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DNA GYRASE – NEW TARGET FOR ANTITUBERCULAR ACTION.

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ABSTRACT

Tuberculosis (TB) is one of the deadliest bacterial diseases in the world. New treatment agents must be used as soon as possible to replace existing drugs for which resistance is a severe problem. DNA topoisomerases are well-known targets for chemotherapies that are antibacterial and anticancer. Topoisomerase I in bacteria hasn't yet been exploited as a target for therapeutic antibiotics, despite DNA gyrase being frequently targeted, notably by the very clinically effective fluoroquinolones that have been employed in TB therapy. While treating Mycobacterium tuberculosis (MTB) infection with antibiotics, DNA gyrase is an important nucleoprotein motor found in all bacteria, is a primary target. Gyrase adds negative supercoils to DNA by hydrolyzing ATP utilising a mechanism known as strand passing that has been studied using biophysical and biochemical methods. Bacterial DNA gyrase is one of the key targets of antibacterial drugs like fluoroquinolones. In the majority of bacterial species, fluoroquinolones disrupt DNA gyrase and topoisomerase IV, which causes bacterial cell death. Novobiocin is just one of many other naturally occurring antibacterial compounds that have been shown to be effective against bacteria. The ATP-dependent enzyme DNA gyrase causes an immediate double-stranded DNA break. Being able to catalyse DNA's negative supercoiling, which is required for efficient DNA replication, transcription, and recombination, makes it unique. The DNA gyrase protein is a tetrameric A2B2 protein. The A subunit carries the breakage-reunion active site, whereas the B subunit allows ATP hydrolysis.

KEYWORDS: DNA gyrase, DNA topoisomerase, Fluoroquinolone, negative supercoiling.

1. INTRODUCTION

Although the access to the information contained in DNA depends on the topology of the DNA, the information itself is essentially independent of how the DNA is knotted or twisted. Numerous enzymes called DNA topoisomerases keep the topological homeostasis of the cell during these DNA transaction processes.[1,2]

Topoisomerases these enzymes catalyse the interconversion of distinct topological states of DNA. It depends Topoisomerases can catalyse DNA relaxation/supercoiling, catenation/ decatenation, and knotting/unknotting reactions, depending on the type of reactants and reaction circumstances.[3] DNA topoisomerases are divided into two groups based on the functional mechanisms they use. One of the two strands of DNA is broken and re-joined during catalysis by type I DNA topoisomerase, whereas type II DNA topoisomerase operates on both strands for each DNA strand-passing reaction and needs ATP for maximal activity.[4]

The constant need for the discovery of new antibacterial medicines is evidenced by the growing threat posed to human health by pathogenic microorganisms that are resistant to antibacterial medications. The World Health Organisation produced a list of priority infections in 2017 for which new antibiotics are urgently required because the current treatment is failing. Pseudomonas aeruginosa, *Acinetobacter baumannii, Staphylococcus aureus, Enterococcus faecium,* and *Enterobacteriaceae* are a few of these that are particularly dangerous due to their resistance.[5] The Mycobacterium tuberculosis is one of the leading bacteria which shows multi-drug resistance in TB. A known target for antibacterial treatment is bacterial DNA gyrase, a type II DNA topoisomerase present in all bacteria.[6] The class of DNA gyrase and topoisomerase IV.[7] In addition to the fluoroquinolones, other bacterial DNA gyrase inhibitors, such the naturally occurring novobiocin, are also known to be efficient antibacterial agents.[8]

Two approaches can be taken in the search for new TB medications that can combat the spread of multidrug-resistant tuberculosis (MDR-TB) and the emerging extremely drug-resistant tuberculosis (XDR-TB): the identification and clinical validation of novel targets for the development of novel antibiotics without preexisting resistance mechanisms; or the exploration of known and clinically validated targets for new chemical series or modification of existing drug classes. Finding newer and more potent generations of compounds based on previous medications from a range of classes, such as macrolides, tetracyclines, cephalosporins, and quinolones, has proven to be a huge success for the second method.

Targeting completely validated molecular targets, whose chemical inhibition is proven to prevent bacterial growth, is another advantage of this strategy.[9] Targeting molecular targets that have undergone thorough validation and whose pharmacological inhibition is known to prevent bacterial growth is another advantage of this strategy. Thus, M. tuberculosis DNA gyrase is a confirmed target for the development of antitubercular drugs; its inhibition produces a high level of mycobactericidal activity. Inhibitors of this enzyme are also effective against persistent, non-replicating mycobacteria, which may be crucial for cutting the length of TB treatment. It is possible that a novel inhibitor of M. tuberculosis DNA gyrase will also be effective against fluoroquinolone-resistant M. tuberculosis and multidrug-resistant (MDR)-TB. Clinical isolates of M. tuberculosis rarely exhibit fluoroquinolone resistance.[10] It is known that amino acid changes in the putative fluoroquinolone-binding area of the M. tuberculosis GyrA-encoded A subunit of DNA gyrase are the cause of high-level resistance to fluoroquinolones in laboratory-produced strains of M. tuberculosis and M. smegmatis.[11] Fluoroquinolones' DNA gyrase inhibitory actions and their antimycobacterial effects seem to be closely related.[12]

2. COMPLICATIONS WITH CURRENT TB DRUGS OR TREATMENTS.

Over the past few decades, there has been an increase in concern regarding the development of drug-resistant (DR) tuberculosis (TB). This is due to a number of factors, including the widespread inappropriate or ineffective use of antimicrobials to treat TB in the absence of drug-susceptibility testing (DST), the inadequate uptake of systematic approaches to the treatment of drug-susceptible (DS) TB and DR-TB, and the introduction of the human immunodeficiency.[13] According to estimates from the World Health Organisation (WHO), more than 500,000 new cases of rifampicin-resistant (RR) and multidrug-resistant (MDR) TB, or sickness brought on by Mycobacterium tuberculosis with resistance to isoniazid and rifampin, are diagnosed each year. The newest figures indicate that just 56% of the 156,000 MDR-TB or RR-TB patients who started therapy globally in 2018 finished it effectively. Using second-line anti-TB drugs (SLDs), which are less efficient and hazardous than the four medications most frequently used to treat DS-TB, for a longer period of time is the cause of such low treatment completion rates. However, under both trial and programmatic situations, it has been demonstrated that treatment performance is significantly greater when regimens are carefully planned and guarantee good retention.[14]

Tuberculosis is an airborne disease transmit through air while breathing. Although TB most frequently affects the lungs, it can also harm the brain, kidneys, or spine. TB is typically treatable and curable, but if it is not treated properly, a person with TB risk of death increases. Bacteria can occasionally develop drug resistance to the medications used to treat tuberculosis (TB), leading to drug-resistant TB. Because of this, the TB bacteria can no longer be killed by the medication.[15,16]

Drug-susceptible TB (DS TB) and drug-resistant TB (DR TB) both spread through the same channels. TB is transferred from one person to another through the air. A person who has TB disease of the lungs or throat coughs, sneezes, speaks, or sings, releasing the TB bacteria into the air. Nearby individuals may inhale these microbes and acquire an infection. It is found that cancer is caused by a several factors and the beginning and the series of cancer is very complex. The establishment of multidrug resistance and recurrence is one of the main issues with the development of anticancer medications. Traditional chemotherapy treatments directly target a cell's DNA, however due to mutations, the cell can become resistant to them.[17]

Globally, tuberculosis (TB) is still a serious health issue. The treatment of TB is hampered in particular by multidrug-resistant TB (MDR-TB), which is defined as TB that demonstrates resistance to both isoniazid and rifampicin. Globally, 3.4% of newly diagnosed TB patients and 20% of those who had previously received TB treatment received an MDR-TB diagnosis. Ineffective and hazardous second-line treatments are used for a lengthy period of time (up to 20–24 months) to treat MDR-TB, and the results are not favourable. However, due to the introduction of a novel agent (bedaquiline), repurposed medications (linezolid, clofazimine, and cycloserine), and technological advancements in rapid drug sensitivity testing, treatment outcomes are anticipated to improve. In 2018, the World Health Organisation (WHO) published a fast communication, and in 2019, it published unified guidelines for the treatment of MDR-TB based on clinical trials and a meta-analysis of individual patient data. The WHO advised oral treatment regimens that incorporated the novel and repurposed compounds as well as the reclassification of second-line anti-TB medications in these guidelines. The purpose of this article is to discuss MDR-TB treatment approaches based on the 2019 WHO guidelines for the management of MDR-TB as well as the diagnostic methods for identifying resistance, including phenotypic and molecular drug sensitivity tests.[13]

3. DNA TOPOISOMERASES

The double-stranded helical structure of the DNA molecule is what makes DNA topoisomerases necessary in nature. The two strands of the DNA helix must be split, either momentarily (during transcription or recombination) or permanently (during replication), for most of the operations that call for access to the individual DNA strands. Due to the circular shape of bacterial chromosomes and the size of eukaryotic chromosomes, this strand separation requirement cannot be met by twisting the strands. Therefore, for the DNA molecule to participate in these crucial cellular processes, topoisomerases must keep the DNA molecule's proper topology.

DNA topoisomerase enzymes modify the architecture of DNA within a cell by allowing one strand of DNA to pass through a break in the other strand or by allowing a section of a duplex from the same or a different molecule to pass through a double-stranded gap in a DNA molecule. The type II subfamily of topoisomerases, which includes DNA gyrase, catalyses the latter mechanism, while the type I family of topoisomerases catalyses the former.

Topoisomerases can introduce either negative supercoils, like bacterial DNA gyrase, or positive supercoils, like reverse gyrase, into the DNA molecule. They can also relax supercoils in both directions or exclusively relax negative supercoils. These topoisomerase activities are used to change a closed DNA domain's topology, encourage the decatenation and catenation of circular DNA molecules (such as the circular bacterial chromosome or plasmid), or untangle interwoven linear chromosomes. The protein's active site tyrosine residue and one of the ends of the broken DNA strand produce a weak phosphodiester link when topoisomerases cleave DNA. The DNA molecule is relegated when the enzyme is released after the topological state of the DNA is changed while this covalent intermediate is kept in place. Type I topoisomerases are those that only cleave one strand of DNA, and type II subfamily topoisomerases are those that cleave both strands to produce staggered double-stranded breaks.[18] The component of the eubacteria called topoisomerase is present in Escherichia coli. The DNA topoisomerase I and III are two of the type I topoisomerases in this organism, and DNA gyrase and DNA topoisomerase IV are the two type II topoisomerases. For the entire bacterial chromosome, DNA gyrase creates negative supercoils, condensing the chromosome for proper partitioning during cell division.[19]

The DNA molecule is decatened during replication by DNA Topoisomerase IV. In order to avoid excessive negative supercoiling that can be brought on by DNA gyrase activity, DNA Topoisomerase IV and I also relax negative supercoils. While DNA gyrase is superior at relaxing positive supercoils than DNA topoisomerase IV at decatenating DNA, both enzymes are much more effective at decatenation.[20,21] The resolution of the positive and negative supercoils that transcription generates in front of and behind the translocating RNA polymerase is carried out by DNA gyrase and DNA polymerase I, respectively. Topoisomerase III helps a circular DNA molecule's replication fork move because it can decatenate the pre-catenanes behind the fork by acting at nicks that are present in the replicating DNA.[22,23,24] This has been assumed to suggest that topoisomerase III and IV have overlapping functions.

There are only two topoisomerase genes encoded in the Myocbacterium TB genome, Campylobacter jejuni, Deinococcus radiodurans, and Treponema pallidum genomes.[25] The E. coli DNA gyrase gene and the E. coli topoisomerase I gene have homologs, however there isn't a gene for E. coli topoisomerase IV. This is assumed to be the bare minimum amount of topoisomerase present in a bacterial cell. DNA gyrase must be in charge of decatenating replicated DNA, relaxing positive supercoils, and negatively supercoiling the DNA in situations where it is the sole type II enzyme present. In such theory, Topoisomerase I would be primarily in charge of reducing the amount of negative supercoils that DNA gyrase creates in order to prevent excessive negative supercoiling.[26]

4. Classification and functions of topoisomerase

Topoisomerase enzymes that only cut one strand of DNA are classed as type I, which is further divided into type IA and type IB subfamilies depending on whether the protein is connected to a DNA phosphate at the 5′ or 3′ position.[27] Type II topoisomerases are topoisomerase enzymes that cleave both strands to produce a staggered double-strand break.[28] The representation of topoisomerase classification in fig.1

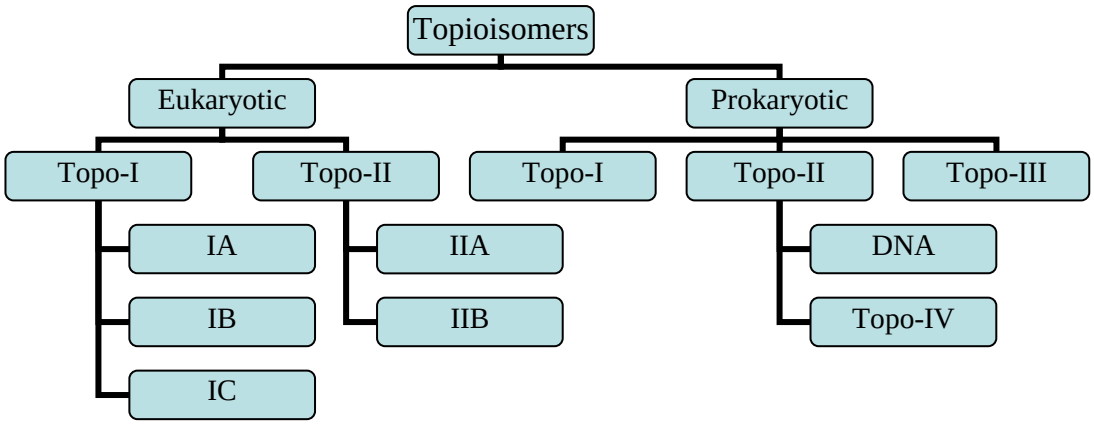


Fig 1: Classification of topoisomerase.

4.1. Eukaryotic topoisomerase enzyme - Both type I and type II eukaryotic topoisomerases are categorised (Fig. 1). Eukaryotes, mammalian cells, yeast, and drosophila are among the organisms that have these enzymes.[23]

4.1.1. Type-I Topoisomerase

This monomeric enzyme (Fig. 2) breaks single strands of double-stranded DNA.[23] Topoisomerases IA and IB are further split into this (based on structure and mechanism). The four domains of type IA topoisomerases are I through IV. While domains III and IV combine to create a helix-turn-helix domain that contains the catalytic tyrosine residue, domain I is made up of TOPRIM (Topoisomerase- Primase). Each of domains III and IV is connected to the other by a flexible bridge from domain II.[29]

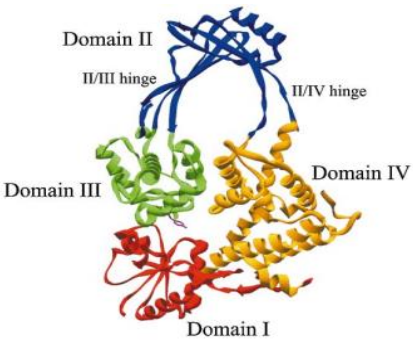


Fig.2 E. coli topiomerase-I[15]

First, only one strand of DNA can bind to domains III and I. A catalytic tyrosine residue causes the DNA backbone to break, resulting in the temporary creation of a 5'-phosphotyrosine intermediate and the subsequent separation of the strands by domain II.A capping and catalytic lobe, an N-terminal breakage- reunion domain, and a C-terminal

domain make up Topo IB.[30] The only member of the type IC class of topoisomerases at this time is topoisomerase V. This was derived from the *Methanopyrus kandleri*, and it works in a manner resembling type IB topoisomerase. To relax DNA's negatively and positively supercoiled forms is the role of type-1 topoisomerase in eukaryotic cells.[31]

4.1.2. Type II topoisomerase

There are two subclasses of this enzyme: type IIA and type IIB topoisomerases. Unlike type IIB topoisomerases, type IIA topoisomerases can simplify DNA topology. Type IIB topoisomerases produce double-stranded breaks with two base overhangs while type IIA topoisomerases generate them with four base pair overhangs. There is no supercoiling activity in them.[28]

4.2.Prokaryotic topoisomerase enzyme

Type-I Topoisomerase -The single chain polypeptide that makes up this enzyme's monomer is most likely. It is produced by the topA gene. Known as the omega protein, E. coli topoisomerase I was first revealed in 1971.[28]

4.2.1 Type II topoisomerase - The enzymes DNA gyrase and topoisomerase IV belong to this class. Two subunits, A and B, make up DNA gyrase (Fig. 3). Two Gyrase A (GyrA) and two Gyrase B (GyrB) subunits combine to create the A2B2 heterotetramer, which is the active holoenzyme. The functional regions involved in DNA binding are found in the 97 kDa A subunit, which is made up of a 59 kDa (GyrA59) N-terminal domain (NTD) and a 38 kDa (GyrA-CTD) [20GyrA's C-terminal domain is crucial for protein interactions, cellular targeting, and substrate recognition. The 90 kDa B-subunits assist DNA binding and are involved in ATP binding and hydrolysis.[32,33]

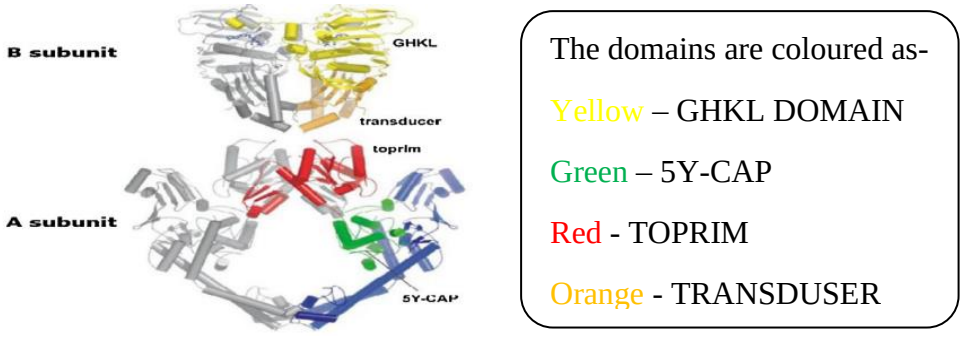


Fig. 3. The structure of type IIA topoisomerase with the domains which comprise the A and B subunits.[34]

The most recent topoisomerase to be identified in E. coli is called topoisomerase IV, and it resembles DNA gyrase in structure. These heterotetrameric enzymes are made up of two C and two E subunits (C2E2) and are encoded by the parC and parE genes. The E-subunit is in charge of the enzyme's ATPase activity, while the C-subunits contain the active site tyrosine. The segregation of catenated DNA rings that are produced during replication is topo IV's primary function. Type II topoisomerase has the role of introducing negative supercoiling, which is necessary for chromosomal replication and segregation. Topoisomerase IV is essential for chromosomal segregation and cell division even though it lacks supercoiling activity.[24,25]

4.2.1. Type III topoisomerase- This monomeric protein enzyme, like type 1 topoisomerase, is encoded by the topB gene.[35] It contributes to plasmid segregation and chromosomal stability.[36]

5. Role of DNA gyrase in DNA synthesis

DNA gyrase is an A2B2 heterotetrameric enzyme composed of two GyrA and two GyrB subunits each. There are two domains in subunit A. The first domain, commonly referred to as the G segment at the DNA gate, is the N-terminal breakage-reunion domain. Its job is to bind the DNA segment, cut it in half, and then reseal it. The second domain is the C-terminal (CTD) domain. ATPase domain and TOPRIM domain make up Subunit B's two domains. The core DNA gate in this enzyme is made up of three protein-protein interfaces that can open and close to let DNA enter through it (Fig.4)

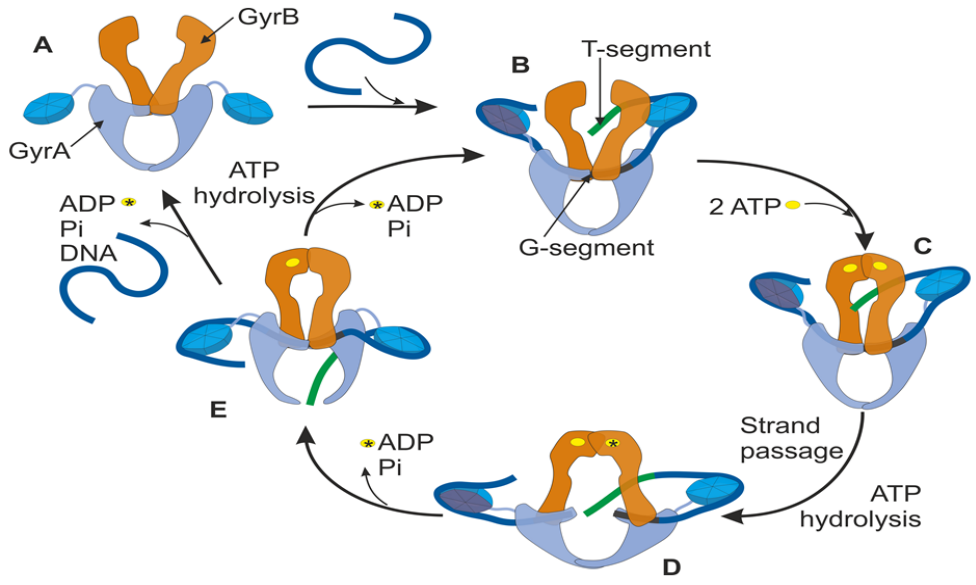


Fig. 4: Role of DNA gyrase in DNA synthesis.

6. DNA GYRASE

GyrB, GyrA, and a C-terminal tail make up the three-domain structure of members of the type II subfamily of topoisomerases, which includes in E. coli DNA gyrase. DNA gyrase's GyrB component is identical to the ParE subunit of topoisomerase IV, as well as the N-terminal half of human and yeast topoisomerase II, with the addition of a 170 amino acid sequence close to the C-terminus. Topoisomerase IV's ParC subunit and the C terminal half of eukaryotic topoisomerase IV are interchangeable with DNA gyrase's GyrA portion. The active site tyrosines are located about 120 amino acids from GyrA's N-terminus. GyrB or ParE's 400 amino acids at the N-terminus contain the ATP binding site. GyrB's C-terminal region, which is thought to be in charge of the interaction between GyrA and GyrB subunits, is located downstream of the ATPase domain.[37] The substrate for negative supercoiling is formed by a region in the DNA gyrase's C-terminal tail domain that coils up roughly 140 base pairs of the bound G-segment DNA into a right-handed supercoil.[38,39]

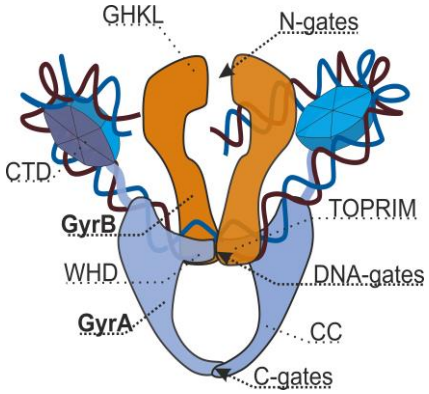


Fig. 5: Scheme of gyrase structure.

7. Mechanism of action and biolochemical features

Numerous excellent reviews have been written about the general structure and reaction mechanism of bacterial DNA gyrase. Two A subunits (encoded by gyrA) and two B subunits (encoded by gyrB) of the enzyme form a tetramer that binds to the DNA molecule to carry out its function. Dimmer gates that regulate the passage of DNA through the holoenzyme are formed by two A and two B subunits.[40]

A positive supercoil formed by a piece of DNA measuring about 140 base pairs is wrapped around the C-terminal tail domain of the GyrA protein when the enzyme binds to DNA. The coupled DNA (the G-segment) is subsequently cut at locations along each strand that are four base pairs apart, leaving the active site tyrosines (Tyr22) from the two A subunits covalently

linked to the 5'-phosphate groups on the cleaved ends. This double stranded break and the enzyme itself are used to transport a different section of DNA (the T-segment). The linking number is lowered by two upon resealing of the cleaved DNA, resulting in the introduction of negative supercoils and changing the structure of the DNA molecule. The ATP hydrolysis and the DNA strand-passage reaction are interconnected, although it is unclear how the energy released during ATP hydrolysis is used to the strand passage process. Nucleotide binding appears to induce one round of supercoiling and ATP hydrolysis is necessary for recycling the enzyme because only little supercoiling happens when ATP is substituted with the non-hydrolyzable analogue, ADPNP (5'-adenylyl-, -imidodiphosphate).[41] Different tasks are carried out by the A and B subunits of the holoenzyme's A2B2 tetramer. The GyrA subunit is composed of a 59 kDa N-terminal domain called GyrA59, which is in charge of DNA breakage and reunion and has amino acid residues that interact with quinolones, and a 38 kDa C-terminal domain called GyrA-CTD, which is involved in wrapping the DNA substrate. The crystal structure of GyrA59, which contains the tyrosine moiety (Tyr122), exhibited a heart-shaped arrangement with two dimmer interfaces. Tyr122's phenolic OH group is the nucleophile that cleaves the phosphodiester bonds of DNA and covalently attaches to the ends of the broken DNA.[42]

The two active-site tyrosine residues are situated close to the core of the amino-terminal contact, which creates a positively charged surface. The G-segment, which would eventually form the DNA gate, is assumed to be bound by this area. The amino acids that can change to cause quinolone resistance are found in another area. This is referred to as the QRDR (quinolone resistance determining region). GyrA-CTD is believed to bind DNA and assist in mediating a favourable superhelical wrap around the protein.[43]

The CTD structure adopts a special fold that resembles a propeller, but it also contains connectivity between strands and a tertiary structure that hasn't been observed in other propellers, leading to the nickname "pinwheel." This domain's perimeter is largely made up of conserved residues, which together form a positively charged patch that may bind and bend DNA.[44] In light of these findings, a possible explanation for how DNA gyrase might be able to add negative supercoils to DNA has been put forth. The B. burgdoferi GryA-CTD and E. coli GyrA-CTD both have circular ß-pinwheel folds that have been solved in their crystal structures, however the B. burgdoferi GryA-CTD is flat and the E. coli GyrACTD is spiral.[45] DNA relaxation assays revealed that the B. burgdorferi GyrA-CTD introduces a

more subdued positive superhelicity compared to the E. coli GyrA-CTD, which wraps DNA producing significant positive superhelicity. GyrA's entire structure has recently been solved at a low resolution. The structure demonstrates that GyrA is a dimeric, non-globular protein with a dimeric GyrA59 core that is closely bordered by two pear-shaped densities, each of which may hold a single GyrA-CTD monomer. The CTDs' location within the GyrA structure revealed that the enzyme underwent a significant conformational shift upon binding DNA.

8. BACTERIAL DNA GYRASE AS NEW TARGET FOR ANTI-TB

Numerous antibiotics have targeted the bacterial enzyme. the quinolones and coumarins being the two most notable types.[46] The DNA gyrase ATPase process is inhibited by coumarins like novobiocin and cyclothialidines, which target the B subunit (GyrB).[47] The DNA breakage-reunion cycle is disrupted by the quinolones, which prevent supercoiling.[48] Experiments in which DNA gyrase is incubated with DNA when quinolones are present, and the reaction is then stopped by the addition of SDS, have shown this to be the case. As a result, DNA experienced double-strand breaks, and the resultant 5'-phosphates were covalently linked to the active site tyrosine residues of the A subunit.[49]

For transcription to be possible, it is essential to be able to relax supercoiled DNA. The transient division of the double strand is necessary for transcription. As it copies each strand, the polymerase moves only in one direction, carrying the bubble containing the single-stranded segments with it. The supercoiling and undercoiling of the strands in front of and behind the flowing bubble cause an increase in resistance to it. Quinolones cause the bubble to freeze and the bacterial cells to die. The fact that DNA gyrase inhibitors are such potent and all-purpose antibiotics is likely due in large part to the fact that inhibiting transcription is a highly conserved and crucial process.[50]

Strand cuts in the DNA are caused by DNA gyrase activity. It appears that there are fatal repercussions for the bacterium if the cut DNA strands are released before the cuts are resealed, according to a convoluted and incompletely understood mechanism.[51,52] Since the inhibition of protein synthesis in some bacteria is partially antagonistic to the lethal effects of quinolones, it appears that this phenomenon requires protein synthesis.[53] A DNA molecule gets wrapped around the enzyme at the AA/BB interface, causing the segment that needs to be cut to enter the AA chamber and the segment that needs to be passed to enter the BB chamber. The AA chamber segment is sliced to create a gate, and the enzyme clings to the

cut ends with covalent connections. After the enzyme separates the broken strands, the molecule's unbroken strand is passed through the gate and into the lower BB chamber. Next, the cut strand is resealed. The uncut strand is discharged from the BB chamber, ending the cycle. In the second or third phase of the cycle, or somewhere in between, quinolone binding causes the DNA-enzyme complex to become frozen. This results in either a cleavable complex or a cut section where the DNA is caught in this transitional state, neither moving forward nor going backward.[54] According to a theory, the ternary cleavable complex either produces or becomes a cellular toxin that quickly destroys the bacterial cell.[55] One explanation for this phenomenon is that the breaks in the DNA induce the biosynthesis of endonucleases that enter into a poorly controlled DNA repair process that results in apoptosis. Quinolones' affinity for the DNA gyrase enzyme by itself is extremely low.[56] They show some affinity towards single stranded DNA[58], however, the presence of double stranded DNA and the DNA gyrase enzyme creates an efficient binding site for the quinolones.[57] This cooperative, reversible binding site can bind four to six quinolone molecules before becoming saturated. Numerous excellent reviews have addressed the biochemistry of the interaction between DNA gyrase and quinolones. One of these is by Mitscher.[58] The widespread use of quinolones has led to the development of resistance to these potent medications, necessitating the search for novel inhibitors of this potent target in other areas. GyrB, the other component of the DNA gyrase machinery that hasn't been used in chemotherapy as much as GyrA, has recently received a lot of interest. The naturally occurring Streptomyces antibioticus product known as coumarins has not yielded any pharmaceutically effective drugs despite having strong in vitro antibacterial activity. Low solubility, limited absorption, and eukaryotic cell toxicity are some of the factors that have prevented this therapeutic family from developing clinically effective molecules.[59] Coumarins are very strong inhibitors of GyrB's ATPase activity.[60] It has been thoroughly characterised how coumarins interact with DNA gyrase, and the crystal structures of GyrB in complex with novobiocin or clorobiocin have been solved.[61] These studies have shown that the coumarin binding sites overlap with the ATP binding site, with the coumarin's noviose moiety overlapping the ATP molecule's adenine ring binding site. Cyclothialidines, as well as the bacterial toxins CcdB and microcin B17, are further GyrB inhibitors.

9. DNA GYRASE FROM *MYCOBACTERIUM TUBERCULOSIS*

The DNA gyrase protein-encoding genes GyrA and GyrB from M. tuberculosis have been identified.[62] It has been determined which M. tuberculosis genes, GyrA and GyrB, encode

the DNA gyrase protein.[63] The M. tuberculosis gyrase shows high homology with topoisomerase IV from E. coli, sharing 62% similarity between ParC and GyrB and 59% similarity between ParE and GyrA. The changes linked to fluoroquinolone resistance in M. tuberculosis clinical isolates are located in codons similar to those in the gyrA genes from other bacteria's fluoroquinolone resistant mutants. The highly conserved N-terminal region of GyrA houses the QRDR of M. tuberculosis, which resembles those from other organisms.[64,65] In M. tuberculosis mutants that have acquired quinolone resistance in the lab, gyrA frequently has one missense mutation for low-level resistance, two mutations for high-level resistance, or one mutation each for gyrA and gyrB.[66] The most isolated gyrA mutants in gyrB are A90V, D94G, N, T, or A, and D472.[67] Furthermore, M. tuberculosis and other bacteria exhibit high conservation in the regions of gyrB that code for common mutations that result in resistance to coumarins and quionoles.

A study analyzing mutations in both gyrA and gyrB associated with quinolone resistance identified a novel N510D mutation in gyrB that was also associated with quinolone resistance. This study also found that while strains that carried the novel GyrA T80A mutation were slightly resistant to quinolones, several isolates carrying a combination of GyrA T80A plus A90G mutations were hypersusceptible to quionolones.[68] Studies looking at the expression of M. tuberculosis DNA gyrase genes showed that the main transcript is dimeric and that the main promoter, PB1, is situated upstream of the gyrB gene. Aside from the primary promoter, it was discovered that the gyr locus has at least two other weaker promoters, PA for gyrA and PR for gyrB, which are likely involved in regulation. Inferring that PR exclusively functions as a regulator, PR is divergently orientated and nearly entirely overlaps PB1. As a result, binding of RNA polymerase to one promoter would block transcription in the opposite direction.

In order to understand the M. tuberculosis DNA gyrase enzyme's mechanism, a number of researchers have tried to purify the enzyme in bulk for in vitro modifications. Recombinant clones have recently been created that enable the synthesis of E. coli and separate separation of recombinant M. tuberculosis GyrA and GyrB subunits. In this manner, a stable pair of the two subunits can be manufactured in vast quantities and reconstructed into a working DNA gyrase enzyme. Using two complementary assays—inhibition of DNA supercoiling and induction of DNA cleavage resulting from stabilisation of the cleavage complex—the reconstituted enzyme has been utilised to examine the interaction of the enzyme with panels

of quinolones.[69] This research has made it possible to demonstrate a link between quinolone structure and action in which DNA supercoiling inhibition and M. tuberculosis growth inhibition are associated. This study found that the quinolone SAR against M. tuberculosis deviated from that observed for other gram-positive organisms, and it attributed this discovery to several potential causes, including that: The peptidic QRDR in both the A and B subunits of DNA gyrase, the sole topoisomerase II in M. tuberculosis, is distinct as described.[70] In contrast to E. coli, where the crucial residue for interaction with quinolones is the extensively conserved Ser-83, M. tuberculosis has the analogous position at Ala-90, which could change how the mycobacterial enzyme interacts with quinolones.

The M. tuberculosis DNA gyrase, the only type II topoisomerase in this bacterium, has been functionally characterised in a study. This study discovered that M. tuberculosis DNA gyrase is more effective in relaxing, cleaving, and decatening DNA than other DNA gyrases are at supercoiling DNA. But compared to a true topoisomerase IV, the DNA gyrase from M. tuberculosis is less effective at decatenating DNA. Similar to other bacterial DNA gyrases, the M. tuberculosis DNA gyrase catalysed ATP-independent DNA relaxing; however, in contrast to topoisomerase IV and eukaryotic topoisomerase II, which need ATP for relaxation, ATP hindered DNA relaxation. The reverse passive strand transfer necessary for relaxation was understood to be hampered by the constricted conformation of the ATP binding site caused by the dimerization of the GyrB subunits. In comparison to other DNA gyrases, the M. tuberculosis enzyme was significantly more effective for the relaxing action, using just half the enzyme for supercoiling. Additionally, compared to other type II topoisomerases, it required less enzyme to cleave DNA.[71,72]

Another work used site-directed mutagenesis to investigate the DNA-binding sites in the M. tuberculosis gyrase GyrA-CTD. Y577, R691 and R745 were identified as important DNA-binding residues in M. tuberculosis GyrA-CTD in this work. It further revealed that the major DNA-binding area in M. tuberculosis DNA gyrase is the 3rd blade of the GyrA-CTD. Y577A, D669A, R691A, R745A, and G729W alterations resulted in the loss of supercoiling and relaxation activities but had no effect on drug-dependent DNA cleavage and decatenation activities and had no effect on ATPase activity. These findings demonstrated that the GyrA-CTD is required for M. tuberculosis DNA gyrase. The DNA-binding sites in GyrA-CTD were discovered for the first time.[73]

10. Inhibitors Of M. Tuberculosis Gyrase

Topo-I have not been actively used as a target for possible antibacterials, but M. TB gyrase (MTgyrase) has. This is partially due to gyrase's high degree of success as an antibiotic target that is effective against a variety of harmful bacteria.[74] Additionally, the vast structural data on gyrase from many bacteria, such as M. TB[75], has enhanced in silico ways to create novel gyrase-specific inhibitors; examples of this strategy are provided below. Perhaps unexpectedly, there isn't a full crystal structure of gyrase (A2B2) for any organism at this time.[76] On the other hand, it is feasible to construct such a structure (in this case for Mtgyrase) utilising the information from domains and protein fragments that are currently available.

The fluoroquinolones are the most effective gyrase-targeted antibiotics, and several of these drugs have been shown to be effective against tuberculosis (TB); these include gatifloxacin and moxifloxacin[77,78], which are being evaluated for both first-line and second-line therapy for multi-drug-resistant (MDR) and extensively drug-resistant (XDR) TB. The outcomes from a recent large-scale Phase-3 trial showed that moxifloxacin, the currently most promising fluoroquinolone targeted to TB, could not replace isoniazid or ethambutol in a 4-month therapy regimen.[79] Moxifloxacin will undoubtedly continue to play a significant position in TB treatment, especially for MDR-TB, it is obvious.

The study of quinolone activity on Mtgyrase and the creation of novel inhibitors have both benefited tremendously from X-ray crystallography. GyrA's N-terminal domain (NTD) and the catalytic core (GyrB's CTD fused to the GyrA NTD) were first identified structurally.[80] This provided some preliminary understanding of drug-protein interactions since these areas include amino acid residues that interact with quinolones. The crystal structures of gyrase and topo IV from different organisms, in particular the structure of the catalytic core fusion of Streptococcus pneumoniae topo IV complexed with moxifloxacin and clinafloxacin[81], have been found to contain bound DNA and quinolones. Staphylococcus aureus gyrase complexed with ciprofloxacin[82] and Acinetobacter baumannii topo IV complexed with moxifloxacin[83] have similar catalytic core fusion structures.

These structures shed light on the quinolones' inhibitory mechanism at the molecular level. Structures of the Mtb GyrB-GyrA catalytic core with moxifloxacin, gatifloxacin, and other quinolones have been discovered more recently.[84] These structures shed information on the specifics of Mtgyrase-quinolone-DNA interactions and demonstrate how they differ from

those observed in other organisms. They will also facilitate the creation of novel drugs with stronger antitubercular effects.

11. CURRENT DRUG DISCOVERY EFFORTS TARGETING M. TUBERCULOSIS DNA GYRASE

A lot of useful information has been accumulated on fluoroquinolones including their activity against their targets, DNA gyrase and topoisomerase IV, mechanism of action and resistance, and clinical efficacy and safety as has been discussed above. The search for newer and better fluoroquinolones continues at various institutions to attempt to address the obvious issues of resistance and some safety concerns for some analogs. Although fluoroquinolones are highly effective antibiotics, their widespread use, and sometimes misuse has increased resistance towards them in both the hospital and in the community. Even though no fluoroquinolone has been specifically registered for use against tuberculosis, many fluoroquinolones have been used in a second line therapy against M. tuberculosis infections including ciprofloxacin, ofloxacin, levofloxacin and sparfloxacin[85] and a number of the newer fluoroquinolones such as gatifloxacin, moxifloxacin, lomefloxacin and sitafloxacin are showing great potential as new additions to the fight against tuberculosis with their much lower MICs and are being evaluated for this purpose.[86,87]

A number of preclinical and clinical studies have evaluated the utility of quinolones against tuberculosis including preclinical combinations with other drugs[88,89], effects of the C-8 substituents on activity[90], activity on non-replicating organisms[91] and organisms in macrophages, efficacy in murine models of infection[92,93] dosage and frequency of dosages, sterilizing activities, efficacy in pulmonary disease[94], and clinical combinations with other first line TB medications.[95]

Fluoroquinolone resistance in mycobacteria is currently uncommon, and other anti-tuberculosis medications have not shown any evidence of cross-resistance. However, due to the high rates of tuberculosis infection around the world, rising rates of MDR-TB, high rates of HIV coinfection, and the recent increase in extremely drug-resistant TB (XDR-TB), there is an urgent need for newer, more potent, non-toxic anti-tuberculosis agents. Fluoroquinolones have become an obvious addition to the drug arsenal against tuberculosis, and they are currently being utilised in cases of MDR-TB. For the same reason, a number of researchers are actively seeking newer and more effective fluoroquinolones that are

optimised against the M. tuberculosis enzyme and may be useful against fluoroquinolone-resistant clinical isolates of M. tuberculosis.

Numerous initiatives are being made to increase the fairly potent fluoroquinolones' effectiveness against tuberculosis. Some gatifloxacin analogues were found to be more active than gatifloxacin against drug sensitive and drug resistant bacteria in one such study that sought to identify better and more effective gatifloxacin by introducing lipophilicity at position 7th in an effort to increase drug uptake by the organism. Numerous novel scaffolds, such as the 3-aminoquinazolinediones and the isothiazolopyridones, have been developed to increase fluoroquinolone activity by creating more efficient analogues.[96,97]

The quinolone project aims to develop a new family of DNA gyrase inhibitors that will be more effective than moxifloxacin at reducing the length of TB treatment while retaining a high level of safety and tolerability. Without harmful drug-drug interactions with anti-retrovirals (ARVs), the new medicines ought to be effective in treating MDR-TB and TB/HIV co-infections.There are several major challenges in order to advance a successful quinolone candidate into clinical development: Whereas the quinolone class has been extensively optimized against many common pathogens, it is obvious that the SAR for M. tuberculosis and other pathogens are significantly different, and a SAR against M. tuberculosis needs to be generated. Whereas traditionally anti-tuberculosis agents are first optimized against M. tuberculosis in the replicating state during the discovery stage, potency against replicating M. tuberculosis appears to have little predictive value for efficacy against tuberculosis in the persistent phase of infection.

11.1. KRQ-10018

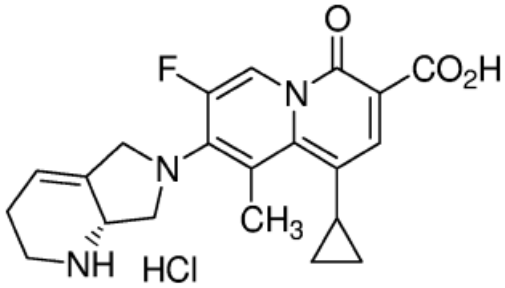
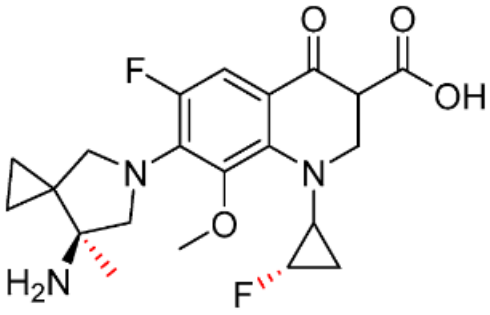


Fig 6: Chemical Structure of KRQ-10018

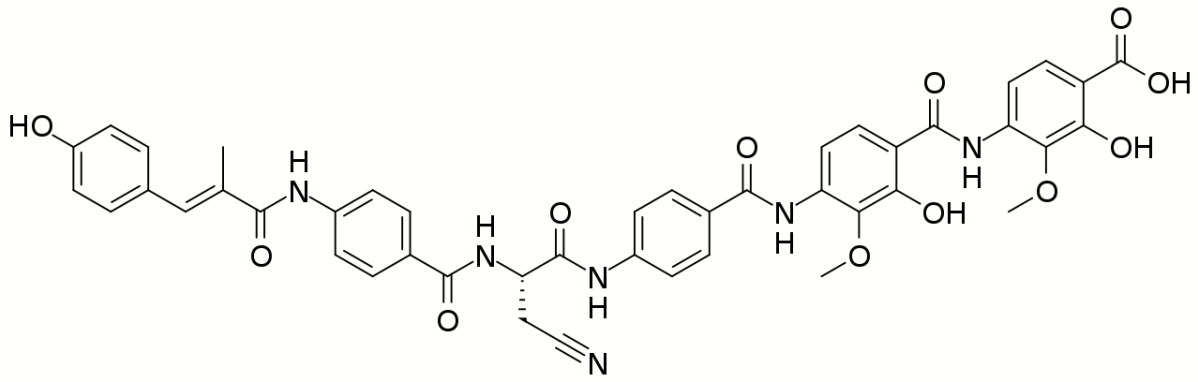
KRQ-10018 was the initial lead compound discovered by the TB Alliance-KRICT programme (ICAAC poster, 2006, San Francisco1) with assistance from the National Institute of Allergy and Infectious Diseases-funded Tuberculosis Antimicrobial Acquisition and Coordination Facility (www.taacf.org). Its effectiveness against replicating M. tuberculosis, non-replicating bacteria, organisms in human macrophages, and a mouse infection model have all been tested.[98]

11.2. DC-159a



The newly developed fluoroquinolone DC-159a from Daiichi-Sankyo, which is being used to treat respiratory infections, has shown excellent activity against M. tuberculosis and non-tuberculous mycobactera as well as activities against quinolone-resistant-MDR-TB (QR-MDR-TB) isolates that were superior to both moxifloxacin and gatifloxacin as well as rifampicin.[99] In comparison to moxifloxacin, DC-159a had MIC90s against QR-MDR-TB isolates that were 8 times lower. In a study that compared the efficacy of DC-159a with that of the other fluoroquinolones in a mouse model of QRMDR-TB infection, the mean survival days of mice treated with DC-159a were higher than those of animals treated with moxifloxacin, levofloxacin, isoniazid, or rifampicin.[100] The efficacy of DC-159a at 50 mg/kg was comparable to that of moxifloxacin at 100 mg/kg in research using a drug-susceptible M. tuberculosis infection model. With a satisfactory safety profile that showed neither phototoxicity or chondrotoxicity, DC- 159a was well tolerated in rats and monkeys.[101] Research is currently being conducted in numerous labs to find effective inhibitors of bacterial DNA gyrase that may one day be turned into antibacterial medications. The majority of these initiatives aren't focused on M. tuberculosis, but the fluoroquinolone example implies that these medications may either be employed right away in treating tuberculosis or they could be further optimised against the M. tuberculosis enzyme to increase their potency against the organism.

11.3. Albicidin



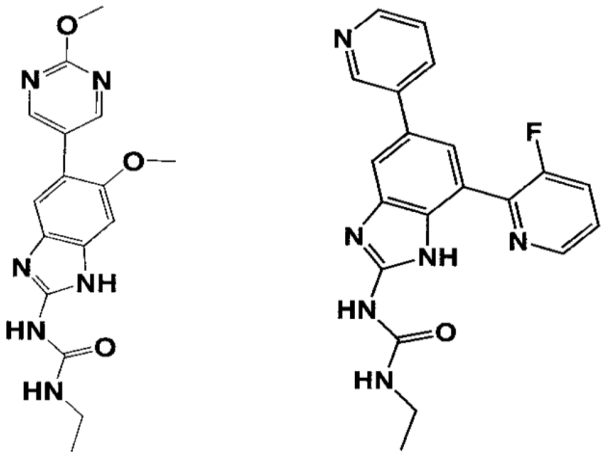
Albicidin, a polyketide-peptide from Xanthomonas albilineans, has an IC50 value of 40–50 nM, making it a more potent inhibitor of the supercoiling activity of bacterial and plant DNA gyrases than the majority of coumarins and quinolones. The mechanisms of action of this natural chemical appear to be the religation of the intermediate generated by DNA cleavage during the catalytic sequence of gyrase and the regulation of the relaxation of supercoiled DNA by gyrase and topoisomerase IV. The amount of albicidin needed to stabilise the gyrase cleavage complex rises 100-fold in the absence of ATP, in contrast to the quinolones. In E. coli, several mutations in gyrA that are known to provide high-level resistance to quinolones or CcdB also confer low-level resistance to or hypersensitivity to albicidin. Gyrase-albicidin interaction has distinct characteristics that point to the potential for new antibacterial medication development.[102]

11.4 Simocyclinones

The new class of antibiotics known as "simocyclinones" was discovered in Streptomyces antibioticus.[103] A crucial component of the architectures of novobiocin, coumermycin A1, and clorobiocin, which likewise target gyrase, is an aminocoumarin moiety. Simocyclinones have a notably distinct behaviour from these substances. Simocyclinones' interaction with bacterial DNA gyrase has recently been described.[104] With an inhibitory concentration 50% lower than that of novobiocin, this study discovered that simocyclinone D8 is a powerful inhibitor of gyrase supercoiling. Although this is thought to be how other aminocoumarins work, it did not competitively inhibit GyrB's DNA-independent ATPase process. Simocyclinone-D8, in contrast to quinolones, prevented DNA relaxing by gyrase and did not promote the development of cleavage complexes. Simocyclinones appear to block the development of cleavage complexes that are generated by both Ca (2+) and quinolones. Simocyclinone-D8 is thought to interact with GyrA's N-terminal domain, according to binding experiments. Together, these results show that simocyclinones suppress the enzyme's

ability to bind to DNA, hence inhibiting an early stage of the gyrase catalytic cycle. This unique gyrase inhibitor mechanism opens up fresh opportunities for the creation of antibacterial medications.

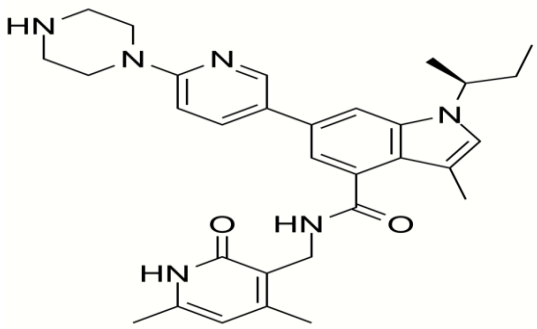
11.5 VRT-125853, VRT-752586 –



VRT-125853 **VRT-752586**

VRT-125853 and VRT-752586 are two compounds that were discovered through an approach used to optimise a novel series of aminobenzimidazoles that block the ATPase activity of bacterial DNA gyrase and topoisomerase IV. The principal target of both drugs was found to be GyrB in Staphylococcus aureus and Haemophilus influenzae, but ParE in Streptococcus pneumonia.[105] Both substances prevented the maintenance of negative supercoils in plasmid DNA in Escherichia coli. VRT-125853's main target in Enterococcus faecalis was ParE, whereas VRT-752586's targeted GyrB. Dual targeting is demonstrated by the fact that gyrB and parE double mutations greatly raised the MICs of VRT-125853 and VRT-752586.

11.6 GSK126



Effects of GSK126 on fluoroquinolone-resistant and wild-type gyrase's capacity to coil DNA. Gyrase's ability to catalyse has been demonstrated to be inhibited by NBTIs, although it is unknown how this effect compares to NBTIs' capacity to promote DNA cleavage in terms of how drugs induce cell death. As a result, GSK126's effects on DNA supercoiling, which is catalysed by the gyrase of B. anthracis, E. coli, and M. tuberculosis, were investigated. Despite having lesser activity against M. tuberculosis gyrase (IC50 values of 0.4 and 2 M, respectively, compared to an IC50 of 75 M), the NBTI was a strong catalytic inhibitor of the B. anthracis and E. coli enzymes. GSK126 could also only reduce the latter enzyme's activity by 60%.[106]

12. CONCLUSION

New pharmacological medications are required to manage tuberculosis because of the rise in MDR-TB cases and the recently discovered XDR-TB strain. In M. tuberculosis, quinolones solely target the one Type II topoisomerase and DNA gyrase is a proven and pharmaceutically efficient target for the development of new antimicrobial drugs. Fluoroquinolones are an excellent class of chemicals with good antimicrobial and pharmacokinetic properties against many bacterial pathogens. Newer fluoroquinolones, such moxifloxacin, have demonstrated good effectiveness against M. tuberculosis despite being significantly less effective against mycobacteria. They are used to treat cases of drug resistance. There is no doubt that topoisomerases have been successful targets for anticancer and antibacterial chemotherapy, and it would seem that there is still a strong case for continuing to look for new drugs that target MTB-gyrase. can be thought of as a "new" target that has yet to be utilised in therapeutic settings. However, it is justified to put more effort into looking for novel inhibitors considering the pressing need to discover new antibacterial drugs and the depth of knowledge about this enzyme. As the given effectiveness of fluoroquinolones and the presence of other exploitable ligand-binding pockets in MTB-gyrase, it is likely that work on finding new gyrase-specific inhibitors will be crucial in the future. It is unclear if these searches are target-based or adhere to phenotypic screening. It's important to note that the majority of these efforts are not focused on tuberculosis, and the usefulness of these new compounds for treating tuberculosis would need to be added to the efforts that appear to be successful by either testing against the entire organism directly or attaching lead optimisation efforts that build in the specificity against the M. tuberculosis gyrase and the entire organism.

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