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Review Article

PROTEIN OPTIMIZATION AND DOCKING STUDIES FOR FLUOROQUINOLONES CLASS OF ANTI-TUBERCULOSIS DRUGS-AN OVERVIEW

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ABSTRACT

Tuberculosis is the contagious disease caused by microorganism called "Mycobacterium tuberculosis". It remains one of the deadliest bacterial infections globally, with raising incidences of multidrug-resistant (MDR), extensively drug-resistant (XDR) and totally drug- resistant (TDR) strains posing critical treatment challenges. Fluoroquinolones are anti-microbial, that inhibits the DNA gyrase A and B enzyme. Despite its efficacy, the limitations of Flu, including potential cardiac toxicity and phospholipidosis, highlight the urgent need for safer and more potent analogs. This review describes how computational methods like molecular docking and protein optimization plays a crucial role in enhancing the effectiveness and safety profiles of anti-TB drugs.

Keywords: Tuberculosis, protein optimization, Fluoroquinolones, Moxifloxacin, Gatifloxacin, Levofloxacin, DNA gyrase enzymes, molecular Docking, mycobacterium tuberculosis.

INTRODUCTION

Mycobacterium tuberculosis, also known as Koch's bacillus, is a pathogenic bacterium in the family of Mycobacteriaceae and the causative agent for tuberculosis. It was first discovered in 1882 by Robert Koch, an infectious disease that continues to be a relevant threat to global public health. The pathogenesis of TB has several risk factors are, HIV infection, malnutrition, air pollution, type 2 DM, smoking, and alcoholism.

Disease Profile

Tuberculosis (TB) is a significant public health concern in many countries with a high disease burden rate. It was encountered as latent TB and active TB. Tuberculosis was the leading cause of death from a single infectious agent, ranking above HIV/AIDS (as per the WHO report, 2022). A total of 13,068 cases of XDR-TB were reported by 81 countries.[4].

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According to WHO (2024), 8.2 million people were notified with TB in 2023, which was the highest in a single year, about 15% higher compared to the 7.5 million in 2022 and the 7.1 million in 2019.

Active infection is also classified depending on the strain: drug-sensitive, multi-drug sensitive (MDR-TB), is resistant to isoniazid and rifampicin, and extensively drug resistant (XDR-TB) is resistant to isoniazid, rifampicin, any fluoroquinolone, and aminoglycosides. [25, 26]

Due to spontaneous mutation in these target genes, such as rpo B, kat G, inh A, emb B, gyr A, gyr B, rrs, eis, tly A, and pnc A results in MDR, Pre-XDR, and XDR-TB (Seung, 2015). [24]

Current Tuberculosis (TB) Management:

One of the major challenges in managing TB is the estimated 3 million "missing" individuals who have developed active infection but remained undetected or undiagnosed. It can be deadly if not treated; with the help of conventional regimens, about

58 million infected people were saved from 2000-2018.[27]

Major types of TB

Pulmonary Tuberculosis (PTB)

This type of TB affects the lungs. It is the most common form and is contagious, spreading through airborne droplets when an infected person coughs (or) sneezes.

Extrapulmonary Tuberculosis (ETB)

This type of TB mainly occurs outside the lungs. Organs like lymph nodes, bones, and brains. It is less contagious and usually spreads within the body from the lungs.

Latent TB infection

Treatment for latent TB infection is only provided for select groups that have a high risk of transitioning to active TB infection, including

- HIV-positive patient
- Patient undergoing dialysis for end-stage renal disease
- Taking anti-tumor necrosis factor medication
- Preparing for transplant surgery

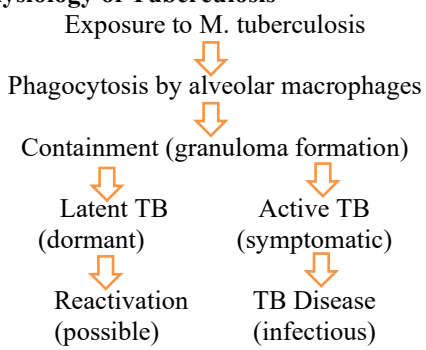
WHO recommended different treatment regimens:3 months of rifapentine and isoniazid, 3-4 months of isoniazid and rifampicin, and 3-4 months of rifampicin and 6-9 months of isoniazid. While all these have established efficacy, poor patient compliance.

Active Drug Sensitive TB

Treatment strategy for activedrug- sensitive TB has not changed from the standard regimen: first- line drugs Rifampicin, Isoniazid, Pyrazinamide and Ethambutol for the first 2 months and then by Isoniazid and Rifampicin for next 4 months, while this treatment is highly efficacious and successful, its long duration primarily leads to poor patient compliance.

In the private sector, nearly 33% (or 8.4 lakhs) of 25.5 lakhs cases were reported in 2024. To compare, only 1.9 lakhs cases were reported in 2015.

Pathophysiology of Tuberculosis



Anti-Tuberculosis Agents

Anti-tuberculosis agents are the agents that are used in the treatment of tuberculosis, which is due to the microorganism "*Mycobacterium tuberculosis.*" They are mainly classified as

Drug Classifications

A) First- Line Drugs
- Isoniazid
- Rifampicin
- Ethambutol
- Pyrazinamide

B) Second- Line Drugs
- Levofloxacin
- Moxifloxacin
- Streptomycin
- Kanamycin
- Amikacin

C) Other Alternative Drugs
- Ethionamide
- Cycloserine
- Amino salicylic acid

D) Newly Approved Drugs
- Bedaquiline
- Delamanid
- Pretomanid

Fluroquinolone Resistance

Fluoroquinolone resistance in bacteria develops primarily through target-site mutation in DNA gyrase and topoisomerase-IV, altering the enzyme drug binding affinity. [31].

1. Drug Profile

Moxifloxacin [7]

Moxifloxacin is a Fluoroquinolones antimicrobial; a broad-spectrum antibiotic, it acts by inhibiting the bacterial DNA gyrase and topoisomerase IV enzyme which is essential for DNA replication and leading to bacterial cell death. It is well absorbed in GIT and its absorption is slightly affected by food. Its exhibit a high plasma protein binding with half-life ranging from 11.5 to 15.6 hrs. This drug has moderate soluble in water and dissolve in DMSO. Metabolism occurs mainly through glucuronidation and sulfate conjugation with the drug excreted unchanged 20% in urine and 25% in faces. It is used in several treatments like sinus and pneumonia and it is also used in the treatment of MDR-TB and XDR-TB.

Gatifloxacin [8]

Gemifloxacin is a Quinolone Antimicrobial and broad-spectrum antibiotic which inhibits the bacterial DNA gyrase and topoisomerase IV enzyme which is essential for DNA replication and leading to bacterial cell death. It is well absorbed in GIT and its absorption is slightly affected by food. Its exhibit a low plasma protein binding with half-life ranging from 7 to 14 hrs. Excretion is predominantly renal, with nearly 80% of the administered dose eliminated unchanged in urine within 24hrs.It is used as the second-line agent in the MDR-TB and XDR-TB.

Levofloxacin [6]

Levofloxacin is a Fluoroquinolone antibiotic, broad-spectrum and concentration dependent antibiotic, which inhibits the bacterial DNA gyrase and topoisomerase IV enzyme which is essential for DNA replication and leading to bacterial cell death. It is completely absorbed through oral administration. It shows moderate plasma protein binding (24-38%), mainly to albumin, independent of plasma concentration, and has half-life of 6-8hrs. The drug is sparingly soluble in water but freely soluble in chloroform. Levofloxacin undergoes limited hepatic metabolism to dimethyl-levofloxacin and levofloxacin-N-oxide, while the majority (-87%) of the administered dose is excreted unchanged in the urine within 48hrs.It is the widely used second line agent in the MDR-TB & XDR-TB.

GEMIFLOXACIN [9]

Gemifloxacin is a Quinolone Antimicrobial, a broad-spectrum and concentration-dependent fluoroquinolone, it produces action by inhibiting the DNA gyrase and topoisomerase IV enzymes, which are essential for DNA replication, transcription, leads to bacterial cell death. It exhibits moderate plasma protein binding of 60-70%, independent of concentration and has an elimination half-life of approximately 7hrs. The drug undergoes limited hepatic metabolism, producing minor metabolites such as N-acetyl Gemifloxacin, the E-isomer of Gemifloxacin and carbamyl glucuronide. Excretion occurs through both urine and faeces, with elimination of parent drug and its metabolites. It shows invitro activity against M. Tuberculosis.

SITAFLOXACIN [5]

Sitafloxacin is abroad spectrum and concentration dependent fluoroquinolone which inhibits bacterial DNA gyrase and topoisomerase IV, enzymes essential for DNA replication, transcription and repair, thereby preventing DNA synthesis leading to bacterial cell death. It is rapidly and extensively absorbed after administration with a half-life of approx. 6.5hrs and around 80% plasma protein binding. The drug undergoes limited metabolism, with about 20% of radio-labelled compound excreted in faeces while renal excretion serves as the primary elimination pathway. It is also having potent in vitro activity against M. Tuberculosis and not take part of WHO recommend regimens.

Ciprofloxacin is a Quinolone antimicrobial, a broad spectrum and concentration dependent fluoroquinolones produces action by inhibiting bacterial DNA gyrase and topoisomerase IV, enzymes essential for DNA replication, transcription and repair, thereby preventing DNA synthesis leading to bacterial cell death.It is generally well absorbed after oral administration, with an oral bioavailability of 70-80%, a half life of 4-6hrs and plasma protein binding of 20-40%. The drug is primarily metabolized by CYP1A2, producing minor metabolites such as oxociprofloxacin and sulfociprofloxacin. Elimination occurs mainly via the kidneys, with approximately 27% of an oral dose recovered unchanged in urine, compared to 46% flowing IV administration.It is an older generation drug that shows activity against M. Tuberculosis.

METHODOLOGY

Docking Studies:

Docking studies is a ***computational method*** that has the ability to interact protein (enzyme) with small molecules to form a supramolecular complex, which may enhance or inhibit its biological function.

The docking process involves two key components:

- Search algorithm
- Scoring function

In the present investigation, we undertake the design of fluoroquinolone class drugs with different substitutions at various levels and subject them for the ADME prediction for the appropriate fluoroquinolones derivatives which is accompanied by the computed- aided drug design.

Preparation of protein:

For the anti-tubercular activity, required proteins are obtained from the RCSB PDB, and the selected protein are prepared by removing water and adding Kollmann charges to the selected protein.

Ligand preparation:

Ligands are prepared by obtaining it from the PubChem or done by using Chemdraw, Chemsketch tools. A converter application (Open Babel) was used to create the 3D structure from the 2D structure using the SMILES structure and to carry out 3D optimization, store it in MDL-MOL file format, and convert it to PDB format

Docking analysis and interpretation:

Molecular interaction analysis was carried out to understand the molecular affinity between the

Fluroquinolone drugs and the DNA gyrase A & B genes of *M. tuberculosis* using AutoDock 4.2

Docking visualization:

The protein-compound interaction, such as bonded and other non-bonded energies of the drug against the target genes of MTB, was depicted by utilizing the Bio-via Discovery Studio visualizer. This software visualizes the molecular interactions, such as hydrogen bonds, hydrophobic interactions and van der Waals interactions.

Molecular Docking:

The 3D structure of target genes (DNA gyrase A and DNA gyrase B) is shown below. Using AutoDock 4.2, the docking study on Fluroquinolones with DNA gyrase A and DNA gyrase B was performed. Numerous conformations of the protein-compound complex have been generated by the docking modelling, depending on the conformation with the lowest binding free energy (ΔG), and the best conformation was picked. The Bio-via Discovery Studio visualizer program is used to show the interaction between Fluroquinolones and the drug target genes.

Table 1:

Kingdom	*Bacillati*
Phylum	*Actinomycetota*
Class	*Actinomycetia*
Order	*Mycobacteriales*
Family	*Mycobacteriaceae*
Genus	*Mycobacterium*
Species	***Mycobacterium tuberculosis***

Table:1
TB cases over India [28, 29]

	2020	**2023**	**2024**
Estimated TB cases	26.9 lakhs	27.4 lakhs	27.8 lakhs
No of cases reported	24.04 lakhs	24.2 lakhs	25.5 lakhs
Reporting from private sector	6.8 lakhs	7.3 lakhs	8.4 lakhs
% cases from private sector	28.20%	30%	32.90%

Table:2

Fluoroquinolones	**Targeted Enzymes**	**Binding Energy**	**Inhibitory Constant**	**Hydrogen Bonding Residue**	**Hydrogen Interaction**
Moxifloxacin	DNA gyrase A	-7.12	6.23µM	Ser83, Asp87	Val89, Ala90
	DNA gyrase B	-6.79	10.12µM	Glu475, Thr500	Phe504, Leu476
Gatifloxacin	DNA gyrase A	-6.89	9.22µM	Ser83, Asp87	Ala90, Val96
	DNA gyrase B	-6.45	15.04µM	Arg482, lu475	Leu476, Phe504
Levofloxacin	DNA gyrase A	-6.95	8.16µM	Ser83, Asp87	Ala90, Ile911
	DNA gyrase B	-6.61	13.49µM	Glu475, Thr500	Phe504, Leu476
Gemifloxacin	DNA gyrase A	-6.75	-10.5µM	Ser83, Asp87	Ala90, Val96
	DNA gyrase B	-6.38	-18.9µM	Glu475, Thr500	Leu476, Phe504
Sitafloxacin	DNA gyrase A	-7.26	5.23µM	Ser83, Asp87	Val89, Ile91
	DNA gyrase B	-6.93	8.81µM	Glu475, Arg482	Phe504, Leu476
	DNA gyrase A	-6.54	16.21µM	Ser83, Asp87	la90, Val96
Ciprofloxacin	DNA gyrase B	-6.31	22.35µM	Glu475, Thr500	Leu476, Phe504

Figure 1: MTB size

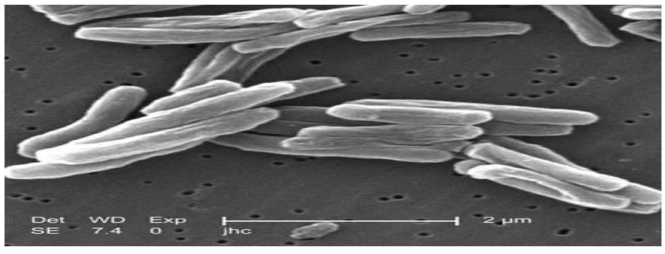


Figure 2: Global trend in case notification of people newly diagnosed with TB 2010-2023.

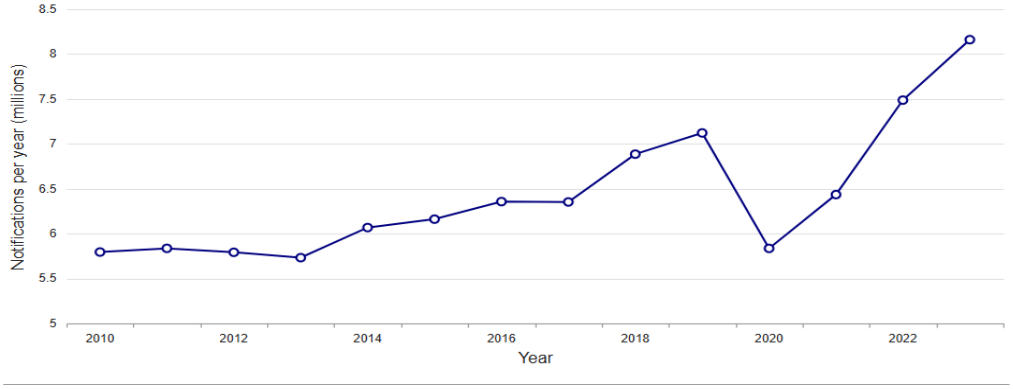


Figure 3: Pathogenesis of Tuberculosis

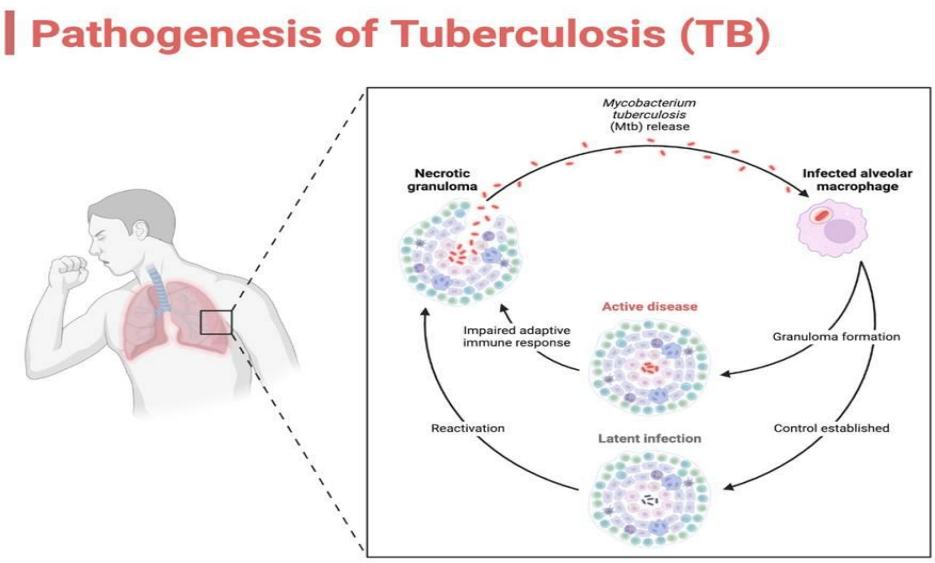


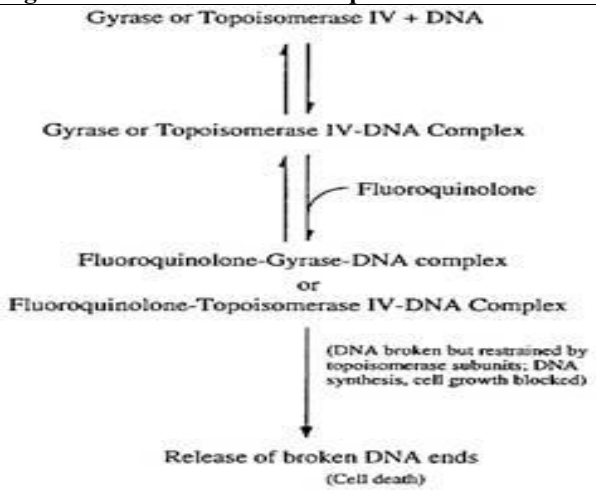
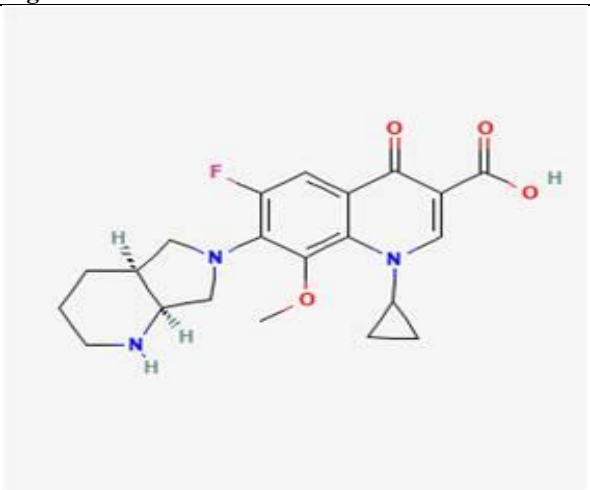
Figure 4: Mechanism of Fluroquinolone	**Figure 5: Moxifloxacin.**
	

Figure 6: Gatifloxacin.

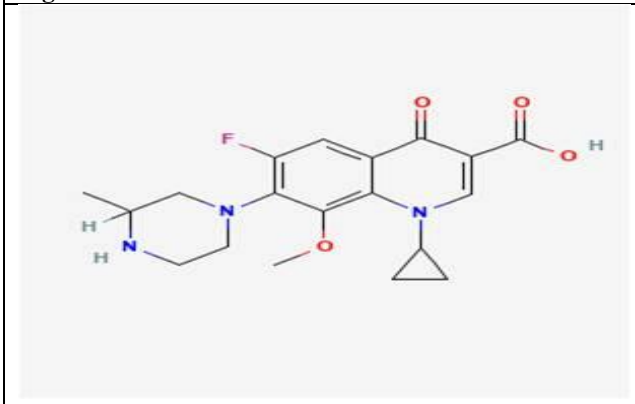


Figure 7: Levofloxacin.

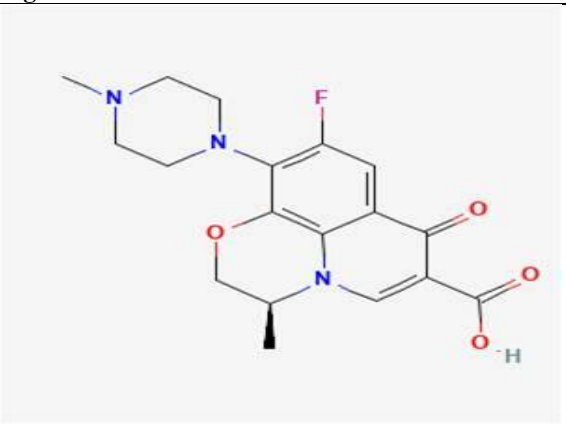


Figure 8: Gemifloxacin.

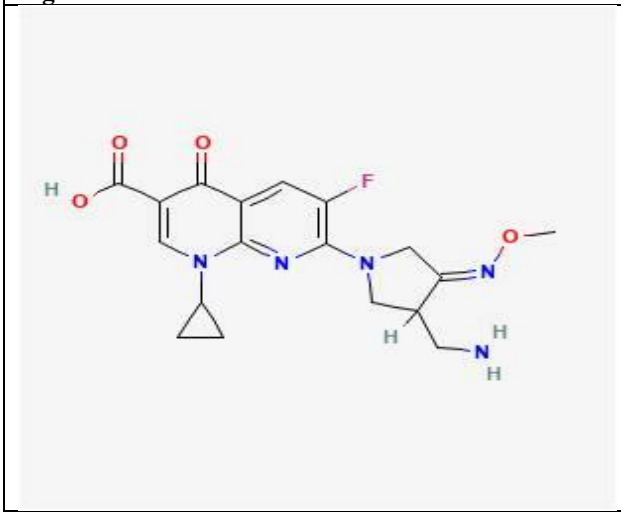


Figure 9: Sitafloxacin.

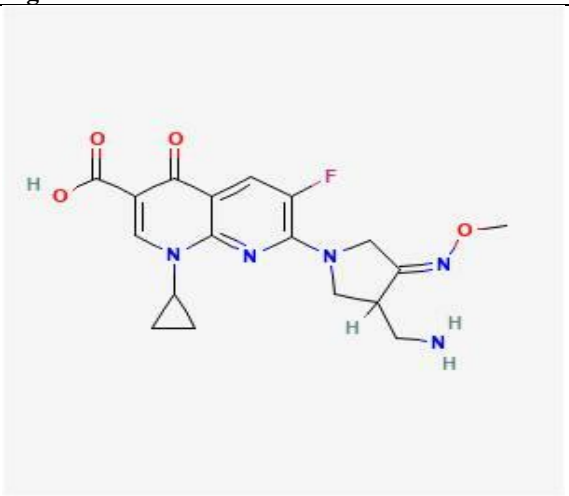


Figure 10: Ciprofloxacin.

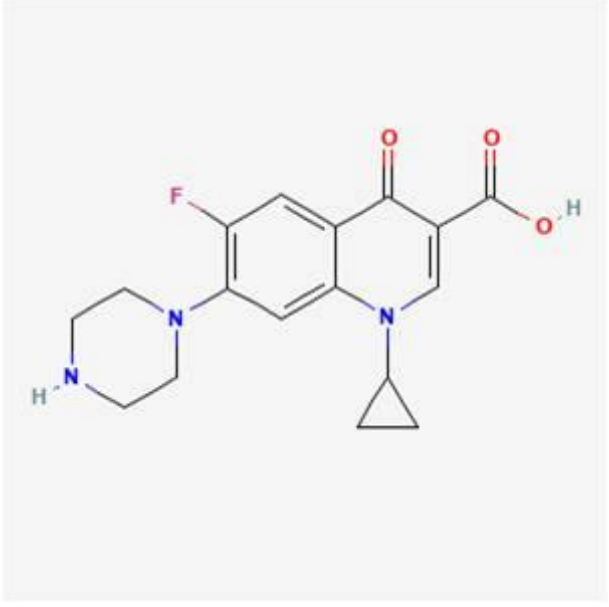


Figure 11: Docking visuals

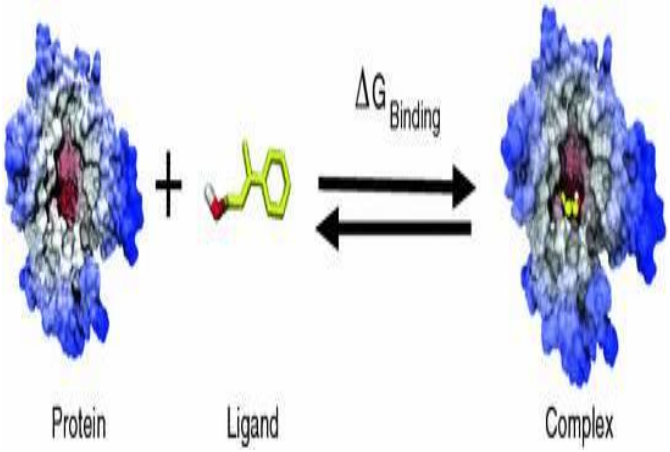
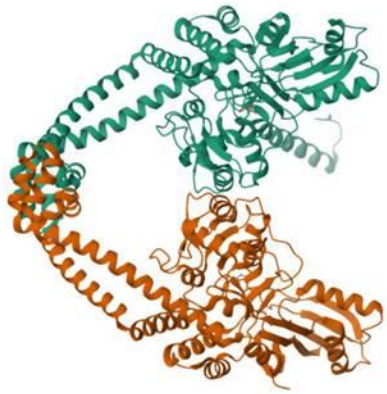
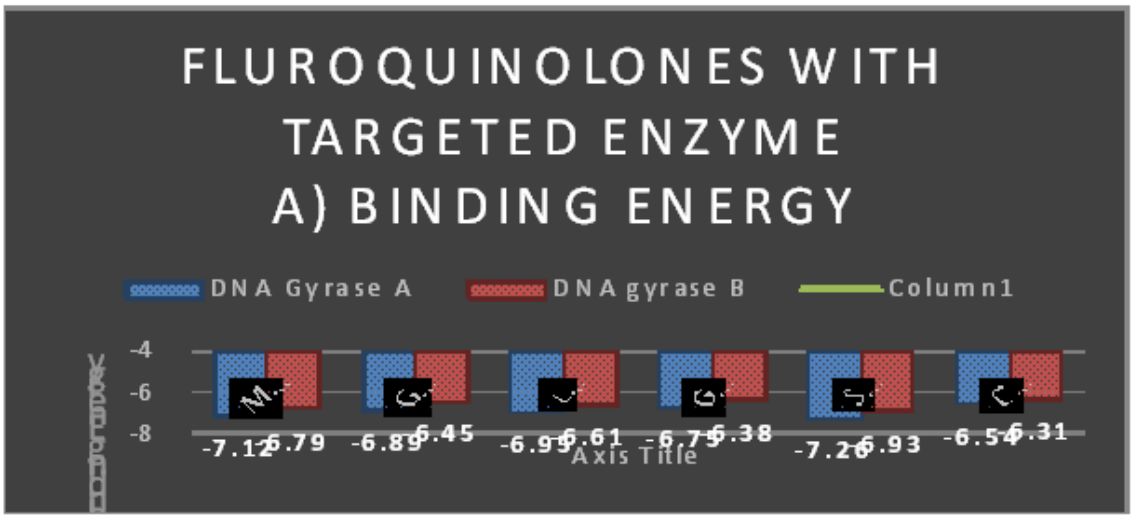
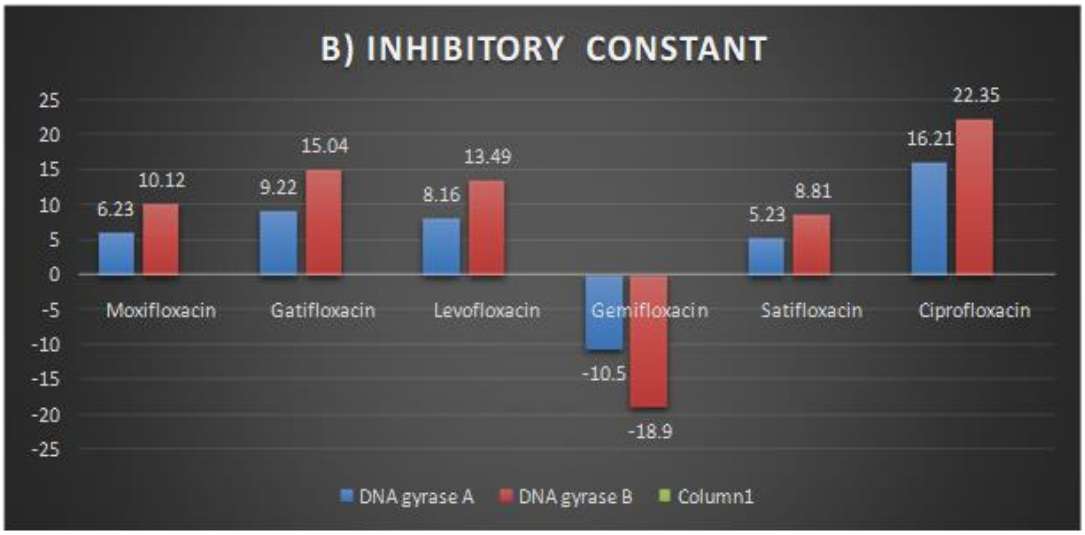


Figure 12: DNA gyrase A	**Figure 13: DNA gyrase B**
	





RESULTS AND DISCUSSION

Based the docking studies of related articles done by several authors that exhibits some relevant binding affinity towards the targeted gene which is listed in table 2. [17, 18, 20, 21].

CONCLUSION

Tuberculosis (TB) continues to be a global health burden, with an increase in the emergence of multidrug-resistant and extensively drug-resistant (XDR) Mycobacterium tuberculosis. With review of this protein optimization and docking studies of Mycobacterium tuberculosis, it states that upon all other enzymes like rpoB, katG, inhA, embB, gyrA, gyrB, rrs, eis, tlyA, and pncA with fluoroquinolone drugs. The DNA gyrase A and B have a higher affinity for the binding of fluoroquinolone antibiotic drugs.

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