SYNTHESIS, DOCKING, AND IN VITRO ANTICOAGULANT ACTIVITY ASSAY OF RHODANINE DERIVATIVES OF PYRROLO[3,2,1-ij]QUINOLIN-2(1H)-ONE AS NEW INHIBITORS OF FACTOR Xa AND FACTOR XIa

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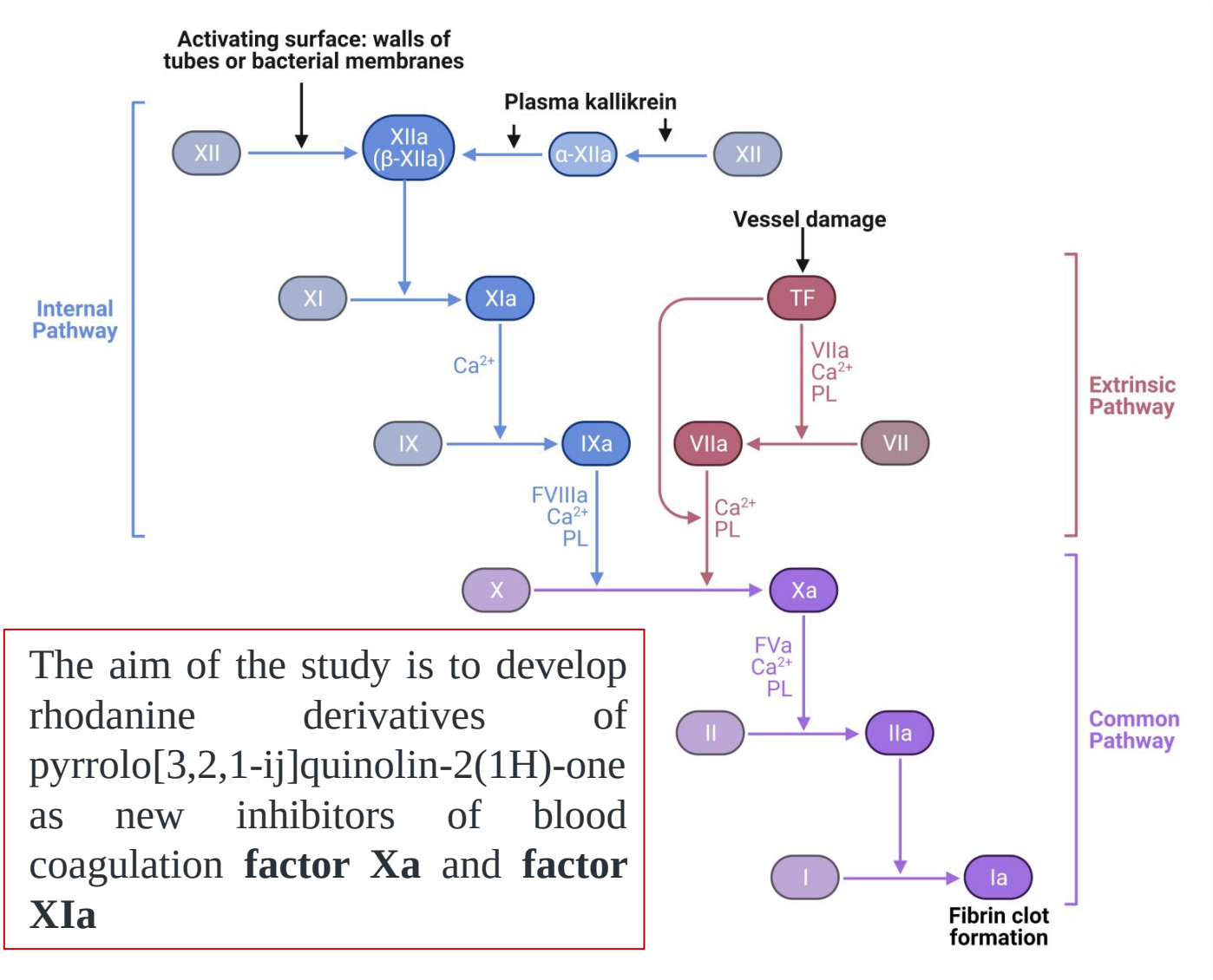
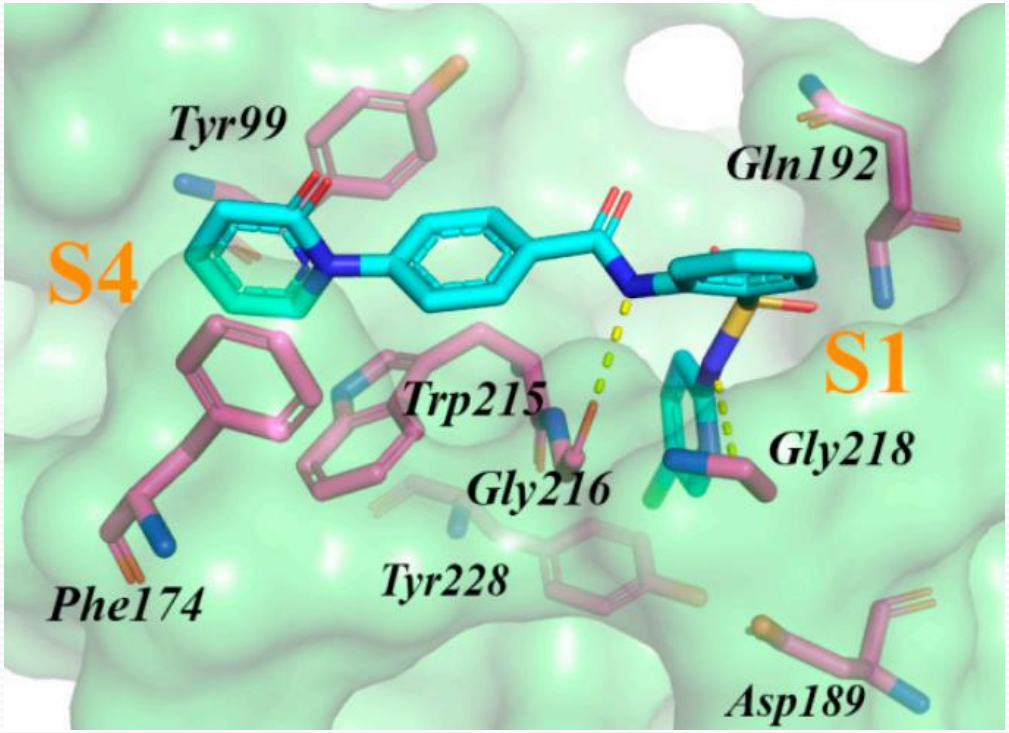
Introduction



The aim of the study is to develop rhodanine derivatives of pyrrolo[3,2,1-ij]quinolin-2(1H)-one as new inhibitors of blood coagulation **factor Xa** and **factor XIa**

Reactions of the plasma blood coagulation system

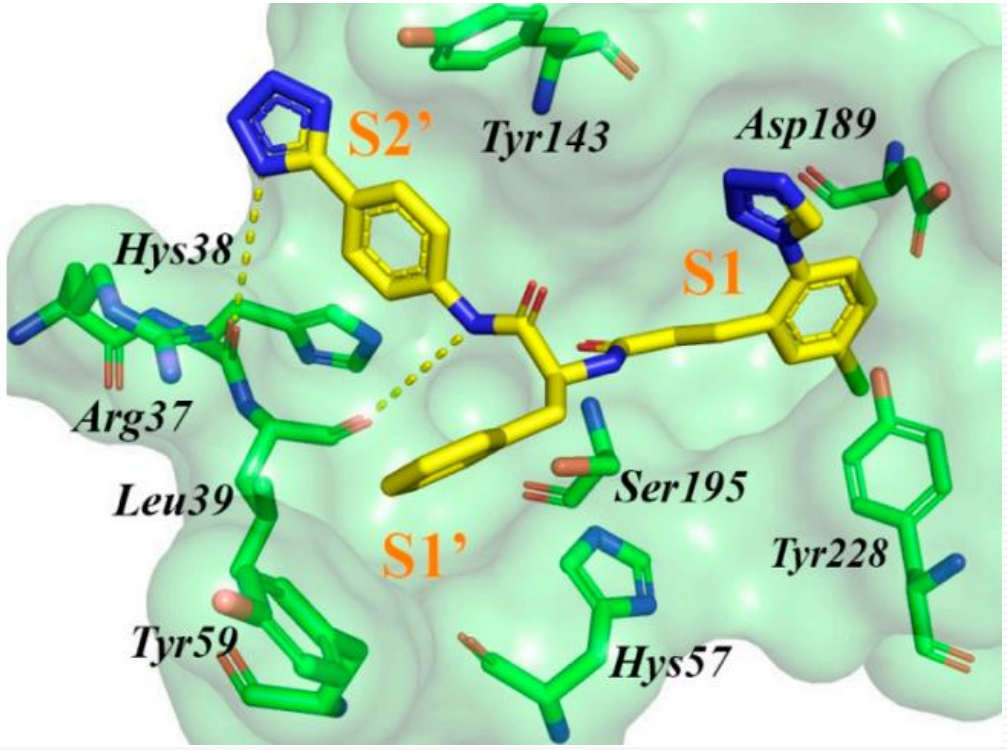
Factor Xa model



The active site of FXa with a bound nanomolar inhibitor (PDB ID: 3CEN). Hydrogen bonds are shown as yellow dotted lines. Buried residues are labeled by the lower font size.

- Selection of the structure of factor Xa from Protein Data Bank ID 3CEN
- adding hydrogen atoms using the APLITE program
- native ligand docking
- cross-docking
- development of selection criteria

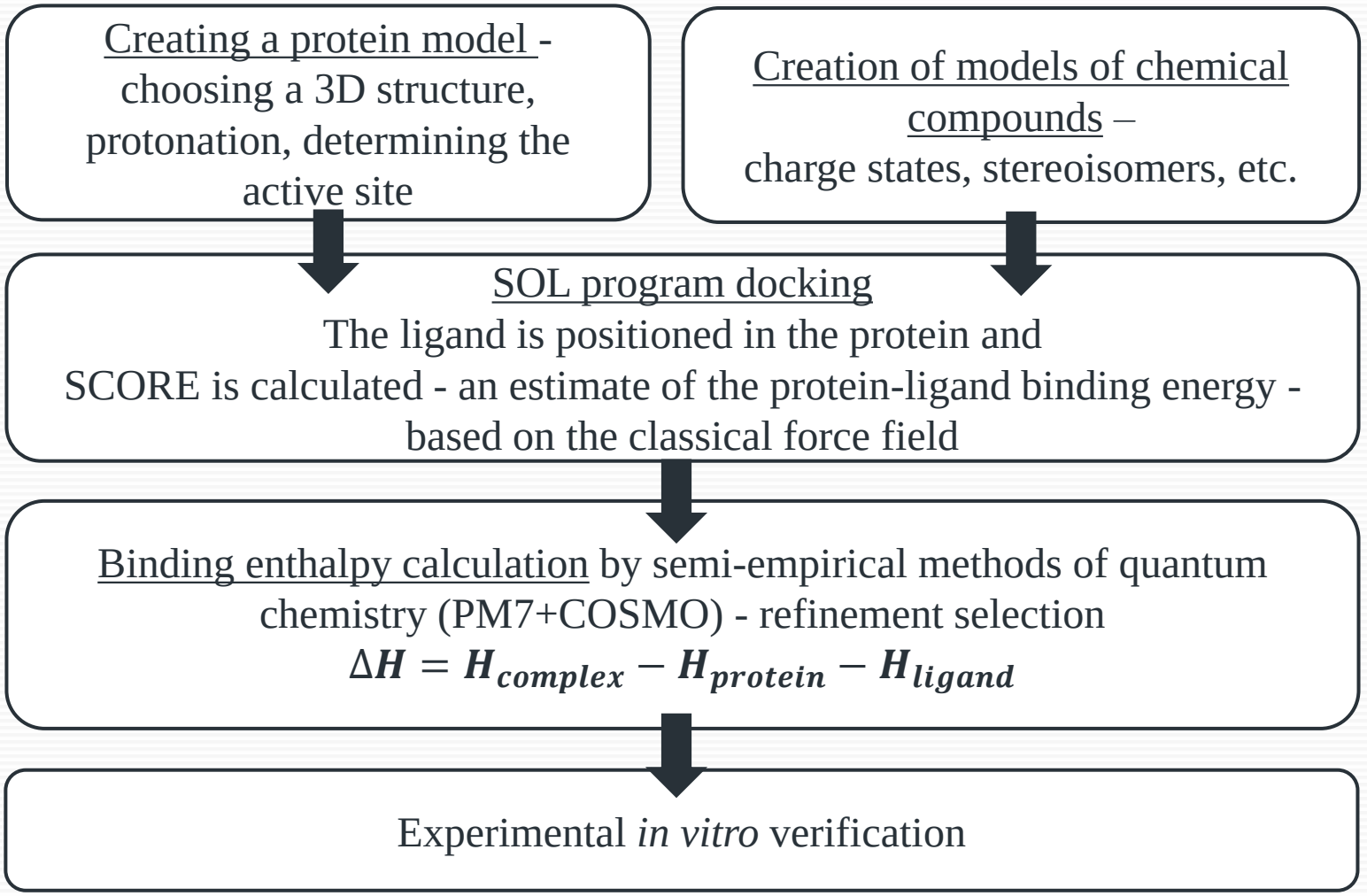
Factor XIa model



The active site of the FXIa with an inhibitor (PDB ID: 4CRC). Hydrogen bonds are shown as yellow dotted lines.

- Selection of the structure of factor XIa from Protein Data Bank ID 4CRC
- adding hydrogen atoms using the APLITE program
- native ligand docking
- development of selection criteria

Virtual screening

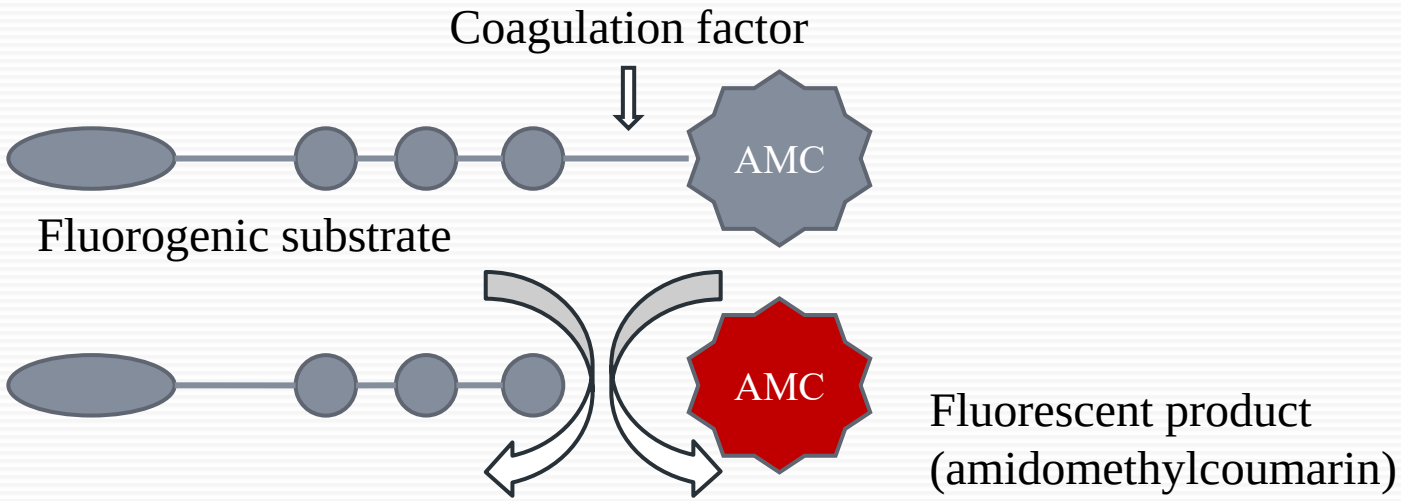


High values of accuracy and specificity, which have a value above 80%

In vitro verification

The experiments were carried out in the laboratory of biophysics of the Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology

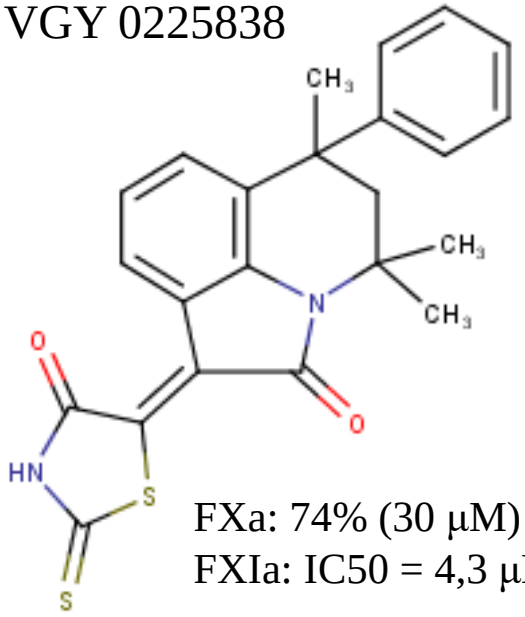
Substrate hydrolysis reaction with coagulation factors XIa and Xa



Experimental validation was carried out for all compounds from four databases. Compounds were considered active if, at a concentration of 30 μM, they reduced the activity of the coagulation factor by at least 50% compared to the basal activity.

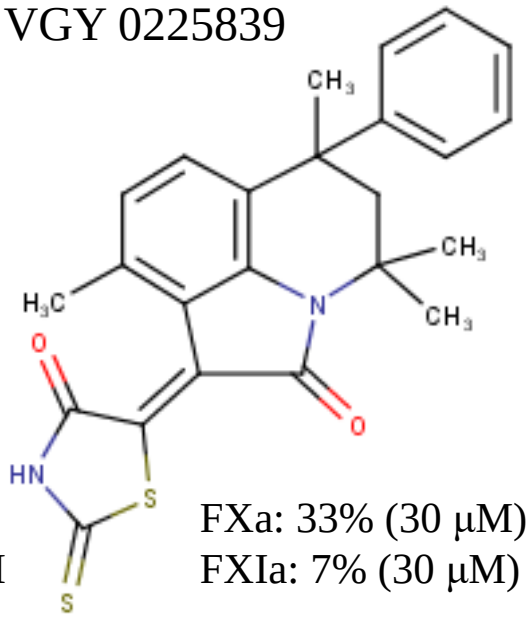
Synthesis of rhodanine derivatives

VGY 0225838



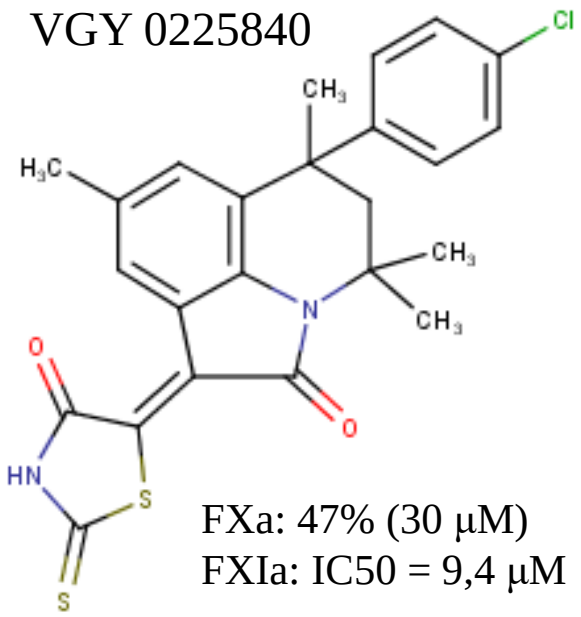
FXa: 74% (30 μM)
FXIa: IC50 = 4,3 μM

VGY 0225839

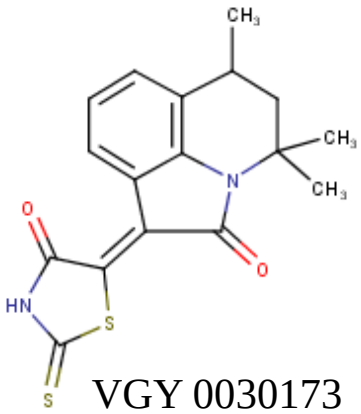


FXa: 33% (30 μM)
FXIa: 7% (30 μM)

VGY 0225840



FXa: 47% (30 μM)
FXIa: IC50 = 9,4 μM



VGY 0030173

The structure without substituents VGY 0030173 inhibits FXa specifically, in contrast to the precursor VGY 0225838, which specifically inhibits FXIa

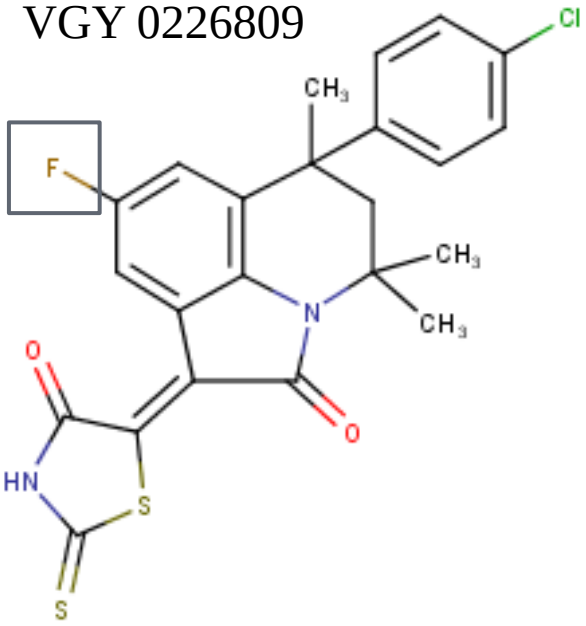
FXa: IC50 = 9,4 μM

FXIa: 22% (30 μM)

Synthesis of rhodanine derivatives

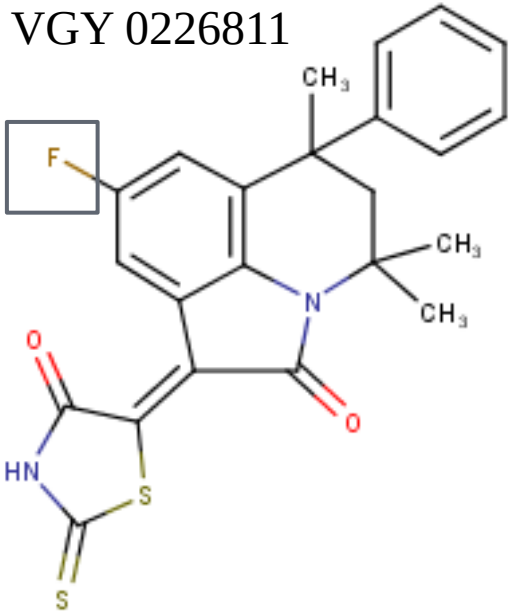
Addition of fluorine (VGY 0226809, VGY 0226811) and an acyloxy group (VGY 0031666) to the aromatic cycle of the tetrahydroquinoline series. This leads to the elimination of specificity, both factors are powerfully inhibited.

VGY 0226809



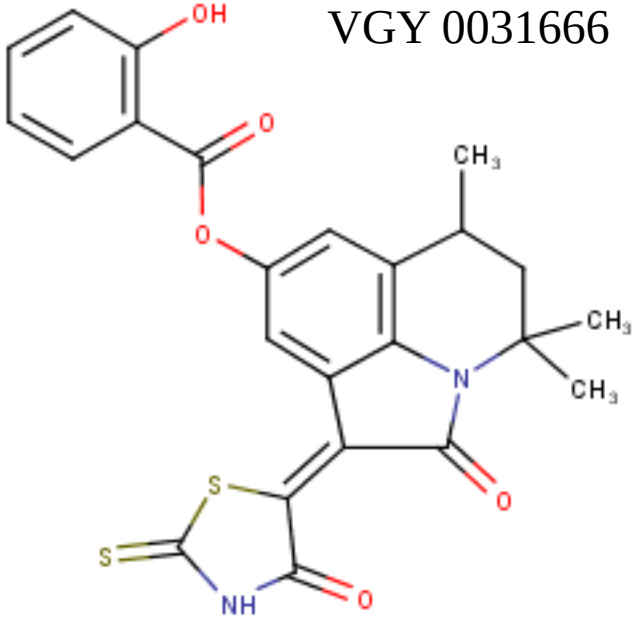
FXa: IC50 = 2,9 µM
FXIa: IC50 = 5,2 µM

VGY 0226811



FXa: IC50 = 3,7 µM
FXIa: IC50 = 5,0 µM

VGY 0031666

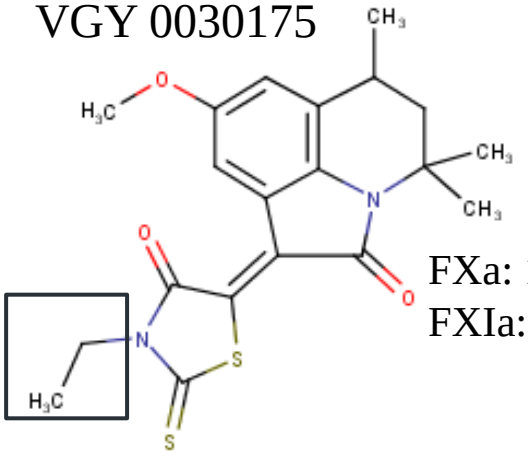


FXa: IC50 = 2,4 µM
FXIa: IC50 = 3,8 µM

Synthesis of rhodanine derivatives

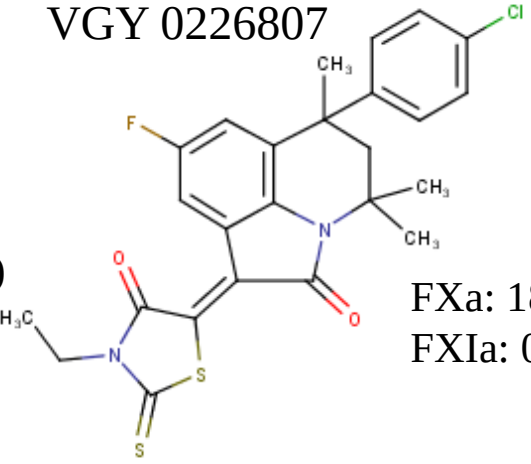
Addition of substituents at the endo-nitrogen atom in the rhodanine (thiazolone) cycle

VGY 0030175



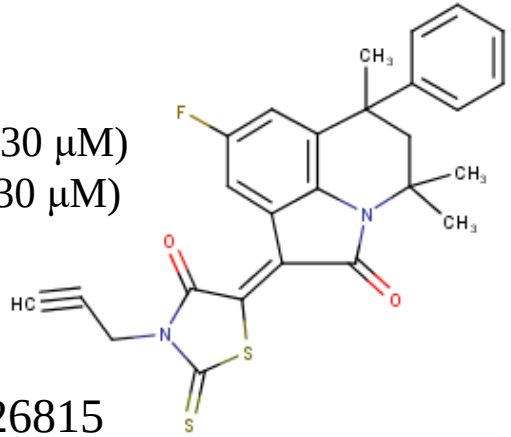
FXa: 18% (30 µM)
FXIa: 5% (30 µM)

VGY 0226807



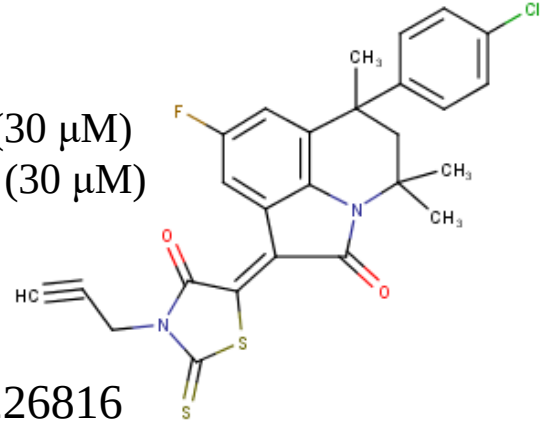
FXa: 18% (30 µM)
FXIa: 0% (30 µM)

FXa: 36% (30 µM)
FXIa: 3% (30 µM)



VGY 0226815

FXa: 49% (30 µM)
FXIa: 17% (30 µM)



VGY 0226816

Conclusion

Synthesis, virtual screening and in vitro testing were carried out for several dozen molecules - derivatives of rhodanines:

- The selection criteria for the experiment for coagulation factors Xa and XIa were validated and various metrics were calculated: accuracy, specificity and sensitivity
- Addition of fluorine and an acyloxy group to the aromatic cycle of the tetrahydroquinoline series leads to the elimination of specificity, both factors are powerfully inhibited
- Verification of inhibitory activity for all molecules against three targets: FXa, FXIa and thrombin, allowed the identification of 6 selective FXa inhibitors and 12 selective FXIa inhibitors