BIOINFORMATICS ANALYSIS OF CYTOCHROME P450 2C FAMILY

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Abstract. Cytochrome P450 enzymes (CYPs) are involved in phase I of metabolism of many xenobiotics, including drugs. The CYPs belonging to the family 2 are known to be responsible for about 20% of the total drugs metabolism and it underlines the importance of understanding the structural properties of these enzymes in correlation with their biological functions. This paper compares structural properties of the members of subfamily 2C of the human cytochrome P450: CYP2C8, CYP2C9 and CYP2C19. It also analyzes the effects of point mutations corresponding to polymorphic variants of these enzymes on the local hydrophobicity and flexibility of the proteins chains. The three enzymes share high sequence identities, similar global physicochemical properties and a high resemblance of their global spatial structures. Despite their global structural similarity, there are local conformational differences concerning the binding sites with direct consequences on the specificity of every enzyme. The genetic polymorphism of the cytochromes belonging to the subfamily 2C is reflected by some amino acid mutations that significantly affect the local hydrophobicity and/or flexibility of the proteins chains and resulting in their poor ability to metabolize some drug or prodrug compounds.

Key words: cytochromes P450 2C8, 2C9 and 2C19, active site cavity, polymorphic variants.

INTRODUCTION

The cytochrome P450 enzymes (CYPs) belong to the family of hem-containing monooxygenases and reach maximum of absorption at 450 nm. In animal organisms these enzymes are mainly found in the liver, but also in extrahepatic tissues, such as in the lungs, duodenum, small intestine, uterus, ovary, brain, epithelial cells, etc. [6]. They catalyze a wide array of biochemical reactions being important for the synthesis and degradation of almost all non-protein ligands that bind to receptors or activate second-messenger pathways. They are involved in

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the phase I of metabolism of endogenous and exogenous compounds by oxidizing steroids, fatty acids and xenobiotics being responsible for about 75% of the total drugs metabolism [12]. They are also involved in hormones synthesis and breakdown, cholesterol synthesis, vitamin D metabolism and in the organism detoxification [26].

CYPs are highly polymorphic enzymes, the impact of the CYPs polymorphism in drug metabolism being widely recognized. Published data suggest that adverse effects of drugs may be avoided through individualization of drug therapies based on genetic polymorphism information [13, 24]. The most common interindividual variability in the activity of CYP enzymes is due to the single nucleotide polymorphisms (SNPs) in CYP genes [16]. CYPs polymorphism may affect enzyme expression and/or its function leading to distinct phenotypes: poor metabolizers (PMs) carrying two nonfunctional genes, intermediate metabolizers (IMs) carrying one functional and one nonfunctional gene, extensive metabolizers (EMs) carrying two copies of functional genes and ultrametabolizers (UMs) carrying more than two copies of functional genes [18]. Knowledge concerning CYPs polymorphism and its effects on drugs metabolism is important for dosing medications.

We focus our attention onto the subfamily 2C of CYPs (CYP2C) that metabolizes about 20% of clinically available drugs, some other xenobiotics and endogenous compounds and has four members in humans: CYP2C8, CYP2C9, CYP2C18, and CYP2C19 [10]. Our group recently reviewed the impacts of amino acid variations in sequences of enzymes belonging to the subfamily CYP2C on the drug metabolism and also discussed the structural bases of molecular mechanisms of amino acid variants of CYP2C members affecting drug metabolism [17]. This study follows the previous one and underlines both the structural properties that are common to all CYP2C members and those that are specific to each member.

CYP2C8 constitutes 26% of the CYP2C subfamily [9]. In humans, CYP2C8 subfamily of enzymes is mainly expressed in the liver but it is also found in the kidney, adrenal gland, brain, uterus, mammary gland, ovary, and duodenum being responsible for the metabolism of endogenous compounds such as retinoic and arachidonic acids and of xenobiotics, such as a wide range of drugs (paclitaxel, amiodarone cerivastatin, repaglinide chloroquine, etc.) [14]. For the CYP2C8 enzyme, 17 allelic variants are known, some of these variants strongly affecting the metabolism of drugs involved in the treatment of cancer, malaria, diabetes and inflammations [17].

CYP2C9 enzyme constitutes about 50% of the CYP2C members being expressed in the liver and duodenum [9]. It metabolizes antidiabetics, anticonvulsants, anti-inflammatories, anti-hypertensives, proton pomp inhibitors and anxiolytics [36] and several endogenous compounds (steroids, melatonin, retinoic and arachidonic acids) [25]. CYP2C9 presents 67 allelic variants affecting the metabolism of many clinically used drugs [17].

CYP2C18 is mainly expressed in the skin and its drug metabolism is not well defined. It has been reported that CYP2C18 efficiently catalyzes the metabolic bioactivation of phenytoin [20].

CYP2C19 is an enzyme found in the liver. It constitutes 16% of the CYP2C members [9] and metabolizes proton pump inhibitors, antiplatelet drugs, antidepressants and anticonvulsants and some endogenous compounds (melatonin, progesterone) [37]. There are 35 allelic variants of the CYP2C19 for humans, several alleles being involved in the alteration of drugs efficacy and/or adverse drug reactions [17].

The aim of this study is to analyze and compare the sequences and structures of the enzymes belonging to the CYP2C subfamily (CYP2C8, CYP2C9 and CYP2C19) in order to better describe their interactions with xenobiotics, especially with drugs, and to understand the substrate specificities of the individual members. Another goal of the study is to analyze how the amino acids variations due to the genetic polymorphism of these enzymes affect the local hydrophobicity and flexibility of their chains in order to understand the impact of the genetic polymorphism on the drugs metabolism.

Taking into account the high percentage of xenobiotics (including the clinically used drugs) that are metabolized by CYP2C enzymes, this study becomes important for understanding the molecular basis of drug recognition and metabolization by the CYP2C subfamily members and by their polymorphic variants, with direct applicability in clinical pharmacology.

MATERIALS AND METHODS

The sequences of the considered enzymes are retrieved from the UniProt database [1]. Starting from the sequences we perform the following analysis: (i) sequences comparison by multiple sequence alignment using the on-line tool CLUSTALOmega [22]; (ii) computation of the global physicochemical properties by means of the ProtParam tool [8]; (iii) study of the effects of amino acids mutations corresponding to the polymorphic variants of CYP2C members on the local hydrophobicity and flexibility of the proteins chains using the ProtScale tool [8]. ProtScale allows computing and representing the profile produced by an amino acid scale for a protein chain. The amino acid scale is obtained by assigning a numerical value to each type of amino acid. In our study we used the hydrophobicity scale produced by Kyte and Doolittle [21] and the average flexibility scale [3]. In order to obtain the profiles of the hydrophobicity and flexibility we established the length of the interval of amino acids to 3 to be used for the profile computation. It means that for the computation of the numerical value for a given residue i, the amino acids in an interval $[i–1, i+1]$ are considered.

Table 1

Structural files for the human CYP2C enzymes

Enzyme	PDB code entry	Resolution (Å)	Ligand	Observations
CYP2C8	1PQ2	2.70	palmitic acid	It illustrates a peripheral binding site for palmitic acid [30].
	2NNH	2.60	retinoic acid, palmitic acid	Two retinoic acid molecules are bound in the active site [31].
	2NNI	2.80	montelukast, palmitic acid	Montelukast is a big molecule and complements the size and shape of the active-site cavity [31].
	2NNJ	2.28	felodipine, palmitic acid	Felodipine is a small molecule positioned with its dichlorophenyl group close to the heme iron [31].
	2VN0	2.70	troglitazone, palmitic acid	Troglitazone occupies only the upper region of the active-site cavity [31].
CYP2C9	1R9O	2.00	flurbiprofen	The file contains the catalytic domain 29–489 with the ILE490VAL mutation. The active site cavity is bigger than the flurbiprofen molecule and it is also occupied by a glycerol and a water molecule [34].
	1OG2	2.60		The file contains the catalytic domain 30–490 of the unbound protein [35].
	1OG5	2.55	S-warfarin	The file contains the catalytic domain 30–490 of the protein in complex with S-warfarin [35].
	4NZ2	2.45	(2R)-N-{4-[(3-bromophenyl) Sulfonyl]-2-Chlorophenyl}-3, 3,3-trifluoro-2-hydroxy-2-methylpropanamide	The file contains the catalytic domain 30–490 of the mutated protein (GLU206LYS, VAL215ILE, TYR216CYS, PRO220SER, ALA221PRO, LEU222ILE, LEU223ILE) in complex with the(2R)-N-{4-[(3-bromophenyl)sulfonyl]-2-chlorophenyl}-3, 3,3-trifluoro-2-hydroxy-2-methylpropanamide [4].
CYP2C19	4GQS	2.87	(4-hydroxy-3,5-dimethyl phenyl) (2-methyl-1-benzofuran-3-yl) methanone, glycerol	The file contains the catalytic domain 21–490 of the CYP2C19 in complex with (4-hydroxy-3,5-dimethylphenyl)(2-methyl-1-benzofuran-3-yl)methanone and glycerol [29].

Spatial structures of CYP2C enzymes are downloaded from Protein Data Bank [2], the codes entry corresponding to their structural files being presented in Table 1. In addition, Table 1 contains the characterization of the structural files. Knowledge concerning the spatial structures of investigated enzymes allows the following analysis: (i) structures comparison and characterization by structural

superposition using Chimera software [27]; (ii) identification and characterization of the active sites cavities by means of the Fpocket online tool [23]; (iii) identification of the residues involved in the interactions with ligands using the ContPro computational tool [7]. For this last purpose, we have used a distance cutoff of 3.5 Å.

We also compute the fractal dimensions of the surfaces of investigated proteins in order to quantitatively describe the complexity and roughness of these surfaces. In order to compute this quantity we use the concepts of the fractal geometry and the methodology is described elsewhere [5].

The polymorphic variants of CYP2C enzymes are identified using two databases: SuperCYP (http://bioinformatics.charite.de/supercyp/) and The Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se/).

RESULTS AND DISCUSSION

Multiple sequence alignment performed using CLUSTALW online tool reveals that CYP2C enzymes have highly similar sequences as it is presented in the Figure 1. The sequence of CYP2C8 shares 77% identity with that of the CYP2C9 and 78% with that of the CYP2C19 and the sequences of CYP2C9 and CYP2C19 share 91% identity. Structural data expose 13 helical regions in the structural arrangement of CYP2C members designated using capital letters from A to L [4, 29, 30, 31, 34, 35] and highlighted in light grey in Figure 1. Furthermore, there are 6 identified substrate recognition sites (SRS) in CYP2C enzymes sequences: 96–117 (SRS1), 198–205 (SRS2), 233–240 (SRS3), 286–304 (SRS4), 359–369 (SRS5) and 470–477 (SRS6) [11, 32] highlighted in dark grey and bold letters in Figure 1.

Even if the sequence similarity between these enzymes is high, Figure 1 reveals that the distinct residues are often found in the SRS regions and they may be responsible for the substrate specificity. Also, the distinct physicochemical properties of these residues modify the geometry of the active site cavity [19]. This observation is important because a single or a few critical residues within an SRS may affect the ability of a particular substrate to bind to a CYP2C enzyme.

ProtParam tool allows computing the global physicochemical properties of CYP2C enzymes and they are presented in Table 2. These properties are quite

similar and it is not unexpected taking into account the high identity of enzymes sequences.

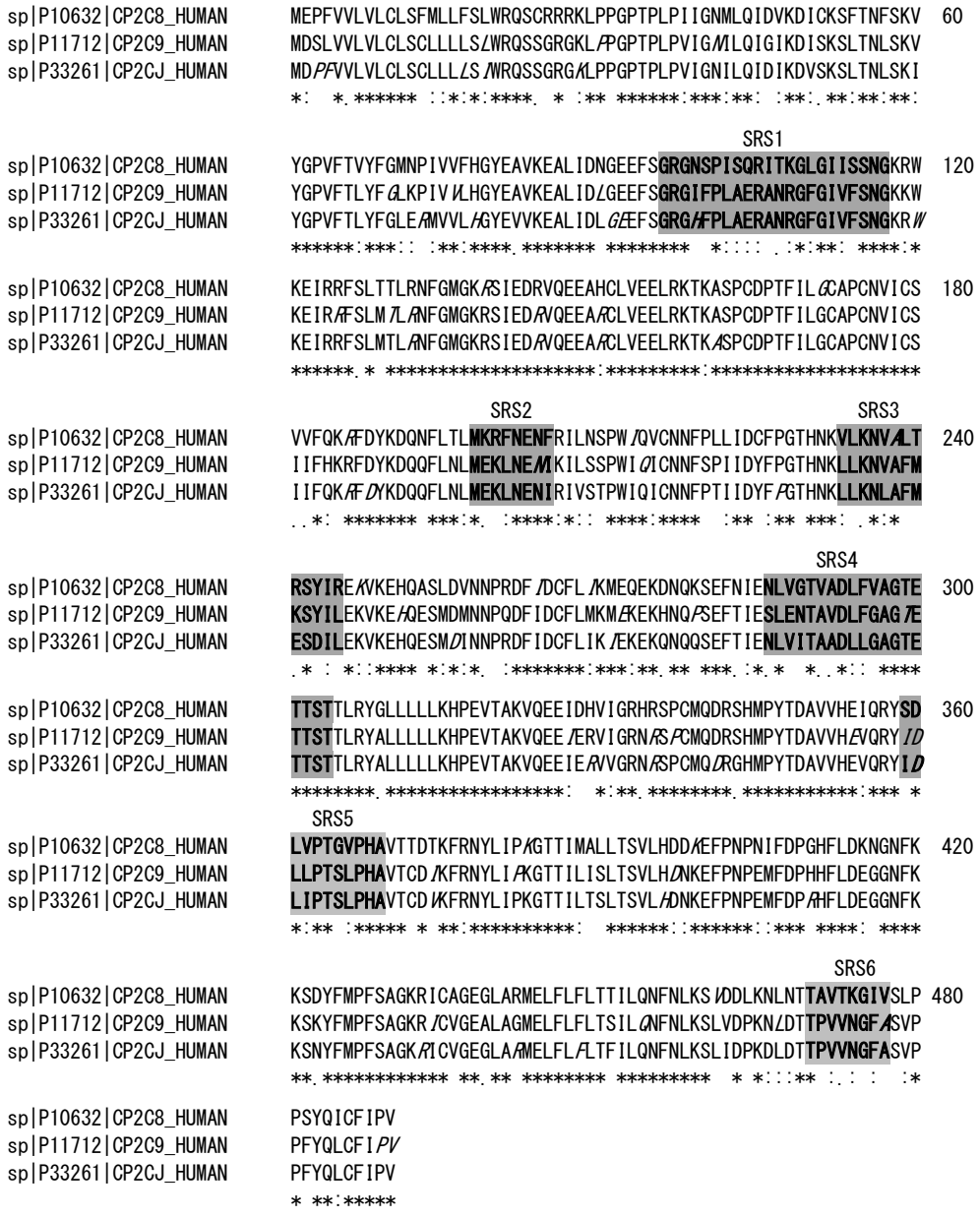


Fig. 1. Sequences alignment for the CYP2C subfamilly of human enzymes: the substrate recognition sites (SRS) are highlighted in dark grey and bold letters and mutated residues are presented in italics.

Table 2

Global physicochemical properties of the human CYP2C enzymes

Property	CYP2C8	CYP2C9	CYP2C19
Theoretical pI	8.80	8.13	7.11
Net charge	9	3	0
Aliphatic index	88.45	94.65	94.84
Hydropathicity index (GRAVY)	–0.167	–0.073	–0.098

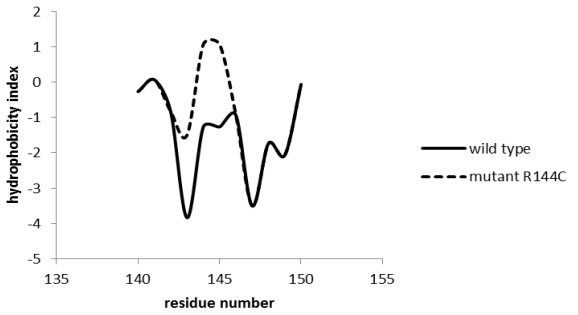
Because some of the charged residues might be either buried or involved in salt bridges, or the local environment of charged residues can affect their pKa, the calculation of the theoretical pI using bioinformatics tools starting from the sequence does not always match the real value in the body. Nonetheless, knowing its value gives suggestions on selecting the correct pH to use for experimental purposes. From this point of view, CYP2C19 reflects a neutral pI and CYP2C8 and CYP2C9 reveal quite basic pI values. These values are correlated with the net charges of these enzymes, CYP2C9 and CYP2C9 having positive net charges in comparison to CYP2C19 that is neutral.

The aliphatic index of a protein is defined as the relative volume of the protein occupied by aliphatic residues (alanine, valine, isoleucine, leucine) [8]. The aliphatic index is considered a positive factor for increasing the thermostability of globular proteins [15] and in our case the values presented in Table 2 reveal that CYP2C9 and CYP2C19 are more stable than CYP2C8.

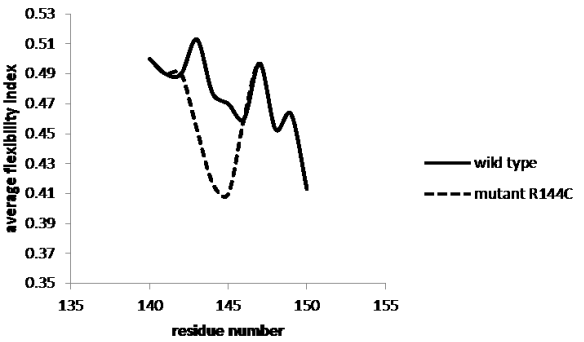
The hydropathicity index is a value characterizing the hydrophobic or hydrophilic properties of the protein [8]. The more positive is the value, the more hydrophobic is the protein. It is important to know this property, as proteins with similar hydropathicity indexes have affinity to each other, informing about the possible partners of interaction. All CYP2C enzymes reflect a low hydrophilic character.

Concerning the polymorphic variants of CYP2C members, there are some mutations in the proteins sequences strongly affecting their interactions with drugs: R139K, G171S, R186G, I223M, A238P, K247R, I264M, I269F, K383N, K399R and P404A for CYP2C8; D49G, V76M, L90P, G96A, G98V, F110S, K119R, R124Q, R125H, T130R, T130M, R132Q, R132W, R144C, A149T, R150H, R150L, P163L, N204H, I207T, Q214L, I222V, P227S, H251R, P279T, I284V, T299A, T299R, I327T, R335W, R335Q, P337T, S343R, I359L, D360E, L361I, P382S, I434F, Q454H, L467F,A477T, P489S for CYP2C9 and L17P, I19L, S51G, G91R, W120R, R132Q, I331V, R144H, R150H, P227L, D256N, R329H, V374I, R433W, F448L for CYP2C19 [17]. Only several residues that are mutated in the polymorphic variants of CYP2C enzymes belong to the SRS regions: one residue for CYP2C8, 5 residues for CYP2C9 and 2 residues for CYP2C19 exposing that mutations seem to not affect the drug recognition.

We have built the hydrophobicity and flexibility profiles for CYP2C enzymes and for their polymorphic variants. Figures 2 illustrates the local hydrophobicity and flexibility profiles around the amino acid arginine 144 of CYP2C9 for the wild type enzyme and for its R144C mutant (a mutation corresponding to the polymorphic variant CYP2C9*2) respectively.



a



b

Fig. 2. Local hydrophobicity (a) and average flexibility (b) profiles for the polymorphic variant CYP2C9*2 (R144C mutant).

Figures 2a,b reveal that R144C mutation produces an increase of the local hydrophobicity and a decrease of the local flexibility. The effects of mutations corresponding to the polymorphic variants of CYP2C8, CYP2C9 and CYP2C19 affecting the drug metabolism [17] on the local hydrophobicity and flexibility are presented in Tables 3–5.

Table 3

Effects of mutations corresponding to polymorphic variants of CYP2C8 on the local flexibility and hydrophobicity of the enzyme chain: white cells – the property is not modified, light grey cells – the property is decreased, dark grey cells – the property is increased

Polymorphic variant	Mutation	Effect on local flexibility of the chain	Effect on the local hydrophobicity of the chain
CYP2C8*2	I269F		
CYP2C8*3	R139K		
	K399R		
CYP2C8*4	I264M		
CYP2C8*6	G171S		
CYP2C8*8	R186G		
CYP2C8*9	K247R		
CYP2C8*10	K383N		
CYP2C8*13	I223M		
CYP2C8*14	A238P		

Table 4

Effects of mutations corresponding to polymorphic variants of CYP2C9 on the local flexibility and hydrophobicity of the enzyme chain: white cells – the property is not modified, light grey cells – the property is decreased, dark grey cells – the property is increased

Polymorphic variant	Mutation	Effect on local flexibility of the chain	Effect on the local hydrophobicity of the chain
CYP2C9*2	R144C		
CYP2C9*3	I359L		
CYP2C9*5	D360E		
CYP2C9*8	R150H		
CYP2C9*9	H251R		
CYP2C9*11	R335W		
CYP2C9*12	P489S		
CYP2C9*13	L90P		
CYP2C9*14	R125H		
CYP2C9*16	T299A		
CYP2C9*17	P382S		
CYP2C9*19	Q454H		
CYP2C9*26	T130R		
CYP2C9*28	Q214L		
CYP2C9*30	A477T		
CYP2C9*33	R132Q		
CYP2C9*57	N204H		
CYP2C9*58	P337T		
CYP2C9*59	I434F		
CYP2C9*60	L467F		

These tables indicate that mutations usually modify the local hydrophobicity and flexibility of the enzymes chains. This observation is in good agreement with another study where we found that these mutations affect the enzymes structures and stability and consequently their functions [17]. Also, it was already proven that the flexibility of the active sites of CYPs caused altered interactions with ligands [19]. Furthermore, the ligands interacting with CYP2C enzymes have been confirmed to be characterized by unrestricted hydropathicity features [28].

Comparison of the structural files of CYP2C enzymes exposes high global structural identities, the superposition of structures (Figure 3) revealing a root mean square deviation of 0.768Å for 409 pairs of carbon alpha atoms (CA) from the total of 460 for the superposition of CYP2C8 with CYP2C9, 0.721 Å for 446 CA pairs from the total of 469 for the superposition of CYP2C8 and CYP2 C19 and 0.708 Å for 408 CA pairs from the total of 459 for the superposition of CYP2C9 and CYP2 C19. In Figure 3, the structure of CYP2C8 (PDB code entry 2NNI) is presented entirely, but for CYP2C9 and CYP2C19 we only illustrate those regions that are dissimilar in structure. Figure 3 reveals that the most divergent in structure are the loop regions. This result is in good agreement with other published data revealing small conformational differences between the backbones of these enzymes and dissimilarities between some loops of the two enzymes [29].

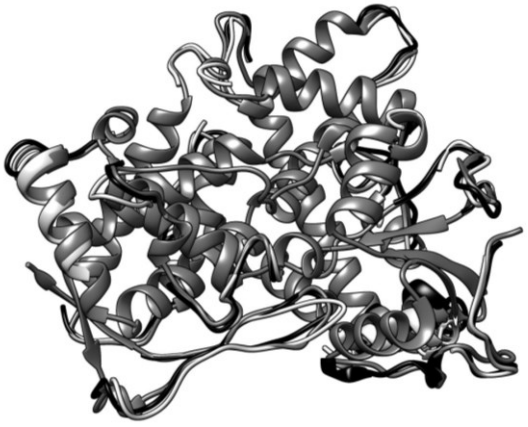


Fig. 3. Structures superposition for CYP2C enzymes: CYP2C8 (PDB code entry 2NNI) is presented as dim grey cartoon, the dissimilar regions of CYP2C9 (PDB code entry 1R90) are presented in light grey and those of CYP2C19 (PDB code entry 4GQS) are presented in black.

The similarity of the global structures is also sustained by the values obtained for the surface fractal dimensions for the three enzymes. The computation of the surface fractal dimension is illustrated in Figure 4 for the structural file corresponding to the complex of CYP2C19 with an inhibitor (PDB code entry 4GQS) and the values obtained for all the structural files considered in this study are presented in Table 6. There are not significant differences between the computed

values of the surface fractal dimensions reflecting the structural similarity of CYP2C enzymes. Also, the values obtained for the surface fractal dimensions are in good agreement with the published data revealing the fractal aspects of other proteins surfaces [4] and strengthens the hypothesis of the applicability of the fractal geometry concepts for studying the structural properties of proteins.

Table 5

Effects of mutations corresponding to polymorphic variants of CYP2C19 on the local flexibility and hydrophobicity of the enzyme chain: white cells – the property is not modified, light grey cells – the property is decreased, dark grey cells – the property is increased

Polymorphic variant	**Mutation**	**Effect on local flexibility of the chain**	**Effect on the local hydrophobicity of the chain**
CYP2C19*4B	I331V		
CYP2C19*5A	R433W		
CYP2C19*6	R132Q		
	I331V		
CYP2C19*8	W120R		
CYP2C19*9	R144H		
	I331V		
CYP2C19*10	P227L		
	I331V		
CYP2C19*11	R150H		
	I331V		
CYP2C19*14	L17P		
	I331V		
CYP2C19*15	I19L		
	I331V		
CYP2C19*16	R442C		
CYP2C19*18	R329H		
CYP2C19*19	S51G		
	I331V		
CYP2C19*22	R186P		
	I331V		
CYP2C19*23	G91R		
	I331V		
CYP2C19*24	R335Q		
	I331V		
CYP2C19*25	F448L		
	I331V		
CYP2C19*26	D256N		
	I331V		
CYP2C19*28	V374I		
	I19L		
	I331V		

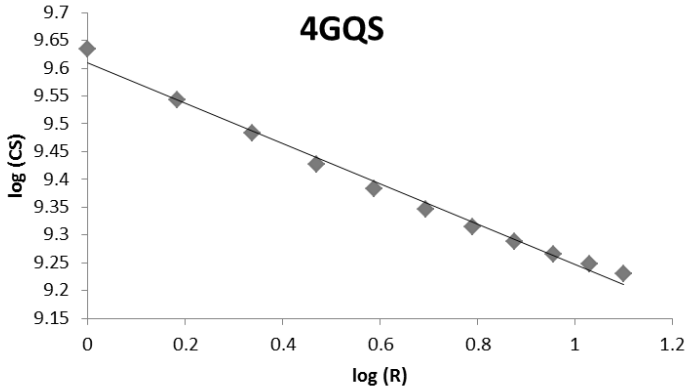


Fig. 4. Determination of the surface fractal dimension for the structure of the complex of CYP2C9 with an inhibitor (PDB code entry 4GQS).

Identification of active site cavities and their characterization has been made using Fpocket tool. The physicochemical characteristics of the active sites cavities of CYP2C enzymes are presented in Table 7 illustrating some distinct properties of the active sites cavities of CYP2C enzymes: CYP2C8 has an active site cavity with a lower hydrophobicity index and CYP2C9 reveals the active site cavity with the highest local hydrophobicity index. Other study illustrated geometric dissimilarities between the active site cavities of these enzymes, CYP2C8 having a larger active site than CYP2C9 and CYP2C19 [29]. These distinct properties of the binding sites cavities may explain and characterize the substrate specificity of these enzymes and they also strengthen the previous results reflecting little commonality in mechanisms relating to the interactions with ligands of CYPs enzymes [33]. Such information is helpful for understanding the regulation of these enzymes, with direct applicability in the clinical pharmacology.

Using ContPro tool we have identified the residues involved in the interactions with ligands for every CYP2C enzyme and these residues are presented in Table 8. Except GLY98 and F110 of the CYP2C9 that are involved in the interaction with S-warfarin and ASN204 of CYP2C9 being involved in the interaction with flurbiprofen, the other residues mentioned in Table 8 are not the subject of mutations conducting to polymorphic variants of CYP2C enzymes that affect drugs metabolism. This observation is in good correlation with the fact that residues that are subject of mutations in the polymorphic variants of CYP2C members do not belong to the SRS (Fig. 1). Moreover, this result emphasizes that residues outside of the active sites are important for the catalytic properties of these enzymes.

Table 6

Surface fractal dimensions obtained for the structures of CYP2C enzymes and of those of their complexes with ligands

Enzyme	PDB code entry	Surface fractal dimension
CYP2C8	1PQ2	2.35±0.24
	2NNI	2.31±0.18
	2NNJ	2.34±0.20
	2NNH	2.32±0.28
	2VN0	2.29±0.31
CYP2C9	1OG2	2.30±0.14
	1OG5	2.30±0.12
	1R9O	2.33±0.21
	4NZ2	2.31±0.19
CYP2C19	4GQS	2.36±0.16

Table 7

Geometric and physicochemical properties of the active sites cavities of CYP2C enzymes

Property	CYP2C8	CYP2C9	CYP2C19
Glogal hydrophobicity index	22.61	32.51	39.63
Local hydrophobicity index	60.08	99.57	77.78
Polarity index	28.00	23.00	27.00

Table 8

Interacting residues with ligands for the CYP2C enzymes (2QJ means (2R)-N-{4-[(3-bromophenyl)sulfonyl]-2-chlorophenyl}- 3,3,3-trifluoro-2-hydroxy-2-methylpropanamide and OXV means (4-hydroxy-3,5-dimethylphenyl)(2-methyl-1-benzofuran-3-yl)methanone

Enzyme	PDB code	Ligand	Interacting residues
CYP2C8	2NNI	montelukast	SER100, SER103, ILE106, THR107, ILE113, SER114, ARG200, PHE201, ASN204, LEU208, ILE213, ASN217, ASN236, VAL237, THR240, VAL296, ALA297, THR301, VAL477
	2NNJ	felodipine	VAL296, THR301, GLY365, VAL366
	2NNH	retinoic acid (2 molecules)	ARG97, GLY98, ASN99, SER100, SER103, ASN204, ASN217, ARG241, VAL296, ALA297, THR301, VAL336, ILE476
	2VN0	troglitazone	ILE106, PHE201, ASN204, PHE205, LEU208, ARG241, VAL296, GLU300
CYP2C9	1R9O	flurbiprofen	ARG108, ASN204, ILE205, LEU208, MET 240, ASP 239, GLY296, ALA297
	1OG5	S-warfarin	ARG97, GLY98, PHE100, LEU102, VAL113, PHE114, LEU208, ASN217, THR364, SER365, LEU366, PRO367, PHE476
	4NZ2	2QJ	ALA106, ASN107, PHE114, LEU233, GLY296, THR301, THR304, LEU362, LEU366, ALA477
CYP2C19	4GQS	0XV	PHE114, ILE205, VAL208, ASP293, ALA297, GLU300, THR301, ILE362, LEU366, PHE476

Table 8 also reflects that the residues involved in the interactions with distinct ligands for the same enzyme are quite different, confirming the distinct binding modes adapted for every ligand. This observation must be taken into account when designing modelization studies concerning the interactions of these enzymes with drugs or other xenobiotics.

CONCLUSION

Our structural bioinformatics study revealed that the three enzymes share high sequence identities, similar global physicochemical properties and a high resemblance of their global spatial structures. Despite the sequence identity and the global structural similarity, our study illustrated that there are local conformational differences especially concerning the loop regions of the CYP2C enzymes. Furthermore, this study revealed distinct physicochemical properties of the active sites cavities of these enzymes. Taking into account both the results obtained in this study and the published data [29, 33], we may conclude that all these dissimilar structural features of the CYP2C enzymes contribute to their specificity and to the distinctive binding modes adopted by every enzyme when interacting with diverse ligands. Revealing new structural features of CYP2C enzymes contributes to improve the knowledge regarding the complex relationships between cytochrome P450 structures and their biological function and to a better understanding of xenobiotics metabolism. Last but not least, such information underlines that choosing the most adequate structure of the CYP2C enzyme is important for structure-based drug design studies.

This study also reveals that amino acid mutations corresponding to the polymorphic variants of the CYP2C enzymes significantly affect the local hydrophobicity and/or flexibility of the proteins chains. Another study made by our group showed that these mutations strongly affect the stability of these enzymes [17]. Molecular docking studies using two polymorphic variants of CY2A6 and CYP3A4 obtained by homology modelling revealed that structural changes due to the punctual mutations in the polymorphic variants are not responsible for their loss of functions when metabolizing coumarin and testosterone [33]. The local changes in the hydrophobicity and/or flexibility of the chain seem to have consequences on the protein ability to metabolize some drugs or prodrug compounds. Determination of structures of these polymorphic variants in complex with some drug molecules could elucidate the effects of mutations on the drug metabolism.

The results of this study highlight the structural dissimilarities between the CYP2C enzymes and the contribution of the local hydrophobicity and/or flexibility of the protein chains to their interactions with specific ligands. This information is important for the understanding of drug and endogenous compound metabolism

and for further modelization studies considering the interactions of CYP2C enzymes with various xenobiotics, especially drugs.

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