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IN-SILICO STRUCTURE-ACTIVITY RELATIONSHIP AND MOLECULAR DOCKING STUDY OF LEVOFLOXACIN AND ITS MONO SUBSTITUTED ANALOGUES AGAINST THE ESCHERICHIA COLI AMINOGLYCOSIDE PHOSPHOTRANSFERASE

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Abstract

Resistance to aminoglycoside antibiotics has had a profound impact on clinical practice. Despite their powerful bactericidal activity, aminoglycosides were one of the first groups of antibiotics to meet the challenge of resistance. The most prevalent source of clinically relevant resistance against these therapeutics is conferred by the enzymatic modification of the antibiotics. Aminoglycoside phosphotransferases provide an important means for high-level resistance to aminoglycoside antibiotics. Levofloxacin is used to treat a number of bacterial infections including acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis, and some types of gastroenteritis. Along with other antibiotics it may be used to treat tuberculosis, meningitis, or pelvic inflammatory disease. Extensive structure activity relationship study was carried out with levofloxacin and its monosubstituted analogues which were designed by substituting the levofloxacin F group for C=O, CH_3, NH_2 and OH functional groups using the Marvin Sketch software. This was followed by a molecular docking study to target the experimental ligands against the E. coli aminoglycoside phosphotransferase active site. The result of the molecular docking study showed that the OH analogue of levofloxacin might be the better antibiotics to combat the resistance exhibited by the E. coli against aminoglycoside antibiotics.

Keywords: *Aminoglycoside; Resistance; Antibiotics; Levofloxacin; E. coli*

Introduction

The Escherichia coli (or E. coli) is the most prevalent infecting organism in the family of gram-negative bacteria known as enterobacteriaceae [1, 2]. E. coli bacteria were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich who also showed that certain strains of the bacterium were responsible for infant diarrhea and gastroenteritis, an important public health discovery [3, 4].

The E. coli that are responsible for the numerous reports of contaminated foods and beverages are those that produce Shiga toxin, so called because the toxin is virtually identical to that produced by Shigella dysenteria type 1 [5, 6]. The best-known and also most notorious E. coli bacteria that produce Shiga toxin is E. coli O157:H7 [7]. Shiga toxin–producing E. coli (STEC) cause approximately 100,000 illnesses, 3,000 hospitalizations, and 90 deaths annually in the United States. Most reported STEC infections are caused by E. coli O157:H7 [8, 9].

Enzymatic modification is the most common type of aminoglycoside resistance. Over 50different enzymes have been identified [10]. Enzymatic modification results in high-level resistance [11, 12]. The genes encoding for aminoglycoside modifying enzymes are usuallyfound on plasmids and transposons. Most enzyme-mediated resistance in gram-negative bacilliis due to multiple genes [13]. It is hypothesized that the enzymes are derived from organismsthat make the aminoglycoside or from the mutation of genes that encode the enzymes involved incellular respiration [14]

Levofloxacin is the levo isomer of the racemate ofloxacin, another quinolone antimicrobial agent [15]. Levofloxacin, a chiral fluorinated carboxyquinolone, is the pure (−)-(S)-enantiomer of the racemic ofloxacin [16]. Distinct functional groups on these molecules include a hydroxyl group, carbonyl group, and an aromatic ring [17]. Since sevofloxacin is the S-enantiomer, it binds more effectively to the DNA gyrase enzyme and topoisomerase IV than its counterpart enantiomer. The substance is used as the hemihydrate, which has the empirical formula $C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2} H_2O$ and a molecular mass of 370.38 g/mol [18].

The aim of this study is to evaluate the inhibitory effect of the modified analogues of levofloxacin against the E. coli aminoglycoside phosphotransferase in order to provide potent alternatives for the aminoglycoside antibiotics resistance exhibited by the E. coli.

Materials and Methods

Protein preparation

The crystal structure of the Escherichia coli aminoglycoside phosphotransferase, was obtained from the Protein Data Bank, PDB 3W0O (Figure 13). The protein structure was subjected to a refinement protocol using the Pymol viewer [19].

Designing of 6-Gingerol structural analogues

The structure of levofloxacin (Figure 1) was drawn with the Marvin Sketch software [20]. The structural analogues of levofloxacin were developed with structural modifications and different substituents [21]. 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 [22, 23].

Molecular docking

Molecular docking was performed using AutoDock Vina Software [24]. Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski drug likeness of levofloxacin and its analogues were determined using SwissADME Server [25].

Results and Discussion

The physicochemical property of proteins is critical for sustainability, efficiency, and stability in a biological system. Various physico-chemical parameters of proteins such as amino acid composition, extinction coefficient, instability index, grand average of hydropathicity (GRAVY), aliphatic index, theoretical pI, atomic composition and molecular weight allows us to understand the stability, activity and nature of the protein [26]. The result from the physiochemical property analysis of the E. coli aminoglycoside phosphotransferase showed that the enzyme is an acidic enzyme with a theoretical pI value in the acidic range of 5.16 [27]. The protein is made up of a total of 349 amino acids

as shown in table 1 with an estimated molecular weight of 39032.87 daltons.

The hydrophobicity scale produces a descriptive value for the relative hydrophobicity of residues of amino acids. The more positive or negative the value, the more hydrophobicity or hydrophilicity of the sequences located in that protein region [28]. The GRAVY calculator used in predicting the hydrophobicity assigned to the protein a value of -0.252.

The docking structures of all the compounds showed that they bind in a very similar pattern with the active site of Escherichia coli aminoglycoside phosphotransferase, as is evident from the superposition of the levofloxacin and all its 4 analogues in Figure 1. The interaction between levofloxacin and the different monosubstituted analogues with Escherichia coli aminoglycoside phosphotransferase shows steric interaction with the amino acid residues. The calculated free energy of binding of the levofloxacin and its analogues were -8.2, -8.7, -8.6, -8.2, and -7.6Kcal/mol (Table 1). This confirms that the structural modification implemented in this study is significantly related to the increased antibiotic activity exhibited by the OH analogue of levofloxacin [29, 30]. This also proved the reliability of the docking results [31].

The solubility of a compound in water could improve its biotransformation and elimination as a drug [32]. 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. A compound can also be considered drug-like if it is characterized by high lipophilicity (less than 5) [33]. This is expressed as Log Po/w. The lipophilicity values of levofloxacin and all the monosubstituted compounds are less than 5 and are most likely to be drugs.

Lipinski'srule of 5 [34] 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 [32]. 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 [35]. 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 [32]. 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.

Conclusion

An In-Silico Structure Activity Relationship and molecular docking study was carried out on the Escherichia coli aminoglycoside phosphotransferase, using levofloxacin and four of its structurally diverse analogues as the experimental compounds. The results obtained indicated that the OH analogue might have a better functional activity having shown a lower binding energy score against 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 the OH analogue of levofloxacin, targeted at the Escherichia coli aminoglycoside phosphotransferase active site is therefore recommended.

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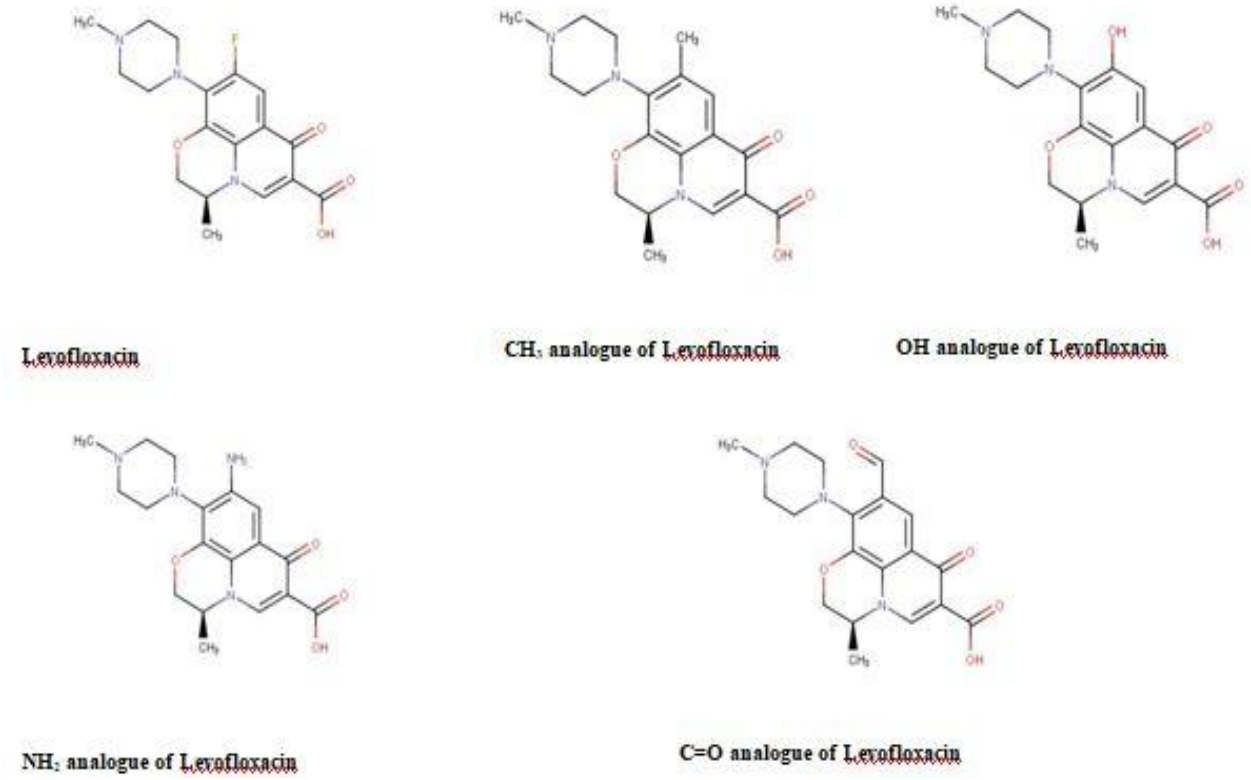
Table 1: Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of Levofloxacin and its monosubstituted analogues

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	-8.2	-8.2	-8.6	-8.2	-7.6
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 Druglikeness	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

Figure 1: Structural formula of levofloxacin and its modified analogues



Structural formula

Figure 2: Crystal structure of the *E. coli* aminoglycoside phosphotransferase PDB 3WoO

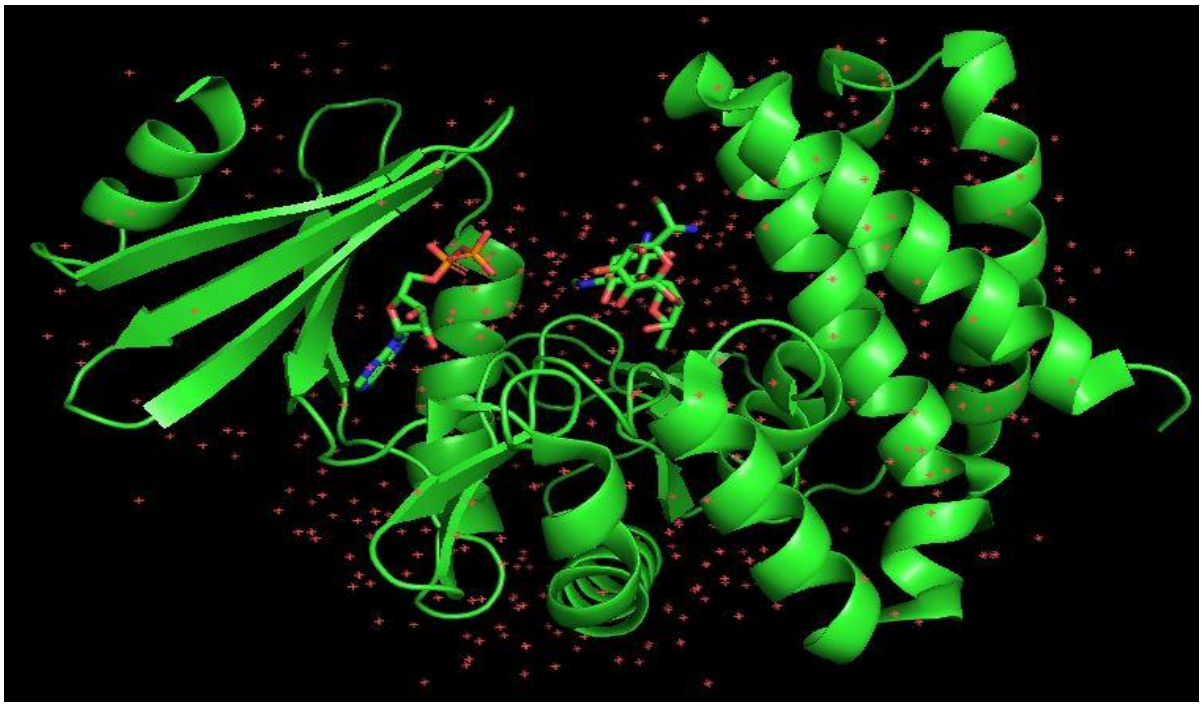


Figure 3: Levofloxacin in complex with *E. coli* aminoglycoside phosphotransferase.

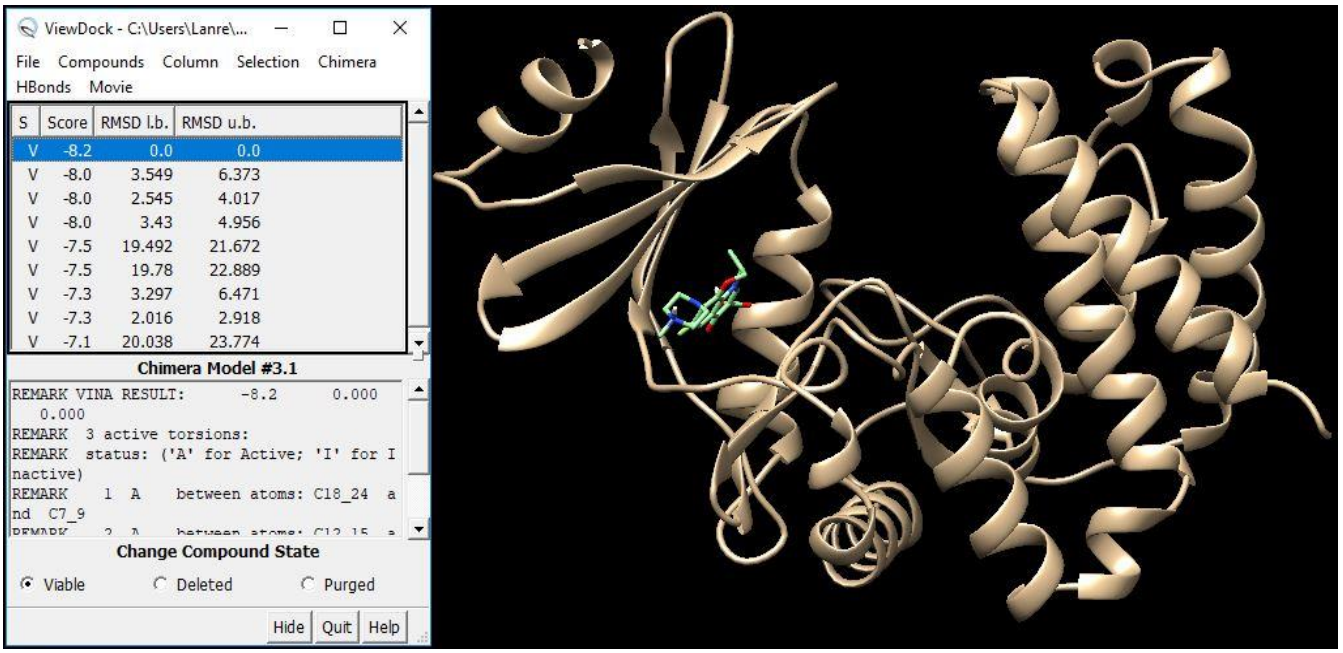


Figure 4: OH analogue of levofloxacin in complex with *E. coli* aminoglycoside phosphotransferase.