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Computer modeling of ferrocene-substituted 3,7-diazabicyclo[3.3.1]nonanes as serine protease inhibitors

Sergey Z. Vatsadze,*[a] Dmitry A. Shulga,[a] Yulia D. Loginova,[a] Irina A. Vatsadze,[b] Li Wang,[c] Haojie Yu[c] and Konstantin V. Kudryavtsev[a]

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. E-mail: szv@org.chem.msu.ru*
[b] *N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation*
[c] *State Key Laboratory of Chemical Engineering, Department of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, P. R. China*

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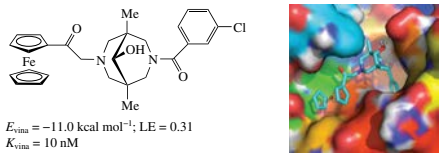
The docking study of ferrocene-substituted bispidines to binding sites of thrombin and factor Xa has shown that bispidine scaffold provides a 3D-arranegment of all substituents and a direction for the ferrocene group to fill the S4 pocket for both thrombin and factor Xa.



Molecular complexity is a key trend in modern science.[1] The study of enzyme–ligand interaction is a step to the next level of complexity compared to the initial structures of low molecular weight compounds and peptides; *i.e.*, this is the complexity of hybrid molecular systems.[2] In modern drug discovery, fragment-based lead discovery has become increasingly popular as it presents a promising alternative to conventional screening approaches.[3]

A search for new anticoagulants is a rapidly growing area, and active research is carried out to identify new promising compounds. Existing therapies, such as orally administered warfarin and subcutaneous injection with low molecular weight heparin (LMWH), are burdened by drug and dietary restrictions, bleeding risk and required monitoring of blood parameters.[4] In the last decade, a new generation of anticoagulants was developed – direct thrombin and factor Xa inhibitors.[5] Compounds with a ferrocene (Fc) moiety have been studied for a large number of activities, and their binding possibilities are often predicted and calculated using computer modeling.[6,7]

Despite the natural substrates and direct inhibitors of thrombin and factor Xa contain a basic amino acid residue, positively charged at physiological pH and making favorable electrostatic interactions with Asp189 at the wall of the S1 pocket, in recent times, it has become an accepted practice to target S1 pocket with non-basic moieties. The latter often contains aromatic fragments substituted with heavy halogens (Cl, Br, I), which presumably favorably participate in so-called halogen bonding[8–10] between a heavy halogen atom and the π-system of Tyr228.

Recent work has revealed the potential of using 3,7-diazabicyclo[3.3.1]nonane (hereafter called bispidine) scaffolds for the design of inhibitors of serine proteases.[8] We have shown that bispidine scaffold can be placed at the binding site of thrombin, and there it directs the substituents to fill the S1, S2 and S4 pockets (see Online Supplementary Materials). The present study is a continuation of the starting work in terms of the computer modeling of interactions of ferrocene-containing bispidine scaffolds with binding sites of serine proteases.

The aim of this work was the computer modeling of substituted 3,7-diazabicyclo[3.3.1]nonanes with a ferrocene moiety to create potential inhibitors of serine proteases. The design of target molecules was carried out by varying substituents at the two positions of bispidine scaffold, namely, two nitrogen atoms N(3) and N(7) (Figure 1). At the N(7) position, the halogen (chlorine) substituted aromatic groups with different linkers were used, which are suitable for the S1 pocket.[8–10] At the N(3) position, a ferrocene moiety with a chain of one to three carbon atoms and a ketone group were placed.

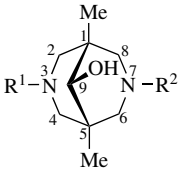


Figure 1 Bispidine scaffold with numbering scheme. Note that the OH group is *syn* to the N(7) atom.

The structures of thrombin (2ZC9) and factor Xa (2W26) were taken from PDB. A regular scenario for protein structure preparation for docking was employed. All structures were stripped from hetero groups and water; in this case, only water that plays a significant role in binding is located at the bottom of the S1 pocket, and our goal was to replace it.[11] Active sites of thrombin and factor Xa are quite rigid, and we used conformations from effective (favorable) complexes. Innate ligands were extracted by means of PyMol[12] and used subsequently for the purposes of docking procedure validation. After that, all of the three protein structures were prepared to docking by means of prepare_receptor4.py from AutoDock Tools.[13] These PDBQT structures were used for studies with AutoDock Vina.[14]

The sequence of ligand preparation was as follows. Innate ligands extracted from the original PDB files were transformed without any change. All structures from the initial set and all of the subsequent modifications were first sketched with MarvinSketch,[15] followed by 3D geometry generation by means of OpenBabel v2.3.2[16] to produce structures in the MOL2 format (used to convert to PDBQT as described above).

After that, the geometries were locally optimized within a UFF force field using its implementation in Avogadro.[17] To guarantee consistency in structure preparation, all the studied structures were subjected to the same geometry optimization. Then, all the ligands were prepared with prepare_ligand4.py from AutoDock Tools to obtain structures in the PDBQT format.

AutoDock Vina (ADV)[14] was used throughout the research. Two parameters were adjusted above the default scenario: the exhaustiveness of search ('exhaustiveness') set to 30, and the number of modes to report ('num_mod') set to 10.

The described protocol was validated to dock innate ligands into receptors. In all cases, the experimental geometries were reproduced closely (RMSD < 0.5 Å). The dispersion in the free energy estimations was higher but still acceptable. Thrombin: E_{exp} = –9.25,[18] ADV = –9.6 kcal mol^{-1}. Factor Xa: E_{exp} = = –12.8,[19] ADV = –9.1 kcal mol^{-1}. Docking results were analyzed with the help of AutoDock Tools and PyMol.

The geometry template for ferrocene moiety was taken from the PDB:1A3L. The remaining part of the ligands was built manually in Avogadro, followed by the molecular mechanics optimization (described above) with the ferrocene fragment frozen, since the assessed force fields do not preserve its correct geometry. During the preparation of PDBQT files, the ferrocene fragment was manually included into the ROOT part of the structure to exclude unwanted torsional rotations between its constituents.

While optimizing the binding of the structures, we tried to achieve optimal interaction with the parts of the binding site (pockets). The main task for all targets was an effective binding with the S1 pocket. In result, energy criteria and ligand efficiency were evaluated numerically, and steric matching and filling the pockets were assessed visually. The presence of hydrophobic contacts and the formation of hydrogen bonds and the alleged halogen–π-interactions at the bottom of the pocket S1 were taken into account.

Sixteen bispidines, in which the ferrocene moiety was linked to the nitrogen atom by a chain of one, two or three atoms (see Online Supplementary Materials), were studied to find the optimal linker. For factor Xa binding energy of the whole series was between –8.4 and –10.5 kcal mol^{-1} [830–25 nM (calculated K_i)] with most promising compound 3-[3-(4-chlorophenyl)-propyl]-7-(2-ferrocenylacetyl)-9-hydroxy-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonane (see Online Supplementary Materials) that had a good position of the scaffold, substantial immersion in both pockets and the formation of a hydrogen bond with GLU192. For thrombin, the results were even better with the energy range between –9.8 and –11.0 kcal mol^{-1} (81–11 nM) with the best structure being 3-(3-chlorobenzoyl)-7-(3-ferrocenyl-2-oxopropyl)-9-hydroxy-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonane shown in Figure 2. A task to have a sufficient immersion into the S1 and S4 pockets at the same time was successfully solved. It also resulted in (i) tight hydrophobic contacts of Fc with the S4 pocket, (ii) halogen–π-interaction in the S1 pocket and (iii) formation of two hydrogen bonds with SER195 and GLU192 (Figure 2). The ligands studied have also promising predicted ligand efficiency values [energy divided by number of heavy atoms (LE)] in the range of 0.3, which emphasizes their potential for further optimization.[20]

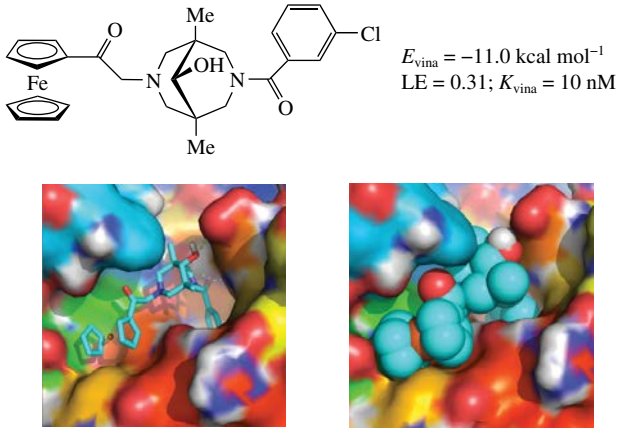


Figure 2 Position of the depicted molecule in the thrombin binding site.

In conclusion, it was found that the ferrocene group is able to fill tightly the S4 pocket for both thrombin and factor Xa. Bispidine scaffold provides the 3D arrengement and a direction for substituents to fill all pockets at the binding site of thrombin. Synthesis of the best compounds and testing of their binding to and/or inhibition of thrombin factor Xa are underway.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2016.04.011.

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