NEW INHIBITORS OF THE COAGULATION FACTOR XIIA: DOCKING AND EXPERIMENTAL VERIFICATION

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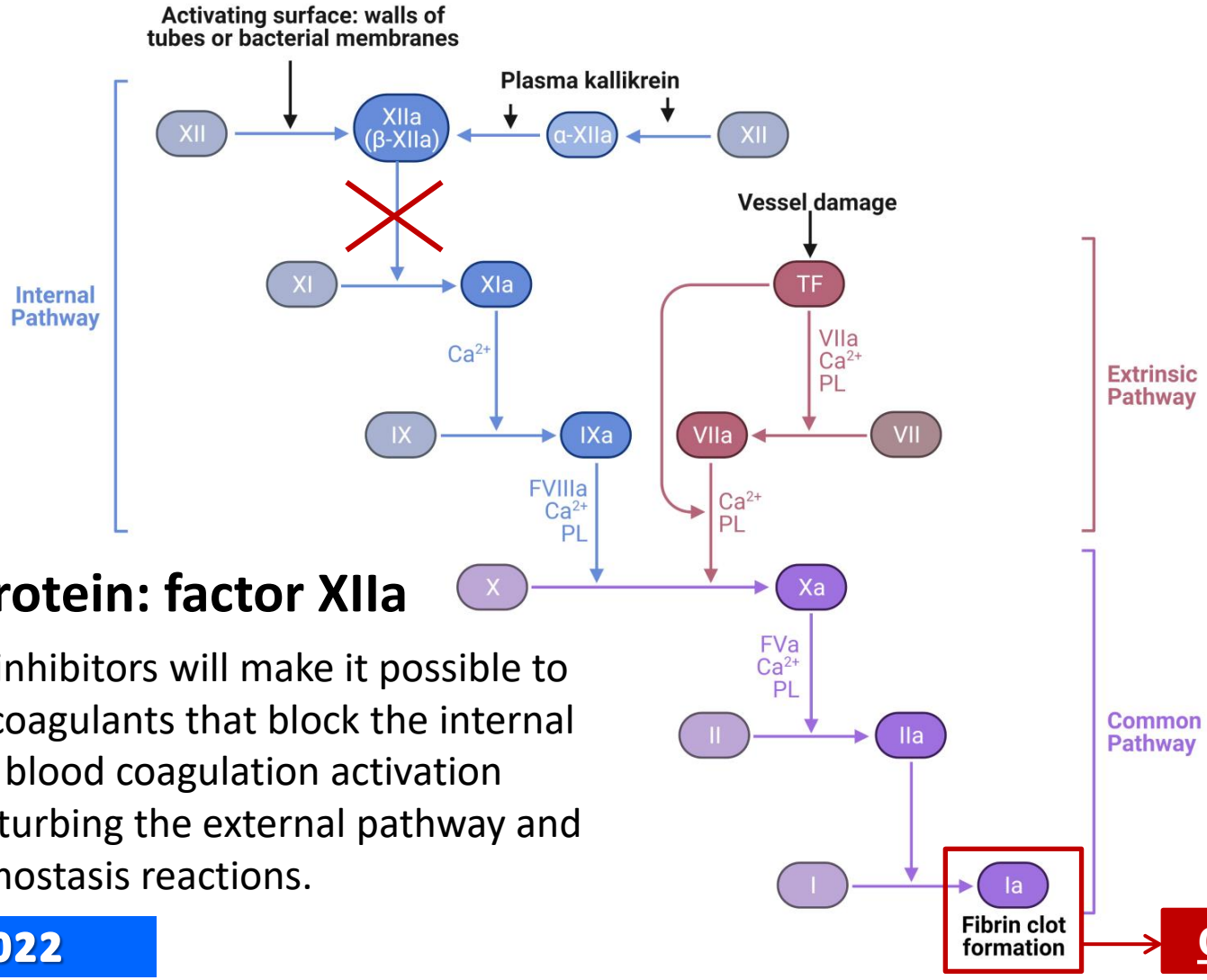
Reactions of the plasma blood coagulation system



Target protein: factor XIIa

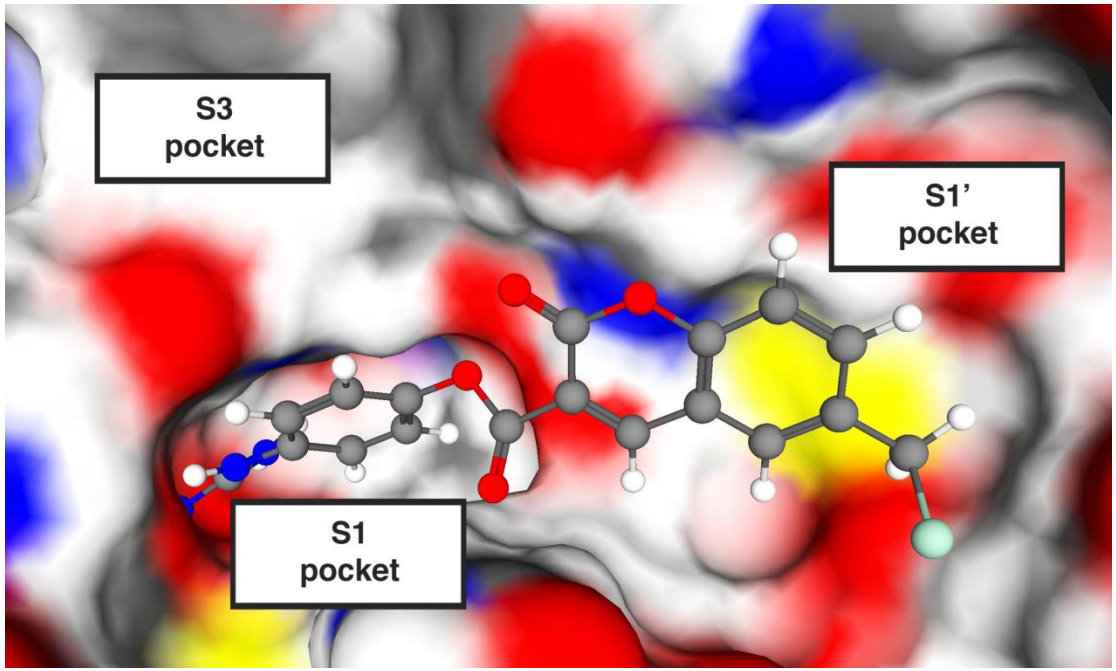
Factor XIIa inhibitors will make it possible to create anticoagulants that block the internal pathway of blood coagulation activation without disturbing the external pathway and plasma hemostasis reactions.

Anticoagulants on the basis of factor XIIa inhibitors

- Blocking the internal pathway of blood coagulation activation without disturbing the external pathway and plasma hemostasis reactions
- With factor XII deficiency, laboratory animals are either completely protected from the development of induced thrombosis or suffer significantly less from its consequences
- FXII-deficient patients demonstrate a normal hemostatic ability
- Factor XII deficiency protects against thrombosis without causing spontaneous bleeding

Factor XIIa model

Development of new inhibitors of blood coagulation factor XIIa using molecular modeling methods

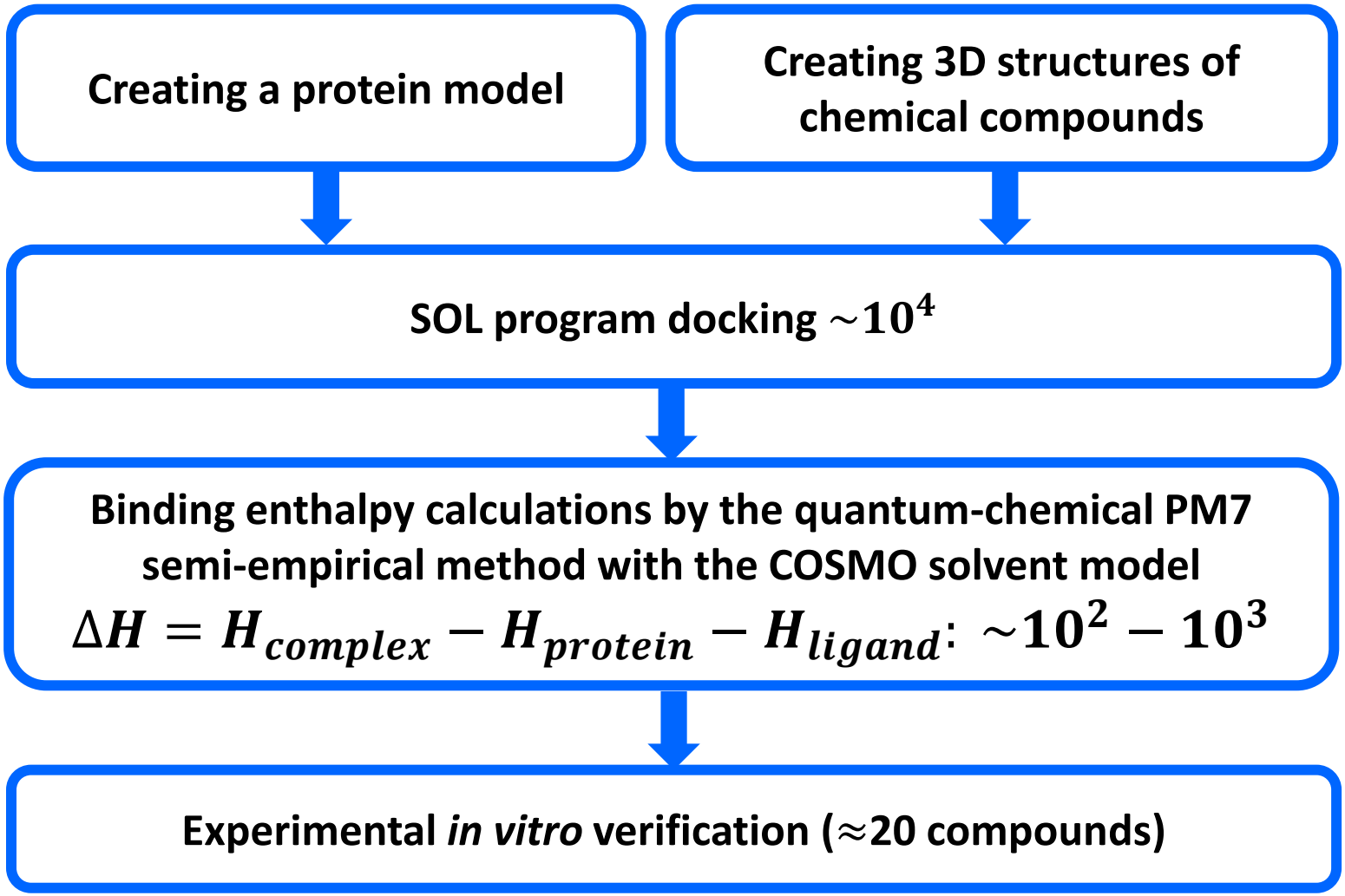


Active site of the protein is represented by binding pockets S1, S1' and S3

Protein model

- Novel PDB structure **6B74** of factor XIIa crystallized with **inhibitor**.
  Resolution = 2.3 Å
- adding hydrogen atoms using the APLITE program
- native ligand docking
- development of selection criteria

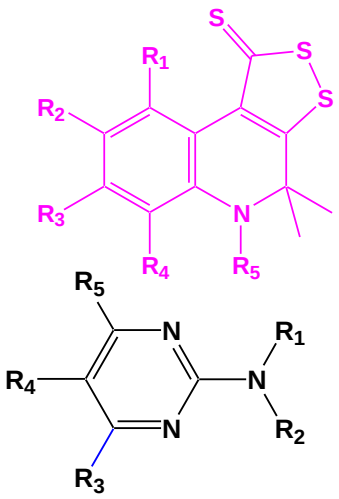
Virtual screening



Database of drug-like compounds of Voronezh State University

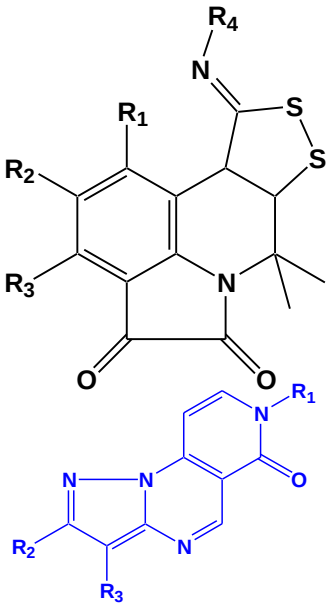
More than 200 000 compounds – virtual, but have been synthesized

Antibacterial, antifungal activity



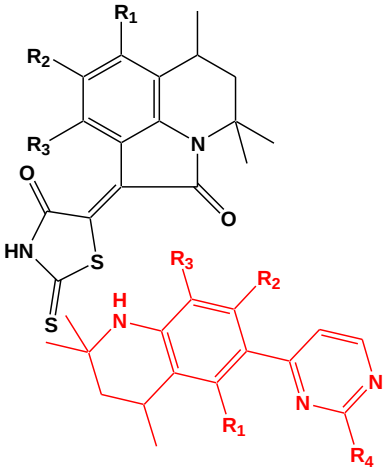
Derivatives of hydroquinoline, quinolinthiones, pyrimidines, quinazolines

Protein kinase inhibitors



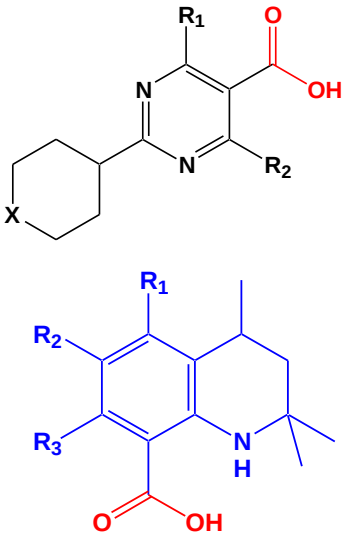
Derivatives of aminopyrimidines, aminoquinazolines, pyrroloquinolines

Anticoagulants



Hydroquinoline derivatives linearly bonded and fused to other heterocycles

Plant growth regulators

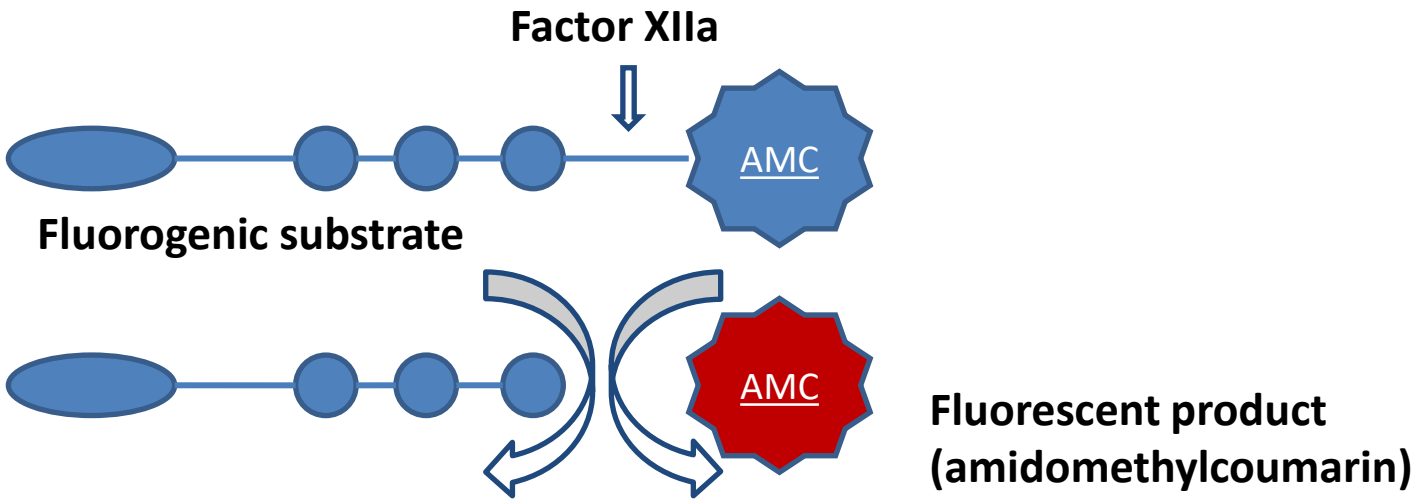


Derivatives of hetarylcarboxylic acids

In vitro verification

Experiments carried out in the laboratory of biophysics of the NMIC DGOI named after Dmitry Rogachev

Substrate hydrolysis reaction with coagulation factor XIIa



Factor XIIa: final concentration 5 nM

Substrate S2302 (Chromogenics, Italy): final concentration 200 μM

Test compounds: concentration 30 μM

A library of chemical compounds

Focused library of chemical compounds - 59 molecules – 326 3D-structures

Selection of compounds for experimental study

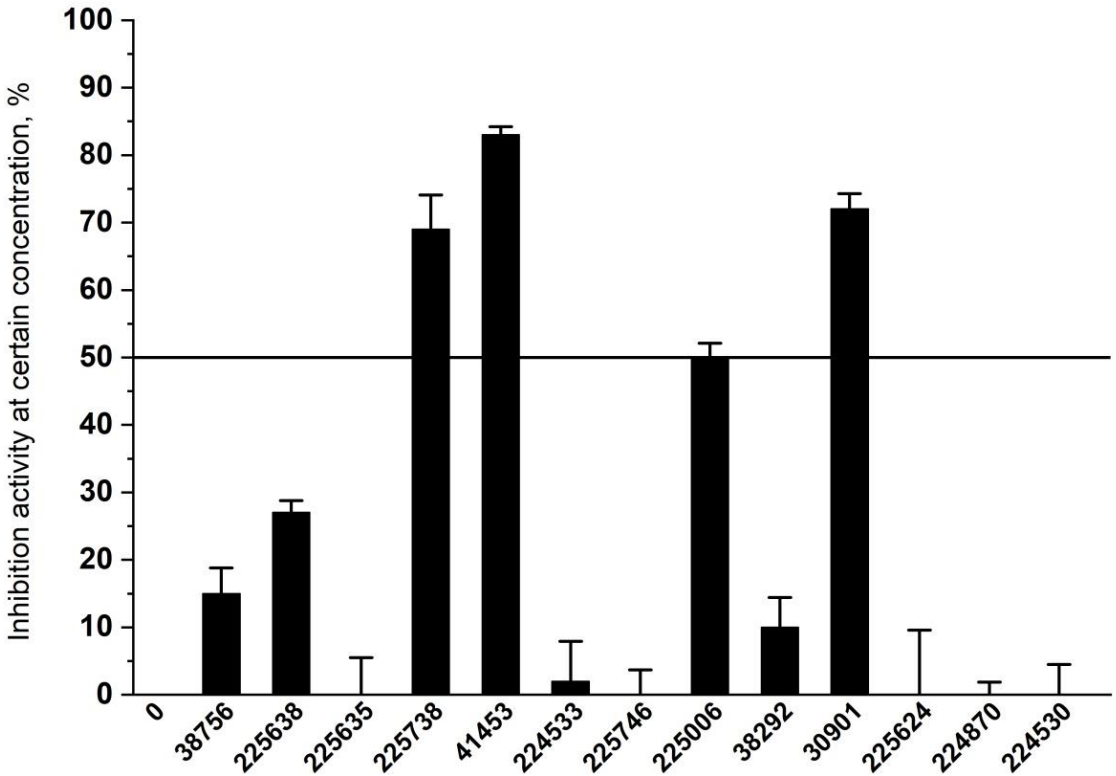
- Docking 326 conformers, 39 ligands were selected for $\boldsymbol{\Delta H}$ calculations, 13 compounds were selected for experiments: 4 compounds confirmed inhibition XIIa in experiments
- Selection criteria: native ligand docking: SOL score = -5.19 kcal/mol

Docking, SOL program: MMFF94 force field, Genetic algorithm

Population size 30 000; number of generations 1 000. For each ligand, 50 independent runs of the genetic global optimization algorithm were performed. The 50 solutions, best ligand poses, were grouped into clusters using a criterion RMSD < 1 Å; a reliable solution must have population of the 1st cluster > 10, 1st cluster contains ligand poses with most negative SOL score.

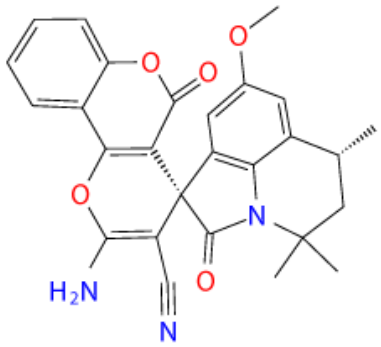
PM7+COSMO binding enthalpy $\boldsymbol{\Delta H}$ must be maximum negative

Experimental results for 13 selected compounds

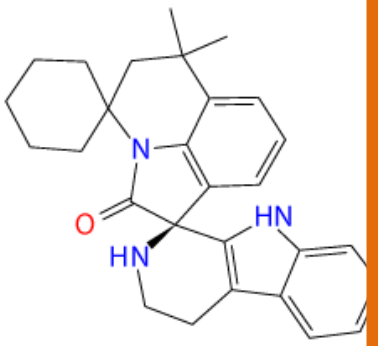


$$\text{Inhibitory activity} = \left(1 - \frac{\text{activity of XIIa with an inhibitor at a concentration 30 } \mu\text{M}}{\text{activity of XIIa without } \textit{inhibitor}}\right) \times 100\%$$

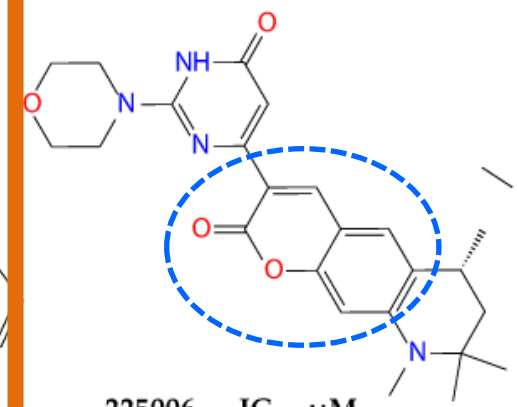
4 confirmed hits



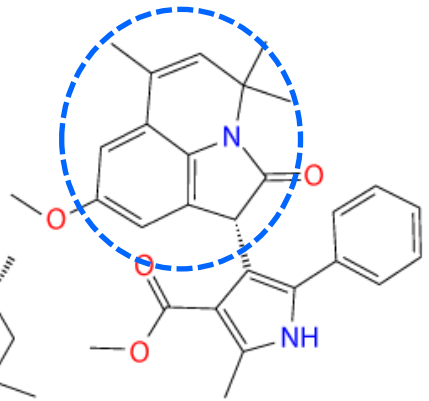
30901	IC_{50}, μM
XIIa	19.67±1.31
XIa	> 30.00
Xa	12.40±0.38



41453	IC_{50}, μM
XIIa	25.88±1.77
XIa	> 30.00
Xa	> 30.00



225006	IC_{50}, μM
XIIa	7.85±0.34
XIa	> 30.00
Xa	> 30.00



225738	IC_{50}, μM
XIIa	12.95±0.32
XIa	> 30.00
Xa	> 30.00

Anna Tashchilova, et al. Molecules (MDPI), 2022, **27**, 1234. https://doi.org/10.3390/molecules27041234

Chemical classes:
225006 – coumarin derivative
225738 – pyrroloquinoline

Database of chemical compounds

Large VSU database - more than 19 000 compounds (30 000 conformers)

Selection of compounds for experimental study

Docking > **30 000** conformers, SOL score < -6.00 kcal/mol; **423** ligands were selected for $\boldsymbol{\Delta H}$ calculations, **41** compounds were selected as best for experiments $\boldsymbol{\Delta H < -53}$ kcal/mol; additional filtering: clustering due to ligand similarity using DataWarrior software (similarity 0.85) ⇒ **25** clusters ⇒ **28** ligands, analysis of interactions, H-bonds, **16** compounds were selected for experiments: **7** compounds are weak inhibitors in experiments,

1 compounds confirmed noticeable inhibition XIIa in experiments:

41% XIIa inhibition at 30 µM

2 H-bonds

3 Halogen bonds

Chemical class: spiro-derivative of succinimide fused with tetrahydrofuran

Analogs of published XIIa inhibitors

100 compounds – analogs of recently published XIIa inhibitors: Marvin Korff, et al. Journal of Medicinal Chemistry 2020, 63 (21), 13159-13186

Selection of compounds for experimental study

- SOL score < -5.1 kcal/mol, 21 selected for experiments
- 3 compounds confirmed inhibitiors XIIa in experiments.
- They belong to two chemical classes:

triazolo-pyrimidine **triazolo-pyridine**

Conclusions

- **The initial search for inhibitors of the coagulation factor XIIa in the available database was carried out using the SOL docking program**
- **Selected compounds were tested in vitro**
- **Several experimentally confirmed hits were discovered**
- **Factor XIIa inhibitors found belong to 5 chemical classes**
  - Coumarin derivative
  - Pyrroloquinoline
  - Spiro-derivative of succinimide fused with tetrahydrofuran
  - Triazolo-pyrimidine
  - Triazolo-pyridine
- **Success of virtual screening using docking is determined by the content of the screened database of compounds**

Thank you for attention!

… Surely every medicine is an innovation; and he that will not apply new remedies, must expect new evils …

Francis Bacon
(1561-1626)
OF INNOVATIONS

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