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► To cite this version:

Francesca Ruggieri, Nina Compagne, Kevin Antraygues, Maxime Eveque, Marion Flipo, et al.. Antibiotics with novel mode of action as new weapons to fight antimicrobial resistance. European Journal of Medicinal Chemistry, 2023, 256, pp.115413. 10.1016/j.ejmech.2023.115413 . hal-04093378

HAL Id: hal-04093378

<https://hal.science/hal-04093378>

Submitted on 10 May 2023

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Antibiotics with novel mode of action as new weapons to fight antimicrobial resistance

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Abstract: Antimicrobial resistance (AMR) is a major public health issue, causing 5 million deaths per year. Without any action plan, AMR will be in a near future the leading cause of death ahead of cancer. AMR comes from the ability of bacteria to rapidly develop and share resistance mechanisms towards current antibiotics, rendering them less effective. To circumvent this issue and avoid the phenomenon of cross-resistance, new antibiotics acting on novel targets or with new modes of action are required. Today, the pipeline of potential new treatments with these characteristics includes promising compounds such as gepotidacin, zoliflodacin, ibezapolstat, MGB-BP-3, CRS-3123, afabacin and TXA-709, which are currently in clinical trials, and lefamulin, which has been recently approved by FDA and EMA. In this review, we report the chemical synthesis, mode of action, structure-activity relationships, *in vitro* and *in vivo* activities as well as clinical data of these eight small molecules listed above.

Keywords: Antimicrobial resistance – New drugs – Novel mode of action – Novel target

Abbreviations: 3-MBA, 3-methoxybenzamide; ABSSSI, acute bacterial skin and skin structure infection; ACP, acyl carrier protein; ADME, absorption, distribution, metabolism, excretion; AE, adverse event; AMR, antimicrobial resistance; ATP, adenosine triphosphate; AUC, area under curve; BCS, biopharmaceutics classification system; CABP, community-acquired bacterial pneumonia; CDAD, *Clostridioides difficile*-associated diarrhea; CDI, 1,1'-carbonyldiimidazole / *C. difficile* infections; CFU, colony-forming unit; dGTP, deoxyguanosine triphosphate; DISARM, developing an innovative strategy for antimicrobial resistant microorganisms; DMF, dimethylformamide; DNSSA, daptomycin non-susceptible *S. aureus*; DPPA, diphenylphosphoryl azide; ED, effective dose; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; ELF, epithelial lining fluid; EMA, european medicines agency; EPI, efflux pump inhibitors; ESBL, extended spectrum β -lactamase; FDA, food and drug administration; HMDS, hexamethyldisilazane; HOBt, hydroxybenzotriazole; Ki, inhibitory constant; LC-MS, liquid chromatography-mass spectrometry; LDA, lithium diisopropylamide; LEAP, lefamulin evaluation against pneumonia; LNSSA, linezolid non-susceptible *S. aureus*; MDR, multidrug-resistant; MetRS1, type 1 methionyl-tRNA synthetase; MGBs, minor groove binders; MIC, minimum inhibitory concentration; MOA, mode of action; MPK, milligrams per kilograms; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; NADH, nicotinamide adenine dinucleotide hydrogen; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NBTI, novel bacterial topoisomerase inhibitors; NCS, N-chlorosuccinimide; PA β N, phenylalanine-arginine- β -naphthylamide; PAE, post-antibiotic effect; PD, pharmacodynamic; pH, potential of hydrogen; PK, pharmacokinetic; PPB, plasma protein binding; PRSP, penicillin-resistant *S. pneumoniae*; PTC, peptidyl transferase center; PTSA, p-toluenesulfonic acid; QIDP, qualified infectious disease product; SAR, structure-activity relationship; S_N2, nucleophilic 2nd order substitution; S_NAr, nucleophilic aromatic substitution; STI, sexually transmitted infections; t_{1/2}, half-life; TEA, triethylamine; THF, tetrahydrofuran; UTI, urinary tract infection; VISA, vancomycin-intermediate *S. aureus*; VRE, vancomycin-resistant *E. faecium*; VRSA, vancomycin-resistant *S. aureus*.

1. Introduction

Antimicrobial resistance (AMR) is one of the major public health problems of this century, with an estimated 10 million people killed annually by 2050 [1]. Recently, predictive statistical models have estimated 5 million AMR-related deaths in the year 2019 [2]. This phenomenon is mainly due to the extensive misuse of antibiotics which leads to the selection of resistant bacteria [3,4]. The resulting infections become more difficult to treat, causing serious illness, with the risk of spreading the disease and facing death.

Drug resistance in bacteria can be classified into four different mechanisms: (1) enzymatic inactivation of the drug, (2) export of the drug by efflux pumps, (3) limitation of drug uptake by changes in membrane permeability, and (4) modification of the drug target [4]. Due to the lack of lipopolysaccharide in the outer membrane, Gram-positive bacteria are more likely to enzymatically inactivate the drug or modify its target rather than limit the uptake or express efflux pumps. This is not the case for Gram-negative bacteria, which can acquire resistance through all the mechanisms described above [5].

Considering the different types of mechanisms that confer drug resistance, the alteration of the drug target is a key feature. Therefore, efforts have been made to develop drugs acting on a novel target and/or with a new mode of action (MOA), especially in academia or in small biotech companies [6,7]. Interestingly, the identification of novel drug targets may come with the discovery and development of first-in-class drugs [8].

In this review, we focus on new drugs in clinical development or recently approved, characterized by novel targets and/or MOA, that are of interest for the treatment of infections caused by multidrug-resistant bacteria (Figure 1 and Table 1). We have deliberately chosen not to discuss compounds at the pre-clinical stage.

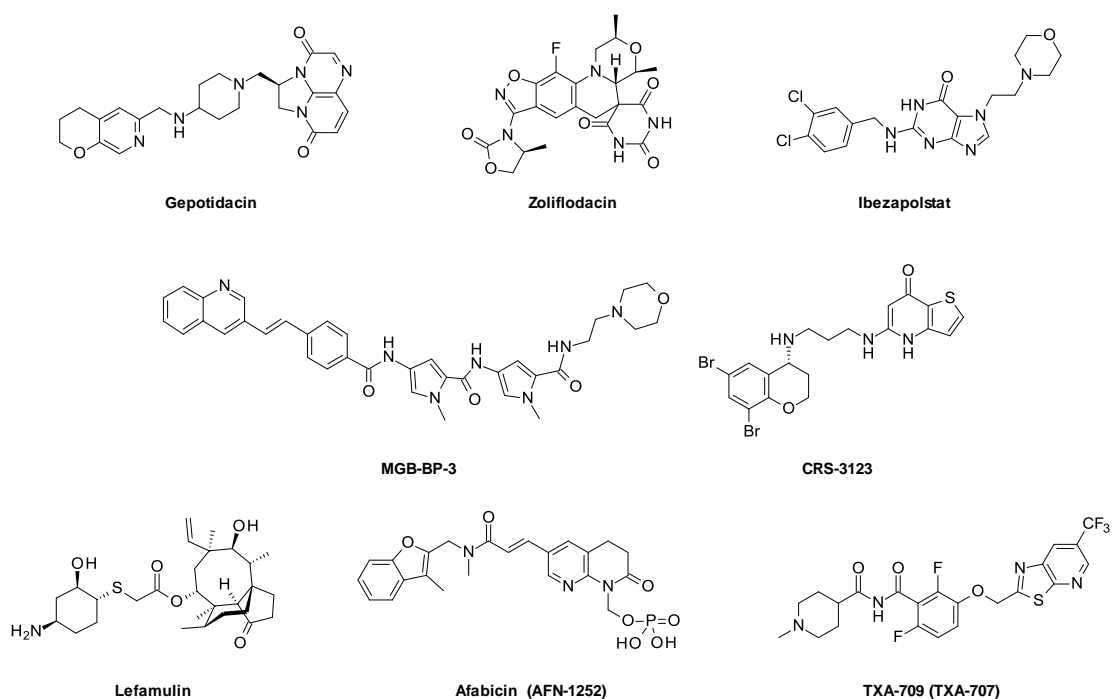


Figure 1. New drugs in the clinical pipeline or recently approved for the treatment of infections caused by resistant bacteria. For prodrugs, the name of the active pharmaceutical ingredient is written into brackets.

Table 1. Main information and calculated chemical properties of the eight drug candidates with their progression toward clinical development.

Name	Gepotidacin	Zoliflodacin	Ibezapolstat	MGB-BP-3
Family	Triazaacenaphthylene/NBTI	Spiropyrimidinetrione	DCBG ([dichlorobenzyl] guanine)	Distamycin
Mode of action	Inhibition of DNA gyrase and topoisomerase IV	Inhibition of topoisomerase II	Inhibition of DNA polymerase IIIC	Binding to the minor groove of DNA
Chemical features	MW: 448.22 cLogP: 0.12 H-bond acc.: 7 H-bond don.: 1 PSA: 90.37	MW: 487.44 cLogP: 1.79 H-bond acc.: 8 H-bond don.: 2 PSA: 143.31	MW: 423.30 cLogP: 2.49 H-bond acc.: 6 H-bond don.: 2 PSA: 83.78	MW: 631.72 cLogP: 3.79 H-bond acc.: 6 H-bond don.: 3 PSA: 122.52
Development phase	Phase III	Phase III	Phase II	Phase II
Relevant references	[9, 14, 17]	[44, 45, 48, 55, 56]	[68, 71, 72, 80, 81]	[83, 85, 86, 87, 89]

Name	CRS-3123	Lefamulin	Afabicin (prodrug of AFN-1252)	TXA-709 (prodrug of TXA-707)
Family	Diaryldiamine	Tricyclic diterpenoid pleuromutilin	Benzofuran naphthyridine	Benzamide
Mode of action	Inhibition of methionyl-tRNA synthetase (MetRS1)	Inhibition of 50S ribosomal subunit	Inhibition of FabI	Favoring uncontrolled FtsZ polymerization
Chemical features	MW: 513.25 cLogP: 4.02 H-bond acc.: 5 H-bond don.: 3 PSA: 90.63	MW: 507.73 cLogP: 2.77 H-bond acc.: 7 H-bond don.: 3 PSA: 135.15	MW: 485.43 cLogP: 2.57 H-bond acc.: 7 H-bond don.: 2 PSA: 143.22	MW: 514.47 cLogP: 3.69 H-bond acc.: 6 H-bond don.: 1 PSA: 112.66
Development phase	Phase II	Approved in 2019 (FDA) and 2020 (EMA)	Phase II	Phase I
Relevant references	[97, 98, 100, 104, 105]	[105, 108, 114, 119, 130]	[140, 141, 143, 145-147, 149]	[153, 156, 157, 161]

2. DNA topoisomerases as drug targets

2.1. Introduction

DNA topoisomerases are enzymes able to induce negative supercoil to compensate positive supercoil occurring during cellular events such as replication, transcription, recombination and DNA

repair processes. Concerning bacteria, there are four types of topoisomerases: DNA gyrases, topoisomerases I, topoisomerases IV and reverse gyrases.

Currently, DNA topoisomerases are targeted by fluoroquinolones, a well-known class of antibiotics. However, more and more fluoroquinolone-resistant strains are being reported. This critical point urges the need to develop topoisomerase inhibitors with a distinct mode of action to avoid cross-resistance. Two of these antibiotics have reached clinical trials: gepotidacin and zoliflodacin.

2.2. Gepotidacin

Gepotidacin (GSK2140944) is a triazaacenaphtylene developed by GSK and belongs to the class of Novel Bacterial Topoisomerase Inhibitors (NBTI). This new antibiotic is currently being investigated in three phase 3 clinical trials.

Gepotidacin is derived from the analogue GSK299423 described by Bax *et al.* [9], which results from a medicinal chemistry program initiated after an unbiased antibacterial screening [10].

2.2.1. Chemical synthesis

The synthesis of gepotidacin has been described in two patents in 2008 and 2016 and comprises 11 steps (Figure 2) [11,12]. First, 2-chloro-6-methoxy-3-nitro-pyridine reacts with 2-amino-propane-1,3-diol through nucleophilic aromatic substitution (S_NAr). The resulting diol is then protected with 2,2-dimethoxypropane in presence of p-toluenesulfonic acid (PTSA) followed by the reduction of the nitro group with hydrogen and 10% Pd/C. The aniline thus formed is then alkylated with ethyl bromoacetate. Cyclization is performed in basic conditions using sodium hydride, followed by oxidation using manganese dioxide. The acetal is then cleaved and the released diol reacts with methanesulfonic anhydride to form the third cycle of the triazaacenaphtylene core. Substitution with Boc-amino-piperidine, followed by deprotection and subsequent purification by chiral chromatography affords the primary amine derivative, which can be condensed by reductive amination with the corresponding aldehyde to give the free base of gepotidacin. The mono-hydrochloride salt is obtained by reaction with one equivalent of HCl 1M in diethylether [13].

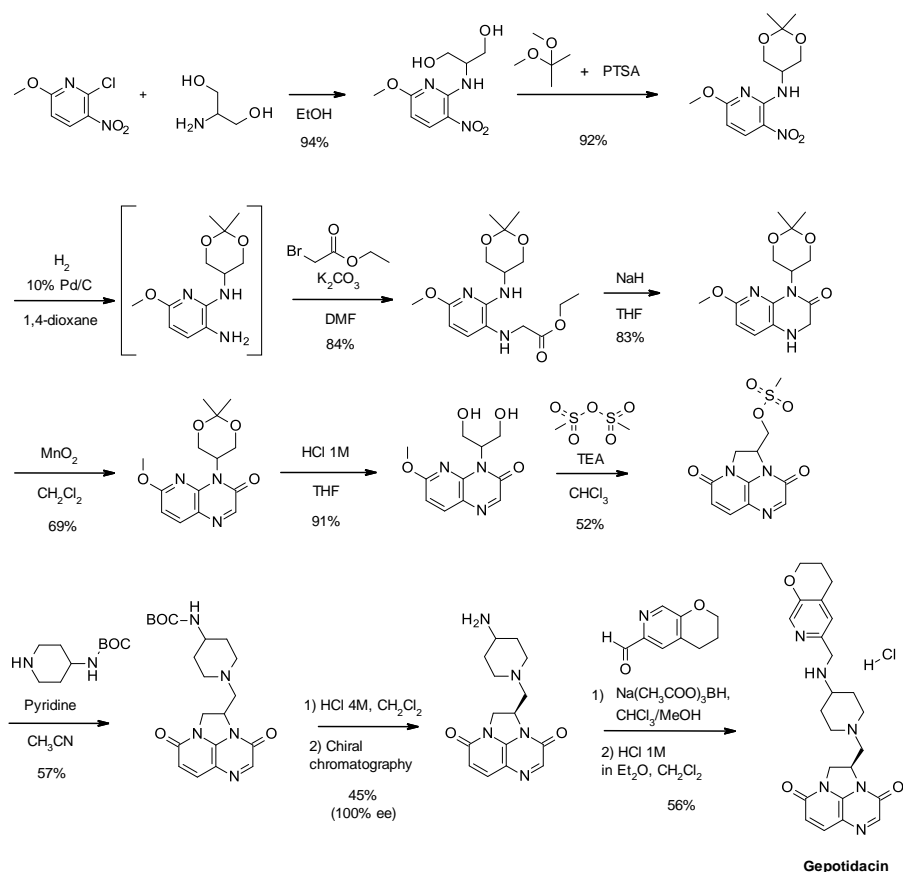


Figure 2. Description of the synthetic pathway leading to gepotidacin [11,12].

2.2.2. Target and mechanism of action

Gepotidacin inhibits both DNA gyrase and topoisomerase IV. More specifically, gepotidacin binds to the GyrA subunit of DNA gyrase and to the ParC subunit of topoisomerase IV. With its novel chemical scaffold, gepotidacin can form a ternary complex with DNA and two GyrA or ParC subunits (Figure 3) [9,14]. The structure of gepotidacin can be divided in three parts: the left-hand side (LHS), the linker and the right-hand side (RHS) (Figure 4). The polar tricyclic core of the LHS intercalates between DNA bases. Its position and structure can affect the spatial orientation of the linker and of the RHS. The linker possesses a basic amine able to interact with both GyrA/ParC subunits of DNA gyrase/topoisomerase IV. Lastly, the RHS binds to a hydrophobic pocket of the bacterial gyrase/topoisomerase IV [15].

With this new binding mode, gepotidacin shows potent antibacterial activity against a broad spectrum of Gram-positive and Gram-negative pathogenic bacteria, including clinical isolates with fluoroquinolone resistance mediated by DNA gyrase and topoisomerase IV mutations.

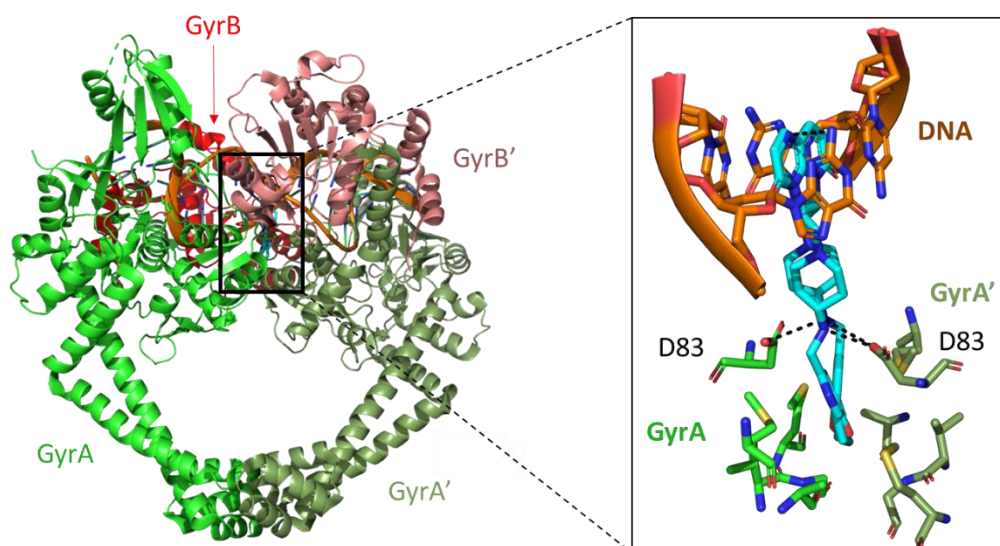


Figure 3. Views of the gepotidacin complex formed with *S. aureus* DNA gyrase and uncleaved DNA (PDB code: 6QTP). Due to a relative movement of the two GyrA subunits, the two existing positions of gepotidacin are represented.

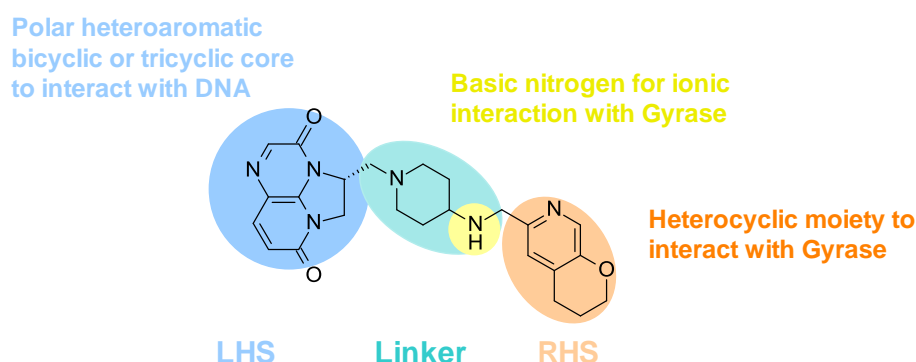


Figure 4. Summary of the key pharmacophore elements of gepotidacin.

2.2.3. *In vitro* activity

Gepotidacin displayed a bactericidal activity against a wide variety of pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant isolates of *Streptococcus pneumoniae* and *Escherichia coli* bacteria expressing extended spectrum β -lactamase (ESBL). Gepotidacin was also found active against different levofloxacin-resistant strains of *S. aureus* and *S. pneumoniae*, with MIC₉₀ of 0.5 μ g/mL. This compound was also potent against levofloxacin-resistant Gram-negative bacteria such as *E. coli* with MIC₉₀ of 4 μ g/mL [16,17].

2.2.4. *In vivo* experiments

The potency of gepotidacin against MRSA was demonstrated in a neutropenic murine lung infection model. Mice received different doses of gepotidacin, ranging from 1.56 to 400 mg/kg, administered every 6 h and starting 3 h post inoculation with six different clinical isolates of MRSA. The maximum decreases in CFU observed after 24 h were 1.4 to 3.1 log₁₀ CFU compared to the initial lung bacterial load in the neutropenic mice (between 5.6 and 6.3 log₁₀ CFU) [18].

Using neutropenic mouse models of thigh and lung infection, the pharmacokinetic-pharmacodynamic (PK-PD) parameters of gepotidacin against *S. aureus* and *S. pneumoniae* were characterized. The free plasma AUC/MIC ratio was identified as the PK-PD index most closely associated with efficacy, implying that gepotidacin activity was concentration dependent [19].

In a recent study, the predicted human exposure was used to assess the efficacy in a rat pyelonephritis model with four multidrug-resistant (MDR) *E. coli* isolates. Overall, treatment with gepotidacin resulted in a significant reduction in CFU in the kidney and bladder [20].

Regarding toxicity, gepotidacin did not demonstrate arthropathy in a juvenile rat model, contrary to fluoroquinolones [21].

2.2.5. Phase 1

Since 2012, gepotidacin has been evaluated in thirteen phase 1 clinical trials [22–34], assessing the safety and tolerability of single and repeated oral or IV doses (100 mg to 6 g per day) in adults and adolescents. In these studies, the authors also evaluated the PK parameters in plasma and lungs, cardiac conduction, food effect, drug-drug interactions and penetration into prostate and tonsil tissue. Finally, the PK was established in patients with various renal and hepatic insufficiencies. Recently, another phase 1 study started to compare the PK properties of the powder for oral suspension to the existing gepotidacin tablets [35].

2.2.6. Phase 2

Gepotidacin was tested against three types of bacterial infections in phase 2 clinical trials. It was studied as a treatment for Gram-positive Acute Bacterial Skin and Skin Structure Infections (ABSSSI), for uncomplicated urogenital gonorrhoea caused by *Neisseria gonorrhoeae*, as well as for uncomplicated Urinary Tract Infections (UTI).

In the ABSSSI phase 2 trial, 122 patients with wound infection, major cutaneous abscess or cellulitis were enrolled. Three dose regimens were evaluated: 750 mg every 12 h (q12h), 1000 mg every 12 h and 1000 mg every 8 h (q8h). Success was observed for the 750 mg q12h and the 1000 mg q8h regimens. The majority of adverse events (AE) declared were gastrointestinal events, principally nausea and diarrhoea [36].

In a second phase 2 trial, 69 participants with urogenital gonorrhoea received a single oral dose of 1500 or 3000 mg. 96% of patients achieved microbiological cure (bacterial eradication of *N. gonorrhoeae*). Of note, 2 out of 3 failures were due to a high-level resistance. AEs were mostly gastrointestinal events (diarrhoea, flatulence, abdominal pain, nausea). Two patients described severe gastrointestinal AE (flatulence or dizziness) but did not discontinue the study. One treatment-related cardiovascular AE (mild tachycardia) was reported by a patient with no underlying cardiovascular history [37].

The goal of the third phase 2 trial was to explore the PK properties in UTI patients. 22 women with uncomplicated UTI received 1500 mg gepotidacin orally twice daily for 5 days. The test of cure was performed from days 10 to 13 and the follow-up visits were planned at day 28 +/- 3. With a dose of 1500 mg twice daily, the maximum plasma concentration was reached 1 h to 1.5 h after dosing. Steady state was reached on day 3. Clinical success was validated for 18 patients (82%) at follow-up visits. 21 patients (95%) experienced mild to moderate AEs, primarily gastrointestinal events. Two patients (9%) reported vulvovaginal mycotic infection after treatment, but no AE prevented treatment [38].

2.2.7. Current advances

Gepotidacin is currently being evaluated in three phase 3 clinical trials. Two studies aim to compare gepotidacin (1500 mg orally twice a day for 5 days) and nitrofurantoin (100 mg orally twice a day for 5 days) for the treatment of uncomplicated UTI in approximately 2500 patients each [39,40]. The third trial will assess the efficacy of gepotidacin (3000 mg orally twice) compared with the combination ceftriaxone/azithromycin (single doses of 500 mg intramuscular and 1000 mg orally, respectively) for the treatment of uncomplicated urogenital gonorrhoea on an estimated 600 patients [41].

In a recent study, gepotidacin was also found efficacious in an inhalational model of pneumonic plague in the African green monkey, a non-human primate, at exposures of the drug equivalent to those achieved in humans. In this experiment, gepotidacin provided a 75 to 100% survival benefit with doses ranging from 28 to 48 mg/kg. These data open up the possibility of also using gepotidacin as a treatment for pneumonic plague caused by *Yersinia pestis* [42].

2.3. Zoliflodacin

Zoliflodacin (previously known as ETX0914 or AZD0914) is a novel spiropyrimidinetrione antibiotic being developed as single oral dose for the treatment of uncomplicated gonorrhoea [43–46].

2.3.1. Spectrum of activity

Zoliflodacin displays activity against Gram-positive bacteria including *S. pneumoniae*, *Streptococcus pyogenes*, and methicillin-resistant fluoroquinolone-resistant *S. aureus*, as well as against fastidious Gram-negative bacteria including *Haemophilus influenzae* and *N. gonorrhoea* [47].

2.3.2. Chemical synthesis

The synthesis of zoliflodacin described below was reported in 2015 [47]. The first step, starting from 2,3,4-trifluorobenzaldehyde, consists of the protection of the aldehyde function to an acetal group. After deprotonation using *n*-BuLi, formylation is performed with DMF to introduce an aldehyde group, which is then converted to oxime using hydroxylamine. Chlorination with *N*-chlorosuccinimide (NCS), followed by reaction with *L*-alaninol and intramolecular S_NAr allows the formation of the benzisoxazole ring. The oxazolidinone moiety is obtained using 1,1'-carbonyldiimidazole (CDI). The deprotection of the aldehyde is then performed in acidic conditions followed by another S_NAr at the *ortho* position of the aldehyde using (2*R*,6*S*)-2,6-dimethylmorpholine. Finally, a Knoevenagel condensation between the aldehyde and hexahydropyrimidine-2,4,6-trione is performed, followed by an intramolecular rearrangement consisting in an [1-5] hydride shift and then intramolecular cyclization leading to zoliflodacin (Figure 5).

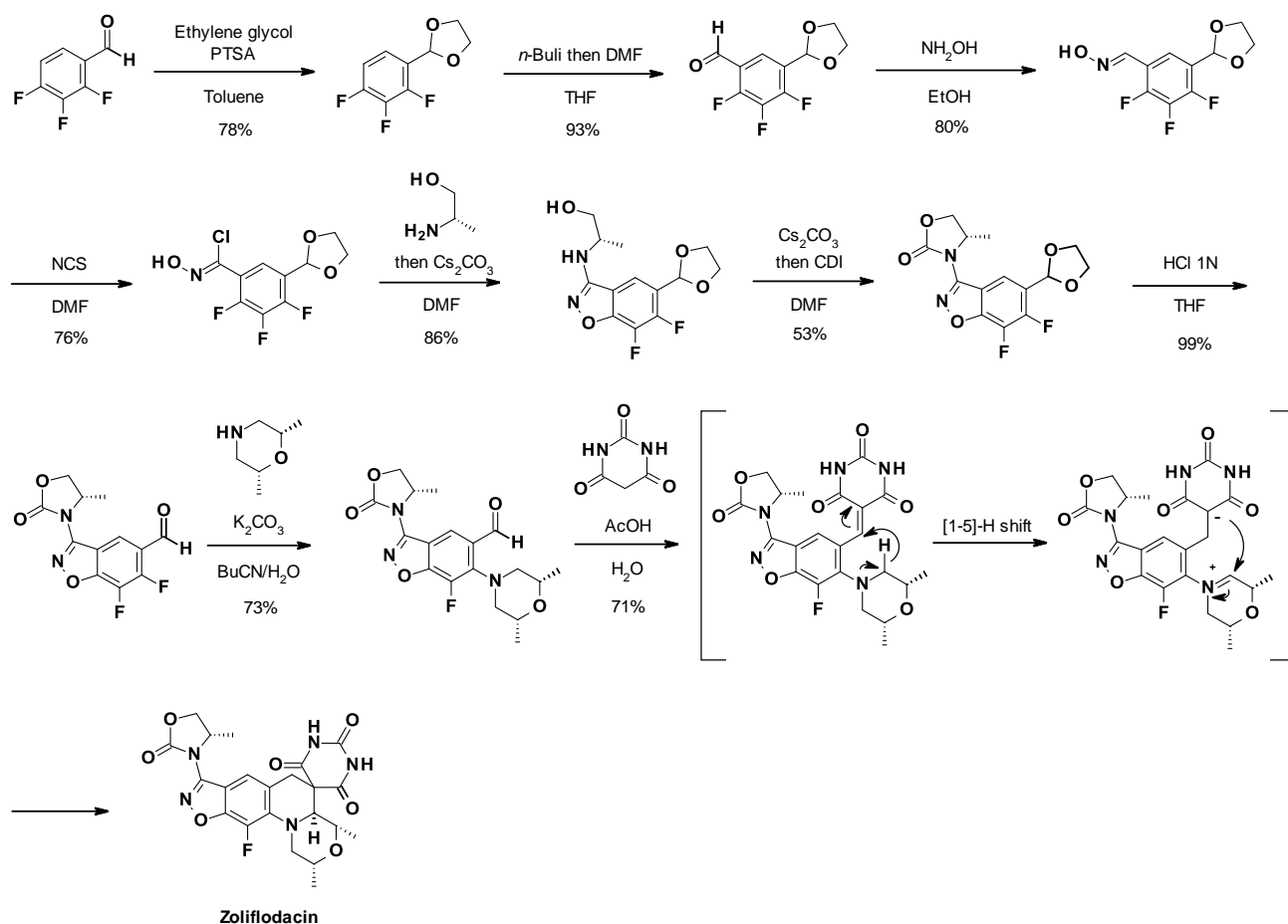


Figure 5. Description of the synthetic pathway leading to zoliflodacin [47].

2.3.3. Target and mechanism of action

Zoliflodacin is a topoisomerase II inhibitor that presents a new mode of action compared with previous inhibitors, such as fluoroquinolones [48]. Zoliflodacin binds to the GyrB subunit of the bacterial DNA gyrase whereas fluoroquinolones target the GyrA subunit [49]. Briefly, the binding enables to stop the cleaved covalent complex of gyrase which thus blocks the religation of the double-strand cleaved DNA to form fused circular DNA. This process leads to the inhibition of DNA biosynthesis and the accumulation of double-strand cleavages in bacteria [50]. Interestingly, there is no cross-resistance with the existing fluoroquinolones [51].

2.3.4. Structure-activity relationship (SAR) studies

After a high-throughput screening at Pharmacia & Upjohn, QPT-1 was found as the first spiropyrimidinetrione inhibiting bacterial DNA gyrase and topoisomerase IV with no cross-resistance with fluoroquinolones (Figure 6). A benzisoxazole scaffold was then introduced by scientists at AstraZeneca [52]. However, due to toxicity and genotoxicity other variations were studied:

- **Spirocyclic pyrimidinetrione:** the pyrimidinetrione was kept constant in all the synthesized analogues.
- **Oxazolidinone introduction:** the oxazolidinone ring leading to zoliflodacin was introduced replacing the methyl substituent of the previous lead to mitigate mammalian toxicity and to obtain compounds characterized by a greater safety [44]. Highest potency was found for derivatives

with substituents at both the 4- and 5- positions orientated below the plane of the oxazolidinone ring [53].

- **Benzisoxazole substitution:** the replacement of the oxazolidinone ring with an ethyloxadiazole and triazole rings led to analogues with higher activity than zoliflodacin. Moreover, the introduction of heterocycles linked to the benzisoxazole through an ether oxygen in a two-atom bridge and the replacement of the fluorine atom with a chlorine resulted in compounds with a similar activity to zoliflodacin [44,54]. In contrast, pyrrolidine benzisoxazole substituents led to derivatives 200-fold less active against *S. aureus*.
- **Morpholine modifications:** overall, the morpholine ring showed little scope for variations and the modifications resulted in less active compounds. The tested analogues were synthesized replacing the morpholine with substituted piperidines and piperazines. However, the introduction of a *N*-cyano piperazine resulted in a *S. aureus* active compound [54]. Furthermore, the biological evaluation of the corresponding enantiomers and diastereoisomers showed that the specific stereochemistry and the two methyl groups around the morpholine are crucial for the antibacterial activity [44,53].

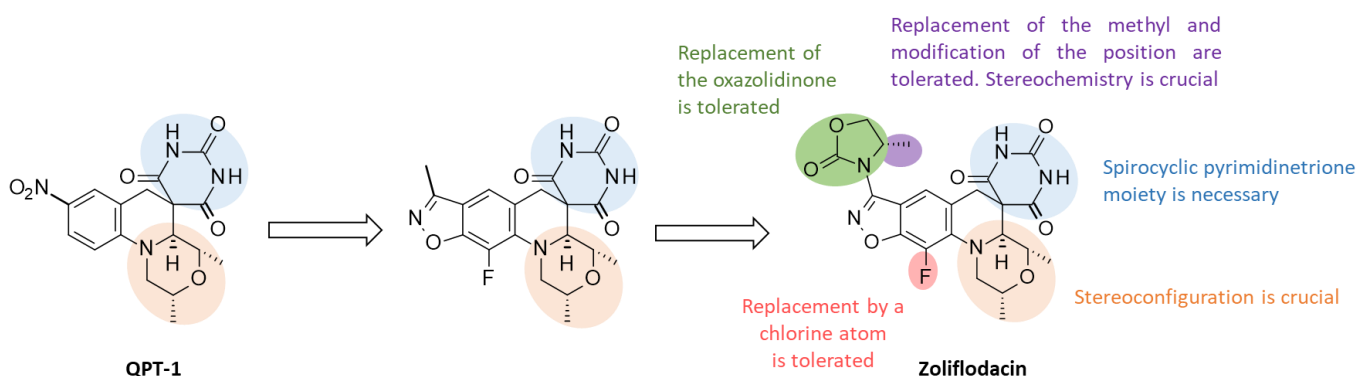


Figure 6. Summary of the SARs of zoliflodacin.

2.3.5. *In vitro* activity

Zoliflodacin is a potent and very selective inhibitor of bacterial topoisomerases. Indeed, *in vitro* assays measuring the growth inhibition of myeloid and erythroid cell lines in mammals showed no genotoxicity or bone marrow toxicity for zoliflodacin at the highest concentrations tested. These data indicate that zoliflodacin presents reduced risks of hematologic toxicity compared with some other fluoroquinolones (such as gemifloxacin, for instance), which have been associated with anemia, leukopenia and bone marrow suppression [48].

Zoliflodacin is bactericidal, with a low frequency of resistance and a potent *in vitro* activity against *N. gonorrhoeae* (MIC₉₀ of 0.125 µg/mL) [45,48]. To date, no clinical zoliflodacin-resistant *N. gonorrhoeae* isolates have been found [48]. However, zoliflodacin resistant mutants have been selected *in vitro*, and all of them contained nonsynonymous mutations in amino acid codons D429 or K450 of GyrB, which leads to increased MICs of zoliflodacin in the mutants [49]. Zoliflodacin also presented potent *in vitro* activity against *Chlamydia pneumoniae*, certain Gram-positive organisms (*S. aureus* and *S. pneumoniae*; including fluoroquinolone- and vancomycin-resistant staphylococci), fastidious Gram-negative organisms (*Moraxella catarrhalis* and *Haemophilus* spp., in particular *H. influenzae*), and atypical anaerobic organisms [44,51].

Zoliflodacin also displayed potent *in vitro* activity against *Mycoplasma genitalium* and 11 azithromycin- and fluoroquinolone-susceptible isolates with MICs < 1 mg/L [55]. Furthermore, zoliflodacin showed activity against *Chlamydia trachomatis* with the highest zoliflodacin MIC of 0.5 mg/L among 10 strains.

Zoliflodacin was also fully active against laboratory strains of *S. aureus* and *S. pneumoniae* resistant to novobiocin. It was also more active against *S. aureus* (including MRSA) than linezolid, which is the current standard of care for skin infections caused by this pathogen [47].

More recently, zoliflodacin demonstrated potent *in vitro* antibacterial activity against a collection of clinical gonococcal isolates from China, including isolates with a high-level of resistance to ciprofloxacin, azithromycin, and extended-spectrum cephalosporins [56]. Due to a distinct mechanism of action, cross-resistance between zoliflodacin and fluoroquinolones has not been observed [57].

2.3.6. *In vivo* experiments

In vitro and *in vivo* studies across species showed that zoliflodacin was extensively metabolized in humans via the cytochrome P450 and non-P450-mediated pathways [58]. The assessment of the *in vivo* efficacy of zoliflodacin was defined using a murine thigh infection model in immunocompetent and neutropenic mice using a methicillin-susceptible strain of *S. aureus* [59]. This study showed that the compound was efficient in a dose-dependent manner in both mouse models and a 3-log reduction in CFU was observed when the exposure (AUC) achieved 70 µg.h/mL in immunocompetent mice and 120 µg.h/mL in immunosuppressed mice.

2.3.7. Phase 1

Two phase 1 studies have been conducted with single oral doses in healthy volunteers [58]. The first study determined the safety, tolerability and PK parameters of zoliflodacin in both fed and fasted states [60], whereas the second study assessed the PK and ADME properties using [¹⁴C] zoliflodacin [61].

In the first study, zoliflodacin showed a rapid absorption, with a T_{max} observed between 1.5 and 2.3 h, and a T_{1/2} range of 5.3 to 6.3 h. Interestingly, the absorption of the drug was delayed (T_{max} at 4 h) in

subjects who were dosed following ingestion of a high-fat meal. Finally, no significant AEs were observed in this study.

In the second study, the ADME properties were defined after a single oral dose of 3000 mg of [¹⁴C] zoliflodacin. The data demonstrated that the PK profile of zoliflodacin (AUC and C_{max}) was similar to the one obtained in the first phase 1 study.

Overall, both phase 1 studies showed linear PK data, good oral bioavailability and no significant safety findings.

2.3.8. Phase 2

The safety and efficacy of zoliflodacin was then assessed in a randomized phase 2 study on 179 participants with uncomplicated urogenital gonorrhea [62]. The subjects were randomized to receive a single oral dose of zoliflodacin (either 2 or 3 g) or a single 500 mg intramuscular dose of ceftriaxone. In summary, oral zoliflodacin has successfully treated most uncomplicated urogenital and rectal gonococcal infections, but has shown little efficacy in treating pharyngeal infections.

2.3.9. Phase 3

Following promising results from one of the phase 2 clinical trials, a phase 3 for the treatment of uncomplicated urogenital gonorrhea is currently underway [63]. The study is expected to include approximately 1000 adults with urogenital gonorrhea from different sites (USA, The Netherlands, Thailand, and South Africa) and results are now expected in 2023 (current status). This open-label trial compares a single 3 g oral dose of zoliflodacin to a combination of ceftriaxone and azithromycin (2:1 randomization). Recently, a parallel study [64] demonstrated that a single oral dose of zoliflodacin was positively evaluated for cardiac safety, supporting further clinical experiments with single 3 g doses in patient with gonorrhea [46].

3. DNA polymerase as a drug target

3.1. Introduction

DNA replication is carried out by the DNA polymerase, a massive enzymatic complex highly organized and therefore susceptible to disruption. Inhibitors of DNA polymerase are widely used to treat viral infections, such as aciclovir for the treatment of herpes virus. However, there is currently no antibiotic in clinic which targets the bacterial DNA polymerase, whereas human and bacterial DNA polymerases have many differences [65]. To date, ibezapolstat is the first bacterial specific DNA polymerase inhibitor in clinical development.

3.2. Ibezapolstat

Ibezapolstat (formerly ACX-362E) is a member of the 6-anilinouracil family of dGTP analogues [66]. Developed by Acurx Pharmaceuticals, it selectively inhibits DNA polymerase III_C of several Gram-positive bacteria, through competition with dGTP [67]. In 2012, Dvoskin *et al.* demonstrated that ibezapolstat was highly effective against *Clostridioides difficile* on *in vitro* and *in vivo* models [68]. The compound is currently being evaluated in a phase 2 clinical study [69].

3.2.1. Chemical synthesis

Ibezapolstat was first synthesized in 2010 in a 3-step procedure, following a protocol described by Wright *et al.* (Figure 7) [70]. Firstly, 2-chloro-6-methoxypurine is alkylated with 1,2-dibromoethane to afford a mixture of 7- and 9- isomers. After isolation of the 7-isomer, a S_N2 reaction with morpholine leads to the corresponding intermediate, which is then subjected to reaction with 3,4-dichlorobenzylamine to give ibezapolstat after the concomitant demethylation due to HCl release.

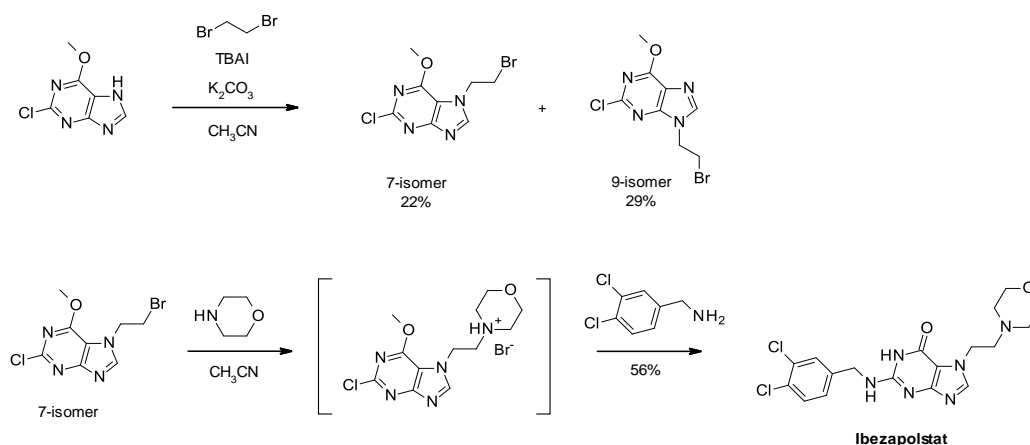


Figure 7. Description of the synthetic pathway leading to ibezapolstat [70].

3.2.2. Target and mechanism of action

Ibezapolstat inhibits the DNA polymerase III_C (DNA pol III_C), an enzyme essential for bacterial DNA replication [71], from low G + C Gram-positive germs (DNA containing less than 50 % of cytosine and guanine). Ibezapolstat is also an analogue of dGTP, allowing the compound to pair with dCTG of DNA. In addition, the aryl part of the molecule can bind to DNA pol III_C. The combination of these two properties leads to the formation of an inactive ternary complex between DNA, ibezapolstat and DNA pol III_C (Figure 8). To note, the compound inhibits the DNA pol III_C of *C. difficile* with a K_i of 0.325 μM [72]. Moreover, there is only little homology between DNA pol III_C and mammalian polymerase [73].

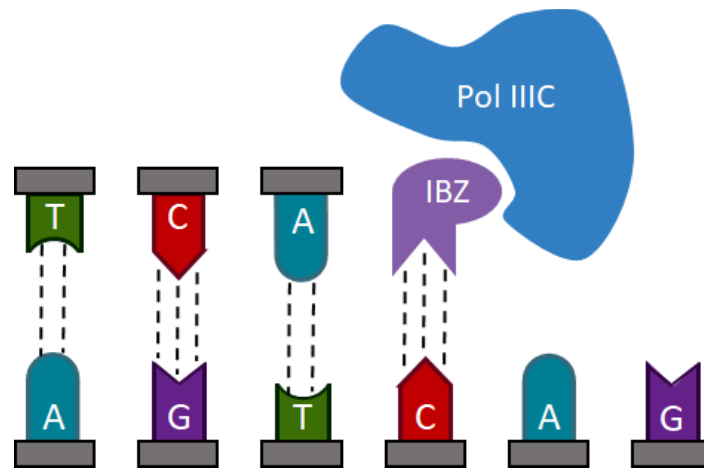


Figure 8. Binding mode of ibezapolstat (IBZ) to dCTP and DNA pol IIIIC. Adapted from Acurx Pharmaceuticals [74].

40 % of the animals died after 7 days of treatment. Administration of ibezapolstat during 14 days did not result in recurrence in any of the animals infected during the 34 days of the experiment.

In the hamster model of *Clostridioides difficile*-associated diarrhea (CDAD), ibezapolstat showed no signs of toxicity at an oral dose of 1 g/kg [68]. Moreover, at the oral dose of 75 mg/kg, only 5 % is absorbed. Because the site of CDI is exclusively located in the colon, a lower absorption results in higher concentrations in the intestine.

3.2.6. Phase 1

Ibezapolstat has completed a phase 1 study in the United States [80]. The randomized, double-blind, placebo-controlled clinical trial involved 62 healthy adults and was divided into three parts.

The first consisted of oral administration of a single ascending dose of ibezapolstat at doses of 150, 300, 600 and 900 mg. In the second part, a single dose of 300 mg was given with a meal. Finally, the third part was a multi-day ascending dose study of ibezapolstat 300 mg and 450 mg given twice daily or vancomycin 125 mg given four times daily.

The drug candidate was found to be safe and well-tolerated. Consistent with the hamster pharmacokinetics, ibezapolstat was poorly absorbed, resulting in no systemic accumulation. Of note, faecal concentrations of ibezapolstat after 4 days of multiple ascending doses were higher than those of vancomycin. Vancomycin also decreased the diversity of the microbiome while ibezapolstat targeted only low G+C organisms. Moreover, vancomycin increased the proportion of proteobacteria, which is associated with susceptibility to CDI [81].

3.2.7. Phase 2

A phase 2a study was conducted in 10 adults with CDI, as a single-arm, open-label multicenter clinical trial [82]. Ibezapolstat was orally given 450 mg twice daily, during 10 days. Safety and pharmacokinetic profiles were consistent with the data from the phase 1 study. *C. difficile* was eradicated after 3 days, resulting in 100 % cure of all 10 patients. In addition, none of the patients experienced a recurrence during the 28-day study.

Regarding the microbiome, an increased proportion of healthy microbiota was observed, including bacteria capable of metabolizing primary bile acids into secondary bile acids [83]. This change in the microbiome resulted in an increased proportion of secondary bile acids, which is associated with protection against CDI [84]. Ibezapolstat is currently being evaluated in phase 2b study to compare efficacy of the drug candidate against vancomycin [69].

4. DNA minor groove as a drug target

4.1. Introduction

Small molecules can manifest their effect on DNA through various binding mechanisms such as intercalation, alkylation as well as major and minor groove binding [85]. These interactions can cause a variety of important biological responses. Minor groove binders (MGBs) are well-known for their applications as anticancer drugs, although their importance as anti-infective agents is growing [86]. The majority of synthetic MGBs are linked to the two natural occurring antibiotics, netropsin and distamycin A, and are generally characterized by the presence of an alkene, a non-polar N-terminal amide isostere, and N-alkyl chains on the pyrrole rings [87].

4.2. MGB-BP-3

MGB-BP-3 is a synthetic polyamide belonging to the large family of minor groove binders, small molecules capable of binding to the minor groove of DNA. Hundreds of MGBs were synthesized and tested for various biological activities by Khalaf *et al.* at the University of Strathclyde. In the end, MGB-BP-3 was found to be the most active against Gram-positive bacteria. Collaboration with MGB Biopharma enabled the preclinical and clinical development of MGB-BP-3 for the treatment of gastrointestinal infections caused by *C. difficile* [87–89].

4.2.1. Chemical Synthesis

MGB-BP-3 was synthesized by Anthony *et al.* by combining two moieties (synthon A and synthon B) [90]. To obtain the synthon A, 1-methyl-4-nitro-pyrrole-2-carboxylic acid is first converted into an acyl chloride which reacts with aminoethyl morpholine to give the corresponding amide. After reduction of the nitro group the intermediate is reacted with the previous acyl chloride to deliver the synthon A. The synthon B is synthesized in three steps starting from methyl 4-(bromomethyl)benzoate. At first, an Arbuzov reaction using triethylphosphite gives the corresponding phosphonate which reacts with quinoline-3-aldehyde *via* a Horner Wadsworth Emmons reaction. Finally, the saponification of the methyl ester delivers the synthon B. Eventually, the reduction of the nitro group of the synthon A and the subsequent coupling with the synthon B lead to MGB-BP-3 (Figure 10).

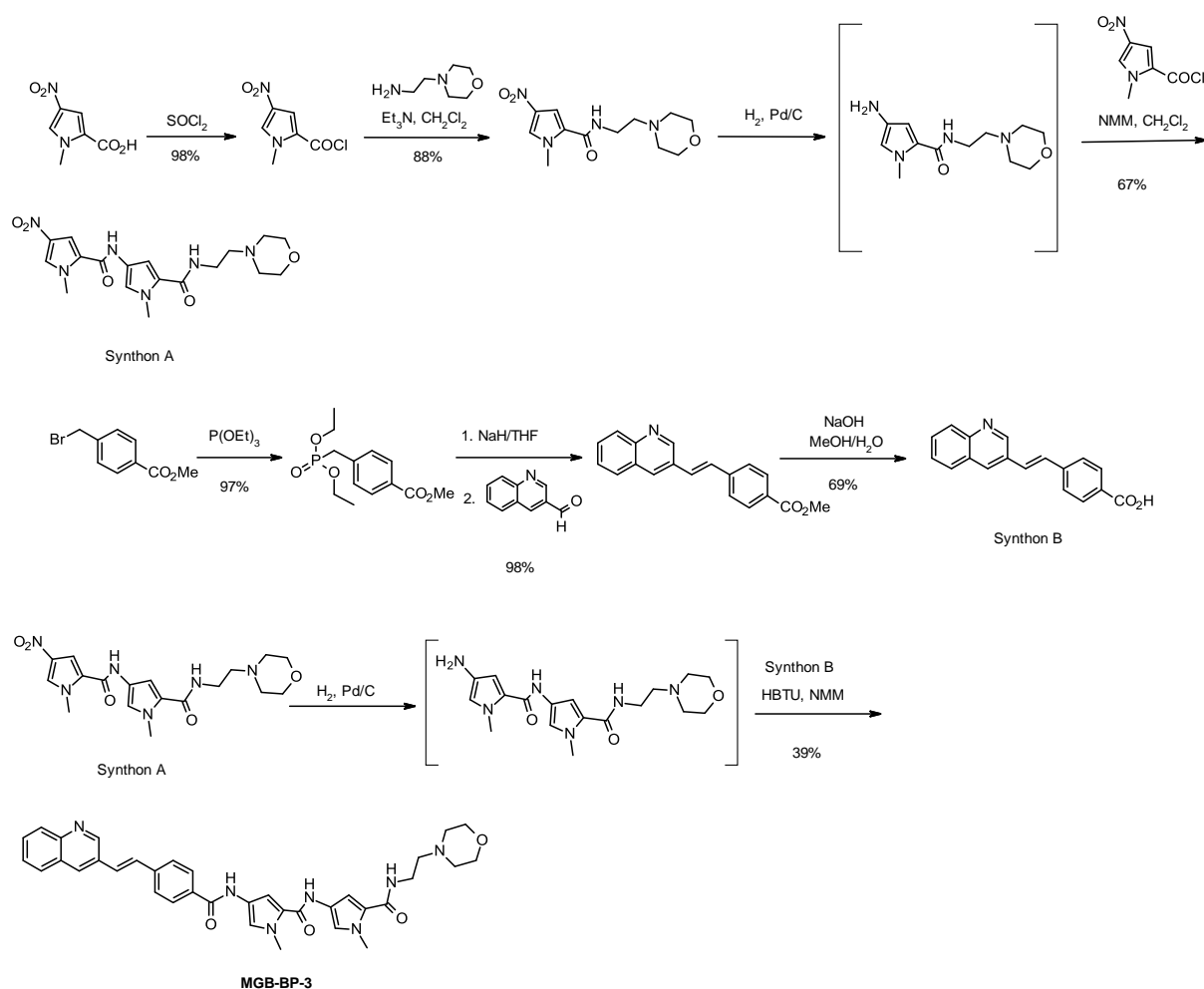


Figure 10. Description of the synthetic pathway leading to MGB-BP-3 [90].

4.2.2. Target and mechanism of action

MGB-BP-3 binds non-covalently to the minor groove of the DNA, most readily to AT-rich regions [89]. Probably, MGB-BP-3 binds as a dimer, with two molecules of ligand in the minor groove with an antiparallel configuration. Thermal melt analysis, capillary electrophoresis, fluorescence intercalator displacement assay, mass spectrometry and NMR techniques have been exploited to investigate and confirm the binding of MGB-BP-3 to DNA [91,92]. MGB-BP-3 binding to DNA interferes with gene expression at the transcription level. Recently, Hind *et al.* found out that MGB-BP-3 interferes with other DNA-centric processes, such as the action of topoisomerase and gyrase, with a different mechanism of action than fluoroquinolones [92].

4.2.3. Structure-activity relationship studies

Suckling *et al.* synthesized several MGB-BP-3-like compounds and reported the following structure-activity relationships against Gram-positive bacteria (Figure 11) [88]:

- **Amino tail group:** substituents such as piperazines, piperidines, pyrrolidines were introduced and led to significantly active but more toxic compounds.
- **Aromatic head group:** aromatic head groups such as meta-methoxybenzene and para-methoxybenzene led to active compounds. However, the introduction of a naphthalene and isoquinoline head groups resulted in less active compounds.
- **Linker:** to increase further the non-polar interactions an alkene between the benzene ring and the quinoline was successfully introduced and opened the way for the discovery of the lead compound MGB-BP-3. In contrast, when an alkene was introduced between the two pyrroles the activity was totally lost.
- **N-substitution:** the introduction of isopropyl or bulkier groups on the nitrogen of pyrrole rings led to active compounds.

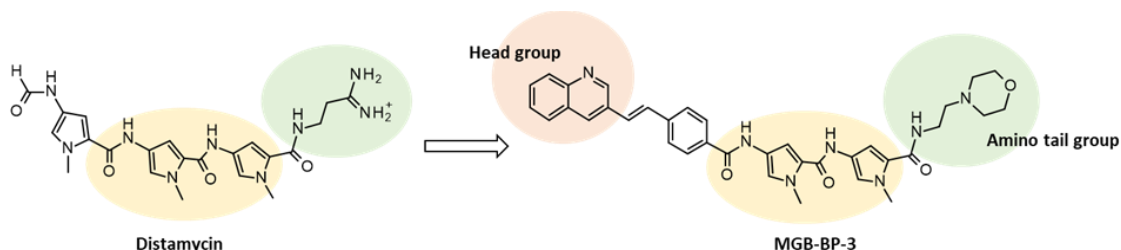


Figure 11. Summary of the SARs of MGB-BP-3 from distamycin.

4.2.4. *In vitro* activity

MGB-BP-3 showed substantial antibacterial activity against many Gram-positive bacteria such as *S. aureus* ($\text{MIC}_{80} < 0.2 \mu\text{M}$) [89]. No evident toxicity was found on a mammalian cell line (HS27 murine fibroblast). Thanks to fluorescence microscopy, Suckling *et al.* showed that there was no penetration of MGB-BP-3 into several mammalian cell lines and concluded that the minor groove binder targeted selectively the bacterial cell [88].

MGB-BP-3 is highly active against *C. difficile*, killing the vegetative bacterium cell before sporulation. In particular, MGB-BP-3 exhibits strong bactericidal activity against BI/NAP1/027, the most virulent strain that is highly resistant to current therapies [93].

In addition, *S. aureus* was grown at sub-MIC₈₀ concentrations of MGB-BP-3 or rifampicin (used as a control) for 80 generations. No resistant strain towards MGB-BP-3 was observed, contrary to rifampicin [89].

MGB-BP-3 did not show significant activity against Gram-negative bacteria. Interestingly, significant activity was found when *Pseudomonas aeruginosa* bacteria were treated with MGB-BP-3 in association with an efflux pump inhibitor (PAβN, Phenylalanine-Arginine-β-naphthylamide) or a membrane permeabilizer (polymyxin B nonapeptide). These results suggest that the major obstacle to the antibacterial activity of MGB-BP-3 is the expression of efflux pumps [89].

4.2.5. *In vivo* experiments

MGB-BP-3 was able to protect against death and prolonged post-treatment survival in a hamster model of CDI [93]. In the study, the efficacy of MGB-BP-3 (free base suspension and colonic delivery microparticles) was compared to that of vancomycin in the treatment of clindamycin-induced *C. difficile* infection in a hamster CDAD model [83].

The animals received 30 mg/kg oral clindamycin, followed 24 hours later by *C. difficile* (strain BI1) by gavage. The treatment started 20 hours post *C. difficile* infection and was administered orally for 5 days. The hamsters received MGB-BP-3 as a free base suspension or as colonic delivery microparticles (10 mg bid), vancomycin (25 mg/kg bid) or a vehicle. The animals were observed for up to 13 days post *C. difficile* administration and sacrificed when severe infection developed.

All untreated and vehicle treated hamster died within 36 hours post infection. On the contrary, MGB-BP-3 free base suspension, MGB-BP-3 microparticle formulation and vancomycin treated hamsters survived throughout the treatment period. 3 days after the end of the treatment, hamsters that received MGB-BP-3 free base deteriorated clinically (wet tail and hypothermia). Conversely, MGB-BP-3 microparticle formulation and vancomycin treated hamsters remained healthy and survived to the end of the study.

Moreover, the rates of regrowth of *C. difficile* isolates in hamster following the end of vancomycin and MGB-BP-3 treatment were compared. *C. difficile* burden in the colon and caecum was much higher in animals treated with vancomycin compared to MGB-BP-3 microparticles. Finally, MIC values of MGB-BP-3 against *C. difficile* before and after infection were measured and no change was observed in their values, indicating that resistance did not develop during therapy.

In another study, the same hamster model of severe CDAD was used to assess the disposition of two MGB-BP-3 formulations (free base suspension and microparticles) in plasma and intestines after oral administration [84]. The results indicated that MGB-BP-3 has a favorable PK profile for the treatment of *C. difficile* infections.

4.2.6. Phase 1

A group of 40 healthy volunteers took part in a phase 1 study in which MGB-BP-3 exhibited an excellent safety and tolerability profile with no serious AEs. Only one subject in the 125 mg dose cohort experienced transient dizziness [94].

4.2.7. Phase 2

A phase 2 study was conducted to assess the safety, tolerability, and efficacy of incremental doses of MGB-BP-3 in patients with CDAD. Three sequential ascending doses of 125 mg, 250 mg, and 500 mg given twice daily were investigated. The study showed that a 10 day-administration of 250 mg of MGB-BP-3 given twice daily resulted in an initial cure and 100% durable cure, with no disease recurrence measured four weeks after treatment. This regimen has been selected for further clinical trials [95].

4.2.8. Current Advances

MGB-BP-3 has been granted “qualified infectious disease product” status (QIDP) by the FDA, allowing it to receive fast track designation and priority review. The molecule will also be eligible to participate in the US program DISARM (Developing an Innovative Strategy for Antimicrobial Resistant Microorganisms), which addresses the lack of financial incentives for pharmaceutical companies to develop new antibiotics [93]. In addition, a phase 3 head-to-head trial has been agreed with the FDA, comparing MGB-BP-3 to vancomycin [89].

5. tRNAs and ribosomes as drug targets

5.1. Introduction

tRNAs are small non-coding RNAs that play a key role in the translation process by delivering the amino acids to the ribosome for incorporation into a growing polypeptide chain during the protein synthesis [96]. Any component of this essential process is thus an interesting target for the development of new antibiotics. This will be highlighted with the development of CRS-3123 and lefamulin.

5.2. CRS-3123

CRS-3123 (formerly REP-3123) targets the methionyl-tRNA synthetase of Gram-positive bacteria, in particular *C. difficile* [97]. The drug candidate is being developed by Creston Inc. and has completed two phase 1 studies in 2022 [98,99].

5.2.1. Chemical synthesis

CRS-3123 was initially synthesized by Replidyne Inc, [100] following a convergent strategy described in Figure 12 [101]. Synthon A is prepared in 6 steps, by first condensing 3-thienyl isocyanate and Meldrum acid through a Knoevenagel-type reaction. The thienolate intermediate is then alkylated with iodomethane. Substitution with 3,3-diethoxypropylamine allows the introduction of the alkyl chain. The obtained intermediate is then heated in presence of HMDS (hexamethyldisilazane) to perform an intramolecular cyclization and obtain thieno[3,2-b]pyridine bicycle, bearing a carboxylic acid. Decarboxylation is achieved with sodium methoxide at reflux to give the pyridinolate intermediate, which is next acylated with benzoic anhydride. Lastly, the acetal group is cleaved in acidic conditions to give the aldehyde A.

In parallel, synthon B is obtained in 3 steps. 6,8-dibromochroman-4-one is stereoselectively reduced using (1S,2S)-(+)-*N*-*p*-tosyl-1,2-diphenylethylene diamine and dichloro(*p*-cymene)-ruthenium (II) dimer to give the S-alcohol intermediate. S_N2 with DPPA (diphenylphosphoryl azide) allows the replacement of the hydroxy group with an azido group, while achieving inversion of configuration of the chiral carbon. The R-azido derivative is then reduced *via* a Staudinger reaction to give the synthon B.

Reductive amination between synthons A and B with sodium triacetoxyborohydride gives the corresponding intermediate, which is then deprotected using ammonia in methanol to cleave the benzoyl group. Finally, salification with HCl in ethanol affords CRS-3123 as a dihydrochloride salt.

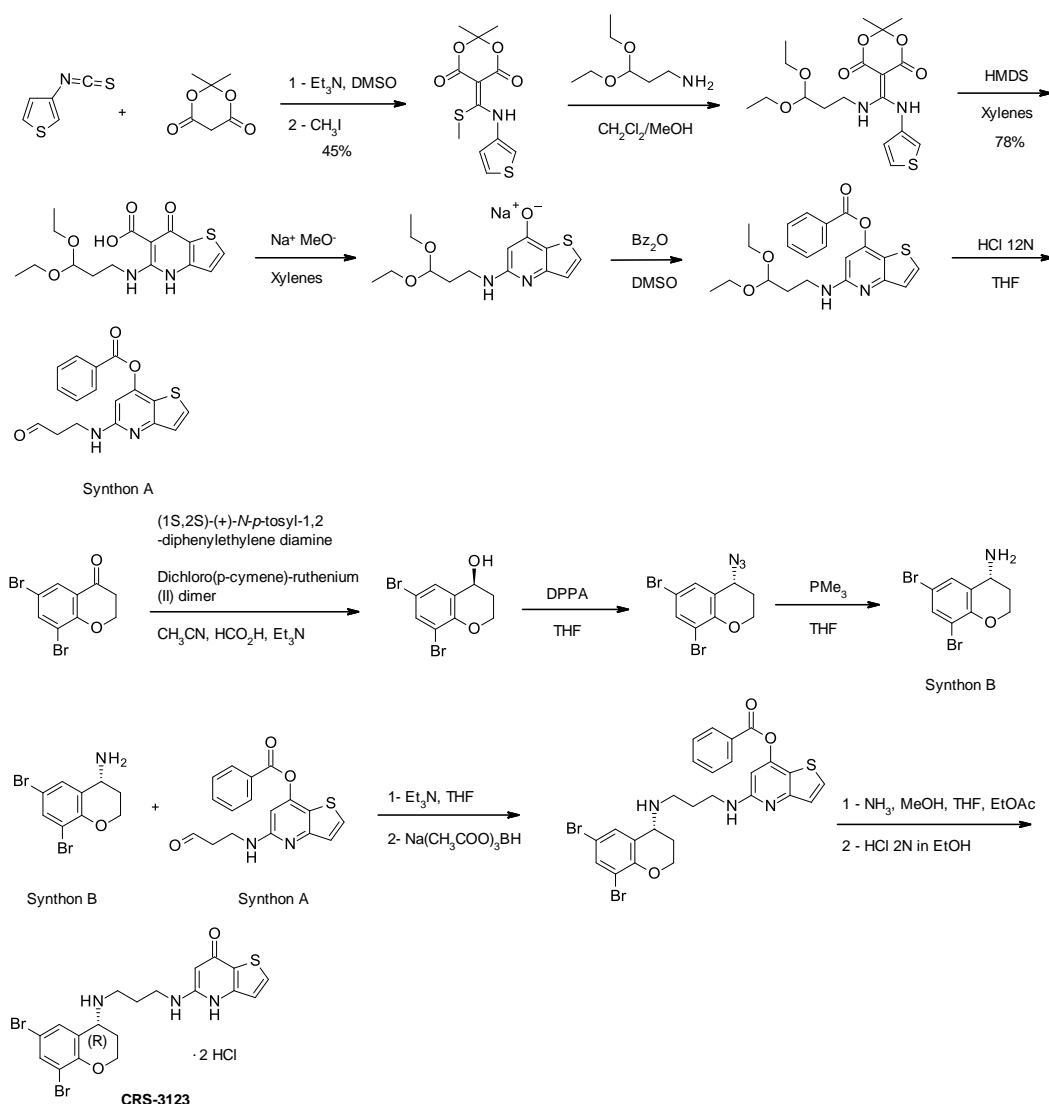


Figure 12. Description of the synthetic pathway leading to CRS-3123 [101]. Where known, yields are indicated.

5.2.2. Target and mechanism of action

CRS-3123 inhibits bacterial protein synthesis by targeting type 1 methionyl-tRNA synthetase (MetRS1) of Gram-positive bacteria [100]. This enzyme catalyzes two consecutive reactions [102]: the formation of the reactive methionyl adenylate species, resulting from the condensation of methionine and ATP, and the transfer of the amino acid on tRNA *via* the activated intermediate. The charged tRNA is then delivered to the ribosome to initiate translation.

Interestingly, the R-enantiomer of CRS-3123 is the most potent stereoisomer against the *C. difficile* MetRS1, with a K_i of 0.02 nM. CRS-3123 inhibits also MetRS1 of other Gram-positive bacteria, such as *S. aureus* ($K_i = 0.017$ nM) and *S. pneumoniae* ($K_i = 0.08$ nM). Notably, the compound is 1000 times less potent on the human mitochondrial MetRS1 ($K_i = 28$ nM). CRS-3123 is less potent against MetRS2, which is present among Gram-negative bacteria, such as *H. influenzae* ($K_i = 178$ nM) and *E. coli* ($K_i = 1900$ nM). To note, the human cytoplasmic type 2 enzyme is not inhibited ($K_i > 20000$ nM).

5.2.3. *In vitro* activity

In agreement with the inhibition data of MetRS1 described above, CRS-3123 is potent against more than 100 isolates of *C. difficile* with MIC ranging from 0.5 to 1 µg/mL [97,100]. Moreover, the compound is as potent as vancomycin (MIC = 0.25-4 µg/mL). CRS-3123 is also active against other Gram-positive bacteria, such as MRSA (MIC = 0.015-0.5 µg/mL) or vancomycin-resistant *Enterococcus faecium* (MIC < 0.004 µg/mL). However, the drug is not potent against Gram-negative bacteria, with MICs > 32 µg/mL for *E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa* or *Acinetobacter baumannii*. Moreover, CRS-3123 is not active against various *Lactobacillus* spp., *Actinomyces* spp. and *Bifidobacterium* spp. (MIC > 32 mg/mL for each) which are Gram-positive anaerobic bacteria of gut microbiota. Even if no pre-existing resistance was observed for *C. difficile*, mutants with decreased susceptibility towards CRS-3123 were selected, with spontaneous resistance frequencies of 10^{-9} - 10^{-8} at 16x MIC and 4x MIC respectively [103].

Contrary to vancomycin and metronidazole, CRS-3123 is able to decrease production of toxins A and B from *C. difficile* [104], which are at the origin of the pathogenicity of the bacterium [105].

Finally, CRS-3123 shows reduction of spore production after 4 days, even at 0.25x MIC [104]. In contrast, metronidazole and vancomycin are not able to decrease the formation of spores, which is even promoted for some strains. The inhibition of spore production observed for CRS-3123 is an interesting property, as spores are very difficult to eliminate and are the main source of new infections and recurrences [105].

5.2.4. *In vivo* experiments

CRS-3123 was shown more effective than vancomycin *in vivo* [104]. In a hamster model of CDI, CRS-3123 at daily doses of 0.5 mg/kg and 5 mg/kg greatly reduced the mortality of the animal, with 62 % and 75 % survival after 33 days, respectively. In this experiment, CRS-3123 was also superior to vancomycin, where no animal survived after 25 days. The increased survival of CRS-3123 is likely due to the inhibition of spore formation, preventing re-infection of animals during the study.

5.2.5. Toxicity and pharmacokinetics

In healthy uninfected hamsters, CRS-3123 displayed a bioavailability of 0.6 %, leading to low systemic exposure and maximal concentrations in the gut [104]. The bioavailability is also low in Sprague-Dawley rats (1 to 10.5 %) and in beagle dogs (< 1 to 7.3 %). CRS-3123 safety was sufficient for progression of the drug into clinical studies [98].

5.2.6. Phase 1

CRS-3123 has completed two phase 1 clinical studies. A double-blind, single-center, placebo-controlled single-ascending dose study was conducted in 40 healthy adults, with a dose of 100, 200, 400, 800 or 1200 mg [98]. The drug candidate was found safe and well tolerated, with the same frequency and severity of AEs between the drug group and the placebo group. Although bioavailability has not been precisely determined, it was found that plasma concentration at higher dose of CRS-3123 were above MICs of Gram-positive pathogenic bacteria. By LC-MS analysis, the *N*-glucuronide metabolite of CRS-3123 was observed in plasma and urine, indicating that the metabolite is excreted in urine. This is also the case for the non-metabolized CRS-3123.

The second phase 1 trial was a double-blind, single-center, placebo-controlled multiple-ascending dose study, conducted in 30 healthy adults with doses of 200, 400 and 600 mg administered twice daily during 10 days [99]. As for the first phase 1 study, CRS-3123 was safe and well tolerated. In particular, no prolongation of the QT interval was noticed. After administration, a large proportion of the drug was retained in the gut and excreted in feces. Indeed, the percentage of free drug and glucuronide metabolite in urine was inferior to 2 %. The low absorption of CRS-3123 leads to no sys-

temic accumulation. Moreover, high concentrations of the drug are recovered in feces at any doses (2115 – 8280 µg/g), which are superior to the MIC₉₀ value against *C. difficile* (1 µg/mL). However, even with high concentrations, CRS-3123 has a minimal effect on the normal microbiota and does not increase the proportion of Proteobacteria. With this narrow spectrum of activity, CRS-3123 is likely to reduce recurrences after treatment of CDI, as it was demonstrated for fidaxomicin [106].

5.2.7. Current advances

With a promising pharmacokinetic and bacterial selectivity profile for therapeutic use, CRS-3123 is currently evaluated in a phase 2 study, to compare efficacy of the drug against vancomycin in adults with CDI [107].

5.3. Lefamulin

Lefamulin is a semisynthetic derivative of the naturally occurring tricyclic diterpenoid pleuromutilin class of antibiotics [108,109]. The compound (formerly known as BC-3781) was developed by Nabriva Therapeutics and is currently marketed under the brand name Xenleta. The drug was approved by the FDA and the EMA in 2019 and 2020, respectively, as a short-course monotherapy for the treatment of community-acquired bacterial pneumonia (CABP) in adults [110–112]. This is the first agent within the Pleuromutilin derivative class to be developed for systemic use in humans by oral and intravenous administration [108,113].

5.3.1. Chemical synthesis

The synthetic route was patented in 2008 by Mang *et al.* [114]. Lefamulin is synthesized in two steps from pleuromutilin thiol [115] and syn-3,4-epoxycyclohexyl-carbamic acid tert-butyl ester [116]. After a S_N2 reaction leading to a mixture of diastereomers (1R, 2R, 4R) and (1S, 2S, 4S), the Boc protecting group is cleaved in acidic conditions. The mixture is then separated by chiral column chromatography to afford lefamulin (Figure 13).

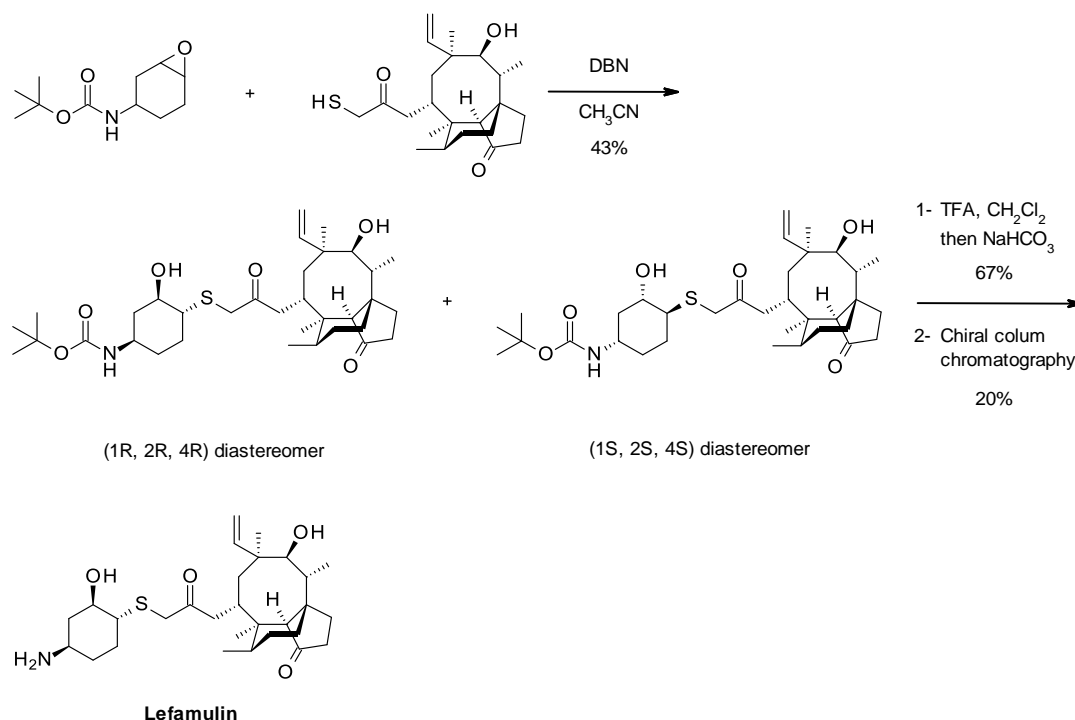


Figure 13. Description of the synthetic pathway leading to Lefamulin [114].

5.3.2. Target and mechanism of action

Lefamulin selectively inhibits bacterial ribosomal translation without affecting the eukaryotic process through a unique mechanism of tight-fit binding to the peptidyl transferase center (PTC) on the 50S ribosomal subunit [108,117]. More precisely, the tricyclic core and the side chain of lefamulin interact respectively with the A (aminoacyl) and P (peptidyl) sites of the subunit, through hydrophobic and hydrogen bond interactions (Figure 14) [108,117,118]. A binding pocket is then formed and the bacterial ribosome closes around the tricyclic core, preventing the correct positioning and binding of tRNA at the A and P sites, which leads to protein synthesis inhibition. However, once elongation has started, lefamulin is ineffective [109,113].

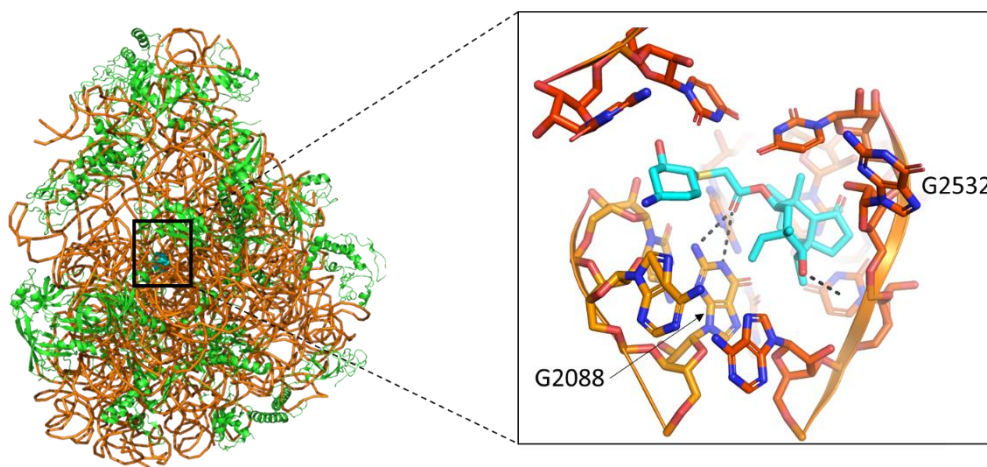


Figure 14. Views of lefamulin complex formed with *S. aureus* DNA gyrase and uncleaved DNA (PDB code: 5HL7).

5.3.3. Structure-activity relationship studies

Lefamulin was designed after a comprehensive study of the possible modifications at the C14-side chain of pleuromutilins [119] (Figure 15).

- **R side chain:** Replacement of the cyclohexyl moiety with an aromatic or non-aromatic heterocycle was tolerated. The thioether function resulted in a gain in potency and water solubility [120].
- **Linker:** the ester linker was favorable for the antibacterial activity. Indeed, the replacement with an amide or a methoxy linker resulted in less potent compounds.
- **Tricyclic core:** the tricyclic moiety together with its hydroxyl and vinyl groups are essential for the antimicrobial activity.

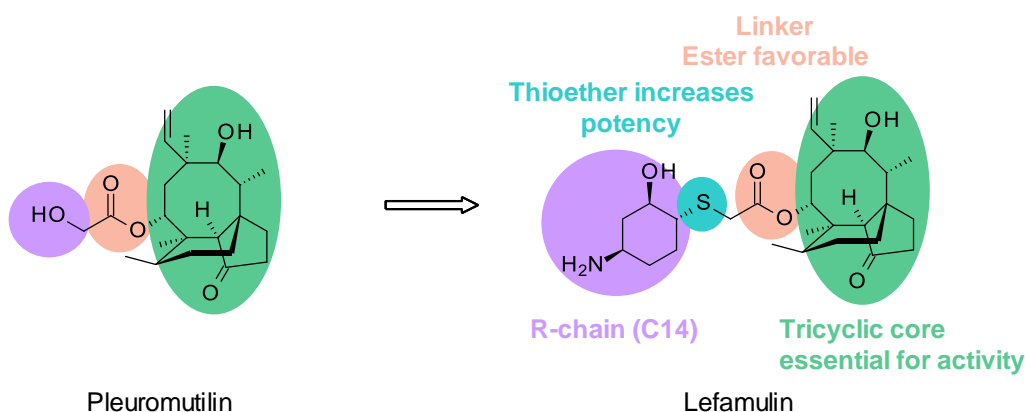


Figure 15. Summary of the SARs of Lefamulin starting from pleuromutilin.

5.3.4. *In vitro* activity

Lefamulin selectively inhibits prokaryotic ribosomal protein synthesis [117]. This selectivity is due to the double layer nucleotides around the pleuromutilin tricyclic core, which creates a structurally and chemically different binding pocket between the prokaryotes and the eukaryotes/archaea. [121] Lefamulin has a potent activity against Gram-positive (*S. pneumoniae*) and resistant Gram-positive (vancomycin-intermediate *S. aureus* (VISA), heterogeneous VISA (hVISA), vancomycin-resistant *S. aureus* (VRSA), penicillin-resistant *S. pneumoniae* (PRSP), MDR *S. pneumoniae*, and vancomycin-resistant *E. faecium* (VRE)) microorganisms. Moreover, the compound shows a potent activity against some Gram-negative pathogens such as *H. influenzae*, *Moraxella catharralis* and *Neisseria* spp.; highly effective against *N. gonorrhoeae*) as well as against mycoplasmas (*Mycoplasma pneumoniae* and *Mycoplasma genitalium*) and intracellular organisms (*Legionella pneumophila*, *Chlamydia trachomatis*, *C. pneumoniae* and spp.) [108,111,117,122,123]. Lefamulin is also fully active against MRSA, β -hemolytic streptococci (group A: *Streptococcus pyogenes* and group B: *Streptococcus agalactiae*) and other drug-resistant organisms such as macrolide-resistant *M. pneumoniae*, for instance [111,117]. Other potent activities against coagulase-negative staphylococci and viridans group streptococci have also been demonstrated [117,122]. Pleuromutilins suppress bacterial protein translation with excellent specificity, but has no effect on eukaryotic protein synthesis, as shown by *in vitro* transcription/translation assays using bacterial ribosomes

In vitro studies revealed that lefamulin is both a substrate and an inhibitor of the CYP3A isoenzyme [124]. Lefamulin has shown *in vitro* activity against aerobic Gram-positive bacteria, a limited number of fastidious Gram-negative bacteria, and atypical bacteria [122,125]. Interestingly, the compound is bactericidal against *S. pneumoniae*, *H. influenzae* and *M. pneumoniae* at concentrations of 0.06, 0.5 and 0.008 $\mu\text{g/ml}$, respectively, and bacteriostatic against *S. aureus* and *S. pyogenes* [118].

In addition, lefamulin exhibits excellent activity against drug-resistant strains of *S. pneumoniae*, the most prevalent bacterial cause of CABP. The drug-resistant strains include isolates resistant to penicillins, cephalosporins, clarithromycin, doxycycline, and trimethoprim–sulfamethoxazole, as well as MDR isolates [126]. Lefamulin also displayed potent *in vitro* activity against Gram-positive organisms commonly associated with ABSSIs, which include *S. pyogenes*, *S. aureus* and MRSA strains [127–129]. Although not indicated for this use, lefamulin has *in vitro* activity against *C. trachomatis*, susceptible and MDR *M. genitalium* and susceptible and resistant *N. gonorrhoeae*, which are the most relevant bacterial pathogens that cause sexually transmitted infections (STI) [130].

5.3.5. *In vivo* experiments

Lefamulin has shown a potent *in vivo* efficacy in pulmonary infection mouse model [131]. The study showed that the compound is not affected by pulmonary surfactant and is able to quickly penetrate and accumulate in J774 mouse macrophages, with a plateau reached in 3 h. The PK parameters were assessed in mice (35 to 70 mg/kg), and demonstrated a fast distribution of lefamulin from the plasma to the target tissue (the epithelial lining fluid (ELF) in lungs), with a 2-fold increase in the drug exposure in the ELF during the 5.5 h period. Moreover, the study displayed desirable PD data in the neutropenic lung infections experiments, where mice received cyclophosphamide before being infected with single *S. pneumoniae* or *S. aureus* strains, and subcutaneous lefamulin (1.25–160 mg/kg) given twice daily post-infection.

The same authors reported that lefamulin exhibits time- and concentration-dependent killing in a neutropenic murine thigh infection model [132]. In this study, free drug values (~20% free fraction) were also established, ranging from 1.98–6.42 and 8.04–16.5 for the *S. pneumoniae* or *S. aureus* strains, respectively.

5.3.6. Phase 1

A four-period crossover study was performed to assess the bioavailability of lefamulin [133]. Moreover, a single-dose study was performed to evaluate the penetration of lefamulin into the ELF. The latter was performed on healthy human volunteers and demonstrated the good penetration into the ELF (fAUC_{0–24} of 8978 ng·h/mL and C_{max} 706 ng/mL) but also in other tissues such as the skeletal muscle and adipose tissue [134]. Last but not least, the PK parameters of IV lefamulin and its major metabolite BC-8041 were assessed in subjects with different degrees of hepatic impairment as well as healthy individuals. Overall, lefamulin was well tolerated, and no significant pharmacokinetic difference was observed between the compound and its metabolite [112].

5.3.7. Phase 2

A double-blind, parallel group, multicenter phase 2 study was performed to compare the efficacy of IV lefamulin to IV vancomycin in 210 adults with ABSSIs. Similar clinical success rates across pathogens were observed between the two groups, including methicillin-susceptible *S. aureus* (MSSA) and MRSA [135]. To date, no subsequent phase 3 trials have been pursued for the treatment of ABSSIs.

5.3.8. Phase 3

Although no phase 2 study for the indication of CABP was performed, two multicentre, randomized, double-blind, double-dummy phase 3 trials were conducted for this infectious disease: the Lefamulin Evaluation Against Pneumonia 1 and 2 (LEAP 1 and LEAP 2). The LEAP 1 trial assessed the

efficacy and safety of IV lefamulin compared to IV moxifloxacin +/- linezolid in 551 adults patients with moderate to severe CABP [136,137]. The LEAP 2 study compared the safety and efficacy of 600mg oral lefamulin twice daily for 5 days versus 400 mg of oral moxifloxacin once daily for 7 days in 738 adults patients with moderate CABP [138,139]. Data from both trials have shown noninferior efficacy with short course therapy compared with moxifloxacin in the treatment of CABP [113].

5.3.9. Safety and tolerability

Intravenous and oral lefamulin was generally well tolerated in phase 1 and phase 2 clinical studies [124,134]. From the phase 2 trial, the most frequently reported AEs were headache, nausea, and diarrhea [135]. In both LEAP 1 and LEAP 2 trials, most AEs were mild or moderate in severity for both arm groups [137,140]. Lefamulin remains contraindicated in patients with known hypersensitivity to pleuromutilins [141]. Interestingly, the concomitant IV or oral administration of lefamulin with strong and/or moderate CYP3A or P-glycoprotein inducers can reduce the efficacy of the drug and should be then avoided. On the other hand, IV or oral coadministration of lefamulin with drugs that strongly or moderately inhibit CYP3A or P-glycoprotein inhibitors might increase the risk of AEs.

5.3.10. Current advances

Lefamulin is currently marketed as treatment for CABP caused by susceptible microorganisms. Moreover, lefamulin could be an option for the treatment of other serious bacterial infections caused by MRSA and vancomycin-resistant *E. faecium*, but also by MDR organisms associated with STI (*N. gonorrhoeae* and *M. genitalium*). The use of lefamulin could be also considered for patients with high-risk/recurrent CDI [109].

6. FabI enzyme as a drug target

6.1. Introduction

FabI is an essential NADH-dependent enzyme which catalyzes the reduction of enoyl-acyl carrier protein (enoyl-ACP) in the last step of the bacterial fatty acid biosynthesis pathway (FAS-II), involved in the synthesis of phospholipids. Contrary to the multifunctional mammalian type I fatty acid synthase (FAS-I), each reaction of FAS-II is catalyzed by a different enzyme [142]. This structural difference between the two pathways is the reason why inhibitors of the FAS-II pathway are selective and safe for use in humans. On the other hand, the complexity in the bacterial fatty acid metabolism can narrow the spectrum of activity of FabI inhibitors [142]. However, their development has continued since FabI represents an essential and validated target in multidrug-resistant *S. aureus*.

FabI inhibitors can be divided in two groups, depending on their ability to form a covalent or non-covalent adduct with the NAD⁺ cofactor. The covalent inhibitors include the diazaborines and the first-line tuberculosis drug isoniazid whereas inhibitors that do not form a covalent adduct include for instance AFN-1252, the preclinical drug candidate fabimycine [143], and the preservation agent triclosan, forming a much broader and heterogeneous group [142].

6.2. AFN-1252

AFN-1252 is a selective inhibitor of *S. aureus* FabI. This molecule belongs to the benzofuran naphthyridines, a novel class of antibiotics. The effort to identify potent and selective FabI inhibitors began with the screening of the GSK proprietary compound collection. From a first high-throughput screening on the *S. aureus* FabI enzyme, Miller *et al.* identified a 1,4-benzodiazepine derivative as a

low micromolar inhibitor. A structure-guided optimization process led to aminopyridine and oxotetrahydronaphthyridine derivatives and to the selection of AFN-1252 as a lead development candidate [144,145].

6.2.1. Chemical Synthesis

The synthesis of AFN-1252 was patented in 2008 by Affinium Pharmaceuticals [145]. The strategy consists of combining two different moieties. The first 3-methylbenzofuran moiety (synthon A) is synthesized starting from 3-methylbenzofuran-2-carboxylic acid. The addition of aqueous methylamine to the acyl chloride intermediate leads to the formation of the corresponding amide, which is then reduced using LiAlH_4 . The second oxotetrahydronaphthyridine moiety (synthon B) is synthesized starting from 6-bromo-3,4-dihydro-1,8-naphthyridin-2(1H)-one bromide. A Heck reaction is performed with *tert*-butylacrylate. The desired carboxylic acid is then obtained as a HBr salt by treating the previously obtained ester with HBr in glacial acetic acid. The coupling between the two moieties by using HOBt and EDCI leads to the formation of AFN-1252 (Figure 16). Afabycin, the corresponding prodrug of AFN-1252, is obtained in three more steps. Firstly, AFN-1252 is reacted with chloromethyl bis(2-trimethylsilylethyl)phosphate, then the two protecting groups are removed in acidic conditions. Finally, the addition of the desired base (ethanolamine, NH_4OH , NaOH) leads to the corresponding salt of afabycin [146].

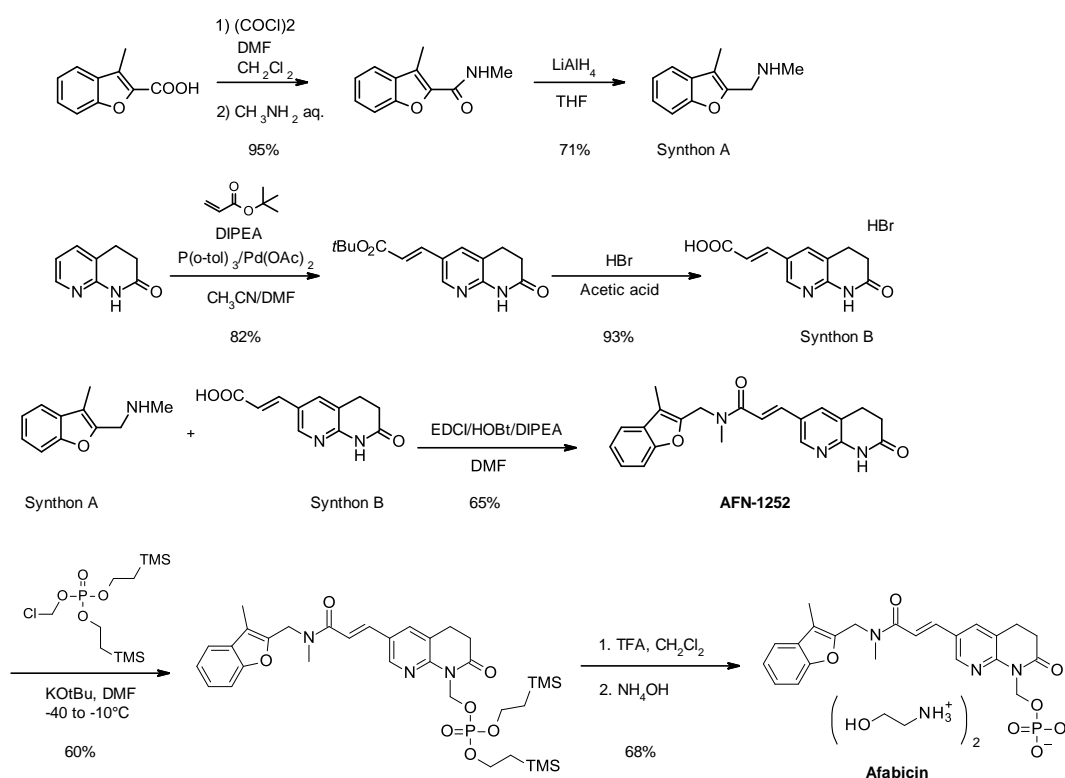


Figure 16. Description of the synthetic pathway leading to AFN-1252 and the diethanol ammonium salt of afabycin [145,146].

6.2.2 Target and mechanism of action

AFN-1252 interrupts fatty acid biosynthesis at the enoyl-acyl carrier protein reductase (FabI) step. FabI was confirmed to be the target of AFN-1252 by using biochemistry, macromolecular synthesis, genetics and co-crystallization of AFN-1252-FabI complex (Figure 17). The 3-methylbenzofuran moiety of AFN-1252 occupies a hydrophobic pocket of the enzyme created by residues F204, Y157 and

Y147. The oxotetrahydronaphtyridine moiety of the molecule occupies a more solvent exposed region and is involved in two hydrogen bond interactions with A97. The carbonyl of the linking cis-amide form two hydrogen bond interactions with the 2'-hydroxyl of the cofactor and the hydroxyl group of Y157 [147].

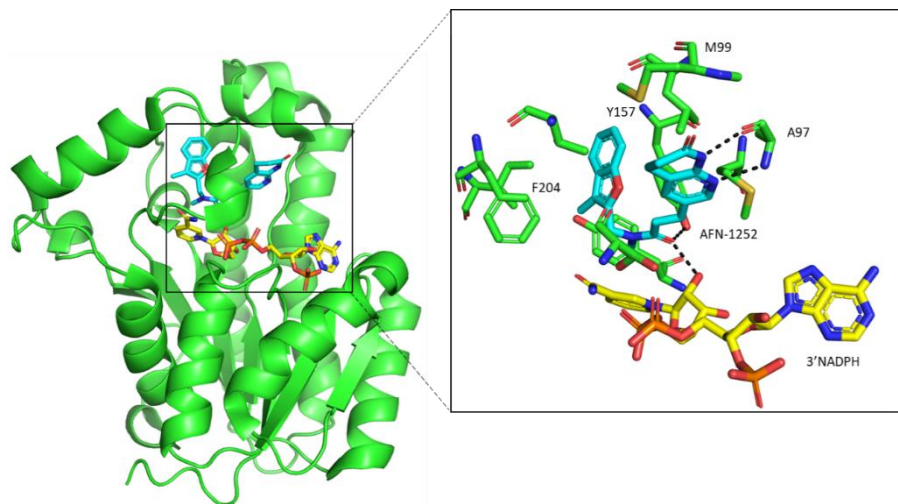


Figure 17. Views of AFN-1252 complex formed with *S. aureus* FabI and the NADPH cofactor (PDB code: 4FS3) .

6.2.3 Structure-activity relationship studies

High-throughput screening of 305,189 compounds from the GSK compound collection identified the benzodiazepine derivative as a low micromolar inhibitor of *S. aureus* FabI [144,148]. In order to improve potency, SAR studies were performed (Figure 18):

- **Removal of the ester chain:** the synthesis of compounds without the ester chain showed that the chain is not important for the inhibition of FabI.
- **Ring-opened variants:** ring-opened variants of the benzodiazepine derivative maintained the activity against *S. aureus*, indicating that the seven membered ring is not necessary for the antibacterial activity.
- **Importance of the *para* amino group:** removal of the *para* amino group caused a substantial decrease in activity.
- **Replacement of the aniline moiety with aminopyridine:** aminopyridine is an essential moiety engaged in two hydrogen-bonding interactions with Ala 97 from the cofactor.
- **Introduction of the unsaturated two-carbon linker:** acrylamide moiety was introduced to maximize the overlapping with previously synthesized promising compounds. The synthesis of the corresponding saturated derivatives led to inactive compounds.

In 2020, Parker *et al.* were able to re-design AFN-1252 into a derivative, AFN-1252-NH₃, that accumulates in *Escherichia coli* and possesses an antibacterial activity against Gram-negative bacteria [143].

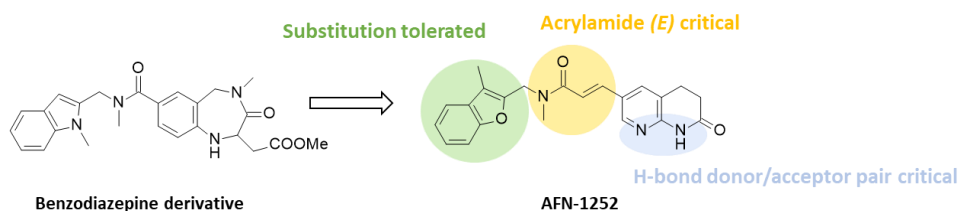


Figure 18. Summary of the SARs of AFN-1252 from the benzodiazepine series.

6.2.4 *In vitro* activity

Kaplan *et al.* showed that AFN-1252 exhibits MIC₉₀ values < 0.015 µg/ml against diverse clinical isolates of *S. aureus*. The research group also reported a high binding (> 95% from equilibrium dialysis) to serum proteins of mouse, rat, dog and humans. This parameter is in line with the eight-fold increase in AFN-1252 MICs observed in the presence of 50% of serum against MSSA, as well as with the long elimination half-life observed in pharmacokinetic studies (mice and early phase 1 studies). Similar MICs were also observed in the presence and absence of lung surfactant. Moreover, AFN-1252 exhibited a short dose-dependent post-antibiotic effect (PAE) of 1.1 hours against both MSSA and MRSA at 128 x MIC, and of 0.1 hour at 32 x MIC, following a 4-hour exposure. AFN-1252 did not show interaction with major antibiotic classes, with the exception of gentamicin, with which it demonstrated synergy [147,149].

6.2.5 *In vivo* experiments

AFN-1252 was orally available in mouse pharmacokinetic studies, where a linear relationship was observed between the administered dose (0.3, 0.6, and 1.0 mg/kg), the observed C_{max} (154, 196, and 304 ng/ml) and AUC_{0-t} (367, 806, and 1.030 ng-h/ml). The long elimination half-life measured (5–7 h) indicated the potential for once- or twice-daily administration in human. Kaplan *et al.* also showed that a single oral dose of 1 mg/kg was efficacious in a mouse model of septicemia, with 100% survival from an otherwise lethal peritoneal infection of *S. aureus*. The group also compared the *in vivo* efficacy of a single oral administration of AFN-1252 to linezolid, and an ED₅₀ of 0.15 mg/kg, suggesting that AFN-1252 was 12 to 24 times more potent than linezolid in this model [147].

6.2.6 Phase 1

A double-blind, randomized, placebo-controlled, two-part study was initiated to assess the safety, tolerability and pharmacokinetic properties of an immediate release tablet formulation of AFN-1252 in healthy subjects, following oral administration in an ascending dose trial [150]. In the first part, single doses of 100, 200, 300, or 400 mg of AFN-1252 were administered. In the second part, subjects received 100, 200, 300 and 400 mg twice a day. This study showed oral doses of AFN-1252 to be safe and well tolerated and highlighted the potential of the drug for once or twice-a-day dosing to treat staphylococcal infections. AFN-1252 was well-absorbed with C_{max} at 3–4 h when given once per day and 2.5–9 h when administered twice daily.

6.2.7 Phase 2

A phase 2 trial was designed to evaluate the efficacy and safety of 200 mg of AFN-1252, given orally twice-a-day in the treatment of ABSSSI. This study showed that AFN-1252 is well tolerated and highly effective in the treatment of ABSSSI caused by *S. aureus*, including MRSA. The overall early re-

response rate at day 3 was 97.3%, where 82.9% of patients had a >20% decrease in the area of erythema, and 77.9% of patients had a >20% decrease in the area of induration. Microbiologic eradication rates for MRSA and MSSA were around 90% at short- and long-term follow-ups. The main drug-related AEs reported, which were mostly mild or moderate, were headache (26.2%) and nausea (21.4%) [151,152].

6.2.8 Prodrug

AFN-1252 belongs to class II biopharmaceutics classification system (BCS), presenting a limited aqueous solubility and oral bioavailability. Therefore, Debiopharm invested in the development of its prodrug Afabacin desphosphono (formerly Debio1450, AFN-1720). As expected, the prodrug showed little to no activity against *S. aureus*, *E. coli* and other species. Afabacin was rapidly converted *in vivo* to the active metabolite AFN-1252 and its antibacterial activity was better than AFN-1252 administered by itself orally [153]. The prodrug was studied in a phase 2 clinical trial to compare its efficacy, safety, and tolerability with those of vancomycin/linezolid in the treatment of ABSSSIs [154]. In a phase 1 clinical trial, the penetration of afabacin into bones in patients undergoing elective hip replacement surgery was evaluated [155]. Moreover, a phase 2 clinical trial is underway to study the safety and efficacy of afabacin in human subjects with bone and joint infections caused *S. aureus*. [156].

7. FtsZ protein as a drug target

7.1. Introduction

FtsZ is a bacterial protein involved in cell division forming single-stranded filaments by guanosine 5'-triphosphate (GTP) dependent polymerization. These FtsZ filaments form the cytokinetic ring (called Z-ring) at midcell, able to recruit other critical elements for peptidoglycan synthesis and septum formation, which leads to the induction of cell division. Because FtsZ is essential for the germ and is widely conserved throughout bacteria, it displays all the attractive features of a new target for antibiotic compounds (Figure 19) [157].

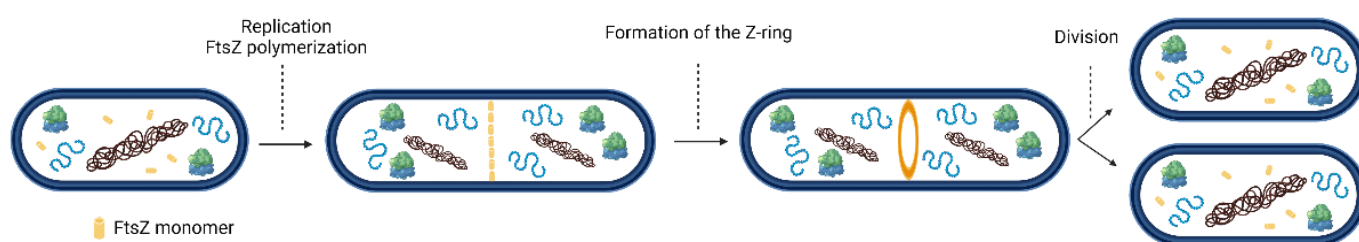


Figure 19. Involvement of the FtsZ protein towards the formation of the Z-ring.

7.2. TXA707/TXA709

TXA707 is the first antibacterial compound targeting FtsZ to reach clinical development. It is being developed as an oral prodrug (TXA709) by Taxis Pharmaceuticals to treat MRSA infections.

TXA707 is derived from PC190723, which is coming from a fragment-based medicinal chemistry program starting from 3-methoxybenzamide (3-MBA), a known FtsZ inhibitor in *Bacillus subtilis* [158]. To improve the pharmacokinetic properties of PC190723, the chlorine atom on the pyridyl ring was replaced with a trifluoromethyl group, resistant to metabolism by cytochrome P450 enzymes.

7.2.1. Chemical synthesis

The commercial synthetic route for TXA709 is a convergent strategy described in a 2018 patent by Taxis Pharmaceuticals [159]. Synthon A is prepared starting from 2-chloro-3-nitro-5-(trifluoromethyl)pyridine. After the substitution of the chlorine atom with a sulfur atom brought by thiourea, the nitro group is reduced to form the aniline. The intermediate finally reacts with 2-chloroacetyl chloride to give the corresponding thiazolo[4,5-*b*]pyridine synthon A. The synthesis of synthon B begins with the nucleophilic substitution between 2,4-difluorophenol and benzyl bromide. After deprotonation with LDA, the carbanion reacts with CO₂ to give the corresponding carboxylic acid, further transformed into primary amide using thionyl chloride and ammonia. The benzyl group is then removed by hydrogenolysis to give synthon B. The phenol ring from synthon B can then react with synthon A to form the active compound TXA707. A coupling reaction is finally performed between the latter and 1-methylpiperidine-4-carboxylic acid using EDCI to obtain the imide prodrug TXA709 (Figure 20).

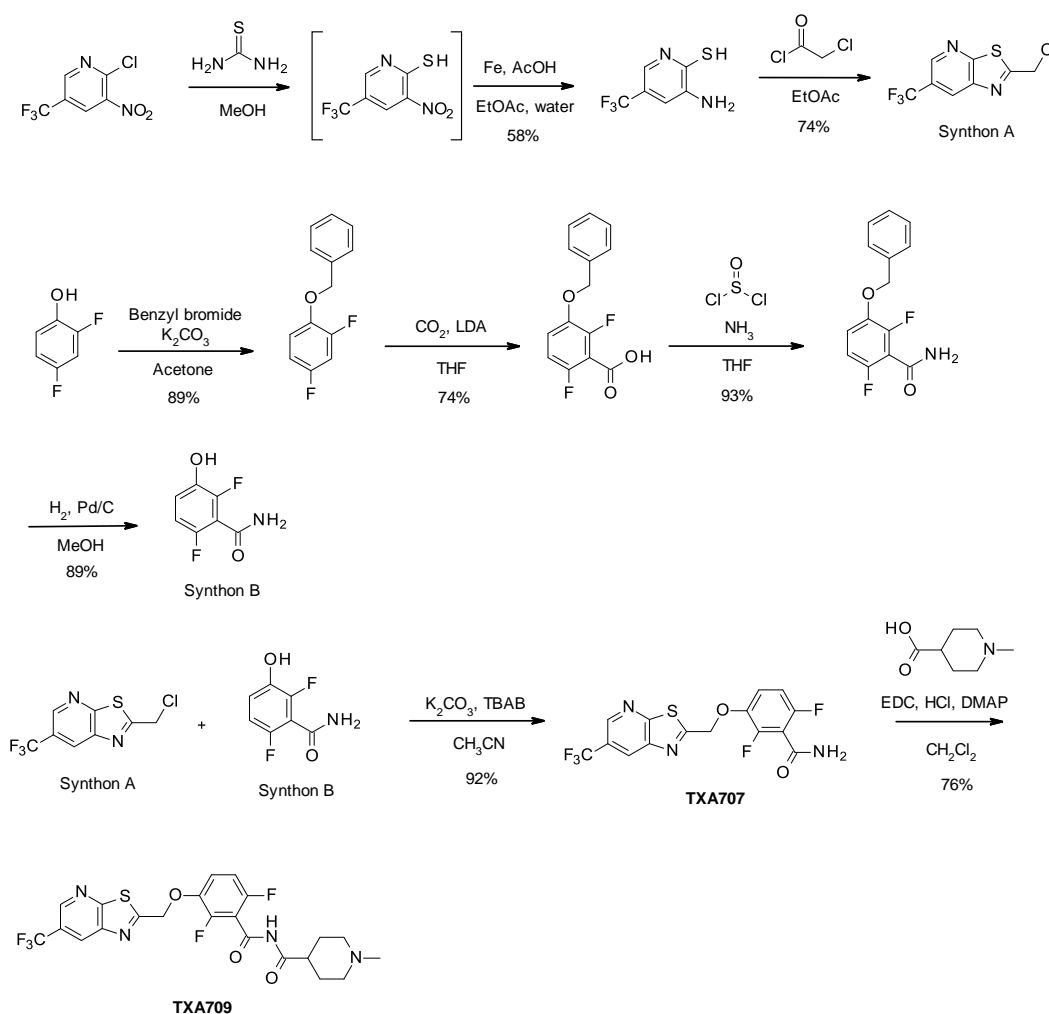


Figure 20. Description of the synthetic pathway leading to TXA709 [159].

7.2.2. Target and mechanism of action

Several biological experiments were performed to validate FtsZ as the target of TXA707. The drug stimulates FtsZ polymerization in a concentration-dependent manner and induces a mislocalization of FtsZ to multiple sites throughout the cell, preventing the formation of the Z-ring.

Transmission electron microscopy studies showed that within 1 hour of treatment with TXA707, *S. aureus* cells became enlarged enough, resulting to cell lysis. Furthermore, TXA707 was co-crystallized in complex with FtsZ (Figure 21) [160]. Eventually, the target was validated with the selection of TXA707-resistant MRSA mutants (frequencies of resistance: $3 \cdot 10^8$ in MRSA ATCC 43300; $1 \cdot 10^8$ in an MRSA clinical isolate). Twenty resistant clones were isolated and the *ftsZ* gene was sequenced. Data showed that all TXA707-resistant strains carried a mutation within this gene [161].

It is worthwhile noting that the mechanism of action of TXA707 on FtsZ echoes that of paclitaxel (tradename TAXOL) on tubulin, the eukaryotic structural homologue of FtsZ. Indeed, both compounds prevent polymers disassembly, eventually leading to cell division halt.

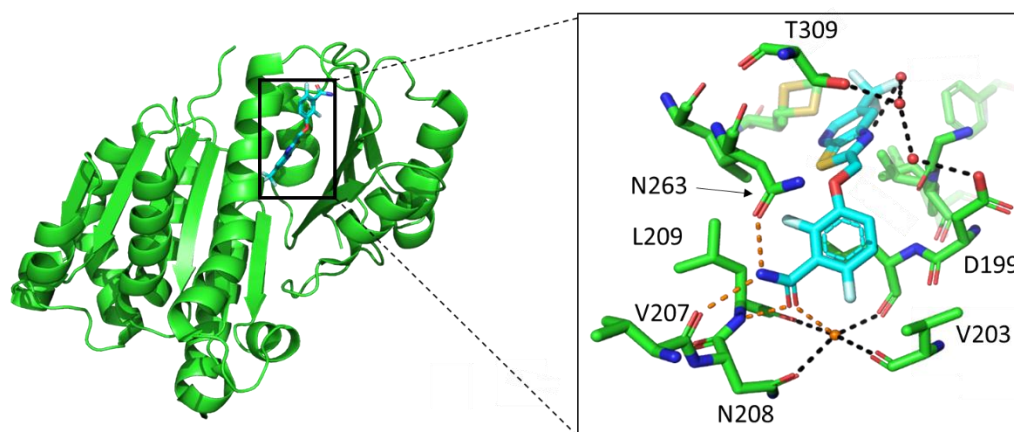


Figure 21. Views of TXA707 complex formed with *S. aureus* FtsZ 12-316 (PDB code: 5XDT).

7.2.3. Structure-activity relationship studies

The starting point for the development of TXA707 was 3-methoxybenzamide, proven to be an FtsZ inhibitor in *B. subtilis* [158]. The methoxy and amide moieties in their respective positions were shown to be essential for the activity on FtsZ (Figure 22). Authors then investigated modifications around the 3-methoxybenzamide core:

- **Substitution of the phenyl ring:** substitution was detrimental, except for the addition of halogen atoms in *ortho* position to the amide.
- **Substitution of the methoxy moiety:** substitution with other alkoxy chains was possible. It was shown that increasing the length of the alkyl chain up to nine carbon atoms allowed an 8000-fold improvement in potency on *B. subtilis* compared to the methoxy moiety [162]. However, this also led to an increase in PPB. Subsequent replacement of the alkyl chain with various heterocycles was investigated, where the benzothiazole gave interesting results.
- **Substitution of the benzothiazole ring:** substitution in position 4 was detrimental. In position 6, substitution was tolerated with small moieties (methyl, chlorine) but larger groups led to inactive compounds (phenyl, methoxy). Lastly, substitution in position 5 was favorable, particularly with a chlorine or bromine atom, phenyl ring, propyl or ethoxy chain.
- **Modification of the benzothiazole ring:** to further decrease the plasma protein binding, thiazolopyridine analogues were synthesized. This allowed the identification of PC190723, which displayed a good potency on *S. aureus* (MIC = 1 $\mu\text{g/mL}$) and a relatively low PPB (85.4% bound)[163].

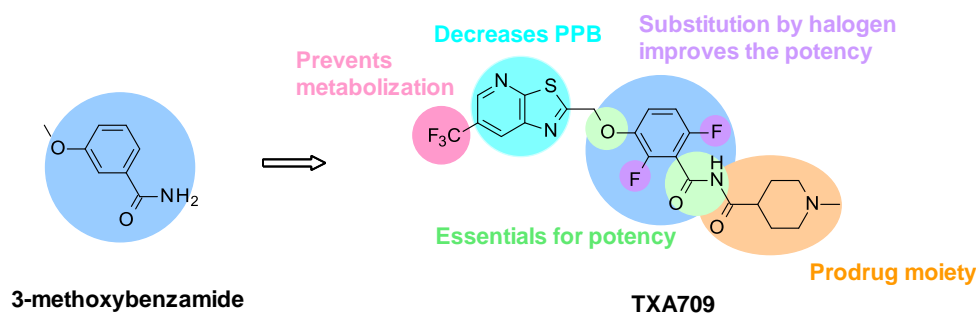


Figure 22. Summary of the SARs of TXA709.

Because PC190723 displayed a low solubility, the maximum dose that could be administered orally was not sufficient to display any efficacy. To circumvent this issue, an imide prodrug with a basic piperidine, TXY541, was synthesized, allowing a great improvement in solubility [164]. Administration of TXY541 proved to be efficacious *in vivo* but required high doses (192 mpk against a MRSA strain). To reduce administered doses, metabolization was decreased by replacement of the chlorine atom on the pyridyl ring with a trifluoromethyl group, yielding TXA707 and the corresponding imide prodrug TXA709 [161].

7.2.4. *In vitro* activity

TXA707 exhibits potent bactericidal activity against a panel of 84 *S. aureus* clinical strains with various levels of resistance against current treatment options. The most prevalent MIC for TXA707 was 1 µg/mL against the five types of clinical isolates tested: MRSA, VISA, VRSA, DNSSA (Daptomycin Non-Susceptible *S. aureus*), and LNSSA (Linezolid Non-Susceptible *S. aureus*). The prodrug TXA709 also showed antibacterial activity against all *S. aureus* strains tested so far, however its modal MICs were 2- to 4-fold higher than those of TXA707. This is explained by a slower conversion from TXA709 to TXA707 in the culture medium [161]. Overall, these results make TXA707 and its prodrug TXA709 a promising alternative for the treatment of multidrug resistant *S. aureus*.

Regarding selectivity, it was shown *in vitro* that PC190723, the parent analogue of TXA707, did not impact positively or negatively the polymerization of tubulin, the eukaryote counterpart of FtsZ [157]. Moreover, TXA709 demonstrated minimal toxicity on mammalian cells (HeLa and MDCK, IC₅₀ > 120 µg/mL) [161].

7.2.5. *In vivo* experiments

Firstly, the pharmacokinetic properties of TXA709 were measured in male BALB/c mice following oral (32 mpk) or IV (24 mpk) administration. TXA709 was not detectable in plasma, indicating a rapid conversion into the active compound TXA707. Maximum plasmatic concentrations were attained after 1h following oral administration, and the bioavailability was calculated to be 95% [161].

The efficacy of TXA709 was demonstrated in a mouse peritonitis model, both with MSSA and MRSA strains. It was demonstrated that a dose of 36 mpk by intravenous administration and a dose of 48 mpk by oral administration were sufficient for the survival of all mice infected by the MRSA strain. TXA709 and TXA707 were further evaluated in a mouse thigh model of MRSA infection. After intravenous administration of TXA709, volume of distribution at steady state for TXA707 was measured at 2.02 L/kg. This value is three times higher than the total water volume of the mouse (0.7 L/kg), indicating that TXA707 is well distributed in the tissues. Moreover, an oral dose of 120 mg/kg of TXA709 in the same mouse thigh infection model resulted in a 2-log reduction in bacterial CFU [161].

Lastly, PK/PD parameters were evaluated in a murine neutropenic thigh infection model and the ratio AUC/MIC was deemed to be the PK/PD index most closely linked to efficacy [165].

7.2.6. Current advances

TXA709 has been evaluated in a non-registered phase 1 clinical trial and no serious AE was reported [166].

8. Conclusion and perspectives

For the first time in decades, pharmaceutical companies are refuelling the pipeline of antibiotics. To date, at least eight compounds with new targets and/or MOA are in clinical trials, including two in phase 3, or have been recently approved. They represent a new hope to fight the bad bugs that are becoming more and more resistant to current antibiotics.

Beside these clinical candidates, other promising antibiotics and/or new targets have been reported in the literature. This is the case for a series of novel tetrazole ligands targeting LpxA, the first enzyme in the lipid A pathway [167]. Moreover, over twenty potential candidates targeting the bacterial RNA polymerase enzyme have been designed [168,169].

In addition, other strategies are being explored such as pathoblockers to disarm bacteria or adjuvants like efflux pump inhibitors (EPI) to potentialize current antibiotics.

Unlike antibiotics, which kill bacteria or inhibit growth and so exert a selection pressure, pathoblockers inhibit virulence factors. Virulence is the ability of bacteria to cause disease and is mediated by factors such as toxins, proteases or cytolysins [170]. Therefore, antivirulence drugs disrupt the relation between the host and the pathogen, causing the disarmament of bacteria which can then be eliminated by the immune system. Because the bacterium viability is not impacted, pathoblockers are believed to reduce resistance development. To date, only one small molecule acting as a pathoblocker has been evaluated in clinical trials: fluorothiazinon (formerly CL-55), developed by the Gamaleya Research Institute of Epidemiology and Microbiology in Russia. Fluorothiazinon inhibits the type III secretion system, a well conserved system among Gram-negative bacteria allowing the secretion of several virulence factors [171–174]. After completing a phase 1 study [175], fluorothiazinon is currently evaluated in a phase 2 clinical study for the treatment of complicated urinary tract infections caused by *P. aeruginosa* [176]. Moreover, recent studies reported quorum sensing inhibitors to reduce virulence in *A. baumannii* [177,178].

EPIs are compounds able to block bacterial multidrug efflux transporters. These pumps are able to extrude several classes of antibiotics from the bacteria such as tetracyclines, macrolides, β -lactams, fluoroquinolones, therefore decreasing their intra-bacterial concentration and eventually rendering them ineffective [179]. (Over)expression of multidrug efflux transporters, notably from the resistance-nodulation-cell division family, is one of the most worrying resistance mechanisms regarding multidrug antibacterial resistance. One strategy to override this defense mechanism is the conception of EPIs [180]. Administering an EPI in combination with an antibiotic effluxed by the targeted bacterial transporters could allow to restore the antibiotic activity and decrease the necessary antibiotic dose, thus reducing the selection pressure [181].

Author Contributions: writing—original draft preparation, F.R., N.C., K.A., M.E; writing—review and editing, F.R., N.C., K.A., M.E, M.F., N.W.; supervision, M.F., N.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Institut National de la Santé et de la Recherche Médicale, Université de Lille, Institut Pasteur de Lille, Région Hauts-de-France.

Conflicts of Interest: The authors declare no conflict of interest.

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