Identification of Potential Inhibitors of Human β-FXIIa as an Alternative to Current Anti-Thrombotic Therapy: A Pharmacophore-based Virtual Screening Approach

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Abstract: Cardiovascular diseases are currently the leading cause of death globally. As such, global efforts have shifted the spotlight to thrombosis which form the underlying pathology of the many cardiovascular disorders. Clinically available anti-coagulant drugs are mostly associated with a high risk of severe bleeding complications and are also shown to interfere with the physiological hemostasis. Several reports have revealed that targeting β-FXIIa with the aim of countering thrombosis without interfering with the homeostatic stability of the body is a viable approach in anticoagulant. The recent release of the X-ray crystal structure of the activated conformation serves as a boost for in silico interventions. In this study we aim to identify potential inhibitors of β-FXIIa that may elicit enhanced inhibitory activity relative to existing anti-thrombotic agents. Molecular docking and pharmacophore-based virtual screening were employed to screen for potential inhibitors from the ZINC database, based on the interacting pharmacophoric features of Benzamidine, a serine protease towards β-FXIIa. Three potential inhibitors ZINC63696130, ZINC63406068 and ZINC64604295 were identified and were shown exhibit favorable binding affinity towards β-FXIIa. Also these compounds also showcased favorable pharmacokinetic properties and passed as drug-like compounds. A further experimental evaluation of these compounds could lead the discovery of novel inhibitors of β-FXIIa with enhanced therapeutic properties.

Keywords: Thrombosis, Hemostasis, Molecular Docking, Virtual Screening, Anti-Coagulants, Pharmacokinetics

Introduction

Globally, there has been a transition from infectious to non-communicable diseases as the major causes of death and disability worldwide which literally affects life expectancy rate and sustainable human development (Hunter and Fineberg, 2014; Jamison et al., 2015; Lozano et al., 2012; Roth et al., 2017). Reports suggests that Cardiovascular Diseases (CVD) are the leading contributors to the burden caused by these non-communicable diseases of which thrombosis forms the underlying pathology of the major cardiovascular disorders including: Ischemic heart disease, stroke and venous thromboembolism (Raskob et al., 2014; Wendelboe and Raskob, 2016). In 2015, 422.7 million cases of CVD were estimated, of which 17.92 million CVD deaths were recorded (Roth et al., 2017). An increased awareness of these global non-communicable disease goals has therefore expanded with attempts to track and benchmark national efforts at reducing CVD and other non-communicable diseases.

Thrombosis as the underlying pathology for the three major cardiovascular diseases results from unwanted blood clot during blood coagulation (Wendelboe and Raskob, 2016). Blood coagulation is essential for the body to maintain a state of homeostatic stability (Colman and Schmaier, 1997; Pathak et al., 2015). Generally, blood coagulation tests are performed in clinical laboratories to assess the efficiency of coagulation in patients (Baeriswyl et al., 2015). In most
Coagulation tests, blood clotting is induced with a strong trigger and the time to coagulation is used as an indication of the coagulation potential. While these tests are useful diagnostically, they are limited in their ability to correlate with the bleeding or thrombotic risk of the patient (Baeriswyl et al., 2015).

To date, anti-coagulant drugs that are clinically available, are associated with a high risk of severe bleeding complications and are also shown to affect the physiological hemostasis. As such new research methods are being developed with the purpose of identifying target molecules (Raskob et al., 2014; Weitz, 2011).

The recent targeting of the human factor β-FXIIa has presented a new scope toward developing active inhibitors for antithrombotic therapy (Dementiev et al., 2018). β-FXIIa is a serine protease that has received a great deal of interest in recent years for the active role it plays towards initiating the cascade of coagulation by contact activation (Colman and Schmaier, 1997; Samuel et al., 1992). This activation requires proteolytic conversion of plasma FXII zymogen to active protease FXIIa on negatively charged surfaces where FXII undergoes conformational changes and small amounts of active FXIIa are formed (Samuel et al., 1992). Previous reports suggest β-FXIIa presents a suitable drug target for the development of safe anti-thrombotic inhibitors that can halt thrombosis without any effect on homeostasis (Weitz, 2011). The X-ray crystal structure of human β-FXIIa was recently resolved in complex with two inhibitors, Benzamidine (BEN), a non-covalent small molecule inhibitor of serine proteases and a covalent aminoisouquinoline containing a boronic acid–reactive group that targets the catalytic serine (Dementiev et al., 2018). Figure 1 shows the X-ray crystal structure of β-FXIIa complexed with BEN while highlighting the interacting binding site residues. Both BEN and the covalent inhibitor bound in a canonical form which is typical of serine protease inhibitors and this forms the basis for β-FXIIa activation and also provides insights into the enzymatic activities of β-FXIIa. The availability of the crystal structure in complex with these inhibitors paves the way for a structure based design of potential inhibitors of β-FXIIa that could possess similar or enhanced inhibitory properties as these known inhibitors. In this study we employed a structure based virtual screening and molecular docking to discover small molecule inhibitors of β-FXIIa based on the essential pharmacophoric moieties of Benzamidine, a known inhibitor of serine protease. Findings in this study seek to identify potential hits that could further be investigated and optimized for use as safe ant-coagulants.

**Figure 1:** 3D X-ray crystal structure of β-FXIIa complex with Benzamidine (BEN). Showing also the binding site region (yellow) and the residues that constitute this binding site region
**Methodology**

**System Preparation**

The 3D structure of human factor β–FXIIa in complex with BenzaMidine (BEN) was retrieved from the Protein Data Bank with ID 6B74. The structure of Human factor β–FXIIa has two distinct chains A and B however due to computational cost and time, the singular chain B of the protein where the BenzaMidine compound is bound to was prepared for a molecular dynamic simulation. The preparation was carried out on UCSF Chimera (Pettersen et al., 2004) interface where hydrogens were added to the protein and removed from the ligand. The single system was subjected to a 20ns MD simulation.

**Pharmacophore Generation**

In order to obtain the bound conformation of the BEN, BEN in complex β–FXIIa was taken through a 20 molecular dynamic simulation. The amino acids that contribute the most towards ligand binding were determined by applying per-residue energy decomposition analysis. A pharmacophore model was then constructed by choosing pharmacophoric moieties that interacted with the high energy contributing residues on the ZINCPharmer online platform. The constructed model was added to ZINCPharmer (Morris et al., 1998; Pettersen et al., 2004) with a distinct criteria (molecular weight of <500 Da, hydrogen bond donors <5, hydrogen bond acceptors <10 and rotatable bonds <6), to screen the ZINC database (Koes and Camacho, 2012).

**Structure-based Virtual Screening**

Virtual screening is a common computational technique used to identify potential inhibitors from large databases of drug like compounds. Based on the generated pharmacophore, the ZINC database was screened to obtain drug-like compounds with similar pharmacophoric moieties as exhibited by BEN upon binding to β–FXIIa. The constructed model was added to ZINCPharmer (Morris et al., 1998; Pettersen et al., 2004) with a distinct criteria (molecular weight of <500 Da, hydrogen bond donors <5, hydrogen bond acceptors <10 and rotatable bonds <6), to screen the ZINC database (Koes and Camacho, 2012) for the potential β–FXIIa inhibitors.

**Preparation of Potential β-FXIIa Inhibitors**

Potential β-FXIIainhibitors obtained from screening on the ZINC database were retrieved in their 2D conformations. Energy minimization was carried out for each of the retrieved compounds on Avogadro using the MMF94 force field (Hanwell et al., 2012). The minimized 3D structures were thereafter individually exported to UCSF Chimera for further preparation prior to molecular docking. Hydrogen atoms were removed and Gasteiger partial charges were allocated to the compounds.

**Molecular Docking**

Molecular docking was performed on all the hits obtained from the ZINC database to predict their binding conformation and affinity within the active site region of β–FXIIa. Docking was carried out using the AutoDockVina software (Morris et al., 1998). The grid box that defines the binding site region of the β–FXIIawas generated using the AutoDock Vina functionality on UCSF Chimera (Pettersen et al., 2004). The grid box size and center coordinates for the protein were x (10, 16.92), y (10, 16.32) and z (10, 46.31) respectively. During the docking process, a maximum of 10 conformers were considered.

**In silico Prediction of Pharmacokinetic Properties**

Obtained potential inhibitors from virtual screening were accessed for their pharmacokinetic properties using the online tool SwissADME (Daina et al., 2017). This tool was used to assess the drug-like qualities of the compounds taken into consideration their molecular weight, logP value, number of hydrogen bond donors and acceptors.

**Binding Interaction of Residues**

The binding interactions of the hits were also used to show the kind of interactions engaged between the inhibitors and β–FXIIa. This was carried out by further uploading the bound complexes onto the Discovery studio software (BIOVIA, 2015). A comparison of residue interaction profiles of the potential inhibitors to that BEN to further assess their inhibitory prospects.

**Results and Discussion**

**Pharmacophore Modelling**

The residue interaction profile of BEN was then accessed after the 20ns simulation using the Discovery studio software as shown in figure. Based on the nature of interactions that existed between the binding site residues of β–FXIIa and BEN, a pharmacophore model as shown in Fig. 2B was generated to screen potential inhibitors of β–FXIIa. As shown in Fig. 2A residues that formed strong interactions with BEN included; Ala195 Gly224, Trp223 and Cys227. These interactions included a conventional hydrogen bond interaction with Cys227 and amide pi-stacked interactions with Gly224 and Trp223 amongst other hydrophobic interactions.

**Fig. 2:** (A) Ligand-residue interaction plot of BEN at the binding site of β–FXIIa. (B) Generated pharmacophore showing an aromatic and hydrophobic moiety (Purple) and two hydrogen donors (yellow)

**Structure-based Virtual Screening**

Current anti-thrombotic agents are usually associating with associated with a high risk of severe bleeding complications with a demonstrable influence on physiological hemostasis. As such this study sort to identify potential inhibitors of β–FXIIa that could serve as alternatives to existing therapeutic options, while forming the basis for the development of safe anti-coagulant agents. Based on the pharmacophore model generated as shown above, virtual screening was performed on the ZINCpharmer compound. This led to the identification of three compounds; ZINC63406068, ZINC63696130 and ZINC64604295 which possessed that ability of becoming potential inhibitors of β–FXIIa upon further exploration based on the criteria of selection employed.

**Molecular Docking of Potential β–FXIIa**

To further explore the prospects of the three identified hit compounds, the binding modes and affinities of each compound was accessing through molecular docking relative to BEN. Scoring functions associated with molecular docking tools permit the prediction of binding affinities. In view of this, the docking scores for the three compounds were recorded after docking as shown in Table 1. Several reports suggests that the more negative the binding affinity of an inhibitor to its target, then the stronger the binding (Abdullahi et al., 2018; Varma et al., 2010). It was observed from the molecular docking results that all three potential inhibitors exhibited favorable binding affinity of -6.3 kcal/mol, -6.6 kcal/mol and -6.1 kcal/mol for ZINC63696130, ZINC63406068 and ZINC64604295 respectively. Amongst all three ZINC63406068 showed the highest binding affinity against β–FXIIa. Overall all the three potential inhibitors elicited a higher binding affinity toward β–FXII areative to the BEN which exhibited a binding affinity of -4.8 kcal/mol as shown on Table 1. This observed favorable binding affinity suggests a level of stability within the binding pocket, which could also infer a possible inhibitory activity on target.

**Comparative Binding Interaction Profiles of Potential Inhibitors**

In ensuring the identified compounds possessed a potential for further exploration as possible inhibitors of β–FXIIa, we analyzed their respective residue interactions profiles at the binding pockets as shown in Fig. 3. This was crucial because, the interaction of these compounds with binding site residues influence the overall binding affinity of the compound within the binding site (Haagsma et al., 2011). Comparatively, all three potential inhibitors exhibited higher number of interactions with binding site residues relative to BEN. As shown in Fig. 3, all three compounds engaged in very strong interactions with some particular residues within the hydrophobic pocket, notably, Ala195, Cys196, SER222, Trp223 and Gly224. ZINC63696130 engaged in similar interactions as BEN by forming intermolecular interactions such van der Waals, conventional hydrogen bonds, Pi–Amide stacked and Pi–alkyl interactions with binding site residues. ZINC64604295 showed slightly different interactions toward the hydrophobic groove of the protein structure with interactions including Halogen (Florine), Pi amide, hydrogen bonds and van der Waals.
Fig. 3: Ligand-residue interaction profiles of all three identified potential inhibitors of β–FXIIa

Table 1: 3D structures of inhibitors (colored by heteroatoms) and their corresponding binding affinities from molecular docking

<table>
<thead>
<tr>
<th>Compound</th>
<th>3D Molecular structure</th>
<th>*ΔG (Kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzamidine</td>
<td></td>
<td>-4.8</td>
</tr>
<tr>
<td>ZINC63696130</td>
<td></td>
<td>-6.3</td>
</tr>
<tr>
<td>ZINC63406068</td>
<td></td>
<td>-6.6</td>
</tr>
<tr>
<td>ZINC64604295</td>
<td></td>
<td>-6.1</td>
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</table>
On the other hand, ZINC63406068 exhibited the most interactions with the hydrophobic groove including a Pi-Pi T shaped, Pi- sulfur and carbon hydrogen bond. This observed high number of strong interactions elicited by the screened compounds could have contributed their high binding affinities estimated in the molecular docking. Relative to the screened compounds, BEN elicited fewer interactions which include; one conventional hydrogens and two amide-pi stacked interactions and one pi-alkyl interaction as shown in Fig. 2. This observed fewer interactions could have accounted for its lower binding affinity.

**Pharmacokinetic Assessment of Potential β–FXIIa Inhibitors**

Using the online platform SwissADME, the pharmacokinetic properties, Absorption, Distribution, Metabolism, Excretion (ADME) properties of potential compounds were predicted. This was necessary to evaluate the prospects n of these compounds for human use. In *silico* ADMET studies are expected to reduce the risk of late-stage attrition of drug development and to optimize screening and testing by looking at only the promising compounds (Yamashita and Hashida, 2004). All the retrieved compounds showed very promising pharmacokinetic properties as shown in Table 2. The pharmacokinetic assessment of these compounds took into the consideration, the Lipinski’s rule of five, which emphasizes on physicochemical properties such as molecular weight, logP value, number of hydrogen donor and number of hydrogen bond acceptors (Lipinski, 2004; 2016). The lipophilicity XlogP, for each of the compounds, was also assessed and provided insights on the membrane permeability of the compounds. A lipophilicity of LogP >5, correlates to a high a metabolic turnover, low solubility and poor oral absorption whereas compounds with a logP greater than 1 or less than 4 (1<<4) are more likely to have optimal physicochemical and ADME properties for an oral drug (Arnott and Planey, 2012; Kah and Brown, 2008; Waring, 2010). As shown in Table 2, ZINC63696130 and ZINC 64604295 exhibited a favorable LogP values of 3.42 and 3.90 respectively, suggesting they possess a huge potential of being oral drugs with optimal physicochemical property upon further investigations. Amongst the potential compounds, ZINC63406068 showed a LogP value of 5.78 indicative of a high lipophilicity and corresponding high metabolic turnover, a high solubility, but a poor oral absorbance. A comparison of the molecular weights of the potential inhibitors were assessed since this could influence the toxicity tendencies of the compound. A high molecular weight compound tend to be toxic than those with lower molecular weight (Omran and Rauch, 2014; Veber et al., 2002). From Table 2, ZINC63406068 displayed the lowest molecular weight of 370.432g/mol amongst the three potential compounds followed by ZINC63696130 (425.48 g/mol) and then ZINC64604295 showed the highest molecular weight of 431.46g/mol. All the compounds including Benzamidine displayed a favorable molecular weight according to the Lipinski’s rule of five suggesting their great potential upon further investigation. Overall, the *in silico* pharmacokinetic analysis performed for these compounds suggests they show a great potential to be very potent inhibitors of β–FXIIa upon further investigation and optimization.

**Conclusion**

Thrombosis remains a serious global threat due to the active role it plays in causing the major cardiovascular disorders. The identification of β–FXIIa as a drug target has paved way for the design of drug molecules that can counter thrombosis. In this study our main aim was to discover new drug compounds based on the pharmacophoric moieties of the already existing serine protease inhibitor, Benzamidine that would serve as an alternative to existing serine protease inhibitors. We employed molecular docking together with structure based virtual screening to screen out best candidates based on a generated pharmacophore model. Findings from this research revealed three compounds, ZINC63696130, ZINC63406068 and ZINC64604295 that possess drug-like properties. These compounds demonstrated stronger binding to the hydrophobic groove of β–FXIIa compared with BEN due to the more hydrogen bonds they form and the high binding affinity estimated. All three compounds showed essential interactions with the active site residues. However, ZINC63406068 exhibited the most interactions of the three, wherein ZINC63696130 and ZINC64604295 engaged in interactions that are similar to BEN. Pharmacokinetic assessment of the compounds revealed these compounds are suitable for use as oral absorbance, low molecular weight and less toxic. The potential

<table>
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<tr>
<th>Compound</th>
<th>ZINC code</th>
<th>xlogP</th>
<th>Molecular weight (g/mol)</th>
<th>Hydrogen Bonds</th>
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</thead>
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<td>431.468</td>
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Table 2: Showing the pharmacokinetic properties of Benzamidine and the screened compounds
inhibitors; ZINC63696130, ZINC63406068 and ZINC64604295 from our study have shown substantial stability and potential to form good oral anti-coagulants based on our analysis. However, discovering a lead compound is only the first step in the long and cumbersome process of getting a drug from concept to the market. As such, findings from this study provides a starting point towards the design of novel anti-coagulant with enhance therapeutic properties with no risk of bleeding.

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Author’s Contributions

Elliasu Y. Salifu: Contributed by compiling literature review, data analysis and interpretation, manuscript writing and preparation of figures whites.

Clement Agoni and Fisayo Olotu: Provided technical support, editing the manuscript and supervised study.

Conflict of Interest

The authors declare no conflict of interest.

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