Small-molecule thrombin inhibitors based on derivatives of N-arylbenzamidines

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Introduction

Thrombin is an enzyme which belongs to the class of hydrolases from the group of serine proteases catalyzing the conversion of fibrinogen to fibrin in blood clotting. Searching for substances that can control the process of blood coagulation, particularly, by means of inhibition of thrombin is one of the main areas of medicinal chemistry.
At present argatroban is the only synthetic direct inhibitor of thrombin, used for intravenous introduction. Another medicine is dabigatran etexilate, a low molecular weight prodrug, which has been recently approved by the US Food and Drug Administration (FDA) for oral use. Thus, the development of new direct thrombin inhibitors is an important task.

Objective

In the present work, our main goal was developing a new class of compounds with anticoagulant activity based on derivatives of N-arylbenzamidines, and conducting preliminary safety and efficacy studies of synthesized compounds

Materials & Methods

1. Computer modeling
Virtual screening was performed using the original docking program FlexX that enables docking of a low molecular weight organic compounds and calculating the energy of inhibitor-enzyme interaction.
The crystal structure of thrombin was used as a target for screening and docking (PDB code 1O2G; http://www.rcsb.org/pdb). Virtual screening was performed using our own library of ligands. The criteria for selection of potential thrombin inhibitors were the highest value scoring function (predicted binding energy, kcal/mol).

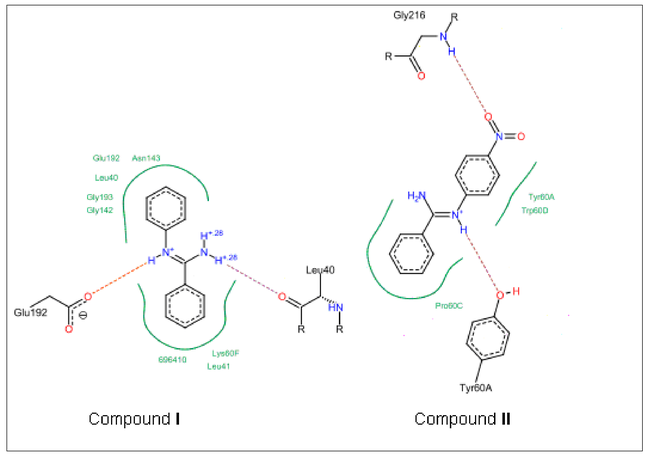
2. Acute toxicity

Acute toxicity was evaluated using express method for determination of LD_{50} on outbred mice.

3. Anticoagulant activity *in vivo*

Measuring of PT and APTT after repeated (5 days) intraperitoneal administration of selected compounds.

Results



After computer simulation several compounds were selected for further chemical synthesis and experimental verification of their activity. Among the selected compounds N-phenylbenzamidine (**I**) and N-4-nitro-phenylbenzamidine (**II**) demonstrated similar values of binding energy.
However, despite the similarity of compounds' **I** and **II** chemical structure, the conformation and location of these compounds regarding the active site of the enzyme differed significantly.

Results (cont.)

Compound **II** showed a similar interaction with the active site of the enzyme due to binding to the hydrophobic side chains of residues Trp 60A, Trp 60D, and also forms hydrogen binding with Gly 216 of thrombin active center. In contrast, the interaction diagram of compound **I** with the enzyme did not contain all of the interactions for the known inhibitors. Analysis of the results allowed us to assume that compound **II**, in contrast to **I,** may demonstrate anticoagulant activity.

To confirm this, compounds **I** and **II** were synthesized and studied for potential anticoagulant activity.

The results of biological studies had demonstrated that only compound **II** exhibited anticoagulant activity. Moreover this compound is less toxic than the ligand **I**. Anticoagulant activity has been manifested in increasing of the PT value among intact laboratory rats after repeated (5 days) administration of this compound.

Summary

The virtual screening allowed the accurate identification of the anticoagulant effect of compound **II**. Its biological activity and safety was confirmed by preliminary studies.

Conclusions

N-4-nitro-phenylbenzamidine (**II**), a compound that selected and synthesized after virtual screening, demonstrated sufficient level of safety and is of practical interest for further evaluation of its efficacy as direct thrombin inhibitor.

Compound I and compound II used for future modeling and structure optimization to understand structure-activity relationship.

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