Chapter 1

Biochemistry and Stereochemistry of Anticoagulants

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Additional information is available at the end of the chapter

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Abstract

The spatial conformation of a molecule, in general, is closely connected to its interaction with the human body, meaning bioreceptors, metabolizing enzymes, transporting proteins, etc. This chapter provides useful information regarding the importance of spatial conformation(s) of anticoagulant molecules in their pharmacological activity. It is divided in several sections: firstly, a short introduction is made into the world of stereochemistry, and the importance of this field to the pharmacotherapy is highlighted. Then, each anticoagulant class is treated regarding their spatial orientations and their significance linked to the mechanism of action, anticoagulant activity, potency, etc.

Keywords: stereochemistry, biochemistry, spatial conformation, enantiomer

1. Introduction

The mechanism of action of anticoagulants is very complex, involving many biochemical reactions. The coagulation cascade which is finally blocked and the formation of a clot is stopped in a direct (e.g. direct Xa factor inhibitors) or indirect way (e.g. vitamin K antagonists) assume many reactions, which involve the activation of factors, cofactors and enzymes. The coagulation cascade involves a series of protein conformation changes, which are called "activations" and which are dependent one to each other leading to this cascade series of reactions. Most of these reactions are calcium-dependent, and some of them are vitamin K-dependent (also known as the anti-haemorrhagic vitamin). The coagulation cascade ends with the formation of a fibrin clot, and it can be stopped by interruption by natural ways, such as genetic disorders (e.g. haemophilia A, B or C) or by extrinsic ways, when administering anticoagulants. As well known, when it comes about natural-occurring compounds, these reactions are very specific, but when it comes about external synthetic derivatives, the

receptors can be "tricked" in different ways. One cause could be the stereochemistry of these compounds, which is also important for these specific interactions.

Stereochemistry can be named the chemistry of 3D compounds and shows the relationship between a spatial orientation of a molecule and its physical and chemical properties. Very often, when a drug molecule can adopt different spatial shapes and forms, its reactivity with the biomolecules can change. Taking into consideration that from its introduction to the human organism until its excretion, a drug will meet tens, hundreds or even more types of biomolecules, its reactivity is translated into its pharmacological effects, but its toxicity is expected to depend also on its spatial orientation.

The aim of this chapter is to highlight the importance of stereochemistry in anticoagulation therapy, following each subclass of anticoagulants. For this purpose, we present basic notions of stereochemistry with details about the importance of spatial conformations of drug molecules related to the biochemistry of these drugs, and in the end of the chapter, we will find out whether the 3D structure of anticoagulants should be thought of in an anticoagulation treatment plan.

2. Basics of stereochemistry

Stereochemistry is defined as chemistry of spatial isomers. When one refers to stereoisomers, generally they are divided into two categories: firstly, there are the *enantiomers*, which are given by the asymmetry of a certain compound, and secondly, there are the *diastereomers*, which include all other conformational isomers which are not enantiomers.

In living organisms, the isometry given by enantiomers, also called *chirality*, is very important. As mentioned before, they are defined by asymmetry. The asymmetry of a molecule can come in two ways:

- When a carbon atom has four different substituents; in this case, the carbon atom is represented by a centre of asymmetry and is called asymmetrical carbon atom; in this case, one can talk about *planar chirality*; there are other types of atoms, such as N or P, which can confer asymmetry to a molecule, but these cases are rarer.
- Usually, in case of macromolecules, such as polypeptides, proteins, DNA, etc., the whole molecule presents a conformational asymmetry; in this case, one can talk about *intrinsic or helicoidal chirality*. We can say that even the human body, if it were regarded as one molecule, has intrinsic chirality, on the inside and also on the outside, as we are not perfectly symmetrical.

The enantiomers of a molecule are represented as the object and its self-mirror-image (**Figure 1**), which are not superimposable.

Under physical and chemical aspects, the two enantiomers of a pair present the same physical properties (same melting point, same boiling point, same density, etc.) and, unlike diaster-

eomers, have the same chemical properties in non-chiral environment. Their properties differ only in chiral environment.

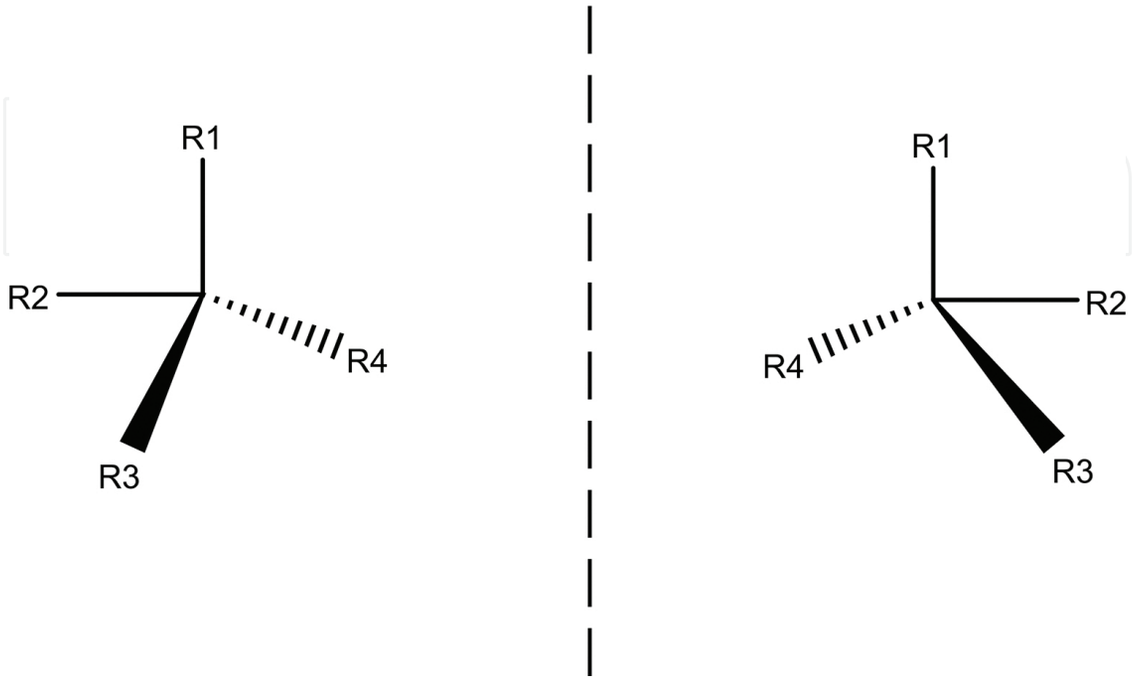

Figure 1. Pair of enantiomers—(S)-enantiomer in the left and (R)-enantiomer in the right—represented as object and its self-mirror-image.

Another different property of enantiomers is their behaviour in polarized light. In one pair of enantiomer, one enantiomer will rotate the plan of polarized light towards the left with a certain angle, while the other one will rotate the plan of polarized light with the same angle but in the other direction. The first enantiomer will be called *levogyre* and is noted (−) or l, and the other one is called *dextrogyre* and is noted (+) or d. The equimolar mixture of the two enantiomers is called *racemic mixture*, is noted with ± and does not affect the plan of polarized light.

Another internationally accepted nomenclature of enantiomers is the Cahn-Ingold-Prelog nomenclature. This uses the absolute configuration of the molecules, which involves the position of the four groups surrounding the asymmetrical carbon atom. They are numbered from the lightest atom directly bonded to the asymmetrical carbon to the heaviest. If the order of reading is towards the right side (or clockwise), the enantiomer is called R (or *rectus*); if the order of reading is towards the left side (or counterclockwise), the enantiomer is called S (or *sinister*) (**Figure 1**). Conventional D or L nomenclature is used for amino acids and saccharides. This should not be confused with the d or l nomenclature. For this reason, to indicate whether an enantiomer is levogyre or dextrogyre, the (+) or (−) notation is preferred.

One molecule will always have a number of 2^{n-1} pairs of enantiomers, where n represents the number of centres of asymmetry of the molecule. For example, the coumarinic anticoagulant warfarin (see **Figure 5**) has one centre of asymmetry represented by one asymmetrical carbon

atom; thus, the molecule will have two enantiomers or one pair of enantiomers. The optical isomers of the molecule which are not enantiomers (one with each other) are called *diastereomers*. In case of a symmetrical molecule where one chiral centre compensates the effect of another one, the enantiomers are practically identical, and they are called *mesomers*.

There are other types of stereoisomerism, where molecules can adopt different spatial conformations. The conformational isomerism of saccharides is worth mentioning. For example, D-glucose can adopt a chair or a boat conformation, a function of the display of the carbon atoms in the hexa-atomic cycle in space. The helicoidal chirality of proteins and other macromolecules should not be left behind.

2.1. Importance of chirality in human life

The reaction between a drug and its receptor is usually a specific reaction between the target molecule and a protein which acts as receptor. As macromolecules, the protein receptors are almost always chiral, so one can expect in case of chiral drugs to meet an enantioselective drug-receptor interaction, which could lead to different effects translated in the pharmacological activity of the molecule.

When it comes about chiral drugs, there can be five cases in which the two enantiomers of a drug can react with the receptors of the human body, translated in their pharmacological or toxicological activities [1]:

- The most common case is when the two enantiomers have the same pharmacological activities. At first sight, the use of a single enantiomer would not be necessary. However, there are many substances which have one enantiomer (eutomer) 5, 10 up to 60 times more active than the other one (distomer). This is the case of adrenaline, omeprazole or coumarinic anticoagulants. In case of omeprazole, it is long time since the eutomer, (S)-(−)-omeprazole, has been introduced on the pharmaceutical market as the single enantiomer (Nexium®). The case of coumarinic anticoagulants will be discussed in Section 4.

- There can be the case when only one of the enantiomer is pharmacologically active, while the other one is inactive. In this case, a separation of the two enantiomers is not strictly necessary. However, in some cases, when the two enantiomers have different metabolic pathways, a chiral separation could prevent some drug-drug interactions.

- When the eutomer and the distomer have totally opposite pharmacodynamic effects, a chiral separation would be necessary. However, in many cases, the racemic mixture is used in therapy, preserving the effect of the most potent enantiomer.

- Another example is the well-known case of thalidomide, in which the eutomer is responsible for the pharmacological activity, while the distomer is responsible for the adverse effects. In this case, the separation of the two enantiomers is mandatory.

- There are cases when using the racemic mixture is more beneficial, compared to the use of the single enantiomers.

As it can be seen, there are some cases when the use of the single enantiomer in drug therapy is mandatory. Moreover, even in other cases, this choice may have many benefits compared to the classical use of the racemic mixture. For these reasons, some drugs which were previously used as racemic mixtures have been "rediscovered" and have been reintroduced in pharmaceutical formulations as their single enantiomer form. This phenomenon is called *chiral switch*, and one of the most recent examples is represented by dexlansoprazole, the eutomer of lansoprazole, commercialized under the name of Dexilant® [2].

All these above-mentioned indicate clearly that the spatial conformation of an active molecule is very important for its pharmacological activity and, moreover, for its possible toxicological effects to the human body.

3. Heparin and related compounds

Heparin is a natural linear polysaccharide, which belongs to the class of glycosaminoglycans, formed of glucosamine units alternating with glucuronic acid and iduronic acid units, bearing N-sulphate, O-sulphate and N-acetyl groups. It acts as an anticoagulant by binding to antithrombin III and changing its conformation, leading to its activation. It is biosynthesized by enzymatic cleavage of the macromolecular heparin, a proteoglycan. Its composition can be very heterogeneous, depending on the type of tissue where it is extracted from and of the species. A very similar polysaccharidic structure is represented by heparan sulphate.

A specific pentasaccharide sequence (**Figure 2**), formed of glucuronic and iduronic sulphated units, has been identified as the active site of heparin for antithrombin III [3].

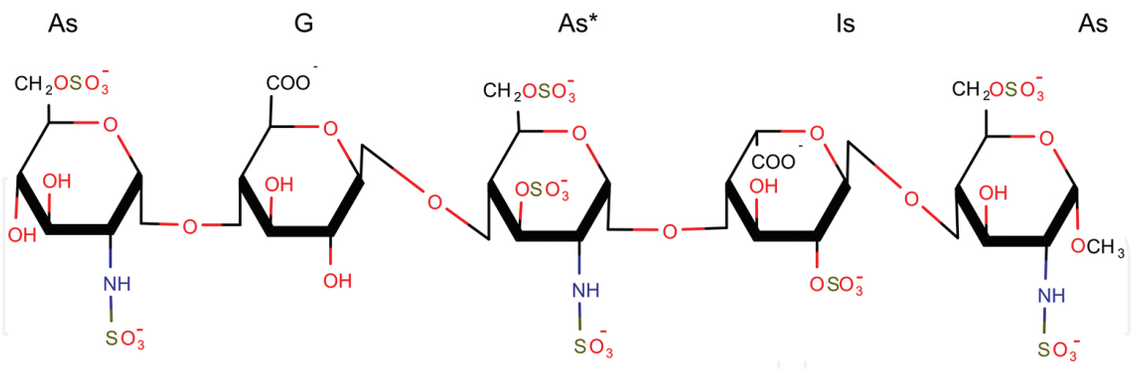

Figure 2. Sequence of the specific pentasaccharide in heparin, which runs as active site for antithrombin III (As, D-glucosamine-N-sulphate; G, α-D-glucuronic acid; As*, D-glucosamine-N-6-disulphate; Is, α-D-iduronic acid).

The conformational studies of the pentasaccharide sequence of heparin realized by ^{1}H-RMN and mechanical calculations [4] have shown that this sequence is formed of a rigid trisaccharide and a more flexible disaccharide. The rigid trisaccharide is based on a glucuronic acid unit, and it is supposed to be responsible for the direct bonding to the active site of antithrombin III, while the flexible disaccharide is based on an iduronic acid unit, it can take several conformations due to the iduronic acid flexibility, which can show different ring conforma-

tions, and the "choice" of a certain conformation can decide the bonding to other sites of the antithrombin III molecule. NMR and molecular modelling studies have shown that the iduronate residue in the pentasaccharide sequence can adopt several conformations of the pyranose ring. Results have shown that the most stable conformations in terms of lowest calculated energies are two chair conformations—$^{1}C_{4}$ and $^{4}C_{1}$—and a twisted conformation, so-called skew-boat conformation, noted $^{2}S_{0}$, and in the heparin polymer, the iduronate unit is found as a mixture of these three conformations. Studies have shown these conformations are interconvertible [4–6] (**Figure 3**).

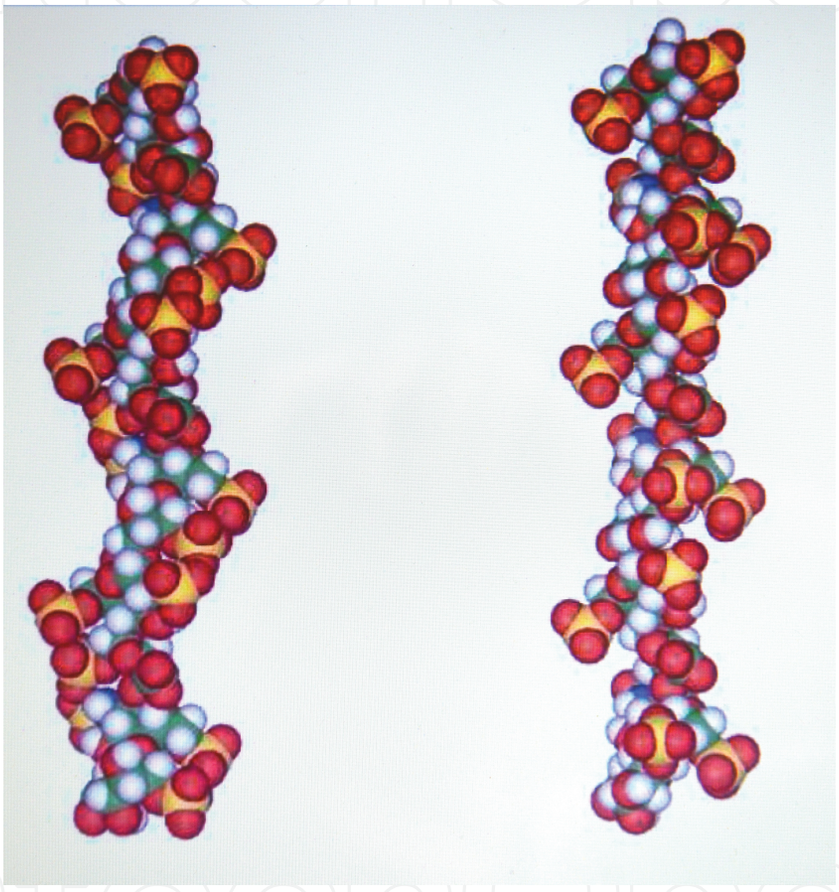

Figure 3. Structures of heparin determined by NMR studies, shown with the iduronic acid unit in the chair $^{1}C_{4}$ conformation (left) and in the skew-boat $^{2}S_{0}$ conformation (right). Reproduced from Mulloy and Forster [6] with permission of Oxford University Press.

We can notice that the spatial conformation is very important in case of heparin starting even from its mechanism of action. Jin et al. [7] have elucidated the mechanism of interaction between the heparin-active pentasaccharide and antithrombin III. The mechanistic study revealed that the antithrombin molecule suffers several conformation rearrangements which allow the pentasaccharide to enter the active site. More specific, the α-helix rearranges allowing the formation of hydrogen bonds with the sulphate groups in the pentasaccharide; the α-sheet is closing, while residues in the active loop are partially expulsed, and the D-helix is extended by one-and-a-half turns (**Figure 4**).

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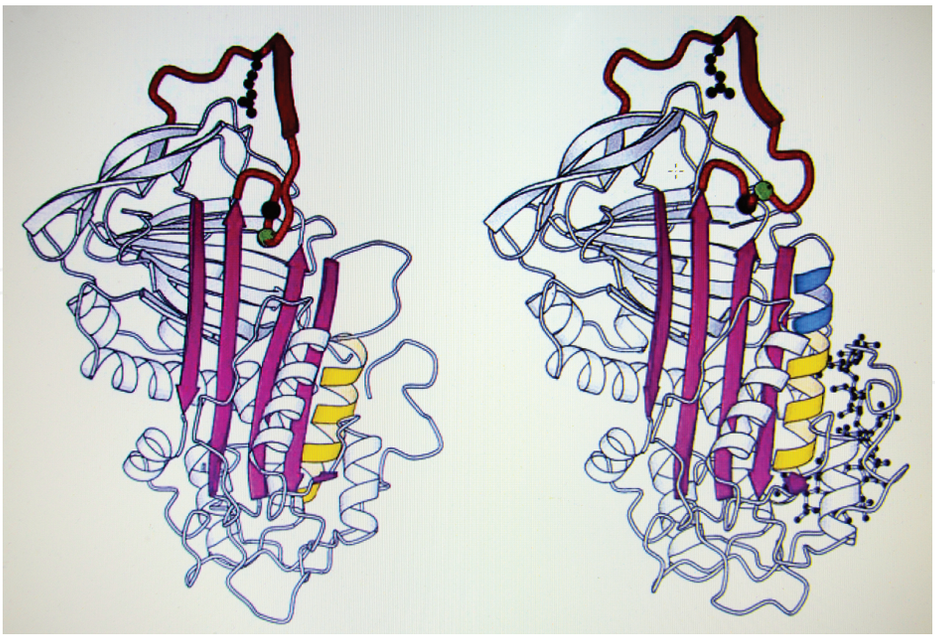

Figure 4. Comparison between free antithrombin III conformation (left) and antithrombin-pentasaccharide complex conformation (right). α-Sheet is closed (magenta), D-helix is extended (blue), and residues in active loop are expulsed (black and green dots). Reproduced from Jin et al. [7] with permission of National Academy of Sciences of the USA.

Thus, the active site loop is partially inserted into the α-sheet, until the antithrombin molecule forms the complex with the pentasaccharide of the heparin polymer. Consequently, the antithrombin-pentasaccharide complex formation and activation model could be extended to the whole heparin polymer. Moreover, by elaborating this model, the authors [7] have demonstrated the importance of conformation changes which take place in antithrombin molecule. A valuable example is the case of natural mutations of antithrombin, which lead to thromboembolic syndrome. Any replacement of any amino acid in the active site of the antithrombin molecule will lead to its inactivity by incapacity of conformation change, because of incomplete or lack of binding sites to the pentasaccharide.

In addition to the conformational change of antithrombin, Verli and Guimarães [8] have described the conformational change of the pentasaccharide, when forming the complex with antithrombin, thus leading to an induced fit mechanism of reaction. A change in the dihedral angles between the glucosamine and iduronic acid unit and, respectively, between glucosamine and glucuronic acid unit, from 20 to 30° was observed, even if the pentasaccharide was in skew-boat $^{2}S_{0}$ conformation or in chair $^{1}C_{4}$ conformation. This gives a new perspective to the mechanism of interaction between heparin and antithrombin, where the conformational flexibility is important on both sides, the ligand and the substrate. Taking a deeper view into the induced fit mechanism of interaction between heparin and antithrombin, it is worth mentioning that there is a need of 7 up to 30 hydrogen bonds for the complex to get formed. This information is essential for the drug development and design for new anticoagulant structures, heparin-related [8]. For example, the highly sulphated saccharidic polymer of heparin was replaced by a highly sulphated lignin, to obtain a new anticoagulant potential

drug [9], with reduced bleeding side effects compared to the low-molecular-weight heparin derivative, enoxaparin.

As the activation of antithrombin was understood as an interaction between certain oligosaccharides and the substrate, thus understanding that not the entire polymer of heparin participates in the activation of the antithrombin, other similar pentasaccharides or other oligosaccharides with potential anticoagulant activity have also been synthesized. In the same time, heparin was fractionated to lower-molecular-weight polymers, which were not as heterogeneous in composition as the original heparin.

The chirality (asymmetry) of heparin has also been used in chiral separations, where heparin has been utilized as a chiral selector. For example, Jin and Stalcup [10] have used heparin as chiral additive to the background electrolyte in capillary electrophoresis (CE), for the separation of several antihistaminic chiral drugs, such as pheniramine, chlorpheniramine, brompheniramine, carbinoxamine and doxylamine. The mechanism of enantioseparation was proposed as being a combination between inclusion complex formation and electrostatic interaction.

4. Coumarinic anticoagulants (vitamin K antagonists)

Unlike heparin and related derivatives, coumarinic anticoagulants have relatively small molecules, and their anticoagulant mechanism of action is totally different. They act by inhibiting the epoxide reductase which is involved in the recovery of vitamin K, after its oxidation.

Warfarin is the most prescribed coumarinic anticoagulant in the USA and UK. Besides warfarin, in other European countries, acenocoumarol and phenprocoumon are used as vitamin K antagonists (**Figure 5**). In all three cases, they present chiral structures as they contain a chiral carbon atom in their molecule, leading to the existence of two enantiomers in each case.

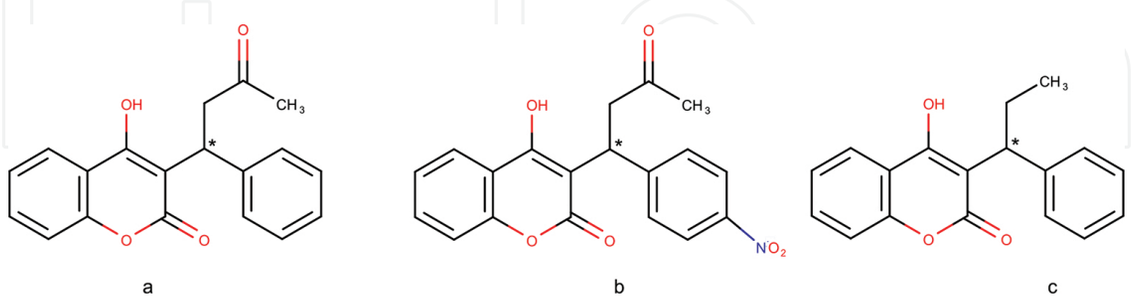

Figure 5. Molecular structures of coumarinic anticoagulants: (a) warfarin, (b) acenocoumarol, and (c) phenprocoumon; chiral centre is indicated with asterisk (C*).

In all three cases, there is a difference in the anticoagulant activity between the two enantiomers of the substance. In case of warfarin and phenprocoumon, their (S)-(−)-enantiomers are two up to five times more active than their optical isomers, (R)-(+)-phenprocoumon, and (R)-(+)-

warfarin, respectively. In case of acenocoumarol, the (R)-(+)-antipode is around five times more active than the (S)-(−)-enantiomer [11].

It is worth mentioning that the difference of only one nitro group between warfarin and acenocoumarol structures leads to the reversal of potency of anticoagulant activity between the two enantiomers; in consequence, (R)-(+)-warfarin and (S)-(−)-acenocoumarol are the more potent ones.

This difference of anticoagulant activity between the enantiomers of coumarinic anticoagulants comes from two directions. Firstly, there are the genetic determinants, and secondly, the different metabolic pathways can explain these differences.

To understand the two types of differences, one should understand the mechanism of action of these anticoagulants. Briefly, they act by interfering in vitamin K-dependent reactions in the organism [11]. Vitamin K, also known as coagulation vitamin or anti-haemorrhagic vitamin, is required for the liver synthesis of some coagulation factors (II, VII, IX and X), protein C and protein S. On the one hand, the above-mentioned molecules become active in γ-carboxylation reaction vitamin K-dependent. Inactive proteins cannot pass through the coagulation cascade. On the other hand, during these reactions, vitamin K suffers an oxidation to a 2,3-epoxide form, which is inactive. The vitamin K 2,3-epoxide reductase (VKOR) is the enzyme which brings vitamin K to its initial active form.

The coumarinic anticoagulants act by inhibiting the 2,3-epoxide reductase. Consequently, vitamin K remains inactive, and so factors II, VII, IX and X remain inactive, and the coagulation cascade is stopped.

Several studies [12–15] reveal that the main genetic polymorphisms responsible for the inter-patient variability are for the VKOR enzyme, under its different VKORC1 haplotypes and the cytochrome P450 2C9 isoenzyme (CYP2C9), involved in the metabolism of these coumarinic anticoagulants. However, a more recent study [16] has proven the significant relationship between VKORC1 genotypes and differences in pharmacodynamics of warfarin enantiomers. The authors have shown that patients with TT genotype of VKORC1 are more sensitive to (S)-warfarin and relatively more sensitive to (R)-warfarin. They have proven a clear anticoagulant contribution of (R)-(+)-warfarin to the racemic mixture, even if the (S)-warfarin is the more potent one. However, when administering the racemic in polytherapy, drug-drug interactions may also occur because of (R)-warfarin; likewise, when administering an inhibitor of (R)-warfarin, such as cimetidine, an increase in the anticoagulant effect is likely to appear. As these interactions do not appear in all populations, it is likely to notice a relationship between (R)-warfarin contribution to drug-drug interactions and the VKORC1 genotype [16].

The genotypes of the CYP2C9 isoenzyme are closely related to the anticoagulant activity of these three molecules and their enantiomers. As this isoenzyme of CYP450 is the main route of metabolism for all three drugs, there is a very close relationship between the pharmacogenetics, pharmacokinetics and in the end pharmacodynamics of the molecule. Thijssen and Ritzen [17] have proven the influence of CYP2C9 polymorphism on the different pharmacokinetics of the two enantiomers of acenocoumarol. The study results revealed that the plasma concentrations of (S)-acenocoumarol at 7 hours after administration of racemic were higher

for patients who presented the CYP2C9*2 or CYP2C9*3 alleles, compared to the control group of CYP2C9*1/*1. In contrast, for (R)-acenocoumarol, it was not observed any significant influence of the CYP2C9 polymorphism over its plasma concentration, when determined at 7 hours or at 24 hours after administration. For (S)-acenocoumarol, the plasma concentration at 24 hours was not detectable (see **Table 1**). These results show the importance of optical conformation of a drug in vivo. In addition to all these data, in some cases, the presence of other CYP2C9 alleles, such as *4, *5 or *6, should be taken in consideration [17]. In case of warfarin and phenprocoumon, there is not such a distinct difference of clearance between the two enantiomers, as can be seen in **Figure 6**.

Genotype	(S)-acenocoumarol (ng/mL)		(R)-acenocoumarol (ng/mL)		
	4 hours after intake	7 hours after intake	4 hours after intake	7 hours after intake	24 hours after intake
*1/*1 (n = 9)	34.9 ± 18.1	5.1 ± 4.2	219 ± 48	146 ± 21	34.5 ± 5.3
*1/*2 (n = 7)	26.3 ± 12.5	9.9 ± 4.9	199 ± 40	135 ± 31	31.7 ± 5.9
*1/*3 (n = 6)	47.7 ± 18.7	14.6 ± 6.6	226 ± 31	151 ± 35	30.7 ± 16.9
*2/*3 (n = 3)	33.2 ± 8.0	16.6 ± 5.0	163 ± 27	136 ± 36	33.9 ± 0.6
*2/*2 (n = 1)	59.6	25.8	180	167	ND

ND, not determined; n, number of patients. Reproduced from Thijssen and Ritzen [17] with permission of John Wiley and Sons, Inc.

Table 1. Plasma (R)- and (S)-acenocoumarol concentrations after oral intake of 8 mg racemic acenocoumarol: effect of CYP2C9 polymorphism.

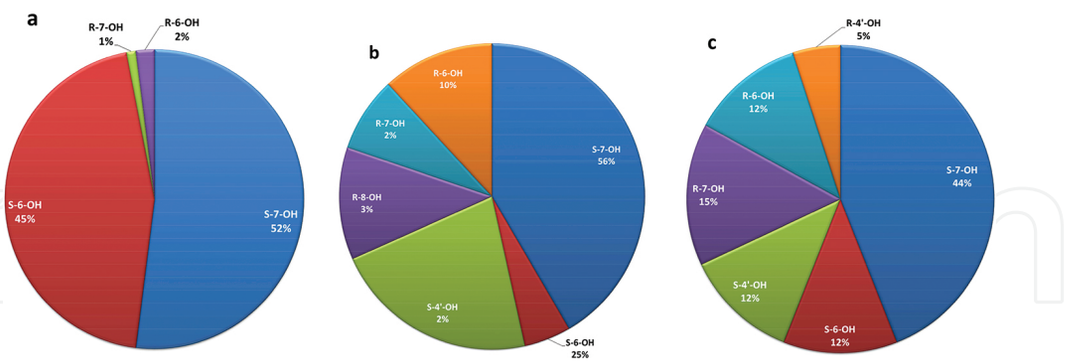

Figure 6. Intrinsic clearances of (a) (S)-acenocoumarol and (R)-acenocoumarol, (b) (S)-warfarin and (R)-warfarin and (c) (S)-phenprocoumon and (R)-phenprocoumon, as calculated from Thijssen et al. [18, 19] and Ufer et al. [20].

As could be seen, the genetic polymorphism leads to a difference in pharmacokinetics of the two enantiomers. In case of acenocoumarol, as it can be seen in **Table 1**, the half-life of (S)-enantiomer is much shorter than of the (R)-enantiomer. In case of CYP2C9*1/*3, the half-life of (S)-acenocoumarol may be even doubled, up to 4 hours, which may lead to an increase in anticoagulant activity. However, even if (S)-acenocoumarol is one of the most potent

anticoagulants in the 4-hydroxycoumarin class [18], its half-life seems to be too short for the (S)-acenocoumarol to interfere in the vitamin K-dependent reactions and to stop the coagulation cascade. Consequently, we can say that generally the effect of the racemic mixture is given by the (R)-acenocoumarol.

Another difference of pharmacokinetics between the two acenocoumarol enantiomers is given by the isoenzymes responsible for their metabolism. While CYP2C9 [18] seems to be the main enzyme to transform (S)-acenocoumarol to its 6- and 7-hydroxy-metabolites, in case of (R)-acenocoumarol, there are several enzymes involved in this process, which can explain the difference in their resulting in vivo half-life. (R)-acenocoumarol is 7-hydroxylated by CYP2C9, but the 6-hydroxylation is mediated, partially by other CYP450 isoenzymes, such as CYP1A2 and CYP2C19. For these enzymes, other drug-drug interactions in polytherapy cases should be predicted.

Phenprocoumon [12] seems to be less affected by CYP2C9, but its metabolism is realized mainly by CYP3A4. Moreover, a significant quantity of this anticoagulant is eliminated under untransformed form, while acenocoumarol and warfarin are almost totally metabolized through hydroxylation. CYP4F2 and CYP2C18 have also been mentioned to participate in the metabolism of coumarinic anticoagulants. Genetic polymorphism should be taken in consideration in this case, too. At the same time, different genetic variants of enzymes involved in the chain of reactions on which the anticoagulant mechanism of these molecules is based should be considered. For example, the different genotypes of epoxide-hydrolase (EPHX1) and γ-glutamyl carboxylase (GGCX) can lead to different anticoagulant potencies [20–22].

Taking all these in consideration, it remains one question: is it worth to introduce the single enantiomer use in therapy for these drugs? The answer is that in all cases, it should at least be reflected about. The different routes of metabolism for the two pair-enantiomers, influenced partially by pharmacogenetic factors as seen, give them not only different half-lives but also different anticoagulant potencies. In addition to these, one should consider that in most cases, these drugs come in polytherapy, exposing the patient to many drug-drug interactions. When eliminating one of the two enantiomers, at least some of these metabolic pathways which lead to drug-drug interactions can be eliminated. In this case, also food-drug interactions should be considered, which can be partially eliminated, and by reducing the dose, the toxicity and probably some of the adverse effects should decrease. So, as in other drug cases (e.g. omeprazole and lansoprazole), a chiral switch may be considered for these coumarinic anticoagulants. However, in case of acenocoumarol, even if the most anticoagulant active enantiomer seems to be (S)-acenocoumarol, its short half-life makes it almost unusable, while the other enantiomer, (R)-acenocoumarol, which could be kept, presents many metabolic pathways which anyways could not be avoided. In this case, other factors, such as economic elements, may be considered, when having a chiral separation or chiral synthesis for the single enantiomer use.

There have been numerous chiral separation methods developed for the separation of these three chiral drugs. These separations can be realized by high-performance liquid chromatography (HPLC), coupled with UV [23, 24] or mass spectrometer (MS) [25] detectors or by capillary electrophoresis (CE) [26, 27]. However, in case of drug control analysis or chiral purity analysis, there is not necessarily the need to effectively separate the two enantiomers but to

know the enantiomeric ratio. For this purpose, p-tert-butylcalix[6]arene derivative has been used as chiral selector for the chiral enantiorecognition of warfarin [28] in a UV-spectral study coupled with the multivariate analysis of the spectral data. The results showed a good capacity of enantiorecognition of the achiral molecule of calixarene towards the enantiomers of warfarin, being able to determine the ratio of one enantiomer when this was in a proportion of up to 5 % in the mixture. The impact of this study relies not only in the determination of the enantiomeric ratio but in the fact that with the aid of multivariate analysis, which can interpret seemingly messy and complicated data, the different behaviour of enantiomers can be discovered even in achiral media, as achiral structures were used as chiral selectors.

5. Direct Xa factor and thrombin inhibitors

As this type of anticoagulants represents recently discovered molecules, their chirality has been already explored in the preclinical phase studies, as regulators in the field (Food and Drug Administration) demand it. To highlight once again the importance of chirality in medical and pharmacological field, it is worth mentioning that among these substances, all which are chiral, are used under their single enantiomer form (**Figure 7**).

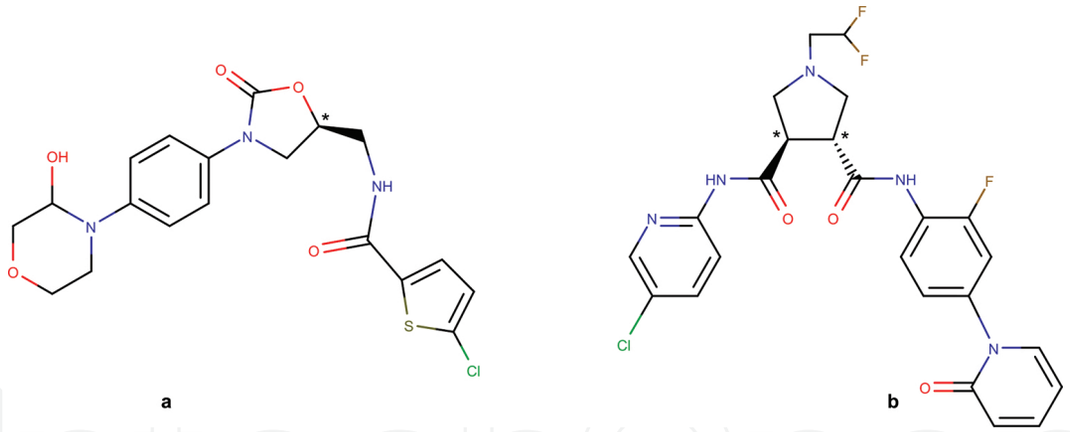

Figure 7. Molecular structures of some direct Xa factor inhibitors: (a) rivaroxaban and (b) R1663, with indication of chiral centre(s).

For example, the (S)-enantiomer of rivaroxaban is used in therapy, as it has a 10,000 times higher affinity towards the active site of Xa factor compared to its pair enantiomer or its diastereomers [29]. In contrast, the (R,R)-enantiomer of otamixaban is preferred as anticoagulant agent [30] as it fits better into the active site of Xa factor. In a similar way, the (R,R)-enantiomer of a novel molecule, (3R,4R)-1-(2,2-difluoro-ethyl)-pyrrolidine-3,4-dicarboxylic acid 3-[(5-chloro-pyridin-2-yl)-amide] 4-{[2-fluoro-4-(2-oxo-2H-pyridin-1-yl)-phenyl]-amide}, named R1663, has already passed phase 1 clinical study for its introduction on the drug market [31]. Edoxaban is another inhibitor of Xa factor used as the single enantiomer in therapy, while it has three asymmetrical carbon atoms in its molecule.

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All these differences in spatial conformation actually exhibit a higher anticoagulant potency or activity by a better interaction with the active site of the target substrate. The interaction with the Xa factor must be seen like a "hand-glove" acceptance, where only one "hand" is most suitable for the "glove".

In a similar way, in case of chiral molecules used in therapy as thrombin inhibitors, one should expect to see a difference in affinity towards the substrate, with a higher anticoagulant activity of one enantiomer compared to the other one. Thus, the thrombin inhibitor argatroban (**Figure 8**) is used in its single enantiomer form as (R,R)-enantiomer [32].

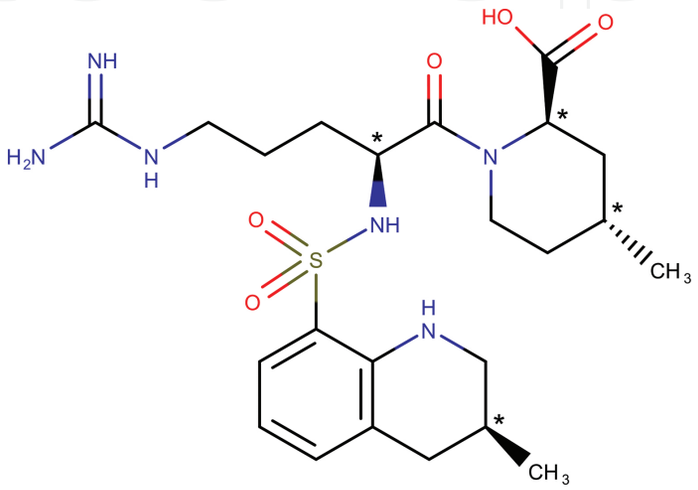

Figure 8. Molecular chiral structure of argatroban, with indication of all chiral centres (C*).

6. Proteic anticoagulants

Proteins, by their nature of macromolecules, are very dependent on their spatial conformation. All natural proteins and polypeptides are formed of natural L-amino acids. The spatial conformation of proteins is crucial for their activity as enzymes, drug receptors, etc.

Thrombin and antithrombin, as it could be seen previously, are two proteins involved in the cascade of coagulation. There are several polypeptidic and proteic anticoagulants used in therapy, which generally target these two molecules. It is worth mentioning the first introduction in therapy, in 2009, of recombinant human antithrombin-α obtained from transgenic goats' milk [33].

Another protein that has given very good results as anticoagulant in deep vein thrombosis is batroxobin, a toxin extracted from *Bothrops atrox* and *Bothrops moojeni* venom [34]. Results showed successful limb salvage for all patients taken in study after they were administered batroxobin, due to anticoagulant and fibrinolytic mechanism of this molecule.

Hirudin is an antithrombin-like protein which possesses anticoagulant effects. It is a smaller proteic molecule which binds to thrombin into its active site. Mengwasser et al. [35] have

studied the hirudin-thrombin complex, in terms of stability, affinity one to each other and conformation. Results showed a higher affinity of hirudin towards the fast (F) allosteric form of thrombin, compared to the slow (S) form. There are common residues responsible for both complex formations, but there are seven residues which are responsible for the more than 10 times higher affinity of hirudin towards the F form of thrombin. The residues Asp221 and Asp222 do not interact directly with hirudin, the effect being probably a stabilization of the S form by Na^+ binding. The same effect could be for Gly-193. The other four residues—Lys36, Leu65, Thr74 and Arg75—do interact directly with hirudin. The preference towards the F form stays probably due to the different spatial orientation of these residues, leading to a better "match" with hirudin in the F form (**Figure 9**).

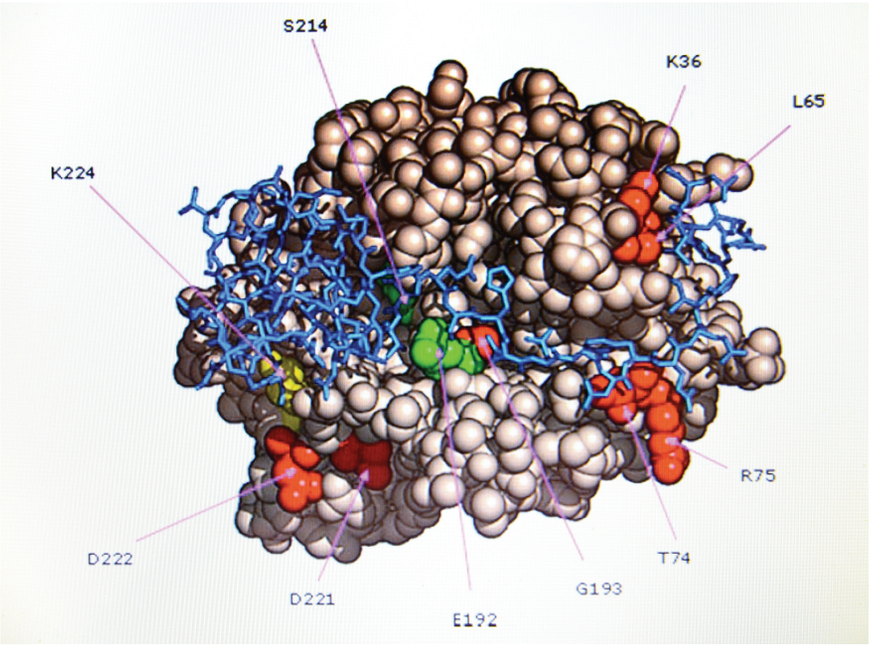

Figure 9. Model of binding of hirudin (marine sticks) to the F (red) and S (green) forms of thrombin. The higher affinity of hirudin towards F thrombin is indicated through the seven residues. The important residue Lys224, which makes an important contribution to hirudin binding, is spotted in yellow. Reproduced from Mengwasser et al. [35] with permission of the American Society for Biochemistry and Molecular Biology.

7. Concluding remarks

Taking all the above-mentioned in consideration, one can say that stereochemistry and the spatial conformations play a key role in the life of a drug molecule, in general, and of an anticoagulant, in special, in terms of pharmacogenetics, pharmacokinetics, pharmacotoxicology and/or pharmacodynamics. When talking about macromolecules, we cannot separate them from their spatial conformation, which is an essential factor which influences their activity. In case of small molecules, the existence of one or more pairs of enantiomers gives them the possibility to act differently with the chiral receptors of the organism, with possible

different effects to be observed. As already seen, these reactions depend also on the flexibility of both the target molecule and the receptor macromolecule, but they usually take place following a "hand-glove" model. Stereochemistry of anticoagulants is important in all aspects and should be considered before establishing a treatment plan.

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