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Structural and Mechanistic Insights Into the Advances of FXIIIa Inhibitors

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Introduction

Cardiovascular diseases (CVDs) represent a major problem to humanity, having the highest mortality rates (above 85% of the deaths caused are due to heart attacks and strokes) in the category of natural causes [1]. The fast treatment with anticoagulants inhibiting the activity of blood clotting factors represents a promising approach to stop the clot formation (thrombosis), and therefore, to protect the patients from fatal consequences. Inhibitors targeting blood clotting factor XIIIa (FXIIIa), that is involved in the last step of coagulation process, have been considered as a new generation of anticoagulants [2]. Plasma FXIIIa is an enzyme, belonging to the family of transglutaminases (TGase), which is primary responsible for stabilization of the fibrin network by stabilizing the preformed clot.

In the current study we aim at:

- Performing a three-step virtual screening (VS) approach in order to estimate binding affinities (steps 1–3, Fig. 1) of a rationally selected set of small drug-like compounds using Single Crystal X-Ray Diffraction (SCXRD) technique in combination with the molecular modelling platform SeeSAR [3].
- Identifying of a set of FXIIIa inhibitors based on their predicted affinity ranges ($K_{i\ HYDE}$ scores), physicochemical and drug-like properties (see Table 1 and 2, Fig. 2 and 3).

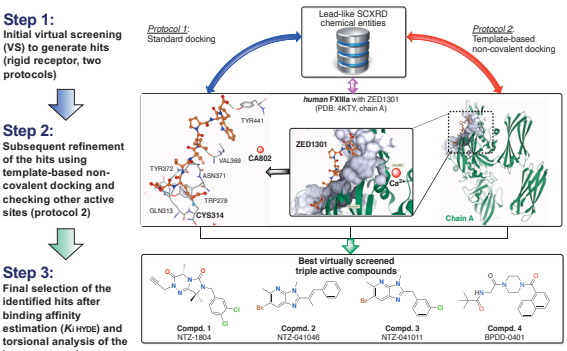

Figure 1. Three-step VS approach of SCXRD chemical entities on human FXIIIa leading to the best scored compounds **1–4**.

SeeSAR visualization and HYDE analysis

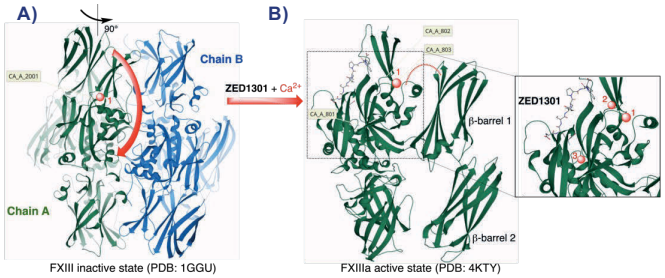

Figure 2: A) SeeSAR visualization of the inactive state of FXIII (PDB: 1GGU) [4], in which FXIII exists as a dimer (Chain A in green and Chain B in blue). B) After binding of the substrate **ZED1301** (off-white, *cf.* Fig. 1) the β-barrel 1 and 2 moved (red arrow) and together with Ca^{2+}-binding (three Ca^{2+} instead one in FXIII) is activated to its active form FXIIIa (PDB: 4KTY) [5].

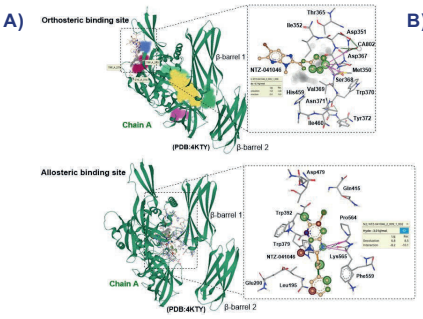

Figure 3: A) SeeSAR visualization of orthosteric and allosteric binding of **NTZ-041046** to *h*FXIIIa. B) HYDE analysis of desolvation effects (lipophilic interactions) and torsions for **NTZ-041046** within the catalytic domain of the activated form of FXIIIa. HYDE coloring and torsion analysis: **green** = good, **red** = bad for affinity.

Modeling of transitional states and predicted energies of FXIII

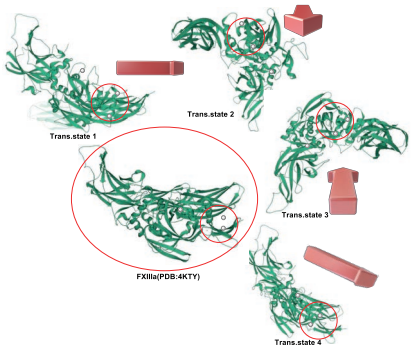

Figure 4. Transitional state models of FXIII generated with HADDOCK 2.4 web server.

ClusterNo	Van Der Waals energy [kcal/mol]	Desolvation energy [kcal/mol]	Electrostatic energy [kcal/mol]	RMSD [Å]	Restraints violation energy [kcal/mol]	HADDOCK score [a.u.]
12.1-12.4	-33.8 ± 11.5	12.1 ± 3.8	-275.0 ± 66.6	32.5 ± 0.1	9.9 ± 9.3	-75.7 ± 17.7

PDB Energy and Energy minimization (kJ/mol)							
Cluster 12.1		Cluster 12.2		Cluster 12.3		Cluster 12.4	
Energy[1]	Minimized[2] Energy	Energy[1]	Minimized[2] Energy	Energy[1]	Minimized[2] Energy	Energy[1]	Minimized[2] Energy
-76.681	-80.806	-65.661	-82.090	-66.514	-81.585	-66.677	-81.897

Transition state models

Table 1. Top scored cluster according to HADDOCK combining the different energies (the more negative the better). Energy optimization was performed to determine the lowest energies of the structures. [1)] initial and [2)] minimized energy.

Ligands	$K_{i\ HYDE}$ lower boundaries (μM) (SeeSAR/HYDE prediction)		
	***h*FXIIIa**	***Trans.state* *h*FXIIIa** (Cl. 12.2)	***TG2*** (2Q3Z)
ZED1301	0.07 **100 [6]**	0.09	2000 **3000 [6]**
Compd. 1	0.75	340	2315
Compd. 2	1.22	31.3	5.56
Compd. 3	1.53	0.03	188
Compd. 4	0.55	336	16.7

Table 2. Receptor binding data and HYDE estimated binding affinity for the human FXIIIa and FXIII generated models as well as the transglutaminase 2 (TG2) enzymes of **ZED1301** inhibitor and ligands **1–4**.

Conclusions

SCXRD/SeeSAR/HYDE concept: An innovative drug design concept combining a X-ray (SCXRD) technique and a protein target (human FXIIIa enzyme) that is related to CVDs pathophysiology with a molecular modeling platform SeeSAR/HYDE was used to identify a small set of promising ligands (compounds **1–4**).

Proof-of-concept: The estimated binding affinities ($K_{i\ HYDE}$ ranges) combining both physicochemical and drug-like properties, as well as torsional and thermodynamic analysis, suggested that these four compounds may be used for further development of new FXIIIa inhibitors as anticoagulants against thrombosis. However, the subsequent experiments partly confirmed their estimated (predicted) inhibitory potencies against FXIIIa.

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Literature

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