



# IN SILICO MOLECULAR DOCKING INTO GATIFLOXACIN DERIVATIVES: STRUCTURAL DETERMINANTS AND ANTIBACTERIAL EFFICACY

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**Abstract:** In this scientific context, Gatifloxacin, a fourth-generation fluoroquinolone antibiotic, demonstrates potent antibacterial activity against a wide range of pathogens. Gatifloxacin and its derivatives are synthetic heterogeneous groups commonly used in hospital and community sectors to treat numerous infections like sexually transmitted diseases, urinary tract infections, respiratory tract infections, conjunctivitis, skin infections, and meningococcal infections. Here, selecting an appropriate antibiotic in light of increasing resistance poses a significant challenge. However, the emergence of resistance to gatifloxacin underscores the need for novel derivatives with enhanced efficacy.

To address this, we employed to leverage computational methods to investigate the structural determinants between gatifloxacin derivatives and their target bacterial enzymes. Our goal was to predict and optimize their binding affinities as antibacterials. Through in silico molecular docking simulations using V-life MDS software, we evaluated the binding modes of diverse gatifloxacin derivatives against key bacterial targets, including DNA gyrase II, topoisomerases II and IV enzymes. (PDB ID: 5CPH).

Here we assessed the binding energies and intermolecular interactions by utilizing state-of-the-art docking algorithms and scoring functions. These analyses provided insights into the structural determinants influencing the potency of these derivatives. Among the compound libraries, gatifloxacin derivatives—namely Gati II, Gati V, Gati VII, Gati IX, Gati XII, and Gati XIV—exhibited the best binding free energy, 2D and 3D interaction images with interlinked amino acids. These findings offer valuable insights that can guide the rational synthesis of new compounds with potentially improved in vitro and in vivo antibacterial efficacy.

**IndexTerms - Gatifloxacin, Docking simulation, 5CPH, Antibacterial activity**

## Introduction

The era of Fluoroquinolones is effective broad-spectrum antibiotics treating various life-threatening diseases. The paper seeks to review the significant progress of fluoroquinolone, from discovery to black box warning. [1] Although it is difficult to choose antibiotics in the face of growing resistance, the introduction of fluoroquinolones has created a new exciting exposure in antimicrobial therapy. It having remarkable highest bacteriologic and clinical cure rates with low resistance. The itinerary of quinolone antibiotics began with the serendipitous discovery of the prototype namely nalidixic acid, in 1960. [2] The novel synthetic agents have been developed extensively day by day. Such synthetic development to combat in healthcare armory to optimize antimicrobial activity with drug safety. [3]

Gatifloxacin is a fourth-generation antimicrobial agent to treat urinary tract infection, respiratory tract infections, skin infections, meningococcal & mycobacterial infections, bone disorder and sexually transmitted diseases. [4] The Indian Drugs Controller General has made it clear that, topical ophthalmic gatifloxacin formulations, tablet dosage forms for 200 mg and 400 mg are banned in India due to dysglycemic adverse effect but eye drops and ointments, are not prohibited in India. [5] The gatifloxacin was available in ophthalmic, tablet and aqueous solutions for intravenous therapy. Nevertheless, the prescribing instructions states that the ophthalmic solution of gatifloxacin was not given the same consideration. [6]

In human medicine, gatifloxacin is a synthetic antibiotic which is frequently used to treat infections brought on by both gram-positive, gram-negative bacteria, aerobic and atypical micro-organisms. [7] These gatifloxacin retain their efficacy against gram-negative pathogens, however their potency against gram-negative bacteria namely Staphylococcus aureus, Pseudomonas aeruginosa is comparatively higher than that of ciprofloxacin. In particular, ciprofloxacin has a lower anti-Pseudomonas aeruginosa effect than these substances. [8] Older and

newer fluoroquinolones show excellent efficacy against various respiratory infections such as *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Legionella pneumophila*.<sup>[9]</sup>

These antimicrobial medications have poor intestinal absorption, which prevents them from being completely digested after administration.<sup>[10]</sup> These medications can be eliminated through the urine and faeces, either as active metabolites or in their unmetabolized forms, contaminating surface water.<sup>[11]</sup> Gatifloxacin exhibit lethality that is dependent on concentration. The metrics that seem to have the strongest correlation with clinical efficacy are the ratios of the peak concentration (C<sub>max</sub>) to the MIC and the area under the curve (AUC) to the MIC. Due to disagreements over the proper end aim to pursue, be it microbiological or clinical, and the absence of a reliable database to precisely determine the predictive value of these pharmacodynamic indicators, there has been debate about which of these best correlates with efficacy.<sup>[12]</sup>

When the pharmacokinetic and pharmacodynamic properties of ciprofloxacin and levofloxacin with those of gatifloxacin, gemifloxacin, and moxifloxacin.<sup>[13]</sup> It should be noted that due to the pharmacokinetic diversity seen in patient groups, simple single-point MIC<sub>90</sub> values may not adequately reflect effective medication activity. This molecule was previously known as AM-1155.2.<sup>[14]</sup> Tokyo, Japan's Kyorin Pharmaceutical Co., Ltd. was responsible for its development. In Japan it is designated as Gatiflo, and in the United States as Zymar. In 2003, Zymar was launched as a therapy for bacterial conjunctivitis. One way to describe bacterial conjunctivitis is as an inflammation of the conjunctiva. Pyogenic bacteria are the cause of it, and symptoms include conjunctival irritation that can often be seen on other areas of the eye, blurred vision, excruciating pain, and a mucopurulent discharge. If left untreated, the infection may result in blindness.<sup>[15]</sup> This illness is treated with strong topical antibiotic medication and occasionally steroid therapy.<sup>[16]</sup>

Fig. 1: Kekule structure of Gatifloxacin

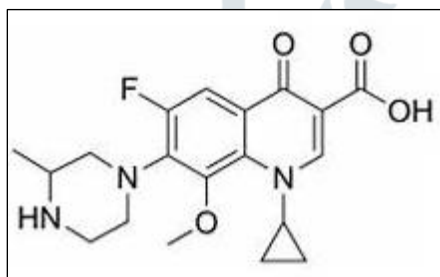
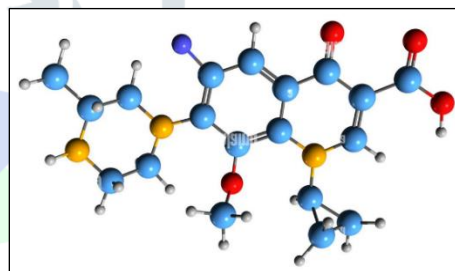


Fig. 2: Ball & Stick model of Gatifloxacin



So, there is need to explore antibacterial weapons to combat numerous infectious diseases by gatifloxacin & its derivatives as most prominent entity.

Here, gatifloxacin exhibit the antimicrobial activity by forming a reversible ternary complex with their bacterial targets. It inhibits DNA gyrase and topoisomerase II, topoisomerase IV and DNA, blocking bacterial growth and chromosome fragmentation which involved in DNA replication, recombination and repair.<sup>[17,18,19]</sup>

The affinity of gatifloxacin with metal ion seems likely to be an important prerequisite of antibacterial action.<sup>[20,21,22]</sup> Therefore, collected evidences suggested that standard doses of the gatifloxacin and gatifloxacin derivatives (3<sup>rd</sup> position) are clinically effective against viral and parasitic infections.<sup>[23,24, 25]</sup>

#### MATERIALS AND METHODS:

The different derivatives of gatifloxacin has been screened for in silico molecular docking by using V- Life MDS software paid version. Here, gatifloxacin derivatives are screened for antibacterial activity. Gatifloxacin contain COOH group at third position which is modified by bulky groups through ester formation. These bulky groups are nothing but the natural chemical constituents isolated from plants. According to literature survey it is reported that, bulky groups or increased molecular weight at third position of gatifloxacin more prone to antibacterial activity. Here, some plantbased constituents are listed namely  $\beta$ -sitosterol, stigmasterol, lanosterol, Quercetin, Kampferol, Bergenin, Rutin. They contain aromatic ring with hydroxyl group which is easy to form ester bond with carboxylic group of gatifloxacin. Table 1 showed the total 14 derivatives of gatifloxacin among that 6 derivatives exhibited best binding energy for antibacterial activity.

Table 1: Gatifloxacin derivatives

Gatifloxacin Derivatives	
Gati I	Gati VIII
Gati II	Gati IX
Gati III	Gati X
Gati IV	Gati XI
Gati V	Gati XII
Gati VI	Gati XIII
Gati VII	Gati XIV

Fig. 3: Ball stick model of Gati II

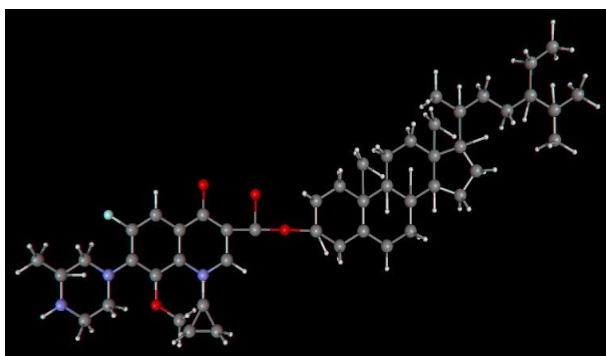


Fig. 4: Ball stick model of Gati V

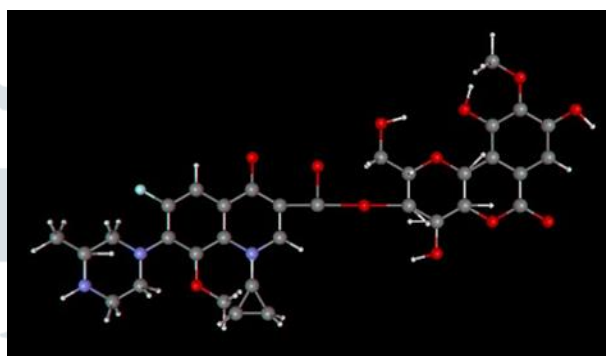


Fig. 5: Ball stick model of Gati VII

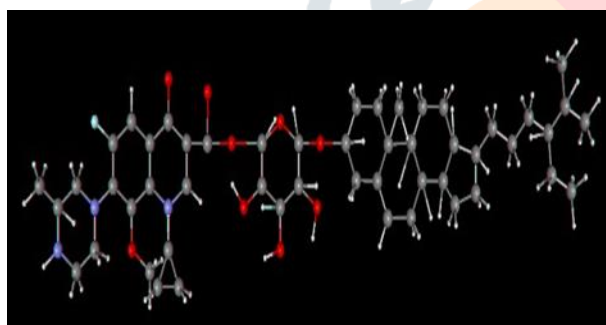


Fig. 6: Ball stick model of Gati IX

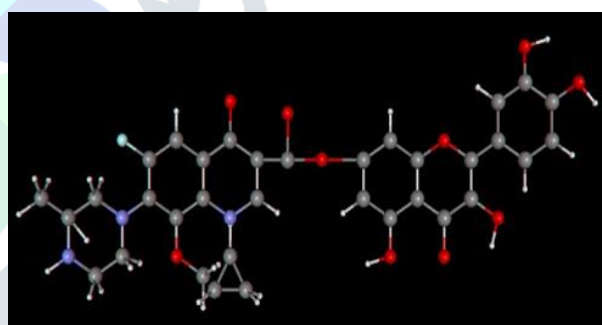


Fig. 7: Ball stick model of Gati XII

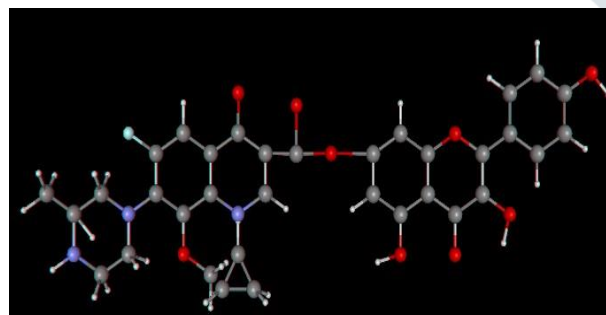
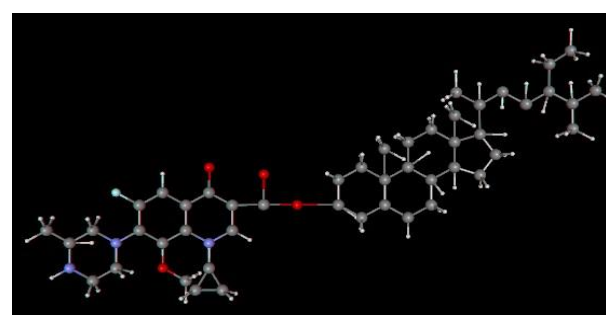



Fig. 8: Ball stick model of Gati XIV



In silico molecular docking achieve an optimized confirmation / orientation when ligand bind to protein surface or receptor to form stable complex in such way that free energy is get minimized in terms of affinity and activity. [26]

Table 2: Macromolecular content

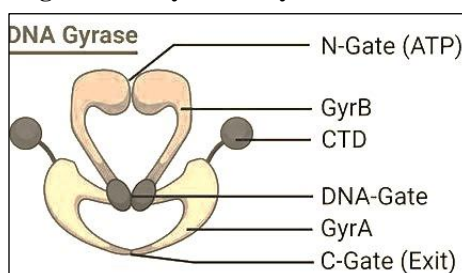
• Structure	
• Classification	Isomerase Inhibitor
• Organism	Staphylococcus aureus
• Molecule	DNA gyrase B
• Chains	Chain A, Chain B
• Sequence Length	212
• Mutation gene name	gyr B EC: 5.99.1.3:
• Expression System	Escherichia coli BL21(DE3)
• Method	X-RAY DIFFRACTION
• Resolution	1.20 Å
• R-Value Free	0.194
• R-Value Work	0.172
• R-Value Observed	0.173
• Total Structure Weight	48.62 kDa
• Atom Count	3,704
• Modelled Residue Count	378
• Deposited Residue Count	424
• Unique protein chains	1

5 CPH belongs to crystal structure of the ATP binding domain of *S. aureus* GyrB complexed with a fragment. [27,28]

#### PDB ID:5CPH

DNA gyrase has two subunits – DNA gyrase subunit A & subunit B. DNA gyrase enzyme belongs to type II Topoisomerase family, it is bacterial enzyme involved in DNA replication & repair. It removes supercoiling of DNA strand by cutting one strand and passing another strand from this generated gap. This negative supercoiling results into to relieve torsional strain, separate DNA is available for DNA replication & repair. Further, steps are continued with transcription and translation process. But, DNA gyrase inhibitor accumulate positive supercoiling will does not allow to separate DNA strand available for DNA replication & repair. [29,30,31]

Fig. 9 DNA Gyrase enzyme



Steps has been performed on V-Life MDS: [32,33,34]

### 1. Selection of Protein:

The suitable protein structure was searched on the Protein Data Bank (PDB) which played vital role in structure-based drug design studies. Here, protein was selected on the basis of enzymes namely DNA gyrase II, topoisomerase II and topoisomerase IV which is responsible for antibacterial activity. The different key factors for selection of targeted protein has been taken into consideration: i) multiple crystal structures existed for the said protein ii) lower resolution of protein iii) data completeness, R-factor, and electron density maps.

### 2. Preparation of Protein

**a. Addition of Hydrogen:** Hydrogen atoms are often involved in hydrogen bonding interactions, is critical in determining the binding affinity, specificity and strength between protein-ligand complex which reflects biological activity. They help maintain proper energetics, geometry and topology during the docking process. Hence, hydrogen atoms are added from right side window, indicating the presence of distinct shell structures.

**b. Incomplete residue:** All proteins has a variety confirmation shows flexible structures. By enabling the incomplete residue to investigate several locations. Hence, selected Biopredicta tools, clicked on Edit, then incomplete residue showed in output window, clicked on Mutate, again same Amino acids selected and finally clicked OK. So, incomplete residues can symbolize this flexibility and possibly result in a more precise prediction of the binding pose during docking.

**c. Missing residue:** Actually, missing residues shows docking simulation gaps due to experimental limitation or incomplete data at active site of protein. To fulfil this criteria, selected Biopredicta Tools, Loop builder showed one window. So, start anchor and end anchor has been selected, so amino acid database showed in output window classified under missing residue Amino acid chain. Then selected same amino acid chain in another box. And clicked for Search. Then it gave one window briefed about numbers of Hit includes similarity & RMS value. So, one Hit was selected based on whose RMS value is less and similarity score is more. After selecting one Hit, went to loop and clicked on Insert. In this way, missing residue has been added to protein.

**d. Chain separation:** Enormous proteins in biological systems consist of multiple chains or subunits explicitly contributing key role in cellular processes, such as signal transduction, enzymatic reactions, and structural support. Chain separation allowed to concentrate on certain binding sites and comprehend the interactions between ligands and these sites. So, to achieve this step, in Biopredicta tools clicked on Edit option. Then whole chain A containing amino acid has been selected. So, chain A was separated and the file was saved Refined-Chain A Mol. In similar way, separated the different chains was present until and unless co-crystalline ligand will remain and saved by Refined-Chain B Mol, Refined-Chain C Mol, etc.

**e. Co-crystalline ligand:** By using a cocrystal as a ligand to be docked in the binding pocket as a substrate/inhibitor of any receptor defeats the underlying purpose, as these cocrystals are not supposed to manifest any pharmacological action. Already chains like chain A, chain B, chain C separated. So, co-crystalline ligands were identified who does not contain amino acids abbreviation. Then applied same procedure to extract and save separate co-crystalline ligand. It was saved as Reference 1, Reference 2, Reference 3 and so on for individual co-crystalline ligand.

**2. Preparation of Ligand:** Here, ligand file in Mole format was opened and hydrogen atoms were added from right side window and saved.

**3. GA Docking:** Now, protein and ligand both was ready to docked. So, docking was processed by clicking GA docking through Biopredicta tools. Afterwards, Reference 1 has been selected and clicked on

Finish. Then energy minimization value was shown in output window. Here, binding of ligand to protein always shows inversely proportional relationship to the energy minimization energy. So, negative energy minimization value is obtained for different Refined chains, co-crystalline ligands with different derivatives.

**4. 2 D Interaction:** After docking opened both files of protein & ligand was opened. Clicked on Tools & select merged molecule option. Further file is saved as merged molecule file. Then clicked on Analyze-Interaction-complex and select merged molecule file. Simultaneously, different parameters like Hydrophobic interaction, Pi interaction, Vander wall forces were selected. Next step was continued with selection of ligand – Untitled 1- pressed on Print interaction. It was reflected & saved as 2 D interaction image in JPEJ format.

**5. 3 D Interaction:** This observation made to see possible the different orientation and geometry of atoms or group of atoms of merged molecules (ligand-protein) in 3D space.

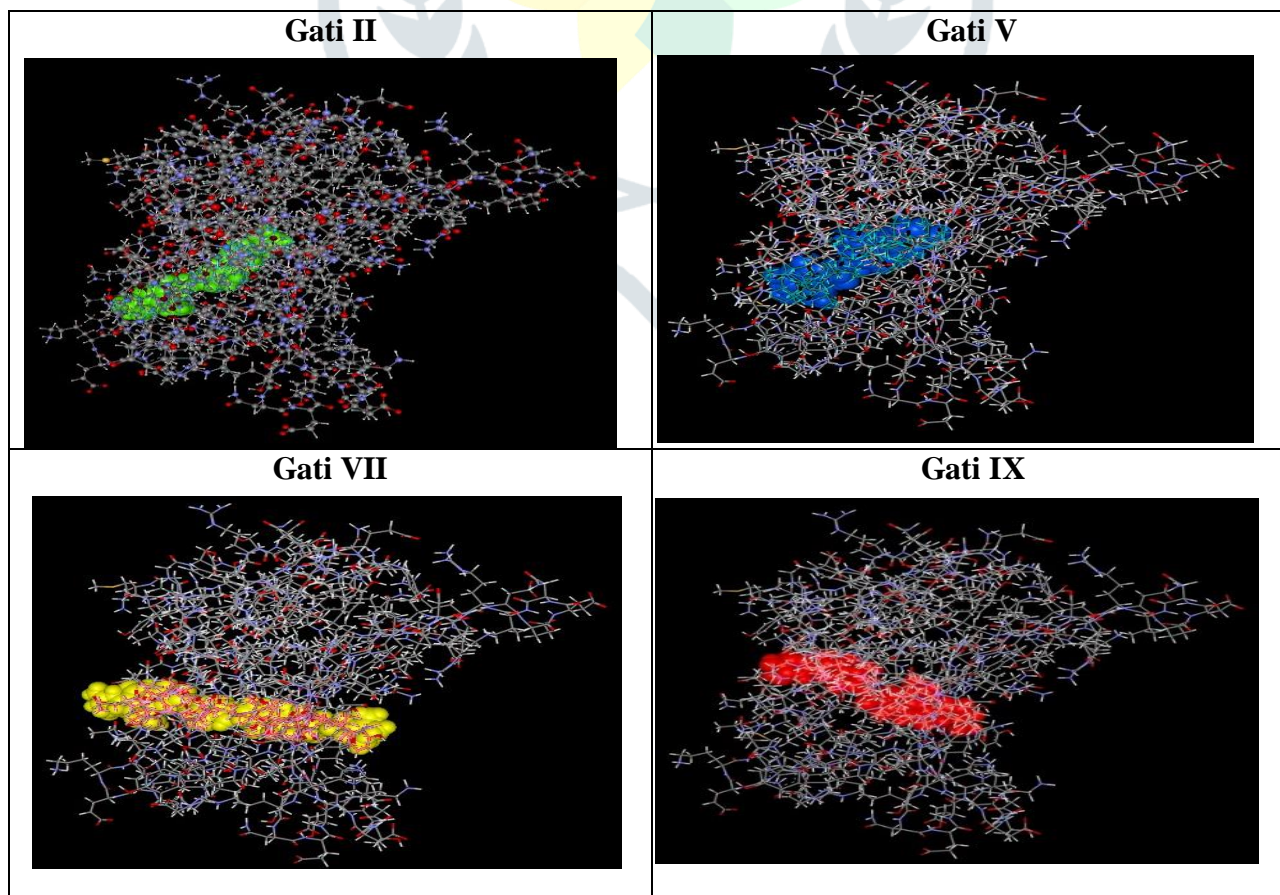
## RESULT AND DISCUSSION:

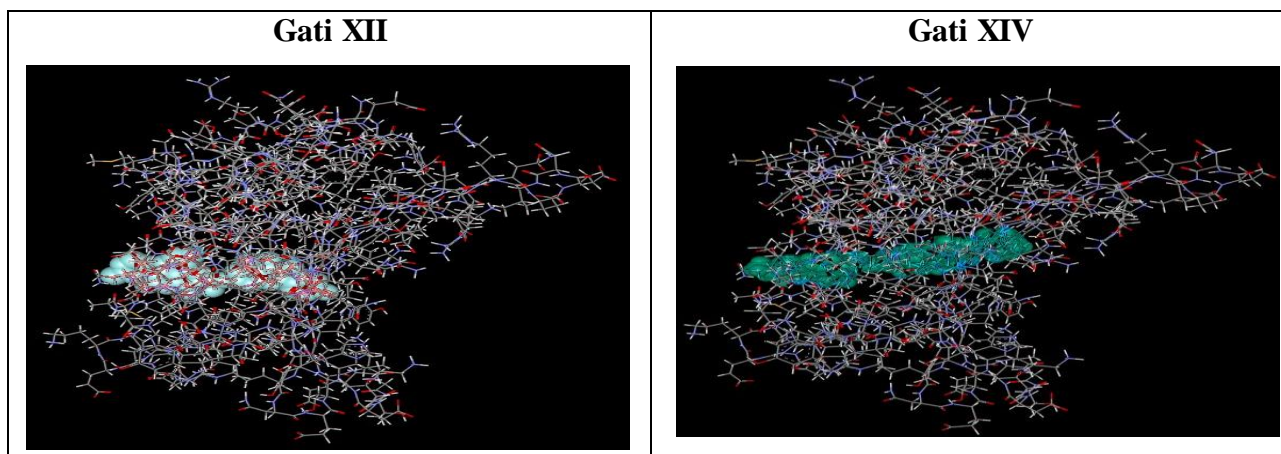
Table 3: Docking score

Sr. no.	Compound	Free binding energy (kcal/mol)	No. of H-bond interaction
1.	Gati II	-10.3	4
2.	Gati V	-10.6	9
3.	Gati VII	-8.6	5
4.	Gati IX	-9.7	4
5.	Gati XII	-10.2	7
6.	Gati XIV	-10.5	6
7.	Std	-7.3	1

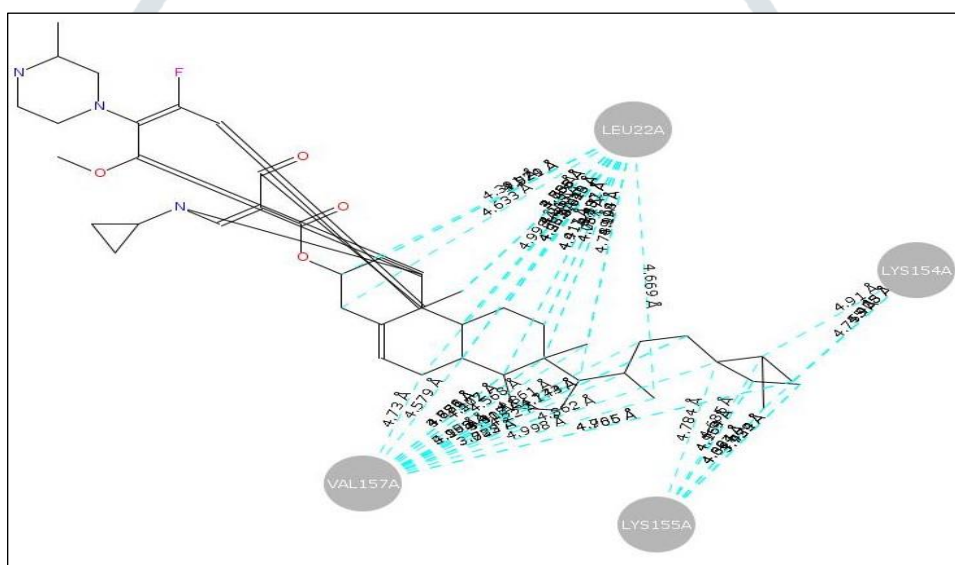
Here, Table 3 showed the interaction of 5CPH which is bacterial targeted protein with different gatifloxacin derivatives present in Table 1. As greater the H interactions with amino acids, more free binding energy explicitly claimed that Gati II, Gati V, Gati VII, Gati IX, Gati XII, Gati XIV having less free binding energy than standard one that is gatifloxacin which is shown in Table 3.

Table 4: 3 D Interactions of Gatifloxacin derivatives with 5CPH protein

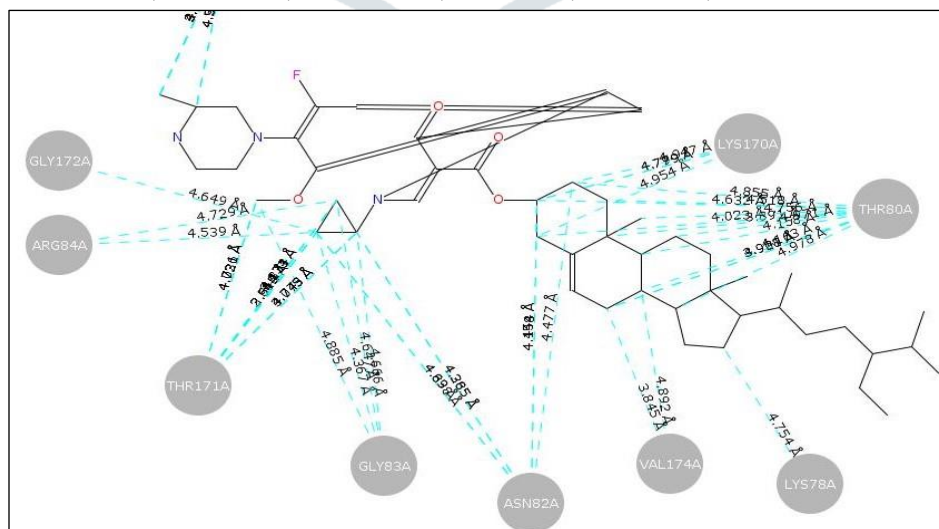




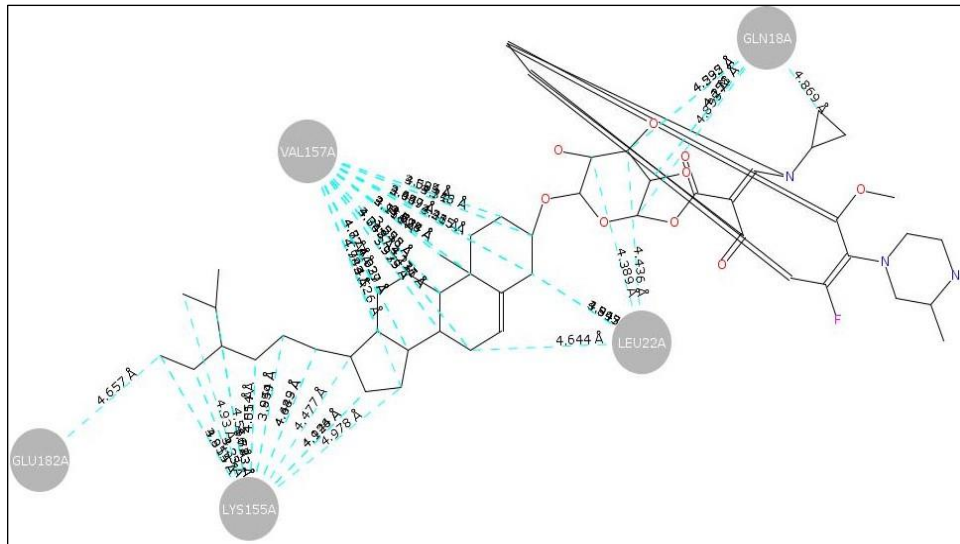
**Fig. 10: Gati II: Free binding energy = - 10.3**  
 Linked Amino acid = VAL157A, LYS55A, LYS154A, LEU22A



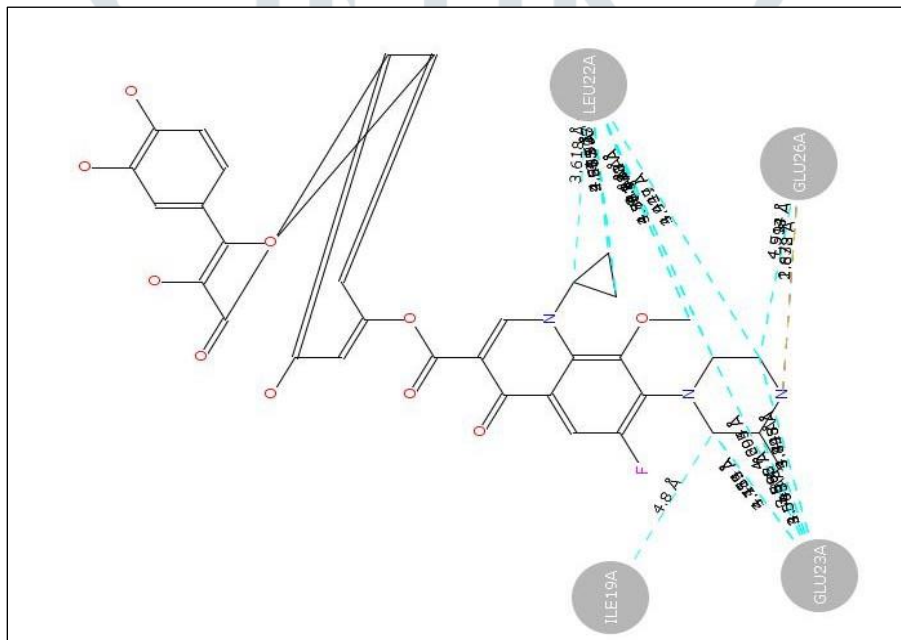
**Fig 11: Gati V: Free binding energy = - 10.6**  
 Linked Amino acid = GLY172A, GLY172A, ARG84A, THR171A, GLY83A, ASN82A, VAL174A, LYS78A, THR80A, LYS170A



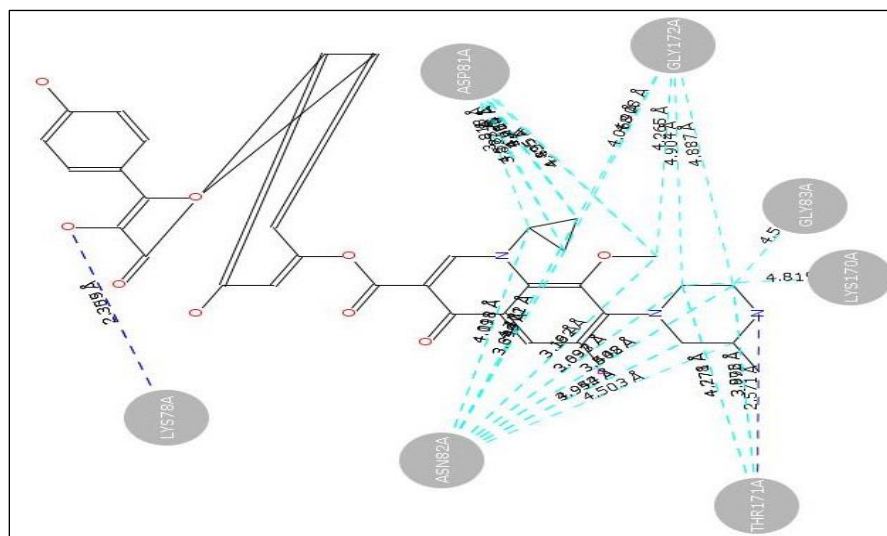
**Fig 12: Gati VII: Free binding energy = - 8.6**  
 Linked Amino acid = VAL157A, GLU182A, LYS155A, LEU22A, GLN18A



**Fig. 13: Gati IX: Free binding energy = - 9.7**  
Linked Amino acid = ILE19A, GLU23A, GLU26A, LEU22A



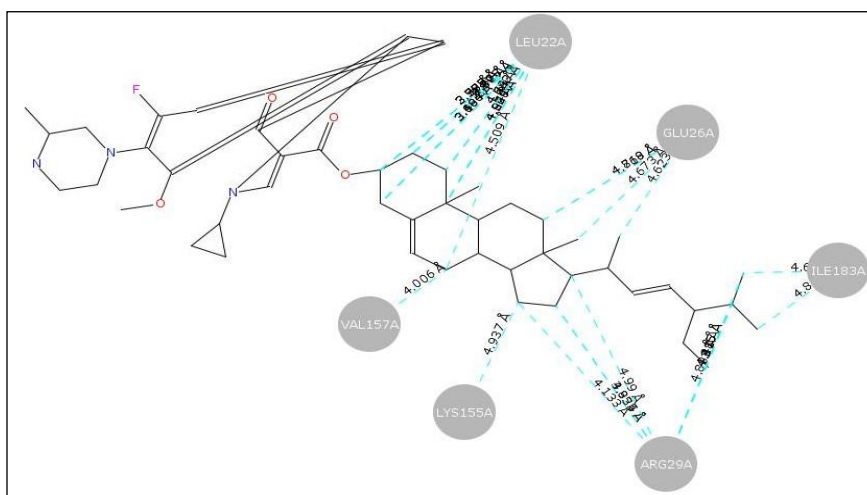
**Fig. 14: Gati XII: Free binding energy = - 10.2**  
Linked Amino acid = LYS78A, ASN182A, THR171A, LYS170A, GLY83A, GLY172A, ASP81A





**Fig 15: Gati XIV: Free binding energy = - 10.5**

Linked Amino acid = VAL157A, LYS155A, ARG29A, ILE183A, GLU26A, LEU22A

**CONCLUSION:**

The molecular docking of gatifloxacin derivatives revealed favorable binding interactions with targeted protein PDB ID: 5CPH. The gatifloxacin derivatives namely Gati II, Gati V, Gati VII, Gati IX, Gati XII, Gati XIV showed best binding free energy among compound database. These endeavors hold the insights gained from molecular docking studies pave the way for the rationale synthesis of novel derivatives could be possible with improved in-vitro & in-vivo therapeutic antibacterial potential. Further exploration of the underlying exploring new targets, optimizing lead compounds, exploring combination therapies of gatifloxacin derivatives.

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