In Silico Design of Novel Quinolone Derivatives against Targeted Enzyme for its Anti-Microbial Activity

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Abstract:

Quinolone antibiotics are broad-spectrum antibiotics that work against Gram-positive and Gram-negative bacteria, as well as anaerobes and mycobacteria. This prevent bacterial DNA replication and transcription by prohibiting bacterial DNA from unwinding and duplicating, interfering with DNA replication, and resulting in cell death. *Staphylococcus aureus* Gyrase B is one of the most investigated and validated targets for the development of new antibacterial agents. Its absence in the mammalian organism and its crucial role in the bacterial DNA replication cycle make this enzyme a suitable target for the development of anti-bacterial agents. This study involves the designing of novel quinolone derivatives and study of its molecular properties to investigate the interaction between the designed derivatives and amino acid residues of *Staphylococcus aureus* Gyrase B using Auto Dock software. All the designed compounds were shown good binding energy than binding energies of standard drug Ciprofloxacin. Compound (3b, 3c, 3d, 3e) substituted with sulphonamido group were shown to produce higher binding affinity towards the target enzyme compared to the standard. Molecular properties of designed compounds were screened using Molinspiration software and the results were according to the Lipinski's rule of five properties.

Keywords: DNA replication, Quinolone , *Staphylococcus aureus*Gyrase B, AutoDock , Ciprofloxacin, N .

1. Introduction:

In recent years, infectious diseases caused by multidrug resistant pathogens have received a significant attention. This is mainly owing to the fact that the spread of resistant bacteria is drastically rising [1-4] and it encourages medicinal chemist to tackle this problem

with a strong tendency to design and develop novel antibacterial agents. The increasing rate of bacterial resistance to clinical antimicrobial agents and its impact on treatment of infectious diseases have begun to present a unique problem throughout the world. Drug resistant, multiple drug resistant (MDR) and extensively drug resistant (XDR) infectious bacterial pathogens put a greater risk on the population at large due to the risk of pandemic illness. The emerging resistance of some microbial species to some synthetic antimicrobial agents makes it necessary to continue the search for new antimicrobial agents. According to WHO, anti−microbial is a urgent global public health threat, killing atleast 1.27 million people worldwide and associated with nearly 5 million deaths in 2019, according to a report released in The Lancet. Quinolone moiety is an important class of nitrogen containing heterocycles widely used as key building block for medicinal agents. It exhibits a wide spectrum of pharmacophores and has bactericidal [5], anti-malarial [6], anti-viral [7] and anti-cancer [8], anti-tubercular [9] activities etc. Antimicrobial resistance (AMR) has been one of the major public health concerns of the twenty-first century [10]. It refers to resistance developed by pathogenic microorganisms to antimicrobial drugs which makes drug ineffective [11]. Developing new anti-microbial agents and increasing the effectiveness of the existing one by structural modification can play profound role to combat multidrug resistance diseases. In this regard, quinolone scaffold has been used to design new prototypes of drug-candidate with potent Anti-microbial properties. Novel quinolone derivatives were designed (Figure 1) using Vilsmeyer Haack Reaction. The proposed chemical structure was given in Table 1. The designed compounds were evaluated for their molecular properties by the Lipinski's rule of 5 and further their ability binding to the active site region of the target protein *Staphylococcus aureus* Gyrase B–anti-bacterial target using Autodock 4. 2. 6.

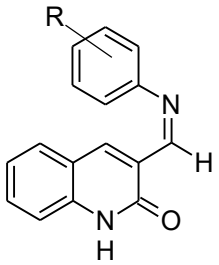


Figure 1: Designed Quinolone Derivative

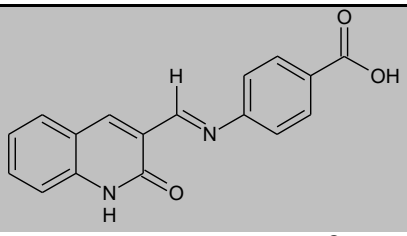
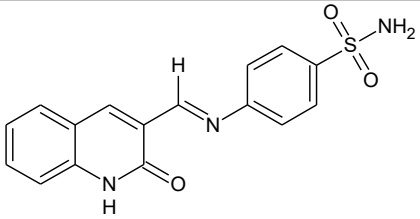
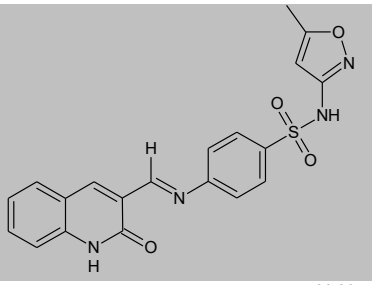
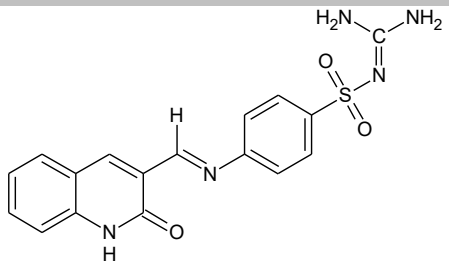
2. Materials and Methods:

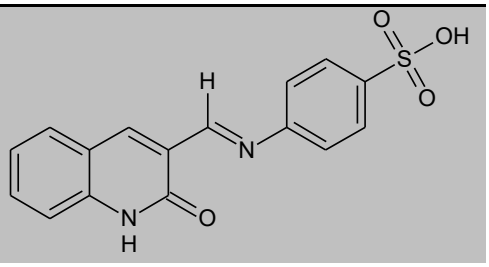
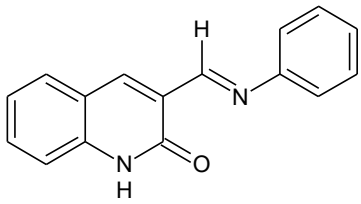
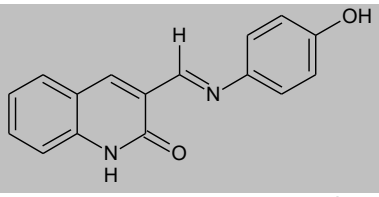
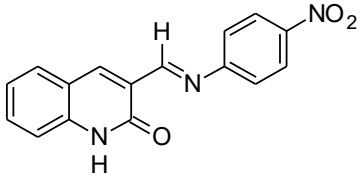
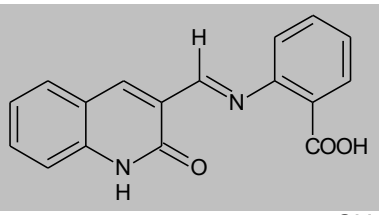
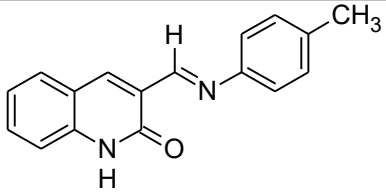
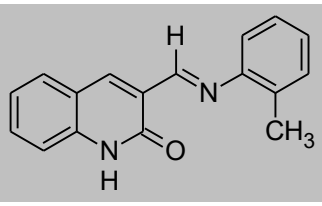
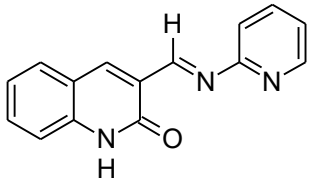
2.1. Designing of Quinolone derivatives:

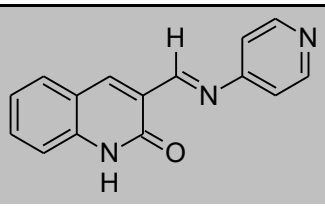
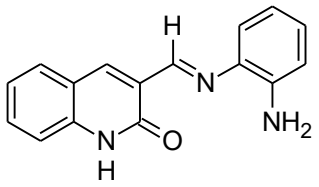
Ligand based drug design is an indirect approach to facilitate the development of pharmacologically active compounds by studying molecules that interact

with the biological target of interest [12].With an aim to develop novel and potent anti-microbial molecules, a novel series of quinolone derivatives were designed by molecular manipulation approach. Fourteen molecules, 3a to 3n were designed with a diverse range of structural substitutions (o/m/p- substituted aryl moiety) at the basic framework of the quinolone scaffold, considering the pharmacodynamic potential of the quinolone imine component and other structural features and property parameters important for biological activity.

Table 1: Structure of the designed compounds

Sl. No.	Compound code	Structure of designed Quinolone derivative
1	3a	
2	3b	
3	3c	
4	3d	

5	3e	
6	3f	
7	3g	
8	3h	
9	3i	
10	3j	
11	3k	
12	3l	

13	3m	
14	3n	

2.2. Preparation of Ligand Molecule:

The two-dimensional (2D) structures of compounds were sketched using Chem Draw Ultra 10.0 (Cambridge Software Co., USA, 2010) and Marvin Sketch (ChemAxon LLC, Cambridge, USA, 2015) software. It is a tool for high-throughput screening which takes description of small molecule in various two-dimensional formats or co-ordinates and generates molecular topologies and energy minimized in variety of formats. Before starting molecular docking process, the active site of the protein was generated. The docking scores of the designed compounds were compared with Ciprofloxacin, a known inhibitor.

2.3. Prediction of drug-likeness and molecular properties:

Molecular properties and drug-likeness parameters were calculated *in silico* for all the designed molecules (3a-3n). It was performed based upon theoretical approaches with an aim to identify the molecules which satisfy the optimum requirements to exhibit as drug-like molecules. The drug-likeness of a chemical substance is defined as a balance between its molecular properties, which has a direct impact on a drug's biological activity, pharmacodynamics, and pharmacokinetics in the human body. The "drug-likeness" test was conducted using Lipinski's "Rule of Five," Ro5 [12]. The distributions of compound molecular weights (MW), calculated lipophilicity (logP), number of hydrogen bond acceptors (HBA), and number of hydrogen bond donors (HBD) were used to assess the "drug-likeness" of compounds. Based on these four chemical descriptors, the technique provides a watchful approach to apparent absorption problems; The rule stipulates that most "druglike" molecules must have log P 5, molecular weight 500, number of hydrogen bond acceptors 10, and number of hydrogen bond donors 5. Molecules that violate more than one of these criteria may impair oral bioavailability. Some other properties were also predicted using open web-based tools like Molinspiration online software (http://www.molinspiration.com/, 2016).

2.4. Molecular Docking Study:

In *in-silico* molecular docking studies of designed quinolone derivatives that exert their action by binding with specific receptor provides evidence on binding conformation, pattern and affinity. In this study, to determine the potential anti-microbial activity of quinolone derivatives, their interaction with known bacterial receptor, *Staphylococcus aureus* Gyrase B (PDB ID: 4URM) were tested.

Ligand preparation:

The designed quinolone derivatives were drawn by using chemsketch free ware and are saved in mol format. Later the ligands were converted to pdb format using open babel software. However, the cocrystalised ligand (Kibdelomycin) was also docked with *Staphylococcus aureus* Gyrase B (PDB Id: 4URM) to compare the docking results.

Protein preparation:

The 3-dimensional structure of *Staphylococcus aureus* Gyrase B (PDB Id: 4URM) was retrieved from the Protein Data Bank database (https://www.rcsb.org/) (Figure 2). The protein was in a complex with an inhibitor Kibdelomycin. Water molecules, inhibitor, and other heteroatoms from the protein removed using Discovery studio and used for molecular docking.

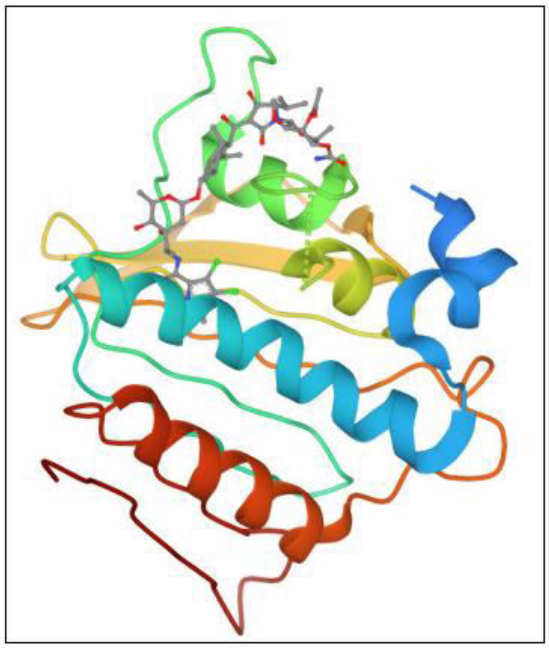


Figure 2: Structure of *Staphylococcus aureus* Gyrase B(4URM) along with its co-crystallized ligand Kibdelomycin

Determining the active site:

The active site amino acid residues of the protein *Staphylococcus aureus* Gyrase B was predicted by estimating the amino acids that are interacting with the co crystallised

ligand. The amino acid residues prediction was done by viewing the 2D interaction diagram of the co crystallised ligand in Discovery studio and then it was compared with the data available in the RCSB data base. Only after this step, molecular docking was succeeded.

Molecular docking using AutoDock 4.2.6:

AutoDock 4.2.6 was downloaded from 'The Scripps Research Institute' official website (http://autodock.scripps.edu/) along with other supporting software viz., Python 3.8.2 and MGLTools 1.5.4. Docking of ligands and *Staphylococcus aureus* Gyrase B was performed indigenously by docking 'one ligand at a time to the protein' manually using AutoDock 4.2.6.

Initializing and preparation of PDBQT files:

Before docking, the starting directory was set to the desired folder. The processed protein molecule was imported into the AutoDock 4.2.6 workspace. The polar hydrogen atoms were added; the Kollman charges were computed for the protein. The protein was then saved in PDBQT format that was then used as the target. The ligand was imported into the workspace; the polar atoms were added; the Gasteiger charges were computed; the torsion tree was defined by choosing the root; the number of rotatable bonds was identified and saved in PDBQT format. The ligand and protein were imported in PDBQT format into the workspace for further simulation process.

Grid parameters:

Assigning the grid parameters is the mostimportant step in molecular docking since itnavigates the ligand to the binding site ofthe *Staphylococcus aureus* Gyrase B. Grid spacing was set to0.400 Å. Centre grid box values were set tox 8.182, y -1.187, and z 61.448. The number of grid points along the x, y, and z dimensions was set as 60 X 60 X 60 respectively. These parameters were set to cover the entire 3-dimensional active site of the *Staphylococcus aureus* Gyrase B. The output was saved in the grid parameter file (GPF) file format.

Running AutoGrid and AutoDock:

The AutoGrid was executed by providingthe AutoGrid executable and GPF files as input and converted to the grid log file (GLG). The grid was then launched. After the successful execution of AutoGrid, the genetic algorithm was set to default and is as follows: i) the number of GA runs: 10; ii) population size: 150; iii) the number of energy evaluations: 2.5 million (2.0 Åclustered tolerance); and iv) the number of generations: 27000. The Lamarckian genetic algorithm was used and the output was saved in docking parameter file (DPF) file format. The AutoDock was executed by providing the AutoDock executable and

DPF files as input, converted to the docking log file (DLG) and docking was launched. The final DLG file contained essential details viz., free binding energies for every run and inhibitory constant. The results were analyzed; ranked based on their binding energies; saved in PDBQT format; the lowest binding energy complex was saved in PDB format for further analysis.

3. Results and Discussion:

Calculation of molecular properties:

The molecular properties were calculated on the basis of simple molecular descriptors used by Lipinski's rule of 5. The five properties consist of Molecular weight, Hydrogen bond donor, Hydrogen bond acceptors, log p and Topological Polar surface area (TPSA) which was calculated using the online cheminformatics tool molinspiration (http:/www.molinspiration.com/) and the results were shown in Table 2.

Druglikeness properties of designed Quinolone Derivatives:

The Molinspiration virtual screening is fast (1,00,000 molecules may be screened in about 30 min) and therefore allows processing of very large molecular libraries. Validation test performed on various targets (including kinase inhibitors, G-protein coupled receptor targets, ion channel modulator, different enzymes etc) show 10-20 fold increases in hit rate in comparison with a standard/random selection of molecules for screening. The data's for drug likeness properties were depicted in Table 3. Based on the result of druglikeness properties of quinolone derivatives, there was no violation in pharmacological action against the tested targets.

Table 2: Molecular descriptor properties of designed compounds:

Compound code	Molecular Formula	Molecular weight	nON	nOHNH	Log P
3a	$C_{17}H_{12}N_2O_3$	292.29	5	2	2.53
3b	$C_{16}H_{13}N_3O_3S$	327.36	6	3	1.31
3c	$C_{20}H_{15}N_4O_4S$	408.44	8	2	2.22
3d	$C_{16}H_{12}N_2O_4S$	371.42	8	4	0.47
3e	$C_{16}H_{12}N_2O_4S$	328.35	6	2	-0.39
3f	$C_{16}H_{12}N_2O$	248.28	3	1	2.62
3g	$C_{16}H_{12}N_2O_2$	264.28	4	2	2.14

3h	$C_{16}H_{11}N_3O_3$	293.28	6	1	2.58
3i	$C_{17}H_{12}N_2O_3$	292.29	5	2	2.67
3j	$C_{17}H_{14}N_2O$	262.31	3	1	3.07
3k	$C_{17}H_{14}N_2O$	262.31	3	1	3.02
3l	$C_{15}H_{11}N_3O$	249.27	4	1	1.72
3m	$C_{15}H_{11}N_3O$	249.27	4	1	1.33
3n	$C_{16}H13N_3O$	263.30	4	3	2.06
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.35	6	2	-0.70

Table 3: Drug-likeness properties of Quinolone Derivatives:

Compound code	GPCR ligand	Ion channel Modulator	Kinase Inhibitor	Nuclear receptor ligand	Protease Inhibitor	Enzyme Inhibitor
3a	-0.41	-0.53	-0.10	-0.28	-0.53	-0.08
3b	-0.49	-0.61	-0.05	-0.66	-0.34	0.00
3c	-0.41	-0.78	-0.22	-0.69	-0.50	-0.27
3d	-0.31	-0.46	-0.09	-0.46	-0.29	0.02
3e	-0.27	-0.41	-0.15	-0.68	-0.30	0.03
3f	-0.60	-0.59	-0.16	-0.64	-0.80	-0.18
3g	-0.48	-0.51	-0.06	-0.39	-0.69	-0.10
3h	-0.59	-0.57	-0.21	-0.55	-0.73	-0.26
3i	-0.38	-0.45	-0.13	-0.24	-0.52	-0.04
3j	-0.58	-0.66	-0.17	-0.60	-0.78	-0.23
3k	-0.56	-0.58	-0.14	-0.54	-0.74	-0.18
3l	-0.53	-0.47	-0.04	-0.79	-0.68	-0.09
3m	-0.49	-0.46	0.04	-0.68	-0.71	-0.05
3n	-0.47	-0.49	0.01	-0.63	-0.59	-0.04
Ciprofloxacin	0.12	-0.04	-0.07	-0.19	-0.20	0.28

Docking analysis:

The newly designed molecules were energy minimized and the resulting molecules were considered for docking analysis for AutoDock 4.0. AutoDock is employed to study the docking molecules within the active site region of the target protein and the H-bond interaction. A docking score, which predicts pharmacological activity, reflects the binding energy required to build a connection between the ligand and the receptor. It also aids in the strengthening of the ligand-receptor connection. Docked scores of newly designed molecules along with inhibition constant, Vdw Desolvation energy, electrostatic energy and hydrogen bonds were represented in tables 4 and 5 respectively. Among the studied compounds, Compound 3d has a highest binding score -8.89 kcal/mol with two hydrogen bonds when compared with the standard drug Ciprofloxacin (-4.63 kcal/mol with two hydrogen bonds), followed by compound 3c with a score of -8.07 kcal/mol with three hydrogen bonds. The docked molecule was visualized using chimera software and the docking pose of the compounds were given in figure 3. Docking results showed that all the designed molecules have similar orientations in the bindingin pocked for the target enzyme *Staphylococcus aureus* GyrB.

Table 4: Hydrogen bond and Hydrophobic Interaction of Ligand against Target enzyme *Staphylococcus aureus* GyrB (Pdb Id: 4URM)

Compound code	No.of Hydrogen Bonds formed	Amino acid Involved in H-bond interaction	H-bond Distance (Å)	Amino acid involved in VanderWalls Interaction
3a	4	Val 88 Arg 144 Arg 223 Asn 145	2.134 1.843 1.655 1.883	Asp 89, Arg 223, Glu193, Arg144
3b	3	Asn 221 Gly 85 Thr 173	2.037 2.152 2.076	Pro 87,Thr 173, Ile 86, Glu 58, Arg 84, Asn 221, Tyr 227, Gln 196
3c	3	Val 88 Asn 221 Arg144	1.609 2.055 1.696	Arg 84, Gln 196, Asp 89, Arg 144
3d	2	Arg223 Gln196	1.996 2.026	Tyr 192, Pro 87, Gly 85, Arg 144, Asn 221, Arg 84

3e	6	Val 88 Arg 144 Arg 223 Asn 145 Glu 193 Arg 223	2.22 1.926 2.003 1.828 2.143 1.754	Asp 89, Glu 193
3f	1	Arg 200	2.153	Ile 102, Ala 98, Gln 91, Gln 196
3g	4	Arg 223 Asn 145 Arg 144 Val 88	2.019 2.031 1.899 1.963	Arg 144, Val 88, Asp 89
3h	0	-	-	Glu 58, Asp 81,Thr 173, Gly 85, Ile 86, Ile102, Ala 98
3i	4	Asp 89 Val 88 Ile 90 Arg 144	2.171 2.135 2.144 1.906	Asn 145, Tyr 149
3j	3	Asn 145 Val 88 Arg 144	1.973 1.954 1.898	Asp 89
3k	1	Arg 200	2.058	Ala 108, Ile 102, Val101, Ala 98, Gln 91,Gln 197, Gln 196
3l	1	Arg 200	2.068	Asp 89, Gln 91, Gln 197, Glu 201
3m	1	Arg 144	2.199	Arg 84, Glu 58, Asp 81, Thr 173, Asn 54, Ile 86, Pro 87,Gly 85
3n	1	Arg 200	2.510	Gln 91, Asp 89, Gln 197
Ciprofloxacin	2	Arg 144 Arg 144	2.080 1.834	Pro 87, Asp 89, Ile 90, Thr 194, Glu 193, Gln 196, Gln 197

Table 5: Energy Minimization table of ligand and *Staphylococcus aureus* GyrB interaction

Compound code	Binding Energy (Kcal/mol)	Inhibition constant	Vander Walls desolvation energy	Intermolecular energy	Electrostatic Energy	Total internal energy
3a	-6.81	10.14	-7.11	-7.71	-0.59	-0.31
3b	-7.35	4.07	-8.11	-8.25	-0.14	**-0.49**
3c	-8.07	1.21	-9.21	-9.57	-0.36	**-0.68**
3d	-8.89	306.49	-8.07	-10.08	-2.01	**-0.89**
3e	-7.22	5.09	-7.23	-8.12	-0.88	**-0.44**
3f	-5.98	41.63	-6.52	-6.57	-0.05	-0.29
3g	-6.44	19.13	-6.75	-7.03	-0.28	-0.26
3h	-6.26	25.99	-6.83	-6.85	-0.02	-0.28
3i	-6.8	10.43	-7.3	-7.69	-0.39	-0.17
3j	-6.29	24.55	-6.8	-6.89	-0.08	-0.36
3k	-6.16	30.3	-6.54	-6.76	-0.23	-0.27
3l	-6.12	32.42	-6.59	-6.72	-0.13	-0.25
3m	-5.72	64.06	-6.24	-6.32	-0.08	-0.15
3n	-6.35	22.31	-6.85	-6.94	-0.09	-0.35
Ciprofloxacin	-4.63	404.1	-5.14	-5.52	-0.39	**-0.05**

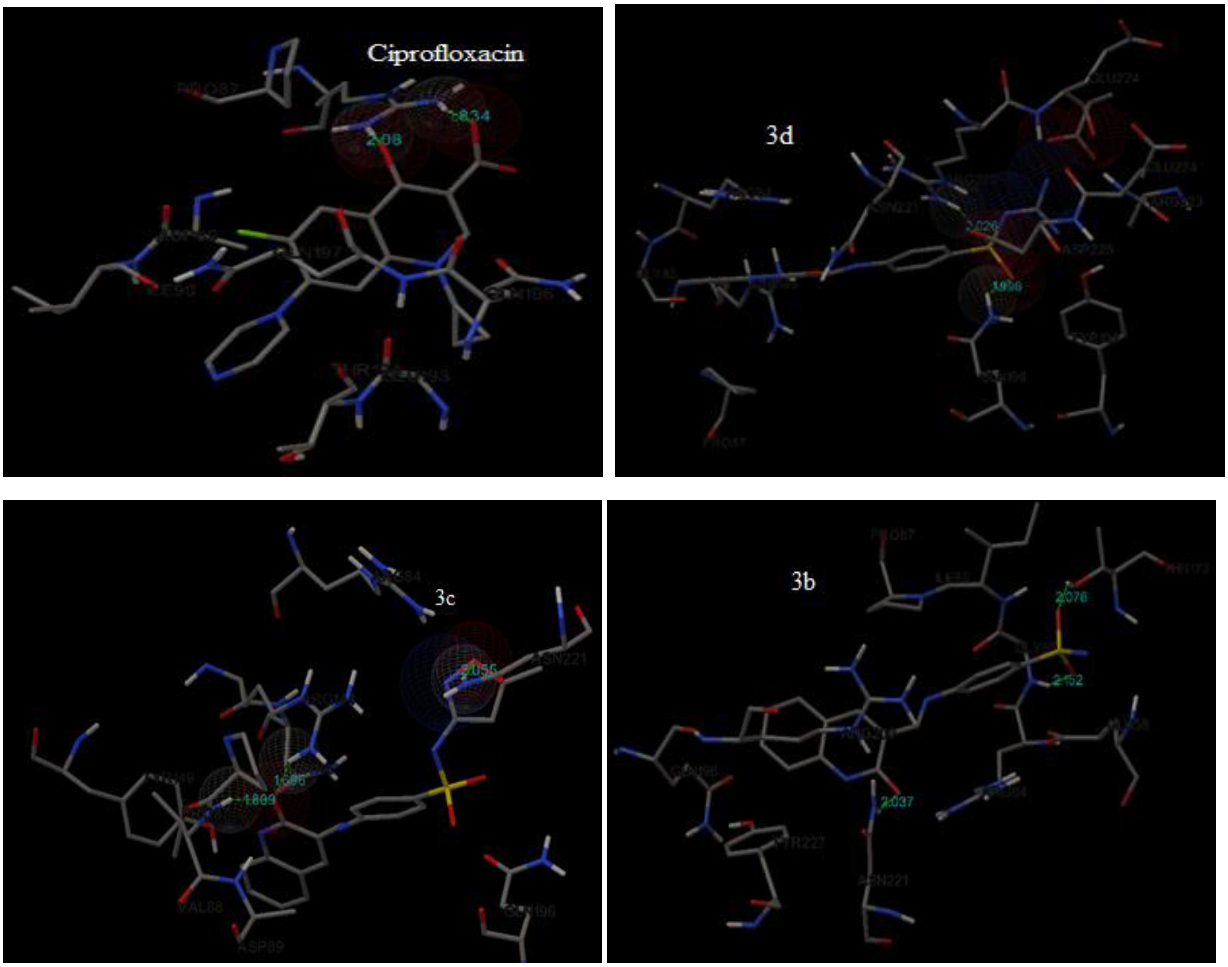


Figure 3: Molecular interactions of compounds Ciprofloxacin, 3d, 3c and 3b in the active site of the protein *Staphylococcus aureus* Gyrase B

4. Conclusion:

In the current study, molecular docking was used to identify the potential inhibitor of *Staphylococcus aureus* Gyrase B by measuring their binding energies. A preliminary QSAR study was carried out for the designed compounds. All the compounds obeys the Lipinski's rule of five properties. Applying the Lipinski's rule of five to these quinolone derivatives to evaluate the drug-likeness, there was no violations of the rule determining drugs pharmacological activity in the body. As per the result of molecular docking study showed that the compound 3b, 3c, 3d and 3epossess potential binding affinity into the binding site of the macromolecule *Staphylococcus aureus* Gyrase B compared to the standard drug Ciprofloxacin. The study may conclude that sulphonamido substitution in quinolone moiety may have higher affinity towards the targeted enzyme and it serves as potential inhibitors of the same. Thus, this study will be useful for the design of novel

inhibitors towards the target enzyme and are suitable candidates for further investigation to evaluate its clinical potency in order to compact the development of antimicrobial resistance.

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