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MODELING, ADME SCREENING & QSAR STUDIES ON FACTOR-XA INHIBITORS

SIBI NARAYANAN[1] AND *D. VELMURUGAN[1,2]

[1]Centre of Advanced Study in Crystallography and Biophysics, University of Madras, Maraimalai Campus (Guindy), Chennai, Tamil Nadu, India.
[2]Bioinformatics Infrastructure Facility, University of Madras,Maraimalai Campus (Guindy), Chennai, Tamil Nadu, India.

ABSTRACT

Thromboembolic disease is caused by improper functioning of the blood coagulation process. The serine protease Factor Xa plays a central role in the coagulation cascade. FXa inhibitors have been identified to maintain a patient's blood level within the therapeutic range and also shown to activate clotting over a much wider concentration range than thrombin in *invitro* assays. Predicting the binding structure of substrate in its receptor (docking simulation) is an effective way, which is used successfully in many applications. Novel and orally active FXa inhibitors incorporating 6-chloro-N-[(3S)-1-substituted-2-oxo-3-pyrrolidinyl]-2-napthalenesulfonamides presented here exhibit good anticoagulant and antithrombotic properties. An ADME (Absorption, Digestion, Metabolism and Excretion) screening was done for second-generation potent drug development. Further, QSAR (Quantity Structure Activity Relationship) activity was also tested using Schrödinger Suite 2009. *Insilico* analysis proved that compound 1F can be used as a lead compound for the anti-coagulant and anti-inflammatory pathways.

KEYWORDS: Factor XA; Glide; Absorption, Digestion, Metabolism and Excretion; Induced Fit Docking; Quantity Structure Activity Relationship; Schrödinger Suite 2009.

D. VELMURUGAN
Centre of Advanced Study in Crystallography and Biophysics, Bioinformatics Infrastructure Facility, University of Madras, Maraimalai Campus (Guindy), Chennai, Tamil Nadu, India.

***Corresponding author**

INTRODUCTION

For the prevention and treatment of ischemic-stroke with Atrial Fibrillation (AF) and recurrent myocardial infarction in patients with acute coronary syndrome, such as unstable angina or non-ST-elevated myocardial infarction, anti-coagulants are being highly recommended[1]. Existing anti-coagulants are very effective, but their use has been limited owing to parenteral administration and subsequent administration or requirement for frequent monitoring. In view of this, there is an urgent need for novel oral agents with a predictable anticoagulant action[2,3,4]. Inhibition of any one enzyme in the coagulation cascade will help in reducing the formation of fibrin polymers from fibrinogen, decreasing the clot formation[5]. Serine proteases are enzymes that cut peptide bonds in proteins in which serine is one of the amino acids at the active site. Found in both single-cell and complex organisms, they function in blood clotting, immune system and inflammation. Factor Xa (FXa) is one such trypsin-like serine protease which exhibits a preference for basic groups to bind in its primary (S1) specificity pocket. FXa has emerged as an attractive target for novel anticoagulants due to its key position in the coagulation cascade and its limited roles outside of coagulation[6,7]. By combining with Factor Va and calcium ions on membrane surfaces, FXa plays a central role in the coagulation cascade by forming the prothrombinase complex, which activates prothrombin to thrombin. To convert fibrinogen into fibrin leading to clot formation is the key action of thrombin[8,9]. At each level of the coagulation cascade, the amount of an activated coagulation factor generated from its inactive precursor increases. One molecule of FXa catalyzes the formation of ~1000 thrombin molecules; it is logical that instead of targeting the downstream thrombin, FXa could be a more effective strategy for anticoagulation[10]. It is easier to maintain a patient's blood level of FXa inhibitor within the therapeutic range, as it has been shown to activate clotting over a much wider concentration range than thrombin in model systems and *in vitro* assays[11]. FXa is essential for both the intrinsic and extrinsic pathways of the coagulation process, and is thought to be a better target of antithrombotic drugs; as many thrombin inhibitors have been shown to increase the risk of abnormal bleeding. It is being secreted into the blood as the zymogen form of the serine protease and is converted to an active form, FXa, by the Factor VIIa / tissue factor complex or by the Factor IXa / Factor VIIIa complex[12,13]. In order to find leads for therapeutic targets, three-dimensional structural information about receptors is one of the modern approaches. Predicting the binding structure of substrate in its receptor (docking simulation) is an effective way, which is used successfully in many applications[14]. Identifying the correct conformation of ligands in the binding pocket of a protein and predicting the affinity between the ligand and the receptor protein are the basic aims in docking procedure. In three-dimensional space, how the receptor protein and the ligand fits well, describes the process well[15]. One of the main goals in drug discovery is the identification of innovative small molecular scaffolds exhibiting high binding affinity and selectivity for the target together with favorable ADME (Absorption, Distribution, Metabolism and Excretion) and QSAR (Quantitative Structure Activity Relationship) studies. All over the world, for drug development, computational modeling has been investigated as a tool for optimized selection of the most suitable candidates[16,17].

Antithrombotics such as aspirin, heparin and warfarin are being used clinically, but these drugs suffer from limited efficacy, low oral bioavailability or a narrow therapeutic window. Therefore the development of antithrombotics is a major focus of pharmaceutical research[18]. Chan *et al.,* (2007) have designed and synthesized a series of novel sulfonamides with different P1 groups that can inhibit Factor Xa. Highly selective and potent thrombin and FXa inhibitors with good anticoagulant properties have been identified and show promise for chronic oral administration. FXa inhibitors having the 3-aminopyrrolidin-2-one scaffold and benzamidine P1 substituents were identified earlier. Nonbasic series of novel and orally active FXa inhibitors incorporating 6-chloro-N-[(3S)-1-substituted-2-oxo-3-pyrrolidinyl]-2-napthalenesulfonamides with this scaffold presented here, exhibit good anticoagulant

and antithrombotic properties[19]. Docking studies of these 25 compounds were carried out using Schrödinger Suite 2009, with FXa as target. Sulfonamide and their analogues were screened for their ADME properties (53 descriptors) using Qikprop module. Using these descriptors as initial properties and with the help of a number of factors, all compounds were tested for their QSAR activity (Strike). The compounds which exhibited good QSAR score along with stable ADME property were introduced into the prestigious Induced Fit Docking.

MATERIALS AND METHODS

The crystal structure of the Human FXa has been determined earlier in complex with GSK (PDB ID: 2CJI)[20]. Protein-ligand complex structure was downloaded from the Protein Data Bank. Firstly, formal charges and bond orders in the ligand, cofactors and nonstandard residues were corrected to start the automated preparation and refinement portions of the protein. X-ray structure analysis cannot distinguish between O and NH_2 groups, therefore terminal amide groups will also be misaligned. Glide helps in properly assigning the required bond orders and ionization states using an all-atom force field. The final structure will be relieved of steric clashes. The entire procedure was being performed using the Protein Preparation Wizard panel of the Suite. Twenty five compounds were taken for our study from the literature. Energy minimization task had to be carried out in order to obtain the three dimensional structure of the molecule, which is free of steric clashes. Three types of algorithms were used to obtain the perfect 3-D structure. *Truncated Newton (TN),* is a very efficient method for producing optimized structures. Here, a short conjugate gradient pre-minimization stage is performed. Later, the *Steepest descent* method, which is a method for initiating a minimization on a starting geometry that contains large steric clashes is followed. Here, the convergence is very poor towards the end of minimization and the *Conjugate gradient* method, which is a very good optimization method, is now followed to improve the convergence of the algorithm. Qikprop is an easy-to-use, accurate and quick module for predicting the physically significant descriptors and pharmaceutically relevant properties of an organic molecule. It provides ranges for comparing a particular molecule's properties with those of 95% of known drugs and flags 30 types of reactive functional groups that may cause false positives in high-throughput screening assays. An evaluation of the acceptability of the inhibitors based on the Lipinski's rule of 5, which is essential for rational drug design, was also carried out. A QSAR hypothesis was run to assess the validity and predictive power of generated QSAR/QSPR models using statistical methods. Such models are later employed as filters and predictive tools, which help in performing similarity analysis in molecular property or 2-dimensional structural space. This could be done using the Strike module, a chemically-aware statistical package which helps in generating basic univariate and bivariate statistics. In standard docking studies, ligands are docked into the active site of the receptor, where the receptor is held rigid and the ligand is free to move. In reality, many proteins undergo side-chain or back-bone movements upon ligand binding, which can give misleading results. Induced fit docking is a method where these changes are allowed in the receptor to alter its binding site so that it closely conforms to the shape and binding mode of the ligand. Amino acid residues which exhibit within 3.5 Å distance of the co-crystal ligand and which also helps in binding or maintain hydrogen bonded interactions with the co-crystal ligand are known as binding site residues. This protocol helps in generating multiple poses of the protein-ligand complex, which includes unique structural modifications of the receptor to fit the ligand and rank the poses based on the GlideScore. Thus it helps in finding the best structure of the docked complex. Using Glide protein preparation, constrained minimization of the receptor is carried out with an RMSD cutoff of 0.18Å. Initial Glide docking was carried out; wherein by default a maximum of 20 conformations per ligand are retained. One round of Prime side-chain prediction and minimization for each complex having residues of default 5 Å of any ligand pose is followed. Finally, Glide re-docking of each complex structure within the default specified energy of the lowest energy structure and

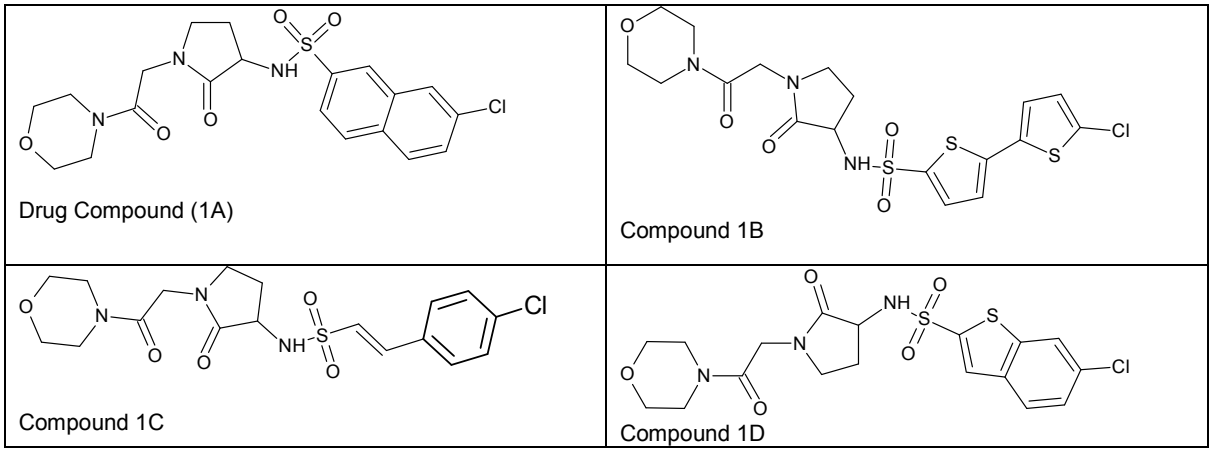
estimation of the binding energy is carried out. All computational work was performed using various modules of Schrödinger Suite 2009 on a Red Hat Enterprise Linux 5.0 interface running on Pentium D workstation.

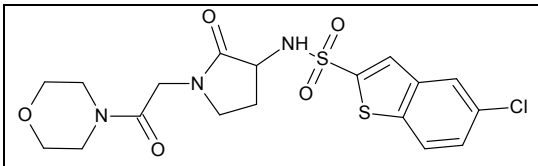
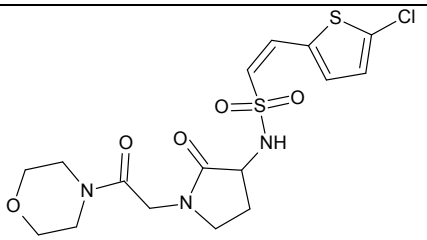
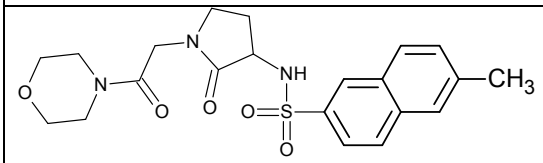
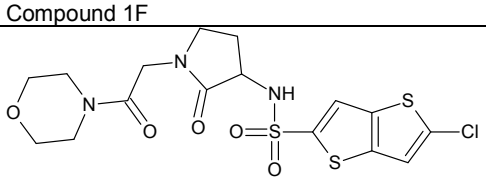
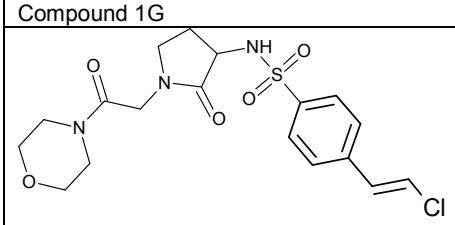
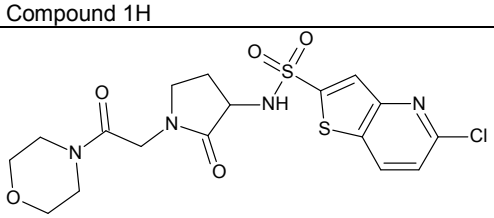
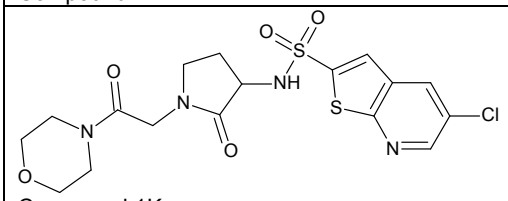
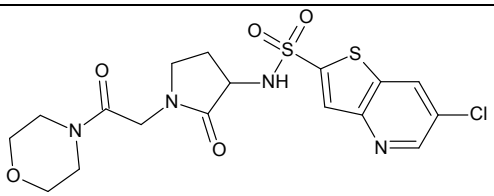
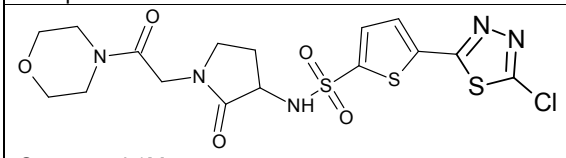
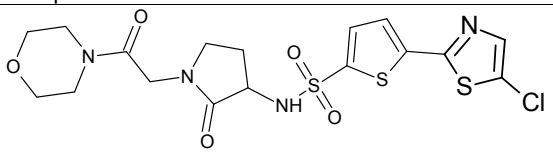
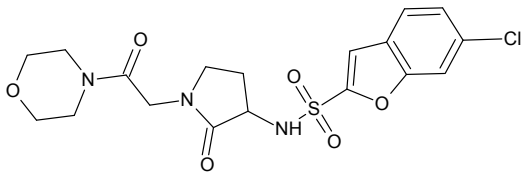
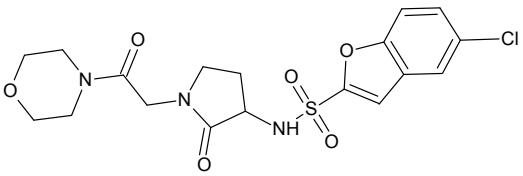
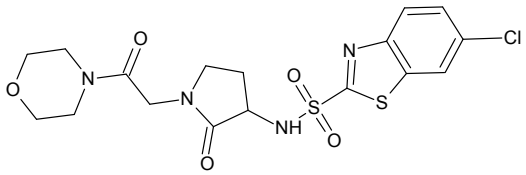
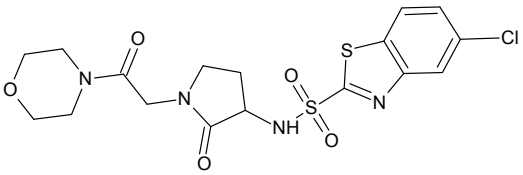
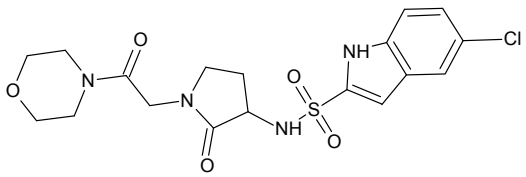
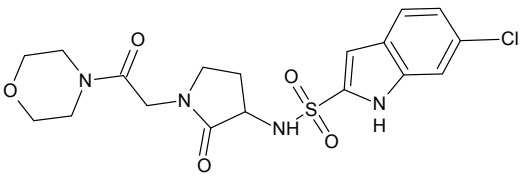
RESULTS AND DISCUSSION

FXa plays a key role in three stages, namely, initiation of coagulation on tissue-factor bearing cells, amplification of the procoagulant signal by thrombin generated on the tissue-factor bearing cell and propagation of thrombin generation on the platelet surface. Activation of coagulation is closely linked to immune and inflammatory responses *in vivo*. Inflammation constitutes the major trigger by which the hemostatic anticoagulant properties of endothelium can be modified into a procoagulant state resulting in pathologic conditions. The crystal structure of the human factor Xa was determined earlier in complex with *6-chloro-n-{(3s)-1-[(1s)-1-methyl-2-(4-morpholinyl)-2-oxoethyl]-2-oxo-3-pyrrolidinyl}-2-naphthalenesulfonamide* (GSK) (PDB ID: 2CJI). The structure of the protein consists of a light chain and a heavy chain linked by a single disulfide bond. The light chain consists of the N-terminal Gla domain and two epidermal growth factor (EGF)-like domains. The Gla domain contains 11 γ-carboxy-glutamic acid residues and mediates binding to the negatively charged phospholipids membrane in the presence of Ca^{2+} ions. The binding of the ligands to FXa involves interaction with residues such as His 57, Gln 61, Tyr 99, Arg 143, Gln 192, Ser 195, Gly 216, Gly 219 and Arg 222.

Target protein structure preparation, ligand structure preparation and ADME analysis

The Protein structure was taken from the Protein Data Bank (2CJI). Twenty five compounds taken from the literature were energy minimized using the various algorithms of the Impact module having default force field OPLS 2001 provided in the suite. These compounds (Figure 1) were later screened for their ADME activity. The program was processed in normal mode, and 53 properties were predicted for the molecules, which consist of principle descriptors and physiochemical properties. Out of 25 compounds, ten of them showed high percentage of human oral absorption (Table 1). All the compounds satisfied the required number of donors and acceptor hydrogen bonds. The molecular weight of each compound was well within range. $Q_p$logKp (predicted skin permeability), $Q_p$logBB (predicted Brain/Blood partition coefficient) and $Q_p$logS (predicted aqueous solubility) of all compounds were also carried out. The total solvent accessible surface area (SASA) in square angstroms using a probe with a 1.4 Å radius should be maintained inside 1000 Å and all the compounds had these values in the range of 560-700 Å. The acceptability of the inhibitors are based on the Lipinski's rule of 5, which is essential for rational drug design. None of the compounds deviated the rule of five. Table 1 shows the ADME properties of selected ten compounds.



 Compound 1E	 Compound 1F
 Compound 1G	 Compound 1H
 Compound 1I	 Compound 1J
 Compound 1K	 Compound 1L
 Compound 1M	 Compound 1N
 Compound 1O	 Compound 1P
 Compound 1Q	 Compound 1R
 Compound 1S	 Compound 1T

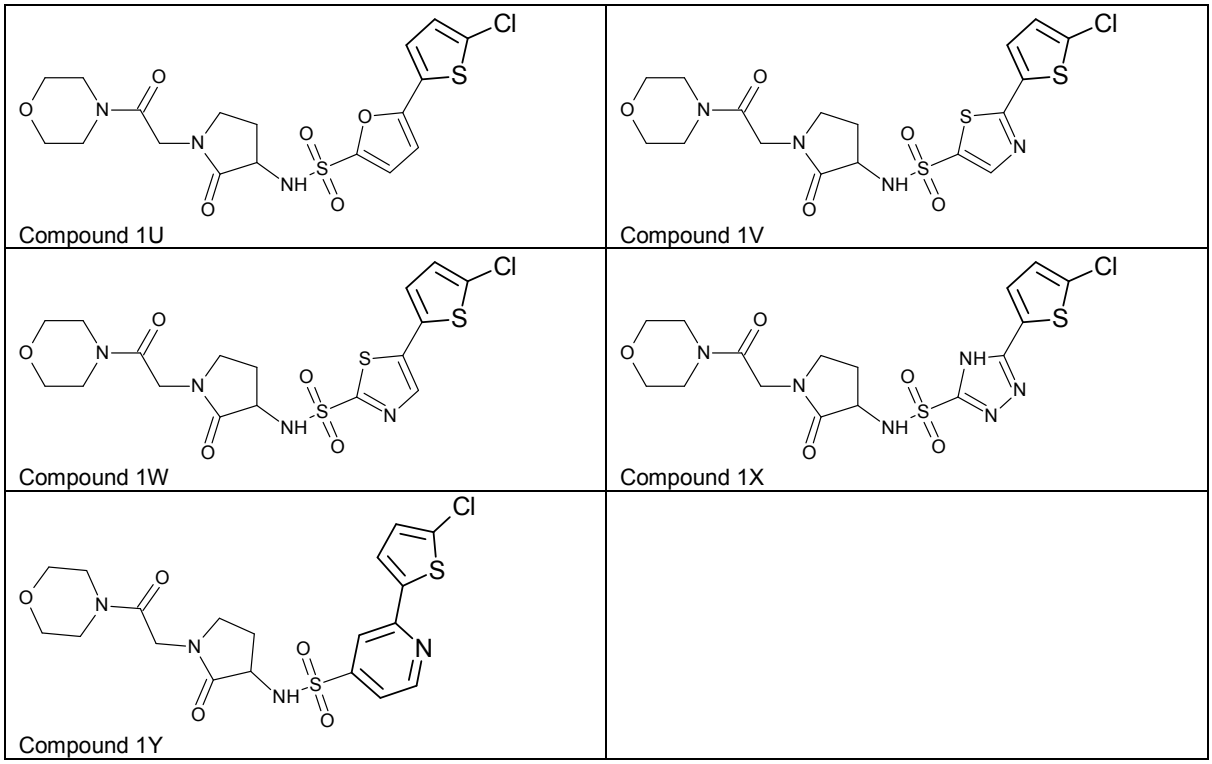


Figure 1
Schematic representation of the compounds taken into study

Table 1
Principle descriptors calculated for all compounds using Qikprop simulation (Glide)

Ligand Name	Molecular Weight (130-725)	SASA (300-1000)	Volume (500-2000)	HB Donors (0-6)	HB Acceptors (2-20)
Drug Compound (1A)	467.9	660	1278.75	0.25	11.45
1B	504.0	643	1279.12	0.25	11.45
1C	441.9	607	1142.56	0.25	11.45
1D	471.9	604	1200.03	0.25	11.45
1E	471.9	597	1191.18	0.25	11.45
1F	447.9	594	1163.88	0.25	11.45
1V	505.0	621	1238.11	0.25	12.95
1X	488.9	628	1225.08	1.25	13.45
1Y	498.9	634	1263.92	0.25	12.45

Ligand Name	QPlogS (-6.5 to 0.5)	QPlogBB (-3.0 to 1.2)	QPlogKp (-8.0 to -1.0)	Percentation of Human Oral Absorbtion	Rule of Five (Max 4)
Drug Compound (1A)	-2.48	-0.49	-2.40	80.52	0
1B	-2.43	-0.38	-2.50	71.46	0
1C	-1.34	-0.59	-2.64	77.79	0
1D	-1.60	-0.41	-2.56	80.56	0
1E	-1.53	-0.36	-2.66	77.84	0
1F	-1.25	-0.53	-2.84	80.67	0
1V	-1.47	-0.45	-4.00	63.04	0
1X	-2.12	-0.93	-2.61	68.19	0
1Y	-1.87	-0.43	-3.14	79.98	0

QSAR Analysis

The output obtained from Qikprop was later given as input to carry out the QSAR study of the compounds. The number of descriptors or properties and the number of factors are taken into consideration to run the program. The workflow of the program consists of mainly data preparation, similarity calculation and

application of calculated similarities. The compounds are differentiated into two sets, a test set and a training set using a random selection method. The random set is chosen from the selected entries of the project table. Linear equations are generated, which describe the relationship between a group of factors (a set of independent descriptors) and a dependent descriptor (the predicted property). The goal of PLS (partial least squares) regression is to find factors that explain the variance in both the independent and the dependent descriptors. A set of diagnostic statistics generated by model X (actual IC_{50}) with Y (predicted IC_{50}) factors is called a predictor (standard deviation, R-squared, F-value, and P-factor). The accuracy of a plot is predicted based on the number of compounds selected (entries) that fall in a straight line. The more the deviation of the compounds from the line, the lesser will be the r-squared value. Standard deviation of the compounds selected should be less and the r-squared value should fall within a range of 0.9 to 0.99 to get the best plot. The more the r-squared value (relativity between the actual and predicted activity) is closer to 1, the better is the plot in terms of accuracy. The F-value and P-factor should also fall to a minimum range. Table2 depicts the QSAR results of FXa inhibitors based on the relativity calculated between the actual IC_{50} obtained from the paper and the predicted IC_{50} value through Strike module of the Suite. Figure 2 shows the XY plot.

Table 2
Table showing the QSAR predicted IC_{50} values (Strike) pitted against the actual IC_{50} values (literature)

Compound	Actual IC_{50} (nM) (Literature) (A)	Predicted IC_{50} (Strike) (B)	Standard Deviation between (A) & (B)	R-squared between (A) & (B)	P-factor between (A) & (B)	F-value between (A) & (B)
1A	6.00	6.04	0.17	0.998	1.28	37.4
1B	4.00	4.09				
1C	11.00	10.98				
1D	47.00	45.97				
1E	15.00	15.72				
1F	4.00	4.05				
1G	109.00	109.01				
1H	165.00	164.78				
1I	314.00	313.97				
1J	163.00	164.67				
1K	2898.00	2897.64				
1L	3670.00	3284.36				
1M	1580.00	1579.91				
1N	3225.00	3225.31				
1O	285.00	285.01				
1P	782.00	781.86				
1Q	4160.00	4159.81				
1R	112.00	111.82				
1S	90.00	90.00				
1T	170.00	169.03				
1U	1200.00	1201.89				
1V	5.00	5.00				
1W	2.00	2.03				
1X	534.00	534.08				
1Y	24.00	24.00				

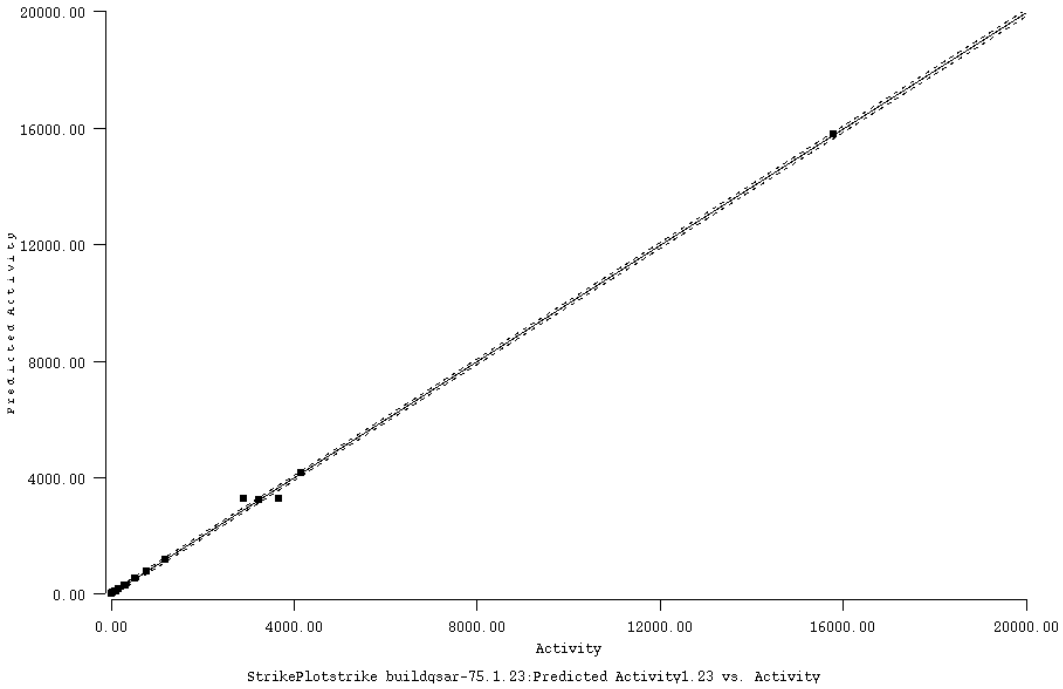


Figure 2
QSAR XY Plot where X(actual IC_{50} values) & Y(predicted IC_{50} values)

Molecular Docking Analysis

The protein and drug compound (GSK) were separately minimized and re-docking of the drug compound was done at the active site of the target protein FXa. Based on the ADME properties, nine compounds were selected for Induced Fit Docking studies. Of all compounds, two main docked poses were selected based on good hydrogen bond interactions, glide score and glide energy for each compound and the best pose of each compound is displayed in the end. Each compound showed good coordination bonds with the active site amino acid residues. In the first compound 1A (drug compound or GSK), oxygen atom of the sulfonamide group interacts with the amide group of Gln 192. Oxygen atom of the morpholine ring present in the analogue maintains a hydrogen bond interaction with a amide group of Arg 143. Amide group of the triazole ring is located at a distance of 3.2 Å from Gly 216. The pose of the compound is exhibited in an 'L' format, where the last five membered ring shifts to a 90° turn from the entire structure, and no interaction was maintained with Ser 214, Ser 195 and Phe 174. The second docked pose of the same compound exhibits in an 'S' shape or conformation. The oxygen of sulfonamide group is situated at a distance of 3.4 Å from Gly 216. The amide group subsequent to the sulfonamide group also maintains an interaction with Gly 216. The oxygen and nitrogen atoms of the morpholine group interact with Gln 192 and Arg 143, situated at the active site of the target protein. The first pose of compound 1B exhibits a parabola or 'U' shape. Thienyl sulfonamido heteroatomic ring in the chlorobithienyl P1 analogue 1B is positioned away from the S1 pocket than the ring distal to the chlorine atom in the benzothienyl analogue. Hydroxyl group of the triazole ring interacts with the nitrogen and oxygen atoms of Gly 193 and Phe 41. The sulfur atom of the thiophene ring adjacent to the sulfonamide group is oriented towards Gly 192. The second pose of the same compound exhibits in an 'L' shape. Hydroxyl group of the triazole ring interacts well with Gly 216 and oxygen atom is situated at 3.1 Å from the same Gly 216. In compound 1C, the non-carbon atoms of the 5-membered or 6-membered ring do not interact with any of the residues at the active site, but as usual there is an interaction with oxygen atom of the sulfonamide group and Gln 192. Compound 1D does not exhibit interactions with atoms of sulfonamide group as the structure is presented in the form of 'U' or parabola where the rings at both the ends face in opposite

directions. In both the docked poses of compound 1E similar interactions viz., oxygen atom of the sulfonamide group with Gly 216 and oxygen atom of the morpholine group with Arg 222 are noted. An interaction with Gln 192 is also observed. Compound 1F assumes a 'hook' shape, wherein interactions with Gln 61, Tyr 99, Gly 216, Ser 195 and His 57 are maintained. Compound 1V also interacts with His 57 at a distance of 2.9 Å and with Tyr 99 at 3.1 Å. Likewise, compounds 1X and 1Y exhibit good interactions with the active site residues. Out of the analogues taken into study, 1A-1F (benzamidines with substituted chloroaromatic groups) show good to excellent FXa inhibitory activities. The combined approach of ADME, QSAR and IFD used in the present work helps in expressing the binding affinity of a series of analogues in the receptor well and also validates them as potential candidates for second generation drug discovery. Tables 3 & 4 show the hydrogen bond interactions of the respective compounds with amino acids at the active site of the target protein FXa and their docked energies. Figures (3-11) show the interactions of the compounds with the amino acid residues at the active site. A compound is considered more stable than the drug or is expressed as a lead, when it forms good hydrogen bond interactions at the active site with the least glide score and glide energy. In the current study, the final binding energy of the complexes after docking, show that the new compounds are more favorable for their anti-coagulant and anti-inflammatory activities.

Table 3
Interactions of the compounds with amino acids at the active site of FXa using Induced Fit Docking module of Glide

Compound with FXa	Interaction at the active site	Distance between Donor and Acceptor (Å)
Drug Compound (1A)	ARG 143 (N-H…O) (N-H…O) GLY 216 GLN 192 (N-H…O)	3.1 3.2 2.6
1B	GLN 192 (O-H…O) (O-H…N) GLY 193 (O-H…O) PHE 41 (O-H…C) SER 214 (O-H…C) SER 195 (O…N-H) ARG 143	3.4 3.4 2.8 3.3 3.4 2.8
1C	(O…O-H) GLN 192 (N-H…O) GLN 192	3.4 3.3
1D	TYR 99 (O-H…O) GLY 216 (N-H…O)	3.3 2.5
1E	GLN 192 (O-H…O) GLY 216 (N-H…O) (N-H…O) GLY 216 (O…N-H) ARG 222	3.2 3.3 3.4 3.0
1F	GLY 216 (N-H…O) (N-H…O) SER 195 HIS 57 (N-H…O) GLN 61 (O-H…O) TYR 99 (O-H…O)	3.0 2.5 2.9 2.5 2.3
1V	HIS 57 (N-H…O) TYR 99 (O-H…O)	2.9 3.1
1X	GLY 216 (N-H…O) GLN 192 (N-H…O) ARG 222 (N-H…N)	3.4 2.5 2.8
1Y	(N-H…O) GLN 192 (O…N-H) ARG 143 GLY 216 (N-H…O)	2.8 3.0 3.2

Table 4
Docking Scores and Docking Energies of the compounds (Glide)

Compound	Glide Score	Glide Energy (Kcal/Mol)
Drug Compound (1A)	-5.39	-46.91
1B	-5.99	-55.74
1C	-6.19	-56.39
1D	-7.17	-57.46
1E	-6.97	-52.25
1F	-7.15	-60.00
1V	-7.35	-57.18
1X	-5.94	-45.62
1Y	-5.84	-52.40

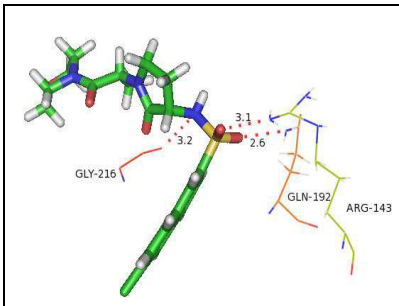


Figure 3
Interaction of Drug compound 1A with amino acids at the active site of FXa

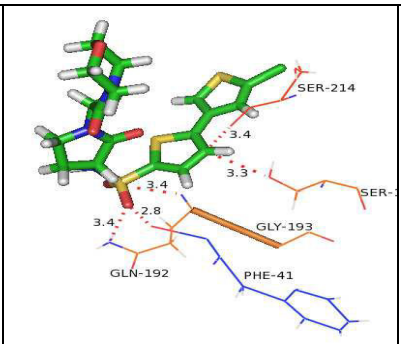


Figure 4
Interaction of compound 1B with amino acids at the active site of FXa

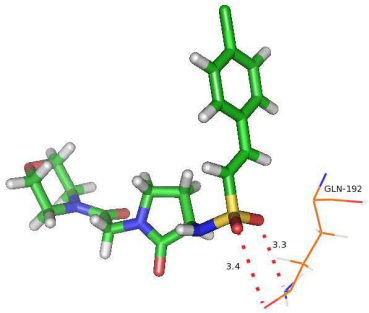


Figure 5
Interaction of compound 1C with amino acids at the active site of FXa

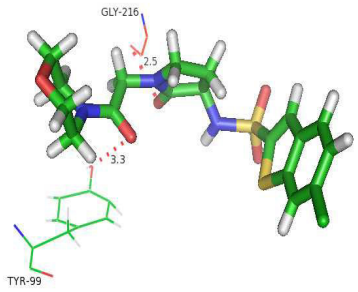


Figure 6
Interaction of compound 1D amino acids at the active site of FXa

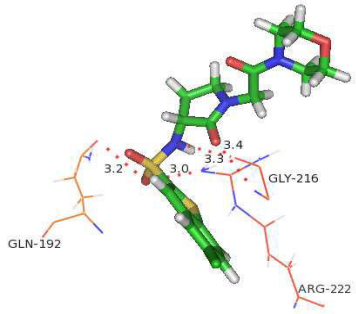


Figure 7
Interaction of compound 1E with amino acids at the active site of FXa

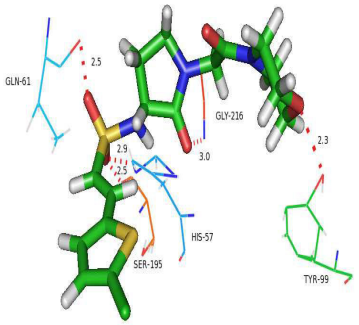


Figure 8
Interaction of compound 1F with amino acids at the active site of FXa

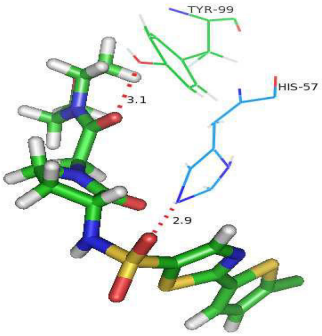


Figure 9
Interaction of compound 1V with amino acids at the active site of FXa

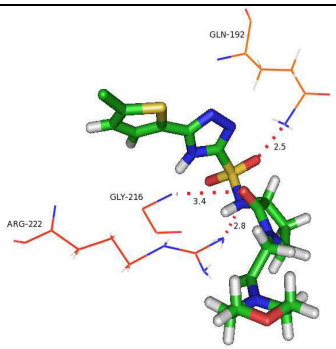


Figure 10
Interaction of compound 1X with amino acids at the active site of FXa

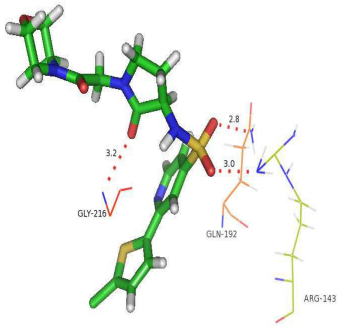


Figure 11
Interaction of compound 1Y with amino acids at the active site of FXa

CONCLUSION

The inhibition of the protein FXa promises to be therapeutic approach in the anti-coagulatory and anti-inflammatory pathways. All the compounds abide the ADME properties and the Lipinski's rule of five. Compounds 1A-1F showed good to excellent FXa inhibitory activities proving them worthy as anti-coagulant agents by exhibiting good glide score, glide energy and hydrogen bond interactions at the active site. All compounds bind well into the S1 pocket, which is essential for good interaction with residues at the active site. Combined analysis of ADME-QSAR-Induced Fit Docking helps in knowing the binding affinity of the analogue, to find suitable inhibitors targeting Factor Xa.

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