Study of the Interaction of Ciprofloxacin and Sparfloxacin with Biomolecules by Spectral, Electrochemical and Molecular Docking Methods

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Abstract. Interactions of ciprofloxacin and sparfloxacin with different biomolecules (DNA, RNA and BSA) are investigated by UV–Visible spectroscopy, fluorescence spectroscopy, cyclic voltammetry and molecular docking methods. Upon increasing the concentration of the biomolecules, the absorption maxima of ciprofloxacin and sparfloxacin are red shifted in the aqueous solutions whereas red or blue shift noticed in the fluorescence spectra. The negative free energy changes suggest that the interaction processes are spontaneous. Cyclic voltammetry results suggested that when the drug concentration is increased, the anodic electrode potential increased. Molecular docking results showed that hydrophobic forces, electrostatic interactions, and hydrogen bonds played vital roles in the interaction drugs with biomolecules. The molecular docking calculation clarifies the binding mode and the binding sites are in good accordance with the experiment results.

1. Introduction

Biomolecules binding has long been considered one of the most important physicochemical characteristics of drugs, playing a potential role in distribution, excretion, and therapeutic effectiveness [1-7]. The study of the interaction of the drug with biomolecules like DNA, RNA and BSA plays a key role in pharmacology and it is of great significance for designing and synthesizing the new drugs targeted to the biomolecules and their effectiveness depends on the mode and affinity of the binding [2, 3]. The interaction of the drugs with DNA is among the most important aspects of biological studies in drug discovery and pharmaceutical development processes. The recognition of DNA binders involves a complex relationship of different interactive forces. It includes the intercalation between adjacent base pairs, intrusion into the minor and major groove and electrostatic interaction [1, 4]. The resulting drug–DNA complex is stabilized by a number of non-covalent (such as van der Waals interactions, hydrophobic forces and hydrogen bonds) and covalent interactions.

The interaction between protein and RNA play an essential role in many cellular processes, such as regulation of gene expression, protein synthesis, as well as replication and assembly of many viruses [5, 6]. Although there are some literatures to investigate the mechanisms by which protein bind to DNA, the identification of RNA-binding proteins, especially their binding sites in residue level is quite poor. The mechanism that RNAs interact with protein and their binding sites is still a major challenge in the post-genome era. The ability to identify the interaction of ciprofloxacin with protein-RNA can broaden our understanding of the molecular recognition, the mechanisms of many important biological processes and guide for the mutant design and drug design [7-9].

Serum albumin (SA) is most abundant serum protein in humans. It binds and transport a large variety of ligands including hormones, fatty acids, drugs, etc. [10-12]. It is a globular, multifunctional protein composed of three structurally similar domains each containing two subdomains and having molecular weight of 67 kD stabilized by 17 disulfide bonds [13]. Apart from ligands binding and transport, it involves in maintaining pH and osmotic pressure, preventing photodegradation of folic acid, and is also a marker of inflammatory state [14, 15].
Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) (Fig. 1a) and sparfloxacin (S-amino-1-cyclopropyl-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-6,8-difluoro-4-oxo-quinoline-3-carboxylic acid) (Fig. 1b) is used to analyzed with biomolecule. Since both drugs has an extended aromatic part and functional groups suitable for hydrogen bonding, it can be expected that this phenolic type molecule is able to interact strongly with biomolecules and that these non-covalent interactions may play a decisive role in its mechanism of action. Both drugs used to treat community-acquired lower respiratory tract infections (acute sinusitis, exacerbations of chronic bronchitis caused by susceptible bacteria, and community-acquired pneumonia) [12]. Because of its pharmacological activity, the investigation of the interactions between this compound and biomolecules are very important [13]. Furthermore, binding of drugs to albumin alters the pattern and volume of distribution, lowers the rate of clearance, and increases the plasma half-life of the drug [12–15].

In the present study we have selected ciprofloxacin and sparfloxacin to DNA, RNA and BSA by means of the following points in mind (i) whether the sensing characteristics of ciprofloxacin and sparfloxacin will be same or different by the presence of DNA, RNA and BSA, (ii) to find out whether the aromatic ring or heterocyclic ring of the drugs interact with DNA, RNA and BSA molecules, (iii) whether the presence of heterocyclic ring in the drugs will increase or decrease the conjugation, (iv) whether or not the oxidation and reduction potential of the drugs affected in the DNA, RNA and BSA molecules and (v) whether the structure of the drugs changed or not in the DNA, RNA and BSA molecules. Thus, in the present work, we utilizing UV-visible absorption spectroscopy, fluorescence spectroscopy, cyclic voltammetry and molecular docking study for the interaction between Ciprofloxacin and sparfloxacin with DNA, RNA and BSA. In this study, the interaction of ciprofloxacin and sparfloxacin to DNA, RNA and BSA was analyzed in UV-visible spectroscopy, fluorescence spectroscopy and cyclic voltammetry methods. Further, molecular docking method was employed to confirm the secondary structural changes upon ciprofloxacin and sparfloxacin binding to the DNA, RNA and BSA.

Our investigation is focused on (i) interaction of ciprofloxacin and sparfloxacin with DNA, RNA and BSA, (ii) determination of binding constant (K_a) and binding sites of the drugs with DNA, RNA and BSA system, (iii) thermodynamic free energy change (ΔG°) and conformation changes of DNA, RNA and BSA upon binding to ciprofloxacin and sparfloxacin. These results should be helpful for understanding the drug – biomolecules interaction at the molecular level and be useful for the design of new drugs binding selectively to predetermined sequences.

2. Experimental

2.1. Materials

Ciprofloxacin, sparfloxacin DNA, RNA and BSA were purchased from Sigma-Aldrich Chemical Company, USA, and used without further purification. Triply distilled water was used for the preparation of aqueous solutions.

2.2. Methods

The concentration of the ciprofloxacin and sparfloxacin stock solution was 2 × 10^{-3} M. 0.2 ml of the ciprofloxacin and sparfloxacin stock solution was transferred into 10 ml volumetric flasks. To this, varying concentrations of the biomolecules (DNA, RNA and BSA) solution were added. DNA, RNA and BSA solutions were prepared in 1 × 10^{-6} M with triply distilled water at pH 7.0. Different volume of the biomolecule solution (0.1, 0.3, 0.5, 0.7, 0.9, 1.0 ml) was added to 10 ml volumetric flask. The solution was diluted to 10 ml with triply distilled water and shaken thoroughly. The final concentration of ciprofloxacin and sparfloxacin drugs in all the flasks was 2 × 10^{-5} M. The experiments were carried out at room temperature. All solutions were stored in a refrigerator at 4°C.
2.3. Instruments

Absorption spectral measurements were carried out with a UV–visible spectrophotometer (model-UV-2600, Shimadzu, Japan). Fluorescence measurements were performed on a spectrofluorophotometer (model- RF-5301PC, Shimadzu, Japan) equipped with 1.0 cm quartz cells. Cyclic voltammetry measurements were performed through an electrochemical work station (model-CHI 620D, CH Instruments, USA) with a three electrode system: surface area 0.1963 cm$^2$ glassy carbon electrode as working electrode, saturated silver electrode as reference electrode and a platinum foil as counter electrode. Prior to use, the working electrode was polished with 0.05 μm alumina and thoroughly washed in an ultrasonic bath for 5 min. Before experiments, the solution within a single compartment cell was deaerated by purging with pure N$_2$ gas for 5 min.

2.4. AutoDock

AutoDock is a software suite for performing automated docking by simulated annealing, local gradient searches and genetic algorithms [16-18]. The combination of a genetic algorithm with inheritance of local optimizations, yielding a Lamarckian genetic algorithm, endows autodock with very good search performance. In addition to an efficient search algorithm, recent versions of autodock include a ΔG bind correlation derived from molecular conformations. As of version 4.2.6, autodock for Linux, Mac OS X and Windows is available under the GNU General Public License (GPL) and can be downloaded free of charge from http://autodock.scripps.edu. At the time of writing, two versions of autodock, autodock 4.2.6 and autodock vina, are available. It should be noted that while the techniques presented in this chapter may be adaptable to autodock vina, these techniques are presented with autodock 4.2.6 in mind. Furthermore, scoring functions from autodock versions 1 to 3 may be adapted for use in either autodock 4.2.6 or autodock vina. To successfully prepare carbohydrate models for use in autodock, several related pieces of software will be useful. If desired, nearly all of the protein and drug preparation can be done using the autodock tools (ADT) available from the same website as autodock. However, the reader may wish to provide his own tools for molecule preparation as appropriate. Gasteiger partial charges were added to the drug atoms. Non-polar hydrogen atoms were merged, rotatable bonds were defined and the docking calculations were carried out on 1bna (DNA), 2ke6 (RNA), 3v03 (BSA), protein model. Hydrogen atoms, solvation parameters and kollman united atom type charges were added with the aid of autodock tools [19]. Affinity (grid) maps of 0.375 Å spacing and 20×20×20 Å grid points were generated using the auto grid program [17]. Autodock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the lamarckian genetic algorithm (LGA) and the solis & wets local search method. Initial position, orientation, and torsions of the drug molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 25 x10$^4$ energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied [20-25].

3. Results and Discussion

3.1. Absorption spectral study

Absorption and fluorescence spectral data of ciprofloxacin and sparfloxacin in different concentrations of DNA, RNA and BSA are summarized in Tables S1 and S2. Figs. 2 and 3 were shows the absorption and fluorescence spectra of ciprofloxacin and sparfloxacin in different concentrations of DNA, RNA and BSA in aqueous solutions (pH ~7.4). In water, absorption maxima of ciprofloxacin appears at 330, 317, 275 and 226 nm and sparfloxacin appears at 366, 290 and 223 nm. The absorption spectra of ciprofloxacin and sparfloxacin can be divided into three regions: (i) the longer wavelength (LW) absorption bands comprising the region at ~330 nm for ciprofloxacin and 366 nm for sparfloxacin, (ii) the middle wavelength (MW) band is around 317 nm
for ciprofloxacin and 290 nm for sparfloxacin, and (iii) the shorter wavelength (SW) absorption band is at 275 nm for ciprofloxacin and 223 nm for sparfloxacin is shown in Figs. 2 and 3.

In aqueous DNA, RNA and BSA solutions, the absorption bands are observed at ~258, ~257 and ~278 nm respectively. With increasing the concentration of the biomolecules, the absorption intensities of DNA and RNA were completely lost whereas in ciprofloxacin, the LW and SW absorption intensities were decreased. However in BSA, the LW absorption intensities were increased while the SW absorption intensities were decreased. In sparfloxacin solution, upon increasing the concentration of the DNA, both the LW and SW absorption intensities were decreased, whereas in RNA and BSA both absorption intensities were increased.

The summarized results of the drugs with the biomolecules are given below: (i) With increasing the DNA concentration, the absorbance of ciprofloxacin and sparfloxacin are decreased, (ii) upon increasing the concentration of RNA and BSA, the absorbance of ciprofloxacin is decreased where as sparfloxacin is increased, (iii) with on increasing the concentration of the DNA and RNA, the absorption maxima of ciprofloxacin is red shifted from 317 nm to 321 nm, while in BSA, it is blue shifted from 317 nm to 311 nm. The inset Figs. 2 and 3 depict the changes in the absorption and fluorescence intensities with the drug concentrations indicating that biomolecules could bind with the ciprofloxacin and sparfloxacin drugs. When compared to biomolecules, the decrease in absorbance is more in ciprofloxacin-DNA than that of the drug - RNA and drug - BSA interactions. The above results indicate that biomolecules are interacted with ciprofloxacin and sparfloxacin drugs.

In general, several driving forces have been postulated for the interaction of the drug with DNA, RNA and BSA compounds [16]. Four main types of non-covalent interactions occur in drug-biomolecules binding: (i) hydrogen bonds, (ii) van der Waals interactions, (iii) electrostatic interactions, and (iv) hydrophobic forces [18]. These results suggest that there is one binding site in DNA, RNA and BSA interacted with ciprofloxacin and sparfloxacin which is further confirmed by a modified Benesi - Hildebrand equation [26]. The above results suggest that the micro-environment around chromophores of DNA, RNA and BSA was changed upon addition of the ciprofloxacin and sparfloxacin drugs and this is confirmed by the following fluorescence spectroscopy studies and its quenching mechanism.

UV–visible absorption measurement is a simple but effective method in detecting complex formation. In general, when a small molecule interacts with biomolecules changes in absorbance and in the position of the band should occur [20-26]. If the binding mode is intercalation, the $\pi^*$ orbital of the intercalated molecule can couple with the $\pi$ orbital of the biomolecules base pairs, thus, decreasing the $\pi \rightarrow \pi^*$ transition energy and resulting in the bathochromism. On the other hand, the coupling $\pi$ orbital is partially filled by electrons, thus, decreasing the transition probabilities and concomitantly resulting in hypochromism [27]. In general, hyperchromism and hypochromism are the spectral features of DNA concerning of its double helix structure; hyperchromism means the breakage of the secondary structure of the DNA and hypochromism means that the DNA-binding mode of the molecule is electrostatic effect or intercalation which can stabilize the DNA duplex, while the existence of a red-shift is indicative of the stabilization of DNA twofold [28].

Upon addition of the drug to the aqueous buffer solution of DNA results in a progressive change in absorbance for ciprofloxacin and sparfloxacin. The difference is initially higher and further additions gradually decrease the absorbance. Compared to ciprofloxacin less change observed in sparfloxacin. On the basis of result, it can be argued that the interaction of ciprofloxacin is higher than that of sparfloxacin with DNA. The changes in the absorption spectra suggest that the drugs interacted with the biomolecules.

After the incremental addition of RNA to the aqueous drug solution results in a progressive decrease in absorbance for ciprofloxacin and increase in absorbance for sparfloxacin. On the basis of the above observation, it can be argued that the interaction of sparfloxacin higher than to ciprofloxacin in RNA. An addition of DNA to the drug solution results in a progressive increase in absorbance noticed at LW and MW and decrease in absorbance at SW for ciprofloxacin whereas
increase in absorbance noticed for sparfloxacin. On the basis of this observation, it can be argued that the interaction of ciprofloxacin is higher than that of sparfloxacin with BSA. The above results indicating that more than one type of interactions present during the binding process.

The binding constant of the drug:biomolecule complexes are calculated by the Benesi–Hildebrand equation. The values of the binding constant (K) for ciprofloxacin-DNA, ciprofloxacin-RNA and ciprofloxacin-BSA were obtained from the absorption wavelength at 317 nm and sparfloxacin-DNA, sparfloxacin-RNA and sparfloxacin-BSA were obtained from the absorption at 366 nm according to the methods published in the literature [29]. For weak binding affinities, the data were treated using linear reciprocal plots based on equation (1) [30]:

$$\frac{1}{(A - A_0)} = \frac{1}{A_\infty - A_0} + \frac{1}{K} \frac{1}{(A - A_0)} \times \frac{1}{[\text{Biomolecules}]}$$

where $A_0$ is absorbance of ciprofloxacin at 317 nm in the absence of biomolecules, $A_\infty$ is final absorbance of the drug-DNA and $A$ is the absorbance recorded at different biomolecule concentrations. The double reciprocal plot of $1/(A - A_0)$ versus $1/ [\text{biomolecules}]$ is linear and the best binding constant (K) can be estimated from the ratio of the intercept and the slope [29].

### 3.2. Fluorescence emission spectrometry

The steady-state emission spectral data of the drug is depicted in Tables S1 and S2. The changes in the fluorescence emission spectra of ciprofloxacin and sparfloxacin in absence and presence of DNA, RNA and BSA are given in Figs. 2 and 3. Changes in the emission spectra of the drugs are likely to occur in response to binding biomolecules [20 - 25]. The emission maximum of DNA, RNA and BSA are noticed at ~364, ~363 and ~340 nm respectively. The emission spectrum of sparfloxacin in aqueous buffer medium is characterized by a two band at 413 nm and 542 nm whereas at ~ 448 nm single emission maximum observed in ciprofloxacin.

Upon increasing the concentration of biomolecules, the emission intensities of DNA, RNA and BSA were completely lost whereas in ciprofloxacin and sparfloxacin the emission intensities are increased or decreased. The results of the interaction of the drugs with the biomolecules are given below:

In the excited state, (i) upon increasing the DNA concentration the emission intensity of ciprofloxacin is decreased and the spectral maximum is blue shifted from 448 nm to 439 nm, (ii) in RNA, the fluorescence intensity of ciprofloxacin is decreased and the fluorescence maximum is red shifted from 448 nm to 452 nm, (iii) Interestingly in BSA, the LW emission intensity is increased while the SW intensity is decreased and the emission maximum is blue shifted from 448 nm to 423 nm.

In sparfloxacin (i) with increasing the DNA concentrations, emission intensity of the LW is increased, while, the SW is decreased, (ii) with RNA, both LW and SW emission intensities are increased, (iii) in BSA, the LW emission intensity is decreased while SW intensity is increased, (iv) in all the three biomolecules, the LW emission maximum is blue shifted from 542 nm to 530 nm, whereas no marginal shift is noticed in the SW and (v) compared to RNA and BSA, the absorption and emission intensity changes in ciprofloxacin and sparfloxacin in DNA is higher than that of RNA and BSA.

The observed spectral changes and the shift in the position of $\lambda_{\text{max}}$ of ciprofloxacin and sparfloxacin are likely to reflect interactions of these biomolecules with the hydrophobic and hydrophilic regions. The fluorescence spectral shifts due to change in polarity of the environment; however the change in fluorescence intensity of the flexible molecules depends not on the polarity of the medium, but also on the constraint provided by the media. The binding constant values were estimated from the fluorescence emission data by using Benesi–Hildebrand equation (2) [26]

$$\frac{1}{(I - I_0)} = \frac{1}{(I_\infty - I_0)} + \frac{1}{K(I_\infty - I_0)} \times \frac{1}{[\text{Biomolecules}]}$$

where $I_0$ is the emission intensities in the absence of biomolecules, I and $I_\infty$ are the emission intensities in the presence of biomolecules respectively. The emission intensities at 448 nm and 542 nm for ciprofloxacin and sparfloxacin respectively used for Benesi–Hildebrand plot (Fig. 2). The binding constant (K) is determined from the ratio of intercept and slope of Benesi–Hildebrand plot.
plot. Using the value of $K$, the free energy change ($\Delta G$ kJ/mol$^{-1}$) for the drug–biomolecule interaction is determined (Tables S1 and S2). From the value of binding constant, it can be seen that drugs binds strongly to DNA, RNA and BSA and the nature of binding with ciprofloxacin is stronger than sparfloxacin. Free energy change indicates spontaneity of complexation process for both cases. The values obtained for $K$ and $\Delta G$ are well in agreement with that obtained for such complexation processes studied earlier [31, 32]. The good linear correlations obtained for $1/(I-I_0)$ versus $1/[\text{biomolecules}]$ plot confirm the formation of 1:1 complex between the drug- biomolecules (Fig. 4). The binding constants thus obtained are $24.9 \times 10^5$ M$^{-1}$, $20.1 \times 10^5$ M$^{-1}$ and $7.0 \times 10^5$ M$^{-1}$, $19.0 \times 10^5$ M$^{-1}$ and $3.6 \times 10^5$ M$^{-1}$ for ciprofloxacin-DNA, sparfloxacin-DNA, ciprofloxacin-RNA, sparfloxacin-RNA, ciprofloxacin-BSA, and sparfloxacin-BSA respectively. The change in free energy for the drug-biomolecule complexation are $-8.78$, $-8.29$ and $-8.05$, $-7.66$, $-7.59$, and $-8.38$ kcal mol$^{-1}$ for ciprofloxacin-DNA, sparfloxacin-DNA, ciprofloxacin-RNA, sparfloxacin-RNA, ciprofloxacin-BSA and sparfloxacin-BSA respectively.

3.3. Cyclic voltammetric study on the interaction between drugs and biomolecules

In order to substantiate the interaction of ciprofloxacin and sparfloxacin drugs with DNA, RNA and BSA on the absorption and fluorescence spectra, we were measured in cyclic voltammetry (CV) method. The application of electrochemical methods to the study of drug interaction to biomolecules provides a useful complement to the previously used methods of investigation, such as UV–visible spectroscopy and fluorescence spectroscopy. Cyclic voltammetry is widely used for the evaluation of mode of action and binding strength of drug–biomolecule interaction. This technique is predominantly useful for chemical compounds due to their accessible redox states. Multiple oxidation states of the same species as well as mixtures of several interacting species can be observed simultaneously [20-25]. Equilibrium constants ($K$) for the interaction of the drug with biomolecules can be obtained from shifts in peak potentials, and the number of base pair sites involved in bindings through intercalative, electrostatic or hydrophobic interactions by the reliance of the current passed during oxidation or reduction of the bound species on the amount of biomolecules added. As in CV the scan is revered so the fate of the species in the backward scan can also be studied. The peak potential and peak current of the compound changes in the presence of biomolecules if the compound interacts with the drugs are discussed. The variation in peak potential and peak current can be exploited for the determination of binding parameters.

Tables S3 and S4 and Figs. 5 to 8 shows the cyclic voltammetry data of ciprofloxacin and sparfloxacin in absence and presence of DNA, RNA and BSA at glassy carbon electrode in Tris-HCl buffer (pH ~ 7). Tables S3 and S4 and Figs. 5 to 8 depict the CV data and anodic and cathodic peaks of ciprofloxacin and sparfloxacin with varying concentration of DNA, RNA and BSA. When ciprofloxacin and sparfloxacin was added to the DNA, RNA and BSA solution, oxidation and reduction peaks shifted towards high and low potentials respectively and increase or decrease of oxidation current was observed.

The results in the Tables S3 and S4 of the interaction of the drugs with the biomolecules are given below: In DNA, (i) the anodic peak potential ($E_{pa}$) and cathodic peak potential ($E_{pc}$) observed at -573, 273 mv and $-228, 602$ mv respectively, (ii) the anodic peak current ($I_{pa}$) and cathodic peak current ($I_{pc}$) observed at $-8.4, -3.3$ mv and $11.0, 10.4$ mv respectively. In RNA, (i) the anodic and cathodic peak potential observed at $-400, 69$ mv and $-296, 508$ mv respectively, (ii) the anodic and cathodic peak current observed at $-5.8, -6.9$ mv and $10.1, 9.1$ mv respectively. In BSA, (i) the anodic and cathodic peak potential observed at $-406, 205$ mv and $-379, 263$ mv respectively, (ii) the anodic and cathodic peak current observed at $-12.4, -9.6$ mv and $48.4, 54.9$ mv respectively.

In ciprofloxacin, (i) the anodic and cathodic peak potential observed at $-641, 158$ mv and $-432, 252$ mv respectively, (ii) the anodic and cathodic peak current observed at $-10.3, -22.1$ mv and $-104.5, 47$ mv respectively. In sparfloxacin, (i) the anodic and cathodic peak potential observed at $-1066, 802$, 116 mv and $-184$ mv respectively, (ii) the anodic and cathodic peak current observed at $-5.7, -0.8, 1.3$ mv and $2.59$ mv respectively.
In ciprofloxacin, when the concentrations of the biomolecules increased (i) the negative anodic peak potentials and anodic peak current are decreased in DNA, (ii) the difference in peak potential ($\Delta E_p$) and difference in current peak ($\Delta I_C$) are increased, (iii) however in RNA, the negative anodic peak potentials and anodic peak current are increased, (iv) the negative anodic peak potentials and anodic current peaks are decreased in BSA.

In sparfloxacin, upon increasing the concentrations of the biomolecules, (i) the negative anodic peak potentials and anodic current peaks are decreased in DNA, (ii) the difference in peak potential ($\Delta E_p$) and difference in current peak ($\Delta I_C$) are decreased, (iii) in RNA, the negative anodic peak potentials and anodic current peaks are decreased, (iv) in BSA, the anodic peak potentials are increased and the negative anodic current peaks are decreased.

The decay in peak current ($I_p$) of the drug by the addition of increasing amount of biomolecules can be used for the determination of binding constant and binding site size, whereas the shift in peak potential can be used to ascertain the mode of interaction. The binding constant is quantified by the following equation (3) [33]:

$$\log \left( \frac{1}{[\text{biomolecule}]} \right) = \log K + \log \left( \frac{I}{I_0 - I} \right),$$

where $K$ is the binding constant, $I_0$ and $I$ are the peak currents of the drug in the absence and presence of biomolecule respectively. The binding constant, $K$ is obtained from the intercept of the plot of $\log \left( \frac{1}{[\text{biomolecule}]} \right)$ vs. $\log \left( \frac{I}{I_0 - I} \right)$. The effect of the addition of biomolecule to the ciprofloxacin and sparfloxacin solution on the wave voltammetry is changed. The current drops on the addition of biomolecule owing to the binding of ciprofloxacin and sparfloxacin. The peak potential shifted to a more positive value in the presence of DNA. The shift in peak potential is typical of the interaction of the drugs with biomolecules. In the presence of nucleic acids, the current is mainly due to free species, as the diffusion rate of bound species is small. The cause for the decrease in the peak current was that the obvious diffusion coefficient and the obvious concentration of electroactive species decreased.

The voltammetric response of the compound changed as is evidenced by the sequential drop in peak current and gradual peak potential shift in positive direction. The shift of peak potential to less negative values is suggesting the interaction of ciprofloxacin and sparfloxacin into the biomolecules. The large peak to peak potential difference ($\Delta E_p$) of -104 mV for ciprofloxacin and -441 mV for sparfloxacin is suggest the electrochemical reaction coupled with a chemical reaction. From the cyclic voltammetry, it can be seen that the electrode reaction of ciprofloxacin and sparfloxacin is a quasi-reversible process. The results indicate that the reaction is a diffusion-controlled process. When DNA, RNA and BSA was added to the ciprofloxacin and sparfloxacin solution, both the oxidation and reduction currents increased (Figs. 7 and 8), indicating that an electrochemically non-active complex could have been formed. The formation of the complex resulted in the decrease of the equilibrium concentration of ciprofloxacin and sparfloxacin in solution, leading to the increase of the peak current.

In order to study the association constant ($K_a$) between ciprofloxacin and sparfloxacin with DNA, RNA and BSA, the cyclic voltammetry of the drugs with DNA, RNA and BSA were recorded at a scan rate of 100-500 mV s$^{-1}$ (Figs. 7 and 8). During the experimental process, the ciprofloxacin and sparfloxacin concentration was kept constant and the concentrations of ciprofloxacin, and sparfloxacin were varied from 2.0 x 10$^{-3}$ to 2.0 x 10$^{-4}$ M.

3.3.1. Stoichiometry of the drug-biomolecules system

Stoichiometry of drug-biomolecule system was determined using voltammetric data. It was assumed that DNA interacted with ciprofloxacin and formed ciprofloxacin-DNA complex. The binding number ($m$) and equilibrium constant ($\beta$) of the binding reaction can be deduced as follows:

$$\text{Ciprofloxacin} + m.\text{DNA} \leftrightarrow \text{Ciprofloxacin-m.DNA}$$

(4)

The equilibrium constant was deduced as follows:

$$B = \frac{[\text{Ciprofloxacin-m.DNA}]}{[\text{Ciprofloxacin}][\text{DNA}]^m}$$

(5)
Since
\[ \Delta I_{\text{max}} = k C_{\text{Ciprofloxacin}} \] (6)
\[ \Delta I = k [\text{ciprofloxacin-m.DNA}] \] (7)
\[ [\text{ciprofloxacin}] + [\text{ciprofloxacin-m.DNA}] = C_{\text{ciprofloxacin}} \] (8)

Therefore,
\[ [\Delta I_{\text{max}} - \Delta I] = k (C_{\text{ciprofloxacin}} - [\text{ciprofloxacin-m.DNA}]) = k [\text{ciprofloxacin}] \] (9)

Equations (5), (7) and (9) give
\[ \log[\Delta I/ (\Delta I_{\text{max}} - \Delta I)] = \log \beta + m \log[\text{DNA}] , \] (10)

where \( \Delta I \) is the difference between the peak current of sample and blank, \( \Delta I_{\text{max}} \) is the maximum value of difference of peak current, \([\text{ciprofloxacin-m.DNA}] \) and \([\text{ciprofloxacin}] \) are the total bound and free concentration of ciprofloxacin in the solution, respectively. The plot of \( E_p \) vs scan rate was linear (Fig. 9). The plot of \( E_p \) vs \( \ln v \) is well-defined straight line and the value can be calculated from the slope and ks from the intercept. The \( E^0 \) values of drugs can be determined from Fig. 10 on the ordinate by extrapolating the line to \( v = 0 \). The plot of log [\( \Delta I/ (\Delta I_{\text{max}} - \Delta I) \)] versus log[\( \text{ciprofloxacin} \)] showed linearity with a correlation coefficient of 0.999 (Fig. 11). The values of \( m \) and \( \beta \) were obtained from the slope and intercept of the plot. The value of \( m \) equal to unity indicated that a stable 1:1 complex is formed.

3.4. Molecular docking study

The computer simulated automated docking studies were performed using the widely distributed molecular docking software autodock 4.2.6. Among the various conformers of docking results, only 10 conformers were taken on the basis of the free energy of binding and score ranking. The minimum binding energy conformer is shown in Figs. 12-15 and all the data related to complexation and binding processes are reported in Tables S5 and S7. We considered the binding free energy of the best ranked conformations as the main parameter for analysis of autodock 4.2.6 result. The autodock study of ciprofloxacin andsparfloxacin was carried out and they were docked in the biomolecules as shown in Tables S5 and S7 their autodock binding free energies \( (\Delta G_b \text{ kcal/mol}) \) and inhibition constants \( (K_i) \) were obtained. Among them all the drug exhibited the lowest free energy between -7.71 and -6.2 kcal/mol. In other words, they possess the highest potential binding affinity into the binding site of the 3D macromolecule. The computationally designed sparfloxacin showed less binding affinity than that of ciprofloxacin. The higher affinity is presumably attributed to the formation of more and/or tighter hydrogen bonds between the several amino acids at the binding site owing to the increased electronegativity of the hydrogen and oxygen. Therefore, ciprofloxacin were docked deeply within the groove of the biomolecules and forming more hydrogen bonds with DA18, DG14, DG24, DG2 for DNA and U9, G6, U43, G10, C35, A32, A33, U13, U8, U15, C27, A30 for RNA. And LYS499, LYS533 for BSA. Further sparfloxacin was docked deeply within the groove of the biomolecules and forming more hydrogen bonds with DA18, DG14, DG24, DG10 for DNA and G2, U4, C47, G6, U16, G10, A39, U8, A45, U17, A32, U15 for RNA and THR495, ALA500, THR419, LYS533, GLN416, LYS533, PRO420, LYS114, ALA128, LYS20, GLN94, LYS136, GLU97, GLU443 for BSA.

Considering the fitting of the ciprofloxacin binding it was demonstrated that their binding energies were higher in comparison with the sparflloxacin. It is noteworthy that the ciprofloxacin exhibited especially stronger binding affinities to possess the lower binding free energy -7.71 kcal/mol and also sparfloxacin showed the binding free energy between -7.25 and -5.47 kcal/mol. It was clarified that the derivatives possessing less binding free energy (-7.71 kcal/mol) are allowed to fit well into the groove of the binding site. The overall good correlation between the growth inhibitory activities of the ciprofloxacin and the binding affinities predicted by autodock was made clear as indicated in (Table 1). A good docking interaction implies
the prediction of drugs confirmation and orientation within targeted binding site and their lower interactions energies [34]. This efficacy and suitability of drugs was determined on the basis of binding energy calculations.

4. Conclusions

UV–Visible, fluorescence, cyclic voltammetry and molecular modeling studies conform both ciprofloxacin and sparfloxacin drugs interacted with the biomolecules. These techniques are used to evaluate the binding mode as well as binding strength of the complex formed between drug and biomolecules. The results shows interaction between drugs and biomolecules was spontaneous and van der Waals interactions, hydrophobic forces, electrostatic interactions, and hydrogen bonds played vital roles which was spontaneous. The docking method provided the participation and interactions of the drug functional groups with biomolecules. From the computational study, we find that negative complexation energy, Gibbs energy, and enthalpy changes for the drugs indicate that the formation of these complexes is spontaneous and exothermic and hydrogen bonding interactions play a major role in the inclusion process.

Table 1. Result of drug receptor interaction.

| Proteins | Drugs      | Binding energy ∆Gb kcal/mol | Intermolec. Energy kcal/mol | Torsional energy | Total Internal energy (kcal/mol) | Inhibition constant (nM) | vdW + H bond + desolv Energy kcal/mol | Electrostatic Energy kcal/mol | Ligand efficiency | Unbond energy kcal/mol | refRMS |
|----------|------------|-----------------------------|-----------------------------|-----------------|---------------------------------|-------------------------|--------------------------------------|-------------------------------|--------------------|----------------------|--------|
| ciprofloxacin | DNA       | -7.71                       | -8.60                       | 0.89            | 0.19                            | 2.23                   | -6.31                               | -2.30                         | -0.28              | 0.19                 | 32.01  |
|          | RNA        | -6.32                       | -7.22                       | 0.89            | -0.30                           | 23.18                  | -5.35                               | -1.87                         | -0.26              | -0.30                | 21.35  |
|          | BSA        | -6.20                       | -7.09                       | 0.89            | -0.32                           | 28.74                  | -5.29                               | -1.80                         | -0.26              | -0.32                | 110.94 |
| sparfloxacin | DNA       | -7.52                       | -8.41                       | 0.89            | -0.32                           | 3.09                   | -6.06                               | -2.36                         | -0.31              | -0.32                | 31.83  |
|          | RNA        | -6.07                       | -6.96                       | 0.89            | -0.40                           | 35.58                  | -5.42                               | -1.54                         | -0.22              | -0.40                | 29.09  |
|          | BSA        | -5.47                       | -6.37                       | 0.89            | -0.42                           | 97.77                  | -5.88                               | -0.48                         | -0.20              | -0.42                | 74.23  |

Figure 1. Chemical structure of ciprofloxacin and sparfloxacin.
Figure 2. Absorbance and fluorescence spectra of drugs (Ciprofloxacin) with different concentrations of biomolecules (M x 10^{-6}): 1) 0, 2) 1, 3) 3, 4) 5, 5) 7 and 6) 9, 7) 10; dotted lines: DNA or RNA or BSA; Inset fig: Absorbance and fluorescence intensity vs. biomolecules concentration. (Ref: DNA or RNA or BSA).
Figure 3. Absorbance and fluorescence spectra of drugs (sparfloxacin) with different concentrations of biomolecules (M x 10^{-6}): 1) 0, 2) 1, 3) 3, 4) 5, 5) 7 and 6) 9, 7) 10; dotted lines: DNA or RNA or BSA; Inset fig: Absorbance and fluorescence intensity vs. Biomolecules concentration. (Ref: DNA or RNA or BSA).
Figure 4. Absorption spectra and fluorescence spectra of Benesi-Hildebrand plot for the complexation of ciprofloxacin and sparfloxacin with DNA, RNA, BSA (Plot of $\frac{1}{[DNA \ or \ RNA \ or \ BSA]} \times 10^5$ vs $\frac{[\text{DNA (or) RNA (or) BSA}]}{[\text{DNA (or) RNA (or) BSA}] - [\text{DNA (or) RNA (or) BSA}]_0}$).
Figure 5. Cyclic voltammograms of a ciprofloxacin with additions of different concentration of biomolecules (in platinum electrode; scan rate, 100 mV s\(^{-1}\); concentration of Ciprofloxacin - 2\(\times\) 10\(^{-4}\) M; Biomolecule concentration (M\(\times\)10\(^{-6}\)) – 0.2, 0.4,0.8, & 1.0).
Figure 6. Cyclic voltammograms of a sparflouxacin with additions of different concentration of Biomolecules (in platinum electrode; scan rate, 100 mV s\(^{-1}\); concentration of sparflouxacin - 2\(\times\) 10\(^{-4}\) M; Biomolecule concentration (M\(\times\)10\(^{-6}\)) – 0.2, 0.4, 0.8, & 1.0).
Figure 7. Cyclic voltammograms of a platinum electrode in ciprofloxacin with successive additions of a final concentration of biomolecules with different scan rate = (100- 500 mV S\(^{-1}\)).
Figure 8. Cyclic voltammograms of a platinum electrode in sparfl oxacin with successive additions of a final concentration of Biomolecules with different scan rate = (100- 500 mV S⁻¹).
Figure 9. Dependence of the peak potential $E_p$ on the potential scan rate ($\nu$) of drugs with DNA, RNA and BSA. [Scan rate - (100 to 500 m V S$^{-1}$)].

Figure 10. Semilogarithmic dependence of the peak potential $E_p$ on the potential scan rate ($\ln \nu$) of drugs with DNA, RNA and BSA. [Scan rate - (100 to 500 m V S$^{-1}$)].
Figure 11. Linear plot of log [Biomolecules] vs log [ΔI/ (ΔI max - ΔI)]. Q- [DNA] (or) [RNA] (or) [BSA].
Figure 12. Results of the GRID analysis searching for regions favorable for interaction of different ciprofloxacin with biomolecules. The protein is showed in white surface format and the drugs is showed in green “ball and stick” format.
Figure 13. Best binding mode between different ciprofloxacin with biomolecules. The protein is showed in backbone format and the drugs is showed in “ball and stick” format.
Figure 14. Results of the GRID analysis searching for regions favorable for interaction of different ciprofloxacin with biomolecules. The protein is showed in white surface format and the drugs is showed in green “ball and stick” format.
Figure 15. Best binding mode between different sparfloxacin with biomolecules. The protein is showed in backbone format and the drugs is showed in “ball and stick” format.
Table S1. Absorption and fluorescence maxima of ciprofloxacin (2 x 10^{-4} M) with different DNA, RNA and BSA concentrations.

| Concentration of Biomolecules (M) | DNA ciprofloxacin | RNA ciprofloxacin | BSA ciprofloxacin |
|----------------------------------|-------------------|-------------------|-------------------|
|                                  | \(\lambda_{\text{abs}}\) | log \(\varepsilon\) | \(\lambda_{\text{flu}}\) | \(\lambda_{\text{abs}}\) | log \(\varepsilon\) | \(\lambda_{\text{flu}}\) | \(\lambda_{\text{abs}}\) | log \(\varepsilon\) | \(\lambda_{\text{flu}}\) |
| Proteins only                    | 258               | 5.68              | 364               | 257               | 5.56              | 363               | 278               | 5.86              | 340               |
| ciprofloxacin only               | 330               | 4.03              | 448               | 330               | 4.11              | 448               | 330               | 3.98              | 448               |
| 0.1                              | 317               | 4.06              | 5.17              | 317               | 4.12              | 317               | 317               | 3.97              | 444               |
| 0.3                              | 275               | 4.55              | 275               | 275               | 4.55              | 275               | 275               | 4.49              | 444               |
| 0.5                              | 226               | 4.15              | 226               | 226               | 4.18              | 226               | 226               | 4.18              | 444               |
| 0.7                              | 332               | 3.90              | 445               | 331               | 4.08              | 452               | 329               | 4.00              | 444               |
| 0.9                              | 319               | 3.94              | 318               | 318               | 4.10              | 316               | 315               | 3.99              | 444               |
| 1                                | 276               | 4.38              | 276               | 276               | 4.53              | 272               | 272               | 4.47              | 444               |
|                                  | 227               | 4.03              | 227               | 227               | 4.14              | 227               | 227               | 4.14              | 444               |

Excitation wavelength (nm)  
K (1:1) \times 10^5 \text{M}^{-1}  
\Delta G (\text{kcalmol}^{-1})

|                 | 330 | 330 | 319 | 22.4 | 24.9 | 1.8 | 7.0 | 2.4 | 3.6 |
|-----------------|-----|-----|-----|------|------|-----|-----|-----|-----|
| K (1:1) \times 10^5 \text{M}^{-1} | -8.70 | -8.78 | -7.26 | -8.05 | -6.05 | -7.59 |
Table S2. Absorption and fluorescence maxima of sparfloxacin \((2 \times 10^{-4} \text{ M})\) with different concentrations of DNA, RNA and BSA

| Concentration of biomolecules (M) | DNA        | RNA        | BSA        |
|----------------------------------|------------|------------|------------|
|                                  | sparfloxacin | sparfloxacin | sparfloxacin |
| Proteins only                    | λ<sub>abs</sub> | log ε | λ<sub>flu</sub> | λ<sub>abs</sub> | log ε | λ<sub>flu</sub> | λ<sub>abs</sub> | log ε | λ<sub>flu</sub> |
| Proteins only                    | 258        | 5.68      | 364        | 257        | 5.56      | 363        | 278        | 5.86      | 340      |
| Sparfloxacin only                | 366        | 3.84      | 542        | 366        | 3.68      | 542        | 366        | 3.71      | 542      |
| Concentration of biomolecules (M) | 290        | 4.19      | 413        | 290        | 4.21      | 413        | 290        | 4.18      | 413      |
| 0.1                              | 223        | 4.06      | 223        | 223        | 4.04      | 223        | 223        | 4.04      |          |
| Sparfloxacin only                | 367        | 3.79      | 533        | 367        | 3.74      | 540        | 365        | 3.76      | 540      |
| Concentration of biomolecules (M) | 291        | 4.00      | 412        | 293        | 4.22      | 414        | 289        | 4.19      | 413      |
| 0.3                              | 224        | 3.89      | 224        | 224        | 4.14      | 224        | 224        | 4.06      |          |
| Sparfloxacin only                | 368        | 3.79      | 531        | 368        | 3.77      | 538        | 364        | 3.79      | 538      |
| Concentration of biomolecules (M) | 292        | 3.94      | 412        | 293        | 4.22      | 415        | 288        | 4.20      | 413      |
| 0.5                              | 225        | 3.72      | 225        | 225        | 4.15      | 225        | 225        | 4.08      |          |
| Sparfloxacin only                | 369        | 3.76      | 530        | 369        | 3.78      | 537        | 363        | 3.80      | 536      |
| Concentration of biomolecules (M) | 293        | 3.94      | 412        | 293        | 4.23      | 416        | 287        | 4.21      | 413      |
| 0.7                              | 226        | 3.69      | 226        | 226        | 4.16      | 226        | 226        | 4.10      |          |
| Sparfloxacin only                | 370        | 3.74      | 529        | 370        | 3.80      | 536        | 362        | 3.18      | 534      |
| Concentration of biomolecules (M) | 294        | 3.92      | 412        | 293        | 4.24      | 417        | 286        | 4.21      | 413      |
| 0.9                              | 227        | 3.65      | 227        | 227        | 4.16      | 227        | 227        | 4.11      |          |
| Sparfloxacin only                | 371        | 3.73      | 527        | 371        | 3.81      | 535        | 361        | 3.83      | 532      |
| Concentration of biomolecules (M) | 295        | 3.90      | 412        | 293        | 4.24      | 418        | 285        | 4.23      | 413      |
| 1                                | 228        | 3.65      | 228        | 228        | 4.17      | 228        | 228        | 4.13      |          |
| Sparfloxacin only                | 372        | 3.70      | 528        | 372        | 3.84      | 534        | 360        | 3.84      | 530      |
| Concentration of biomolecules (M) | 296        | 3.89      | 412        | 293        | 4.24      | 419        | 284        | 4.23      | 413      |
| 1                                | 229        | 3.61      | 229        | 229        | 4.18      | 229        | 229        | 4.14      |          |

Excitation wavelength (nm) | 360 | 360 | 360 |

K (1:1) x10^5 M^-1 | 18.3 | 20.1 | 16.1 | 19.8 | 17.0 | 20.8 |

ΔG (kcal mol^-1) | -7.49 | -8.29 | -7.49 | -7.66 | -7.75 | -8.38 |
| Drug/ biomolecule | Drugs / biomolecule concentration (× 10⁻⁶) | $E_{pa}$ (mV) | $I_{pa}$ (μA) | $E_{pc}$ (mV) | $I_{pc}$ (μA) | $E_{pa}-E_{pc}/2$ | $I_{pa}/I_{pc}$ |
|------------------|------------------------------------------|---------------|---------------|---------------|---------------|-----------------|----------------|
| DNA only         | 2                                        | -573          | -8.405        | -228          | 11.09         | -172            | -0.7578        |
|                  |                                          | 273           | -3.264        | 602           | 10.38         | -164            | -0.3144        |
| RNA only         | 2                                        | -400          | -5.809        | -296          | 10.14         | -52             | -0.5728        |
|                  |                                          | 69            | -6.873        | 508           | 9.16          | -219            | -0.7503        |
| BSA only         | 2                                        | -406          | -12.38        | -379          | 48.38         | -13             | -0.2588        |
|                  |                                          | 205           | -9.600        | 263           | 54.96         | -29             | -0.1746        |
| ciprofloxacin    | 2                                        | -641          | -10.32        | -432          | 33.22         | -104.5          | -0.3106        |
| only             |                                          | 158           | -22.14        | 252           | 31.54         | -47             | 0.7019         |
|                  | ciprofloxacin-DNA                        | -474          | -5.578        | -197          | 12.35         | -138            | -0.4516        |
|                  | 4                                        | -421          | -5.578        | -183          | 16.39         | -119            | -0.3403        |
|                  |                                          | 135           | -6.175        | 346           | 10.21         | -105            | -0.6048        |
|                  | 8                                        | -391          | -5.578        | -171          | 19.52         | -110            | -0.2857        |
|                  |                                          | 158           | -5.763        | 323           | 14.37         | -82             | 0.4010         |
|                  | 10                                       | -364          | -5.578        | -160          | 21.33         | -102            | -0.2615        |
|                  |                                          | 168           | -4.341        | 299           | 19.43         | -65             | -0.2234        |
|                  | ciprofloxacin-RNA                        | -480          | -14.32        | -596          | 14.50         | 58              | 0.9875         |
|                  | 4                                        | -568          | -16.17        | -550          | 17.87         | -9              | -0.9048        |
|                  |                                          | 103           | -21.30        | 293           | 12.16         | -95             | -1.7516        |
|                  | 8                                        | -648          | -18.83        | -535          | 23.53         | -56             | -0.8002        |
|                  |                                          | 145           | -24.80        | 315           | 15.31         | -85             | -1.6198        |
|                  | 10                                       | -697          | -21.77        | -508          | 28.75         | -94             | -0.7572        |
|                  |                                          | 185           | -27.78        | 334           | 18.49         | -74             | -1.5024        |
|                  | ciprofloxacin-BSA                        | -409          | -8.309        | -324          | 15.82         | -42             | -0.5252        |
|                  | 4                                        | -410          | -7.290        | -335          | 13.79         | -37             | -0.5286        |
|                  |                                          | 123           | -6.874        | 406           | 18.31         | -141            | -0.3754        |
|                  | 8                                        | -411          | -6.189        | -343          | 11.41         | -34             | -0.5424        |
|                  |                                          | 90            | -5.389        | 424           | 15.79         | -167            | -0.3412        |
|                  | 10                                       | -412          | -5.894        | -358          | 9.13          | -27             | -0.6455        |
|                  |                                          | 69            | -4.405        | 436           | 13.46         | -183            | -0.3272        |
Table S4. CV for sparfloxacin with biomolecule (scan rate, 100 mV s$^{-1}$, concentration of drugs $\sim 2 \times 10^{-4}$ M; biomolecule concentration $\sim M \times 10^{-6}$).

| Drug-biomolecule | Drugs-biomolecule concentration ($\times 10^{-6}$) | $E_{pa}$ (mV) | $I_{pa}$ ($\mu$A) | $E_{pc}$ (mV) | $I_{pc}$ ($\mu$A) | $E_{pa}$- $E_{pc}$/2 | $I_{pa}$/I_{pc} |
|-----------------|-----------------------------------------------|--------------|----------------|-------------|----------------|-----------------|----------------|
| DNA only        | 2                                             | -573         | -8.405          | -228        | 11.09          | -172            | -0.7578        |
| RNA only        | 2                                             | -400         | -5.809          | -296        | 10.14          | -52             | -0.5728        |
| BSA only        | 2                                             | -406         | -12.38          | -379        | 48.38          | -13             | -0.2588        |
| sparfloxacin only| 2                                             | -1066        | -5.669          | -184        | 2.586          | -441            | -2.1921        |
| sparfloxacin-DNA | 2                                             | -881         | -5.885          | -138        | 4.570          | -371            | -1.2877        |
| sparfloxacin-RNA | 4                                             | -853         | -5.473          | -103        | 3.119          | -375            | -1.7547        |
| sparfloxacin-BSA | 8                                             | -836         | -4.077          | -077        | 2.132          | -379            | -1.9122        |
| sparfloxacin-BSA | 10                                            | -812         | -3.088          | -039        | 1.027          | -386            | -3.0068        |

Table S5. Result of drug receptor interaction

| Proteins | Drugs | Binding energy (kcal/mol) | Intermolec. Energy kcal/mol | Torsional energy | Total Internal energy | Inhibition constant (uM) | vdW + H bond + desolv Energy kcal/mol | Electrostatic Energy kcal/mol | Ligand efficiency | Unbond energy | refRMS |
|----------|-------|---------------------------|-----------------------------|-----------------|----------------------|-------------------------|--------------------------------------|-------------------------------|-------------------|--------------|--------|
| DNA      | -7.71 | -8.60                     | 0.89                        | 0.19            | 2.23                 | -6.31                   | -2.30                               | -0.28                         | 0.19              | 32.01       |
| RNA      | -6.32 | -7.22                     | 0.89                        | -0.30           | 23.18                | -5.35                   | -1.87                               | -0.26                         | -0.30             | 21.35       |
| BSA      | -6.20 | -7.09                     | 0.89                        | -0.32           | 28.74                | -5.29                   | -1.80                               | -0.26                         | -0.32             | 110.94      |
| DNA      | -7.52 | -8.41                     | 0.89                        | -0.32           | 3.09                 | -6.06                   | -2.36                               | -0.31                         | -0.32             | 31.83       |
| RNA      | -6.07 | -6.96                     | 0.89                        | -0.40           | 35.58                | -5.42                   | -1.54                               | -0.22                         | -0.40             | 29.09       |
| BSA      | -5.47 | -6.37                     | 0.89                        | -0.42           | 97.77                | -5.88                   | -0.48                               | -0.20                         | -0.42             | 74.23       |
Table S6. List of interactions of ciprofloxacin with biomolecules.

| Ciprofloxacin | DNA   | ciprofloxacin | RNA   | Ciprofloxacin | BSA      |
|---------------|-------|---------------|-------|---------------|----------|
| :H            | DA18:O1 | :H            | U9:O1 | :O            | LYS499:HZ3 |
| :O            | DG14:H22 | :O            | G6:H22 | :O            | LYS533:HZ2 |
| :O            | DG24:H3 | :O            | U43:PHO2 | :O            |          |
| :O            | DG24:H21 | :O           | G10:H22 | :O            |          |
| :O            | DG2:H21 | :O            | G10:PHO2 | :O            |          |
| :O            | DG2:H21 | :O            | G10:H3  | :O            |          |
| :O            | DG2:H21 | :O            | C35:O1  | :O            |          |
| :O            | DG2:H21 | :O            | A32:H3  | :O            |          |
| :O            | DG2:H21 | :O            | A33:PHO2 | :O            |          |
| :O            | DG2:H21 | :O            | U13:O2P | :O            |          |
| :O            | DG2:H21 | :O            | U8:OP2  | :O            |          |
| :O            | DG2:H21 | :O            | C35:H42 | :O            |          |
| :O            | DG2:H21 | :O            | U15:O2,OP2 | :O            |          |
| :O            | DG2:H21 | :O            | C27:OP2 | :O            |          |
| :O            | DG2:H21 | :O            | A30:H62 | :O            |          |
| :O            | DG2:H21 | :O            | A30:H7  | :O            |          |

Table S7. List of interactions of sparfloxacin with biomolecules.

| Sparfloxacin | DNA   | Sparfloxacin | RNA   | Sparfloxacin | BSA      |
|--------------|-------|--------------|-------|--------------|----------|
| :H           | DA18:OP1 | :O           | G2:H22 | :H           | THR495:OG1 |
| :O           | DG14:H22 | :H           | U4:O3P | :O           | ALA500:HN  |
| :O           | DG24:H21 | :H           | C47:O2 | :O           | THR419:HN  |
| :O           | DG24:H3 | :O           | G6:H13 | :O           | LYS533:HZ2 |
| :O           | DG10:OP1 | :H           | U16:O2 | :H           | GLN416:OE1 |
| :O           | DG10:OP1 | :O           | G10:H3  | :O           | LYS533:HZ2 |
| :O           | DG10:OP1 | :O           | G10:PHO2 | :O           | PRO420:O  |
| :O           | DG10:OP1 | :O           | A39:OP1 | :O           | LYS114:HZ2 |
| :O           | DG10:OP1 | :O           | U8:OP2  | :O           | ALA128:O  |
| :O           | DG10:OP1 | :O           | A45:H3  | :O           | LYS20:HZ1  |
| :O           | DG10:OP1 | :O           | A45:PHO2 | :O           | GLN94:HE22 |
| :O           | DG10:OP1 | :O           | U17:O2  | :O           | LYS136:HZ1 |
| :O           | DG10:OP1 | :O           | A32:O4P | :O           | GLU97:OE2  |
| :O           | DG10:OP1 | :O           | U15:O2P | :O           | GLU443:OE2 |
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