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Computational Calculations of Molecular Properties and Molecular Docking of New and Reference Cephalosporins on Penicillin Binding Proteins and Various β-Lactamases

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Abstract: An approach of using molinspiration calculations and molecular docking on PBPs (penicillin-binding proteins) and certain β-lactamases is employed to predict the molecular properties, bioactivity and resistance of newer and reference cephalosporins. The previously synthesized cephalosporins 1-8 and reference cephalosporins were subjected to extensive evaluations by calculating the molecular properties, drug-likeness scores on the bases of Lipinski's rule and bioactivity prediction using the method of molinspiration web-based software. The TPSA (topological polar surface area), OH-NH interactions, n-violation and the molinspiration Log partition coefficient (miLogP) values were also calculated. The investigated cephalosporins were subjected to molecular docking study on PBPs (1pyy) and on β-lactamases produced by S. aureus, K. pneumonia, E. coli and P. auroginosa using 1-click-docking website. Molecular properties of 1-8 recorded higher TPSA than cephalexin and were lower than the reference cephalosporins and do not fulfill the requirements for Lipinski's rule. Bioactivities of 1-8 were predicted to be less and their docking scores on PBPs were comparable to those of the reference cephalosporins, particularly ceftobiprole. The references recorded various docking scores on the above β-lactamases and as expected, ceftobiprole recorded the lowest scores on all β-lactamases. Cephalosporins 1-8 recorded various docking scores on β-lactamases. Molecular docking studies on PBPs and β-lactamases are considered as very useful, reliable and practical approach for predicting the bioactivity scores and to afford some information about the stability and selectivity of the newly proposed cephalosporins against β-lactamases of certain pathogenic microbes, such as P. auroginosa and MRSA, by recording the relative docking scores in comparison with those of reference cephalosporins.

Key words: Cephalosporins, Molinspiration, Molecular docking, β-lactamases, Lipinski's rule.

1. Introduction

Drug resistance is a serious situation that limits the treatment choices of infections caused by MRSA (methicillin-resistant *Staphylococcus aureus*). MRSA strains have spread worldwide and are considered as the most dangerous threat to humans [1-4] and the outbreaks of MRSA in the community have increased alarmingly. The widespread resistance of MRSA to β-lactam antibiotics has made treatment of infections by these antibiotics extremely difficult [5]. Besides, *strains of S. aureus* have also become resistant to so-called "drugs of last choice" including vancomycin, linezolid and daptomycin [6-8]. The development of potent new drugs is one of the most difficult and complicated processes in pharmaceutical industry.

Cephalosporins are the most widely used β-lactam antibiotics for treatment of bacterial infections and perform their action through covalent binding with PBPs, thus inhibiting the final step in cell wall biosynthesis. The development of bacterial resistance is a major concern that encourages the development of new resistant antibiotics towards bacterial β-lactamases. Cephalosporins have different

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antibacterial spectra, β-lactamase sensitivity/ resistance and pharmacokinetic properties [9]. The fifth generation cephalosporins, ceftobiprole [10] and ceftaroline [11] are characterized by having unique spectrum on G (-) bacteria (powerful anti-Pseudomonal activity) and an expanded spectrum against G (+) bacteria include MRSA. Their activities are beyond all other cephalosporins and appear to be less susceptible to develop resistance. Ceftobiprole exhibits a high level of affinity for PBPs of MRSA [12].

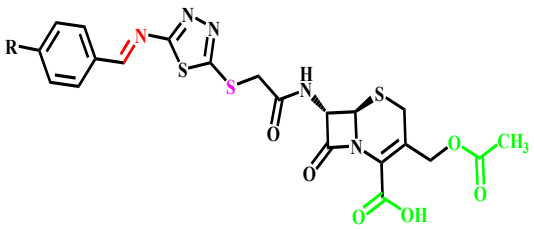
CADD (computer-aided drug design) approach aims to shorten the time and tedious procedures and provide better efficiency in the processes of drug discovery. This approach provides more details and aid to coordinate the information to make the drug design more rational [13-15]. Rational drug design helps to facilitate and fasten the procedures of drug designing process, which includes various methods to identify and select the novel potent compounds. Molecular docking of a drug molecule with a certain receptor is one of these approaches [16].Docking is the binding or interaction of a ligand with targeted receptor in the 3-D (three dimensional structure) spaces in order to study the molecular properties and degree of binding that reflect its bioactivity [17-19]. There is an increasing interest and potential application of this approach in the field of drug design and discovery. The antibacterial activity of novel α-amino acid functionalized fluoroquinolones is validated by molecular docking studies and is in good correlation with the experimental results [20]. Docking study of polycyclic quinolone-based molecules revealed that these compounds recorded weak cytotoxic effects and poor binding affinity to human topoisomerase [21]. Results of docking paclitaxel and its analogues on target proteins tubulin B-1 chain and B-c1-2 showed various docking scores and predicted only two analogues as more suitable than paclitaxel [22]. Molecular docking studies of cyclic octapeptide (D-proline-incorporated wainunuamide) revealed more potent affinity for HPV18-2IOI (HeLa cancer cell lines) and exhibited better antitumor activity [23].

In view of the cumulative information, it was considered that CADD may be useful to design new cephalosporins of great potential and have better activities and properties, particularly β-lactamase resistant and antipseudomonal activity. Molecular docking as one of the CADD strategies was used for providing extensive molecular modeling calculations, bioactivity prediction and docking scores of cephalosporins to PBPs and β-lactamases from different sources. This approach may aid in the discovery of novel potent cephalosporins that are resistant to β-lactamases. Based on the author's knowledge, no docking study arising from the use of cephalosporins on β-lactamases have been reported, so far. Application of this approach was attempted on the previously synthesized cephalosporins [24], and selected reference cephalosporins representing the five generations that are susceptible or resistant to β-lactamases to confirm its validity.

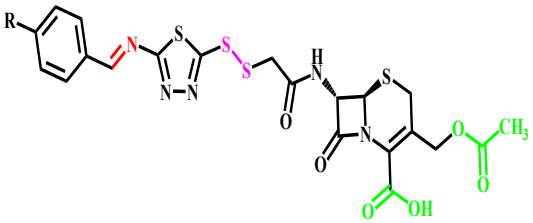
2. Experimental

2.1 The Investigated Cephalosporins

Cephalosporins 1-8 were previously synthesized, characterized and identified and evaluated for their antimicrobial activities [24].The chemical structures of 1-8 are given as supplementary information on Fig. 1. Reference cephalosporins selected from the five generations, that are of various degree of stability against β-lactamases were used, such as, cephalexin and cefuroxime (susceptible to hydrolysis by β-lactamases) and ceftazidime, ceftriaxone, cefozopran, and ceftobiprole (resistant to β-lactamases). Using one of the chemoffice softwares (ChemDraw Ultra 10.0 program), the chemical structures of all the investigated cephalosporins and their SMILES notation were obtained. Their chemical structures are illustrated on Fig. 1 and chemical nomenclature are as follows; compounds 1-4 [Substitiuted-3-(acetoxymethyl)-7-(2-(5-benzylidenea



R= H, cephalosporins 1-5
R= Br, cephalosporins 3 and 7



R= Cl, cephalosporins 2 and 6
R= NO_2, cephalosporins 4 and 8

Fig. 1 General structures of the investigated cephalosporins 1-8.

mino)-1,3,4-thiadiazol-2-yl-thio)-acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid] and compounds 5-8 [Substituted-3-(acetoxymethyl)-7-(2-((5-benzylideneamino)-1,3,4-thiadiazol-2-yl)-disulfanyl)acetamido)-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid].

2.2 Calculation of Molecular Properties and Bioactivity Scores

Lipinski's rule of five [25, 26] was used to evaluate the drug-likeness and calculate the molecular properties that are essential factors for a drug pharmacokinetics, including ADME (absorption, distribution, metabolism and excretion). Molinspiration website-based software (www.molinspiration.com) was employed to obtain certain molecular parameters. The values of miLogP, as (octanol/water partition coefficient) and TPSA of the investigated cephalosporins were determined using the method developed by molinspiration [27]. Drug-likeness scores were calculated to represent the amount of fragments based on contributions and correction factors [28]. The prediction of bioactivity scores of these cephalosporins were calculated by recording the activity scores of GPCR (G-protein coupled receptors ligand), KI (kinase inhibitor), PI (protease inhibitor), EI (enzyme inhibitor), ICM (ion channel modulator) and NRL (nuclear receptor ligand).

2.3 Docking Study on PBPs (Penicillin Binding Proteins)

The docking study of the investigated cephalosporins on PBP_2 (1pyy) was conducted to confirm and support the antimicrobial activities of cephalosporins 1-8 [24] and to be compared with reference cephalosporins of various activities to validate this approach. This study was conducted using 1-click-docking software (mcule.com) and the chemical structures of PBPs were retrieved from the protein data bank (PDB, www.rcsb.org (DOI:10.2210/pdb3b60/pdb).

2.4 Docking Study on Certain β-Lactamases

The structures of the bacterial β-lactamases were retrieved from PDB. Different β-lactamases of various bacteria were used for docking study of these cephalosporins to calculate the docking scores of the binding energies and consequently, investigate the possibility of resistance toward the above types of β-lactamases. These types of β-lactamases are; PDB (ID: 1xgj) of *E. coli*, PDB (ID: 3q6x) of *K. Pneumonia*, PDB (ID: 1ome) of *S. aureus* and PDB (ID: 2wzz) of *P. auroginosa*

3. Results and Discussion

3.1 Molinspiration Calculations

Molecular properties were calculated on the bases of Lipinski's rule and its components. The cephalosporins 1-8 have higher TPSA than cephalexin, particularly, 4 and 8 (196.98), which do not comply with Lipinski's rule (Table 1). Furthermore, TPSA values of the reference cephalosporins are higher (except for cephalexin) and increasing with advancing in generations, particularly, ceftriaxone (221.61) and ceftobiprole (203.18) and these do not comply with

Lipinski's rule (Table 1). These cephalosporins largely differ in their ADME properties. The values of OH-NH polar fragments representing the proton donors and proton acceptors of 1-8 and the reference cephalosporins were 2 and 3 to 6 respectively (Table 1). The OH-NH values centered polar fragments should be <5 and ≤ 10 respectively, based on Lipinski's rule. Accordingly, cephalosporins 1-8 do not fulfill the requirements for Lipinski's rule. TPSA is a very useful descriptor used to characterize drug absorption and bioavailability, permeability through Caco-2 cells and transport across blood brain barriers [25, 26]. The higher values of TPSA and OH-NH interactions indicate that these compounds may have smooth and efficient binding to receptor, as compared with the reference cephalosporins (Table 1). However, drug molecules with TPSA values of 140 Å or higher are expected to have very low absorption [26]. Lipophilicity (miLogP) and TPSA values are essential factors for the prediction of oral bioavailability of drugs [29]. Molinspiration cheminformatics are available from http://www.molinspiration.com. TPSA values are high for ceftazidime, ceftriaxone and ceftobiprole (Table 1), which do not comply with Lipinski's rule, and as expected, since all these are administered parenterally. The n-violation values of 1-8 were 2, which is comparable with the reference cephalosporins.

3.2 Prediction of bioactivity scores

The prediction of bioactivity scores of cephalosporins 1-8 were recorded by calculating the activity scores of GPCR ligand, ICM, NRL, KI, PI and EI (Table 2). Their predicted drug likeness scores were much less than those of the reference cephalosporins (Table 2). Likewise, 1-8 have consistent negative values in all categories and numerical values for those of the reference cephalosporins. Accordingly, all the synthesized cephalosporins 1-8 are predicted to have less activity than the reference cephalosporins used based upon those categories and these results comply with the previously reported activity evaluation [24]. The n-violation of cephalosporins 1-8 was 2, as compared with cephalexin which has zero violation and complies with Lipinski's rule. Cephalexin has good bioactivity and excellent absorption. The other reference cephalosporins, which are only used parenterally, have n-violation of 1-3 (Table 1). Drug likeness scores predicted poor bioactivities for the cephalosporins 1-8 and the results showed various values and are summarized on Table 2.

Table 1 Molinspiration calculations of the synthesized and reference cephalosporins.

Compound	Molinspiration Calculations					
	MW	miLogP	TPSA	OH-NH Interaction	n Violation	Volume
1	533.6	1.458	151.16	2	2	421.85
2	568.1	2.136	151.16	2	2	435.38
3	612.5	2.213	151.16	2	2	439.73
4	578.6	1.417	196.98	2	2	445.18
5	565.7	1.958	151.20	2	2	439.97
6	600.1	2.636	151.16	2	2	453.51
7	644.7	2.767	151.16	2	2	457.86
8	610.6	1.917	196.98	2	2	463.31
Cephalexin	347.4	-1.486	112.73	4	0	293.2
Cefuroxime	424.39	-0.978	173.77	4	1	334.95
Ceftazidime	547.6	-0.630	189.45	5	2	442.52
Ceftriaxone	540.6	-2.110	221.61	5	2	405.45
Cefozopran	515.5	-5.51	184.87	3	2	400.75
Ceftobiprole	534.58	-1.504	203.18	6	3	424.33

Key notes: MW= molecular weight, miLogP = molinspiration Log partition coefficient, TPSA = topological polar surface area.

Table 2 Molinspiration drug-likeness of the synthesized and reference cephalosporins.

Compound	Drug-Likeness					
	GPCR	ICM	KI	NRL	PI	EI
1	-1.05	-1.22	-1.3	-1.14	-0.18	-0.27
2	-1.02	-1.21	-1.28	-1.12	-0.20	-0.29
3	-1.09	-1.26	-1.30	-1.19	-0.26	-0.31
4	-1.07	-1.26	-1.01	-1.13	-0.27	-0.34
5	-0.79	-1.10	-1.01	-1.01	-0.06	-0.01
6	-0.77	-1.11	-1.00	-0.99	-0.09	-0.04
7	-0.84	-1.15	-1.02	-1.06	-0.14	-0.06
8	-0.83	-1.18	-1.05	-1.03	-0.15	-0.12
Cephalexin	-0.35	-0.73	-1.03	-0.98	0.5	0.13
Cefuroxime	-0.28	-0.83	-0.91	-0.57	0.43	0.15
Ceftazidime	-0.23	-0.75	-0.57	-0.70	0.24	0.29
Ceftriaxone	-0.18	-0.73	-0.80	-1.07	0.03	0.33
Cefozopran	0.071	-0.498	-0.75	-1.16	0.19	0.39
Ceftobiprole	-0.21	-0.499	-0.62	-1.02	0.294	0.412

Key notes: GPCR = G-protein coupled receptor, ICM = Ion channel modulator, KI =Kinase inhibitor, NRL= Nuclear receptor ligand, PI = Protease inhibitor, EI = Enzyme inhibitor.

3.3 Docking Scores of Cephalosporins on PBP2

Docking of a compound on a macromolecule allows recording a database and predicting the strongest and more potent binding affinity to a certain binding site. The antibacterial activity of cephalosporins against certain pathogenic microbes depends on its degree of binding affinity to specific PBPs in that microbe. There are a number of PBPs in each microbe, represented as PBP 1-6 with additional subtypes. The high or potent affinity towards only one type of PBPs is enough to show antibacterial action. Docking of 1-8 on PBPs were conducted to confirm and support the evaluation of their antimicrobial activities mentioned earlier [24]. This docking approach was also employed for the reference cephalosporins of various activities for comparison and to establish a relationship or a correlation. It may be suitable for predicting activity and stability against β-lactamases and anti-pseudomonal activities of the newly proposed cephalosporins. The docking scores of cephalosporins 1-8 on PBPs were -6.90 to -7.37 (Table 3). Moreover, compounds 2, 3, 4, and 6 recorded the lowest docking scores of -7.125, -7.025, -7.37 and -7.075 respectively, which may indicate that these have higher activities than the others and comparable with the reference cephalosporins, particularly, ceftobiprole (Table 3). The docking pose of 4 (-7.37) is shown on Fig. 2. Docking pose of cephalexin on PBP2 (1pyy) was recorded on Fig. 3 to show the position of its functional groups on the surface of PBP2 and for comparison with other cephalosporins.

The docking score of 4 is very close to those of ceftazidime, ceftriaxone and ceftobiprole, which may indicate that it has strong affinity binding to PBPs and hence is equally potent. Interestingly, and as expected the docking scores of the reference cephalosporins are decreasing to lower values (Table 3), when advancing in generations, indicating potent affinity binding and

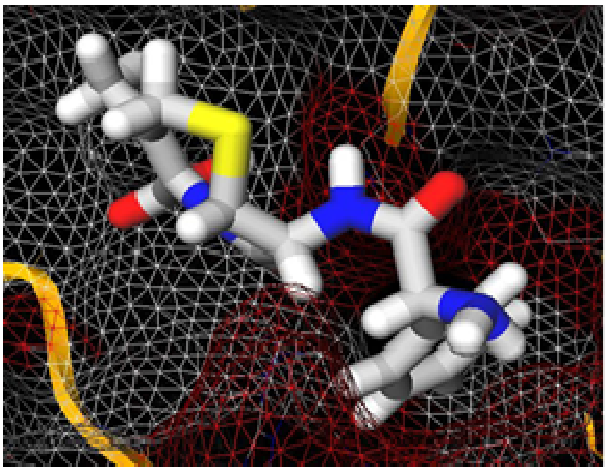


Fig. 2 Docking pose of cephalosporin 4 on pbp2 (1pyy).

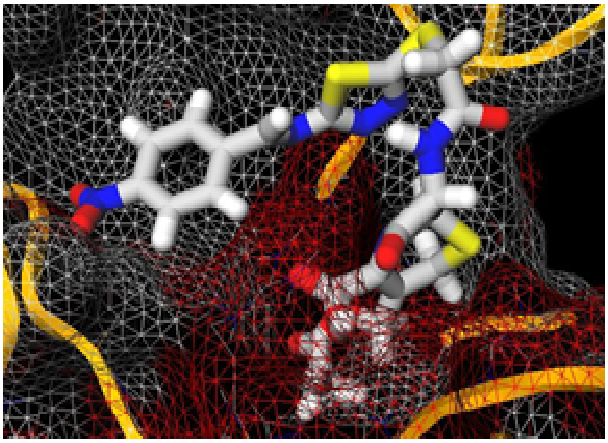


Fig. 3 Docking pose of cephalexin on pbp2 (1pyy).

more activities. Ceftobiprole recorded the lowest docking score of -7.47, while cephalexin has higher docking score of -5.75 and this obviously means that ceftobiprole has higher binding affinity towards PBPs and much better activity than cephalexin. The detailed results are given on Table 3. Various β-lactam antibiotics exhibited different binding affinities for the PBPs of *E. coli* K-12 [30]. The affinities of these compounds for PBPs revealed close agreement with their antibacterial activities. Previous report [31] indicated that there is no correlation between the minimum inhibitory concentrations (MICs) and the binding affinity of certain cephalosporins, such as cefozopran for PBPs 1, 2 or 6. The results suggested that cefozopran has good activity against *E. faecalis* TN2005 by binding to PBP 5, while, ceftazidime and cefmenoxime have no affinity for PBP 5. Furthermore, ampicillin, benzyl penicillin and imipenem recorded higher affinity for PBP 3-5 than cefozopran, while their experimental activities were lower than that of cefozopran [31]. Ceftobiprole and ceftriaxone have good affinity for the PBP 2 with distinguished antibacterial activities [32, 33]. However, it was reported that there is no direct relationship between the amounts of expressed PBP 2a in different strains of MRSA and the β-lactam MICs [34]. Moreover, it was shown that ceftobiprole exhibited potent binding to PBP2x of penicillin-resistant *S. pneumoniae,* PBP2a of MRSA and PBP3 and other essential PBPs of methicillin susceptible *S. aureus, E. coli*, and *P. aeruginosa* [35]. Ceftobiprole also bind effectively to PBP2 in the latter organisms, contributing to its antibacterial activity against G (-) and G (+) bacteria [31]. Ceftaroline exhibited potent binding to many PBPs in *S. aureus* (PBP 2a), responsible for methicillin resistance of MRSA and to PBPs in *S. pneumonia*, PBP 2b, PBP 2x and PBP 1a, which is an important factor in penicillin resistance [36]. Moreover, it was confirmed that the binding affinities of several β-lactams correlated well with their respective MICs for various strains of MRSA [29, 37, 38]. β-Lactam antibiotics, which are substrate analogues, covalently bind to PBPs and inactivate them at concentrations that are approximately equal to their MICs [31]. The PBPs (1a, 1b) and PBP2 (2a, 2b, 2x) of *S. pneumonia* have high affinity for most β-lactams [31]. Certain β-lactam antibiotics were subjected to affinity binding study and were found to have a close correlation between their MICs and the concentrations required to saturate 50% of PBP 2b [39, 40]. However, this correlation was not apparent when the bacteria were previously exposed to these antibiotics at their MICs. Accordingly and based on all these studies and observations, it is concluded that correlation between MICs and the binding affinity is not a decisive matter to predict the activity and could not be relied on to find a link between activity (MICs) and binding affinity to PBPs. It is worth suggesting that docking of cephalosporins on PBPs and measuring the docking scores may have an excellent direct correlation with the activity of these antibiotics, when compared with docking results of the known reference antibiotics.

3.4 Docking Scores of the Investigated Cephalosporins on β-Lactamases

The docking results of cephalosporins 1-8 on various β-lactamases were compared with those of the reference cephalosporins (Table 3). These recorded low docking scores on β-lactamase of *E. coli* (-7.15 to -7.85), which were within the same range of the docking scores of the reference cephalosporins, except

for ceftriaxone (-8.47) and ceftobiprole (-8.65). However, cephalosporins 2 and 3 recorded the lowest scores of (-7.85 for both) and this result may suggest that their binding affinities are higher than cephalexin, cefuroxime or ceftazidime toward *E. coli,* and consequently, may be more resistant to this β-lactamase. Cephalosporins 1 and 4 have the lowest docking scores of -7.07 and -7.40 on β-lactamase of *K. pneumonia*, respectively, which may suggest that these two cephalosporins are more resistant than cephalexin, cefuroxime or ceftazidime (Table 3). Docking of 5 and 6 on β-lactamase of *P. auroginosa* recorded the lowest docking scores of -8.65 and -8.85, respectively, and these may be more resistant than cephalexin, cefuroxime, or ceftazidime (Table 3). Docking scores of 2, 6 and 7 on β-lactamase of *S. aureus* were the lowest (-7.07), as shown on Table 3, and these cephalosporins are presumed to be more stable than all reference cephalosporins, except ceftobiprole (-7.3). These cephalosporins are also assumed to have potent interaction on the binding sites and their 3-dimentional structure may have great effect on the stability towards β-lactamases. The presence of Schiff bases and sulfide or disulfide bonds on either side of the 1,3,4-thiadiazole ring in the acyl side chain may contribute to the functionality of the structures that provide strong interaction with the binding sites rather than the active sites.

In these docking studies the red net that appeared on the surface of the β-lactamases in the diagrams (Figs. 4-10) represent the polar sites of the enzyme that aid in binding and catalysis of substrates. The white net represents the non-polar sites that do not contribute to any catalysis. It is obvious that when the β-lactam ring is accommodated within the polar active site, means the enzyme is capable of hydrolyzing this antibiotic. The docking of various cephalosporins presented as their 3D structures on β-lactamases (Figs. 4-10) showed clearly the positioning of the β-lactam ring on the surface of the enzyme. The β-lactam ring of these cephalosporins was projected away from the polar active site, while the polar groupings on either side of the cephem nucleus of the molecule interacted with the polar binding sites, and provided some degree of stability against β-lactamases. Docking poses of compounds 2-4 and 6 on β-lactamases were taken for comparison (Figs. 4-7), since those showed low docking scores (Table 3).

Table 3 Docking scores of the synthesized and reference cephalosporins.

Compound	Docking Scores* (Docking energy, Kcal/mol)				
	β-lactamases				PBPs
	E. coli *1xgj*	*K. Pneumonia* *3q6x*	*S. aureus* *1ome*	*P. auroginosa* *2wzz*	1pyy
1	-7.80	-7.07	-6.85	-8.50	- 6.90
2	-7.85	-6.95	-7.07	-8.55	-7.12
3	-7.85	-5.80	-6.95	-8.52	-7.02
4	-7.47	-7.40	-6.9	-8.42	-7.37
5	-7.77	-6.42	-6.7	-8.65	-6.97
6	-7.50	-5.72	-7.07	-8.85	-7.07
7	-7.15	-5.70	-7.07	-7.85	-6.95
8	-7.47	-6.95	-7.0	-7.60	-6.85
cephalexin	-6.95	-6.35	-6.35	-7.60	-5.75
cefuroxime	-7.33	-6.02	-6.17	-8.02	-6.1
ceftazidime	-7.40	-6.80	-6.75	-8.17	-7.37
ceftriaxone	-8.47	-7.20	-6.92	-9.32	-7.27
cefozopran	-8.3	-7.0	-6.2	-9.0	-7.40
ceftobiprole^{+}	-8.65	-7.90	-7.3	-9.40	-7.47

*More negative values indicate higher binding affinity. Four docking poses were taken for each compound on each enzyme and scores represent the average. + Two docking poses appeared on *P. auroginosa* and *K. pneumonia* and only one pose appeared on *E. coli* and *S. aureus*.

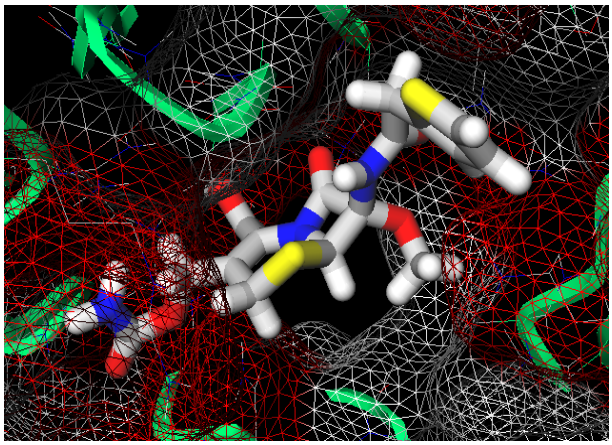


Fig. 4 The best docking pose of cephalosporin 3 on 1xgj.

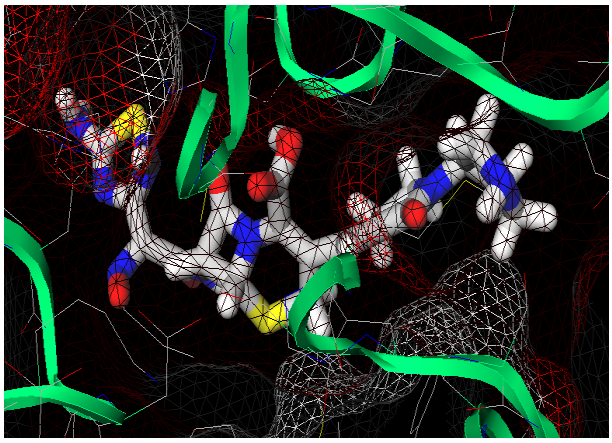


Fig. 5 The best docking pose of cephalosporin 4 on 3q6x.

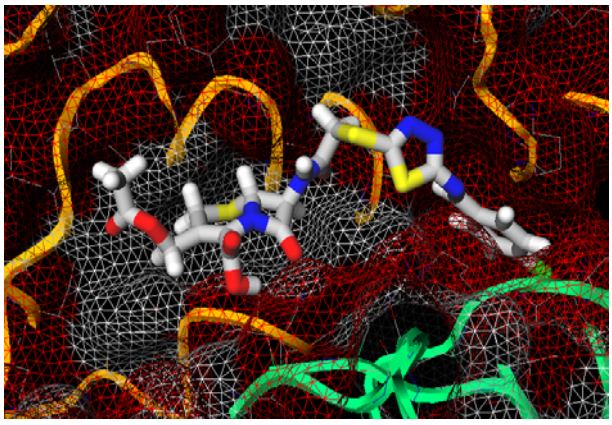


Fig. 6 The best docking pose of cephalosporin 2 on 1ome.

3.5 Docking Scores of the Reference Cephalosporins on β-Lactamases

Cephalexin and cefuroxime recorded high docking scores on all types of β-lactamases (-6.35 to -6.95) and (-6.02 to -7.33) and this means that they needed high

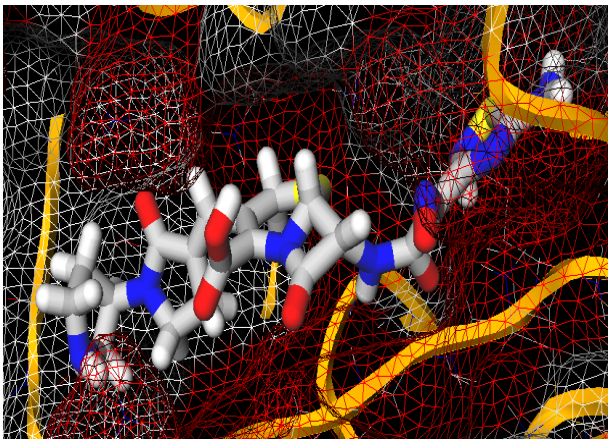


Fig. 7 The best docking pose of cephalosporin 6 on 2wzz.

energy of binding. These two cephalosporins are susceptible to hydrolysis by all β-lactamases and recorded high docking scores (Table 3). The resistant cephalosporins (ceftazidime, ceftriaxone, cefozopran and ceftobiprole) recorded lower docking scores on all β-lactamases (Table 3) and ceftobiprole has always the lowest values (-7.3 to -9.4). Moreover, these cephalosporins recorded very low docking scores (-7.6 to -9.4) on *P. auroginosa*, which are supported by the known fact that these cephalosporins have antipseudomonal activities. However, the docking scores of the reference cephalosporins on β-lactamases of *S. aureus, K. pneumonia* and *E. coli* were -6.17 to -7.3, -6.02 to -7.9, and -6.95 to -8.65, respectively (Table 3). Ceftobiprole recorded the lowest docking scores on all types of β-lactamases (Table 3), and this is an expected result due to the fact that it is much more resistant. However, ceftriaxone showed comparable results to ceftobiprole with respect to *E. coli, S. aureus* and *P. auroginosa* (Table 3). These results indicated that the resistant cephalosporins have potent binding affinity to the binding sites of the β-lactamases but not on the active site of these enzymes. There is a clear evidence that docking of these resistant cephalosporins on β-lactamases showed that the β-lactam ring was projected away or flipped from the active site, and therefore is not hydrolyzed and consequently, showed greater stability against β-lactamases (Figs. 9 and 10). However, the β-lactam ring of cephalexin was

accommodated in a dense polar area, the red net (presumably the active site), which explains its susceptibility to hydrolysis by β-lactamases (Fig. 8). The docking scores on β-lactamases indicated that there is a direct relationship between the energy of the binding affinity, referring to the lowest docking scores and the stability. This means that these cephalosporins have more potent binding with much more stability, as shown for ceftobiprole (Table 3). The more convincing explanation for the strong binding of resistant cephalosporins with β-lactamases without suffering hydrolysis is that these cephalosporins interact with β-lactamases to form an acyl adduct or a complex. But once in this form, it is presumed that the polar substituents on either C7 side chain or C3 position force the electrophilic acyl group to rotate away or flipped and thus displayed from the site of hydrolysis by the active site of the enzyme. Furthermore, these polar substituents provide strong interaction to the binding sites (Figs. 9 and 10). An interesting finding was observed in the comparison of the structures of complexes of AmpC β-lactamase with ceftazidime and loracarbef (a substrate of β-lactamase), which illustrated that the structural conformation of ceftazidime in the active site differs from that of substrate [41]. This study suggested that ceftazidime inhibit the formation of the tetrahedral transition state, indicating that it is an inhibitor of AmpC β-lactamase, without suffering hydrolysis by this β-lactamase. Ceftazidime structure is not exposed into a conformation that is liable for hydrolysis due to steric factors [38]. Extensive structure-activity studies suggest a role for many of the binding sites present on surface and a detailed explanation was reported [42-44]. A similar phenomena and explanation was outlined for imipenem, which acts as an inhibitor of AmpC β-lactamase and at the same time it is resistant to hydrolysis by this enzyme [45].

The potent binding affinity of the resistant cephalosporins on various β-lactamases indicated, through their low docking scores (Table 3), the strong binding to these enzymes on certain binding sites located on their surfaces with very low level of binding energy. This is supported by the observation that many

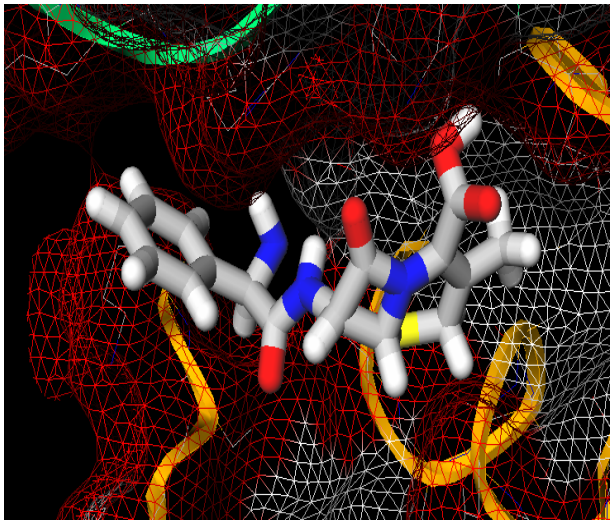


Fig. 8 Docking pose of cephalexin on 1ome.

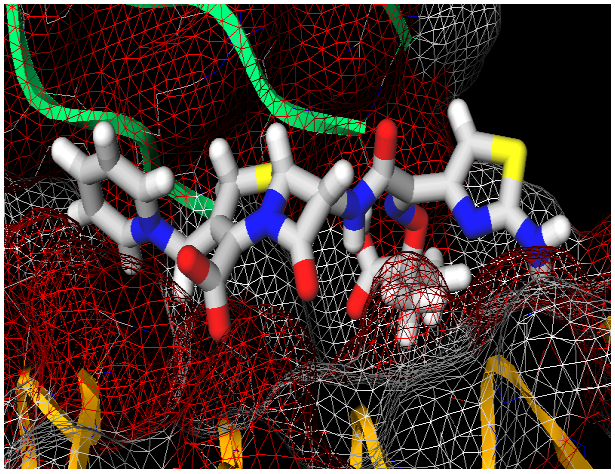


Fig. 9 Docking pose of ceftazidime on 1ome.

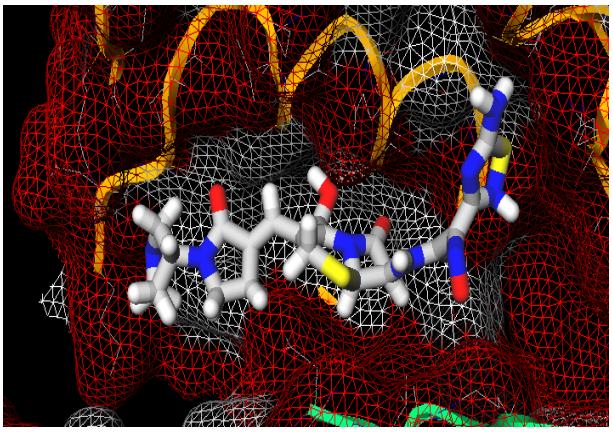


Fig. 10 Docking pose of ceftobiprole on 1ome.

binding sites were identified on β-lactamases, particularly AmpC β-lactamase and several water sites [44, 46]. There are well-defined positions in the binding sites that may accommodate ligand functional groups. The binding affinity of a cephalosporin with a β-lactamase may be a distinguishing factor in the evaluation of β-lactamase-resistance, and have an important role in the susceptibility to highly β-lactamase-resistant cephalosporins [47]. A similar phenomenon was also observed in this study, when ceftazidime and ceftobiprole were docked on β-lactamases, which showed that their β-lactam rings were not accommodated in the active site of β-lactamases to be hydrolyzed (Figs. 9 and 10), but projected away or flipped from the active site. The resistant cephalosporins were bound effectively to the binding sites of the enzyme, as noticed from the docking scores on β-lactamases (Table 3). The relative docking scores of all the investigated cephalosporins were illustrated on a histogram (Fig. 11) showing the main differences in activity and binding affinity to β-lactamases.

3.6 Validity of the Docking Study on PBPs and β-Lactamases

The application of this molecular docking approach on PBPs and β-lactamases was validated for its reliability as an important source of database screening, prediction and selection of the most potent cephalosporin. Three different methods of information were used for the validation of this approach. The first is based on comparism of the docking scores of the reference cephalosporins selected from different generations of various activities and stabilities. The results have indicated that there was a noticeable decline in the docking scores of the reference cephalosporins as advancing in generations towards the fifth generation. Cephalexin recorded high docking score, while ceftobiprole showed the lowest docking scores. It is well-known that ceftobiprole is the most active with broader spectrum, as indicated from its very low MIC values against various microbes [30]. The second method has included the experimental data of the antibacterial activity of the investigated cephalosporins [24], which have indicated that they

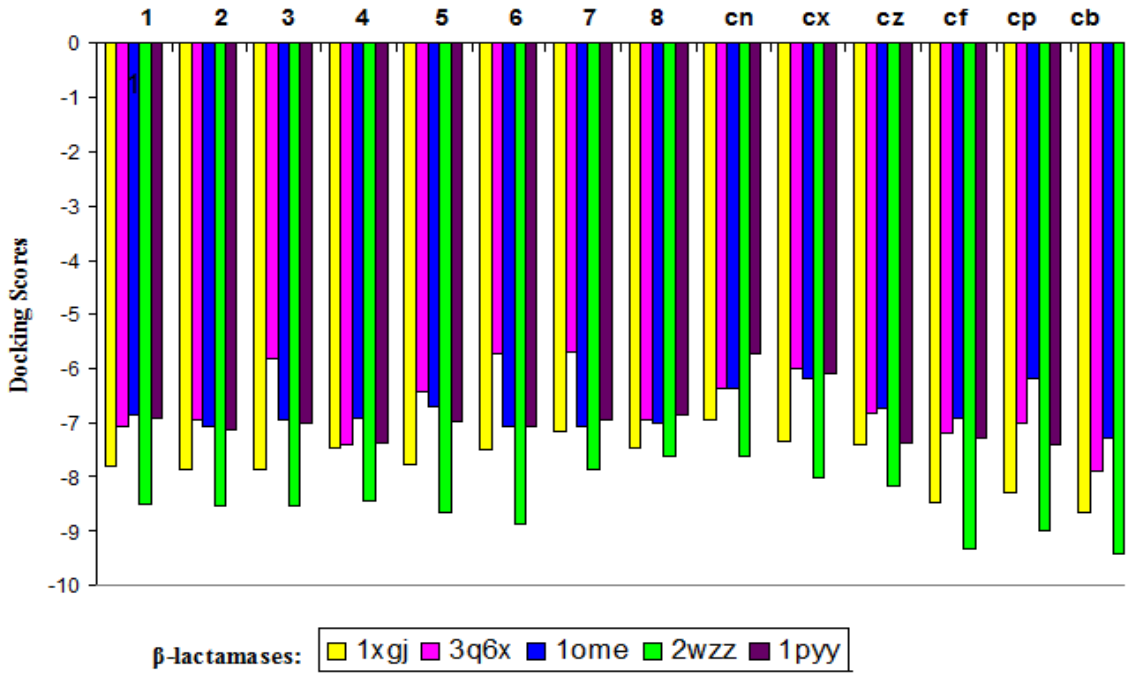


Fig. 11 Histogram of the docking scores of the investigated cephalosporins.

Keynote: Synthesized cephalosporins (1-8), Cephalexin (cn), Cefuroxime (cx), Ceftazidime (cz), Ceftriaxone (cf), Cefozopran (cp) and Ceftobiprole (cb).

comply with the docking scores. The third method is based on the results of the docking study on β-lactamases, which predicted the possible stability of the newer cephalosporins based on docking scores and positioning on surface of β-lactamases. The docking scores of the susceptible cephalosporins, such as cephalexin and cefuroxime were much higher than those of the resistant cephalosporins. The resistant cephalosporins have shown very low docking scores and the lowest was recorded for ceftobiprole. This indicates that these cephalosporins strongly bound to β-lactamases, but are not hydrolyzed. This comparison is a relative study based on docking scores of the resistant and the more potent cephalosporins with the newly synthesized cephalosporins. It is presumed that there is no limitation for such study, as the molecular docking approach can be applied to all types of antibacterial agents that perform their action through affinity binding to PBPs to predict their bioactivity in comparison with the most potent cephalosporins.

5. Conclusion

The application of molinspiration calculations and molecular docking studies of cephalosporins on β-lactamases and PBPs is considered very useful and practical approach to calculate the molecular properties and predict the bioactivity scores against certain microbes. Molecular docking of the proposed cephalosporins on PBPs could be a reliable and rapid approach for determining the bioactivity scores and thereby selecting the most potent cephalosporin. This approach is also very useful to predict the stability and selectivity of the investigational cephalosporins against β-lactamases of various origins against certain pathogenic microbes including *P. auroginos*a and / or MRSA. The prediction of docking scores and consequently, the degree of stabilities are recorded as relative values in comparison with reference cephalosporins. Therefore, this method is suggested as a very useful new approach that could be used prior the chemical synthesis of newer cephalosporins.

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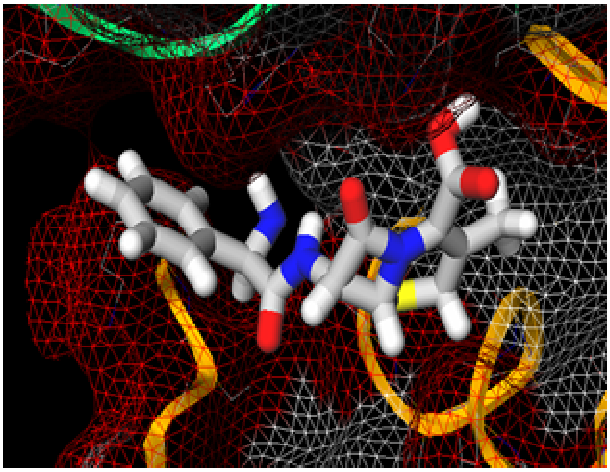
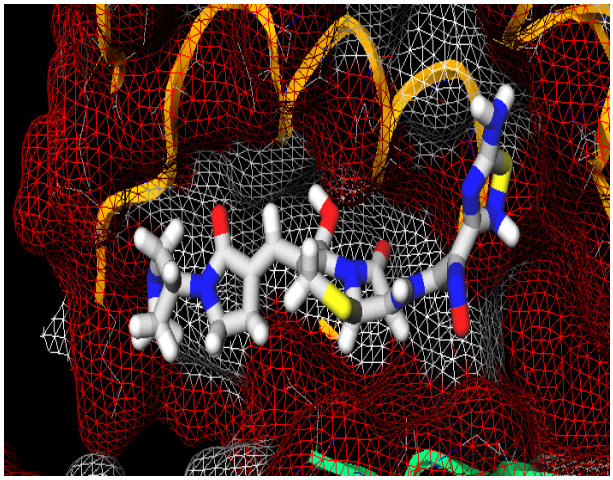
Graphical Abstract

A new approach of using molinspiration calculations and molecular docking on PBPs (penicillin-binding proteins) and certain β-lactamases is employed to predict the activity and resistance of cephalosporins. Cephalosporins are subjected to extensive evaluations on the bases of Lipinski's rule, bioactivity prediction by molinspiration and docking study on PBPs. Docking study on β-lactamases of *E. coli, K. pneumonia, S. aureus* and *P. auroginosa* is also conducted. Docking studies on PBPs and β-lactamases are considered as very useful, reliable and practical for calculating molecular properties, bioactivity scores against certain pathogens, such as *P. auroginosa* and MRSA and to determine the stability of the newer cephalosporins against β-lactamases.



Docking pose of cephalexin on β-lactamase 1ome



Docking pose of ceftobiprole on β-lactamase 1ome