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# *In-Silico* Identification and Molecular docking studies of Quinolone resistance determining region (QRDR) of e.*coli* DNA Gyrase-a with n-substituted Piperazinyl Schiff bases of Gatifloxacin

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## Abstract:

A series of N-substituted piperazinyl Schiff bases of gatifloxacin were designed and were docked within the "Quinolone Resistance Determining Region" (QRDR) of *E. coli* DNA Gyrase-A (EcGyr-A) chain (QRDR-A), to evaluate the possible relationship between docking scores and their contribution to biological activity, along with the interaction with target residues. The obtained docking scores of analogues were compared with score of reference ligand gatifloxacin, under identical experimental sets. The analogue with 2-(pyridin-4-ylcarbonyl) hydrazinylidene substituents, **1h** showed highest docking score (-167.66 kcal.mol$^{-1}$). Compounds with substituents 2-hydroxyimino, **1b** and 2-carbamothioylhydrazinylidene, **1d** showed moderate docking score (-161.32 kcal.mol$^{-1}$ and -158.64 kcal.mol$^{-1}$ respectively) against QRDR-A. Among the eight analogues selected for docking studies, a moderate correlation was also observed between docking scores and experimental biological activity reported in our previous work. Further structural analysis of docking studies on our compounds suggests attractive starting point to find new lead compounds with potential improvements.

**Keywords:** Gatifloxacin, docking, N-substituted, QRDR, *E. coli*, DNA Gyrase-A.



## Introduction

*Escherichia coli* (*E. coli*) is one of the most frequent causes of many common bacterial infections, including gastroenteritis, cholecystitis, bacteremia, cholangitis, urinary tract infection, and traveler's diarrhea, as well as other clinical infections such as neonatal meningitis and hemolytic-uremic syndrome [1]. In rarer cases, virulent strains are also responsible for peritonitis, mastitis, septicemia and Gram-negative (Gm-ve) pneumonia [2]. Fluoroquinolones are broad-spectrum antimicrobials of quinolone class of drug, used in the treatment of infectious diseases caused by enteric bacteria such as *E. coli*. Unfortunately frequent use and misuse of fluoroquinolones leads to emergence of fluoroquinolone-resistant bacteria, especially in Gm-ve bacteria such as *E. coli* [3].

The major target of fluoroquinolone in *E. coli* is DNA gyrase (type IIA topoisomerase), which plays essential roles in bacterial DNA replication [4,5]. DNA gyrase is a heterotetrameric structure, consisting of two proteins Gyrase-A (GyrA) and Gyrase-B (GyrB), which form an $A_2B_2$ complex in the active enzyme. Gyrase introduces change in the topology of closed circular DNA by cleaving the helix in both strands and passing another segment of DNA through the break and finally resealing the broken ends. The double-stranded breaks in DNA that are created by GyrA are stabilized by quinolones. The quinolones exert the antibacterial activity by giving unfavorable conditions for DNA ligation and thereby blocking

DNA replication [6]. The resistance against quinolones is mutation in two short regions, known as "Quinolone Resistance Determining Region" (QRDR) in the GyrA subunit (region 67 to 106) and in the GyrB subunit (region 426 to 464) in *E.coli* [7,8]. Mutations conferring bacterial resistance to quinolones which occurs in QRDR region are located in the breakage-reunion domain of GyrA subunit (QRDR-A) and less frequently in the Toprim domain of GyrB (QRDR-B) [9,10]. However, the mutation in GyrA leads to a 20 fold resistance, while in GyrB results only a 4-fold resistance. Further, in GyrB region where mutations are reported is in fact distal (40 Å) to the active site; while the QRDR, where mutations are seen in GyrA is proximal to the active site [11]. Therefore, any slight conformational change in the QRDR-A results in drastic change in the cellular function of gyrase. This suggests that mutation in QRDR-A plays a crucial role as compared to QRDR-B in causing resistance. Fluoroquinolones resistance in *Escherichia coli* is most commonly associated with amino acid substitutions at Ser83 and Asp87 in QRDR-A, which map to the putative DNA binding surface of α-helix 4 [12].

The inhibition of DNA gyrase and cell permeability of the quinolones is greatly influenced by the nature of C-7 substituents on the standard structure of 4-quinolones-3-carboxylic acid. In addition, the substitution of bulky group is permitted at the C-7 position [13,14]. Furthermore, it has been proposed that for Gram-positive (Gm+ve) organism, that increasing molecular mass and bulkiness of substituents at the C-7 position are not barriers to penetration. Considering this in mind previously several N-(2-oxo-2-(4-substituted phenyl) ethyl derivatives with different quinolones including norfloxacin and 6,8-difluoro quinolones have been designed for enhanced antibacterial activity against some Gm+ve and Gm-ve organism as compared to the parent quinolone [15]. Gatifloxacin is a novel extended spectrum (fourth generation) fluoroquinolones with improved Gm+ve and anaerobes coverage compared to the older agents such as norfloxacin and ciprofloxacin [16]. However, dysglycemia has been noted as the life-threatening adverse effect of gatifloxacin, which led to its withdrawal from the market in the United States in 2006 [17]. Thus there exists continuous need for novel gatifloxacin derivatives with better activity profile and tolerability to overcome the limitations. Schiff bases are the important compounds owing to their wide range of biological activities such as antimalarial [18], anticancer [19], antitumor [20], antibacterial [21], antifungal [22], antitubercular [23], anti-HIV [24], antimicrobial [25] and antiviral [26] etc.

Because of the lack of data in the literature, concerning with the analogues of gatifloxacin, we have previously reported the same, by introducing some new functionality as Schiff bases (hydrazones, oximes and semicarbazones) against *E. coli* [27]. With the increasing number and accuracy of crystal structures in recent years, molecular docking has become an important tool for the synthetic elaboration of novel therapeutics based on chemical scaffolds [28]. Taking into account, the accuracy aspect of molecular docking, important biological activities of Schiff bases and crucial role of QRDR-A, recent efforts have been directed towards docking same series of gatifloxacin, with QRDR-A, aimed to evaluate the possible relationship between docking score and their contribution to biological activity, along with the interaction with their residues.

## Experimental section

### Material and methods

The molecular docking study of gatifloxacin analogues with well established structure of EcGyr-A was done using MolDock docking engine of Molegro Virtual Docker, version 5.5.0 **(**MVD) software from CLC Bio (http://www.clcbio.com/products/molegro, Aarhus, Denmark) [29]. All calculations were conducted on IntellCore2 Duo T6400, 1.20 GHz dual processing machine. Docking of gatifloxacin and it's analogues with EcGyr-A proceeds in three steps; the first is ligand preparation; second is retrieval, preparation and validation of 3D X-ray crystal structure of EcGyr-A and third is identification of QRDR-A along with molecular docking of reference ligand and designed analogues to QRDR-A.

### Lig and Preparation

The two-dimensional (2D) structures of gatifloxacin analogues were drawn using ChemDraw ultra 10.0 (Cambridge software) and was saved as MDL Mol files. The three-dimensional structures (3D) were generated using GlycoBioChem PRODRG2 online server (http://davapc1.bioch.dundee.ac.uk/prodrg/) [30]. To obtain 3D structure as PDB file format, the 2D structure (MDL Mol files) were used as input files for PRODRG. The finally obtained 3D structures were energy minimized using Hyperchem's MM+ force field (http://www.hyper.com/) [31]. The minimization was executed until the root mean square (r.m.s) gradient value reached a value smaller than 0.001kcal.mol$^{-1}$. Such energy minimized structures of gatifloxacin analogues were considered for molecular docking studies.

### Retrieval and preparation of 3D-structure of EcGyr-A

The 3D X-ray crystal structure of target protein EcGyr-A was retrieved from Brook Heaven Protein Data Bank (PDB database) (http://www.rcsb.org/pdb) (PDBID: 1AB4) at 1.60 Å RMSD resolution. Identification and analysis of protein template i.e. QRDR-A was considered as standard, reported by Yoshida, Conrad and Friedman *et al* [32-34].

### Molecular docking with gatifloxacin derivatives and scoring

Molecular Docking is the process in which two molecules fit together in 3D space. It is a key tool in structural biology and computer-aided drug design. The goal of ligand and protein docking is mainly to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure [35]. In MVD the receptor and ligand coordinates were used in PDB format. MolDock docking engine of MVD automatically identifies potential binding sites, (hereafter referred to as cavity) using the cavity detection algorithm. During Docking at first the molecules were prepared and bonds, bond orders, explicit hydrogens, charges, flexible torsions, were assigned if they were missing, by the MVD program to both the protein and ligands. From the docking wizard, ligands were selected and the docking was performed in the QRDR-A including Ser83 and Asp87, taking bound gatifloxacin molecule as standard ligand [36]. An exhaustive systemic search of the conformational space was performed with the help of heuristic search algorithm to locate the possible position of ligand in the QRDR-A during docking simulation. The QRDR-A is defined as a spherical region, surface area: 305.92 Å$^2$, coordinates dimensions X (68.08 Å), Y (76.18 Å), Z (25.01 Å) axes, respectively. The





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Int. J. Drug Dev. & Res., October -December 2013, 5 (4): 203-210

potential binding site within QRDR-A; a cavity of volume 67.58 Å$^3$ was observed close to amino acid residue Asp82, Ser83, Ala84, Tyr86, Asp87, Val90, Arg91, Gln94, Phe96 and Ser97 located within the constraints 17 Å **(Figure 1)**. The search algorithm was taken as Moldock SE and docking was performed using a grid resolution of 0.3 Å. For each of the 10 independent runs; a maximum number of 1500 iterations were executed on a single population of 50 individuals. Side chain flexibility of the amino acids present in the binding site of QRDR-A was incorporated during docking run was performed. For each benchmark complex, 10 independent runs were conducted and each of these runs returning one solution (pose). These 10 solutions were then re-ranked and the highest ranked (ranked by the lowest docking energy) solution was compared with the reference ligand, along with their docking score.

## Results and discussion

### Docking analysis

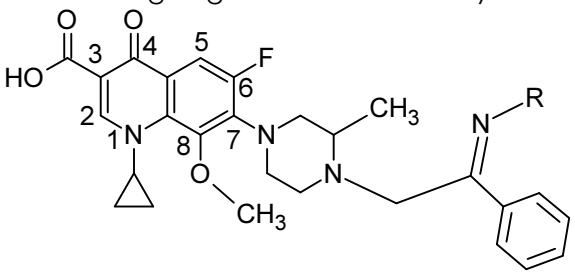
Molecular docking is a powerful tool in drug design, which could predict the best mode by which a given compound fits well into a binding site of a macromolecular target [37]. With *in vitro* antimicrobial result in hand, we thought it worthwhile to perform *in silico* studies to support the result [38]. The docked binding mode is used to establish a link between the MolDock score and biological activity. Table 1 presents the experimental values of the inhibitory activity (MIC) of *E. coli* and the interaction energy between inhibitor (synthesized analogues) and QRDR-A obtained after docking. The theoretical results obtained in the molecular docking were compared with the experimental results (MIC) [39,40]. One can observe the result in table 1, that the theoretical results obtained by the docking study of gatifloxacin analogues with QRDR-A were moderately correlated ($r^2$ = 0.648; n= 8) with the experimental results. Thus it confirms that, the experimental values moderately agree with theoretical values, which suggest that the parameters for docking simulation are optimum in reproducing experimental orientation of these compounds.

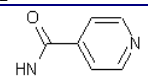
### Protein-ligand molecular docking with QRDR-A and interaction analysis

The main aim of docking study is to predict the orientation into the QRDR-A and interaction of analogues with their residues including Ser83 and Asp87, which are commonly altered in fluoroquinolones resistant *E. coli*. Evaluation of the docking results was based on protein-ligand complementarities considering steric and electrostatic properties as well as calculated potential interaction energy in the complex. After docking calculations, it is verified that the orientation of the derivatives into the active site QRDR-A was similar to that of gatifloxacin (reference ligand). It is evident from the table 1 that a group of residue located in the QRDR-A binding cavity such as Asp87, Arg91, Gln94 and Ser97 plays an important role in the ligand recognition and affinity. Our docking results with experimental compounds showed that almost all the compounds interact with QRDR-A through hydrogen bonds. The docking of all gatifloxacin derivatives with QRDR-A showed improved docking score, when compared with the reference drug gatifloxacin (-138.38 kcal.mol$^{-1}$) except compound **1a** (-124.34 kcal.mol$^{-1}$), **1c** (-125.57 kcal.mol$^{-1}$), **1e** (-120.07 kcal.mol$^{-1}$) and **1f** (-122.31 kcal.mol$^{-1}$), which have docking score less than reference ligand gatifloxacin. Compound **1h** exhibited highest docking score (-167.66 kcal.mol-

[1]), interacts with the QRDR residue Arg91 and Ser97. On interaction analysis of the same compound, the C=O and =N- of imine were found to interact with Arg91, two H-bonds, bond length 2.66 Å and 2.48 Å respectively, as well as =N-NH- group with Ser97, two H-bonds, bond length 2.24 Å and 2.12 Å respectively. Compounds **1b** showed docking score -161.32 kcal.mol$^{-1}$ and ranked second. The –C=O group of –COOH was found to interact with Arg91, H-bond, bond length 2.06 Å and N-4 of piperizinyl group with residue Asp87, H-bond, bond length 2.18 Å. Compounds **1d** (-158.64 kcal.mol$^{-1}$) ranked third on the basis of docking score and >C=O of quinolone ring and –C=O of –COOH were found to interact with amino-acid residue Arg91, H-bond, bond length 1.88 Å and 2.45 Å respectively. The N-4 of piperizinyl group interacts with residue Asp87, H-bond, bond length 2.38 Å **(Figure 2)**. Finally compound **1g** showed docking score -143.57 kcal.mol$^{-1}$, which is more than reference ligand. In addition with compounds **1b** and **1d**, the compounds **1a**, **1c** and **1f** were also found to interact with Asp87, which is the main residue which plays a major role in fluoroquinolone resistance **(Figure 3)**. Compounds **1a** were found to interact with Ser83 with single H-bond, bond length 2.75 Å. Reference ligand (gatifloxacin) were found to interact with Arg91 of QRDR-A residue, H-bond, bond length 2.32 Å **(Figure 4)**. So it may be speculated, that the presented gatifloxacin derivatives, especially compounds **1b**, **1d** and **1h** may be a successful drug candidates and can play major role to combat bacterial resistance. These derivatives may be an attractive starting point as new lead compounds with potential improvements.

**Table 1:** Docking results of N-substituted piperazinyl Schiff bases of gatifloxacin with Quinolone Resistance Determining Region of *E. coli* DNA Gyrase-A.

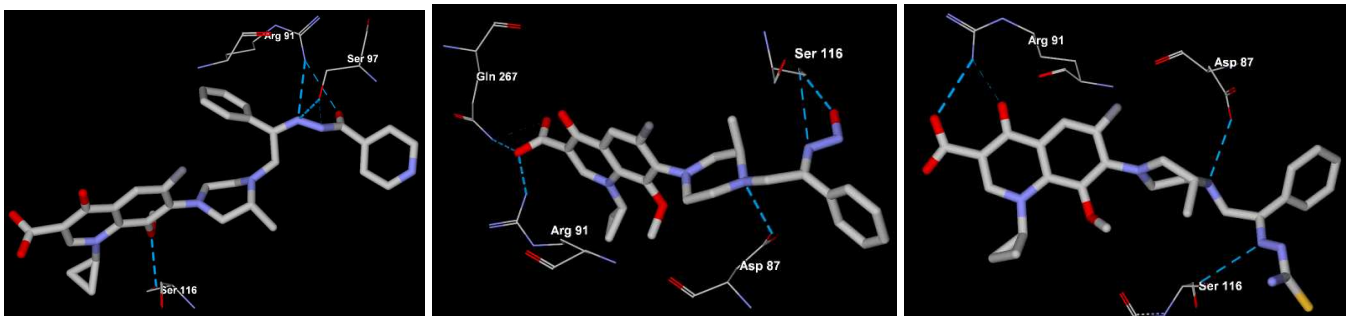



| Compounds | R | MIC | Docking Score [a] (kcal.mol$^{-1}$) | Interacting QRDR-A residue(s) with gatifloxacin analogues. |
|---|---|---|---|---|
| 1a | $NH_2$ | 0.78 | -124.34 | Asp 87, Gln 94, Ser 97 |
| 1b | OH | 0.19 | -161.32 | Asp 87, Arg 91 |
| 1c | $NHC(=O)NH_2$ | 1.56 | -125.57 | Asp 87, Arg 91, Ser 97 |
| 1d | $NHC(=S)NH_2$ | 0.39 | -158.64 | Asp 87, Arg 91 |
| 1e | Ph | 1.56 | -120.07 | Arg 91, Gln 94, Ser 97 |
| 1f | NHPh | 1.56 | -122.31 | Asp 87 |
| 1g | $NHPh(NO_2)_2$ | 0.78 | -143.57 | Ser 97 |
| 1h |  | 0.78 | -167.66 | Arg 91, Ser 97 |
| GATI[b] | ----- | 0.04 | -138.38 | Arg 91 |



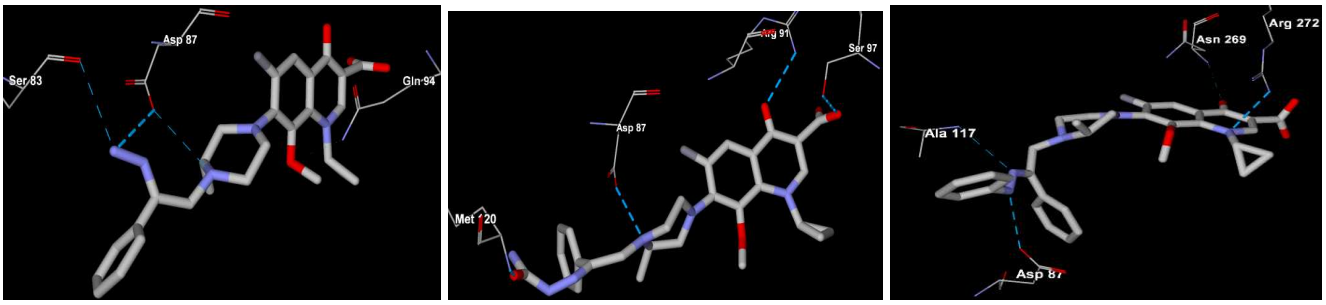
[a] Based on MolDock score [b] GATI = Gatifloxacin (reference ligand)


**Figure 1**: Binding pocket targeting QRDR of *E. coli* DNA Gyrase-A




**1h (A)** **1b (B)** **1d (C)**

**Figure 2:** Interaction of compounds **1h (A)**, **1b (B)** and **1d (C)** with QRDR-A residues.




**1a (A)** **1c (B)** **1f (C)**

**Figure 3:** Interaction of compounds **1a (A)**, **1c (B)** and **1f (C)** with QRDR-A residue Asp87.

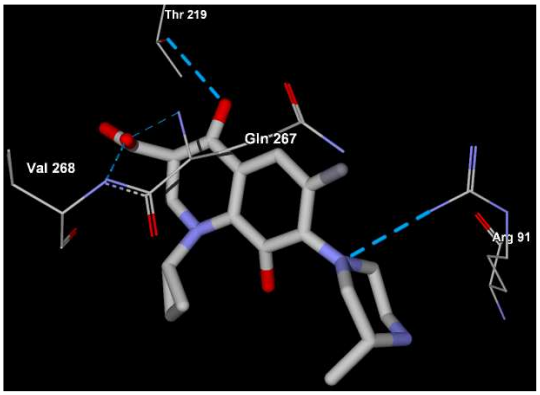



**Figure 4:** Interaction of reference ligand (gatifloxacin) with QRDR-A residue.



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## Conclusion

Finally it may be concluded, that a series of N-piperazinyl substituted gatifloxacin Schiff bases have been docked successfully and analyzed to investigate the role of these derivatives, which indicates the importance of oximes, hydrazones and semicarbazones moieties. The docking scores showed significance in prediction of inhibition of EcGyr-A. Thus it is summarized that derivatization of N-piperazinyl position in gatifloxacin as Schiff bases are optimum and a determinant for generation of bio-activity with regard to structure-activity relationships. The findings of this work should be helpful to medicinal chemists involved in further drug development of novel antimicrobials against *E.coli*.


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## Conflict of interest: Nil

## Source of Support: None


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