Exploring Topoisomerase as a Drug Target: Molecular Docking Analysis for TUBERCULOSIS Disease Drug Repurposing

A Dissertation

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LIST OF ABBREVIATIONS

MT	Microbacterium Tuberculosis
TB	Tuberculosis
HbhA	Heparin binding hemagglutinin Adhesin
TNF	tumor necrosis factor
WHD	winged-helix domain
PDB	Protein Data Bank
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
FDA	Food and Drug Administration
MDR-TB	Multidrug-resistant Tuberculosis
NRAMP	Natural resistance associated macrophages
IFNG	Interferon-gamma
NOS2	Nitric oxide synthase 2
IGR	Intergenic region
VDR	Vitamin D receptor
TYK2	Tyrosine kinase 2
TLR	Toll like receptor

ABSTRACT

Aim:

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), remains a leading cause of global mortality, particularly in high-burden countries. Despite decades of research, TB treatment continues to face major challenges due to the rise of multidrug-resistant strains and limitations in current therapeutic options. This study aimed to identify novel therapeutic strategies through the repurposing of FDA-approved drugs by targeting bacterial topoisomerases—essential enzymes involved in DNA replication, transcription, and cell survival. The specific objective was to use a computational approach, including molecular docking and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis, to screen FDA-approved compounds for their structural and functional similarity to Levofloxacin, a known topoisomerase inhibitor, and to assess their potential as anti-tubercular agents with favorable safety and efficacy profiles.

Result:

The molecular docking analysis was performed on a dataset of 388 FDA-approved drugs to evaluate their potential binding affinities with topoisomerase enzymes implicated in MTB survival. Out of these, 48 compounds fulfilled the required pharmacokinetic and drug-likeness

properties, such as Lipinski's Rule of Five, blood-brain barrier permeability, and minimal PAINS (Pan Assay Interference Compounds) alerts. Among them, 14 compounds exhibited high binding affinities with docking scores better than -8.0 kcal/mol, suggesting a strong and stable interaction with the target protein. Notably, Quarfloxacin, Finafloxacin, Ofloxacin, D-levofloxacin, and Sitafloxacin emerged as the top-performing candidates based on both docking scores and favorable ADMET profiles. These compounds demonstrated high water solubility, significant gastrointestinal absorption, and minimal predicted toxicity, including low hepatotoxicity and no major immunotoxic or carcinogenic risks. The structural interactions revealed key residues involved in ligand binding, highlighting the potential for these compounds to effectively inhibit topoisomerase activity in MTB.

Conclusion:

This study reinforces the potential of drug repurposing as a viable strategy to accelerate the discovery of new treatments for tuberculosis, especially in the face of increasing drug resistance. The identification of several fluoroquinolone derivatives with strong topoisomerase binding affinity and acceptable safety profiles suggests they could serve as promising candidates for further preclinical and clinical evaluation. Specifically, Quarfloxacin and Finafloxacin demonstrated optimal pharmacokinetic and toxicity profiles, making them suitable for oral administration and further investigation. By leveraging computational tools such as molecular docking and ADMET analysis, this study demonstrates a time- and cost-effective approach to identifying new therapeutic avenues. Overall, targeting bacterial topoisomerases using repurposed drugs opens a promising path toward the development of more effective TB treatments and supports future translational research in this critical area of global healt

Chapter 1 Introduction

One of the long-standing disease that affects people is tuberculosis (TB), which is caused by the strain of Mycobacterium tuberculosis. There are several ways that tuberculosis can present itself, but the most prevalent one is pulmonary TB.[1] In addition to the lungs, other organs such as the central nervous system and bones (Pott's disease) can be impacted by TB. Since TB is an organism that targets several systems, it will cause serious health complications. Everything starts with aerosol droplets that carry strains of MT and land on the lung's alveolar surface.[2] Once infected, it will initiate the infiltration of inflammatory cells, which attempt to keep the bacteria in one area and build a main Ghon complex that stops the organism from spreading to other parts of the body. If the initial Ghon complex ruptures and secondary TB develops, it may stay latent or become reactive. Because of its physiological and anatomical characteristics, Mycobacterium TB is virulent even though it does not generate any toxins like other organisms. Factors such as intracellular growth, oxygen-mediated radical detoxification, cord factor, high lipid density in the cell wall, Erp surface-located protein, mycocerosic acid, and adherence, including heparin-binding hemagglutinin adhesin (HbhA), are among the structural and physiological factors that are most likely to be the cause. To cure TB patients, appropriate research and treatment must be carried out.[3] Tuberculosis (TB) was still a major worldwide health concern in 2023. The World Health Organization's Worldwide TB Report 2024 estimates that 10.8 million people, including six million men, more than three million women, and more than one million children, developed TB. An estimated 1.25 million people died from tuberculosis (TB), including 161,000 who also had HIV. With around 1.3 million children getting TB and almost 191,000 fatalities among those under 15, including 25,000 children with HIV, children were particularly impacted.[4] Only over 40% of those with multidrug-resistant tuberculosis (MDR-TB) received treatment in 2023, indicating that the disease still poses a hazard to public health. The majority of TB cases were concentrated in South-East Asia (45%), Africa (24%), and the Western Pacific (17%). India continues to bear the highest burden of TB globally, accounting for 26% of all cases, followed by Indonesia, China, the Philippines, and Pakistan. While TB-related deaths decreased from 1.32 million in 2022 to 1.25

million in 2023, the global TB incidence rate experienced a slight increase in recent years, reversing previous declines.[5] Achieving global TB elimination goals will require increased investment in TB prevention, diagnosis, treatment, and research, particularly in high-burden countries like India. About 90% of persons who get Mycobacterium tuberculosis acquire latent TB, which is defined by the presence of dormant bacilli that do not cause any symptoms. On the other hand, about 10% of infected people develop active TB disease.[6] About 5% of patients have active disease within the first two years after infection; the remaining 5% may reactivate later in life, frequently due to immune suppression or underlying comorbidities. Numerous risk factors that impact general health or host immunity have a substantial impact on the progression of latent to active TB.[7]

HIV infection, age-related immune senescence, genetically based intrinsic immunodeficiencies, and immunosuppressive conditions associated with solid organ donation are significant immunological risk factors. Additionally, it is well recognized that immune surveillance is compromised by cytotoxic chemotherapy, long-term corticosteroid treatment, and the use of tumor necrosis factor (TNF) antagonists, which are frequently used to treat autoimmune diseases. One becomes more vulnerable to active TB as a result. This risk is increased by metabolic diseases including diabetes mellitus and nutritional deficits, especially protein-energy malnutrition.[8]

Lifestyle and behavioral factors are significant in addition to clinical immunosuppression. Heavy drinking and smoking have been independently associated with an elevated risk of active tuberculosis due to their negative effects on pulmonary and systemic immunity. Together, these factors demonstrate how important a comprehensive risk assessment is for individuals with latent tuberculosis infection to prevent the disease from worsening.[9] Global TB eradicated campaigns have been significantly impacted by the COVID-19 pandemic, which has led to a decrease in latent and active cases of TB and infections in the year 2020. However, it also contributed to an estimated half million more TB-related deaths between 2020 and 2022. The increased incidence of TB infections since 2021 or 2022 is most likely due to a backlog of patients with delayed diagnoses.[10] Drug-resistant TB is a significant public health issue, accounting for 13% of new infections and 17% of treated cases that are susceptible to rifampin and isoniazid.[11]

Chapter 2

Literature review

2.1Tuberculosis

2.1.1 History

In the early 18th century, English physician Benjamin Marten proposed an infectious etiology for TB. During the industrial revolution, the illness was associated with socioeconomic circumstances and spread quickly, especially among young people.[12] The phrase "white plague" was initially used to refer to the pallor and severe anemia of TB patients. Scrofula, TB, and phthisis were all treated as separate disease entities or symptoms of the same illness, raising questions about the etiopathological genesis of phthisis.[13] Following the identification of the TB bacillus in Guinea pigs in 1867, anti-tuberculous drugs were created, and the World Health Organization pledged to eradicate M. tuberculosis by 2050. [14]

2.1.2 Genetic and Metabolic Interactions in the Pathophysiology of Tuberculosis: Consequences for the Development of Therapeutics

Because of its complicated pathophysiology and the growing threat of multidrug-resistant strains, tuberculosis (TB), a chronic infectious illness caused by Mycobacterium tuberculosis (MTB), continues to be a worldwide health problem.[15] [16]

Important Host Genes

TNF-

This gene produces the cytokine tumor necrosis factor alpha, which is implicated in immunological responses and inflammation. Granulomas are structures that hold the TB germs and stop them from spreading, and their development and maintenance depend on TNF.[17] Additionally, TNF stimulates macrophages, which are cells capable of eliminating the germs. Nevertheless, tissue injury and fibrosis can also result from excessive TNF production.[18]

NRAMP

Macrophages and other cells express a protein called natural resistance associated macrophage protein 1, which is encoded by this gene. The development and survival of TB bacteria depend on the movement of iron and other metals, which is facilitated by NRAMP1. [19]Antimicrobial compounds called reactive oxygen and nitrogen species are also produced in response to NRAMP1 modulation. There is evidence linking NRAMP1 mutations to heightened vulnerability to tuberculosis.[20], [21]

IFNG

The gene IFNG produces the cytokine interferon gamma (IFN-γ), which is essential for the immune system's defense against tuberculosis (TB). IFN γ causes macrophages, which are cells that can destroy TB germs, to produce more antibiotic chemicals. Additionally, T cells—immune cells that aid in the activation and regulation of other immune cells—are encouraged to differentiate by IFN-γ.[22] Additionally, IFN-γ collaborates with TNF, another cytokine that is necessary for granuloma development and stopping the spread of TB germs. (IFN-γR1) is a protein that attaches itself to IFN-γ and sends a signal to the cell. The activation and functionality of T cells and macrophages in response to TB infection depend on IFN-γR1.[23] Defects or mutations in IFN-γR1 can raise the chance of contracting TB and weaken the immune system's defenses against it.[24]

NOS2

Nitric oxide synthase 2, an enzyme that generates nitric oxide (NO), a reactive nitrogen molecule that can destroy TB bacteria, is encoded by this gene. IFN-γ and other cytokines cause macrophages and other cells to produce NOS2.[25] In addition, NOS2 controls the expression of additional genes related to immunity and inflammation. Environmental and genetic variables, including oxygen levels and food, affect NOS2 activity.[26]

IGR

The intergenic region between NRAMP1 and DC SIGN, two genes important in the identification and phagocytosis of TB germs, contains this set of genes. NRAMP1 and DC SIGN expression and function may be impacted by polymorphism IGR genes. [27] Additionally, IGR genes may affect a person's susceptibility and resistance to tuberculosis and other infectious illnesses.[28]

VDR

This gene generates a vitamin D receptor protein, is binds to vitamin D and regulates the expression of many genes involved in calcium metabolism, immunity, and bone health.VDR regulates the synthesis of antimicrobials and is expressed in macrophages and other cells.[29] peptides that have the ability to eradicate TB germs, like cathelicidin. Additionally, VDR controls the expression of NOS2 and other genes related to immunity and inflammation.[30] Increased vulnerability to tuberculosis has been linked to vitamin D insufficiency and VDR mutations.[31]

TYK2

Tyrosine kinase 2, an enzyme implicated in the signaling pathways of several cytokines, including IFN-γ, IL-6, IL-10, and IL-12, is encoded by this gene. The transcription factors STAT1 and STAT3, which control the expression of several genes related to inflammation and immunology, are activated by TYK2. [32]Additionally, TYK2 controls the synthesis of antibacterial compounds called reactive oxygen and nitrogen species. Increased vulnerability to TB and other infectious illnesses has been linked to mutations in TYK2. [33]

TLR

These genes produce toll-like receptors, which are proteins that can identify and attach to a variety of microbial substances, including mycobacterial DNA, peptidoglycan, and lipopolysaccharides (LPS).[34] TLRs trigger the production of pro-inflammatory cytokines including TNF, IL-6, and

IL-12 and activate the innate immune system. By promoting the maturation and migration of dendritic cells, which deliver antigens to T cells, TLRs help strengthen the adaptive immune system.[35] Additionally, TLRs control the expression of SARS-CoV-2 receptors, which may have an impact on TB patients' vulnerability and severity of COVID-19.[36]

2.2.3 Crucial MTB Metabolic Routes for Infection and Durability

MTB's metabolic flexibility contributes to its capacity to endure in the host and avoid immune clearance. MTB survival during infection has been found to depend on a number of metabolic pathways, especially when host macrophages are immune-stressed and nutrient-limited.[37]

Amino Acid and Nucleotide Biosynthesis

The fragility of MTB's reliance on certain metabolic pathways may be used therapeutically. For example, intracellular development and virulence depend on the phenylalanine biosynthesis route, which involves the gene pheA, and the de novo purine biosynthesis pathway, which involves purF.[38] In animal models, MTB has been shown to be significantly attenuated when these pathways are disrupted by genetic mutations or pharmacologic inhibitors. These results highlight the pathogen's dependence on endogenous biosynthesis since it has limited access to metabolites originating from the host.[39]

Signaling Routes Mediated by Cytokines

Modification of host signaling networks, especially those linked to cytokine responses, is another aspect of host-pathogen interactions in tuberculosis. The key role in coordinating immunological response to the infection is suggested by the enrichment of pathways such cytokine–cytokine receptor interaction, NF-Kb activation, and JAK-STAT signaling.[40] These pathways control the production of inflammatory mediators (including IL-6, IFN-γ, and TNF-α), which are essential for the development of granulomas and the control of infection. Prolonged activation, however, may

also lead to tissue damage and immunopathology, suggesting a fine balance in immunological signaling.[41]

2.2.4 Implications for Novel Therapeutic Strategies

A strategic foundation for the creation of novel therapies is provided by the discovery of important host and pathogen genes implicated in TB pathogenesis:

Targeted Drug Development

Monoclonal antibodies or small chemical inhibitors that target important metabolic enzymes (such as PurF or PheA) may be able to specifically reduce MTB viability without having an impact on host cells. By targeting hitherto undiscovered weaknesses, these targets could provide a remedy for drug-resistant TB strains.[42]

Immunomodulatory Therapies

Increasing cytotoxic responses via the GZMB and PRF1 pathways or altering hub genes like CTLA4 may improve host immunity, particularly in immunocompromised people. Restoring immunological responses during a persistent tuberculosis infection may be possible with immune checkpoint blockade, which is now used in cancer.[43]

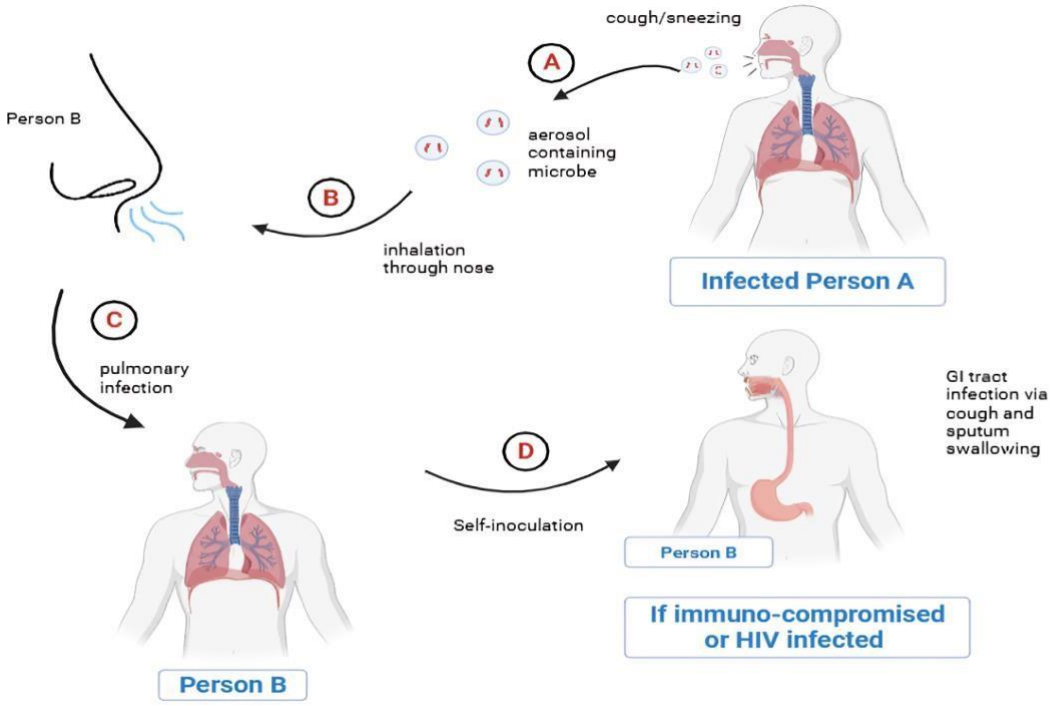

Fig 1. Various ways the disease spreads.

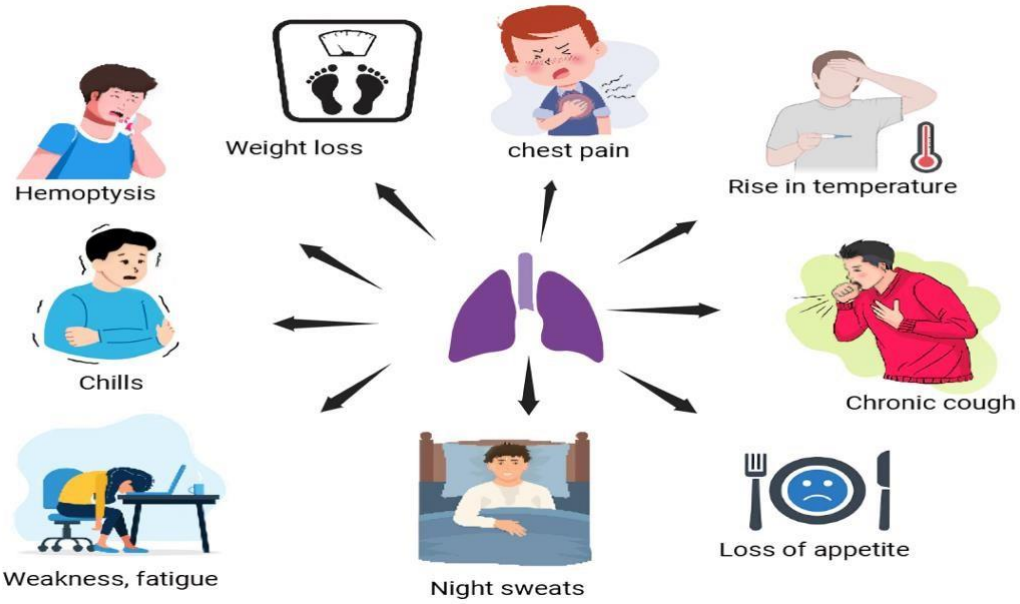

Fig.2 Symptoms of TB

2.2 TOPOISOMERASE

2.2.1 Essentiality of Topoisomerase I

Topoisomerases are a significant class of enzymes that preserve topological equilibrium across several DNA transaction processes, including transcription, chromosomal segregation, DNA replication.[45] Type I and type II topoisomerases have been distinguished by their structure and mode of activity. Despite having distinct modes of action, type I and type II enzymes work in tandem to preserve the cell's topological equilibrium. Topoisomerases have been investigated as potential therapeutic targets due to their crucial role in cellular processes in a variety of animals.[46] It is true that human type I and type II topoisomerases are widely used in anticancer treatments. Numerous antibacterial drugs have been effective in targeting DNA gyrase, the bacterial type II enzyme. The Mtb genome encodes just one single copy of type I and a single copy of the type II topoisomerase.[47] The chemotherapeutic potential of the Mtb DNA gyrase has garnered significant interest, and newer fluoroquinolones that target DNA gyrase, such as moxifloxacin and gatifloxacin, show promise in reducing the length of time needed to cure tuberculosis. On the other hand, the potential of topoisomerase I, a member of the type I class, as

a possible therapeutic target has not been investigated. [48]According to high-throughput saturation mutagenesis research, TopoIMt (Rv3646c) is necessary for Mtb growth and survival. Although TopoIMt is biochemically described, its significance in Mtb development and in vivo function has not yet been validated.[49]

2.2.2 Mechanism of action

Cellular functions of topoisomerases

(1). Type IB topoisomerases

Eukaryotic Top 1's swivel function is crucial for releasing superhelical tension in DNA that develops during transcription and replication. Interestingly, Top 2β's topoisomerase activity is necessary for transcription, although Top 1's catalytic activity is irrelevant for transcription activation.[50] RNA polymerase II and hTop 1 activity are correlated in human cells. Research on the effects of Top 1 poisons like camptothecin has shown that both Top 1 and Top 2β are necessary for the effective expression of long genes. [51]The only topoisomerase that specifically targets mitochondria is mTop I. However, mitochondria have also been shown to contain Top 2α and Top 2β as well as a long Top 3α isoform produced by a different, upstream translation start point. Crucial functions of mTop 1 include transcription and circular mitochondrial DNA replication. Cells can live without mTop 1, however losing mTop 1 has a negative impact on energy consumption and mitochondrial integrity. Nevertheless, Top 3α in mitochondria probably helps cells survive.[52]

(2). Type IIA topoisomerases

The only topoisomerase that can introduce negative supercoils is DNA gyrase, whereas Top 4 is mostly in charge of decatenating daughter chromosomes. The balance between DNA gyrase's supercoiling activity and Top 1's relaxing activity maintains the bacterial chromosome at a certain negative superhelicity.[53] There is just one type IIA topoisomerase found in lower eukaryotes, Top 2, whose structure and function are comparable to those of Top 4 (notably, Top 2 and Top 4 have different C-terminal domains). Bacterial and eukaryotic type IIA topoisomerases vary

primarily in that the former are homodimers, while the latter are A2B2 heterotetramers.[54] When the two subunits are combined to form a single peptide, bacterial type IIA topoisomerases have been demonstrated to remain active . A heterotetrametric type II topoisomerase can therefore be transformed via gene fusion into a homodimeric type II topoisomerase. Mammals contain two isoforms of Top 2, Top 2α, and Top 2β, in contrast to lesser eukaryotes.[55] These isoforms have different physiological roles while having comparable catalytic activity and about 70% amino acid sequence similarity.[56] Top 2α expression is markedly increased in cancerous and growing cells, and it is necessary for DNA replication and chromosomal segregation. Consequently, hTop 2α functions as a biomarker for some types of cancer. On the other hand, Top 2β is essential for transcription in both cancerous and normal mitotic and postmitotic cells. Additionally, it is necessary for some brain cells to form and survive.[57]

2.2.3 Structural studies of topoisomerases

(1). Type IB topoisomerases

Because of the torsional stresses present in these structures, Relaxed DNA in a low energy shape, while supercoiled DNA either positive or negative are in high energy states. In a simple three-step process that eliminates the need for additional energy input, type IB topoisomerases efficiently gather the available energy held in DNA supercoils to return these highly strained molecules of DNA to their stable forms.[58] [59] This relaxation event is triggered by a nucleophilic attack initiated by a catalytic tyrosine residue on a cleavable phosphodiester bond in a double stranded DNA, resulting in a transient single-strand break composed of a 3′-linked phosphotyrosyl bond and released 5′-OH. [60] By rotating the DNA molecule around the break, the strain caused by DNA supercoiling may be released . The enzyme is then reset for the subsequent relaxation event by resealing the cleaved phosphodiester bond and regenerating the catalytic tyrosine. The structural analysis of hTop 1 in non-covalent and covalent complexes with DNA indicates that a "controlled rotation mechanism" is most likely responsible for the expected DNA rotation throughout the catalytic cycle. [61] CAT domain, which was initially discovered in tyrosine recombinases[62], make up the catalytic core of type IB topoisomerases. Both of these regions clamp around the DNA duplex upstream of the cleavage site. Due to a number of DNA- proteins

interaction between DNA and the core of Top 1, the DNA located upstream of single-strand break is firmly bonded, primarily to the phosphate backbone of the DNA, and cannot spin. In contrast, the CAT domain's linker sections and the Cap domain's nose cone only loosely hold the DNA downstream of the break.[63] Theoretically, the accumulated superhelical strain can easily penetrate this weak protein-DNA interface, enabling the downstream DNA to rotate one or more times around the cleavage point until all torsional stresses are eliminated or the split is sealed once again. In the presence of significant superhelical tension, the enzyme seems to have trouble capturing the downstream DNA due to the small amount of DNA-interacting residues.[64] However, at lower superhelical densities, this interface can be restored, stopping the revolving DNA and putting the 5′-OH in good position to participate in the ligation event[65]. Instead of random rotation, which would complicate the ligation activity, the enzyme "controls" the orientation and rotation of downstream DNA.[66] For their catalytic activity, topoisomerases of types IA, IB, and IIA have different needs for Mg2+. The reason type IB topoisomerases do not need a divalent cation to function was discovered by structural study of the enzyme-DNA covalent complex.[67] It is known that topoisomerase-catalyzed transesterification reactions go through a high-energy bipyramidal transition state where the pentavalent phosphorus is joined to two oxyanions. To electrostatically stabilize the transition state, type IB topoisomerases and tyrosine recombinase family members need positively charged Arg and Lys residues, while types IA and IIA topoisomerases require Mg2+ as a required cofactor.[68]

(2). Type IIA topoisomerases

Earlier then the first crystal structure of type IIA topoisomerases were present, a number of advanced biochemical research showed that the type II topoisomerases is changed the linking number of DNA via “two-gate" method. This theory states that the construction of the so-called "DNA gate" corresponds to the binding of G-segment DNA to enzyme, which initiates the catalytic cycle of type IIA topoisomerases.[69] Through Mg2+-dependent transesterification processes that occur between catalytic tyrosine residues and the phosphodiester bonds of the DNA backbone, such a topoisomerase-DNA binary complex facilitates the cleavage of both G-segment strands. The G-segment experiences a reversible DSB as a consequence. When the double- strand DNA breaks (DSB) is produced, the sugar phosphate backbones release and two parts of the G-

segment may split away.[70] The strand-passage channel is amplified to achieve an relaxed state when the gap between the ends of the cleaved DNA is sufficiently large to allow the Transported DNA segment to pass through. The approaching T-segment enters the strand-passage channel by traveling through the gate of entry on side of type IIA topoisomerase (the N-terminal end of eukaryotic Top 2) then following the DNA-conducting pathway that the G-segment travels to reach at its binding surface. Once the Transported DNA segment has passed through the DNA gate, it is aimed at and eventually released from exit point at the other end of the topoisomerase (the Cterminal end for eukaryotic Top 2). [71]Because T-segment entry and departure are regulated by two different protein gates of type IIA topoisomerases, this unidirectional DNA transport method is known as "two-gated. The structural consideration of the following elements would be necessary for a more thorough illustration of the catalytic processes of type IIA topoisomerases: (1) the general structure and spatial domain organization of the enzyme; (2)creation of a DNA gate that is connected to proteins; (3) the liberation of the DNA backbones through the strand breaking of a bound G-segment; (4) the T-segment's capture; (5) DNA gate-related conformational alterations; (6) the split G-segment's resealing and the release of the T-segment; (7) For the next catalytic cycle, the enzyme's conformation is reset. The several crystal structures we have discovered for various sections of type IIA topoisomerase as well as two catalytically competent type IIA topoisomerases have given us valuable insights into each of these stages. At different phases of the catalytic cycle, these crystal structures show structural snapshots of distinct type IIA topoisomerase areas.[72]

The constituent domains of all type IIA topoisomerases exist in pairs, exhibiting a two-fold symmetric design. The access gate is made up of two GHKL ATPase domains, that are acknowledge to dimerize when ATP is attached but separated when nucleotide-free. The gate seems to function as to act like a protein operated by nucleotide clamps appropriate for transported DNA segment capture since ATP controls its opening and shutting.[73] According to the structural analysis, components from both ATPase domains create each nucleotide binding pocket; ATP would thus encourage dimerization by "gluing" the two domains together. Even though every kind of IIA topoisomerase has two mechanically similar sites for ATP binding, it was found that a ATP is sufficient to close the gate of entry in the closed form.This result implies that entrance gate would not open again once it had been closed until both of the bound ATP molecules had been hydrolyzed at the end of the catalytic cycle.[74] G-segment DNA and type IIA topoisomerase

combine to generate the DNA gate. Two copies of each of the following domains make up the protein portion of the DNA gate: the winged-helix domain (WHD), topoisomerase-primase (Toprim) domain, and a so-called "tower" domain. Transesterification process depends on the Mg2+-coordinating DXD motif found in the Toprim domain, which takes on a Rossmann-like shape.[75] Because it contains the catalytic tyrosine residue and a helix-turn-helix DNA-binding motif, the WHD domain is essential to type II topoisomerase activity. The tower domain is a crucial component of the DNA gate and supplies extra DNA-binding residues. According to the DNA gate's closed crystal structures, all of these regions combine to form a G-segment interaction groove, with the WHD motifs at the bottom and the towering and Toprim motifs at the sides.[76] In addition to being bent into a U shape by two homologous isoleucine residues that merge into DNA at locations 12 base pairs apart, the bound G-segment is mainly stabilized by association with the helix-turn-helix patterns of the WHD domains.[77] The interaction between the DNA backbone and basic residues from the tower domains is thought to maintain the G-segment's bent shape. The active site tyrosine residues, the catalytic Mg2+ ions, as well as the scissile phosphodiester linkages are most likely oriented in the best possible way to take part in the transesterification events due to the two intercalating residues' distortion of the DNA duplex toward the A-form. Strand breaking and religation activities require the catalytic tyrosine of a single subunit to work in tandem with the magnesium ion provided by the Toprim motif of the antagonistic subunit. A structural feature unique to the DNA opening is this one. For the phosphotyrosyl linkage to be reactive, DNA gate must close since decoupling both catalytic parts would stop the covalent constrained from being attacked by adjacent nucleophiles.[79]The Cterminal dimerization interface, which is often seen in the closed condition, is represented by the exit gate. The opening of the exit gate is anticipated to be a brief occurrence within the catalytic cycle, with the conformational balance of the gate leaning towards the closed state. This is because an early opening of the gate could drastically change the overall structure of a type IIA topoisomerase and potentially prevent the cleaved G-segment from being rejoined.[80] However, crystallography has shown that the exit gate opens even when there is no T-segment. The DNA gate is positioned between the entrance and exit gates, as demonstrated by the structures of a fully functioning type IIA topoisomerase. This arrangement fully conforms to the proposed two-gate system. Additionally, the DNA gate has a hollow on both sides, suggesting that the T-segment may become briefly caught inside the enzyme during transit.[81] Additionally, these structures

demonstrated that the transducer domains and coiled-coil sections, respectively, connect the DNA gate to the entrance and exit gates. It is believed that allosteric connections between the gates are mediated by these bridge components. For instance, the transducer domain may reorganize as a result of conformational changes brought on by ATP hydrolysis, changing the structure of a DNA gate.[82] Additionally, it has been demonstrated that by modifying the kink of the coiled-coil region, the DNA and exit gate conformations may be connected. In order to understand the coordination of these gates' opening and closing, more positional states of IIA topoisomerases, like the DNA gate's opening, would be necessary.[83]

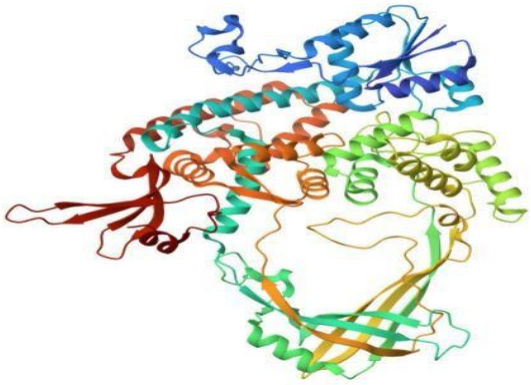

Fig. 3 **Structure of topoisomerase diagram**

2.3.Pathways involved in tuberculosis

2.3.1. Hijacking phagosomes as a replicative niche

It was suggested that M. tuberculosis uses the organelle as an intracellular replicative niche by preventing phagosome maturation. Phagosomes frequently engage with the endosomal segment to attract V-ATPase, which consistently transfers protons into the phagosome and produces a strongly acidic lumen necessary for later joined with lysosomes and the action of the antimicrobial agents that they supply.[84]

Tyrosine phosphatase PtpA, a secreted mycobacterial protein, enters the cytosol through the phagosome membrane and attaches itself to host V-ATPase subunit H, according to recent research. This interaction not only locates PtpA into its catalytic substrate, vacuolar protein sorting (VPS)33B, which is essential for maintaining endocytic membrane fusion, but it also prevents VATPase from attaching to the phagosome membrane.[85] PtpA must dephosphorylate VPS33B and interfere with V-ATPase recruitment in order to prevent phagosome acidification and phagosomelysosome fusion. It's interesting to note that whereas PtpA deletion limits microbial growth in human THP-1 cells, mutant with a deletion did not exhibit reduced growth or pathogenicity when infected in the mice. This disparity was ascribed to possible species variations influencing PtpA activity, meaning that decreased activity in a certain species would mask any flaw brought about its deletion.[86] It is equally probable that certain mycobacterial virulence factors have some functional redundancy that only manifests themselves after in vivo infection. Many virulence factors, such as SecA2, PknG, elements of the ESX-1 secretion system, and different glycolipids, have been shown to disrupt phagosome-lysosome fusion in vitro. In animal models, it was discovered that deleting several of these virulence factors reduced bacterial growth and illness.[87] Although this may suggest that some virulence factors have multiple functions in preventing the formation of phagolysosomes in vivo, it is challenging to ascertain the degree to which their phagolysosome-inhibitory activity alone affects the pathophysiology of the disease due to the functionally diverse roles of many of the effectors of this inhibition. [88]

2.3.2. The phagosome—a Trojan horse?

Previously, it was thought that Mycobacterium TB lived inside the macrophages' phagosomal compartment over the whole infection cycle and was only liberated when the host cell underwent lytic death. But new data has revealed that M. tuberculosis may go into the cytosol and cause the phagosomal membrane to break, which has caused a paradigm change. This phagosomal escape seems to depend on the ESX-1 secretion system as well as certain lipids in the bacterial outer membrane, including phthiocerol dimycocerosates (PDIMs).[89] It has been shown that the secondary messenger cyclic GMP-AMP (cGAMP) is produced when the cytosolic DNA sensor

cyclic GMP-AMP synthase (cGAS) attaches to M. tuberculosis DNA. The stimulator of interferon genes (STING) is then activated, which sets off the STING-TBK1-IRF3 signaling pathway and causes type I interferons, particularly IFN-β, to be transcriptionally induced. IFN-β release after M. tuberculosis infection was eliminated in vitro when the cGAS-STING pathway in macrophages was deleted.[90] In vivo investigations employing $Cgas^{-/-}$ and $Sting^{-/-}$ mice, however, only partially reflected this phenotype: other inflammatory indicators did not alter, despite a considerable decrease in blood IFN-β levels. Additionally, at all time periods, the bacterial burdens in different organs were similar in wild-type and mutant animals, suggesting that the decreased IFN-β levels did not compromise resistance to M. tuberculosis infection.[91]

2.3.3. Modulation of type I IFN signaling

Despite being strong antiviral immunity mediators, type I IFNs are frequently linked to the advancement of bacterial infections. Except for a decrease in the splenic bacterial load, C57BL/6 mice lacking the type I IFN receptor (IFNAR) are identical to wild-type mouse after being infected with M. tuberculosis.[92] However, animals with significantly lower bacterial loads survive when IFNAR is deleted in more vulnerable mouse strains. Together with findings from people, these murine model studies provide credence to the idea that type I IFNs are linked to the advancement of tuberculosis. Although the exact mechanism is still unknown, new research indicates that it may entail the activation of immunosuppressive IL-10, blunting of IFN-γ responsiveness, and suppression of host-protective cytokines (IL-1β TNF, IL-12,). [93],[94]In example, Wassermann and colleagues[95]demonstrated that infected cGAS- and STING-deficient macrophages had decreased IL-10 production. They also showed that cGAS or STING deletion improved the survival and decreased the generation of IL-10 in macrophages contaminated with the hypervirulent HN878 strain, which triggers a higher type I IFN response. Examining the results of infecting Cgas−/− animals with HN878 and, in fact, cGAS deficiency in a more vulnerable mouse strain would be intriguing. A working ESX-1 releasing system is necessary for the generation of IFN-β after MT infection,[96] demonstrating that the mycobacterial DNA that induce cGAS comes from live bacteria instead of dead or decaying ones. It is tempting to think that M. tuberculosis purposefully transfers its DNA in the cytoplasm in order to appoint host signaling in favor of IFNβ synthesis, even if the precise mechanism is yet unknown. However, later research revealed that

strain-dependent variations in IFN-β production were not caused by variations in bacterial access to the cytosol or bacterial DNA shedding, but rather was at minimum linked to the degree of mitochondrial stress and mitochondrial DNA released into the cytosol as a result of M. tuberculosis infection.[97] Thus, type I IFN production may be aided by cGAS's recognition of host mitochondrial DNA. Although the precise cause of strains' differences in their capacity to cause mitochondrial tension or DNA produced unknown, it is likely related to variations in the expression of certain lethal factors. However, since mitochondrial involvement cannot fully explain the changes in IFN-β production between strains, it is nearly a given that other components or pathways play a role.[98]

2.3.4. Short-circuiting signal transduction pathways

Several cytokines are produced by M. tuberculosis-infected macrophages to coordinate a successful immune response against the disease. The MAPK and NF-κB signaling cycles control the synthesis of many host-protective cytokines. It may come as no surprise that the regulatory pathways of several of these cytokines, including TNF, IL-1β, and IL-6, are appealing targets for reducing the host immunological response to infection, as they are strong, nonredundant mediators of anti-TB immunity. For example, the virulence protein PtpA of M. tuberculosis inhibits the NFκB, p38, and JNK pathways in macrophages.[99] To repress the JNK and p38 MAPK pathways, PtpA's phosphatase activity is required, since it dephosphorylates phospho-JNK and phospho-p38. It's interesting to note that this activity is also triggered when host ubiquitin binds to the ubiquitininteracting motif-like region of PtpA. This may limit the protein's phosphatase activity until it is released into the cytoplasmFurthermore, PtpA partially disrupts NF-κB activation by binding to TAB3 in a competitive way, which stops it from attaching to K63 ubiquitin chains. According to another study, PtpA also targets host TRIM27's RING domain.[100]To limit M. tuberculosis, TRIM27 stimulates the activation of the JNK/p38 pathway and possesses E3 ubiquitin ligase activity. By removing PtpA from Mycobacterium bovis bacillus Calmette-Guérin (BCG), M. tuberculosis has a number of other virulence factors (such ESX-1) that BCG does not have, which might make PtpA's role in vivo unnecessary. Because PtpA inhibits MAPK, especially JNK and p38 but not ERK1/2, it disrupts NF-κB signalling, which appears to be the main mechanism by which it suppresses cytokine production.[101]There have also been reports of other

M. tuberculosis virulence factors interfering with NF-κB induction. Purified early released antigenic target 6 (ESAT6), the primary ESX-1 substrate, for instance, blocks NF-κB activation downstream of TLRs, reducing the release of IL-6 and TNF.[102] Interestingly, although while ESAT-6 only binds directly to TLR2, it can block TLR-controlled NF-κB induced downstream of all TLRs. The activation of cytoplasmic Akt kinase following the ligation of additional TLRs seems to avoid the synthesis of MyD88 signalling fragment, thus, NF-κB induction. The mce3 operon encodes the mammalian cell entry protein 3E (Mce3E), which is secreted by phagocytosed M. tuberculosis and produced during human infection.[103]

It was recently demonstrated that MceE3 enters the cytosol and localizes in the endoplasmic reticulum (ER) to disrupt the ERK1/2 MAPK signaling pathway. [104]Here, it binds to ER by interaction with ERK1/2. This prevents the nuclear transfer of phospho-ERK 2/1 and the phosphorylation of ERK 2/1 by MEK1. In Mycobacterium smegmatis and BCG, Mce3E expression increased intracellular growth and inhibited the generation of TNF and IL-6 by infected macrophages. However, it has been observed that cytokine induction and intracellular survival in macrophages were unaffected by loss of the whole mce3 operon in M. tuberculosis.[105] Mice infected with the mce3-deleted strain of M. tuberculosis lived a little longer than those infected with the wild-type strain, despite the fact that their lung bacterial loads remained same.

2.3.5. Host cell metabolic reprogramming

Because of its metabolic adaptability, M. tuberculosis may develop on a range of carbon sources. Based on the research that is now available, the pathogen predominantly depends on the metabolism of fatty acids and cholesterol ester during intracellular growth. [106]The "foamy" macrophages that are typically observed at the interface of core necrotic zones inside granulomas are the result of Mycobacterium TB modifying macrophage metabolism under hypoxic circumstances to encourage the deposition of lipid aggregates.[107] It has been shown that phagosomes filled with Mycobacterium TB connect with and release bacilli into host lipid bodies.

These interactions give phagosomes which would otherwise be nutritionally inadequate a vital supply of nutrients. Additionally, this association with host lipid bodies creates a safe haven where the virus is shielded from bactericidal processes like respiratory burst. Furthermore, mycobacteria that are encased in lipid bodies have a dormant phenotype that allows them to withstand a number of first-line antibiotics.[108]

Even while the benefits of lipid body buildup for M. tuberculosis are well known, it has only now become clear how the pathogen co-opts the macrophage to produce the foamy appearance. ESAT6 causes GLUT-1 glucose transporters to migrate through the cytoplasm to the cell membrane, which greatly increases glucose metabolism and absorption and causes macrophages to enter an anabolic state.[109] ESAT-6 seems to increase the working of a number of glycolyticproteins, which disrupts the regular flow between the tricarboxylic acid cycle and glycolysis. As a result, dihydroxyacetone phosphate builds up and is utilized as a substrate for the production of triacylglycerol (TAG). [110]

While part of this acetyl CoA can potentially be moved toward initial lipid synthesis, concurrently rising intracellular concentrations of acetyl CoA also encourage ketogenesis and cause acetyl CoA to be shunted toward the formation of the ketone body d-3-hydroxybutyrate (3HB), [111] The antilipolytic G protein-coupled receptor GPR109A is induced by secreted 3HB, blocking the process that phosphorylates perilipin.[112] When phosphorylated perilipin is absent, hormonesensitive lipase cannot translocate to lipid bodies, which stops stored TAG from being mobilized. As a result, ESAT-6 alters host glucose metabolism to both stop lipid catabolism and encourage the formation of lipid bodies and, consequently, "foamy" macrophages. However, this increase in glycolysis also reduces IL-10, which is necessary to regulate intracellular replication, and promotes IL-1β . [113]Furthermore, research revealed that lipid body formation may be partly TLR2-dependently induced by mycobacterial cell wall components such trehalose dimycolate and lipoarabinomannan, and does not require live bacilli. [114] This would imply that ESAT-6mediated glycolysis regulation might not be the only or primary factor influencing "foamy" macrophages. It has been found that elevated levels of miR-33 inhibit key activator of autophagy, a process that degrades intracellular part by enveloping them in vesicles bounded with membrane and transporting them to lysosomes. By breaking down TAG and cholesterol esters inside lipid bodies, autophagy is known to encourage lipid catabolism, or lipophagy. [115] Thus, it should

come as no surprise that miR-33-mediated autophagy suppression reduced cellular fatty acid oxidation and increased the quantity and size of aggregates lipid, which facilitate intracellular mycobacteria growth and metabolism. [116] Numerous investigations have shown that intracellular M. tuberculosis incorporates host fatty acids, which are then either used as a source of nutrition or stored as TAG. [117] The way the virus mobilizes host TAG reserves to import the fatty acids into the bacterium, however, has received little study. M. tuberculosis release hydrolytic protein (Msh1) an associated membrane- into macrophages when they are under the hypoxic tension, according to recent research. [118] Msh1 catalyzes the hydrolysis of host lipids and is expressed during infection in vivo. Therefore, M. tuberculosis has developed ways to both store vital macromolecule in the type of lipid bodies and draw from them when needed, maybe during latency reactivation.[119]

2.3.6. Autophagy and antigen presentation inhibition

It is wise to draw attention to the long-running debate about autophagy's function in the pathophysiology of M. tuberculosis illness. At first, it was believed that the macrophage enclosing intracellular mycobacteria in membranes for lysosomal destruction was a necessary host defense mechanism. By using gene-targeted mice, were able to provide compelling evidence that autophagy does not play a role in the regulation of M. tuberculosis in vivo. [120] It is still possible, nevertheless, that M. tuberculosis suppresses the autophagic process . Numerous investigations that identify different mycobacterial virulence factors as mediators of this inhibition lend credence to this idea.[121] restricted autophagic pathway is thought to help intracellular infections, such as M. tuberculosis, by reducing the display of their antigens to T cells by MHC class II, in addition to encouraging the accumulation of nutrients. [122] In a recent genomewide search for M. TB genetic loci that disrupt MHC class II limited antigen presentation, the M. tuberculosis protein PE_PGRS47A was discovered. [123] PE-PGRS47A is secreted by Mycobacterium TB into the cytoplasm of contaminated dendritic cells (DCs) and macrophages, where it inhibits the development of autophagosomes that encapsulate intracellular bacilli. In vitro contamination of DCs with a PE-PGRS47A deletion transformant decreased intracellular bacterial populations and improved their ability to deliver M. tuberculosis antigens to T cells.[124] This is supported by the fact that mice infected with the mutant strain had a much greater CD4+ T-cell

response specific to M. tuberculosis, which greatly reduced tissue damage and bacterial growth in all organs during the chronic phases of infection. However, animals infected with the deletion mutant lived longer than mice with severe combined immunodeficiency, which is unable to mount adaptive immune responses. This suggests that PE-PGRS47A also disrupts innate immunity. Therefore, M. tuberculosis may effectively influence both innate killing and adaptive immunity by blocking autophagy. [125]

2.4 Drug repurposing

It can cost a lot of money and take ten to fifteen years for a new medicine to be authorized. This lengthy discovery process enables drug repurposing, also known as repositioning, which is an alternative method of decreasing the time necessary to make a medication. Repurposing a pharmaceutical entails employing drugs that have been approved by regulatory agencies such as the Medicines and Healthcare Products Regulatory Agency (MHRA), the European Medicines Agency (EMA), the FDA, and others for a new use.[126] Because of the tremendous potential of a decreased development process, several drug enterprises are already embracing medication repurposing to turn some of its FDA-approved and previously unsuccessful pipeline drug into new medications for a range of medical diseases. The current review provides a summary of current repurposing methodologies and examines case studies that demonstrate the effectiveness and value of drug repurposing, as evidenced by the significant reduction in drug development time due to the availability of all pertinent clinical and toxicological data. [127]

2.4.1 Significance of drug repurposing

A new medication must adhere to strict standards in order to be released onto the market. Due mainly to the difficulties of scaling up production and the many physicochemical qualities of the chemical entities, it takes a substantial expenditure to find a medicine and subsequently develop it. [128] Additionally, this limitation allows academic institutions or pharmaceutical companies to adequately and efficiently employ approved drug for a new symptom that diseased individual with

that situation do not already have access to. Repurposing the experimental drug that don't work for a specified indication usually gives them a strong start toward a comeback. They can eventually be developed into effective medicines, especially in cases of uncommon illnesses that pose considerable hurdles in diagnosis, therapy, and resource availability.[129] They can also be further rediscovered for a new use or indications. Certain autoimmune diseases, bacterial infections, and uncommon malignancies, for example, are idiopathic in nature and hence more challenging to treat since they are not inherited Compared to laborious traditional research and development methods, drug repurposing offers patients effective medications at a lower cost and in a shorter amount of time. Additionally, by addressing the rising expenses of drug research, this strategy lowers patients' out-of-pocket expenses and, eventually, the true cost of treatment. [130]

Due to the lack of safety and effectiveness data for new investigational molecules, attrition throughout the drug discovery process is higher and results in the greatest number of safety or efficacy failures.[131] A repositioned medicine can be effectively brought to market with less effort if past knowledge about safety, effectiveness, and the best route of administration is available. This decreases development costs and time dramatically. [132] Starting with an established medicine makes drug development significantly more cost-effective due to the introduction of new technology and the availability of computational tools. About one -third novel authorized medications by the FDA in the upcoming times have been derived from this strategy. Because these medications have qualities that appeal to potential consumers, drug repositioning also presents a significant opportunity for out-licensing.[133] While appealing, it's crucial that finding a new illness target doesn't impair the drug's ability to be marketed for its original purpose**.** [134]

2.4.2 Challenges for drug repurposing

Despite the recent increase in interest in medication repurposing, fewer applications exist than anticipated due to a number of implementation-related issues. It is difficult for new start-ups to give regulatory agencies pertinent information since there are no clear-cut regulations for repurposing medicinal prospects. Furthermore, using a repurposed drug for a unique purpose may seem to be related to the Orphan Drug Act and patent exclusivity. However, these restrictions could not stop a doctor from non-regulated use of the drug.[135] A unexpected change in regulations has given some drugs, including rapamycin and thalidomide, more exclusivity, allowing patients to

receive vital therapies at a lower cost. However, using repurposed medications is still very difficult because to the absence of a defined exclusivity pathway. Repurposing a candidate usually entails a possible time risk, particularly if it hasn't worked for an intended indication in the past.[136] It is wise to create a branching development session in this situation, where the main molecule or medication is assessed for many signs at once. This method lowers the danger of time and potential immediate intellectual property expiration, which would normally necessitate a large reinvestment of resources in order to re-profile the same molecule. [137] Pharmaceutical firms give lucrative and cost-effective discovery areas a lot of thought. However, there is no guarantee that the financial gains from repurposing a medication for uncommon and neglected illnesses will be significant .Therefore, focusing on a more specific and established research directive is more practical for an industry. Another issue that pharmaceutical businesses face is a lack of financing for research and financial incentives.[138] When using a repurposed candidate in clinical trials, there may be obstacles to overcome. Preclinical and proof-of-concept research may not be sufficient to demonstrate scientifically verified efficacy, and initiating Phase I clinical trials may necessitate a substantial financial outlay.[139] Due to a lack of patients, there may also be issues with the restricted patient recruitment for extensive clinical investigations needed for specific uncommon illnesses. Furthermore, product safety in older patients and other unique patient groups with comorbidities has to be proven.[140] Finding a new indication for a medication or dosage form whose intellectual property rights have expired will usually yield little return on investment and may also present legal challenges for the developer. Before generic medications hit the market, the developer may have a very brief window of time to recover development expenses, much alone earn a profit.[141]

2.5. MOLECULAR DOCKING

A computational approach that has significant ramifications for drug development and discovery that uses advanced algorithms to predict how medicines will interact with target proteins. This method might reduce the time and expenses involved in drug development by streamlining the screening of new compounds and optimizing currently available medications (Jorgensen,

2004).[142] Since the early 1980s, when the human genome project was completed and several crystallography, nuclear magnetic resonance, and high throughput protein purification spectroscopy techniques were made, this computational approach has dominated structure-based drug design. These developments have pushed and enabled research and development in the creation of new therapeutic targets for drug discovery. [143] While early knowledge was predicated on Fisher's lock-and-key theory, the ligand-receptor binding mechanism operates on the same basis as Koshland's induced-fit theory. [144] Our understanding of molecular docking has improved significantly as a result of both hypotheses. Researchers can learn more about the mechanisms of small compounds with potential drug properties within the binding site of particular proteins and the clarification of crucial biochemical mechanisms necessary for improving drug design and efficacy by looking at the interactions between the ligand and receptor at the molecular level. [145] The ultimate goal is to use a computational approach to investigate the structure of the ligand-receptor complex interaction. This can be accomplished in two interconnected steps: first, ligand conformations from the protein's active site are chosen, and each conformation is then given a score. [146]

2.6.ADMET ANALYSIS

ADSORPTION

A chemical must first enter the circulation before it can reach a tissue. Before a medicine is absorbed by the target cells, it is usually administered through mucous membranes such as the digestive tract, especially through intestinal absorption. The amount of drug absorption after oral ingestion is reduced by a number of factors, including the compound's low solubility, the length of time it takes for it to pass through the intestines, the rate at which the stomach empties, its incapacity to cross the intestinal barrier, and chemical instability in the stomach.[147]

DISTRIBUTION

The creation of extremely effective medications requires uniform drug delivery. A drug's distribution qualities dictate how it moves through the body from the site of administration to the desired area. Smaller molecules with lower molecular weights are typically believed to be simpler

to distribute since they can move more readily throughout the body. The FDA's clearance of several medications with molecular weights between 500 and 2000 Dalton, however, has challenged this notion.[148]

METABOLISM

A medicine must go through the proper metabolic processes, which mostly take place in the liver, in order to exhibit its pharmacological effects.[149]

EXCRETION

Excretion is the process by which the medication is removed from the body by the excretory system after it has been active, usually by urine. Negative effects on the host's body may result from ineffective medication excretion after exercise.[150]

TOXICITY

It is imperative to carefully and precisely address the most important property in question. Toxicity can be defined as any impact of a medication on the recipient's body that deviates from its intended therapeutic effects. [151]

Chapter 3.

Methodology

3.1. Preparation of Target Protein

Molecular docking begins with the target protein being prepared, usually from a structural database like the Protein Data Bank (PDB). Unless they are functionally important, unnecessary molecules like ions, water, and co-crystallized ligands are eliminated. The protein structure is stabilized by the addition of hydrogen atoms, particularly polar hydrogens. The protein's 3D conformation is subsequently optimized by energy reduction using programs like AutoDock Tools, PyMOL, or Chimera.

3.2. Preparing Ligands

The ligand, which is often a tiny molecule, is made with molecular sketching programs like ChemDraw or MarvinSketch or is sourced from chemical databases like PubChem. If necessary, the structure is transformed from two-dimensional to three-dimensional, and protonation states or tautomeric forms are modified to correspond with physiological pH levels. After that, energy minimization is applied to the ligand to get its most stable shape for docking experiments.

3.3.The Binding Site Definition

Previous experimental data or literature is used to determine the protein's binding or active site. Computational techniques like CASTp, SiteMap, or DoGSite can forecast possible binding pockets if the binding location is unknown. To limit the docking simulations to a pertinent area, a grid box or search space is established around the active site after the binding site has been determined.

3.4. Simulation of Docking

Specialized software like AutoDock, AutoDock Vina, Schrödinger Glide, MOE, or Gold is used to carry out the actual docking operation. Within the protein's binding site, these programs produce a variety of ligand conformations and orientations, or poses. The interactions are assessed using

scoring systems that take into account several kinds of molecular interactions, such as electrostatic forces, hydrophobic contacts, and hydrogen bonds.

3.5. Ranking and Scoring

A binding affinity score is given to each produced posture; a lower or more negative value generally denotes a stronger bond. For a single ligand, several postures might be produced; they are then sorted based on their scores. Based on their binding energies and interactions with important amino acid residues in the binding pocket, the optimal postures are chosen.

3.6. Analysis of Post-Docking

Following docking, molecular visualization software like PyMOL, Chimera, or Discovery Studio is used to display the top-ranked protein-ligand complexes. Finding important interactions between the ligand and active site residues, such as salt bridges, π–π stacking, hydrophobic interactions, and hydrogen bonds, is the main goal of the investigation. Comparisons with previously confirmed binding modes are done if they are known.

3.7. Verification

There may be validation procedures implemented to guarantee the accuracy of the docking findings. In order to determine if the docking tool can replicate the experimentally reported binding posture, redocking entails docking a known ligand again into the protein. Consistency can also be tested by cross-docking with other proteins or other structures. To validate the computational predictions, in vitro binding tests or other experimental validation should ideally come next.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results of molecular docking

Only 48 of the 388 FDA-approved medications that were first chosen due to their structural resemblance to Levofloxacin satisfied the necessary pharmacokinetic requirements, which included permeability of the blood-brain barrier (BBB), the lack of PAINS (Pan-Assay Interference Compounds) alerts, and compliance with Lipinski's Rule of Five. The bulk of these compounds demonstrated substantial binding affinities, according to subsequent molecular docking experiments using the Topoisomerase. Of them, 14 medicines had docking scores better than -8.0 kcal/mol, which is typically seen as a sign of favorable and stable binding.

Four of these compounds showed remarkably high binding affinities (binding energy < 8.5kcal/mol), indicating strong and long-lasting interactions inside Topoisomerase active region. Notably, the reference chemical Levofloxacin, which had a binding energy of roughly -8.0 kcal/mol, was outperformed by Quarfloxacin (-9.4 kcal/mol) and Finafloxacin (-8.8 kcal/mol), which had the strongest binding. Furthermore, 18 substances shown moderate-to-significant binding affinities ranging from -7.5 to -8.4 kcal/mol, further supporting their potential as repurposable TOPOISOMERASE, while two medications showed binding energies precisely at -8.0 kcal/mol, which is equivalent to that of Levofloxacin

The remaining drugs provided important insights into the structural features that can influence TOPOISIMERASE-ligand interactions, even if their binding energies were lower. All of these results point to the potential of certain FDA-approved drugs, such as Quarfloxacin, Finafloxacin, Pradofloxacin, Ofloxacin, and others, as therapeutic options for altering TOPOISIMERASE activity in Tuberculosis. Because of their higher or comparable binding affinities to Levofloxacin, these compounds demand further experimental validation and optimization for the repurposing of topoisomerase-targeted medications.

TABLE 3. LIST OF DRUGS WITH THEIR ESTIMATED ΔG (KCAL/MOL)

S.no	Drugs	Estimated ΔG (kcal/mol)
1.	Levofloxacin (reference drug)	-8.0
2.	Quarfloxacin	-9.4
3.	Finafloxacin	-8.8
4.	Pradofloxacin	-8.5
5.	Ofloxacin	-8.4
6.	D-levofloxacin	-8.3
7.	Sitafloxacin	-8.3
8.	Besifloxacin	-8.3
9.	Sparfloxacin	-8.2
10.	Rufloxacin	-8.2
11.	Cadazolid	-8.2
12.	Trovafloxacin	-8.2
13.	Alatrofloxacin	-8.2
14.	Gemifloxacin	-8.1
15.	Delafloxacin	-8.1
16.	Nemonoxacin	-8.0
17.	Orbifloxacin	-8.0
18.	Clinafloxacin	-7.9
19.	Prulifloxacin	-7.9
20.	Enrofloxacin	-7.8
21.	Levonadifloxacin	-7.8
22.	Danofloxacin	-7.6

4.2 Visualization of interactions

Following molecular docking of the selected ligands with the topoisomerase, the two-dimensional (2D) and three-dimensional (3D) binding conformations of the top-performing compounds were analyzed using BIOVIA Discovery Studio. The kind and intensity of the interactions between the ligands and the topoisomerase active site residues were shown in this picture. Drugs with higher docking scores than the reference molecule Levofloxacin, such as Quarfloxacin, Finafloxacin, Pradofloxacin, Ofloxacin, and D-levofloxacin, were found to have several stabilizing interactions. These included π–π stacking, hydrogen bonding, hydrophobic interactions, which enabled strong and accurate binding inside the active region. The 2D interaction graphs clearly showed important contact residues, which are crucial components of the catalytic site. The 3D visualizations provided additional confirmation of these ligands' correct alignment with the substrate-binding groove, close proximity to the catalytic core, and good fit into the binding pocket. These results provide credence to the compounds' potential as promising inducer for the Topoisomerase, which calls for more experimental verification.

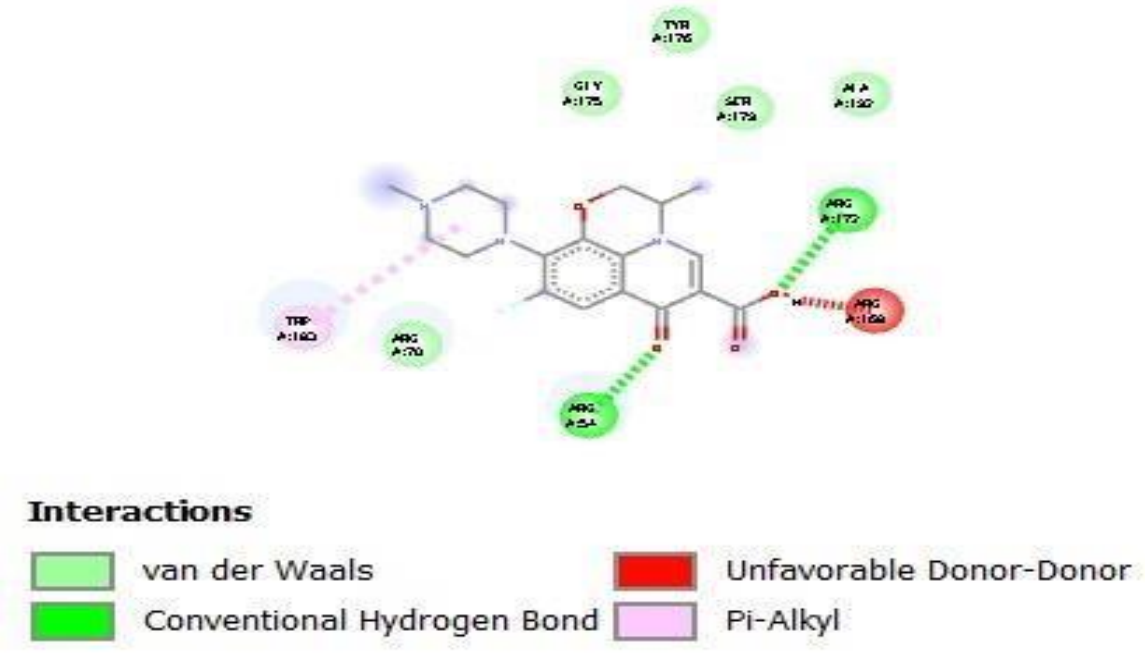

Fig.4 Demonstrates different interactions between LEVOFLOXACIN and the Topoisomerase receptor in a twodimensional graphical representation

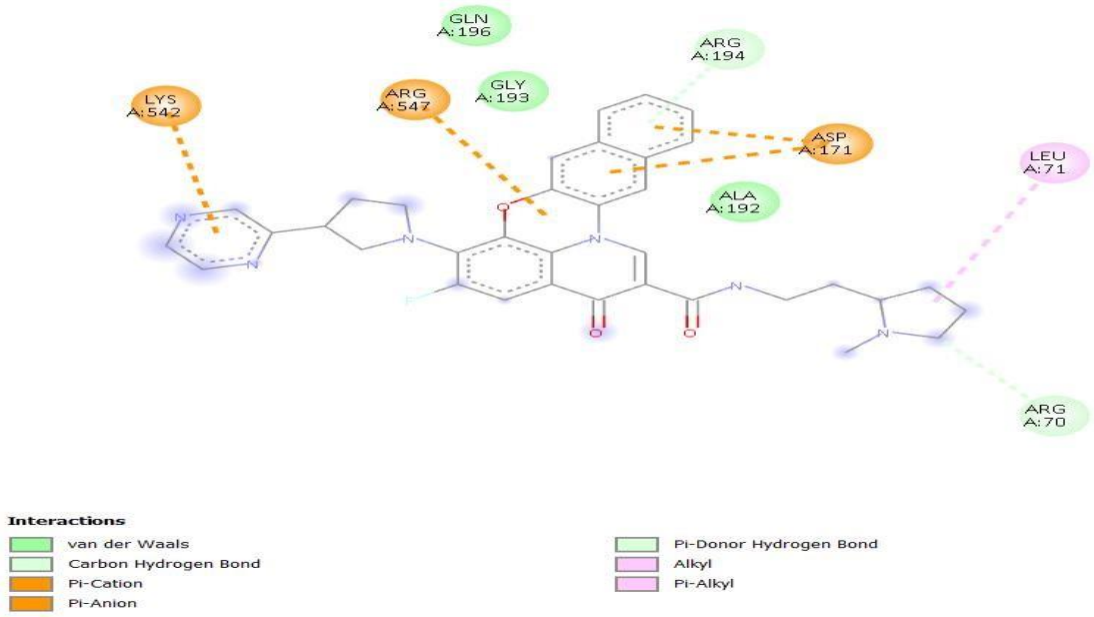

Fig.5 Demonstrates different interactions between QUARFLOXACIN and the topoisomerase receptor in a twodimensional graphical representation

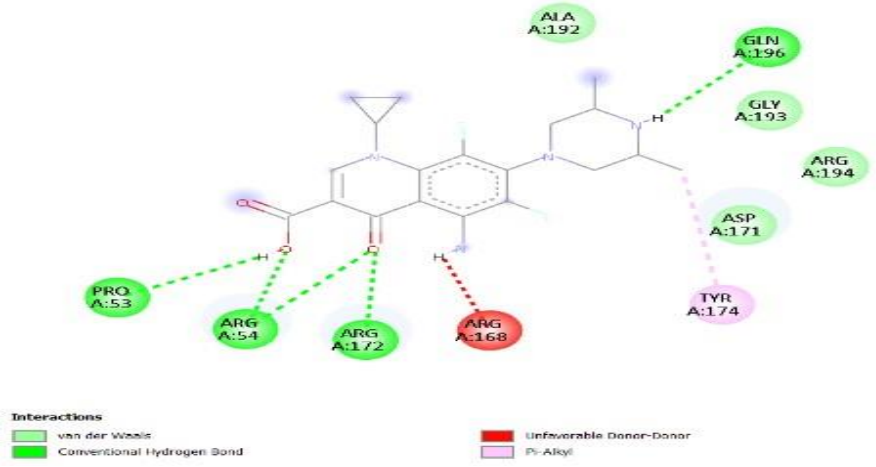

Fig.6 Demonstrates different interactions between SPARFLOXACIN and the topoisomerase receptor in a twodimensional graphical representation

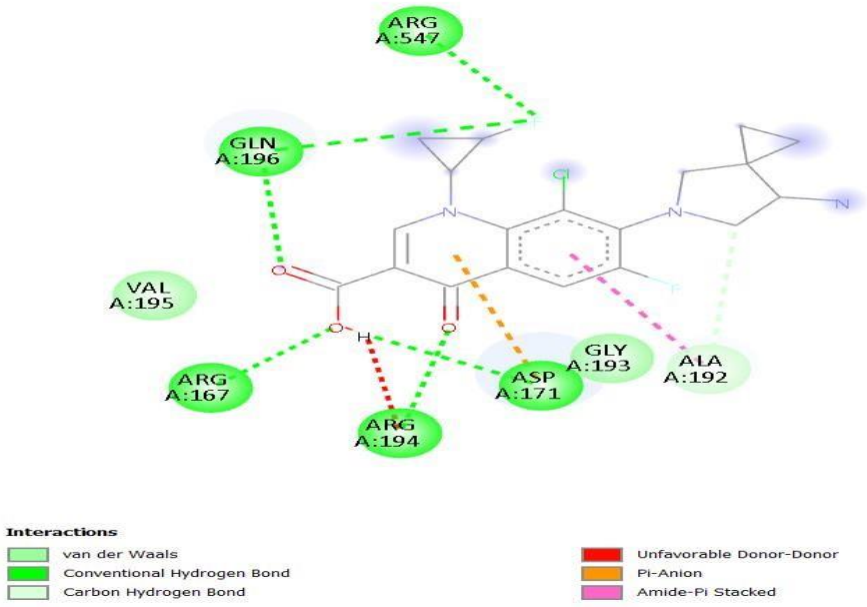

Fig.7 Demonstrates different interactions between SITAFLOXACIN and the topoisomerase receptor in a twodimensional graphical representation

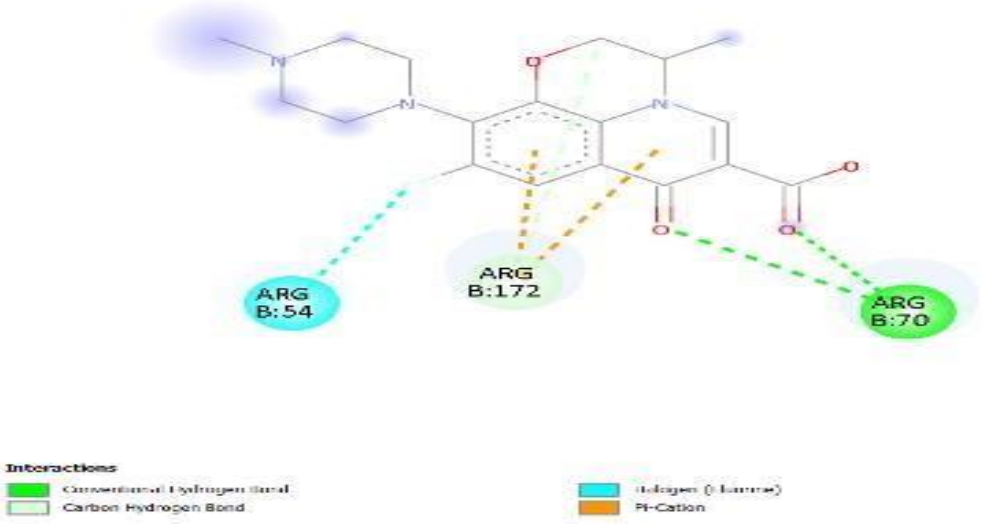

Fig.8 Demonstrates different interactions between D-LEVOFLOXACIN and the topoisomerase receptor in a twodimensional graphical representation

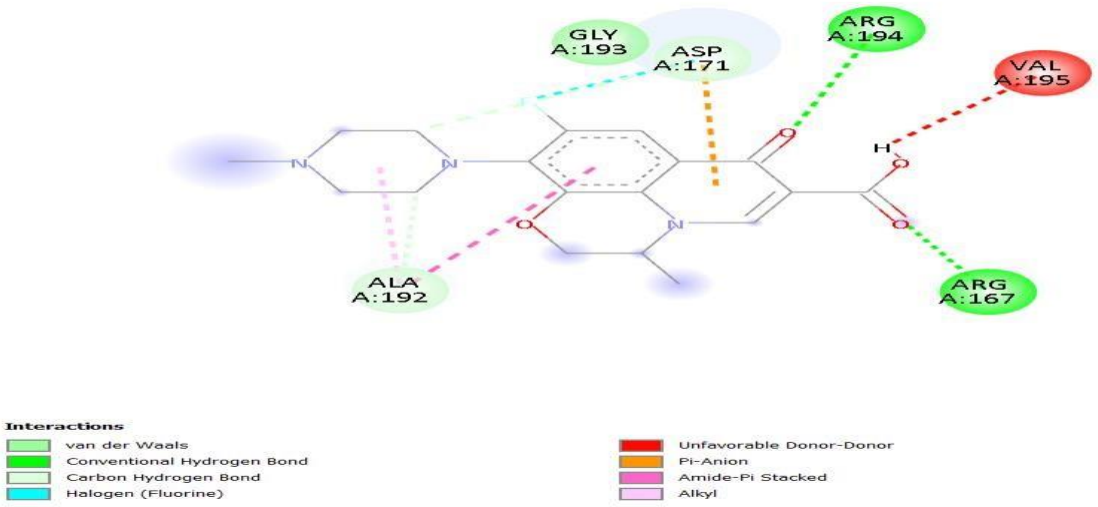

Fig.9 Demonstrates different interactions between OFLOXACIN and the topoisomerase receptor in a two-dimensional graphical representation

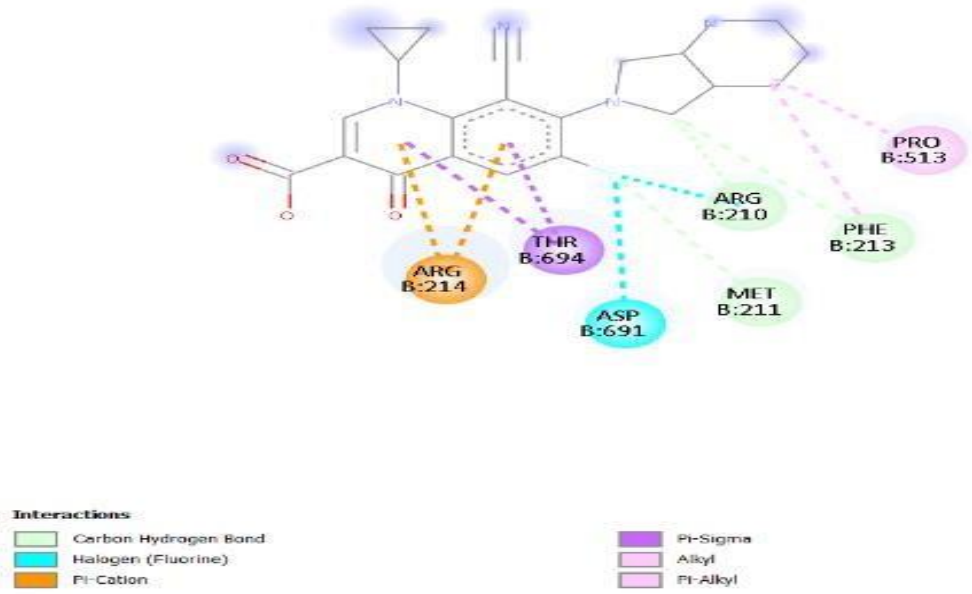

Fig.10 Demonstrates different interactions between PRADOFLOXACIN and the topoisomerase receptor in a twodimensional graphical representation

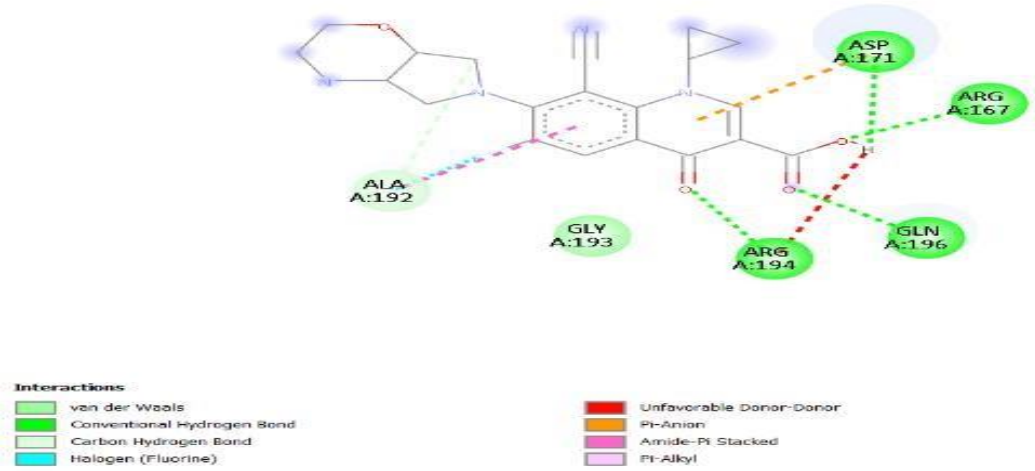

Fig.11 Demonstrates different interactions between FINOFLOXACIN and the topoisomerase receptor in a twodimensional graphical representation

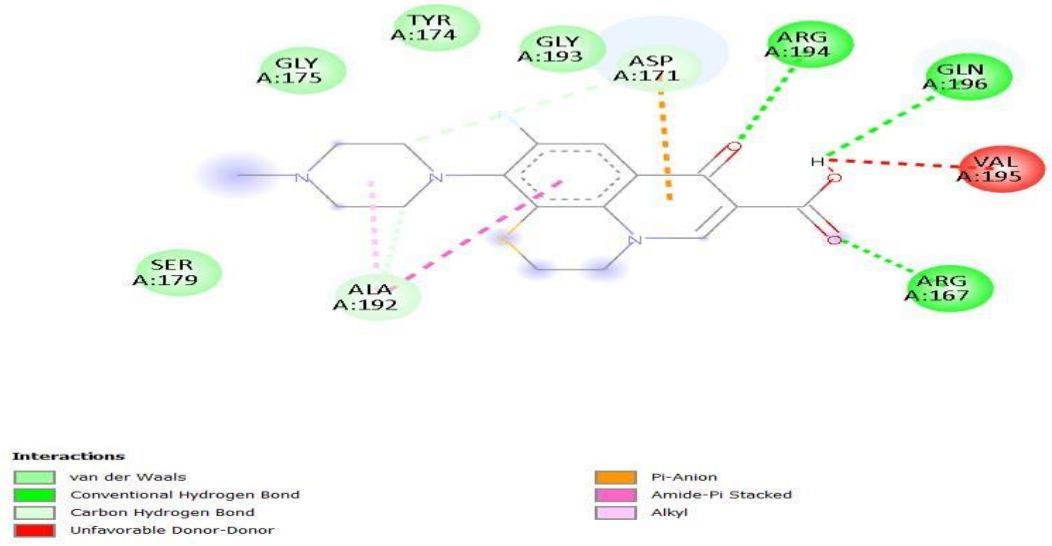

Fig.12 Demonstrates different interactions between RUFLOXACIN and the topoisomerase receptor in a twodimensional graphical representation

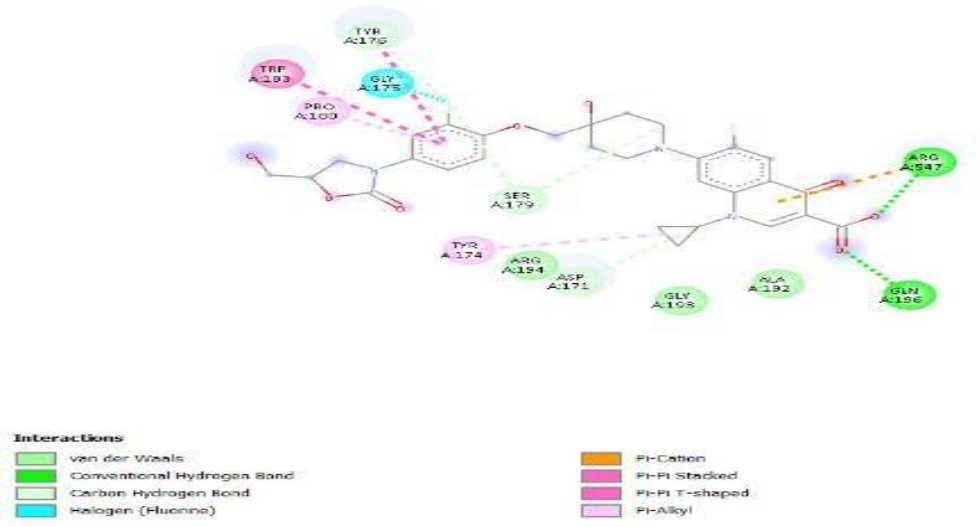

Fig.13 Demonstrates different interactions between CADAZOLID and the topoisomerase receptor in a two-dimensional graphical representation

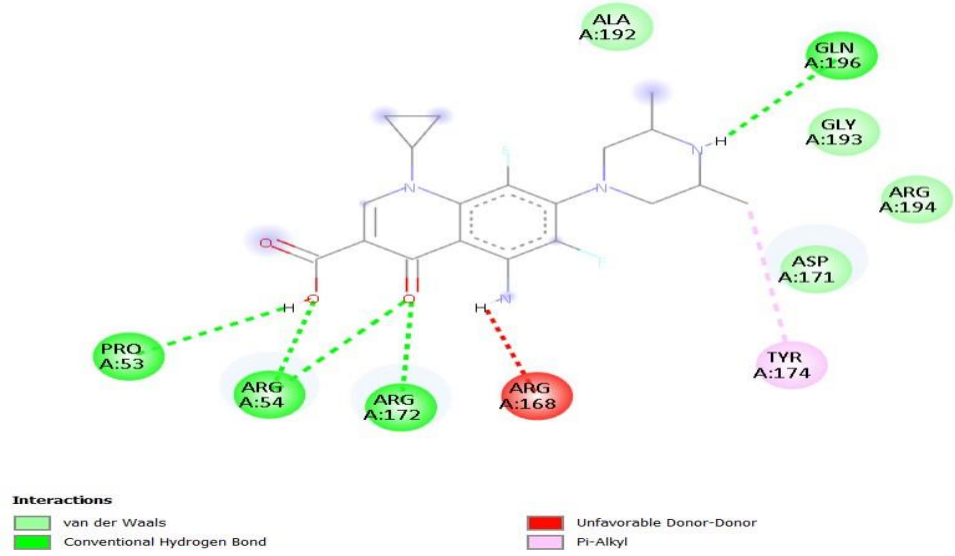

Fig.4 Demonstrates different interactions between SPARFLOXACIN and the topoisomerase receptor in a twodimensional graphical representation

4.3 ADMET analysis

After the molecular docking tests, the top-performing chemical drug candidates with the highest binding affinities for Topoisomerase were evaluated for pharmacokinetic suitability using ADME (Absorption, Distribution, Metabolism, and Excretion) analysis. The majority of the 48 nominated compounds had strong water solubility and high gastrointestinal (GI) absorption, suggesting significant potential for development as oral accessible medications. Following oral administration, these drugs would most likely be efficacious in the systemic circulation because their bioavailability ratings were within a reasonable range. Importantly, most of the selected candidates did not violate Lipinski's Rule of Five, indicating that they met the required criteria for drug-likeness. These findings support the hypothesis that the drugs have favorable pharmacokinetic and safety characteristics in addition to significant biological activity against the topoisomerase. The combination of these FDA-approved drugs' strong binding affinity, structural compatibility, and acceptable ADME qualities supports the case for repurposing them as potential therapeutic agents for Tuberculosis disease.

ADME ANALYSIS OF ALL BINDING DRUGS

S.no	Drugs	Consensus Log P value	GI absorption rate	TPS A value	Lipinski violation
1.	Quarfloxacin	4.29	“High”	95.98	0
2.	Finafloxacin	0.84	“High”	107.59	0
3.	Pradofloxacin	1.59	“High”	98.36	0
4.	Ofloxacin	1.15	“High”	75.01	0
5.	D-levofloxacin	1.15	“High”	75.01	0
6.	Sitafloxacin	2.18	“High”	88.56	0
7.	Besifloxacin	2.25	“High”	88.56	0
8.	Sparfloxacin	1.53	“High”	100.59	0
9.	Rufloxacin	1.27	“High”	91.08	0

10.	Cadazolid	2.74	Low	141.77	No
11.	Trovafloxacin	2.14	High	101.45	0
12.	Alatrofloxacin	1.54	Low	159.65	No
13.	Gemifloxacin	0.90	High	123.04	0
14.	Delafloxacin	2.28	High	121.68	0
15.	Nemonoxacin	1.42	High	97.79	0
16.	Orbifloxacin	2.30	High	74.57	0

4.4 Toxicity assessment using ProTox II (version 3.0) server

The ProTox II server was used to assess the toxicity profile of the top ten binding medications. The server forecasts the hazardous tendencies based on LD50 values using a variety of machine learning (ML) techniques that are quite useful in biological research these days. These machine learning algorithms are trained to identify trends and correlations between different chemical structures and toxicity profiles. A '+' sign in Table 4 indicates an active toxicity outcome, whereas a '-' sign indicates an inactive toxicity outcome. Table 3 is a tabulation of the toxicity data.

Drug	**LD50 value (mg/kg)**	**Hepatotoxicity status**	**Carcinogenicity status**	**Immunotoxicity status**	**Cytotoxicity status**	**Toxicity class predicted**
Quarfloxacin	2000	-	-	+	-	“Class 4”
Pradofloxacin	3500	-	-	-	-	“Class 5”
Ofloxacin	1478	-	-	-	-	“Class 4”
D-levofloxacin	1478	-	-	+	-	“Class 4”
Sitafloxacin	3500	+	-	-	-	“Class 5”
Besifloxacin	3800	-	-	-	-	“Class 5”
Sparfloxacin	2000	-	-	-	-	“Class 4”
Rufloxacin	501	-	-	-	-	“Class 4”

To assess the safety profiles of the best binding medications, toxicity prediction was done using LD50 values, hepatotoxicity, carcinogenicity, immunotoxicity, cytotoxicity, and expected toxicity class. The LD50 values of the selected medication ranged from 500 mg/kg to 3800 mg/kg,

indicating varying degrees of acute toxicity. While other chemicals were classified under Toxicity Class 4, which indicates that they are deadly if swallowed, Pradofloxacin, with an LD50 of 3500 mg/kg, Sitafloxacin, with an LD50 of 3500 mg/kg, Besifloxacin, with an LD50 of 3800 mg/kg was classified under Class 5, which indicates reduced toxicity.

Interestingly, none of the substances were hepatotoxic, carcinogenic, mutagenic, or cytotoxic. These findings demonstrate that comprehensive toxicity testing is required to ensure that drug candidates are appropriate for repurposing in the treatment of AD, even if they have strong binding affinities and good ADME profiles. Overall, the information shows that the majority of the selected medications have sufficient safety margins; nevertheless, Quarfloxacin, Finafloxacin, Pradofloxacin, Ofloxacin, and D-levofloxacin are particularly noteworthy as prospective choices with minimal toxicity issues.

4.5 Selection of potential drugs

Interestingly, 19 drugs showed greater binding affinities than the reference medication i.e. Levofloxacin. Based on their docking scores and important interactions with the catalytic residues inside the Topoisomerases active site and toxicity analysis, the most promising candidates were found, particularly the top hits, , Quarfloxacin, Finafloxacin, Pradofloxacin, Ofloxacin, and Dlevofloxacin. These results imply that the drugs on the shortlist have a great deal of promise for use as topoisomerase enhancers in treatment of TB.

CHAPTER 5

CONCLUSION

TB is one of the most challenging and complex illnesses, with a complex etiology and few treatment options. Among the several molecular targets, Topoisomerase induction has emerged as a strong contender since it is necessary for the. Finding new and focused pathways that could serve as the basis for novel medications is vital and urgent, especially because all pharmaceutical therapies for TB have failed thus far. To successfully control Topoisomerase activity in both healthy and pathological conditions, it will be essential to comprehend how it is regulated.

We used a computational medication repurposing approach to find FDA-approved drugs that share structural similarities with the well-known Topoisomerase enhancer Levofloxacin. Using Auto Dock Vina for molecular docking and virtual screening, we found many candidates that had a high binding affinity for the Topoisomerase active site. Notably, Quarfloxacin, Finafloxacin, Ofloxacin, D-levofloxacin, and Sitafloxacin showed best stronger binding affinities, established long-lasting bonds with important catalytic residues in the active site and adequate safety margins in toxicity analysis, suggesting that they may be able to modify Topoisomerase activity. These results demonstrate how useful molecular docking is for quickly and affordably finding novel therapeutic candidates, particularly from among medications that have already received approval, greatly speeding up the drug development process. The docking results are merely the initial stage of drug validation, even though they offer important insights into ligand-receptor interactions. Validating the biological efficacy of these drugs requires experimental research, such as in vivo studies in TB models and in vitro enzymatic assays to evaluate protein activation. Future research should also use molecular dynamics simulations to investigate the stability, conformational behavior, and longterm binding properties of these ligand-protein complexes in physiological settings. All things considered, this study demonstrates the viability and potential of drug repurposing using computational methods as a means of discovering new TB treatments, opening the door for further translational studies that focus on Topoisomerase.

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