CHAPTER TWENTY-ONE

# Nonfluoroquinolone-Based Inhibitors of Mycobacterial Type II Topoisomerase as Potential Therapeutic Agents for TB

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## 1. INTRODUCTION

Tuberculosis (TB) is more prevalent in the world today than at any other time in human history.[1] *Mycobacterium tuberculosis* (Mtb), the pathogen responsible for TB, uses diverse strategies to survive in a variety of host lesions and to evade immune surveillance.[1] Few novel drug targets for Mtb have been identified, in spite of the genomic sequence having been known for more than a decade and the proliferation of new genetic technologies.[2] Extensive research has elucidated the Mtb *genes* that are required for growth and pathogenicity, but very few new drug targets have been validated using small-molecule inhibitors.[2] The development of novel treatments for TB is further complicated by the requirement that a new drug

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must provide an improved standard of care over the current multidrug, directly observed treatment short course regimen, which has proven to be quite effective.[3] Thus, it is imperative to validate new targets with potent small-molecule inhibitors and demonstrate their potential for therapeutic value in the context of new TB treatment regimens.[3]

One promising target for antimycobacterials is DNA gyrase, which belongs to a class of enzymes known as topoisomerases which are involved in the vital processes of DNA replication, transcription, translation, and recombination in prokaryotic and eukaryotic cells.[4,5] Two types of the topoisomerases are known: type I topoisomerases change the degree of supercoiling of DNA by causing single-strand breaks and religation and type II topoisomerases (including bacterial DNA gyrase) cause double-strand breaks.[4,5] DNA gyrase, unique to prokaryotes, binds DNA as a tetramer in which two A and two B subunits (GyrA and GyrB, respectively) combine with an appropriately displayed DNA leading strand that becomes cleaved. Subsequent passage of a lagging DNA strand through the interior of the enzyme complex and through the DNA cleavage site is followed by religation with hydrolysis of ATP to drive the catalytic cycles and produce a negative supercoil in the DNA.[6–8]

DNA gyrase has many of the ideal attributes required for an attractive antibiotic target. It is an essential gene for bacterial viability, it is present in a single copy, and its inhibition results in significant cell death because there are no viable alternative mechanisms for performing this function.[2] Gyrase genes from among a variety of Mtb isolates that have been sequenced are nearly 99.9% homologous, further signifying its broad applicability as a drug target.[2] The B subunit of DNA gyrase (GyrB) contains the ATP-binding pocket. Tetrameric topoisomerase IV (topoIV) is closely related to DNA gyrase and consists of two subunits of ParC and two of ParE, with ParE containing the ATP-binding pocket (Fig. 21.1) analogous to GyrB. The A subunit of DNA gyrase (GyrA) is responsible for DNA cleavage where covalent bonds to each of the cleaved DNA strands are made into active-site tyrosine residues. Analogously, the ParC subunits of topoIV execute DNA strand cleavage and religation.

To date, no advanced inhibitors of bacterial type I topoisomerases have advanced into the clinic.[9] In contrast, clinically valuable inhibitors of type II topoisomerases are abundant, from the well-established fluoroquinolone class to a variety of emerging classes in various stages of clinical or preclinical evaluation. Fluoroquinolones principally bind GyrA near the intersection of the GyrB subunits and the associated DNA strand of the functional DNA

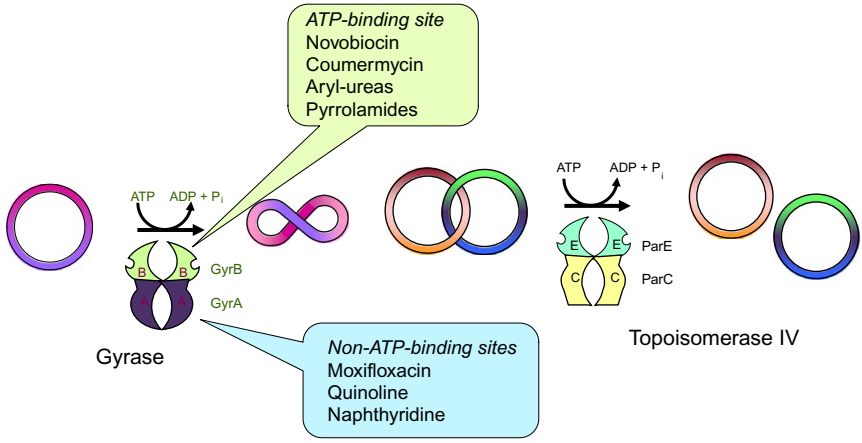
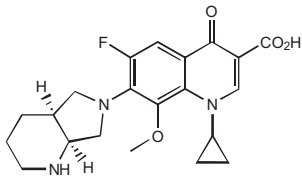



**Figure 21.1** Schematic diagram of bacterial type II topoisomerases.

gyrase heterotetramer (A2B2) complex. They analogously bind to the ParC units of topoIV, at the interface with ParE and DNA.[5] Resistance to fluoroquinolones is typically (though not exclusively) generated by point mutations in the *gyrA* gene, which gives rise to resistance against the entire class of fluoroquinolones, thus embodying class resistance.[5,6,10] As fluoroquinolones [e.g., in a recent Phase II clinical trial, moxifloxacin (**1**) was used in place of ethambutol (in combination with isoniazid, rifampicin, and pyrazinamide) and demonstrated improved culture conversion in the initial phase of TB treatment[11]] become incorporated into clinical TB drug treatment regimens, alternative drug combinations will be required to combat the emergence of fluoroquinolone-resistant Mtb strains. However, if GyrB is targeted, it still exerts the same phenotypic effects on bacterial viability as do the fluoroquinolones. Compounds such as the coumarin antibiotics novobiocin (**2**), chlorobiocin, and coumermycin that target GyrB and ParE by binding to the ATP sites also kill bacteria, thus establishing both the GyrA and GyrB subunits of DNA gyrase complex as worthwhile objectives for inhibitor and drug development against TB.

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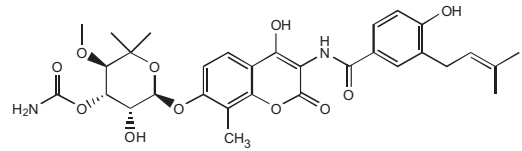


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This review largely focuses on small-molecule inhibitors of mycobacterial type II topoisomerase that bind at GyrB having ATP sites and non-fluoroquinolone inhibitors that bind at GyrA having other than ATP sites within the enzyme tetramers.

## 2. INHIBITION AT THE ATP-BINDING SITE

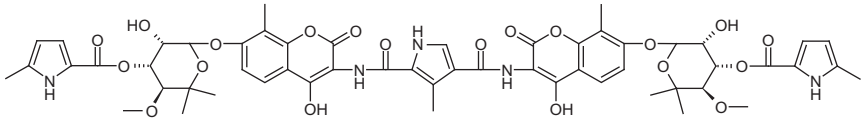
### 2.1. Novobiocin

As mentioned, the natural antibiotic novobiocin is member of aminocoumarin class which was described in 1955,[1] and its mode of action was demonstrated to be the inhibition of bacterial topoisomerase, specifically DNA gyrase, in 1970.[4,5] Novobiocin inhibits DNA gyrase and topoIV by binding to the ATP pocket of GyrB and ParE, respectively.[2,12–19] The *Streptomyces* strain that produces this and related antibiotics, and its biosynthetic pathways have been elucidated and characterized.[20] GyrB has been genetically demonstrated to be essential for Mtb viability,[2] but there have not been any effective drug developed against this target for TB. Novobiocin has been shown to be a potent inhibitor of GyrB, with enzyme inhibition ($K_i$) and binding ($K_d$) constants in the low nanomolar range (7–15 nM) as well as the ability to inhibit DNA supercoiling *in vitro*.[2] It was originally approved for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections, but has since been withdrawn from the market due to poor pharmacological properties and safety concerns.[2,12–19]

### 2.2. Coumermycin analogs

The antibiotic coumermycin Al (**3**) was first isolated from the fermentation broths of *Streptomyces rishiriensis* by Kawaguchi and co-workers.[21] It is an acidic antibiotic with *in vitro* activity against Gram-positive as well as some Gram-negative bacteria.[22,23] The spectrum of microorganisms inhibited by this compound includes *streptococci*, *pneumococci*, *bacillus*, and *mycobacteria* species, as well as a number of *enterobacteriaceae* strains. Kawaguchi *et al.* demonstrated *in vitro* activity of coumermycin A1 against three

*Mycobacterium* species; however, in mice experimentally infected with the $H_{37}Rv$ strain of Mtb, *in vivo* activity was not observed.[24] Although the antibiotic's subcutaneous $LD_{60}$ of 250–380 mg/kg and oral $LD_{50}$ of $>2000$ mg/kg[24,25] indicate only a moderate level of toxicity, the compound does have several undesirable characteristics. Among these are low oral bioavailability and an irritating effect on tissues at the site of parenteral administration. Coumermycin has several structural moieties in common with novobiocin[26,27] (**1**). Structural modification of the latter antibiotic by Walton *et al.*[28] failed to increase antibacterial potency or spectrum.



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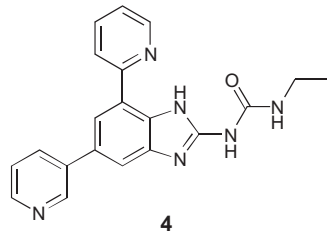
## 2.3. Aryl-ureas

As reported in the literature, aryl-ureas containing an ethyl-urea pharmacophore represent a novel class of GyrB inhibitors. This class was first identified in a high-throughput assay targeting the ATPase activity of the *S. aureus* GyrB.[29] The potent activity of this class has generated considerable attention by a variety of pharmaceutical companies through a series of scaffold-hopping approaches. Herein we describe some of the derivatives of aryl-urea scaffold that have demonstrated Mtb activity.

### *2.3.1 Aminobenzimidazole derivatives*

The aminobenzimidazole (e.g., **4**) was developed as a GyrB inhibitors for the treatment of MRSA.[29,30] This and related compounds are much more potent and less toxic as compared to novobiocin.[2] Recently, a representative from this class was compared with novobiocin to validate the GyrB target in Mtb[2] as an opportunity for a first-line drug therapy. The enzyme potency [inhibition ($K_i$) and binding ($K_d$) constants] of aminobenzimidazole to GyrB is in the low nanomolar range, making them more potent than novobiocin. The

aminobenzimidazoles also inhibit DNA supercoiling activity *in vitro*[29,31] and demonstrate cidality with an excellent activity against drug-resistant Mtb strains, including fluoroquinolone-resistant strains. These GyrB inhibitors do not exhibit antagonism against rifampicin and isoniazid[2,29,30] supporting their use in anti-TB multidrug cocktails. With GyrB as the target, aminobenzimidazole exhibits potent activity against nonreplicating Mtb expanding the attractiveness of the compounds for the treatment of TB.[32,33]



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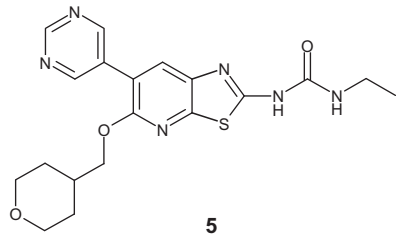
Based on the structural work with the aminobenzimidazoles, the urea portion of the molecule makes a critical hydrogen bonding interactions with an aspartic acid residue and thus the urea is essential for activity.[29,30] Therefore, any changes to the residues surrounding the urea region of the GyrB-binding pocket would result in a significant loss of activity. Because of mutations to these residues, not all mycobacteria are equally susceptible to these aminobenzimidazoles.[2] Another notable hydrogen bonding interaction is seen from the structural work between the pyridine ring of aminobenzimidazoles and an arginine residue similar to that seen for coumarin hydroxyl of aminocoumarins.[29] Mutation of the arginine residue, which is located on the edge of the ATP-binding pocket, resulted in resistance to novobiocin. However, aminobenzimidazoles do not significantly lose their potency against Mtb strains containing this novobiocin-resistant mutant, perhaps due to dual targeting of the topoIV DNA gyrase by aminobenzimidazoles.[2]

### 2.3.2 Other aryl ureas

The thiazolopyridine urea series was discovered at AstraZeneca through a scaffold-hopping approach combining benzimidazole and benzothiazole urea cores reported in the literature.[29] Compound (**5**) from this series displayed excellent biochemical potency by inhibiting *Mycobacterium smegmatis* (Msm) GyrB isozymes at 2.5 nM and good antimycobacterial activity (Mtu MIC 0.06 μg/ml).[34]



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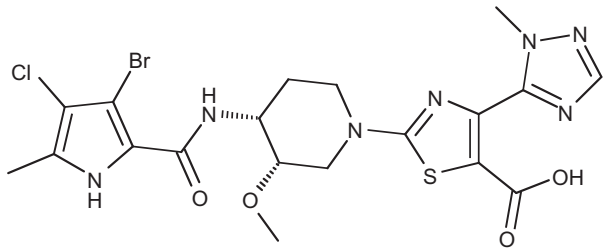
## 2.4. Miscellaneous compounds or inhibitors

### 2.4.1 Pyrrolamides

A novel class of bacterial DNA GyrB inhibitors, the pyrrolamides were discovered using fragment-based screening reported earlier.[35] Our group at AstraZeneca has profiled this novel class of inhibitors for their antimycobacterial properties with an objective of developing an orally active anti-TB agent. Initial screening and subsequent lead optimization lead to pyrrolamide (**6**), which has excellent enzyme and cellular activity against the Msm GyrB enzyme and Mtb $H_{37}Rv$ respectively.[36] Compound (**6**) also shows > 70% oral bioavailability and efficacy of 1 log kill in an acute mouse model.



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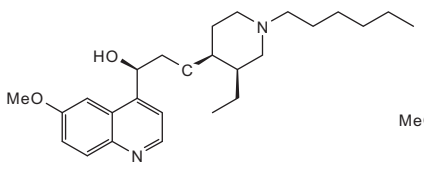
# 3. INHIBITION AT THE NON-ATP-BINDING SITE

## 3.1. Piperidinyl quinoline and naphthyridines

Without any indication of mode of action, a series of piperidinyl alkyl quinoline derivatives as antibacterials was first reported by GSK in 1999.[37] Compound **7** from this initial disclosure was reported to have an MIC of 4 μg/ml against *E. coli*. Subsequently, there has been a large body of work reported around this scaffold in an effort to develop novel antibacterial agents and establish new IP space. Novexel reported the target information for a clinical candidate **8** (NXL101), as inhibition of type II topoisomerases (both topoIV and gyrase) in *E. coli* and *S. pneumoniae*. NXL101 also showed good activity

against fluoroquinolone-resistant strains of *S. aureus* with known mutations in the quinolone-resistance determining region (QRDR). This indicates that mechanism of inhibition of topoisomerases by NXL-101 is very different as that of fluoroquinolones mechanism.[38] Compound **8** was advanced to Phase I clinical studies, but was discontinued due to QT prolongation signals in the healthy volunteers.[39] Despite numerous patents covering quinoline- and naphthyridine-based gyrase inhibitors as broad spectrum antibacterial agents, only GSK has reported antimycobacterial activity.[40–44] Analogs from quinolone (**9,10)**, pyridopyrazinone (**11**), and pyridopyrazinedione (**12**) series with bicyclic right-hand side (RHS) fragments showed Mtb MICs ranging from 0.3 to 2 μg/ml, though there is no data related to antimycobacterial mode of action reported for this set of compounds. It is likely that the Mtb activity is due to gyrase inhibition with the structural resemblance to **8** and the reported inhibition of type II topoisomerases in *E. coli* and *S. pneumonia*.[38] Further medicinal chemistry optimization for Mtb activity led to additional series with monocyclic RHS fragments as seen in compounds **13**–**16**, reported to demonstrate MICs of 0.01–0.3 μg/ml against Mtb.[45–47] Compound **14** and its closely related analogs maintain wild-type Mtb MICs and improve MICs versus fluoroquinolone-resistant strains with well-characterized mutations in the QRDR region (S91P, A90V, and D94G).[48,49] These compounds also retained Mtb MICs against extensively drug-resistant clinical isolates, which are completely resistant to first-line and second-line TB agents including ciprofloxacin and moxifloxacin. The above study reinforces that these antimycobacterial mechanisms of gyrase inhibition are distinctly different from the fluoroquinolone mode of inhibition.

Preliminary pharmacokinetic profiling of compound **13** in mouse showed 28% bioavailability and also demonstrated excellent efficacy with clear dose response (limit of quantification at 75 mg/kg BID, s.c.) in an acute mouse model with BID dosing through a subcutaneous route of administration. However, the series was reported to inhibit the hERG cardiac ion channel (hERG), and optimization of the series toward reducing the hERG liability is underway.[48,49]



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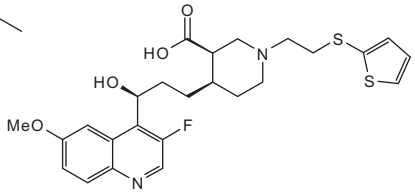

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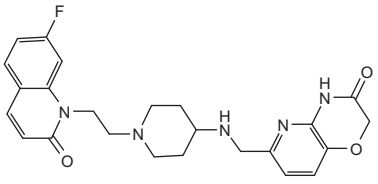

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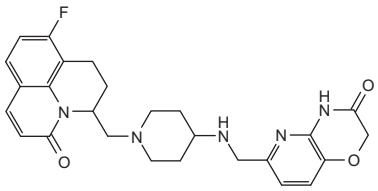

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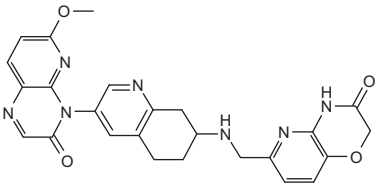

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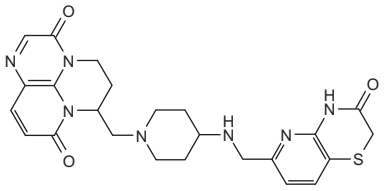

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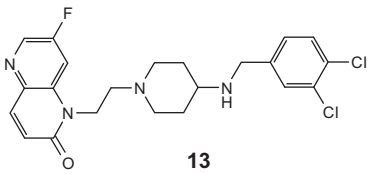

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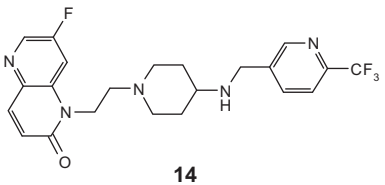

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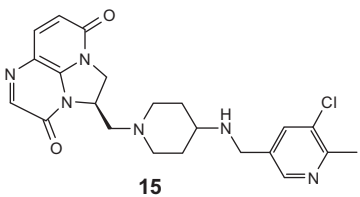

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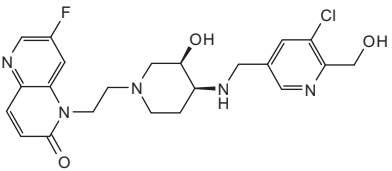

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## 4. CONCLUSIONS

In today's advanced multidisciplinary drug discovery, TB still remains a challenging endeavor at every level. DNA gyrase remains sole target for quinolones and continues to be pharmaceutically effective target for drug discovery against Mtb. The ATPase activity of bacterial DNA gyrase that resides in the B subunit is emerging as a novel target and lot of efforts are being put by various groups with encouraging results. Based on this progress, if we can successfully leverage the opportunities in this target, there is hope that we will be able to raise novel gyrase inhibitor in earnest in the long struggle against TB.

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