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In-silico Identification and Molecular Docking Studies of Quinolone Resistance Determining Region (QRDR) of E. coli DNA Gyrase-A with Ofloxacin Schiff Bases

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Abstract: A series of ofloxacin Schiff bases 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 ofloxacin, under identical experimental sets. The analogue with -NHC(=O)Ph$(NO_2)_2$ substituents, **OFX-10** showed highest docking score (-154.62 kcal.mol^{-1}) along with interaction with Asp87. Two more compounds with substituents hydrazinylidene (=N-NH_2), **OFX-1** and 2-carbamoylhydrazinylidene (=N-NHC(=O)NH_2), **OFX-6** showed moderate docking scores -131.85 kcal.mol^{-1} and -129.88 kcal.mol^{-1} respectively, against QRDR-A along with interaction with Asp87. Among the ten synthesized analogues selected for docking studies, a good correlation was also observed between docking scores and experimental biological activity.
Key words: Ofloxacin, docking, *E. coli*, DNA Gyrase-A, QRDR-A.

Introduction

German pediatrician and bacteriologist, Theodor Escherich discovered *Escherichia coli* (*E. coli*) bacterium in 1885, which is now classified as part of the Enterobacteriaceae family of gamma-proteobacteria. *E. coli* is a species that occurs normally in the intestines of humans and other vertebrates, is widely distributed in nature, and is a frequent cause of infections of the 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. Enteropathogenic strains of *E. coli* cause diarrhea due to enterotoxin, the production of which seems to be associated with a transferable episome. Most *E. coli* strains pose no harm to human health, except for serotype O157:H7, which can cause food poisoning in humans and can become life-threatening. Other less common serotypes, such as O104:H4, O121, O26, O103, O111, O145 and O104:H21

can also cause serious infection.[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] 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] Ofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class considered to be a second-generation fluoroquinolone. Like other quinolones, ofloxacin has been associated with a significant number of serious adverse drug reactions, such as tendon damage and peripheral neuropathy; such reactions may manifest long after therapy had been completed, and, in severe cases, may result in lifelong disabilities. Hepatotoxicity has also been reported with the use of ofloxacin. Case reports of hepatitis have been published for the older fluoroquinolones including ciprofloxacin, ofloxacin, and norfloxacin.[16,17,18] Thus there exists continuous need for novel ofloxacin 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 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 ofloxacin and for other fluoroquinolones, we have previously synthesized some novel derivatives by introducing new functionalities (hydrazones, oximes and semicarbazones) as Schiff bases 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 of previously designed and biologically evaluated series of ofloxacin, 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

Materials and methods

The molecular docking study of ofloxacin 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 ofloxacin 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.

Ligand Preparation

The two-dimensional (2D) structures of ofloxacin 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 0.001 kcal.mol^{-1}. Such energy minimized structures of ofloxacin 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 Á RMSD resolution. Identification and analysis of protein template i.e. QRDR-A was considered as standard, reported by Yoshida, Conrad and Friedman *et al.*[32,33,34]

Molecular docking with ofloxacin 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 fluoroquinolone 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 Á^2, coordinates dimensions X (68.08 Á), Y (76.18 Á), Z (25.01 Á) axes, respectively. The potential binding site within QRDR-A; a cavity of volume 67.58 Á^3 was observed close to amino acid residue Asp82, Ser83, Ala84, Tyr86, Asp87, Val90, Arg91, Gln94, Phe96 and Ser97 located within the constraints 17 Á **(Figure 1).** The search algorithm was taken as Moldock SE, using default parameters in the docking simulations with a grid resolution of 0.3 Á **(Table 1).** 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.

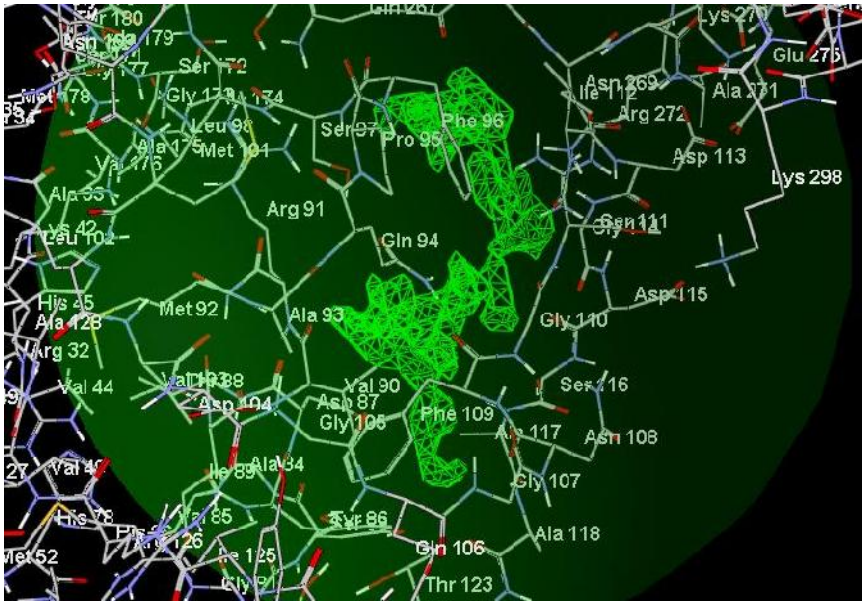


Figure 1 Binding pocket targeting QRDR of *E. coli* DNA Gyrase-A.

Table 1 Default Parameters Used in the Docking Simulations with MolDock SE [MVD]

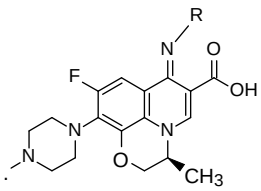
Scoring function Score: MolDock Score(GRID) Grid resolution (Å): 0.30 Ligand evaluation: yes
Binding site Origin: Reference ligand Center: X (68.08 Å), Y (76.18 Å), Z (25.01 Å) Radius: 17 Å
Search algorithm Algorithm: MolDock SE Number of runs: 10 Constrain poses to cavity: yes After docking: Optimize H-bonds
Parameter settings Max iterations: 1500 Max population size: 50
Pose generation Energy threshold: 100.0 Tries. Min: 10 Quick: 10 Max: 30
Simplex evolution Max steps: 300 Neighbor distance factor: 1.00
Return multiple poses for each run Max number of poses returned: 5 Enable energy threshold: No Cluster similar poses. RMSD threshold: 1.00 Ignore similar poses (for multiple runs only). RMSD threshold: 1.00

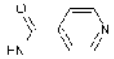
Results and discussion

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 2** presents the experimental values of the minimum inhibitory concentration (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 2**, that the theoretical results obtained after docking of ofloxacin analogues with QRDR-A, showed good correlation (r^2 = 0.857; n= 10) with the experimental results **(Figure 2)**. 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.

Table 2 Docking result of ofloxacin Schiff bases.



Compd.	R	MIC (µg.mL^{-1}) against *E. coli*	Docking Score [a] (kcal.mol^{-1})	Interacting EcQRDR-A residues with OFX 1-10
OFX-1	NH_2	1.56	-131.85	Asp87, Arg91, Gln94, Ser97
OFX-2	NHPh	6.25	-101.86	Asp87, Arg91, Gln94, Ser97
OFX-3	NHPh $(NO_2)_2$	0.19	-152.51	Arg91, Ser97
OFX-4	OH	0.78	-144.15	Arg91, Ser97
OFX-5	$NHC(=S)NH_2$	3.12	-116.97	Asp87, Arg91, Ser97
OFX-6	$NHC(=O)NH_2$	1.56	-129.88	Asp87, Arg91, Ser97
OFX-7		0.78	-123.34	Arg91, Gln94, Ser97
OFX-8	NHC(=O)Ph	6.25	-84.124	Ala84, Thr88, Arg91, Gln94, Ser97
OFX-9	NHC(=O)PhCl	0.39	-143.24	Arg91
OFX-10	$NHC(=O)Ph(NO_2)_2$	0.39	-154.62	Asp87, Arg91, Gln94, Ser97
[b]OFX	= O	0.19	-124.74	Arg91, Ser97

[a] Based on MolDock score,
[b] OFX = ofloxacin (Reference ligand)

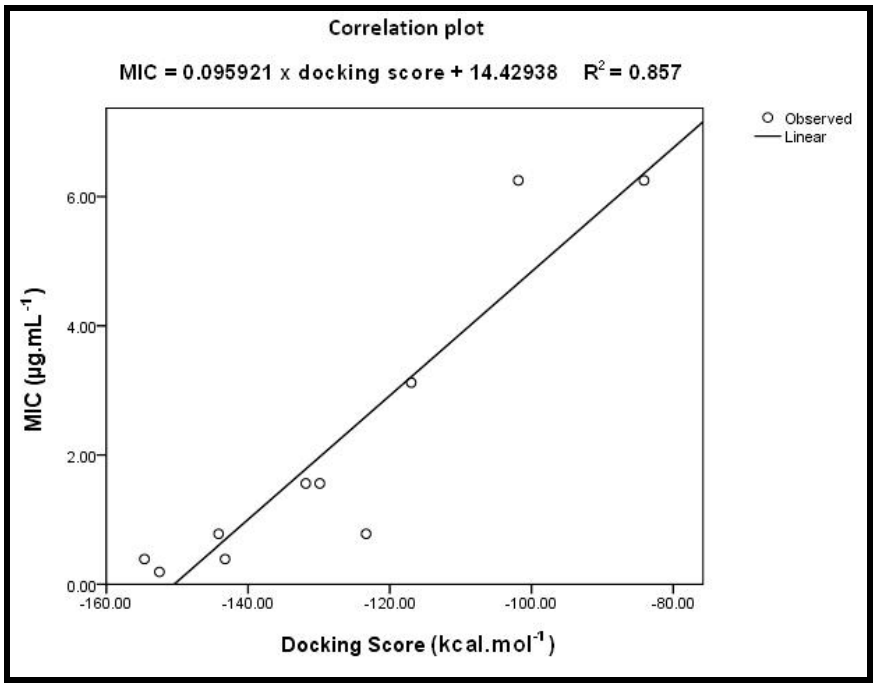
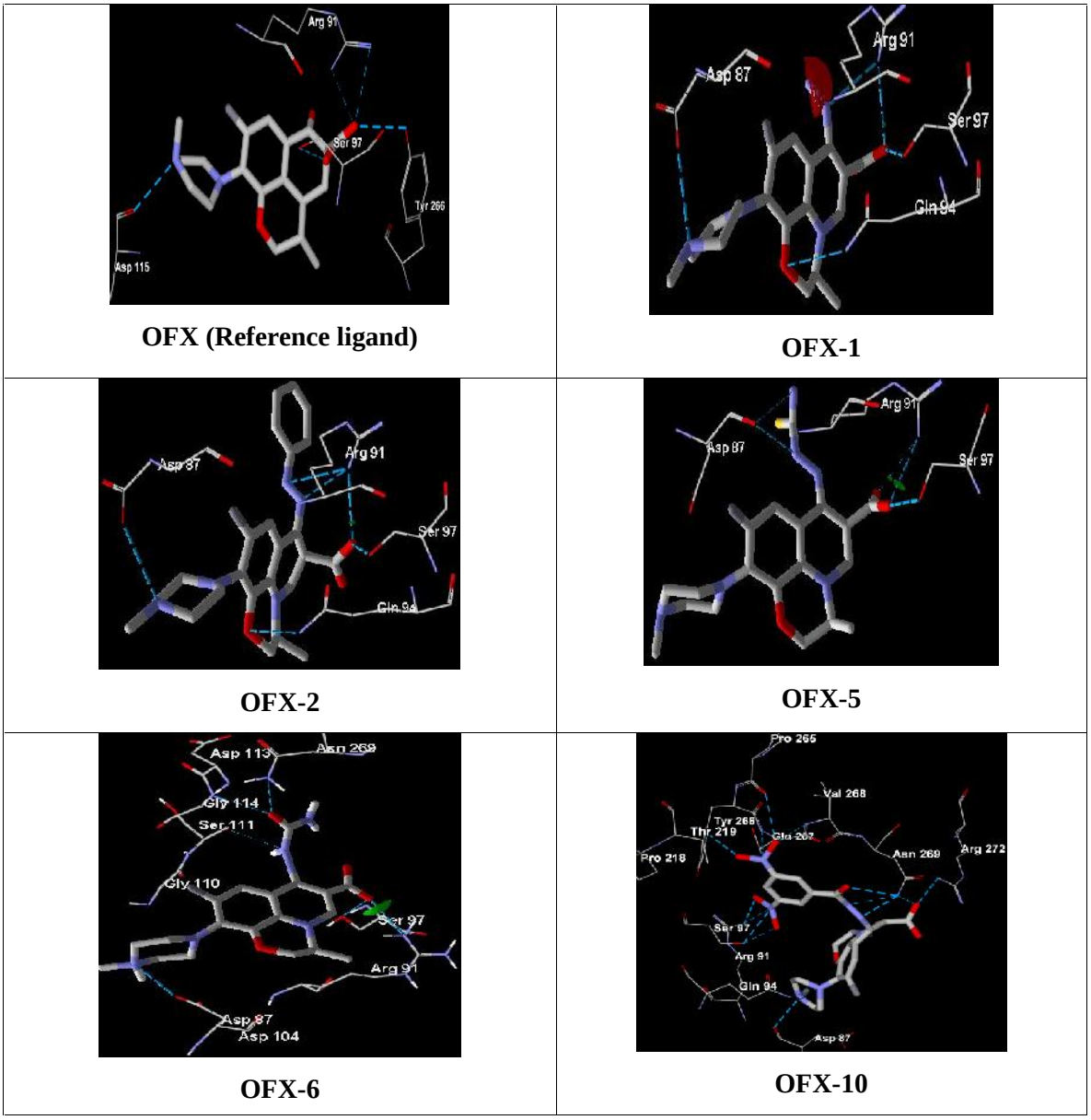


Figure 2 Correlation plot between $MIC_{E.c}$ (µg.mL^{-1}) and docking scores (kcal.mol^{-1}) of OFX 1-10

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

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 simulation, it is evident from the **table 2** that a group of residue located in the QRDR-A binding cavity such as Ala84, Asp87, Thr88, Arg91, Gln94 and Ser97 plays an important role in the ligand recognition and affinity. Our docking results with experimental compounds showed that almost all compounds were involved in hydrogen bonding with Arg91 and Ser97, except compound **OFX-9** with Ser97. Compound **OFX-1, OFX-3, OFX-4, OFX-6, OFX-9** and **OFX-10** showed improved docking score than ofloxacin (reference ligand). Compound **OFX-1, OFX-2, OFX-5, OFX-6** and **OFX-10** were found to interact with Asp87. Result illustrates that the compound **OFX-10** showed highest docking score (-154.62 kcal.mol^{-1}), interact with QRDR-A residues Asp87, Arg91, Gln94 and Ser97. On further analysis of the same compound, N-1 and N-4 of piperazine ring formed H-bonding with Gln94 and Ap87, bond length 2.73 Å and 2.32 Å respectively. Further analysis of same compound, it was found that substituent -NO_2 were found to interact with Ser97 with two H-bonding (bond length 2.53 Å and 2.15 Å) and Arg91 with two H-bonding, bond length 2.28 Å and 2.68 Å. Compound **OFX-3**, ranked second on the basis of docking score (-152.51 kcal.mol^{-1}) and the 2-NO_2 group showed two H-bonds with Arg91 and Ser97, bond length 2.25 Å and 2.14 Å. Introduction of hydroxyimino substituents in compound **OFX-4** at position C-4, exhibited docking score -144.15 kcal.mol^{-1}. The -OH of =N-OH and -C=O group of -COOH exhibited two H-bonding with Arg91, bond length 2.39 Å and 3.43 Å respectively in compound **OFX-4**. The -OH of -COOH of same derivative interact with Ser97, H-bond, bond length 2.28 Å **(Figure 3)**. Compound **OFX-9** showed docking score -143.24 kcal.mol^{-1}, and H-bonding with Arg91 as same binding pattern as in compound **OFX-4**. Finally the compound **OFX-1** (-131.85 kcal.mol^{-1}) and **OFX-6** (-129.88 kcal.mol^{-1}) have moderate docking score which is more than reference drug ofloxacin, but found to interact with Asp87. Compounds **OFX-2, OFX-5, OFX-7** and **OFX-8** have docking score less than reference drug, but compound **OFX-2** and **OFX-5** were found to interact with Asp87 and Ser97. None of the compounds were found to interact with Ser83. Parent drug ofloxacin interacts with Arg91 and Ser97 of QRDR-A residues. So it may be speculated that the presented ofloxacin derivatives, especially compounds **OFX-10, OFX-1** and **OFX-6** 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.



OFX (Reference ligand) | **OFX-1**

OFX-2 | **OFX-5**

OFX-6 | **OFX-10**

Figure 3 Interaction of ofloxacin Schiff bases with QRDR-A along with other residues

Conclusion

Finally it may be concluded, that a series of ofloxacin 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 4-oxo position in ofloxacin 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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