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DNA Gyrase as a Prime Antibacterial Target: Mechanisms, Resistance, and Therapeutic Innovations

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Abstract

DNA gyrase, a bacterial type IIA topoisomerase, remains a validated and privileged antibacterial target due to its essentiality in bacteria and absence in humans. Gyrase introduces negative supercoils into DNA via an ATP-dependent strand-passage cycle mediated by GyrA (DNA cleavage/re-ligation) and GyrB (ATPase), creating multiple pharmacological intervention points. Resistance to gyrase-targeting drugs arises from mutations in the quinolone resistance–determining region (QRDR), alterations in GyrB's ATP pocket, plasmid-encoded protection (e.g., Qnr), drug-modifying enzymes, and efflux 4. This mini-review focuses on (i) gyrase biology and catalytic cycle; (ii) resistance mechanisms; and (iii) medicinal chemistry progress across four major inhibitor spaces: fluoroquinolones (poisons), GyrB ATP-competitive inhibitors (aminocoumarins and synthetics), novel bacterial topoisomerase inhibitors (NBTIs; e.g., zoliflodacin; gepotidacin), and natural/allosteric classes (simocyclinone, albicidin; recent allosteric series). We emphasize strategies that mitigate resistance, including dual targeting of gyrase and topoisomerase IV, distinct binding footprints orthogonal to QRDR, and optimization to evade efflux while improving safety.

Introduction

The clinical success of fluoroquinolones (FQs) confirmed DNA gyrase as a premier antibacterial target; their ability to poison the gyrase–DNA cleavage complex yields potent, broad-spectrum bactericidal activity [4-6]. Yet widespread use selected for target-site mutations, efflux, and plasmid-mediated protection, motivating new chemotypes that retain activity against resistant pathogens [5-7]. Because gyrase (AB) actively underwinds DNA, a function not present in human topoisomerases-its bacterial selectivity is inherently attractive for therapy [1–3]. Current discovery efforts span ATP-competitive GyrB ligands, NBTIs with non-quinolone binding modes, DNA-gate binders, and emerging allosteric inhibitors.

Biology and Function of DNA Gyrase

Gyrase's AB complex uses a two-gate mechanism: DNA binding and cleavage at

the GyrA "DNA gate," capture of a transported segment (T-segment) upon ATP-driven GyrB dimerization, passage of the T-segment through the transient double-strand break, and re-ligation with supercoil introduction [1–3,10,11]. Each cycle consumes ATP and introduces negative supercoils essential for replication/transcriptional homeostasis [1–3]. The cycle affords distinct druggable sites: (i) the DNA cleavage complex (GyrA/DNA interface), targeted by FQs; (ii) the GyrB N-terminal GHKL ATP pocket, targeted by aminocoumarins and synthetics; and (iii) the DNA-binding surface at the DNA gate (e.g., simocyclinone), as well as newly revealed allosteric pockets [4–6,10–13,20].

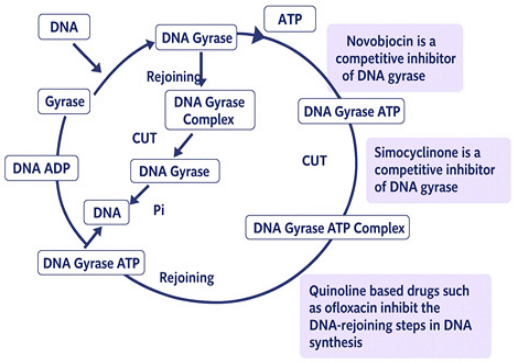

Figure 1. DNA gyrase catalytic cycle and inhibitor intervention points.

FQs stabilize the cleaved DNA at the GyrA/DNA interface (poisons) [4–6]; aminocoumarins/synthetic GyrB ligands block ATP turnover [10–12,15]; simocyclinone blocks DNA binding at the DNA gate [13]; and allosteric series modulate conformational cycling via GyrA pockets [20].

Mechanisms of Resistance

Target-site mutation- QRDR substitutions in GyrA (e.g., Ser83, Asp87; E. coli numbering) and in ParC (topoisomerase IV) reduce FQ binding and elevate MICs; GyrB mutations reduce affinity of ATP-site inhibitors [4–9]

Plasmid-encoded protection or modification- Qnr proteins shield gyrase/topo IV; AAC(62)-Ib-cr acetylates certain FQs; QepA and related pumps actively efflux quinolones [5,7–9].

Efflux/Permeability- AcrAB-TolC and other pumps lower intracellular drug exposure; porin changes further reduce entry in Gram-negatives [8].

Target mimicry- DNA-mimic proteins (e.g., MfpA) can occlude inhibitor action at gyrase [5].

These mechanisms motivate dual targeting (balanced inhibition of gyrase and topoisomerase IV) and orthogonal footprints (NBTIs, allosteric binders; DNA-gate blockers) to suppress single-step resistance and avoid QRDR liabilities [15].

Major Inhibitor Classes

1) Fluoroquinolones (Gyrase Poisons)

FQs intercalate at the DNA-cleavage site and coordinate a water–metal bridge with conserved GyrA residues, stabilizing the cleaved complex and causing lethal double-strand DNA breaks [4–6]. Optimized side-chain patterns expanded spectra and PK (e.g., C-7 piperazine; C-8 methoxy; delafloxacin's anionic profile) while maintaining dual targeting of gyrase and topo IV 4–64–64–6. Pervasive resistance (QRDR/ParC mutations, efflux, Qnr) and class toxicities compel next-gen chemotypes [9].

2) GyrB ATP-Competitive Inhibitors (Aminocoumarins and Synthetics)

Aminocoumarins (novobiocin, clorobiocin) occupy the N-terminal ATP pocket of

GyrB, halting energy transduction [10,11,15]. Clinical use of novobiocin was curtailed by solubility/toxicity and limited Gram-negative activity [12]. Synthetic ATP-competitive scaffolds—including pyrrolamides, benzothiazoles, and related series—deliver low-nanomolar enzyme inhibition and improved bacterial spectra, often with GyrB/ParE dual inhibition to mirror FQ dual targeting [21-24]. Key challenges remain Gram-negative penetration and efflux evasion, but iterative SAR has produced leads with better permeability and reduced off-target liabilities [23].

3) Novel Bacterial Topoisomerase Inhibitors (NBTIs)

NBTIs bind the gyrase–DNA complex with non-quinolone footprints, commonly bridging DNA with pockets formed at the GyrA/GyrB interface and often stabilizing single-strand rather than double-strand breaks [17,18].

- Zoliflodacin (ETX0914), a spiropyrimidinetrione targeting N. gonorrhoeae, demonstrated high cure rates in Phase 2 and is in Phase 3 for drug-resistant gonorrhea [16].
- Gepotidacin, a triazaacenaphthylene, exhibits balanced inhibition of gyrase and topo IV and activity against common FQ-resistance genotypes (discussed across reviews and SAR series) [5,16,19,25]
- Early NBTIs suffered hERG liabilities; modern analogs incorporate heterocycles/ linker redesign to reduce cardiotoxicity while retaining on-target potency and Gram-negative coverage [17,18].

4) Natural Product and Allosteric Inhibitors

Simocyclinone D8 binds the DNA-gate on GyrA and precludes DNA binding (catalytic inhibition), providing a mechanism orthogonal to quinolones; structural work defined its bifunctional engagement at the GyrA dimer interface [13].

Albicidin, a linear oligoaryl peptide, bridges the cleavage site and extends into GyrA/GyrB, poisoning the cleavage complex with a geometry distinct from FQs; synthetic analogs improve stability and show Gram-negative efficacy in models [26].

Allosteric isoquinoline-sulfonamide series (and related efforts) bind non-classical GyrA pockets revealed by structure-guided design, inhibiting conformational cycling and retaining activity against FQ-resistant isolates 202020. These orthogonal sites offer combinability with active-site binders and reduced cross-resistance risk [20].

Table 1. Representative DNA gyrase inhibitor classes, binding sites, and salient features.

Class (example)	Binding site	Mechanistic note & development highlights
Fluoroquinolones (ciprofloxacin, moxifloxacin, delafloxacin)	GyrA–DNA cleavage complex (DNA gate)	Poisons that stabilize cleaved DNA (DSBs); broad spectrum; dual gyrase/topo IV; resistance via QRDR/efflux/Qnr [4–9]
Aminocoumarins (novobiocin)	GyrB N-terminal ATP pocket	ATP-competitive; Gram-positive focus; limited by solubility/toxicity and Gram-negative penetration [10–12]
Synthetic GyrB inhibitors (pyrrolamides, benzothiazoles)	GyrB ATP pocket (±ParE)	Low-nM enzyme inhibition; GyrB/ParE dual action; SAR improves Gram-negative exposure/efflux evasion [21,23,24].
NBTIs (zoliflodacin, gepotidacin)	GyrA/GyrB–DNA interface (non-quinolone)	Stabilize primarily single-strand breaks; retain activity vs FQ-resistant targets; Phase 2/3 clinical progress [16–19,25]
DNA-gate blockers (simocyclinone D8)	GyrA DNA-binding surface	Prevent DNA binding (catalytic inhibition); orthogonal to QRDR; inspires gate-blocking analogs [13].
Peptidic poisons (albicidin analogs)	Bridging GyrA/GyrB at cleavage site	Novel bicentric poisoning; potent vs Gram-negatives; active analogs emerging [26].
Allosteric GyrA binders	Non-classical GyrA pockets	Conformation modulators; activity against FQ-resistant strains; early lead series [20].

Future Perspectives

Resistance-aware design- Balanced dual targeting of gyrase and topo IV reduces single-step resistance pathways; spanning interfaces or combining ATP-site and DNA-site interactions increases the mutational barrier [5,19,25].

Gram-negative penetration- Fine-tuning polarity, size, and hydrogen-bond topology is improving uptake and efflux avoidance for GyrB ligands and NBTIs [21–24].

Orthogonal binding sites- DNA-gate blockers and allosteric GyrA pockets provide non-overlapping liabilities with QRDR, enabling combinations that suppress resistance emergence [13,20].
Safety. Iterative SAR to minimize hERG/CNS liabilities (early NBTIs) while preserving potency is paying off (e.g., zoliflodacin clinical trajectory) [16–19].

Natural product revival- Albicidin/simocyclinone exemplify nature-derived mechanisms translatable into drug-like entities; semi-synthetic analogs continue to mature [13,26]. Overall, gyrase remains a fertile target space where orthogonal mechanisms and dual-target strategies can deliver durable activity against resistant pathogens.

Conclusion

DNA gyrase remains one of the most validated and exploited antibacterial targets in modern drug discovery. Despite decades of clinical reliance on fluoroquinolones, bacterial adaptability through QRDR mutations, efflux mechanisms, and plasmid-borne protection has diminished their long-term efficacy. Yet, these very resistance challenges have driven remarkable innovation in medicinal chemistry. From ATP-competitive GyrB inhibitors and DNA-gate blockers like simocyclinone to next-generation scaffolds such as NBTIs (gepotidacin, zoliflodacin) and peptide-based inhibitors like albicidin, research has expanded far beyond the classical quinolone paradigm. Each class has introduced novel binding sites, alternative cleavage stabilization profiles, or dual-targeting properties that collectively reduce the probability of cross-resistance.

Future antibacterial success will depend on integrating these mechanistic innovations with careful pharmacokinetic optimization, toxicity minimization, and resistance surveillance. The emergence of allosteric GyrA inhibitors demonstrates that even an exhaustively studied enzyme like gyrase still harbors unexploited druggable regions. Moreover, rational dual inhibitors and combination therapies promise to extend the clinical lifetime of existing antibiotics. As new structural, computational, and synthetic tools converge, the next generation of gyrase-targeted agents will likely exhibit not only superior potency but also resilience against evolving bacterial defenses. In summary, DNA gyrase continues to serve as both a historical and forward-looking cornerstone for antibacterial research, bridging foundational enzymology with next-wave medicinal innovation aimed at restoring the effectiveness of antibiotic therapy in the resistance era.

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