IJBPAS, September, 2025, 14(9): 4991-5005

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

IN SILICO MOLECULAR DOCKING OF GATIFLOXACIN ANALOGUES AS ANTIBACTERIAL SCAFFOLDS

MAJALEKAR P*[1] AND SHIROTE P[2]

1: Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India

2: Director, Sarojini College of Pharmacy, Kolhapur

***Corresponding Author: Dr. Priyanka Majalekar: E Mail: majalekarpriyanka@gmail.com**

Received 15th April 2024; Revised 20th Aug. 2024; Accepted 15th Sept. 2024; Available online 1st Sept. 2025

https://doi.org/10.31032/IJBPAS/2025/14.9.9382

ABSTRACT

In this scientific context, Gatifloxacin, a fourth-generation fluoroquinolone antibiotic, demonstrates potent antibacterial activity against a wide range of pathogens. Gatifloxacin and it's 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.

Keywords: 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 iternrary 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. **[9]** Theses antimicrobial medications have poor intestinal absorption, which prevents them from being completely digested after administration. **[10]** These medications can

be eliminated through the urine and faeces, either as active metabolites or in their unmetabolized forms, contaminating surface water. **[11]** 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 (Cmax) 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. **[12]**

When the pharmacokinetic and pharmacodynamic properties of ciprofloxacin and levofloxacin with those of gatifloxacin, gemifloxacin, and moxifloxacin. **[13]** It should be noted that due to the pharmacokinetic diversity seen in patient groups, simple single-point MIC90 values may not adequately reflect effective medication activity. This molecule was previously known as AM-1155.2. **[14]** 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. **[15]** This illness is treated with strong topical antibiotic medication and occasionally steroid therapy **[16]**.

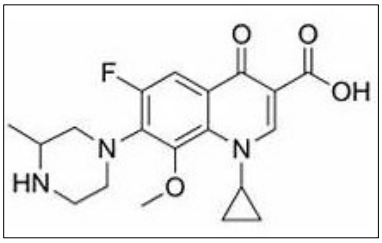


Figure 1: Kekule structure of Gatifloxacin

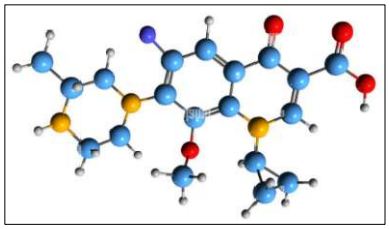


Figure 2: Ball & Stick model of Gatifloxacin

So, there is need to explore antibacterial weapons to combat numerous infectious diseases by gatifloxacin & it's 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 **[17, 18, 19]**.

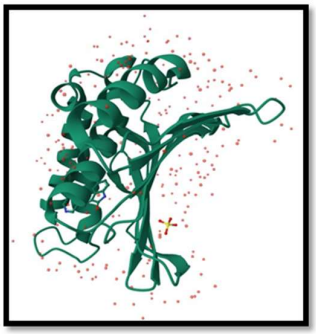
The affinity of gatifloxacin with metal ion seems likely to be an important prerequisite of antibacterial action **[20, 21, 22]**. Therefore, collected evidences suggested that standard doses of the gatifloxacin and gatifloxacin derivatives (3rd position) are clinically effective against viral and parasitic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

MATERIAL METHOD:

The different derivatives of gatifloxacin has been screened for in silico molecular docking by using V- Life MDS software. Here, 6 gatifloxacin derivatives were hypppothesizd in which substitution at 3rd position of gatifloxacin is engaged with bulky aromatic rings with hydroxyl groups. So, some are listed here like β-sitosterol, stigmasterol, lanosterol, Quercetin, Kampferol, Bergenin.

In silico molecular docking achieved 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 1: Macromolecular Content

• **Structure**	
• **Classification**	**Isomerase Inhibitor**
• **Organism(s)**	**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 relive 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]**.

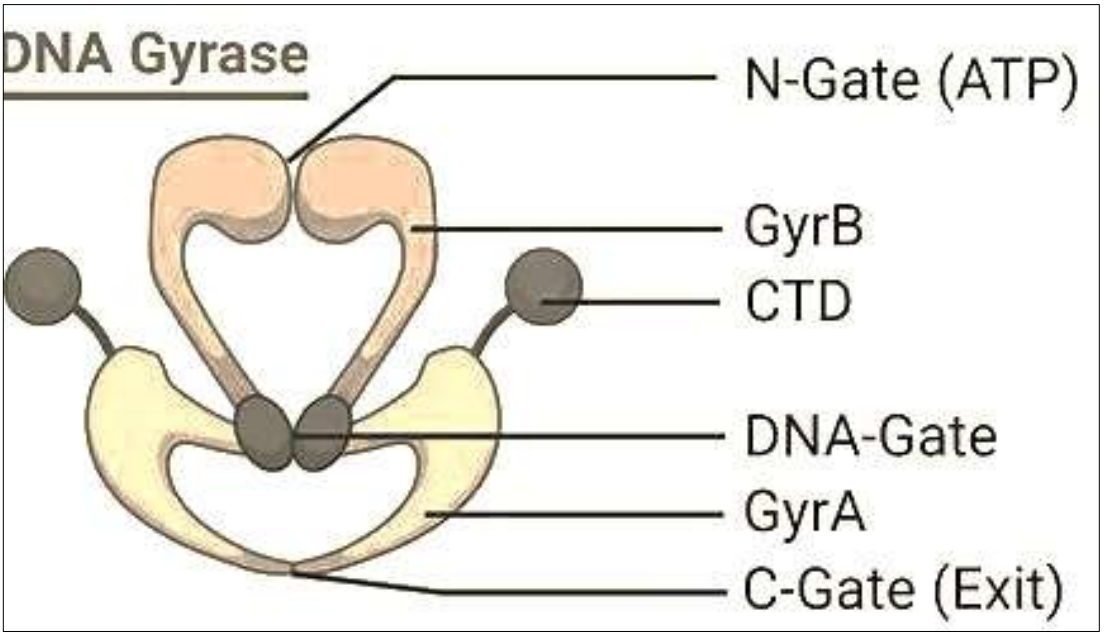


Figure 3: DNA Gyrase enzyme

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 targated 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.

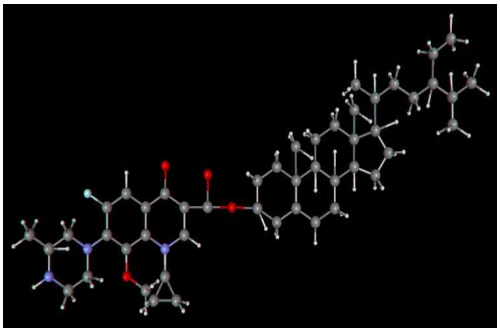


Figure 4: Ball stick model of Gati I

Figure 5: Ball stick model of Gati II

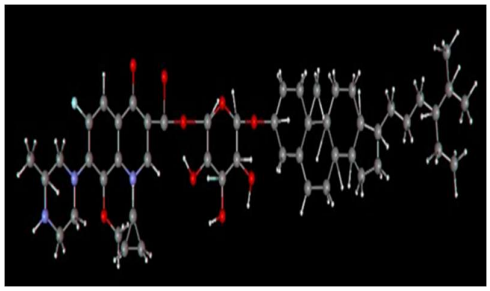


Figure 6: Ball stick model of Gati III

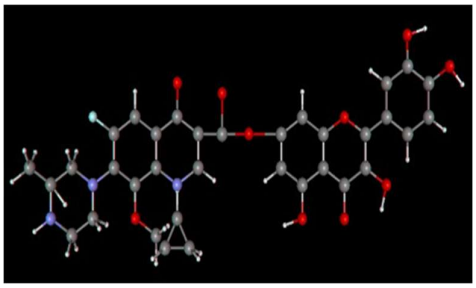


Figure 7: Ball stick model of Gati IV

Figure 8: Ball stick model of Gati V

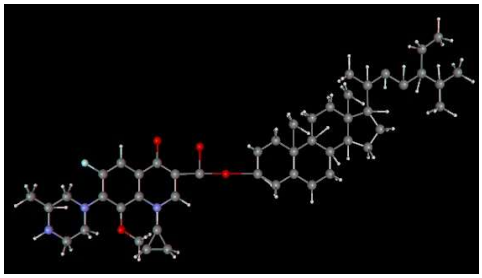


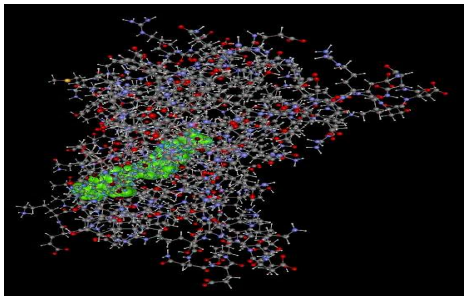
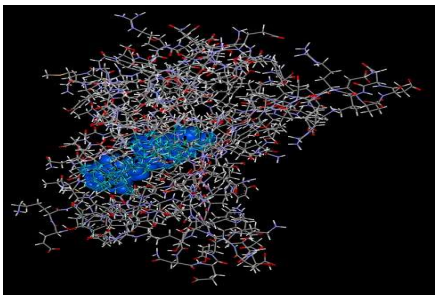
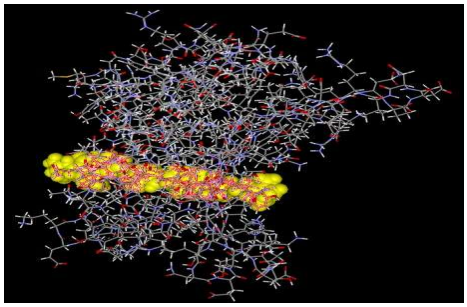
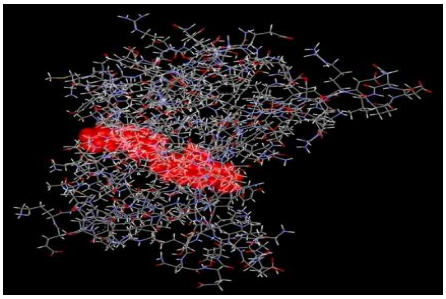
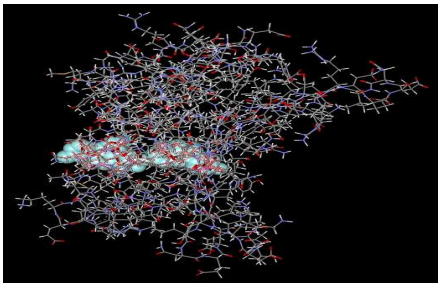
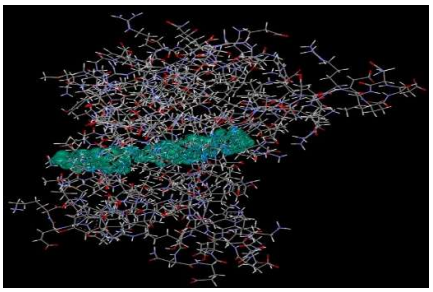
Figure 9: Ball stick model of Gati VI

RESULT AND DISCUSSION:

Table 2: Docking score

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

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

 **Gati I**	 **Gati II**
 **Gati III**	 **Gati IV**
 **Gati V**	 **Gati VI**

As greater the H interactions with amino acids, more free binding energy explicitly claimed that Gati I, Gati II, Gati III, Gati IV, Gati V, Gati VI having less free binding energy than standard one that is gatifloxacin which is shown in **Table 3**.

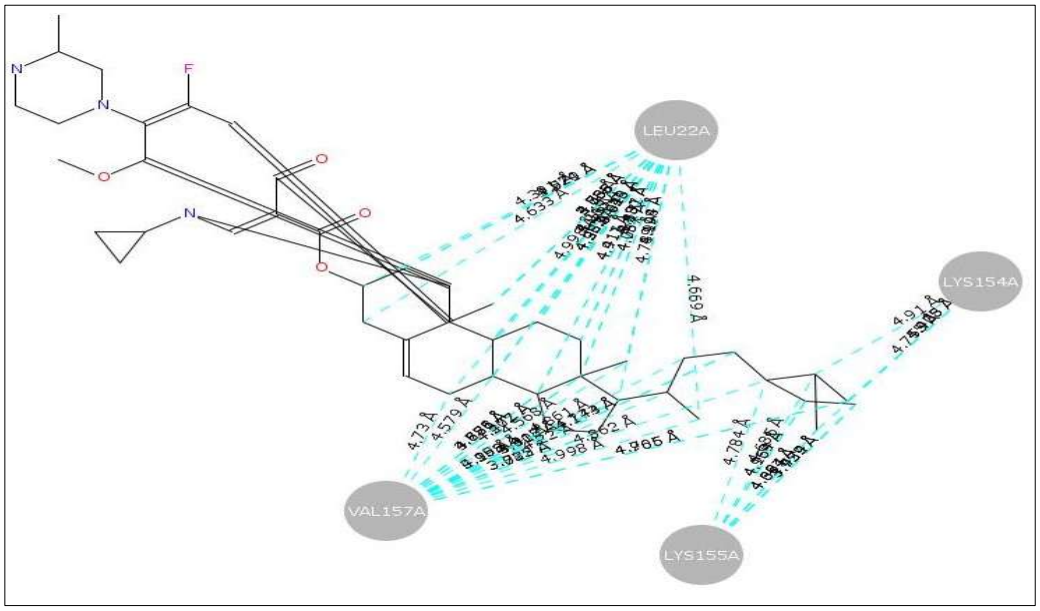


Figure 10: Gati I: Free binding energy = - 10.3
Linked Amino acid = VAL157A, LYS55A, LYS154A, LEU22A

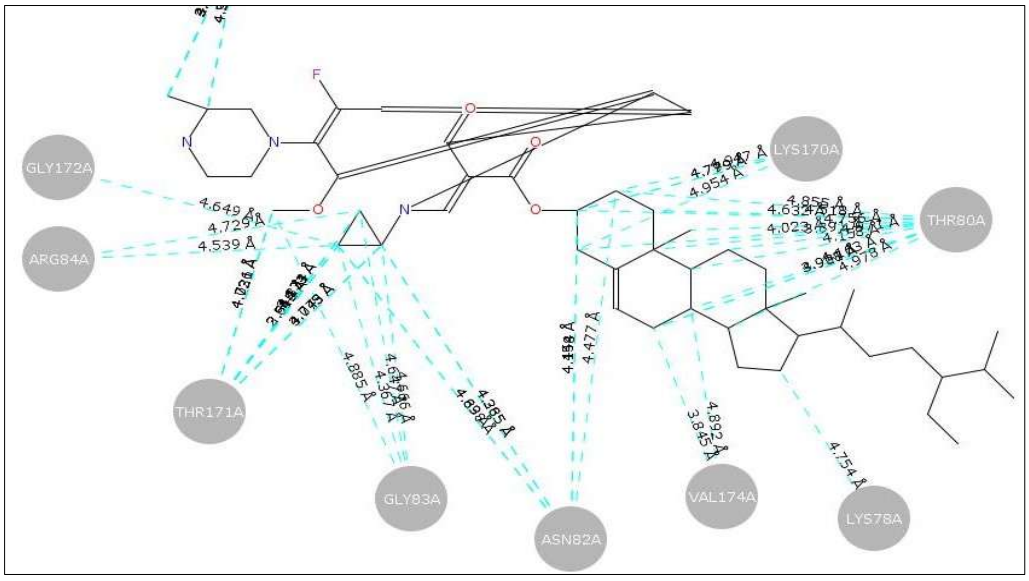


Figure 11: Gati II: Free binding energy = - 10.6
Linked Amino acid = GLY172A, GLY172A, ARG84A, THR171A, GLY83A, ASN82A, VAL174A, LYS78A, THR80A, LYS170A

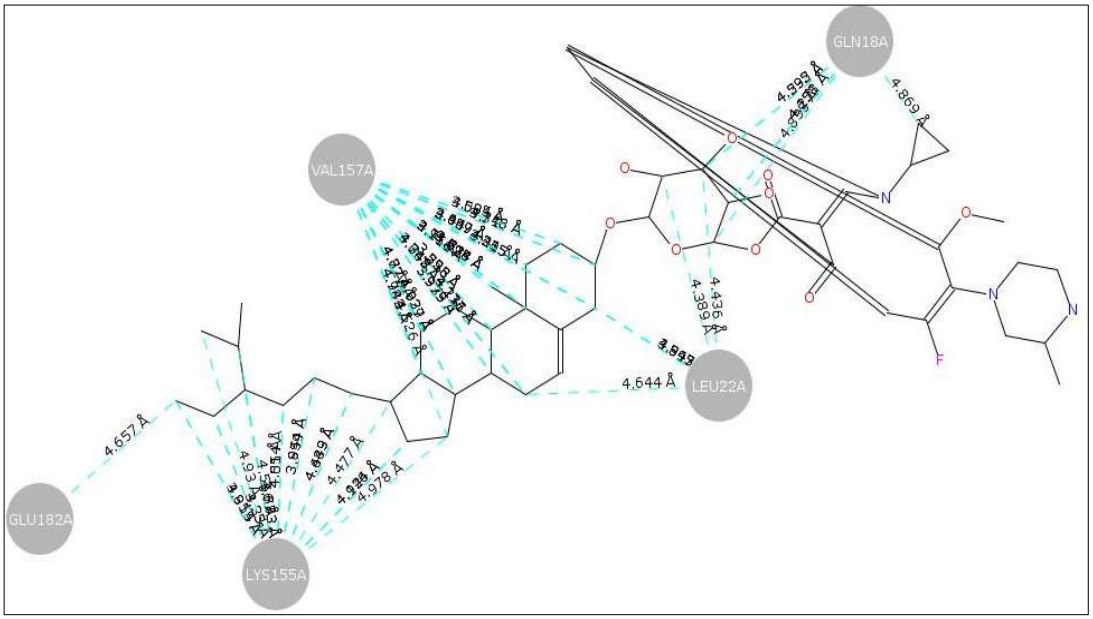


Figure 12: Gati III: Free binding energy = - 8.6
Linked Amino acid = VAL157A, GLU182A, LYS155A, LEU22A, GLN18A

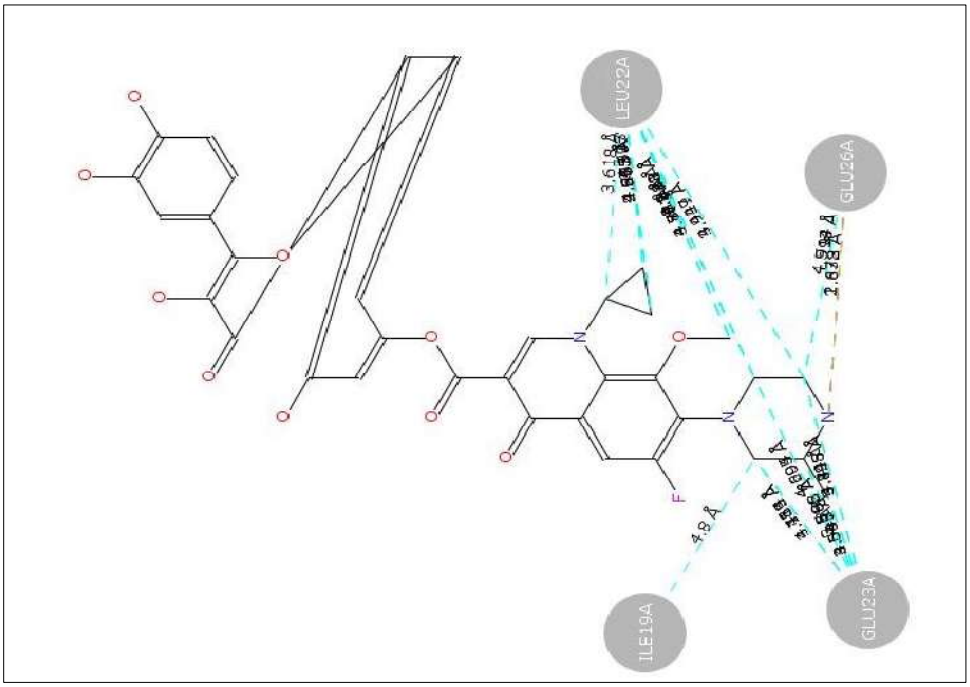


Figure 13: Gati IV: Free binding energy = - 9.7
Linked Amino acid = ILE19A, GLU23A, GLU26A, LEU22A

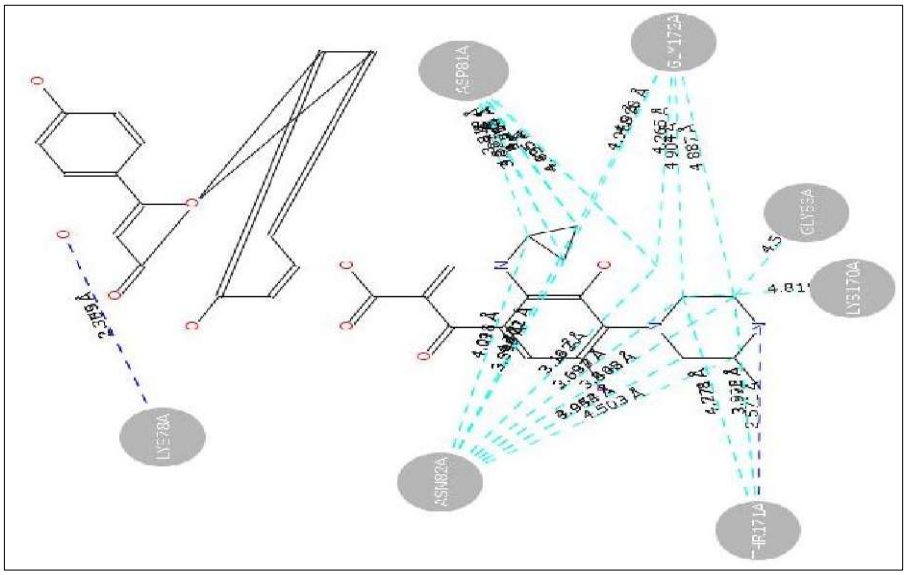


Figure 14: Gati V: Free binding energy = - 10.2

Linked Amino acid = LYS78A, ASN182A, THR171A, LYS170A, GLY83A, GLY172A, ASP81A

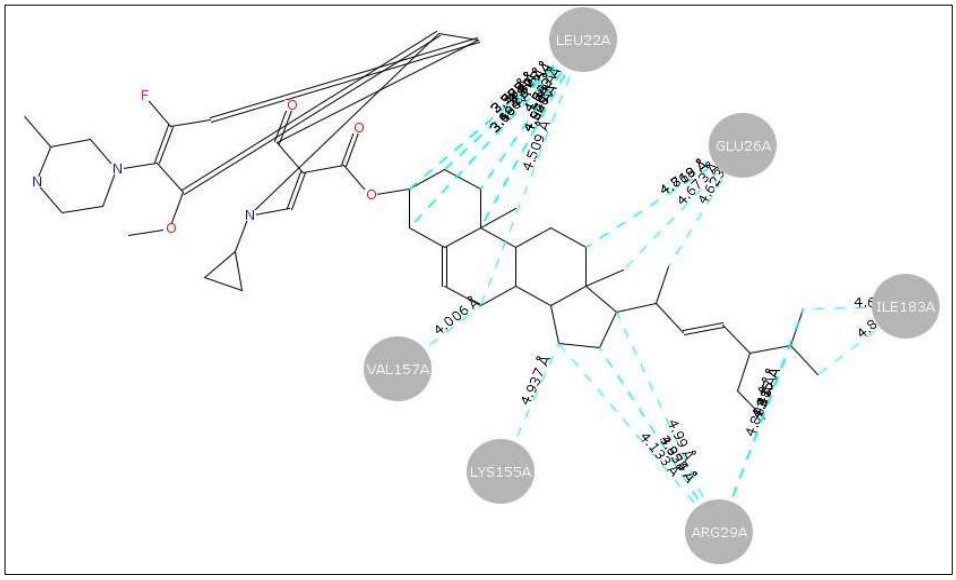


Figure 15: Gati VI: Free binding energy = - 10.5
Linked Amino acid = VAL157A, LYS155A, ARG29A, ILE183A, GLU26A, LEU22A

The different 2 D and 3 D images of gatifloxacin and it's derivatives are shown in **Table 3**.

The Gati I, Gati III, Gati IV, Gati V, Gati VI has lowest binding free energy score as compared to standard gatifloxacin which is shown in **Table 2** The Gati II has binding free energy is -10.6 exhibits which is good compared to standard gatifloxacin showed -7.3 binding free energy.

CONCLUSION:

The molecular docking of gatifloxacin derivatives revealed favorable binding interactions with targeted protein PDB ID: 5CPH. The gatifloxacin derivatives namely Gati I, Gati II, Gati III, Gati IV, Gati V, Gati VI showed best binding free energy among compound database. These endeavors holds 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.

ACKNOWLEDGEMENT:

The authors are acknowledged the support received from research center Appasaheb Birnale College of Pharmacy, Sangli.

DECLARATION:

Conflict of interest

The authors state that there is no conflict of interest with this study.

Funding

None declared.

REFERENCES:

[1] Zhanel C, Walkty A, Vercaigne L, et al, 1999, The new fluoroquinolones: A critical review, Can J Infect Dis, 10(3), pp 207-38.

[2] Bertino J, Fish D, 2000, The Safety Profile of the Fluoroquinolones, Clinical Therapeutics, 22(7), pp 798-817.

[3] Randall C, Walker M, 1994 The Fluoroquinolones, Mayo Clin Proc, 74, 1030-37.

[4] Cunha B, 1998, Quinolones: Clinical use and formulary considerations, Adv Ther, 15(5), pp 277 – 87.

[5] Notification: Determination that TEQUIN (Gatifloxacin) was withdrawn from sale for Reasons of Safety Effectiveness by Department of Health and Human Services, FDA (US), Docket no. FDA, 2006, 0081.

[6] Gajjar D, Kollia G, Lacreta F, 2000, Effect of multiple- dose gatifloxacin or ciprofloxacin on glucose homeostasis and insulin production in patients with noninsulin-dependent diabetes mellitus maintained with diet and exercise. Pharmacother, 20(6 Pt 2), 76– 86.

[7] Kenton I, McKinnon P, Okhuysen P, Peymann P, Septimus E, 2005, A retrospective, comparative evaluation of dysglycemias in hospitalized patients receiving gatifloxacin, levofloxacin, ciprofloxacin, Sparfloxacin, or ceftriaxone, Pharmacother, 25(10), pp 1303 – 09.

[8] Alangaden G, Lerner S, 1997, The clinical use of fluoroquinolones for the treatment of mycobacterial diseases, Clin Infect Dis, 25(5), pp 1213-21.

[9] Sharma P, Jain A, Jain S, 2009, Fluoroquinolone Antibacterials: A Review on Chemistry, Microbiology and Therapeutic Prospects, Acta Poloniae Pharmaceutica and Drug Research, 66 (6), pp 587-604.

[10] Owens R, 2005, Fluoroquinolone-associated dysglycemias: a tale of two toxicities. Pharmacother, 25(10), pp 1291 – 95.

[11] Chock A, Faulkner M, Skrabal M, Stading J, 2005, Development of diabetes after gatifloxacin therapy, Am J Health Syst Pharm, 62(21), pp 2293 – 95.

[12] Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J, 2018, Insilico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats, Toxicology Mechanism and Method, pp 1-48.

[13] Bagri P, Ali M, Aeri V, Bhowmik M, 2016, Isolation And Antidiabetic Activity of New Lanostenoids From The Leaves of psidium Guajava L, International Journal of Pharmacy and Pharmaceutical Sciences, 8(9), pp 1-5.

[14] Wang J, Huang M, Yang J, 2017, Anti-diabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter, Food and Nutrition Research, 61, pp 1-14.

[15] Bulea M, Abdurahmand A, Nikfara S, 2019, Antidiabetic effect of quercetin: A systematic review and meta-analysis of animal studies, Food and Chemical Toxicology, 125, pp 494-502.

[16] Ibitoye O, Uwazie J, Ajiboye T, 2017, Bioactivity-guided isolation of kaempferol as the antidiabetic principle from Cucumis sativus L. fruits, J Food Biochem, pp 1-7.

[17] Blondeau J, 2004, Fluoroquinolones: mechanism of action, classification, and development of resistance Surv Ophthalmol, 49 Suppl 2, pp 73 - 78.

[18] Blanche F, Cameron B, Crouzet J, et al, 1994, Cloning and primary structure of Staphylococcus aureus DNA topoisomerase IV: a primary target of - fluoroquinolones. Mol. Microbiol, 13, 641– 53.

[19] Aldred K, Kerns R, Osheroff N, 2014, Mechanism of quinolone action and resistance, Biochem, 53(10), 1565 – 74.

[20] Chandra R., Kumar N, Sakharkar M, Singh A, et al, 2018, Antibacterial and pharmacological evaluation of fluoroquinolones: A chemoinformatics approach., Genomics Inform, 16(3), pp 44 – 51.

[21] Vincent A, 2000, The quinolones: Past, present, future, Supplement article, Overview of quinolone development, Clin Infect Dis, (41 Supp 2), pp 113 – 19.

[22] Schimizu M, 1995, Quinolone antibacterial agents: Their past, present, future, J Infect Chemother, 1, pp 16 – 29.

[23] Manda H, Takei M, Tsurumaki Y, 1998, Inhibitory activities of gatifloxacin (AM-1155) a newly developed fluoroquinolone, against bacterial and mammalian type II topoisomerases, Antimicrob Agents Chemother, 42(10), pp 2678 – 81.

[24] Bonner D, Fung-Tomc J, Gradelski E, Kolek B, 2002, Bactericidal mechanism of gatifloxacin compared with other quinolones, J Antimicrob Chemother, 49, pp 185 – 88.

[25] Figgitt D, Hurst M, Lamb H, Scoot L, 2003, Leavofloxacin: an updated review of it's use in the treatment of bacterial infections in the United States, Drugs, 62(14), pp 2127 – 67.

[26] Agu P, Afiukwa C, Orji U, *et al*, 2003, Molecular docking as a tool for the discovery of molecular targets of neutraceuticals in diseases management, 13, pp 1 -18.

[27] https://www.rcsb.org/structure/5CPH

[28] Mesleh M, Cross J, Zhang J, *et al*, 2016, Crystal structure of the ATP binding domain of S. aureus Gyr B complexd with a fragment, Bioorg Med Chem Lett, 26, pp 1314-18.

[29] Collin F, Karkare S, Maxwell A, 2011, Exploiting bacterial DNA gyrase as a drug target: current state and perspectives, Appl Microbiol Biotechnol, 92(3), pp 479 – 97.

[30] Khan T, Sankhe K, Suvarna V, *et al*, 2018, DNA gyrase inhibitors: Progress and synthesis of potent compounds as antibacterial agents, Biomedicine & pharmacotherapy, 103, pp 923 – 38.

[31] Kumar A, Prasun C, Rathi E, et al, 2023, Identification of potential DNA gyrase inhibitors: virtual screening, extra-precision docking and molecular dynamics simulation study, Chemical Papers, 77, pp 6717 – 27.

[32] *https://www.vlifesciences.com/products/VLifeMDS/Product_VLifeMDS.php*

[33] *https://www.vlifesciences.com/support/request_demo.php*

[34] *https://www.vlifesciences.com/products/Functional_products/VLifeDock.php*