Mycobacterium tuberculosis DNA Gyrase as a Target for Drug Discovery

Khisimuzi Mdluli* and Zhenkun Ma

Global Alliance for Tuberculosis Drug Development, 80 Broad Street, 31st Floor. New York, NY. 10004

Abstract: Bacterial DNA gyrase is an important target of antibacterial agents, including fluoroquinolones. In most bacterial species, fluoroquinolones inhibit DNA gyrase and topoisomerase IV and cause bacterial cell death. Other naturally occurring bacterial DNA gyrase inhibitors, such as novobiocin, are also known to be effective as antibacterial agents. DNA gyrase is an ATP-dependent enzyme that acts by creating a transient double-stranded DNA break. It is unique in catalyzing the negative supercoiling of DNA and is essential for efficient DNA replication, transcription, and recombination. DNA gyrase is a tetrameric A_2B_2 protein. The A subunit carries the breakage-reunion active site, whereas the B subunit promotes ATP hydrolysis. The *M. tuberculosis* genome analysis has identified a *gyrB-gyrA* contig in which *gyrA* and *gyrB* encode the A and B subunits, respectively. There is no evidence that *M. tuberculosis* has homologs of the topoisomerase IV, *parC* and *parE* genes, which are present in most other bacteria. Newer fluoroquinolones, including moxifloxacin and gatifloxacin, exhibit potent activity against *M. tuberculosis*, and show potential to shorten the duration for TB treatment. Resistance to fluoroquinolones remains uncommon in clinical isolates of *M. tuberculosis*. *M. tuberculosis* DNA gyrase is thus a validated target for anti-tubercular drug discovery. Inhibitors of this enzyme are also active against non-replicating mycobacteria, which might be important for the eradication of persistent organisms. A novel inhibitor of *M. tuberculosis* DNA gyrase would be effective against multi-drug resistant (MDR)-TB, and it could also be effective against fluoroquinolone-resistant *M. tuberculosis*.

Keywords: Type II topoisomerase, supercoiling, decatenate, quinolones, coumarin, simocyclinones, aminobenzimidazoles, indazole.

INTRODUCTION

The search for new TB drugs that can overcome the increasing spread of multidrug resistant tuberculosis (MDR-TB) and emerging extremely drug resistant tuberculosis (XDR-TB) can be approached from two directions: the identification and validation of novel targets for the development of novel antibiotics with no preexisting resistance mechanisms; or exploration of known and clinically validated targets for new chemical series or modification of existing drug classes to eliminate the possibility of cross resistance with existing drugs for which resistances have developed. The first approach has been tried for over a decade of antibiotic discovery efforts fueled by multiple bacterial genome analyses, and high throughput screening and has had limited success due to low hit rates from high throughput screens, and the difficulties associated with attempting to validate novel targets without inhibitors that can be tested in infection models [1]. The second approach has had significant successes identifying newer and more effective generations of compounds based on older drugs from a variety of classes including macrolides, tetracyclines, cephalosporins and quinolones. This approach also benefits from targeting fully validated molecular targets whose chemical inhibition is known to cause inhibition of bacterial growth.

Bacterial DNA gyrase, a type II DNA topoisomerase found in all bacteria, is a proven target for antibacterial chemotherapy [2]. Fluoroquinolones, a class of synthetic antimicrobial agents, inhibit bacterial DNA gyrase and topoisomerase IV in most bacterial species and cause bacterial cell death [3]. Besides the fluoroquinolones, other bacterial DNA gyrase inhibitors, such as naturally occurring novobiocin, are known to be effective as antibacterial agents [4].

Fluoroquinolones exert their powerful antibacterial activity by interacting with DNA gyrase and DNA topoisomerase IV [5]. Specifically, they bind to the enzyme-DNA complex and thereby stabilizing the covalent enzyme tyrosyl-DNA phosphate ester (a transient intermediate in the topoisomerase reaction). Hydrolysis of this linkage leads to the accumulation of double-stranded DNA fragments, which probably accounts for the bactericidal activity of fluoroquinolone treatment. Newer fluoroquinolones, including moxifloxacin and gatifloxacin, exhibit effective *in vitro* activity against mycobacteria [6,7], and have the potential, based on animal data, to reduce TB treatment regimens from 6 to 4 months or less when substituted for isoniazid in a standard regimen [8]. Since DNA gyrase is the sole Type II topoisomerase in *M. tuberculosis*, it is likely the only target for fluoroquinolones in this organism [9,10].

Resistance to fluoroquinolones remains uncommon in clinical isolates of *M. tuberculosis* [11]. High-level resistance to fluoroquinolones in laboratory generated strains of *M. tuberculosis* and *M. smegmatis* is known to result from amino acid substitutions in the putative fluoroquinolone-binding region of the *M. tuberculosis gyrA*-encoded A subunit of DNA gyrase [12,13]. The antimycobacterial activities of fluoroquinolones appear to correlate well with their DNA gyrase inhibitory activities [14].

M. tuberculosis DNA gyrase is thus a validated target for anti-tubercular drug discovery; its inhibition results in highly mycobactericidal activity. Inhibitors of this enzyme are also active against non-replicating, persistent mycobacteria, which might be important for shortening the duration of TB therapy. A novel inhibitor of *M. tuberculosis* DNA gyrase would also be effective against multi-drug resistant (MDR)-TB, and will likely be effective against fluoroquinolone-resistant *M. tuberculosis*.

Of all the antimicrobials that have progressed to the point of human testing for the treatment of tuberculosis, the quinolone class holds a great potential for shortening treatment duration, overcoming multidrug-resistant tuberculosis (MDR-TB) and improving therapy of TB-HIV co-infections.

DNA TOPOISOMERASES

The reason that DNA topoisomerases are required in nature is the double-stranded helical nature of the DNA molecule. For most of the processes for which access to the individual DNA strands is required, the two strands of the helix need to be separated either temporarily (transcription, recombination), or permanently (replication). This strand separation requirement cannot be accomplished through the winding of the strands due to the circular nature of the bacterial chromosome and the large size of the eukaryotic chromosomes. Topoisomerases are therefore required to maintain a proper topology of the DNA molecule for it to participate in these essential cellular functions.

DNA topoisomerase enzymes control the topology of DNA in the cell by passing one strand of the DNA through a break in the opposing strand, or by passing a region of duplex from the same or

*Address correspondence to this author at the Global Alliance for Tuberculosis Drug Development, 80 Broad Street, 31st Floor, New York, NY. 10004; E-mail: khisi.mdluli@tballiance.org

1871-5265/07 $50.00+.00

a different molecule through a double-stranded gap in a DNA molecule. The type I family of topoisomerases catalyzes the former mechanism, and the type II subfamily (including DNA gyrase) catalyzes the latter. Topoisomerases can either only relax the negative supercoils, relax supercoils of both directions, or introduce either negative supercoils like bacterial DNA gyrase, or positive supercoils like reverse gyrase, into the DNA molecule. These topoisomerase activities serve to alter the topology of a closed DNA domain, promote the catenation and decatenation of circular DNA molecules (like the circular bacterial chromosome or plasmid), or separate intertwined linear chromosomes.

When topoisomerases cleave DNA they form a transient phosphodiester bond between the active site tyrosine residue in the protein and one of the ends of the broken DNA strand. The topological state of the DNA is then altered while this covalent intermediate is maintained, and the DNA molecule is religated when the enzyme is released. Topoisomerases that cleave only one strand of the DNA are classified as the type I, and enzymes that cleave both strands to generate staggered double-stranded breaks are classified as the type II subfamily of topoisomerases.

Escherichia coli provides the best example of the topoisomerase component in the eubacteria. The topoisomerases in this organism consist of two type I enzymes, DNA topoisomerase I and III, and the two type II enzymes, DNA gyrase and DNA topoisomerase IV. DNA gyrase generates negative supercoils for the entire bacterial chromosome which results in a condensed chromosome for proper partitioning during cell division [15,16]. DNA Topoisomerase IV decatenates the DNA molecule during replication. DNA Topoisomerase IV and topoisomerase I also relax negative supercoils to prevent excessive negative supercoiling that maybe caused by DNA gyrase activity. DNA topoisomerase IV is much more effective at decatenating DNA than at relaxing positive supercoils, and DNA gyrase is better at relaxing positive supercoils than at decatenation [17-19]. Transcription generates positive supercoils ahead of and negative supercoils behind the translocating RNA polymerase, and these are resolved by DNA gyrase and DNA polymerase I, respectively. Topoisomerase III supports the movement of the replication fork on a circular DNA molecule due to its ability to decatenate the precatenanes behind the fork by acting at nicks that are present in the replicating DNA [20-22]. This has been assumed to suggest that topoisomerase III and IV have overlapping functions.

The *Myocbacterium tuberculosis* genome, as well as those of *Campylobacter jejuni, Deinococcus radiodurans,* and *Treponema pallidum,* encodes only two topoisomerase genes [23]. There is both a homolog of the *E. coli* DNA gyrase gene, and a homolog of the *E. coli* topoisomerase I gene, but no homolog of the *E. coli* topoisomerase IV. This is thought to represent the minimal topoisomerase component for a bacterial cell. In such cases where DNA gyrase is the only type II enzyme it must be responsible for negatively supercoiling the DNA, relaxing positive supercoils, and decatenating replicated DNA. In that case Topoisomerase I would minimally be responsible for relaxing negative supercoils to prevent excessive negative supercoiling by DNA gyrase.

DNA GYRASE

Members of the type II subfamily of topoisomerases as represented by *E. coli* DNA gyrase have a three domain structure: the GyrB domain, the GyrA domain, and a C-terminal tail. The GyrB portion of DNA gyrase corresponds to the ParE subunit of topoisomerase IV, and to the N-terminal half of human and yeast topoisomerase II, with an insertion of a 170 amino acid fragment close to the C-terminus of GyrB. The GyrA portion of DNA gyrase corresponds with the ParC subunit of topoisomerase IV and the C-terminal half of the eukaryotic topoisomerase IV. The active site tyrosines are located at about 120 amino acids from the N-terminus of GyrA. The ATP binding site is located within the N-terminal 400 amino acids of GyrB or ParE. Downstream of the ATPase domain is the C-teminal region of GyrB that is predicted to be responsible for the interaction between GyrA and GyrB subunits [24]. A region that is located in the C-terminal tail domain of the DNA gyrase is responsible for wrapping about 140 base pairs of the bound G-segment DNA into a right-handed supercoil forming the substrate that will undergo negative supercoiling [25-27].

STRUCTURE AND MECHANISM OF ACTION

The general structure and reaction mechanism of bacterial DNA gyrase has been the subject of a number of excellent reviews [23,28-31]. The enzyme functions as a tetramer in which two A subunits (encoded by *gyrA*) and two B subunits (encoded by *gyrB*) bind to the DNA molecule. Within the holoenzyme, two A and two B subunits form dimmer gates that control the passage of DNA through the enzyme. The enzyme binds to DNA, and a segment of about 140 base pairs is wrapped around the C-terminal tail domain of the GyrA protein into a positive supercoil. The bound DNA (the G-segment) is then cleaved in each strand at sites separated by 4 base pairs leaving the active site tyrosines (Tyr22) from the two A subunits covalently attached to the 5'-phosphate groups on the cleaved ends. Another segment of DNA (the T-segment) is transported through this double-stranded break and through the enzyme itself. Upon resealing of the cleaved DNA, the linking number is reduced by two which results in the introduction of negative supercoils, altering the topology of the DNA molecule. The DNA strand-passage reaction is coupled to the hydrolysis of ATP, but exactly how the energy from the ATP hydrolysis is used in the strand passage process is not clear. If ATP is replaced by the non-hydrolyzable analog, ADPNP (5'-adenylyl β,γ-imidodiphosphate), only limited supercoiling occurs, suggesting that nucleotide binding promotes one round of supercoiling, and ATP hydrolysis is required for recycling the enzyme [32]. The A and B subunits that form the A_2B_2 tetramer in the holoenzyme perform distinct functions. The GyrA subunit is made up of a 59 kDa N-terminal domain (GyrA59) which is responsible for the DNA breakage-reunion and contains amino acid residues that interact with quinolones, and a 38 kDa C-terminal domain (GyrA-CTD) involved in the wrapping of the DNA substrate [30,33]. GyrA59 contains the tyrosine moiety (Tyr122) whose phenolic OH group is the nucleophile that cleaves the phosphodiester bonds of DNA and covalently binds to the ends of the cleaved DNA, and it's crystal has been solved and it revealed a heart-shaped arrangement with two dimmer interfaces [34]. The amino-terminal interface forms a positively charged surface with the two active-site tyrosine residues located near the center. This is thought to be the region that binds the G-segment that will become the DNA gate. Another region contains those amino acids whose mutation leads to quinolone resistance. This is called the quinolone resistance-determining region (QRDR). The GyrA-CTD is thought to bind DNA and help mediate a positive superhelical wrap about the protein [35-38]. The wrapping allows the protein to use closely spaced segments of the same DNA molecule as the G and T segments. First, the wrapping serves to constrain a local positive supercoil, and then turns it into a negative supercoil after the T segment has been passed through, causing a net change in the linking number of -2 [39,40]. The crystal structure of the 35 kDa GyrA-CTD domain from *Borrelia* burgdoferi has been solved to 1.75 A resolution [41]. The CTD structure adopts a unique fold that resembles a β-propeller, but has β-strand connectivity and a tertiary structure not previously seen in other β-propellers and has been termed a β-pinwheel. A large fraction of the perimeter of this domain that consists of conserved residues forms a positively charged patch that might bind and bend DNA. These observations have suggested an explanation for how DNA gyrase might be able to introduce negative supercoils into DNA. The *E. coli* GyrA-CTD crystal structure has also been solved and revealed a circular-shaped β-pinwheel fold, similar to that of *B. burgdoferi* GryA-CTD, except that whereas the *B. burgdoferi*

GryA-CTD is flat, the *E. coli* GyrA-CTD is spiral [42]. DNA relaxation assays revealed that the *E. coli* GyrA-CTD wraps DNA inducing substantial positive superhelicity, while the *B. burgdorferi* GyrA-CTD introduces a more modest positive superhelicity. Recently a low-resolution structure of the full-length GyrA has been solved [43]. The structure reveals GyrA as a dimeric and non-globular protein with a dimeric GyrA59 core that is closely flanked by two pear-shaped densities, each of which can accommodate a GyrA-CTD monomer. The position of the CTDs within the GyrA structure suggested a large conformational change of the enzyme upon DNA binding.

GyrA amino acids changes that result in resistance to fluoroquinolones are predominantly in the above mentioned quinolone-resistance determining region (QRDR). This region extends from amino acid 67 to amino acid 106 in the N-terminal portion of the GyrA protein, and the most frequently altered residues include numbers 67, 81, 82, 83, 84, 87 and 106 in *E. coli* [44-52]. The presence of a single mutation in the QRDR usually results in low level resistance to fluoroquinolones, and to obtain high levels of resistance, additional mutations in *gyrA* and/or in *parC* are required [45,53]. The most frequent mutation observed in both clinical and laboratory derived isolates that have quinolone resistance is at amino acid residue 83 of the GyrA protein [44-47,49,50,53]. The second most clinically relevant mutation involves amino acid residue 87, and strains with a double mutation at both these codons have higher MICs for fluoroquinolones [44,45]. Different amino acid substitutions at the same position result in different quinolone susceptibility levels, and this has been suspected to be due to the mechanism of interaction between the quinolones and their targets [50]. It has been suggested that amino acid residue 83 interacts with the group in position 1 of quinolones, whereas amino acid residue 87interacts with the group in position 7 [45].

The GyrB protein comprises a 43 kDa N-terminal domain (NTD) which contains the ATPase site and dimerizes upon ATP binding. This subunit also contains a 47 kDa C-terminal domain (CTD) which consists of a coumarin drug-binding site, and is involved in the interaction with the GyrA protein and DNA [54]. ATP binding and hydrolysis seems to be required for protein-protein interactions and recycling of the enzyme [55]. The B subunit also forms the entry site for the transfer (T) segment of DNA during the supercoiling process [56-58]. It has also been shown to perform as an ATP-operated clamp that binds DNA during the supercoiling process [59]. Most of the amino acid residues that bind ATP are in the 24-kDa N-terminal domain between residues 1-220 (NTD), but two residues Gln335, and Lys337 in the C-terminal domain (CTD), residues 220-392, also make contact with the ATP molecule. These residues have been implicated in the hydrolysis of ATP and they are thought to transmit conformational changes upon ATP hydrolysis [60,61]. There is a loop that is thought to close the active site and it contains amino acid residues 98-118. This loop is also implicated in the binding and hydrolyis of ATP, and is composed of a number of conserved residues which are involved in the catalytic mechanism [62-64]. The crystal structure of the NTD of the B subunit from different bacteria has been solved in complex with several inhibitors, mainly of the coumarin class [65,66]. Crystallographic studies using GyrB from the thermophilic bacterium *Thermus thermophilus,* revealed a novobiocin molecule trapped in the dimeric conformation of the ATP-binding 43kDa NTD. This structure suggested that the conformation trapped by novobiocin represents an open conformational state adopted by the enzyme immediately after ATP hydrolysis and product release [67]. The coumarins, like novobiocin, are naturally occurring compounds isolated from certain strains of *Streptomyces*, and the cyclothialidines are naturally occurring cyclic peptides, and both these compound classes inhibit the ATPase activity of the B subunit of DNA gyrase [68,69]. Since both antibiotic classes inhibit the ATPase activity of the B subunit they are assumed to bind at or near the ATP binding site. Both antibiotic classes are thought to be competitive inhibitors for the binding of the ATP substrate [70,71] due to their strong inhibition of the holoenzyme, but studies of the B subunit alone [72,73] and the 43 kDa NTD fragment of subunit B [74] have challenged the validity of these compounds being competitive inhibitors. Neither compound class inhibits other ATP dependent enzymes as well as they inhibit DNA gyrase, and neither compound class shares much structural resemblance with ATP, possibly ruling out competitive inhibition. Finally, point mutations in the B subunit that confer resistance to coumarins are outside the ATP binding site [75,76].

Substitutions in GyrB resulting in resistance to quinolones have been described at positions 426 and 447 [77]. Substitutions at position 426 seem to confer resistance to all quinolones, whereas those at position 447 result in an increased level to older quinolones like nalidixic acid, but a greater susceptibility to newer fluoroquinolones [78].

BACTERIAL DNA GYRASE AS A DRUG TARGET

The bacterial enzyme has been the target of many antibiotics the most notable being the quinolones and the coumarins [79-82]. The cyclothialidines and the coumarins like novobiocin inhibit the ATPase reaction of DNA gyrase, and they target the B subunit (GyrB) [83]. The quinolones inhibit supercoiling by interrupting the DNA breakage-reunion cycle [84,85]. This has been demonstrated by experiments where DNA gyrase is incubated with DNA in the presence of quinolones and then the reaction is terminated by the addition of SDS. This resulted in double-stranded breaks in DNA with the active site tyrosine residues of the A subunit covalently attached to the resulting 5'-phosphates [86-88].

The ability to relax supercoiled DNA is crucial for allowing transcription. Transcription requires temporary separation of the double strand. The polymerase moves in only one direction as it copies each strand, moving the bubble that contains the single stranded segments with it. The moving bubble meets increasing resistance from the supercoiling of the strands ahead of it, and from the undercoiling of the strands behind. Quinolones freeze the bubble, and produce bacterial cell death. Transcription is a highly conserved and essential activity and its inhibition is likely one major reason that inhibitors of DNA gyrase are such effective and broad spectrum antibiotics.

DNA gyrase activity introduces strand cuts in the DNA. In a complex and not yet completely understood mechanism, it seems that if the cut DNA strands are released prior to the resealing of the cuts there are lethal consequences for the bacterium [89,90]. This phenomenon seems to require protein synthesis since inhibition of protein synthesis is partially antagonistic to the lethal action of quinolones in some bacteria [91,92]. The DNA gyrase reaction mechanism involves a DNA molecule getting wrapped around the enzyme at the AA/BB interface such that the segment to be cut enters the AA chamber and the segment to be passed enters the BB chamber. The segment in the AA chamber is cut to produce a gate, and the enzyme holds onto the cut ends through covalent bonds. The enzyme then separates the cut strands and the uncut strand of the molecule is passed through the gate into the lower BB chamber. The cut strand is then resealed. The cycle is complete when the uncut strand is expelled from the BB chamber. Quinolone binding freezes the DNA-enzyme complex somewhere at or between the second or third phases of the cycle. This produces either a cleavable complex or a cut segment in which the DNA becomes trapped in this transitional state, neither progressing nor reverting [93]. It has been speculated that the ternary cleavable complex becomes or induces a cellular poison that rapidly kills the bacterial cell [94]. 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. It has also

been postulated that an unidentified toxic protein is synthesized or released due to the interference of quinolones with the DNA gyrase processes.

Quinolones have very little affinity for the DNA gyrase enzyme alone [95]. They have some affinity for single stranded DNA [93], but when the DNA gyrase enzyme and double stranded DNA are both present they create an effective binding site for the quinolones [96]. This binding site is reversible and cooperative and can be saturated with about four quinolone molecules [93]. The biochemistry of the interaction of DNA gyrase with quinolones has been the subject of many excellent reviews including that by Mitscher [97].

Widespread use of quinolones has resulted in the generation of resistances to these powerful drugs that have made it necessary to look elsewhere for novel inhibitors of this highly effective target. Much attention has turned to GyrB, the other half of the DNA gyrase machinery that has not been pharmaceutically exploited as much as GyrA for chemotherapy. Coumarins are naturally occurring products of *Streptomyces antibioticus* which, despite their potent *in vitro* antibacterial activity, have produced no pharmaceutically useful drug. The reasons for the failure of this drug class to produce clinically useful compounds include low solubility, poor uptake, and eukaryotic cell toxicity [98]. Coumarins are extremely potent inhibitors of the ATPase activity of GyrB [99]. The interaction of coumarins with DNA gyrase has been well characterized, and crystal structures of GyrB in complex with novobiocin or clorobiocin have been solved [100,101]. These studies have revealed the binding sites for the coumarins as overlapping with the ATP binding site, with the noviose moiety of the coumarin overlapping the binding site for the adenine ring of the ATP molecule. Other GyrB inhibitors include the cyclothialidines and the bacterial toxins CcdB and microcin B17.

DNA GYRASE FROM *MYCOBACTERIUM TUBERCULOSIS*

The genes that encode the DNA gyrase proteins (GyrA and GyrB) from *M. tuberculosis* have been cloned and their sequences have been determined [102,103]. The *gyrA* gene is located 36 base pairs downstream of *gyrB*. The *M. tuberculosis* GyrB and GyrA proteins share 63% and 69% similarity with the *E. coli* enzymes, respectively [**Error! Bookmark not defined.**]. The *M. tuberculosis* gyrase shows considerable homology with topoisomerase IV from *E. coli* with 62% similarity between ParC and GyrB, and 59% similarity between ParE and GyrA [102]. The QRDR of *M. tuberculosis* is similar to those from other organisms, and is located at the highly conserved N-terminal region of GyrA, and the mutations associated with fluoroquinolone resistance in clinical isolates of *M. tuberculosis* are located in codons equivalent to those in the *gyrA* genes from fluoroquinolone-resistant mutants from other bacteria [104-108]. Laboratory generated quinolone resistant mutants of *M. tuberculosis* usually have single missense mutations in *gyrA* for low-level resistance, and two mutations in *gyrA*, or one mutation in *gyrA* and one in *gyrB* for high-level resistance [109]. The most isolated *gyrA* mutants are A90V, and D94G, N, T, or A, and D472 mutations in *gyrB* [109]. The regions of *gyrB* that encode common mutations that lead to resistance to coumarins and quionoles are also highly conserved between *M. tuberculosis* and other bacteria [102].

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 [110].

Studies analyzing the expression of the *M. tuberculosis* DNA gyrase genes revealed that the primary transcript is dicistronic and that the primary promoter, P_{B1}, is located upstream of the g*yrB* gene [111]. It was also found that apart from the primary promoter, the *gyr* locus has at least two other promoters, P_A for *gyrA*, and P_R for *gyrB,* that are weaker and probably play a regulatory role [111]. P_R is divergently oriented and almost completely overlaps P_{B1} such that the binding of RNA polymerase to one promoter would prevent transcription in the opposite direction, suggesting that P_R only plays a regulatory role [111].

To enhance the understanding of the mechanism of the *M. tuberculosis* DNA gyrase enzyme, a number of investigators have attempted to purify the enzyme in bulk for *in vitro* manipulations. Recently, recombinant clones have been developed that allow the production in *E. coli* and purification of recombinant *M. tuberculosis* GyrA and GyrB subunits separately [14]. This way large quantities of the two subunits can be produced that are stable and can be reconstituted into a functional DNA gyrase enzyme. The reconstituted enzyme has been used to investigate the interaction of the enzyme with panels of quinolones using two complimentary assays, inhibition of DNA supercoiling, and induction of DNA cleavage arising from stabilization of the cleavage complex. These studies have allowed the establishment of a quinolone structure-activity relationship in which inhibition of DNA supercoiling is correlated with *M. tuberculosis* growth inhibition [14]. This study concluded that the quinolone SAR against *M. tuberculosis* did not follow that established for other gram-positive organisms and attributed this observation to a number of possible factors including that: DNA gyrase is the sole topoisomerase II in *M. tuberculosis*; the peptidic QRDR in both the A and B subunits is unique as described [112]; and whereas the widely conserved Ser-83 is the key residue for interaction with quinolones in *E. coli*, the equivalent position in *M. tuberculosis* is Ala-90, all of which may affect the manner in which the mycobacterial enzyme interacts with quinolones.

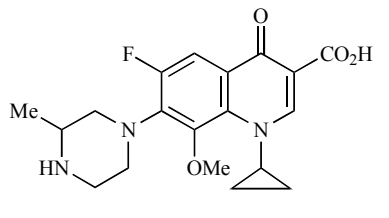
Due to these differences in the molecular targets in *M. tuberculosis* compared with other organisms, and potentially also to differences in cell wall structure and efflux systems, the anti-tuberculosis activity of quinolones correlates poorly with activities against other common pathogens, such as *S. aureus* or *E. coli* (Table **1**) [113]. As a consequence, the potency of quinolones against other organisms has little predictive value for their potency against *M. tuberculosis*. This poor predictability illustrates the need for a dedicated quinolone program that specifically targets *M. tuberculosis*. Thus, quinolones primarily targeting topoisomerase IV will be less effective against *M. tuberculosis*. In addition, the unique cell wall structure and efflux systems of *M. tuberculosis* probably add more layers of complexity.

A study has functionally characterized the *M. tuberculosis* DNA gyrase that is unique in being the only type II topoisomerase in this organism [114]. This study found that *M. tuberculosis* DNA gyrase supercoils DNA with an efficiency similar to that of other DNA gyrases, but more efficient in its relaxation, DNA cleavage, and decatenation activities. On the other hand, the *M. tuberculosis* DNA gyrase does not decatenate DNA as efficiently as a true topoisomerase IV [114]. The *M. tuberculosis* DNA gyrase was similar to other bacterial DNA gyrases in catalyzing ATP-independent DNA relaxation, but unlike topoisomerase IV and eukaryotic topoisomerase II that require ATP for relaxation, ATP inhibited DNA relaxation. This was interpreted to mean that turning the ATP binding site into a closed conformation that leads to the dimerization of the GyrB subunits hampers the reverse passive strand transfer required for relaxation [114]. The *M. tuberculosis* enzyme was much more efficient for the relaxation activity requiring twice the amount of enzyme needed for supercoiling whereas other DNA gyrases require 50 times more [115-117]. It also required less enzyme to cleave DNA than observed for other type II topoisomerases [115,117].

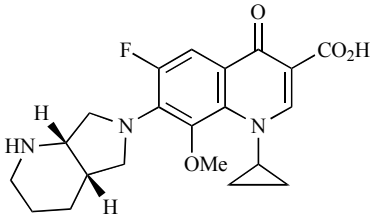
Another study has investigated the DNA-binding sites in the GyrA-CTD of *M. tuberculosis* gyrase through site-directed

Table 1. Comparison of Quinolone Compounds in Antibacterial Activities and Activities Against *M. tuberculosis* DNA Gyrase

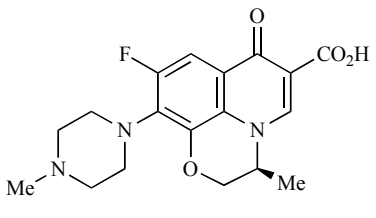
Quinolones	*S. aureus*	*E. coli*	*M. tuberculosis*	*M. tuberculosis* DNA gyrase	
	MIC (μg/ml)	MIC (μg/ml)	MIC (μg/ml)	IC_{50} (μg/ml)	CC_{50} (μg/ml)
Gatifloxacin	0.05	0.02	0.12	3	4
Moxifloxacin	0.03	0.015	0.5	4.5	4
Levofloxacin	0.12	0.008	0.5	5	12
Garenofloxacin	0.01	0.015	2	13	15
Gemifloxacin	0.01	0.13	4	11	6
Trovafloxacin	0.03	0.02	16	15	25



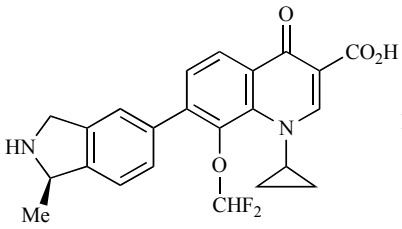
Gatifloxacin



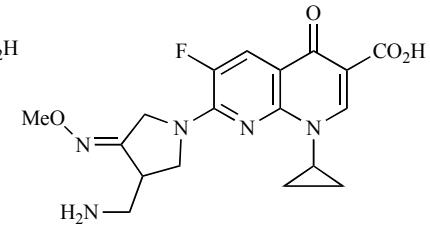
Moxifloxacin



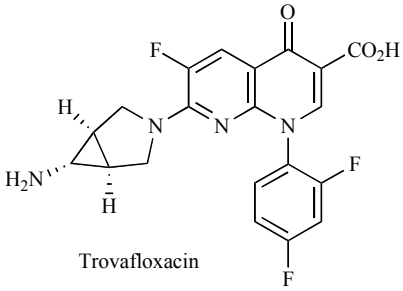
Levofloxacin



Garenofloxacin



Gemifloxacin



Trovafloxacin

mutagenesis [118]. This study identified Y577, R691 and R745 as being among the key DNA-binding residues in *M. tuberculosis* GyrA-CTD. It also showed that the third blade of the GyrA-CTD is the main DNA-binding region in *M. tuberculosis* DNA gyrase. The substitutions of Y577A, D669A, R691A, R745A and G729W led to the loss of supercoiling and relaxation activities, although they had a little effect on the drug-dependent DNA cleavage and decatenation activities, and had no effect on the ATPase activity. These findings showed that the GyrA-CTD is essential to DNA gyrase of *M. tuberculosis.* This was the first time that the DNA-binding sites in GyrA-CTD had been identified.

Another study investigated the enzymatic efficiency and inhibition by quinolones of *M. tuberculosis* GyrA carrying a mutation at position G88 [119]. G88 in *M. tuberculosis* GyrA corresponds to G81 in *E. coli*, and has been predicted to be involved in the quinolone binding site. This study clearly established that GyrA G88C and G88A mutations conferred resistance to fluoroquinolones in *M. tuberculosis* by decreasing gyrase inhibition. Interestingly, this study further found that quinolones that do not have either an R7 ring, or a fluorine in R6 had MICs for the G88A mutants that were similar to wild type, and the IC50s for the mutant enzymes with both the G88C and G88A were similar to the IC50 for the wild type enzyme. These results were consistent with the previous observation that a G81D mutation in the *E. coli* enzyme conferred resistance to ciprofloxacin but not to nalidixic acid [47], while mutations at positions 83, 84, and 87 conferred resistance to both compounds [78].

THE ROLE OF QUINOLONES IN TUBERCULOSIS

Fluoroquinolones have a broad-spectrum of activity that covers many important bacterial pathogens. Their use against respiratory pathogens has widely increased as various resistances have continued to develop against other antibiotics. Ofloxacin was the first fluoroquinolone to show activity against pulmonary tuberculosis [120]. Strains of *M. tuberculosis* have been isolated that are resistant to ofloxacin and ciprofloxacin [121]. It has also been recognized that other more effective quinolones such as sparfloxacin can block the development of resistant mutants [122,123], suggesting that more active fluoroquinolones could be designed that could have better activity against resistant organisms. C-8-methoxy fluoroquinolones (moxifloxacin and gatifloxacin) were introduced onto the market having been developed for use against a wide variety of pathogens. These agents exhibited excellent activity against *M. tuberculosis* [124].

Even though *M. tuberculosis* belongs in that select list of bacterial organisms whose genomes does not encode DNA topoisomerase IV, making DNA gyrase the only type II topoisomerase [125], and thus the sole target for fluoroquinolones, bacterial topoisomerases tend to be highly conserved even between various species, making it possible to transfer most of the knowledge that

has been learned from studies using the *E. coli* enzyme onto the understanding of the mechanisms of the *M. tuberculosis* enzyme. To that extent the expected DNA gyrase mechanistic properties have been confirmed with the mycobacterial enzyme [126,127]. Similarly, quinolone resistant mutations are found in a region of the protein homologous to the QRDR of the *E. coli* enzyme [128,129]. There is the obvious difference that the *M. tuberculosis* GyrA protein contains an alanine at the position homologous to serine 83 in the *E. coli* enzyme where a substitution to a hydrophobic residue leads to drug resistance as in S83W [130,131]. But the most common mutations in fluoroquinolone resistant *M. tuberculosis* clinical isolates are still due to substitutions at codons 90 and 94 of the *gyrA,* whose *E. coli* equivalents are also resistance determinants. Various alterations in the QRDR result in various levels of resistance, and similar to other pathogens, high levels of resistance develop in a stepwise manner as mutations accumulate in the QRDR [132]**Error! Bookmark not defined.**.

Fluoroquinolones demonstrate high potency against *M. tuberculosis* and the newer derivatives like sparfloxacin, gatifloxacin and moxifloxacin are the most active [133-139]. They are also able to penetrate the mammalian host cell and exert their antimycobacterial effects inside infected macrophages [140,141], and their activity can be enhanced by combining them with the first line antituberculosis drugs [142]. These compounds have also demonstrated exquisite *in vivo* mycobactericidal activity that for gatifloxacin and moxifloxacin is comparable to that of isoniazid [143]. A number of clinical trials have been conducted with various fluoroquinolones of various potencies, in various drug combinations on various populations that have proven difficult to evaluate in a comparative manner (reviewed in [144]). Observations from such studies have resulted in the recommendation that fluoroquinolones be used as second line drugs for the treatment of multidrug-resistant tuberculosis by the WHO [145].

Moxifloxacin and gatifloxacin are two of the newer 8-methoxyquinolones with high bactericidal activity against both replicating [146] and non-replicating [147] *M. tuberculosis*, and favorable pharmacokinetics in humans [148-153] that have been prioritized for concerted clinical evaluation for the treatment of both drug susceptible and drug resistant tuberculosis. They are effective against quinolone-resistant clinical isolates [154], and the enhanced bactericidal activity of moxifloxacin in the mouse model at high doses indicated that if used in proper combinations with other drugs it might shorten the duration of therapy, or allow once weekly dosing [155]. Follow-up studies with various drug combinations show that a combination of moxifloxacin, rifampin and pyrazinamide reduced the time needed to eradicate *M. tuberculosis* from the lungs of infected mice by up to 2 months when compared with the standard regimen of isoniazid, rifampin, and pyrazinamide, further confirming the hypothesis that such a regimen could potentially shorten the duration of therapy needed to cure human tuberculosis [156]. A number of clinical trials are currently ongoing to evaluate the multidrug usage of both moxifloxacin and gatifloxacin. Results from one such study that compared the substitution of moxifloxacin for ethambutol with the standard regimen shows that while moxifloxacin use did not affect the 2 month sputum culture status, it did show increased activity at earlier time points [157].

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 [158] 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 [159-161]. A number of preclinical and clinical studies have evaluated the utility of quinolones against tuberculosis including preclinical combinations with other drugs [162-164], effects of the C-8 substituents on activity [165,166], activity on non-replicating organisms [167] and organisms in macrophages, efficacy in murine models of infection [168,169], dosage and frequency of dosages, sterilizing activities, efficacy in pulmonary disease [170,171], and clinical combinations with other first line TB medications [172,173]. The incidence of mycobacterial resistance to fluoroquinolones is currently relatively low, and there has been no cross-resistance observed with other anti-tuberculosis drugs. But with the high infection rates with tuberculosis globally, the increasing rates of multidrug-resistant tuberculosis (MDR-TB), the high rate of co-infection with HIV, and the recent surge in extremely drug-resistant TB (XDR-TB), there is an urgent need for newer and more effective, non-toxic anti-tuberculosis agents. Fluoroquinolones have become an obvious addition to the drug armamentarium against tuberculosis and they are currently being used in cases of MDR-TB. For the same reason a number of investigators are actively pursuing newer and more effective fluoroquinolones that are optimized against the *M. tuberculosis* enzyme, and may be effective against fluoroquinolone-resistant clinical isolates of *M. tuberculosis.*

Numerous efforts are aimed at improving the efficacy against tuberculosis of the already fairly potent fluoroquinolones. One such study that sought to identify better and more efficacious gatifloxacin by introducing lipophilicity at position #7 in an attempt to increase drug uptake by the organism [174], identified some gatifloxacin analogs that were more active than gatifloxacin against drug sensitive and drug resistant bacteria. Improving fluoroquinolone activity by developing more effective analogues has introduced a number of novel scaffolds including, for example, the 3-aminoquinazolinediones [175-177], and the isothiazolopyridones [178-181].

The TB Alliance has a collaborative program with Korea Research Institute of Chemical Technology (KRICT) aimed at identifying a quinolone that is optimized and dedicated to the treatment of tuberculosis. Several members of this drug class have also shown favorable long-term safety in the treatment of tuberculosis, even though the quinolones as a class had not been extensively optimized for a TB indication before. The objective of the quinolone project is to develop a new generation of DNA gyrase inhibitors that will be more effective in shortening TB therapy than moxifloxacin, while maintaining an excellent safety and tolerability profile. The new agents should also be suitable for the treatment of MDR-TB and TB/HIV co-infections without prohibitive drug-drug interactions with anti-retrovirals (ARVs). 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. Anti-tuberculosis agents

developed in such a fashion are not optimized for shortening the duration of therapy. In this program, new discovery platforms utilizing a series of *in vitro* and *in vivo* models based on non-replicating *M. tuberculosis* are being used routinely to evaluate new compounds for their potential for shortening the duration of therapy. Since some members of the quinolone family have been associated with certain toxicities and adverse events, a discovery program for an anti-tuberculosis quinolone must continuously monitor prioritized compounds for toxicity to ensure safety as a long-term agent (2 months or longer). The most important safety concerns for the quinolone class are genotoxicity, phototoxicity, QT prolongation and drug-drug interactions, each of which can be routinely monitored.

KRQ-10018 was the initial lead compound that was identified by the TB Alliance-KRICT program (ICAAC poster, 2006, San Francisco[1]) with the support of the Tuberculosis Antimicrobial Acquisition and Coordination Facility (www.taacf.org) that is funded by the National Institute of Allergy and Infectious Diseases. This compound has been evaluated for potency against replicating *M. tuberculosis*, non-replicating bacteria, organisms in human macrophages, and in a mouse infection model. KRQ-10018 is more potent than other fluoroquinolones, including moxifloxacin against *M. tuberculosis*, with an MIC of 0.05 μg/ml compared to 0.25 μg/ml for moxifloxacin. Preliminary data indicated that at a 10 μg/ml concentration, KRQ-10018 was similar to moxifloxacin and rifampin in its activity against *M. tuberculosis* in the non-replicating state induced under anaerobic conditions. KRQ-10018 demonstrated better activity than moxifloxacin against *M. tuberculosis* grown intracellularly in macrophages and was less active than moxifloxacin in an *in vivo* efficacy mouse model. Other members of this group of compounds are 6-10 fold more potent than moxifloxacin against *M. tuberculosis* under anaerobic conditions and in macrophages, and taken together, this preliminary data suggests that this series has great potential to shorten the duration of therapy.

DC-159a, a new fluoroquinolone from Daiichi-Sankyo that is being developed for respiratory infections, exhibited excellent activities against *M. tuberculosis* and non-tuberculous mycobacteria, and had activities against quinolone-resistant-MDR-TB (QR-MDR-TB) isolates that were better than both moxifloxacin and gatifloxacin and also better than rifampicin [182]. The MIC90s of DC-159a against QR-MDR-TB isolates were 8-fold lower than the MIC90 of moxifloxacin. In a study that compared the efficacy of DC-159a with that of the other fluoroquinolones in a mouse QR-MDR-TB infection model, the mean survival days of DC-159a treated animals was higher than for animals treated with moxifloxacin, levofloxacin, isoniazid or rifampicin [183]. In a study with a drug-susceptible *M. tuberculosis* infection model, DC-159a at 50mg/kg had an equivalent efficacy to that of moxifloxacin at 100mg/kg. In rats and monkeys DC- 159a was well tolerated with an acceptable safety profile showing no phototoxicity or chondrotoxicity [184].

Various efforts are on-going in a number of laboratories to identify potent inhibitors of bacterial DNA gyrase that may become antibacterial drugs of the future. Most of these efforts are not directed towards *M. tuberculosis,* but the example of the fluoroquinolones suggests that such drugs could either be used directly in tuberculosis care, or they can be further optimized against the *M. tuberculosis* enzyme to derive sufficient potency against the organism.

Albicidin, a polyketide-peptide from *Xanthomonas albilineans* is a potent inhibitor of the supercoiling activity of bacterial and plant DNA gyrases, with IC50s of 40 to 50 nM, more potent than those of most coumarins and quinolones [185]. The mechanism of action of this natural compound seems to be the blockage of the religation of the cleaved DNA intermediate during the gyrase catalytic sequence and the inhibition of the relaxation of supercoiled DNA by gyrase and topoisomerase IV. Unlike the coumarins, albicidin does not inhibit the ATPase activity of gyrase. In contrast to the quinolones, the albicidin concentration required to stabilize the gyrase cleavage complex increases 100-fold in the absence of ATP. Some mutations in *gyrA*, known to confer high-level resistance to quinolones or CcdB, confer low-level resistance or hypersensitivity to albicidin in *E. coli*. The novel features of the gyrase-albicidin interaction indicate the potential for the development of new antibacterial drugs.

Taking advantage of the available detailed 3D structural information of the targeted ATP binding site, a study that combined an in silico screening for potential low molecular weight inhibitors, a focused high throughput DNA gyrase screen, validation of the screening hits by biophysical methods, and a 3D guided optimization process, identified a new class of GyrB inhibitors described as indazoles that could be validated as true, novel DNA gyrase inhibitors that act by binding to the ATP binding site located on subunit B [186,187]. The 3D guided optimization provided a highly potent 3,4-disubstituted indazole as being 10 times more potent at DNA gyrase inhibition than novobiocin.

Simocyclinones are a class of new antibiotics that were isolated from *Streptomyces antibioticus* Tu 6040 [188-190]. Their structures include an aminocoumarin moiety, a key feature of novobiocin, coumermycin A_1, and clorobiocin, which also target gyrase. Simocyclinones behave strikingly differently from these compounds. A recent study has characterized the interaction of simocyclinones with bacterial DNA gyrase [191]. This study found that simocyclinone D8 is a potent inhibitor of gyrase supercoiling with a 50% inhibitory concentration lower than that of novobiocin. However, it did not competitively inhibit the DNA-independent ATPase reaction of GyrB, which is a suspected mode of action for other aminocoumarins. Simocyclinone D8 also inhibited DNA relaxation by gyrase but did not stimulate cleavage complex formation, unlike quinolones. Instead, simocyclinones seem to abrogate both Ca(2+)- and quinolone-induced cleavage complex formation. Binding studies suggested that simocyclinone D8 interacts with the N-terminal domain of GyrA. Taken together, these findings demonstrate that simocyclinones inhibit an early step of the gyrase catalytic cycle by preventing binding of the enzyme to DNA. This is a novel mechanism for a gyrase inhibitor and presents new possibilities for antibacterial drug development.

A strategy used to optimize a novel series of aminobenzimidazoles that inhibit the ATPase activity of bacterial DNA gyrase and topoisomerase IV has identified two compounds, VRT-125853 and VRT-752586 [192]. Both compounds inhibited the maintenance of negative supercoils in plasmid DNA in Escherichia coli, and their primary target was revealed to be GyrB in both *Staphylococcus aureus* and *Haemophilus influenzae*, but ParE in *Streptococcus pneumoniae*. In *Enterococcus faecalis*, the primary target of VRT-125853 was ParE, whereas for VRT-752586 it was GyrB. *gyrB* and *parE* double mutations increased the MICs of VRT-125853 and VRT-752586 significantly, providing evidence of dual targeting.

A whole cell screen to identify inhibitors of chromosome partitioning in *E. coli* against a random compound library identified a new class of DNA gyrase inhibitors, the pyrazoles [193,194]. These inhibitors were similarly active against fluoroquinolone-sensitive and resistant bacteria even though they had weak antibacterial activity due to poor enzyme inhibition.

Using the above mentioned crystal structure of the 43 kDa N-terminal fragment of GyrB in complex with the nonhydrolyzable

[1] 46th International Conference on Antimicrobial Agents and Chemotherapy. Abstract F1372: Preclinical Testing of the Quinolone KRQ-10018 for Activity against *Mycobacterium tuberculosis* in a Series of In Vitro and In Vivo models, A. J. Lenaerts, V. Gruppo, K. S. Marietta, C. M. Johnson, I. M. Orme.

form of ATP, ADPNP, as the starting point, an in silico-based fragment drug design identified two low-molecular weight fragments, 2-aminobenzimidazole and indolin-2-one [195]. Subsequent in silico optimization of the indolin-2-one led to the discovery of potent compound, HTS05063 that inhibited the DNA gyrase supercoiling activity.

CONCLUSIONS

The increasing incidence of MDR-TB and the recently reported XDR-TB poses dangerous public health problems around the world, and new pharmaceutical agents are needed to control tuberculosis. DNA gyrase, the only Type II topoisomerase and sole target for quinolones in *M. tuberculosis*, is a validated, and pharmaceutically effective target for drug discovery against this important pathogen. Fluoroquinolones are a superb class of compounds with good antimicrobial and pharmacokinetic properties against many bacterial pathogens, and even though they are relatively less effective against mycobacteria, newer fluoroquinolones like moxifloxacin have demonstrated good activity against *M. tuberculosis,* and have found use in treating drug resistant cases. Useful SAR is developing when comparing the older, less effective analogs with the newer, more effective fluoroquinolones and the much studied *E. coli* DNA gyrase with the enzyme from *M. tuberculosis,* revealing a potential to develop new fluoroquinolones with enhanced activity against tuberculosis, and the ability to minimize resistance developed against the existing drugs. Various groups are dedicating much effort to the search for better fluoroquinolones and related compounds that would be more effective than the current drugs, but not extend cross-resistance to existing fluoroquinolones. The ATPase activity of bacterial DNA gyrase that resides in the B subunit remains pharmaceutically under-exploited even though it is an effective target of coumarins and related compounds. A lot of effort is also being diverted to that part of the molecule by various groups with encouraging results.

Importantly, most of these efforts are not directed towards tuberculosis, and utility of these new compounds for tuberculosis care would have to be added onto the seemingly successful efforts by either directly testing against the whole organism, or attaching lead optimization efforts that build in the specificity against the *M. tuberculosis* gyrase and the whole organism.

ABBREVIATIONS

DNA	=	Deoxyribonucleic acid
RNA	=	Ribonucleic acid
ATP	=	Adenosine triphosphate
MDR-TB	=	multiple drug resistant tuberculosis
XDR-TB	=	Extremely/extensively drug resistant tuberculosis
QR-MDR-TB	=	Quinolone resistance- multiple drug resistant tuberculosis
TB-HIV	=	Tuberculosis/Human immunodeficiency virus
ADPNP	=	5'-adenylyl β,γ-imidodiphosphate
GyrA-NTD	=	N-terminal domain of DNA gyrase subunit A
GyrA-CTD	=	C- terminal domain of DNA gyrase subunit A
QRDR	=	Quinolone resistance determining region
ARV	=	Anti-retroviral
SAR	=	Structure/Activity relationship
SDS	=	Sodium dodecyl sulphate

REFERENCES

[1] Payne. D. J.; Gwynn, M. N.; Holmes, D. J.; Pompliano, D. L.; *Nat Rev Drug Discov*. **2007**, *6*, 29.

[2] Ferrero, L.; Cameron, B.; Manse, B.; Lagneaux, D.; Crouzet, J.; Famechon, A.; Blanche, F. *Mol. Microbiol.* **1994,** *13*, 641.

[3] Miyamoto, T.; Matsumoto, J.; Chiba, K.; Egawa, H.; Shibamori, K.; Minamida, A.; Nishimura, Y.; Okada, H.; Kataoka, M.; Fujita, M.; Hirose T.; Nakano, J. *J. Med. Chem.* **1990,** *33*, 1645.

[4] Maxwell, A. *Mol. Microbiol.* **1993,** *9*, 681.

[5] Drlica, K.; Malik, M.*Curr. Top. Med. Chem.* **2003,** *3*, 249.

[6] Crofton, J.; Chaulet, P.; Maher**,** D. Report WHO/TB/96.210. **1997.** World Health Organization, Geneva, Switzerland

[7] Ji, B.; Lounis, N.; Truffot-Pernot, C.; Bonnafous, P.; Grosset, J. *Antimicrob. Agents Chemother.* **1998***, 42*, 2066.

[8] Nuermberger, E.L.; Yoshimatsu, T.; Tyagi, S.; Williams, K.; Rosenthal, I.; O'Brien, R. J.; Vernon, A. A.; Chaisson, R. E.; Bishai, W. R.; Grosset, J. H. *Am. J. Respir. Crit. Care Med.* **2004,** *170*, 1131.

[9] Aubry, A.; Pan, X.-S.; Fisher, L. M.; Jarlier, V.; Cambau, E. *Antimicrob. Agents Chemother.* **2004,** *48*,1281.

[10] Guillemin I.; Jarlier, V.; Cambau, E**.** *Antimicrob. Agents Chemother.* **1998,** *42*, 2084.

[11] Sullivan, E. A.; Kreiswirth, B. N.; Palumbo, L.; Kapur, V. Musser JM, Ebrahimzadeh A, Frieden TR. City *Lancet* **1995,** *345*, 1148.

[12] Takiff, H. E.; Salazar, L.; Guerrero, C.; Philipp, W.; Huang, W. M.; Kreiswirth, B.; Cole, S. T.; Jacobs, W. R., Jr.; Telenti, A. *Antimicrob. Agents Chemother.* **1994,** *38,* 773.

[13] Cambau, E.; Sougakoff, W.; Besson, M.; Truffot-Pernot, C.; Grosset, J.; Jarlier, V. *J. Infect. Dis.* **1994,** *170*, 479.

[14] Aubry, A.; Pan, X. S.; Fisher, L. M.; Jarlier, V.; Cambau, E. *Antimicrob Agents Chemother*. **2004,** *48,* 1281.

[15] Holmes, V. F.; Cozzarelli, N. R. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 1322.

[16] Sawitzke, J. A. Austin S. *Proc. Natl. Acad. Sci. USA* **2000** , *97*, 1671.

[17] Levine, C.; Hiasa, H.; Marians, K. J. *Biochim. Biophys. Acta* **1998**, *1400*, 29.

[18] Hiasa, H.; Marians, K. J. *J. Biol. Chem.* **1996**, *271*, 31529.

[19] Ullsperger, C.; Cozzarelli N. R. *J. Biol. Chem.* **1996**, *271*, 31549.

[20] Hiasa,.H.; Marians, K. J. *J. Biol. Chem.* **1994**, *269*, 32655

[21] DiGate, R. J.; Marians, K. J. *J. Biol. Chem.* **1988**, *263*, 13366.

[22] Hiasa, H.; DiGate, R. J.; Marians, K. J. *J. Biol. Chem.* **1994,** *264*, 2093.

[23] Champoux, J. J. *Annu. Rev. Biochem.* **2001**, *70*, 369.

[24] Bjergbaek, L.; Kingma, P.; Nielsen, I. S.; Wang, Y.; Westergaard, O.; Osheroff, N, and Andersen, A. H. *J. Biol. Chem.* **2000**, *275*, 13041.

[25] Wang, J. C. *Q. Rev. Biophys*. **1998,** *31*, 107.

[26] Kampranis, S. C.; Maxwell, A. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 14416.

[27] Reece, R. J.; Maxwell, A. *Nucleic Acids Res*. **1991**, *19,* 405.

[28] Drlica, K.; Zhao X. *Microbiol, and Mol. Biol. Reviews.* **1997**, *61*, 377.

[29] Schoeffler, A. J.; Berger, J. M. *Biochemical Society Transactios*. **2005**, *33*, 1464.

[30] Reece, R.; Maxwell, A. *Crit. Rev. Biochem. Mol Biol*. **1991**, *26*, 335.

[31] Roca, J. *Trends Biochem. Sci*. **1995**, *20*, 156.

[32] Critchlow, S. E.; Maxwell, A. *Biochem.* **1996** , *35*, 7387.

[33] Reece, R. J.; Maxwell, A. *J. Biol. Chem.* **1989**, *264*, 19648.

[34] Morais Cabral, J. H.; Jackson, A. P.; Smith, C. V.; Shikotra, N.; Maxwell, A.; Liddington, R.C..*Nature*. **1997**, *388* (6645), 903.

[35] Reece, R. J.; Maxwell A. *J Biol Chem*. **1989**, *264*, 19648.

[36] Reece, R. J.; Maxwell A. *J Biol Chem*. **1991**, *266*, 3540.

[37] Liu, L. F.; Wang, J. C. *Proc Natl Acad Sci U S A*. **1978** , *75*, 2098.

[38] Liu, L. F.;. Wang, J. C. *Cell*. **1978**, *15*(3), 979.

[39] Brown, P. O.; Cozzarelli N. R. *Science*. **1979**, *206*, 1081.

[40] Kampranis, S. C.; Bates, A. D.; Maxwell, A. *Proc Natl Acad Sci* U S A. **1999** , *96*, 8414.

[41] Corbett, K. D.; Shultzaberger, R. K.; Berger, J. M. *Proc Natl Acad Sci* U S A. **2004** , *101*, 7293.

[42] Ruthenburg, A. J.; Graybosch, D. M.; Huetsch, J. C.; Verdine, G. L. *J Biol Chem*. **2005**, *280*, 26177.

[43] Costenaro, L.; Grossmann, J. G.; Ebel, C.; Maxwell, A. *Structure*. **2005** , *13*, 287.

[44] Everett, M. J.; Jin, Y. F.; Ricci, V.; Piddock L J. *Antimicrob Agents Chemother*. **1996** , *40*, 2380.

[45] Vila, J.; Ruiz, J.; Marco, F.; Barcelo, A.; Goni, P.; Giralt, E.; Jimenez de Anta, T. *Antimicrob Agents Chemother*. **1994** , *38*, 2477.

[46] Yoshida, H.; Bogaki, M.; Nakamura, M.; Nakamura, S. *Antimicrob Agents Chemother*. **1990** , *34*, 1271.

[47] Cambau, E.; Bordon, F.; Collatz, E.; Gutmann, L. *Antimicrob Agents Chemother*. **1993** , *37*, 1247.

[48] Hallett, P.; Maxwell, A. *Antimicrob Agents Chemother*. **1991**, *35*, 335.

[49] Oram. M.; Fisher, L. M. *Antimicrob Agents Chemother*. **1991,** *35*, 387.

[50] Tavio, M. M.; Vila, J.; Ruiz, J.; Ruiz, J.; Martin-Sanchez, A. M.;, Jimenez de Anta, M. T. *J Antimicrob Chemother*. **1999**, *44*, 735.

[51] Truong, Q. C.; Nguyen Van, J. C.; Shlaes, D.; Gutmann, L; Moreau, N. J..*Antimicrob Agents Chemother*. **1997**, *41*, 85.

[52] Yoshida, H.; Kojima, T.; Yamagishi, J.; Nakamura, S. *Mol Gen Genet*. **1988**, *211*, 1.

[53] Ruiz, J.; Gomez, J.; Navia, M. M.; Ribera, A.; Sierra, J. M.; Marco, F.; Mensa, J.; Vila, J. *Diagn Microbiol Infect Dis*. **2002**, *42*, 257.

[54] Adachi, T.; Mizuuchi, M.; Robinson, E. A.; Appella, E.; O'dea, M. H.; Gellert, M.; Mizuuchi, K.; *Nucleic Aids Res* **1987***, 15*, 771.

[55] Brino, L.; Urzhumtsev, A.; Mousli, M.; Bronner, C.; Mitschler, A.; Oudet, P.; Moras, D. *J Biol Chem*. **2000**, *275*, 9468.

[56] Williams, N. L.; Maxwell, A. *Biochemistry*. **1999**, *38*, 13502.
[57] Williams, N. L.; Howells, A. J.; Maxwell, A. *J Mol Biol*. **2001**, *306*, 969.
[58] Kampranis, S. C.; Bates, A. D.; Maxwell, A. *Proc Natl Acad Sci USA*. **1999**, *96*, 8414.
[59] Tingey, A. P.; Maxwell, A. *Nucleic Acids Res.* **1996**, *24*, 4868.
[60] Smith, C. V.; Maxwell, A. *Biochemistry*. **1998,** *37*, 9658.
[61] Hu, T.; Chang, S.; Hsieh, T. *J Biol Chem*. **1998**, *273*, 9586.
[62] Lee, M. P.; Hsieh, T. S. *J Mol Biol*. **1994**, *235*, 436.
[63] Li, W.; Wang, J. C. *J Biol Chem*. **1997**, *272*, 31190.
[64] Tamura, J. K.; Gellert, M. *J Biol Chem*. **1990,** *265*, 21342.
[65] Lewis, R. J.; Singh, O. M.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; Wonacott,, A. J.; Wigley, D. B. *EMBO J.* **1996**, *15*, 1412.
[66] Tsai, F. T.; Singh, O. M.; Skarzynski, T.; Wonacott, A. J.; Weston, S.; Tucker, A.; Pauptit, R. A.; Breeze, A. L, Poyser, J. P.; O'Brien, R.; Ladbury, J. E.; Wigley, D. B. *Proteins*. **1997**, *28*, 41.
[67] Lamour, V.; Hoermann, L.; Jeltsch, J. M.; Oudet, P.; Moras, D. *J Biol Chem*. **2002**, *277*, 18947.
[68] Hoeksema, H.; Johnson, J. L.; Hinman, J. W. *J. Am. Chem. Soc.* **1955**, *78*, 6710.
[69] Gellert, M.; O'Dea, M. H.; Itoh, T.; Tomizawa, J. *Proc Natl Acad Sci* U S A. **1976**, *73*, 4474-8.
[70] Sugino, A.; Higgins, N. P.; Brown, P. O.; Peebles, C. L.; Cozzarelli, N. R. *Proc Natl Acad Sci* U S A. **1978**, *75*, 4838.
[71] Sugino, A.; Cozzarelli, N. R.; *J Biol Chem*. **1980**, *255*, 6299.
[72] Staudenbauer, W. L.; Orr, E. *Nucleic Acids Res* **1981**, *9*, 3589.
[73] Maxwell, A.; Gellert, M. *J Biol Chem*. **1984**, *259*, 14472.
[74] Ali, J. A.; Jackson, A. P.; Howells, A. J.; Maxwell, A. *Biochemistry*. **1993**, *32*, 2717.
[75] del Castillo, I.; Vizan, J. L.; Rodriguez-Sainz, M. C.; Moreno, F. *Proc Natl Acad Sci* U S A. **1991,** *88*, 8860.
[76] Holmes, M. L.; Dyall-Smith, M. L. *J Bacteriol*. **1991,** *173*, 642.
[77] Yoshida, H.; Bogaki, M.; Nakamura, M.; Yamanaka, L. M.; Nakamura, S. *Antimicrob Agents Chemother*. **1991**, *35*, 1647.
[78] Ruiz, J. *J Antimicrob Chemother*. **2003,** *51*, 1109.
[79] Drlica, K.; Coughlin, S. *Pharmacol. Ther*. **1989**, *44*, 107.
[80] Radl, S. *Pharmacol. Ther*. **1990**, *48*, 1.
[81] Reece, R. J.; Maxwell, A. *CRC Crit. Rev. Biochem. Mol Biol*. **1991**, *26*, 335.
[82] Maxwell, A. *Mol. Microbiol*. **1993**, *9*, 681.
[83] Mizuuchi, K.; O'Dea, M. H.; Gellert, M. *Proc. Natl. Acad. Sci. USA*. **1978**, *75*, 5960.
[84] Liu, L. F. *Annu. Rev. Biochem*. **1989**, *58*, 351.
[85] Corbett, A. H.; Osheroff, N. *Chem. Res. Toxicol*. **1993**, *6*, 585.
[86] Gellert, M.; Mizuuchi, K.; O'Dea, M. H.; Itoh, T.; Tomizawa, J. *Proc. Natl. Acad. Sci*. USA **1977**, *74*, 4772.
[87] Sugino, A.; Peebles, C. L.; Kruezer, K. N.; Cozzarelli, N. R. *Proc. Natl. Acad. Sci*. USA. **1977**, *74*, 4767.
[88] Tse, Y. C.; Kirkegaard, K.; Wang, J. C. *J Biol. Chem*. **1980**, *255*, 5560.
[89] Chen, C. R.; Malik, M.; Snyder, M.; Drlica, K. *J Mol. Biol*. **1996**, *258*, 627.
[90] Krasin, F.; Hutchinson, F. *J. Mol. Biol*. **1977**, *116*, 81.
[91] Mouton, R. P.; Koelman, A. *Chemotherapy* **1966**, *11*, 10.
[92] Gradelski, E.; Kolek, B.; Bonner, D. P.; Valera, L.; Minassian, B.; Fung-Tomc, J. *Int. J. Antimicrob. Agents*. **2001**, *17*, 103.
[93] Shen, L. L.; Mitscher, L. A.; Sharma, P.N.; O'Donnell, T. J.; Chu, D. W.; Cooper, C. S.; Rosen, T.; Pernet, A. G. *Biochemistry*. **1989**, *28*, 3886.
[94] Elsea, S. H.; Osheroff, N.; Nitiss, J. L. *J. Biol. Chem*. **1992**, *267*, 13150.
[95] Shen, L. L.; Pernet, A. G. *Proc. Natl. Acad. Sci*. USA. **1985**, *82*, 307.
[96] Shen, L. L. *Methods Mol. Biol*. **2001**, *95*, 171.
[97] Mitscher, L. A. *Chem Rev*. **2005**, *105*, 559.
[98] Maxwell, A.; Lawson, D. M. *Curr Top Med Chem*. **2003,** *3*, 283.
[99] Gormley, N. A.; Orphanides, G.; Meyer, A.; Cullis, P. M.; Maxwell, A. *Biochemistry*. **1996**, *35*, 5083.
[100] Holdgate, G. A.; Tunnicliffe, A.; Ward, W. H.; Weston, S. A.; Rosenbrock, G.; Barth, P. T.; Taylor, I. W.; Pauptit, R. A.; Timms, D. *Biochemistry*. **1997**, *36*, 9663.
[101] Lewis, R. J.; Singh, O. M.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B. *EMBO J.* **1996**, *15*, 1412.
[102] Takiff, H. E.; Salazar, L.; Guerrero, C.; Philipp, W.; Huang, W. M.; Kreiswirth, B.; Cole, S. T.; Jacobs, WR Jr.; Telenti, A. *Antimicrob Agents Chemother*. **1994**, *38*, 773.
[103] Madhusudan, K.; Ramesh, V.; Nagaraja, V. *Biochem Mol Biol Int.* **1994**, *33*, 651.
[104] Goswitz, J. J.; Willard, K. E.; Fasching, C. E.; Peterson, L R. *Antimicrob Agents Chemother*. **1992**, *36*, 1166.
[105] Hopewell, R.; Oram, M.; Briesewitz, R.; Fisher, L M. *J Bacteriol*. **1990**, *172*, 3481.
[106] Sreedharan, S.; Oram, M.; Jensen, B.; Peterson, L. R.; Fisher, L. M. *J Bacteriol*. **1990**, *172*, 7260.
[107] Wang, Y.; Huang, W. M.; Taylor, D. E.; *Antimicrob Agents Chemother*. **1993**, *37*, 457.
[108] Yoshida, H.; Bogaki, M.; Nakamura, M.; Nakamura, S. *Antimicrob Agents Chemother*. **1990**, *34*, 1271.
[109] Kocagoz, T.; Hackbarth, C. J.; Unsal, I.; Rosenberg, E. Y.; Nikaido, H.; Chambers, H. F. *Antimicrob Agents Chemother*. **1996**, *40*, 1768.
[110] Aubry, A.; Veziris, N.; Cambau, E.; Truffot-Pernot, C.; Jarlier, V.; Fisher, L. M. *Antimicrob Agents Chemother.* **2006**, *50*, 104.
[111] Unniraman, S.; Chatterji, M.; Nagaraja, V. *J Bacteriol*. **2002**, *184*, 5449.
[112] Guillemin, I.; Jarlier, V.; Cambau, E. *Antimicrob Agents Chemother*. **1998**, *42*, 2084.
[113] Ma, Z.; Ginsberg, A.; Spigelman, M. Antimycobacteriun Agents in Comprehensive Medicinal Chemistry II. Elsevier: Oxford, 2007.
[114] Aubry, A.; Fisher, L. M.; Jarlier, V.; Cambau, E. *Biochem Biophys Res Commun*. **2006,** *348*(1), 158.
[115] Levine, C.; Hiasa, H.; Marians, K. J. *Biochim Biophys Acta*. **1998**, *1400*, 29.
[116] Blanche, F.; Cameron, B.; Bernard, F. X.; Maton, L.; Manse, B.; Ferrero, L.; Ratet, N.; Lecoq, C.; Goniot, A.; Bisch, D.; Crouzet, J. *Antimicrob Agents Chemother*. **1996**, *40*, 2714.
[117] Pan, X. S.; Fisher, L. M. *Antimicrob Agents Chemother*. **1999**, *43*, 1129.
[118] Huang, Y. Y.; Deng, J. Y.; Gu, J.; Zhang, Z. P.; Maxwell, A.; Bi, L. J.; Chen, Y. Y.; Zhou, Y. F.; Yu, Z. N.; Zhang, X. E. *Nucleic Acids Res*. **2006**, *34*, 5650.
[119] Matrat, S.; Veziris, N.; Mayer, C.; Jarlier, V.; Truffot-Pernot, C.; Camuset, J.; Bouvet, E.; Cambau, E.; Aubry, A. *Antimicrob Agents Chemother*. **2006**, *50*, 4170.
[120] Tsukamura, M. *Am Rev Respir Dis*. **1985**, 132, 915.
[121] Sullivan, E. A.; Kreiswirth, B. N.; Palumbo, L.; Kapur, V.; Musser, J. M.; Ebrahimzadeh, A.; Frieden, T. R.*Lancet*. **1995**, *345*, 1148.
[122] Xu, C.; Kreiswirth, B. N.; Sreevatsan, S.; Musser, J. M.; Drlica, K. *J Infect Dis*. **1996**, 174, 1127. Erratum in: J Infect Dis **1997**, *175*,1027.
[123] Dong, Y.; Xu, C.; Zhao, X.; Domagala, J.; Drlica, K. *Antimicrob Agents Chemother*. **1998,** *42*, 2978.
[124] Dong, Y.; Zhao, X.; Kreiswirth, B. N.; Drlica, K. *Antimicrob Agents Chemother*. **2000**, *44*, 2581.
[125] Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry, C. E 3rd.; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davies, R.; Devlin, K.;, Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Krogh, A.; McLean, J.; Moule, S.; Murphy, L.; Oliver, K.; Osborne, J.; Quail, M. A.; Rajandream, M. A.; Rogers, J.; Rutter, S.; Seeger, K.; Skelton, J.; Squares, R.; Squares, S.; Sulston, J. E.; Taylor, K.; Whitehead, S.; Barrell, B. G. *Nature*. **1998,** *393*, 537.
[126] Drlica, K.; Xu, C.; Wang, J. Y.; Burger, R. M.; Malik, M. *Antimicrob Agents Chemother*. **1996**, *40*, 1594.
[127] Onodera, Y.; Tanaka, M.; Sato, K.; *J Antimicrob Chemother*. **2001**, *47*, 447.
[128] Takiff, H. E.; Salazar, L.; Guerrero, C.; Philipp, W.; Huang, W. M.; Kreiswirth, B.; Cole, S. T.; Jacobs W. R. Jr.; Telenti, A. *Antimicrob Agents Chemother*. **1994,** *38*, 773.
[129] Zhou, J.; Dong, Y.; Zhao, X.; Lee, S.; Amin, A.; Ramaswamy, S.; Domagala, J.; Musser, J. M.; Drlica, K. *J Infect Dis*. **2000**, *182*, 517.
[130] Guillemin, I.; Jarlier, V.; Cambau, E. *Antimicrob Agents Chemother*. **1998,** *42*, 2084.
[131] Guillemin, I.; Sougakoff, W.; Cambau, E.; Revel-Viravau, V.; Moreau, N.; Jarlier, V..*Microbiology*. **1999**, *145*, 2527.
[132] Kocagoz, T.; Hackbarth, C. J.; Unsal, I.; Rosenberg, E. Y.; Nikaido, H.; Chambers, H. F. *Antimicrob Agents Chemother*. **1996**, *40*, 1768.
[133] Ji, B.; Lounis, N.; Maslo, C.; Truffot-Pernot, C.; Bonnafous, P.; Grosset, J. *Antimicrob Agents Chemother*. **1998**, *42*, 2066.
[134] Ji, B.; Lounis, N.; Truffot-Pernot, C.; Grosset. J. *Antimicrob Agents Chemother*. **1995**, *39*(6), 1341.
[135] Tomioka, H.; Sato, K.; Akaki, T.; Kajitani, H.; Kawahara, S.; Sakatani, M. *Antimicrob Agents Chemother*. **1999,** *43*, 3001.
[136] Ruiz-Serrano, M. J.; Alcala, L.; Martinez, L.; Diaz, M.; Marin, M.; Gonzalez-Abad, M. J.; Bouza, E. *Antimicrob Agents Chemother*. **2000**, *44*, 2567.
[137] Tomioka, H.; Sato, K.; Kajitani, H.; Akaki, T.; Shishido, S. *Antimicrob Agents Chemother*. **2000**, *44*, 283.
[138] Rodriguez, J. C.; Ruiz, M.; Lopez, M.; Royo, G. *Int J Antimicrob Agents*. **2002**, *20*, 464.
[139] Truffot-Pernot, C.; Ji, B.; Grosset, J. *Tubercle*. **1991**, *72*, 57-64.
[140] Van der Auwera, P.; Matsumoto, T.; Husson, M. *J Antimicrob Chemother*. **1988**, *22*, 185.
[141] Rastogi, N.; Labrousse, V.; Goh, K. S. *Curr Microbiol*. **1996**, *33*, 167.
[142] Tomioka, H.; Sato, K.; Shimizu, T.; Sano. C. *J Infect*. **2002**, *44*, 160.
[143] Alvirez-Freites, E. J.; Carter, J. L.; Cynamon, M. H. *Antimicrob Agents Chemother*. **2002**, *46*, 1022.
[144] Ginsburg, A. S.; Grosset, J. H.; Bishai, W. R. *Lancet Infect Dis*. **2003**, *3*, 432.
[145] Crofton, J.; Choculet, P.; Maher, D. WHO/TB/96-210 (rev 1). **1997**, Geneva. World Health Organization.
[146] Sulochana, S.; Rahman, F.; Paramasivan, C. N. *J Chemother*. **2005**, *17*, 169.
[147] Hu, Y.; Coates, A. R.; Mitchison, D. A. *Antimicrob Agents Chemother*. **2003**, *47*, 653.
[148] Ballow, C.; Lettieri, J.; Agarwal, V.; Liu, P.; Stass, H.; Sullivan, J. T. *Clin Ther*. **1999,** *21*, 513.
[149] Lubasch, A.; Keller, I.; Borner, K.; Koeppe, P.; Lode, H. *Antimicrob Agents Chemother*. **2000,** *44*, 2600.
[150] Stass, H.; Dalhoff, A.; Kubitza, D.; Schuhly, U. *Antimicrob Agents Chemother*. **1998,** *42*, 2060.
[151] Stass, H.; Kubitza D. *J Antimicrob Chemother.* **1999**, *43*, 83.
[152] Sullivan, J. T.; Woodruff, M.; Lettieri, J.; Agarwal, V.; Krol, G. J.; Leese, P. T.; Watson, S.; Heller, A. H. *Antimicrob Agents Chemother*. **1999**, *43*, 2793.
[153] Wise, R.; Andrews, J. M.; Marshall, G.; Hartman, G. *Antimicrob Agents Chemother*. **1999**, *43*, 1508.

[154] Zhao, B. Y.; Pine, R.; Domagala, J.; Drlica, K. *Antimicrob Agents Chemother*. **1999**, *43*, 661.

[155] Yoshimatsu, T.; Nuermberger, E.; Tyagi, S.; Chaisson, R. ; Bishai, W.; Grosset, J. *Antimicrob Agents Chemother*. **2002**, *46*, 1875.

[156] Nuermberger, E. L.; Yoshimatsu, T.; Tyagi, S.; Williams, K.; Rosenthal, I.; O'Brien, R. J.; Vernon, A. A.; Chaisson, R. E.; Bishai, W. R.; Grosset. J. H. *Am J Respir Crit Care Med*. **2004**, *170*, 1131.

[157] Burman, W. J.; Goldberg, S.; Johnson, J. L.; Muzanye, G.; Engle, M.; Mosher, A. W.; Choudhri, S.; Daley, C. L.; Munsiff, S. S.; Zhao, Z.; Vernon, A.; Chaisson, R. E. *Am J Respir Crit Care Med*. **2006,** *174*, 331.

[158] Gillespie, S. H.; Kennedy, N. *Int J Tuberc Lung Dis*. **1998,** *2*, 265.

[159] Ji, B.; Lounis, N.; Maslo, C.; Truffot-Pernot, C.; Bonnafous, P.; Grosset, J. *Antimicrob Agents Chemother*. **1998**, *42*, 2066.

[160] Tomioka, H.; Sato, K.; Akaki, T.; Kajitani, H.; Kawahara, S.; Sakatani, M.; *Antimicrob Agents Chemother*. **1999**, *43*, 3001.

[161] Piersimoni, C.; Morbiducci, V.; Bornigia, S.; De Sio, G.; Scalise, G. *Am Rev Respir Dis*. **1992**, *146*, 1445.

[162] Rastogi, N.; Goh, K. S.; Bryskier, A.; Devallois, A. *Antimicrob Agents Chemother*. **1996**, *40*, 1610.

[163] Nuermberger, E. L.; Yoshimatsu, T. ; Tyagi, S.; O'Brien, R. J.; Vernon, A. N.; Chaisson, R. E.; Bishai, W. R.; Grosset, J. H. Am J Respir Crit Care Med. **2004**, *169*, 421.

[164] Nuermberger, E. L.; Yoshimatsu, T. ; Tyagi, S.; Williams, K.; Rosenthal, I.; O'Brien, R. J.; Vernon, A. A.; Chaisson, R. E., Bishai , W. R.; Grosset , J. H. *Am J Respir Crit Care Med*. **2004**, *170*, 1131.

[165] Dong, Y.; Xu, C.; Zhao ,X,; Domagala, J.; Drlica K. *Antimicrob Agents Chemother*. **1998**, *42*, 2978.

[166] Zhao, B. Y, Pine, R.; Domagala, J.; Drlica K. *Antimicrob Agents Chemother*. **1999**, *43*, 661.

[167] Hu, Y.; Coates, A. R.; Mitchison, D. A. *Antimicrob Agents Chemother*. **2003**, *47*, 653.

[168] Alvirez-Freites, E. J.; Carter, J. L.; Cynamon, M. H. *Antimicrob Agents Chemother*. **2002**, *46*, 1022.

[169] Yoshimatsu, T.; Nuermberger, E.; Tyagi, S.; Chaisson, R.; Bishai, W.; Grosset, J. *Antimicrob Agents Chemother*. **2002**, *46*, 1875.

[170] Gosling, R. D.; Uiso, L. O.; Sam, N. E.; Bongard, E.; Kanduma, E. G.; Nyindo, M.; Morris, R. W.; Gillespie, S. H. *Am J Respir Crit Care Med*. **2003**, *168*, 1342.

[171] Pletz, M. W.; De Roux, A.; Roth, A.; Neumann, K. H.; Mauch, H.; Lode, H. *Antimicrob Agents Chemother*. **2004**, *48*, 780.

[172] Burman, W. J.; Goldberg, S.; Johnson, J. L.; Muzanye, G.; Engle, M.; Mosher, A. W.; Choudhri, S.; Daley, C. L.; Munsiff, S. S.; Zhao, Z.; Vernon, A.; Chaisson, R. E. *Am J Respir Crit Care Med*. **2006**, *174*, 331.

[173] Johnson, J. L.; Hadad, D. J.; Boom, W. H.; Daley, C. L.; Peloquin, C. A.; Eisenach, K. D.; Jankus, D. D.; Debanne, S. M.; Charlebois, E. D.; Maciel, E.; Palaci, M.; Dietze, R. *Int J Tuberc Lung Dis*. **2006**, *10*, 605.

[174] Sriram, D.; Aubry, A.; Yogeeswari, P.; Fisher, L. M. *Bioorg Med Chem Lett*. **2006**, *16*, 2982.

[175] Tran, T. P.; Ellsworth, E. L.; Stier, M. A.; Domagala, J. M.; Hollis Showalter, H. D.; Gracheck, S. J.; Shapiro, M. A.; Joannides, T. E.; Singh, R. *Bioorg Med Chem Lett*. **2004**, *14*, 4405.

[176] Ellsworth, E. L.; Tran, T. P.; Showalter, H. D.; Sanchez, J. P.; Watson, B. M.; Stier, M. A.; Domagala, J. M.; Gracheck, S. J.; Joannides, E. T.; Shapiro, M. A.; Dunham, S. A.; Hanna, D. L.; Huband, M. D.; Gage, J. W.; Bronstein. J. C.; Liu, J. Y.; Nguyen, D. Q.; Singh, R. *J Med Chem*. **2006**, *49*, 6435.

[177] Tran, T. P.; Ellsworth, E. L.; Sanchez, J. P.; Watson, B. M.; Stier, M. A.; Showalter, H. D.; Domagala, J. M.; Shapiro, M. A.; Joannides, E. T.; Gracheck, S. J.; Nguyen, D. Q.; Bird, P.; Yip, J.; Sharadendu, A.; Ha, C.; Ramezani, S.; Wu, X.; Singh, R. *Bioorg Med Chem Lett*. **2007**, *17*, 1312.

[178] Wiles, J. A.; Song, Y.; Wang, Q.; Lucien, E.; Hashimoto, A.; Cheng, J.; Marlor, C. W.; Ou, Y.; Podos, S. D.; Thanassi, J. A.; Thoma, C. L.; Deshpande, M.; Pucci, M. J.; Bradbury, B. J. *Bioorg Med Chem Lett*. **2006**, *16*, 1277.

[179] Wiles, J. A.; Wang, Q.; Lucien, E.; Hashimoto, A.; Song, Y.; Cheng, J.; Marlor, C. W.; Ou, Y.; Podos, S. D.; Thanassi, J. A.; Thoma, C. L.; Deshpande, M.; Pucci, M. J.; Bradbury, B. J. *Bioorg Med Chem Lett*. **2006,** *16*, 1272.

[180] Wiles, J. A.; Hashimoto, A.; Thanassi, J. A.; Cheng, J.; Incarvito, C. D.; Deshpande, M.; Pucci, M. J.; Bradbury, B. J. *J Med Chem*. **2006**, *49*, 39.

[181] Wang, Q.; Lucien, E.; Hashimoto, A.; Pais, G. C.; Nelson, D. M.; Song, Y.; Thanassi, J. A.; Marlor, C. W.; Thoma, C. L.; Cheng, J.; Podos, S. D.; Ou, Y.; Deshpande, M.; Pucci, M. J.; Buechter, D. D.; Bradbury, B. J.; Wiles, J. A *J Med Chem*. **2007** *50*, 199.

[182] Doi, N and Disratthakit, A. **2006**. Poster F1-0491 ICAAC, San Francisco.

[183] Doi, N.; Disratthakit, A.; Ogiso, S.; Uoyama, S.; Kurosawa, Y. **2006**. Poster F1-0492 ICAAC. San Francisco.

[184] Takasuna, K.; Itoh, S.; Kitano, Y.; Jindo, T.; Furuhama, K. **2006**. Poster F1-0494. ICAAC San Francisco.

[185] Hashimi, S. M.; Wall, M. K.; Smith, A. B.; Maxwell, A.; Birch, R. G. *Antimicrob Agents Chemother*. **2007**, *51*, 181.

[186] Boehm, H. J.; Boehringer, M.; Bur, D.; Gmuender, H.; Huber, W.; Klaus, W.; Kostrewa, D.; Kuehne, H.; Luebbers, T.; Meunier-Keller, N.; Mueller, F. *J Med Chem*. **2000**, *43*, 2664.

[187] Lubbers, T.; Angehrn, P.; Gmunder, H.; Herzig, S.; Kulhanek, J. *Bioorg Med Chem Lett*. **2000,** *10*, 821.

[188] Emmerson, A. M.; Jones, A. M. *J Antimicrob Chemother*. **2003**, *51*, 13.

[189] Schimana, J.; Fiedler, H. P.; Groth, I.; Sussmuth, R.; Beil, W.; Walker, M.; Zeeck, A. *J Antibiot* (Tokyo). **2000**, *53*, 779.

[190] Theobald, U.; Schimana, J.; Fiedler, H. P. *Antonie Van Leeuwenhoek*. **2000**, *78*, 307.

[191] Flatman, R. H.; Howells, A. J.; Heide, L.; Fiedler, H. P.; Maxwell, A. *Antimicrob Agents Chemother*. **2005**, *49*, 1093.

[192] Grossman, T. H.; Bartels, D. J.; Mullin, S.; Gross, C. H.; Parsons, J. D.; Liao, Y.; Grillot, A. L.; Stamos, D.; Olson, E. R.; Charifson, P. S.; Mani, N. *Antimicrob Agents Chemother*. **2007**, *51*, 657.

[193] Tanitame, A.; Oyamada, Y.; Ofuji, K.; Fujimoto, M.; Iwai, N.; Hiyama, Y.; Suzuki, K.; Ito, H.; Terauchi, H.; Kawasaki, M.; Nagai, K.; Wachi, M.; Yamagishi, J. *J Med Chem*. **2004**, *47*, 3693.

[194] Tanitame, A.; Oyamada, Y.; Ofuji, K.; Terauchi, H.; Kawasaki, M.; Wachi, M.; Yamagishi, J.*Bioorg Med Chem Lett*. **2005**, *15*, 4299.

[195] Oblak, M.; Grdadolnik, S. G.; Kotnik, M.; Jerala, R.; Filipic, M.; Solmajer, T. *Bioorg Med Chem Lett*. **2005**, *15*, 5207.