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An *In-Silico* Approach towards Drug Discovery against Bacterial DNA Gyrase using Nalidixic Acid

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Abstract

Escherichia coli is a gram- negative bacteria and is common for bacterial disease that affects the intestinal tract. DNA gyrase A was an enzyme which is responsible for DNA breakage and reunion are mostly released by *Escherichia coli* bacteria in human gastrointestinal tract, causing a situation called gastroenteritis. Normally in native DNA gyrase A protein, the 83th position is occupied by serine residue which is mutated to phenylalanine making the enzyme as antibiotic resistant against the known antibacterial drugs. Nalidixic acid is mainly used in the treatment for urinary tract infections caused by gram-negative bacteria. Here, according to our present study, we compared binding affinities of native protein structure and mutated protein structure of *E. coli* DNA gyrase A against the Nalidixic acid. ADMET properties, bioactive scores and other parameters of the ligand structure were calculated using various tools including SwissADME, pkCSM software as well as PreADMET web tool. Lastly molecular docking study was carried out using AutoDock Vina software and the results were evaluated on the basis of iMod server. After comparing the docking scores, it was observed that the mutated protein (-5.5 kCal/mol.) shows more binding affinity towards nalidixic acid than the native protein (-5.3 kCal/mol.) Although, more *in-vitro* and *in-vivo* studies need to be performed to get a satisfactory conclusion.

Keywords: Bacterial DNA Gyrase, Drug discovery, Nalidixic acid, Molecular docking interaction.

Introduction

DNA gyrase is an enzyme which belongs to class of Topoisomerase enzyme; more specifically Topoisomerase II. This enzyme mainly catalyses the ATP-dependent negative super-coiling of double-stranded closed-circular DNA (1,2). It is divided into two subunits preferentially known as Gyrase A and Gyrase B; among which DNA Gyrase A (GyrA) is made up of two functional groups like N-terminal and C-terminal. DNA-protein bridges which are formed by N-terminal responsible for breaking and re-joining function whereas C-terminal was responsible for DNA-binding non-specifically (3-5). GyrA was used for DNA cleavage and ligation for the tyrosine which is an active site of GyrA. This enzyme is resistant to multiple fluoroquinolones in enteric bacteria because of the spontaneous mutation happens in GyrA enzyme. *'Escherichia coli releases the DNA Gyrase A enzyme in human gastrointestinal tract that may not be directly related to gastroenteritis.* As per the literature study it has been noted that mutation occurs at 83rd position of GyrA making the bacterial genome as antibiotic resistant. One more such variation occurs where serine at 83rd position was mutated to phenylalanine (S83F). "Sandhya Bansal; Vibha Tandon. (2011). Contribution of mutations in DNA gyrase

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and topoisomerase IV genes to ciprofloxacin resistance in Escherichia coli clinical isolates. , 37(3), 253–255"

In case of drug discovery for such disorder, we need to have mutated GyrA structure which is not available in the database. So, this sequence structure gap in the study will be very useful for understanding the drug discovery approach (6, 7). In account of drug discovery Nalidixic acid ($C_{12}H_{12}N_2O_3$) is such a synthetic quinolone and antibacterial agent that can be active against mostly gram-negative organisms (8-11). Due to mutation in the GyrA sequence, it has become antibiotic resistance which destabilizes the structure upon binding. "Pourahmad Jaktaji R, Mohiti E. Study of Mutations in the DNA gyrase gyrA Gene of Escherichia coli. Iran J Pharm Res. 2010 Winter;9(1):43-8" To find the protein-ligand interaction stability between the native and the mutant structure by using sequence analysis and molecular docking, we have taken Nalidixic acid as the ligand, which could show the stability result upon binding with our desired protein (Native and Mutant) structure so that we could determine that weather Nalidixic acid has any positive role against the disease gastroenteritis or not (Fig 1). The direct and rational process for drug discovery was molecular docking and here the protein-ligand binding energies calculated in kilo calories per mole (Kcal/mol).

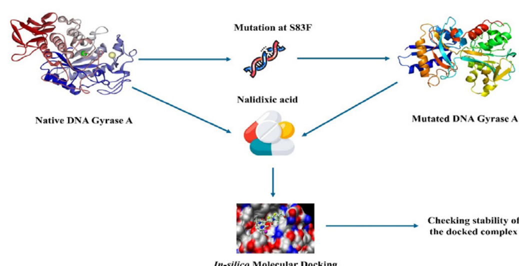

Fig. 1: The Graphical Abstract

Materials and Methods

Selection and preparation of Receptor or Protein

For the purpose of protein selection, 6RKU protein has been selected. This protein denotes the crystal structure of *E. coli* DNA Gyrase A subunit, belonging to ligase family. From Protein Databank (http://www.rcsb.org/), the 3D structure of 6RKU (Fig 2) has been obtained. Then to stabilize the receptor structures, the already attached ligands and water molecules were removed by BIOVIA Discovery Studio 2020 software (https://discover.3ds.com/discovery-studio-visualizer-download/) (12). In order to prepare the mutant version of the protein structure, SPDBV software (https://spdbv.unil.ch/) has been used. By using this software, the serine residue at 83rd position of the native protein was mutated to phenylalanine.

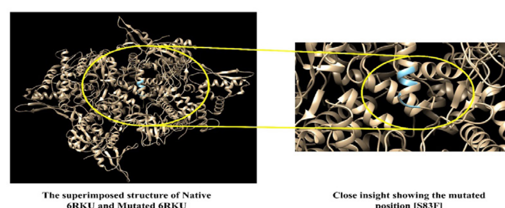

Fig. 2: The Superimposed Structure of Both the Native 6RKU and Mutated 6RKU

Validation of protein structure

The newly generated protein PDB structure was then undergone through a series of quality analyses including ERRAT, Procheck using SAVES 6.0 (https://saves.mbi.ucla.edu/) (13, 14), and ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php/) (15).

Selection and preparation of the ligands

Chemical compounds, or more precisely, bioactive ligands i.e., Nalidixic acid, was chosen on the basis of literature study (Fig 3). 2D Structure Data Format (SDF) of all chemical compounds or ligand files were retrieved from PubChem (www.pubchem.ncbi.nlm.nih.gov/) and later translated to their 3D PDB format using Open Babel software. A PDBQT format file was created after adding (16) hydrogenating atoms and the desired torsion to a PDB format

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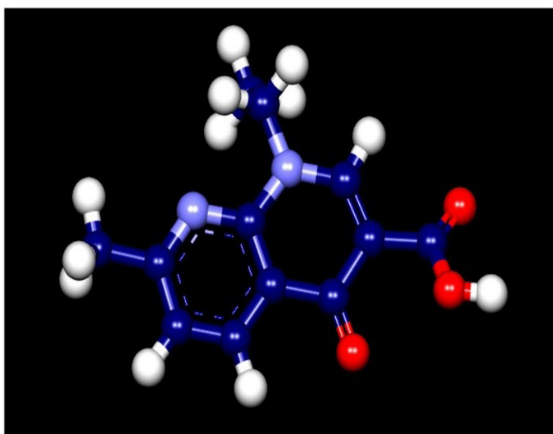

file.

Fig. 3: The 3D Chemical Structure of Nalidixic acid

Validation of compound structures

SwissADME prediction of the compounds

Adsorption, Distribution, Metabolism and Excretion all-together termed as ADME is a very profitable process to assess all those previously mentioned parameters of the ligands using the server of SwissADME website (https://www.swissadme.ch/) (17, 18).

Toxicity prediction of the compounds

In case of drug designing and establishment of a suitable drug compound, it is a very necessary step to predict the toxicity level of the small compounds or rather ligands before investigating their endurance capacity when ingested into any animal model like mouse, rat as well as in human too. There are two online servers available for these purposes, they are: PreADMET server (https://preadmet.bmdrc.kr/) (19, 20) and pkCSM (Predicting Small-Molecule Pharmacokinetic Properties Using Graph-Based Signature) (http://biosig.unimelb.edu.au/pkcsm/) (21). In case of PreADMET server, first the SDF structures retrieved from the "Pourahmad Jaktaji R, Mohiti E. Study of Mutations in the DNA gyrase gyrA Gene of Escherichia coli. Iran J Pharm Res. 2010 Winter;9(1):43-8" Pubchem were converted to mol2 format using Open babel software and then submitted to the online server for toxicity prediction: mutagenicity (AMES test), carcinogenicity (for rat and mouse) and hERG inhibition. On the other hand, SMILEs structure of each ligand, derived from Pubchem database were directly submitted to the pkCSM server to analyse the results of LD50 (mol/kg) and highest permissible dosage for human (log mg/kg/day).

Molecular docking interaction using AutoDock Vina

AutoDock Vina software (http://vina.scripps.edu/) (22) for molecular docking and virtual screening that significantly improves efficient binding mode predictions, thereafter gives more accuracy in protein-ligand interaction. AutoDock Vina works by calculating the grid maps and clusters. Before proceeding to final docking step, Kollman charges and other modifications were added to the purified form of protein and converted into a proper readable PDBQT file format. Similarly, ligand is also transformed into PDBQT file. A grid box on active residues of protein was generated with different grid dimensions and centres but with similar spacing i.e., 0.375. The exhaustiveness was set at 8 and binding energy affinity was predicted with AutoDock Vina software. The final visualization of docked structure was performed using BIOVIA Discovery Studio 2020 (https://discover.3ds.com/discovery-studio-visualizer-download/) and PYMOL software (https://pymol.org/) (23).

Assessment of structural hotspots and binding pockets on the receptor protein

An online server called CASTp 3.0 (http://sts.bioe.uic.edu/) (24) is used to predict active amino acid residues, or alternatively structural hotspots on the receptor protein. A systematic quantitative characterisation of the surface topography of proteins is often provided by the Computer Atlas Surface Topography of Protein (CASTp).

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Results and Discussion

Validation of protein structure

According to the predictions made by the aforementioned web tools, Fig 4 depicts the overall quality of recognition of the 3D protein PDB structure native 6RKU as well as mutant 6RKU. The process of approving an ideal protein structure involves confirming the protein PDB model using a number of quality control metrics. According to ERRAT's results, both the native and mutant the proteins exhibit a quality score of 85%, indicating that the protein is well-modelled. The "overall quality factor" for non-bonded atomic interactions is displayed, with higher values denoting higher quality. Moving on, the ProSA-web result displayed the protein's total z score. In this case, the scores are -7.95 (native) and -7.88 (mutant), indicating that both the structures are located within the X-ray region. The Ramachandran plot of the protein models then showed that, in accordance with the PROCHECK result, 86% of residues were found in the most preferred regions, followed by 13.0% in additional allowed and 0 % in generously allowed and disallowed regions. The collective outcomes derived from the previously mentioned attributes indicate that native and mutant version of 6RKU protein is of high quality and appropriate for additional molecular interaction analysis.

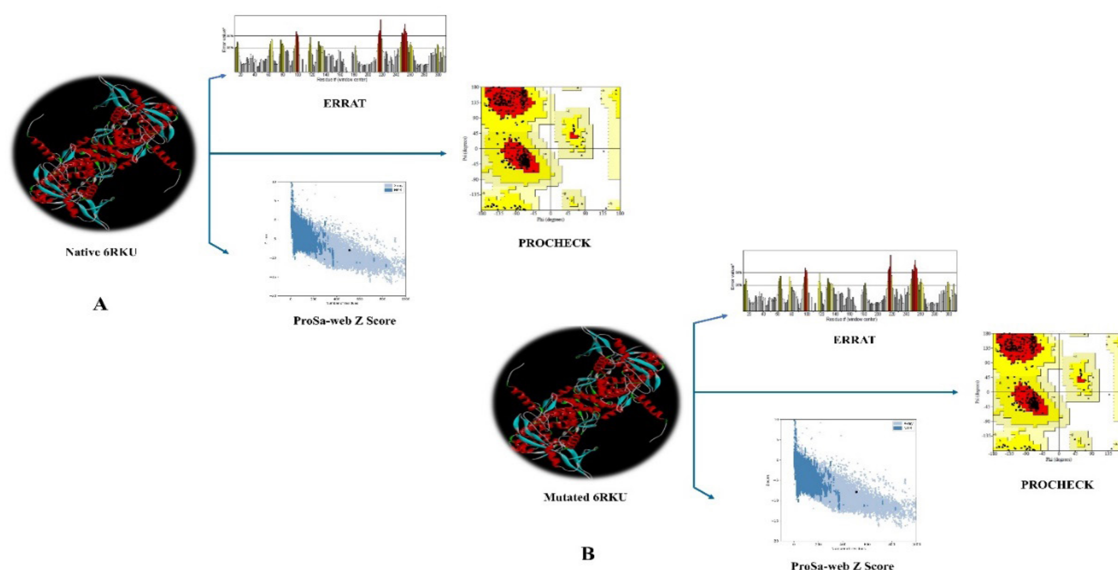

Fig. 4: Results Showing Quality Checking Parameters of [A] Native 6RKU and [B] Mutated 6RKU

Validation of compound structures

SwissADME prediction of the compounds

It is intended for the study to apply a variety of *in-silico* techniques to assess the computational aspects of the therapeutically active elements. Upon submission of ligand structure in SMILEs format, SwissADME results are generated based on ADME/toxicity analysis and Lipinski filter analysis upon submission of the ligand structure in SMILEs format. Here in our result, we have represented the outcome of the drug likeliness data in a tabulated manner. (Table 1)

According to the SwissADME server derived drug likeliness result, from Table 1, Nalidixic acid shows satisfactory result with 0 violations.That means, nalidixic acid follow Lipinski's rule 5 completely (Molecular weight must not exceed 500 Dalton; Hydrogen bond donors and

acceptors must not exceed 5 and 10 respectively and last but not least, the octanol-water partition coefficient should not cross 5) (25). In addition, bioavailability scores describe how quickly and how much a molecule enters the bloodstream after oral delivery, eventually reaching the desired areas, according to Table 1, all selected compounds show the similar score i.e., 0.85. This value implies that the compounds have 85% probability of being bioavailable (26).

Table 1: Showing Results of Drug Likeliness of Nalidixic acid

Name of the Compound	Lipinski's Rule		
	Satisfactory	No. of Violations	Bioavailability Score
Nalidixic acid	Yes	0	0.85

Toxicity prediction of the compounds

Assessing the toxicity of small compounds is an essential stage in the drug discovery approach. The PreADMET server's toxicological prediction result, which includes the chemicals' hERG inhibition, mutagenicity, and carcinogenicity are shown in Table 2. According to the result, the negative prediction translates carcinogenic activity whereas positive means the compound possess no carcinogenic activity (18). Talking about the mutagenic characteristics, all compounds are mutagenic in nature. In case of hERG inhibition, most of the compounds show medium to low probabilities of blogging hERG gene that often associated with sudden heart attacks in humans (27). The result obtained from pkCSM server is given in Table 3. The term "LD50" refers to the concentration of a test substance deemed to be lethal for 50% of the test subjects in the treated group; in this case, the lethal dose for rats has been determined and is stated in terms of mol/kg. It is one method of determining a compound's acute toxicity, or short-term poisoning potential.

The highest dose or quantity of a medicine or test substance that does not manifest any undesirable side effects is referred to as the maximum tolerated dose. It is identified through multiple clinical trials that involve gradually raising the dosage in various human populations until and unless a tolerable adverse impact is observed. The unit of expression is log mg/kg/day (28).

Table 2: Showing Results of Toxicity Analysis of Nalidixic acid

Name of the compound	Toxicity			
	Mutagenicity	Carcinogenicity		
		Rat	Mouse	hERG inhibition
Nalidixic acid	Mutagen	Negative	Negative	Low risk

Table 3: Showing Results of LD 50 Value and Maximum Tolerated Dose for Human of Nalidixic acid

Name of the Compound	Oral Rat Acute Toxicity (LD 50)	Maximum Tolerated Dose (Human)
Nalidixic acid	2.48	1.644

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Molecular docking interaction using AutoDock Vina

The binding affinity of nalidixic acid with the necessary protein crystal structure of E. coli DNA Gyrase A subunit (6RKU, 6RKU_m) is ascertained based on the docking analysis performed by AutoDock Vina. The ligand with the highest binding affinity to the proteins is the one with greater negative binding energy. We have chosen nalidixic acid based on our research, and it exhibits a unique set of results due to variations in its binding capacity with the target protein receptors (6RKU, 6RKU_m). As reported by the docking result, nalidixic acid shows the maximum binding affinity i.e., -5.5 kcal/ mol. with 6RKU_m followed by -5.3 kcal/mol. against 6RKU. Replace this line with Fig 5A-5B show the 3D and 2D interactions mode between the protein and the ligand molecule.

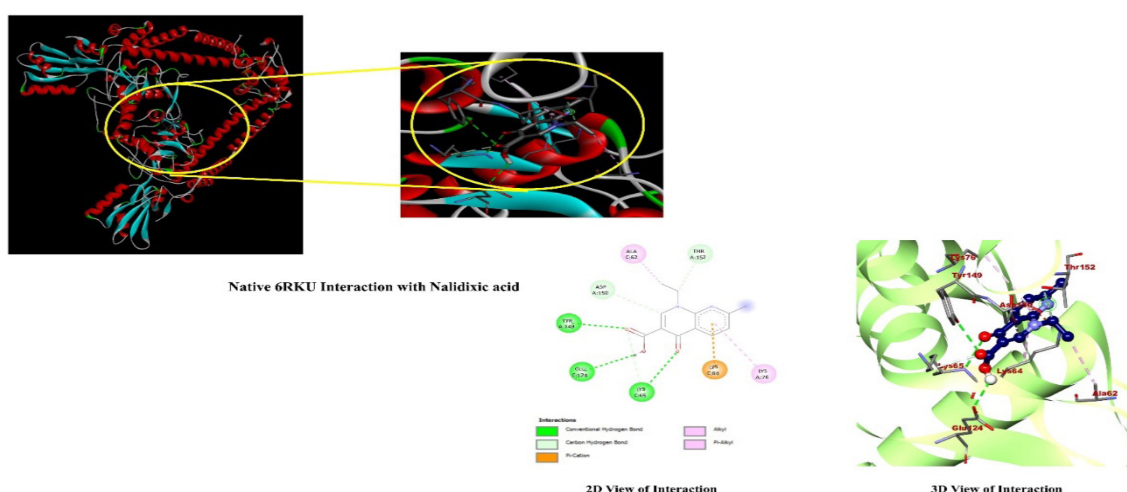

Fig. 5A: Figure Showing Molecular Docking Interaction Between Native 6RKU And Nalidixic Acid with Respective 2D and 3D View of Interactions

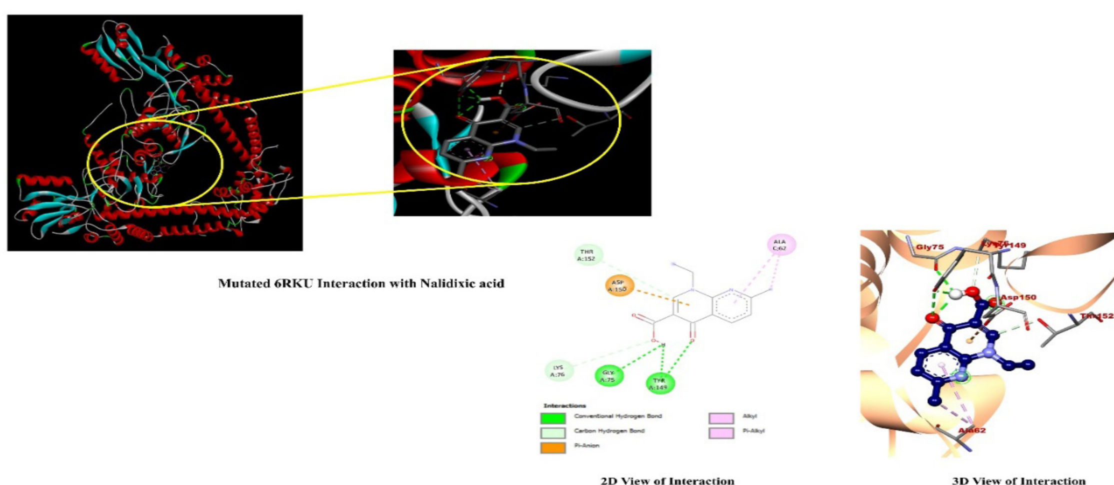

Fig. 5B: Figure Showing Molecular Docking Interaction Between Mutated 6RKU And Nalidixic Acid with Respective 2D and 3D View of Interactions

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Assessment of structural hotspots and binding pockets on the receptor protein

Table 4 displays the findings for the 3D protein PDB structure 6RKU and 6RKU_m. obtained from the CASTp 3.0 online server. This outcome demonstrates the key amino acids involved in the particular protein-ligand interaction.

Table 4: Table Showing Active Amino Acid Residues Obtained via Molecular Docking Interaction for Each of the Receptor Proteins [6RKU and 6RKU_m] Along with the Ligand

Name of the compound	Name of the protein	Active Amino Acid Residues
Nalidixic acid	6RKU	ALA 62, GLY 75, LYS 76, TYR 149, ASP 150, THR 152
	6RKU_m	ALA 62, LYS 64, LYS 65, LYS 76, GLU 124, TYR 149, ASP 150, THR 152

Fig 6 shows the active binding pockets which are present in the 3D protein (6RKU). Each binding pockets represent the ligand attachment region on the protein. Binding pockets (1-5) are differentiated on the basis of their size and volume.

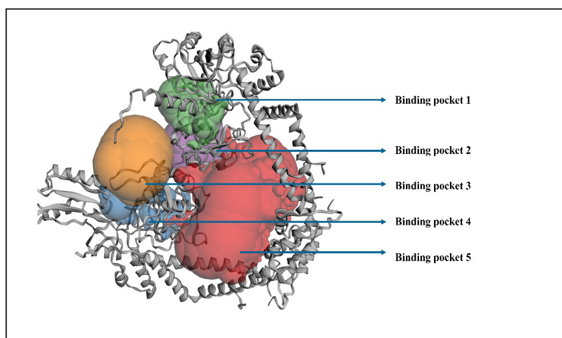

Fig. 6: Figure Showing the Active Binding Pockets on The Protein 6RKU

Conclusion

Virtual screening via molecular docking is one of the most popular approaches to evaluate the binding possibility between a receptor protein and ligand structures. Generally, molecular interaction study shows that protein-ligand binding only happens when free energy change is negative along with increasing negative value of the binding energy corresponds the increased stability between protein-ligand complex. According to our study, we have performed docking interactions between the native and mutant form of bacterial DNA gyrase with a common quinolone antibiotic Nalidixic acid. Upon carrying out and alongside validating all the necessary variables for *in-silico* screening, it was revealed that nalidixic acid showed more stable interaction with the mutant version of bacterial DNA gyrase than the native one. From this initial virtual work, we can conclude that nalidixic acid showed active target towards the E. coli DNAs, by exhibiting positive interaction against mutated version of the target protein. Furthermore, to establish this hypothesis into original theory, more *in-vivo* and *in-vitro* studies are required.

Acknowledgement

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Conflict of Interest

There are no conflicts of interest, the authors declare.

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