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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, afabycin 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  $\beta$ -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 $\beta$ N, phenylalanine-arginine- $\beta$ -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<sub>N</sub>2, nucleophilic 2<sup>nd</sup> order substitution; S<sub>N</sub>Ar, nucleophilic aromatic substitution; STI, sexually transmitted infections; t<sub>1/2</sub>, 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*.

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## 1. Introduction

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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.

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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].

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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].

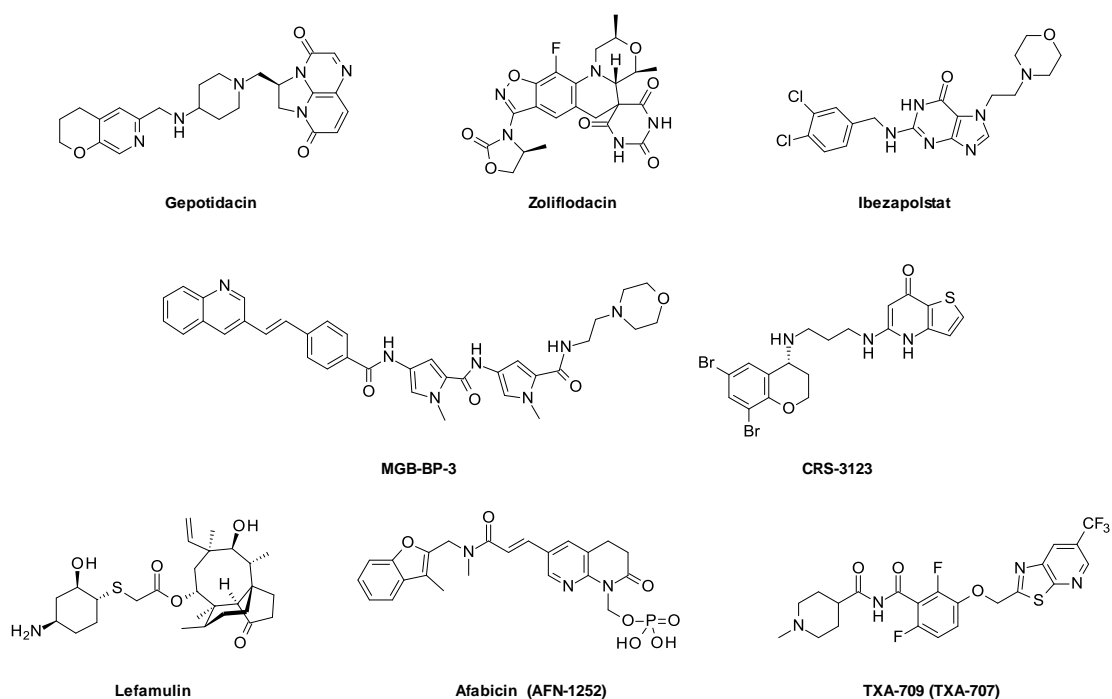
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In this review, we focus on new drugs in clinical development or recently approved, characterized by novel targets and/or MOA, that are of interest for the treatment of infections caused by multidrug-resistant bacteria (Figure 1 and Table 1). We have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



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**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
<b>Family</b>	Triazaacenaphthylene/NBTI	Spiropyrimidinetrione	DCBG ([dichlorobenzyl] guanine)	Distamycin
<b>Mode of action</b>	Inhibition of DNA gyrase and topoisomerase IV	Inhibition of topoisomerase II	Inhibition of DNA polymerase III C	Binding to the minor groove of DNA
<b>Chemical features</b>	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
<b>Development phase</b>	Phase III	Phase III	Phase II	Phase II
<b>Relevant references</b>	[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)
<b>Family</b>	Diaryldiamine	Tricyclic diterpenoid pleuromutilin	Benzofuran naphthyridine	Benzamide
<b>Mode of action</b>	Inhibition of methionyl-tRNA synthetase (MetRS1)	Inhibition of 50S ribosomal subunit	Inhibition of FabI	Favoring uncontrolled FtsZ polymerization
<b>Chemical features</b>	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
<b>Development phase</b>	Phase II	Approved in 2019 (FDA) and 2020 (EMA)	Phase II	Phase I
<b>Relevant references</b>	[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

89 repair processes. Concerning bacteria, there are four types of topoisomerases: DNA gyrases, topoi-  
90 somerases I, topoisomerases IV and reverse gyrases.

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

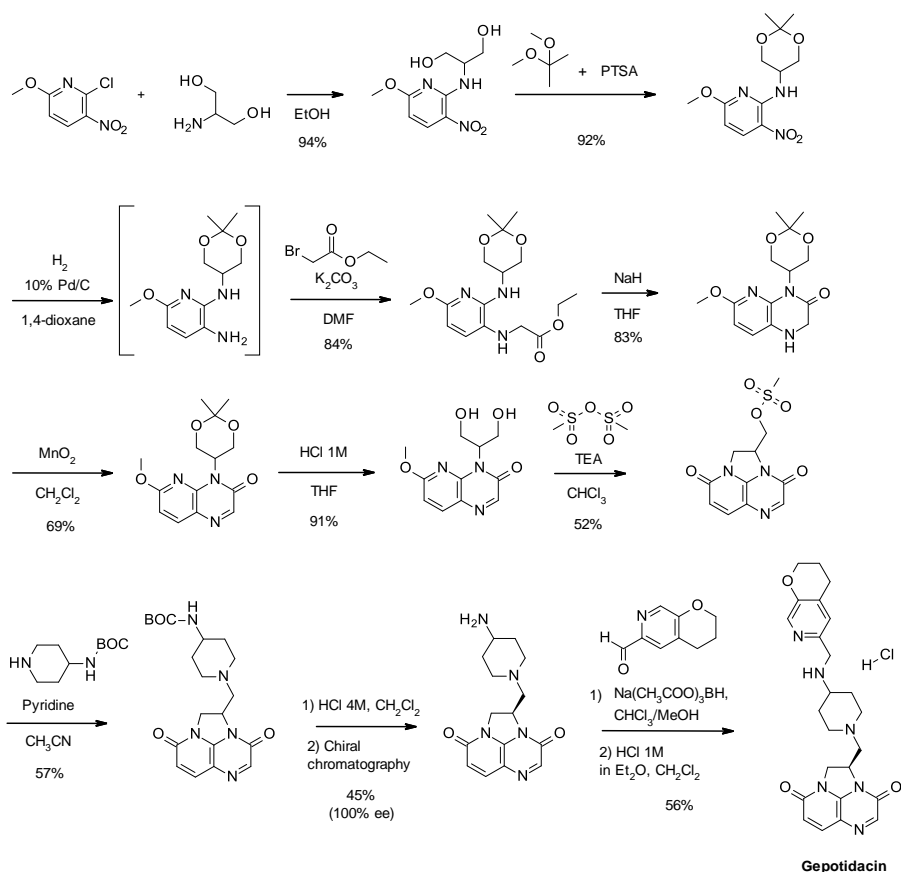
## 95 **2.2. Gepotidacin**

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

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

### 103 **2.2.1. Chemical synthesis**

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106 The synthesis of gepotidacin has been described in two patents in 2008 and 2016 and comprises  
107 11 steps (Figure 2) [11,12]. First, 2-chloro-6-methoxy-3-nitro-pyridine reacts with  
108 2-amino-propane-1,3-diol through nucleophilic aromatic substitution ( $S_NAr$ ). The resulting diol is then  
109 protected with 2,2-dimethoxypropane in presence of p-toluenesulfonic acid (PTSA) followed by the  
110 reduction of the nitro group with hydrogen and 10% Pd/C. The aniline thus formed is then alkylated  
111 with ethyl bromoacetate. Cyclization is performed in basic conditions using sodium hydride, followed  
112 by oxidation using manganese dioxide. The acetal is then cleaved and the released diol reacts with  
113 methanesulfonic anhydride to form the third cycle of the triazaacenaphtylene core. Substitution with  
114 Boc-amino-piperidine, followed by deprotection and subsequent purification by chiral chromatogra-  
115 phy affords the primary amine derivative, which can be condensed by reductive amination with the  
116 corresponding aldehyde to give the free base of gepotidacin. The mono-hydrochloride salt is ob-  
117 tained by reaction with one equivalent of HCl 1M in diethylether [13].

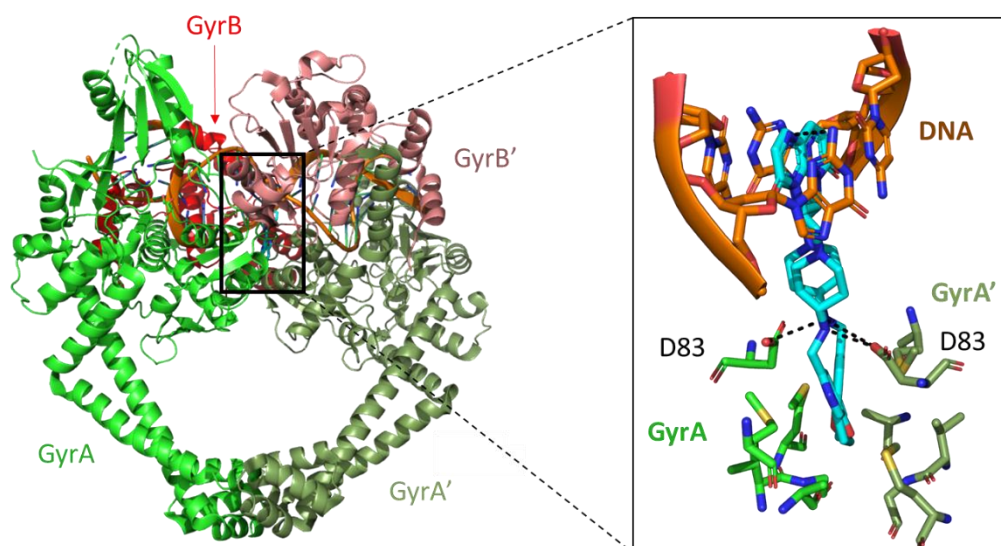


**Figure 2.** Description of the synthetic pathway leading to gepotidacin [11,12].

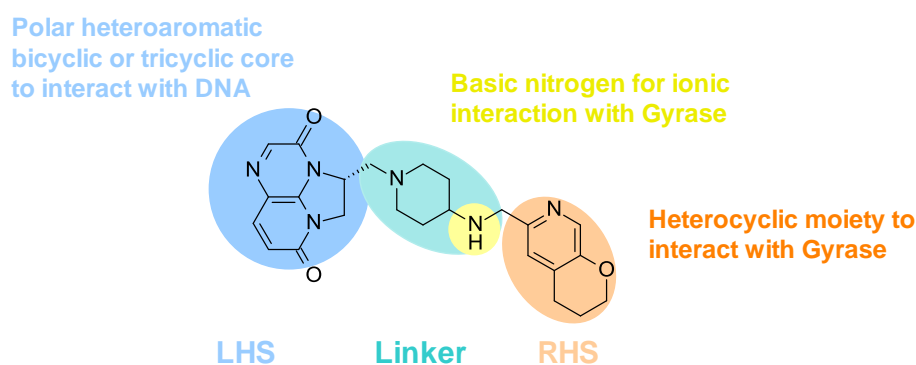
### 2.2.2. Target and mechanism of action

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  $\beta$ -lactamase (ESBL). Gepotidacin was also found active against different levofloxacin-resistant strains of *S. aureus* and *S. pneumoniae*, with MIC<sub>90</sub> of 0.5  $\mu$ g/mL. This compound was also potent against levofloxacin-resistant Gram-negative bacteria such as *E. coli* with MIC<sub>90</sub> of 4  $\mu$ 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 log<sub>10</sub> CFU compared to the initial lung bacterial load in the neutropenic mice (between 5.6 and 6.3 log<sub>10</sub> CFU) [18].

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

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

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

#### 168 2.2.5. Phase 1

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

#### 178 2.2.6. Phase 2

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

185 In the ABSSSI phase 2 trial, 122 patients with wound infection, major cutaneous abscess or cellu-  
186 litis were enrolled. Three dose regimens were evaluated: 750 mg every 12 h (q12h), 1000 mg every  
187 12 h and 1000 mg every 8 h (q8h). Success was observed for the 750 mg q12h and the 1000 mg q8h  
188 regimens. The majority of adverse events (AE) declared were gastrointestinal events, principally  
189 nausea and diarrhoea [36].

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

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

#### 205 2.2.7. Current advances



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

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

### 220 **2.3. Zoliflodacin**

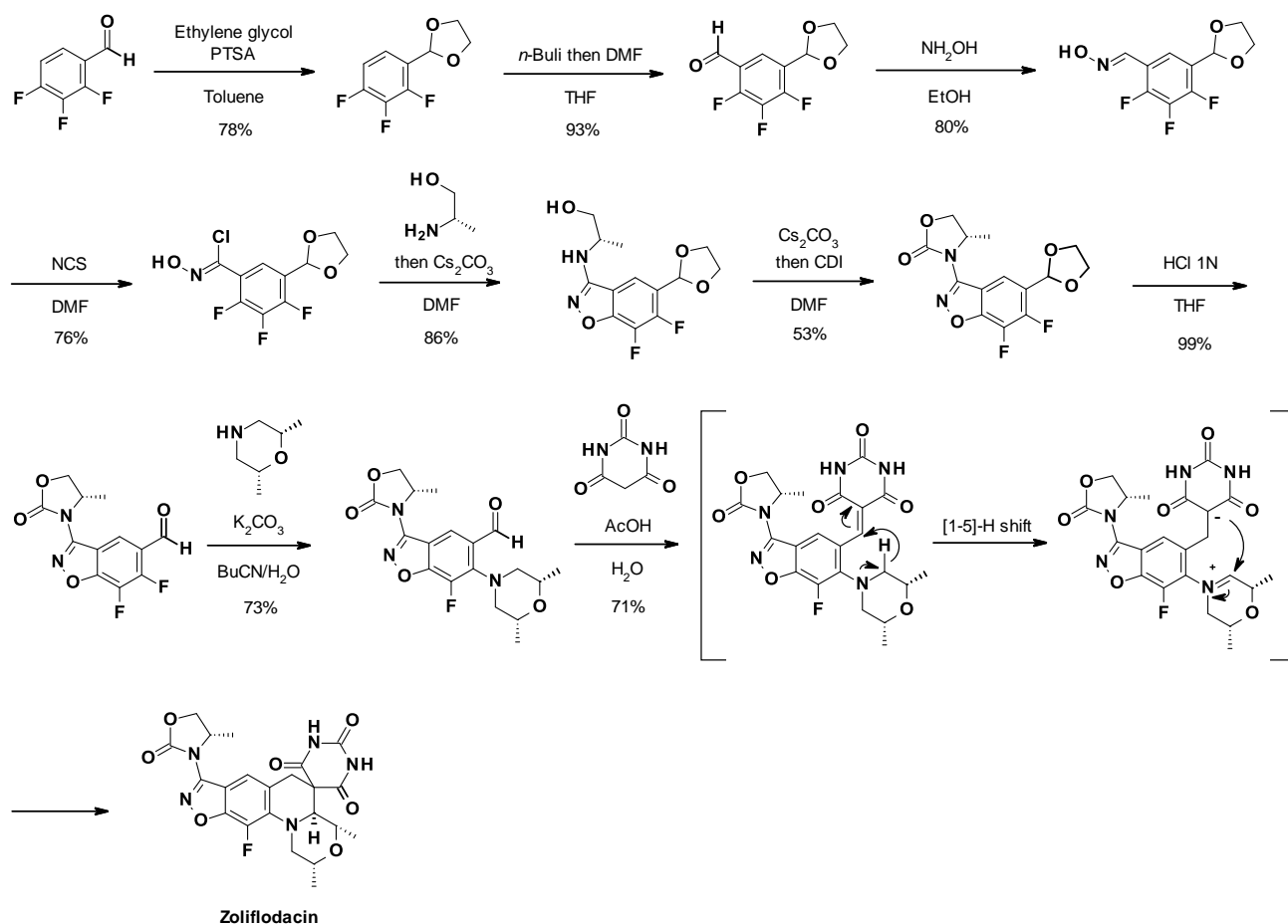
221  
222 Zoliflodacin (previously known as ETX0914 or AZD0914) is a novel spiropyrimidinetrione antibi-  
223 otic being developed as single oral dose for the treatment of uncomplicated gonorrhoea [43–46].

#### 225 **2.3.1. Spectrum of activity**

226  
227 Zoliflodacin displays activity against Gram-positive bacteria including *S. pneumoniae*, *Strepto-*  
228 *coccus pyogenes*, and methicillin-resistant fluoroquinolone-resistant *S. aureus*, as well as against fas-  
229 tidious Gram-negative bacteria including *Haemophilus influenzae* and *N. gonorrhoea* [47].

#### 231 **2.3.2. Chemical synthesis**

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233 The synthesis of zoliflodacin described below was reported in 2015 [47]. The first step, starting  
234 from 2,3,4-trifluorobenzaldehyde, consists of the protection of the aldehyde function to an acetal  
235 group. After deprotonation using *n*-BuLi, formylation is performed with DMF to introduce an alde-  
236 hyde group, which is then converted to oxime using hydroxylamine. Chlorination with  
237 *N*-chlorosuccinimide (NCS), followed by reaction with *L*-alaninol and intramolecular S<sub>N</sub>Ar allows the  
238 formation of the benzisoxazole ring. The oxazolidinone moiety is obtained using  
239 1,1'-carbonyldiimidazole (CDI). The deprotection of the aldehyde is then preformed in acidic condi-  
240 tions followed by another S<sub>N</sub>Ar at the *ortho* position of the aldehyde using  
241 (2*R*,6*S*)-2,6-dimethylmorpholine. Finally, a Knoevenagel condensation between the aldehyde and  
242 hexahydropyrimidine-2,4,6-trione is performed, followed by an intramolecular rearrangement con-  
243 sisting 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].

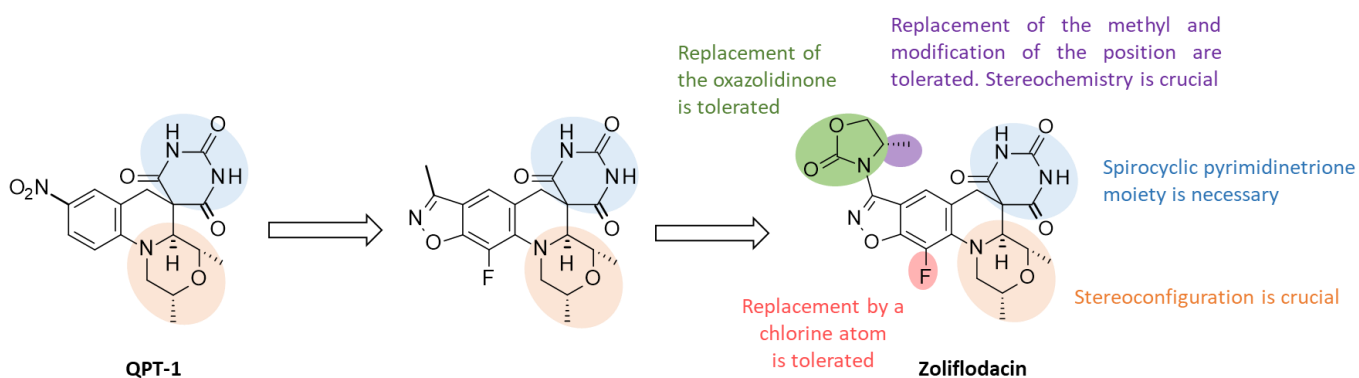
### 2.3.4. Structure-activity relationship (SAR) studies

After a high-throughput screening at Pharmacia & Upjohn, QPT-1 was found as the first spiroimidinetrone 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 pyrimidinetrone:** the pyrimidinetrone 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* (MIC<sub>90</sub> 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 [<sup>14</sup>C] zoliflodacin [61].

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

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

338 In the second study, the ADME properties were defined after a single oral dose of 3000 mg of [<sup>14</sup>C]  
339 zoliflodacin. The data demonstrated that the PK profile of zoliflodacin (AUC and C<sub>max</sub>) was similar to  
340 the one obtained in the first phase 1 study.

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

### 344 2.3.8. Phase 2

345  
346 The safety and efficacy of zoliflodacin was then assessed in a randomized phase 2 study on 179  
347 participants with uncomplicated urogenital gonorrhoea [62]. The subjects were randomized to receive  
348 a single oral dose of zoliflodacin (either 2 or 3 g) or a single 500 mg intramuscular dose of ceftriax-  
349 one. In summary, oral zoliflodacin has successfully treated most uncomplicated urogenital and rectal  
350 gonococcal infections, but has shown little efficacy in treating pharyngeal infections.

### 352 2.3.9. Phase 3

353  
354 Following promising results from one of the phase 2 clinical trials, a phase 3 for the treatment of  
355 uncomplicated urogenital gonorrhoea is currently underway [63]. The study is expected to include ap-  
356 proximately 1000 adults with urogenital gonorrhoea from different sites (USA, The Netherlands, Thai-  
357 land, and South Africa) and results are now expected in 2023 (current status). This open-label trial  
358 compares a single 3 g oral dose of zoliflodacin to a combination of ceftriaxone and azithromycin (2:1  
359 randomization). Recently, a parallel study [64] demonstrated that a single oral dose of zoliflodacin  
360 was positively evaluated for cardiac safety, supporting further clinical experiments with single 3 g  
361 doses in patient with gonorrhoea [46].

## 363 3. DNA polymerase as a drug target

### 365 3.1. Introduction

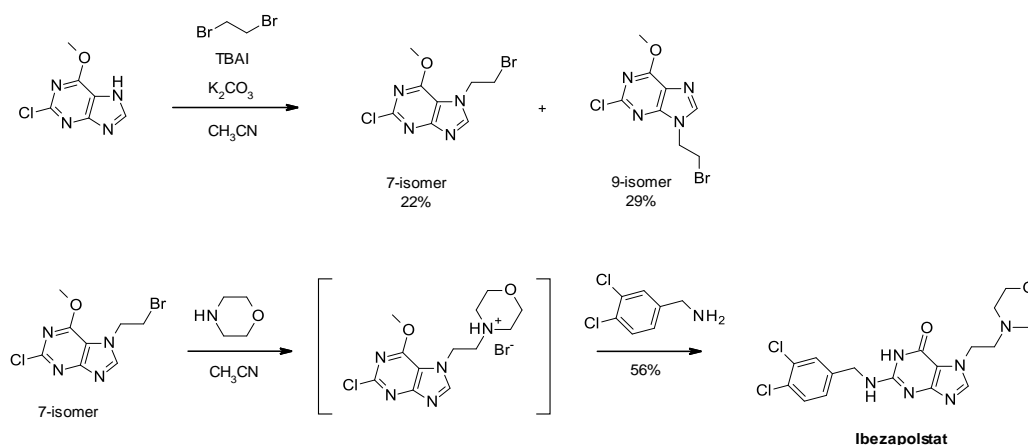
366  
367 DNA replication is carried out by the DNA polymerase, a massive enzymatic complex highly orga-  
368 nized and therefore susceptible to disruption. Inhibitors of DNA polymerase are widely used to treat  
369 viral infections, such as aciclovir for the treatment of herpes virus. However, there is currently no an-  
370 tibiotic in clinic which targets the bacterial DNA polymerase, whereas human and bacterial DNA  
371 polymerases have many differences [65]. To date, ibezapolstat is the first bacterial specific DNA  
372 polymerase inhibitor in clinical development.

### 374 3.2. Ibezapolstat

375  
376 Ibezapolstat (formerly ACX-362E) is a member of the 6-anilinouracil family of dGTP analogues  
377 [66]. Developed by Acurx Pharmaceuticals, it selectively inhibits DNA polymerase III $\epsilon$  of several  
378 Gram-positive bacteria, through competition with dGTP [67]. In 2012, Dvoskin *et al.* demonstrated  
379 that ibezapolstat was highly effective against *Clostridioides difficile* on *in vitro* and *in vivo* models  
380 [68]. The compound is currently being evaluated in a phase 2 clinical study [69].

#### 382 3.2.1. Chemical synthesis

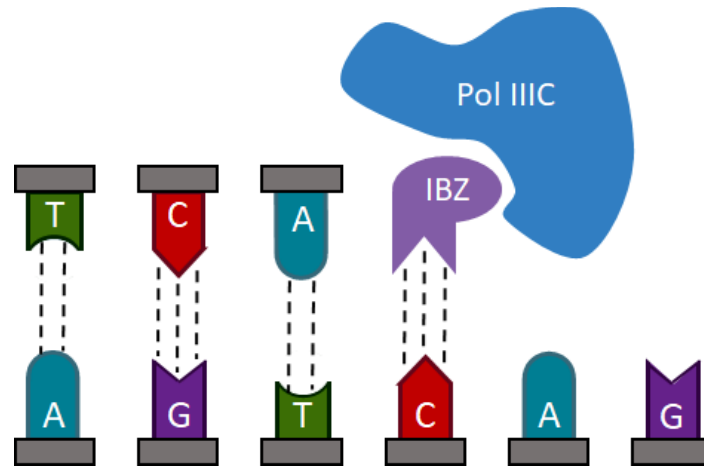
Ibezapolstat was first synthesized in 2010 in a 3-step procedure, following a protocol described by Wright *et al.* (Figure 7) [70]. Firstly, 2-chloro-6-methoxypurine is alkylated with 1,2-dibromoethane to afford a mixture of 7- and 9- isomers. After isolation of the 7-isomer, a S<sub>N</sub>2 reaction 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<sub>C</sub> (DNA pol III<sub>C</sub>), 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<sub>C</sub>. The combination of these two properties leads to the formation of an inactive ternary complex between DNA, ibezapolstat and DNA pol III<sub>C</sub> (Figure 8). To note, the compound inhibits the DNA pol III<sub>C</sub> of *C. difficile* with a K<sub>i</sub> of 0.325 μM [72]. Moreover, there is only little homology between DNA pol III<sub>C</sub> and mammalian polymerase [73].



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

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### 3.2.3. Structure-activity relationship studies

Ibezapolstat is a 6-anilinouracil derived from 6-(p-hydroxyphenylhydrazino)-uracil (H<sub>2</sub>-HPUra), itself identified in the late 1970's as an inhibitor of DNA pol IIIc. In order to improve potency, structure-activity relationship studies were performed [75,76] (Figure 9):

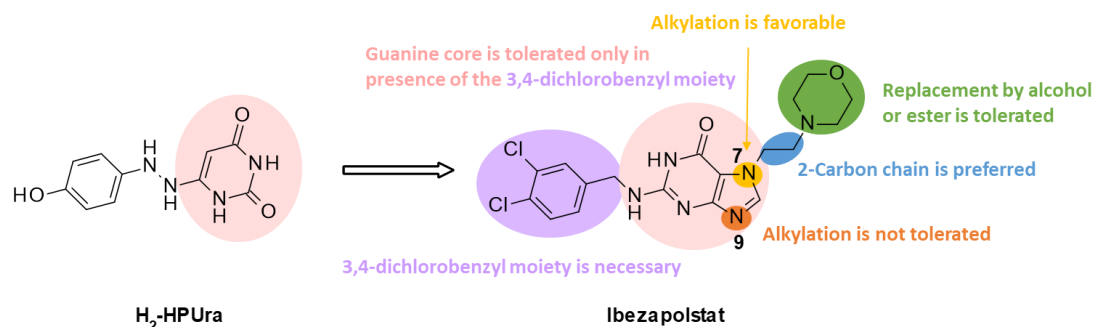
- **Replacement of the uracile core by a guanine core:** H<sub>2</sub>-HPUra binds to cytosine via its uracil core. The latter was replaced by the natural DNA base guanine. A loss of activity was observed, except when the 3,4-dichlorobenzyl moiety was introduced.

- **Modification of the 3,4-dichlorobenzyl moiety:** replacement by other groups and/or modification of the position of the two chlorine atoms led to less potent compounds.

- **Alkylation of the guanine:** the alkylation of the nitrogen at position 7 resulted in more potent derivatives. On the contrary, the alkylation of the nitrogen at position 9 led to a total loss of potency.

- **Modification of the linker:** even if compounds with a longer linker are active, the ethylene linker results in the most potent compound.

- **Modification of the morpholino part:** replacement of the morpholino part by esters or alcohols is tolerated. However, the presence of a protonated nitrogen at physiological pH probably explains why the morpholine ring was kept.



**Figure 9.** Summary of the SARs of ibezapolstat from H<sub>2</sub>-HPUra.

### 3.2.4. *In vitro* activity

Against 23 isolates of *C. difficile*, ibezapolstat (MIC<sub>50</sub> of 2 µg/mL) was shown as potent as metronidazole and vancomycin (MIC<sub>50</sub> of 1 µg/mL for both compounds), which are the treatment of choice for *C. difficile* infections (CDI) [77]. More recently, ibezapolstat was evaluated against 104 isolates of *C. difficile* [71]. MIC range of the molecule (1-8 µg/mL) was higher than metronidazole (0.25-16 µg/mL), vancomycin (0.5-4 µg/mL) and fidaxomicin (0.015-1 µg/mL). However, due to the mechanism of action of ibezapolstat targeting DNA pol IIIc, which is not expressed by Gram-negative bacteria, the compound has a narrow spectrum compared to metronidazole and vancomycin. Indeed, ibezapolstat has low activity against representatives of the normal colonic flora, such as *Bacteroides fragilis*, while metronidazole is active against some of them [72]. Overall, ibezapolstat exhibits an interesting spectrum profile, as the compound is unlikely to disrupt the endogenous bacterial flora, a property that is preferable for avoiding recurrence of CDI [78,79].

### 3.2.5. *In vivo* experiments

An efficacy study was performed in a golden Syrian hamster model by calculating the percentage of survivors after 5 days. Animals were completely protected at a dose of 12.5 mg/kg administered twice daily for 3 days. However, 67 % of the animals died after 15 days, due to recurrence of the infection. The recurrence was less pronounced with animals treated for more than 3 days. Indeed, only



447 40 % of the animals died after 7 days of treatment. Administration of ibezapolstat during 14 days did  
448 not result in recurrence in any of the animals infected during the 34 days of the experiment.

449 In the hamster model of *Clostridioides difficile*-associated diarrhea (CDAD), ibezapolstat showed  
450 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  
451 absorbed. Because the site of CDI is exclusively located in the colon, a lower absorption results in  
452 higher concentrations in the intestine.

### 453 454 3.2.6. Phase 1

455  
456 Ibezapolstat has completed a phase 1 study in the United States [80]. The randomized, dou-  
457 ble-blind, placebo-controlled clinical trial involved 62 healthy adults and was divided into three parts.

458 The first consisted of oral administration of a single ascending dose of ibezapolstat at doses of  
459 150, 300, 600 and 900 mg. In the second part, a single dose of 300 mg was given with a meal. Finally,  
460 the third part was a multi-day ascending dose study of ibezapolstat 300 mg and 450 mg given twice  
461 daily or vancomycin 125 mg given four times daily.

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

### 468 469 3.2.7. Phase 2

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

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

## 481 482 4. DNA minor groove as a drug target

### 483 484 4.1. Introduction

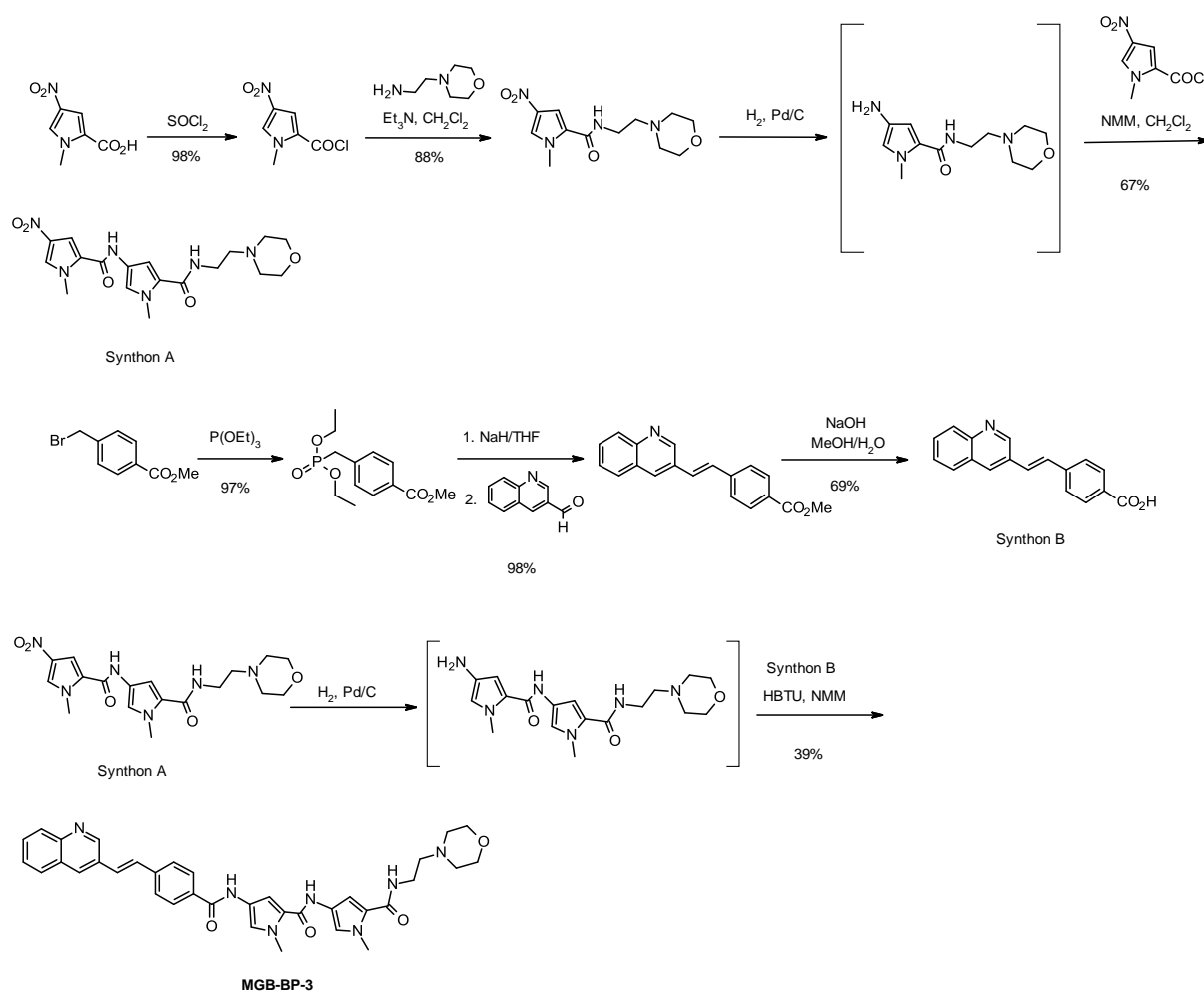
485  
486 Small molecules can manifest their effect on DNA through various binding mechanisms such as  
487 intercalation, alkylation as well as major and minor groove binding [85]. These interactions can cause  
488 a variety of important biological responses. Minor groove binders (MGBs) are well-known for their  
489 applications as anticancer drugs, although their importance as anti-infective agents is growing [86].  
490 The majority of synthetic MGBs are linked to the two natural occurring antibiotics, netropsin and  
491 distamycin A, and are generally characterized by the presence of an alkene, a non-polar N-terminal  
492 amide isostere, and N-alkyl chains on the pyrrole rings [87].

### 493 494 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].

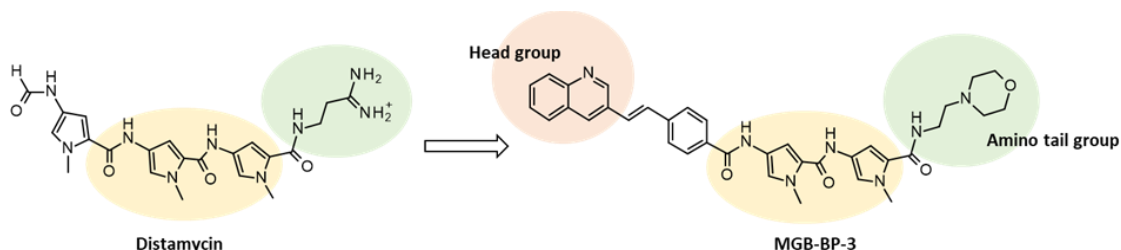
#### 4.2.2. Target and mechanism of action

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* ( $MIC_{80} < 0.2 \mu M$ ) [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].

555 In addition, *S. aureus* was grown at sub-MIC<sub>80</sub> concentrations of MGB-BP-3 or rifampicin (used as  
556 a control) for 80 generations. No resistant strain towards MGB-BP-3 was observed, contrary to ri-  
557 fampicin [89].

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

#### 563 4.2.5. *In vivo* experiments

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

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

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

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

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

#### 590 4.2.6. Phase 1

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

#### 596 4.2.7. Phase 2

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599 A phase 2 study was conducted to assess the safety, tolerability, and efficacy of incremental  
600 doses of MGB-BP-3 in patients with CDAD. Three sequential ascending doses of 125 mg, 250 mg, and  
601 500 mg given twice daily were investigated. The study showed that a 10 day-administration of 250  
602 mg of MGB-BP-3 given twice daily resulted in an initial cure and 100% durable cure, with no disease  
603 recurrence measured four weeks after treatment. This regimen has been selected for further clinical  
604 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

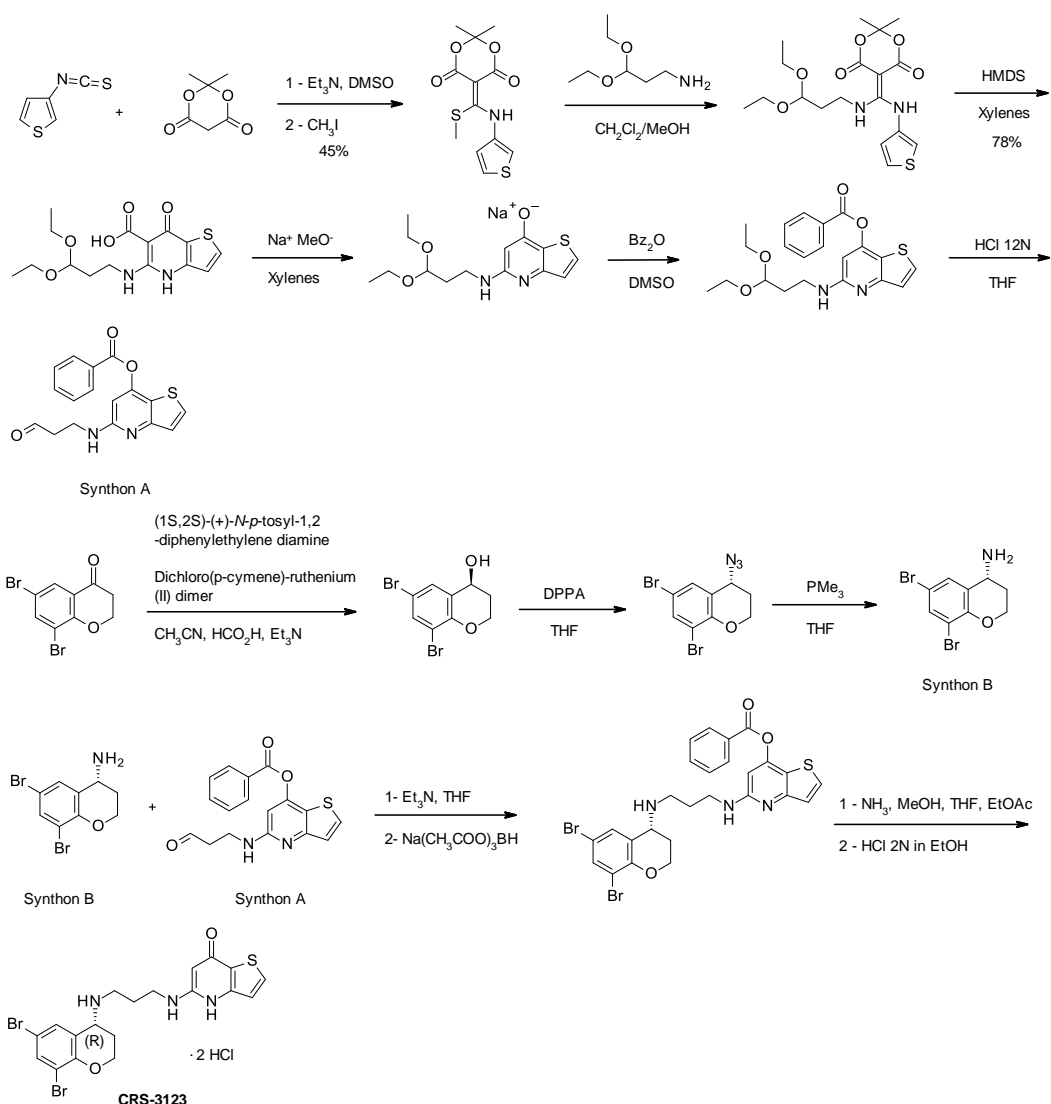
CRS-3123 (formerly REP-3123) targets the methionyl-tRNA synthetase of Gram-positive bacteria, in particular *C. difficile* [97]. The drug candidate is being developed by Creston Inc. and has completed two phase 1 studies in 2022 [98,99].

##### 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<sub>N</sub>2 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

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

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

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

#### 687 5.2.4. *In vivo* experiments

689 CRS-3123 was shown more effective than vancomycin *in vivo* [104]. In a hamster model of CDI,  
690 CRS-3123 at daily doses of 0.5 mg/kg and 5 mg/kg greatly reduced the mortality of the animal, with  
691 62 % and 75 % survival after 33 days, respectively. In this experiment, CRS-3123 was also superior to  
692 vancomycin, where no animal survived after 25 days. The increased survival of CRS-3123 is likely due  
693 to the inhibition of spore formation, preventing re-infection of animals during the study.

#### 694 5.2.5. Toxicity and pharmacokinetics

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

#### 699 5.2.6. Phase 1

700 CRS-3123 has completed two phase 1 clinical studies. A double-blind, single-center, placebo-  
701 controlled single-ascending dose study was conducted in 40 healthy adults, with a dose of 100,  
702 200, 400, 800 or 1200 mg [98]. The drug candidate was found safe and well tolerated, with the same  
703 frequency and severity of AEs between the drug group and the placebo group. Although bioavailabil-  
704 ity has not been precisely determined, it was found that plasma concentration at higher dose of  
705 CRS-3123 were above MICs of Gram-positive pathogenic bacteria. By LC-MS analysis, the  
706 *N*-glucuronide metabolite of CRS-3123 was observed in plasma and urine, indicating that the metab-  
707 olite is excreted in urine. This is also the case for the non-metabolized CRS-3123.

708 The second phase 1 trial was a double-blind, single-center, placebo-controlled multiple-ascending  
709 dose study, conducted in 30 healthy adults with doses of 200, 400 and 600 mg administered twice  
710 daily during 10 days [99]. As for the first phase 1 study, CRS-3123 was safe and well tolerated. In par-  
711 ticular, no prolongation of the QT interval was noticed. After administration, a large proportion of  
712 the drug was retained in the gut and excreted in feces. Indeed, the percentage of free drug and  
713 glucuronide metabolite in urine was inferior to 2 %. The low absorption of CRS-3123 leads to no sys-  
714

719 temic accumulation. Moreover, high concentrations of the drug are recovered in feces at any doses  
720 (2115 – 8280 µg/g), which are superior to the MIC<sub>90</sub> value against *C. difficile* (1 µg/mL). However,  
721 even with high concentrations, CRS-3123 has a minimal effect on the normal microbiota and does  
722 not increase the proportion of Proteobacteria. With this narrow spectrum of activity, CRS-3123 is  
723 likely to reduce recurrences after treatment of CDI, as it was demonstrated for fidaxomicin [106].

#### 724 725 5.2.7. Current advances

726  
727 With a promising pharmacokinetic and bacterial selectivity profile for therapeutic use, CRS-3123  
728 is currently evaluated in a phase 2 study, to compare efficacy of the drug against vancomycin in  
729 adults with CDI [107].

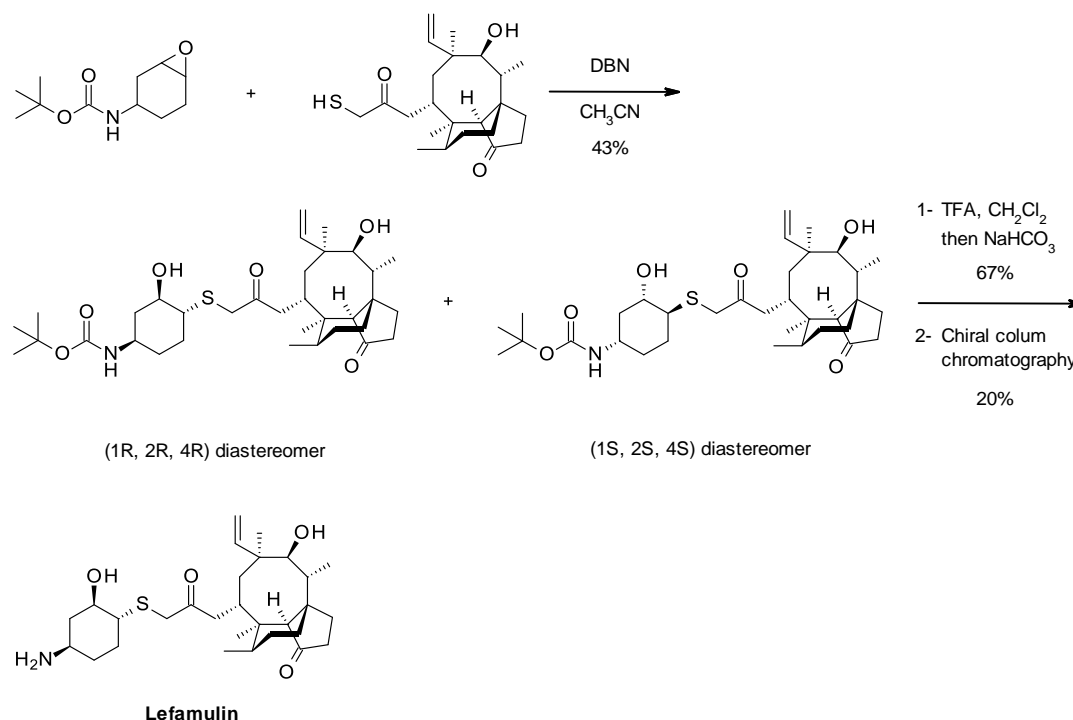
### 730 731 **5.3. Lefamulin**

732  
733 Lefamulin is a semisynthetic derivative of the naturally occurring tricyclic diterpenoid  
734 pleuromutilin class of antibiotics [108,109]. The compound (formerly known as BC-3781) was devel-  
735 oped by Nabriva Therapeutics and is currently marketed under the brand name Xenleta. The drug  
736 was approved by the FDA and the EMA in 2019 and 2020, respectively, as a short-course  
737 monotherapy for the treatment of community-acquired bacterial pneumonia (CABP) in adults  
738 [110–112]. This is the first agent within the Pleuromutilin derivative class to be developed for sys-  
739 temic use in humans by oral and intravenous administration [108,113].

#### 740 741 5.3.1. Chemical synthesis

742  
743 The synthetic route was patented in 2008 by Mang *et al.* [114]. Lefamulin is synthesized in two  
744 step from pleuromutilin thiol [115] and syn-3,4-epoxycyclohexyl-carbamic acid tert-butyl ester [116].  
745 After a S<sub>N</sub>2 reaction leading to a mixture of diastereomers (1R, 2R, 4R) and (1S, 2S, 4S), the Boc pro-  
746 tecting group is cleaved in acidic conditions. The mixture is then separated by chiral column chroma-  
747 tography to afford lefamulin (Figure 13).

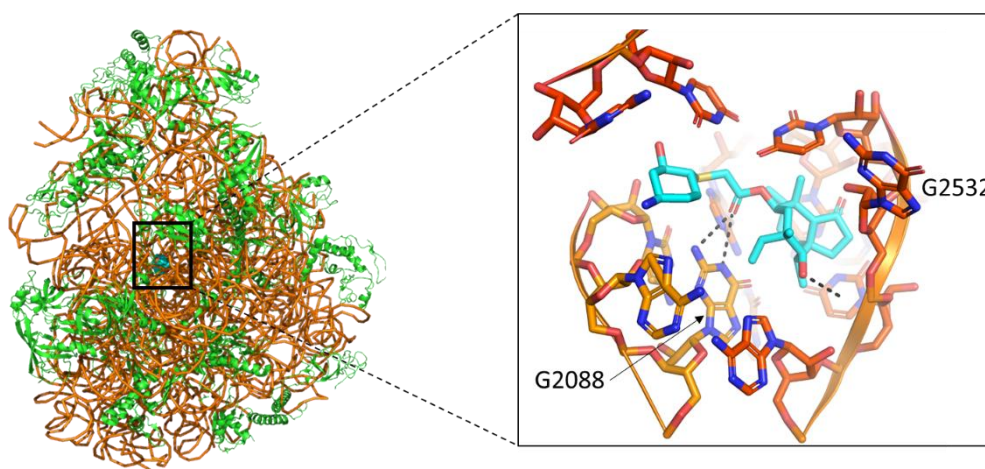




**Figure 13.** Description of the synthetic pathway leading to Lefamulin [114].

### 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].

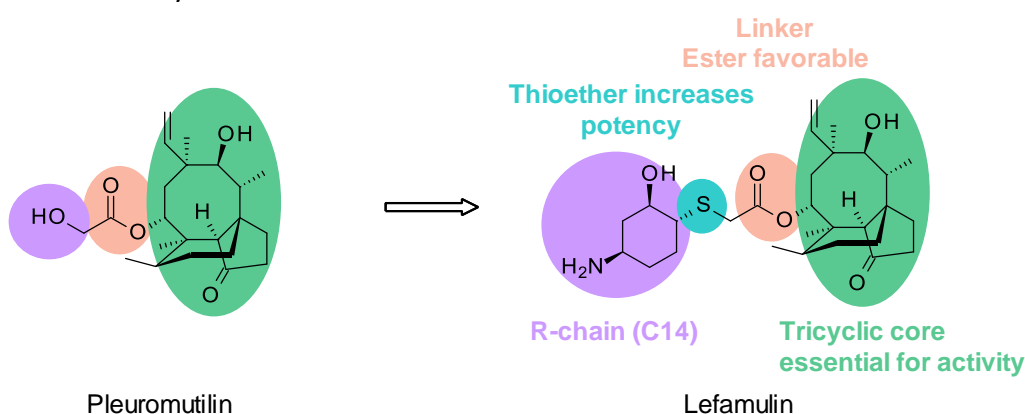


**Figure 14.** Views of lefamulin complex formed with *S. aureus* DNA gyrase and uncleaved DNA (PDB code: 5HL7).

### 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,  $\beta$ -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<sub>0–24</sub> of 8978 ng·h/mL and C<sub>max</sub> 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

850 efficacy and safety of IV lefamulin compared to IV moxifloxacin +/- linezolid in 551 adults patients  
851 with moderate to severe CABP [136,137]. The LEAP 2 study compared the safety and efficacy of  
852 600mg oral lefamulin twice daily for 5 days versus 400 mg of oral moxifloxacin once daily for 7 days  
853 in 738 adults patients with moderate CABP [138,139]. Data from both trials have shown noninferior  
854 efficacy with short course therapy compared with moxifloxacin in the treatment of CABP [113].

### 855 5.3.9. Safety and tolerability

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857  
858 Intravenous and oral lefamulin was generally well tolerated in phase 1 and phase 2 clinical stud-  
859 ies [124,134]. From the phase 2 trial, the most frequently reported AEs were headache, nausea, and  
860 diarrhea [135]. In both LEAP 1 and LEAP 2 trials, most AEs were mild or moderate in severity for both  
861 arm groups [137,140]. Lefamulin remains contraindicated in patients with known hypersensitivity to  
862 pleuromutilins [141]. Interestingly, the concomitant IV or oral administration of lefamulin with strong  
863 and/or moderate CYP3A or P-glycoprotein inducers can reduce the efficacy of the drug and should be  
864 then avoided. On the other hand, IV or oral coadministration of lefamulin with drugs that strongly or  
865 moderately inhibit CYP3A or P-glycoprotein inhibitors might increase the risk of AEs.

### 866 5.3.10. Current advances

867  
868  
869 Lefamulin is currently marketed as treatment for CABP caused by susceptible microorganisms.  
870 Moreover, lefamulin could be an option for the treatment of other serious bacterial infections  
871 caused by MRSA and vancomycin-resistant *E. faecium*, but also by MDR organisms associated with  
872 STI (*N. gonorrhoeae* and *M. genitalium*). The use of lefamulin could be also considered for patients  
873 with high-risk/recurrent CDI [109].

## 874 6. FabI enzyme as a drug target

### 875 6.1. Introduction

876  
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878  
879 FabI is an essential NADH-dependent enzyme which catalyzes the reduction of enoyl-acyl carrier  
880 protein (enoyl-ACP) in the last step of the bacterial fatty acid biosynthesis pathway (FAS-II), involved  
881 in the synthesis of phospholipids. Contrary to the multifunctional mammalian type I fatty acid syn-  
882 thase (FAS-I), each reaction of FAS-II is catalyzed by a different enzyme [142]. This structural differ-  
883 ence between the two pathways is the reason why inhibitors of the FAS-II pathway are selective and  
884 safe for use in humans. On the other hand, the complexity in the bacterial fatty acid metabolism can  
885 narrow the spectrum of activity of FabI inhibitors [142]. However, their development has continued  
886 since FabI represents an essential and validated target in multidrug-resistant *S. aureus*.

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

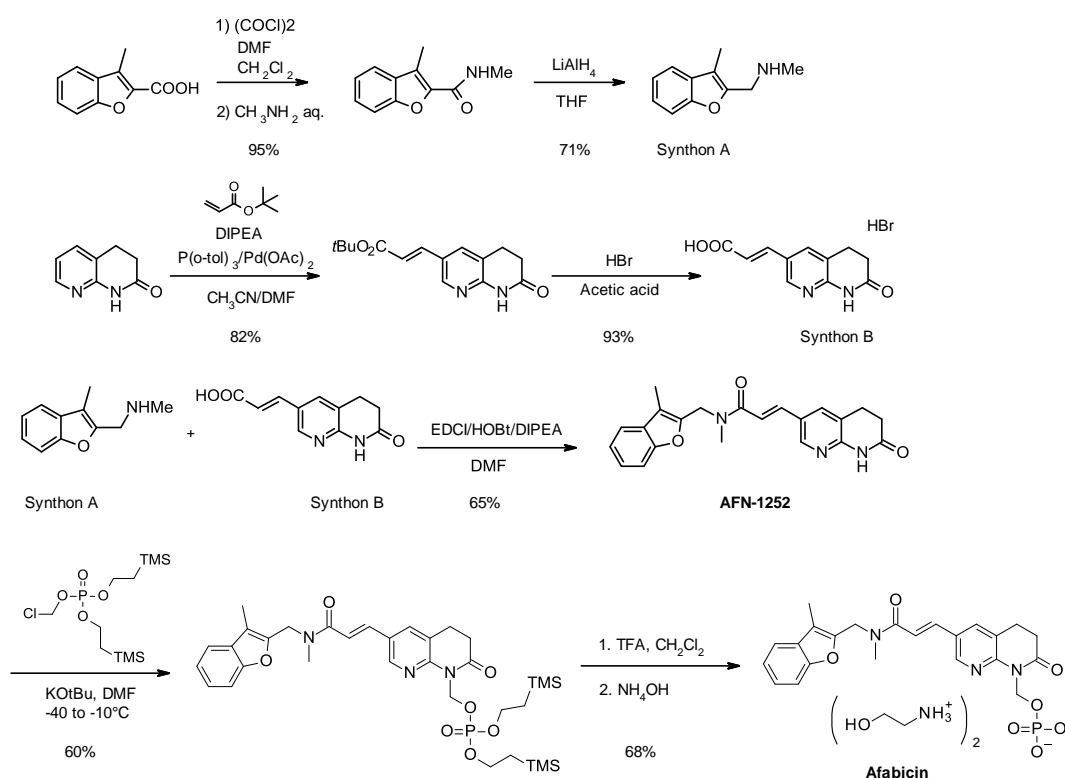
### 892 6.2. AFN-1252

893  
894  
895 AFN-1252 is a selective inhibitor of *S. aureus* FabI. This molecule belongs to the benzofuran  
896 naphthyridines, a novel class of antibiotics. The effort to identify potent and selective FabI inhibitors  
897 began with the screening of the GSK proprietary compound collection. From a first high-throughput  
898 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  $\text{LiAlH}_4$ . 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,  $\text{NH}_4\text{OH}$ , NaOH) leads to the corresponding salt of afabycin [146].

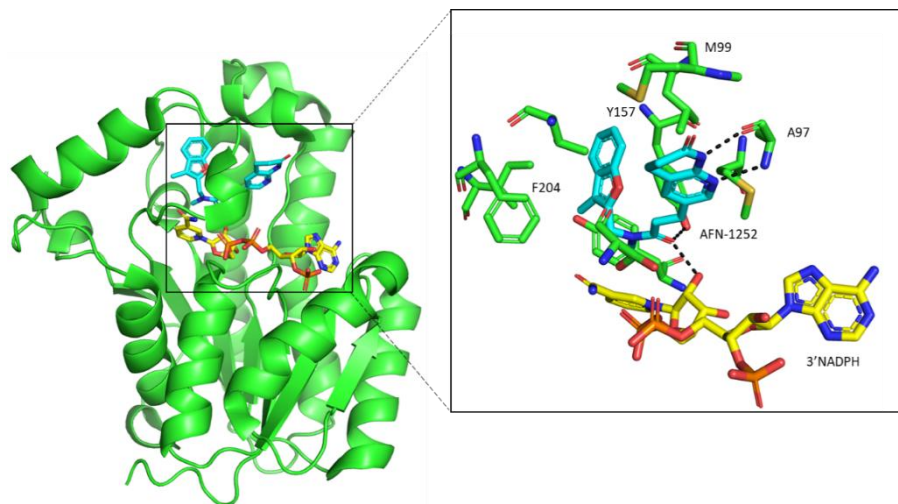


**Figure 16.** Description of the synthetic pathway leading to AFN-1252 and the diethanol ammonium salt of afabycin [145,146].

### 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 oxotetrahydronaphthyridine 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].



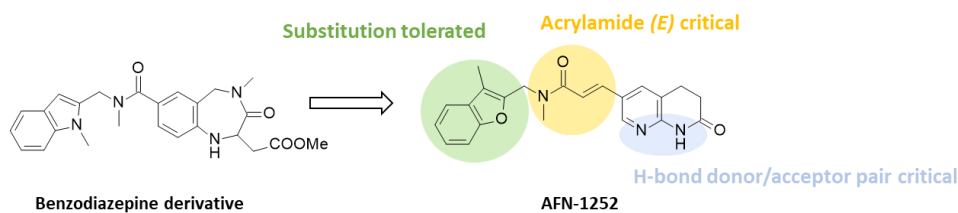
**Figure 17.** Views of AFN-1252 complex formed with *S. aureus* FabI and the NADPH cofactor (PDB code: 4FS3) .

### 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-NH<sub>3</sub>, 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 MIC<sub>90</sub> 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<sub>max</sub> (154, 196, and 304 ng/ml) and AUC<sub>0-t</sub> (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 ED<sub>50</sub> 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<sub>max</sub> 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-

995 response rate at day 3 was 97.3%, where 82.9% of patients had a >20% decrease in the area of ery-  
 996 thema, and 77.9% of patients had a >20% decrease in the area of induration. Microbiologic eradica-  
 997 tion rates for MRSA and MSSA were around 90% at short- and long-term follow-ups. The main  
 998 drug-related AEs reported, which were mostly mild or moderate, were headache (26.2%) and nausea  
 999 (21.4%) [151,152].

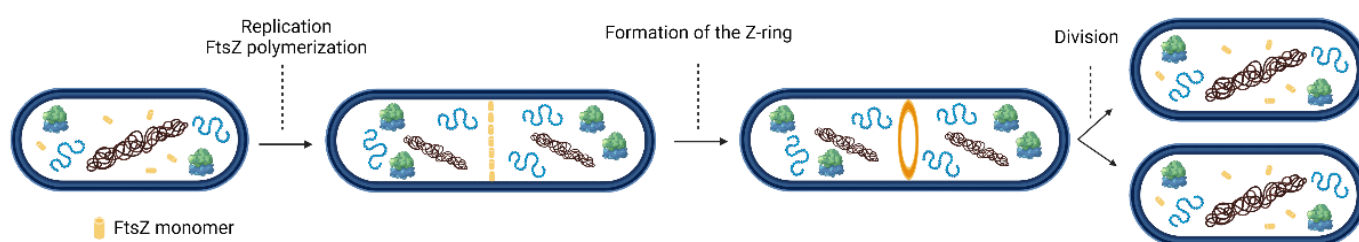
## 1000 6.2.8 Prodrug

1003 AFN-1252 belongs to class II biopharmaceutics classification system (BCS), presenting a limited  
 1004 aqueous solubility and oral bioavailability. Therefore, Debiopharm invested in the development of its  
 1005 prodrug Afabacin desphosphono (formerly Debio1450, AFN-1720). As expected, the prodrug showed  
 1006 little to no activity against *S. aureus*, *E. coli* and other species. Afabacin was rapidly converted *in vivo*  
 1007 to the active metabolite AFN-1252 and its antibacterial activity was better than AFN-1252 adminis-  
 1008 tered by itself orally [153]. The prodrug was studied in a phase 2 clinical trial to compare its efficacy,  
 1009 safety, and tolerability with those of vancomycin/linezolid in the treatment of ABSSSIs [154]. In a  
 1010 phase 1 clinical trial, the penetration of afabacin into bones in patients undergoing elective hip re-  
 1011 placement surgery was evaluated [155]. Moreover, a phase 2 clinical trial is underway to study the  
 1012 safety and efficacy of afabacin in human subjects with bone and joint infections caused *S. aureus*.  
 1013 [156].

## 1014 7. FtsZ protein as a drug target

### 1015 7.1. Introduction

1018 FtsZ is a bacterial protein involved in cell division forming single-stranded filaments by guanosine  
 1019 5'-triphosphate (GTP) dependent polymerization. These FtsZ filaments form the cytokinetic ring  
 1020 (called Z-ring) at midcell, able to recruit other critical elements for peptidoglycan synthesis and sep-  
 1021 tum formation, which leads to the induction of cell division. Because FtsZ is essential for the germ  
 1022 and is widely conserved throughout bacteria, it displays all the attractive features of a new target for  
 1023 antibiotic compounds (Figure 19) [157].



1024 **Figure 19.** Involvement of the FtsZ protein towards the formation of the Z-ring.

### 1025 7.2. TXA707/TXA709

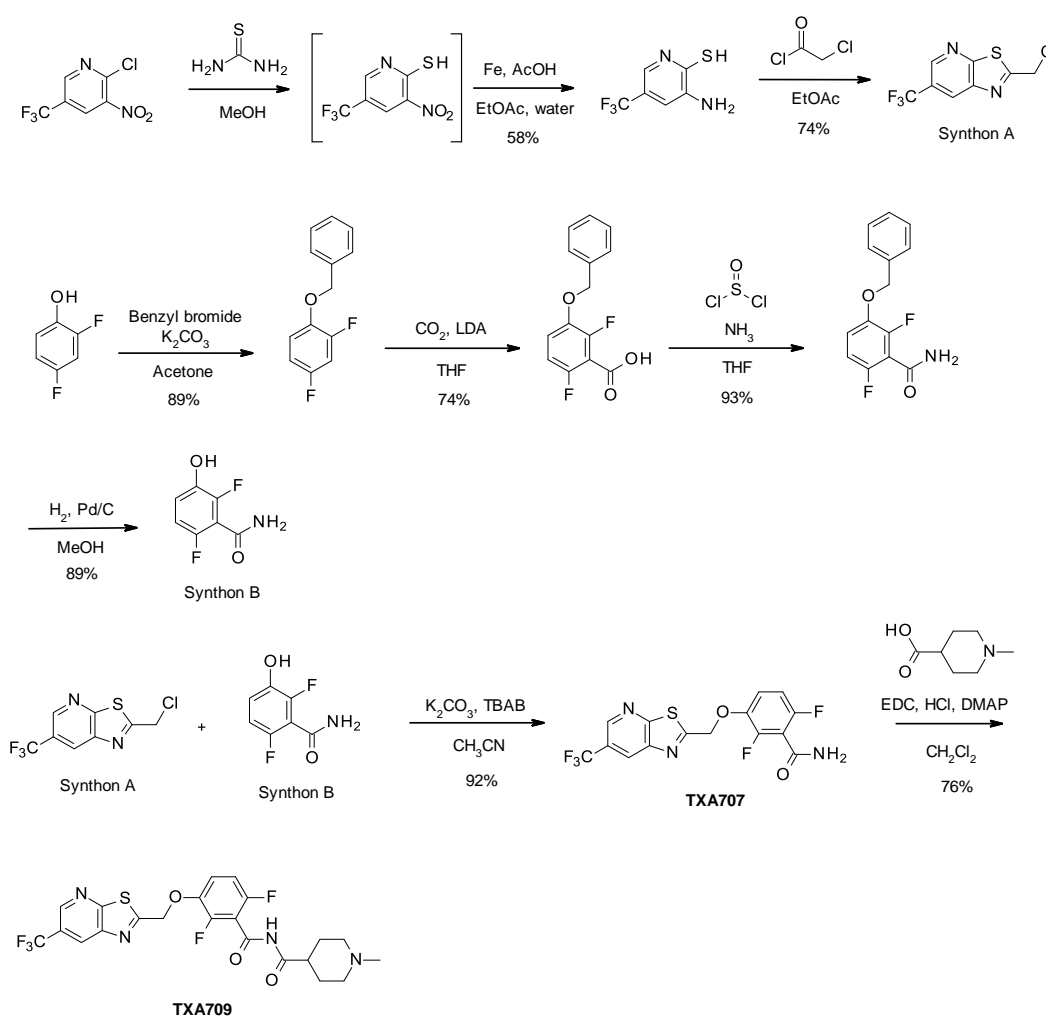
1026 TXA707 is the first antibacterial compound targeting FtsZ to reach clinical development. It is be-  
 1027 ing developed as an oral prodrug (TXA709) by Taxis Pharmaceuticals to treat MRSA infections.

1028 TXA707 is derived from PC190723, which is coming from a fragment-based medicinal chemistry  
 1029 program starting from 3-methoxybenzamide (3-MBA), a known FtsZ inhibitor in *Bacillus subtilis* [158].  
 1030 To improve the pharmacokinetic properties of PC190723, the chlorine atom on the pyridyl ring was  
 1031 replaced with a trifluoromethyl group, resistant to metabolism by cytochrome P450 enzymes.  
 1032  
 1033  
 1034



### 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 CO<sub>2</sub> 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).



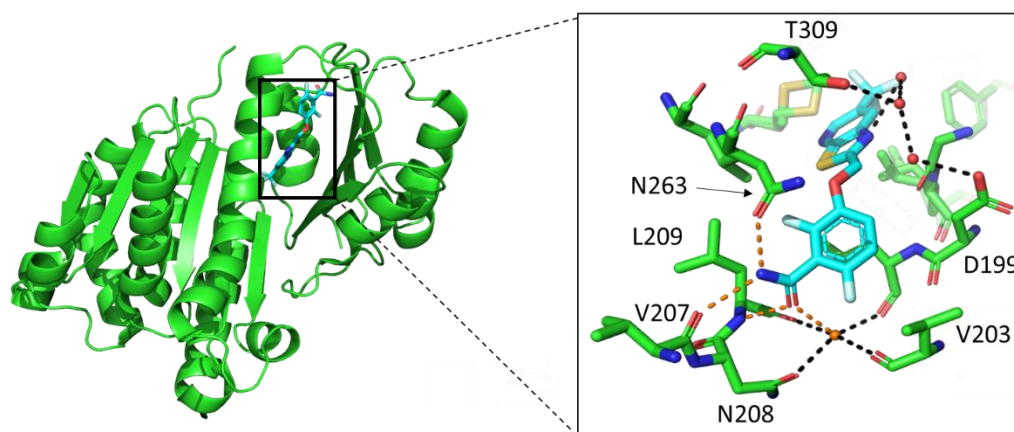
**Figure 20.** Description of the synthetic pathway leading to TXA709 [159].

### 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.

1057 Transmission electron microscopy studies showed that within 1 hour of treatment with TXA707, *S.*  
 1058 *aureus* cells became enlarged enough, resulting to cell lysis. Furthermore, TXA707 was co-crystallized  
 1059 in complex with FtsZ (Figure 21) [160]. Eventually, the target was validated with the selection of  
 1060 TXA707-resistant MRSA mutants (frequencies of resistance:  $3 \cdot 10^8$  in MRSA ATCC 43300;  $1 \cdot 10^8$  in an  
 1061 MRSA clinical isolate). Twenty resistant clones were isolated and the *ftsZ* gene was sequenced. Data  
 1062 showed that all TXA707-resistant strains carried a mutation within this gene [161].

1063 It is worthwhile noting that the mechanism of action of TXA707 on FtsZ echoes that of paclitaxel  
 1064 (tradename TAXOL) on tubulin, the eukaryotic structural homologue of FtsZ. Indeed, both com-  
 1065 pounds prevent polymers disassembly, eventually leading to cell division halt.

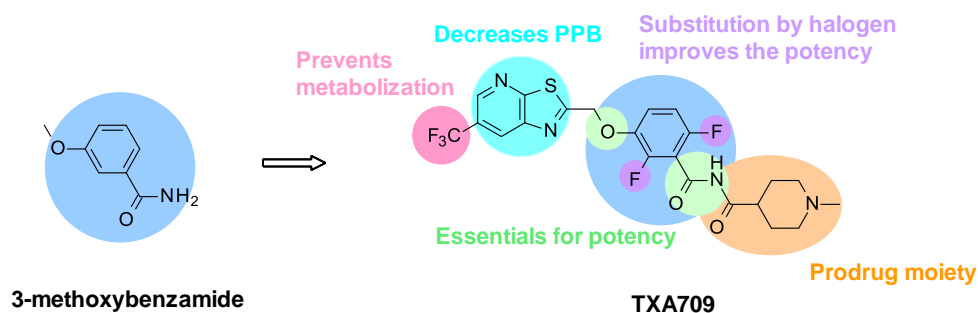


1066  
 1067  
 1068 **Figure 21.** Views of TXA707 complex formed with *S. aureus* FtsZ 12-316 (PDB code: 5XDT).

### 1069 7.2.3. Structure-activity relationship studies

1070 The starting point for the development of TXA707 was 3-methoxybenzamide, proven to be an FtsZ  
 1071 inhibitor in *B. subtilis* [158]. The methoxy and amide moieties in their respective positions were shown  
 1072 to be essential for the activity on FtsZ (Figure 22). Authors then investigated modifications around the  
 1073 3-methoxybenzamide core:

- 1074 • **Substitution of the phenyl ring:** substitution was detrimental, except for the addition of halogen  
 1075 atoms in *ortho* position to the amide.
- 1076 • **Substitution of the methoxy moiety:** substitution with other alkoxy chains was possible. It was  
 1077 shown that increasing the length of the alkyl chain up to nine carbon atoms allowed an 8000-fold  
 1078 improvement in potency on *B. subtilis* compared to the methoxy moiety [162]. However, this also  
 1079 led to an increase in PPB. Subsequent replacement of the alkyl chain with various heterocycles was  
 1080 investigated, where the benzothiazole gave interesting results.
- 1081 • **Substitution of the benzothiazole ring:** substitution in position 4 was detrimental. In position 6,  
 1082 substitution was tolerated with small moieties (methyl, chlorine) but larger groups led to inactive  
 1083 compounds (phenyl, methoxy). Lastly, substitution in position 5 was favorable, particularly with a  
 1084 chlorine or bromine atom, phenyl ring, propyl or ethoxy chain.
- 1085 • **Modification of the benzothiazole ring:** to further decrease the plasma protein binding,  
 1086 thiazolopyridine analogues were synthesized. This allowed the identification of PC190723, which  
 1087 displayed a good potency on *S. aureus* (MIC = 1  $\mu\text{g}/\text{mL}$ ) and a relatively low PPB (85.4%  
 1088 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,  $IC_{50} > 120$  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].

1129 Lastly, PK/PD parameters were evaluated in a murine neutropenic thigh infection model and the  
1130 ratio AUC/MIC was deemed to be the PK/PD index most closely linked to efficacy [165].  
1131

#### 1132 7.2.6. Current advances 1133

1134 TXA709 has been evaluated in a non-registered phase 1 clinical trial and no serious AE was re-  
1135 ported [166].  
1136

### 1137 8. Conclusion and perspectives 1138

1139 For the first time in decades, pharmaceutical companies are refuelling the pipeline of antibiotics.  
1140 To date, at least eight compounds with new targets and/or MOA are in clinical trials, including two in  
1141 phase 3, or have been recently approved. They represent a new hope to fight the bad bugs that are  
1142 becoming more and more resistant to current antibiotics.

1143 Beside these clinical candidates, other promising antibiotics and/or new targets have been re-  
1144 ported in the literature. This is the case for a series of novel tetrazole ligands targeting LpxA, the first  
1145 enzyme in the lipid A pathway [167]. Moreover, over twenty potential candidates targeting the bac-  
1146 terial RNA polymerase enzyme have been designed [168,169].

1147 In addition, other strategies are being explored such as pathoblockers to disarm bacteria or ad-  
1148 juvants like efflux pump inhibitors (EPI) to potentialize current antibiotics.

1149 Unlike antibiotics, which kill bacteria or inhibit growth and so exert a selection pressure,  
1150 pathoblockers inhibit virulence factors. Virulence is the ability of bacteria to cause disease and is me-  
1151 diated by factors such as toxins, proteases or cytolysins [170]. Therefore, antivirulence drugs disrupt  
1152 the relation between the host and the pathogen, causing the disarmament of bacteria which can  
1153 then be eliminated by the immune system. Because the bacterium viability is not impacted,  
1154 pathoblockers are believed to reduce resistance development. To date, only one small molecule act-  
1155 ing as a pathoblocker has been evaluated in clinical trials: fluorothiazinon (formerly CL-55), devel-  
1156 oped by the Gamaleya Research Institute of Epidemiology and Microbiology in Russia. Fluorothiazinon  
1157 inhibits the type III secretion system, a well conserved system among Gram-negative  
1158 bacteria allowing the secretion of several virulence factors [171–174]. After completing a phase 1  
1159 study [175], fluorothiazinon is currently evaluated in a phase 2 clinical study for the treatment of  
1160 complicated urinary tract infections caused by *P. aeruginosa* [176]. Moreover, recent studies report-  
1161 ed quorum sensing inhibitors to reduce virulence in *A. baumannii* [177,178].

1162 EPIs are compounds able to block bacterial multidrug efflux transporters. These pumps are able  
1163 to extrude several classes of antibiotics from the bacteria such as tetracyclines, macrolides,  
1164  $\beta$ -lactams, fluoroquinolones, therefore decreasing their intra-bacterial concentration and eventually  
1165 rendering them ineffective [179]. (Over)expression of multidrug efflux transporters, notably from the  
1166 resistance-nodulation-cell division family, is one of the most worrying resistance mechanisms re-  
1167 garding multidrug antibacterial resistance. One strategy to override this defense mechanism is the  
1168 conception of EPIs [180]. Administrating an EPI in combination with an antibiotic effluxed by the tar-  
1169 geted bacterial transporters could allow to restore the antibiotic activity and decrease the necessary  
1170 antibiotic dose, thus reducing the selection pressure [181].  
1171

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1173 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  
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## References

- [1] J. O'Neill, Tackling drug-resistant infections globally: final report and recommendations, 2016.
- [2] C.J. Murray, K.S. Ikuta, F. Sharara, L. Swetschinski, G. Robles Aguilar, A. Gray, C. Han, C. Bisignano, P. Rao, E. Wool, S.C. Johnson, A.J. Browne, M.G. Chipeta, F. Fell, S. Hackett, G. Haines-Woodhouse, B.H. Kashef Hamadani, E.A.P. Kumaran, B. McManigal, R. Agarwal, S. Akech, S. Albertson, J. Amuasi, J. Andrews, A. Aravkin, E. Ashley, F. Bailey, S. Baker, B. Basnyat, A. Bekker, R. Bender, A. Bethou, J. Bielicki, S. Boonkasidecha, J. Bukosia, C. Carvalheiro, C. Castañeda-Orjuela, V. Chansamouth, S. Chaurasia, S. Chiurchiù, F. Chowdhury, A.J. Cook, B. Cooper, T.R. Cressey, E. Criollo-Mora, M. Cunningham, S. Darboe, N.P.J. Day, M. De Luca, K. Dokova, A. Dramowski, S.J. Dunachie, T. Eckmanns, D. Eibach, A. Emami, N. Feasey, N. Fisher-Pearson, K. Forrest, D. Garrett, P. Gastmeier, A.Z. Giref, R.C. Greer, V. Gupta, S. Haller, A. Haselbeck, S.I. Hay, M. Holm, S. Hopkins, K.C. Iregbu, J. Jacobs, D. Jarovsky, F. Javanmardi, M. Khorana, N. Kisoona, E. Kobeissi, T. Kostyanov, F. Krapp, R. Krumkamp, A. Kumar, H.H. Kyu, C. Lim, D. Limmathurotsakul, M.J. Loftus, M. Lunn, J. Ma, N. Mturi, T. Munera-Huertas, P. Musicha, M.M. Mussi-Pinhata, T. Nakamura, R. Nanavati, S. Nangia, P. Newton, C. Ngoun, A. Novotney, D. Nwakanma, C.W. Obiero, A. Olivas-Martinez, P. Olliaro, E. Ooko, E. Ortiz-Brizuela, A.Y. Peleg, C. Perrone, N. Plakkal, A. Ponce-de-Leon, M. Raad, T. Ramdin, A. Riddell, T. Roberts, J.V. Robotham, A. Roca, K.E. Rudd, N. Russell, J. Schnall, J.A.G. Scott, M. Shivamallappa, J. Sifuentes-Osornio, N. Steenkeste, A.J. Stewardson, T. Stoeva, N. Tasak, A. Thaiprakong, G. Thwaites, C. Turner, P. Turner, H.R. van Doorn, S. Velaphi, A. Vongpradith, H. Vu, T. Walsh, S. Waner, T. Wangrangsimakul, T. Wozniak, P. Zheng, B. Sartorius, A.D. Lopez, A. Stergachis, C. Moore, C. Dolecek, M. Naghavi, Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, *The Lancet*. 399 (2022) 629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0).
- [3] S. Harbarth, H.H. Balkhy, H. Goossens, V. Jarlier, J. Kluytmans, R. Laxminarayan, M. Saam, A. Van Belkum, D. Pittet, Antimicrobial resistance: one world, one fight!, *Antimicrob. Resist. Infect. Control*. 4 (2015) 49. <https://doi.org/10.1186/s13756-015-0091-2>.
- [4] Annunziato, Strategies to Overcome Antimicrobial Resistance (AMR) Making Use of Non-Essential Target Inhibitors: A Review, *Int. J. Mol. Sci.* 20 (2019) 5844. <https://doi.org/10.3390/ijms20235844>.
- [5] W. C Reygaert, Department of Biomedical Sciences, Oakland University William Beaumont School of Medicine, Rochester, MI, USA, An overview of the antimicrobial resistance mechanisms of bacteria, *AIMS Microbiol.* 4 (2018) 482–501. <https://doi.org/10.3934/microbiol.2018.3.482>.
- [6] J.R. Everett, Academic drug discovery: current status and prospects, *Expert Opin. Drug Discov.* 10 (2015) 937–944. <https://doi.org/10.1517/17460441.2015.1059816>.
- [7] J. Frearson, P. Wyatt, Drug discovery in academia: the third way?, *Expert Opin. Drug Discov.* 5 (2010) 909–919. <https://doi.org/10.1517/17460441.2010.506508>.
- [8] C.H. Emmerich, L.M. Gamboa, M.C.J. Hofmann, M. Bonin-Andresen, O. Arbach, P. Schendel, B. Gerlach, K. Hempel, A. Bespalov, U. Dirnagl, M.J. Parnham, Improving target assessment in biomedical research: the GOT-IT recommendations, *Nat. Rev. Drug Discov.* 20 (2021) 64–81. <https://doi.org/10.1038/s41573-020-0087-3>.
- [9] B.D. Bax, P.F. Chan, D.S. Eggleston, A. Fosberry, D.R. Gentry, F. Gorrec, I. Giordano, M.M. Hann, A. Hennessy, M. Hibbs, J. Huang, E. Jones, J. Jones, K.K. Brown, C.J. Lewis, E.W. May, M.R. Saunders, O. Singh, C.E. Spitzfaden, C. Shen, A. Shillings, A.J. Theobald, A. Wohlkonig, N.D. Pearson, M.N. Gwynn, Type IIA topoisomerase inhibition by a new class of antibacterial agents, *Nature*. 466 (2010) 935–940. <https://doi.org/10.1038/nature09197>.

- 1221 [10] W.J. Coates, M.N. Gwynn, I.K. Hatton, P.J. Masters, N.D. Pearson, S.S. Rahman, B. Slocombe, J.D. Warrack,  
1222 Quinoline derivatives as antibacterials. WO1999037635, 1999., WO1999037635A1, n.d.  
1223 <https://patents.google.com/patent/WO1999037635A1/en> (accessed April 19, 2022).
- 1224 [11] D.E. Davies, D.T. Davies, I. Giordano, A.J. Hennessy, N.D. Pearson, Tricyclic nitrogen containing compounds  
1225 as antibacterial agents. WO2008128942, 2008., WO2008128942A1, n.d.  
1226 <https://patents.google.com/patent/WO2008128942A1/en> (accessed August 24, 2022).
- 1227 [12] K.L. Widdowson, Tricyclic nitrogen containing compounds for treating neisseria gonorrhoea infection.  
1228 WO2016027249, 2016., WO2016027249A1, n.d.  
1229 <https://patents.google.com/patent/WO2016027249A1/fr?q=wo+2016%2f027249> (accessed August 24, 2022).
- 1230 [13] C.W. Barfoot, P. Brown, S. Dabbs, D.T. Davies, A.J. Hennessy, T.J. Miles, N.D. Pearson, The design of efficient  
1231 and selective routes to pyridyl analogues of 2,3-dihydro-1,4-benzodioxin-6-carbaldehyde, *Tetrahedron Lett.* 51  
1232 (2010) 5038–5040. <https://doi.org/10.1016/j.tetlet.2010.07.099>.
- 1233 [14] E.G. Gibson, B. Bax, P.F. Chan, N. Osheroff, Mechanistic and Structural Basis for the Actions of the  
1234 Antibacterial Gepotidacin against *Staphylococcus aureus* Gyrase, *ACS Infect. Dis.* 5 (2019) 570–581.  
1235 <https://doi.org/10.1021/acsinfecdis.8b00315>.
- 1236 [15] M. Kokot, M. Anderluh, M. Hrast, N. Minovski, The Structural Features of Novel Bacterial Topoisomerase  
1237 Inhibitors That Define Their Activity on Topoisomerase IV, *J. Med. Chem.* 65 (2022) 6431–6440.  
1238 <https://doi.org/10.1021/acs.jmedchem.2c00039>.
- 1239 [16] D.J. Biedenbach, S.K. Bouchillon, M. Hackel, L.A. Miller, N.E. Scangarella-Oman, C. Jakielaszek, D.F. Sahm, *In*  
1240 *Vitro* Activity of Gepotidacin, a Novel Triazaacenaphthylene Bacterial Topoisomerase Inhibitor, against a  
1241 Broad Spectrum of Bacterial Pathogens, *Antimicrob. Agents Chemother.* 60 (2016) 1918–1923.  
1242 <https://doi.org/10.1128/AAC.02820-15>.
- 1243 [17] R.K. Flamm, D.J. Farrell, P.R. Rhomberg, N.E. Scangarella-Oman, H.S. Sader, Gepotidacin (GSK2140944) In  
1244 *Vitro* Activity against Gram-Positive and Gram-Negative Bacteria, *Antimicrob. Agents Chemother.* 61 (2017).  
1245 <https://doi.org/10.1128/AAC.00468-17>.
- 1246 [18] W. So, J.L. Crandon, D.P. Nicolau, Pharmacodynamic Profile of GSK2140944 against Methicillin-Resistant  
1247 *Staphylococcus aureus* in a Murine Lung Infection Model, *Antimicrob. Agents Chemother.* 59 (2015) 4956–4961.  
1248 <https://doi.org/10.1128/AAC.00625-15>.
- 1249 [19] C.C. Bulik, Ó.O. Okusanya, E.A. Lakota, A. Forrest, S.M. Bhavnani, J.L. Hoover, D.R. Andes, P.G. Ambrose,  
1250 Pharmacokinetic-Pharmacodynamic Evaluation of Gepotidacin against Gram-Positive Organisms Using Data  
1251 from Murine Infection Models, *Antimicrob. Agents Chemother.* 61 (2017) e00115-16.  
1252 <https://doi.org/10.1128/AAC.00115-16>.
- 1253 [20] J.L. Hoover, C.M. Singley, P. Elefante, S. Rittenhouse, Efficacy of Human Exposures of Gepotidacin  
1254 (GSK2140944) against *Escherichia coli* in a Rat Pyelonephritis Model, *Antimicrob. Agents Chemother.* 63 (2019).  
1255 <https://doi.org/10.1128/AAC.00086-19>.
- 1256 [21] C. Fishman, J.M. Caverly Rae, L.M. Posobiec, S.B. Laffan, S.A. Lerman, N. Pearson, S. Janmohamed, E. Dumont,  
1257 D. Nunn-Floyd, D.J. Stanislaus, Novel Bacterial Topoisomerase Inhibitor Gepotidacin Demonstrates Absence of  
1258 Fluoroquinolone-Like Arthropathy in Juvenile Rats, *Antimicrob. Agents Chemother.* 66 (2022) e00483-22.  
1259 <https://doi.org/10.1128/aac.00483-22>.
- 1260 [22] GlaxoSmithKline, A Phase I, Randomized, Double-Blinded, Placebo- and Moxifloxacin-Controlled, 4-Period  
1261 Crossover Study to Evaluate the Effect of GSK2140944 on Cardiac Conduction as Assessed by 12-lead  
1262 Electrocardiogram in Healthy Volunteers (NCT02257398), [clinicaltrials.gov](https://clinicaltrials.gov), 2017.  
1263 <https://clinicaltrials.gov/ct2/show/NCT02257398> (accessed June 9, 2022).

- 1264 [23] GlaxoSmithKline, A Randomized, Single Blind, Placebo Controlled Study to Evaluate Safety, Tolerability, and  
1265 Pharmacokinetics of Single Escalating Oral Doses of GSK2140944 in Healthy Adult Subjects (NCT02202187),  
1266 clinicaltrials.gov, 2017. <https://clinicaltrials.gov/ct2/show/NCT02202187> (accessed June 9, 2022).
- 1267 [24] GlaxoSmithKline, A Single-Center, Three-Part, Open Label Study to Evaluate the Relative Bioavailability of  
1268 Two Formulations, Food Effect, and Interaction With Itraconazole Following Single Dose of GSK2140944 in  
1269 Healthy Subjects and Effect of Food on Safety, Tolerability, and Pharmacokinetics Following Multiple Doses of  
1270 GSK2140944 in Healthy Elderly Subjects (NCT02045849), clinicaltrials.gov, 2017.  
1271 <https://clinicaltrials.gov/ct2/show/NCT02045849> (accessed June 9, 2022).
- 1272 [25] GlaxoSmithKline, A Two-Part Study to Evaluate Safety, Tolerability, and Pharmacokinetics of Single and  
1273 Repeat IV Doses of GSK2140944 in Healthy Adult Subjects. (NCT01615796), clinicaltrials.gov, 2017.  
1274 <https://clinicaltrials.gov/ct2/show/NCT01615796> (accessed June 9, 2022).
- 1275 [26] GlaxoSmithKline, An Open-label Study To Evaluate Plasma and Pulmonary Pharmacokinetics Following  
1276 Intravenous Administration of GSK2140944 in Healthy Adult Subjects (NCT01934205), clinicaltrials.gov, 2017.  
1277 <https://clinicaltrials.gov/ct2/show/NCT01934205> (accessed June 9, 2022).
- 1278 [27] GlaxoSmithKline, An Open-Label, Non-Randomized, Two-Period, Cross-Over, Mass Balance Study to  
1279 Investigate the Recovery, Excretion and Pharmacokinetics of <sup>14</sup>C-GSK2140944 Administered as a Single  
1280 Intravenous and Single Oral Dose to Healthy Adult Male Subjects (NCT02000765), clinicaltrials.gov, 2017.  
1281 <https://clinicaltrials.gov/ct2/show/NCT02000765> (accessed June 9, 2022).
- 1282 [28] GlaxoSmithKline, A Randomized, Single Blind, Placebo-Controlled Study to Evaluate Safety, Tolerability, and  
1283 Pharmacokinetics of Repeat Escalating Oral Doses of GSK2140944 in Healthy Adult Subjects (BTZ116778)  
1284 (NCT01706315), clinicaltrials.gov, 2017. <https://clinicaltrials.gov/ct2/show/NCT01706315> (accessed August 24,  
1285 2022).
- 1286 [29] GlaxoSmithKline, A Phase I, Double-Blind, Two-Part, Sequential Study to Evaluate the Pharmacokinetics of  
1287 Gepotidacin Tablets in Healthy Adult and Adolescent Participants (NCT04079790), clinicaltrials.gov, 2020.  
1288 <https://clinicaltrials.gov/ct2/show/NCT04079790> (accessed October 21, 2021).
- 1289 [30] GlaxoSmithKline, A Phase I, Open-Label, Single-Dose, Multi-Part Study to Assess the Pharmacokinetics of  
1290 Gepotidacin (GSK2140944) in Male and Female Adult Subjects With Varying Degrees of Renal Impairment and  
1291 in Matched Control Subjects With Normal Renal Function (NCT02729038), clinicaltrials.gov, 2020.  
1292 <https://clinicaltrials.gov/ct2/show/NCT02729038> (accessed June 9, 2022).
- 1293 [31] GlaxoSmithKline, A Phase I; Multi-Center; Open-Label (Parts 1 and 2); Randomized, Double-Blind,  
1294 Placebo-Controlled (Part 3); Single-Dose; 3-Part Study to Evaluate the Relative Bioavailability of Three  
1295 Formulations in Healthy Subjects, Food Effect on Tablet Formulation in Healthy Subjects, and  
1296 Pharmacokinetics of Gepotidacin (GSK2140944) in Japanese Subjects in Fasted and Fed States (NCT02853435),  
1297 clinicaltrials.gov, 2020. <https://clinicaltrials.gov/ct2/show/NCT02853435> (accessed June 9, 2022).
- 1298 [32] Institut National de la Santé Et de la Recherche Médicale, France, Penetration of the Innovative Antibiotic  
1299 Gepotidacin Into Prostate and Tonsillar Tissue. (NCT04484740), clinicaltrials.gov, 2020.  
1300 <https://clinicaltrials.gov/ct2/show/NCT04484740> (accessed June 9, 2022).
- 1301 [33] GlaxoSmithKline, A Pharmacokinetic, Multi-cohort Study in Healthy Adult Subjects to Assess Gepotidacin as  
1302 Victim and as Perpetrator of Drug-Drug Interactions Via CYP450, Renal and Intestinal Transporters, and to  
1303 Assess Gepotidacin Pharmacokinetics in Japanese Healthy Adults (NCT04493931), clinicaltrials.gov, 2021.  
1304 <https://clinicaltrials.gov/ct2/show/NCT04493931> (accessed June 9, 2022).
- 1305 [34] GlaxoSmithKline, A Phase I, Open-Label, Single-Dose, Two-Part Study to Assess the Pharmacokinetics of  
1306 Gepotidacin (GSK2140944) in Male and Female Adult Participants With Varying Degrees of Hepatic

- 1307 Impairment and in Matched Control Participants With Normal Hepatic Function (NCT03562117),  
1308 [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/NCT03562117), 2021. <https://clinicaltrials.gov/ct2/show/NCT03562117> (accessed June 9, 2022).
- 1309 [35] GlaxoSmithKline, A Randomized, Open-Label, Single-Dose, 2-Period, Crossover Study to Compare  
1310 Gepotidacin Powder for Oral Suspension With the Adult Tablet Formulation in Healthy Male and Female  
1311 Participants Aged 18 to 50 Years (NCT05271799), [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/NCT05271799), 2022.  
1312 <https://clinicaltrials.gov/ct2/show/NCT05271799> (accessed June 9, 2022).
- 1313 [36] W. O’Riordan, C. Tiffany, N. Scangarella-Oman, C. Perry, M. Hossain, T. Ashton, E. Dumont, Efficacy, Safety,  
1314 and Tolerability of Gepotidacin (GSK2140944) in the Treatment of Patients with Suspected or Confirmed  
1315 Gram-Positive Acute Bacterial Skin and Skin Structure Infections, *Antimicrob. Agents Chemother.* 61 (2017)  
1316 e02095-16. <https://doi.org/10.1128/AAC.02095-16>.
- 1317 [37] S.N. Taylor, D.H. Morris, A.K. Avery, K.A. Workowski, B.E. Batteiger, C.A. Tiffany, C.R. Perry, A.  
1318 Raychaudhuri, N.E. Scangarella-Oman, M. Hossain, E.F. Dumont, Gepotidacin for the Treatment of  
1319 Uncomplicated Urogenital Gonorrhea: A Phase 2, Randomized, Dose-Ranging, Single-Oral Dose Evaluation,  
1320 *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* 67 (2018) 504–512. <https://doi.org/10.1093/cid/ciy145>.
- 1321 [38] J.S. Overcash, C.A. Tiffany, N.E. Scangarella-Oman, C.R. Perry, Y. Tao, M. Hossain, A. Barth, E.F. Dumont,  
1322 Phase 2a Pharmacokinetic, Safety, and Exploratory Efficacy Evaluation of Oral Gepotidacin (GSK2140944) in  
1323 Female Participants with Uncomplicated Urinary Tract Infection (Acute Uncomplicated Cystitis), *Antimicrob.*  
1324 *Agents Chemother.* 64 (2020) e00199-20. <https://doi.org/10.1128/AAC.00199-20>.
- 1325 [39] GlaxoSmithKline, A Phase III, Randomized, Multicenter, Parallel-Group, Double-Blind, Double-Dummy Study  
1326 in Adolescent and Adult Female Participants Comparing the Efficacy and Safety of Gepotidacin to  
1327 Nitrofurantoin in the Treatment of Uncomplicated Urinary Tract Infection (Acute Cystitis) (NCT04187144),  
1328 [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/NCT04187144), 2020. <https://clinicaltrials.gov/ct2/show/NCT04187144> (accessed February 11, 2021).
- 1329 [40] GlaxoSmithKline, A Phase III, Randomized, Multicenter, Parallel-Group, Double-Blind, Double-Dummy Study  
1330 in Adolescent and Adult Female Participants Comparing the Efficacy and Safety of Gepotidacin to  
1331 Nitrofurantoin in the Treatment of Uncomplicated Urinary Tract Infection (Acute Cystitis) (NCT04020341),  
1332 [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/NCT04020341), 2022. <https://clinicaltrials.gov/ct2/show/NCT04020341> (accessed April 11, 2022).
- 1333 [41] GlaxoSmithKline, A Phase III, Randomized, Multicenter, Open-Label Study in Adolescent and Adult  
1334 Participants Comparing the Efficacy and Safety of Gepotidacin to Ceftriaxone Plus Azithromycin in the  
1335 Treatment of Uncomplicated Urogenital Gonorrhea Caused by *Neisseria Gonorrhoeae* (NCT04010539),  
1336 [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/NCT04010539), 2022. <https://clinicaltrials.gov/ct2/show/NCT04010539> (accessed April 11, 2022).
- 1337 [42] C. Jakielaszek, M. Hossain, L. Qian, C. Fishman, K. Widdowson, J.J. Hilliard, F. Mannino, A. Raychaudhuri, E.  
1338 Carniel, S. Demons, H.S. Heine, J. Hershfield, R. Russo, W.M. Mega, D. Revelli, K. O’Dwyer, Gepotidacin is  
1339 efficacious in a nonhuman primate model of pneumonic plague, *Sci. Transl. Med.* 14 (2022) eabg1787.  
1340 <https://doi.org/10.1126/scitranslmed.abg1787>.
- 1341 [43] M. Unemo, C.S. Bradshaw, J.S. Hocking, H.J.C. de Vries, S.C. Francis, D. Mabey, J.M. Marrazzo, G.J.B. Sonder,  
1342 J.R. Schwebke, E. Hoornenborg, R.W. Peeling, S.S. Philip, N. Low, C.K. Fairley, Sexually transmitted infections:  
1343 challenges ahead, *Lancet Infect. Dis.* 17 (2017) e235–e279. [https://doi.org/10.1016/S1473-3099\(17\)30310-9](https://doi.org/10.1016/S1473-3099(17)30310-9).
- 1344 [44] G.S. Basarab, G.H. Kern, J. McNulty, J.P. Mueller, K. Lawrence, K. Vishwanathan, R.A. Alm, K. Barvian, P.  
1345 Doig, V. Galullo, H. Gardner, M. Gowravaram, M. Huband, A. Kimzey, M. Morningstar, A. Kutschke, S.D.  
1346 Lahiri, M. Perros, R. Singh, V.J.A. Schuck, R. Tommasi, G. Walkup, J.V. Newman, Responding to the challenge  
1347 of untreatable gonorrhea: ETX0914, a first-in-class agent with a distinct mechanism-of-action against bacterial  
1348 Type II topoisomerases, *Sci. Rep.* 5 (2015) 11827. <https://doi.org/10.1038/srep11827>.



- 1349 [45] A.A. Miller, M.M. Traczewski, M.D. Huband, P.A. Bradford, J.P. Mueller, Determination of MIC Quality  
1350 Control Ranges for the Novel Gyrase Inhibitor Zoliflodacin, *J. Clin. Microbiol.* 57 (2019).  
1351 <https://doi.org/10.1128/JCM.00567-19>.
- 1352 [46] L.M. Newman, M. Kankam, A. Nakamura, T. Conrad, J. Mueller, J. O'Donnell, B.L. Osborn, K. Gu, G.A.  
1353 Saviolakis, Thorough QT Study To Evaluate the Effect of Zoliflodacin, a Novel Therapeutic for Gonorrhea, on  
1354 Cardiac Repolarization in Healthy Adults, *Antimicrob. Agents Chemother.* 65 (2021) e01292-21.  
1355 <https://doi.org/10.1128/AAC.01292-21>.
- 1356 [47] G.S. Basarab, P. Doig, V. Galullo, G. Kern, A. Kimzey, A. Kutschke, J.P. Newman, M. Morningstar, J. Mueller, L.  
1357 Otterson, K. Vishwanathan, F. Zhou, M. Gowravaram, Discovery of Novel DNA Gyrase Inhibiting  
1358 Spiropyrimidinetriones: Benzisoxazole Fusion with N-Linked Oxazolidinone Substituents Leading to a Clinical  
1359 Candidate (ETX0914), *J. Med. Chem.* 58 (2015) 6264–6282. <https://doi.org/10.1021/acs.jmedchem.5b00863>.
- 1360 [48] P.A. Bradford, A.A. Miller, J. O'Donnell, J.P. Mueller, Zoliflodacin: An Oral Spiropyrimidinetrione Antibiotic  
1361 for the Treatment of *Neisseria gonorrhoeae*, Including Multi-Drug-Resistant Isolates, *ACS Infect. Dis.* 6 (2020)  
1362 1332–1345. <https://doi.org/10.1021/acsinfecdis.0c00021>.
- 1363 [49] S. Jacobsson, D. Golparian, J. Oxelbark, E. Alirol, F. Franceschi, T.N. Gustafsson, D. Brown, A. Louie, G.  
1364 Drusano, M. Unemo, Pharmacodynamic Evaluation of Dosing, Bacterial Kill, and Resistance Suppression for  
1365 Zoliflodacin Against *Neisseria gonorrhoeae* in a Dynamic Hollow Fiber Infection Model, *Front. Pharmacol.* 12  
1366 (2021) 682135. <https://doi.org/10.3389/fphar.2021.682135>.
- 1367 [50] M.D. Huband, P.A. Bradford, L.G. Otterson, G.S. Basarab, A.C. Kutschke, R.A. Giacobbe, S.A. Patey, R.A. Alm,  
1368 M.R. Johnstone, M.E. Potter, P.F. Miller, J.P. Mueller, *In Vitro* Antibacterial Activity of AZD0914, a New  
1369 Spiropyrimidinetrione DNA Gyrase/Topoisomerase Inhibitor with Potent Activity against Gram-Positive,  
1370 Fastidious Gram-Negative, and Atypical Bacteria, *Antimicrob. Agents Chemother.* 59 (2015) 467–474.  
1371 <https://doi.org/10.1128/AAC.04124-14>.
- 1372 [51] S.N. Taylor, J. Marrazzo, B.E. Batteiger, E.W. Hook, A.C. Seña, J. Long, M.R. Wierzbicki, H. Kwak, S.M.  
1373 Johnson, K. Lawrence, J. Mueller, Single-Dose Zoliflodacin (ETX0914) for Treatment of Urogenital Gonorrhea,  
1374 *N. Engl. J. Med.* 379 (2018) 1835–1845. <https://doi.org/10.1056/NEJMoa1706988>.
- 1375 [52] C. Shi, Y. Zhang, T. Wang, W. Lu, S. Zhang, B. Guo, Q. Chen, C. Luo, X. Zhou, Y. Yang, Design, Synthesis, and  
1376 Biological Evaluation of Novel DNA Gyrase-Inhibiting Spiropyrimidinetriones as Potent Antibiotics for  
1377 Treatment of Infections Caused by Multidrug-Resistant Gram-Positive Bacteria, *J. Med. Chem.* 62 (2019)  
1378 2950–2973. <https://doi.org/10.1021/acs.jmedchem.8b01750>.
- 1379 [53] G.S. Basarab, P. Doig, V. Galullo, G. Kern, A. Kimzey, A. Kutschke, J.P. Newman, M. Morningstar, J. Mueller, L.  
1380 Otterson, K. Vishwanathan, F. Zhou, M. Gowravaram, Discovery of Novel DNA Gyrase Inhibiting  
1381 Spiropyrimidinetriones: Benzisoxazole Fusion with N-Linked Oxazolidinone Substituents Leading to a Clinical  
1382 Candidate (ETX0914), *J. Med. Chem.* 58 (2015) 6264–6282. <https://doi.org/10.1021/acs.jmedchem.5b00863>.
- 1383 [54] P. Govender, R. Müller, K. Singh, V. Reddy, C.J. Eyermann, S. Fienberg, S.R. Ghorpade, L. Koekemoer, A.  
1384 Myrick, D. Schnappinger, C. Engelhart, J. Meshanni, J.A.W. Byl, N. Osheroff, V. Singh, K. Chibale, G.S. Basarab,  
1385 Spiropyrimidinetrione DNA Gyrase Inhibitors with Potent and Selective Antituberculosis Activity, *J. Med.*  
1386 *Chem.* 65 (2022) 6903–6925. <https://doi.org/10.1021/acs.jmedchem.2c00266>.
- 1387 [55] A.C. Damião Gouveia, M. Unemo, J.S. Jensen, In vitro activity of zoliflodacin (ETX0914) against  
1388 macrolide-resistant, fluoroquinolone-resistant and antimicrobial-susceptible *Mycoplasma genitalium* strains, *J.*  
1389 *Antimicrob. Chemother.* 73 (2018) 1291–1294. <https://doi.org/10.1093/jac/dky022>.
- 1390 [56] W. Le, X. Su, X. Lou, X. Li, X. Gong, B. Wang, C.A. Genco, J.P. Mueller, P.A. Rice, Susceptibility Trends of  
1391 Zoliflodacin against Multidrug-Resistant *Neisseria gonorrhoeae* Clinical Isolates in Nanjing, China, 2014 to 2018,  
1392 *Antimicrob. Agents Chemother.* 65 (2021). <https://doi.org/10.1128/AAC.00863-20>.

- 1393 [57] R.A. Alm, S.D. Lahiri, A. Kutschke, L.G. Otterson, R.E. McLaughlin, J.D. Whiteaker, L.A. Lewis, X. Su, M.D.  
1394 Huband, H. Gardner, J.P. Mueller, Characterization of the Novel DNA Gyrase Inhibitor AZD0914: Low  
1395 Resistance Potential and Lack of Cross-Resistance in *Neisseria gonorrhoeae*, *Antimicrob. Agents Chemother.*  
1396 59 (2015) 1478–1486. <https://doi.org/10.1128/AAC.04456-14>.
- 1397 [58] J. O'Donnell, K. Lawrence, K. Vishwanathan, V. Hosagrahara, J.P. Mueller, Single-Dose Pharmacokinetics,  
1398 Excretion, and Metabolism of Zoliflodacin, a Novel Spiropyrimidinetrione Antibiotic, in Healthy Volunteers,  
1399 *Antimicrob. Agents Chemother.* 63 (2019). <https://doi.org/10.1128/AAC.01808-18>.
- 1400 [59] G.S. Basarab, P. Brassil, P. Doig, V. Galullo, H.B. Haimes, G. Kern, A. Kutschke, J. McNulty, V.J.A. Schuck, G.  
1401 Stone, M. Gowravaram, Novel DNA Gyrase Inhibiting Spiropyrimidinetriones with a Benzisoxazole Scaffold:  
1402 SAR and in Vivo Characterization, *J. Med. Chem.* 57 (2014) 9078–9095. <https://doi.org/10.1021/jm501174m>.
- 1403 [60] AstraZeneca, A Phase I, Randomized, Placebo-controlled, Single-center Study to Assess the Safety, Tolerability,  
1404 and Pharmacokinetics of AZD0914 After a Single Oral Administration and to Assess the Effect of Food in  
1405 Healthy Adult Volunteers (NCT01929629), [clinicaltrials.gov](https://clinicaltrials.gov), 2014.  
1406 <https://clinicaltrials.gov/ct2/show/NCT01929629> (accessed August 24, 2022).
- 1407 [61] Entasis Therapeutics, A Phase I, Open-Label Study to Assess the Absorption, Metabolism, Excretion, Safety  
1408 and Tolerability of a Single Oral Dose of Radiolabeled [14C]-AZD0914 in Healthy Male Subjects  
1409 (NCT02298920), [clinicaltrials.gov](https://clinicaltrials.gov), 2015. <https://clinicaltrials.gov/ct2/show/NCT02298920> (accessed August 24,  
1410 2022).
- 1411 [62] National Institute of Allergy and Infectious Diseases (NIAID), A Randomized, Open-label Phase 2 Study to  
1412 Evaluate the Efficacy and Safety of a Single Dose of Oral AZD0914 Compared to Intramuscular Ceftriaxone in  
1413 the Treatment of Male and Female Subjects With Uncomplicated Gonorrhoea (NCT02257918), [clinicaltrials.gov](https://clinicaltrials.gov),  
1414 2017. <https://clinicaltrials.gov/ct2/show/NCT02257918> (accessed August 24, 2022).
- 1415 [63] Global Antibiotics Research and Development Partnership, A Multi-center, Randomized, Open-label, Non  
1416 Inferiority Trial to Evaluate the Efficacy and Safety of a Single, Oral Dose of Zoliflodacin Compared to a  
1417 Combination of a Single Intramuscular Dose of Ceftriaxone and a Single Oral Dose of Azithromycin in the  
1418 Treatment of Patients With Uncomplicated Gonorrhoea (NCT03959527), [clinicaltrials.gov](https://clinicaltrials.gov), 2021.  
1419 <https://clinicaltrials.gov/ct2/show/NCT03959527> (accessed August 24, 2022).
- 1420 [64] National Institute of Allergy and Infectious Diseases (NIAID), A Phase 1, Randomized, Double-blinded,  
1421 Four-period Crossover, Thorough QT/QTc (TQT) Clinical Trial to Evaluate the Effect of Zoliflodacin on  
1422 Cardiac Repolarization in Healthy Male and Female Subjects (NCT03613649), [clinicaltrials.gov](https://clinicaltrials.gov), 2020.  
1423 <https://clinicaltrials.gov/ct2/show/NCT03613649> (accessed August 24, 2022).
- 1424 [65] J.A. Santos, M.H. Lamers, Novel Antibiotics Targeting Bacterial Replicative DNA Polymerases, *Antibiotics.* 9  
1425 (2020) 776. <https://doi.org/10.3390/antibiotics9110776>.
- 1426 [66] P.M. Tarantino, C. Zhi, J.J. Gambino, G.E. Wright, N.C. Brown, 6-Anilinouracil-based inhibitors of *Bacillus*  
1427 *subtilis* DNA polymerase III: antipolymerase and antimicrobial structure-activity relationships based on  
1428 substitution at uracil N3, *J. Med. Chem.* 42 (1999) 2035–2040. <https://doi.org/10.1021/jm980693i>.
- 1429 [67] J.S. Daly, T.J. Giehl, N.C. Brown, C. Zhi, G.E. Wright, R.T. Ellison, In vitro antimicrobial activities of novel  
1430 anilinouracils which selectively inhibit DNA polymerase III of gram-positive bacteria, *Antimicrob. Agents*  
1431 *Chemother.* 44 (2000) 2217–2221. <https://doi.org/10.1128/AAC.44.8.2217-2221.2000>.
- 1432 [68] S. Dvoskin, W.-C. Xu, N.C. Brown, I.B. Yanachkov, M. Yanachkova, G.E. Wright, A novel agent effective  
1433 against *Clostridium difficile* infection, *Antimicrob. Agents Chemother.* 56 (2012) 1624–1626.  
1434 <https://doi.org/10.1128/AAC.06097-11>.

- 1435 [69] Acurx Pharmaceuticals Inc., ACX-362E [Ibezapolstat] for Oral Treatment of Clostridioides Difficile Infection: A  
1436 Phase 2A Open-Label Segment Followed by a Phase 2B Double-Blind Vancomycin-Controlled Segment,  
1437 clinicaltrials.gov, 2022. <https://clinicaltrials.gov/ct2/show/NCT04247542> (accessed March 10, 2022).
- 1438 [70] G.E. Wright, W.-C. Xu, Selective antibacterials for clostridium difficile infections. Coates, US2010048379, n.d.  
1439 <https://patents.google.com/patent/US8796292/en> (accessed September 8, 2022).
- 1440 [71] B. Murray, C. Wolfe, A. Marra, C. Pillar, D. Shinabarger, In vitro activity of the novel antibacterial agent  
1441 ibezapolstat (ACX-362E) against Clostridioides difficile, J. Antimicrob. Chemother. 75 (2020) 2149–2155.  
1442 <https://doi.org/10.1093/jac/dkaa134>.
- 1443 [72] A. Torti, A. Lossani, L. Savi, F. Focher, G.E. Wright, N.C. Brown, W.-C. Xu, Clostridium difficile DNA  
1444 polymerase IIIc: basis for activity of antibacterial compounds, Curr. Enzyme Inhib. 7 (2011) 147–153.  
1445 <https://doi.org/10.2174/157340811798807597>.
- 1446 [73] W.-C. Xu, M.H. Silverman, X.Y. Yu, G. Wright, N. Brown, Discovery and development of DNA polymerase  
1447 IIIc inhibitors to treat Gram-positive infections, Bioorg. Med. Chem. 27 (2019) 3209–3217.  
1448 <https://doi.org/10.1016/j.bmc.2019.06.017>.
- 1449 [74] Acurx Pharmaceuticals., Advancing a promising antibacterial against Clostridioides difficile, Biopharm. Deal.  
1450 (2021). <https://www.nature.com/articles/d43747-021-00149-0> (accessed January 18, 2023).
- 1451 [75] M.M. Butler, L.W. Dudycz, N.N. Khan, G.E. Wright, N.C. Brown, Development of novel inhibitor probes of  
1452 DNA polymerase III based on dGTP analogs of the HPUra type: base, nucleoside and nucleotide derivatives of  
1453 N<sup>2</sup>-(3,4-dichlorobenzyl)guanine, Nucleic Acids Res. 18 (1990) 7381–7387.  
1454 <https://doi.org/10.1093/nar/18.24.7381>.
- 1455 [76] G.E. Wright, N.C. Brown, W.-C. Xu, Z.-Y. Long, C. Zhi, J.J. Gambino, M.H. Barnes, M.M. Butler, Active site  
1456 directed inhibitors of replication-specific bacterial DNA polymerases, Bioorg. Med. Chem. Lett. 15 (2005)  
1457 729–732. <https://doi.org/10.1016/j.bmcl.2004.11.016>.
- 1458 [77] Z. Peng, L. Ling, C.W. Stratton, C. Li, C.R. Polage, B. Wu, Y.-W. Tang, Advances in the diagnosis and treatment  
1459 of Clostridium difficile infections, Emerg. Microbes Infect. 7 (2018) 1–13.  
1460 <https://doi.org/10.1038/s41426-017-0019-4>.
- 1461 [78] M.C. Rea, A. Dobson, O. O’Sullivan, F. Crispie, F. Fouhy, P.D. Cotter, F. Shanahan, B. Kiely, C. Hill, R.P. Ross,  
1462 Effect of broad- and narrow-spectrum antimicrobials on Clostridium difficile and microbial diversity in a  
1463 model of the distal colon, Proc. Natl. Acad. Sci. 108 (2011) 4639–4644. <https://doi.org/10.1073/pnas.1001224107>.
- 1464 [79] C. Tsigrelis, Recurrent Clostridioides difficile infection: Recognition, management, prevention, Cleve. Clin. J.  
1465 Med. 87 (2020) 347–359. <https://doi.org/10.3949/ccjm.87gr.20001>.
- 1466 [80] K.W. Garey, K. Begum, C. Lancaster, A. Gonzales-Luna, D. Bui, J. Mercier, C. Seng Yue, M.P. Ducharme, M.  
1467 Hu, B. Vince, M.H. Silverman, M.J. Alam, M. Kankam, A randomized, double-blind, placebo-controlled, single  
1468 and multiple ascending dose Phase 1 study to determine the safety, pharmacokinetics and food and faecal  
1469 microbiome effects of ibezapolstat administered orally to healthy subjects, J. Antimicrob. Chemother. 75 (2020)  
1470 3635–3643. <https://doi.org/10.1093/jac/dkaa364>.
- 1471 [81] J.A. Winston, C.M. Theriot, Impact of microbial derived secondary bile acids on colonization resistance against  
1472 Clostridium difficile in the gastrointestinal tract, Anaerobe. 41 (2016) 44–50.  
1473 <https://doi.org/10.1016/j.anaerobe.2016.05.003>.
- 1474 [82] K.W. Garey, J. McPherson, A.Q. Dinh, C. Hu, J. Jo, W. Wang, C.K. Lancaster, A.J. Gonzales-Luna, C. Loveall, K.  
1475 Begum, M.J. Alam, M.H. Silverman, B. Hanson, Efficacy, Safety, Pharmacokinetics, and Microbiome Changes  
1476 of Ibezapolstat in Adults with Clostridioides difficile Infection: A Phase 2a Multicenter Clinical Trial, Clin.  
1477 Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. (2022) ciac096. <https://doi.org/10.1093/cid/ciac096>.

- 1478 [83] J.M. Ridlon, D.-J. Kang, P.B. Hylemon, Bile salt biotransformations by human intestinal bacteria, *J. Lipid Res.*  
1479 47 (2006) 241–259. <https://doi.org/10.1194/jlr.R500013-JLR200>.
- 1480 [84] J.R. Allegretti, S. Kearney, N. Li, E. Bogart, K. Bullock, G.K. Gerber, L. Bry, C.B. Clish, E. Alm, J. Korzenik,  
1481 Recurrent *Clostridium difficile* Infection Associates with Distinct Bile Acid and Microbiome Profiles, *Aliment.*  
1482 *Pharmacol. Ther.* 43 (2016) 1142. <https://doi.org/10.1111/apt.13616>.
- 1483 [85] G.S. Khan, A. Shah, Zia-ur-Rehman, D. Barker, Chemistry of DNA minor groove binding agents, *J. Photochem.*  
1484 *Photobiol. B.* 115 (2012) 105–118. <https://doi.org/10.1016/j.jphotobiol.2012.07.003>.
- 1485 [86] L. Hlaka, M.-J. Rosslee, M. Ozturk, S. Kumar, S.P. Parihar, F. Brombacher, A.I. Khalaf, K.C. Carter, F.J. Scott, C.J.  
1486 Suckling, R. Guler, Evaluation of minor groove binders (MGBs) as novel anti-mycobacterial agents and the  
1487 effect of using non-ionic surfactant vesicles as a delivery system to improve their efficacy, *J. Antimicrob.*  
1488 *Chemother.* 72 (2017) 3334–3341. <https://doi.org/10.1093/jac/dkx326>.
- 1489 [87] A.I. Khalaf, N. Anthony, D. Breen, G. Donoghue, S.P. Mackay, F.J. Scott, C.J. Suckling, Amide isosteres in  
1490 structure-activity studies of antibacterial minor groove binders, *Eur. J. Med. Chem.* 46 (2011) 5343–5355.  
1491 <https://doi.org/10.1016/j.ejmech.2011.08.035>.
- 1492 [88] C.J. Suckling, The Antibacterial Drug MGB-BP3: from discovery to clinical trial, *Chem. Biol. Interface.* 5 (2015)  
1493 166–174.
- 1494 [89] C.J. Suckling, I.S. Hunter, F.J. Scott, Multitargeted anti-infective drugs: resilience to resistance in the  
1495 antimicrobial resistance era, *Future Drug Discov.* (2022) FDD73. <https://doi.org/10.4155/fdd-2022-0001>.
- 1496 [90] N.G. Anthony, D. Breen, J. Clarke, G. Donoghue, A.J. Drummond, E.M. Ellis, C.G. Gemmell, J.-J. Helesbeux, I.S.  
1497 Hunter, A.I. Khalaf, S.P. Mackay, J.A. Parkinson, C.J. Suckling, R.D. Waigh, Antimicrobial Lexitropsins  
1498 Containing Amide, Amidine, and Alkene Linking Groups, *J. Med. Chem.* 50 (2007) 6116–6125.  
1499 <https://doi.org/10.1021/jm070831g>.
- 1500 [91] A.I. Khalaf, N. Anthony, D. Breen, G. Donoghue, S.P. Mackay, F.J. Scott, C.J. Suckling, Amide isosteres in  
1501 structure-activity studies of antibacterial minor groove binders, *Eur. J. Med. Chem.* 46 (2011) 5343–5355.  
1502 <https://doi.org/10.1016/j.ejmech.2011.08.035>.
- 1503 [92] C. Hind, M. Clifford, C. Woolley, J. Harmer, L.M.C. McGee, I. Tyson-Hirst, H.J. Tait, D.P. Brooke, S.J. Dancer,  
1504 I.S. Hunter, C.J. Suckling, R. Beveridge, J.A. Parkinson, J.M. Sutton, F.J. Scott, Insights into the Spectrum of  
1505 Activity and Mechanism of Action of MGB-BP-3, *ACS Infect. Dis.* 8 (2022) 2552–2563.  
1506 <https://doi.org/10.1021/acsinfecdis.2c00445>.
- 1507 [93] R. Kullar, M.-C.N. Tran, E.J. Goldstein, Investigational Treatment Agents for Recurrent *Clostridioides difficile*  
1508 Infection (rCDI), *J. Exp. Pharmacol.* Volume 12 (2020) 371–384. <https://doi.org/10.2147/JEP.S242959>.
- 1509 [94] MGB Biopharma Limited, A Single-centre, Double-blind, Placebo-controlled, Study in Healthy Men to Assess  
1510 the Safety and Tolerability of Single and Repeated Ascending Doses of MGB-BP-3, [clinicaltrials.gov](https://clinicaltrials.gov), 2016.  
1511 <https://clinicaltrials.gov/ct2/show/NCT02518607> (accessed June 21, 2022).
- 1512 [95] MGB Biopharma Limited, An Exploratory, Open Labelled, Phase IIa Study to Assess Safety, Tolerability and  
1513 Efficacy of Incremental Doses of MGB-BP-3 in Patients With *Clostridium Difficile*-associated Diarrhea (CDAD),  
1514 [clinicaltrials.gov](https://clinicaltrials.gov), 2020. <https://clinicaltrials.gov/ct2/show/NCT03824795> (accessed June 21, 2022).
- 1515 [96] I. Avcilar-Kucukgoze, A. Kashina, Hijacking tRNAs From Translation: Regulatory Functions of tRNAs in  
1516 Mammalian Cell Physiology, *Front. Mol. Biosci.* 7 (2020) 610617. <https://doi.org/10.3389/fmolb.2020.610617>.
- 1517 [97] D.M. Citron, Y.A. Warren, K.L. Tyrrell, V. Merriam, E.J.C. Goldstein, Comparative in vitro activity of REP3123  
1518 against *Clostridium difficile* and other anaerobic intestinal bacteria, *J. Antimicrob. Chemother.* 63 (2009)  
1519 972–976. <https://doi.org/10.1093/jac/dkp037>.
- 1520 [98] S.U. Nayak, J.M. Griffiss, J. Blumer, M.A. O’Riordan, W. Gray, R. McKenzie, R.A. Jurao, A.T. An, M. Le, S.J.  
1521 Bell, U.A. Ochsner, T.C. Jarvis, N. Janjic, J.M. Zenilman, Safety, Tolerability, Systemic Exposure, and

- 1522 Metabolism of CRS3123, a Methionyl-tRNA Synthetase Inhibitor Developed for Treatment of *Clostridium*  
1523 *difficile*, in a Phase 1 Study, *Antimicrob. Agents Chemother.* 61 (2017) e02760-16.  
1524 <https://doi.org/10.1128/AAC.02760-16>.
- 1525 [99] B.K. Lomeli, H. Galbraith, J. Schettler, G.A. Saviolakis, W. El-Amin, B. Osborn, J. Ravel, K. Hazleton, C.A.  
1526 Lozupone, R.J. Evans, S.J. Bell, U.A. Ochsner, T.C. Jarvis, S. Baqar, N. Janjic, Multiple-Ascending-Dose Phase 1  
1527 Clinical Study of the Safety, Tolerability, and Pharmacokinetics of CRS3123, a Narrow-Spectrum Agent with  
1528 Minimal Disruption of Normal Gut Microbiota, *Antimicrob. Agents Chemother.* 64 (2019) e01395-19.  
1529 <https://doi.org/10.1128/AAC.01395-19>.
- 1530 [100] I.A. Critchley, L.S. Green, C.L. Young, J.M. Bullard, R.J. Evans, M. Price, T.C. Jarvis, J.W. Guiles, N. Janjic, U.A.  
1531 Ochsner, Spectrum of activity and mode of action of REP3123, a new antibiotic to treat *Clostridium difficile*  
1532 infections, *J. Antimicrob. Chemother.* 63 (2009) 954–963. <https://doi.org/10.1093/jac/dkp041>.
- 1533 [101] J. Guiles, X. Sun, N. Janjic, S. Strong, A. Gyorkos, Enantiomeric Compounds with Antibacterial Activity., n.d.  
1534 <https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2008039640> (accessed March 15, 2022).
- 1535 [102] O. Faghhi, Z. Zhang, R.M. Ranade, J.R. Gillespie, S.A. Creason, W. Huang, S. Shibata, X. Barros-Álvarez,  
1536 C.L.M.J. Verlinde, W.G.J. Hol, E. Fan, F.S. Buckner, Development of Methionyl-tRNA Synthetase Inhibitors as  
1537 Antibiotics for Gram-Positive Bacterial Infections, *Antimicrob. Agents Chemother.* 61 (n.d.) e00999-17.  
1538 <https://doi.org/10.1128/AAC.00999-17>.
- 1539 [103] E. Rinninella, P. Raoul, M. Cintoni, F. Franceschi, G.A.D. Miggiano, A. Gasbarrini, M.C. Mele, What is the  
1540 Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases,  
1541 *Microorganisms*. 7 (2019). <https://doi.org/10.3390/microorganisms7010014>.
- 1542 [104] U.A. Ochsner, S.J. Bell, A.L. O’Leary, T. Hoang, K.C. Stone, C.L. Young, I.A. Critchley, N. Janjic, Inhibitory  
1543 effect of REP3123 on toxin and spore formation in *Clostridium difficile*, and in vivo efficacy in a hamster  
1544 gastrointestinal infection model, *J. Antimicrob. Chemother.* 63 (2009) 964–971.  
1545 <https://doi.org/10.1093/jac/dkp042>.
- 1546 [105] D.A. Leffler, J.T. Lamont, *Clostridium difficile* Infection, *N. Engl. J. Med.* 372 (2015) 1539–1548.  
1547 <https://doi.org/10.1056/NEJMra1403772>.
- 1548 [106] T.J. Louie, K. Cannon, B. Byrne, J. Emery, L. Ward, M. Eyben, W. Krulicki, Fidaxomicin preserves the intestinal  
1549 microbiome during and after treatment of *Clostridium difficile* infection (CDI) and reduces both toxin  
1550 reexpression and recurrence of CDI, *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* 55 Suppl 2 (2012) S132-142.  
1551 <https://doi.org/10.1093/cid/cis338>.
- 1552 [107] Crestone, Inc, A Phase 2, Randomized, Double-Blind, Comparator-Controlled, Multicenter Study to Evaluate  
1553 the Safety and Efficacy of CRS3123 Compared With Oral Vancomycin in Adults With *Clostridioides Difficile*  
1554 Infection, *clinicaltrials.gov*, 2022. <https://clinicaltrials.gov/ct2/show/NCT04781387> (accessed March 14, 2022).
- 1555 [108] V. Falcó, J. Burgos, B. Almirante, An overview of lefamulin for the treatment of community acquired bacterial  
1556 pneumonia, *Expert Opin. Pharmacother.* 21 (2020) 629–636. <https://doi.org/10.1080/14656566.2020.1714592>.
- 1557 [109] N.J. Mercurio, M.P. Veve, Clinical Utility of Lefamulin: If Not Now, When?, *Curr. Infect. Dis. Rep.* 22 (2020) 25.  
1558 <https://doi.org/10.1007/s11908-020-00732-z>.
- 1559 [110] S. Wu, Y. Zheng, Y. Guo, D. Yin, D. Zhu, F. Hu, In vitro Activity of Lefamulin Against the Common  
1560 Respiratory Pathogens Isolated From Mainland China During 2017–2019, *Front. Microbiol.* 11 (2020) 578824.  
1561 <https://doi.org/10.3389/fmicb.2020.578824>.
- 1562 [111] J.R. Covvey, A.J. Guarascio, Clinical use of lefamulin: A first-in-class semisynthetic pleuromutilin antibiotic, *J.*  
1563 *Intern. Med.* (2021) joim.13378. <https://doi.org/10.1111/joim.13378>.

- 1564 [112] W.W. Wicha, T.C. Marbury, J.A. Dowell, J.L. Crandon, C. Leister, J. Ermer, S.P. Gelone, Pharmacokinetics and  
1565 safety of lefamulin after single intravenous dose administration in subjects with impaired-hepatic function,  
1566 *Pharmacother. J. Hum. Pharmacol. Drug Ther.* 41 (2021) 457–462. <https://doi.org/10.1002/phar.2522>.
- 1567 [113] W. Perry, Y. Golan, Therapeutic potential of lefamulin in the treatment of community-acquired pneumonia,  
1568 *Future Microbiol.* 14 (2019) 927–939. <https://doi.org/10.2217/fmb-2019-0027>.
- 1569 [114] R. Mang, W. Heilmayer, R. Badegruber, D. Strickmann, R. Novak, M. Ferencic, A.R.C.M. Bulusu, Pleuromutilin  
1570 derivatives for the treatment of diseases mediated by microbes., WO2008113089, n.d.  
1571 <https://patents.google.com/patent/WO2008113089A1/en?q=Preparation+of+pleuromutilin+derivatives+for+the>  
1572 [+treatment+of+diseases+mediated+by+microbes](https://patents.google.com/patent/WO2008113089A1/en?q=Preparation+of+pleuromutilin+derivatives+for+the+treatment+of+diseases+mediated+by+microbes) (accessed September 8, 2022).
- 1573 [115] R. Nagarajan, Pleuromutilin glycoside derivatives., US4130709A, n.d.  
1574 <https://patents.google.com/patent/US4130709A/en?q=US4%2c130%2c709> (accessed September 8, 2022).
- 1575 [116] E. Gómez-Sánchez, J. Marco-Contelles, Synthesis and transformations of alkyl N-(1-cyclohex-3-enyl)carbamates  
1576 prepared from cyclohex-3-ene carboxylic acid via Curtius rearrangement, *Tetrahedron.* 61 (2005) 1207–1219.  
1577 <https://doi.org/10.1016/j.tet.2004.11.037>.
- 1578 [117] K.A. Rodvold, Introduction: lefamulin and pharmacokinetic/pharmacodynamic rationale to support the dose  
1579 selection of lefamulin, *J. Antimicrob. Chemother.* 74 (2019) iii2–iii4. <https://doi.org/10.1093/jac/dkz084>.
- 1580 [118] N. Eraikhuemen, D. Julien, A. Kelly, T. Lindsay, D. Lazaridis, Treatment of Community-Acquired Pneumonia:  
1581 A Focus on Lefamulin, *Infect. Dis. Ther.* 10 (2021) 149–163. <https://doi.org/10.1007/s40121-020-00378-3>.
- 1582 [119] S. Adhikary, M.K. Duggal, S. Nagendran, M. Chintamaneni, H.S. Tuli, G. Kaur, Lefamulin: a New Hope in the  
1583 Field of Community-Acquired Bacterial Pneumonia, *Curr. Pharmacol. Rep.* 8 (2022) 418–426.  
1584 <https://doi.org/10.1007/s40495-022-00297-6>.
- 1585 [120] Y.-G. Li, J.-X. Wang, G.-N. Zhang, M. Zhu, X.-F. You, Y.-C. Wang, F. Zhang, Design, synthesis, and biological  
1586 activity evaluation of a series of pleuromutilin derivatives with novel C14 side chains, *Bioorg. Med. Chem. Lett.*  
1587 30 (2020) 126969. <https://doi.org/10.1016/j.bmcl.2020.126969>.
- 1588 [121] Z. Eyal, D. Matzov, M. Krupkin, S. Paukner, R. Riedl, H. Rozenberg, E. Zimmerman, A. Bashan, A. Yonath, A  
1589 novel pleuromutilin antibacterial compound, its binding mode and selectivity mechanism, *Sci. Rep.* 6 (2016)  
1590 39004. <https://doi.org/10.1038/srep39004>.
- 1591 [122] C. Dillon, A.J. Guarascio, J.R. Covvey, Lefamulin: a promising new pleuromutilin antibiotic in the pipeline,  
1592 *Expert Rev. Anti Infect. Ther.* 17 (2019) 5–15. <https://doi.org/10.1080/14787210.2019.1554431>.
- 1593 [123] M.P. Veve, J.L. Wagner, Lefamulin: Review of a Promising Novel Pleuromutilin Antibiotic, *Pharmacother. J.*  
1594 *Hum. Pharmacol. Drug Ther.* 38 (2018) 935–946. <https://doi.org/10.1002/phar.2166>.
- 1595 [124] W.W. Wicha, W.T. Prince, C. Lell, W. Heilmayer, S.P. Gelone, Pharmacokinetics and tolerability of lefamulin  
1596 following intravenous and oral dosing, *J. Antimicrob. Chemother.* 74 (2019) iii19–iii26.  
1597 <https://doi.org/10.1093/jac/dkz087>.
- 1598 [125] E.B. Chahine, A.J. Sucher, Lefamulin: The First Systemic Pleuromutilin Antibiotic, *Ann. Pharmacother.* 54 (2020)  
1599 1203–1214. <https://doi.org/10.1177/1060028020932521>.
- 1600 [126] R.M. Taylor, J.A. Karlowsky, M.R. Baxter, H.J. Adam, A. Walkty, P. Lagacé-Wiens, G.G. Zhanel, In vitro  
1601 susceptibility of common bacterial pathogens causing respiratory tract infections in Canada to lefamulin, a new  
1602 pleuromutilin, *Off. J. Assoc. Med. Microbiol. Infect. Dis. Can.* 6 (2021) 149–162.  
1603 <https://doi.org/10.3138/jammi-2020-0043>.
- 1604 [127] S. Paukner, H.S. Sader, Z. Ivezic-Schoenfeld, R.N. Jones, Antimicrobial Activity of the Pleuromutilin Antibiotic  
1605 BC-3781 against Bacterial Pathogens Isolated in the SENTRY Antimicrobial Surveillance Program in 2010,  
1606 *Antimicrob. Agents Chemother.* 57 (2013) 4489–4495. <https://doi.org/10.1128/AAC.00358-13>.

- [128] H.S. Sader, D.J. Biedenbach, S. Paukner, Z. Ivezic-Schoenfeld, R.N. Jones, Antimicrobial Activity of the Investigational Pleuromutilin Compound BC-3781 Tested against Gram-Positive Organisms Commonly Associated with Acute Bacterial Skin and Skin Structure Infections, *Antimicrob. Agents Chemother.* 56 (2012) 1619–1623. <https://doi.org/10.1128/AAC.05789-11>.
- [129] C.V. Pollack, A. Amin, W.T. Ford, R. Finley, K.S. Kaye, H.H. Nguyen, M.J. Rybak, D. Talan, Acute Bacterial Skin and Skin Structure Infections (ABSSSI): Practice Guidelines for Management and Care Transitions in the Emergency Department and Hospital, *J. Emerg. Med.* 48 (2015) 508–519. <https://doi.org/10.1016/j.jemermed.2014.12.001>.
- [130] S. Paukner, A. Gruss, J.S. Jensen, *In Vitro* Activity of Lefamulin against Sexually Transmitted Bacterial Pathogens, *Antimicrob. Agents Chemother.* 62 (2018). <https://doi.org/10.1128/AAC.02380-17>.
- [131] W.W. Wicha, D.B. Strickmann, S. Paukner, Pharmacokinetics/pharmacodynamics of lefamulin in a neutropenic murine pneumonia model with *Staphylococcus aureus* and *Streptococcus pneumoniae*, *J. Antimicrob. Chemother.* 74 (2019) iii11–iii18. <https://doi.org/10.1093/jac/dkz086>.
- [132] W.W. Wicha, W.A. Craig, D. Andes, *In vivo* pharmacodynamics of lefamulin, the first systemic pleuromutilin for human use, in a neutropenic murine thigh infection model, *J. Antimicrob. Chemother.* 74 (2019) iii5–iii10. <https://doi.org/10.1093/jac/dkz085>.
- [133] Nabriva Therapeutics AG, A Study to Assess the Bioavailability and Pharmacokinetics of an Immediate Release Tablet Formulation of Lefamulin When Administered to Fed and Fasted Healthy Subjects in Comparison to an Intravenous Formulation and a Capsule Formulation (NCT02557789), [clinicaltrials.gov](https://clinicaltrials.gov), 2015. <https://clinicaltrials.gov/ct2/show/NCT02557789> (accessed June 26, 2022).
- [134] M. Zeitlinger, R. Schwameis, A. Burian, B. Burian, P. Matzneller, M. Müller, W.W. Wicha, D.B. Strickmann, W. Prince, Simultaneous assessment of the pharmacokinetics of a pleuromutilin, lefamulin, in plasma, soft tissues and pulmonary epithelial lining fluid, *J. Antimicrob. Chemother.* 71 (2016) 1022–1026. <https://doi.org/10.1093/jac/dkv442>.
- [135] W.T. Prince, Z. Ivezic-Schoenfeld, C. Lell, K.J. Tack, R. Novak, F. Obermayr, G.H. Talbot, Phase II Clinical Study of BC-3781, a Pleuromutilin Antibiotic, in Treatment of Patients with Acute Bacterial Skin and Skin Structure Infections, *Antimicrob. Agents Chemother.* 57 (2013) 2087–2094. <https://doi.org/10.1128/AAC.02106-12>.
- [136] Nabriva Therapeutics AG, A Phase 3, Randomized, Double-Blind, Double-Dummy Study to Compare the Efficacy and Safety of Lefamulin (BC 3781) Versus Moxifloxacin (With or Without Adjunctive Linezolid) in Adults With Community-Acquired Bacterial Pneumonia (NCT02559310), [clinicaltrials.gov](https://clinicaltrials.gov), 2019. <https://clinicaltrials.gov/ct2/show/NCT02559310> (accessed June 26, 2022).
- [137] T.M. File, L. Goldberg, A. Das, C. Sweeney, J. Saviski, S.P. Gelone, E. Seltzer, S. Paukner, W.W. Wicha, G.H. Talbot, L.B. Gasink, Efficacy and Safety of Intravenous-to-oral Lefamulin, a Pleuromutilin Antibiotic, for the Treatment of Community-acquired Bacterial Pneumonia: The Phase III Lefamulin Evaluation Against Pneumonia (LEAP 1) Trial, *Clin. Infect. Dis.* 69 (2019) 1856–1867. <https://doi.org/10.1093/cid/ciz090>.
- [138] Nabriva Therapeutics AG, A Phase 3, Randomized, Double-Blind, Double-Dummy Study to Compare the Efficacy and Safety of Oral Lefamulin (BC 3781) Versus Oral Moxifloxacin in Adults With Community-Acquired Bacterial Pneumonia (NCT02813694), [clinicaltrials.gov](https://clinicaltrials.gov), 2019. <https://clinicaltrials.gov/ct2/show/NCT02813694> (accessed June 26, 2022).
- [139] T.M. File, E. Alexander, L. Goldberg, A.F. Das, C. Sandrock, S. Paukner, G.J. Moran, Lefamulin efficacy and safety in a pooled phase 3 clinical trial population with community-acquired bacterial pneumonia and common clinical comorbidities, *BMC Pulm. Med.* 21 (2021) 154. <https://doi.org/10.1186/s12890-021-01472-z>.

- 1650 [140] E. Alexander, L. Goldberg, A.F. Das, G.J. Moran, C. Sandrock, L.B. Gasink, P. Spera, C. Sweeney, S. Paukner,  
1651 W.W. Wicha, S.P. Gelone, J. Schranz, Oral Lefamulin vs Moxifloxacin for Early Clinical Response Among  
1652 Adults With Community-Acquired Bacterial Pneumonia: The LEAP 2 Randomized Clinical Trial, *JAMA*. 322  
1653 (2019) 1661. <https://doi.org/10.1001/jama.2019.15468>.
- 1654 [141] XENLETA® (lefamulin) Safety and Tolerability, XENLETA™ Lefamulin Website Healthc. Prof. (n.d.).  
1655 <https://www.xenleta.com/safety-tolerability> (accessed June 28, 2022).
- 1656 [142] H. Lu, P.J. Tonge, Inhibitors of FabI, an Enzyme Drug Target in the Bacterial Fatty Acid Biosynthesis Pathway,  
1657 *Acc. Chem. Res.* 41 (2008) 11–20. <https://doi.org/10.1021/ar700156e>.
- 1658 [143] E.N. Parker, B.N. Cain, B. Hajian, R.J. Ulrich, E.J. Geddes, S. Barkho, H.Y. Lee, J.D. Williams, M. Raynor, D.  
1659 Caridha, A. Zaino, M. Shekhar, K.A. Muñoz, K.M. Rzasas, E.R. Temple, D. Hunt, X. Jin, C. Vuong, K. Pannone,  
1660 A.M. Kelly, M.P. Mulligan, K.K. Lee, G.W. Lau, D.T. Hung, P.J. Hergenrother, An Iterative Approach Guides  
1661 Discovery of the FabI Inhibitor Fabimycin, a Late-Stage Antibiotic Candidate with *In Vivo* Efficacy against  
1662 Drug-Resistant Gram-Negative Infections, *ACS Cent. Sci.* 8 (2022) 1145–1158.  
1663 <https://doi.org/10.1021/acscentsci.2c00598>.
- 1664 [144] D.J. Payne, W.H. Miller, V. Berry, J. Brosky, W.J. Burgess, E. Chen, W.E. DeWolf, A.P. Fosberry, R. Greenwood,  
1665 M.S. Head, D.A. Heerding, C.A. Janson, D.D. Jaworski, P.M. Keller, P.J. Manley, T.D. Moore, K.A. Newlander,  
1666 S. Pearson, B.J. Polizzi, X. Qiu, S.F. Rittenhouse, C. Slater-Radosti, K.L. Salyers, M.A. Seefeld, M.G. Smyth, D.T.  
1667 Takata, I.N. Uzinskas, K. Vaidya, N.G. Wallis, S.B. Winram, C.C.K. Yuan, W.F. Huffman, Discovery of a Novel  
1668 and Potent Class of FabI-Directed Antibacterial Agents, *Antimicrob. Agents Chemother.* 46 (2002) 3118–3124.  
1669 <https://doi.org/10.1128/AAC.46.10.3118-3124.2002>.
- 1670 [145] H. Pauls, J. Ramnauth, Salts, Prodrugs and Polymorphs of Fab I Inhibitors. WO2008098374, 2008.,  
1671 WO2008098374A1, n.d. <https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2008098374> (accessed June  
1672 28, 2022).
- 1673 [146] J. Partridge, J. Colucci, Y. Gareau, M. Therien, R. Zamboni, B. Hafkin, A. Marfat, H. Zaghdane, Prodrug  
1674 Derivatives of  
1675 (e)-N-Methyl-N-((3-Methylbenzofuran-2-Yl)methyl)-3-(7-Oxo-5,6,7,8-Tetrahydro-1,8-Naphthyridin-3-Yl)acryla  
1676 mide. WO2013190384, 2013., WO2013190384A1, n.d.  
1677 <https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2013190384> (accessed September 20, 2022).
- 1678 [147] N. Kaplan, M. Albert, D. Awrey, E. Bardouniotis, J. Berman, T. Clarke, M. Dorsey, B. Hafkin, J. Ramnauth, V.  
1679 Romanov, M.B. Schmid, R. Thalakada, J. Yethon, H.W. Pauls, Mode of Action, *In Vitro* Activity, and *In Vivo*  
1680 Efficacy of AFN-1252, a Selective Antistaphylococcal FabI Inhibitor, *Antimicrob. Agents Chemother.* 56 (2012)  
1681 5865–5874. <https://doi.org/10.1128/AAC.01411-12>.
- 1682 [148] W.H. Miller, M.A. Seefeld, K.A. Newlander, I.N. Uzinskas, W.J. Burgess, D.A. Heerding, C.C.K. Yuan, M.S.  
1683 Head, D.J. Payne, S.F. Rittenhouse, T.D. Moore, S.C. Pearson, V. Berry, W.E. DeWolf, P.M. Keller, B.J. Polizzi, X.  
1684 Qiu, C.A. Janson, W.F. Huffman, Discovery of Aminopyridine-Based Inhibitors of Bacterial Enoyl-ACP  
1685 Reductase (FabI), *J. Med. Chem.* 45 (2002) 3246–3256. <https://doi.org/10.1021/jm020050+>.
- 1686 [149] N. Kaplan, D. Awrey, E. Bardouniotis, J. Berman, J. Yethon, H.W. Pauls, B. Hafkin, *In vitro* activity (MICs and  
1687 rate of kill) of AFN-1252, a novel FabI inhibitor, in the presence of serum and in combination with other  
1688 antibiotics, *J. Chemother.* 25 (2013) 18–25. <https://doi.org/10.1179/1973947812Y.0000000063>.
- 1689 [150] B. Hafkin, N. Kaplan, T.L. Hunt, Safety, tolerability and pharmacokinetics of AFN-1252 administered as  
1690 immediate release tablets in healthy subjects, *Future Microbiol.* 10 (2015) 1805–1813.  
1691 <https://doi.org/10.2217/fmb.15.101>.



- 1692 [151] Affinium Pharmaceuticals, Ltd, A Phase 2, Open-Label, Multi-Center Study of Safety, Tolerability, and Efficacy  
1693 of AFN-12520000 in the Treatment of Acute Bacterial Skin and Skin Structure Infections (ABSSSI) Due to  
1694 Staphylococci, *clinicaltrials.gov*, 2012. <https://clinicaltrials.gov/ct2/show/NCT01519492> (accessed June 27, 2022).
- 1695 [152] B. Hafkin, N. Kaplan, B. Murphy, Efficacy and Safety of AFN-1252, the First Staphylococcus-Specific  
1696 Antibacterial Agent, in the Treatment of Acute Bacterial Skin and Skin Structure Infections, Including Those in  
1697 Patients with Significant Comorbidities, *Antimicrob. Agents Chemother.* 60 (2015) 1695–1701.  
1698 <https://doi.org/10.1128/AAC.01741-15>.
- 1699 [153] F. Zheng, S. Wang, W. Hou, Y. Xiao, P. Liu, X. Shi, M. Shen, Comparative study of resazurin reduction and  
1700 MTT assays for cytocompatibility evaluation of nanofibrous materials, *Anal. Methods.* 11 (2019) 483–489.  
1701 <https://doi.org/10.1039/C8AY02310G>.
- 1702 [154] Debiopharm International SA, A Phase 2, Randomized, Double-Blind, Multicenter Study of Safety, Tolerability,  
1703 and Efficacy of Debio 1450 vs Vancomycin (IV)/Linezolid (Oral) in the Treatment of Acute Bacterial Skin and  
1704 Skin Structure Infections (ABSSSI) Due to Staphylococcus Sensitive or Resistant to Methicillin, *clinicaltrials.gov*,  
1705 2019. <https://clinicaltrials.gov/ct2/show/NCT02426918> (accessed August 23, 2022).
- 1706 [155] Debiopharm International SA, A Phase 1, Open-Label Pharmacokinetic Study to Investigate Drug Penetration  
1707 in the Bone After Repeated Oral Administration of Debio 1450 to Patients Undergoing Hip Replacement  
1708 Surgery, *clinicaltrials.gov*, 2017. <https://clinicaltrials.gov/ct2/show/NCT02726438> (accessed August 23, 2022).
- 1709 [156] Debiopharm International SA, Randomized Open-label Active-controlled Study to Assess the Safety,  
1710 Tolerability, and Efficacy of Afabicin IV/Oral in the Treatment of Patients With Bone or Joint Infection Due to  
1711 Staphylococcus, *clinicaltrials.gov*, 2022. <https://clinicaltrials.gov/ct2/show/NCT03723551> (accessed August 23,  
1712 2022).
- 1713 [157] D.J. Haydon, N.R. Stokes, R. Ure, G. Galbraith, J.M. Bennett, D.R. Brown, P.J. Baker, V.V. Barynin, D.W. Rice,  
1714 S.E. Sedelnikova, J.R. Heal, J.M. Sheridan, S.T. Aiwale, P.K. Chauhan, A. Srivastava, A. Taneja, I. Collins, J.  
1715 Errington, L.G. Czaplewski, An Inhibitor of FtsZ with Potent and Selective Anti-Staphylococcal Activity,  
1716 *Science.* 321 (2008) 1673–1675. <https://doi.org/10.1126/science.1159961>.
- 1717 [158] Y. Ohashi, Y. Chijiwa, K. Suzuki, K. Takahashi, H. Nanamiya, T. Sato, Y. Hosoya, K. Ochi, F. Kawamura, The  
1718 lethal effect of a benzamide derivative, 3-methoxybenzamide, can be suppressed by mutations within a cell  
1719 division gene, *ftsZ*, in *Bacillus subtilis*, *J. Bacteriol.* 181 (1999) 1348–1351.  
1720 <https://doi.org/10.1128/JB.181.4.1348-1351.1999>.
- 1721 [159] S. Ghone, F.-A. Kang, N. Jain, A. Parhi, R. Ponnaiah, A.K. Soni, S.R.R. Athunuri, T. Korrakuti, P. Seelam,  
1722 *Procédés De Synthèse Et Intermédiaires De Synthèse*, 2018.  
1723 <https://patentscope.wipo.int/search/fr/detail.jsf?docId=WO2018183917> (accessed April 22, 2022).
- 1724 [160] J. Fujita, Y. Maeda, E. Mizohata, T. Inoue, M. Kaul, A.K. Parhi, E.J. LaVoie, D.S. Pilch, H. Matsumura,  
1725 Structural Flexibility of an Inhibitor Overcomes Drug Resistance Mutations in *Staphylococcus aureus* FtsZ, *ACS*  
1726 *Chem. Biol.* 12 (2017) 1947–1955. <https://doi.org/10.1021/acscchembio.7b00323>.
- 1727 [161] M. Kaul, L. Mark, Y. Zhang, A.K. Parhi, Y.L. Lyu, J. Pawlak, S. Saravolatz, L.D. Saravolatz, M.P. Weinstein, E.J.  
1728 LaVoie, D.S. Pilch, TXA709, an FtsZ-Targeting Benzamide Prodrug with Improved Pharmacokinetics and  
1729 Enhanced *In Vivo* Efficacy against Methicillin-Resistant *Staphylococcus aureus*, *Antimicrob. Agents Chemother.*  
1730 59 (2015) 4845–4855. <https://doi.org/10.1128/AAC.00708-15>.
- 1731 [162] L.G. Czaplewski, I. Collins, E.A. Boyd, D. Brown, S.P. East, M. Gardiner, R. Fletcher, D.J. Haydon, V. Henstock,  
1732 P. Ingram, C. Jones, C. Noula, L. Kennison, C. Rockley, V. Rose, H.B. Thomaidis-Brears, R. Ure, M. Whittaker,  
1733 N.R. Stokes, Antibacterial alkoxybenzamide inhibitors of the essential bacterial cell division protein FtsZ,  
1734 *Bioorg. Med. Chem. Lett.* 19 (2009) 524–527. <https://doi.org/10.1016/j.bmcl.2008.11.021>.

- [163] D.J. Haydon, J.M. Bennett, D. Brown, I. Collins, G. Galbraith, P. Lancett, R. Macdonald, N.R. Stokes, P.K. Chauhan, J.K. Sutariya, N. Nayal, A. Srivastava, J. Beanland, R. Hall, V. Henstock, C. Noula, C. Rockley, L. Czaplewski, Creating an Antibacterial with in Vivo Efficacy: Synthesis and Characterization of Potent Inhibitors of the Bacterial Cell Division Protein FtsZ with Improved Pharmaceutical Properties, *J. Med. Chem.* 53 (2010) 3927–3936. <https://doi.org/10.1021/jm9016366>.
- [164] M. Kaul, L. Mark, Y. Zhang, A.K. Parhi, E.J. LaVoie, D.S. Pilch, Pharmacokinetics and in vivo antistaphylococcal efficacy of TXY541, a 1-methylpiperidine-4-carboxamide prodrug of PC190723, *Biochem. Pharmacol.* 86 (2013) 1699–1707. <https://doi.org/10.1016/j.bcp.2013.10.010>.
- [165] A.J. Lepak, A. Parhi, M. Madison, K. Marchillo, J. VanHecker, D.R. Andes, *In Vivo* Pharmacodynamic Evaluation of an FtsZ Inhibitor, TXA-709, and Its Active Metabolite, TXA-707, in a Murine Neutropenic Thigh Infection Model, *Antimicrob. Agents Chemother.* 59 (2015) 6568–6574. <https://doi.org/10.1128/AAC.01464-15>.
- [166] Our Pipeline – TAXIS Pharmaceutical, (n.d.). <https://www.taxispharma.com/research-development/our-pipeline/> (accessed October 27, 2022).
- [167] M.D. Sacco, K. Defrees, X. Zhang, W. Lawless, E. Nwanochie, A. Balsizer, S.E. Darch, A.R. Renslo, Y. Chen, Structure-Based Ligand Design Targeting *Pseudomonas aeruginosa* LpxA in Lipid A Biosynthesis, *ACS Infect. Dis.* 8 (2022) 1231–1240. <https://doi.org/10.1021/acsinfecdis.1c00650>.
- [168] S. Sartini, E. Levati, M. Maccesi, M. Guerra, G. Spadoni, S. Bach, M. Benincasa, M. Scocchi, S. Ottonello, S. Rivara, B. Montanini, New Antimicrobials Targeting Bacterial RNA Polymerase Holoenzyme Assembly Identified with an *in Vivo* BRET-Based Discovery Platform, *ACS Chem. Biol.* 14 (2019) 1727–1736. <https://doi.org/10.1021/acscchembio.9b00178>.
- [169] S.H. Kirsch, F.P.J. Haeckl, R. Müller, Beyond the approved: target sites and inhibitors of bacterial RNA polymerase from bacteria and fungi, *Nat. Prod. Rep.* 39 (2022) 1226–1263. <https://doi.org/10.1039/D1NP00067E>.
- [170] A.E. Clatworthy, E. Pierson, D.T. Hung, Targeting virulence: a new paradigm for antimicrobial therapy, *Nat. Chem. Biol.* 3 (2007) 541–548. <https://doi.org/10.1038/nchembio.2007.24>.
- [171] N.A. Zigangirova, L.N. Nesterenko, A.B. Sheremet, A.V. Soloveva, S.I. Luyksaar, E.S. Zayakin, D.V. Balunets, A.L. Gintsburg, Fluorothiazinon, a small-molecular inhibitor of T3SS, suppresses salmonella oral infection in mice, *J. Antibiot. (Tokyo)*. 74 (2021) 244–254. <https://doi.org/10.1038/s41429-020-00396-w>.
- [172] L.N. Nesterenko, N.A. Zigangirova, E.S. Zayakin, S.I. Luyksaar, N.V. Kobets, D.V. Balunets, L.A. Shabalina, T.N. Bolshakova, O.Y. Dobrynina, A.L. Gintsburg, A small-molecule compound belonging to a class of 2,4-disubstituted 1,3,4-thiadiazine-5-ones suppresses Salmonella infection in vivo, *J. Antibiot. (Tokyo)*. 69 (2016) 422–427. <https://doi.org/10.1038/ja.2015.131>.
- [173] A.B. Sheremet, N.A. Zigangirova, E.S. Zayakin, S.I. Luyksaar, L.N. Kapotina, L.N. Nesterenko, N.V. Kobets, A.L. Gintsburg, Small Molecule Inhibitor of Type Three Secretion System Belonging to a Class 2,4-disubstituted-4H-[1,3,4]-thiadiazine-5-ones Improves Survival and Decreases Bacterial Loads in an Airway *Pseudomonas aeruginosa* Infection in Mice, *BioMed Res. Int.* 2018 (2018) 5810767. <https://doi.org/10.1155/2018/5810767>.
- [174] N.E. Bondareva, A.V. Soloveva, A.B. Sheremet, E.A. Koroleva, L.N. Kapotina, E.Y. Morgunova, S.I. Luyksaar, E.S. Zayakin, N.A. Zigangirova, Preventative treatment with Fluorothiazinon suppressed Acinetobacter baumannii-associated septicemia in mice, *J. Antibiot. (Tokyo)*. 75 (2022) 155–163. <https://doi.org/10.1038/s41429-022-00504-y>.
- [175] Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation, An Open Study to Assess the Safety and Pharmacokinetics of Fluorothiazinone Used as a Single-Dose Administration or a Treatment Course in Healthy Volunteers, *clinicaltrials.gov*, 2018. <https://clinicaltrials.gov/ct2/show/NCT03205462> (accessed June 15, 2022).

- 1779 [176] Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation,  
1780 Randomized, Blind, Placebo-controlled Study to Evaluate Safety and Efficacy of Ftortiazinon in Combination  
1781 With Maxipime®, in Comparison With Placebo in Combination With Maxipime®, in the Treatment of Patients  
1782 With Complicated Urinary Tract Infections Caused by *P. Aeruginosa*, *clinicaltrials.gov*, 2021.  
1783 <https://clinicaltrials.gov/ct2/show/NCT03638830> (accessed June 15, 2022).
- 1784 [177] N.M. Seleem, H.K. Abd El Latif, M.A. Shaldam, A. El-Ganiny, Drugs with new lease of life as quorum sensing  
1785 inhibitors: for combating MDR *Acinetobacter baumannii* infections, *Eur. J. Clin. Microbiol. Infect. Dis.* 39 (2020)  
1786 1687–1702. <https://doi.org/10.1007/s10096-020-03882-z>.
- 1787 [178] S. Zhong, S. He, Quorum Sensing Inhibition or Quenching in *Acinetobacter baumannii*: The Novel Therapeutic  
1788 Strategies for New Drug Development, *Front. Microbiol.* 12 (2021) 558003.  
1789 <https://doi.org/10.3389/fmicb.2021.558003>.
- 1790 [179] X.-Z. Li, P. Plésiat, H. Nikaido, The Challenge of Efflux-Mediated Antibiotic Resistance in Gram-Negative  
1791 Bacteria, *Clin. Microbiol. Rev.* 28 (2015) 337–418. <https://doi.org/10.1128/CMR.00117-14>.
- 1792 [180] N. Compagne, A. Vieira Da Cruz, R.T. Müller, R.C. Hartkoorn, M. Flipo, K.M. Pos, Update on the Discovery of  
1793 Efflux Pump Inhibitors against Critical Priority Gram-Negative Bacteria, *Antibiotics.* 12 (2023) 180.  
1794 <https://doi.org/10.3390/antibiotics12010180>.
- 1795 [181] P. Blanco, F. Sanz-García, S. Hernando-Amado, J.L. Martínez, M. Alcalde-Rico, The development of efflux  
1796 pump inhibitors to treat Gram-negative infections, *Expert Opin. Drug Discov.* 13 (2018) 919–931.  
1797 <https://doi.org/10.1080/17460441.2018.1514386>.  
1798