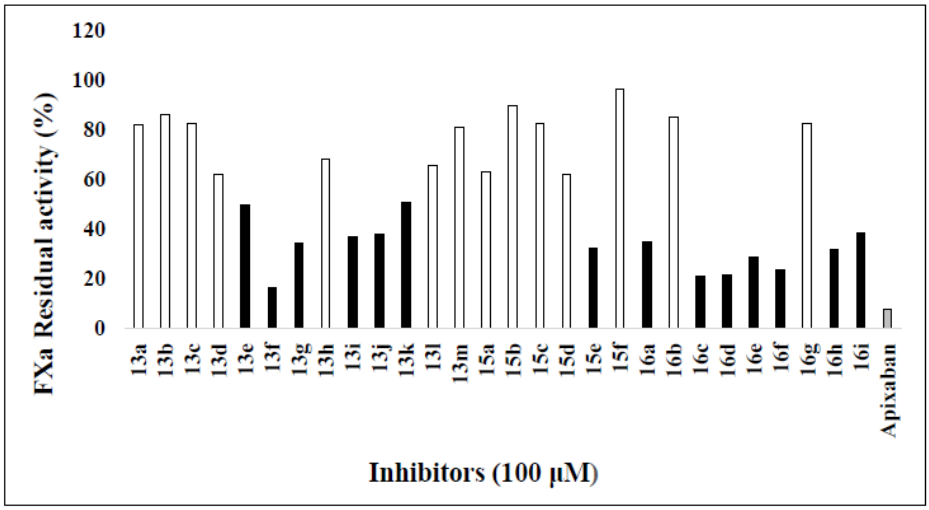


Figure 5. FXa residual activity (%) after treatment with the synthesized compounds. Experiments were performed at 100 µM in duplicate. Mean of % FXa residual activity values are represented (SE < 20 %).

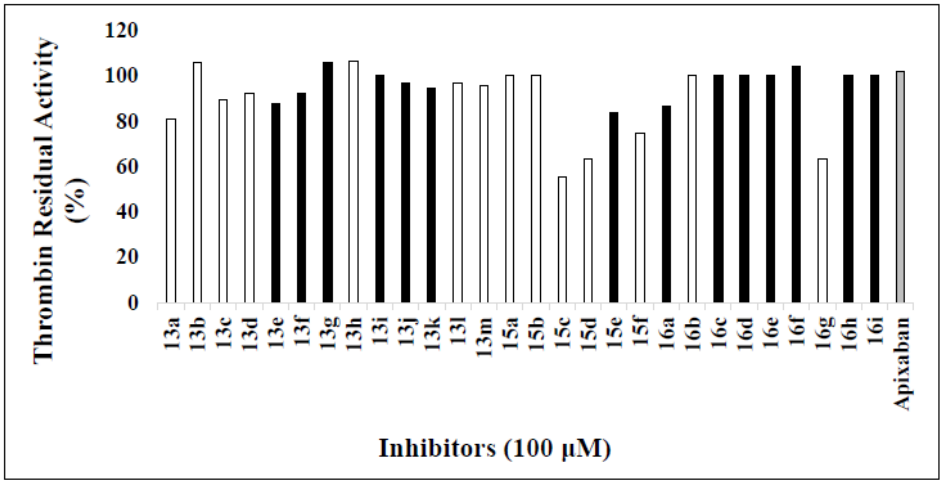


Figure 6. Thrombin residual activity (%) after treatment with the synthesized compounds. Experiments were performed at 100 µM in duplicate. Mean of % FXa residual activity values are represented (SE < 20 %).

Ex vivo PT prolongation and clotting time

Compounds showing significant inhibition of the enzyme in the preliminary screening were evaluated further using *ex vivo* measurements of prothrombin time and clotting time (for more details, refer supporting information). The PT prolonging activity and clotting time of compounds **13e-13g**, **13i-13k**, **15e** and **16a**, **16c-16f**, **16h** and **16i** along with control group and standard drug apixaban were determined at 2 h after oral administration in rat (30 mg/kg dose) and the data are shown in **Table 1**. Most of the tested compounds indicated slightly higher

prolongation in prothrombin time than that of control (7.7 sec). Compounds **16c** (9.8 sec), **16d** (9.9 sec), **16f** (10.1 sec) and **13j (**9.9 sec) showed significant change in the prothrombin time. The selected test compounds also showed much higher clotting time than the control (12.5 sec). Compound **16f** (45 sec) exhibited significant change in clotting time offering the highest value among the selected compound. However, this was lesser than the standard drug apixaban (60 sec).

***In vivo* $FeCl_3$ induced arterial thrombosis**

Based on *in vitro* FXa inhibitory activity and *ex vivo* PT prolongation time of compound **16f**, it was selected for *in vivo* evaluation of antithrombotic potential by $FeCl_3$ induced arterial thrombosis model in rats. The reduction in thrombus weight was considered as a preventive measure for *in vivo* efficacy of a compound. Compound **16f** reduced thrombus weight by 46 % at 30 mg/kg in rats. In the case of standard drug (apixaban) at dose of 30 mg/kg, the reduction in thrombus weight was found to be 69 %. (Figure 7)

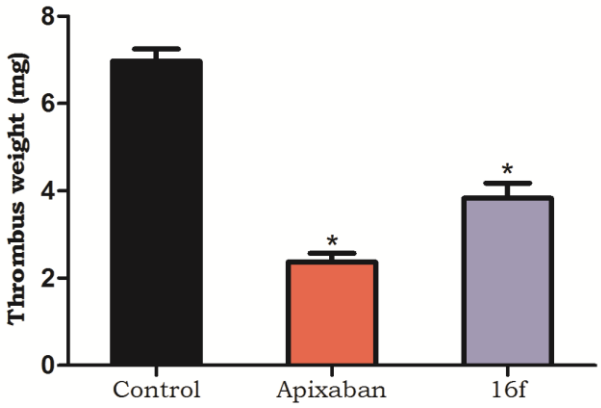


Figure 7. Effect of **16f** and apixaban (30 mg/kg) on thrombus weight ($FeCl_3$ induced arterial thrombosis model).

Table 1. IC_{50} values, PT time and Clotting time for the test compounds

Compound	IC_{50} value (μM)[a]		PT time (sec)[b]	Clotting time (sec)[b]
	FXa	Thrombin		
13e	23.7 ± 3.4	>100	9.2	15
13f	18.4 ± 2.6	>100	8.7	30
13g	11.5 ± 1.3	>100	8.9	30
13i	41.0 ± 3.2	>100	8.6	30
13j	48.3 ± 2.9	>100	9.9	30
13k	23.2 ± 8.4	>100	9.4	37.5
15e	57.5 ± 8.6	>100	9.4	30
16a	35.3 ± 2.3	>100	8.8	35
16c	5.4 ± 1.0	>100	9.8	40
16d	1.3 ± 0.8	>100	9.9	40
16e	12.5 ± 2.2	>100	9.1	35
16f	**0.7 ± 0.2**	**>100**	**10.1**	**45**
16h	30.0 ± 6.4	>100	8.7	30
16i	52.5 ± 5.7	>100	9.5	35
Apixaban	0.35 ± 0.1	>100	12.8	60
Control	-	-	7.7	12.5

[a] IC_{50} values shown are the mean of duplicate or triplicate measurements.

[b] The *ex vivo* PT time and Clotting time were determined 2 h after oral administration of the test compounds to rats at a dose of 30 mg/kg (n = 3).

Part-II: 1,3,4-Thiadiazole based FXa inhibitors

The multireceptor based virtual screening approach was employed to identify novel FXa inhibitors. After multi-stage virtual screening several hits were generated from Zinc library. Based on persistent high performance, novelty and synthetic feasibility, compound (A) was selected as the hit compound. To generate lead molecule (B), p-chlorophenyl was inroduced at S1 binding site of the enzyme as a neutral haloaromatic group, particularly the chlorinated ones have proven their worth as successful P1 motifs.

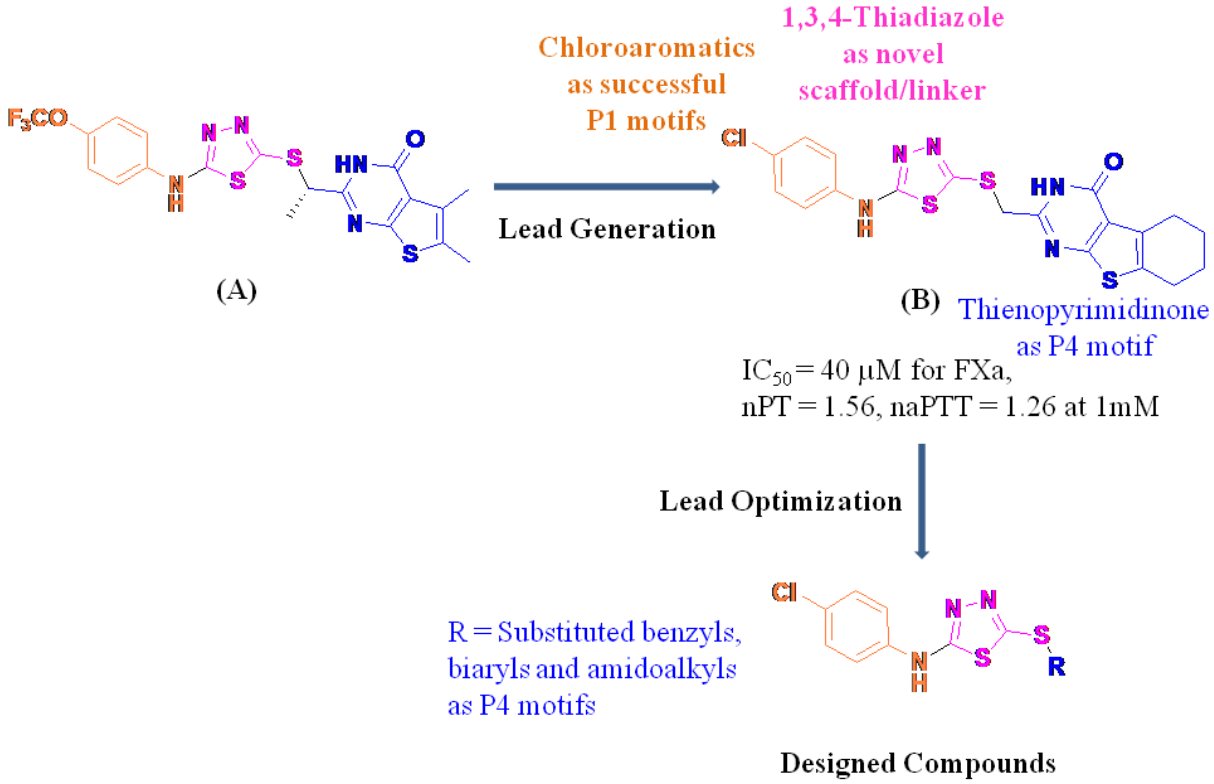


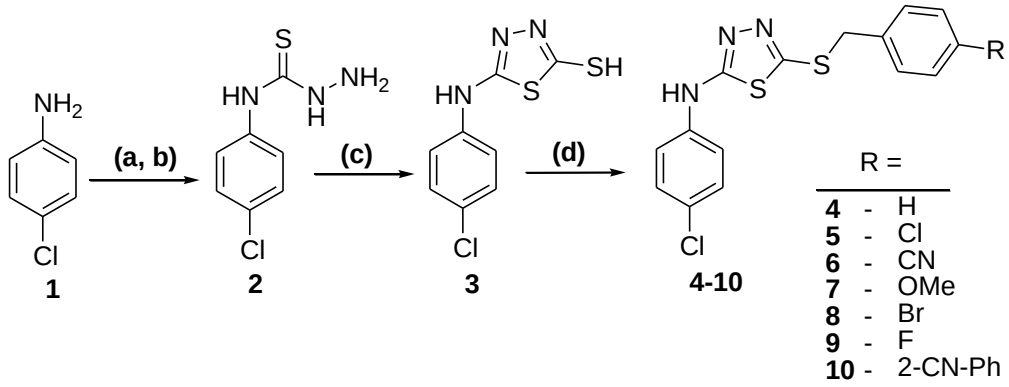
Figure 8. Designing of 1,3,4-thiadiazole derivatives as Factor Xa inhibitors

Considering the activity observed for the lead compound (B), it was contemplated to introduce substituted benzyls, biaryls or amidoalkyls as S4 binding ligands in novel 1,3,4-thiadiazole scaffold and maintain the chloroaromatic group as such as the S1 binding ligand as represented in Figure 8.

Chemistry

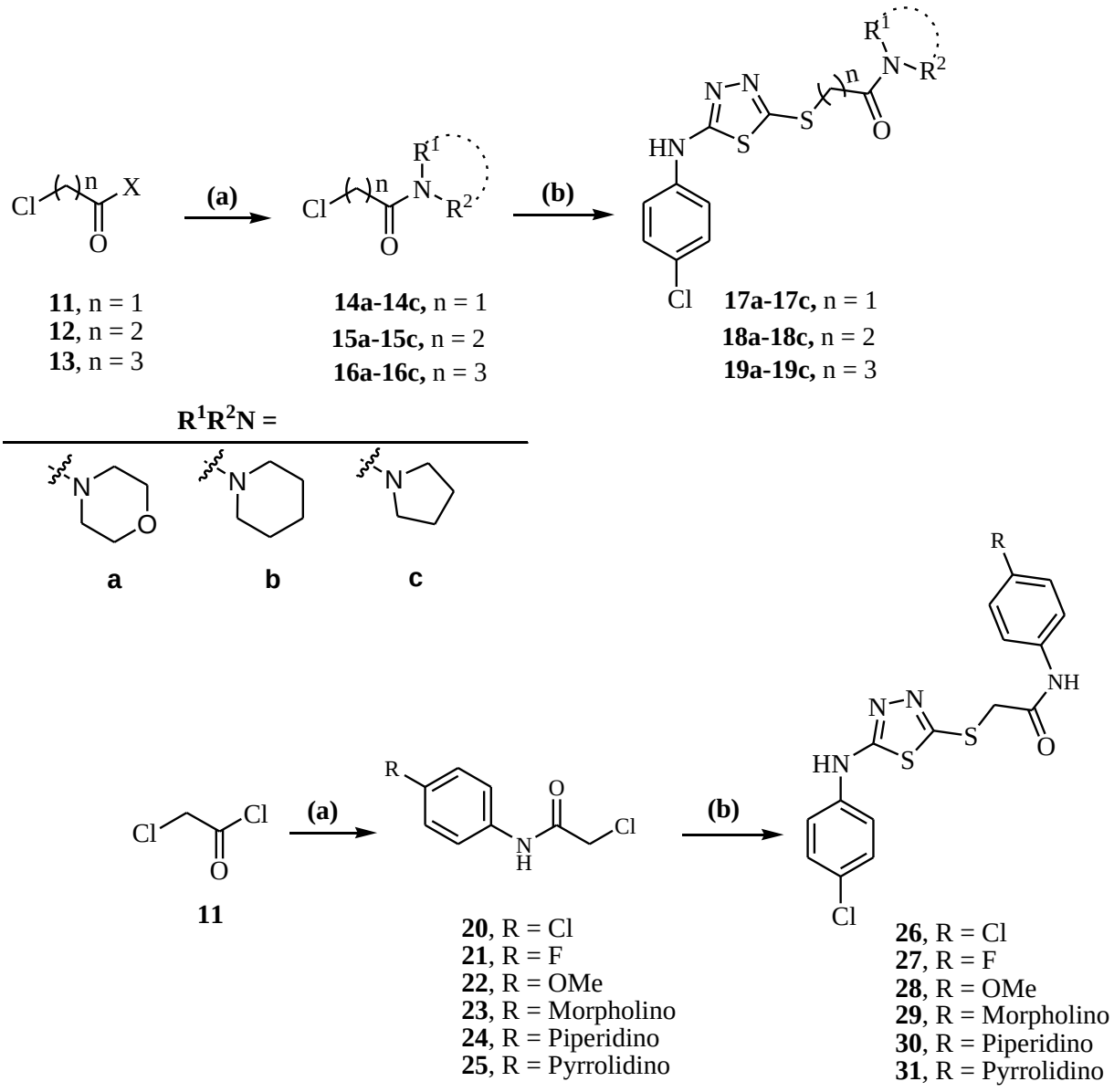
The designed 1,3,4-thiadiazole derivatives were synthesized by adopting Schemes **I**-**III**. The key intermediate **3** required for the synthesis of final compounds were prepared as per the reported procedure.[34] The intermediate **3** was further reacted with different benzyl halides in the presence of base in DMF to get the aspired compounds **4-10** (scheme **I**). The intermediates **14a-16c** and **20-25** were synthesized by reacting acid chloride with various primary and secondary amines. These intermediates **14a-16c** and **20-25** were reacted with compound (**3**) to get the desired 5-thiosubstituted thiadiazoles **17a-19c** and **26-31** (Scheme **II**).

Scheme I



SCHEME I: Reagents and Conditions: (a) Carbon disulphide, sodium hydroxide, DMF, 20-30 °C, 2-3 hrs; (b) Hydrazine hydrate,70 °C, 2-3 hrs; (c) Carbon disulphide, potassium hydroxide, methanol, 2M HCl, Reflux, 5-6 hrs. (d) Substituted benzyl halides, potassium carbonate, DMF, rt, 3-4hr.

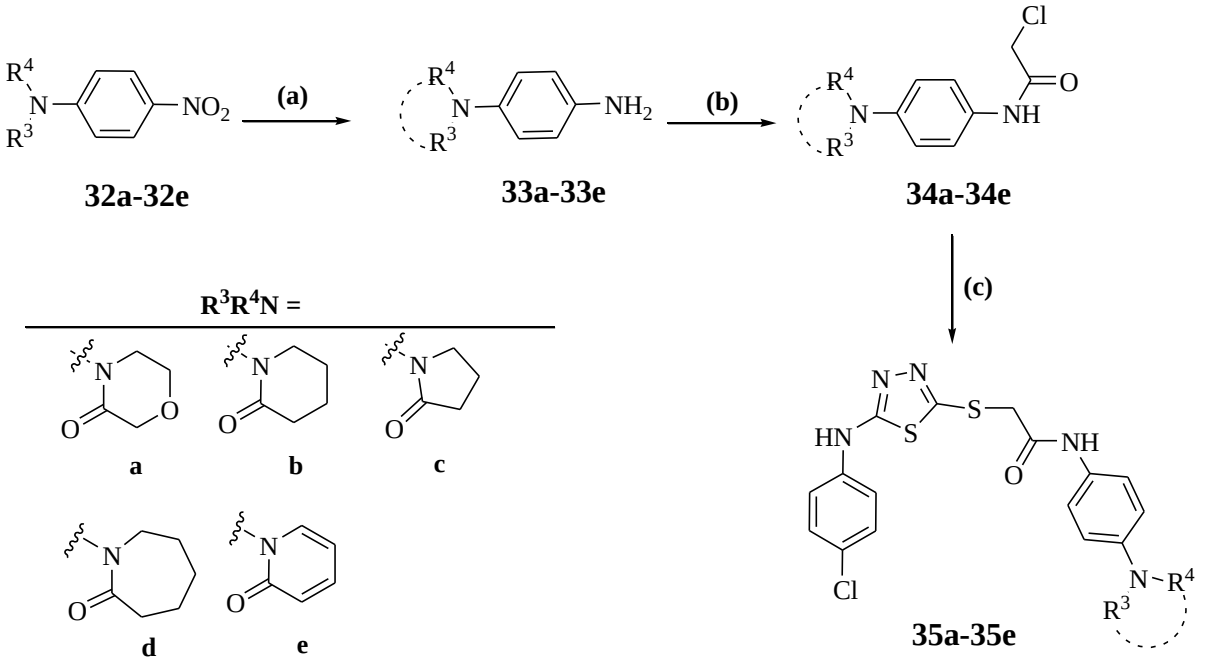
Scheme II



SCHEME II: Reagents and Conditions: (a) Substituted anilines/cyclic amines, anhydrous potassium carbonate, dry DCM, rt, 6-7 hrs; (b) Compound (**3**), potassium carbonate, DMF, rt, 3-4 hrs.

The synthetic routes for the preparation of compounds **35a-35e** are also illustrated in **Scheme III**. Commercially available secondary amides were used to obtain intermediates **32a-32e** as per the reported procedure.[35] Hydrogenation of compounds **32a-32e** was done using hydrazine hydrate (80 %) and Pd/C (10 %) in ethanol to get the aniline derivatives **33a-33e** which were further acylated to obtain **34a-34e**. These acylated intermediates **34a-34e** were used to get desired compounds **35a-35e (**Scheme **III).**

Scheme III



SCHEME II: Reagents and Conditions: (a) Hydrazine hydrate (80 %), Pd/C (10 %), EtOH, reflux 1.5 hrs; (b) Chloroacetyl chloride, anhydrous potassium carbonate, dry DCM, rt, 6-7 hrs; (b) Compound (**3**), potassium carbonate, DMF, rt, 3-4 hrs

All the synthesized compounds were characterized by IR, NMR and Mass spectroscopy and evaluated for their antithrombotic activity.

References:

[1] a) F. J. Vazquez, M. L. Posadas-Martinez, J. Vicens, F. Gonzalez Bernaldo de Quiros, D. H. Giunta, *Thromb J* **2013**, *11*, 16; b) G. E. Raskob, P. Angchaisuksiri, A. N. Blanco, H. Buller, A. Gallus, B. J. Hunt, E. M. Hylek, A. Kakkar, S. V. Konstantinides, M. McCumber, Y. Ozaki, A. Wendelboe, J. I. Weitz, *Arter Thromb Vasc Biol* **2014**, *34*, 2363-2371.

[2] https://www.isth.org (Accessed on 23rd November 2018)

[3] a) P. Alonso-Coello, S. Bellmunt, C. McGorrian, S. S. Anand, R. Guzman, M. H. Criqui, E. A. Akl, P. Olav Vandvik, M. G. Lansberg, G. H. Guyatt, F. A. Spencer, *Chest* **2012**, *141*, 669S-690S; b) J. Fareed, *Nat. Rev. Cardiol.* **2015**, *12*, 70-71; c) C. Kearon, E. A. Akl, A. J. Comerota, P. Prandoni, H. Bounameaux, S. Z. Goldhaber, M. E. Nelson, P. S. Wells, M. K. Gould, F. Dentali, M. Crowther, S. R. Kahn, *Chest* **2012**, *141*, 419S-494S; d) W. M. Lijfering, L. E. Flinterman, J. P. Vandenbroucke, F. R. Rosendaal, S. C. Cannegieter, in *Seminars in thrombosis and hemostasis, Vol. 37*, **2011**, p. 885; e) J. I. Weitz, J. W. Eikelboom, M. M. Samama, *Chest* **2012**, *141*, 120S-151S; f) X. Z. Yang, W. H. Yang, Y. G. Xu, X. J. Diao, G. W. He, G. Q. Gong, *Eur. J. Med. Chem.* **2012**, *57*, 21-28; g) J. J. You, D. E. Singer, P. A. Howard, D. A. Lane, M. H. Eckman, M. C. Fang, E. M. Hylek, S. Schulman, A. S. Go, M. Hughes, F. A. Spencer, W. J. Manning, J. L. Halperin, G. Y. H. Lip, *Chest* **2012**, *141*, 531S-575S.

[4] G. Lippi, M. Franchini, G. Targher, *Nat. Rev. Cardiol.* **2011**, *8*, 502-512.

[5] S. J. McRae, J. S. Ginsberg, *Circulation* **2004**, *110*, I-3-I-9.

[6] a) D. E. Becker, *Anesth. Prog.* **2013**, *60*, 72-80; b) J. L. Mega, T. Simon, *The Lancet*, *386*, 281-291; c) M. M. Shahpouri, S. Mousavi, F. Khorvash, S. M. Mousavi, T. Hoseini, *J. Res. Med. Sci.* **2012**, *17*, 396-401.

[7] P. D. Stein, D. Grandison, T. A. Hua, P. M. Slettehaugh, J. W. Henry, P. Turlapaty, R. Kerwin, *Postgrad. Med. J.* **1994**, *70 Suppl 1*, S72-83.

[8] S. A. Spinler, V. J. Willey, *Circulation* **2011**, *124*, 209-211.

[9] D. B. Blossom, A. J. Kallen, P. R. Patel, A. Elward, L. Robinson, G. Gao, R. Langer, K. M. Perkins, J. L. Jaeger, K. M. Kurkjian, M. Jones, S. F. Schillie, N. Shehab, D. Ketterer, G. Venkataraman, T. K. Kishimoto, Z. Shriver, A. W. McMahon, K. F. Austen, S.

Kozlowski, A. Srinivasan, G. Turabelidze, C. V. Gould, M. J. Arduino, R. Sasisekharan, *N. Engl. J. Med.* **2008**, *359*, 2674-2684.

[10] S. Krishnaswamy, K. G. Mann, M. E. Nesheim, *J. Biol. Chem.* **1986**, *261*, 8977-8984.

[11] T. Hara, A. Yokoyama, K. Tanabe, H. Ishihara, M. Iwamoto, *Thromb Haemost:* **1995**, *74*, 635-639.

[12] J. Graff, S. Harder, *Clinl Pharmacokinet* **2013**, *52*, 243-254.

[13] N. B. Norgard, J. J. DiNicolantonio, T. J. Topping, B. Wee, *Ther Adv Chronic Dis* **2012**, *3*, 123-136.

[14] E. Perzborn, S. Roehrig, A. Straub, D. Kubitza, F. Misselwitz, *Nature reviews. Drug discovery* **2011**, *10*, 61-75.

[15] M. R. Lassen, B. L. Davidson, A. Gallus, G. Pineo, J. Ansell, D. Deitchman, *J Thromb Haemost* **2007**, *5*, 2368-2375.

[16] G. Y. Lip, G. Agnelli, *Eur Heart J* **2014**, *35*, 1844-1855.

[17] A. T. Cohen, R. A. Harrington, C. M. Gibson, *N Engl J Med* **2016**, *375*, e50.

[18] Y. H. Mekaj, A. Y. Mekaj, S. B. Duci, E. I. Miftari, *Ther Clin Risk Manag* **2015**, *11*, 967-977.

[19] J. Lutz, K. Jurk, H. Schinzel, *Int J Nephrol Renovasc Dis* **2017**, *10*, 135-143.

[20] R. Trikha, P. R. Kowey, *Cardiology* **2017**, *136*, 115-124.

[21] a) D. Mendel, A. L. Marquart, S. Joseph, P. Waid, Y. K. Yee, A. L. Tebbe, A. M. Ratz, D. K. Herron, T. Goodson, J. J. Masters, J. B. Franciskovich, J. M. Tinsley, M. R. Wiley, L. C. Weir, J. A. Kyle, V. J. Klimkowski, G. F. Smith, R. D. Towner, L. L. Froelich, J. Buben, T. J. Craft, *Bioorg Med Chem Lett* **2007**, *17*, 4832-4836; b) J. Yang, G. Su, Y. Ren, Y. Chen, *Eur J Med Chem* **2015**, *101*, 41-51.

[22] a) L. Anselm, D. W. Banner, J. Benz, K. G. Zbinden, J. Himber, H. Hilpert, W. Huber, B. Kuhn, J. L. Mary, M. B. Otteneder, N. Panday, F. Ricklin, M. Stahl, S. Thomi, W. Haap, *Bioorg Med Chem Lett* **2010**, *20*, 5313-5319; b) R. J. Young, C. Adams, M. Blows, D. Brown, C. L. Burns-Kurtis, C. Chan, L. Chaudry, M. A. Convery, D. E. Davies, A. M. Exall, G. Foster, J. D. Harling, E. Hortense, S. Irvine, W. R. Irving, S. Jackson, S. Kleanthous, A. J. Pateman, A. N. Patikis, T. J. Roethka, S. Senger, G. J. Stelman, J. R. Toomey, R. I. West, C. Whittaker, P. Zhou, N. S. Watson, *Bioorg Med Chem Lett* **2011**, *21*, 1582-1587.

[23] a) M. J. Orwat, J. X. Qiao, K. He, A. R. Rendina, J. M. Luettgen, K. A. Rossi, B. Xin, R. M. Knabb, R. R. Wexler, P. Y. Lam, D. J. Pinto, *Bioorg Med Chem Lett* **2014**, *24*, 3341-3345; b) M. L. Quan, D. J. Pinto, K. A. Rossi, S. Sheriff, R. S. Alexander, E. Amparo, K. Kish, R. M. Knabb, J. M. Luettgen, P. Morin, A. Smallwood, F. J. Woerner, R. R. Wexler, *Bioorg Med Chem Lett* **2010**, *20*, 1373-1377.

[24] T. Xue, S. Ding, B. Guo, Y. Zhou, P. Sun, H. Wang, W. Chu, G. Gong, Y. Wang, X. Chen, Y. Yang, *J Med Chem* **2014**, *57*, 7770-7791.

[25] H. Matter, E. Defossa, U. Heinelt, P.-M. Blohm, D. Schneider, A. Müller, S. Herok, H. Schreuder, A. Liesum, V. Brachvogel, *J Med Chem* **2002**, *45*, 2749-2769.

[26] R. A. Al-Horani, A. Y. Mehta, U. R. Desai, *Eur J Med Chem* **2012**, *54*, 771-783.

[27] K. M. Amin, N. M. Abdel Gawad, D. E. Abdel Rahman, M. K. El Ashry, *Bioorg Chem* **2014**, *52*, 31-43.

[28] Y. Shi, S. P. O'Connor, D. Sitkoff, J. Zhang, M. Shi, S. N. Bisaha, Y. Wang, C. Li, Z. Ruan, R. M. Lawrence, H. E. Klei, K. Kish, E. C. Liu, S. M. Seiler, L. Schweizer, T. E. Steinbacher, W. A. Schumacher, J. A. Robl, J. E. Macor, K. S. Atwal, P. D. Stein, *Bioorg Med Chem Lett* **2011**, *21*, 7516-7521.

[29] J. T. Kohrt, K. J. Filipski, W. L. Cody, C. F. Bigge, F. La, K. Welch, T. Dahring, J. W. Bryant, D. Leonard, G. Bolton, L. Narasimhan, E. Zhang, J. T. Peterson, S. Haarer, V. Sahasrabudhe, N. Janiczek, S. Desiraju, M. Hena, C. Fiakpui, N. Saraswat, R. Sharma, S. Sun, S. N. Maiti, R. Leadley, J. J. Edmunds, *Bioorg Med Chem* **2006**, *14*, 4379-4392.

[30] N. R. Patel, D. V. Patel, P. R. Murumkar, M. R. Yadav, *Eur J Med Chem* **2016**, *121*, 671-698.

[31] D. J. Pinto, J. M. Smallheer, D. L. Cheney, R. M. Knabb, R. R. Wexler, *J Med Chem* **2010**, *53*, 6243-6274.

[32] a) A. M. D'Souza, N. Spiccia, J. Basutto, P. Jokisz, L. S. Wong, A. G. Meyer, A. B. Holmes, J. M. White, J. H. Ryan, *Organic letters* **2011**, *13*, 486-489; b) J. Fensholdt, J. Thorhauge, B. Nørremark, Google Patents, **2011**; c) G. E. Hardtmann, G. Koletar, O. R. Pfister, *J Heterocycl Chem* **1975**, *12*, 565-572; d) A. A. Wube, F. Bucar, C. Hochfellner, M. Blunder, R. Bauer, A. Hüfner, *Eur J Med Chem* **2011**, *46*, 2091-2101; e) W. Z. Zhang, N. Zhang, Y. Q. Sun, Y. W. Ding, X. B. Lu, *ACS Catalysis* **2017**, *7*, 8072-8076.

[33] a) M. de Candia, F. Liantonio, A. Carotti, R. De Cristofaro, C. Altomare, *J Med Chem* **2009**, *52*, 1018-1028; b) F. Fringuelli, R. Girotti, O. Piermatti, F. Pizzo, L. Vaccaro, *Org lett* **2006**, *8*, 5741-5744; c) L. Liu, Y. Zhang, Y. Wang, *J Org Chem* **2005**, *70*, 6122-6125; d) S. Pradhan, R. P. John, *New J Chem* **2015**, *39*, 5759-5766.

[34] A. Karaburun, U. Acar Çevik, D. Osmaniye, B. Sağlık, B. Kaya Çavuşoğlu, S. Levent, Y. Özkay, A. Koparal, M. Behçet, Z. Kaplancıklı, *Molecules* **2018**, *23*, 3129.

[35] J. Xing, L. Yang, H. Li, Q. Li, L. Zhao, X. Wang, Y. Zhang, M. Zhou, J. Zhou, H. Zhang, *Eur J Med Chem* **2015**, *95*, 388-399.