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# An overview on antitubercular activities of fluoroquinolones and other related analogues

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#### ABSTRACT

The reappearance of tuberculosis (TB) and resistant of Mycobacterium strains is a crucial public health concern for the treatment of TB. Some findings on the fluoroquinolone (FQs) derivatives are being developed as effective anti-TB agents. Some FQs antibiotics like ofloxacin, moxifloxacin, gatifloxacin, sparfloxacin, ciprofloxacin, levofloxacin are act as bactericidal with exceptional activity against Mtb and are presently used as second-line anti-TB drugs. The FQs exert their antibacterial effects by trapping gyrase and topoisomerase-IV enzymes on deoxyribonucleic acid (DNA) and blocking the replication and transcriptions. Unlike most other bacteriums, Mtb lacks topoisomerase-IV, but contains the genes gyrA and gyrB encoding the A and B subunits of DNA gyrase. Various new-generation FQs are under clinical trials with the aim of reducing the time periods of TB treatment while others are considered to be capable candidates for future drug development.

Keywords: Mycobacteria, tuberculosis, drug-combinations, infectious disease.

#### **INTRODUCTION**

Tuberculosis (TB) is a serious health problem worldwide and its condition is worsened by the existence of multidrug resistant tuberculosis (MDR-TB) and extensive drug resistant tuberculosis (XDR-TB) strains. Mycobacterium tuberculosis (Mtb) is the main causative agent of the TB. Recently, even more serious forms of drug resistance (latent-TB) have been reported. Effective expected treatment of TB became available in the mid-1940s with the introduction of streptomycin [1,2]. The human immune deficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS) resulted in a global resurgence of TB. The emergence and global presence of MDR-TB and XDR-TB are highly lethal, very expensive and complex to treat and causing health concern worldwide [3,4]. In spite of being a world public health crisis, TB has remained a mistreated disease. Since the beginning of rifampicin, anti-TB drug discovery has been lethargic. Since then, no new drug has become present that can be contrast to rifampicin in conditions of utility and safety. The treatment of MDR-TB/XDR-TB, there is an urgent need for novel

anti-TB drugs that are more potent and have less toxicity [5,6]. The rise in tuberculosis (TB), due in particular to the increased incidence of *Mtb* infections in HIV-infected individuals has prompted a strong search for new anti-TB drugs for the treatment of TB. Increased infection with the *M. avium* complex (MAC) is contributing to the morbidity and mortality in AIDS patients. The most urgent aim of treatment of TB and MAC infections should be the improvement of extremely active and low-cost anti-TB drugs [7-9]. The MAC shows intrinsic resistance to the various common anti-TB drugs, in several cases due to poor uptake of these drugs [9]. The immunological deterioration seen in AIDS patients is frequently attended by opportunistic infections counting *Mtb* and non-tuberculosis (*M. avium*) mycobacterium diseases. Treatment of these infections is often complicated by patient intolerance of the drugs used or pathogen resistance to usual drug therapy.

The main drugs presently used to treat TB are isoniazid (INH), rifampicin (RIF), ethambutol (EMB), pyrazinamide (PZA) and streptomycin (SM), or most of them. Some studies reported that about 19% of TB isolates in a hospital were resistant to RIF and INH, most common anti-TB drugs. Normally, the resistance to INH and SM is more common than resistance to RIF, EMB and PZA. For empiric treatment of mycobacterium infections, even if drug resistance is not suspected, the 4-drugs regimen of INH, RIF, PZA, and EMB (or SM) is recommended [8]. This review is an attempt to summarize evidence regarding the efficacy and potentials of various existing fluoroquinolones (FQs) as anti-TB drugs and emerging new FQs in the treatment of active TB [10-13].

The search for new drugs is imperative and the strategies followed to generate new TB therapies may involve [14]:

• Developing new drugs from existing lead molecules used to treat other bacterial infections (like. fluoroquinolones).

• Modifying an existing drug to improve its anti-TB activity and its pharmacokinetic properties to make it less susceptible to the known mechanism of resistance. This is the strategy adopted in developing new anti-TB drug analogues.

• Discovering new drugs either by random screening or if a specific target is known, by a rational design. New quinolones act as active agents. Since resistance will likely develop upon prolonged treatment, such agents will always be used in conjunction with one (or perhaps more) other anti-TB drugs to which mycobacteria is susceptible. Regarding the FQs, they have activity against *Mtb* and penetrate human macrophages in which mycobacteria survive. Among the FQs, levofloxacin and ciprofloxacin have better pharmacokinetics [15]. Other new agents also appear to have promising activity against TB. Macrolides, like clarithromycin, azithromycin [16,17] and Rifapentine, were less active *in vitro* against *Mtb* than the FQs. Generally, they are used in combination with at least one other drug to avoid resistance [18]. The search for new, effective agents with a different mechanism of action is the most challenging, but the approach for discovering new agents that may shorten the duration of treatment and provide clarification to both the drug intolerance and drug-resistance harms [19].

**Fluoroquinolones:** The fluoroquinolones (FQs) are synthetic antibacterial drugs and in 1962, discovered by Sterling-Winthrop Institute, as an impurity during synthesis of the anti-malarial drug chloroquine [20]. This byproduct, nalidixic acid is used to treat Gram-negative urinary tract infections.



Fluoroquinolones (FQs) are deoxyribonucleic acid gyrase (DNA gyrase) inhibitors. These drugs are also effective against non-replicating, persistent mycobacterium. They are potentially useful and important for shortening the duration of TB treatment. Among the newer FQs, there have been several clinical phase trials assessing the utility of moxifloxacin (400 mg/day) in place of any of the fist-line anti-TB drugs with different results. Addition of moxifloxacin to INH, RIF and PZA did not affect 2-month sputum culture conversion. A small but non significant increase in the week 8 culture negativity was reported. A trial conducted in diagnosed sputum smear-positive pulmonary TB patients with moxifloxacin, gatifloxacin or high-dose levofloxacin compared with INH for 7 days has

shown good antibacterial activity that was almost comparable to that of INH. The utility of substituting gatifloxacin or moxifloxacin for EMB or INH for reduced the time of treatment from the standard 6 months to 4 months. The results of the clinical trial comparing the potential of two moxifloxacin-containing therapy to reduced treatment in pulmonary TB is likely to clarify the status of moxifloxacin in the TB treatment [21-26].

Fluoroquinolones (FQ) like ciprofloxacin, ofloxacin and moxifloxacin are second line anti-TB drugs used in combination with first line anti-TB drugs to treat MDR-TB. The MIC of these FQs ranges from 0.12 to 2µg/mL. Levofloxacin, L-isomer of ofloxacin is two time more active as the parent drug. They compounds cause side effects as central nervous system disturbances, gastrointestinal reactions and skin reactions. While gatifloxacin and moxifloxacin are new FQ that offer advantages over ofloxacin and ciprofloxacin. These new FQs moxifloxacin and gatifloxacin are the most advanced anti-TB compounds in development and showed promise to be the first new anti-TB drugs [27-29].



Fig. 1: First- and second-generation fluoroquinolones compounds

In spite of good bioavailability and simple synthesis, nalidixic acid has limited clinical use due to its poor pharmacokinetic profile and narrow antibacterial range [30]. The discovery of the first reported antibacterial FQs was norfloxacin [31] and it showed 1,000-fold greater antibacterial activity than nalidixic acid [32,33] with improved pharmacokinetic, longer half-life and improved solubility profile [32-34]. Norfloxacin and some other second generation FQs like ciprofloxacin [35], ofloxacin [36], and levofloxacin, which is S-isomer of racemic mixture of ofloxacin [36] and have relatively safe and frequently prescribed drugs [30]. The development of various analogs with broader antibacterial activity, better solubility and long half-lives [30,33], the third and fourth generations FQs, moxifloxacin [37], which has a bulky hydrophobic alteration at C(7), has been the most successful. Unfortunately, some third and fourth generation FQs have been limited or withdrawn due to rigorous adverse effects (Fig. 2) with temafloxacin, trovafloxacin, grepafloxacin and clinafloxacin [38,39]. Several new FQs are in development such as gemifloxacin [40], and sitafloxacin [41] which showed activity against respiratory pathogens [30].





Fig. 2: Third- and fourth-generation fluoroquinolone compounds

Recently new bacterial topoisomerase inhibitors (NBTIs) with similar modes of action like previously reported FQs, including GSK 299423 [42, NXL101 [43], and a series of tetrahydroindazoles [44,45]. These new FQs were showed good in vitro activity against both Gram-positive and Gram-negative bacteria plus FQs-resistant strains; they also exhibited activity against *Mtb*.



Fig. 3: New DNA gyrase inhibitors

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99

### Asif Husain et al

While the activity profile of the FQs has not been analyzed particularly for *M.tb*, it is assume that many relationships were found in other bacteria that will be applicable to *Mtb* (Fig. 3). Alteration at N<sub>1</sub> control activity, with electrondeficient and sterically strained cyclopropyl ring being optimal, trailed by 2,4-difluoro-phenyl and t-butyl [46]. This substituent controls Gram-negative and Gram-positive effectiveness, and 2,4-difluoro-phenyl group enhanced activity against anaerobic bacteria. The C<sub>2</sub> position is near the DNA gyrase binding site, and sterically undemanding H atom at R<sub>2</sub> is optimal for activity [47]. The dicarbonyl group is required for binding to DNA gyrase and is critical for activity. Alteration at C<sub>5</sub> control activity [33,46,49] and active groups are small electron-rich such as -NH<sub>2</sub>, -OH, and -CH<sub>3</sub> [46].



In addition,  $C_5$  alterations affect activity against Gram-negative and Gram-positive microbes. The F atom at  $C_6$  improves DNA gyrase inhibition effect [30,47] and can increase the MIC value of the compound 100-time more than other substitutions [46]. The most active substituents at  $C_7$  have 5 and 6 membered nitrogen heterocycles, with pyrrolidines rising activity against Gram-negative bacteria and piperazines affecting activity against Gram-positive bacteria. The  $C_8$  position controls absorption and half-life. The optimal alterations for in vivo effectiveness include groups that cause an electron deficient  $\pi$  system, i.e., N, CF, and CCI [49]. Various alterations that generate a  $N_1$  to  $C_8$  bridges have been successful, i.e., ofloxacin and levofloxacin, which both exhibit considerable gyrase inhibiting activity [47].

Mechanism of resistance: Fluoroquinolone resistance (FQ-R) in Mtb is mainly connected with mutations in preserved quinolone resistance-determining region (QRDR) of gyrA and gyrB concerned with interaction between drug molecule and DNA gyrase [11]. The degree of FQ-R is stated by definite amino acid substitution in QRDR. The mutations in gyrA may give low-level resistance (MIC>2mg/l) [50], high-level resistance to FQs regularly requires multiple mutations in gyrA, or simultaneous mutations in gyrA and gyrB [50,51]. The most often observed mutations linked with FQ-R in Mtb are at positions Ala-90 and Asp-94 in gyrA gene. Interestingly, mutations of gyrA have been accounted to cause hyper susceptibility to FQs, mainly when present with other resistance mutations [52]. While mutations in the QRDR region of gyrA are recognized in only 42-85% of FQ-R clinical isolates, option mechanisms of resistance are supposed to be present, including the potential participation of efflux pumps [53]. The FQs modify DNA topology and block replication by inhibiting two crucial bacterial enzymes, DNA gyrase or topoisomerase II and topoisomerase IV. The DNA gyrase, encoded by gyrA and gyrB, retain the intensity of supercoiled DNA vital for efficient replication and is a primary target for the FQs in the majority Gram-negative bacteria [54]. Topoisomerase-IV, encoded by parC and parE, is accountable for decatenation of DNA subsequent replication and is the main target of the FQs in several Gram-positive bacteria [33,55]. Mycobacteria are unique in that genome sequence analyses have failed to recognize DNA topoisomerase-IV [33]. The gyrA and gyrB are the merely targets of the FQs in Mtb. The MIC for various FQs has determined for both Mtb H37Rv and clinical isolates of Mtb. The MIC values against Mtb H37Rv for the clinically relevant FQs are showed in Table 1 and range from 0.1 to 5 mM [56].

FQs	MIC (mM)	FQs	MIC (mM)	FQs	MIC (mM)
Ciprofloxacin	1.51	Moxifloxacin	0.16	Levofloxacin	1.25
Gatifloxacin	1.25	Ofloxacin	2.5	Lomefloxacin	5
Sparfloxacin	0.08				

Table 1. MIC data for fluoroquinolones (FQs) used in treatment of *Mtb* [56]

The FQs are well tolerated and causing mild adverse effects and seldom require discontinuation or changes in therapy [39,57] (Table 2). The most common adverse effects accounted include gastrointestinal upset, disturbances of the CNS, and some skin reactions [30]. Some more serious adverse effects were reported with FQs. The FQs were linked with tendonitis and tendon rupture due to collagen damage, in 2008 impelled a black box warning for all available drugs of this class and all FQs may cause photosensitivity [38]. For example, the existence of halogen atoms at  $C_5$  or  $C_8$  and a bulky side chain or methyl group at  $C_5$  showed the maximum potential for this effect [46]. The FQs can cause QTc interval prolongation by blocking voltage-gated K<sup>+</sup> channels, which has connected with torsades de pointes syndrome, arrhythmia, cardio-toxicities and death. The severity varies on the basis of structural changes and the amount of dose administered. Other adverse effects are hepatotoxicity, kidney and liver dysfunction and dysglycemia [30,39].

Table 2	. Notable	side ef	fects of	some	fluoroc	uinolo	nes [30	,38]
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FQs	Adverse effect	Implication	FQs	Adverse effects	Implication
Ciprofloxacin	Tendonitis or tendon rupture	Black box warning	Grepafloxacin	Cardiotoxicity	Withdrawn
Clinafloxacin	Phototoxicity, hypoglycemia	Development stopped	Ofloxacin	Tendonitis and tendon rupture	Black box warning
Gatifloxacin	Dysglycemia	Oral and injectable, not used in USA	Temafloxacin	hemolytic, renal failure, clotting abnormalities	Withdrawn
Levofloxacin	Tendonitis/ tendon rupture	Black box warning	Trovafloxacin	Hepatotoxicity	Withdrawn/ limited use
Sparfloxacin	Phototoxicity, QTc interval prolongation	Not used in USA	Moxifloxacin	QTc-interval pro-longation, tendonitis/tendon rupture	Black box warning
Lomefloxacin	Phototoxicity, CNS effects	Black box warning			

**Clinical Uses:** The fluoroquinolones (FQs) have several pharmacokinetic features that have valuable for treating TB. The oral bioavailability of many FQs is good, ranging from 70-100 %, and the levels in the blood peaking soon after taken [58]. The FQs are cell permeable and extensively distributed in all part of body, which is essential for killing intracellular microbes and treating pathogenic disease. The later generation FQs has long serum half-lives, but these vary broadly, from 5.4 h for ciprofloxacin to 18.30 h for sparfloxacin [49]. Most FQs are cleared via the kidneys [57].



From a chemical feature, levofloxacin and ciprofloxacin have no substituents at  $C_8$ . Quinolones reveal that the  $C_7$  ring,  $C_6$  fluorine,  $N_1$  cyclopropyl and the  $C_8$  substituents are attractive chemical features in targeting *Mtb* gyrase [59]. Newer FQs, are  $C_8$  methoxy or chloro substituted. Quinolones with  $C_8$  methoxy groups showed greater

synergistic effect than those lacking this group. Gatifloxacin and moxifloxacin will be discussed, together with sitafloxacin, a  $C_8$  chloro derivative. They have lower MICs for *Mtb* than previous quinolones.

Tubercular patients with MDR-TB used one of FQs as second-line ant-TB agents in the treatment of TB, including gatifloxacin, levofloxacin, moxifloxacin, or ofloxacin [6,60]. In murine model studies [61-63], the most effective FQs are: moxifloxacin ¼ gatifloxacin> levofloxacin>ofloxacin [64]. The aforementioned FQs, some studies have examined the efficacy of sparfloxacin and lomefloxacin [65], while sparfloxacin emerge efficient for treating MDR-TB, role for lomefloxacin in TB is unknown [65]. In a mouse studies, moxifloxacin and gatifloxacin are clinical trials to find out whether they can reduce the duration of therapy [66].

Effect of fluoroquinolones with other antitubercular drugs: Quinolones act by inhibiting DNA super coiling and causing interference with transcription and DNA synthesis. They inhibiting cell division and leading to cell death by inhibiting the topoisomerase enzymes [59]. High-level resistance to quinolones occurs in many species, including mycobacteria, via mutations in a short region of 2517 bp *gyrA* gene [67]. Strains with lower levels of resistance did not have mutations in gyrase. Mycobacteria are usually less susceptible to quinolones than most of other bacteria [68] and new quinolones are adequately active against *Mtb* infections [69]. Newer FQs have important advances in therapy. In spite of this, some quinolones are used as second line therapeutics against TB [70-72] and their activity against MAC are relatively weak. The incidence of *Mtb* resistance to FQs is relatively low and there is no cross resistance to other anti-TB drugs. Quinolones are orally bioavailable and their penetration into tissues and host macrophages. Because of the low occurrence of serious adverse effects, FQs may be used for long-term therapy, especially for the patients coinfected with HIV. They will have to be used in combination with other anti-TB drugs to evade development of resistance. The multidrug therapy viz. ofloxacin plus PZA, ciprofloxacin plus RIF or PZA, ofloxacin plus RIF plus INH given 50-100 % positive response in the control of TB [72].

• Gatifloxacin is a  $C_8$  methoxy substituted 6-FQ and exhibits excellent activity, particularly against gyrase resistance mutants. Gatifloxacin was tested alone and in combination with several anti-TB drugs (EMBl, PZA and ethionamide (ETA) and compared to INH and RIF using short (4-week) and long (12-week) long treatment period [61].

• Combination with EMB, PZA and ETA, gatifloxacin emerges to have satisfactory activity alone and in combination with ETA with or without PZA to have concern in the treatment of TB [73].

• Combination with macrolides against extracellular MAC, gatifloxacin was tested in combination with RIF, SM, and CAM and the activity against extracellular MAC was considerably attenuated by combination with RIF and CAM. The same action was obtained for ciprofloxacin and levofloxacin. The macrolides are protein synthesis inhibitors, the antagonistic action between these quinolones and CAM would be explained by the inhibitors of protein synthesis interfered with the lethal activity of FQs [74,75]. The observed antagonism between FQs and RIF might be connected to each drugs mode of inhibiting RNA synthesis [76]. These results suggest that such combinations may not be effective in eliminating extracellular MAC organisms growing in the lesions of patients.

• Combination with macrolides against intra-macrophage MAC, the antagonistic effect was not seen against intra macrophage MAC infection. The combination of FQs, especially gatifloxacin and levofloxacin, and RIF revealed additive effects. This combination could be used in treatment of MAC infections without lesions [76]. Compare the activity of  $C_8$  methoxy (gatifloxacin and moxifloxacin) to that of  $C_8$ -halogeno quinolones (sitafloxacin), gatifloxacin and sitafloxacin exhibited same levels of bacteriostatic and/ or bactericidal effects against extracellular and intra macrophage MAC.

• Quinolone-resistant gyrase mutants revealed that  $C_8$ -methoxy derivatives were more bactericidal than  $C_8$ -bromo or  $C_8$ -h quinolones. The gatifloxacin and sitafloxacin have the same MICs for quinolone resistant isolates of *Mtb*, it depends on the substituent at  $C_7$  of the structure and not on the  $C_8$  substituent [73].

• Low concentrations of moxifloxacin killed *Mtb* more comprehensively than did gatifloxacin [77] and both FQs were more active than levofloxacin. Alone and in combination with INH, macrolides, cycloserine and EMB or three drug combinations containing moxifloxacin or gatifloxacin plus INH and RIF.

• Combination with isoniazid exhibited bactericidal activity higher than either compounds separately used or the effects change little between the concentration of 0.2 mg/l and 2 mg/l, becoming more lethal when the concentration of INH increases.

• Combination with macrolides: the RIF-*moxifloxacin* combination was more lethal than RIF alone, but only when the amount of RIF was low (0.1-0.5 mg/ml) [77].

#### Asif Husain et al

• Combination with cycloserine and capreomycin: the effects of moxifloxacin-capreomycin combination exhibit greater activity than either alone, while cycloserine had modest effect on moxifloxacin activity.

• The combination with EMB, that alone has a little effect against *Mtb*, reduces the lethal activity of moxifloxacin by about 80%. In a murine model, neither capreomycin nor cycloserine affected the activity of moxifloxacin [78]. This interference is not limited to *Mtb* but has little effect on bacteriostatic activity of FQs against *M. smegmatis*.

• Three drug combinations containing moxifloxacin or gatifloxacin plus INH and RIF: the  $C_8$  methoxy FQs contribute lethal activity to combination treatment. In spite of the interfering of RIF with moxifloxacin lethality, three drug combinations containing moxifloxacin or gatifloxacin plus INH and RIF reduced the number of *Mtb* cells by 4-10 times over the two drug combination of RIF and INH.

• Sitafloxacin is a  $C_8$ -chloro quinolone [76], the activities of Sitafloxacin were exhibited activity in combination with other anti-TB drugs against extracellular and intra-macrophage *Mtb*, and *M. avium* complex.

- Combination with RIF against extracellular Mtb, increased the activity against Mtb.
- Combination with Rifalazil (KRM) against intra-macrophage Mtb enhanced the activity against Mtb.
- Combination with INH against intra-macrophage *Mtb*, Sitafloxacin potentiates the activity of INH.
- Combination with CAM and RIF against extracellular MAC, sitafloxacin was significantly attenuated by combination with RIF and CAM.

• Combination with macrolides against intra-macrophage MAC, the antagonistic effect was not noticed against intra-macrophage MAC infection.

- Combination with SM against extracellular *Mtb* decreased the activity against *Mtb*.
- Combination with RIF against intra-macrophage *Mtb* potentiated the activity of Rifampicin.

The activity of sitafloxacin against *Mtb* replicating within intracellular Mono Mac 6-Macrofage (MM6-M $\varphi$ s) a human monocytic cell line and A-549 type II human lung epithelial alveolar cell line (A-549 cells) in comparison with other FQs [79]. The MM6-M $\varphi$ s and A-549 type II are the cells that initially encounter the pathogen, and they represent a highly predictive test for activity [76].

Antimicrobial activities against intra-macrophage *Mtb*: the antimicrobial activities of sitafloxacin, levofloxacin and gatifloxacin against intra-macrophage *Mtb* were considerably, dependent on their activities against extracellular *Mtb* based on the MIC values (0.125 mg/mL, 0.06 mg/mL, 0.25 mg/mL for gatifloxacin, Sitafloxacin and levofloxacin, respectively), the order of activity is Sitafloxacin>gatifloxacin>levofloxacin. Sitafloxacin causes complete inhibition of bacterial growth. These quinolones were added at the Cmax in the blood (1 mg/l, 1.7 mg/l, and 2 mg/l for sitafloxacin, gatifloxacin and levofloxacin), they exhibited bactericidal activity against intra-macrophage *Mtb* and the efficacy order was gatifloxacin>Sitafloxacin>levofloxacin. If the test drugs were added at lower concentrations (1/8 Cmax to 1/2 Cmax) the efficiency was in the order Sitafloxacin>gatifloxacin>levofloxacin, as in the case based on the MIC values [80]. The MIC values of FQs are not always predictive of their activity against intracellular *Mtb*.

Efficacy of Sitafloxacin on intracellular mycobacteria in A-549 cells, compared with gatifloxacin and levofloxacin. If the test quinolones were added at the MIC, sitafloxacin and gatifloxacin caused growth inhibition but not levofloxacin. The order was Sitafloxacin> gatifloxacin> levofloxacin. When the quinolones were added at the Cmax, the bactericidal activity was in the order gatifloxacin>sitafloxacin> levofloxacin, and if they were added at lower concentrations the order was sitafloxacin>gatifloxacin> levofloxacin. Both sitafloxacin and gatifloxacin at Cmax caused the complete elimination of intracellular *Mtb*.

**Other Quinolones:** The introduction of nalidixic acid during 1962 has shown the new path for bacterial infections. The effort to increase the efficacy against bacterial strains has led to identify new model quinolones. The introduction of norfloxacin, a FQ derivative has changed the background of antibacterial therapy. The quinolone drugs like ofloxacin, moxifloxacin, gatifloxacin and levofloxacin are used as second line ant-TB drugs. Many researchers were evaluated quinolones for their anti-TB activity. In this direction, a series of 1-ethyl- and 1-aryl-6-fluoro-1,4-dihydroquinol-4-ones were evaluated for anti-TB and cytotoxic activities. One compound (1) was exhibited the preeminent MIC of  $1.56\mu$ g/mL against *Mtb* and good selectivity index (SI=>40.06). Compound 1 has potent anti-TB agent with an EC<sub>90</sub> value of  $5.75\mu$ g/ml [81]. Several 1-(cyclopropyl/2,4-difluorophenyl/t-butyl)-1,4-dihydro-6-fluoro-7-(substituted secondary amino)-4-oxoquinoline-3-carboxylic acids were found potent anti-TB agent (2), with MIC of  $0.09\mu$ M against *Mtb*. Compound 2 decreased the mycobacterial load in lung and spleen tissues at a dose of 50 mg/kg body weight [82-84].



Some quinolones were observed for their potency against mycobacterium species. Series of pyridobenzoxazines by replacement of the *N*-methylpiperazinyl group of Levofloxacin with different basic substituents to examined the anti-TB activities. Compound **3**, which was a 2,8-diazabicyclo [4.3.0]nonanyl derivative with relatively low lipophilic and showed the most potent activity anti-TB activity and activity was 4- to 32- times more potent than that of levofloxacin. The increase in the lipophilicity of levofloxacin analogues contributed to enhancement of anti-TB activity against *M. kansasii*, levofloxacin showed MIC value 0.12-0.25µg/mL while moxifloxacin showed the range of MIC=  $\leq 0.06-0.12\mu$ g/mL. A series of lamivudine, prodrugs bearing fluoroquinolones (4) was evaluated for their efficacy against *Mtb*. All compounds were exhibited an inhibition of 92-100% at a dose of 6.25µg/mL [86,87]. While one compound (**5**) showed in vivo anti-TB activity by reducing the bacterial load in spleen tissue and showed moderately active in reducing bacterial count in spleen [86,87]. Gatifloxacin derivatives (**6**) were found more potent in comparison to compound **5**. Compound **6** decreased the bacterial load in lung and spleen tissues [88,89]. Most potent compound (**7**) which reduced bacterial load in lung and spleen tissues, at 25 mg/kg body weight [88,89. The 7-[4-(5-amino-1,3,4 thiadiazole-2-sulfonyl)]-1-piperazinyl-fluoroquinolone derivatives (**8a** and **8b**) were showed moderate anti-TB activity at MIC of 10µg/mL compared to INH [90].





The effect of nitro substitution on quinoline ring, a series of 2-(sub)-3-fluoro/nitro-5,12-dihydro-5oxobenzothiazolo[3,2-a]quinoline-6-carboxylic acids were evaluated for anti-TB activities against *Mtb*, MDR-TB, and *M. smegmatis*. Among these compounds, 2-(3-(diethyl carbamoyl) piperidin-1-yl)-)-3-fluoro-5,12-dihydro-5oxobenzothiazolo[3,2-a]quinoline-6-carboxylic acid (9) was found to be the most active with MIC of 0.18 and 0.08 $\mu$ M against *Mtb* and MDR-TB. Compound 9 decreased the bacterial load in lung and spleen tissues at the dose of 50 mg/kg body weight [91-93]. The 6-nitroquinolone (10) was found to be the most active compound in vitro with MIC of 0.08 and 0.16  $\mu$ M against *Mtb* and MDR-TB. Compound 10 reduced the bacterial load in lung and spleen tissues at the dose of 50 mg/kg body weight [94].



A series of [1,2,3]Triazolo[4,5-h]quinolones were evaluated for their anti-TB activity against *Mtb* and some other clinically isolated strains of *Mtb* able with different drug resistance. Among all, compound **11** was exhibited highest activity against all strains with a MIC of  $0.5\mu$ g/ml [95]. A series of [1,2,3]Triazolo[4,5-h]quinolones, compounds **12a** and **12b** were exhibited better activity with MIC in the range  $0.125-16.0 \mu$ g/mL against *Mtb* and other clinical isolates of MDR-TB. The results exhibited that [1,2,3]-triazolo[4,5-h]quinolones were able with an tremendous activity against MDR-TB strains with no cytotoxicity [95].



New quinolones as anti-TB agents, many quinolone derivatives were evaluated for their in vitro activity against *Mtb* and MDR-TB. The most potent compound **13** was exhibited MIC value 0.19  $\mu$ M and 0.09 $\mu$ M against *Mtb* and MDR-TB and decreased the bacterial load in lung and spleen tissues at a dose of 50 mg/kg body weight [91-93]. Compound **14** reduced the bacterial load in lung and spleen tissues [94], while compound **15** reduced the bacterial load in lung and spleen tissues [94], while compound **15** reduced the bacterial load by 30% and 42%, respectively, at a dose of 50 mg/kg body weight [91-93]. The 1-(cyclopropyl/2,4-difluorophenyl/ tert-butyl)-1,4-dihydro-8-methyl-6-nitro-4-oxo-7-(substituted-secondary-amino) quinoline-3-carboxylic acids, most active compound (**16**) was showed MIC of 0.42  $\mu$ M and 0.09  $\mu$ M against *Mtb* and MDR-TB [94] and 7-(3-(diethylcarbamoyl)piperidin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**17**) was exhibited promising MIC of 0.09  $\mu$ M against *Mtb* and MDR-TB. Compound **17** decreased the mycobacterial load in lung and spleen tissues respectively at a dose of 50 mg/kg body weight [94]. Moxifloxacin and gatifloxacin derivatives were evaluated against *Mtb* and the most active compound (**18**) was exhibited MIC value 0.31 $\mu$ g/mL [96].



Fluoroquinolones (FQs) are used as second-line ant-TB drugs in the treatment of MDR-TB. Ciprofloxacin and ofloxacin are derivatives of the parent compound nalidixic acid, discovered as a by-product of the antimalarial chloroquine. Newer-generation quinolones like moxifloxacin and gatifloxacin were evaluated in clinical trials and proposed as first-line antibiotics with the purpose of reducing the length of treatment of TB [97,98]. The FQs are acts by inhibiting the topoisomerase-II (DNA gyrase) and topoisomerase-IV (critical enzymes for bacterial viability). The proteins are encoded by the genes *gyrA*, *gyrB*, *parC* and *parE*. In *Mtb*, only DNA gyrase is present and thus it is the only target of FQ activity [59]. DNA gyrase is a tetramer formed by two  $\alpha$  and  $\beta$  subunits, coded by *gyrA* and *gyrB*, which catalyzes the super-coiling of DNA [67]. The mechanism of development of FQ-resistance in *Mtb* is by chromosomal mutations in quinolone-resistance-determining region of *gyrA* or *gyrB* [99,100]. The FQ-resistance-linked gyrase mutations in *Mtb* have been reported. The *Mtb* is the presence of a natural polymorphism in *gyrA* that is not related to FQ-resistance and FQ-susceptible strains. The concurrent event of mutations T80A and A90G in *gyrA* led to hyper susceptibility to various quinolones [59]. This finding point out that problem of FQ-resistance in *Mtb* might be more complex than was thought initially [53,101].

Drug resistance emerges as a result of spontaneous gene mutations in *M. tuberculosis* that render the bacteria resistant to the commonly used anti-TB drugs. The standard treatment of TB calls for a 6 month therapy of four drugs that in the case of MDR-TB is extended to 18–24 months involving second-line drugs. This makes fulfillment with the treatment therapy very challenging and the rates of non-adherence could be high, resulting in poor outcomes and further spreading of MDR strains [102]. A better knowledge of the mechanisms of drug resistance of *Mtb* and the relevant molecular mechanisms involved will improve the available techniques for rapid drug resistance detection and will help to explore new targets for drug activity and development.

### CONCLUSION

Tuberculosis (TB) has been a leading cause of death. The emergence of multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) TB has been threatening to destabilize TB control. There is an urgent need for

new anti-TB drugs that are more effective and have less toxicity. Newer fluoroquinolones and related compounds have been shown to improve the activity of standard anti-TB treatment regimen when substituted for first line anti-TB ethambutol and to shorten the treatment time in drug-susceptible TB. There is a great hope in getting promising antitubercular agents in near future as the current research focuses on developing novel agents having potential, selective and newer mechanisms of action.

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