Molecular insight of the possible inhibition mechanism of therapeutic cephalosporin derivatives against human glutathione reductase enzyme

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Abstract

Glutathione reductase is a key enzyme for glutathione metabolism. Inhibition of the enzyme activity related to various health problems. Therefore, determination of inhibitors of the enzyme and its possible inhibition mechanism are quite important. Some cephalosporins have exhibited potent inhibitory effect against human glutathione reductase (hGR). In order to understand the inhibition mechanism of the cephalosporins, we carried out molecular docking studies with Glide docking and Induced-fit Docking methods. Binding sites of hGR were predicted and the best suitable binding site of the drugs was identified with the Glide docking method. The binding affinity of the drugs was calculated with the induced-fit docking method. The best binding site of the drugs was detected as a part of the catalytic active site for Cefoperazone, Cefodizime, and Ceftazidime, dimerization site for Cefotaxime, Ceftriaxone, and Cefuroxime, and aromate binding site for Ceftizoxime. The Binding affinity of the Cefoperazone was calculated as -10.643 kcal/mol. The results have indicated that hGR enzyme would be inhibited with different mechanisms because of its several druggable sites. These findings would be helpful for designing new inhibitors for hGR enzyme and understanding of potential inhibition mechanism of its other known inhibitors.

1. Introduction

Antibiotics are chemical substances naturally produced by bacteria and plant as well as synthesis in the laboratory. The chemical substances exhibit antibacterial activity by killing or growth arresting of bacteria [1] through inhibition mechanisms of cell wall synthesis [2], cell membrane function [3], ribosome function [4], nucleic acid synthesis [5, 6] and folate metabolism [7]. Physical, chemical, microbiological, pharmacological and finally clinical properties of antibacterial agents depend on its chemical structure [8] and they can be classified as macrolides and lincosamides, quinolones, trimethoprim, tetracyclines, cephalosporins, penicillinase-resistant penicillins, narrow-spectrum penicillins and broad-spectrum penicillins according to the chemical structure.

Cephalosporins are derived from cephalosporin C which is a natural product of *Cephalosporium acremonium* [9]. The molecular structures of cephalosporins are closely related to penicillin with a β-lactam ring. They exhibit antibacterial activity by inhibiting bacterial cell wall synthesis like other antibiotics that have a β-lactam ring [10]. The cephalosporins can be classified in different ways. However, the most favourite classification is based on generation. The classification is made according to a general spectrum of the drugs' activity [11]. First-generation cephalosporins are used for the treatment of infection caused by Gram-positive bacteria such as *Streptococci* and *Staphylococci*. But they have a relatively narrow spectrum of activity against them [12]. Second-generation cephalosporins have broader activity against the Gram-negative bacteria, unlike the first generation [11] and they also exhibit resistance to β-lactamase [13]. The activity of third-generation cephalosporins is improved against Gram-negative bacteria compared to the second-generation. In addition to this, fourth-generation cephalosporins have the broadest spectrum of activity against both Gram-positive and Gram-negative bacteria. Finally, the fifth-generation cephalosporins have enhanced activity against multidrug-resistant gram-positive cocci [11]. More than fifty cephalosporins have been discovered involving all generations since 1945 [14]. It is known that cefalosporins may cause side effects such as reversible thrombocytopenia, hemolytic anemia, neutropenia, interstitial nephritis or abnormal liver

function depending on using more than two weeks [10].

Glutathione (GSH) is an essential molecule in all mammalian cell and is the most abounded non-protein sulfhydryl group [15]. It has several critical cellular functions such as participating as a coenzyme in some enzymatic reactions, protection of the thiol moieties of proteins and low molecular weight compounds, detoxification of toxic compounds and also protecting against oxidative damage [16]. Glutathione reductase (GR) plays a key role in glutathione metabolism because it catalyzes the reduction of glutathione disulfide (GSSG) in the presence of NADPH [17]. Naturally, intracellular concentrations of glutathione change between 0.5 and 10 mM in the mammalian cell [18] and the ratio of [GSH]/[GSSG] exceeds 100 in the resting cell. However, the reduced form of glutathione (GSH) is converted to oxidized form (GSSG) because of oxidative stress and therefore the ratio decreases in the cell [19]. In tissues, GSH concentration should be kept constant at the mentioned ratio above due to it is one of the most important components of the overall antioxidant defense system in the body. This flavin enzyme ensures the maintaining of high GSH and low GSSG levels. Therefore it prevents interrupting of glutathione-related functions in the cell [20]. Many researchers have investigated the effects of some antibiotics on GR enzyme activities in human erythrocyte as *in vitro* [21-23].

In the literature, there are many studies which detected the inhibitory effect of drugs against human glutathione reductase (hGR) enzyme. However, there is not much information about their inhibition mechanisms on hGR enzyme. Our study shed some light on the explanation of the mechanisms using computational methods. It has identified that cefoperazone, cefodizime, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone and cefuroxime are the potent inhibitors of hGR at previously reported *in vitro* studies [21, 22, 24]. In order to explore the possible inhibition mechanism of the drugs against hGR enzyme, we performed the Glide docking and the induced fit docking (IFD) studies with these drugs against the three-dimensional (3D) structure of the enzyme.

2. Material and Method

2.1. Protein target selection and processing

The X-ray crystal structure of hGR enzyme (PDB code: 3DK9) has been chosen due to its near 1Å resolution and good percentile ranks. The structure also has co-crystallized ligand which can be used in the study of docking validation. The structure was downloaded from RCSB Protein Data Bank and

prepared using the protein preparation wizard module because of was not suitable for immediate use in molecular modelling calculations [25]. The protein preparation workflow which detailed described in previous studies was incrementally introduced as a summary. (I) Bond order and charges have been assigned and then missing hydrogen atoms have been added to the structure. (II) Missing side chains have been filled. Amino acids have been ionized at physiological pH. (III) Water molecules have been removed. (IV) Energy minimization and geometry optimization have also been performed [26, 27].

2.2. Binding sites prediction

Binding sites of the prepared structure have been predicted using the SiteMap module of Mastro [28]. SiteScore and Dscore of the sites have been calculated using the default parameter of top-ranked potential protein binding site. Active site and druggable site properties of predicted binding sites have been determined by analyzing SiteScore and Dscore, respectively. The binding sites have also used at target selection and docking hits evaluation [29].

2.3. Ligands preparation

3D structures of the drugs have been downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov). A suitable structure for molecular docking studies of the drugs has been prepared using the LigPrep module [30]. Briefly, their correct molecular geometries and protonation state at pH 7.0±2.0 have been prepared [31].

2.4. Molecular docking studies

In order to identify binding affinity and possible inhibition mechanism of the cephalosporin drugs have been carried out Glide docking and Induced-fit docking studies. Glide docking studies have been performed detection binding site where cephalosporin drugs are best fitted using Glide module of Maestro [32]. Validation of the Glide docking method has been performed with the re-docking procedure by extracting co-crystallized ligand in the structure before the drugs were docked into binding sites of the enzyme. Briefly, the grid box has been generated by selecting the predicted binding site using the Receptor Grid Generation module. Following grid generation, the cephalosporin drugs have been docked into all predicted binding site by setting Extra Precision (XP). After the docking process, XP scores have been evaluated for the detection of the best suitable binding site [33, 34].

After detection of the best suitable binding site, the drugs have been docked into the best suitable binding site for each drugs using induced-fit docking module

of Maestro [35]. Validation of induced-fit docking method has been performed with the re-docking procedure by extracting co-crystallized ligand in the crystal structure of the enzyme before the drugs docked into their best suitable the binding site. Reliability of induced-fit docking method has been evaluated on the basis of RMSD value which calculated with Atom pair method in Superposition panel, between co-crystallized ligand and re-docked ligand. All drugs have been docked into their suitable binding site with the same procedures. For this purpose, the centroid of the residues has been generated around the predicted binding site in the binding site of the enzyme. After that, side chains have been automatically trimmed based on B-factor, closest residues to the ligand have been refined within 3.4 Å of ligand pose in prime refinement. After the docking process, the binding affinity of the drugs has been determined by analyzing the Docking score and Emodel score [36].

3. Results and Discussion

GR is the key enzyme responsible for the maintenance of reduced glutathione in cells. Therefore, the enzyme plays a regulating role in glutathione related metabolism. Loss of the enzyme activity causes accumulation of toxic compound, oxidative stress and reduction of thiol moieties of proteins and low molecular weight compounds. So protection of the enzyme activity is very important. However, it has been reported that the hGR enzyme has been inhibited by some drugs and molecules such as cefotaxime, cefodizime [22], melatonin [37], imipenem, rifamycin, sulfanylacetamide, ceftazidime, chloramphenicol, ceftriaxon, vancomycin, cefuroxime, ornidazole [21], dantrolene [38], diclofenac sodium, ketoprofen, lornoxicam, tenoxicam etomidate, morphine, propofol [39], dacarbazine, thiocolchicoside, methotrexate, olanzapine, pantoprazole sodium, 5-fluorouracil [40], ketotifen, meloxicam, phenyramidol–HCl, gadopentetic acid [17], cefuroxime, ceftriaxone, ceftizoxime and cefoperazone [24]. The enzyme kinetics experiments are very important clarifying inhibition type, IC_{50}-value, and Ki-value of molecules against their targets. However, these results may be inadequate explaining the binding mechanism between molecules and their targets in detail. In the previous studies, IC_{50}-value and Ki-value, as well as inhibition types of cephalosporins drugs against hGR enzyme, have been reported. In order to examine the possible inhibition mechanism of the drugs against their targets, we selected seven cephalosporins including cefoperazone, cefodizime, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone and cefuroxime with potent inhibitory effect against hGR *in vitro* studies. Then, we have performed *in silico* evaluation of the drugs towards hGR.

In the beginning, we have predicted 5 different binding sites of the enzyme structure. The predicted sites have been illustrated in Figure 1. After that, in order to detect the best suitable binding site for the drugs, they have been docked into the predicted sites using Glide docking method. The best suitable binding sites for the drugs have been identified using their docking scores. The scores have been presented in Table 1. According to Docking scores, while Cefoperazone, Cefodizime, and Ceftazidime have located into the Site1. Cefotaxime, Ceftriaxone, and Cefuroxime have located in the Site3. Ceftizoxime has located into the Site5, unlike other drugs. However, their possible inhibition mechanism has not evaluated with the Glide docking. Because the docking method uses searching for possible locations of the ligand in the binding sites of the receptor [41].

Table 1. The glide docking scores of the drugs into predicted binding sites of hGR enzyme

Name	XP Score (kcal/mol)				
	Site 1	Site 2	Site 3	Site 4	Site 5
Cefoperazone	-7.026*	-1.911	-4.857	-2.727	-6.122
Cefodizime	-6.495*	-3.843	-4.955	-3.019	-5.894
Cefotaxime	-5.148	-3.078	-5.391*	-2.926	-4.649
Ceftazidime	-5.730*	-2.169	-4.723	-5.105	-5.186
Ceftizoxime	-4.390	-1.346	-3.643	-2.913	-4.633*
Ceftriaxone	-4.668	-2.707	-5.926*	-3.122	-5.046
Cefuroxime	-4.898	-2.573	-5.213*	-3.477	-4.652

*Best binging sites for docked drugs

Following detection of the best suitable binding sites for each drug, the drugs have docked into their binding site using the induced-fit docking method. Induced-fit docking method provides fully flexibility for protein structure by relaxing small backbone in the receptor structure as well as significant side-chain conformational changes [42], unlike the Glide docking method. Therefore, the method has been used for detecting of possible inhibition mechanism of the drugs.
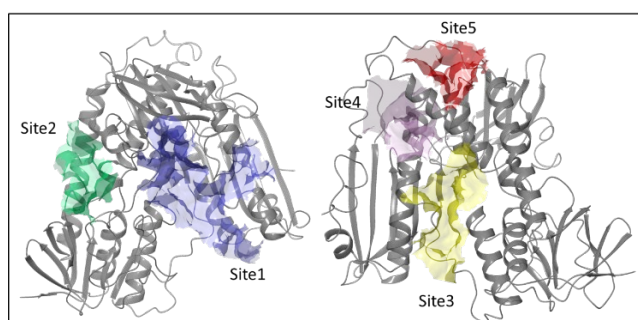

Figure 1. Predicted binding sites of the hGR enzyme.

The human glutathione reductase is a flavoenzyme which uses flavin adenine dinucleotide (FAD) as a prostatic group in catalysis [43]. When NADPH binds to the enzyme, FAD acts as a bridge which transferred electron to Cys58-Cys63 disulfide bond from NADPH [44]. Reduced Cys58 residue causes two GSH molecules by attacking GSSG [43]. Therefore, after best suitable binding sites detection, the drugs have been docked into their best suitable binding sites using the induced-fit docking method. Fallowing docking procedure, docking hits for each drug have been evaluated on the bases of Docking scores. The scores have been presented in Table 2. Pose with the highest score in the negative direction has been chosen as the best-pose. Then, in order to detect possible inhibition mechanism of the drugs, their 2D interaction modes and 3D detailed binding modes have examined. Interacted residues with them have been presented in Table 3.
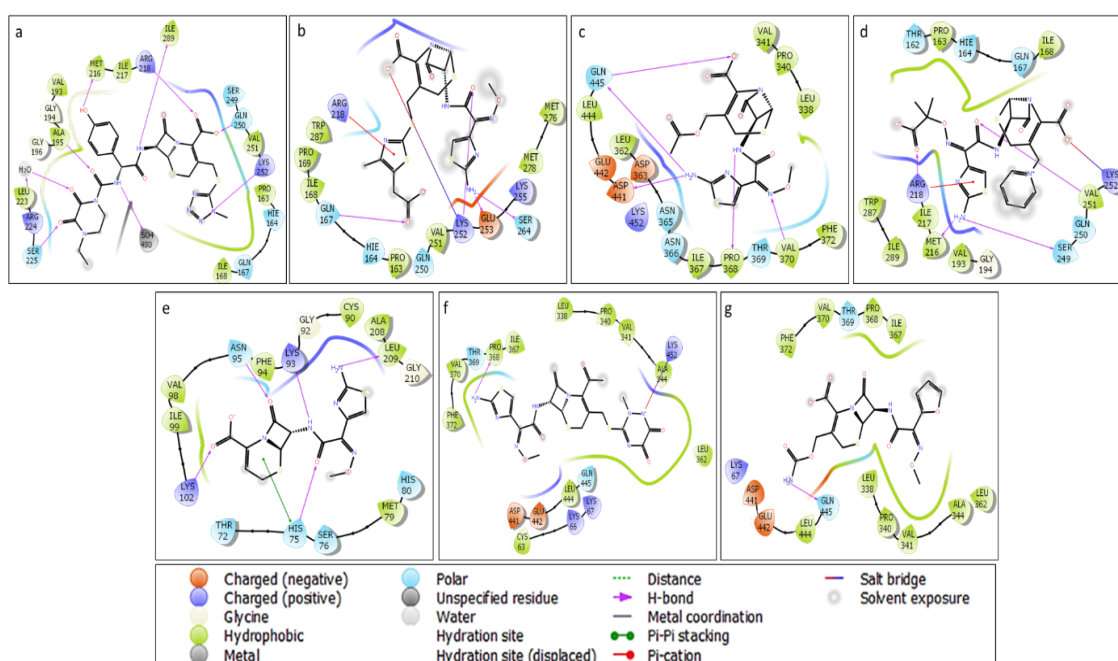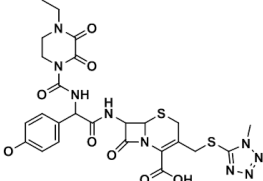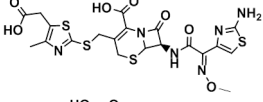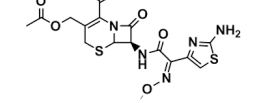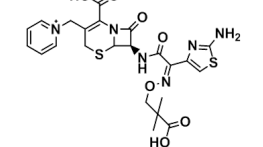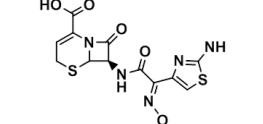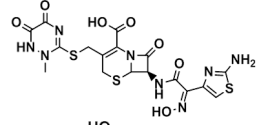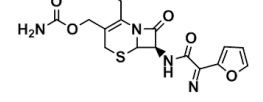

Figure 2. 2D interaction mode of best-posed drugs. (a) Cefoperazone, (b) Cefodizime, (c) Cefotaxime, (d) Ceftazidime, (e) Ceftizoxime, (f) Ceftriaxone and (g) Cefuroxime into their best suitable binding site.

The drugs have strong binding affinity due to the construction of a good few hydrogen bonds and aromatic hydrogen bonds as seen in Figure 2 and Figure 3. Cefoperazone, Cefodizime, and Ceftazidime interacted with Site1 residues have constituted more hydrogen bonds with the residues than those of other drugs. π-π Stacking, π-cation or salt bridge interaction have also contributed to the binding affinity of Cefodizime, Ceftazidime, Ceftizoxime, and Ceftriaxone. The drugs fitted into Site1 and Site3 have similar interaction with those of NADPH and FAD because the site has constituted parts of catalytic active sites. 2D interactions of NADPH and FAD have been shown in Figure 5. Nordhoff *et. al.* [45] have expressed that 436-459 residues responsible for dimerization of hGR enzyme monomers. Site3 residues have included some of the 436-459 residues.

Table 2. Induced fit docking scores of the drugs into their best binding site.

Name	Structure	IFD Docking Score (kcal/mol)	Experimental K_i (mM)	Inhibition Type
Cefaperazone		-10.643	0.029 [24]	Non-Competitive
Cefodizime		-6.120	0.745 ± 0.222 [22]	Competitive
Cefotaxime		-8.035	6.512 ± 4.114 [22]	Non-Competitive
Ceftazidime		-6.480	3.590 ± 0.510 [21]	Competitive
Ceftizoxime		-7.852	22.760 [24]	Non-Competitive
Ceftriaxone		-6.079	3.710 ± 0.600 [24]	Non-Competitive
Cefuroxime		-7.690	23.500 ± 2.940 [21, 24]	Competitive

Cefotaxime, Ceftriaxone, and Cefuroxime have formed hydrogen bond, salt bridge, and negative interactions with the dimerization residues as seen in Figure 2-c-f-g. Therefore, they can cause inhibition of the enzyme by hindering dimerization. Unlike the others, Ceftizoxime has interacted with residues of the different binding site, site5, as seen in Figure 2-e and 3-e. The site has named as aromate binding site by Kasozi *et. al.* [46]. They have identified that pyocyanin molecule inhibited the hGR enzyme by interacting with the residues of the aromate binding site. According to the docking scores and the interaction modes of the drugs, we have explored that they exhibit strong binding affinity and are potent inhibitor for hGR as indicated previously *in vitro* studies.
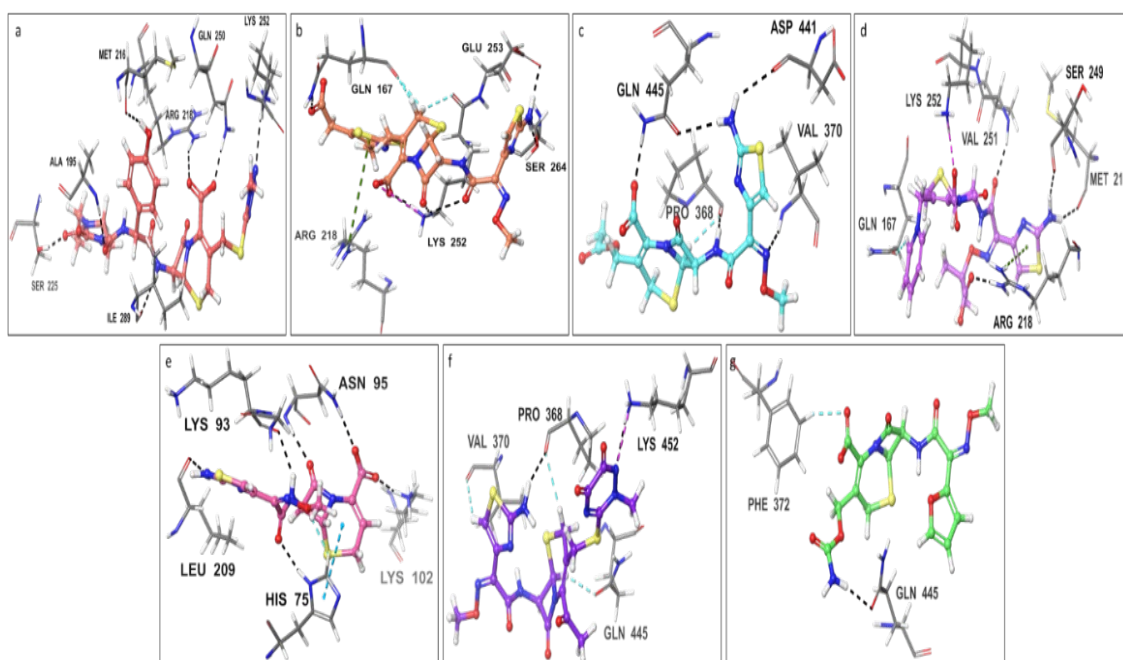
Figure 3. 3D detailed binding mode of best-posed drugs. (a) Cefoperazone, (b) Cefodizime, (c) Cefotaxime, (d) Ceftazidime, (e) Ceftizoxime, (f) Ceftriaxone and (g) Cefuroxime into their best suitable binding site. Drugs have been represented as colored ball-stick model and amino acid residues have been represented as grey thick-tube model.

Table 3. Interacted residues with the drugs into hGR enzyme.

Name	Hydrogen Bond	π-π Stacking and π-cation	Salt Bridge
Cefaperazone	Ala195, Met216, Arg218, Ser225, Gln250, Lys252, Ile289	-	-
Cefodizime	Gln167, Lye252, Glu253, Ser264	Arg218	Lys252
Cefotaxime	Pro368, Val370, Asp441, Gln445	-	-
Ceftazidime	Gln167, Met216, Arg218, Ser249, Val251, Lys252	Arg218	
Ceftizoxime	His75, Lys93, Asn95, Lys102, Leu209	His75	-
Ceftriaxone	Pro368, Val370, Gln445, Lys452	-	Lys452
Cefuroxime	Phe372, Gln445	-	-

In order to give a more detailed explanation for their possible inhibition mechanism, we also compared confirmation of important catalytic active site residue for Native hGR (hGR$_{native}$), NADPH docked hGR (hGR$_{NADPH}$) and Cefoperazone docked hGR (hGR$_{Cefoperazone}$). These structures have been illustrated in Figure 4. For the comparison, we have used Cys58, Cys63, Tyr197, and Phe327 residues. Because Cys58 and Cys63 residues directly participate in the catalytic activity of the enzyme and conformations of Tyr197 and Phe327 residues have changed with NADPH binding [43]. According to Figure 4, the position of hGR$_{NADPH}$ Cys58 and Cys63 residues significantly differ from those of hGR$_{native}$ end hGR$_{Cefoperazone}$ and the distance between the residues has calculated as 0.646 Å. These results have indicated that NADPH bonded structure get active form. Alongside the residues, the position of Tyr197 and Phe327 residues of hGR$_{Cefoperazone}$ differ from hGR$_{NADPH}$ structure and the distance between the residues has calculated as 0.322 Å. The results have also indicated that Cefoperazone causes to remain at the inactive form of the enzyme by effecting conformation of the catalytic active site.
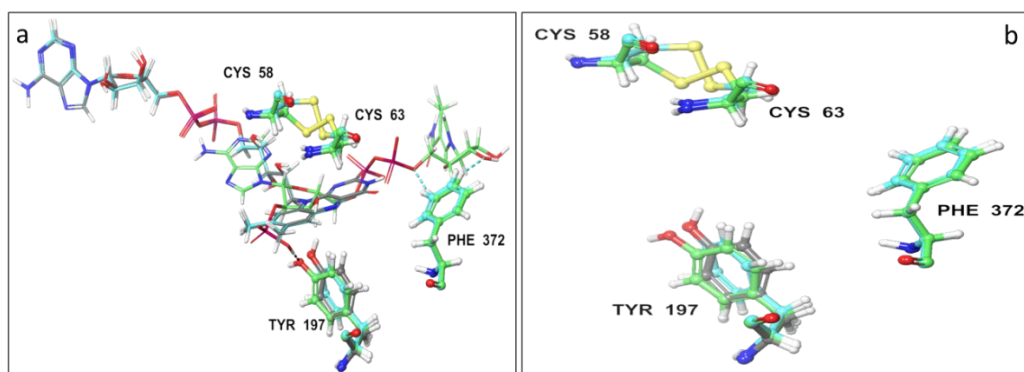
Figure 4. Conformation of active site residues. (a) FAD and residues and (b) active site residues. FAD and residues of hGR_native have been represented grey color, FAD and residues of hGR_Cefoperazone have been represented turquoise color, and residues of hGR_NADPH has been represented green color.
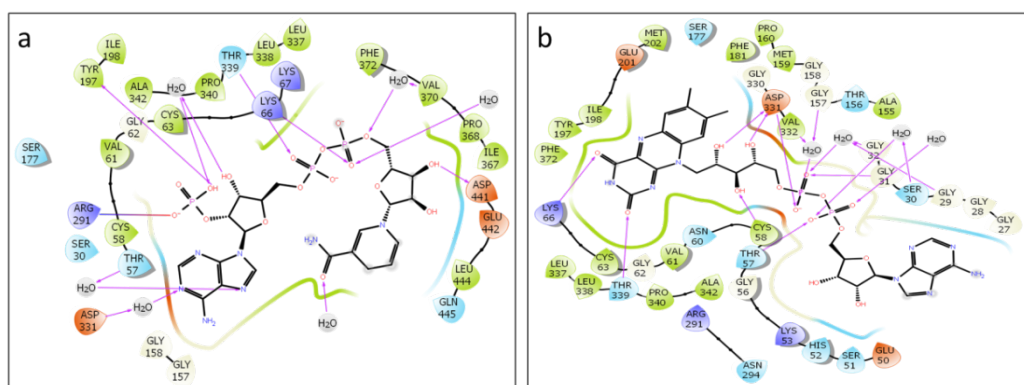

Figure 5. 2D interaction mode of the best-posed (a) NADPH and (b)FAD into catalytic active site of hGR enzyme.

4. Conclusions

In this study, we determined theoretically for the first time that the certain seven cephalosporins have an inhibitory effect against human glutathione reductase. Molecular docking results have shown that the drugs have exhibited potent inhibition compatible with previously reported *in vitro* experiments. The results have also shown that the drugs inhibit the enzyme with different mechanisms including structural conformational changing into the catalytic active site and hindering dimerization. Thus, our findings provide new insights into the potential inhibition mechanism of these inhibitors of hGR enzyme. The present study gives us a new perspective for understanding how cephalosporins inhibit the hGR enzyme. These results would be helpful for designing new inhibitors and understanding of potential inhibition mechanism of the other known inhibitors.

Acknowledgments

Conflicts of interest

The authors state that did not have a conflict of interests

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