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Molecular docking analysis on the interaction between bovine serum albumin and three commercial fluoroquinolones: Ciprofloxacin, enrofloxacin and pefloxacin

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ABSTRACT

Fluoroquinolones are a family of broad spectrum, systemic antibacterial agents that have been used as therapy for infections in the respiratory and alimentary tract in animals. The pharmacodynamic of this class is widely described, predominantly to the commercial drugs ciprofloxacin (CIP), enrofloxacin (ENR), and pefloxacin (PEF). Bovine serum albumin (BSA) is the main endogenous carrier in the bovine bloodstream, being responsible for the biodistribution of different classes of molecules and drugs, including fluoroquinolones. The molecular features and interaction between BSA and fluoroquinolones are not fully described, thus, the present work enlightens the intimacy of the interaction of BSA with CIP, ENR, PEF through structural modeling and molecular docking calculation approaches. The role of key amino acid residues was assessed, indicating that the main protein binding pocket is composed by Trp-212 residue playing an important stabilization for the three fluoroquinolones through both hydrogen bonding and van der Waals forces, where reside the individual structural differences observed among the three fluoroquinolones and BSA. There is a descriptive protagonism of carboxyl group on the ENR interaction which traps the molecule and avoids the deep communication in the protein binding pocket, as well as the ligands CIP and PEF showed an interface amino acid residue interaction profile higher than 70%.

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1. Introduction

The fluoroquinolones are a drug class that targets the bacterial DNA gyrase and topoisomerase IV varying the efficiency between low and high concentrations [1]. The fluoroquinolones are extensively used for the treatment of sick animals by bacteria, whether in the rapid growth or stationary microorganism phase [2]. Due to the uncontrolled used of some fluoroquinolones, not only as antibacterial agents but also as growth promoters and prophylactic agents in lactating animals, it has been detecting the presence of fluoroquinolones in commercial milk, being a problem for human health [3-5]. The ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl) -1,4-dihydroquinoline-3-carboxylic acid, Figure 1A), enrofloxacin (1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, Figure 1B) and pefloxacin (1-ethyl-6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, Figure 1C) are one of the most common synthetic fluoroquinolones widely used as antimicrobial agents [6]. The **CIP**, **ENR**, and **PEF** are characterized by two pK_a values, *e.g.,* pK_1 = 6.16 and pK_2 = 8.63 for **CIP** [7], pK_1 = 6.00 and pK_2 = 8.72 for **ENR** [8] and pK_1 = 6.30 and pK_2 = 7.60 for **PEF** [9], showing their internal versatile structural change according to the pH of the different biological medium.

In terms of infections in the respiratory and alimentary tracts, generally the fluoroquinolones are administered in veterinary medicine via subcutaneous in cattle, or even intramuscular administration to pigs and orally to turkeys and chickens [10]. Particularly, the **ENR** injection is the main veterinary conduct for use in the treatment of bovine respiratory illness caused by susceptible microorganisms, including *Mannheimia* (*Pasteurella*) *haemolytica*, *Histophilus somni* and *Pasteurella multocida* [11,12]. Alternatively, the available biological assay data of **CIP** for the treatment of acute mastitis in dairy cows showed a rate of 86.0% of success, compared with 72.0% of the control penicillin-streptomycin [13]. In its turn, the **PEF** performance showed substantial activity against gram-negative bacteria including *Aeromonas hydrophila*, *Capnocytophaga*, *Haemophilus*, *Neisseria,* and *Legionella*, however, **PEF** is less active than **CIP** towards *Pseudomonas aeruginosa* [14]. In cattle, has already been described the detection of **ENR** + **CIP** deposit, being detected in about 65.0%, 80.0%, 88.0%, and 50.0% in liver, kidney, muscle, and fat, respectively [5].

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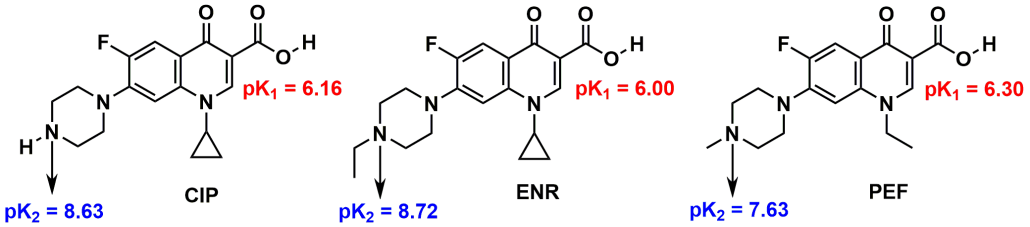

Figure 1. The chemical structure of the three fluoroquinolones under study: ciprofloxacin (**CIP**), enrofloxacin (**ENR**), and pefloxacin (**PEF**).

The pharmacokinetics of this class is mainly driven by serum albumin (SA), which is the most abundant protein present in the circulatory system, being about 60% of the total plasma proteins [15]. Its main function is associated with the distribution and excretion of several endogenous and exogenous compounds, *e.g.,* fatty acids, bilirubin, prostaglandins, steroids, cholesterol, hormones, vitamins, and drugs [16]. In this case, bovine serum albumin (BSA) is the main transporting protein synthesized in the cattle liver and also the most representative globular protein for preliminary pharmacokinetic studies. [17]. From a structural point of view, BSA is a single-chain composed of 583 amino acid residues, having 20 tyrosine and 2 tryptophan residues (Trp-134 and Trp-212) which play an important role in the interaction with endogenous and exogenous compounds. The secondary structure of BSA consists of nine loops held together by 17 disulfide bonds, resulting in tertiary structure of three domains (I, II, and III) separated into two subdomains (A and B). These disulfide bonds carry a rigidity to the helices, but at the same time allow sufficient flexibility for the protein to suffer conformational changes based on the experimental conditions [6]. Due to a large number of negative charged (Glu and Asp) and positively charged (Lys and Arg) residues, BSA is highly soluble in aqueous media [17].

Extensive investigations on the interaction between commercial drugs with SA have been reported [18-20]. Since SA assists as the main transport carrier of drugs and plays a prevailing role on their bioavailability, having a significant impact on the pharmacokinetics of drugs, the profound study of the behavior on the relationship between albumin and drugs is particularly important, affecting the effectiveness of pharmaceutical agents [21]. Despite the numerous reports of the therapeutic importance of **CIP**, **ENR**, and **PEF** on the treatment of diverse animal illness, the specialized literature reports a spectroscopic analysis on the understanding of the interactions between albumin: **CIP**/**ENR**/**PEF** [6,22-31]. In silico approach *via* molecular docking calculations is a very effective tool which explores at both molecular and atomic levels the behavior on the ability to estimate the participation of specific chemical groups and their interactions in complex stabilization. The lack of this kind of data for the three cited fluoroquinolones drove the work to fill this blank. Thus, to complement the studies of biodistribution of **CIP**, **ENR** and **PEF** in the bovine blood plasma, molecular docking calculations under physiological conditions (pH = 7.4) were carried. For the present study, we were chosen **CIP**/**ENR**/**PEF** as three representative commercial fluoroquinolones, due to their high importance and wide application in veterinary medicine.

2. Experimental

2.1. Molecular descriptor calculation

The crystallographic structure of BSA was obtained from the Protein Data Bank (PDB) with access code 4F5S [32]. This structure has a resolution of 2.47 Å. The **CIP**, **ENR**, and **PEF** structures were built and energy minimized at physiological pH (pH = 7.40) with the Density Functional Theory (DFT) method B3LYP/6-31G* available at the Spartan'14 software (Wavefunction, Inc.). The molecular docking was performed with the GOLD 5.6 software (Cambridge Crystallographic Data Centre - CCDC). The crystallographic hydration molecules were deleted and hydrogen atoms were added to the protein according to the data inferred by GOLD 5.6 software on the ionization and tautomeric states [33]. To evaluate the best scoring function that will be used in the molecular docking run (*ChemPLP*, *GoldScore*, *ChemScore,* or *ASP*), redocking study was carried out with the crystallographic ligand naproxen inside BSA binding pocket (Trp-212-containing binding site – PDB code: 4OR0) [34]. The Root Mean Square Deviation (RMSD) obtained for *ChemPLP*, *GoldScore*, *ChemScore,* and *ASP* was 0.512, 1.103, 0.811, and 1.415, respectively. Since *ChemPLP* function provided the lowest RMSD value, there is a clear indication that this scoring function is the best choice to be applied in the molecular docking studies for the three fluoroquinolones.

The data available from literature by spectroscopic assays (fluorescence quenching studies) described that **CIP**, **ENR** and **PEF** interact with BSA possibly bound next to one of the internal tryptophans (Trp-134 or Trp-212) [6,22-31]. From this data, to identify the main binding site of BSA, it was defined 10 Å spherical radius around each tryptophan residue to molecular docking calculations. The docking score of each pose was obtained through the sum of a series of energy terms involved in the protein-ligand interaction process, so a more positive score indicates a better interaction [35]. The number of genetic operations (crossover, migration, mutation) in each docking run used in the searching procedure was set to 100,000. It was used Student's *t*-test to determine statistical significance between the data obtained for the two binding sites. The figures of the docking poses with the highest docking score value were generated with the PyMOL Delano Scientific LLC software [36]. Additionally, in order to verify the frequency calculation, the molecular docking calculations were carried out three times selecting the same binding region and using the same spherical radius. In all cases, the best docking pose was obtained to the same site of the albumin structure, as well as the ligand assumed a quite similar docking pose.

3. Results and discussion

The BSA structure is didactically divided in three homologous helical domains: I (1-179), II (180-384), and III (385-583), which show different binding abilities toward small molecules [19,21,32,37]. Is shown in Figure 2, the BSA structure presenting two internal tryptophan residues: Trp-134 located in domain I (generally known as site III) and Trp-212 located in the domain II (generally known as site I) [32,35]. From the literature it is known that the fluoroquinolones **CIP**, **ENR** and **PEF** are able to quench the BSA intrinsic fluorescence, indicating that probably these ligands might be bound next one of the two tryptophan residues [22-31].

Table 1. Fitness scores (dimensionless) for the best docking poses in the Trp-134- and Trp-212-containing binding sites for BSA: **CIP**, BSA: **ENR**, and BSA: **PEF** (*ChemPLP* function).

BSA:CIP		BSA:ENR		BSA:PEF	
Trp-134	**Trp-212**	**Trp-134**	**Trp-212**	**Trp-134**	**Trp-212**
42.61	48.87	34.49	49.36	43.93	49.32
43.14	53.99	35.98	45.14	39.64	49.72
40.26	52.96	39.46	49.97	42.99	46.85
40.24	44.88	39.87	40.07	37.27	45.57
44.13	43.32	44.71	40.19	48.59	50.33
44.12	53.50	39.56	50.00	43.01	45.54
42.42*	49.59 *	39.01*	45.79 *	42.57 *	47.89 *

* Docking score average value.

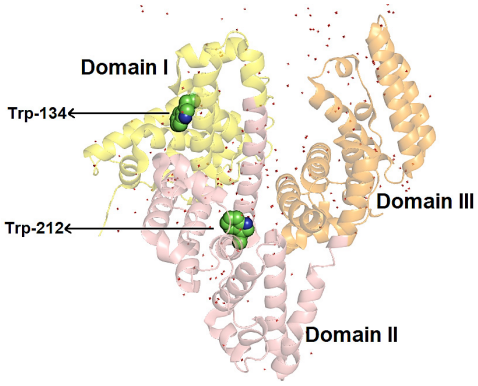

Figure 2. Crystallographic BSA structure (PDB: 4F5S) highlighted the three domains: I (yellow), II (beige), and III (orange). The internal tryptophan residues were represented as green spheres and hydration molecules are represented as red dots.

In addition, the experimental number of binding sites ($n \sim 1.00$) indicate that each fluoroquinolone interacts in the ratio of 1:1, suggesting just one main binding site in the BSA structure for **CIP**, **ENR** or **PEF** [22-30]. From these experimental parameters, the molecular docking calculations were carried out for BSA: **CIP**, BSA: **ENR** and BSA: **PEF** in the Trp-134 and Trp-212-containing binding sites.

The approach used was to split the protein into multiple binding sites and carry out individual docking experiments in each binding pocket. The molecular docking calculations were carried out for Trp-134- and Trp-212-containing binding sites. Table 1, shows the fitness scores for the ten best docking poses in both Trp-134- and Trp-212-containing binding sites (*ChemPLP* function) performed on **CIP**, **ENR** and **PEF**. The docking score average suggests that all three fluoroquinolones interact satisfactorily with BSA into the Trp-212-containing binding site than in the Trp-134-containing binding site.

To find a statistically significant difference from the theoretical results, it was applied Student's *t*-test: as the *p* values for the three fluoroquinolones (1.24×10^{-2}, 2.07×10^{-2}, and 1.89×10^{-2}, for **CIP**, **ENR** and **PEF**, respectively) are less than 5.00×10^{-2} (95.0% confidence interval), can reject the null hypothesis, indicating a statistically significant difference between the two protein binding sites (Trp-134- and Trp-212-containing binding sites) [37]. Usually, the literature reveals that the ligands which interact preferentially in the site I are composed by carboxylic acid groups and/or bulky heterocyclic molecules dominated by a negative charge or containing azo and/or sulfur groups (*e.g.* phenylbutazone, azapropazone, tolbutamide, bucolome and sulfisoxazole) rich in non-ligand *p* pair and π electrons [38,39], whose shows structural similarity characteristics to those in the three fluoroquinolones under study.

With the intention of offer a molecular description of the binding between BSA and fluoroquinolones inside the Trp-212-containing binding site, as well as suggesting the main amino acid residues and the main chemical binding force directly involved on the interaction, the highest docking score value of each fluoroquinolone was analyzed individually. Figure 3 depicts the best docking pose for the three fluoroquinolones into site I and Table 2 shows the main amino acid residues involved in the interaction of BSA: **CIP**, BSA: **ENR**, and BSA: **PEF**. Molecular docking results suggested hydrogen bonding and van der Waals interactions as the main intermolecular forces responsible for the complex stabilization for all studied fluoroquinolones. In general, van der Waals and hydrogen bonding are the main binding forces which contribute to interactions between proteins and small organic molecules. However, the first one is usually estimated based on idealized models of the molecular geometry, *e.g.*, spheres or spheroids, involving less energy when compared to hydrogen bonding (energies associated with hydrogen bonds are in the range of 6-30 kJ/mol (≈ 2-12 k_BT) [40-43]. Since **ENR** presented just one interaction *via* hydrogen bonding (with Arg-194 residue in a distance of 3.10 Å) compared to three for the other fluoroquinolones (Arg-194, Arg-217, and Ser-343 residues for **CIP** within a distance of 3.30, 3.30, and 1.70 Å, respectively, and Arg-198, Trp-212, and Ser-343 residues for **PEF** within a distance of 3.80, 3.20, and 2.80 Å, respectively), there is an indicative that **ENR** has weaker interaction toward BSA than **CIP** and **PEF** [44].

To clarify, the hydrogen from guanidinium group of the Arg-194 and Arg-217 residues are potential donors to form hydrogen bonding appropriately with the nitrogen atoms from **CIP** structure, within a distance of 3.30 Å in both cases. The particular interaction of hydrogen from -OH group in the Ser-343 residue is also a strong potential donor for hydrogen bonding with oxygen from carboxyl group of **CIP** structure within a distance of 1.70 Å, showing a strong covalent character [43].

The description of the interaction *via* van der Waals forces was also suggested between **CIP** and four kinds of amino acid residues: Leu-197, Arg-198, Trp-212, and Lys-294, within a distance of 2.80, 3.50, 2.00, and 2.60 Å, respectively. Overall, all the fluoroquinolones studied in this work showed remarkable theoretical binding capacity mainly related with Trp-212 residue in domain II, however, the slight structural differences presented among the three fluoroquinolones drove influences on the interaction toward BSA, *e.g.* the carboxyl group of **ENR** is not fully buried in the protein binding pocket, and the ligands

Table 2. Amino acid residues involved in the interaction BSA:**CIP**, BSA:**ENR**, and BSA:**PEF** in the Trp-212-containing binding site (*ChemPLP* function).

Sample	Amino acid residue	Interaction	Distance (Å)
BSA:**CIP**	Arg-194	Hydrogen bonding	3.30
	Leu-197	Van der Waals	2.80
	Arg-198	Van der Waals	3.50
	Trp-212	Van der Waals	2.00
	Arg-217	Hydrogen bonding	3.30
	Lys-294	Van der Waals	2.60
	Ser-343	Hydrogen bonding	1.70
BSA:**ENR**	Arg-194	Hydrogen bonding	3.10
	Leu-197	Van der Waals	3.40
	Trp-212	Van der Waals	2.50
	Arg-217	Van der Waals	2.80
	Gln-220	Van der Waals	2.60
	Lys-294	Van der Waals	3.60
	Val-342	Van der Waals	1.90
BSA:**PEF**	Arg-194	Van der Waals	3.40
	Leu-197	Van der Waals	2.50
	Arg-198	Hydrogen bonding	3.80
	Trp-212	Hydrogen bonding	3.20
	Arg-217	Van der Waals	2.30
	Ala-341	Van der Waals	3.70
	Val-342	Van der Waals	1.20
	Ser-343	Hydrogen bonding	2.90

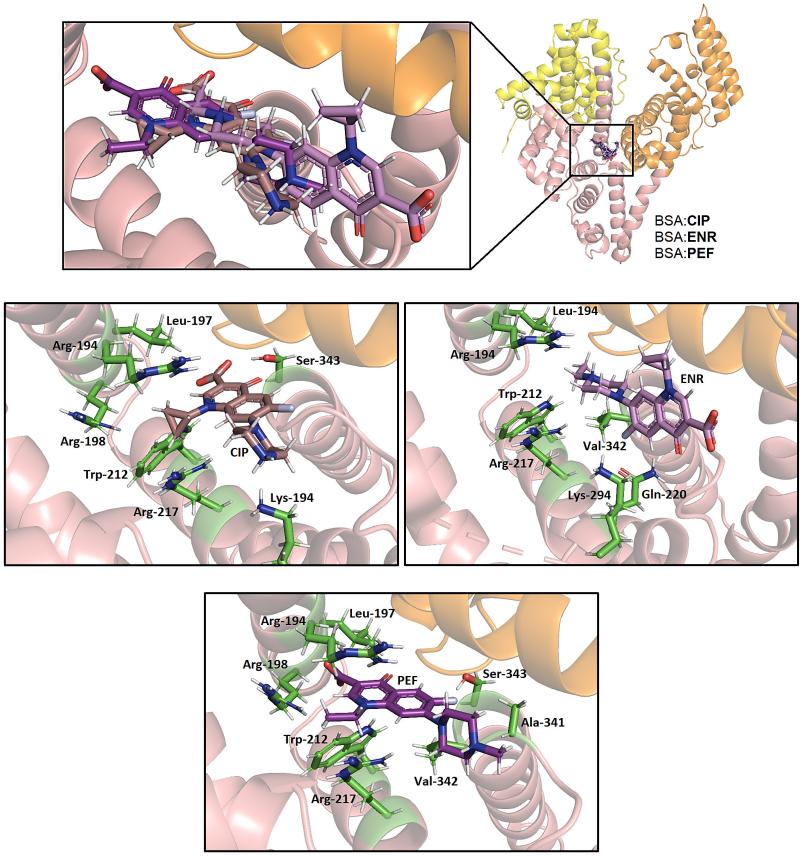

Figure 3. Best docking pose for the interaction between BSA and the three fluoroquinolones in the Trp-212-containing binding site. For each case was showed the zoom representation corresponding to the interaction BSA: **CIP**, BSA: **ENR**, and BSA: **PEF**. Domains I, II, and III are represented as cartoon in yellow, beige and orange, respectively, while the selected amino acid residues, **CIP**, **ENR** and **PEF** are represented as sticks in green, brown, magenta and purple, respectively. Hydrogen: white; oxygen: red; fluorine: light blue and nitrogen: dark blue.

CIP and **PEF** presented qualitatively interaction similarity higher than 70%. Additionally, since bovine serum albumin (BSA) and human serum albumin (HSA) share 76% identity and 88% similarity [35,45,46], the results obtained for BSA might be correlated with HSA binding capacity.

4. Conclusions

The molecular docking scores suggested the Trp-212-containing binding site (site I - domain II) as the main binding pocket for the three commercial fluoroquinolones (**CIP**, **ENR**, and **PEF**). Student's *t*-test indicated a statistically significant difference between the two main protein binding sites (Trp-134- and Trp-212-containing binding sites). The hydrogen bonding in a minor way and van der Waals forces in a major way are the main chemical interactions involved between BSA and fluoroquinolones. Overall, all fluoroquinolones presented in this study had a significant theoretical binding capacity in the Trp-212-containing binding site, attributed mainly by the slight structural differences among the three ligands. There were

some particular features on the interaction toward BSA, *e.g*. the carboxyl group of **ENR** is not entirely buried in the protein binding pocket, as well as this ligand showed lower binding capacity compared to **CIP** and **PEF**, suggesting the behaviour of higher partition of **ENR** to the animal bloodstream compared to other fluoroquinolones.

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Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: Otávio Augusto Chaves conducted the molecular docking calculations. Otávio Augusto Chaves and Leonardo Vazquez wrote the manuscript.
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