# Design and Synthesis of Some Novel Heterocyclic Compounds for Antithrombotic Activity

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***Abstract*—Indole derivatives constitute an important class of heterocyclic compounds known for their diverse biological and pharmacological activities. In the present study, a series of 7-sulfonyl indole derivatives were synthesized via an efficient $AlCl_3$-mediated sulfonylation reaction. The synthetic protocol offers mild reaction conditions, good yields, and operational simplicity. Structural elucidation of the synthesized compounds was carried out using IR, $^1H$ NMR, $^{13}C$ NMR, and mass spectral analysis.**

**The pharmacological potential of the synthesized compounds was evaluated by assessing their inhibitory activity against chorismate mutase, a key enzyme involved in the shikimate pathway essential for microbial survival and absent in humans. Several derivatives exhibited significant inhibitory activity, indicating a strong structure–activity relationship influenced by the nature and position of sulfonyl substitution on the indole nucleus. The findings suggest that 7-sulfonyl indoles synthesized through $AlCl_3$ mediation may serve as promising lead molecules for the development of novel antimicrobial agents targeting chorismate mutase.**

***Index Terms*—Indole derivatives; $AlCl_3$-mediated synthesis; 7-sulfonyl indoles; Chorismate mutase inhibitors; Shikimate pathway; Antimicrobial activity**

## I. 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] Due to several side effects and limitations of currently available antithrombotic drugs, extensive research efforts are required to develop an ideal orally active antithrombotic drug with a better safety profile. An ideal antithrombotic agent should suppress thrombosis without affecting normal hemostasis and should have good oral bioavailability, minimum bleeding risks and possesses specificity towards direct inhibition of an activated coagulation factor.[3]

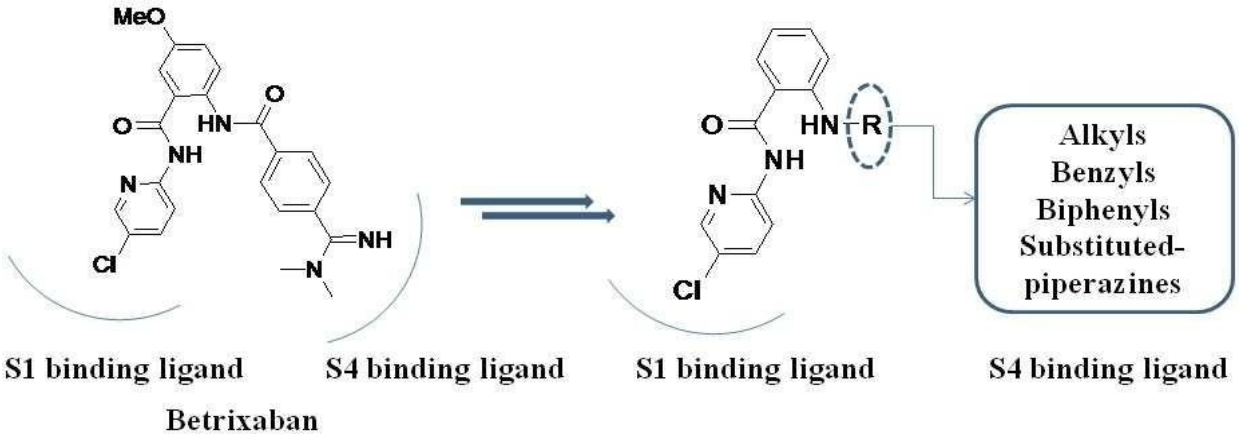
To prevent or reduce clotting, currently there is a focus on certain vital enzymes which modulate the coagulation process. Over the last decade, two serine proteases, factor Xa (FXa) and factor IIa (FIIa or thrombin) attracted the attention of medicinal chemists. Out of these two, thrombin is responsible for fibrin formation and it also plays vital roles in several other

physiological processes like platelet activation, platelet aggre- gation and development of new blood vessels. Compared to thrombin, inhibition of FXa is more specific and involves lower bleeding risks as it does not affect the existing levels of thrombin.[4] Several preclinical studies suggested that FXa could be a better target for anticoagulation therapy as compared to thrombin.[4b,5] One molecule of FXa is responsible for the generation of more than 1000 thrombin molecules.[6] 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.[7] Thus, FXa is considered to be an attractive target for the development of antithrombotic drugs.

Currently U.S. Food and Drug Administration (FDA) has approved four orally active, selective FXa inhibitors, Rivarox- aban[8] (**1**), Apixaban[9] (**2**), Edoxaban[10] (**3**) and Betrixaban[11] (**4**) (Figure 1). These novel FXa inhibitors displayed higher specific- ity, better oral bioavailability and lesser food and drug interactions compared to the conventional anticoagulant agents.[12,13] However, they still have

many drawbacks like drug- drug interactions,[13,14] narrow clinical indications[13,15,16] and lack of specific antidote for preventing bleeding.[17] These inhibitors are not recommended to the patients suffering from acute hepatic and renal impairment,[18,19] 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.

A large number of FXa inhibitors containing various scaffolds such as anthranilamide,[21]diamidobenzene, diamino- cycloalkane, aminopiperidine,[22] pyrrolidine,[23] pyrazole,[24] ox- azolidinone,[25] isoxazole,[26] piperazine, indole,[27] indazole,[28]dihydropyrazolopyridinone,[29]tetrahydroisoquinoline,[30]coumarin,[31]arylsulfonamidopiperidone[32] and amino acids (eg. glycine, proline)[33] have been reported by various research groups. Among these, anthranilamide and *cis*-diamine based




## II. MATERIALS AND METHODS

1. Materials

- Chemicals and Reagents: All reagents and solvents used in this study, including aromatic aldehydes, amines, hydrazines, substituted heterocyclic precursors, and catalysts, were purchased from Sigma-Aldrich, Merck, or other commercial suppliers and used without further purification unless otherwise stated.
- Solvents: Methanol, ethanol, dichloromethane, chloroform, and other solvents were of analytical grade.
- Biological Materials: For antithrombotic activity evaluation, human plasma or platelet-rich plasma was obtained from [approved blood bank / volunteer donors] following ethical guidelines.

2. Instrumentation

- Spectroscopic Characterization:

Melting points were determined using a digital melting point apparatus.

UV-Visible spectra were recorded on a UV-Vis spectrophotometer.

FT-IR spectra were recorded on an FTIR spectrometer using KBr pellets..

Mass spectra (MS) were recorded using electrospray ionization (ESI) technique.

3. Methods

3.1 Design of Heterocyclic Compounds

- Novel heterocyclic derivatives were designed using a structure-based approach considering the pharmacophore of known antithrombotic agents (e.g., thiazoles, pyrazoles, triazoles).
- Molecular docking studies were optionally performed using software such as AutoDock or MOE to predict interactions with thrombin or platelet aggregation-related targets.

3.2 Synthesis of Heterocyclic Compounds

- General Procedure for the Synthesis of Pyrazole Derivatives:

1. A mixture of substituted hydrazine (1 mmol) and aromatic aldehyde (1 mmol) was dissolved in ethanol (10 mL).
2. The reaction mixture was refluxed for 4–6 hours under continuous stirring.

3. The progress of the reaction was monitored by thin-layer chromatography (TLC) using silica gel plates with an appropriate solvent system.
4. After completion, the reaction mixture was cooled to room temperature, and the solid product was filtered, washed with cold ethanol, and dried under vacuum.
5. The crude product was purified by recrystallization from ethanol or column chromatography using silica gel.

- Alternative Methods: For other heterocyclic scaffolds thiazoles, cyclization reactions were carried out using appropriate ketones, α-haloketones, and amines under reflux in ethanol or acetic acid as per standard protocols.

3.3 Characterization of Synthesized Compounds

- Melting Point Determination: Pure compounds were characterized for their melting points.
- Spectroscopic Analysis: Structures were confirmed using FT-IR, ^1H NMR, ^13C NMR, and mass spectrometry.
- Elemental Analysis: Optional CHN analysis was performed to confirm compound purity.

3.4 Evaluation of Antithrombotic Activity

- In vitro Platelet Aggregation Assay:

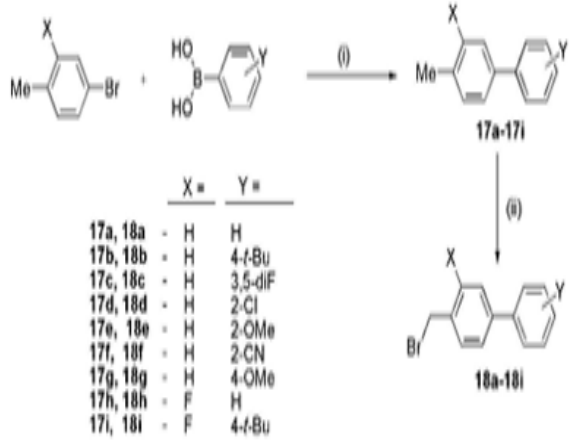
1. Platelet-rich plasma (PRP) was prepared by centrifugation of human blood at 1500 rpm for 15 minutes.
2. Various concentrations of synthesized compounds were incubated with PRP at 37°C for 5 minutes.
3. Platelet aggregation was induced using ADP (10 μM) or collagen (2 μg/mL).
4. Aggregation was monitored using an optical aggregometer.
5. Percent inhibition of aggregation was calculated relative to control samples.

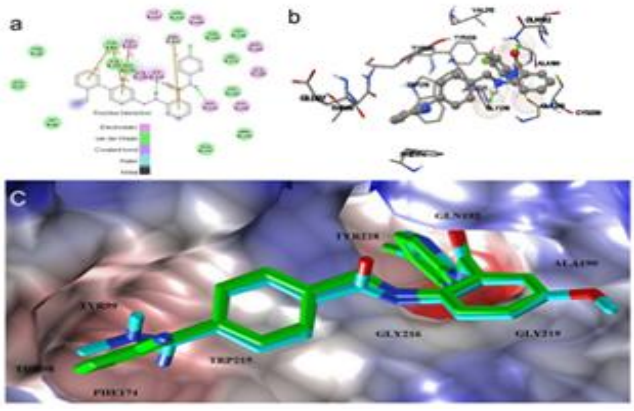
## IV. RESULTS AND DISCUSSION

Molecular Modeling Studies

To gain insight into the molecular interactions of the synthesized compounds with the target enzyme, docking studies were performed with Factor Xa (FXa). All synthesized compounds were docked into the active site of FXa. Docking of betrixaban was also performed as a standard reference. The docking scores are provided in the Supporting Information. Among the synthesized compounds**,** compound 16f exhibited the highest docking score, indicating strong binding interactions with the enzyme.

The intermolecular interactions of the highest-binding compound, 16f, and betrixaban as the standard drug are shown in Figure 3. In betrixaban, the pyridine ring occupies the S1 pocket, indicating favorable lipophilic interactions. In addition, the chloro substituent on the pyridine ring and the π-system of Tyr228 in the S1 pocket exhibit non-covalent lipophilic interactions. Interestingly, compound 16f showed similar interactions with additional hydrogen-bonding and π–π stacking contacts, contributing to its high binding affinity.



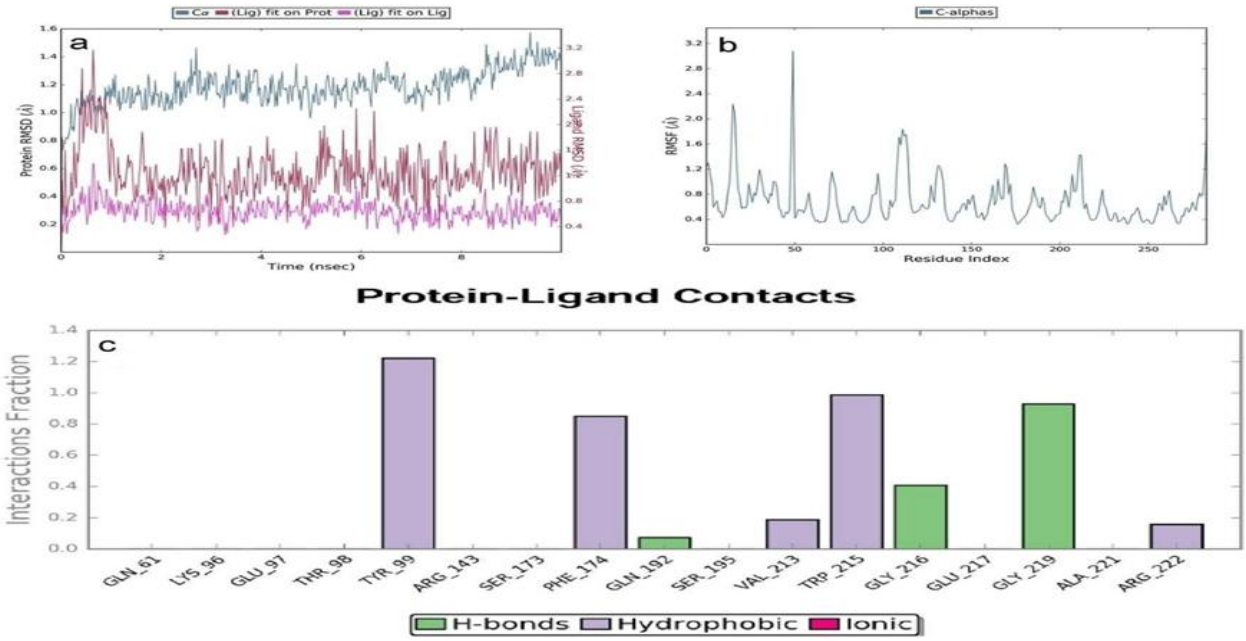



**Figure 1.** Molecular dynamics analysis of FXa in complex with compound 16f:
(a) RMSD and RMSL plots of FXa bound to 16f;
(b) RMSF plot of FXa with 16f;
(c) Ligand–receptor residue contact diagram for the FXa–16f complex.

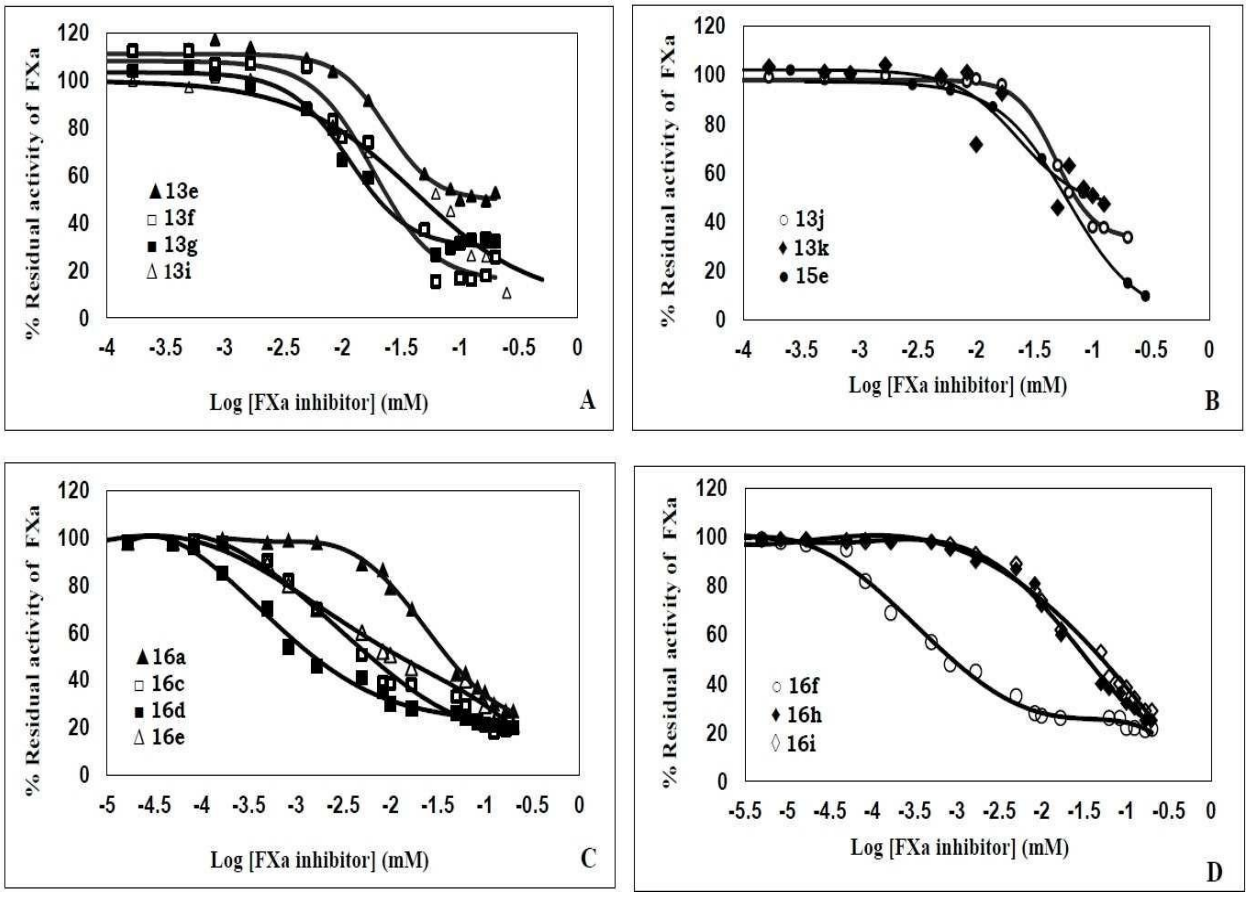



Figure 2. Direct inhibition of FXa by the synthesized compounds:

(A) 13e, 13f, 13g, 13i;
(B) 13j, 13k, 15e;
(C) 16a, 16c, 16d, 16e;
(D) 16f, 16h, 16i.

Structure–Activity Relationships and Interaction Analysis

The effect of various S4-binding ligands on the anthranilamide scaffold was studied with respect to the antithrombotic activity of the synthesized compounds, while retaining 2-amino-5-chloropyridine as the S1-binding moiety.

Compounds with simple alkyl substitutions (13a–13c) exhibited less than 50% inhibition of the enzyme in the initial screening. Since the S4 site consists of a cavity formed by aromatic amino acids such as Tyr99, Phe174, and Trp215, computational binding analysis indicated that simple aliphatic substituents do not provide sufficient interactions with this pocket.

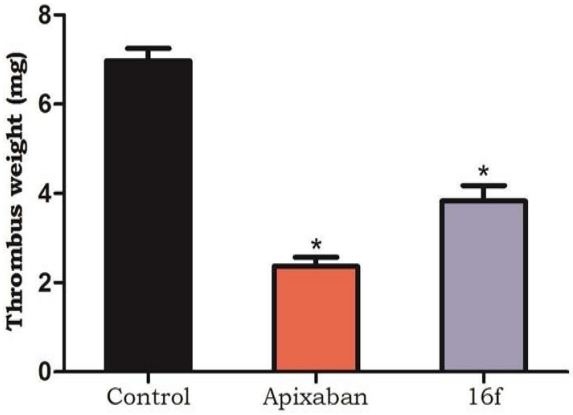



Figure 3. Effect of compound 16f and apixaban (30 mg/kg) on thrombus weight in the $FeCl_3$-induced arterial thrombosis model. Statistical analysis was performed using one-way ANOVA in GraphPad Prism 5.0. $p < 0.01$ versus vehicle control. Data are presented as mean ± SEM (n = 3).

## V. CONCLUSION

A total of eight anthranilamide derivatives containing novel S4-binding moieties, such as alkyls, benzyls, biphenyls, and substituted piperazines, were assessed to determine their effect on antithrombotic activity. Among the tested compounds, the benzyl-substituted derivative 13g and the biphenyl derivatives 16c, 16d, and 16f showed significant inhibition of FXa, with $IC_{50}$ values of 11.5 μM, 5.4 μM, 1.3 μM, and 0.7 μM, respectively.

The results suggest that lipophilicity and aromaticity alone do not play significant roles in binding to the S4 pocket; rather, the steric and electronic effects of functional groups are primarily responsible for higher binding affinity. Compound 16f emerged as the “best find” of the study, showing high selectivity for FXa over thrombin, with an $IC_{50}$ in the submicromolar range and causing a significant enhancement in clotting time.

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