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Fluoroquinolones as versatile scaffolds: Potential for targeting classical and novel mechanisms to combat antibacterial resistance

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ABSTRACT

Antibiotics play an essential role in combating infectious diseases. Due to the emergence of multidrug-resistant bacteria, the efficacy of antibiotic therapy is continually decreasing. Consequently, there is an urgent need for the development of novel antibiotics, preferably with novel targets that have not yet been clinically exploited and/or multiple mechanisms of action, reducing the probability of fast resistance development. Recently, several new promising antibacterial targets have been identified, including *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate synthase, metal-dependent deacetylase, and carbonic anhydrase. Additionally, inhibition of biofilm formation enhances bacterial susceptibility to antibiotics and potentially minimizes the risk of resistance development. This review discusses fluoroquinolones as versatile scaffolds, covering their structure-activity relationships, recent modifications and their role in inhibiting multiple bacterial targets. Multi-target fluoroquinolone derivatives exhibit enhanced activity against multidrug-resistant bacteria, including Gram-positive, Gram-negative, and mycobacterial species. Therefore, the continued optimization of fluoroquinolone structures represents an attractive approach to combat antibacterial resistance and achieve better therapeutic outcomes.

1. Introduction

1.1. Challenges of multidrug resistance

Multidrug resistance (MDR) is defined as insensitivity of a microorganism to several antimicrobial agents which are structurally unrelated and have different molecular targets, despite earlier sensitivity to them (Magiorakos et al., 2012, Nikaido, 2009). Bacterial strains are considered multidrug-resistant, if they are resistant to three or more classes of antimicrobials (van Duin and Paterson, 2016). Studies and reports from the World Health Organization (WHO) have indicated particularly high rates of resistance in *Escherichia coli* against cephalosporin and fluoroquinolones, *Klebsiella pneumoniae* against cephalosporin and carbapenems, *Staphylococcus aureus* against methicillin, *Streptococcus pneumoniae* against penicillin, and *Mycobacterium tuberculosis* against rifampicin, isoniazid, and fluoroquinolone (Tanwar et al., 2014). Currently, approximately 70 percent of bacteria that cause hospital-acquired infections are resistant to at least one of the most commonly used drugs, representing an escalating public health threat (Van Goethem et al., 2024). A concerning rise in resistance among bacteria causing community-acquired infections has also been observed, particularly in staphylococci and pneumococci. Multidrug resistance can arise from two key mechanisms: accumulation of resistance genes on plasmids and overexpression of efflux pumps that expel a broad range of antibiotics. Additionally, biofilm formation contributes to bacterial resistance by providing a protective environment (Nikaido, 2009, Uddin et al., 2021). Understanding these mechanisms is important to understand the adaptation and survival of bacteria against antimicrobial agents and to improve the task of treating infectious diseases worldwide. Given these challenges, there is an urgent need for innovative antibiotic strategies. This review highlights fluoroquinolones—known for targeting DNA gyrase—as promising scaffolds for the development of multi-target antibacterial agents. We explore how structural modifications to fluoroquinolones enable activity against both classical and emerging bacterial targets, offering new approaches to overcome multidrug resistance. The review begins with a summary of resistance

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mechanisms, followed by discussion of novel targets, and concludes with strategies for optimizing fluoroquinolones as multi-target agents.

1.2. Mechanisms of bacterial resistance

1.2.1. Intrinsic resistance

Intrinsic resistance arises from the inherent biological characteristics of bacteria and is not directly related to the exposure to antibiotics. It can be due to the absence of the target of the antibiotic, or to antibiotics not reaching their target (Van Goethem et al., 2024). This type of resistance reflects evolutionary traits that are encoded in the bacterial genome, rather than responses to environmental stress or specific antibiotic classes. An example of intrinsic resistance is *Pseudomonas aeruginosa*, whose low membrane permeability is likely to be a main reason for its innate resistance to many antimicrobials. Similarly, enterococci are intrinsically resistant to β-lactams, as their penicillin binding proteins (PBP) have a naturally low affinity for β-lactam antibiotics (Kakoullis et al., 2021).

1.2.2. Acquired resistance

Acquired antibiotic resistance is the result of a specific selection pressure that promotes selection of molecular mechanisms that enable a previously sensitive bacterial population to become resistant against the selecting agent (Magiorakos et al., 2012, Almagor et al., 2018). As illustrated in Fig. 1, acquired resistance mechanisms can be classified into four main types: limited uptake of a drug, modifying a drug target, inactivating a drug, and active drug efflux (Uddin et al., 2021, Reygaert, 2018, Varela et al., 2021, Devi et al., 2024).

1.3. Multidrug resistance in Gram-positive and Gram-negative bacteria

Undoubtedly, one of the most common challenges is multidrug resistance in Gram-positive pathogens. Numerous studies have shown the wide spread of antibiotic-resistant clones of Gram-positive organisms such as β-lactamase-resistant *S. pneumoniae*, methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant enterococci (VRE) (Cornaglia, 2009). Resistance in Gram-positive bacteria arises primarily through two mechanisms: enzymatic destruction of antibiotics via β-lactamase production and the acquisition of alternative target enzymes, such as the *mecA* gene in *Staphylococcus aureus*, which encodes PBP2a—a penicillin-binding protein with reduced affinity for β-lactam antibiotics, allowing continued cell wall synthesis even in the presence of these antibiotics. A recent study detected the *mecA* gene in both clinical and environmental MRSA isolates and demonstrated its potential inhibition using selected phytochemicals through *in vitro* and *in silico* methods (Mohammed et al., 2025). Similarly, VRE acquire *vanA* or *vanB* operons, which alter the D-Ala-D-Ala terminus of peptidoglycan precursors to D-Ala-D-Lac, reducing binding affinity for vancomycin. Notably, a clinical *Enterococcus faecium* isolate was recently identified that had both *vanA* and *vanB* genes and exhibited high-level resistance not only to vancomycin, but also to linezolid and tigecycline (Wardal et al., 2023).

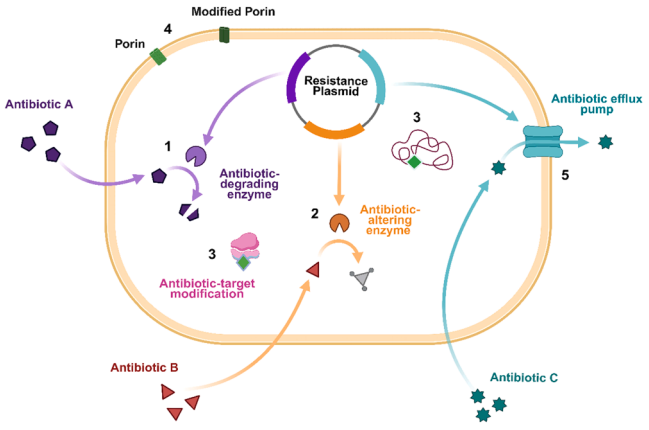


Fig. 1. The major acquired-resistance mechanisms in bacteria: (**1, 2**) enzymatic degradation or modification. (**3**) modification of antibiotic target. (**4**) decrease of cell envelope permeability, and (**5**) antibiotic export by efflux pumps (Varela et al., 2021). Created with BioRender (**2025**). https://BioRender.com/.

Gram-negative bacteria such as *E. coli, K. pneumoniae* or *P. aeruginosa* present an even more complex scenario than Gram-positive bacteria due to their unique structure and resistance mechanisms. Nosocomial infections caused by Gram-negative bacilli represent a major challenge for the healthcare system due to their resistance to antibiotics (Doi et al., 2017). Antibiotic resistance in Gram-negative bacteria mainly arises from the expression of antibiotic-inactivating enzymes, such as extended-spectrum β-lactamases (ESBLs) like CTX-M-15 commonly found in *E. coli* (Doerr et al., 2023). Alterations in outer membrane permeability, such as loss of porins OmpK35/36 in *K. pneumoniae*, decrease antibiotic influx and contribute to resistance (Elías-López et al., 2024). Modifications of bacterial targets, including mutations in *gyrA* and *parC* genes, result in fluoroquinolone resistance in *E. coli* (Slettemeås et al., 2024). Additionally, resistance can be further exacerbated by increased intrinsic resistance due to chromosomal changes (e.g., enhanced expression of antibiotic-inactivating enzymes, efflux pumps, or target modifications) or by the acquisition of mobile genetic elements, such as plasmids encoding β-lactamases and aminoglycoside-modifying enzymes (Reygaert, 2018).

1.4. Multidrug resistance in mycobacteria

Mycobacterium tuberculosis (*Mtb*) is the causative agent of tuberculosis (TB), which remains one of the top ten causes of death and is the first-leading cause of death from a single infectious agent, surpassing both COVID-19 and HIV/AIDS (Iacobino et al., 2020, Berida and Lindsley, 2024). In 2023, the WHO estimates of the global burden of TB were 7.5 million cases and 1.3 million deaths. Furthermore, about 1.7 billion people are known to be latently infected with *Mtb*, with about 10% of them reactivating to active TB in their lifetime (Iacobino et al., 2020). Isoniazid, rifampicin, pyrazinamide, and ethambutol are potent first-line antitubercular drugs (Singh et al., 2015). However, the treatment of TB with first-line antitubercular drugs is becoming ineffective because of the emergence of multidrug-resistant tuberculosis (MDR-TB), resistant to at least isoniazid and rifampicin, and extensively drug-resistant strains of *Mycobacterium* (XDR-TB) resistant to isoniazid and rifampicin, as well as to any fluoroquinolone and at least one of three injectable second-line drugs (amikacin, kanamycin, or capreomycin). Thus, the development of new antimycobacterial drugs based on scaffolds having the ability to inhibit the growth of *Mtb* is of great interest (Singh et al., 2015, Pecora et al., 2021).

1.4.1. Mechanisms of multidrug resistance in mycobacteria

Drug resistance in mycobacteria arises through both intrinsic and acquired mechanisms, enabling *M. tuberculosis* to withstand various antimicrobial agents. **Intrinsic drug resistance** in *M. tuberculosis* is attributed to the unique properties of its cell envelope (Fig. 2), which contains mycolic acids, i.e., high-molecular-weight α-alkyl-β-hydroxy fatty acids covalently bound to arabinogalactan. This structure forms a highly hydrophobic barrier that contributes to the resistance of the bacterium (Abrahams and Besra, 2018).

In contrast, **acquired drug resistance** in *M. tuberculosis* is primarily due to spontaneous mutations in genes encoding specific drug targets in the chromosomal DNA (Aldred et al., 2016). For instance, fluoroquinolones, such as levofloxacin and moxifloxacin, are crucial therapeutic agents for treating multidrug-resistant TB that inhibit the Mtb DNA gyrase enzyme, which is essential for DNA replication. Mutations in the genes encoding DNA gyrase (*gyrA* and *gyrB*) can alter the enzyme's structure, making the bacterium resistant to fluoroquinolones and

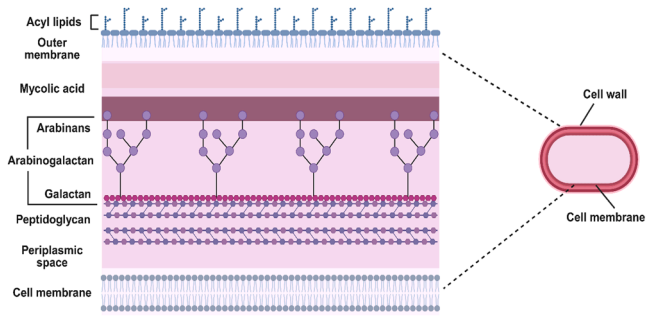


Fig. 2. Diagram of the basic components of the mycobacterial cell envelope (Abrahams and Besra, 2018). The envelope consists of a cell membrane, peptidoglycan, arabinogalactan, mycolic acids, and surface acyl lipids. Created with BioRender (**2025**). https://BioRender.com/.

leading to broad cross-resistance to this class of drugs (Aldred et al., 2016, Von Groll et al., 2009).

1.4.2. Current drug targets in M. tuberculosis

Despite advances in understanding the biology of *M. tuberculosis*, no new drug has been approved for the treatment of tuberculosis in the last three decades, except for bedaquiline, which received the U.S. Food and Drug Administration (FDA) approval in 2012. Therefore, it is necessary to identify new drug targets against *Mtb* to develop more effective treatments (Zhang et al., 2024, Desai et al., 2016). Extensive efforts in the past decade have been directed towards the discovery of novel anti-TB drugs, with special emphasis on targeting key and common biological processes, including DNA replication, protein synthesis, cell wall biosynthesis, and energy metabolism (Shetye et al., 2020, Bhat et al., 2018).

Fluoroquinolones, including moxifloxacin, levofloxacin, ofloxacin, and gatifloxacin, are broad-spectrum antimicrobial agents that inhibit mycobacterial DNA replication. These antibiotics act by inhibiting DNA gyrase (topoisomerase II), a key enzyme responsible for relieving supercoils and maintaining DNA integrity in *Mtb*. By interfering with DNA replication and repair, fluoroquinolones effectively hinder bacterial growth, making them essential in the treatment of MDR-TB and XDR-TB (Shetye et al., 2020, Bhat et al., 2018).

Disrupting ribosomal assembly or any step involved in protein synthesis is another key strategy in antimycobacterial drug discovery (Yin et al., 2019). Several classes of antibiotics that inhibit mycobacterial protein synthesis include aminoglycosides, such as amikacin, capreomycin, kanamycin, and streptomycin, as well as oxazolidinones like linezolid. Aminoglycosides exert their bactericidal effect by binding to the 30S ribosomal subunit, leading to misreading of mRNA and inhibition of protein synthesis, eventually causing bacterial cell death. Capreomycin, although structurally different, has similar effects by disrupting ribosomal function. Linezolid targets the 50S ribosomal subunit, preventing the formation of the initiation complex that is necessary for protein synthesis (Bhat et al., 2018, Bandodkar et al., 2020).

The cell envelope of *Mtb* is uniquely structured with a high content of lipids and carbohydrates, which act as an impermeable barrier against hydrophilic antimicrobial agents (Vincent et al., 2018, Chiaradia et al., 2017). Antibiotics that target the mycobacterial cell envelope include isoniazid, ethambutol and cycloserine. Isoniazid interferes with biosynthesis of mycolic acid, a crucial component of the mycobacterial cell envelope. Isoniazid is a prodrug that is activated by the mycobacterial catalase-peroxidase enzyme KatG. Once activated, it forms a complex with NAD^+, which inhibits the enoyl-ACP reductase InhA, a key enzyme in the fatty acid elongation cycle of mycolic acid synthesis. Ethambutol inhibits arabinogalactan synthesis, an important polymer connecting the peptidoglycan and mycolic acid layers. It inhibits the arabinosyl transferases, enzymes responsible for polymerizing arabinose residues during arabinogalactan biosynthesis. Cycloserine disrupts peptidoglycan formation by inhibiting enzymes that are involved in the synthesis of cell wall precursors: alanine racemase, which converts L-alanine to D-alanine, and D-alanine–D-alanine ligase, which forms the D-Ala-D-Ala dipeptide necessary for peptidoglycan cross-linking (Bhat et al., 2018).

Targeting energy metabolism in *Mtb* also offers a promising approach for drug development (Bald et al., 2017). Key antibiotics targeting mycobacterial energy metabolism include pyrazinamide and bedaquiline. Pyrazinamide disrupts energy production by inhibiting the fatty acid synthase I system and interfering with membrane potential. Bedaquiline, a novel diarylquinoline, specifically targets the mycobacterial ATP synthase, disrupting ATP production and resulting in bacterial cell death (Shetye et al., 2020, Bhat et al., 2018, Bandodkar et al., 2020).

2. Novel antibacterial targets

As multidrug resistance continues to compromise the efficacy of existing antibiotics, the identification of novel bacterial targets has become a key approach in developing more effective antibiotics. Fluoroquinolones, known for their broad-spectrum activity and synthetically facile core structure, offer a strong foundation for designing new agents aimed at both classical and emerging targets. In this chapter, we first explore promising novel bacterial targets essential for survival and pathogenicity. This is followed by a discussion of fluoroquinolones more broadly, including their structure–activity relationships (SAR) and important modifications that highlight their potential as scaffolds for the next generation of multifunctional antibiotics incorporating pharmacophores that allow them to exert activity against novel targets in addition to their classical topoisomerase inhibition capabilities.

2.1. N-Acetylglucosamine-6-phosphate deacetylase (NagA)

The cell wall of bacteria consists of a peptidoglycan known as murein that surrounds the cytoplasmic membrane and that is decorated with other glycopolymers, such as teichoic acids or polysaccharides, and proteins. The cell wall serves several functions during bacterial growth, such as preserving the integrity and shape of the bacterial cell and withstanding osmotic pressure. Furthermore, it must remain flexible to accommodate the remodeling that is required for cell division and growth (Chapot-Chartier and Kulakauskas, 2014, Dörr et al., 2019). The peptidoglycan layer contains a glycan backbone, which is made up of both *N*-acetylated muramic acid and glucosamine, and highly cross-linked with oligopeptides (Reith and Mayer, 2011, Tian et al., 2022).

N-Acetylglucosamine-6-phosphate deacetylase (NagA) belongs to the amidohydrolase superfamily and catalyzes the deacetylation of *N*-acetylglucosamine-6-phosphate (GlcNAc6P) to glucosamine-6-phosphate (GlcN6P). GlcN6P is a precursor in the biosynthesis of peptidoglycan, a major component of the bacterial cell wall, which consists primarily of repeating units of MurNAc (*N*-acetylmuramic acid) and GlcNAc (*N*-acetylglucosamine) (Yadav et al., 2011, Ferreira et al., 2006). NagA catalyzes a key enzymatic step in the recycling of peptidoglycan fragments, producing essential amino–sugar precursors required for new cell wall biosynthesis. Fig. 3 illustrates the broader amino sugar metabolism pathway for context, as this review focuses specifically on the role of NagA in catalyzing the deacetylation of GlcNAc6P, highlighted in red (Ahangar et al., 2018). Consequently, NagA is considered as a promising antibacterial and antimycobacterial drug target, as its inhibition blocks the biosynthesis of peptidoglycan, making the bacterial cell wall fragile and increasing the susceptibility of bacteria, including *Mtb*, to antimicrobial agents (Abrahams and Besra, 2021).

2.1.1. 3D Structure of N-acetylglucosamine-6-phosphate deacetylase enzyme (NagA)

The structure of NagA is exemplified by *Mycolicibacterium smegmatis*

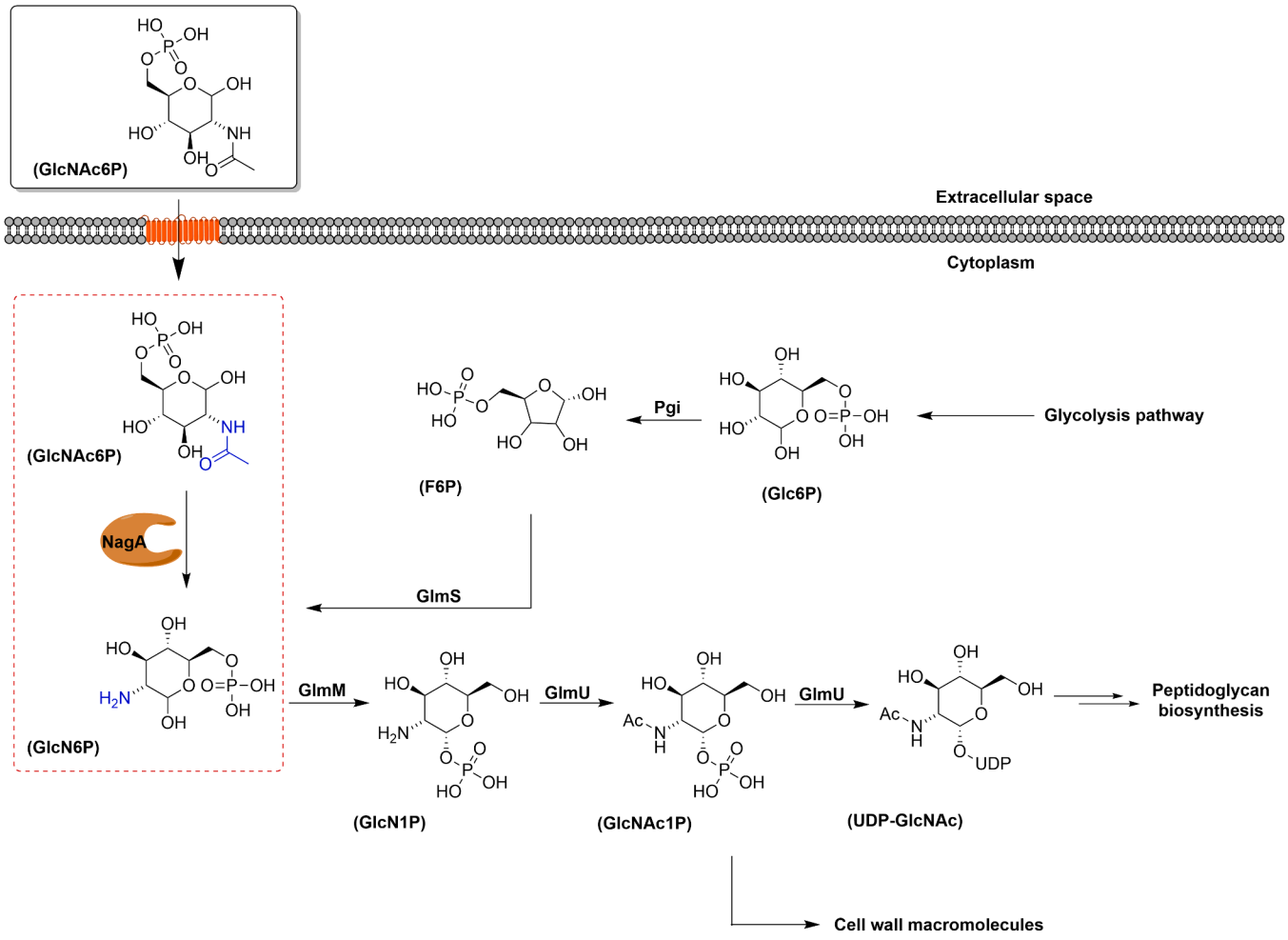


Fig. 3. The pathways of amino sugar metabolism, with the NagA-catalyzed deacetylation of GlcNAc6P to GlcN6P highlighted in red (Ahangar et al., 2018). Created by ChemDraw, version 23.1.1.3. Abbreviations: F6P, fructose-6-phosphate; Glc6P, glucose-6-phosphate; GlcN1P, glucosamine-1-phosphate; GlcN6P, glucosamine-6-phosphate; GlcNAc1P, *N*-acetylglucosamine-1-phosphate; GlcNAc6P, *N*-acetylglucosamine-6-phosphate; GlmM, phosphoglucosamine mutase; GlmS, glutamine—fructose-6-phosphate aminotransferase; GlmU, bifunctional *N*-acetyltransferase/uridyltransferase; Pgi, phosphoglucose isomerase; UDP-GlcNAc, UDP-*N*-acetylglucosamine.

NagA, a metal-dependent amidohydrolase, which is very similar to other NagA enzymes. The structure can be divided into two chains and each one consists of two domains. Domain I (residues 52–344) is a twisted $(\beta/\alpha)_8$-barrel that is comprised of eight alternating β-strands and α-helices. This domain encloses the catalytic site, which contains both Zn^{2+} and Cd^{2+} ions as observed in the crystal structure, with Cd^{2+} identified as the catalytically active metal ion responsible for enzymatic activity, whereas Zn^{2+} is not catalytically active in this enzyme. Domain II is a small β-barrel comprising eight β-strands contributed from the N and C termini of the *Mycobacterium smegmatis* NagA protein (amino acids 1–51 and 344–382) as shown in Fig. 4 (Ahangar et al., 2018). Although NagA is recognized as a critical metabolic checkpoint in bacterial pathogens like *M. tuberculosis* and *E. coli*, no well-defined clinically validated inhibitors targeting this enzyme have been reported to date. Most studies focus on its structural and functional properties, emphasizing the enzyme's active site, the role of divalent metal ions, and substrate analogs, to guide preliminary inhibitor design efforts (Ramharack et al., 2023).

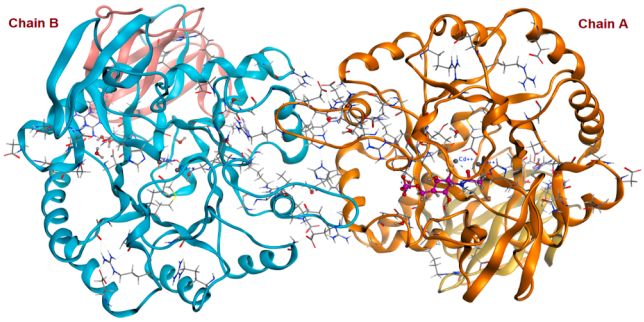


Fig. 4. *Mycobacterium smegmatis* NagA structure (PDB code: 6fv4) with two subunits (chains A and B). Domain I is colored brown (chain A) and cyan (chain B), and domain II is colored yellow (chain A) and pink (chain B). The metal ions are represented as gray spheres, and the GlcNAc6P ligand (magenta) is shown in ball and stick representation (Ahangar et al., 2018). Created by Molecular Operating Environment program.

2.2. Glucosamine-6-phosphate synthase

L-Glutamine: glucosamine-6-phosphate synthase (G6P synthase), also known as D-fructose 6-phosphate amidotransferase, catalyzes the first step in hexosamine biosynthesis by converting fructose-6-phosphate (F6P) into glucosamine-6-phosphate (GlcN6P), which is a precursor of uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), as shown in Fig. 5. *N*-Acetylglucosamine is an essential constituent of the peptidoglycan layer of the microbial cell wall (Stefaniak et al., 2022, Lather et al., 2020). Inactivation of G6P synthase is lethal to fungi and

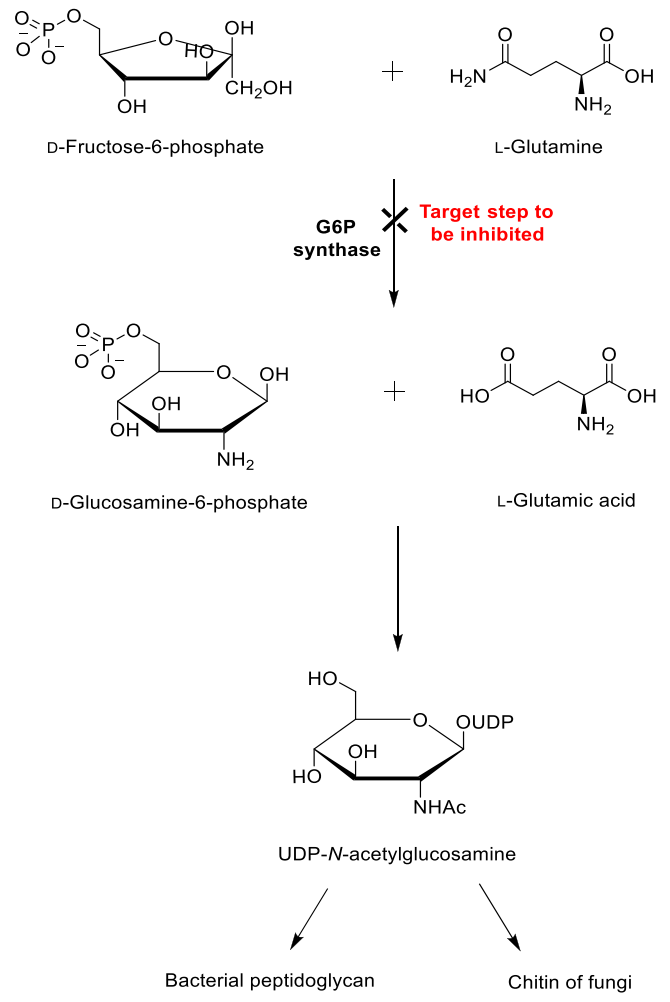


Fig. 5. The role of G6P synthase in cell wall biosynthesis (Lather et al., 2021). Created by ChemDraw, version 23.1.1.3.

bacteria because these organisms have rapid growth rates and short doubling times, requiring continuous synthesis of cell wall components like peptidoglycan for successful cell division. Inhibiting this enzyme quickly disrupts cell wall biosynthesis, leading to fungicidal and bactericidal effects. However, a temporary reduction in the amino sugar pool does not represent a threat to mammals, because mammalian cells have a longer life span, and the G6P synthase has a longer half-life. In addition, the expression of the mammalian gene encoding the enzyme G6P synthase is very rapid (Stefaniak et al., 2022, Wojciechowski et al., 2005). Accordingly, G6P synthase offers a potential target for the action of antimicrobial agents through disruption the cell wall biosynthesis.

Although no clinically approved inhibitors of glucosamine-6-phosphate synthase are available, several compounds have been investigated as possible inhibitors of this enzyme. These include glucosamine, 2-deoxyglucose, and fructose-6-phosphate analogs, which have shown different efficiencies in modulating G6P synthase activity. Despite these preliminary efforts, G6P synthase inhibition remains an underexplored area with high therapeutic potential, especially in diseases where the hexosamine biosynthetic pathway plays a crucial role, such as infectious diseases, cancer, diabetes, and inflammation. Further studies on the identification and optimization of selective inhibitors may provide new avenues to novel therapeutic strategies against this key enzyme (Andrade et al., 2014, Chittur and Griffith, 2002).

2.3. Metal-dependent deacetylase (LpxC)

Gram-negative bacteria are characterized by a unique three-layered envelope (Fig. 6) (Miller et al., 2016). The outer membrane (OM) consists of phospholipids in the inner leaflet of the membrane, while the outer leaflet is primarily composed of lipopolysaccharides (LPS), which are a hallmark feature of Gram-negative bacteria, distinguishing them from other species. Beneath the outer membrane lies the peptidoglycan cell wall, a rigid structure that provides shape and stability to the bacterial cell. The innermost layer is the inner membrane, a phospholipid bilayer responsible for a variety of vital processes, including structural integrity, nutrient transport, and biosynthetic processes. The space between the outer and inner membrane is referred to as periplasm and constitutes a distinct cellular compartment (Devi et al., 2024, Slettemeås et al., 2024).

2.3.1. Biosynthesis of lipid A

LPS consists of three major parts: lipid A, core oligosaccharide, and an O-antigen polysaccharide (MacNair et al., 2020). The biosynthesis of lipid A (Fig. 7) is known as the 'Raetz pathway' and is catalyzed by conserved enzymes, which are located in the cytoplasm or on the inner surface of the inner membrane (Barb and Zhou, 2008, Kalinin and Holl, 2017, Zuo et al., 2017). In *E. coli*, at first, UDP-*N*-acetylglucosamine (UDP-GlcNAc, **A**) reversibly undergoes a selective LpxA-catalyzed 3-O-acylation to form UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine (UDP-3-*O*-(acyl)-GlcNAc, **B**). The subsequent deacetylation of *N*-acetylglucosamine derivative **B** to form UDP-3-*O*-(acyl)-GlcN (**C**) is catalyzed by the Zn^{2+}-dependent enzyme LpxC in *E. coli* and the vast majority of other Gram-negative bacteria (Barb and Zhou, 2008, Kalinin and Holl, 2017, Zuo et al., 2017). Some LpxC enzymes are Mg^{2+}-dependent, for example, in *P. aeruginosa* (PDB code: 6mod) (Protein Data, 2025). The deacetylase LpxC is highly conserved in almost all Gram-negative bacteria and shows no sequence similarity to any mammalian proteins. Due to its key role in lipid A biosynthesis, LpxC is considered a promising target for the development of antibiotics that specifically target Gram-negative bacteria (Kalinin and Holl, 2017).

LpxC is a tightly regulated enzyme whose proper expression is critical for bacterial survival. Inhibition of LpxC disrupts outer membrane integrity, increasing permeability and weakening the bacterium to antimicrobials and attack by the host immune system. Conversely, overexpression leads to toxic accumulation of lipid A in the inner membrane, causing membrane rupture and cell death. This makes LpxC a promising target—not only for traditional inhibition but also for inducing its dysregulation. Such effects can be achieved by blocking downstream enzymes or inhibiting FtsH, the key protease that maintains LpxC at non-toxic levels through regulated proteolysis (Fivenson and Bernhardt, 2020).

2.3.2. 3D Structure of metal-dependent deacetylase (LpxC)

The LpxC enzyme of the hyperthermophilic bacterium *Aquifex aeolicus* serves as a representative structural example. It consists of two homologous domains, each one containing two α-helices sandwiched between two five-stranded β-sheets. The active-site cleft is located at the interface of the two domains and contains a Zn^{2+} or Mg^{2+}-binding motif and a hydrophobic tunnel formed by a βαβ subdomain. A catalytic magnesium or zinc ion at the base of the cleft is in a square pyramidal coordination by a solvent molecule and the residues His79, His238, and Asp242. The tunnel is required for efficient catalysis and can accommodate a long (C14) fatty acid side chain attached to the *N*-acetylglucosamine group of the UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine substrate (Fig. 8) (Whittington et al., 2003, Mochalkin et al., 2008).

2.3.3. Examples of reported LpxC inhibitors

The history of LpxC as a drug target started in the mid-1980s, before the discovery of the enzyme itself. Antibacterial LpxC inhibitors were

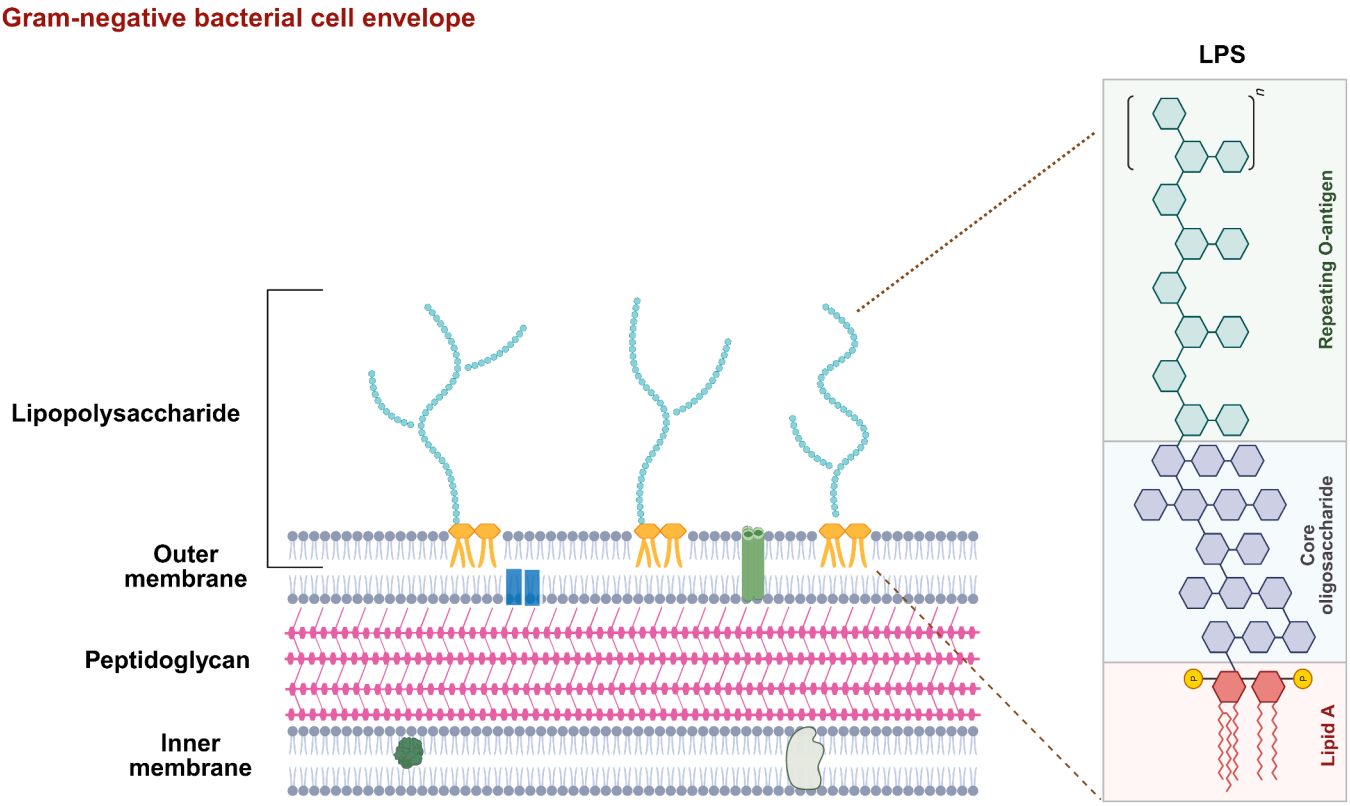


Fig. 6. Structure of the Gram-negative bacterial cell envelope (Bidne et al., 2018), highlighting the inner membrane, peptidoglycan, the outer membrane, as well as the molecular structure of LPS. Created with BioRender (**2025**). https://BioRender.com/.

originally discovered by random screening against *E. coli*. The development of LpxC inhibitors started with the discovery of L-573,655 (lead compound **1**) in 1996 (Fig. 9**A**). This compound had a minimal inhibitory concentration (MIC) for wild-type *E. coli* cultures of 200–400 mg/mL, but was later found to inhibit the *E. coli* LpxC enzyme with an IC_{50} of 8.5 μM. Approximately 200 analogs were synthesized, increasing potency 100-fold, concomitantly improving antibacterial activity (Erwin, 2016, Ding et al., 2018). However, despite extensive research, few inhibitors have reached clinic trials and none of them have reached the market. The most well-studied LpxC inhibitor is CHIR-090 **2**. It displayed high affinity for both *E. coli* and *P. aeruginosa* LpxC (IC_{50} ~4 nM) and remarkable antibacterial activity against a wide range of Gram-negative pathogens (MIC 0.2–2 μg/mL) (Erwin, 2016). Other LpxC inhibitors like BB-78485 **3** (IC_{50} 20 nM) and TU-514 **4** (IC_{50} 650 nM) bind and inhibit LpxC at nanomolar concentration *in vitro* and also exhibit antibacterial activity (Clayton et al., 2013). Later, Lee *et al.* and Liang *et al.* synthesized and investigated a series of compounds bearing linear diphenylacetylene scaffolds, such as LPC-058 **5** (IC_{50} 0.0035 nM), LPC-09 **6** (IC_{50} 2 nM), and compound **7** (IC_{50} 4 nM). Some of these compounds, especially LPC-058 **5**, exhibited excellent therapeutic potential against MDR/XDR strains *in vitro*, with MIC values typically ranging from 0.015 to 0.5 μg/mL. In 2012, Pfizer reported a series of novel LpxC inhibitors with a methylsulfone moiety, exemplified by compounds **8, 9** and **12** (IC_{50} 13.8, 3.6, and 2.8 nM, respectively). Compound ACHN-975 (**10**, IC_{50} 0.02 nM), developed by Achaogen, is the only LpxC inhibitor that reached phase I clinical trials, but has not progressed further (Ding et al., 2018, Lee et al., 2018). LpxC inhibitors have been widely reported with much effort focused on the hydroxamate class of inhibitors, where inclusion of a zinc binding hydroxamate group greatly increased binding to LpxC. Initial reports from Merck revealed an oxazoline (**11**, IC_{50} 50 nM) with a hydroxamic acid group as a potent inhibitor of *E. coli* LpxC (ecLpxC), but it showed weaker *in vitro* inhibition of *P. aeruginosa* LpxC (paLpxC) (Warmus et al., 2012).

Most LpxC inhibitors share the same pharmacophoric features, namely a hydroxamate head group, a linker, and a lipophilic tail (Lee et al., 2018). The active center of LpxC contains a catalytic zinc or magnesium ion, along with a hydrophobic tunnel that accommodates a myristate fatty acid side chain of the natural substrate. Accordingly, most LpxC inhibitors have a zinc-chelating group and a lipophilic tail that occupies the hydrophobic tunnel. The lipophilic tails of the LpxC inhibitors mediate important van der Waals interactions with the hydrophobic tunnel of LpxC, which is critical for their inhibition activity (Zuo et al., 2017, Ding et al., 2018, Warmus et al., 2012). The structure of a typical LpxC inhibitor can be divided into five parts as exemplified by CHIR-090 in Fig. 9**B**.

For the successful rational design of a novel antibacterial agent with the potential to inhibit the LpxC enzyme, it is crucial to consider these pharmacophoric features, particularly the metal-chelating group and the lipophilic tail, which are essential for binding to and interacting with the active site of LpxC. Optimizing these features can enhance the efficacy and selectivity of LpxC inhibitors, ultimately contributing to the development of potent antibacterial therapeutics.

2.4. Carbonic anhydrases

Carbonic anhydrases (CAs) are metalloenzymes, present in nearly all living organisms and encoded by eight evolutionarily unrelated gene families: the α-, β-, γ-, δ-, ζ-, η-, θ-, and ι-CAs (Supuran and Capasso, 2017, Supuran and Capasso, 2020). All these enzymes play a crucial role in the reversible hydration of carbon dioxide, transforming it into a bicarbonate ion and a proton. This process is vital for several physiological functions. It has been observed that abnormal levels or activities of these enzymes are often associated with different human diseases (Supuran and Capasso, 2020). All human CAs (hCAs) are members of the α-class, and there are currently 15 isoforms known to exist. These are distinguished by their molecular characteristics, oligomeric configuration,

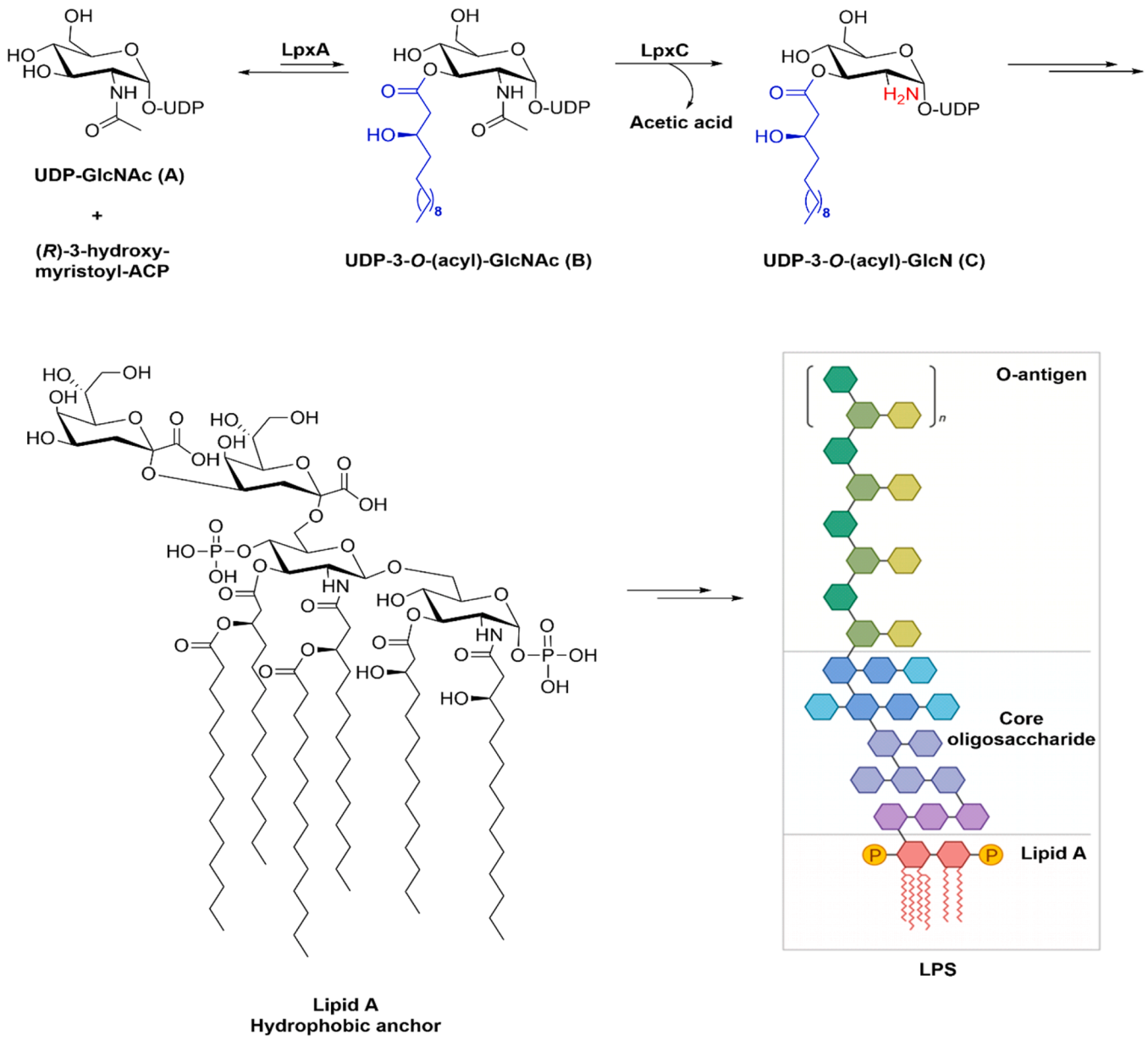


Fig. 7. Biosynthesis and structure of lipid A in *E. coli* (Whittington et al., 2003). Created by ChemDraw, version 23.1.1.3 and with BioRender (**2025**). https://BioRender.com/. Abbreviations: (*R*)-3-hydroxymyristoyl-ACP, (*R*)-3-hydroxymyristoyl-acyl carrier protein; UDP-3-*O*-(acyl)-GlcN, UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-glucosamine; UDP-3-*O*-(acyl)-GlcNAc, UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine; UDP-GlcNAc, UDP-*N*-acetylglucosamine.

cellular location, organ and tissue distribution, expression levels, kinetic characteristics, and reaction to various inhibitor classes (Alterio et al., 2012). Conversely, bacteria exhibit notable structural variations from the human isoforms, expressing the β- and γ-classes in addition to the α-class. Sequence identity between bacterial β- or γ-CAs and human α-CAs is generally below 25%, reflecting significant differences in both sequence and three-dimensional structure. By the same mechanism, CAs catalyze the straightforward but physiologically crucial hydration reaction of carbon dioxide to bicarbonate and protons in bacteria (Annunziato et al., 2016, Cheah et al., 2016). They also have a role in photosynthesis, electrolyte secretion, biosynthesis, and the transport and supply of CO_2 or HCO_3^- in maintaining pH homeostasis. Additionally, several antibacterial medications, including sulfanilamide, target CAs. Bacterial CAs are therefore of special interest since their inhibition lowers the production of virulence factors, impairs bacterial growth (bacteriostatic or bactericidal effects), and increases the susceptibility of the bacteria to host defense mechanisms. Because of all of this, CA is considered as a promising bacterial target for the development of new agents capable of combating multi-drug-resistant bacteria (Nishimori et al., 2008).

2.4.1. *Classes of bacterial CAs*

While the α- and β-CAs contain Zn^{2+} as catalytic metal, the γ-CAs are Fe^{2+}-dependent enzymes that are also active with bound Zn^{2+} or Co^{2+} (Pinard et al., 2015). The metal ion at the CA active site is coordinated by three His residues in the α- and γ-classes, and by one His and two Cys residues in the β-class. The fourth ligand is a water molecule or hydroxide ion, which functions as a nucleophile in the enzyme's catalytic cycle (Capasso and Supuran, 2015). In contrast to the mammalian α-CAs, bacterial α-CAs have only been inadequately characterized. Actually, the CAs from *Neisseria gonorrhoeae, Helicobacter pylori, Sulfurihydrogenibium yellowstonense, Sulfurihydrogenibium azorense*, and *Thermovibrio ammonificans* are the only bacterial α-CAs having a known three-dimensional structure (Kim et al., 2019). Many β-CAs, including

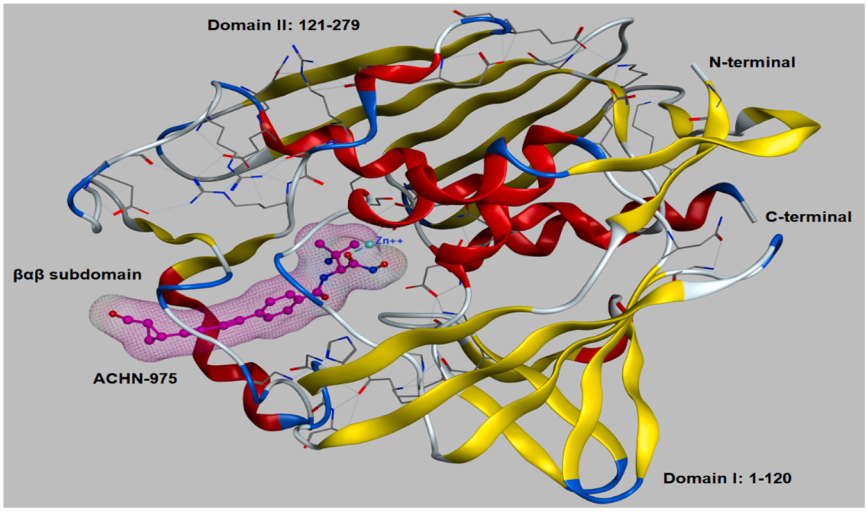


Fig. 8. 3D structure of *A. aeolicus* LpxC enzyme in complex with ACHN-975 inhibitor (PDB code: 6IH0) (Protein Data, 2024). ACHN-975 inhibitor is represented using a ball-and-stick model, with atoms color-coded as follows: magenta for carbon, blue for nitrogen, red for oxygen, and white for hydrogen. Created by Molecular Operating Environment program.

those from *Salmonella enterica, Pseudomonas aeruginosa, Haemophilus influenzae, H. pylori, Mycobacterium tuberculosis, Escherichia coli, Vibrio cholerae*, and *Streptococcus mutans*, have X-ray crystal structures available (Cronk et al., 2000, Covarrubias et al., 2006). CAM (Carbonic Anhydrase Methanosarcina) from *Methanosarcina thermophila* stands out as the only γ-class carbonic anhydrase enzyme that has been successfully crystallized so far (Supuran and Capasso, 2017). The two isoforms encoded by *Helicobacter pylori*, namely the cytosolic HpβCA and the periplasmic HpαCA, have been extensively studied and documented. Together with urease, both isoforms function as a buffering system to keep cells at a neutral pH and guarantee the pathogen's survival at the low pH of the stomach (Campestre et al., 2021).

2.4.2. 3D crystal structure of carbonic anhydrase

As a representative example, the *E. coli* β-CA enzyme functions as a tetramer, consisting of two homodimers. Every monomer, as revealed by the crystal structure (PDB ID: 1I6P), contributes to a long, narrow channel leading to the active site, where the catalytic zinc ion is located. The dimerization is stabilized by interactions between the N-terminal arms and the zinc-binding cores of adjacent monomers, creating a saddle-shaped groove at the dimer interface (Fig. 10) (Cronk et al., 2001).

The zinc ion at the active site is tetrahedrally coordinated by three amino acid residues: Cys42, His98, and Cys101. Notably, the fourth coordination position is occupied by Asp44, replacing the water molecule normally present in active carbonic anhydrases. This configuration corresponds to the "closed" conformation (type II β-CA), which is catalytically inactive due to the absence of the zinc-bound hydroxide nucleophile necessary for CO_2 hydration. The enzyme displays pH-dependent conformational changes. At pH values above 8.3, a water molecule takes the place of the Asp44 side chain, shifting the active site into what is known as the "open" conformation (type I β-CA), which is catalytically active. This pH-induced switch controls the enzyme's activity by modulating the coordination environment of the zinc ion (Nocentini et al., 2020). Near the active site, a non-catalytic bicarbonate binding pocket has also been discovered. This pocket, located approximately 8 Å from the zinc ion, contains residues such as Trp39, Gly41, Val47, Arg64, and Tyr181. It is believed that bicarbonate binding at this location stabilizes the enzyme's closed conformation, which helps control its catalytic activity (Del Prete et al., 2020).

2.4.3. Reported CA inhibitors (CAIs)

Carbonic anhydrase inhibitors (CAIs) fall into one of four categories: (1) metal ion binders (anion, sulfonamides, and their bioisosteres, dithiocarbamates, xanthates); (2) compounds that bind to the zinc-coordinated water molecule/hydroxide ion (phenols, polyamines, thioxocoumarins, sulfocoumarins); (3) compounds that block the active site entrance, such as coumarins and their isosteres; and (4) compounds that bind out of the active site (Fig. 11). This subdivision has been made based on the mechanism, by which the inhibitors bind the catalytic metal ion, the metal coordinated-water molecule, and the blockage of the active site (Supuran, 2017, Gumus et al., 2024, Pichake et al., 2014). The anions and sulfonamides are the most studied CAIs. Domagk discovered the first antibacterial medications, sulfonamides, in 1935. The first sulfonamide demonstrating effective antibacterial activity was Prontosil, a sulfanilamide prodrug isosteric/isostructural with *p*-aminobenzoic acid (PABA) (Duysak et al., 2023). Following this, a variety of analogues that make up the so-called sulfa drug class of antibacterials entered clinical use, and many of these compounds are still widely used. Moreover, some CA inhibitors are already clinically used drugs such as acetazolamide, hydrochlorothiazide (diuretic), celecoxib (selective cyclooxygenase-2 inhibitor) and topiramate (antiepileptic) (Supuran and Capasso, 2017).

Nevertheless, because CAs are not species-specific enzymes, their inhibitors may also affect the host CAs. This can result in adverse effects due to inhibition of the host over the pathogen enzyme. However, there are examples among the antibacterial agents that can assist rationalization of this phenomenon. For instance, the enzyme dihydrofolate reductase (DHFR) is found in many bacteria and is the target for a number of medications, including the widely used antibacterial trimethoprim (Supuran and Capasso, 2020). This enzyme catalyzes the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reduction of 5,6-dihydrofolate to 5,6,7,8-tetrahydrofolate, a crucial cofactor involved in the biosynthetic pathways of purines, thymidylate, methionine, glycine, pantothenic acid, and *N*-methionyl-tRNA (Supuran and Capasso, 2020). The bacterial DHFR amino acid sequence has a similarity of 30% with the corresponding human protein (sequence identity between bacterial β- or γ-CAs and human α-CAs is generally below 25%). Trimethoprim specifically inhibits the bacterial enzyme, but not human DHFR (Covarrubias et al., 2006). Since it was feasible to create specific and selective inhibitors for the bacterial over human DHFR enzyme, we hypothesize that the same might be true for pathogenic CAs for the development of selective inhibitors.

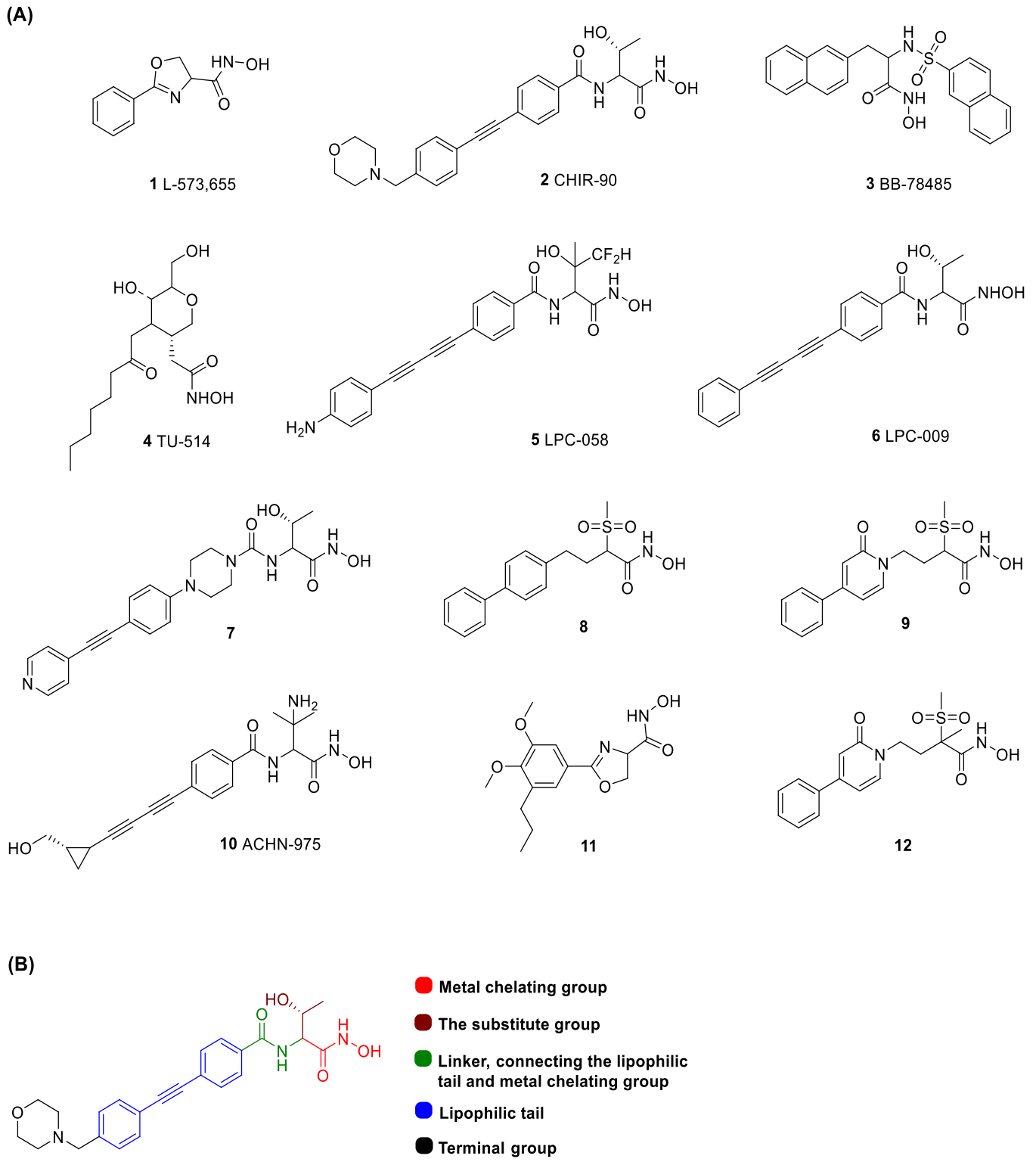


Fig. 9. (**A**) Representative examples of different LpxC enzyme inhibitors. (**B**) The structural features of LpxC inhibitors outlined on the example of CHIR-090 (Ding et al., 2018). Created by ChemDraw, version 23.1.1.3.

2.5. Inhibition of biofilm formation

Biofilms are multicellular bacterial communities embedded in an extracellular polymeric substance (EPS) matrix consisting of proteins, polysaccharide, and nucleic acids. The polymeric matrix does not only provide a protective shield, but also serves as a survival strategy, helping bacteria evade the host defense systems. They may cause local tissue damage and an acute infection by staying dormant and hidden from the immune system (Vestby et al., 2020). The biofilm enables the bacteria to adapt to environmental anoxia and nutrient deficiency by exhibiting a modified metabolism, gene expression, and protein production, which may lead to a decreased metabolic rate and slower cell division. In addition, these changes make bacteria more resistant to antimicrobial agents by decreasing penetration of antibiotics, causing mutations in the

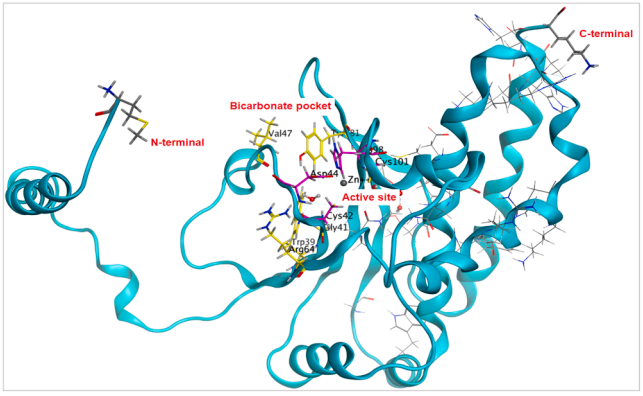


Fig. 10. 3D structure of *E. coli* β-CA (PDB ID: 1I6P) (RCSB Protein Data Bank, 2025), showing the active site (magenta) and the bicarbonate pocket (yellow). Created by Molecular Operating Environment program.

antimicrobial targets, or reducing the requirements for the cellular function that the antimicrobials disrupt (Hall-Stoodley and Stoodley, 2009, Donlan and Costerton, 2002).

Biofilm formation is a multi-step process (Fig. 12) regulated by quorum-sensing, a cell-to cell communication system that plays a major role in biofilm development. This process is mediated by the production, release, and detection of signaling molecules called autoinducers (Preda and Săndulescu, 2019). When an autoinducer reaches a critical concentration, bacteria recognize and respond to this signal by modifying their gene expression. Bacterial quorum-sensing (QS) signaling systems can generally be divided into three main categories. Gram-positive bacteria specifically produce autoinducing peptides as signaling molecules, whereas Gram-negative bacteria communicate using small molecules such as *N*-acyl homoserine lactones. The autoinducer-2 signal molecule has been detected in both Gram-negative and Gram-positive bacteria (Tsai and Winans, 2010, Sepandj et al., 2007, Miller and Bassler, 2001). Currently, more than 70 species of bacteria with quorum-sensing abilities are known, including *P. aeruginosa, E. coli*, and *S. aureus*. According to research conducted by the National Institutes of Health, biofilms are associated with 80% of all bacterial infections and increase antibiotic resistance and bacterial pathogenicity by a factor of 1000 compared to planktonic bacteria. Biofilms are particularly relevant in the context of medical devices, such as catheters and implants, where their formation poses significant challenges, making this a major focus area of current biomedical research (Sambanthamoorthy et al., 2012, Hall-Stoodley et al., 2004, Zhao et al., 2023).

Development of antibiofilm agents as an adjuvant therapy is an attractive field of research, and an effective strategy for the control and prevention of bacterial infections. Disruption of biofilms increases the susceptibility of bacteria to antibiotics, thus reducing the dose needed of these drugs to be effective. In addition, suppression of biofilm formation without affecting bacterial growth minimizes the risks of resistance development (Piewngam et al., 2020). Numerous efforts have been made to identify novel agents that disrupt the bacterial biofilm cycle, which is a critical challenge in treating persistent infections and combating antibiotic resistance. Promising strategies target various stages of biofilm development, including QS inhibitors to block cell aggregation, enzymes such as DNases and proteases to degrade the protective extracellular matrix, and small molecules or repurposed drugs to disperse biofilms or enhance antibiotic effectiveness (Fig. 13) (Hassan et al., 2023, Sully et al., 2014).

3. Quinolones and fluoroquinolones

While the identification of novel bacterial targets holds great promise in the fight against multidrug-resistant infections, fluoroquinolones stand out as a unique class of antibiotics due to their broad-spectrum activity, favorable pharmacokinetics, and highly adaptable structures. These properties do not only distinguish them from other widely used scaffolds but also position them as an ideal foundation for designing novel derivatives acting on both classical targets, such as their established target enzymes DNA gyrase and topoisomerase IV, and newly identified targets such as NagA, LpxC, G-6-P synthase and

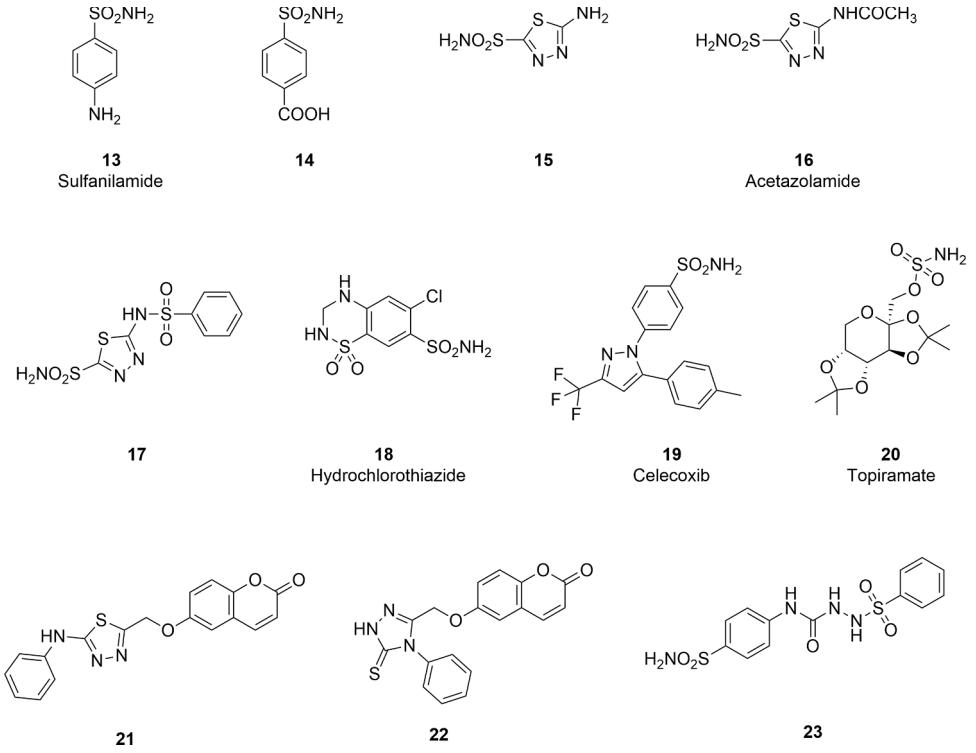


Fig. 11. Representative chemical structures of reported CA inhibitors.

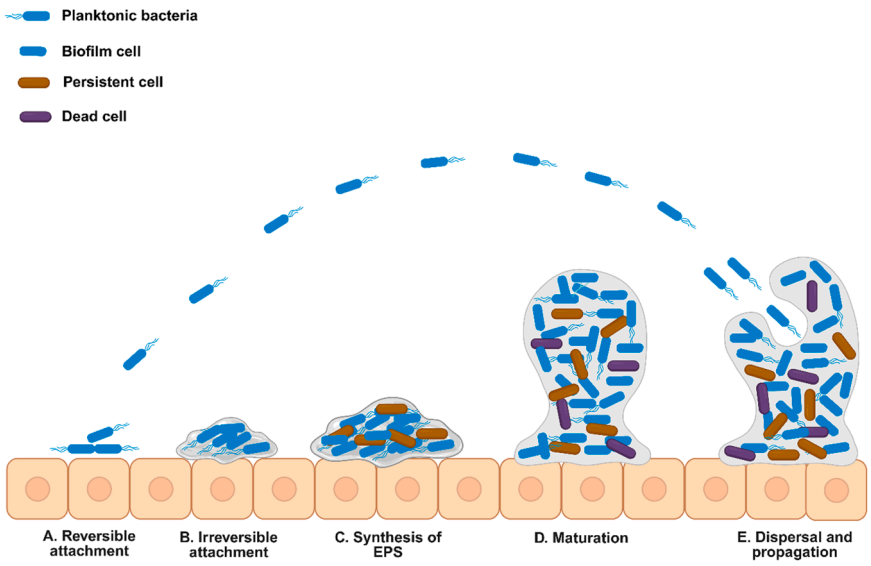


Fig. 12. Stages of biofilm formation, illustrating reversible attachment, irreversible attachment, synthesis of EPS, maturation, and dispersal/propagation (Zhao et al., 2023). Created with BioRender (**2025**). https://BioRender.com/.

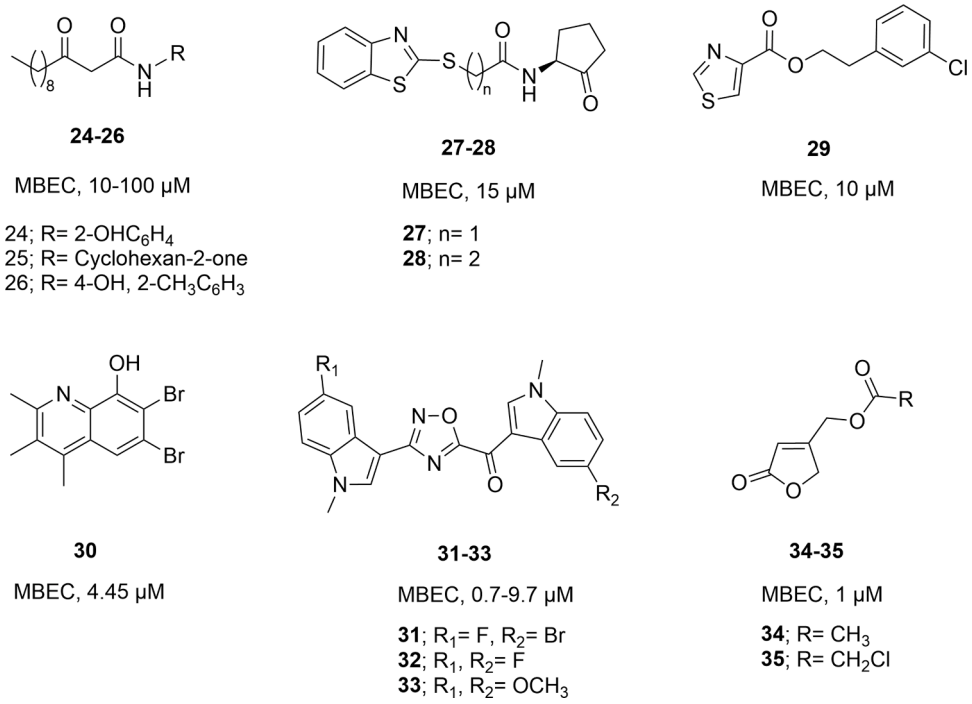


Fig. 13. Representative examples of antibiofilm agents. MBEC: Minimum Biofilm Eradication Concentration.

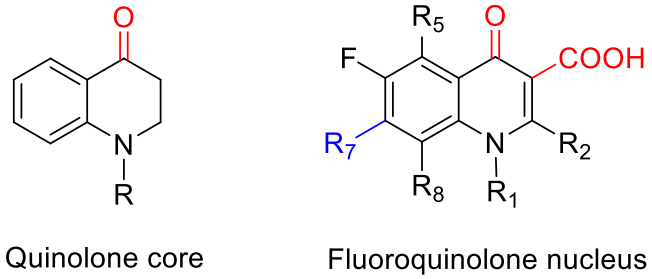


Fig. 14. The basic structure of quinolone antibacterials.

carbonic anhydrase enzymes, as well as inhibition of biofilm formation.

Quinolones are a group of synthetic antibacterial compounds containing a bicyclic core structure belonging to the 4-quinolone class as shown in Fig. 14. Following their discovery in the early 1960s, they have gained high importance as key therapies to treat both community-acquired and severe nosocomial infections (Pham et al., 2019). Quinolones and fluoroquinolones are known for their strong bactericidal effects and broad-spectrum activity against numerous pathogens responsible for infections such as urinary tract infections, gastrointestinal infections, respiratory tract infections, sexually transmitted diseases and skin infections (Sharma et al., 2009, Bisacchi, 2015). Ciprofloxacin, one of the most widely used fluoroquinolones, is the first-line treatment for bacterial infections of the urinary, biliary, and

respiratory tracts, as well as sexually transmitted infections. It was patented in 1983 and approved by the FDA in 1987 (Suaifan and Mohammed, 2019).

3.1. Development of quinolones

The first quinolone discovered in 1962 was formed as a by-product of the commercial preparation of the antimalarial chloroquine. This by-product was found to have antibacterial activity and was subsequently modified to yield nalidixic acid (Bush et al., 2020). Nalidixic acid was well-tolerated in the treatment of urinary tract infections. Through the 1960s and 1970s, a number of related agents were developed producing first-generation quinolones (Fig. 15), reflecting the early experimentation stage with the original nalidixic acid structure (Millanao et al., 2021). These compounds generally displayed increased Gram-negative activity over nalidixic acid, but lacked useful activity against Gram-positive cocci, *P. aeruginosa*, and anaerobes. Generally, they were well absorbed after oral administration and attained high concentrations in the urinary tract, making them useful for treatment of urinary tract infections (Pham et al., 2019, Sharma et al., 2009). However, due to their low tissue penetration, these first-generation quinolones are not appropriate for the treatment of systemic infections.

Structurally, nalidixic acid is a naphthyridone derivative. The switch to the quinolone core and introduction of a fluorine atom at *C*-6 and piperazine at *C*-7 created second-generation derivatives with a broader spectrum of activity (Fig. 16). By the late 1970s, ciprofloxacin and norfloxacin were developed, combining cyclopropyl and ethyl groups in their structures, respectively. They proved highly active against Enterobacteriaceae, *H. influenzae*, and aminoglycoside resistant *P. aeruginosa* and marginally active against streptococci, staphylococci and β-lactamase producing bacteria (Andersson and MacGowan, 2003). Generally, the introduction of piperazine to the *C*-7 position enhances pharmacokinetic properties and effectiveness against Gram-negative organisms. It is reported that fluoroquinolones such as ciprofloxacin, norfloxacin and levofloxacin can inhibit urease activity in *Helicobacter pylori* and *Proteus mirabilis* at sub-inhibitory concentrations (Alkhalil, 2024).

Further development of second-generation derivatives with the introduction of new *C*-7 rings or chiral resolution of existing agents led to third and fourth-generation derivatives (Figs. 17-18). These derivatives possess enhanced activity against Gram-positive bacteria and some penicillin resistant bacteria. Moreover, they are characterized by increased activity against anaerobes and some ciprofloxacin resistant bacteria (Mohammed et al., 2019). The latest approved fluoroquinolone, delafloxacin, was developed by Melinta Therapeutics (formerly Rib-X Pharmaceuticals). Delafloxacin is a broad-spectrum fluoroquinolone antibacterial with activity against methicillin-resistant *S. aureus* (MRSA) and was FDA-approved in 2017 for the treatment of acute bacterial skin infections (Markham, 2017).

Fluoroquinolones, despite their broad-spectrum activity, have faced increasing regulatory restrictions due to safety concerns. The FDA, European Medicines Agency (EMA), and other health authorities have issued warnings regarding serious adverse effects, including tendinitis, tendon rupture, QT interval prolongation, aortic aneurysm, central nervous system toxicity (such as seizures and peripheral neuropathy), and dysglycemia. Consequently, fluoroquinolones are now reserved for treating severe infections when alternative treatment options are not effective or available (Xie et al., 2024, Kuula et al., 2019).

Several fluoroquinolones have been withdrawn from the market due to significant safety concerns. For instance, trovafloxacin was withdrawn due to reports of fatal hepatotoxicity, while gatifloxacin was discontinued because it caused serious disturbances in glucose metabolism, leading to both hyperglycemia and hypoglycemia. Other fluoroquinolones, such as grepafloxacin, and sparfloxacin, were removed due to cardiotoxicity and hemolytic complications. Currently, the FDA guidelines recommend that fluoroquinolones should not be used for uncomplicated infections (e.g., sinusitis, bronchitis, and urinary tract infections) unless no other treatments are available. The EMA has also limited their use, especially among elderly patients and those with a history of cardiovascular or neurological disorders (Rusu et al., 2023, Kuula et al., 2022). These regulatory measures underscore the need for fluoroquinolone derivatives with improved safety profiles.

3.2. Mechanism of action of quinolones

Quinolones target two essential bacterial enzymes. In Gram-negative bacteria such as *E. coli* and *P. aeruginosa*, the main target is DNA gyrase, while the main target in Gram-positive bacteria, such as *S. aureus* and *S. pneumoniae* is DNA topoisomerase IV (Fàbrega et al., 2009). Gyrase controls DNA supercoiling and relieves topological stress arising from the translocation of transcription and replication complexes along DNA; topoisomerase IV is a decatenating enzyme that separates interlinked daughter chromosomes following DNA replication. However, quinolones do not merely eliminate topoisomerase function; they likely cause the lethal release of DNA double strand breaks by trapping gyrase and topoisomerase IV on the DNA (Aldred et al., 2014, Mustaev et al., 2014). Gyrase can also remove positive supercoils in the presence of ATP and relax negatively (but not positively) supercoiled DNA in the absence of ATP (Ashley et al., 2017). Quinolones stabilize a conformational change in the gyrase-DNA complex through forming a non-functional ternary complex involving enzyme, DNA and drug that can neither dissociate back to its components nor progress to final products preventing DNA synthesis and replication, which ultimately results in rapid cell death (Fig. 19) (Drlica and Zhao, 2021). Quinolones inhibit the *in vitro* activities of DNA topoisomerase IV by interrupting separation of replicated chromosomal DNA into the respective daughter cells during cell division. This action is believed to be the primary target in Gram-positive bacteria. As a result, the net effect of quinolone treatment is to

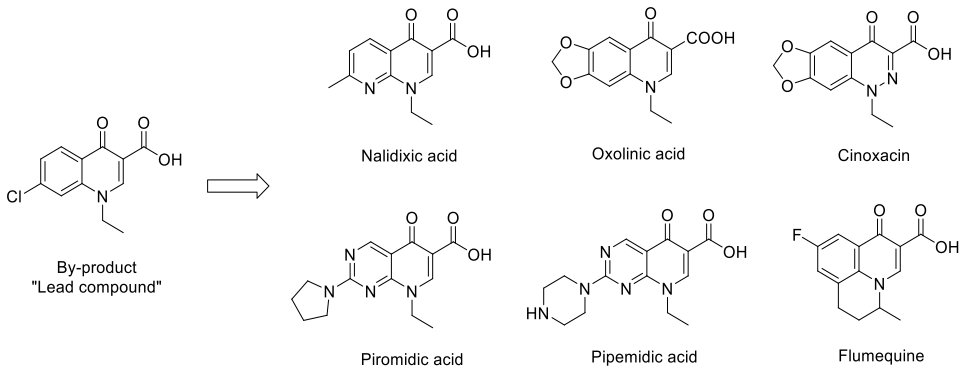


Fig. 15. First-generation quinolones.

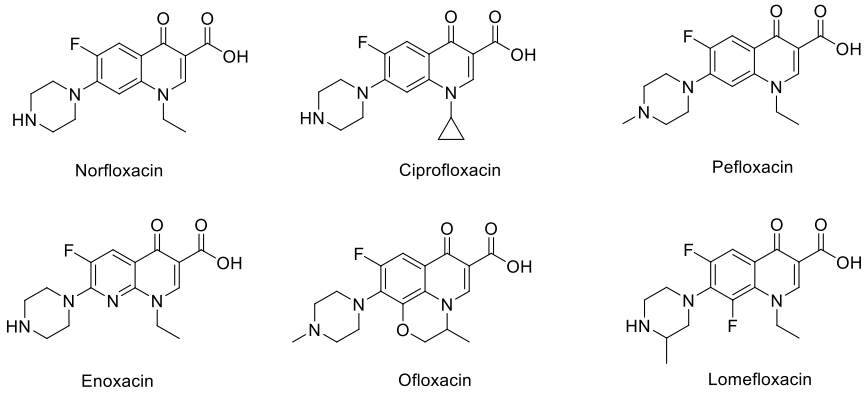


Fig. 16. Second-generation quinolones.

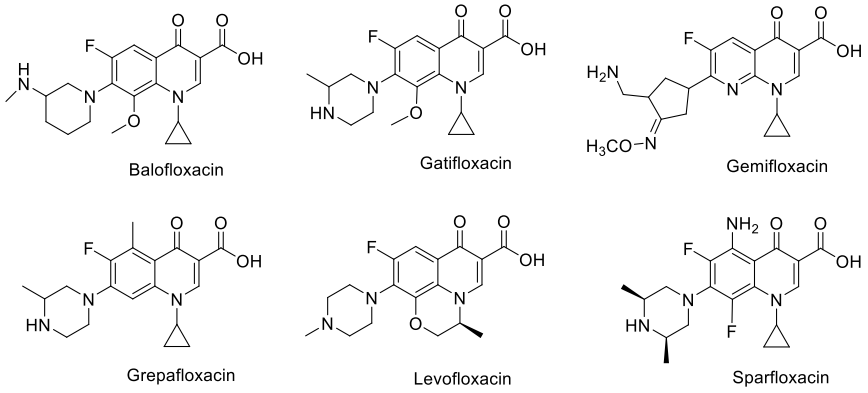


Fig. 17. Third-generation quinolones.

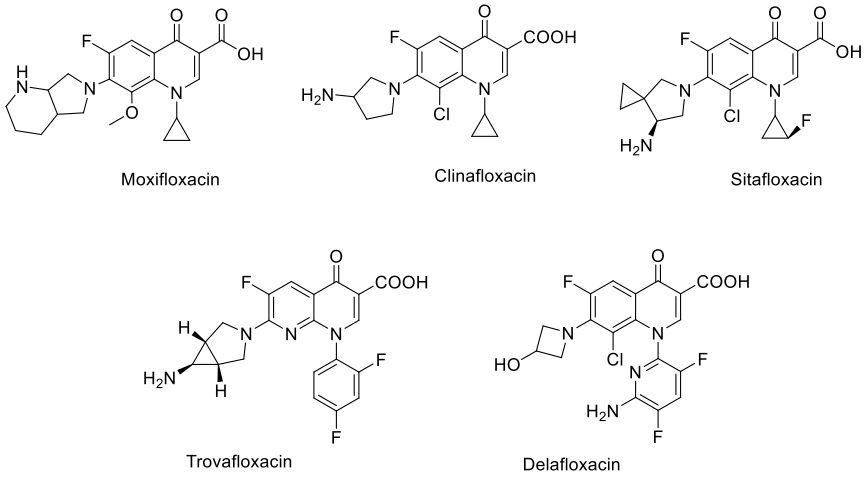


Fig. 18. Fourth-generation quinolones.

generate DNA double-stranded breaks that are trapped by covalently linked topoisomerases. As a result of quinolone–topoisomerase–DNA complex formation, the DNA replication becomes arrested at blocked replication forks, leading to inhibition of DNA synthesis, bacteriostasis and cell death (Kohanski et al., 2010, Drlica et al., 2008).

The affinity of quinolones to metal ions seems to be an important mechanism of their antibacterial activity, where quinolones bind to the DNA-gyrase-complex via a magnesium ion (Arabiyat et al., 2024). Quinolone–topoisomerase binding was recently demonstrated to occur through a water–metal ion bridge, where a noncatalytic Mg^{2+} ion coordinated with four water molecules forming a bridge for hydrogen bonding between the quinolone and the serine and acidic residues. These interactions serve as anchor points for the enzyme (Bush et al., 2020, Correia et al., 2017), (Fig. 20).

The amino acids that are usually accompanied with quinolone-resistance are GyrA-Ser-83 and GyrA-Asp-87 in *E. coli*. Mutation of these two amino acids to alanine leads to resistance to quinolones, which showed the importance of Ser83 and Asp87 in drug interaction. Consequently, it has been assumed that these two amino acids play a vital role in quinolone-enzyme interactions (Naeem et al., 2016). It was

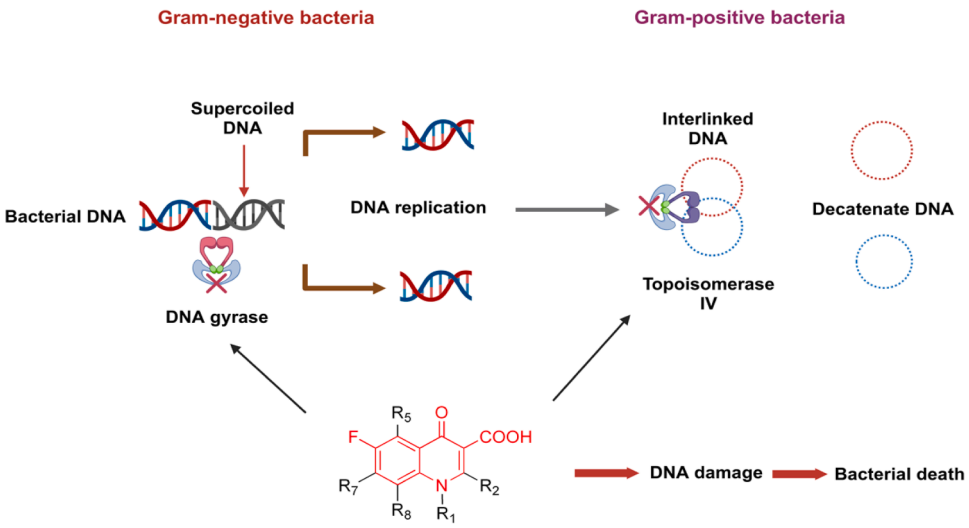


Fig. 19. The overall mechanism of action of fluoroquinolone antibacterials (Rose et al., 2022). Created with BioRender (**2025**). https://BioRender.com/.

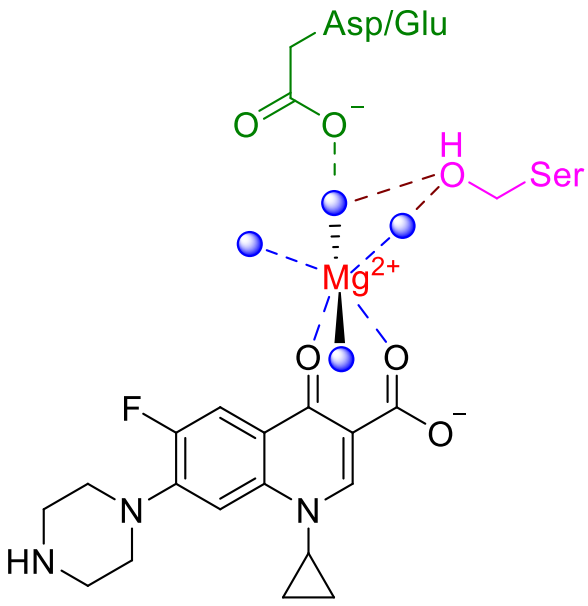


Fig. 20. Quinolone-gyrase interactions via water–metal ion bridge (Naeem et al., 2016). Created by ChemDraw, version 23.1.1.3.

found that the type II topoisomerase of humans lacks serine and acidic residues to form the water-metal ion bridge and is therefore not capable of utilizing this critical mechanism to interact with quinolones. Therefore, the topoisomerase-mediated toxicity of quinolones is minor for humans. These differences in topoisomerase structure constitute the molecular basis, on which quinolones differentiate between bacterial and human topoisomerases (Naeem et al., 2016).

The key molecular interactions responsible for quinolone binding and inhibition of DNA gyrase and topoisomerase IV were further elucidated by investigating the co-crystal structures of moxifloxacin with these enzymes. The co-crystal structure of moxifloxacin with DNA gyrase and DNA (PDB code 5cdq) (Protein Data, 2024), and the co-crystal structure of moxifloxacin, DNA, and topoisomerase IV (PDB code 2xkk) (RCSB Protein Data Bank, 2024), were obtained from the Protein Data Bank (Kamal El-sagheir et al., 2023).

Regarding DNA gyrase, the amino acid residues and nitrogenous bases of DNA involved in interaction at the binding site with co-crystallized moxifloxacin are Arg A122, Ser B84, DA E2013 (deoxyadenosine), DT3 D8 (deoxythymidine), DG D2009 (deoxyguanosine) and Mg^{2+} (Chan et al., 2015), where the main interactions are H-bonding with amino acid residues and nitrogenous bases as well as a coordination bond with the Mg^{2+} metal through the oxygen of keto carbonyl and carboxyl carbonyl groups with bond lengths equal to 2.05 and 1.98 Å, respectively (Fig. 21**A**). In topoisomerase IV, the active site interactions with co-crystallized moxifloxacin involve Arg A1123, DT3 D15, DT C19, DA C20, and Mg^{2+} (Wohlkonig et al., 2010). Key interactions include a coordination bond with the Mg^{2+} metal through the oxygen of keto carbonyl and carboxyl carbonyl groups with a bond length equal to 1.91 and 1.99 Å, respectively. Additionally, H-bonding occurs between the oxygen of carboxyl carbonyl and the Arg A1123 residue with a bond length of 3.59 Å. Moreover, π-π stacking and π-H bonding are observed between the quinolone ring and DT3 D15, DT C19 and DA C20 (Fig. 21**B**).

3.3. *Mechanisms of bacterial resistance to fluoroquinolones*

Like other antibiotics, the extensive use of quinolones has led to resistance in infectious bacteria. Bacterial resistance to quinolones is increasing and is commonly reported worldwide, particularly among critical pathogens such as vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *S. aureus* (MRSA), *K. pneumoniae*, and *P. aeruginosa*. In urinary tract infections, resistance in *E. coli* strains responsible for community-acquired infections has been observed to exceed 50% in certain regions, especially in Asia. Respiratory tract infections caused by *S. pneumoniae* and *H. influenzae* generally show lower resistance rates, with about 10% to 30% of these isolates being fluoroquinolone-resistant (Pham et al., 2019). Similarly, fluoroquinolone resistance among extended-spectrum β-lactamase-producing Enterobacteriaceae is quite high, with approximately one-third to two-thirds of isolates exhibiting resistance, which complicates treatment. Moreover, in tuberculosis cases, fluoroquinolone resistance among non-MDR strains has been reported at 5.4%, significantly higher than the global estimate of 0.8% (Nehru et al., 2024).

The responsible resistance mechanisms include target-site gene mutations, other chromosome-mediated resistance mechanisms (reduced membrane permeability and downregulation of porin expression), and plasmid-mediated resistance, all of which reduce the efficacy of quinolones (Eliopoulos and Gold, 2001, Hashem et al., 2013) (Fig. 22).

Target-site gene mutations

The most common mechanism of resistance is due to mutations

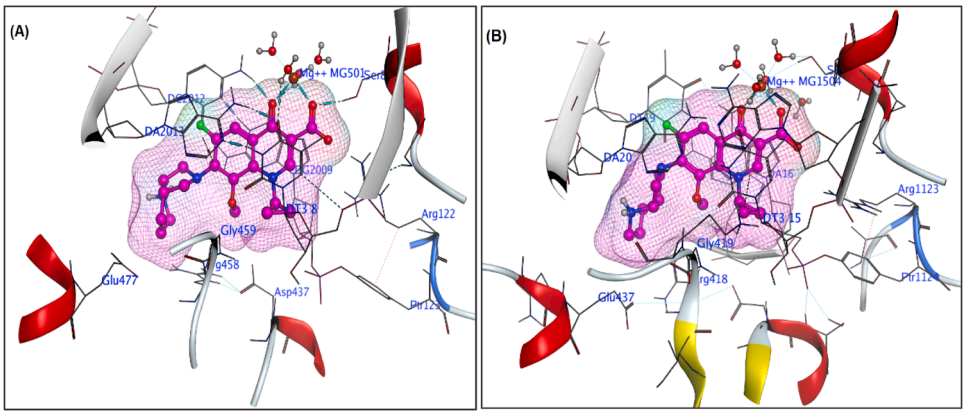


Fig. 21. 3D representations of moxifloxacin within DNA gyrase of *S. aureus* (PDB ID: 5cdq) (**A**), and Topo IV active site of *A. baumannii* (PDB ID: 2xkk) (**B**) are shown. Key molecular interactions are highlighted: H-bonds are depicted in cyan, H-π bonds in red, and van der Waals clashes in orange. Moxifloxacin is represented using a ball-and-stick model, with atoms color-coded as follows: magenta for carbon, blue for nitrogen, red for oxygen, and white for hydrogen (created by Molecular Operating Environment program).

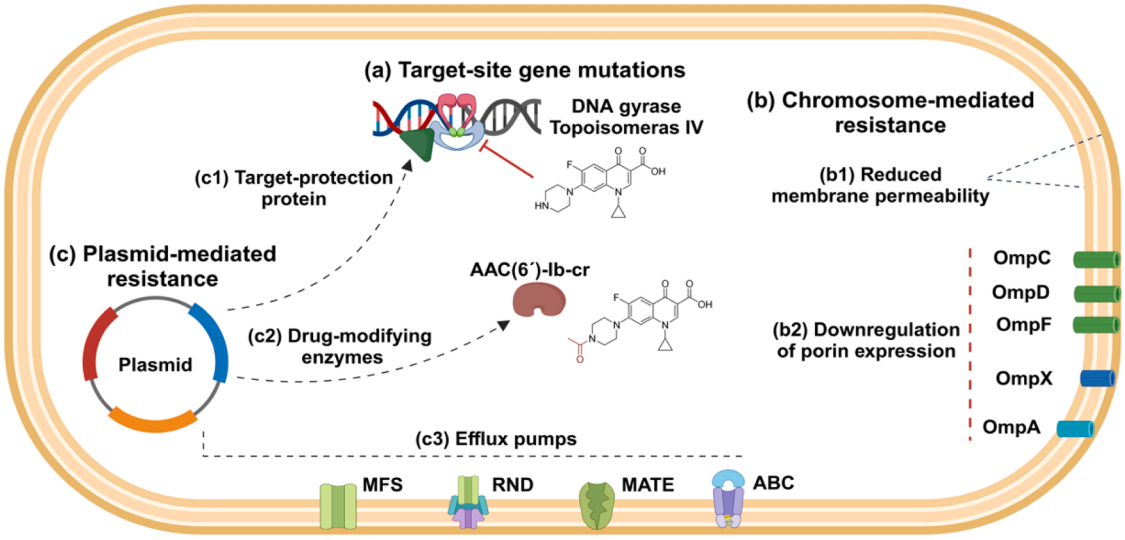


Fig. 22. Mechanisms of bacterial resistance to quinolones: (**a**) target-site gene mutation, (**b**) chromosome-mediated resistance, and (**c**) plasmid-mediated resistance (Correia et al., 2017). Efflux pumps involved include MFS (Major Facilitator Superfamily), RND (Resistance-Nodulation-Division Superfamily), MATE (Multidrug and Toxic Compound Extrusion family), and ABC (ATP-Binding Cassette family). Created with BioRender (**2025**). https://BioRender.com/.

within the quinolone resistance determining regions (QRDRs) of at least one of the genes that encode the primary and secondary targets, the type II topoisomerases (*gyrA, gyrB, parC* and *parE*). Mutations in the QRDRs of these genes result in amino acid alterations that structurally change the target protein and, subsequently, the drug-binding affinity of the enzyme (Correia et al., 2017, Vila et al., 2006, Jacoby, 2005). The serine and acidic residues that anchor the water–metal ion bridge represent the most common single amino acid substitutions that cause quinolone resistance. This mutation frequently results in ten times lower binding affinity for quinolones (Naeem et al., 2016).

Chromosome-mediated resistance

The intracellular concentration of quinolones is controlled by drug uptake through diffusion and their removal via efflux pumps. For a drug to diffuse into cells of Gram-negative bacteria, it must pass through the outer membrane. Therefore, the influx of drugs into Gram-negative species is mediated by protein channels called porins. The down-regulation of porin expression leads to low level quinolone resistance. Similarly, increased expression of chromosome or plasmid-encoded efflux pumps lead to low-level resistance (Correia et al., 2017, Vila et al., 2006, Jacoby, 2005).

Plasmid-mediated resistance

This type of bacterial resistance to quinolones is mediated by plasmids that carry specific quinolone resistance genes. The transfer of plasmid-mediated quinolone resistance from one bacterial generation to another can occur vertically or horizontally through conjugation. Plasmid-mediated quinolone resistance is associated with three groups of genes. The first group, quinolone resistance (*qnr*) genes, encodes Qnr proteins (e.g., QnrA, QnrB, QnrS), members of the pentapeptide repeat protein family, which bind directly to DNA gyrase and topoisomerase IV, thereby preventing quinolones from binding to their targets. The second group includes ciprofloxacin-resistant acetylating aminoglycoside 6′-*N*-acetyltransferase type Ib variant AAC(6′)-Ib-cr, an antibiotic-modifying enzyme that reduces quinolone activity by acetylating the unsubstituted nitrogen on the *C*-7 piperazine ring. The third group consists of efflux pump genes, which actively expel quinolones from bacterial cells (Correia et al., 2017, Naeem et al., 2016).

4. Structure-activity relationships of quinolones

The mechanisms by which fluoroquinolones inhibit DNA gyrase and topoisomerase IV are intrinsically related to their structure. These interactions emphasize the importance of understanding the structure-activity relationship to guide the development of more potent fluoroquinolone derivatives. This section explores how specific modifications to the fluoroquinolone scaffold enhance their antibacterial activity, broaden their activity and target spectra, and overcome resistance mechanisms.

4.1. Basic quinolone pharmacophore

The quinolone pharmacophore consists of the pyridone ring and the associated carboxyl group. To achieve antibacterial activity, the minimal chemical structure must include an unaltered double bond between positions 2-3, and a free ketone carbonyl group at position 4. A free carboxyl group in position 3 was claimed to be essential, as it facilitates binding to both DNA and DNA gyrase and contributes to the overall antibacterial efficacy (Bush et al., 2020, Dube et al., 2023). Fig. 23 shows the general structure of the quinolone molecule and the positions at which key changes are engineered. Some of these molecular features should not be altered as they would considerably reduce the basic mode of action of the drug. Positions 2, 3, and 4 are critical for enzyme and DNA binding. At position 2, a hydrogen moiety is optimal, and any larger molecular additions may cause a steric hindrance at the adjacent positions 3 and 4. The moiety at position 6 should be small; a fluorine atom is optimal as it confers between five- and 100-fold greater potency than any other halogen substituents. Positions 5, 7 and 8 can accommodate a wide range of potential substituents (Bisacchi, 2015, Peterson, 2001).

4.2. The impact of specific substituents

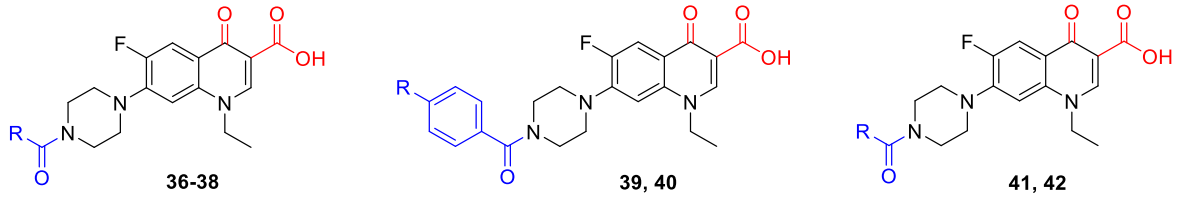
The antibacterial activity of fluoroquinolones is primarily determined by their bicyclic heteroaromatic structure, which integrates the pyridone-β-carboxylic acid nucleus with an aromatic ring. However, it is not just this core structure that governs their effectiveness. The nature, position, and spatial arrangement of peripheral substituents on the quinolone ring influence key factors such as binding affinity to DNA gyrase and topoisomerase IV, DNA interaction, and cellular uptake, thereby enhancing the antibacterial potency, spectrum of activity, and pharmacokinetic properties of fluoroquinolone antibiotics, leading to more efficient treatments against a wide range of bacterial infections (Medellín-Luna et al., 2023). Table 1 outlines how specific structural modifications at various positions impact fluoroquinolone activity.

5. Modified fluoroquinolone derivatives with enhanced activity

In response to the growing challenge of quinolone resistance, researchers have focused on designing and synthesizing modified fluoroquinolones with altered structures to improve their antibacterial and antimycobacterial properties. These structural modifications are aimed at enhancing binding affinity and inhibition of classical targets, as well as acting on novel targets including peptidoglycan biosynthesis, outer membrane integrity, and biofilms. These are aimed at overcoming common resistance mechanisms, such as efflux pump activity, target site mutations, and reduced drug permeability. This section provides examples of the latest advancements in the development of novel fluoroquinolone derivatives, showing their enhanced efficacy against resistant pathogens, and their potential to address critical gaps in current antimicrobial therapies. While some of the bacterial species mentioned in these studies—such as *Xanthomonas oryzae, Xanthomonas axonopodis, Erwinia aroideae*, and *Rhizoctonia solani*—are primarily plant pathogens, and *Aggregatibacter actinomycetemcomitans* is a less common human pathogen, these organisms are frequently employed in antimicrobial screening and SAR studies to evaluate the broader potential of novel fluoroquinolone scaffolds.

5.1. Modification at N4 of the piperazine ring

Khan *et al.* synthesized various *N*4-piperazinyl derivatives of norfloxacin and evaluated them for antibacterial, antifungal, and cytotoxic activities. Compounds **36-38** demonstrated activity comparable to or better than norfloxacin against both Gram-positive and Gram-negative bacteria (Khan et al., 2012). Similarly, a series of 1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)quinoline-3-carboxylic acid (norfloxacin) derivatives **39-42** were synthesized by Yu *et al.* and evaluated for their *in vitro* activities against five pathogenic bacteria and three fungi. Preliminary bioassays showed that the synthesized compounds retained the antibacterial activity of norfloxacin against *Xanthomonas oryzae, Xanthomonas axonopodis* and *Erwinia aroideae* and exhibited better antifungal activity against *Rhizoctonia solani* (Yu et al., 2009).



36-38

39, 40

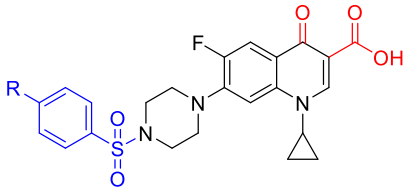
41, 42

36: R =CH_3
37: R =CH_2Cl
38: R =Ph

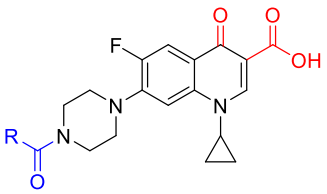
39; R =Cl
40; R =CH_3

41; R =3-Pyridyl
42; R =Furyl

Nieto *et al.* synthesized a new series of benzenesulfonamide fluoroquinolones **43-46** and evaluated their *in vitro* antibacterial activities. The introduction of benzenesulfonylamido groups to the piperazine ring enhanced the activity against both Gram-negative and Gram-positive strains, outperforming ciprofloxacin (Nieto et al., 2005). Moreover, a library of N-acylated ciprofloxacin analogues **47-50** demonstrated broad efficacy against Gram-positive and Gram-negative bacteria, with MICs ≤ 1 μg/mL against methicillin-resistant *S. aureus* and *Bartonella* species (Cormier et al., 2012).



43-46



47-50

43: R = H
44: R = CH_3
45: R = Cl
46: R = NO_2

47: R = CH_3
48: R = CH_2CH_3
49: R = $CH_2CH_2CH_3$
50: R = Ph

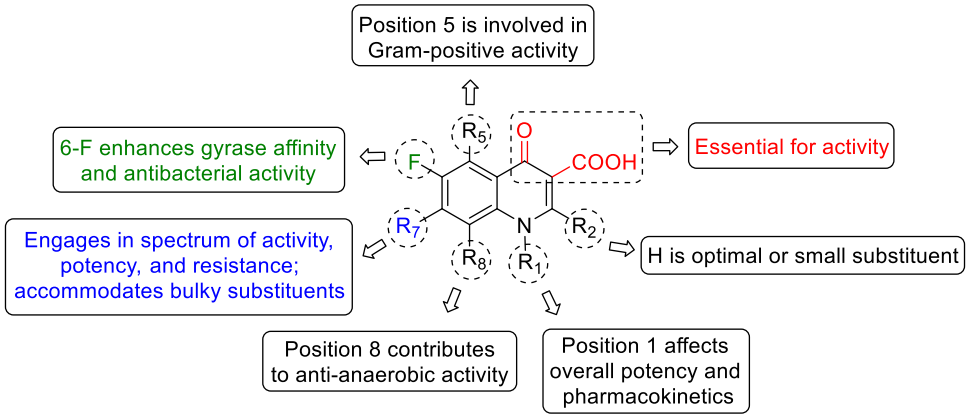


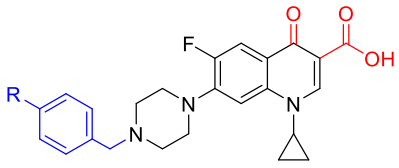
Fig. 23. SAR of fluoroquinolones, R(s) indicate possible sites for structural modification (Kamal El-sagheir et al., 2023). Created by ChemDraw, version 23.1.1.3.

Table 1
Structural modifications and their impact on the antibacterial activity of fluoroquinolones.

Position	Possible impacts on the activity	References
1	This position is part of the enzyme-DNA binding complex and has a hydrophobic contact with the main groove of DNA. A cyclopropyl substituent is the most potent modification, followed by a 2,4-difluorophenyl group, which is a nonclassical bioisosteric substitute for cyclopropyl. The ethyl analogues are found more active than analogues with smaller or larger *N*-l alkyl substituents.	(Pham et al., 2019, Minovski et al., 2011)
2	The *C*-2 position is left unsubstituted because of its proximity to the enzyme binding site. It is considered that any bulky substituents interfere with access and lead to a lower binding affinity and decreased antibacterial activity. Only a sulfur, integrated into a small ring, has been able to replace hydrogen at the R-2 location.	(Peterson, 2001, Gao et al., 2018)
3, 4	These two positions on the quinolone nucleus are considered crucial for interacting with target enzymes. Consequently, the 3-carboxylate and 4-carbonyl groups are necessary for antibacterial action. Moreover, they mediate binding by creating strong hydrogen bonds with DNA bases. Chelating activity with a Mg(II) cation that binds to the DNA phosphate backbone may also be significant for fluoroquinolone penetration into the bacterial cell. Notably, some studies showed that replacement of carboxyl group with classical or nonclassical isosteres maintain the biological activity such as hydroxamic, hydrazide, tetrazole, triazole and oxadiazole moieties.	(Correia et al., 2017, Medellín-Luna et al., 2023)
5	Substituents at this position appear to have the ability to alter the planar structure of the molecule, which is how changes here are thought to affect activity. Considerable alterations have been done at this location to increase the activity of the fluoroquinolones against Gram-positive bacteria. Modestly sized additions, such as an amino, hydroxyl, or methyl groups can strongly enhance *in vitro* activity against Gram-positive bacteria, whereas halide and methoxy substituents tend to diminish activity.	(Peterson, 2001, Gao et al., 2018)
6	The incorporation of a fluorine atom here substantially increased antibacterial activity compared to the original quinolone agents and gave rise to the now widely used and clinically successful fluoroquinolone compounds. Generally, the presence of a *C*-6 fluorine atom boosts the binding affinity to and the inhibition of the gyrase enzyme, as well as cell penetration.	(Medellín-Luna et al., 2023)
7	This position directly interacts with DNA gyrase, or topoisomerase IV. Moieties with at least a 5- or 6-membered nitrogen heterocycle have been determined to be the optimal substituents at this position. The most widely used of these are aminopyrrolidines and piperazines, where the addition of piperazine significantly increases potency against Gram-negative bacteria. Methylation of the 5- or 6-membered heterocycle enhances activity against Gram-positive bacteria.	(Pham et al., 2019, Kamal El-sagheir et al., 2023)
8	Similar to position 5, this position is thought to influence the overall planar structure of the molecule. Therefore, changes introduced here affect target affinity, probably by modifying drug access to the enzyme or DNA binding sites. The activity against anaerobes might be strengthened by a free halogen (F or Cl). Methoxy or methyl substituents, together with halogen substituents, also increase the *in vitro* activity against Gram-positive cocci.	(Peterson, 2001, Minovski et al., 2011)

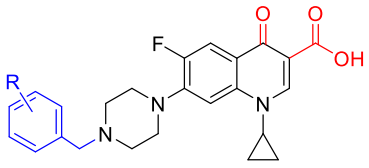
A group of novel ciprofloxacin derivatives **51-54** exhibited significant improvement in lipophilicity by introducing a substituted benzyl moiety to the *N*4 position of the piperazine ring, enhancing their antimycobacterial and antibacterial activity. Compound **52** showed strong *in vitro* activity against all tested Gram-positive strains, including MRSA and MRSE with MIC values of 0.06–32 μg/mL. Additionally, it showed considerable potency against *P. aeruginosa* (MICs 0.5–4 μg/mL), demonstrating two to eight times greater efficacy than, or comparable to, ciprofloxacin (MICs 0.25–128 μg/mL) (Wang et al., 2012).

A series of new 1,2,4-triazole and 1,3,4-oxadiazole hybrid derivatives **59-66** were screened for their antimicrobial activity. These compounds exhibited excellent antimicrobial activity, with MIC values of 0.125 μg/mL against *E. coli* and 0.25 μg/mL against *K. pneumoniae*. Some of these derivatives also demonstrated excellent inhibition of DNA gyrase and topoisomerase IV with IC_{50} of 0.88 μM and 6.78 μM, respectively (Mermer et al., 2019). Additionally, a series of *N*-ciprofloxacin-isatin Mannich bases of **67** and **68** were found to be more potent than parent ciprofloxacin, showing excellent activity against *Salmonella typhi, Vibrio cholerae*, and *K. pneumoniae* with MIC values of 0.19, 0.10, and 0.15 μg/mL, respectively (Guo, 2019).



51, 52

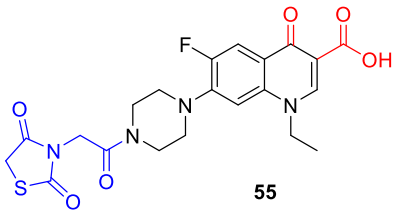
51; R= H
52; R= OCH_3



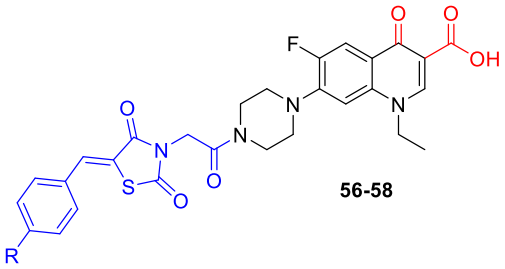
53, 54

53; R= 3,5-dimethoxy
54; R= 2,5-dimethoxy

Marc *et al.* synthesized a series of hybrid norfloxacin–thiazolidinedione compounds **55-58** and screened them for their antimicrobial and anti-biofilm properties. The new hybrids were designed to bind DNA gyrase in a novel way, offering potent antibacterial activity and effectiveness against quinolone-resistant bacterial strains. Compound **56** exhibited excellent activity against *E. coli* with an MIC of 0.125 μg/mL, while compound **57** showed promising activity against *P. aeruginosa* (MIC 0.5 μg/mL) (Marc et al., 2019).

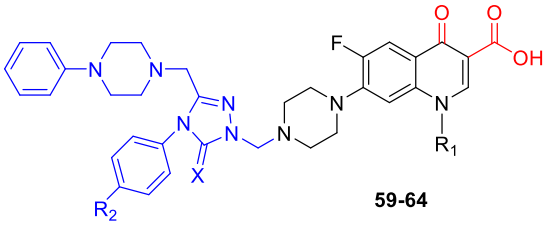


55



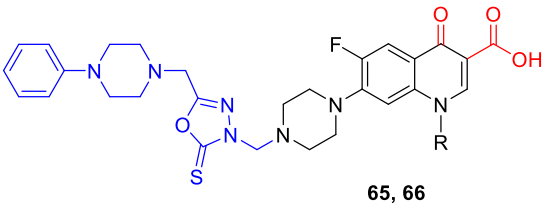
56-58

56: R = H
57: R = OCH_3
58: R = Cl



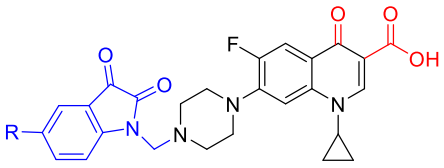
59-64

59: R_1=Ethyl, R_2 = H, X =S
60: R_1=Ethyl, R_2 =F, X =S
61: R_1=Cyclopropyl, R_2 =H, X =S
62: R_1=Cyclopropyl, R_2 =F, X =S
63: R_1=Ethyl, X =O
64: R_1=Cyclopropyl, X =O



65, 66

65: R=Ethyl
66: R=Cyclopropyl



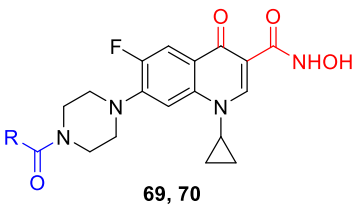
67, 68

67: R = H
68: R = CH_3

5.2. Modification at the carboxyl group

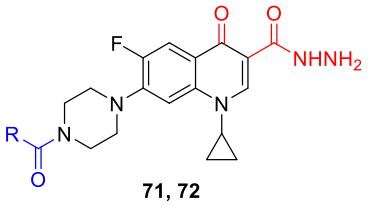
Abdullah *et al.* synthesized new hydroxamic acid and hydrazide derivatives of ciprofloxacin, along with their levofloxacin analogues **69-74**. Some of the synthesized compounds showed promising activity against urease-producing *P. mirabilis*. Notably, the ciprofloxacin hydrazide derivative **71** and levofloxacin hydroxamic acid derivative **73** exhibited the highest inhibitory activity against urease enzyme with IC_{50} values of 1.22 μM and 2.20 μM, respectively (Abdullah et al., 2017).

A series of triazolylnaphthyridinones **75-76** were developed as new derivatives of nalidixic acid. These compounds were evaluated *in vitro* for antimicrobial activity against resistant strains of Gram-positive and Gram-negative bacteria. The results showed selective activity against *B. subtilis* and *Aggregatibacter actinomycetemcomitans*, which are resistant to nalidixic acid, with growth inhibition zones ranging from 20 to 40 mm at 10 mg/mL and MIC values of approximately 3.68–6.3 μM (Mohamed et al., 2018). Additionally, new naphthyridine-3-(1,3,4-oxadiazole) derivatives **77-78** were synthesized and screened for antibacterial activity against Gram-positive and Gram-negative strains, with compounds **77a** and **77c** displaying the highest activity against



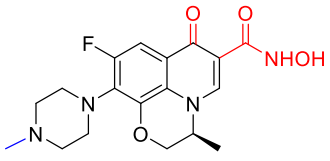
69, 70

69: R = CH_3
70: R = Ph

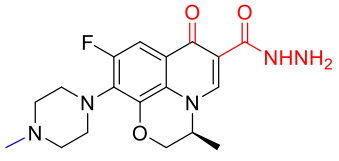


71, 72

71: R = CH_3
72: R = Ph

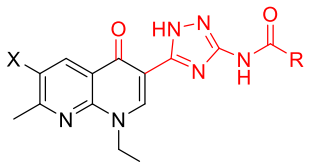


73



74

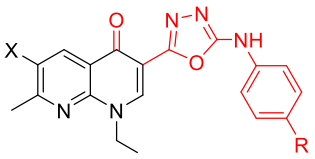
S. aureus (MICs 6–7 mM) and brominated oxadiazoles **78a, 78c**, and **78d** showing activity against *Bacillus cereus* (MICs 5.5–5.9 mM). Further enzyme assays revealed that oxadiazoles **77a** and **78c** effectively inhibited DNA gyrase, with IC_{50} values of 3.36 and 3.89 μg/mL, respectively (Omar et al., 2018).



75, 76(a-e)

75: X=H; **76**: X=Br

R= a: $-CH_2Cl$
b: $-C_6H_5$
c: $4\text{-}ClC_6H_4$
d: $4\text{-}FC_6H_4$
e: $4\text{-}CH_3OC_6H_4$



77, 78(a-d)

77: X=H; **78**: X=Br

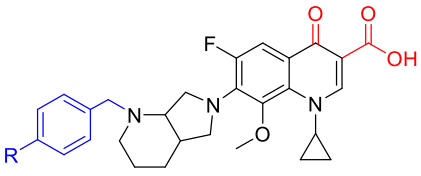
R= a: H
b: Cl
c: CH_3
d: OCH_3

5.3. Fluoroquinolone derivatives with antimycobacterial activity

Wang *et al*. synthesized a series of novel ciprofloxacin derivatives **51-54** with enhanced lipophilicity by introducing a substituted benzyl moiety to the *N*4 of the piperazine ring. Compound **52** demonstrated good antimycobacterial activity, with an MIC of 1 μg/mL against *M. tuberculosis* H37Rv ATCC 27294 (Wang et al., 2012).

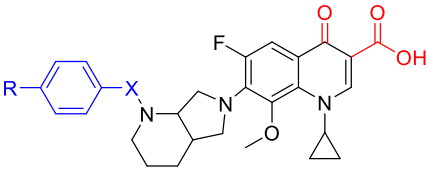
Novel alkyl-, acyl-, and sulfonyl-substituted moxifloxacin derivatives **79-82** were developed and evaluated for their antimycobacterial and antibacterial activity. The synthesized derivatives showed enhanced activity against *M. tuberculosis* H37Rv and MRSA with MIC values ranging from 0.39 to 25 μg/mL (Türe et al., 2019).

Pandeya *et al*. synthesized a series of norfloxacin Mannich bases **83-86** that were investigated *in vitro* against *M. tuberculosis*. Compound **85** showed promising activity, with 100% inhibition at a concentration lower than 6.25 μg/mL (Pandeya et al., 2001). Additionally, 4-substituted piperazinyl derivatives **87-91** were synthesized and evaluated for their *in vitro* antimycobacterial activity. Many of these compounds had MIC values in the range of 7.32–136.10 μM against *M. tuberculosis* H37Rv (Suresh et al., 2014).



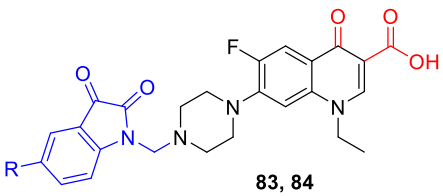
79, 80

79; R=H
80; R=CH_3



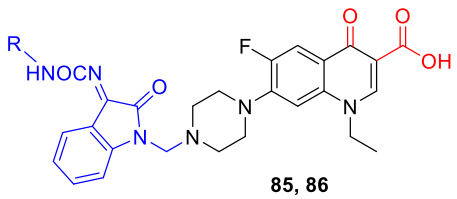
81, 82

81; R=NO_2, X=CO
82; R=H, X=SO_2



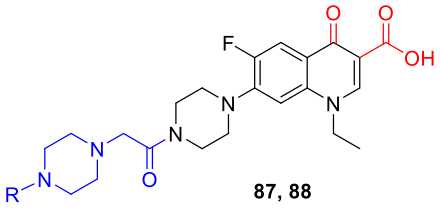
83, 84

83; R=Br
84; R=Cl



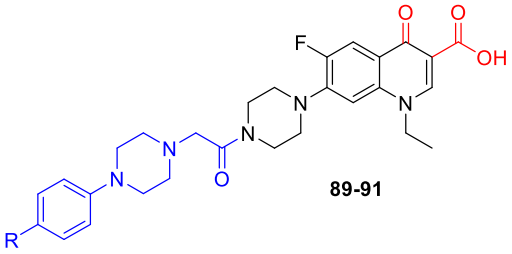
85, 86

85; R= Pyridyl
86; R= H



87, 88

87; R= CH_3
88; R= $COCH_3$



89-91

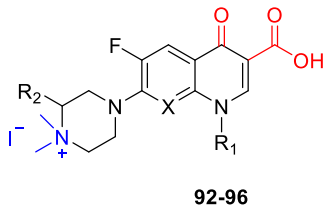
89: R = Ph
90; R= 4-NO_2Ph
91; R= 4-ClPh

5.4. *Fluoroquinolone derivatives with antibiofilm activity*

A series of hybrid norfloxacin–thiazolidinedione molecules **55-58** was synthesized and screened by Marc *et al.* for their antibiofilm properties, demonstrating promising minimal biofilm eradication concentrations (MBECs) of 4.9-39 μg/mL against different Gram-positive and Gram-negative strains, including *S. aureus, E. coli* and *P. aeruginosa* (Marc et al., 2019).

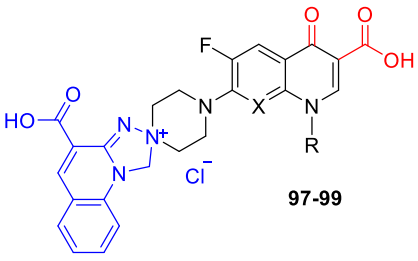
Fedorowicz *et al.* synthesized a series of quaternary ammonium fluoroquinolones **92-99**, including ciprofloxacin and norfloxacin, and tested their antibacterial and antibiofilm activities against Gram-positive and Gram-negative pathogens. The most active quaternary ammonium fluoroquinolones inhibited over 90% of biofilm formation and reduced the total biomass of *P. aeruginosa* (at concentrations of 20-80 μM) and *S. aureus* (at concentrations of 2.5-5 μM) (Fedorowicz et al., 2023).

A series of norfloxacin conjugates coupled with nitrogen-containing heterocycles (triazole and thiazole) **100-107** were prepared and screened for antibacterial and antibiofilm activities. Some derivatives showed excellent MIC values, ranging from 0.05 to 0.4 μg/mL, against various Gram-positive and Gram-negative bacteria. Compounds **104** and **105** inhibited biofilm formation by *S. aureus* with EC_{50} values of 0.11 and 0.562 μg/mL, respectively (Roszkowski et al., 2024). EC_{50} (half maximal effective concentration) is the compound concentration needed to inhibit 50% of biofilm formation.



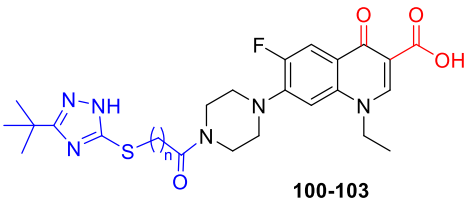
92-96

92; R_1= Cyclopropyl, R_2= H, X= CH
93; R_1= Ethyl, R_2= H, X= CH
94; R_1= Ethyl, R_2= H, X= N
95; R_1= Cyclopropyl, R_2= Me, X= COMe
96; R_1= Ethyl, R_2= H, X= CF



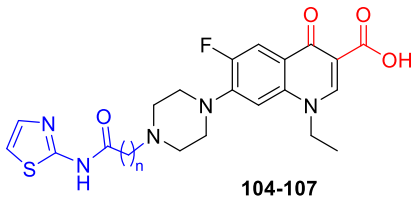
97-99

97; R= Cyclopropyl, X= CH
98; R= Ethyl, X= CH
99; R= Ethyl, X= N



100-103

100; n= 1
101; n= 2
102; n= 3
103; n= 4

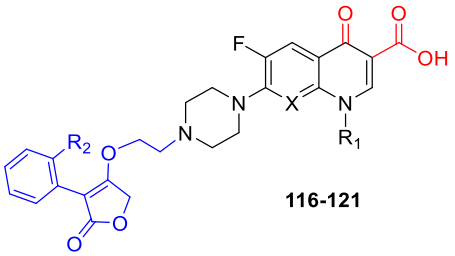


104-107

104; n= 1
105; n= 2
106; n= 3
107; n= 4

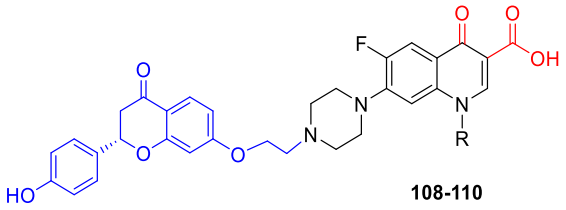
5.5. *Multi-target fluoroquinolone derivatives*

Multi-target topoisomerase inhibitors of fluoroquinolone-flavonoid hybrids **108-115** were designed and synthesized by Xiao *et al.* Some hybrids showed excellent antibacterial activity against drug-resistant strains. Specifically, the naringenin-ciprofloxacin hybrid **109** exhibited the best activity, with MICs of 0.71, 0.062, and 0.29 μg/mL against *E. coli* ATCC 35218, *B. subtilis* ATCC 6633, and *S. aureus* ATCC 25923, respectively. In addition to strong DNA gyrase inhibition, compounds **109** and **110** also demonstrated effective efflux pump inhibition. Quantitative analysis revealed that ciprofloxacin exhibited a high efflux rate (46.7%) in MRSA strains, whereas compounds **109** and **110** showed significantly reduced efflux (~10%), with intracellular accumulation levels similar to those observed when the efflux inhibitor carbonyl cyanide 3-chlorophenylhydrazone is present. This suggests that inhibition of efflux pumps contributed significantly to the observed antibacterial potency (Xiao et al., 2014).



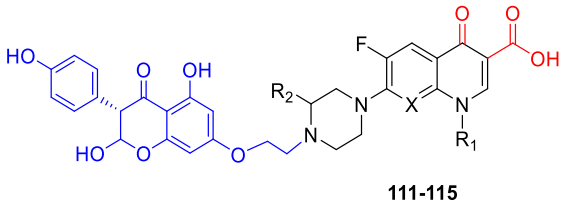
116-121

116; R_1= Et, R_2= H, X= CH
117; R_1= Cyclopropyl, R_2= H, X= CH
118; R_1= 4-FPh, R_2= H, X= CH
119; R_1= Et, R_2= Cl, X= CH
120; R_1= Cyclopropyl, R_2= F, X= CH
121; R_1= Et, R_2= H, X= N



108-110

108; R= Et
109; R= Cyclopropyl
110; R= 4-FPh

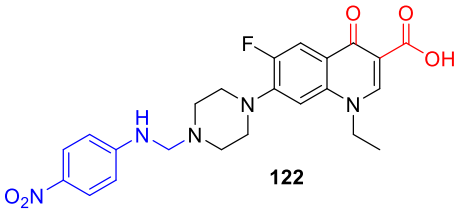


111-115

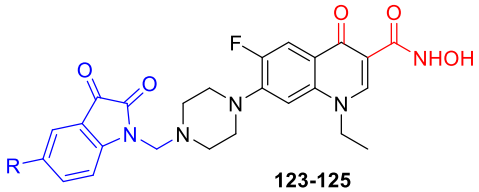
111; R_1= Et, R_2= H, X= CH
112; R_1= Cyclopropyl, R_2= H, X= CH
113; R_1= 4-FPh, R_2= H, X= CH
114; R_1= Et, R_2= CH_3, X= CF
115; R_1= Et, R_2= H, X= N

Novel hybrids of 3-arylfuran-2(5*H*)-one-fluoroquinolones, specifically compounds **116-121**, were synthesized and tested for their antimicrobial properties. Several of these hybrids demonstrated a wide range of effectiveness against resistant bacterial strains, including both Gram-negative and Gram-positive bacteria. Compound **120** was the most effective in antibacterial testing, with a MIC of 0.11 μg/mL against multidrug-resistant *E. coli*, making it approximately 51 times more potent than ciprofloxacin. Enzyme assays revealed that compound **120** could be a strong multi-target inhibitor with IC_{50} of 1.15 ± 0.07 μM for DNA gyrase and 0.12 ± 0.04 μM for tyrosyl-tRNA synthetase (Wang et al., 2014).

Kamal *et al.* synthesized and investigated novel multi-targeted norfloxacin derivatives **122-132**. These derivatives exerted additional inhibitory effects, including disruption of outer membrane integrity (checkerboard assay) and interference with peptidoglycan integrity and biosynthesis (bubble assay, MurG localization and MreB mobility). Phenotypic analysis confirmed that the enhanced antibacterial and antimycobacterial activities of these derivatives arise from a combination of mechanisms. Some derivatives showed promising inhibitory activity against Gram-positive bacteria including *S. aureus* and *E. faecalis* (MIC 1.89–8.61 μM), Gram-negative bacteria including *E. coli, P. aeruginosa*, and *K. pneumoniae* (MIC 0.18–7.20 μM) as well as *M. tuberculosis* (MIC 1.19–6.39 μM). Most tested derivatives were more potent inhibitors of DNA gyrase than norfloxacin (4-7 times lower IC_{50} values) (Kamal El-sagheir et al., 2023, Kamal El-sagheir et al., 2023).

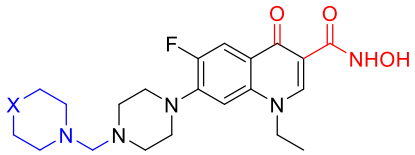


122



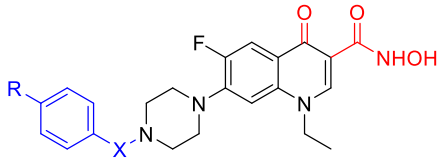
123-125

123; R= H
124; R= Br
125; R= OCH_3



126, 127

126; X= CH_2
127; X= O



128-132

128; R= H, X= C=O
129; R= OCH_3, X= C=O
130; R= F, X= C=O
131; R= H, X= CH_2
132; R= Cl, X= CH_2

Recent studies highlight the multi-targeting capabilities of metal coordination complexes of fluoroquinolones, presenting a promising new strategy to address antibiotic resistance.

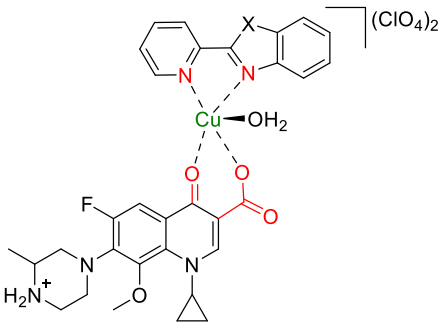
Three novel antibacterial copper complexes derived from gatifloxacin **133–135** have been developed, exhibiting remarkable antibacterial activity. Among them, complex **133** showed the most potent effect, with a MIC of 0.063 μg/mL against *S. aureus*, demonstrating strong bacteriostatic properties. Further investigations into complex **133**'s mode of action revealed its multiple mechanisms: it inhibits DNA gyrase with an IC_{50} of 4.7 μM (compared to 5.1 μM for gatifloxacin), significantly suppresses biofilm formation (43%, 56%, and 81% reduction at 0.0158, 0.0315, and 0.063 μg/mL after 24 hours, respectively, as shown by crystal violet staining), and disrupts cell membrane integrity. Scanning electron microscopy analysis confirmed this, showing membrane damage—wrinkles, indentations, and ruptures—after 4-hour treatment with **133**, in contrast to the smooth, intact membranes observed with gatifloxacin (Wu et al., 2024).

Ziga *et al.* synthesized two novel metallo-antibiotics derived from ciprofloxacin (**136** and **137**) and subjected them to a detailed biological evaluation, including proteomic analysis. In this study, *S. aureus* cells were exposed to the complexes to elucidate their mode of action. These compounds exhibited antibacterial activity against both *S. aureus* and methicillin-resistant *S. aureus* (MRSA), with MICs ranging from 1.95 to 15.63 μg/mL. Furthermore, they showed good *in vivo* tolerability in *Galleria mellonella* larvae, an established model due to its functional and structural similarities to the mammalian innate immune system. Proteomic analysis revealed that, unlike ciprofloxacin alone, both complexes disrupted additional cellular processes in *S. aureus*, including catabolic pathways, sugar transport systems, arginine degradation, and cleavage of DNA hairpin structures. Complex **136** further affected amino acid biosynthesis, cell wall degradation, metal ion binding, and rRNA processing, while complex **137** had a more pronounced impact on proteins

Table 2
Biological activities of fluoroquinolone derivatives with antibiofilm or multi-target effects.

Cmpd	Structural Class	Biofilm Activity	MIC (μg/mL)	Molecular Target(s)
55–58	Norfloxacin–thiazolidinedione hybrids	MBECs: 4.9–39 μg/mL (*S. aureus, E. coli, P. aeruginosa*)	–	DNA gyrase inhibition
92–99	Quaternary ammonium fluoroquinolones	>90% biofilm inhibition: *P. aeruginosa* (20–80 μM) *S. aureus* (2.5–5 μM)	–	DNA gyrase inhibition
100–107	Norfloxacin + *N*-heterocycles (triazole/thiazole)	EC_{50} (biofilm, *S. aureus*): **104**: 0.11 μg/mL **105**: 0.562 μg/mL	MIC: 0.05–0.4 (various strains)	DNA gyrase inhibition
109	Ciprofloxacin–naringenin hybrid	–	*E. coli*: 0.71 *B. subtilis*: 0.062 *S. aureus*: 0.29	DNA gyrase inhibition; efflux pump inhibition (~10% efflux vs. 46.7% for CIP)
110	Ciprofloxacin–naringenin hybrid	–	–	DNA gyrase & efflux pump inhibition
120	3-Arylfuran-2(5*H*)-one-FQ hybrid	–	*E. coli* (MDR): 0.11	DNA gyrase (1.15 μM), Tyrosyl-tRNA synthetase (0.12 μM)
122–132	Norfloxacin derivatives	–	GPB: 1.89–8.61 GNB: 0.18–7.20 *Mtb*: 1.19–6.39	DNA gyrase inhibition; peptidoglycan & membrane disruption
133	Cu–Gatifloxacin complex	Biofilm inhibition: 43–81% (0.0158–0.063 μg/mL)	*S. aureus*: 0.063	DNA gyrase (4.7 μM); membrane damage (SEM)
136–137	Cu–Ciprofloxacin complexes	–	MRSA: 1.95–15.63	Disrupt metabolic, stress, and virulence pathways; proteomic effects

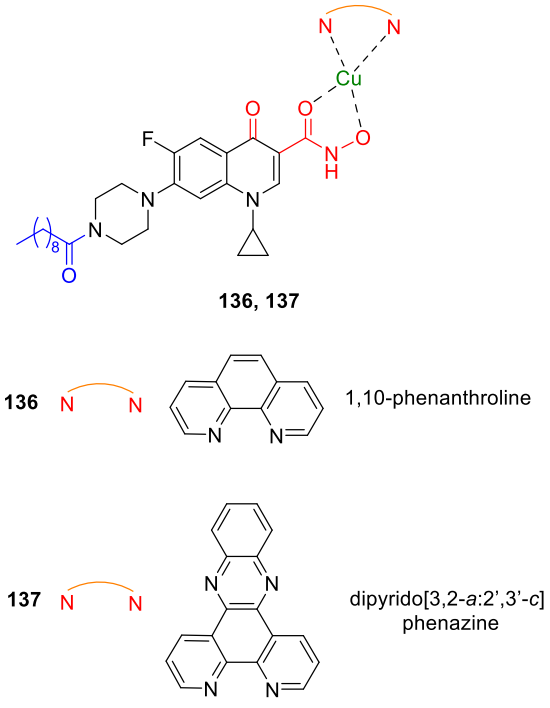
involved in stress response and virulence. These findings highlight the potential of such Cu-based hybrids to overcome resistance by engaging multiple non-traditional targets beyond those affected by ciprofloxacin alone (Ude et al., 2021).



133-135

133; X= S
134; X= NH
135; X= O

moieties increases hydrophilicity, preventing excessive CNS accumulation. Designing hybrid fluoroquinolones with efflux pump inhibitors or agents that disrupt biofilms and outer membrane integrity allows for lower drug concentrations, thus reducing systemic toxicity (Pham et al., 2019, Haddad et al., 2022).



136, 137

136 1,10-phenanthroline

137 dipyrido[3,2-*a*:2',3'-*c*] phenazine

To provide a clear comparison of key activities, Ta/ble 2 summarizes the antimicrobial, antibiofilm, and mechanistic data of fluoroquinolone derivatives with either antibiofilm or multi-target properties. This table highlights their potency across various assays, including MICs, biofilm inhibition, and target-specific interactions.

5.6. Design strategies to minimize fluoroquinolone toxicity

Structural modifications can not only enhance the activity but also improve the safety profile and reduce toxicity of fluoroquinolone derivatives. This approach addresses issues like QT interval prolongation, musculoskeletal side effects, and neurotoxicity. To minimize cardiotoxicity, replacing the *C*-7 piperazine with groups that have lower affinity for the hERG K^+ channel, such as aminopyrrolidine, or adding bulky groups at *N*4 of piperazine or *N*-1 decreases hERG binding and the risk of QT interval prolongation. Tendinopathy risk is lessened by altering the *C*-7 piperazine ring, which is associated with mitochondrial dysfunction and the production of reactive oxygen species (ROS). This can be achieved by introducing bulkier substituents at *N*4 of piperazine that induce less oxidative stress. Incorporating antioxidant moieties, such as phenolic or thiol groups, can counteract the activation of ROS-mediated matrix metalloproteinase (MMP). Modifying the *C*-3 carboxyl minimizes chelation with Mg^{2+} and Zn^{2+} metals, which are crucial for collagen stability and tendon integrity (Wempe, 2022, Duman et al., 2025). To tackle neurotoxicity, reducing GABA-A receptor binding and optimizing lipophilicity limit CNS penetration. Replacing piperazine at *C*-7 with less lipophilic or bulkier groups like aminopyrrolidine or alkylated amines, increasing steric bulk at *N*-1, and adding methoxy or alkoxy groups at *C*-8 help lower GABA-A binding. Incorporating polar groups such as hydroxyl, carboxyl, or heterocyclic

5.7. Future perspectives on modified fluoroquinolones

The development of modified fluoroquinolones holds significant potential for overcoming the resistance challenges associated with traditional quinolone therapies. Key structural innovations, including modifications to the *N*4 of piperazine ring, carboxyl group, and the incorporation of hybrid compounds or metal complexes, have yielded derivatives with superior antibacterial, antifungal, and antitubercular properties (Fig. 24). The inherent flexibility of the quinolone core allows broad derivatization, enabling the creation of multi-functional hybrids that combine antimicrobial, anti-biofilm, and efflux pump-inhibitory effects. This adaptability gives fluoroquinolones a distinct advantage over some rigid scaffolds such as β-lactams and macrolides, which are more constrained with respect to chemical modification (Shi et al., 2023).

Several fluoroquinolone derivatives have demonstrated biological activities extending beyond classical DNA gyrase and topoisomerase IV inhibition. These include inhibiting biofilm formation, disrupting outer membrane integrity, interfering with peptidoglycan biosynthesis, and blocking efflux pump function—key mechanisms contributing to bacterial pathogenicity and resistance. These effects strategically align with novel targets reviewed in Section 2 (e.g., LpxC, NagA, CA), underscoring the structural and functional adaptability of fluoroquinolones as promising scaffolds for the next-generation antibacterial agents. Future fluoroquinolone (FQ) optimization should focus on rational scaffold engineering to deliberately engage these novel bacterial mechanisms. Strategic chemical modifications can convert FQs into multi-target agents by incorporating features such as lipophilic extensions that enhance Gram-negative bacterial penetration and bind the hydrophobic tunnel of LpxC, inhibiting lipid A biosynthesis; metal-chelating groups (e.g., carboxy, hydrazide, hydroxamic) that interact with catalytic

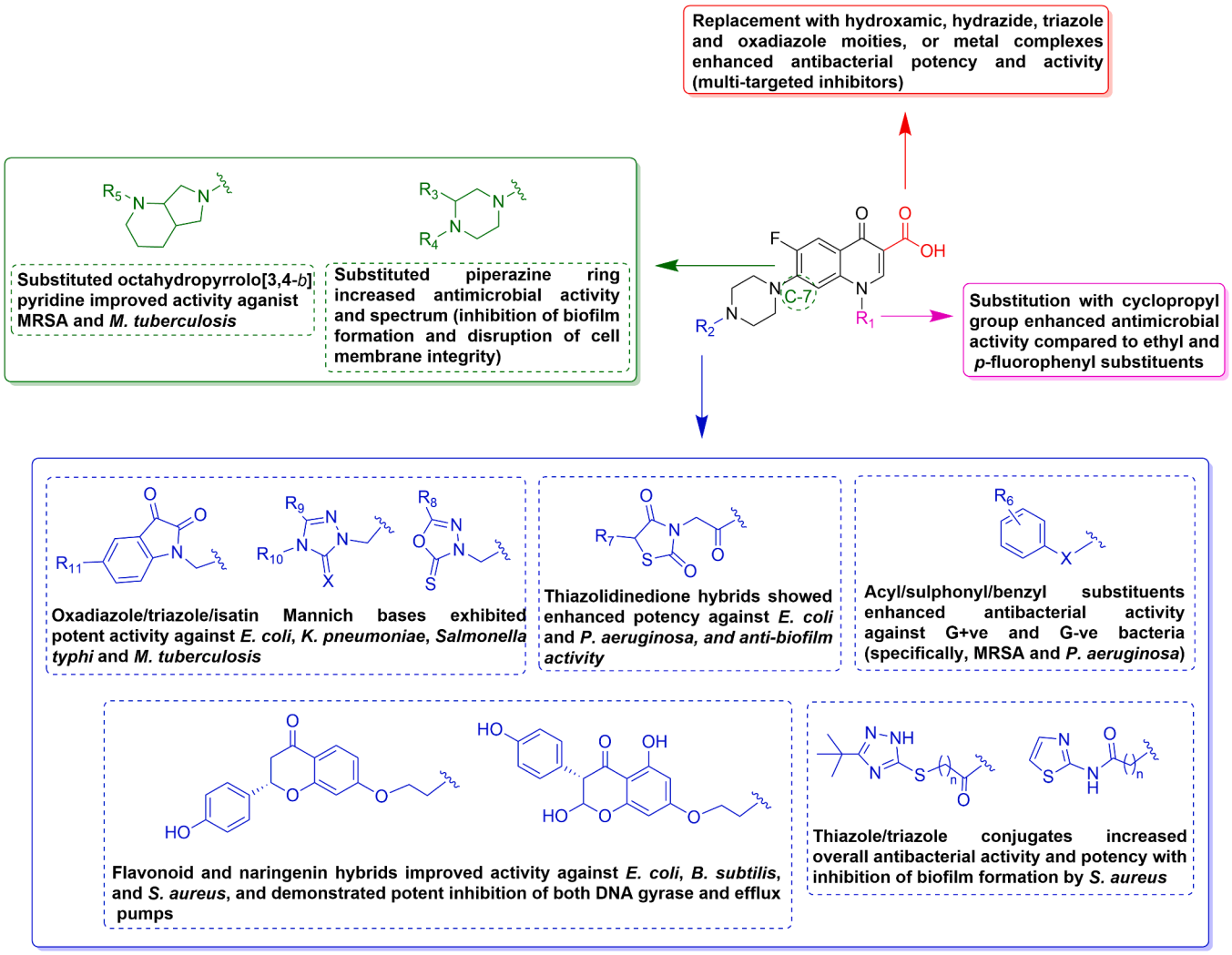


Fig. 24. SAR of the potent fluoroquinolone derivatives.

metals to inhibit metal-dependent enzymes such as NagA, LpxC, and carbonic anhydrase; and sulfonamide or heterocycle hybrids that mimic carbonic anhydrase inhibitors, disrupting pH homeostasis and virulence. Additionally, the inclusion of cationic moieties like quaternary ammoniums or metal complexes could break down biofilms via electrostatic interactions. Combining these targeted designs with AI-driven SAR analysis and hybrid strategies can accelerate the development of fluoroquinolones with enhanced efficacy and lower resistance potential.

Although there is growing interest in multifunctional fluoroquinolones, the precise role of each biological activity often remains undefined due to insufficient mechanistic deconvolution. This represents a significant gap in the literature. Future studies should apply orthogonal and quantitative methods—such as biochemical assays, genetic knockouts, and proteomics—to better define individual mechanisms and guide rational drug design.

Despite advances in fluoroquinolone modifications, their clinical success remains limited by bacterial resistance, toxicity—especially in long-term treatments like tuberculosis—and poor activity against Gram-negative bacteria due to low membrane permeability. Effective clinical translation requires mitigating toxicity in chronic regimens, enhancing membrane penetration, and improving activity against persistent *M. tuberculosis* infections (Anwar et al., 2024, Kherroubi et al., 2024).

6. Conclusion

In this review, we explored the versatility of fluoroquinolones as scaffolds for the development of multi-targeting antimicrobial agents, focusing on both classical and novel bacterial targets to address the challenge of multidrug-resistant bacteria. As synthetic medications, fluoroquinolones can be readily modified at nearly every position on the quinolone ring, enabling the production of diverse and potent derivatives. Fluoroquinolone hybrid compounds, designed with multiple pharmacophores to act on several targets, represent a promising strategy. This innovative approach, known as polypharmacology, offers a significant advantage by promising a lower rate of resistance development compared to traditional single-target antibiotics.

These advancements introduced fluoroquinolone derivatives with enhanced antimicrobial activity and novel mechanisms of action, such as outer membrane disruption, and inhibition of cell wall biosynthesis and biofilm formation. Such compounds can be considered as lead structures for further optimization, tackling the global crisis of antimicrobial resistance. The structural versatility of fluoroquinolones also facilitates targeting both classical and emerging bacterial pathways. Additionally, utilizing computational drug design and AI can speed up the discovery of fluoroquinolones with improved pharmacokinetics and specificity. Hybrid fluoroquinolones combined with efflux pump inhibitors and β-lactamase inhibitors could further enhance their effectiveness. Despite these promising developments and promising *in vitro*

activity of fluoroquinolone derivatives, further *in vivo* studies and clinical trials are needed to assess safety profiles, long-term efficacy, and regulatory hurdles to bring these innovations into practical applications. Ultimately, the progress achieved thus far offers substantial promise for combating the escalating global problem of antimicrobial resistance and paving the way for the next generation of more potent and versatile antimicrobial agents.

CRediT authorship contribution statement

Ahmed M. Kamal El-sagheir: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Michaela Wenzel:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Jari Yli-Kauhaluoma:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have declared no conflict of interest.

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Data availability

No data was used for the research described in the article.

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