Drug-Repurposing of Varespladib: Coagulation Activity Analysis Using In-Silico Pharmacokinetic, Pharmacological Network, and Molecular Docking Analysis

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Abstract. Pathological bleeding presents a significant clinical challenge due to limited effective anti-hemorrhagic therapies. Varespladib, originally developed as an anti-inflammatory agent has shown potential as a repurposed anti-hemorrhagic drug. Our study aimed to explore varespladib's coagulation-modulating potential using in silico pharmacokinetics, pharmacological network, and molecular docking analysis. ADME profiles were evaluated via SwissADME, and target prediction was conducted using SwissTargetPrediction, GeneCards, and OMIM databases. Overlapping targets between varespladib and hemorrhage-associated genes were identified and analyzed using STRING and Cytoscape, with core proteins prioritized via the MCC (Maximal Clique Centrality) algorithm. Enrichment analyses using KEGG and Reactome confirmed strong associations between varespladib with coagulation-related pathways. Biological activity predictions via PASS indicated high phospholipase A2 inhibitory potential (Pa>0.9), while molecular docking showed strong binding affinities to key coagulation proteins, particularly FGA (Fibrinogen Alpha Chain) (–8.2 kcal·mol^{-1}) and F2 (Coagulation Factor II) (–8.1 kcal·mol^{-1}). Overall, the results support the potential of varespladib as a repurposed therapeutic candidate for hemorrhagic conditions, with further in vivo studies and clinical trials needed to confirm its efficacy and safety.

Keywords: Drug Repurposing, Hemorrhage, Network Pharmacology, Molecular Docking, Varespladib.

1 Backgrounds

Pathological bleeding is a critical factor in many clinical conditions, with hemorrhage being one of the most severe forms. Hemorrhage is characterized by pathological condition that occurs when the amount of oxygen delivered to tissues is insuffient to support normal aerobic energy production [1]. Haemorrhage is identified as the cause of 25 % – 30 % of people died and more than 80 % in preventable postinjury deaths, either in the military and civilian environment. Death caused by haemorrhage is one of the acute tragedies, happening in the first 24 hours postinjury [2].

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Various conditions can lead to hemorrhage, including snake envenomation, which is often associated with extensive tissue damage and persistent local or systemic bleeding. Hemorrhage can progress to ischemia, resulting in acute kidney injury, multi-organ failure, and even death [3]. It is also known to be a rapidly progressing condition in vivo, with drastic structural damage occurring within minutes [4]. Despite its severity, effective, rapid, and accessible anti-hemorrhagic therapies remain limited, especially those targeting the specific molecular mechanisms of hemorrhage. One major contributor to hemorrhage in snake envenomation is snake venom phospholipase A2 (svPLA2), an enzyme that plays a key role in vascular damage and bleeding [5].

Originally developed as an anti-inflammatory drug for coronary artery disease, varespladib has also been preclinically tested as a repurposed therapeutic agent for snake envenomation, based on its ability to inhibit key venom components [6]. Numerous clinical studies have demonstrated its safety, pharmacokinetics (PK), and efficacy in pathological conditions unrelated to snake envenomation, involving approximately 4,600 human subjects across Phase 1, Phase 2, and Phase 3 clinical trials [6]. These findings suggest that varespladib may have potential effects on signaling pathways associated with hemorrhage. Varespladib (LY315920), or its methyl ester prodrug varespladib-methyl (LY333013), is a synthetic indole-substituted compound with a molecular weight of approximately 402.38 $g \cdot mol^{-1}$, formulated as a sodium salt of the pharmaceutically active substance [6]. A comprehensive understanding of the mechanisms by which varespladib acts in the context of hemorrhage requires a systematic and comprehensive investigative approach that integrates multiple in-silico methodologies. The methodologies consist of pharmacokinetic analysis, network pharmacology, and molecular docking analysis. Pharmacokinetic analysis aims to evaluate the drug-likeness and ADME profiles. Network pharmacology serves an effective and integrative method for mapping the interactions between drugs, targets, and biological pathways. By leveraging bioinformatics data, this approach enables the prediction of potential targets and signaling pathways modulated by varespladib under hemorrhagic conditions. To complement these analyses, molecular docking is conducted to simulate and quantify the binding interaction between varespladib and hemorrhagic-related proteins. Based on this background, the present study aims to repurpose varespladib as an anti-hemorrhagic agent using pharmacokinetic analysis, network pharmacology and molecular docking analysis, focusing on key targets involved in the pathophysiology of hemorrhage.

2 Methods

2.1 Computer Spesification

Computational analysis and prediction simulation was performed with Windows 11 operating system with 16,0 GB of RAM and AMD Ryzen 7 7730U with Radeon Graphics, 2000 Mhz, 8 Core (s), 16 Logical Processsors as a processor.

2.2 Drug-likeness

The drug-likeness of varespladib was evaluated using SwissADME v1.0 (http://www.swissadme.ch/). We analyzed its Absorption, Distribution, Metabolism, and Excretion (ADME) properties, as these are key characteristics of a potential drug candidate.

2.3 Data Collection

The targets of varespladib were collected from the SwissTargetPrediction database, Nucl. Acids Res. (2019) (http://www.swisstargetprediction.ch/). Meanwhile, hemorrhage-related targets were obtained from the GeneCards database 5.24 version (https://www.genecards.org/) and the OMIM database with its last updated is July, 2025 (https://www.omim.org/) using the keyword "haemorrhage."

2.4 Network Pharmacology and Protein-protein Interactions

The pharmacological network and protein-protein interactions are key components of this study, aimed at predicting the function of each protein target and the therapeutic potential of the drug candidate. In the first step, the targets of varespladib and hemorrhage-related targets were compared using VENNY v2.1 (https://bioinfogp.cnb.csic.es/tools/venny/). Subsequently, STRING v11.0 (https://stringdb.org/) was used to map the interactions among overlapping protein targets between varespladib and hemorrhage. Cytoscape v3.10.3 was utilized for network visualization, and the CytoHubba plug-in was employed to identify core proteins using the MCC (Maximal Clique Centrality) ranking algorithm.

2.5 Enrichment Analysis

Enrichment analysis was performed on the overlapping core proteins associated with hemorrhagic activity to evaluate their statistical significance by assessing the False Discovery Rate (FDR). In this analysis, the KEGG Pathway and Reactome Pathway databases were used as the basis for enrichment. ShinyGO v0.8 (https://bioinformatics.sdstate.edu/go/) was then used to visualize the gene set enrichment graphically.

2.6 Prediction of Activity Spectra for Substances Prediction

The secondary structure of varespladib (CID: 155815) was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The biological activity of varespladib was predicted using the Prediction of Activity Spectra for Substances (PASS) software (https://way2drug.com/passonline/predict.php) v2.0 © 2011–2025. The results were considered significant if the probability of activity (Pa) was greater than the probability of inactivity (Pi), and Pa was greater than 0.7 (Pa>0.7).

2.7 Molecular Docking

The core proteins identified from Cytoscape and the most significant interactions between varespladib and human proteins predicted by PASS were selected for docking studies. The conformation of the compound varespladib (PubChem CID: 155815) was downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The 3D structures of the proteins PLA2 (PDB ID: 3u8d), VWF (6n29), F2 (1bhx), FGA (1bbr), PROC (1aut), F10 (1ezq), and F5 (1czt) were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/) and saved in PDB (.pdb) format. Protein preparation was carried out using Chimera 1.17.3 to remove all native ligands (non-standard residues), and AutoDockTools 1.5.7 was used to remove water molecules, add hydrogen atoms and AD4 types, and calculate Gasteiger charges. Molecular docking was performed using AutoDock Vina via the PyRx 0.8 software. Protein binding sites were optimized for surface area coverage using a grid box. The data with the highest binding affinity and RMSD upper and lower values of 0.00 were presented in tabular form. The molecular docking results were further analyzed using PyMOL 3.1.3.1 and visualized with BIOVIA Discovery Studio v25.1.0.24284.

Table 1. Dimension of Docking in PyRx of Each Molecules.

Macromolecule	Dimension (Amstrong) (Å)		
	X	Y	Z
PLA2-Varespladib	44.4410	44.1574	55.0124
VWF-Varespladib	122.2524	120.1138	113.4081
F2-Varespladib	47.7073	47.7234	50.7027
FGA-Varespladib	100.3940	81.8977	92.4965
PROC-Varespladib	63.6280	74.3615	53.2832
F10-Varespladib	63.0513	48.9628	41.5762
F5-Varespladib	45.1056	37.8776	46.2790

Based on Table 1., presents the docking grid dimensions (in angstroms) used for molecular docking simulations in PyRx for each macromolecule complexed with varespladib. The dimensions along the X, Y, and Z axes vary depending on the size and structure of each macromolecule (Table 1).

3 Results

3.1 Drug Likeness from Swiss ADME Analysis

Table 2. Physicochemical and Pharmacokinetic Properties.

Property	Value
Formula	$C_{21}H_{20}N_2O_5$
Molecular Weight	380.39 $g \cdot mol^{-1}$
Number of H-bond Acceptors	5
Number of H-bond Donors	2
Topological Polar Surface Area (TPSA)	111.62 $Å^2$
WLOGP	2.38
Solubility	2.58×10^{-3} $mg \cdot mL^{-1}$; 6.79×10^{-6} $mol \cdot L^{-1}$
Lipinski's Rule	Yes; 0 violations
Ghose Rule	Yes
Veber Rule	Yes
Egan Rule	Yes
Muegge Rule	Yes
PAINS	0 Alert
Brenk	1 alert: diketo_group

Synthetic Accessibility	3.04
Blood-Brain Barrier (BBB) Permeant	No
Gastrointestinal (GI) Absorption	High
CYP1A2 Inhibitor	No
CYP2C19 Inhibitor	No
CYP2C9 Inhibitor	Yes
CYP2D6 Inhibitor	No
CYP3A4 Inhibitor	No

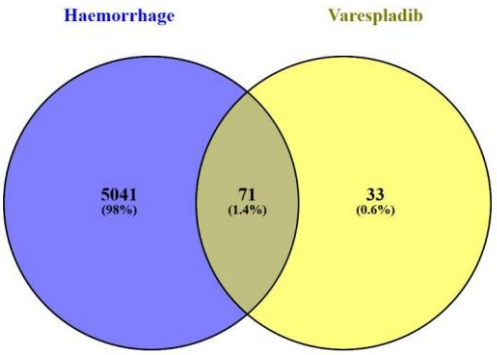


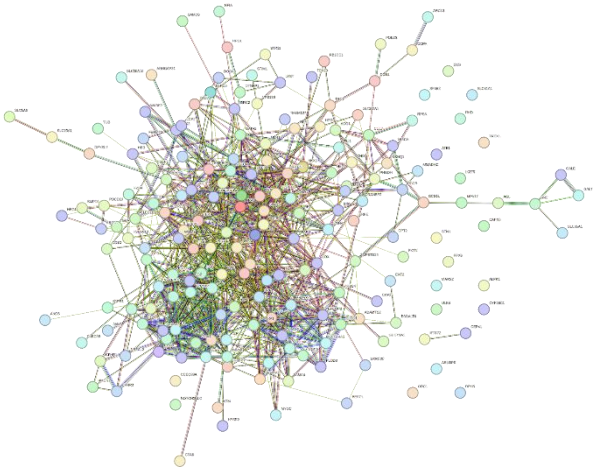
Fig. 1. Venn diagram showing the overlap between hemorrhage-related proteins and predicted targets of Varespladib.

Based on ADME analysis (Table 2.), varespladib exhibits a promising drug-like physicochemical profile, with high predicted absorption and an acceptable molecular size. The lack of blood-brain barrier (BBB) permeation is advantageous in minimizing potential central nervous system (CNS) side effects. However, inhibition of CYP2C9 should be carefully evaluated to assess possible adverse effects associated with this compound.

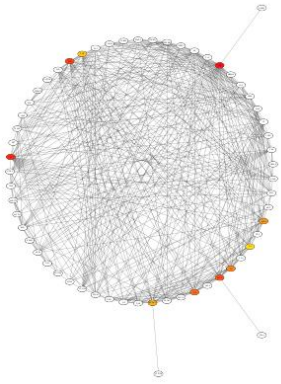
3.2 Data Collection

Among 5,112 proteins involved in hemorrhagic activity and 104 proteins potentially targeted by varespladib, VENNY analysis identified 71 shared target genes (1.4 %) between varespladib and hemorrhage-related pathways, indicating a potential mechanistic link (Fig. 1).

3.3 Protein-Protein Interactions



(A)



(B)

Fig. 2. Protein–protein interaction (PPI) analysis was visualized using STRING and Cytoscape. A total of 71 proteins were found to interact, forming a network comprising 192 nodes and 961 edges.

The upper image, generated using STRING (A), displays The PPI network, comprising 192 nodes and 961 edges, which is more than twice the 399 edges expected in a random network of similar size (Fig. 2). The lower image, visualized using Cytoscape (B**)**,

provides a topological layout of the same network, hihglighting key hub proteins. It is resulting in an average node degree of 10 and a local clustering coefficient of 0.474, combined with a PPI enrichment p-value $<1\times10^{-16}$, these metrics indicate a highly modular, non-random organization reflective of biologically significant interactions.

Based on the MCC (Maximal Clique Centrality) algorithm, the core proteins are identified, such as VWF (Von Willebrand Factor), F2 (Coagulation Factor II), FGA (Fibrinogen Alpha Chain), PROC (Protein C), F10 (Coagulation Factor X) and F5 (Coagulation Factor V), emphasizing their important roles in their network and potential participation linked to haemorrhage (Table 3).

Table 3. Top 10 Key Proteins in Network Analysis MCC Method based on PPI network.

Rank	Protein Name	MCC Score
1	VWF	2.59×10^{16}
2	F2	2.59×10^{16}
3	FGA	2.59×10^{16}
4	PROC	2.59×10^{16}
5	F10	2.59×10^{16}
6	F5	2.59×10^{16}
7	KNG1	2.51×10^{16}
8	SERPINC1	2.44×10^{16}
9	FGB	2.12×10^{16}
10	F11	1.74×10^{15}

3.4 Enrichment Analysis

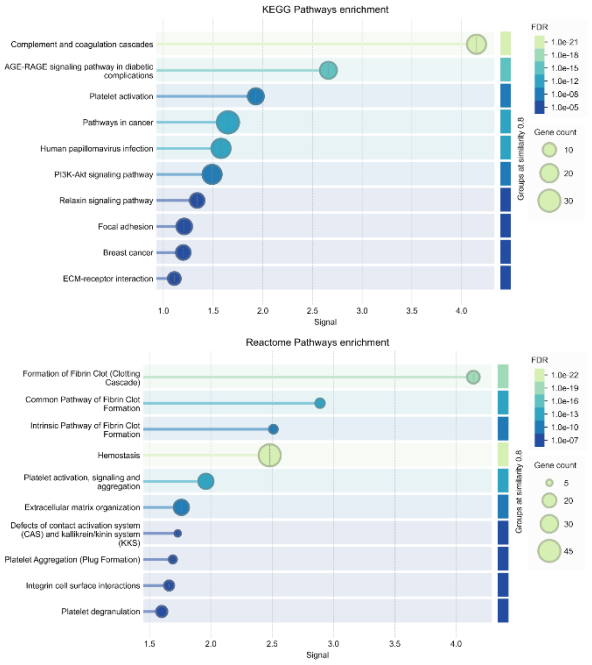


Fig. 3. Enrichment Analysis of Core Protein using KEGG Pathway and Reactome Pathway.

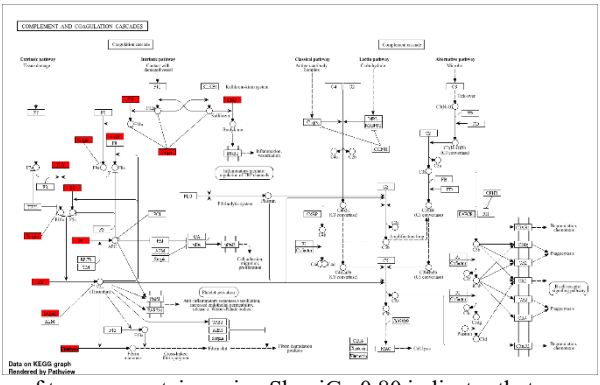


Fig. 4. Visualization of ten core proteins using ShyniGo 0.80 indicates that varespladib has multi activity in complement and coagulation cascades.

Varespladib-targeted proteins were significantly associated with coagulation-related pathways. KEGG revealed strongest association in complement and coagulation cascades (FDR: 1.0×10^{-21}), while Reactome pathway revealed with formation of fibrin clot (FDR: 1.0×10^{-19}). Mapped for the KEGG pathway and Reactome pathway, key proteins such as F2, F5, F10, FGA and VWF were clearly supporting their role in adjusting haemostatic balance and potentially reducing haemorrhagic activity (Fig. 3 and Fig. 4).

3.5 Prediction of Activity Spectra for Substances prediction (PASS)

Table 4. List of Prediction of Activity Spectra for Subtances prediction (PASS).

Pa	Pi	Activity
0.954	0.001	Phospholipase inhibitor
0.949	0.001	Phospholipase A2 inhibitor
0.844	0.001	Phospholipase A2 IIa inhibitor
0.813	0.001	1-Alkyl-2-acetylglycerophosphocholine esterase inhibitor
0.825	0.031	Membrane integrity agonist
0.785	0.001	Secretory phospholipase A2 inhibitor
0.721	0.047	Mucomembranous protector

PASS analysis of the varespladib compound shows high probabilities for phospholipase inhibition activities, particularly as a phospholipase A2 inhibitor, which is strongly associated with anti-inflammatory and anticoagulant effects. Additionally, other predicted activities include membrane integrity agonism and mucomembranous protection, suggesting possible cytoprotective effects (Table 4).

3.6 Molecular Docking Analysis

Table 5. Molecular docking between Varespladib and each macromolecules.

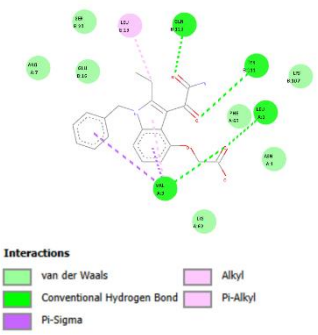
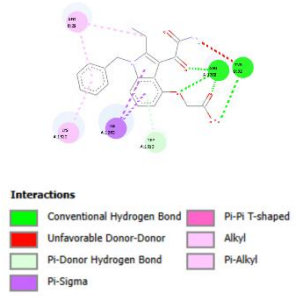
Macromolecule	Binding Affinity	RMSD/ub	RMSD/lb
PLA2-Varespladib	-6,9	0.0	0.0
	-6,5	6.668	3.814
	-6.4	20.1	16.58
	-6.4	13.775	10.529
	-6.4	21.198	18.417
	-6.4	12.365	9.31
	-6.3	6.552	3.672
	-6.2	10.425	6.859
	-6.2	16.095	12.644
VWF-Varespladib	-7.6	0.0	0.0
	-7.4	1.843	1.329
	-7.2	6.457	3.053
	-7.1	64.867	62.458
	-7.0	6.632	2.771
	-6.8	32.888	31.266
	-6.7	31.323	29.157
	-6.6	7.006	3.274
	-6.6	33.88	31.78
	-8.1	0.0	0.0
	-7.5	3.823	1.503

	-7.2	3.887	1.702
	-7.0	2.014	1.624
F2-Varespladib	-6.5	3.431	2.219
	-6.3	6.981	3.323
	-6.3	24.074	21.633
	-6.1	6.201	2.734
	-6.1	7.032	3.547
	-8.2	0.0	0.0
	-7.7	6.437	3.189
	-7.6	7.433	4.479
FGA-Varespladib	-7.6	4.386	2.196
	-7.3	6.218	2.936
	-7.2	8.226	5.424
	-7.2	6.963	3.521
	-7.1	7.826	4.584
	-7.1	7.436	4.71
PROC-Varespladib	-7.2	0.0	0.0
	-6.5	7.374	4.476
	-6.4	8.11	4.619
	-6.4	33.337	30.65
	-6.2	32.911	30.515
	-6.1	49.821	47.262
	-6.0	12.885	9.235
	-6.0	3.721	1.625
	-5.9	50.493	47.921
	-7.4	0.0	0.0
	-7.0	7.18	3.498
	-6.4	8.913	5.648
	-6.3	6.739	3.281
F10-Varespladib	-6.3	23.703	20.791
	-6.3	6.015	2.81
	-6.3	6.331	3.374
	-6.2	23.13	20.732
	-6.1	8.561	5.405
	-5.6	0.0	0.0
	-5.5	32.754	31.214
	-5.4	19.081	15.244
	-5.3	31.985	30.369
F5-Varespladib	-5.3	2.822	1.904
	-5.2	18.419	14.554
	-5.1	18.633	15.466
	-5.1	22.037	18.77
	-5.0	21.729	19.072

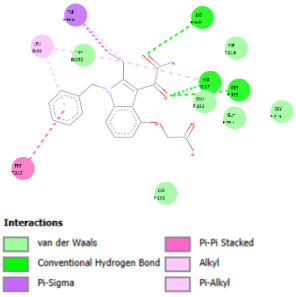
Table 5. displays the molecular docking results between varespladib and several molecules, including PLA2, VWF, F2, FGA, PROC, F10, and F5. The parameters presented include binding affinity (in kcal·mol^{-1}), and the root-mean-square deviation values for both upper bound (RMSD/ub) and lower bond (RMSD/lb), which indicate the reliability and accuracy of docking poses. The highest binding strength is -8.2 kcal/mol from FGA-Varespladib complex with RSMD values of 0.0 Å, suggesting a highly accurate pose at that energy level. Following this, the next highest binding

strength are observed in the complexes with F2 (–8.1 kcal·mol^{-1}), VWF (–7.6 kcal·mol^{-1}), F10 (–7.2 kcal·mol^{-1}), PROC (–6.9 kcal·mol^{-1}), PLA2 (–6.9 kcal·mol^{-1}), and F5 (–6.6 kcal·mol^{-1}).

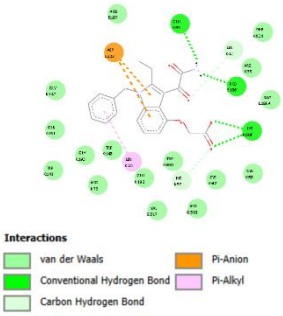
Table 6. Varespladib–Target Protein Interactions: Complexes & 2D Structures.

Complex of Ligand-Receptor	2D Structure
PLA2-Varespladib	
VWF-Varespladib	

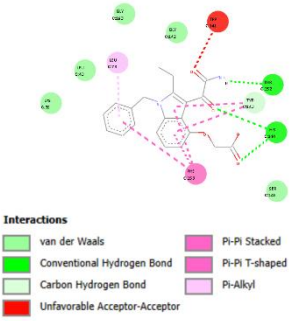
F2-Varespladib



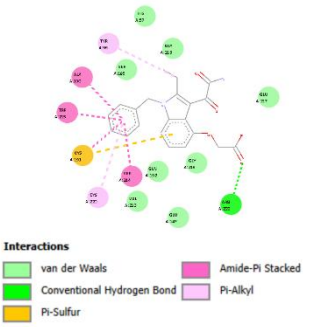
FGA-Varespladib



PROC-Varespladib



F10-Varespladib



F5-Varespladib

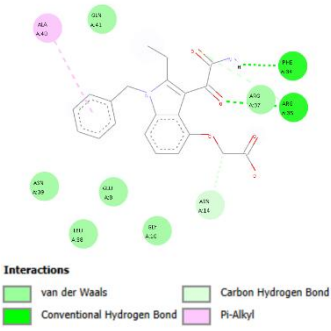


Table 6 depicts the interaction between varespladib and various target proteins (PLA2, VWF, F2, FGA, PROC, F10, and F5). It consists of the ligand-receptor complexes, the 3D structural visualization of each complex, and the 2D interaction diagrams showing the types of molecular interactions involved.

Table 7. Comparison of Amino Acid Residues in Complexes: Varespladib-PLA2, Varespladib-VWF, Varespladib-F2, Varespladib-FGA, Varespladib-PROC, Varespladib-F10, and Varespladib-F5.

Compound	*Binding Affinity* (kcal/mol)	Interaction with Amino Acids		
		Hydrogen Bonds	Van der Waals	Others

PLA2-Varespladib	-6.9	VAL3 LEU2 GLN110 TYR111	ARG7 GLU16 SER20 PHE63 LYS62 ASN1 LYS107	LEU19
VWF-Varespladib	-7.6	ASP864 HIS1114 THR866	ASP1076 PRO1079 VAL1075 SER868 TYR1080 SER981 GLN1113 HIS1109 VAL983 PRO1166 VAL1167 GLY880 PHE878 ALA865	LEU876 VAL1110
F2-Varespladib	-8.1	LYS60F HIS57 SER195	TRP60D CYS191 GLU192 SER214 GLY193 GLY216	TRP215 LEU99 TYR60A

FGA-Varespladib	-8.2	GLU39 PRO186 LYS6OF	GLY142 GLN151 TRP141 GLY193 ARG73 TRP148 GLU192 VAL317 TRP6OD ARG318 CYS42 CYS58 GLY186A ARG35 PHE6OH ARG187	ASP222 LEU4O HIS57 LEU41
PROC-Varespladib	-7.2	THR152 HIS144	SER145 LYS39 LEU40 GLY193 GLY142	LEU73 PHE153 TRP141 TYR143
F10-Varespladib	-7.4	ARG222	HIS57 GLY216 SER195 GLU217 GLY219 GLN192 VAL213 GLU147	TYR99 ALA190 TRP215 CYS191 CYS220 SER214
F5-Varespladib	-5.6	PHE34 ARG35	GLN41 ASN39 GLU8 LEU38 GLY10 ARG35	ASN14 ALA40

The interactions with amino acids in the complexes–Varespladib-PLA2, Varespladib-VWF, Varespladib-F2, Varespladib-FGA, Varespladib-PROC, Varespladib-F10, and Varespladib-F5–consists of hydrogen bonds, Van der Waals interactions, and other types of bonds such as alkyl and pi-alkyl interactions (Table 7).

4 Discussion

In this study, we analyze the potential of varespladib as a coagulant drug candidate using pharmacokinetic analysis, network pharmacology, and molecular docking

analysis. Varespladib physicochemical and pharmacokinetics characteristics suit as a haemorrhagic's drug since it has high solubility, low lipophilicity, and low possibility of side-effects to pass Blood Brain Barrier (BBB) and doesn't break any rules (Table 2). Complementing these findings (Table 2), the molecular weight of varespladib is 380.39 $g \cdot mol^{-1,}$ which falls within the acceptable range for oral drugs (<500 $g \cdot mol^{-1}$) as suggested by Lipinski's Rule of Five [7]. Notably, the compound demonstrates high solubility (Table 2) 2.58×10^{-3} $mg \cdot mL^{-1}$; 6.79×10^{-6} $mol \cdot L^{-1}$, which is a crucial parameter for effective absorption. According to current FDA biowaiver guidelines, a drug is classified as highly permeable if more than 90 % of its orally administered dose is absorbed into the bloodstream. Additionally, a drug is considered highly soluble when its highest dose strength can dissolve in 250 mL or less of aqueous solution across a pH range of 1.0 to 7.5 [8]. Furthermore, AlogP value below 1 is generally considered as low lipophilicity, which may affect membrane permeability and oral bioavailability [9]. Considering its ADME analysis which is high aqueous solubility, low lipophilicity, and favorable physicochemical profile, varespladib is suitable for oral administration. The inhibition activity for CYP2C9 needs to be considered since CYP2C9 is a responsible enzyme for metabolizing many common drugs, including warfarin as an anticoagulant[10]. This potency could lead to elevated plasma levels of co-administered CYP2C9 substrates and paradoxically increase bleeding risk rather than ameliorate it if the drug combination is false. In vitro and in vivo analysis of inhibition constants against CYP2C9 and drug-drug interactions need to be studied. Moreover, varespladib shows a Synthetic Accessibility (SA) score 3.04, indicating that it is relatively easy to synthesize using standard laboratory methods. This score suggests a favorable balance between molecular complexity and the presence of commonly used chemical fragments, making it a practical candidate for further drug development efforts [11].

From 71 shared-proteins targets between varespladib and haemorrhagic activity, the protein interaction comprises 192 nodes connected by 961 edges (Fig. 2). This network explains the high connection between each protein. The PPI enrichment p-value of less than 1.0×10^{-16} also provides strong statistical support that the observed connectivity is not a product of random sampling. The clustering coefficient is 0.747, indicating the strong interconnection between proteins of each in the cluster. The results of STRING analysis will reveal the specific biological processes that connect these proteins.

The enrichment analysis revealed that the "Complement and Coagulation Cascades" (KEGG) and "Formation of Fibrin Clot (Clotting Cascade" (Reactome) pathways were among the most significantly enriched, with extremely low FDR values of approximately 1.0e–21 and 1.0e–22 (Fig. 3), respectively. These pathways play central roles in blood coagulation by regulating the sequential activation of coagulation factors, culminating in the generation thrombin (F2) and the conversion of fibrinogen (FGA) into fibrin, which forms the structural framework of blood clots [12]. Varespladib demonstrated strong predicted binding affinities to both F2 and FGA, suggesting its potential to directly interfere with the terminal steps of clot formation. This is consistent with its mechanism of action as an inhibitor of phopholipase A2 (PLA2), which has also been implicated in modulating coagulation and vascular permeability during pathological bleeding (Table 4). These findings indicate that varespladib may exert anti-hemorrhagic effects not only through PLA2 inhibition but also by engaging directly with key proteins in the coagulation cascade.

The ten core protein was found using the MCC algorithm by CytoHubba (Table 3). The ten proteins are VWF (Von Willebrand Factor), F2 (Coagulation Factor II), FGA (Fibrinogen Alpha Chain), PROC (Protein C), F10 (Coagulation Factor X), F5 (Coagulation Factor V), KNG1 (Kininogen-1), SERPINC1 (Serpin Family C Number 1), FGB (Fibrinogen Beta Chain), and F11 (Coagulation factor XI). The results of mapping the KEGG and Reactome pathway enrichment, confirm the involvement of the ten core proteins in modulating haemostatic balance and possibly reducing haemorrhagic activities. For the examples, VWF protein facilitates platelet adhesion to sites of vascular injury and stabilizes coagulation factor VIII, making the clotting process start (13). F2 or commonly called as thrombin is a key enzyme that transforms fibrinogen to fibrin and forms the blood clots [13]. FGA, with FGB, are a component of fibrinogen, which is cleaved by thrombin to generate the fibrin matrix [14]. PROC is an anticoagulant, inactivating factors Va and VIIIa, thus regulating clot formation and preventing excessive coagulation [15].

In the context of blood coagulation, numerous proteins play critical roles in the regulation and execution of coagulation activity. One of the core proteins involved in this process is Factor X (F10), which is essential for the conversion of prothrombin into thrombin. The activation of Factor X, resulting in its active form F10a, occurs via two distinct pathways: the extrinsic pathway, which involves tissue factor (TF) and Factor VIIa, and the intrinsic pathway, which involves Factor VIIIa and Factor IXa [16]. During the coagulation process, F10 functions in concert with Factor V (F5) to form the prothrombinase complex, which catalyzes the generation of thrombin and facilitates the formation of a blood clot composed of fibrin and platelets. Factor V is a multifunctional protein that contributes to both the initiation and modulation of blood clotting, depending on the specific enzymes with which it interacts. This protein can be activated by F10a, thrombin, or activated protein C (aPC) [17][18]. Another important coagulation protein is Factor XI (F11), an inactive zymogen in the bloodstream that is converted into its active form, FXIa, during the coagulation cascade. Once activated, FXIa contributes to thrombin generation. Structurally and functionally, FXIa shares similarities with prekallikrein (PK), a component of the kallikrein-kinin system [19]. The plasma levels of F11 are regulated by the *KNG1* gene, which encodes high-molecular-weight kininogen (HK), a protein that plays a pivotal role in the intrinsic pathway of coagulation [20]. In contrast to the coagulation-promoting factors, the *SERPINC1* gene encodes antithrombin III, a glycoprotein that acts as a natural anticoagulant. Antithrombin III plays a critical regulatory role by inhibiting thrombin and Factor Xa, thereby preventing excessive clot formation [21]. In the final stages of the coagulation cascade and wound healing, pleiotropic proteins encoded by the γ fibrinogen-chain genes are essential. Among these, the *FGB* gene encodes the γ chain of fibrinogen. Mutations in *FGB* are associated with fibrinogen deficiencies, which can significantly impair the coagulation process [22] (Fig. 4). Furthermore, Using PASS, our study indicates varespladib plays a significant role in coagulation activity by inhibiting the PLA2-family activity (Table 4).

Molecular docking analysis revealed that varespladib exhibits binding affinity toward several proteins associated with hemorrhagic activity (Table 6 and Table 7). The proteins ranked 1st to 6th in the MCC analysis were selected because they had the highest MCC values, indicating the need for further molecular docking analysis. Among the six proteins tested, FGA (with key residues GLU39, PRO186, and LYS60F)

and F2 (with residues LYS60F, HIS57, and SER195) demonstrated the strongest binding affinities. FGA (Fibrinogen Alpha Chain) showed the lowest binding affinity value at –8.2 kcal·mol^{-1}, indicating the strongest predicted protein–ligand interaction. FGA is one of the three polypeptide chains that constitute fibrinogen and consists of 610 amino acid residues. This polypeptide chain gradually assembles into Bβ-γ and Aα-γ intermediates, which in turn form the hexameric glycoprotein fibrinogen. Indirectly, FGA plays a critical role in providing structural integrity to blood clots during the wound healing process by working in conjunction with the β and γ chains of the fibrinogen complex [23]. F2 (Coagulation Factor II) exhibited the second strongest binding affinity with varespladib (–8.1 kcal.mol^{-1}). F2 plays a crucial role in the vascular injury response, in which prothrombin is activated into thrombin by the prothrombinase complex, comprising Factor Xa, Factor Va, calcium ions, and phospholipids [24]. Thrombin subsequently executes several essential functions, including the conversion of fibrinogen to fibrin, activation of platelets, and enhancement of endothelial permeability. These processes are central to coagulation and represent critical targets for anticoagulant therapy [25]. Another key parameter in molecular docking analysis is the evaluation of hydrogen bonds. Hydrogen bonding is a significant non-covalent interaction that contributes to the stability of biomolecular complexes. It occurs between hydrogen atoms carrying a partial positive charge and electronegative atoms such as oxygen, nitrogen, or fluorine [26]. The PLA2–varespladib complex formed the highest number of hydrogen bonds (VAL3, LEU2, GLN110, TYR111); however, it exhibited a weaker binding affinity, suggesting that the number of hydrogen bonds alone does not necessarily predict binding strength. Both the F2–varespladib and FGA–varespladib complexes (with residues LYS60F, HIS57, SER195 and GLU39, PRO186, LYS60F, respectively) demonstrated an equal number of hydrogen bonds. Nonetheless, the FGA–varespladib complex showed the most favorable binding affinity (–8.2 kcal·mol^{-1}), indicating that FGA may represent the most promising target within the coagulation pathway.

In this study, varespladib showed stronger binding affinity toward F2 and FGA, compared to other coagulation protein such as F5, F10, and PROC. This difference can be expolained by the structural roles of these proteins in the coagulation cascade. F2 acts as a proteolytic enzyme that directly converts fibrinogen into fibrin [25]. On the other hand, FGA is one of the key fibrinogen subunits cleaved during clot formation [23]. These proteins provide accessible and well-defined active sites, which are structurally favorable for small-molecule binding. In contrast, F5, F10, and PROC function mainly as cofactors or regulatory components within large multi-subunit complexes such as the prothrombinase complex [17][18]. Their binding pockets may be buried or structurally less accessible, which could reduce varespladib's ability to interact effectively. Morover, since varespladib was originally designed as phospholipase A2 (PLA2) inhibitor, it is more likely to engage enzymatic or substrate-based targets rather than non-enzymatic cofactors [6]. This specifity supports its preferential interaction with catalytic proteins like F2, rather than structural regulators like F5 or PROC. Based on the strong binding affinity of varespladib toward F2 and FGA (Table 7) suggests its potential to interfere with the final step of coagulation, potentially reducing fibrin formation and clot stability. This depending on the biological context and dosage. Conversely, the lower binding affinity of varespladib to uppstream factors such as F10 and F5 (Table 7) indicates that it does not significantly disrupt the

early activation steps of thrombin formation [27]. This selectivity may be advantageous in avoiding broad suppression of the coagulation system, but it also suggests that varespladib may have limited impact if upstream inhibition is required. Following the analysis of binding affinity, the accuracy of the predicted binding poses was further validated using RMSD values (Table 5). As detailed in Table 5, all target proteins exhibited RMSD values of zero at their lowest binding energy, for both the lower bound (RMSD/lb) and upper bound (RMSD/ub) measurements. RMSD serves as an indicator of the reliability of the predicted ligand-protein binding poses. RMSD/lb denotes the minimum deviation in the predicted atomic positions of the ligand, while RMSD/ub measures the atomic distance variation between two conformations. In general, an RMSD values below 2 Å is considered acceptable, indicating a reliable prediction, whereas higher RMSD values suggest less accurate dosking results [28]. However, further studies are necessary to elucidate the specific interactions involved and to evaluate the stability of the protein–ligand complex under physiological conditions.

Although varespladib was not originally developed as an anticoagulant, its ability to bind directly to thrombin (F2) and fibrinogen alpha chain (FGA) indicates potential anticoagulant-like effects. This pattern of interaction resembles dabigatran, a clinically used direct thrombin inhibitor that targets F2 to prevent fibrin formation [29]. In contrast, other anticoagulants such as heparin and warfarin act directly-heparin enhances antithrombin III to inactivate F2 and F10a [30]. Compared to these agents, varespladib shows selective affinity toward F2 and FGA, suggesting it may interfere specifically with the final steps of the coagulation cascade where fibrin clots are formed.

This study is limited by its reliance solely on in silico methods, without experimental validation through in vitro or in vivo models. While network pharmacology and molecular docking provide valuable predictions regarding potential targets and binding interactions, these findings must be interpreted with caution. The absence of pharacodynamic assessment and toxicity profiling further limits the translational significance of the results. Moreover, molecular docking simulations do not account for biological complexity, such as dynamic protein conformations or systemic physiological responses, which may influence the actual drug-target interaction in vivo. Therefore, further experimental studies are essential to validate the efficacy and safety of varespladib as an anti-hemorrhagic agent.

5 Conclusion

Our studies conclude that varespladib exhibits a potential activity as an anti-haemorrhagic candidate due to the interaction between varespladib and coagulation factors, such as FGA and F2. Network pharmacology, enrichment analysis, and molecular docking suggest that varespladib may modulate coagulation pathways. However, the CYP2C9 inhibition activity needs to be evaluated. In vivo studies and clinical trials need to be studied to confirm its therapeutic potential and safety.

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References

1. Convertino VA, Koons NJ, Suresh MR. Physiology of Human Hemorrhage and Compensation. Compr Physiol [Internet]. 2021 Apr [cited 2025 Jul 18];11(2):1531–74. Available from: https://onlinelibrary.wiley.com/doi/10.1002/j.2040-4603.2021.tb00162.x
2. Sauaia A, Moore EE, Wade CE, Holcomb JB. Epidemiology of Hemorrhage-Related Mortality. In: Moore HB, Neal MD, Moore EE, editors. Trauma Induced Coagulopathy [Internet]. Cham: Springer International Publishing; 2021 [cited 2025 May 24]. p. 13–27. Available from: https://link.springer.com/10.1007/978-3-030-53606-0_2
3. Gutiérrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, Warrell DA. Snakebite envenoming. Nat Rev Dis Primer [Internet]. 2017 Sep 14 [cited 2025 May 18];3(1):17063. Available from: https://www.nature.com/articles/nrdp201763
4. Gutiérrez J, Escalante T, Rucavado A, Herrera C. Hemorrhage Caused by Snake Venom Metalloproteinases: A Journey of Discovery and Understanding. Toxins [Internet]. 2016 Mar 26 [cited 2025 May 18];8(4):93. Available from: https://www.mdpi.com/2072-6651/8/4/93
5. Castro-Amorim J, Novo De Oliveira A, Da Silva SL, Soares AM, Mukherjee AK, Ramos MJ, et al. Catalytically Active Snake Venom PLA2 Enzymes: An Overview of Its Elusive Mechanisms of Reaction: Miniperspective. J Med Chem [Internet]. 2023 Apr 27 [cited 2025 May 18];66(8):5364–76. Available from: https://pubs.acs.org/doi/10.1021/acs.jmedchem.3c00097
6. Lewin MR, Carter RW, Matteo IA, Samuel SP, Rao S, Fry BG, et al. Varespladib in the Treatment of Snakebite Envenoming: Development History and Preclinical Evidence Supporting Advancement to Clinical Trials in Patients Bitten by Venomous Snakes. Toxins [Internet]. 2022 Nov 11 [cited 2025 May 18];14(11):783. Available from: https://www.mdpi.com/2072-6651/14/11/783
7. Ibrahim ZY, Uzairu A, Shallangwa GA, Abechi SE. Pharmacokinetic predictions and docking studies of substituted aryl amine-based triazolopyrimidine designed inhibitors of Plasmodium falciparum dihydroorotate dehydrogenase (PfDHODH). Future J Pharm Sci [Internet]. 2021 Dec [cited 2025 Jul 18];7(1). Available from: https://fjps.springeropen.com/articles/10.1186/s43094-021-00288-2
8. Yazdanian M, Briggs K, Jankovsky C, Hawi A. The "High Solubility" Definition of the Current FDA Guidance on Biopharmaceutical Classification System May Be Too Strict for Acidic Drugs. Pharm Res [Internet]. 2004 Feb [cited 2025 Jul 18];21(2):293–9. Available from: http://link.springer.com/10.1023/B:PHAM.0000016242.48642.71
9. Shaikh MissTT, Shaikh MissTT, Adnaik DrRS, Patil MissNB. Swiss ADME Predictions of Pharmacokinetics and Drug-Likeness Properties of Chemical Constituents Present in Embelia Ribes. Int J Pharm Pharm Res [Internet]. 2025 [cited 2025 Jul 18];31(1):68–76. Available from: https://ijppr.humanjournals.com/wp-content/uploads/2025/01/10.Miss_.-Tasmeem-T.-Shaikh-Dr.-Rahul-S.-Adnaik-Miss.-Neha-B.-Patil.pdf
10. Ramamoorthy A, Sissung T, Pacanowski M. Clinical pharmacogenetics. In: Atkinson's Principles of Clinical Pharmacology [Internet]. Elsevier; 2022 [cited 2025 May 24]. p. 189–212. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128198698000161

11. Ertl P, Schuffenhauer A. Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions. J Cheminformatics [Internet]. 2009 Dec [cited 2025 Jul 18];1(1). Available from: https://jcheminf.biomedcentral.com/articles/10.1186/1758-2946-1-8
12. Heurich M, McCluskey G. Complement and coagulation crosstalk – Factor H in the spotlight. Immunobiology [Internet]. 2023 Nov [cited 2025 Jul 18];228(6):152707. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0171298523045096
13. Naito K, Fujikawa K. Activation of human blood coagulation factor XI independent of factor XII. J Biol Chem [Internet]. 1991 Apr [cited 2025 May 24];266(12):7353–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925820894538
14. Suh TT, Holmbäck K, Jensen NJ, Daugherty CC, Small K, Simon DI, et al. Resolution of spontaneous bleeding events but failure of pregnancy in fibrinogen-deficient mice. Genes Dev [Internet]. 1995 Aug 15 [cited 2025 May 24];9(16):2020–33. Available from: http://genesdev.cshlp.org/lookup/doi/10.1101/gad.9.16.2020
15. Kovács KB, Pataki I, Bárdos H, Fekete A, Pfliegler G, Haramura G, et al. Molecular characterization of p.Asp77Gly and the novel p.Ala163Val and p.Ala163Glu mutations causing protein C deficiency. Thromb Res [Internet]. 2015 Apr [cited 2025 May 24];135(4):718–26. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0049384815000298
16. Hertzberg M. Biochemistry of factor X. Blood Rev [Internet]. 1994 Mar [cited 2025 May 23];8(1):56–62. Available from: https://linkinghub.elsevier.com/retrieve/pii/0268960X94900078
17. Smith SA, Travers RJ, Morrissey JH. How it all starts: Initiation of the clotting cascade. Crit Rev Biochem Mol Biol [Internet]. 2015 Jul 4 [cited 2025 May 24];50(4):326–36. Available from: http://www.tandfonline.com/doi/full/10.3109/10409238.2015.1050550
18. Dahlbäck B. Pro- and anticoagulant properties of factor V in pathogenesis of thrombosis and bleeding disorders. Int J Lab Hematol [Internet]. 2016 May [cited 2025 May 24];38(S1):4–11. Available from: https://onlinelibrary.wiley.com/doi/10.1111/ijlh.12508
19. Mohammed BM, Matafonov A, Ivanov I, Sun M fu, Cheng Q, Dickeson SK, et al. An update on factor XI structure and function. Thromb Res [Internet]. 2018 Jan [cited 2025 May 24];161:94–105. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0049384817305248
20. Lv X, Li P, Gui Y, Qin C, Huang S, Qi Y, et al. KNG1 mutations (c.618 T > G and c.1165C > T) cause disruption of the Cys206-Cys218 disulfide bond and truncation of the D5 domain leading to hereditary high molecular weight kininogen deficiency. Clin Biochem [Internet]. 2025 Mar [cited 2025 May 24];136:110877. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0009912025000062
21. Riju Aikkal. SERPINC1: Structure, Function, and Therapeutic Implications. 2025 [cited 2025 May 23]; Available from: https://rgdoi.net/10.13140/RG.2.2.13801.40801
22. Paraboschi E, Duga S, Asselta R. Fibrinogen as a Pleiotropic Protein Causing Human Diseases: The Mutational Burden of Aα, Bβ, and γ Chains. Int J Mol Sci [Internet]. 2017 Dec 14 [cited 2025 May 24];18(12):2711. Available from: https://www.mdpi.com/1422-0067/18/12/2711
23. Wolberg AS. Fibrinogen and fibrin: synthesis, structure, and function in health and disease. J Thromb Haemost [Internet]. 2023 Nov [cited 2025 May 24];21(11):3005–15. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1538783623006426
24. Krishnaswamy S. The transition of prothrombin to thrombin. J Thromb Haemost [Internet]. 2013 Jun [cited 2025 May 24];11:265–76. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1538783622051273

25. Chinnaraj M, Planer W, Pozzi N. Structure of Coagulation Factor II: Molecular Mechanism of Thrombin Generation and Development of Next-Generation Anticoagulants. Front Med [Internet]. 2018 Oct 2 [cited 2025 May 24];5:281. Available from: https://www.frontiersin.org/article/10.3389/fmed.2018.00281/full
26. Frimayanti N, Nasution MR, Etavianti E. Molecular docking and molecular dynamic simulation of 1,5-benzothiazepine chalcone derivative compounds as potential inhibitors for Zika virus helicase. J Ris Kim [Internet]. 2021 Apr 3 [cited 2025 May 24];12(1):44–52. Available from: http://jrk.fmipa.unand.ac.id/index.php/jrk/article/view/365
27. Xie C, Albulescu LO, Still KBM, Slagboom J, Zhao Y, Jiang Z, et al. Varespladib Inhibits the Phospholipase A2 and Coagulopathic Activities of Venom Components from Hemotoxic Snakes. Biomedicines [Internet]. 2020 Jun 17 [cited 2025 Jul 18];8(6):165. Available from: https://www.mdpi.com/2227-9059/8/6/165
28. Shamsian S, Sokouti B, Dastmalchi S. Benchmarking different docking protocols for predicting the binding poses of ligands complexed with cyclooxygenase enzymes and screening chemical libraries. BioImpacts [Internet]. 2023 Sep 12 [cited 2025 Jul 19];14:29955. Available from: https://bi.tbzmed.ac.ir/Article/bi-29955
29. Sanda T, Yoshimura M, Hyodo K, Ishii H, Yamashita T. Effects of Long-term Thrombin Inhibition (Dabigatran Etexilate) on Spontaneous Thrombolytic Activity during the Progression of Atherosclerosis in ApoE−/−–LDLR−/− Double-Knockout Mice. Korean Circ J [Internet]. 2020 [cited 2025 Jul 18];50(9):804. Available from: https://e-kcj.org/DOIx.php?id=10.4070/kcj.2020.0055
30. Qiu M, Huang S, Luo C, Wu Z, Liang B, Huang H, et al. Pharmacological and clinical application of heparin progress: An essential drug for modern medicine. Biomed Pharmacother [Internet]. 2021 Jul [cited 2025 Jul 18];139:111561. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0753332221003462