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R E S E A E C H A R T I C L E

In-Silico Structure-Activity Relationship and Molecular Docking Study of Levofloxacin and its Mono substituted Analogues against the *Escherichia coli* DNA Gyrase

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A B S T R A C T

Background: Escherichia coli is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), traveler's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia. Levofloxacin, a chiralfluorinated carboxyquinoloneis used to treat a variety of bacterial infections. This medication belongs to a class of drugs known as quinolone antibiotics. It functions by inhibiting the DNA gyrase and topoisomerase IV of both Gram-positive and Gram-negative bacteria. **Materials and Methods:** Molecular docking study was carried out on four analogous structurally diverse levofloxacin against Escherichia coli DNA Gyrase using the Autodock Vina software. Extensive structure activity relationship study was also carried out with these molecules. The physiochemical analysis, lipophilicity, solubility, pharmacokinetics and Lipinski drug likeness of levofloxacin and its mono substituted analogues were evaluated. These molecules were designed by substituting the fluorine (F) attachment of the levofloxacin with CH_3, OH, NH_2 and C=O groups. The scoring function (empirical binding free energy) and hydrogen bond formation was used to estimate the inhibitory effect of the protein-ligand complex. **Results:** The binding energy of levofloxacin was -7.2kcal/ mol. The free binding energies of the CH_3, OH, NH_2 and C=O analogues of levofloxacin were -7.7, -6.5, -6.8 and -6.4Kcal/mol respectively. Levofloxacin also formed 4 hydrogen bonds with the Escherichia coli DNA Gyrase while it's CH_3, OH, NH_2 and C=O analogues formed 8, 8, 13, and 2 hydrogen bonds respectively. All other mono substituted analogues except the CH3 analogue of levofloxacin, showed slightly higher values than the non substituted levofloxacin. The CH_3, OH and NH_2 analogues also formed more hydrogen bonds with the target enzyme than levofloxacin. The lower free binding energy value (more negative value) displayed by the CH_3 analogue means it show a better antimicrobial activity than levofloxacin. The higher number of hydrogen bonds formed by the CH_3 and NH_2 analogues also indicates a higher binding affinity with the target enzyme. The two analogues do not cross the blood brain barrier (BBB). This also shows that they cannot cause problem to the brain. **Conclusion:** These results indicated that the CH_3 and NH_2 analogues may be better antimicrobial agents. Synthesis and pre-clinical studies of these mono substituted derivatives with Escherichia coli DNA Gyrase is recommended in order to confirm their new potentials as better antimicrobial agents than the un substituted analogue.

Keywords: Docking, Levofloxacin, Escherichia coli DNA Gyrase, Pharmacokinetics, Blood Brain Barrier.

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C O N T E N T S

1. Introduction

The gram-negative bacteria *Escherichia coli* are the most numerous aerobic commensal inhabitants of the large intestine [3]. Certain strains cause diarrhea, and all can cause infections when they invade sterile sites (eg, the urinary tract) [11]. Besides being resistant to ampicillin and tetracycline, *E. coli* have become increasingly resistant to TMP/SMX and fluoro quinolones [20]. Levofloxacin is a synthetic broad-spectrum antibacterial agent for oral administration. Chemically, levofloxacin, a chiral fluorinated carboxyquinolone, is the pure (-)-(S)-enantiomer of the racemic drug substance ofloxacin. The chemical name is (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrates [15]. Levofloxacin is an antibiotic which is used in the treatment of a number of bacterial infections including acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis, and some types of gastroenteritis [31].

Just like every other antibiotics, levofloxacin may be used to treat tuberculosis, meningitis, or pelvic inflammatory disease [30]. Levofloxacin is also used as antibiotic eye drops to prevent bacterial infection [14]. Levofloxacin interferes with the bacterial DNA synthesis via inhibition of the DNA gyrase or topoisomerase IV [9]. DNA gyrase is an enzyme within the class of topoisomerase (Type II topoisomerase) [12] that relieve strain while double-stranded DNA is being unwound by helicase [*22*].

The enzyme causes negative supercoiling of the DNA or relaxes positive supercoils. DNA gyrase does so by looping the template so as to form a crossing, then cutting one of the double helices and passing the other through it before releasing the break, changing the linking number by two in each enzymatic step [27]. This process occurs in prokaryotes (in particular, in bacteria), whose single circular DNA is cut by DNA gyrase and the two ends are then twisted around each other to form supercoils. Gyrase has been found in the apicoplast of the malaria parasite *Plasmodium falciparum*, a unicellular eukaryote [7].

Bacterial DNA gyrase is the target of many antibiotics, including nalidixic acid, novobiocin, and ciprofloxacin [6]. DNA gyrase has two subunits, which in turn have two subunits each, i.e. the 2A and 2B subunits. The A and B subunits of DNA gyrase together binds to the DNA, hydrolyze ATP, and introduce negative supertwists. The A subunit carries out nicking of DNA, B subunit introduces negative supercoils, and then A subunit reseals the strands [10]. In this study, the In-Silico Structure-Activity Relationship and molecular docking study was directed at investigating the inhibitory effect of levofloxacin and its monosubstituted analogues on the structure and function of the *Escherichia coli* DNA Gyrase, by predicting the binding energies, number of hydrogen bonds formed and various pharmacokinetics parameters necessary for computational drug design.

2. Materials and Methods

Protein preparation

The crystal structure of the *Escherichia coli* DNA Gyrase, was obtained from the Protein Data Bank, PDB 5L3J (Figure 13). The protein structure was subjected to a refinement protocol using the Pymol viewer [8].

Designing of 6-Gingerol structural analogues

The structure of levofloxacin (Figure 1) was drawn with the Marvin Sketch software [24]. The structural analogues of levofloxacin were developed with structural modifications and different substituents [19]. The F substituent of levofloxacin was replaced with CH_3, OH, NH_2 and C=O groups. The structures were built with the Marvin Sketch software and minimized using the Chimera software [13, 23].

Molecular docking

Molecular docking was performed using AutoDock Vina Software [25]. Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of 6-Gingerol and its analogues were determined using SwissADME Server [5].

3. Results and Discussions

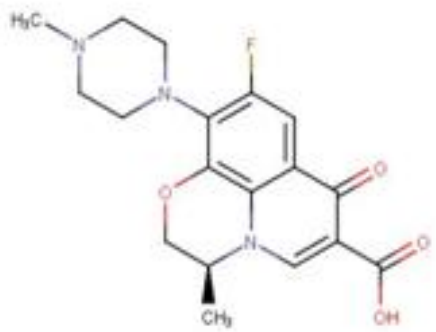

Figure 1: Levofloxacin structural formula

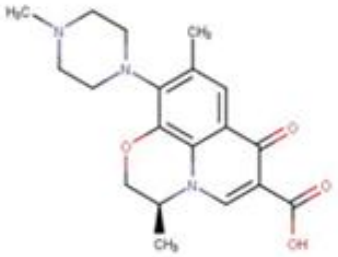

Figure 2: CH_3 analogue of Levofloxacin.

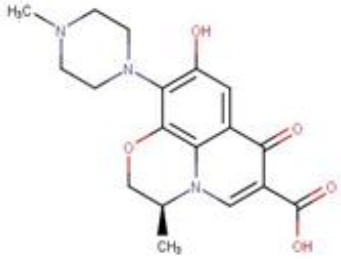

Figure 3: OH analogue of Levofloxacin

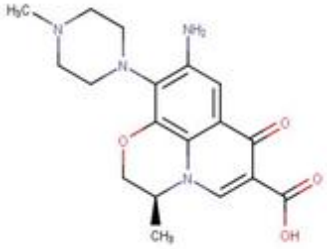

Figure 4: NH_2 analogue of Levofloxacin

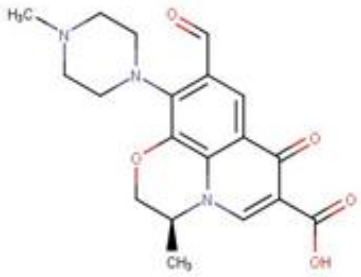

Figure 5: C=O analogue of Levofloxacin

Docking results

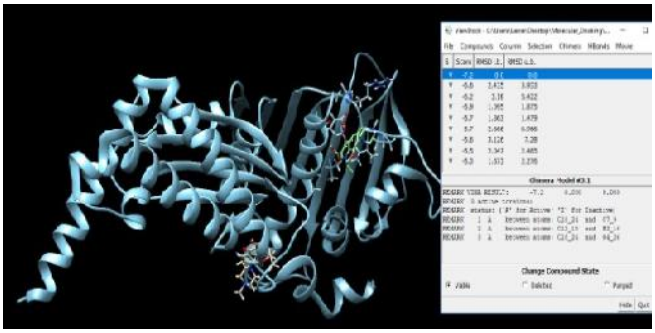

Figure 6: Levofloxacin in complex with *E. coli* DNA Gyrase

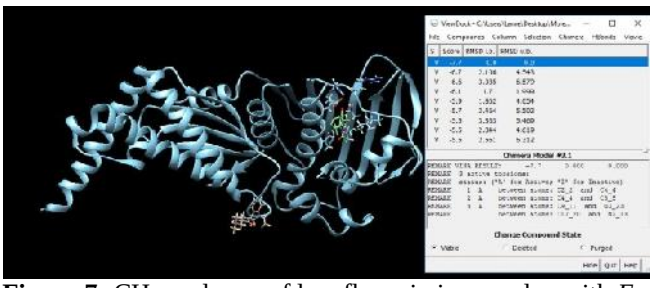

Figure 7: CH_3 analogue of levofloxacin in complex with *E. coli* DNA Gyrase.

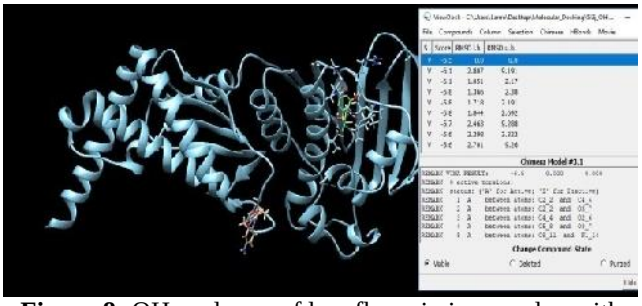

Figure 8: OH analogue of levofloxacin in complex with *E. coli* DNA Gyrase.

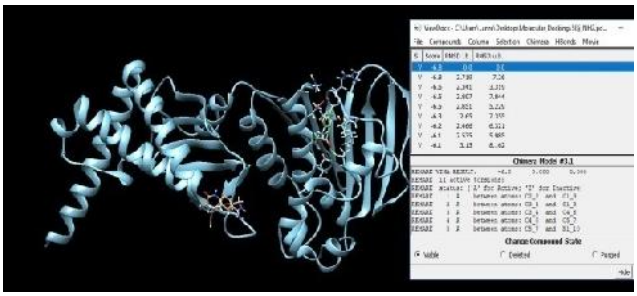

Figure 9: NH_2 analogue of levofloxacin in complex with *E. coli* DNA Gyrase.

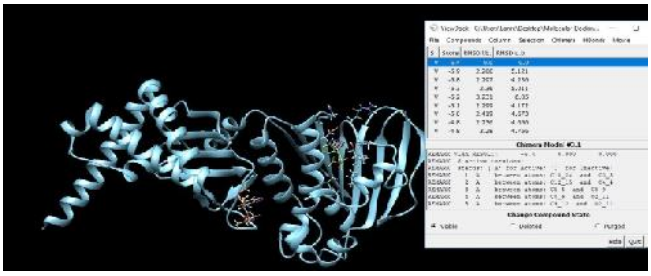

Figure 10: C=O analogue of levofloxacin in complex with E. coli DNA Gyrase.

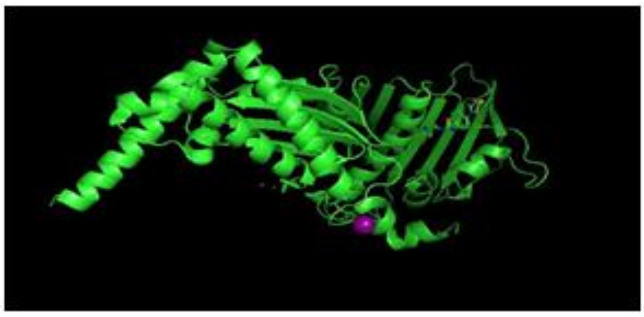

Figure 11: Crystal structure of the *E. coli* DNA Gyrase PDB 5L3J.

Discussion

Escherichia coli DNA Gyrase contains 378 amino acid residues. The docking structures of all the compounds showed that they bind in a very similar pattern with the active site of *Escherichia coli* DNA Gyrase, as is evident from the superposition of the levofloxacin and all its 4 analogues in Figures 6-10. The interaction between levofloxacin and the different monosubstituted analogues with *Escherichia coli* DNA Gyrase shows steric interaction with the amino acid residues. The calculated free energy of binding of the levofloxacin and its analogues were -7.2, -7.7, -6.5, -6.8, and -6.4Kcal/mol (Table 1). This confirms that the structural modification implemented in this study is significantly related to their activity [17, 21]. Also, this proved the reliability of the docking results [26]. Hydrogen-bonds play a crucial role in determining the specificity of ligand binding [28]. Their important contribution is explicitly incorporated into a computational method called GRID. This has been designed to detect energetically favorable ligand binding sites on a chosen target molecule of known structure [29]. It can be observed that substitution of the F substituent of levofloxacin with the CH3 and NH2 analogues led to an increase in the binding affinity of the modified analogues. The solubility ofa compound in water could improve its biotransformation and elimination as a drug [16]. Levofloxacin and all the substituted analogues were soluble in water (Table 1). The molecular weight of all the substituted derivatives including levofloxacin were less than 500g/mol, showing that they can be considered as drug [2]. A compound can also be considered drug-like if it is characterized by high lipophilicity (less than 5) [1]. This is expressed as Log Po/w. The lipophilicity values of levofloxacin and all the mono substituted compounds are less than 5 and are most likely to be drugs.

Lipinski'srule of 5 [18] helps in distinguishing between drug-like and non drug-like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules: Molecular mass less than 500g/mol; High lipophilicity (expressed as Log Po/w less than 5); Less than 5 hydrogen bond donors; Less than 10 hydrogen bond acceptors; Molar refractivity should be between 40-130. These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures [16]. Levofloxacin and all the monosubstituted analogues violated none of the Lipinski's rule and therefore are likely to be drugs (Table 1).

High penetration is needed for most of the drugs targeting the central nervous system (CNS), whereas blood brain barrier (BBB) penetration should be minimized for non-CNS drugs to avoid undesired side-effects [4]. Pharmacokinetically, the gastrointestinal drug absorption of all the substituents was high and could not cross the blood brain barrier (BBB). This shows that they cannot cause any problem to the brain. For synthetic accessibility, values of 5 to10 means that the drug could be synthesized [16]. Levofloxacin and all it analogues showed values less than 4. This means that the compounds can easily be synthesized in the laboratory. Synthetic studies followed by pre- clinical studies are further recommended.

Table 1: Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski drug likeness of Levofloxacin and its mono substituted analogues

Parameters	Levofloxacin	CH_3 analogue of Levofloxacin	OH analogue of Levofloxacin	NH_2 analogue of Levofloxacin	C=O analogue of Levofloxacin
Molecular weight g/mol	361.37	357.40	359.38	358.39	371.39
Docking score Kcal/mol	-7.2	-7.7	-6.5	-6.8	-6.4
Num. H-Bond formed with protein	4	8	8	13	2
Num. H-Bond acceptors	6	5	6	5	6
Num. H-Bond donors	1	1	2	2	1
Molar Refractivity	101.83	106.84	103.90	106.28	107.26
Lipophilicity Consensus Log $P_{o/w}$	1.10	1.12	0.45	0.29	0.52
Water Solubility Class	Very Soluble	Soluble	Very Soluble	Very Soluble	Very Soluble
GI absorption	High	High	High	High	High
BBB permeant	No	No	No	No	No
P-gp substrate	Yes	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	No	Yes	No	No	No
CYP3A4 inhibitor	No	No	No	No	No

Lipinski Drug likeness	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Synthetic accessibility	3.63	3.66	3.65	3.66	3.67

4. Conclusion

We carried out an In-Silico Structure Activity Relationship and molecular docking study on *Escherichia coli* DNA Gyrase, using levofloxacin and four of its structurally diverse analogues as the experimental compounds. The results obtained indicated that the CH_3 and NH_2 analogues may have a better functional activity having shown a high binding energy value and exhibited a higher level of specificity and affinity through the number of hydrogen bonds formed with the target enzyme. These analogues also pose no threat to the Central Nervous System (CNS) as they do not penetrate the blood brain barrier. Synthesis and pre-clinical studies of these monosubstituted derivatives with *Escherichia coli* DNA Gyrase is recommended. **Abbreviations:** PDB: Protein Data Bank; BBB: Blood Brain Barrier; CNS: Central Nervous System; UTI: Urinary Tract Infection; DNA: Deoxyribonucleic Acid.

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