ISSN 0006-2979, Biochemistry (Moscow), 2019, Vol. 84, No. 2, pp. 119-136. © Pleiades Publishing, Ltd., 2019.
Russian Text © A. S. Kabankin, E. I. Sinauridze, E. N. Lipets, F. I. Ataullakhanov, 2019, published in Biokhimiya, 2019, Vol. 84, No. 2, pp. 191-211.

REVIEW

Computer Design of Low-Molecular-Weight Inhibitors of Coagulation Factors

A. S. Kabankin[1,a], E. I. Sinauridze[1,2], E. N. Lipets[1,2], and F. I. Ataullakhanov[1,2,3,4,b]*

[1]*Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences, 119991 Moscow, Russia*
[2]*Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, 117997 Moscow, Russia*
[3]*Lomonosov Moscow State University, Faculty of Physics, 119991 Moscow, Russia*
[4]*Moscow Institute of Physics and Technology, 141701 Dolgoprudny, Moscow Region, Russia*
[a]*e-mail: rla2001@mail.ru*
[b]*e-mail: ataullakhanov.fazly@gmail.com*

Received September 5, 2018
Revised October 19, 2018
Accepted October 19, 2018

Abstract—The review discusses main approaches to searching for new low-molecular-weight inhibitors of coagulation factors IIa, Xa, IXa, and XIa and the results of such studies conducted from 2015 to 2018. For each of these factors, several inhibitors with IC_{50} < 10 nM have been found, some of which are now tested in clinical trials. However, none of the identified inhibitors meets the requirements for an "ideal" anticoagulant, so further studies are required.

DOI: 10.1134/S0006297919020032

Keywords: anticoagulant, low-molecular-weight inhibitor, coagulation factor, computer molecular design, molecular docking

Coagulation system is responsible for the maintenance of blood fluidity under physiological conditions, hemostasis (physiologic blood clotting) at the site of blood vessel injury, and lysis of clots after they fulfilled their role. Coagulation system disorders are one of the main causes of mortality and disability worldwide. Impairments of coagulation can trigger either uncontrolled bleeding, or thrombosis that might hinder blood supply and elicit multiple organ failure. Thrombosis is associated with various diseases, such as atherosclerosis, heart attack, stroke, trauma, surgery, etc. It has become increasingly evident now that coagulation system disorders require more attention because of increasing lifespan and population aging. In view of this, development of efficacious and safe anticoagulant agents has become a priority issue in medicine.

Coagulation system involves a complex cascade of enzymatic reactions that can be activated via both intrinsic and extrinsic pathways [1-3]. Proteins participating in coagulation reactions are called blood coagulation factors and designated by Roman numerals. Normally, they are present in the plasma in inert state and get activated via cleavage of one or more peptide bonds by an active factor that is higher in the cascade. Both pathways converge at factor Xa and then proceed by the same mechanism. Hemostasis (arrest of bleeding) occurs via the fibrin clot formation by polymerization of fibrin monomers generated by fibrinogen cleavage. Fibrinogen is hydrolyzed by thrombin (factor IIa) formed from prothrombin in a reaction catalyzed by factor Xa. Factor X is activated either by factor IXa or factor VIIa complexed with the tissue factor (VIIa–TF) (via extrinsic pathway). The VIIa–TF complex also activates factor IX. Tissue factor (TF) acts as a primary physiological activator in the coagulation system. It is present in the perivascular tissues and gets exposed to the blood upon the endothelial barrier damage – a process known as the extrinsic pathway. The intrinsic

Abbreviations: ADME, absorption, distribution, metabolism, excretion; ADMET (ADME/Tox), absorption, distribution, metabolism, excretion, toxicity; ATIII, antithrombin III; CoMFA, comparative molecular field analysis; CoMSIA, comparative molecular similarity indices analysis; DOACs, direct oral anticoagulants; DTIs, direct thrombin inhibitors; LBDD, ligand-based drug design; PASS, prediction of activity spectra for substances; QSAR, quantitative structure-activity relationships; SBDD, structure-based drug design; TF, tissue factor; TFPI, tissue factor pathway inhibitor.

* To whom correspondence should be addressed.

pathway is triggered when factor XII is exposed to foreign surfaces. Activated factor XIIa interacts with high-molecular-weight kininogen and induces further cascade of reactions resulting in sequential activation of factors XI, IX and X via their conversion into XIa, IXa and Xa, respectively.

Thrombin is an essential enzyme of the coagulation system. Besides being involved in fibrin formation, thrombin activates factors V, VIII, XI, XIII, protein C, and platelets and takes part in both positive and negative feedback loops. Thrombin accelerates its own production by 3-5 orders of magnitude by converting factors V and VIII to their active forms that interact with factors Xa and IXa, respectively, with the formation of prothrombinase (Xa–Va, phospholipid surface, Ca^{2+}) and intrinsic tenase (VIIIa–IXa, phospholipid surface, Ca^{2+}). However, thrombin complexed with thrombomodulin slows its own formation by activating protein C, an inhibitor of factors Va and VIIIa.

All active factors should be rapidly and irreversibly neutralized after coagulation is complete in order to avoid full blood clotting and to keep blood in a liquid state. This process is mediated by specific blood coagulation inhibitors (anticoagulants) targeting active coagulation factors. Among most significant physiological anticoagulants are antithrombin III (ATIII), heparin, tissue factor pathway inhibitor (TFPI), and proteins C and S. At present, several major anticoagulant drugs with different modes of action are used in clinical practice, including polymeric glycosaminoglycans heparins (low-molecular-weight and unfractionated) that augment the activity of the natural inhibitor ATIII; aspirin acting as a platelet aggregation inhibitor; hirudin and hirudin-like peptide bivalirudin, both inhibiting thrombin; vitamin K antagonists (VKAs), the most known of which is warfarin lowering the levels of vitamin K required for production of coagulation factors in the liver; and various low-molecular-weight inhibitors, such as argatroban, dabigatran, rivaroxaban, etc. that inhibit specific coagulation factors (Fig. 1). These compounds are quite efficient, but each of them has some serious limitations, which promotes continuous studies aimed at identification and development of efficacious and safe new anticoagulants [4, 5].

Heparins and warfarin act on multiple molecular targets, which is considered as their disadvantage because it does not allow to assess and predict their action on certain sections of the coagulation process. It is now commonly accepted that the priority in the anticoagulant development should be given to inhibitors selectively acting on individual coagulation factors. This is why the majority of researchers conduct studies in this direction.

The following low-molecular-weight anticoagulants have been approved for the use in clinical practice: dabigatran etexilate, rivaroxaban, apixaban, edoxaban, and betrixaban [6-12] (Fig. 1). The last four components on this list are direct oral selective inhibitors of factor Xa. Dabigatran etexilate is a prodrug that is converted into direct thrombin inhibitor dabigatran upon oral administration. Direct oral anticoagulants (DOACs) are superior to the well-known and broadly used warfarin because of the ease of administration, limited interference with other drugs and food, and lack of need for continuous laboratory monitoring. However, even these compounds are not ideal and should be used with certain limitations [8, 9, 12]. In addition, their administration is associated with an increased risk of gastrointestinal bleeding compared to warfarin. Although DOACs do not interact with the majority of drugs, they serve as substrates for cytochrome P450 (CYP3A4) and P-glycoprotein (P-gp). Inhibitors of these proteins elevate the DOAC concentration in the plasma strongly increasing the risk of hemorrhages. DOACs should not be used at all or administered with great caution in patients with renal and hepatic failure, individuals with artificial heart valves, children under 18 years old, and pregnant women. In the cases when DOACs still have to be used in such patients, their administration requires dose titration and laboratory monitoring, which may also pose some issues due to inapplicability of generally accepted methods for determining the anti-coagulant activity of DOACs and measuring their concentration in the blood plasma. Another disadvantage of DOACs is the lack of specific antidotes. However, it should be mentioned that idarucizumab (a monoclonal antibody fragment) was recently approved by the US Food and Drug Administration as a specific reversal agent for dabigatran [13]. Finally, the high cost of DOACs also prevents their use in medicine.

COMPUTER DESIGN AND DEVELOPMENT OF NEW DRUGS

The first stage in the design of any drug (including anticoagulants) involves discovery of an essentially novel basic chemical structure (lead compound) with the required biological activity. Then, the basic structure is optimized by synthesizing various derivatives of the lead compound and introducing modifications in order to augment the biological activity and to improve the pharmacological profile of the resulting substance. Today, the search for new basic structures is carried out mostly by *in silico* screening (molecular design). Depending on the available information, *in silico* screening may be performed in a number of ways [14-16].

Knowledge of the 3D structure of the active site in the target macromolecule (usually by X-ray structure analysis) allows us to directly search for or design potential ligands (new basic structures) that would fit the active site in accordance with the lock-and-key model. This approach, called the structure-based drug design (SBDD), relies on relatively complex molecular docking computational algorithms [17-20]. Molecular docking is

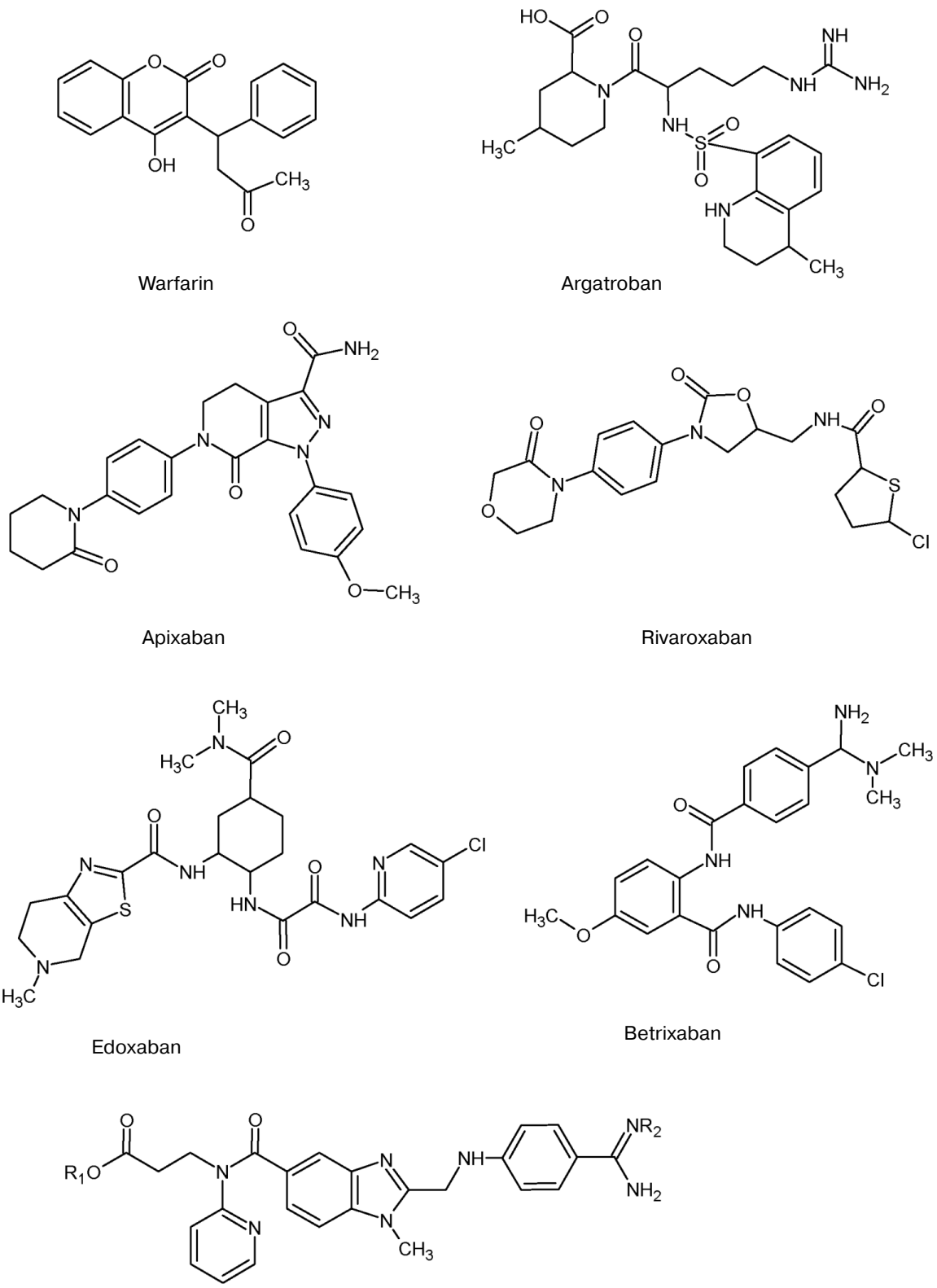


Fig. 1. Low-molecular-weight anticoagulants approved for clinical practice.

aimed at determining preferential conformation of a ligand molecule, as well as its most beneficial position in the active site, and estimating the ligand–receptor binding energy. Current docking algorithms account for the conformational mobilities of the protein and its ligands. One of the criteria for choosing the software for computer-aided molecular docking is the opportunity to mimic ligand orientation in the protein active site determined by the X-ray structure analysis of the protein–ligand complex. Estimation of the ligand–protein binding energy represents the most difficult task in the design of potential ligands. Despite existing methods for calculating the free energy based on molecular dynamics [21] and currently developed approaches that use statistical mechanics and quantum chemistry [22], the binding energy is more often approximated by using the scoring functions, such as empirical, force field-based, and knowledge-based (based on the X-ray structural data for the ligand–protein complexes). In the case of empirical scoring function, the binding energy is calculated as a sum of an arbitrary number of members reflecting relevant impact from each interaction involved in the ligand binding (hydrogen bonds, ionic interactions, lipophilic and aromatic interactions, desolvation, etc.). The weight coefficients for these members are calculated by multiple linear regression analysis using a training set of ligand–protein complexes with experimentally determined binding constants and structure. However, such approach represents the main drawback of using empirical scoring functions, since they might be correct only for proteins resembling those used in the training set. The force field-based scoring functions rely on the force fields used in molecular dynamics. Van der Waals interactions are described either by the Lennard–Jones potential or similar functions, whereas the Coulomb's law models describe electrostatic interactions. Lately, the knowledge-based scoring functions have become increasingly more common for evaluating the binding energy. The knowledge-based scoring functions allow us to avoid the fitting of the binding energy estimate to the experimental data and rely only on the structural data on the protein–ligand complexes. These data may be applied to evaluate the frequency of various types of atom–atom contacts at a given distance between the ligand and protein in all available data sets and to determine the pairwise interaction potentials between *i*-type-atoms of protein and *j*-type-atoms of ligand using statistical mechanics. Therefore, the total energy of interaction can be calculated as a sum of all energies of atom–atom interactions in the protein–ligand complex. The impacts of solvation and entropy are accounted for implicitly. Compared to the empirical and force-field-based scoring functions, knowledge-based scoring functions provide much broader opportunities to estimate the free binding energy in various protein–ligand complexes. Nonetheless, when molecular docking is used for screening of large datasets containing high variety of compounds and targets not used for calibrating the scoring functions, the overall accuracy of analysis is limited. Additional issues in molecular docking might be posed by the question whether bound water molecules, cofactors, and metal ions in the active site should be taken into consideration or ignored.

When the data on the spatial structure of a biological target are unavailable, but a large number of ligands for this target are known, it is possible to search for the new ligands indirectly, based on the assumption that the structure of a chemical compound is related to its biological activity. This relationship may be evaluated by analyzing the structure and biological activity of the known compounds with further extrapolation of the obtained data to new compounds in a process called ligand-based drug design (LBDD). Since the biological activity represents a cumulative effect of multiple processes, it is difficult to explicitly describe the relationship between the biological activity and molecular structure. As a rule, a simple mathematical function (e.g., polynomial) with coefficients calculated by the multivariate statistical analysis is used. In particular, a search for a function linking biological activity expressed in certain units (e.g., IC_{50}, a half maximal inhibitory concentration) to molecular structure relies on selection of means for presenting molecular structure of investigated compounds (molecular descriptors) and a mathematical method suitable for describing such relationship. By now, several thousands of various molecular descriptors and numerous mathematical methods have been proposed to describe the quantitative structure-activity relationships (QSARs) [23, 24]. Molecular descriptors are numerical characteristics reflecting structural features of various compounds, such as physical and chemical parameters obtained experimentally (e.g., solubility, partition coefficient, melting point, NMR chemical shift, etc.). However, molecular descriptors cannot be obtained for *any* possible settings. Therefore, the most common descriptors are theoretically calculated ones that can be predicted for all existing and hypothetical molecules. Theoretical descriptors are divided into several groups corresponding to different levels of molecular structure description: structural fragments, topological descriptors, quantum-chemical descriptors, spatial structural descriptors, and descriptors of intermolecular forces.

We would like to mention the PASS software (Prediction of Activity Spectra for Substances) developed at the Orekhovich Institute of Biomedical Chemistry, Russian Academy of Sciences, which provides an unparalleled array of evaluated biological activities for various drug-like molecules [25-27]. Based on the analysis of the structure-activity relationships in the training set containing the data on the structure and biological activity for more than 950,000 drugs and biologically active compounds, PASS software can predict more than 7000 types of biological activities (average accuracy, ~95%) for novel

untested organic substances. No data on the spatial conformation of investigated molecules are required, since routine structural formulas used in analysis are sufficient to automatically calculate individual molecular descriptors. Classification models used for description of biological effects linked to certain types of biological activity or differentiation of various types of biological activity hold an important place in computer-aided molecular design [23, 24]. In this case, the initial training set consists of groups (classes) of mutually exclusive chemical substances. The substances are grouped according to qualitative characteristics, e.g., active and inactive substances; or highly active, moderate, low active, and inactive substances. Other examples might be carcinogens and non-carcinogens; or highly toxic, low toxic and non-toxic substances, etc. Classification algorithms based on the training set analysis allow determining efficient decision rules for assigning new substances to the certain groups. In many cases, such methods are more convenient than regression because they do not require precise experimental biological activity data for classification of substances. Currently, a broad arsenal of classification methods exists: naive Bayes classifier, linear discriminant analysis, K-nearest neighbor classification, artificial neural networks, classification and regression tree, support vector machines (SVMs), decision trees, random forest, etc. [23, 24]. Resulting from the binary classification, the empirical Lipinski's rule of five was proposed that allows distinguishing between medicinal and non-medicinal substances [28]. This rule states that a drug-like molecule should meet to the following criteria: molecular weight less than 500 Da; the logarithm of the octanol–water partition coefficient (logP) no greater than 5; no more than 5 hydrogen bond donors in the molecule; no more than 10 hydrogen bond acceptors; no more than 10 free rotatable bonds; the polar surface area no greater than 140 Å^2. Today, Lipinski's rule of five and similar rules are commonly used as the cutoff criteria for excluding unsuitable molecules from the available chemical compound datasets.

Uncovering new chemical substances with a high affinity, required activity, and selectivity toward molecular targets is not sufficient *per se* to develop high-quality drug candidates for testing in preclinical studies. The selected substances should also have a set of physical, chemical, and pharmacokinetic properties ensuring their bioavailability. To evaluate the drug performance *in vivo*, it is crucial to assess the organism's response to this compound, including its absorption, distribution, metabolism, and excretion, collectively known as ADME properties. Later, toxicity was added, giving rise to ADMET (or ADME/Tox). At least 50% new chemical substances are withdrawn from the preclinical studies due to unsatisfactory ADMET properties. Low water solubility may limit drug absorption and result in the toxicity if the compound precipitates in the kidneys. Drugs should be lipophilic enough to penetrate through the membranes to reach the site of action. On the other hand, excessive lipophilicity implies low solubility, strong binding to proteins, and accumulation in body tissues and cells, which increases the risk of unwanted toxic effects. Prediction of compound properties as early as possible can substantially reduce financial expenses and time spent to design new drugs. This is why starting from the mid-1990s, special attention has been focused on creating computer-aided models utilizing physical, chemical, and structural parameters to predict water solubility, lipophilicity, absorption, metabolism, penetration through the blood-brain barrier, and the other parameters, underlying drug delivery to the site of action and drug toxicity [29-31]. The results and the perspectives gathered from such studies have been discussed elsewhere [32, 33].

To conclude the above-mentioned, none of the virtual screening techniques (SBDD and LBDD) provides unprecedented efficacy or demonstrates unequivocal superiority; both these approaches have advantages and flaws. At present, multicriteria optimization is considered as the most rational strategy that implies that the drug affinity to its biological target should be optimized simultaneously with the optimization of ADMET properties.

Computer design has been successfully used in searching for inhibitors of blood coagulation factors. Because factor Xa and thrombin play a central role in the coagulation cascade, most attention has been paid to the development of their selective inhibitors. As early as in 1990s, studies using computer-aided design to find thrombin inhibitors were published [34]. In our review, we will focus only on the recent studies describing low-molecular-weight inhibitors of blood coagulation factors.

NEW THROMBIN INHIBITORS

Thrombin belongs to serine protease family; its active site has three pockets – S1, S2, and S3. Negatively charged aspartate residue (Asp189) is located at the bottom of the deep and narrow S1 pocket. The S2 pocket contains proline and glycine residues, whereas the flat S3 pocket has Leu99, Ile174, and Trp215 and is accessible to solvents. Inhibitor molecule fragments (called motifs, similarly to protein structures) residing in these pockets are designated as P1, P2, and P3, respectively. Along with the active site, thrombin also contains two anion-binding sites, one of which (exosite 1) is located close to the catalytic portion of the active site; it accounts for fibrinogen binding and ensures thrombin high proteolytic activity. The second site (exosite 2) is located on the opposite side of the protein globule and binds heparin and other polysaccharides, as well as fibrinogen γ′-chains.

The review of Kong et al. published in 2014 presented patent information on direct thrombin inhibitors (DTIs) proposed in 2002-2012 [35]. It was found that the

greatest activity was exhibited by low-molecular-weight inhibitors containing the D-Phe-Pro-Arg (P3-P2-P1) tripeptide analogs that could bind to the amino acid residues in the thrombin active site. Typical DTIs contain highly basic functional groups in the P1 position: guanidine (in argatroban), benzamide (in dabigatran), alkylamine, or 4-aminopyridine. However, these compounds have low oral bioavailability because basic amines are strongly protonated at low gastric pH values. The strategy to overcome this drawback includes administration of pro-drugs (e.g., dabigatran etexilate) bearing weakly basic or nonbasic groups in P1 position, Later, it was shown that the presence of highly basic functional group in P1 is not a necessary condition for the anti-thrombin activity. Introduction of heterocycle-substituted chlorophenyl moiety into P1 motif resulted in active compound **1** (Table 1) with IC_{50} of 4 nM. The P2 motif in the tripeptide also plays an important role in thrombin inhibition, as well as in the compound oral bioavailability. Introduction of 3-aminopyrazinone in the P2 motif resulted in compound **2** (Table 1) with the inhibition constant K_i = 5.2 nM. The inhibitory action of this compound involved hydrogen bond formation between 3-aminopyrazinone and thrombin Gly216 residue. In their review, Kong et al. [35] mostly discussed thrombin peptide inhibitors isolated from blood-sucking insects, ticks, leeches, snake and wasp venoms, and skin secretions of toads.

He et al. [36] summarized the data on orally active thrombin inhibitors developed since 2010 by chemical synthesis or modification of natural flavonoids extracted from plants. The structural formulas and activities for the top thrombin inhibitor compounds **3-7** mentioned by He et al. are shown in Table 1. Compounds **4-6** selectively inhibited thrombin over trypsin (compounds **4** and **5**) or factors VIIa, IXa, Xa, and XIIa (compound **6**). Orally active compound **3** (RWJ-671818) selectively inhibiting thrombin (K_i = 1.3 nM) over other coagulation factors was tested in clinical trial.

He et al. [36] also discussed allosteric thrombin inhibitors. It was shown that ligand binding to the thrombin exosites 1 or 2 might induce conformational changes in the enzyme active site. Thus, thrombomodulin binding to the exosite 1 shifts thrombin substrate specificity from fibrinogen to protein C. Because the use of available thrombin inhibitors is associated with the risk of developing hemorrhages, allosteric thrombin inhibition via its exosites 1 or 2 might be an alternative of direct competitive inhibition by providing the opportunity of control by incomplete inhibition of the exosite. Several dozens of monosulfated derivatives of benzofuran and its di-, tri-, and tetrameric homologs have been synthesized and tested as allosteric thrombin inhibitors targeting the exosite 2, the most active being the benzofuran trimeric homolog (IC_{50} = 0.67 μM). However, neither of these allosteric inhibitors was orally active [36].

Numerous chemical compounds capable of thrombin inhibition have been identified. These compounds possess the same or very close molecular scaffolds but different substituents, so that replacement of one or several of these substituents might significantly alter the antithrombin activity of the compound without markedly affecting its overall molecular structure. Therefore, elucidation of the molecular structure features responsible for the biological activity is essential for successful molecular design of active compounds.

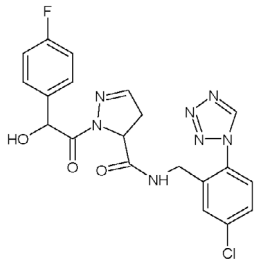
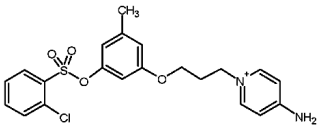
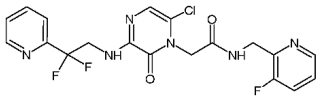
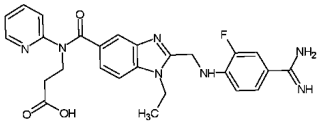
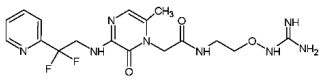
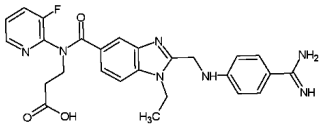
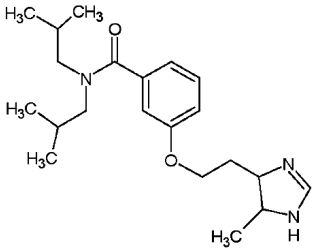
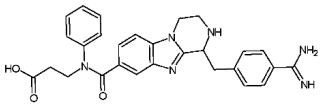
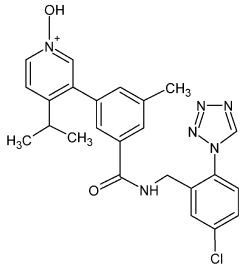
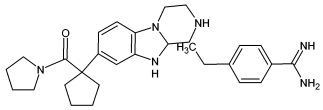
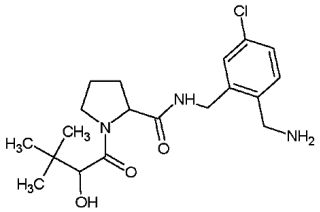
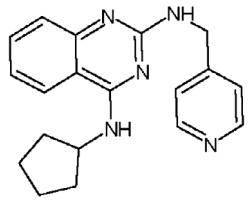
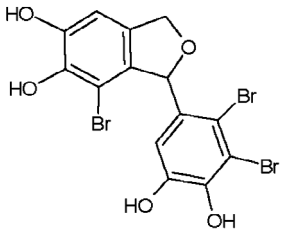
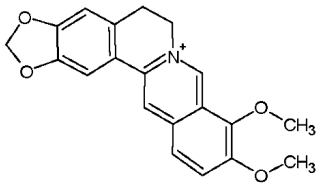
Mena-Ulecia et al. [37] examined differential activity of 177 non-peptide thrombin inhibitors with eight distinct molecular scaffolds based on the molecular structure and activity data obtained in earlier studies. The authors first confirmed that the Glide software for molecular docking was able to correctly reproduce the crystal structures of five known thrombin–ligand complexes (PDB IDs: 1T4U, 1T4V, 3C27, 2R2M and 3LDX). Using the Glide software, preferred conformation and best-energy orientation in the thrombin active site were determined for each of the remaining 172 inhibitors. Then, using the optimal ligand conformations determined by molecular docking, the predictive QSAR model was built using the CoMSIA (Comparative Molecular Similarity Indices Analysis) module of the Sybyl 7.3 software suite. For this, analyzed compounds were randomly divided into the training (134 compounds) and testing (34 compounds) sets. The activity of the test compounds was predicted based on the developed model and depicted as a plot showing the relation between calculated and experimental (published before) activities of the test compounds [37].

Sinauridze et al. [38] used the earlier determined orcinol scaffold [39] to ideintify a group of novel highly efficient thrombin inhibitors by selecting the P1 fragment for the binding to the thrombin S1 pocket using the SOL docking software. As the charged P1 fragment, the tested molecules contained isothiouronium, 4-aminopyridinium, or 2-aminothiazolinium [38]. The K_i values for these compounds in buffered solution ranged from 0.21 to 5.7 nM; the IC_{50} values in the plasma – from 0.1 to 1.24 μM. The structure of the most active compound (compound **8**) is shown in Table 1.

Many drugs developed in recent years contain fluorine atoms or fluorine-containing groups [40]. Fluorination changes the pharmacokinetic properties of compounds, mainly resulting in their higher bioavailability due to the increased permeability across the cell membrane. Fluorination can block drug metabolic pathways at various levels by altering the metabolism rate, as well as improving the metabolic stability.

Li et al. synthesized a series of new 2,5-substituted 1-ethyl-1H-benzimidazole fluorinated derivatives [41] and experimentally demonstrated that these compounds inhibited thrombin *in vitro*. Moreover, some of them were more active than argatroban, with the highest activity

Table 1. Novel inhibitors of thrombin

No.	Structural formula	Activity	References	No.	Structural formula	Activity	References
1		IC_{50} = 4 nM	[35]	8		K_i = 0.21 nM	[39]
2		K_i = 5.2 nM	[35]	9		IC_{50} = 3.39 nM	[41]
3		K_i = 1.3 nM	[36]	10		IC_{50} = 3.52 nM	[42]
4		K_i = 3.5 nM	[36]	11		IC_{50} = 7.48 nM	[43]
5		K_i = 0.77 nM	[36]	12		IC_{50} = 82.8 nM	[44]
6		K_i = 1.5 nM	[36]	13		IC_{50} = 0.019 μM	[45]
7		IC_{50} = 1.03 nM	[36]	14		IC_{50} = 2.92 μM	[46]

found for compound **9** (Table 1), whose molecular structure differs from dabigatran structure by the presence of fluorine atom in the benzene ring. The IC_{50} value for compound **9** was 3.39 nM, which was comparable to that of dabigatran (IC_{50} = 2.61 nM). Most synthesized active compounds contained benzene ring instead of the pyrimidine ring. Introduction of electron-donor substituents into this ring decreased the anticoagulant activity of the resulting compounds, while introduction of electron-acceptor substituents increased it. The position of the substituent in the ring also affected the compound activity, which declined as follows: *meta-* > *ortho-* > *para-*.

Chen et al. [42] synthesized a series of dabigatran structural analogs, in which pyridine ring was replaced by the benzene ring with fluorine atoms introduced at various positions. Evaluation of the *in vitro* activity of the obtained compounds revealed that the most active among them (IC_{50} = 3.52 nM) was compound **10** (Table 1) containing fluorine atom at the *ortho*-position of the benzene ring. When fluorine was introduced at the *meta*-position, the inhibitory activity of the resulting compound decreased approximately 2-fold, while introduction of fluorine at the *para*-position produced virtually no effect on the compound activity. Introduction of the -CF_3 group into the benzene ring at the *ortho-*, *meta-*, and *para*-positions reduced the inhibitory activity 2-fold, 7-fold, and up to 60-fold, respectively [42].

Chen et al. [43] also analyzed the antithrombin activity of dabigatran analogs, in which the benzamidine was replaced with the tricyclic scaffold. The IC_{50} value of the most active compound **11** was 7.48 nM. Based on this compound, 22 derivatives were synthesized and examined, some of which exhibited sufficiently strong inhibitory effect on the thrombin-induced platelet aggregation [43].

The antithrombin inhibitory activity was assessed for 10 derivatives of 4-((1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyrazin-1-yl)methyl)benzimidamide [44]. Compound **12** with IC_{50} = 82.8 nM was chosen as the lead compound to synthesize a series of derivatives, most of which inhibited thrombin-induced platelet aggregation (max. IC_{50} = 1.95 μM).

Lee et al. [45] used the thrombin structure (PDB ID: 2CF9-H) to perform the high-throughput virtual screening of ZINC15 database with the DOCK3.6 software. The authors identified new compound **13** (JJ1) that displayed the antithrombin activity and high selectivity both *in vitro* and *in vivo*. Compound **13** had a novel scaffold that differed from those previously described for thrombin inhibitors. Comparing molecular structures of compound **13** and 2038 non-peptide thrombin inhibitors retrieved from the Binding DB database revealed that the average chemical similarity between compound **13** and these inhibitors assessed using the pairwise Tanimoto coefficient (Tc) was 0.12 ± 0.03, thus evidencing a high novelty of compound **13** [45].

New thrombin inhibitors have been also searched for among compounds isolated from natural sources, e.g., herbs used in traditional Chinese medicine (TCM). Wang et al. [46] applied a pharmacophore model (a set of stereo-electronic parameters necessary for optimal ligand–target interaction) and molecular docking for virtual screening of a library containing 23,023 compounds used in TCM. Using the pharmacophore generation module, 10 models were generated based on six moderately different compounds with IC_{50} ranging from 0.1 to 73 nM. The pharmacophore models were validated by using a database including 100 experimentally identified thrombin inhibitors and 256 inactive compounds. The pharmacophore model that most accurately identified the active compounds (92%) contained one H-bond acceptor, one aromatic ring, and one hydrophobic group. Using this model to screen the TCMD 2009 database (23,023 compounds) allowed to identify 93 compounds, which were then tested by molecular docking (Surflex-Dock software) using the thrombin crystal structure. Twenty-three compounds were synthesized and assayed for the antithrombin activity. The compound with the highest inhibitory activity was berberine (**14**) with IC_{50} = 2.92 μM [46].

Based on the above data, it can be concluded that thrombin inhibitors from different classes of chemical compounds are characterized by specific structure-activity relations or the impact produced by substituents in certain groups on the compound activity, thus allowing optimization of a search for the most promising compounds. However, the same molecular groups might have different impact on the compound properties and activity when introduced in different molecular environment. Therefore, it is impossible to design a novel active inhibitor based on individual conclusions regarding the effects of various chemical groups in a particular environment. This problem can be solved either by using previously identified structure-activity relationships in a certain class of chemical molecules and then seeking for novel more active compounds within the same class or by searching for new scaffolds in available compound databases by using high-throughput virtual screening followed by structural modification of the hit compounds for identification and optimization of the most active among them.

NEW FACTOR Xa INHIBITORS

Factor Xa is a serine protease consisting of the light (139 a.a.) and heavy (303 a.a.) chains bound via a disulfide link. The ligand-binding site is located in the heavy chain and composed of four pockets (S1, S2, S3 and S4) that bind the P1, P2, P3, and P4 inhibitor motifs, respectively. Because factor Xa acts upstream of thrombin in the coagulation cascade, its specific inhibitors might downregulate coagulation more efficiently compared to throm-

bin inhibitors. Currently, four factor Xa inhibitors [6-10, 47] have been approved for clinical practice: rivaroxaban, apixaban, edoxaban, and betrixaban. However, we have already mentioned that these drugs exhibit side effects and have contraindications, thus instigating a search for new efficient and safe factor Xa inhibitors.

Patel et al. [48] comprehensively reviewed the studies conducted mainly before 2015 and aimed at the development of new factor Xa inhibitors. In particular, the authors discussed structural formulas of chemical groups used as the central scaffold (11 groups), P1 motif (25 groups), and P4 motif (25 groups). They also presented the data deposited since 2010 in the PDB on the crystal structures of factor Xa complexes with 33 different inhibitors [48]. For this reason, we will discuss the studies of factor Xa inhibitors published after 2015.

Virtual screening of two molecular libraries, NCI Diversity database (USA) containing 1888 compounds and the database of the Voronezh State University, Faculty of Chemistry, containing 14,271 compounds, was performed by using the SOL docking and DISCORE post-docking software [49]. The structure of factor Xa in its complex was retrieved from the PDB (PDB ID: 3IIT). The majority of compounds (12 out of 17) selected as a result of screening inhibited factor Xa at the micromolar concentrations *in vitro*. Based on the obtained data, a new compound (**15**) was synthesized (Table 2) that inhibited factor Xa *in vitro* with IC_{50} = 0.7 μM. In addition, the nature of compounds interacting with the S1, S2, S3 and S4 pockets in the factor Xa binding site was discussed.

Based on the data of earlier studies, Yang et al. [50] synthesized a new series of 3,4-diaminobenzoyl derivatives and tested *in vitro* activity against factor Xa. It was found that the majority of these compounds exhibited good or excellent inhibitory activity. Analysis of the structure-activity relationship in the obtained inhibitors allowed to uncover the role of the chemical structure of P1 and P4 on the compound activity, resulting in the discovery of a new active highly selective direct inhibitor of Xa (compound **16**) (Table 2). The *in vivo* activity of compound **16** in rats was comparable to that of rivaroxaban. The activity of the tested compounds increased with the increasing volume of the P1 fragment and decreased upon substitution of the benzene ring with pyridine or thiophene ring. Substitution of the pyridine ring in P4 with lactam ring also decreased the anti-coagulant activity of the resulting compound. The carboxamide group position in the central ring of compound **16** strongly affected its activity. The most beneficial location was at position 5 (Table 2), which ensured formation of the hydrogen bonds between this group and Glu146, Gly216, and Gly218 residues. Substitution of the carboxamide group with ester or carboxylic acid groups reduced the anti-coagulant activity.

Similar analysis of the structure-activity relationship for synthesized anthranilamide derivatives resulted in the discovery of an active oral factor Xa inhibitor (**17**) with IC_{50} = 8.7 nM [51]. The impact of the chemical structure and position of substituents in P1 (Table 2) on the compound ability to inhibit factor Xa was analyzed as well. It was shown that 4-substituted vs. 3-substituted derivatives possessed higher inhibitory activity. Compounds containing small lipophilic substituents at position 4 (chlorine, methyl or methoxyl groups) were highly active, whereas compounds with bulky substituents (ethyl or isopropyl groups) demonstrated significantly lower activity. Substitution of 2-aminopyridine with other cyclic systems (3-aminopyridines, 5-membered heterocycles, bicyclic groups) resulted in a considerably reduced inhibitory activity.

Using experimentally determined activities of tricyclic oxazolidinone inhibitors of factor Xa with the general formula shown in Fig. 2, Xu et al. [52] developed and examined a 3D model for quantitative structure-activity relationship (QSAR – 3D-QSAR) based on the combination of various molecular modeling approaches (CoMFA, CoMSIA, molecular docking, molecular dynamics). The initial data set (38 compounds) was divided into two groups: training set (31 compounds) used to create the model and testing set (7 compounds). The model built using the CoMFA (Comparative Molecular Field Analysis) approach was characterized by the statistical parameters R^2 = 0.984 and Q^2 = 0.511, whereas the model developed with CoMSIA (Comparative Molecular Similarity Indices Analysis) gave rise to R^2 = 0.993 and Q^2 = 0.700, where R^2 is the correlation coefficient for the training set and Q^2 is the correlation coefficient calculated by the leave-one-out cross-validation. Both models satisfactorily reproduce experimental pIC_{50} ($-\log IC_{50}$) values for the testing set compounds. The authors also used molecular docking and molecular dynamics methods to thoroughly examine probable ways for ligand binding in the protein active site (protein structure: PDB ID 2W26).

Wang et al. [53] synthesized four series of factor Xa inhibitors by substituting P1, P2, and P4 groups in apixaban. The inhibitory activity of these compounds was

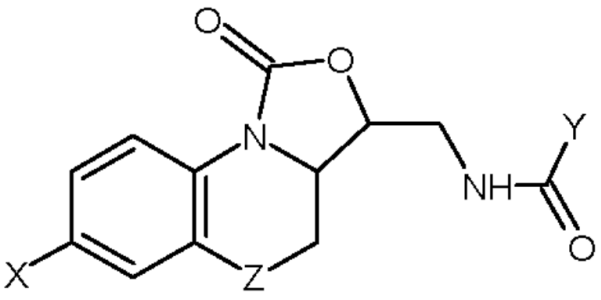
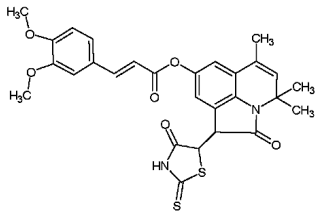
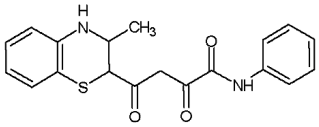
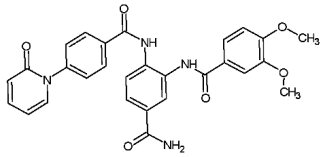
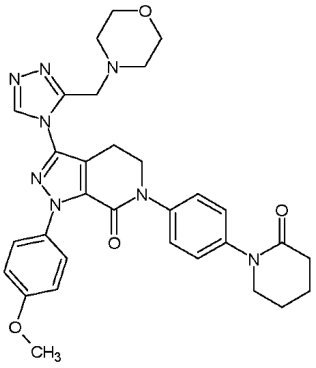
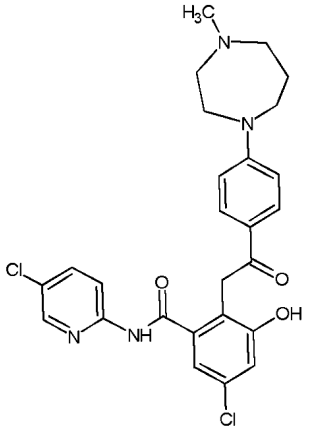
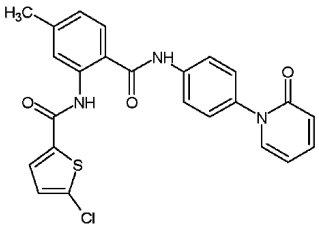
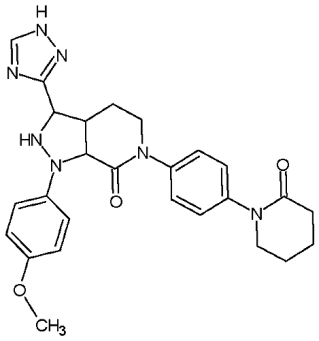
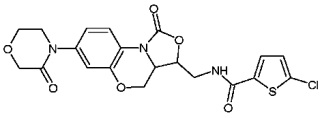
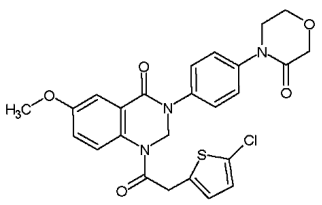


Fig. 2. General formula of tricyclic oxazolidinone inhibitors of factor Xa.

Table 2. Novel inhibitors of factor Xa

No.	Structural formula	Activity	References	No.	Structural formula	Activity	References
15		IC_{50} = 0.7 μM	[49]	20		IC_{50} = 2.02 nM	[56]
16		IC_{50} = 17.1 nM	[50]	21		IC_{50} = 0.14 μM	[57]
17		IC_{50} = 8.7 nM	[51]	22		IC_{50} = 25 nM	[58]
18		IC_{50} = 0.15 μM	[53]	23		IC_{50} = 3.33 nM	[59]
19		IC_{50} = 21 nM	[54]				

assessed *in vitro* in human and rabbit plasma. The most active inhibitor was compound **18** (Table 2) with IC_{50} = 0.15 μM. Substitution of the P1 methoxyl group with various electron-acceptor groups resulted in reduced inhibitory activity in the following order: $-OCH_3 \rightarrow -F \rightarrow -OCHF_2 \rightarrow -Cl \rightarrow OCF_3 \rightarrow -Br$. As a rule, introduction of substituents at various positions into the triazole ring in P2 also decreased the inhibitory activity.

A series of novel 2,3-dihydroquinazolin-4(1H)-one derivatives was synthesized in [54]. The majority of these compounds exhibited a pronounced *in vitro* inhibitory activity against factor Xa, the most active being compound **19** (Table 2) with IC_{50} = 21 nM (the IC_{50} value of compound **19** for thrombin was as high as 67 μM, which indicated its high selectivity to factor Xa).

In order to identify new factor Xa inhibitors, Pu et al. [55] conducted virtual screening of the SPECS database containing ~220,000 compounds. At the first stage, the authors applied the Lipinski's rule of five to distinguish between drug-like vs. non-drug-like substances (see above), which resulted in a hit list containing only 75,671 compounds that were then screened with a pharmacophore model. A pharmacophore is a speculative stereochemical structure complimentary to the active site of a particular receptor and adopted by active molecules for the optimal interaction with this receptor. The pharmacophore module from commercially available Discovery Studio Version 4.0 (DS 4.0) package was used to generate the pharmacophore model. A set consisting 24 factor Xa inhibitors retrieved from available publications was built: the training set contained 10 compounds, whereas the remaining 14 compounds were included into the testing set to generate 10 pharmacophore models. The use of the test compounds allowed choosing the best model to be applied in the virtual screening. As a result, only 10,000 compounds were chosen for molecular docking (with GOLD 5.2.2 package). After this procedure, 200 compounds remained, of which, analyzing the modes of ligand binding and its affinity for the active center of factor Xa (SYBYL, DS 4.0, PyMOL programs), the authors, without going into detail, suggested 10 different basic structures for experimental research as new potential factor Xa inhibitors. Structural formulas of these compounds were published in [55].

Virtual screening of the NCI Open database (260,000 compounds) using a combination of various approaches allowed to identify novel lead structures as potential factor Xa inhibitors in [56]. In particular, compound **20** (Table 2) with IC_{50} = 2.02 nM was found to be the most active out of 30 compounds selected for experimental testing *in vitro*.

To find novel factor Xa inhibitors based on previously identified structure-activity relationship, a series of tetrahydropyrazolopyridone derivatives containing various groups in P2 (1,3,4-triazole, triazolylmethyl moieties, etc.) was designed and synthesized [57]. All compounds exhibited *in vitro* inhibitory activity against factor Xa; the most active among them was compound **21** (Table 2) with IC_{50} = 0.14 μM.

Wang et al. [58] used anthranilamide as a lead candidate. It was found that its three derivatives demonstrated high inhibitory activity *in vitro* against factor Xa and high selectivity over thrombin. The highest activity was observed for compound **22** with IC_{50} = 25 nM (Table 2). Molecular docking using the Glide software demonstrated that compound **22** binding to the factor Xa active site was similar to that of rivaroxaban. Therefore, compound **22** can be a lead candidate for subsequent modification to enhance its anticoagulant activity.

Hu et al. [59] designed and synthesized more than 100 novel compounds based on the structures of several factor Xa inhibitors and factor Xa itself. By using various screen methods, the authors chose compound **23** (ID DJT06001; Table 2). Its anticoagulant activity was examined *in vitro* and *in vivo* in more detail. It was found that compound **23** prevented thrombosis via direct specific inhibition of factor Xa. The risk of bleeding after its administration was similar or even lower than upon rivaroxaban application. Moreover, compound **23** exhibited higher solubility and bioavailability compared to rivaroxaban. Finally, compound **23** was able to inhibit free Xa in the plasma with IC_{50} = 3.33 nM, whereas for factor Xa bound to prothrombinase, IC_{50} was 2.53 nM.

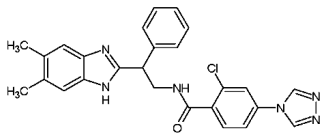
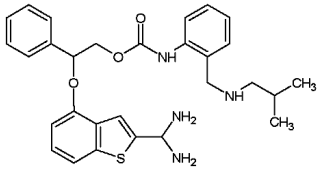
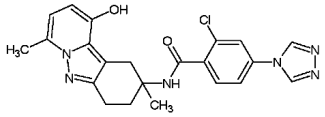
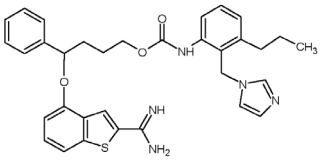
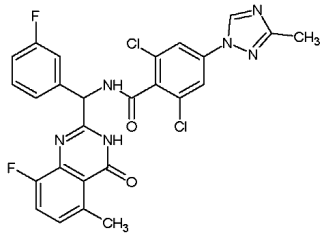
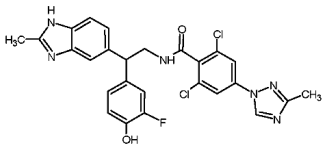
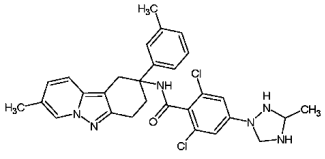
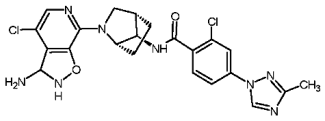
NOVEL FACTOR IXa INHIBITORS

As mentioned above, external vascular damage results in the formation of extrinsic tenase complex (TF–VIIa), which activates factors IX and X. Factor X conversion into Xa can be catalyzed by factor IXa and intrinsic tenase (IXa–VIIIa); the latter reaction being almost 10^5-times more efficient. Factor Xa is unstable in the plasma; it is rapidly inhibited by antithrombin and TFPI. In contrast, factor IXa, is relatively stable and diffuses away from the TF-bearing cells to activated platelets. Therefore, factor IXa is a very promising target for designing novel efficient anticoagulants [60].

Numerous studies have been published that described various structures such as monoclonal antibodies, DNA aptamers, and synthetic low-molecular-weight compounds (e.g., polysubstituted benzothiophenes) as factor IXa inhibitors [61-63]. Here, we will only discuss the papers published after 2015.

Various derivatives of substituted benzimidazoles (class A) and pyrazolopyridines (class B) have been synthesized based on two molecular structure hits with the micromolar activity against factor IXa identified by high-throughput screening [64] and their activity against factor IXa vs. factor Xa was assessed. Structural formulas for the most active compounds **24** and **25** from these two classes are shown in Table 3. In particular, it was found that com-

Table 3. Novel inhibitors of factor IXa

No.	Structural formula	Activity	References	No.	Structural formula	Activity	References
24		K_i = 0.016 μM	[64]	28		pIC_{50} = 8.70	[67]
25		K_i = 0.09 μM	[64]	29		pIC_{50} = 10.50 (CoMFA) 10.66 (CoMSIA)	[67]
26		K_i = 1.0 nM	[65]	30		IC_{50} = 1.86 nM	[68]
27		K_i = 1.8 nM	[66]	31		IC_{50} = 4.9 nM	[69]

pounds **24** and **25** were 24- and 120-times, respectively, more active against factor IXa than against factor Xa. Structure-activity relationship analysis was performed in order to search further for factor IXa inhibitors. Removal of both methyl groups in the benzimidazole ring of compound **24** resulted in the compound decreased inhibitory activity. The transfer of the triazole ring from *para*-position to *ortho*- or *meta*-positions relatively to the amide group reduced the activity of both compounds, respectively. The authors also investigated the impact of the alkyl chain length in the central portion of compound **24**, as well as of various substitutions, on the factor IXa inhibition. Introduction of substituents at the position adjacent to the benzamide group decreased the inhibitory activity, while introduction of substituents at the position adjacent to the benzimidazole ring increased the activity. Moreover, substitution of the phenyl ring with methyl group also decreased the activity of the resulting compound. Both decreasing the chain length to one C atom or increasing it up to three C atoms decreased the activity of compound **24**. Compound **24** is an R-enantiomer that exhibits 30 times higher inhibitory activity compared to the S-enantiomer.

Zhang et al. synthesized a series of compound **24** analogs as candidates for the factor IXa inhibitors [65]. *In vitro* testing showed that more than a half of the synthesized compounds exhibited very high selectivity toward factor IXa over factor Xa. The most promising inhibitor among them was compound **26** (Table 3) with K_i = 1.0 nM, that was almost 30,000-times more selective toward factor IXa over factor Xa.

Meng et al. [66] synthesized about 40 tricyclic structural analogs of compound **25** (Table 3). Some of them demonstrated remarkable inhibitory activity and high selectivity toward factor IXa over factor Xa. Although not the most potent inhibitor, compound **27** (K_i = 1.8 nM; Table 3) displayed the highest selectivity among the tested compounds: its inhibitory activity toward factor IXa was 300-times higher than toward factor Xa. The authors also presented detailed description of the effects of introduction of substituents of different chemical nature at various positions on the anticoagulant activity. However, it is difficult to make unambiguous conclusions, as an impact of each substituent at a certain position strongly depends on the chemical nature of substituents introduced at other positions.

Based on the experimental data for 84 amidinobenzothiophene derivatives [62, 63], Gao et al. used CoMFA and CoMSIA to determine the 3D quantitative structure-activity relationships (QSAR – 3D-QSAR) and 3D quan-

titative structure-selectivity relationships (3D-QSSR) for this type of factor IXa inhibitors [67]. To evaluate the QSAR, the $pIC_{50} = -logIC_{50}$ values were used as dependent variables. The initial array (84 compounds) was randomly divided into test (12 compounds) and training (72 compounds) sets. When constructing the 3D-QSSR, the ratio of $-logIC_{50}$ values for factors IXa and Xa was used as the selectivity (S) index. In this case, the initial array contained 73 compounds (64 in the training set and 9 in the test set). The highest activity among the compounds in the initial array was observed for compound **28** (Table 3): pIC_{50} (factor IXa) = 8.70, S = 2.56. Using the above-mentioned methods, the authors developed equations relating the pIC_{50} and S values to the parameters of examined compounds of the training set. After verifying the validity of the obtained equations, Gao et al. designed 16 novel derivatives in the examined chemical class and predicted their activity and selectivity. In particular, all these compounds were predicted to have higher inhibitory activity and selectivity toward factor IXa as compared to the compounds in the initial array. The following parameters were predicted for the most active novel compound **29** (Table 3): pIC_{50} (factor IXa) = 10.50, S = 4.34 (CoMFA); and pIC_{50} (factor IXa) = 10.66, S = 4.41 (CoMSIA). Unfortunately, no data regarding experimental testing of these compounds were provided [67].

By incubating of one of factor IXa active inhibitors (IC_{50} = 9.99 nM) with human and rat liver microsomes, Zhang et al. [68] were able to isolate and identify three metabolites, one of which (compound **30**) was more potent inhibitor of factor IXa (IC_{50} = 1.86 nM) compared to the initial compound [68].

Tricyclic pyrazinopyridine derivatives were identified as factor IXa inhibitors in [64, 66]. Replacing the tricyclic fragment with simpler chemical groups, Sakurada et al. [69] synthesized and tested a series of aminobenzisoxazole derivatives as potential factor IXa inhibitors. It was found that the most active compound **31** (Table 3) possessed both high inhibitory activity against factor IXa (IC_{50} = 4.9 nM) and high selectivity (i.e., it inhibited factor IXa 6300-times more efficiently that factor Xa). This compound was characterized by moderate water solubility, good membrane permeability, microsomal stability, and reasonable half-life (0.8 h in rats and 4.7 h in dogs). Currently, this compound, designated CFM-184, is considered as a candidate for preclinical studies [69].

NOVEL FACTOR XIa INHIBITORS

Besides the VIIa–TF complex, factor IX can be activated by factor XIa. Despite the fact that under normal conditions, factor XIa has the minimal impact on hemostasis, its activation plays an important role in developing thrombotic complications. According to the laboratory research, epidemiological studies, and clinical observations, factor XIa can be considered as the major and most promising target for developing anticoagulant drugs with a low risk of bleeding [70, 71]. Starting from 2010, the number of patents and publications related to the development of factor XIa inhibitors has significantly increased. Bane et al. [70] and Al-Horani et al. [71] reviewed the main groups of substances proposed as factor XIa inhibitors: 1) monoclonal antibodies; 2) antisense oligonucleotides (chemically modified single-stranded nucleotide sequences capable of complimentary binding to specific mRNA motifs and regulating the target gene expression and production of the corresponding proteins); 3) polypeptides; 4) synthetic low-molecular-weight compounds (acyclic, monocyclic, bicyclic, macrocyclic) binding directly to the factor XIa active site; 5) synthetic allosteric inhibitors; 6) compounds isolated from natural source (bromophenyl carbamate) [70, 71]. Here, as above, we will focus on synthetic low-molecular-weight factor XIa inhibitors developed after 2015. The structural formulas for the most efficient novel factor XIa inhibitors are presented in Table 4.

As a part of the program on studying structurally diverse factor XIa inhibitors based on previously isolated compounds, Corte et al. synthesized a series of novel derivatives by introducing various substituents into the imidazole ring of initial compounds and examined their activity [72]. Two derivatives with the maximal inhibitory activity against factor XIa (compounds **32** and **33**) are shown in Table 4. Compound **32** is an S-enantiomer, which is markedly more active (K_i = 8.4 nM) than the existing racemate. Compound **33** has K_i = 3.7 nM. The crystal structure of compound **32** (S-enantiomer) complexed with factor XIa was examined, and the binding of the synthesized compounds to distinct areas of factor XIa active site was described in detail.

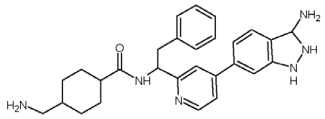
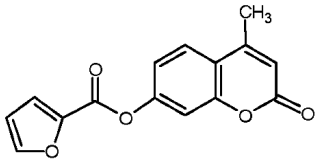
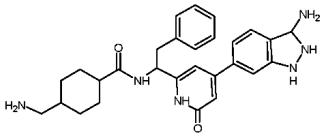
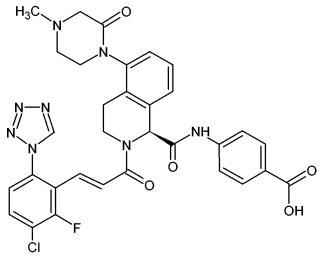
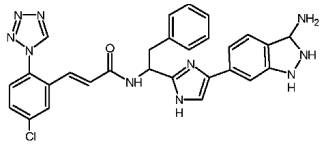
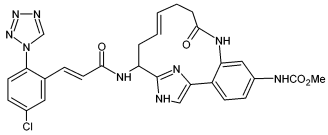
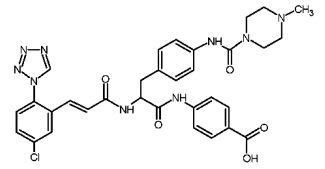
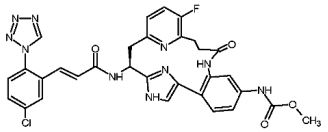
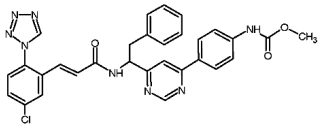
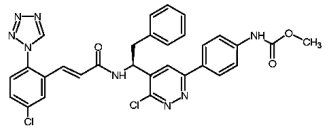
Pinto et al. modified the P1 and P2 motifs in the earlier proposed factor XIa active inhibitors and examined the derivatives for improved selectivity profile and oral bioavailability [73]. The most active and highly selective inhibitor was compound **34** with K_i = 6.7 nM.

Smith et al. investigated the effect of various substitutions in the P1 and P2 motifs of phenylalanine derived diamides on the factor Xa inhibition, resulting in the discovery of compound **35** with a high affinity to factor XIa (K_i = 2.0 nM) [74].

Corte et al. [75] continued their studies on developing new factor XIa inhibitors [72] in order to improve oral bioavailability of the newly synthesized derivatives. They identified compound **36** (K_i = 6.7 nM), an S-enantiomer with high activity and good bioavailability.

Compound **37** (coumarin derivative) efficiently inhibited factor XIa (IC_{50} = 0.77 μM) and displayed moderate-to-high selectivity for factor XIa over related serine proteases [76]. Compound **37** can be used as a central scaffold for developing efficient factor XIa inhibitors having no or limited risk of internal hemorrhages.

Table 4. Novel inhibitors of factor XIa

No.	Structural formula	Activity	References	No.	Structural formula	Activity	References
32		K_i = 8.4 nM	[72]	37		IC_{50} = 0.77 μM	[76]
33		K_i = 3.7 nM	[72]	38		K_i = 0.7 nM	[77]
34		K_i = 6.7 nM	[73]	39		K_i = 0.16 nM	[78]
35		K_i = 2.0 nM	[74]	40		K_i = 0.02 nM	[79]
36		K_i = 6.7 nM	[75]	41		K_i = 1.9 nM	[80]

Sequential optimization of compounds with phenylalanine and tetrahydroisoquinoline scaffolds [77] allowed to obtain highly selective direct inhibitor of factor Xa (compound **38**) designated as BMS-96221. This inhibitor has a good water solubility and short half-life and is suitable for parenteral administration according to its pharmacokinetics parameters. BMS-96221 has passed phase I clinical trial.

Corte et al. used molecular modeling and conformational analysis based on acyclic phenylimidazole compounds to design and examine a series of 11-, 12-, 13- and 14-membered macrocyclic inhibitors of factor XIa [78]. Theoretical analysis revealed that 12- and 13-membered macrocycles fit the factor XIa active site best. These compounds were synthesized by opening the benzyl ring of the P1 motif and connecting it to the P2 phenyl at the *ortho*-position via alkyl, ether or amide linkers of varying length. One of such 13-membered macrocycles (compound **39**), being an E-isomer, demonstrated high activity and selectivity against factor XIa (K_i = 0.16 nM). At the same time, it showed very low oral bioavailability, presumably due to high molecule polarity. In 2017, the same group used different macrocyclization strategy to obtain another active (K_i = 0.02 nM) highly selective factor XIa inhibitor (compound **40**); unfortunately, its oral bioavailability was low [79].

Hu et al. [80] synthesized and examined pyridazine derivatives as potential active and orally bioavailable factor XIa inhibitors [80]. Overall, the obtained compounds were active and selective inhibitors of factor XIa based on the pharmacokinetics studies in dogs; the most active among them was compound **41** (K_i = 1.9 nM) that exhibited moderate oral bioavailability.

None of the compounds presented in this review can be called an "ideal" anticoagulant. Al-Horani and Desai

[71] formulated main requirements for such "ideal" anticoagulant: (i) predictable pharmacokinetics; (ii) lack of toxicity in liver (hepatotoxicity), bones (osteoporosis), and platelets (thrombocytopenia); (iii) possibility of rapid reversal of action by efficient and inexpensive antidotes; (iv) no requirements for continuous monitoring, assessment, and dose adjustment; (v) safety in compromised patients (e.g., pregnant women, cancer patients, children, elderly individuals, etc.); (vi) availability in a relatively inexpensive oral and/or parenteral form; (vii) efficacy and safety in the case of any elevated risk of internal bleeding.

Thus, despite certain progress in the development of anticoagulant therapy, the search for novel anticoagulants still continues, because no ideal anticoagulant agent for preventing and treating thromboembolic diseases is currently available in clinical practice.

Because thrombosis is a complex process occurring via multiple mechanisms, we should mention the studies using a combination of two or more antithrombotic drugs to treat thrombosis. In particular, Neves et al. [81] discussed the structure-activity relationships and provided structural formulas for 163 chemical compounds acting as dual inhibitors against coagulation factors: thrombin and Xa; thrombin and VIIa, Xa, or IXa; Xa and VIIa, Xa, or plasma kallikrein, etc. For instance, compound EP217609 that has the bulkiest structural formula among the examined structures was found to be a direct inhibitor for thrombin (IC_{50} = 11.5 nM) and indirect inhibitor for factor Xa (IC_{50} = 11.5 nM); this compound was approved for clinical trial. The key issue in searching for such inhibitors is reaching a compromise between simultaneous activities toward several targets and acceptable pharmacokinetics profiles.

Funding

The study was supported by the Russian Science Foundation (project 16-14-00224, section "Novel Thrombin Inhibitors") and the Program for Fundamental Research of the Presidium of the Russian Academy of Sciences (Basic Technologies of Physiological Adaptations).

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. Panteleev, M. A., and Ataullahanov, F. I. (2008) Blood coagulation: biochemical basics, *Klin. Onkogematol.*, **1**, 50-62.
2. Panteleev, M. A., Vasil'ev, S. A., Sinauridze, E. I., Vorob'ev, A. I., and Ataullakhanov, F. I. (2012) *Practical Coaguology* [in Russian], Prakticheskaya Meditsina, Moscow.
3. Panteleev, M., Kotova, Ya., and Tokarev, A. (2008) Mechanisms regulating blood coagulation, *Terap. Arkhiv*, **7**, 88-91.
4. Sinauridze, E. I., Panteleev, M. A., and Ataullakhanov, F. I. (2012) Anticoagulant therapy: basic principles, classic approaches and recent developments, *Blood Coagul. Fibrinolysis*, **23**, 482-493.
5. Broussalis, E., Anna, W., Trinka, E., Mutzenbach, S., and Killer, M. (2014) Latest developments in anticoagulant drug discovery, *Drug Discov. Today*, **19**, 921-935.
6. Ahrens, I., Peter, K., Lip, G. Y. H., and Bode, C. (2012) Development and clinical applications of novel oral anticoagulants. Part I. Clinically approved drugs, *Discov. Med.*, **13**, 433-443.
7. Roca, B., and Roca, M. (2015) The new oral anticoagulants: reasonable alternatives to warfarin, *Cleve Clin. J. Med.*, **82**, 847-854.
8. Adcock, D. M., and Gosselin, R. (2015) Direct oral anticoagulants (DOACs) in the laboratory: 2015 review, *Thromb. Res.*, **136**, 7-12.
9. Mekaj, Y. H., Mekaj, A. Y., Duci, S. B., and Miftari, E. I. (2015) New oral anticoagulants: their advantages and disadvantages compared with vitamin K antagonists in the prevention and treatment of patients with thromboembolic events, *Ther. Clin. Risk. Manag.*, **11**, 967-977.
10. Gomez-Outes, A., Suarez-Gea, M. L., Lecumberri, R., Terleira-Fernandez, A. I., and Vargas-Castrillon, E. (2015) Direct-acting oral anticoagulants: pharmacology, indications, management, and future perspectives, *Eur. J. Haematol.*, **95**, 389-404.
11. Sinauridze, E., Vuymo, T., and Ataullakhanov, F. (2017) Dabigatran etexilate: a novel oral coagulant, *Vopr. Gematol./Onkol. Immunol. Pediatr.*, **16**, 1-15.
12. Joppa, S. A., Salciccioli, J., Adamski, J., Patel, S., Wysokinski, W., McBane, R., Al-Saffar, F., Esser, H., and Shamoun, F. (2018) A practical review of the emerging direct anticoagulants, laboratory monitoring, and reversal agents, *J. Clin. Med.*, **7**, E29.
13. Pollack, C. V., Reilly, P. A., van Ryn, J., Eikelboom, J. W., Glund, S., Bernstein, R. A., Dubiel, R., Huisman, M. V., Hylek, E. M., Kam, C. W., Kamphuisen, P. W., Kreuzer, J., Levy, J. H., Royle, G., Sellke, F. W., Stangier, J., Steiner, T., Verhamme, P., Wang, B., Young, L., and Weitz, J. I. (2017) Idarucizumab for dabigatran reversal – full cohort analysis, *N. Engl. J. Med.*, **377**, 431-441.
14. Hung, C.-L., and Chen, C.-C. (2014) Computational approaches for drug discovery, *Drug Dev. Res.*, **75**, 412-418.
15. Lill, M. (2013) Virtual screening in drug design, *Methods Mol. Biol.*, **993**, 1-12.
16. Baron, R. (2012) *Computational Drug Discovery and Design*, Springer New York, NY.
17. Khel't'e, Kh.-D., Zippl', V., Ron'yan, D., and Fol'kers, G. (2015) *Molecular Modeling. Theory and Practice* [in Russian], BINOM. Laboratoriya Znanii, Moscow.
18. De Ruyck, J., Brysbaert, G., Blossey, R., and Lensink, M. F. (2016) Molecular docking as a popular tool in drug design, an *in silico* travel, *Adv. Appl. Bioinform. Chem.*, **9**, 1-11.
19. Chen, Y.-C. (2015) Beware of docking! *Trends Pharmacol. Sci.*, **36**, 78-95.

20. Sulimov, V. B., and Sulimov, A. V. (2017) *Docking: Molecular Modeling for Drug Design* [in Russian], IIntell, Moscow.
21. Klimovich, P. V., Shirts, M. R., and Mobley, D. L. (2015) Guidelines for the analysis of free energy calculations, *J. Comput. Aided. Mol. Des.*, **29**, 397-411.
22. Sulimov, A. V., Kutov, D. C., Katkova, E. V., Ilin, I. S., and Sulimov, V. B. (2017) New generation of docking programs: supercomputer validation of force fields and quantum-chemical methods for docking, *J. Mol. Graph. Model.*, **78**, 139-147.
23. Raevskiy, O. (2013) *Structure-Based Properties of Chemical Compounds and Drugs* [in Russian], Dobrsovet, KDU, Moscow.
24. Raevskiy, O. (2015) *Modeling Structure–Property Relationship* [in Russian], Dobrsovet, KDU, Moscow.
25. Filimonov, D., and Poroykov, V. (2006) Predicting a spectrum of biological properties in organic compounds, *Ross. Khim. Zh.*, **50**, 66-75.
26. Poroykov, V., Filimonov, D., Gloriozova, T., Lagunin, A., Druzhilovskiy, D., and Stepanchikova, A. (2009) A computer-aided prediction of structure–activty relationship: virtual chemogenomics, *Inform. Vestnik VOGiS*, **13**, 137-142.
27. Filimonov, D. A., Lagunin, A. A., Gloriozova, T. A., Rudik, A. V., Druzhilovskiy, D. S., Pogodin, P. V., and Poroykov, V. V. (2014) Prediction of structure–activty relationship by using PASS online web resource, *Khim. Geterotsikl. Soedin.*, **3**, 483-499.
28. Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeney, P. J. (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, *Adv. Drug Deliv. Rev.*, **46**, 3-26.
29. Shen, J., Cheng, F., Xu, Y., Li, W., and Tang, Y. (2010) Estimation of ADME properties with substructure pattern recognition, *J. Chem. Inf. Model.*, **50**, 1034-1041.
30. Khakar, P. S. (2010) Two-dimensional (2D) *in silico* models for absorption, distribution, metabolism, excretion and toxicity (ADME/T) in drug discovery, *Curr. Top. Med. Chem.*, **10**, 116-126.
31. Kujawski, J., Bernard, M. K., Janusz, A., and Kuzma, W. (2012) Prediction of log P: ALOGPS application in medicinal chemistry education, *J. Chem. Educ.*, **89**, 64-67.
32. Matter, H., and Schmider, W. (2006) *In-silico* ADME modelling, in *Drug Discovery and Evaluation*, *Safety and Pharmacokinetic Assays* (Vogel, H. G., ed.), Springer, Heidelberg, pp. 409-436.
33. Wang, Y., Xing, J., Xu, Y., Zhou, N., Peng, J., Xiong, Z., Liu, X., Luo, X., Luo, C., Chen, K., Zheng, M., and Jiang, H. (2015) *In silico* ADME/T modelling for rational drug design, *.Q Rev. Biophys.*, **48**, 488-515.
34. Obst, U., Banner, D. W., Weber, L., and Diederich, F. (1997) Molecular recognition at the thrombin active site: structure-based design and synthesis of potent and selective thrombin inhibitors and the X-ray crystal structures of two thrombin–inhibitor complexes, *Chem. Biol.*, **4**, 287-295.
35. Kong, Y., Chen, H., Wang, Y.-Q., Meng, L., and Wei, J.-F. (2014) Direct thrombin inhibitors: patents 2002-2012 (review), *Mol. Med. Rep.*, **9**, 1506-1514.
36. He, L.-W., Dai, W.-C., and Li, N.-G. (2015) Development of orally active thrombin inhibitors for the treatment of thrombotic disorder diseases, *Molecules*, **20**, 11046-11062.
37. Mena-Ulecia, K., Tiznado, W., and Caballero, J. (2015) Study of the differential activity of thrombin inhibitors using docking, QSAR, molecular dynamics, and MM-GBSA, *PLoS One*, **10**, e0142774.
38. Sinauridze, E. I., Romanov, A. N., Gribkova, I. V., Kondakova, O. A., Surov, S. S., Gorbatenko, A. S., Butylin, A. A., Monakov, M. Y., Bogolyubov, A. A., Kuznetsov, Y. V., Sulimov, V. B., and Ataullakhanov, F. I. (2011) New synthetic thrombin inhibitors: molecular design and experimental verification, *PLoS One*, **6**, e19969.
39. Lu, T., Tomczuk, B., Illig, C. R., Bone, R., Murphy, L., Spurlino, J., Salemme, F. R., and Soll, R. M. (1998) *In vitro* evaluation and crystallographic analysis of a new class of selective, non-amide-based thrombin inhibitors, *Bioorg. Med. Chem. Lett.*, **8**, 1595-1600.
40. Hagmann, W. K. (2008) The many roles for fluorine in medicinal chemistry, *J. Med. Chem.*, **51**, 4359-4369.
41. Li, M., and Ren, Y. (2015) Synthesis and biological evaluation of some new 2,5-substituted 1-ethyl-1H-benzoimidazole fluorinated derivatives as direct thrombin inhibitors, *Arch. Pharm. (Weinheim)*, **348**, 353-365.
42. Chen, H., and Ren, Y. (2015) Design, synthesis, and antithrombotic evaluation of some novel fluorinated thrombin inhibitor derivatives, *Arch. Pharm. (Weinheim)*, **348**, 408-420.
43. Chen, D., Wang, S., Diao, X., Zhu, Q., Shen, H., Han, X., Wang, Y., Gong, G., and Xu, Y. (2015) Design, synthesis and antithrombotic evaluation of novel dabigatran etexilate analogs, a new series of non-peptides thrombin inhibitors, *Bioorg. Med. Chem.*, **23**, 7405-7416.
44. Chen, D., Shi, J., Liu, J., Zhang, X., Deng, X., Yang, Y., Cui, S., Zhu, Q., Gong, G., and Xu, Y. (2017) Design, synthesis and antithrombotic evaluation of novel non-peptide thrombin inhibitors, *Bioorg. Med. Chem.*, **25**, 458-470.
45. Lee, W., Lee, S., Choi, J., Park, J.-H., Kim, K.-M., Jee, J.-G., and Bae, J.-S. (2017) Antithrombotic properties of JJ1, a potent and novel thrombin inhibitor, *Sci. Rep.*, **7**, 14862.
46. Wang, X., Zhang, Y., Yang, Y., Wu, X., Fan, H., and Qiao, Y. (2017) Identification of berberine as a direct thrombin inhibitor from traditional Chinese medicine through structural, functional and binding studies, *Sci. Rep.*, **7**, 44040.
47. Levy, J. H., Spyropoulos, A. C., Samama, C. M., and Douketis, J. (2014) Direct oral anticoagulants: new drugs and new concepts, *JACC Cardiovasc. Interv.*, **7**, 1333-1351.
48. Patel, N. R., Patel, D. V., Murumkar, P. R., and Yadav, M. R. (2016) Contemporary developments in the discovery of selective factor Xa inhibitors: a review, *Eur. J. Med. Chem.*, **121**, 671-698.
49. Sulimov, V. B., Gribkova, I. V., Kochugaeva, M. P., Katkova, E. V., Sulimov, A. V., Kutov, D. C., Shikhaliev, K. S., Medvedeva, S. M., Krysin, M. Y., Sinauridze, E. I., and Ataullakhanov, F. I. (2015) Application of molecular modeling to development of new factor Xa inhibitors, *Biomed. Res. Int.*, **2015**, 120802.
50. Yang, J., Su, G., Ren, Y., and Chen, Y. (2015) Synthesis of 3,4-diaminobenzoyl derivatives as factor Xa inhibitors, *Eur. J. Med. Chem.*, **101**, 41-51.
51. Ishihara, T., Koga, Y., Iwatsuki, Y., and Hirayama, F. (2015) Identification of potent orally active factor Xa inhibitors based on conjugation strategy and application of predictable fragment recommender system, *Bioorg. Med. Chem.*, **23**, 277-289.

52. Xu, C., and Ren, Y. (2015) Molecular modeling studies of [6,6,5] tricyclic fused oxazolidinones as fXa inhibitors using 3D-QSAR, topomer CoMFA, molecular docking and molecular dynamics simulations, *Bioorg. Med. Chem. Lett.*, **25**, 4522-4528.
53. Wang, Y., Sun, X., Yang, D., Guo, Z., Fan, X., Nie, M., Zhang, F., Liu, Y., Li, Y., Wang, Y., Gong, P., and Liu, Y. (2016) Design, synthesis, and structure–activity relationship of novel and effective apixaban derivatives as FXa inhibitors containing 1,2,4-triazole/pyrrole derivatives as P2 binding element, *Bioorg. Med. Chem.*, **24**, 5646-5661.
54. Xing, J., Yang, L., Yang, Y., Zhao, L., Wei, Q., Zhang, J., Zhou, J., and Zhang, H. (2017) Design, synthesis and biological evaluation of novel 2,3-dihydroquinazolin- 4(1H)-one derivatives as potential fXa inhibitors, *Eur. J. Med. Chem.*, **125**, 411-422.
55. Pu, Y., Liu, H., Zhou, Y., Peng, J., Li, Y., Li, P., Li, Y., Liu, X., and Zhang, L. (2017) *In silico* discovery of novel FXa inhibitors by pharmacophore modeling and molecular docking, *Nat. Products Bioprospect.*, **7**, 249-256.
56. Lagos, C. F., Segovia, G. F., Nunez-Navarro, N., Faundez, M. A., and Zacconi, F. C. (2017) Novel FXa inhibitor identification through integration of ligand- and structure-based approaches, *Molecules*, **22**, E1588.
57. Sun, X., Hong, Z., Liu, M., Guo, S., Yang, D., Wang, Y., Lan, T., Gao, L., Qi, H., Gong, P., and Liu, Y. (2017) Design, synthesis, and biological activity of novel tetrahydropyrazolopyridone derivatives as FXa inhibitors with potent anticoagulant activity, *Bioorg. Med. Chem.*, **25**, 2800-2810.
58. Wang, W., Yuan, J., Fu, X., Meng, F., Zhang, S., Xu, W., Xu, Y., and Huang, C. (2016) Novel anthranilamide-based FXa inhibitors: drug design, synthesis and biological evaluation, *Molecules*, **21**, 491.
59. Hu, X., Xiao, Y., Yu, C., Zuo, Y., Yang, W., Wang, X., Gu, B., and Li, J. (2018) Characterization of a novel selective factor Xa inhibitor, DJT06001, which reduces thrombus formation with low risk of bleeding, *Eur. J. Pharmacol.*, **825**, 85-91.
60. Smiley, D. A., and Becker, R. C. (2014) Factor IXa as a target for anticoagulation in thrombotic disorders and conditions, *Drug Discov. Today*, **19**, 1445-1453.
61. Choudhari, P., and Bhatia, M. (2012) 3D QSAR, pharmacophore identification studies on series of 4-substituted benzothiophene analogs as factor IXa inhibitors, *Pharmacophore*, **3**, 189-198.
62. Wang, S., Beck, R., Blench, T., Burd, A., Buxton, S., Malic, M., Ayele, T., Shaikh, S., Chahwala, S., Chander, C., Holland, R., Merette, S., Zhao, L., Blackney, M., and Watts, A. (2010) Studies of benzothiophene template as potent factor IXa (FIXa) inhibitors in thrombosis, *J. Med. Chem.*, **53**, 1465-1472.
63. Wang, S., Beck, R., Burd, A., Blench, T., Marlin, F., Ayele, T., Buxton, S., Dagostin, C., Malic, M., Joshi, R., Barry, J., Sajad, M., Cheung, C., Shaikh, S., Chahwala, S., Chander, C., Baumgartner, C., Holthoff, H. P., Murray, E., Blackney, M., and Giddings, A. (2010) Structure based drug design: development of potent and selective factor IXa (FIXa) inhibitors, *J. Med. Chem.*, **53**, 1473-1482.
64. Parker, D. L., Jr., Walsh, S., Li, B., Kim, E., Sharipour, A., Smith, C., Chen, Y. H., Berger, R., Harper, B., Zhang, T., Park, M., Shu, M., Wu, J., Xu, J., Dewnani, S., Sherer, E. C., Hruza, A., Reichert, P., Geissler, W., Sonatore, L., Ellsworth, K., Balkovec, J., Greenlee, W., and Wood, H. B. (2015) Rapid development of two factor IXa inhibitors from hit to lead, *Bioorg. Med. Chem. Lett.*, **25**, 2321-2325.
65. Zhang, T., Andre, P., Bateman, T. J., Chen, Y.-H., Desai, K., Ellsworth, K., Geissler, W. M., Guo, L., Hruza, A., Jian, T., Meng, D., Parker, D. L., Jr., Qian, X., Reichert, P., Sherer, E. C., Shu, M., Smith, C. J., Sonatore, L. M., Tschirret-Guth, R., Nolting, A. F., Orr, R., Campeau, L. C., Araki, K., Nishimura, T., Sakurada, I., and Wood, H. B. (2015) Development of a novel class of potent and selective FIXa inhibitors, *Bioorg. Med. Chem. Lett.*, **25**, 4945-4949.
66. Meng, D., Andre, P., Bateman, T. J., Berger, R., Chen, Y. H., Desai, K., Dewnani, S., Ellsworth, K., Feng, D., Geissler, W. M., Guo, L., Hruza, A., Jian, T., Li, H., Metzger, J., Parker, D. L., Reichert, P., Sherer, E. C., Smith, C. J., Sonatore, L. M., Tschirret-Guth, R., Wu, J., Xu, J., Zhang, T., Campeau, L. C., Orr, R., Poirier, M., McCabe-Dunn, J., Araki, K., Nishimura, T., Sakurada, I., Hirabayashi, T., and Wood, H. B. (2015) Development of a novel tricyclic class of potent and selective FIXa inhibitors, *Bioorg. Med. Chem. Lett.*, **25**, 5437-5443.
67. Gao, J.-S., Tong, X.-P., Chang, Y.-Q., He, Y.-X., Mei, Y.-D., Tan, P.-H., Guo, J.-L., Liao, G.-C., Xiao, G.-K., Chen, W.-M., Zhou, S.-F., and Sun, P.-H. (2015) Design and prediction of new anticoagulants as a selective factor IXa inhibitor via three-dimensional quantitative structure–property relationships of amidinobenzothiophene derivatives, *Drug Des. Devel. Ther.*, **9**, 1743-1759.
68. Zhang, T., Liu, Y., Yang, X., Martin, G. E., Yao, H., Shang, J., Bugianesi, R. M., Ellsworth, K. P., Sonatore, L. M., Nizner, P., Sherer, E. C., Hill, S. E., Knemeyer, I. W., Geissler, W. M., Dandliker, P. J., Helmy, R., and Wood, H. B. (2016) Definitive metabolite identification coupled with automated ligand identification system (ALIS) technology: a novel approach to uncover structure–activity relationships and guide drug design in a factor IXa inhibitor program, *J. Med. Chem.*, **59**, 1818-1829.
69. Sakurada, I., Endo, T., Hikita, K., Hirabayashi, T., Hosaka, Y., Kato, Y., Maeda, Y., Matsumoto, S., Mizuno, T., Nagasue, H., Nishimura, T., Shimada, S., Shinozaki, M., Taguchi, K., Takeuchi, K., Yokoyama, T., Hruza, A., Reichert, P., Zhang, T., Wood, H. B., Nakao, K., and Furusako, S. (2017) Discovery of novel aminobenzisoxazole derivatives as orally available factor IXa inhibitors, *Bioorg. Med. Chem. Lett.*, **27**, 2622-2628.
70. Bane, C. E., and Gailani, D. (2014) Factor XI as a target for antithrombotic therapy, *Drug Discov. Today*, **19**, 1454-1458.
71. Al-Horani, R. A., and Desai, U. R. (2016) Factor XIa inhibitors: a review of the patent literature, *Expert. Opin. Ther. Pat.*, **26**, 323-345.
72. Corte, J. R., Fang, T., Hangeland, J. J., Friends, T. J., Rendina, A. R., Luettgen, J. M., Bozarth, J. M., Barbera, F. A., Rossi, K. A., Wei, A., Ramamurthy, V., Morin, P. E., Seiffert, D. A., Wexler, R. R., and Quan, M. L. (2015) Pyridine and pyridinone-based factor XIa inhibitors, *Bioorg. Med. Chem. Lett.*, **25**, 925-930.
73. Pinto, D. J. P., Smallheer, J. M., Corte, J. R., Austin, E. J. D., Wang, C., Fang, T., Smith, L. M., 2nd, Rossi, K. A., Rendina, A. R., Bozarth, J. M., Zhang, G., Wei, A.,

Ramamurthy, V., Sheriff, S., Myers, J. E., Jr., Morin, P. E., Luettgen, J. M., Seiffert, D. A., Quan, M. L., and Wexler, R. R. (2015) Structure-based design of inhibitors of coagulation factor XIa with novel P1 moieties, *Bioorg. Med. Chem. Lett.*, **25**, 1635-1642.

74. Smith, L. M., Orwat, M. J., Hu, Z., Han, W., Wang, C., Rossi, K. A., Gilligan, P. J., Pabbisetty, K. B., Osuna, H., Corte, J. R., Rendina, A. R., Luettgen, J. M., Wong, P. C., Narayanan, R., Harper, T. W., Bozarth, J. M., Crain, E. J., Wei, A., Ramamurthy, V., Morin, P. E., Xin, B., Zheng, J., Seiffert, D. A., Quan, M. L., Lam, P. Y. S., Wexler, R. R., and Pinto, D. J. P. (2016) Novel phenylalanine derived diamides as factor XIa inhibitors, *Bioorg. Med. Chem. Lett.*, **26**, 472-478.
75. Corte, J. R., Fang, T., Pinto, D. J. P. P., Orwat, M. J., Rendina, A. R., Luettgen, J. M., Rossi, K. A., Wei, A., Ramamurthy, V., Myers, J. E., Sheriff, S., Narayanan, R., Harper, T. W., Zheng, J. J., Li, Y.-X. X., Seiffert, D. A., Wexler, R. R., and Quan, M. L. (2016) Orally bioavailable pyridine and pyrimidine-based factor XIa inhibitors: discovery of the methyl *N*-phenyl carbamate P2 prime group, *Bioorg. Med. Chem.*, **24**, 2257-2272.
76. Obaidullah, A. J., and Al-Horani, R. A. (2017) Discovery of chromen-7-yl furan-2-carboxylate as a potent and selective factor XIa inhibitor, *Cardiovasc. Hematol. Agents Med. Chem.*, **15**, 40-48.
77. Pinto, D. J. P., Orwat, M. J., Smith, L. M., 2nd, Quan, M. L., Lam, P. Y. S., Rossi, K. A., Apedo, A., Bozarth, J. M., Wu, Y., Zheng, J. J., Xin, B., Toussaint, N., Stetsko, P., Gudmundsson, O., Maxwell, B., Crain, E. J., Wong, P. C., Lou, Z., Harper, T. W., Chacko, S. A., Myers, J. E., Jr., Sheriff, S., Zhang, H., Hou, X., Mathur, A., Seiffert, D. A., Wexler, R. R., Luettgen, J. M., and Ewing, W. R. (2017) Discovery of a parenteral small molecule coagulation factor XIa inhibitor clinical candidate (BMS-962212), *J. Med. Chem.*, **60**, 9703-9723.
78. Corte, J. R., Fang, T., Osuna, H., Pinto, D. J. P., Rossi, K. A., Myers, J. E., Sheriff, S., Lou, Z., Zheng, J. J., Harper, T. W., Bozarth, J. M., Wu, Y., Luettgen, J. M., Seiffert, D. A., Decicco, C. P., Wexler, R. R., and Quan, M. L. (2017) Structure-based design of macrocyclic factor XIa inhibitors: discovery of the macrocyclic amide linker, *J. Med. Chem.*, **60**, 1060-1075.
79. Wang, C., Corte, J. R., Rossi, K. A., Bozarth, J. M., Wu, Y., Sheriff, S., Myers, J. E., Luettgen, J. M., Seiffert, D. A., Wexler, R. R., and Quan, M. L. (2017) Macrocyclic factor XIa inhibitors, *Bioorg. Med. Chem. Lett.*, **27**, 4056-4060.
80. Hu, Z., Wang, C., Han, W., Rossi, K. A., Bozarth, J. M., Wu, Y., Sheriff, S., Myers, J. E., Luettgen, J. M., Seiffert, D. A., Wexler, R. R., and Quan, M. L. (2018) Pyridazine and pyridazinone derivatives as potent and selective factor XIa inhibitors, *Bioorg. Med. Chem. Lett.*, **28**, 987-992.
81. Neves, A. R., Correia-da-Silva, M., Sousa, E., and Pinto, M. (2016) Structure–activity relationship studies for multitarget antithrombotic drugs, *Future Med. Chem.*, **8**, 2305-2355.