2.1 HISTORY AND OVERVIEW OF QUINOLONES

The quinolone anti-infective agents are of wholly synthetic origin and are not modeled knowingly after any natural antibiotic. Several ring systems are or have been involved. The numbering system is illustrated as follows:

![Chemical Structures](image)

4-Oxo-1,4-dihydro-quinolone  
4-Oxo-1,4-dihydro-[1,8]-naphthyridine

Of all the totally synthetic antimicrobial agents, the (fluoro)quinolones have proven to be the most successful economically and clinically. They are orally and parenterally active, have a broad antimicrobial spectrum that includes many frequently encountered pathogens, are bactericidal in clinically achievable doses, generate comparatively tolerable resistance levels, possess a fascinating molecular mode of action, are comparatively easily synthesized, and with a few notable exceptions are safe. That is not to say that they are perfect drugs and cannot be improved but rather that they are important weapons in the ongoing struggle against morbidity and mortality caused by microbial pathogens.

The first antimicrobial quinolone was discovered about 50 years ago, in 1960s, as an impurity in the chemical manufacture of a batch of the antimalarial agent chloroquine.
It demonstrated anti Gram-negative antibacterial activity, but its potency and antimicrobial spectrum were not significant enough to be useful in therapy. Building on this lead, however, subsequently nalidixic acid was commercialized. Nalidixic acid remains on the market today and represents the so-called first generation quinolones. Its antibacterial properties appeared immediately to be very interesting [Sissi, C., et al., 2003]. Despite its convenient oral activity, bactericidal action, and ease of synthesis, its limited antimicrobial spectrum (primarily activity against *Escherichia coli*) and poor pharmacokinetic characteristics limit its use primarily to treatment of sensitive community-acquired urinary tract infections.

The first of the second-generation family of quinolones, norfloxacin (NOR), had dramatically enhanced and broader spectrum anti Gram-negative activity and possessed significant anti Gram-positive activity as well. The potency of NOR was in the same range as that of many fermentation-derived antibiotics, and its comparative structural simplicity and synthetic accessibility lead to a very significant effort to find even more improved analogues.

Shortly thereafter, ciprofloxacin (CIP) and OFLO, as well as its optically active form LVFX, were introduced. The second-generation agents have significant broad-spectrum antimicrobial activity including important Gram-positive pathogens. This is coupled with gratifying safety and pharmacokinetic characteristics.

A wide variety of clinical indications have been approved for quinolones including many infections commonly encountered in community practice including upper and lower respiratory infections, gynecologic infections, sexually transmitted diseases, prostatitis, and some skin, bone and soft tissue infections.
Recently introduced members of the fluoroquinolone family belong to the third generation. These include GAT and MXFX, which possess further enhanced activity against Gram-positive infections, and anti-anaerobic coverage is now present although at present only trovafloxacin is approved for this indication. (Table 2.1). [Mitscher, A. L., 2005]

2.1.1 Development of Quinolones

Quinolones were derived from quinine [Andersson, M. I., et al., 2003]. A most successful achievement was the preparation of fluoroquinolones. In fact, the introduction of a fluorine atom at the C₆ position increased several folds the activity of the drug and led to compounds active against a broader spectrum of bacteria [Sissi, C., et al., 2003; Andersson, M. I., et al., 2003]. The first fluoroquinolone clinically used was NOR. Its poor tissue distribution limited its applications to the treatment of urinary tract infections. Modification of the substituent at N₁ position produced CIP. This drug presents an excellent systemic activity upon oral administration and has become indeed one of the most frequently prescribed antibiotics. Fluoroquinolones have been further optimized to improve both pharmacokinetic and pharmacodynamic properties like favorable bioavailability allowing oral administration, good tolerability, high tissue concentrations as well as superior bactericidal activity against a broad spectrum of clinically relevant pathogens. Thus, new generations of fluoroquinolones are now available. They preserve excellent potency against Gram-negative bacteria and, at the same time, they show an increased activity towards Gram-positive bacteria compared to CIP. At the moment, the main interest for these compounds is related to the treatment of respiratory diseases [Sissi, C., et al., 2003]. Two major groups have been developed from the basic structure: quinolones and naphthyridones. The presence of nitrogen at position 8 identifies the naphthyridones, a carbon and associated group at position 8 identifies the quinolones [Andersson, M. I., et al., 2003] (Figure 2.1).
Table 2.1: The various generations of the family of quinolones.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First-generation compounds</strong> (often all included as 4-quinolones)</td>
<td></td>
</tr>
<tr>
<td>1,8 naphthyridine (carboxylic acid)</td>
<td>Nalidixic acid</td>
</tr>
<tr>
<td>7-methyl, 7-pyrrole</td>
<td>Piromidic acid</td>
</tr>
<tr>
<td>1,2-cinnoline (carboxylic acid)</td>
<td>Cinoxacin</td>
</tr>
<tr>
<td>4-quinolone (carboxylic acid)</td>
<td>Oxolinic acid</td>
</tr>
<tr>
<td>7-piperazine (pyrido-pyrimidine)</td>
<td>Pipemidic acid</td>
</tr>
<tr>
<td><strong>6,7,8 side chain substituents</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Second-generation compounds (IIA)</strong></td>
<td></td>
</tr>
<tr>
<td>A. Fluoroquinolones with enhanced but predominantly Gram-negative activity</td>
<td></td>
</tr>
<tr>
<td>6-Fluoro</td>
<td>Flumequine</td>
</tr>
<tr>
<td>6-Fluoro-7-piperazinyl</td>
<td>Ciprofloxacin, Pefloxacin, Norfloxacin</td>
</tr>
<tr>
<td>Ofloxacin, Levofloxacin, Rufloxacin</td>
<td></td>
</tr>
<tr>
<td>6,8-difluoro-7-piperazinyl</td>
<td>Lomefloxacin, Fleroxacin</td>
</tr>
<tr>
<td><strong>Second-generation compounds (IIB)</strong></td>
<td></td>
</tr>
<tr>
<td>B. Fluoroquinolones with balanced broad spectrum activity</td>
<td></td>
</tr>
<tr>
<td>6-fluoro-7-piperazinyl</td>
<td>Temafloxacin</td>
</tr>
<tr>
<td>6,8-difluoro-7-dimethylpiperazinyl</td>
<td>Grepafloxacin</td>
</tr>
<tr>
<td>6-fluoro-8-chloro-7-pyrrolodinyl</td>
<td>Sparfloxacin</td>
</tr>
<tr>
<td>6-fluoro-7-pyrrolodinyl naphthyridone</td>
<td>Clinafloxacin, Sitafoxacin, Tosufloxacin</td>
</tr>
<tr>
<td><strong>Third-generation compounds</strong></td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolones with enhanced Gram-positive activity</td>
<td></td>
</tr>
<tr>
<td>6-fluoro-7-azabicyclo naphthyridone</td>
<td>Trovafloxacin</td>
</tr>
<tr>
<td>6-fluoro-8-methoxy-7-azabicyclo</td>
<td>Moxifloxacin</td>
</tr>
<tr>
<td>6-fluoro-8-methoxy-7-piperazinyl</td>
<td>Gatifloxacin</td>
</tr>
<tr>
<td>6-fluoro-7-methoxyiminonaphthyridone</td>
<td>Gemifloxacin</td>
</tr>
</tbody>
</table>
**Figure 2.1:** Development of quinolones.
Fluoroquinolones exhibit potent *in vitro* and *in vivo* antimycobacterial activity [Shandil, R. K., *et al.*, 2007]. The quinolones would appear to fulfill most of the criteria for an ideal class of antimycobacterial drugs. The *in vitro* activity of quinolones against mycobacteria and the efficacy of these drugs in murine models of mycobacterial infection have been documented in various studies and reviews [Sato, K., *et al.*, 2003; Aubry, A., *et al.*, 2004; Alangaden, G. J., *et al.*, 1997]. Quinolones have shown excellent bactericidal activity against several mycobacteria. Most strains of *MTB*, *M. leprae*, *M. bovis*, *M. kansasii*, *M. marinum* and *M. xenopi* were inhibited *in vitro* by CIP or OFLO at concentrations ranging between 0.5 mg/L and 2.0 mg/L [Sato, K., *et al.*, 2003; Aubry, A., *et al.*, 2004].

Quinolones can be administered orally with good absorption, and favorable pharmacokinetics which permit once- or twice-daily administration [Hooper, D. C., *et al.*, 1985]. Quinolones have excellent oral bioavailability and penetrate extremely well into tissues and into host cells, such as macrophages, in which mycobacteria often reside [von Rosenstiel, N., *et al.*, 1994; Takemura, M., *et al.*, 2001]. The incidence and severity of side effects of the quinolone are generally low [von Rosenstiel, N., *et al.*, 1994]. The incidence of mycobacterial resistance to quinolone is relatively low at the present time [Frieden, T. R., *et al.*, 1993], and there is no cross-resistance [Cambau, E., *et al.*, 1994] or antagonism with other classes of antimycobacterial agents [Grange, J. M., *et al.*, 1994], so they may be used for long-term therapy in combination with other antimycobacterial agents and with the diverse array of drugs that patients with HIV infection may be receiving.

Although quinolones have several advantages, pre-marketing trials showed the fluoroquinolone agents to have a favorable side-effect profile, with treatment-related adverse events comprising gastrointestinal, central nervous system and dermatologic effects that were generally mild and reversible on cessation of treatment. However, post-marketing surveillance studies have identified severe adverse events, including severe anaphylaxis, QTc-interval prolongation by SPFX and grepafloxacin [Andersson, M. I., *et al.*, 2003], and potential cardiotoxicity, associated with 3 quinolone agents that either
resulted in the removal of the agent from the market (temafloxacin and grepafloxacin) or significantly restricted its use due to substantial mortality and morbidity associated with liver toxicity (trovafloxacin). To date, there have been no such significant adverse events associated with the older fluoroquinolone agents, including CIP, OFLO, NOR, and LXFX [Bertino, J. Jr., et al., 2000; Stratton, C. W., 1998]. Lomefloxacin, fluoroquinolone containing fluorine group at C₈ position, shows phototoxicity towards (Ultraviolet) UV radiation [Man, I., et al., 1999]. NOR, CIP and enoxacin are very photohemolytic, but sparfloxacin (SPFX) was not, indicating that the in vivo phototoxic potencies of fluoroquinolones might not be predictable by the photohemolysis study. GAT is a non-phototoxic quinolone [Yamamoto, T., et al., 2001]. Quinolones are shown to produce convulsions when administered orally or intracerebrally in mice along with non-steroidal anti-inflammatory drugs like fenbufen. [Hirai, S., et al., 1989; Furuhama, K., et al., 1997]. Other effects include a haemolytic uraemic-like syndrome with temafloxacin, a metallic taste with grepafloxacin, hepatitis with trovafloxacin, unexpected hypoglycaemia with CNFX and temafloxacin, a number of immunologically mediated adverse events with tosufloxacin, and an immune-mediated rash in young women with gemifloxacin. To date there are little toxicological data on garenoxacin [Andersson, M. I., et al., 2003].

2.1.2 Mode of Action

Forty years of research in the quinolone field has allowed to elucidating some key aspects of their mechanism of action. Their main biological targets are the DNA gyrase and topoisomerase IV, two enzymes belonging to the type II topoisomerase family. These enzymes are present in all bacteria and are expressed in prokaryotic organisms only, features that make them ideal antibacterial drug targets. Indeed, the prominent selectivity of quinolones in poisoning the bacterial type II topoisomerases, as the corresponding mammalian enzymes represents the rational basis for their safe use in antibacterial chemotherapy [Sissi, C., et al., 2003; Maxwell., 1997].

Quinolone antibacterials, by inhibiting bacterial topoisomerase II (DNA gyrase) and topoisomerase IV [Maxwell., 1997] in Gram-positive species, inhibit tertiary negative
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supercoiling of bacterial DNA. This effect, probably associated with binding of quinolones to a DNA gyrase complex, is rapidly bactericidal. The minimum bactericidal concentration is usually only two- to four folds the MIC, and a prolonged post-antibiotic effect is produced at concentrations exceeding the MIC. Fluoroquinolones only rarely demonstrate synergy or antagonism with other agents. [Andriole, V. T., 2000]

The target for quinolone action in Gram-negative bacteria is the A subunit of DNA gyrase. DNA gyrase is a bacterial type II topoisomerase. It is made up of a tetramer of two parts, A2 and B2. This protein converts relaxed DNA into supercoiled DNA. The A subunit is responsible for breakage and resealing of chromosomal DNA. The B subunit is responsible for energy transduction from ATP hydrolysis [Bonomo, R. A., 1998]. The mechanism of action responsible for the bactericidal activity of quinolone antimycobacterial appears to be an inhibition of bacterial DNA gyrase. Quinolones interfere with the action of DNA gyrase, preventing closure of the double stranded nicks produced by the A subunits [Dougherty, T. J., et al., 1985] by forming a quinolone-gyrase-DNA ternary complex [Bonomo, R. A., 1998]. Failure to close these nicks inhibits supercoiling and results in the degradation of chromosomal DNA into fragments by exonucleases [Fairweather, N. F., et al., 1980], leading to termination of chromosomal replication and interference with cell division and gene expression [Fairweather, N. F., et al., 1980], penetration through macrophages and phagosome walls especially for lomefloxacin [Mozhokina, G. N., et al., 1998]. Topoisomerase IV is responsible for the separation of daughter DNA strands during bacterial cell division in Gram-positive bacteria. Topoisomerase IV is likely to be the primary target for quinolone action in Gram-positive bacteria. In addition to attacking DNA gyrase and topoisomerase IV, quinolones are bactericidal by other mechanisms. Three mechanisms A, B, and C have been proposed. Mechanism A requires RNA and protein synthesis as well as cell division for bactericidal action. Mechanism B is the ability to kill non-dividing cells without concomitant protein or RNA synthesis. Mechanism C is the bactericidal activity that occurs in the absence of multiplication, yet in the presence of protein and RNA synthesis. The action of these mechanisms is organism specific [Bonomo, R. A., 1998].
2.1.3 Quinolones and Immune Function

Quinolone antibiotics diffuse into human polymorphonuclear leukocytes (PMNs) and monocytes in concentrations one to five times greater than into the extracellular compartment. Intracellular quinolones may be bactericidal and may exert effects against *Staphylococcus aureus*, *Serratia marcescens*, *Mycobacterium fortuitum*, and *Salmonella typhimurium*. Although there is no effect on chemotaxis of PMNs, low concentrations have been reported to enhance phagocytosis [Bonomo, R. A., 1998].

2.1.3.1 Resistance Mechanism

Resistance to quinolone antibiotics is generally mediated by alterations in the chromosomal DNA of bacteria. In the main, most bacteria accumulate several mutations that affect both DNA gyrase and permeability.

Mutations in the regulatory genes that govern permeability channels or porins and efflux pumps are common. The expression of *marA*, a transcriptional activator involved in multiple antibiotic resistance, results in diminished entry of quinolones into cells. Mutations that lead to a decrease porin expression of the bacterial outer membrane of Gram-negative bacteria also limit entry. Efflux pumps responsible for quinolone resistance have been characterized in both Gram-positive and Gram-negative bacteria. The physical properties of the quinolone determine whether it is pumped out.

Point mutations in residues 67 to 106 of the A subunit of DNA gyrase, the quinolone-resistance determining region (QRDR), also result in resistance. Mutations in this region are associated with increased resistance to all quinolones.

Resistance to quinolone agents may emerge during therapy e.g., CIP-resistant *pseudomonas aeruginosa*, *S. aureus* and *S. epidermidis*. In addition, strains of ciprofloxacin-resistant *Neisseria gonorrhoeae* have been reported from Southeast Asia, Australia, Africa, and the United Kingdom and from Ohio, California, Hawaii, and
Washington. In one sexually transmitted disease clinic in Ohio, 145 gonococcal isolates had reduced susceptibility to ciprofloxacin. Treatment failures have been reported with single dose regimens if the MIC of CIP for a strain of *N. gonorrhoeae* was $\geq 1\mu g/mL$ [Bonomo, R. A., 1998].

### 2.1.4 BACTERIAL RESISTANCE TO QUINOLONES

Fluoroquinolone resistance may result from chromosomal mutations coding for modifications in target subunits (primarily *gyr A*, but also *gyr B*) of bacterial topoisomerase II, alterations in expression of outer membrane proteins — most importantly OmpF— and, in Gram-positive species, by variations in the uptake/efflux processes and mutations in topoisomerase IV. Thus, resistance in the pneumococci requires mutations in both *par C* and *gyr A* configurations. Plasmid-mediated resistance has not been confirmed to occur. [Andriole, V. T., 2000]

1. **Chromosomal mutations** in the genes encoding DNA gyrase and Topo IV
   - Changes the target region where the drug binds to the enzyme
   - The drug exhibits reduced affinity for the target site, now ineffective

2. **Porin mutations**
   - Alteration of the outer membrane porin proteins of Gram-negative organisms lead to decreased permeability of the drug through the outer membrane so less drug reaches the target enzyme

3. **Efflux mutations**
   - Enhance the organism's efflux capability, increasing the amount of drug pumped out of the cell.

### 2.1.5 STRUCTURE - ACTIVITY RELATIONSHIP (SAR): Quinolone molecule

Recently, understanding of how molecular modifications of the core quinolone structure affect(s) antimicrobial agent activity has progressed rapidly. Three positions (2, 3, and 4) cannot be changed without a significant loss of biological activity. Furthermore, it appears that a cyclopropyl group is optimal at position 1. Substituents at positions 5 and 8
affect planar configuration, and either a methyl or methoxy appear optimal at these sites. Hydrogen and amino groups have been investigated as useful substituents at position 6, replacing the fluorine of the fluoroquinolones. Interestingly, *in vitro* activity Enhancement observed with alterations at positions 5 and 6 is not always accompanied by improved *in vivo* action. For all these modifications, the substituents at positions 7 and 8 are critical for potent antimicrobial activity. Optimizing overall molecular configuration enhances the number of intracellular targets for antimicrobial action (R-8) and impedes the efficiency of efflux proteins (R-7) that diminish intracellular penetration. [Peterson, L. R., 2001]

- Position 6 fluorine results in more than 10-fold increase in gyrase inhibition
- C7 substituents - associated with increased potency against Gram-positive bacteria
  - Cyclic amino groups…
    - Piperazine rings = increase potency against Gram-bacteria
    - Pyrrolidine rings = increased potency against Gram-positive bacteria, yet lower water solubility
  - Methyl groups shown to help
- C8 position - alkylation increases activity against Gram-positive bacteria and tissue penetration

![Diagram](image)

**Figure 2.2:** Overview of SAR of quinolone and naphthyridone molecule. In molecules where X is a carbon atom, the molecule is a quinolone (cinoxacin, NOR, OFLO, CIP, temafloxacin, SPFX, grepafloxacin, LVFX, CLFX, MXFX, GAT). Where X is a nitrogen atom the molecule is a naphthyridone (nalidixic acid, enoxacin, tosufloxacin, trovafloxacin, gemifloxacin). [Andersson, M. I., *et al.*, 2003]
2.2 ANTITUBERCULAR QUINOLONES

CIP, OFLO and NOR were ineffective at clinically relevant concentrations against the *Mycobacterium avium* complex (MAC) *in vitro* as measured by radiometric respirometry, when administered alone. But good antimycobacterial activity was obtained when any of the quinolones was combined with ethambutol. The synergistic effect was most pronounced for the combination of EMB and CIP. This suggests that the synergism is based on an enhanced penetration of the quinolones by EMB [Hoffner, S. E., *et al.*, 1989]. SPFX was also tested against 30 strains of *Mycobacterium avium* complex (MAC) isolated from patients with acquired immune deficiency syndrome, alone and in combination with various antitubercular drugs. [Yajko, D. M., *et al.*, 1990]

In 1991, Rastogi, N., and Goh, K. S., determined the MICs of the new fluoroquinolone drugs, OFLO, CIP, and SPFX (AT-4140) for 10 strains of MTB by using both a BACTEC radiometric method and testing on solid 7H11 agar medium. Radiometric MICs by 7H12 broth testing ranged from 0.5 to 1.0, 0.25 to 0.5, and 0.1 to 0.2 µg/ml for OFLO, CIP, and SPFX respectively, whereas MICs in solid medium ranged from 0.5 to 1.0, 0.5 to 1.0, and 0.2 to 0.5 µg/ml, respectively. The bactericidal action of the quinolones compared with their reported peak concentrations in human serum showed that SPFX is the most bactericidal, followed by CIP and OFLO. The results suggested the potential of the new difluorinated quinolone SPFX for use against the tubercle bacillus and its antimycobacterial spectrum. [Rastogi, N., *et al.*, 1991]

Ji, B., et al., reported *in vitro* and *in vivo* activities of LVFX against MTB. In tests with 18 drug-susceptible strains of MTB, the MIC at which 50% of the strains are inhibited by LVFX was one dilution less than that at which 50% of the strains are inhibited by OFLO, but the MICs at which 90% of the strains are inhibited were similar. The *in vivo* activity of LVFX against MTB was compared with the activities of INH, OFLO, and SPFX. Mice were inoculated intravenously with $1.74 \times 10^6$ CFU of H37Rv, and treatments began the next day and were carried out six times weekly for 4 weeks. The severity of infection and effectiveness of treatment were assessed by survival rate, spleen weights, gross lung lesions, and enumeration of CFU in the spleen. In terms of CFU counts, the ranking of the anti-MTB activities of the treatments used ran in the following order: LVFX (300 mg/kg of body weight) = SPFX (100 mg/kg) > INH (50 mg/kg) > SPFX (50 mg/kg) > OFLO (300 mg/kg) = LVFX (150 mg/kg) > OFLO (150 mg/kg) = LVFX (50 mg/kg). It seems, therefore, that the *in vivo* activity of LVFX is comparable to that produced by a two fold - greater dosage of OFLO. It is assumed that the maximal clinically tolerated dosage of LVFX is similar to that of OFLO, i.e., 800 mg daily, which is equivalent to 300 mg of LVFX per kg in mice. Because LVFX displayed powerful bactericidal activity, promising effects against human tuberculosis may be achieved if patients are treated with the maximal clinically tolerated dosage of LVFX. [Ji, B., *et al.*, 1995]

The fluoroquinolones have been shown to be highly active *in vitro* against many mycobacterial species, including most strains of MTB and *M. fortuitum*, and some strains of *M. kansasii, M. avium*-intracellulare (MAI) complex and *M. leprae*. CIP, OFLO and SPFX are the best studied of this class of drugs to date, and they are among the most active of these against MTB and other mycobacteria. The use of ofloxacin in the treatment of patients with multidrug-resistant pulmonary tuberculosis has resulted in the selection of quinolone - resistant mutants in a few patients [Jacobs, M. R., 1995]. The dramatic increase in drug resistant MTB has caused resurgence in research targeted toward these organisms. As part of a systematic study to optimize the quinolone antibacterials against mycobacteria, Renau, T. E., *et al.*, have prepared a series of N-l-phenyl-substituted derivatives to explore the effect of increasing lipophilicity on potency.
at this position. The compounds, synthesized by the modification of a literature procedure, were evaluated for *Mycobacterium fortuitum* and activity against Gram-negative and Gram-positive bacteria, *Mycobacterium smegmatis* (MC²), and the results correlated with log P, pKa, and other attributes. The activity of the compounds against the rapidly growing, less hazardous organism *M. fortuitum* was used as a measure of MTB activity. The results demonstrate that increasing lipophilic character by itself does not correlate with increased potency against mycobacteria. Rather, intrinsic activity against Gram-negative and/or Gram-positive bacteria is the governing factor for corresponding activity against mycobacteria. [Renau, T. E., *et al.*, 1995]

As part of a study to optimize the quinolone antibacterials against MTB, Renau, T. E., *et al.*, have prepared a series of N₁– and C₇– substituted quinolones to examine specific structure - activity relationships between modifications of the quinolone at these two positions and activity against mycobacteria. The compounds were evaluated for activity against *Mycobacterium fortuitum* and MC² as well as Gram - negative and Gram - positive bacteria. The activity of the compounds against *M. fortuitum* was used as a barometer of MTB activity. The results demonstrated that (i) the activity against mycobacteria was related more to antibacterial activity than to changes in the lipophilicity of the compounds, (ii) the antimycobacterial activity imparted by the N₁ substituent was in the order tert – butyl > cyclopropyl > 2,4 - difluorophenyl > ethyl ≈ cyclobutyl > isopropyl, and (iii) substitution with either piperazine or pyrrolidine heterocycles at C₇ afforded similar activity against mycobacteria. [Renau, T. E., *et al.*, 1996a]

Renau, T. E., *et al.*, reported a series of quinolones with substitutions at the 8th position to examine the relationship between structural modifications at this position and activity against mycobacteria. The compounds were prepared by procedures described in the literature and were evaluated for their activities against *Mycobacterium fortuitum* and MC². The activities of the compounds against these two organisms were used as a measure of MTB activity. The results demonstrate that the contribution of the 8th position to antimycobacterial activity was dependent on the substituent at N₁ and was in the order
(i) COMe ≈ CBr > CCI > CH ≈ CF ≈ COEt > N > CCF₃ when N₁ was cyclopropyl; (ii) N ≈ CH > CF > COMe when N₁ was 2,4-difluorophenyl; (iii) N > or = CH when N₁ was tert-butyl; and (iv) N > CH when N₁ was ethyl. In general, derivatives with piperazine substitutions at C₇ were slightly less active against mycobacteria than the analogs with pyrrolidine substitutions, regardless of the pattern of substitution at the 8 position. Several of the best compounds were evaluated for their potential side effects as well as their activities against *Mycobacterium aurum*, *Mycobacterium avium*, *M. intracellularare*, and MTB. These agents exhibited biological profiles similar to or better than those of the positive controls CIP and SPFX. [Renau, T. E., *et al.*, 1996b]

In 1996, Klopman, G., *et al.*, showed the importance of tert-butyl group at N₁ position and said that tert-butyl substituents at least as good as cyclopropyl in rendering high levels of antimycobacterial activity. The 63 synthesized molecules were tested against *Mycobacterium avium*, *Mycobacterium intracellularare* complex. [Klopman, G., *et al.*, 1996]

Sbarbaro, J. A., *et al.*, reported that clinically achievable level of PZA enhances the antimycobacterial effect of low, non-bactericidal levels of OFLO and does not impede the bactericidal effect of a higher clinically effective level of OFLO. Unlike the combination of PZA and rifampin, these interactive effects are not affected by the sequence of drug administration. Findings support the use of these agents as a potentially effective preventive therapy combination for individuals exposed to multi-drug resistant tuberculous organisms. [Sbarbaro, J. A., *et al.*, 1996]

Ji, B., *et al.*, On 10 % oleic acid – albumin – dextrose – catalase - enriched 7H11 agar medium, the MIC at which 90 % of the isolates are inhibited for 20 strains of MTB was 0.5 µg of SPFX or MXFX per ml and 1.0 µg of clinafloxacin (CNFX) per ml, indicating that the *in vitro* activities of SPFX and MXFX were virtually identical and were slightly greater than that of CNFX. However, the *in vivo* activities of these drugs in a murine tuberculosis model differed considerably. Female Swiss mice were infected intravenously with $6.2 \times 10^6$ CFU of the H37Rv strain and treated for 4 weeks, beginning the next day
after infection, with INH serving as the positive control. By the criteria of 30-day survival rate, spleen weight, gross lung lesion, and mean number of CFU in the spleen, treatment with CNFX at up to 100 mg/kg of body weight six times weekly displayed no measurable effect against MTB, whereas both SPFX and MXFX were effective; administration six times weekly of either of the latter two drugs demonstrated dosage-dependent bactericidal effects, as measured by enumeration of CFU in the spleens, and MXFX appeared more bactericidal than the same dosage of SPFX. Of the three fluoroquinolones, only MXFX at 100 mg/kg six times weekly appeared as bactericidal as INH at 25 mg/kg six times weekly. Thus, MXFX may be an important component of the newer combined regimens for treatment of tuberculosis. [Ji, B., et al., 1998]

In 1999, Vacher, S., et al., compared the antimycobacterial activities of fluoroquinolones viz., ofloxacin, ciprofloxacin and grepafloxacin against three complex strains of *Mycobacterium avium*, *Mycobacterium kansasii*, *Mycobacterium marinum* and *Mycobacterium tuberculosis*. [Vacher, S., et al., 1999]

Bermudez, L. E., et al., evaluated MXFX activity against MAC *in vitro* against 25 strains. The results showed that MXFX, EMB, and azithromycin were active as single agents in liver, spleen, and blood. Rifabutin showed inhibitory activity only in the blood. Two-drug combinations containing azithromycin were no more active than azithromycin alone. Similarly, the three-drug combination was not more active than azithromycin alone in the spleen. Rifabutin did not add to the activity of any other single agent or two-drug combination. MXFX at both the concentrations in combination with ethambutol was significantly more active than each drug alone. [Bermudez, L. E., et al., 2001]

The *in vitro* inhibitory activities of quinolones against supercoiling activity of MTB DNA gyrase were measured by Onodera, Y., et al. The various quinolones studied were sitafloxacin (DU-6859a), SPFX, CIP and LVFX. Two altered proteins of *GyrA* containing Ala-90Val, or Ala-90Val and Asp-94Gly were also purified and the inhibitory activities were studied and were found to be weaker than those against the wild-type
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enzyme. These results suggested that mutations in the corresponding genes confer quinolone resistance. [Onodera, Y., et al., 2001]

Savini, L., et al., reported the synthesis of a series of 4-quinolylhydrazones and tested against MTB H37Rv. For the most of derivatives interesting antitubercular properties were showed; two compounds (1 and 2), identified as the most active, were tested also against Mycobacterium avium. Both compounds (1 and 2), with a high SI value (11.68 and 10.24 respectively), were then tested for efficacy in vitro in a TB-infected macrophage model, showing good values of EC_{90} and EC_{99}. Furthermore, these compounds were evaluated for their inhibitory activity against a single strain of M. avium, an opportunistic pathogen which has been associated with tuberculosis in patients infected by HIV. As for compounds 1 and 2, which showed a SI of 23.32 and 20.45 respectively. It is important to point out the low toxicity particularly shown by quinolylhydrazones 1 and 2 active against MTB H37Rv and M. avium. [Savini, L., et al., 2002]

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\text{\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Compd} & \textbf{R} & \textbf{R'} & \textbf{Ar} \\
\hline
1 & H & 7-OCH_3 & 2-OCH_3-Naphthyl \\
2 & CH_3 & 6-Cyclopropyl & C_6H_5 \\
\hline
\end{tabular}}
\]

Rodríguez, J. C., et al., in 2002 evaluated the in vitro activity of MXFX, GAT, LVFX and linezolid against 234 strains of MTB isolated in the Southeast of Spain. All drugs tested showed good activity, with an MIC_{90} of less than 1 mg/l, and were active against INH and RIF resistant strains. [Rodríguez, J. C., et al., 2002]

Alvirez-Freites, E. J., et al., evaluated GAT and MXFX in vitro and in vivo to determine their activities against MTB. GAT was subsequently compared in a dose range study to INH in a murine tuberculosis model. They found that GAT was somewhat less active
than INH. GAT and MXFX were found to have similar activities. GAT was studied alone and in combination with EMB, ETH, and PZA and compared to INH and RIF. [Alvirez-Freites, E. J., et al., 2002]

Foroumadi, A., et al., reported a series of N-[2-oxo-2-(4-substitutedphenyl)ethyl]piperazinyl quinolones and N-[2-hydroxyimino-2-(4-substitutedphenyl)ethyl]piperazinyl quinolones for antituberculosis activity against MTB H37R. Active compounds were also screened by serial dilution to assess toxicity to a Vero cell line. Nine compounds were efficient antimycobacterial agents showing MIC values ranging from 0.78 to 6.25 µg/ml. Generally, CIP derivatives were more active than NOR and enoxacin derivatives and the oxime analogues were less active than corresponding ketones [Foroumadi, A., et al., 2002a]. Various CIP derivatives containing 2-(2-furyl)-2-oxoethyl group [Foroumadi, A., et al., 2002b], 2-phenyl-2-axoethyl and 2-(4-fluorophenyl)-2-axoethyl groups [Foroumadi, A., et al., 2003a] at N₄ position of piperazine ring was tested for efficacy in vitro in TB-infected macrophage model.

Jain, R., et al., reported the synthesis and antituberculosis activities of a series of novel ring-substituted quinolines. The most effective compound of the series 3 (MIC = 6.25 mg/mL, MTB H37Rv strain) was synthesized in one step; thus is an attractive lead molecule for antituberculosis drug development. The results of this study represent the discovery of ring-substituted 4-methylquinolines as new class of potential antituberculosis agents. [Jain, R., et al., 2003]

![Chemical Structure](image)

Ciccone, R., et al., reported the antimycobacterial activity of NCX 976 (4), a new molecule obtained adding a NO moiety to the fluoroquinolone CIP, on MTB H37Rv
strain, both in a cell-free model and in infected human macrophages. Unlike unaltered ciprofloxacin, 4 displayed a marked activity also at low-nanomolar concentrations. [Ciccone, R., et al., 2003]

![Chemical Structure](image)

4

Foroumadi, A., et al., reported a series of N-[2-(2-furyl)-2-oxoethyl], N-[2-(2-furyl)-2-oxyiminoethyl], N-[2-oxo-2-(2-thienyl)ethyl] and N-[2-oxyimino-2-(2-thienyl)ethyl] piperazinyl quinolones and evaluated for antituberculosis activity against MTB H37Rv using the BACTEC 460 radiometric system and BACTEC 12B medium. CIP derivatives were more active than NOR derivatives and the oxime analogues were less active than the corresponding ketones. [Foroumadi, A., et al., 2003b]

Sbardella, G., et al., synthesized novel 1,7-disubstituted-6-nitroquinolones which were tested against MTB and MAC as well as against both Gram-positive and Gram-negative bacteria. *In vitro* assays showed some derivatives were endowed with good inhibiting activities against tested mycobacteria. Some derivatives were also found more potent than CIP and OFLO against Gram-positive bacteria. [Sbardella, G., et al., 2004]

Vaitilingam, B., et al., synthesized four new series of ring-substituted quinolinecarboxylic acids/esters constituting 45 analogues from the structural optimization of recently discovered new chemical entity, 2,8-dicyclopentyl-4-methylquinoline (MIC= 6.25 µg/mL, MTB H37Rv). All new derivatives were evaluated for *in vitro* antimycobacterial activities against MTB H37Rv. Certain ring-substituted-2-quinolinecarboxylic acid ester and ring-substituted-2-quinoline acetic acid ester analogues described showed moderate to good inhibitory activity. In particular, three analogues methyl 4,5-dicyclopentyl-2-quinolinecarboxylate (5), methyl 4,8-
dicyclopentyl-2-quinolinecarboxylate and ethyl 2-(2,8-dicyclopentyl-4-quinolyl)acetate exhibited excellent MIC values of 1.00, 2.00 and 4.00 µg/mL, respectively. Results obtained indicated that substitution of the quinoline ring with dicyclopentyl substituent presumably enhances the antimycobacterial activities in the quinoline analogues described herein. [Vaitilingam, B., et al., 2004]

Vangapandu, S., et al., report in vitro antimycobacterial properties of ring substituted quinolines constituting 56 analogues against drug-sensitive and drug-resistant MTB H37Rv strains. The most effective compounds 6 and 7 have exhibited an MIC value of 1 µg/mL against drug-sensitive MTB H37Rv strain that is comparable to first line anti-tuberculosis drug, INH. Selected analogues (with MIC: 6.25 µg/mL) upon further evaluation against single-drug-resistant (SDR) strains of MTB H37Rv have produced potent efficacy in the range between 6.25 and 50 µg/mL. [Vangapandu, S., et al., 2004]

Monga, V., et al., performed additional structural modifications of the new chemical entity, 2,8-dicyclopentyl-4-methylquinoline (MIC = 6.25 µg/mL, M. tuberculosis H37Rv) resulted in the synthesis of four new series of the ring-substituted quinolinecarbohydrazides constituting 22 analogues. All new derivatives were evaluated for in vitro antimycobacterial activities against drug-sensitive MTB H37Rv strain. Certain ring substituted 2-quinolinecarbohydrazide analogues described herein showed good inhibitory activity. In particular, analogues 4-(1-adamantyl)-2-quinolinecarbohydrazide, 4,5-dicyclopentyl-2-quinoline-carbohydrazide, 4,8-
dicyclopentyl-2-quinolinecarbohydrazide and 4,5-dicyclohexyl-2-quinolinecarbohydrazide have exhibited the MIC value of 6.25 µg/mL. Further investigation of the most suitable lead prototype, 4-(1-adamantyl)-2-quinolinecarbohydrazide led to the synthesis of N\textsubscript{2}-alkyl/N\textsubscript{2}, N\textsubscript{2}-dialkyl/N\textsubscript{2}-aryl-4-(1-adamantyl)-2-quinolinecarboxamides consisting of 13 analogues. Some of the synthesized carboxamides 8 and 9 reported herein have exhibited excellent antmycobacterial activities in the range of 6.25 - 3.125 µg/mL against drug-sensitive and drug-resistant MTB H37Rv strains. [Monga, V., et al., 2004]


Zhao, Y. L., et al., reported synthesis and antmycobacterial activity of a number of fluoroquinolone derivatives. Preliminary results were (1) for 1-aryl fluoroquinolones, 1-(4-nitrophenyl) derivatives were inactive while their 1-(2-fluoro-4-nitrophenyl) counterparts were active anti-TB agents (10 vs 12; 9 vs 11) indicated the fluoro substituent at C\textsubscript{2} position is important. For the 1-(2-fluoro-4-nitrophenyl)quinolones, 7-piperidinyl derivative 12 and 7-(3,5-dimethylpiperazinyl) derivative 16, which exhibited 97 % and 98 % inhibition, respectively, were more active than their 7-morpholinyl, 7-(4-methylpiperazinyl) and 7-piperazinyl congeners, 13, 14 and 15, respectively. In addition,
7-[4-(8-hydroxyquinolin-2-ylmethyl)piperazin-1-yl] derivative 20 exhibited 44% inhibition on the growth of MTB while its 7-(4-methylpiperazin-1-yl) counterpart was inactive implied the metal-chelating 8-hydroxyquinoline moiety was capable of enhancing the anti-TB activity, (2) for the bifunctional fluoroquinolone-hydroxyquinoline complexes, CIP and OFLO derivatives, which exhibited the same anti-TB activity (98% inhibition), are more potent than norfloxacin counterpart, which in turn is more potent than 1-aryl congeners (18, 19 > 17 > 20, 21). [Zhao, Y. L., et al., 2005]

Shindikar, A. V., et al., reported that novel 6,8-difluoro-1-alkyl-5-amino-1,4-dihydro-4-oxo-7-{4-substituted piperazin-1-yl}-quinoline-3-carboxylic acids, with the substituents at 4th position of piperazine being -[2(pyridine-4-carbonyl) hydrazono]propyl and -2[(pyrazine-2-carbonyl) amino] ethyl, were synthesized and evaluated in vivo against
MTB H37Rv in Swiss albino mice. Test compounds exhibited activity comparable to that of SPFX (survival rate, reduction of splenomegaly and reduced tubercular lesions) at a dose of 200 mg/kg. Compound 22 with 100% survival rate, absence of lung lesions, and 75% inhibition of CFUs emerged as the most potent compound. [Shindikar, A. V., et al., 2005]

Sriram, D., et al., synthesized various 7-substituted CIP derivatives and evaluated for antimycobacterial activity in vitro and in vivo against MTB and for inhibition of the supercoiling activity of DNA gyrase from MC². Preliminary results indicated that most of the compounds demonstrated better in vitro antimycobacterial activity against MTB than CIP. Compound 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[[N4-[1’-(5-methylisatinyl-beta-semicarbazo)]methyl]N1-piperazinyl]-3-quinoline carboxylic acid (23) decreased the bacterial load in spleen tissue with 0.76-log10 protections and was considered to be moderately active in reducing bacterial count in spleen. The results demonstrated the potential and importance of developing new quinolone derivatives against mycobacterial infections. [Sriram, D., et al., 2005]

Zhao, G., et al., described the syntheses and anti-tuberculosis activity of quinolone-cephalosporin conjugates (24 and 25). Both showed broad-spectrum antibacterial activity
and significant anti-TB activity. The carbamate-linked quinolone-cephem 25 showed better antimycobacterial activity, including anti-TB activity, than the direct amine-linked quinolone-cephem 24, while quinolone-cephem 25 was slightly more effective against some Gram-negative bacterial strains. [Zhao, G., et al., 2006]

Schwartz, Y. S., et al., prepared the MXFX-conjugated dansylated carboxymethylglucan (M-DCMG) conjugate by chemically linking dansylcadaverine (D) and MXFX to carboxymethylglucan (CMG), a known ligand of macrophage scavenger receptors. The targeted delivery to macrophages and the antituberculosis activity of the conjugate MXFX-DCMG were studied in vitro and in vivo. Using fluorescence microscopy, fluorimetry, and the J774 macrophage cell line, MXFX-DCMG was shown to accumulate in macrophages through scavenger receptors in a dose-dependent (1 to 50 µg/ml) manner. After intravenous administration of MXFX-DCMG into C57BL/6 mice, the fluorescent conjugate was concentrated in the macrophages of the lungs and spleen. Analyses of the pharmacokinetics of the conjugate demonstrated that MXFX-DCMG was more rapidly accumulated and more persistent in tissues than free MXFX. Importantly, therapeutic studies of mycobacterial growth in C57BL/6 mice showed that the MXFX-DCMG conjugate was significantly more potent than free MXFX. [Schwartz, Y. S., et al., 2006]
Foroumadi, A., et al., synthesized a number of N-substituted piperazinylquinolone derivatives and evaluated for antibacterial activity against Gram-positive and Gram-negative bacteria. Preliminary results indicated that most compounds tested in this study demonstrated comparable or better activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* than their parent piperazinylquinolones as reference drugs. Among these derivatives, CIP derivative 26, containing N-[2-[5-(methylthio)thiophen-2-yl]-2-oxoethyl] residue, showed significant improvement of potency against staphylococci, maintaining Gram-negative coverage. [Foroumadi, A., et al., 2006]

Nayyar, A., et al., have previously identified ring substituted quinolines as a new structural class of antituberculosis agents. In this article, efforts at structural optimization of this class, four series of ring-substituted-2/4-quinolinecarbaldehyde derivatives were synthesized. All twenty four compounds were synthesized using short and convenient one to two high yielding steps. The newly synthesized compounds were tested *in vitro* against drug-sensitive MTB H37Rv strain. Several derivatives were found to be promising inhibitors of MTB displaying > 90% inhibition at 6.25 µg/mL in the primary assay. The most active compounds, N-(2-fluorophenyl)-N-quinolin-2-ylmethylene-hydrazine (27), N-(2-adamantan-1-yl-quinolin-4-ylmethylene)-N-(4-fluorophenyl)hydrazine (28), and N-(2-cyclohexyl-quinolin-4-ylmethylene)-N-(2-fluorophenyl)hydrazine (29), exhibited 99% inhibition at the lowest tested concentration of 3.125 µg/mL against drug-sensitive MTB H37Rv strain. The similarity index based on steric and electrostatic features of the molecules was used, in conjunction with principal component analysis and linear discriminant analysis, successively to classify the molecules based on their activity into two classes. This classification method gives confidence in predicting the activity class of any new unsynthesized molecule belonging to these series. [Nayyar, A., et al., 2006]
Anquetin, G., et al., reports on the rational design of a series of new 6-fluoroquinolones by QSAR analysis against *Toxoplasma gondii*, their synthesis, their biological evaluation against *T. gondii* and *Plasmodium* spp., and their effect on MTB DNA gyrase and growth inhibition. Of the 12 computer-designed 8-ethyl(or methoxy)- and 5-ethyl-8-methoxy-6-fluoroquinolones predicted to be active against *T. gondii*, they synthesized four 6-fluoro-8-methoxy-quinolones. The four 6-fluoro-8-methoxy-quinolones were active on *T. gondii* but only one is as active as predicted. One of these four compounds appears to be an antiparasitical drug of great potential with inhibitory activities comparable to or higher than that of trovafloxacin, GAT, and MXFX. They also inhibit DNA supercoiling by MTB gyrase with an efficiency comparable to that of the most active quinolones but were poor inhibitors of MTB growth. Compounds 30 and 31 inhibited DNA supercoiling by MTB gyrase with IC$_{50}$ of < 6 µg/ml. [Anquetin, G., et al., 2006]

Sriram, D., et al., synthesized sixteen 7-substituted GAT derivatives and evaluated for antimycobacterial activity in vitro and in vivo against MTB H37Rv and multi-drug resistant *M. tuberculosis* (MDR-TB), and also tested for the ability to inhibit the supercoiling activity of DNA gyrase from MTB. Among the synthesized compounds, 1-cyclopropyl-6-fluoro-8-methoxy-7-[[[N$^1$-[1'-(5-isatinyl-β-semicarbazo)]methyl]-3-methyl]N$^1$-piperazinyl]-4-oxo-1,4-dihydro-3-quinoline carboxylic acid (32) was found to
be the most active compound *in vitro* with an MIC of 0.0125 µg/mL against MTB and MDR-TB. In the *in vivo* animal model 32 decreased the bacterial load in lung and spleen tissues with 3.62- and 3.76-log10 protections, respectively. Compound 32 was also found to be equally active as gatifloxacin in the inhibition of the supercoiling activity of wild-type MTB DNA gyrase with an IC\(_{50}\) of 3.0 µg/mL. The results demonstrate the potential and importance of developing new quinolone derivatives against mycobacterial infections. [Sriram, D., *et al.*, 2006a]

Sriram, D., *et al.*, tested the antimycobacterial activity (both *in vitro* and *in vivo*) and DNA gyrase inhibition of newly synthesized fluoroquinolone derivatives against MTB H37Rv and MC\(^2\), respectively. Among the synthesized compounds, compound 33 was found to exhibit the most potent *in vitro* antimycobacterial activity with a MIC value of 0.78 µg/ml, and a selectivity index of more than 80 while not being cytotoxic to the Vero cell line up to 62.5 µg/ml. When evaluated for *in vivo* antimycobacterial activity, compound 33 demonstrated a paramount decrease of bacterial load in lung and spleen tissues compared to the control and better than the standard drug CIP. [Sriram, D., *et al.*, 2006b]
Sriram, D., et al., designed an isatinimino lead compound as a novel non-nucleoside reverse transcriptase inhibitor with antimycobacterial properties for the effective treatment of AIDS and AIDS-related tuberculosis. Among the compounds synthesized, 1-cyclopropyl-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-7\([N^4\cdot[3'\cdot{(4,6\text{-dimethylpyrimidin-2-yl)}\text{benzenesulfonyl-4-yl}]}\text{imino-1'\cdot{(5-fluoroisatinyi)}]}\text{methyl}]-3\text{methyl N}^1\text{-piperazinyl}-3\text{quinoline carboxylic acid (34)} emerged as the most potent broad-spectrum chemotherapeutic agent active against HIV (EC\(_{50}\) : 12.1 µg/ml), and MTB (MIC: 1.22 µg/ml). [Sriram, D., et al., 2006c]
Sriram, D., *et al.*, synthesized a series of PZA manich bases by reacting PAZ, formaldehyde, and various substituted piperazines using microwave irradiation with the yield ranging from 46% to 86%. The synthesized compounds were evaluated for antimycobacterial activity *in vitro* and *in vivo* against MTB H37Rv (MTB). Among the synthesized compounds, 1-cyclopropyl-6-fluoro,1,4-dihydro-8-methoxy-7-(3-methyl-4-((pyrazine-2-carboxamido)methyl)piperazin-1-yl)-4-oxoquinoline-3-carboxylic acid (35) was found to be the most active compound *in vitro* with MIC of 0.39 and 0.2 µg/mL against MTB and MDR-TB, respectively. In the *in vivo* animal model 35 decreased the bacterial load in lung and spleen tissues with 1.86 and 1.66-log10 protections, respectively. [Sriram, D., *et al.*, 2006d]

![Chemical structure of compound 35](image)

de Almeida, M. V., *et al.*, reported the synthesis and biological evaluation of 12 lipophilic MXFX or GAT derivatives, by reaction of 1-cyclopropyl-6,7-difluoro,1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid with several N-monoalkyl 1,2-ethanediame or 1,3-propanediamine. Compound 36 inhibited growth at 0.31 µg/ml. The author states that the antitubercular activity depends on the alkyl chains, size or ramification. Ideal chain contains 10 carbon atoms. [de Almeida, M. V., *et al.*, 2007]

![Chemical structure of compound 36](image)
Nayyar, A., *et al.*, based on there previously identified molecule, designed two series of 4-(adamantan-1-yl)-2-substituted quinolines. All new derivatives were evaluated *in vitro* for antimycobacterial activities against drug-sensitive MTB H37Rv strain. Several 4-adamantan-1-yl-quinoline-2-carboxylic acid *N*-alkylhydrazides (AQCH) described showed promising inhibitory activity. In particular, few analogs displayed MIC of 3.125 µg/mL. Further investigation of AQCH by its reaction with various aliphatic, aromatic, and heteroaromatic aldehydes led to the synthesis of 4-adamantan-1-yl-quinoline-2-carboxylic acid alkylidene hydrazides which have produced promising antimycobacterial activities (99 % inhibition) at 3.125 µg/mL against drug-sensitive MTB H37Rv strain. The most potent analog 37 of the series produced 99 % inhibition at 1.00 µg/mL against drug-sensitive strain, and MIC of 3.125 µg/mL against isoniazid-resistant TB strain. To understand the relationship between structure and activity, a 3D-QSAR analysis has been carried out by three methods –comparative molecular field analysis (CoMFA), CoMFA with inclusion of a hydropathy field (HINT), and comparative molecular similarity indices analysis (CoMSIA). Several statistically significant CoMFA, CoMFA with HINT, and CoMSIA models were generated. Prediction of the activity of a test set of molecules was the best for the CoMFA model generated with database alignment. Based on the CoMFA contours, the authors have tried to explain the structure – activity relationships of the compounds reported. [Nayyar, A., *et al.*, 2007]
2.3 ANTIMICROBIAL QUINOLONES

Lesher, G. Y., *et al.*, prepared a series of 1-alkyl-1,8-naphthyridin-4-one-3-carboxylic acid derivatives. Several members of the series were found to be highly effective antibacterial agents both *in vitro* and *in vivo*. [Lesher, G. Y., *et al.*, 1962]

Santilli, A. A., *et al.*, reported the synthesis of a series of 1,2,3,4-tetrahydro-4-oxo-1,8-naphthyridine-3-carboxylic acid esters, carbonitriles, and carboxamides and evaluated (dose range 50 - 400 mg/kg) in mice infected with *Escherichia coli*. Only two derivatives, the ethyl and butyl esters of 1-ethyl-1,2-dihydro-4-hydroxy-7-methyl-1,8-naphthyridine-3-carboxylic acid, protected the animals against *E. coli* and several other Gram-negative bacterial pathogenic infections. A pro-drug type of mechanism was suggested to be operable. [Santilli, A. A., *et al.*, 1975]

Mitscher, L. A., *et al.*, developed a flexible reaction sequence starting with anthranilic acids or isatoic anhydrides and leads regiospecifically to 1-alkyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids after reaction with 1,3-dicarbonyl compounds. A number of new and known antimicrobial agents were prepared and tested in vitro, demonstrating, inter alia, that substitution of the H at C\textsubscript{2} abolished antibacterial activity. [Mitscher, L. A., *et al.*, 1978]

Curran, D. P., *et al.*, gave a novel method for synthesis of quinolones by employment of polyphosphoric acid as the dehydrating reagent as one method for cyclization of the ring. [Curran, D. P., *et al.*, 1984]

Chu, D. T. W., *et al.*, prepared a series of novel arylfluoroquinolones having a fluorine atom at the 6\textsuperscript{th} position, substituted amino groups at the 7-position, and substituted phenyl groups at the 1\textsuperscript{st} position. SAR studies indicate that the *in vitro* antibacterial potency is greatest when the 1-substituent is either p-fluorophenyl or p-hydroxyphenyl and the 7-substituent is either 1-piperazinyl, 4-methyl-1-piperazinyl, or 3-amino-1-pyrrolidinyl. The electronic and spatial properties of the 1-substituent, as well as the steric bulk, play
important roles in the antimicrobial potency in this class of antibacterials. [Chu, D. T. W., et al., 1985]

Miyamoto, H., et al., prepared a series of substituted 4-oxoquinoline-3-carboxylic acids having a methyl group at the 8th position and tested for their antibacterial activity. 7-(trans-3-Amino-4-methyl-1-pyrrolidinyl)-1-cyclopropyl-1,4-dihydro-6-fluoro-8-methyl-4-oxoquinoline-3-carboxylic acid (38) exhibited highly potent antibacterial activity against both Gram-positive and Gram-negative bacteria, including Pseudomonas aeruginosa. [Miyamoto, H., et al., 1990]

![Chemical Structure](image)

Xin, T., et al., synthesized twenty four 1-amino-6-fluoro-1,4-dihydro-4-oxo-7-(substituted) piperazinyl-3-quinoline carboxylic acids and evaluated for there in vitro antibacterial activity. [Xin, T., et al., 1993]

The 6-aminoquinolone had previously been identified as a new class of quinolone antibacterial agents. To continue SAR study in this series, Cecchetti, V., et al., synthesized novel 6-amino-8-methylquinolone derivatives and evaluated for in vitro antibacterial activity. The coupled presence of a methyl group at the C8 position with an amino group at C6 is effective for enhancing antibacterial activity, particularly against Gram-positive bacteria. [Cecchetti, V., et al., 1996a]

In a furtherance of SAR study on the C6 position of quinolone antibacterials, a series of 6-desfluoro-8-methylquinolones were synthesized by Cecchetti, V., et al., and evaluated for their in vitro antimicrobial activity. As a result of this study, compounds with strong activity against Gram-positive bacteria, including ciprofloxacin-resistant and methicillin-resistant Staphylococcus aureus, were identified. The best Gram-positive antibacterial
activity was exhibited by piperidinyl derivative 39, which was 17 times more potent than CIP and displayed extremely high activity against *Streptococcus pneumoniae* with an MIC value of $< 0.016 \mu g/mL$. Thus, it was shown that substituent combinations in the quinolone ring, excluding the C$_6$ fluorine atom, might produce powerful antibacterial agents. [Cecchetti, V., *et al.*, 1996b]

![39]

New pyrrolidine derivatives, which bear an alkyloxime substituent in the 4-position and an aminomethyl substituent in the 3-position of the pyrrolidine ring, have been synthesized by Hong, C. Y., *et al.*, and coupled with various quinolone carboxylic acids to produce a series of new fluoroquinolone antibacterials. These fluoroquinolones were found to possess potent antimicrobial activity against both Gram-negative and Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA). Variations at the C$_8$ position of the quinolone nucleus included fluorine, chlorine, nitrogen, methoxy, and hydrogen atom substitution. The activity imparted to the substituted quinolone nucleus by the C$_8$ substituent was in the order F (C$_5$-NH$_2$) > F (C$_5$-H) > naphthyridine > Cl = OMe = H against Gram-positive organisms. In the case of Gram-negative strains, activity was in the order F (C$_5$-NH$_2$) > naphthyridine = F (C$_5$-H) > H > Cl > OMe. [Hong, C. Y., *et al.*, 1997]

Topoisomerase IV is the primary cellular target for most quinolones in Gram-positive bacteria; however, its interaction with these agents is poorly understood. Therefore, the effects of four clinically relevant antibacterial quinolones (CIP, and three new generation quinolones: trovafloxacin, LVFX, and SPFX) on the DNA cleavage / religation reaction of *Staphylococcus aureus* topoisomerase IV were characterized by Anderson, V. E., *et al.* These quinolones stimulated enzyme-mediated DNA scission to a similar extent, but their potencies varied significantly. Drug order in the absence of ATP was trovafloxacin
The most striking correlation, however, was between quinolone potency and Inhibition of enzyme-mediated DNA religation: the greater the potency, the stronger the inhibition. [Anderson, V. E., et al., 2000]

Fang, K. C., et al., report herein the synthesis and biological evaluation of two series of 7-substituted norfloxacin derivatives. Most compounds tested in this study demonstrated better activity against MRSA than NOR. Preliminary in vitro evaluation indicated that the 7-[4-(2-hydroxyiminoethyl)piperazin-1-yl] derivatives possess distinct cytotoxicity profiles as compared with their alpha-methylene-gamma-butyrolactone counterparts, i.e., excellent activities against the renal cancer subpanel. Among them, 1-ethyl-6-fluoro-7-[4-[2-(4-chlorophenyl)-2-hydroxyiminoethyl]-1-piperazinyl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (40) demonstrated the most significant activities against renal cancer cell lines, with log GI50 values of -6.40 against CAK-1, -6.14 against RXF 393, and -7.54 against UO-31, compared with a mean log GI50 value of -5.03. [Fang, K. C., et al., 2000]

Kawakami, K., et al., synthesized a series of 8-methoxyquinolones bearing 3-amino-4-methylpyrrolidines or 3-amino-4-fluoromethylpyrrolidines at the C7 position and evaluated for their physicochemical and biological properties. All of the compounds synthesized showed more potent activity than LVFX against both Gram-positive and negative bacteria. Increases in lipophilicity of these compounds had desirable effects on their potency of single intravenous toxicity and pharmacokinetic profiles in animals. Among the compounds synthesized, 1-fluorocyclopropyl derivatives, and 7-(cis-3-amino-4-fluoromethylpyrrolidinyl) derivative showed negative responses in the micronucleus test in mice while 1-cyclopropyl-7-(3-aminopyrrolidinyl) derivative showed a positive
response. These results suggested that the introduction of a fluorine atom into the 3-aminopyrrolidinyl substituent resulted in favorable influence on genetic toxicity as well as into the N_1 cyclopropyl substituent. [Kawakami, K., et al., 2000]

Chen, Y. L., et al., reported the synthesis of a number of 7-substituted quinolone derivatives and evaluated for antibacterial and cytotoxic activities. Preliminary results indicated that most compounds tested in this study demonstrated better activity against MRSA than NOR. Among them, 1-(4-amino-2-fluorophenyl)-6-fluoro-1,4-dihydro-7-[4-[2-(4-methoxyphenyl)-2-hydroxyiminoethyl]-1-piperazinyl]-4-oxo-3-quinolinecarboxylic acid (41) and its ketone precursor exhibited significant activities against Klebsiella pneumoniae, MRSA, erythromycin- and ampicillin-resistant Streptococcus pneumoniae, and vancomycin-resistant Enterococcus faecalis. Due to strong cytotoxicities of 41 (a mean log GI<sub>50</sub> of -5.40), with good antibacterial activities and low cytotoxicities (a mean log GI<sub>50</sub> of -4.67), is a more potential drug candidate. [Chen, Y. L., et al., 2001]

![Chemical Structure of 41](image-url)

Herczegh, P., et al., reported that bisphosphonates conjugated to fluoroquinolone (42) antibacterials through an intermediate carbon had better activity than conjugates lacking the carbon. Virtually all molar-based activity of these esterified bisphosphonate derivatives was identical to that of its parent. De-esterified free-acid forms retained good activity against most Gram-negative bacteria, but not against Gram-positives. A free-acid derivative remained bound to washed bone and completely inhibited Staphylococcus aureus growth. The more potent parent CIP, failed to bind significantly, resulting in the occurrence of bacterial growth. [Herczegh, P., et al., 2002]
Miolo, G., et al., investigated a representative set of potent antibacterial 6-desfluoro-8-methylquinolones, in which the C$_6$ fluorine atom is replaced by -NH$_2$ or -H, and their 6-fluoro counterparts, to evaluate their phototoxic potential and to explore the mechanism behind their phototoxicity. The capacity to photosensitize biological substrates (lipids, proteins, DNA) has been analyzed, as well as their photocytotoxicity on red blood cells and 3T3 murine fibroblasts. The results obtained show that a major correlation with phototoxicity lies in the structure of the individual antibacterials and their hydrophobicity; in particular, 6-amino derivatives are less phototoxic than corresponding unsubstituted and fluorinated compounds. Cellular phototoxicity was inhibited by the addition of free radical and hydroxyl radical scavengers (BHA, GSH and DMTU), suggesting the involvement of a radical mechanism in their cytotoxicity. A good correlation was observed between lipid peroxidation and phototoxicity, indicating that the test compounds exert their toxic effects mainly in the cellular membrane. [Miolo, G., et al., 2002]

Hu, X. E., et al., designed novel quinolone antibacterial agents bearing (3S)-amino-(4R)-ethylpiperidines by using low energy conformation analysis and synthesized by applying a conventional coupling reaction of the quinolone nuclei with new piperidine side chains. These compounds were tested in MIC assays and found to be highly potent against Gram-positive and Gram-negative organisms. In particular, the new compounds exhibited high activity against the resistant pathogens *Staphylococcus aureus* (MRSA) and *Streptococcus pneumoniae* (penicillin resistant). Importantly, when the (3S)-amino-(4R)-ethylpiperidinyl quinolones (43) were compared with marketed quinolones sharing the
same quinolone nuclei but different side chains at the C$_7$ position, the new quinolones showed superior activity against Gram-positive organisms, including resistant pathogens. [Hu, X. E., et al., 2003]

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Inagaki, H., et al., synthesized a series of novel 5-amino-6-fluoro-1-[(1R,2S)-2-fluorocyclopropan-1-yl]-8-methylquinolones bearing fluorinated (3R)-3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl substituents at the C$_7$ position to obtain potent drugs for infections caused by Gram-positive pathogens, which include resistant strains such as MRSA, penicillin-resistant \textit{Streptococcus pneumoniae} (PRSP), and vancomycin-resistant enterococci (VRE). These fluorinated compounds exhibited potent antibacterial activity comparable with that of a compound bearing a non-fluorinated (3R)-3-(1-aminocyclopropan-1-yl)pyrrolidine moiety at the C$_7$ position (\textbf{44}) and had at least 4 times more potent activity against representative Gram-positive bacteria than CIP, GAT, or MXFX. Among them, the 7-[(3S,4R)-4-(1-aminocyclopropan-1-yl)-3-fluoropyrrolidin-1-yl] derivative (\textbf{45}), which showed favorable profiles in preliminary toxicological and nonclinical pharmacokinetic studies, exhibited potent antibacterial activity against clinically isolated resistant Gram-positive pathogens. [Inagaki, H., et al., 2003]

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\text{\textbf{44} } R_1 = R_2 = H \\
\text{\textbf{45} } R_1 = F; R_2 = H
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Kuramoto, Y., et al., designed \( m \)-aminophenyl groups as novel \( N_1 \) substituents of naphthyridones and quinolones. Among newly synthesized compounds, 7-(3-aminoazetidin-1-yl)-1-(5-amino-2,4-difluorophenyl)-8-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (46) has extremely potent antibacterial activities against Gram-positive as well as Gram-negative bacteria. This compound is significantly more potent than trovafloxacin against clinical isolates: 30 times against *Streptococcus pneumoniae* and 128 times against MRSA. The SAR study revealed that a limited combination of 1-(5-amino-2,4-difluorophenyl) group, 7-(azetidin-1-yl) group, and 8-Cl atom (or Br atom or Me group) gave potent antibacterial activity. An X-ray crystallographic study of a 7-(3-ethylaminoazetidin-1-yl)-8-chloro derivative demonstrated that the \( N_1 \) aromatic group was remarkably distorted out of the core quinolone plane by steric repulsion between the \( C_8 \) chlorine atom and the \( N_1 \) substituent.

Furthermore, a molecular modeling study of 46 and its analogues demonstrated that a highly distorted orientation was induced by a steric hindrance of the \( C_8 \) substituent, such as chlorine, bromine, or a methyl group. Thus, their highly strained conformation should be a key factor for the potent antibacterial activity. [Kuramoto, Y., et al., 2003]

A series of N-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl]piperazinyl quinolones were synthesized by Foroumadi, A., et al., and evaluated for *in vitro* antibacterial activity against some Gram-positive and Gram-negative bacteria. Compound 47 (ciprofloxacin analogue) was the most active compound against Gram-positive bacteria (MIC = 0.008 - 0.015 \( \mu g \text{ mL}^{-1} \)). [Foroumadi, A., et al., 2003c]
Baker, W. R., et al., prepared a fluoroquinolone prodrug, PA2808 (49), and shown to convert to the highly active parent drug PA2789 (48). *In vitro* and *in vivo* activation of 49 by alkaline phosphatase was demonstrated using disk diffusion and rat lung infection models. The water solubility of 49 showed a marked increase compared to 48 over a pH range suitable for aerosol drug delivery. A total of forty eight analogues based on 48 were prepared and screened against a panel of Gram-positive and Gram-negative pathogens. Incorporating a cyclopropane-fused pyrrolidine (amine) at C7 resulted in some of the most active analogues. [Baker, W. R., *et al.*, 2004]

Asahina, Y., *et al.*, synthesized novel 1-trifluoromethyl-4-quinolone derivative and evaluated the antibacterial activity of each. An oxidative desulfurization – fluorination reaction was employed to introduce a trifluoromethyl group at the N1 position as a key step. Among the derivatives, 50 was found to exhibit antibacterial activity comparable to that of NOR against *Staphylococcus aureus*, *Streptococcus pneumoniae* IID1210, and *Escherichia coli* NIHJ JC-2. [Asahina, Y., *et al.*, 2005a]
Asahina, Y., et al., synthesized novel 1-(2-fluorovinyl)-4-quinolone-3-carboxylic acid derivatives, conformationally restricted analogues of fleroxacin and evaluated their *in vitro* antibacterial activity. A dehydrosulfenylation of a 2-fluoro-2-[(4-methoxyphenyl)sulfinyl]ethyl group was employed as a key step for the construction of a 2-fluorovinyl group at the N₁ position. It appeared evident that the Z-isomers exhibited 2- to 32- fold more potent *in vitro* antibacterial activity than the corresponding E- isomers. Furthermore, since 51 showed *in vitro* antibacterial activity and DNA gyrase inhibition comparable to that of fleroxacin, it was hypothesized that the conformation of 51 would be equivalent to the active conformer of fleroxacin. The results revealed that the antibacterial Z-1-(2-fluorovinyl)quinolone derivatives carry the novel N₁ substituent of the fluoroquinolones. [Asahina, Y., *et al.*, 2005b]

Dizman, B., *et al.*, synthesized a novel methacrylate monomer containing a quinolone moiety and homopolymerized in N,N-dimethylformamide (DMF) by using azobisisobutyronitrile (AIBN) as an initiator. The new monomer was copolymerized with poly(ethylene glycol) methyl ether methacrylate (MPEGMA) in DMF using the same initiator. The monomer, homopolymer, and copolymer were characterized by elemental analysis, thermo gravimetric analysis (TGA), differential scanning calorimetry (DSC), size exclusion chromatography (SEC), FTIR, $^{13}$C NMR, and $^1$H NMR. The antibacterial activities of the monomer as well as polymers were investigated against *Staphylococcus*
aureus and Escherichia coli, which are representative of Gram-positive and Gram-negative bacteria, respectively. All compounds showed excellent antibacterial activities against these two types of bacteria. The antibacterial activities were determined using the shaking flask method, where 25 mg/mL concentrations of each compound were tested against $10^5$ CFU/mL bacteria solutions. The number of viable bacteria was calculated by using the spread plate method, where 100 µL of the incubated antibacterial agent in bacteria solutions were spread on agar plates and the number of viable bacteria was counted after 24 h of incubation period at 37 °C.

philic polymer. The monomer and polymers showed excellent antimicrobial activities against S. aureus and E. coli. These results indicate that the new monomer and polymers have potential as potent antimicrobial agents although mode of activity is not clear. Since these agents are relatively stable to high temperatures, they can be used for medical and biomaterial applications requiring thermal sterilization. [Dizman, B., et al., 2005]

Hansen, T. M., et al., prepared a series of 5-methoxy- and 5-hydroxy-6-fluoro-1,8-naphthyridone-3-carboxylic acid derivatives and evaluated for cell-free bacterial protein synthesis inhibition and whole cell antibacterial activity. When compared to the analogous 5-hydrogen compounds, the presence of the 5-hydroxyl group negatively affects biochemical potency. [Hansen, T. M., et al., 2005]

Nieto, M. J., et al., studied the SAR of new antibacterial benzenesulfonamide fluoroquinolones (BSFQs, 52), from derivatization of N^4-piperazinyl of CIP. The behavior of the new BSFQ series was similar to the NOR analogs, making possible a Quantitative Structure - Activity Relationships (QSAR) analysis of the complete set of BSFQs. The presence of the benzenesulfonylamido (BS) groups shifted the activity of classic antimicrobial fluoroquinolones from being more active against Gram-negative to Gram-positive strains. QSAR studies through Hansch analysis showed a linear correlation of the activity with electronic and steric parameters. Small electron-donor groups would increase the in vitro activity against Gram-positive bacteria. Hydrophobic properties played a minor role when activity is measured as MIC. [Nieto, M. J., et al., 2005]
Zhi, C., *et al.*, reported novel Gram-positive antibacterial compounds consisting of a DNA polymerase IIIC (pol IIIC) inhibitor covalently connected to a topoisomerase/gyrase inhibitor. Specifically, 3-substituted 6-(3-ethyl-4-methylanilino)uracils (EMAUs) in which the 3-substituent is a fluoroquinolone moiety (FQ) connected by various linkers were synthesized. The resulting "AU-FQ" hybrid (53) compounds were significantly more potent than the parent EMAU compounds as inhibitors of pol IIIC and were up to 64 - fold more potent as antibacterials *in vitro* against Gram-positive bacteria. The hybrids inhibited the FQ targets, topoisomerase IV and gyrase, with potencies similar to NOR but 10 - fold lower than newer agents, for example, CIP and SPFX. Representative hybrids protected mice from lethal *Staphylococcus aureus* infection after intravenous dosing, and one compound showed protective effect against several antibiotic-sensitive and -resistant Gram-positive infections in mice. The AU - FQ hybrids are a promising new family of antibacterials for treatment of antibiotic-resistant Gram-positive infections. [Zhi, C., *et al.*, 2006]