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Polypharmacological assessment of Amoxicillin and its analogues against the bacterial DNA gyrase B using molecular docking, DFT and molecular dynamics simulation

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ABSTRACT

There has been an increase in the emergence and spread of drug-resistant pathogens, leading to a steep incline in the cases of antimicrobial resistance. Due to this, there is an imperative need for the development and identification of new antimicrobials to combat this menace of antimicrobial resistance. But this need is not being completely fulfilled by conventional drug discovery focused on a one molecule-one target approach. Polypharmacology, i.e., designing a drug in a way that acts on multiple cellular or molecular targets, a new approach for the identification of antimicrobial compounds, has been gaining attention. DNA gyrase B is one of the critical proteins involved in DNA replication and cell division in *E. coli*. In this study, the polypharmacological effect of amoxicillin and its analogues was studied on the DNA gyrase B and various other proteins of *E. coli*, using multiple in silico approaches like molecular docking, structural similarity, DFT, and molecular dynamics simulation. Both amoxicillin and its analogue, Cefaclor, tend to disrupt bacterial cell wall synthesis, but this study, based on in silico analysis, suggests a probable additional mode of action involving DNA gyrase B of *E. coli* which can be further explored to design novel dual-target inhibitors.

1. Introduction

The increasing use of anti-bacterial agents such as beta-lactams, macrolides, vancomycin, or quinolones has resulted in the emergence of multi-drug resistant pathogens, especially gram-positive bacteria (Shiroya and Patel, 2013). Widespread emergence of bacterial resistance to current drugs represents a serious problem in treatment of bacterial infections. Modern approaches towards the development of new potential inhibitors are based on the knowledge of structure and function of proteins specific to bacteria. One of these proteins is DNA gyrase, an essential bacterial enzyme, the inactivation of which leads to bacterial death. For this reason, gyrases have been chosen as targets for anti-bacterial agents (Li et al., 2015). DNA-gyrase has drawn much attention as a selected target for development and identification of potent anti-bacterial agents against multi-drug resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and penicillin-resistant *S. pneumoniae* (PRSP). DNA-gyrase, a typical type-II topoisomerase,

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Table 1
Selected target proteins and the corresponding CB-DOCK score of Amoxicillin docked on them.

S. No.	Target Name and Organism	PDB ID	CB-DOCK Score (kcal/mol)
1.	DNA gyrase B (*E. coli*)	3G7E	−7.0
2.	Protease DegS (*E. coli*)	3LGI	−6.9
3.	Protease DegQ (*E. coli*)	3STJ	−6.7
4.	Cell-division protein ZIPA (*E. coli*)	1F46	−6.4

has been known to be involved in DNA replication, transcription, and recombination processes (Shiroya and Patel, 2013).

Recent knowledge and modern advancements in tools and techniques have enabled the identification of polypharmacological activities of currently used antibiotics and other drugs (Miethke et al., 2021; Takeuchi et al., 2023). Recently, Wetzel et al., have shown the polypharmacological effects of a group of FDA approved antibiotics against different bacterial target proteins (Wetzel et al., 2021). In this study, we attempted to determine the polypharmacological effect of amoxicillin (which originally acts on Penicillin-Binding Proteins) (Nagai et al., 2002), on different proteins of *E. coli*, out of which DNA Gyrase B was identified as the most suitable target.

Amoxicillin is a moderate-spectrum, semisynthetic β-lactam compound, active against a wide range of Gram-positive and a limited range of Gram-negative bacteria. It is one of the most utilized drugs in this class because its oral absorption is better when compared with other β-lactam antibiotics (Salvo et al., 2009). However, during the past decades, an increasing number of bacteria have become resistant to routinely used antibiotics, making bacterial resistance one of the major global public health concerns. β-Lactamase production is one of the most common mechanisms of bacterial resistance; these enzymes, that cleave the β-lactam ring, are produced by several Gram-positive bacteria (including *Staphylococci* spp.), Gram-negative bacteria (*Escherichia coli, Neisseria gonorrhoeae, Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas and Klebsiella* spp.), and anaerobic bacteria (*Bacteroides* spp.) (Wilke et al., 2005; Lima et al., 2020; Tooke et al., 2019; Abeylath and Turos, 2008). To overcome this problem, in the 1970s, a new area of research was focused on identifying compounds able to inhibit β-lactamase, and in 1972 clavulanic acid (CA) was identified. CA is structurally related to penicillin; it prevents inactivation of antibiotics, thus increasing their effectiveness when combined. The association of amoxicillin/clavulanate (AMC) was first marketed in 1981, and it is the only penicillin combined with a β-lactamase inhibitor available in oral formulation (Salvo et al., 2009).

Amoxicillin is an antibacterial prescription medicine approved by the U.S. Food and Drug Administration (FDA) on 18th January 1974 (https://pubchem.ncbi.nlm.nih.gov/compound/33613) for the treatment of certain bacterial infections, such as community-acquired pneumonia; infections of the ear, nose and throat; infections of the

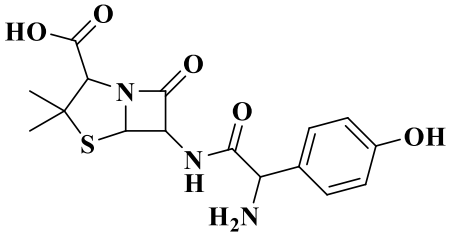


Fig. 2. Structure of user compound (Amoxicillin) (Drug Bank ID: DB01060).

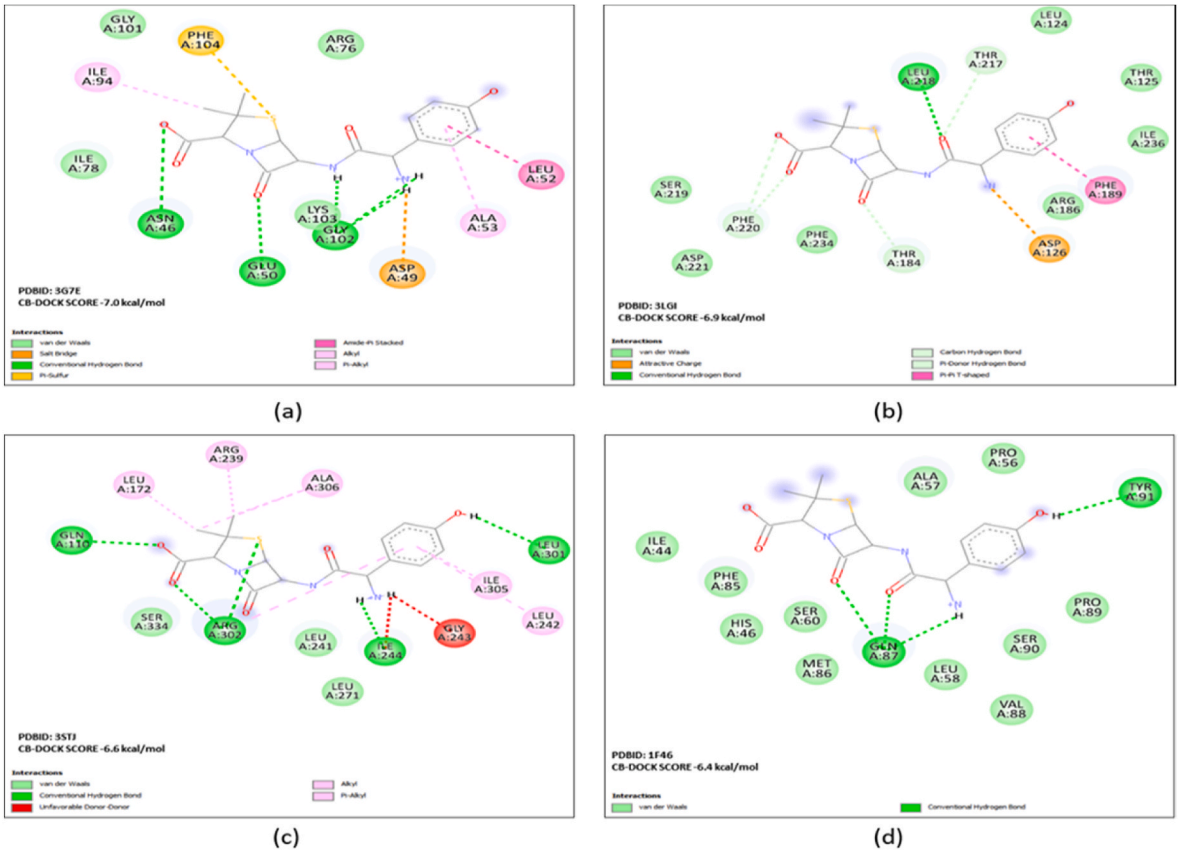
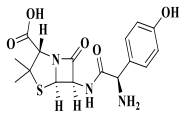
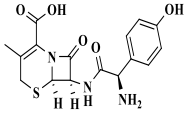
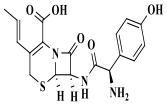
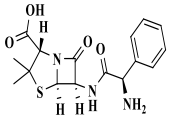
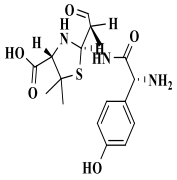
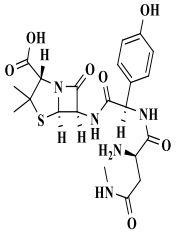
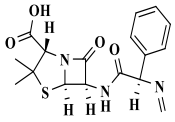
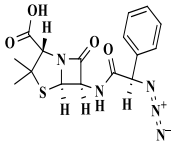
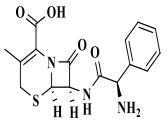
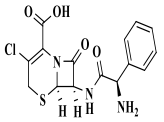
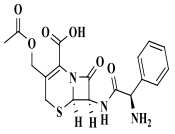


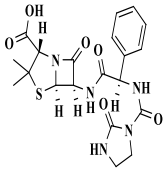
Fig. 1. The 2D interactions of Amoxicillin docked on the *E. coli* target proteins (a) DNA gyrase B, (b) Protease degS, (c) Protease degQ and (d) Cell-division protein ZIPA.

Table 2
Selected Amoxicillin analogues from drug bank database using the Swiss Similarity web tool.

Druk Bank ID	Similarity Score	Name	Structure	MW	Type	Group
DB01060	1.000	Amoxicillin		365.404	Small Molecule	Approved, Vet approved
DB01140	0.822	Cefadroxil		363.388	Small Molecule	Approved, Vet approved, Withdrawn
DB0110	0.822	Cefprozil		389.426	Small Molecule	Approved
DB00415	0.610	Ampicillin		349.405	Small Molecule	Approved, Vet approved
DB03658	0.569	(2R,4S)-2-[(1R)-1-{[(2R)-2-Amino-2-(4-hydroxyphenyl) acetyl]amino}-2-oxoethyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid		367.42	Small Molecule	Experimental
DB13816	0.566	Aspoxicillin		493.54	Small Molecule	Experimental
DB13836	0.524	Metampicillin		361.42	Small Molecule	Experimental
DB08795	0.524	Azidocillin		375.402	Small Molecule	Experimental
DB00567	0.518	Cephalexin		347.389	Small Molecule	Approved, Investigational, Vet approved
DB00833	0.518	Cefaclor		367.807	Small Molecule	Approved
DB00689	0.509	Cephaloglycin		405.425	Small Molecule	Approved

(*continued on next page*)

Table 2 (*continued*)

Druk Bank ID	Similarity Score	Name	Structure	MW	Type	Group
DB01061	0.507	Azlocillin		461.492	Small Molecule	Approved

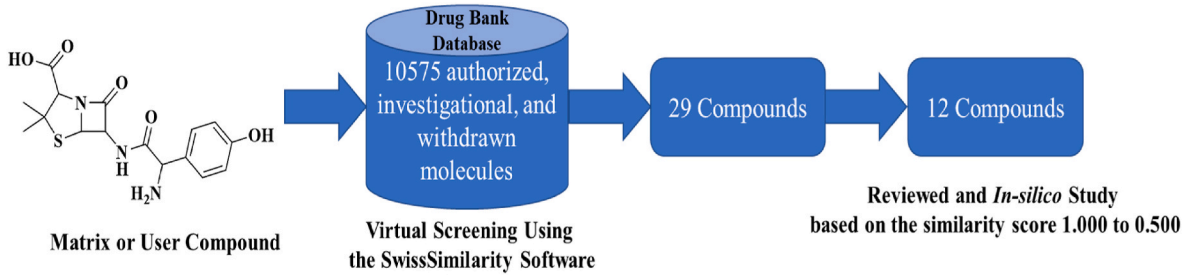


Fig. 3. The procedure of amoxicillin analogues selection method.

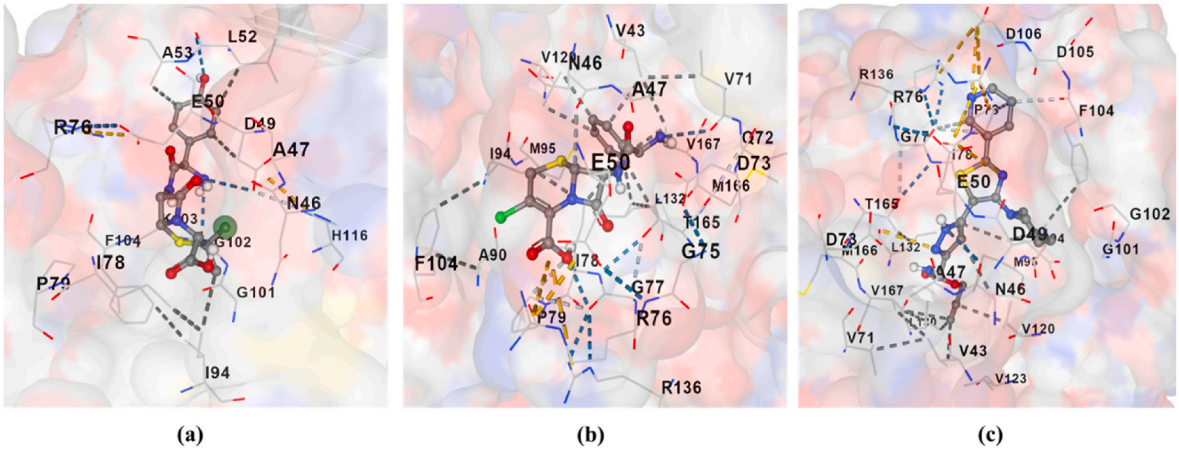


Fig. 4. Molecular docking interaction results of DNA gyrase B (PDB ID: 3G7E) with the ligands, (a) Amoxicillin, (b) Cefaclor and (c) co-crystal ligand B46 using the CB-Dock Software.

Table 3
Molecular docking results and interactions of Amoxicillin and Cefaclor with the *E. coli* DNA gyrase B (PDB ID: 3G7E) using the CB-Dock Software.

Drug Bank ID	Cavity Score	AutoDock Vina Score (Kcal/mol)	No. Of H–B	Bound Amino Acid Residues
DB01060 (Amoxicillin)	945	−7.0	6	Asp73, Gly75, Thr165, Glu50, Gly102, Gly77 (H–B), Ile78, Pro79, Ile94, Lys103, Asn46, Glu50 (C–H), Arg76 (ionic)
DB00833 (Cefaclor)	945	−8.4	6	Asp73, Thr165, Val71, Glu50, Gly77, Asn46 (H–B), Asn46, Val120, Ile94, Ala90, Ile78, Leu132, Thr165, Gly77, Val143 (C–H), Arg76, Arg136 (ionic)

genitourinary tract and infections of the skin and respiratory tract (Lipsky, 2005). The association of amoxicillin/clavulanate (AMC) was first marketed in 1981, and it is the only penicillin combined with a β-lactamase inhibitor available in oral formulation (Salvo et al., 2009). According to Durgadasheemi and Kolageri (2023), Amoxicillin showed the binding affinity value of −7.11 kcal/mol with the DNA gyrase B (PDB ID: 5D7R) and exhibited interactions with Arg84, Ser55, Glu58, Ile86 and Ile179 residues. Moreover, Amoxicillin showed the binding affinity of −6.7 kcal/mol with the DNA gyrase A and interacted with Asp576, Ile578 and Arg612 as H–B (Philip et al., 2023). Furthermore, Amoxicillin showed anti-bacterial activity against gram positive bacteria (*S. Pneumoniae*, *S. epidermidis* and *S. aureus*) with the IC_{50} values of 7.8 ± 0.25, 15.6 ± 0.20 and 15.6 ± 0.01 μg/mL as well as against gram negative bacteria (*E. coli*, *K. pneumoniae* and *S. paratyphi*) with the IC_{50} values of 0.01 ± 15.6, 7.8 ± 0.25 and 15.6 ± 0.30 μg/mL (Fayed et al., 2021).

Drug Bank is a comprehensive, freely available web resource containing detailed drug, drug-target, drug action and drug interaction information about FDA-approved drugs as well as experimental drugs going through the FDA approval process. The rich, high quality,

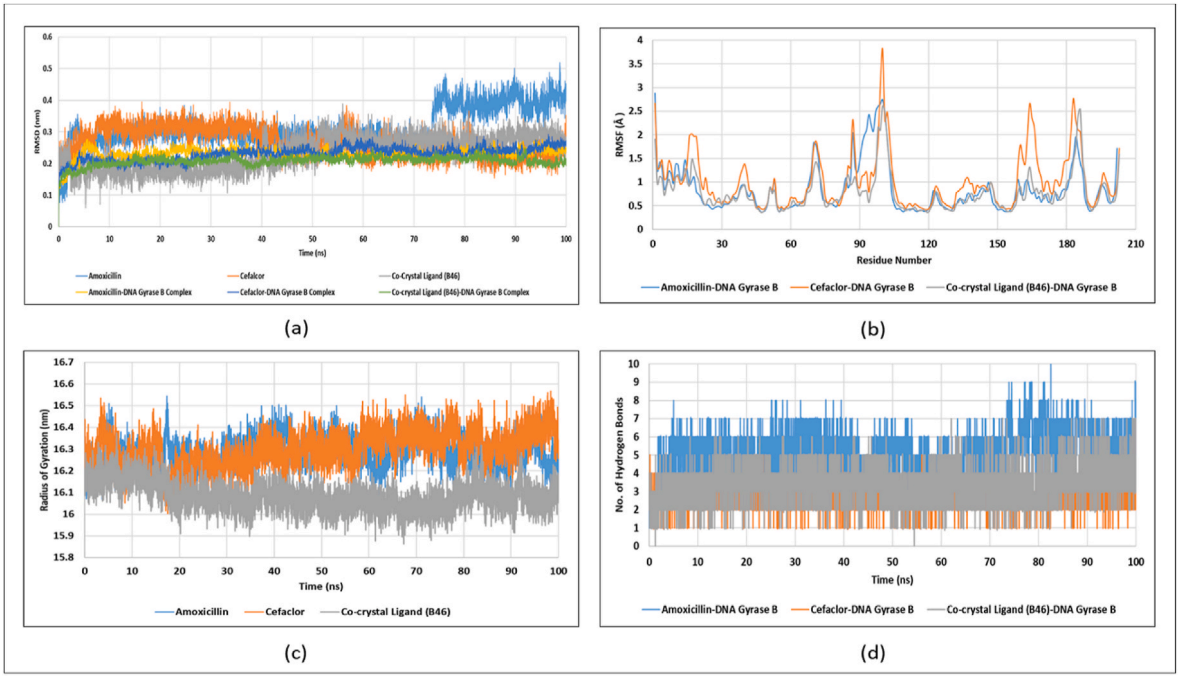


Fig. 5. The analysis of the molecular dynamics simulation trajectories of *E. coli* DNA gyrase B in complex with Amoxicillin, cefaclor and co-crystal ligand B46 respectively. (a) The RMSD analysis of ligands and protein-ligand complexes, (b) The RMSF analysis of ligand bound DNA gyrase B, (c) The change in radius of gyration of DNA gyrase B in complex with the ligands and (d) The number of hydrogen bonds formed between DNA gyrase B and the corresponding ligands.

primary-sourced content found in Drug Bank has allowed it to become one of the world's most widely used reference drug resources. The public, educators, pharmacists, pharmacologists, medicinal chemists, pharmaceutical researchers and the pharmaceutical industry (Wishart et al., 2018) routinely use it.

In the present study, Amoxicillin (DB01060) was used as the user or matrix compound for screening the drug bank database for the discovery of anti-bacterial drug against DNA gyrase B using the Swiss Similarity software. Similar compounds with the similarity score of 1.000 to 0.500 were selected for further in-silico studies such as docking, molecular dynamics simulation and density functional theory study. The SwissSimilarity programme was used to gather twelve (12) compounds from the drug bank database and perform in-silico docking against DNA gyrase B. To clarify the stability of dockedcefaclor-DNA gyrase B and amoxicillin-DNA gyrase B complexes, MD simulation was carried out. In order to determine the stability and reactivity of chemical species for the lead compound cefaclor, a DFT investigation was lastly conducted.

2. Methodology

2.1. Selection and structure preparation of target proteins

The crystal structures of *E. coli* target proteins DNA Gyrase B (PDB ID: 3G7E), Protease DegS (PDB ID: 3LGI), Protease DegQ (PDB ID: 3STJ) and cell-division protein ZIPA (PDB ID: 1F46) with their corresponding PDB IDs were retrieved from the RCSB Protein Data Bank. The proteins were processed by removing co-crystal waters, ions, and heteroatoms. Polar hydrogens were added using Biovia Discovery Studio Visualizer (Zaelani et al., 2021) and saved the structures in .pdb format.

2.2. Preparation of Amoxicillin structure and selection of its analogues

The 2D structure of Amoxicillin was drawn and then converted into the SMILES format using ChemDraw Professional 16.0. The amoxicillin molecule was used to search for new similar molecules using Swiss Similarity web tool (Anukanon and Teerapattarakarn, 2022) to search the similar compounds from the drug bank database for the discovery of anti-bacterial agents against DNA gyrase B.

In this study, the compounds with the similarity scores 1.000 to 0.500 were chosen, and these amoxicillin analogues were then subjected to docking simulation in CB-Dock (https://cadd.labshare.cn/cb-dock/php/index.php) to determine their affinity for binding and interactions with the target protein's amino acid residues.

2.3. Molecular docking of Amoxicillin onto the selected target proteins of *E. coli*

The protein-ligand docking web tool CB-Dock (Cavity Detection Blind Docking) was used to dock amoxicillin into the active site of the selected target proteins (Roney et al., 2023; Roney et al., 2023). This method uses AutoDock Vina to carry out molecular docking and automatically detects the binding sites, determines the center and size, modifies the docking box size based on the query ligands (Liu et al., 2020). Using this method, several top cavities were automatically chosen for additional study (cavity sorting), and molecular docking was carried out at each one. The ideal binding posture for the query ligand is thought to be in the first conformation, and the matching position is thought to be the best binding site. Similarly, CB-DOCK was used to dock the analogues of Amoxicillin onto DNA gyrase B using default parameters.

2.4. MD simulation and binding free-energy calculations

All-atom MD simulations were used to assess the stability of the compounds docked in DNA gyrase B's active region. The topology of DNA gyrase B and the screened ligands were each constructed using AMBER99SB and GAFF forcefields, whilst ligand charge calculations were performed using the bcc technique (Roney et al., 2023). The protein-ligand complexes were solvated in TIP3P water-filled cubes, and the system was subsequently neutralized by adding enough Na^+ and Cl^- ions. The two periodic pictures were kept apart by a spacing of 2 nm. The systems were subjected to a 2000-step steepest descent energy minimization process, followed by a 100-step conjugate gradient energy minimization, and then they achieved equilibrium for 1 ns under NVT

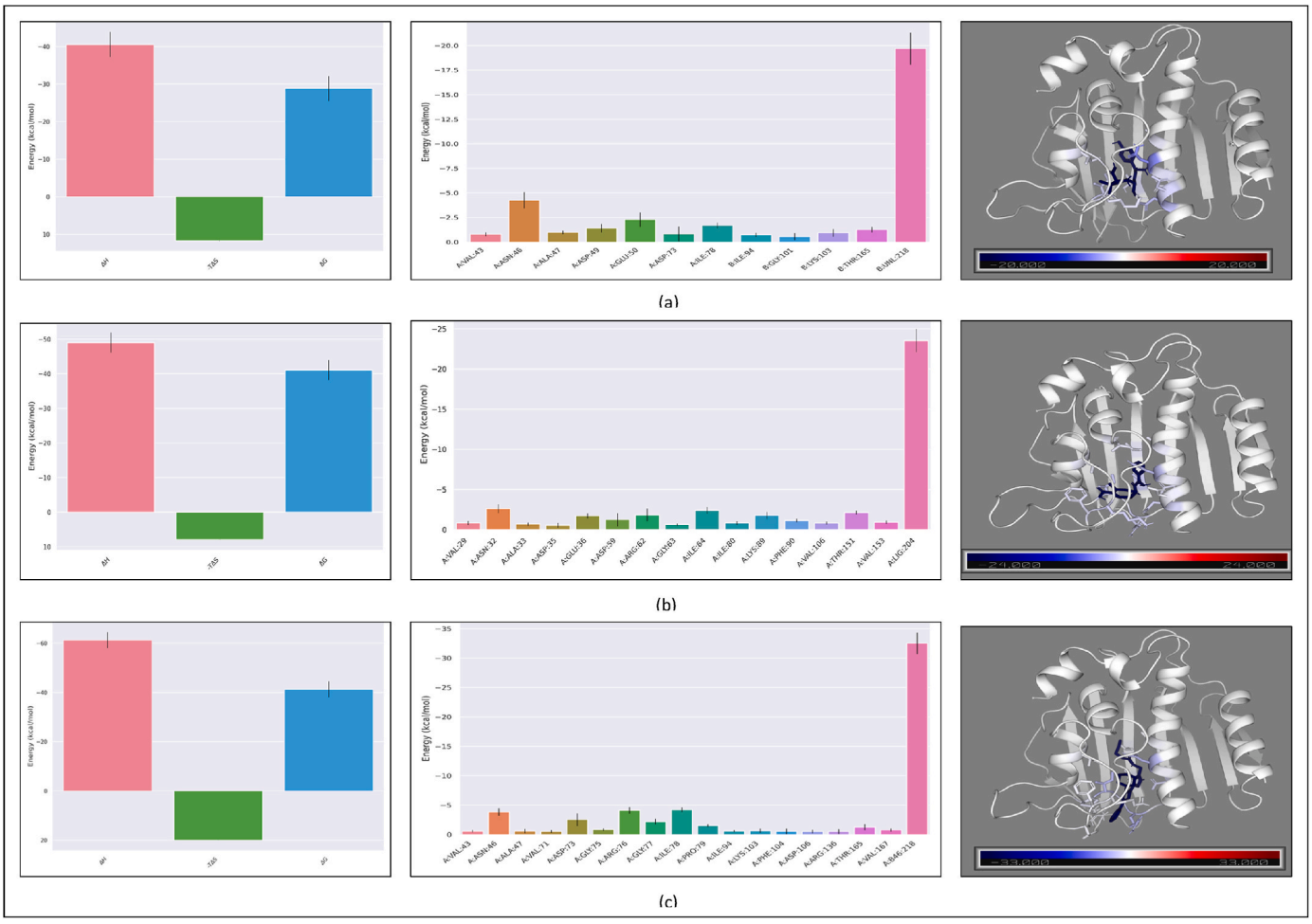


Fig. 6. The total binding free-energy values, per-residue contribution to the binding energy and the corresponding 3D interaction depiction of DNA gyrase B with the ligands, (a) Amoxicillin, (b) Cefaclor and (c) co-crystal ligand B46.

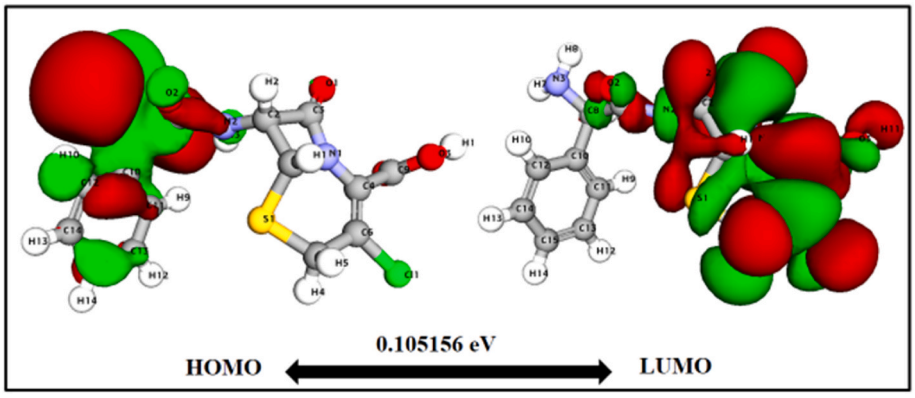


Fig. 7. Cefaclor HOMO-LUMO Map with energy gap.

and NPT ensembles at 310.15 K and 1 bar using modified Berendsen thermostat and Parrinello-Rahman barostat, respectively. Following this, 100-ns production runs at the same temperature and pressure were performed on the equilibrated systems. The frames were changed every 1 ps, with a 2-fs time-step appended. The study of the trajectory was carried out using standard GROMACS methods, and the trajectory visualisation was done using PyMol.

The gmx_MMPBSA v.1.5.2 tool (Bhardwaj et al., 2020) was used to calculate the end-state MMGBSA binding free energy. With an internal and external dielectric constant of 1 and 80, respectively, all 10,000 frames were collected for the calculations of binding free energy, and iGB model 2 was chosen for the analyses. For the previous 250 frames, the interaction entropy was determined. The binding free energy is defined by Eq. (1):

$$\Delta G_{gas} + \Delta G_{solv} = \Delta G_{total} \quad (1)$$

Where ΔG_{gas} constitutes the gas phase binding energy contributions, including electrostatic and van der Waals interactions, ΔG_{solv} marks the

Table 4
Density Function Theory calculations with other descriptors.

Name	Cefaclor
Total energy	−1892.02
Binding Energy	−7.465
HOMO Energy	−0.193663
LUMO Energy	−0.0885073
Band Gap Energy	0.105156
Dipole moment	1.23097
Hardness	0.052578
Softness	19.01936171022100498307 27
Electronegativity	0.14108515
Electrophilicity	0.0005232

solvation free energy contribution, and ΔG_{total} represents the total binding free energy.

2.5. Density functional theory (DFT) calculations

DFT, one of the best methods for analyzing the stability and reactivity of chemical species, was used for computational chemistry calculations. The Gaussian 09 software and the Gauss View 5 program were used to sketch the input molecules for the DFT quantum computations for the Cefaclor (DB00833) drug ground state, electrical, and reactive characteristics. Fig. 7 shows a 3D representation of the optimal molecular structure of the current molecule as determined by DFT using the B3LYP/6-311++G (d, p) basis set. The Lee-Yang-Parr gradient corrected correlation functional (LYP) (Ditchfield et al., 1971) and the three-parameter functional (B3) (Becke, 2003) for the exchange component and Becke's (Becke, 1993; Lee et al., 1988). A B3LYP/6-311++G (d, p) (Ditchfield et al., 1971) polarized base was utilized to create a safe basis set. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) are computed to get the surfaces of the frontier molecular orbitals (FMO). The calculation of the vibrational and statistical thermodynamic analysis at B3LYP/6-311G (d, p) computational level, which was the same condition for the fundamental vibrational frequencies and optimization calculations, yielded the thermodynamic data, internal energy (U), enthalpy (H), entropy (S), and Gibbs free energy (G) (Grimme, 2012). The energy values of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) were used to compute the descriptors of Cefaclor (Kumar et al., 2015; Pereira et al., 2016). The ionization energy (I) and electron affinity (A) of a compound reacted to the energy of the HOMO orbital and the energy of the LUMO orbital, respectively, in accordance with the DFT theorem put forth by Koopmans (1934) and other parameters (Bharadwaj et al., 2021a,b; Bharadwaj et al., 2021a,b; Deswal et al., 2022; Kumar et al., 2023). Calculations of molecular characteristics including chemical hardness, softness, electronegativity, and electrophilicity index were done using the HOMO and LUMO energies. Mulliken's Hirshfeld, ESP, and atom charges have all been estimated. Certain atomic charge patterns may be revealed via Mulliken's DFT atomic charges.

2.6. Molecular electrostatic potential (MESP) calculations

The optimized structure of Cefaclor was used for molecular electrostatic potential calculations and the energies of the compound using the Gaussian 09 software package and the density functional theory at the B3LYP/6-31G (d, p) level of theory (Abu-Melha, 2018).

3. Results and discussion

3.1. Selection of best identified target protein of Amoxicillin

According to the CB-DOCK score (Table 1) and the interactions of Amoxicillin with the respective target proteins (Fig. 1), Amoxicillin showed highest affinity with the active site of DNA gyrase B (−7.0 kcal/mol) followed by Protease DegS (−6.9 kcal/mol), Protease DegQ (−6.7 kcal/mol) and cell division protein ZIPA (−6.4 kcal/mol) (Table 1). Based on these values, DNA gyrase B was selected as suitable target protein amongst the list of target proteins in consideration and further screening and docking of Amoxicillin analogues were carried out on *E. coli* DNA gyrase B.

3.2. Selection and preparation of Amoxicillin analogues

According to the ligand-based virtual screening theory, similar molecules are likely to have similar biological activity, which is the foundation of Swiss Similarity software. With the use of this technology, vast drug libraries can be quickly screened in more than 30 chemical databases, which contain more than 205 million virtual compounds that can be synthesized using readily available reagents (Zoete et al., 2016). The Swiss Similarity web tool produced four kinds of compounds (more than 48486099 compounds) to put it succinctly. Drugs (about 13935 molecules), Bioactive (roughly 3464715 compounds from multiple databases), Commercials (more than 24923383 compounds from various databases), and Synthesizable (nearly 24923383 compounds from various databases). This technology has been utilized in in-silico drug planning investigations (Majumder and Mandal, 2022).

Firstly, we chose Amoxicillin (Drug Bank ID: DB01060) (Fig. 2), which is Food and Drug Administration (FDA) agency approved on 18 January 1974 ("Amoxil: FDA-Approved Drugs". U.S. Food and Drug Administration (FDA). Retrieved 8 October 2022) as an antibacterial drug for the treatment of infections caused by Gram-positive bacteria, in particular Streptococcal bacteria causing upper respiratory tract infections (Holten and Onusko, 2000; Woodnutt and Berry, 1999). This substance was used in the current study as a matrix or user compound.

The Swiss Similarity web tool (http://www.swisssimilarity.ch/) was then used to look for substances that were structurally related to amoxicillin. In this study, the Drug Bank (10575 authorized,

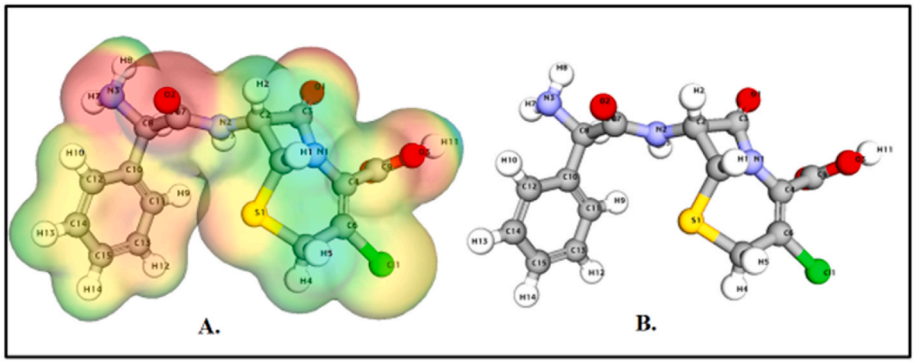


Fig. 8. A. Molecular electrostatic potential of Cefaclor and B. Optimized structure of Cefaclor.

investigational, and withdrawn molecules) database was screened using the pharmacophore recognition of the user compound. Twenty-nine (29) compounds were looked up based on the virtual screening using the pharmacophore recognition of the Drug Bank database with a similarity score in the range of 1.000–0.353 (Table 2). Moreover, in this work, the compounds that had a similarity score of more than 0.500 were selected and these amoxicillin analogues were reviewed based on the mechanism of action and then submitted into the CB-Dock software to compute their binding interaction and energy. As we can see in Table 2, Twelve (12) compounds based on the similarity score (1.000–0.500) were structurally similar to the user compound, which contains 4 compounds in approved; 2 compounds in approved and vet approved; 1 compound in approved, vet approved and withdrawn; 1 compound in approved, investigational and vet approved; 4 compounds in experimental category. The compounds selection method is shown in Fig. 3.

3.2.1. Approved

Cefaclor, like penicillin, is a β-lactam antibiotic. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, it inhibits the third and last stage of bacterial cell wall synthesis (Petri, 2006). Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins. It is possible that cefaclor interferes with an autolysin inhibitor (Ethiraj et al., 2019). The bactericidal activity of cephaloglycin results from the inhibition of cell wall synthesis via affinity for penicillin-binding proteins (PBPs) (Waxman and Strominger, 1983; Frère and Page, 2014). By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, azlocillin inhibits the third and last stage of bacterial cell wall synthesis (Sharan and Carlson, 2022). Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that azlocillin interferes with an autolysin inhibitor (Karpova, 2018).

3.2.2. Approved and vet approved

Amoxicillin competitively inhibits penicillin-binding protein 1 and other high molecular weight penicillin binding proteins (Nagai et al., 2002). Penicillin bind proteins are responsible for glycosyltransferase and transpeptidase reactions that lead to cross-linking of D-alanine and D-aspartic acid in bacterial cell walls (Hancock et al., 2014), without the action of penicillin binding proteins, bacteria upregulate autolytic enzymes and are unable to build and repair the cell wall, leading to bacteriocidal action (Cho et al., 2014; Tomasz, 1979). By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, Ampicillin inhibits the third and last stage of bacterial cell wall synthesis (Leclercq et al., 2017). Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Ampicillin interferes with an autolysin inhibitor (Ginsburg, 2002).

3.2.3. Approved, vet approved and withdrawn

Like all beta-lactam antibiotics, cefadroxil binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefadroxil interferes with an autolysin inhibitor (Jain et al., 2018).

3.2.4. Approved, investigational and vet approved

Cephalexin is a first-generation cephalosporin antibiotic (Nowakowski et al., 2000). Cephalosporins contain a beta lactam and dihydrothiazide (O'Callaghan et al., 1972). Unlike penicillins, cephalosprins are more resistant to the action of beta lactamase. Cephalexin inhibits bacterial cell wall synthesis, leading breakdown and eventually cell death (Petri, 2006; Spratt, 1978).

3.2.5. Experimental

(2R,4S)-2-[(1R)-1-{[(2R)-2-Amino-2-(4-hydroxyphenyl)acetyl] amino}-2-oxoethyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid is β-lactamase inhibitor of *Escherichia coli* (strain K12). Aspoxicillin is a broad-spectrum, semisynthetic penicillin derivative with antibacterial activity. Aspoxicillin binds to and inactivates penicillin-binding proteins (PBPs) located on the inner membrane of the bacterial cell wall. Inactivation of PBPs interferes with the cross-linkage of peptidoglycan chains necessary for bacterial cell wall strength and rigidity. This interrupts bacterial cell wall synthesis and results in the weakening of the bacterial cell wall, eventually causing cell lysis (Scheinin et al., 1994). Metampicillin is a penicillin antibiotic prepared by the reaction of ampicillin with formaldehyde. It is hydrolysed in aqueous solution to form ampicillin. Hydrolysis is rapid under acid conditions like the stomach (Bush, 2010). By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, Azidocillin inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Azidocillin interferes with an autolysin inhibitor (Sahoo et al., 2014).

3.3. Molecular docking of Amoxicillin and its analogues onto the DNA gyrase B

To investigate potential binding interactions and compare the binding patterns of the reference compound (Co-crystal), molecular docking analysis of the chosen compounds into the active site of DNA gyrase B was conducted using the CD-Dock software. Molecular docking of Amoxicillin and its analogues onto the DNA gyrase B showed that, although all molecules have properties that result in comparable energetically favorable interactions (−7.0 kcal/mol to −8.9 kcal/mol) (Table S1) in the active binding region, Cefaclor showed the better binding energy. The control compound (Co-crystal) has a binding energy of −9.7 kcal/mol and exhibited hydrogen bonds with Glu50, Asn46, Gly77, Phe104 and Thr165 residues as well as hydrophobic interactions with the residues Val43, Val167, Leu130, Val120, Leu132, Asn46, Ile78, Ile94 and Pro79 (Fig. 4c; Table 3).

Amoxicillin formed hydrogen bonds with the amino acid residues Asp73, Gly75, Thr165, Glu50, Gly102 and Gly77 on active site of DNA gyrase B, with a binding energy of −7.0 kcal/mol, Ile78, Pro79, Ile94, Lys103, Asn46 and Glu50 residues are all participated in additional hydrophobic interactions (Fig. 4a; Table 3). Cefaclor established hydrogen bonds with Asp73, Thr165, Val71, Glu50, Gly77 and Asn46 residues in the active site of DNA gyrase B with the comparable predicted binding energy of (8.4 kcal/mol. The good binding energy of Cefaclor appeared to be influenced by additional interactions with the hydrophobic residues of Asn46, Val120, Ile94, Ala90, Ile78, Leu132, Thr165, Gly77 and Val143 (Fig. 4b; Table 3).

These findings suggest that the Cefaclor was successfully bound within the binding pocket of DNA gyrase B, and the optimum binding pattern was found. Cefaclor demonstrated strong quantitative docking findings when compared to the control medication when docking with DNA gyrase B. A thorough study of the data revealed several interactions between the surrounding residues and the ligand, including hydrogen bonds, hydrophobic contacts, ionic, and Pi interactions. Furthermore, Cefaclor produced more hydrogen bonds and hydrophobic interactions with the target protein, which may have contributed to their increased binding affinities compared to the control medication. Cefaclor was likely promiscuous and might be possible lead against DNA gyrase B, according to the considerable docking score on the corresponding target.

3.4. Molecular dynamics simulation results

The explicit molecular dynamics simulation takes into consideration the flexible nature of the protein and ligand complex as well as the effect of hydration on the stability and interaction of the complex. The assessment of the critical interactions of the active site residues of DNA gyrase B with Amoxicillin, Cefaclor and co-crystal ligand (B46) along

with the overall stability of these complexes were monitored as shown in Figs. 5 and 6. From the RMSD analysis (Fig. 5a), the RMSD of Amoxicillin remained up to 3.2 Å till 75ns and reached 5 Å by the last quarter of the trajectory. Cefaclor showed RMSD up to 3.5 Å till 45 ns and then showed reduction in RMSD till 0.2 Å by the end of the trajectory. On the other hand, the co-crystal ligand (B46) showed an increase in RMSD till 42 ns and remained constant with RMSD up to 3.2 Å till the end of the trajectory. The overall RMSD of all the complexes remained well within 3 Å indicating the stable complexation over the length of the simulation. The RMSF (Fig. 5b) showed higher fluctuations in the loop encompassed by the residues 95-110 and β-sheet strand coded by the residues 160-172. Cefaclor bound DNA gyrase B showed comparatively higher fluctuations in the interactive residues sequence followed by Amoxicillin and co-crystal ligand (B46). In terms of radius of gyration (Fig. 5c), a slight compaction was observed in the structure of DNA gyrase B in complex with the co-crystal ligand B46 as compared to Amoxicillin and Cefaclor. For the interactions in terms of number of hydrogen bonds (Fig. 5d), Amoxicillin formed 7-10 hydrogen bonds while Cefaclor and B46 formed 3-5 and 5-7 hydrogen bonds with the interacting residues of DNA gyrase B respectively. The overall complexes reached the metastable binding states by the end of the simulation.

The relative end-state MMGBSA binding energy calculations were also estimated for the last 1000 frames of the trajectories between DNA gyrase B and ligands (Fig. 6). Cefaclor showed binding affinity (−41.08 ± 2.91 kcal/mol) similar to that of co-crystal ligand B46 (−41.24 ± 3.23 kcal/mol) while Amoxicillin showed slightly lower binding affinity (−28.83 ± 3.32 kcal/mol) with the active site residues of DNA gyrase B. All three ligands showed different binding conformations in the ATP binding site of DNA gyrase B showing interaction with multiple different residues and some overlapping residues as shown in Fig. 6a–c. Both Amoxicillin and B46 showed interaction with ATP binding residues (R46, D73 and K103) along with other residues lining the ATP binding site.

3.5. Density function theory (DFT) calculations

The Cefaclor (DB00833) drug determined reactivity descriptors are displayed in Table 4. A higher value of energy for the HOMO orbital indicates a reasonable ability of the chemical species to donate electronic density, while a lower value of energy for the LUMO orbital indicates a decent inclination to receive electronic density. This leads to a direct correlation between the frontier orbitals and chemical reactivity, with large values of the energy gap suggesting lower reactivity and short values suggesting higher reactivity. The energy gap (0.105156 gap), which is defined as the difference between the energies for the LUMO-HOMO orbitals (Fig. 7). According to Pearson's HSAB hypothesis (Zeroka et al., 1999; Zacharias et al., 2018), two chemicals interact well when both are hard or soft molecules (Pearson, 1990), hence Cefaclor is deemed soft because it has a low global hardness (eV) or high global softness (eV) value (0.4667 eV). In addition to the energy gap, there are other measurements known as reactivity descriptors that aid computational chemists in forecasting how one chemical species will behave in relation to another. The energy of the HOMO and LUMO orbitals was also used to compute chemical reactivity characteristics for the examined molecule, including chemical softness (S), chemical potential (m), electrophilicity index (u), and chemical hardness (h). The Cefaclor drug is a viable option for usage as a chelating agent due to its narrow energy gap, high softness, and low chemical hardness. The Cefaclor electrophilicity and electronegativity indices both have higher values. As a result, the Cefaclor molecule is more able to receive electrical density, making it an excellent electrophilic species.

3.6. Molecular electrostatic potential (MESP) calculations

The significance of MESP maps displays the color marking system's design, size, negative, positive, and neutral electrostatic potential zones. The graphic depicts the MESP of the Cefaclor. The blue color scheme area reflects the positive substantial electrostatic potential of the compounds (showing a notably electron-deficient region), whereas the red color scheme area shows the particles' greatest electronegative potential (indicating an electron-rich region). In the case of Cefaclor, highly negative points were localized at atoms O1, O2, O3, H7, H8 and N3 whereas the highly positive points were located throughout H1, H2, H4, H5, H11 and N1 atoms (Fig. 8).

The capacity of the Cefaclor to give electrons to the approaching electrophile is strongly related to the degree of negative Hirshfeld charge on it, indicating nucleophilicity, and electronic charges play an essential role in determining a Cefaclor bonding capacity. Mulliken charge values for the component atoms of the investigated Cefaclor is presented in (Table S2). The degree of negative Hirshfeld charge on the targeted Cefaclor, displaying nucleophilicity, strongly correlates with its ability to donate electrons to the approaching electrophile. The computed bond properties are shown in Table S3.

4. Conclusions

In the present study, we employed a combined structure-based and ligand-based approach to identify the polypharmacological effect of Amoxicillin on various target proteins of *E. coli* and screen out the potential analogues of Amoxicillin that might possess higher inhibitory activity compared to Amoxicillin and known co-crystallized inhibitor (B46) of *E. coli* DNA gyrase B. The study integrated molecular docking, structural similarity, DFT and molecular dynamics simulation-based validation of the binding interactions of Amoxicillin and its analogues on DNA gyrase B of *E. coli*. Amoxicillin and the best identified analogue Cefaclor are both known to interfere with bacterial cell-wall synthesis, however, our study proposes their probable polypharmacological mode of action by targeting bacterial DNA gyrases as well. Further in-vitro validations are required to confirm their polypharmacological behavior.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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