Journal of Antimicrobial Chemotherapy 50 years

J Antimicrob Chemother
https://doi.org/10.1093/jac/dkaf460

In vitro activity of gepotidacin against *Chlamydia trachomatis*

Arabella Touati [1]*, Olivia Peuchant [1,2], Chase A. Weikel[3] and Cécile Bébéar [1,2]

[1]*Department of Bacteriology, National Reference Centre for Bacterial Sexually Transmitted Infections, CHU Bordeaux, Bordeaux, France;* [2]*CNRS, Microbiologie Fondamentale et Pathogénicité (MFP), UMR 5334 ARMYNE, Univ. Bordeaux, Bordeaux, France;* [3]*Infectious Disease Research, GSK, Collegeville, PA, USA*

*Corresponding author. E-mail: arabella.touati@chu-bordeaux.fr

Gepotidacin is a novel, first-in-class, triazaacenaphthylene antibiotic that selectively inhibits type IIA topoisomerases through a distinct binding site and a unique mechanism of action not used by any other current therapeutic agent.[1,2] In the era of increasing antimicrobial resistance (AMR), new therapeutic options are needed. This is especially true for *Neisseria gonorrhoeae* and *Mycoplasma genitalium*, two bacteria responsible for sexually transmissible infections (STIs). The rise in AMR among these two pathogens in recent years is a significant concern. Additionally, there have been instances of incurable infections caused by *M. genitalium*.[3] Gepotidacin has proven to be active *in vitro* against *N. gonorrhoeae*, including MDR and XDR isolates, and more recently, was shown to be efficacious and well tolerated in a Phase 3 uncomplicated urogenital gonorrhoea clinical trial (EAGLE-1).[4,5] Gepotidacin is also effective *in vitro* against *M. genitalium* isolates resistant to macrolides and fluoroquinolones.[6] However, no data on clinical efficacy are currently available for *M. genitalium*.

Also of concern is *Chlamydia trachomatis* as it is the most prevalent bacterial STI globally. Moreover, various studies report the incidence of *C. trachomatis* coinfection in patients with *N. gonorrhoeae* at 18%–58%.[7] However, gepotidacin activity against *C. trachomatis* has yet to be investigated.

The aim of this study was to assess the *in vitro* activity of gepotidacin against various strains of *C. trachomatis,* compared with azithromycin, doxycycline and levofloxacin.

Eight *C. trachomatis* strains/isolates were tested: two reference strains [D/UW-3/Cx (ATCC VR-885) and L2/434/Bu (ATCC VR-902B)], five clinical isolates collected between 2016 and 2021, and one ofloxacin-resistant mutant of the L2/434/Bu reference strain (OFX-R) selected *in vitro* (Table 1).[8] The MIC was obtained by inoculation of *C. trachomatis* on McCoy cell monolayers in 12 mm coverslips in shell vials in the presence of increasing concentrations of antibiotics.[9] The following dilution scheme was used: 1–256 mg/L for gepotidacin, 0.0075–1 mg/L for doxycycline, 0.0075–1 mg/L for azithromycin, 0.03–4 mg/L for levofloxacin (1–128 mg/L for OFX-R strain). Chlamydial inclusions were detected using IMAGEN Chlamydia (OXOID) using a fluorescein-conjugated monoclonal antibody. The inoculum size of chlamydia was 10^5 inclusion-forming-units in each vial resulting in the infection of approximately 10% to 50% of the cells in the monolayers. For each strain/antibiotic series, a positive control containing antibiotic-free McCoy cells infected by *C. trachomatis* was used for visual estimation of the size and morphology of inclusions and to ensure that 10% to 50% of the cells were infected (corresponding to 5 to 10 inclusions minimum per field under the fluorescence microscope). The entire monolayer of the positive control was scanned first and served as point of comparison with the infected monolayers in contact with the antibiotic, which were scanned next in increasing order. For interpretation of the results, the transition point MIC (MIC_{TP}) was defined as the concentration of drug in which 90% or more of the inclusions were altered in size and morphology.[9] The MIC was defined as the concentration of drug that was one 2-fold dilution more concentrated than the MIC_{TP}. Results are shown in Table 1. Doxycycline MICs were ≤0.03 mg/L, and those for azithromycin and levofloxacin were ≤0.5 mg/L, except for the mutant L2/434/Bu OFX-R, which displayed a levofloxacin MIC of 64 mg/L. For gepotidacin, all MICs were off scale at >256 mg/L. Sequencing of the quinolone-resistance determining region (QRDR) of GyrA revealed no amino acid change (relative to consensus sequence for this species) in two isolates, and V61A and H129Q (*Escherichia coli* numbering) changes in three isolates.[8] Regarding the QRDR of ParC, an R97G (*E. coli* numbering) change was found in two isolates.[8] We cannot attribute gepotidacin lack of activity against *C. trachomatis* to the GyrA and ParC mutations identified in this study as none of these substitutions have been associated with a change in susceptibility to gepotidacin. In particular, none of the isolates contained either an A92T GyrA or a D86N ParC substitution, which, when present together, were associated with elevated gepotidacin MIC in *N. gonorrhoeae*.[10]

Our study demonstrates that gepotidacin is not active *in vitro* against *C. trachomatis,* suggesting that there is no potential for therapeutic use against these infections. These findings align with those from previous internal studies showing that against *Chlamydia pneumoniae*, gepotidacin had poor inhibition of DNA gyrase and lack of *in vitro* activity (unpublished data). Given the conservation of key GyrA residues between *C. pneumoniae* and *C. trachomatis*, we hypothesize that similar poor inhibition of DNA gyrase could be contributing to the lack of activity against

Table 1. MIC values for azithromycin, doxycycline, levofloxacin and gepotidacin for *C. trachomatis* and sequencing results for GyrA and ParC QRDRs

			MIC, mg/L				Sequencing results for QRDR of[b]	
Strain[a]	Year	Origin	Azithromycin	Doxycycline	Levofloxacin	Gepotidacin	GyrA	ParC
D1772	2018	Cervix	0.25	0.03	0.25	>256	WT (silent:C237T)	WT
Da1775	2018	Cervix	0.125	0.03	0.5	>256	V61A-H129Q	R97G
E1759	2018	Urethral	0.25	0.015	0.25	>256	V61A-H129Q	WT
E1991	2021	Cervix	0.125	0.03	0.5	>256	V61A-H129Q	WT
L2b-IG16	2016	Anorectal	0.125	0.015	0.5	>256	WT	WT
L2/434/Bu OFX-R	1998	NA	0.5	0.03	64	>256	S83I	R97G
L2/434/Bu (ATCC VR-902B)	NA	Bubo	0.25	0.03	0.25	>256	WT	WT
D/UW-3/Cx (ATCC VR-885)	NA	Cervix	0.125	0.03	0.5	>256	WT (silent:C237T)	WT

NA, not available.
[a]Strains are named by their genovar followed by the number of collection at the French National Reference Centre (NRC) for bacterial STIs.
[b]*E. coli* numbering.

C. trachomatis. However, additional studies would be needed to elucidate the exact mechanisms driving the lack of gepotidacin activity observed against *C. trachomatis*.

It is important to emphasize that this lack of activity does not pose a major clinical issue given that *C. trachomatis* remains susceptible to first-line treatments with no significant resistance reported to date.

Funding

GSK provided gepotidacin powder and supported this study. The funder had no influence on the interpretation of the results.

Transparency declarations

C.A.W. is an employee of, and shareholder in, GSK. All other authors: none to declare.

References

1 Bax BD, Chan PF, Eggleston DS *et al*. Type IIA topoisomerase inhibition by a new class of antibacterial agents. *Nature* 2010; **466**: 935–40. https://doi.org/10.1038/nature09197

2 Gibson EG, Bax B, Chan PF *et al*. Mechanistic and structural basis for the actions of the antibacterial gepotidacin against *Staphylococcus aureus* gyrase. *ACS Infect Dis* 2019; **5**: 570–81. https://doi.org/10.1021/acsinfecdis.8b00315

3 Jensen JS, Unemo M. Antimicrobial treatment and resistance in sexually transmitted bacterial infections. *Nat Rev Microbiol* 2024; **22**: 435–50. https://doi.org/10.1038/s41579-024-01023-3

4 Jacobsson S, Golparian D, Scangarella-Oman N *et al*. *In vitro* activity of the novel triazaacenaphthylene gepotidacin (GSK2140944) against MDR *Neisseria gonorrhoeae*. *J Antimicrobial Chemother* 2018; **73**: 2072–7. https://doi.org/10.1093/jac/dky162

5 Ross JDC, Wilson J, Workowski KA *et al*. Oral gepotidacin for the treatment of uncomplicated urogenital gonorrhoea (EAGLE-1): a phase 3 randomised, open-label, non-inferiority, multicentre study. *Lancet* 2025; **405**: 1608–20. https://doi.org/10.1016/S0140-6736(25)00628-2

6 Jensen JS, Norgaard C, Scangarella-Oman N *et al*. *In vitro* activity of the first-in-class triazaacenaphthylene gepotidacin alone and in combination with doxycycline against drug-resistant and -susceptible *Mycoplasma genitalium*. *Emerg Microbes Infect* 2020; **9**: 1388–92. https://doi.org/10.1080/22221751.2020.1775498

7 Ball LM, Bronstein E, Liechti GW *et al*. *Neisseria gonorrhoeae* drives *Chlamydia trachomatis* into a persistence-like state during in vitro co-infection. *Infect Immun* 2024; **92**: e0017923. https://doi.org/10.1128/iai.00179-23

8 Dessus-Babus S, Bebear CM, Charron A *et al*. Sequencing of gyrase and topoisomerase IV quinolone-resistance-determining regions of *Chlamydia trachomatis* and characterization of quinolone-resistant mutants obtained *in vitro*. *Antimicrob Agents Chemother* 1998; **42**: 2474–81. https://doi.org/10.1128/AAC.42.10.2474

9 Suchland RJ, Geisler WM, Stamm WE. Methodologies and cell lines used for antimicrobial susceptibility testing of *Chlamydia* spp. *Antimicrob Agents Chemother* 2003; **47**: 636–42. https://doi.org/10.1128/AAC.47.2.636-642.2003

10 Scangarella-Oman NE, Hossain M, Dixon PB *et al*. Microbiological analysis from a phase 2 randomized study in adults evaluating single oral doses of gepotidacin in the treatment of uncomplicated urogenital gonorrhea caused by *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 2018; **62**: e01221-18. https://doi.org/10.1128/AAC.01221-18