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Molecular structure, lipophilicity, solubility, absorption, and polar surface area of novel anticoagulant agents

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ABSTRACT

The methods of theoretical chemistry have been used to elucidate molecular properties of factor Xa inhibitors (rivaroxaban, apixaban, otamixaban, betrixaban, razaxaban, and DX-9065a) and direct inhibitor of thrombin (dabigatran). The geometries and energies of these drugs have been computed using HF/6-31G(d), Becke3LYP/6-31G(d) and Becke3LYP/6-31++G(d,p) model chemistries. In the case of the Xa inhibitors (rivaroxaban, apixaban, otamixaban, betrixaban, razaxaban, and DX-9065a) the fully optimized most stable conformers possess characteristic L-shape structure. Water has a remarkable effect on the geometry of the anticoagulants studied. The anticoagulant drugs exhibit the largest stability in solvent as expected. Computed partition coefficients (ALOGPS method) for drugs studied varied between 1.7 and 3.9. Neutral compounds are described as lipophilic drugs. Rivaroxaban is drug with lowest lipophilicity. The anticoagulants studied are only slightly soluble in water, their computed solubilities from interval between 5 and 70 mg/L are sufficient for fast absorption. Experimentally determined solubility of rivaroxaban (8 mg/L) is very well interpreted by calculation. Rivaroxaban with PSA value 88 belongs to the anticoagulants with increased absorption. Direct thrombin inhibitor dabigatran is molecule with high total number of proton donor and proton acceptor groups (15), high PSA (150) and lowest absorption of the compounds studied.

1. Introduction

Anticoagulants are key drugs for the prophylaxis and treatment of thromboembolic disorders [1–5]. Commonly used anticoagulants include parenterally administered unfractionated heparin and low molecular weight heparins, and the orally administered vitamin K antagonists (warfarin) [1,6]. These drugs are not targeted, i.e. they inhibit more than one enzyme in the coagulation cascade [1–6]. Heparin-based anticoagulants are indirect inhibitors that enhance the proteinase inhibitory activity of a natural anticoagulant, antithrombin [6]. Although effective, their use has been hampered by numerous limitations [4]. There is a growing interest in new, orally active anticoagulants with significant advantages to current agents such as heparin and warfarin for the treatment and prevention of thrombotic diseases. The new anticoagulants under investigation for venous thromboembolism treatment target factor Xa (fXa) or thrombin [1–5]. Inhibitors of factor Xa block thrombin generation, whereas thrombin inhibitors block the activity of thrombin, the enzyme that catalyses the conversion of fibrinogen to fibrin [1,3]. Besides synthetic indirect factor Xa inhibitors from the family of glycosaminoglycans (fondaparinux, idraparinux) numerous direct, selective factor Xa inhibitors are currently at various stages of development in different therapeutical indications [1–4]. Small-molecule synthetic compounds such as rivaroxaban, razaxaban, apixaban, betrixaban are members of a new class of orally available active-site-directed factor Xa inhibitors. Small-molecule direct thrombin inhibitors (dabigatran etexilate) inhibit thrombin directly by directly binding to the active catalytic site [1–3].

Despite a great deal of experimental evidence for the relationship between the chemical and pharmaceutical properties of new anticoagulants targeting factor Xa or thrombin and their biological activity, there is no single experimental study concerned with the systematic comparative experimental investigation of the physico-chemical and pharmacokinetic parameters of these medicinally useful new anticoagulants. Quantitative structure activity relationships of factor Xa inhibitors were discussed quite recently [7], and the X-ray crystal structures of rivaroxaban [8], DX-9065a [9], apixaban [10], otamixaban [11] and razaxaban [12] in complexes with factor Xa were used to clarify the binding mode of these ligands. The molecular structure of six monomeric structural units (1-OMe ΔIdoA-2SNa$_2$ (unit A), 1-OMe GlcN-S6SNa$_2$ (unit D), 1,4-DiOMe GlcNa (unit E), 1,4-DiOMe GlcN-S3S6SNa$_3$ (unit F), 1,4-DiOMe IdoA-2SNa$_2$ (unit G), and 1,4-DiOMe GlcN-S6SNa$_2$ (unit H)), four dimeric structural units (D–E, E–F, F–G, and G–H), two trimeric structural units (D–E–F, and F–G–H) and pentamer D–E–F–G–H

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(fondaparinux) of heparin has been previously investigated [13–17] using the density functional theory.

The absence of experimental data of novel synthetic anticoagulants targeting factor Xa or thrombin presents a challenge to the application of computational modeling techniques in order to enhance our understanding of the subtle biological effects of these anticoagulants. In this paper we have used the results of large-scale theoretical calculations for the study of the molecular structure, pK_a, lipophilicity, solubility, absorption, and polar surface area of factor Xa inhibitors (rivaroxaban, apixaban, otamixaban, betrixaban, razaxaban, and DX-9065a) and direct inhibitors of thrombin (dabigatran). The results of theoretical studies of these drugs were compared with the available experimental data and discussed in relation to the present theories of action of these agents.

2. Computational details

Theoretical calculations of the rivaroxaban, apixaban, otamixaban, betrixaban, razaxaban, DX-9065a, dabigatran, and dabigatran etexilate (Fig. 1) were carried out with the Gaussian 03 computer code [18] at the *ab initio* SCF (HF [19]) and density functional theory (DFT, Becke3LYP [20–24]) levels of theory using the 6–31G(d) and 6–31++G(d,p) basis sets. In order to evaluate the conformational behavior of these systems in solvent, we carried out optimization calculations in the presence of water. The methodology used in this work is centered on two solvation methods, PCM [25,26] and Onsager [27] models. The structures of all gas-phase species were fully optimized at the HF/6–31G(d) and Becke3LYP/6–31G(d) levels of theory without any geometrical constraint. In order to check the correctness of the B3LYP calculated relative energies using the double-ζ basis set, we also performed calculations of the anticoagulant species, using the larger basis set 6–31++G(d,p) implemented in the Gaussian 03 package of computer codes [14,15]. The structures of all condensed-phase (SCRF) species were fully optimized without any geometrical constraint at the DFT level of theory applied. Lipophilicity and water solubility calculations were carried out using web-based VCCLAB [28–30]. For calculations of molecular polar surface areas the fragment-based method of Ertl and coworkers [31,32] incorporated in the Molinspiration Cheminformatics software [33] was used.

3. Results and discussion

3.1. Molecular structures

Conformational search using theoretical methods for such large systems was in the past limited to use some of the available force-field methods [34]. Rapid advances in computer hardware and software and in quantum medicinal chemistry have brought high-performance computing and graphic tools within the reach of most academic and industrial laboratories, thus facilitating the development of useful approaches to rational drug design. Quantum chemical calculations are now applied successfully in medicinal chemistry and drug design to determine accurately molecular structures and properties for use in a wide variety of CADD studies [35]. It is common in the computational study of drugs to use structural data obtained from X-ray crystallography or NMR spectroscopy as guides to the quality of theoretical computations. In the absence of the experimental published data about molecular conformations of drugs, as an alternative to analyzing small molecule crystal structures the conformations of drugs bound to their protein targets can be examined [36]. Rivaroxaban, otamixaban and DX-9065a are chiral molecules and may be present as racemates indicating a specific interaction with fXa. The calculations for these drugs were carried out with the biologically most active enantiomers only. In the absence of the small molecule crystal structures, we examined the conformations of ligands bound to their fXa and trombin targets by studying the macromolecular crystal structures deposited in the Protein Data Bank. In the context of drug design, the conformation a small molecule adopts when bound to a pharmaceutical target is of fundamental importance. The relative orientation of anticoagulant moiety defined by individual dihedral angles (α, β, γ, δ, ε, ζ, η, and θ, Fig. 1) was taken from the experimental data for available X-ray data of the crystal structures deposited in the Protein Data Bank (PDB) [37] (complexes of the Xa factor with rivaroxaban (PDB: 2W26), apixaban (PDB: 2P16), otamixaban (PDB: 1KSN), razaxaban (PDB: 1Z6E), DX-9065a (PDB: 1FAX), and complex of the ethylester of dabigatran with trombin (PDB: 1HTS). A particular ligand conformations observed in these complexes may be due to an intermolecular interaction that is not present in solution or vapor phases. All compounds, but apixaban possess amide functionality, which imparts certain conformational rigidity to the overall structure of molecules studied. The possible relaxation of the geometry of a ligand upon dissociation from the receptor may bring new information about the conformation of drug in isolated state. An analysis of the harmonic vibrational frequencies at the HF level of theory of the optimized species revealed that all the structures obtained were minima (no imaginary frequencies). The Cartesian coordinates (Å) of all gas-phase drugs investigated, optimized at the B3LYP/6–31++G(d,p) level of theory, are given in Table A of the electronic Supporting Information. The geometries optimized at the B3LYP/6–31++G(d,p) level of theory are shown in Fig. 2. In the case of the Xa inhibitors (rivaroxaban, apixaban, otamixaban, betrixaban, razaxaban, and DX-9065a) the fully optimized most stable conformers possess characteristic L-shape structure. Examination of the space models of the B3LYP computed structures using two basis sets of the drugs investigated shows that the increase of the basis set gives essentially the same results. The effect of bulk solvent is treated with two solvation methods (the Onsager [27] and PCM [25,26]) for comparison. Initial calculations were carried out, for computational reasons, using the SCRF formalism of Wong et al. [38–41]. The radii of the cavities used in this approach were chosen after a volume calculation of each molecule, and the dielectric constant of water ($\varepsilon = 78.5$) was used. The placing of the isolated molecules into a spherical cavity within a dielectric medium of the Onsager model of solvation does not represent the realistic situation in the biological medium; it seems helpful in revealing the main role of the solvent in intermolecular electrostatic interactions. The second, PCM (conductor-like polarizable model), defines the cavity by the envelope of spheres centered on the atoms or the atomic groups [25,26]. The whole concept of using such macroscopic properties as dielectric constants in microscopic computations has been criticized [42,43]. Despite all these valid criticisms, continuum-based methods of solvation are used extensively and successfully in a variety of problems [44,45]. It has been shown previously [46] that the conductor-like polarizable method reproduces hydration energies with accuracy in the order of a few kcal/mol but mostly (70% of the cases) even better than one kcal/mol.

The factor Xa inhibitors (rivaroxaban, apixaban, otamixaban, betrixaban, razaxaban, and DX-9065a) studied do not possess common pharmacophore functionality. However, the intense research in this field accumulated new results, which have been summarized in a number of publications [1–12]. The binding of inhibitor to fXa is characterized by two general interaction sites S1 and S4 (Fig. 3). Based on the analysis of the binding in the S1 pocket fXa inhibitors are categorized into two classes. One class of early inhibitors mimics

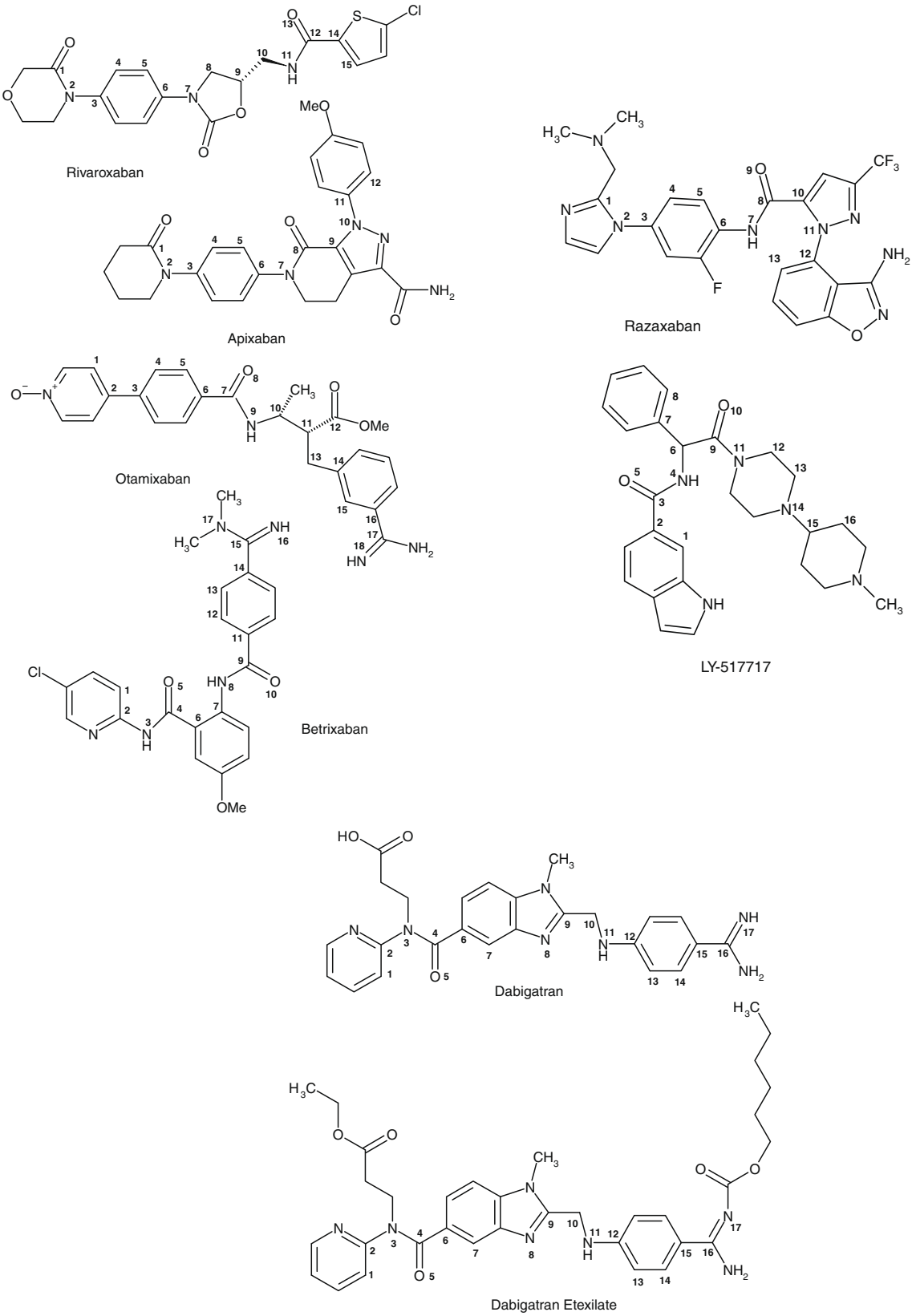

Fig. 1. Structure and atom labeling in the anticoagulant drugs studied.

often contain phenylamidine group mimicking this interaction (otamixaban, betrixaban, DX-9065a). The second category of com- aminobenzisoxazole for razaxaban, chlorothiophene moiety of rivaroxaban). In addition to the S1 pocket, a second major binding site of

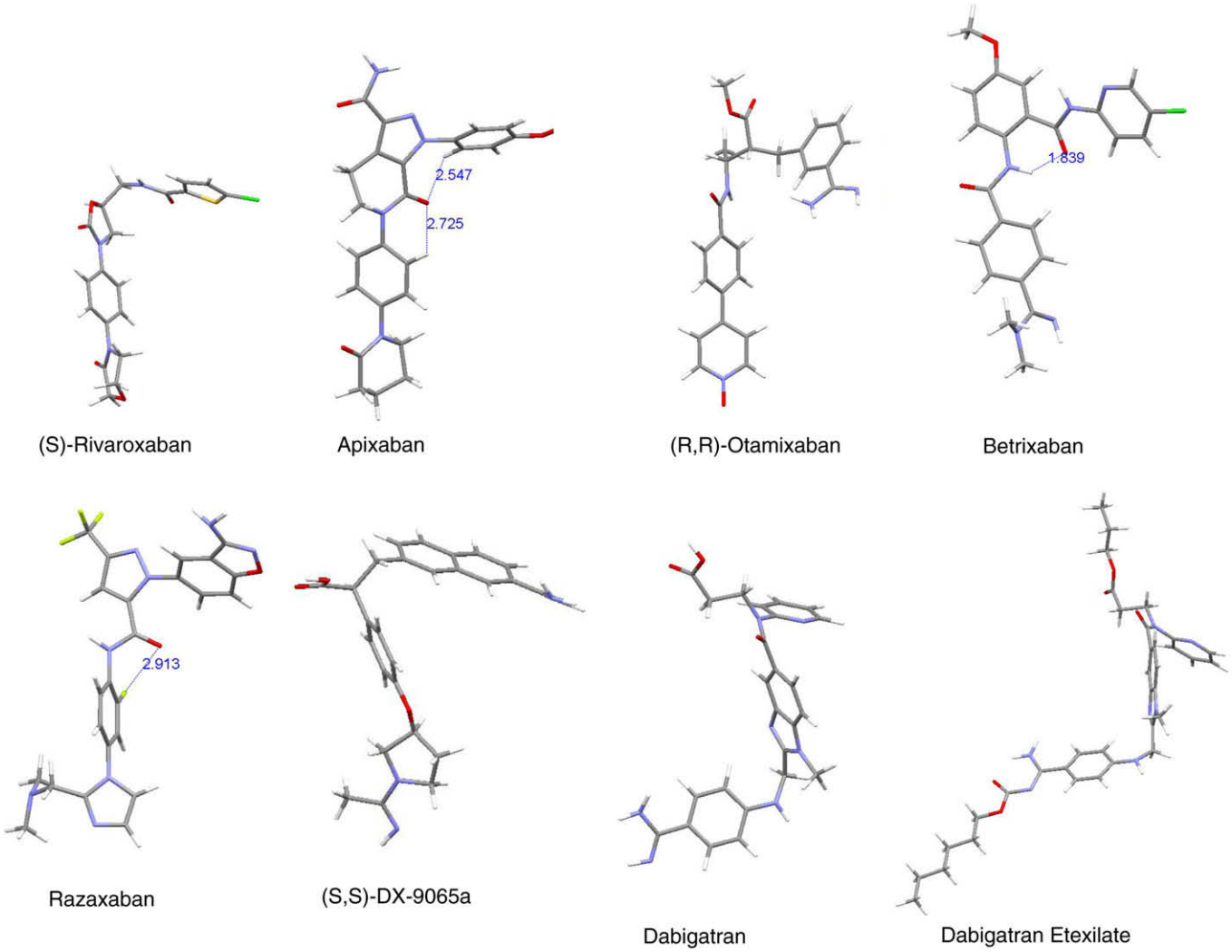

Fig. 2. B3LYP/6–31++G(d,p) optimized structures of the anticoagulants investigated.

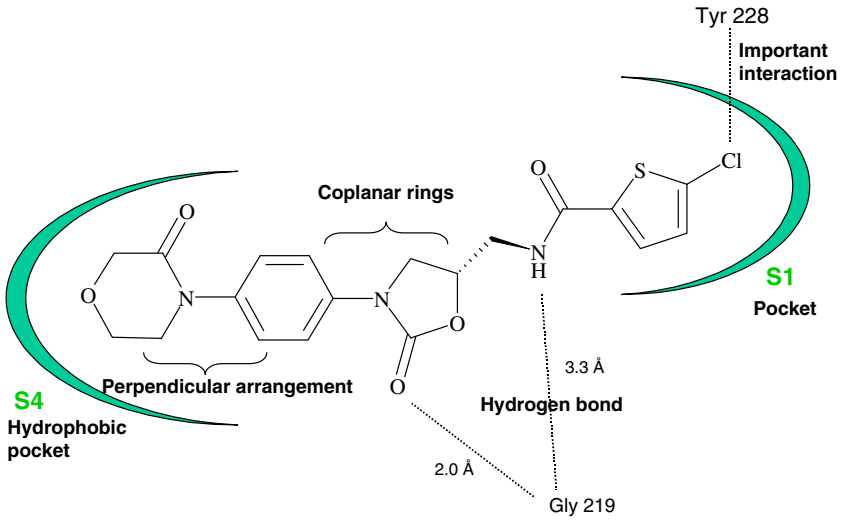

Fig. 3. Binding model of rivaroxaban. S1 and S4 pocket represents binding sites typical for synthetic direct fXa inhibitors.

aromatic rings of Tyr99, Phe174, and Trp215 [8]. Dabigatran is a no-

and distal (D) pockets. The amidine group of dabigatran interacts

interaction with the S2 pocket, and the 2-pyridyl group is positioned between Leu99 and Ile174 in the D-pocket [47].

The relative molecular orientation of individual drugs studied is described by different number of rotatable bonds. These dihedral angles of the fully optimized anticoagulants are given in Table 1 together with the available X-ray structures of these drugs in the bound state (at the receptor). Values for these dihedral angles for individual drugs are different (Table 1), and no general conclusions about pharmacophore functionality can be deduced. Thus, the structure of drugs studied will be discussed individually. According to our calculations in the gas state, the equilibrium geometries computed at the HF level of theory are in general agreement with the DFT results obtained with the "standard" 6-31G(d) basis set. However, the *ab initio* SCF and DFT optimized dihedral angles of some drugs studied exhibit large differences (within the 10°–20°). In order to study the basis set effect on the geometry of the anticoagulants investigated within the DFT theory we also carried out calculations using the larger basis set 6–31++G(d,p). The extension of the basis set in the DFT calculations resulted in only small changes in the equilibrium geometry of the drugs studied. The optimized dihedral angles using two basis sets within the DFT theory fit one another to within about 2°–5° (Table 1).

Water has a remarkable effect on the geometry of the anticoagulants studied (Table 1). Table 2 shows the results obtained for calculations performed in both, vacuum and that based on the solvation method used. The anticoagulant drugs exhibit the largest stability in solvent as expected, since they hold considerable dipole moment (Table 2). The energy difference between gas phase and solvated phase was significant for the both solvation models employed in this work. The solvated phase energies within the Onsager model were obtained after full geometry optimization in water. However, some anticoagulants failed to optimize geometries in water within the PCM formalism. Table 2 contains water stabilization energies using single-point PCM calculations and in vacuo fully optimized geometries. The comparison of the single-point PCM and available water stabilization energies obtained after full geometry optimization in water indicate that in the case of neutral drugs the optimization of geometry in aqueous medium does not significantly change the solvation energy. The difference in water stabilization energy is very small (around 5 kJ/mol). The PCM provided substantially more stable structures than Osanger's model.

Experimentally, small molecule drug conformations are commonly studied using X-ray crystallography. The absence of the published experimental X-ray structural data of anticoagulants studied presents a challenge to the application quantum chemical methods in order to obtain information about the stable conformations of these drugs in the gas phase and in solution. A comparison of the *ab initio* SCF calculated conformational energies of drug molecules with the conformer distribution in the solid state routinely show a good correlation [48]. Moreover, previous investigations of the protein–ligand complexes revealed similar torsion angles distributions for fragments when the bound and unbound distributions were compared [49]. For the reason of comparison and analysis of theoretically determined conformations and protein-bound conformations of anticoagulants studied we also present the available structural data for bound anticoagulants on the factor Xa receptor.

3.1.1. Rivaroxaban

The 1,3-oxazolidin-ring system of rivaroxaban (5-chloro-*N*-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]thiophene-2-carboxamide) has in position 5 a chiral carbon atom and possesses two enantiomers termed *R* and *S* with a clear preference for (*S*)-configuration [8]. Optimized molecular 3D structure of the (*S*)-rivaroxaban studied corresponds to the bound molecule at the protein, therefore the general structural motifs of drug can be compared with results for isolated molecule from theoretical methods only. The experimental values for the dihedral angles in the rivaroxaban–fXa complex are well interpreted by the corresponding angles computed for the solvated (*S*)-rivaroxaban (Fig. 4). The main difference in the molecular structure of bound and unbound rivaroxaban arises from the position of the morpholinone end moiety, dihedral angle α[C(1)–N(2)–C(3)–C(4)]. The carbonyl group of this moiety effects mainly a planarization of the morpholinone ring and brings it into a rather perpendicular arrangement to the aryl ring [8]. The DFT calculations suggest more planar arrangement of the morpholine and aryl rings. The dihedral angle α[C(1)–N(2)–C(3)–C(4)] is about 51°–55° (DFT method), and for (*S*)-rivaroxaban in water solution, and/or at receptor increases to about 67°–77° (Table 1). Oxazolidone and aryl rings are almost coplanar (the dihedral angle β[C(5)–C(6)–N(7)–C(8)]) (Table 1).

3.1.2. Apixaban

Apixaban (1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5-dihydropyrazolo[5,4-c]pyridine-3-carboxamide) is one of the most rigid molecules of the anticoagulants studied. Its 3D structure (Fig. 2) is governed by three dihedral angles (α, β, and γ), Table 1. The 3D structure of the bound apixaban at the factor Xa receptor, the gas-phase structure and solvated apixaban are substantially different. In general, the coordination of the apixaban to its fXa receptor (PDB file 2P16) leads to the perpendicular arrangement of the phenyllactam moiety (dihedral angle α[C(1)–N(2)–C(3)–C(4)]). The same perpendicular arrangement forms also bicyclic pyrazole scaffold and p-methoxyphenyl moiety of bound apixaban (dihedral angle γ[C(9)–N(10)–C(11)–C(12)]. The phenyllactam and the bicyclic pyrazole scaffold of bound apixaban are also in almost perpendicular arrangement (dihedral angle β[C(5)-C(6)-N(7)-C(8)]), Table 1. Values of these dihedral angles in the gas-phase structure and solvated apixaban are quite different. The unbound apixaban is in gas phase and in water solution substantially more planar, the planarization effect is especially considerable for the moiety containing phenyl ring, bicyclic pyrazole scaffold and p-methoxyphenyl group (dihedral angles β, and γ). The central part in the planarization of this apixaban moiety plays the carbonyl group of the central lactam group of the bicyclic pyrazole scaffold. This group effects a planarization of whole moiety via electrostatic intramolecular interaction with acidic hydrogen atoms of the neighboring rings (Fig. 2). The C-3 carboxamido substituent and the pyrazole scaffold are in mutual planar arrangement.

3.1.3. Otamixaban

(*R*,*R*)-Otamixaban (methyl (2*R*,3*R*)-2-(3-carbamimidoylbenzyl)-3-{[4-(1-oxidopyridin-4-yl)benzoyl]amino}butanoate) is structurally very flexible molecule, and its space arrangement is defined by 8 dihedral angles (Fig. 1). The P4 phenylpyridyl-*N*-oxide and benzamidine groups, responsible for the interaction of otamixaban with the fXa receptor, are connected by the system of single bonds enable large structural flexibility of this drug on receptor. A comparison of the fully optimized isolated molecule and the bound otaximaban (PDB file 1K3N) indicates that the largest structural rearrangement resulting in the biologically active conformation is related with the dihedral angles ε, ζ, and η (Table 1). The gas-phase conformations of the phenylpyridyl-*N*-oxide and benzamidine groups of otamixaban are also preserved in bound drug. The amidine group of the benzamidine moiety is twisted out of the aro-

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