J Antimicrob Chemother 2020; **75**: 3244–3247
doi:10.1093/jac/dkaa322 Advance Access publication 26 July 2020

Journal of Antimicrobial Chemotherapy

High *in vitro* activity of DIS-73285, a novel antimicrobial with a new mechanism of action, against MDR and XDR *Neisseria gonorrhoeae*

Susanne Jacobsson[1], Clive Mason[2], Nawaz Khan[2], Paul Meo[2] and Magnus Unemo [1]*

[1]*WHO Collaborating Centre for Gonorrhoea and Other Sexually Transmitted Infections, National Reference Laboratory for Sexually Transmitted Infections, Department of Laboratory Medicine, Faculty of Medicine and Health, Örebro University, Örebro, Sweden;* [2]*Summit Therapeutics, Merrifield Centre, Rosemary Lane, Cambridge, UK*

*Corresponding author. E-mail: magnus.unemo@regionorebrolan.se

Received 9 April 2020; accepted 22 June 2020

Background: The rising incidence of antimicrobial resistance in *Neisseria gonorrhoeae* may result in untreatable gonorrhoea in certain circumstances and development of novel antimicrobials is urgently needed.

Objectives: To evaluate the *in vitro* activity of a novel small-molecule antimicrobial with a new mechanism of action, DIS-73285, against a large geographically, temporally and genetically diverse collection of clinical *N. gonorrhoeae* isolates and reference strains, including various types of high-level resistant, MDR and XDR gonococcal isolates ($n = 262$).

Methods: MICs (mg/L) of DIS-73285 were determined by agar dilution and by Etest for ceftriaxone, cefixime, azithromycin, ciprofloxacin, ampicillin, spectinomycin and tetracycline.

Results: DIS-73285 was substantially more potent than any of the currently or previously used therapeutic antimicrobials, with MICs ranging from $\leq$0.001 to 0.004 mg/L, and the MIC_{50}, MIC_{90} and modal MIC all $\leq$0.001 mg/L (lowest MIC tested). No correlation with the MICs of DIS-73285 and the MICs of any of the currently or previously used antimicrobials was observed.

Conclusions: The novel chemotype, small-molecule antimicrobial DIS-73285, demonstrated high *in vitro* potency against all tested *N. gonorrhoeae* isolates. Further *in vitro* and *in vivo* studies, evaluating efficacy, resistance emergence, pharmacokinetic/pharmacodynamic parameters, toxicity and safety, should be conducted to evaluate DIS-73285 as a therapy specifically for urogenital and extra-genital gonorrhoea.

Introduction

The rising incidence of antimicrobial resistance (AMR) in *Neisseria gonorrhoeae*, the causative agent of gonorrhoea, may result in untreatable gonococcal infections in certain circumstances. The bacterium has evolved from a pathogen highly susceptible to most antimicrobials into a superbug, displaying both MDR and XDR phenotypes.[1–3] The extended-spectrum cephalosporin (ESC) ceftriaxone is currently the last remaining effective option for empirical first-line monotherapy. Current gonorrhoea management guidelines by the WHO, in Europe and in most high-income countries worldwide recommend empirical dual treatment with ceftriaxone (250–500 mg) intramuscularly plus oral azithromycin (1–2 g).[4–8] However, some countries recommend high-dose (1 g) ceftriaxone monotherapy when *Chlamydia trachomatis* infection has been excluded.[9–11] Worryingly, failure in treating pharyngeal gonorrhoea with dual therapy was reported in 2016,[12] a ceftriaxone-resistant gonococcal strain has been spreading globally since 2015,[13–19] and the first strain with resistance to ceftriaxone and high-level azithromycin resistance was isolated in England and Australia in 2018.[20–22]

As a response to this AMR development, the WHO has stressed the crucial need for new treatments for gonorrhoea, and *N. gonorrhoeae* was recognized as a priority pathogen for research and development of new antimicrobial agents in 2017.[23] Since then, novel antibacterial compounds that selectively target *N. gonorrhoeae* have been discovered; one of these is DIS-73285, a novel highly potent and selective novel small-molecule antimicrobial belonging to the DDS-03 series.[24–26] The discovery and development of the DDS-03 series has been enabled by the Summit Therapeutics' Discuva Platform. This platform uses proprietary high-density transposon libraries that identify an antibacterial compound's mechanism of action and routes to resistance.[24,25] The target and mechanism of action of DIS-73285 involve the

electron transfer proteins (ETFs) A/B/D in *N. gonorrhoeae*.[25,26] DIS-73285 has shown low MICs in single *N. gonorrhoeae* and *Neisseria meningitidis* reference strains, but very high MICs in single reference strains of 28 other bacterial species.[25,26] *In vitro* absorption, distribution, metabolism and excretion (ADME) and toxicological assays have also been performed using standard protocols, showing a clean ADME and toxicology profile.[26]

The aim of the present study was to comprehensively investigate the *in vitro* activity of the new therapeutic compound DIS-73285 against a large collection of clinical *N. gonorrhoeae* isolates ($n = 228$) and international reference strains ($n = 34$), including numerous MDR and XDR gonococcal isolates.

Materials and methods

Neisseria gonorrhoeae isolates

A geographically (from 23 countries mainly globally), temporally, phenotypically and genetically diverse selection of 262 *N. gonorrhoeae* isolates were investigated. The collection consisted of 34 international gonococcal reference strains originally isolated from 1991 to 2013, 100 consecutive clinical Swedish gonococcal isolates cultured in 2016 (from 15 July 2016 to 26 September 2016) and 128 gonococcal clinical isolates selected for their AMR phenotype (obtained from 2000 to 2018). The international gonococcal reference strains included the 2016 WHO reference strains ($n = 14$),[27,28] WHO A-E, WHO I, WHO J, CCUG 41810-41813, A02, A17, A25, G07-700, A04, G07-672, G06-1153, FA1090 and MS11. The selected AMR isolates included isolates with *in vitro* or clinical resistance to ESCs (15 ceftriaxone-resistant isolates and 31 cefixime-resistant isolates), high-level clinical resistance to all other antimicrobials previously used for treatment of gonorrhoea and a large number of MDR ($n = 57$) and XDR ($n = 14$) gonococcal isolates. MDR and XDR *N. gonorrhoeae* isolates were defined as previously described.[29]

Antimicrobial susceptibility testing

The MICs (mg/L) of DIS-73285 (Summit Therapeutics, Cambridge, UK), dissolved and diluted in DMSO (<1% in all agar plates used for testing), were determined by the agar dilution technique, according to current CLSI guidelines.[30] The MICs (mg/L) of ceftriaxone, cefixime, azithromycin, ciprofloxacin, spectinomycin, tetracycline and ampicillin were determined using the Etest method (AB bioMérieux, Marcy l'Etoile, France), in accordance with the manufacturer's instructions. Only whole MIC dilutions (half MIC dilutions were rounded up to the next whole MIC dilutions) are reported in the present study. All MICs, except for DIS-73285, were interpreted as susceptibility (S); susceptibility, increased exposure (I); and resistance (R) using current breakpoints stated by EUCAST (www.eucast.org/clinical_breakpoints). For azithromycin and ampicillin, no clinical breakpoints are stated by EUCAST. Consequently, the azithromycin epidemiological cut-off of MIC >1 mg/L was used to indicate isolates with azithromycin resistance determinants (referred to as resistant in this study) and the clinical breakpoints for benzylpenicillin were used for ampicillin (www.eucast.org/clinical_breakpoints).

Results

The results of the antimicrobial susceptibility testing for DIS-73285 and the seven antimicrobials currently or previously used for the treatment of gonorrhoea are summarized in Table 1.

DIS-73285 showed highly potent *in vitro* activity against all *N. gonorrhoeae* isolates tested ($n = 262$), with an MIC range of ≤0.001–0.004 mg/L (MIC ≤ 0.001 mg/L, $n = 235$; MIC = 0.002 mg/L, $n = 26$; MIC = 0.004 mg/L, $n = 1$). The MIC_{50}, MIC_{90} and modal MIC were all ≤0.001 mg/L (lowest MIC tested), which is substantially lower than those observed for all the other antimicrobials tested (Table 1). No cross-resistance or correlation between the MICs of DIS-73285 and the MICs of any of the currently or previously used therapeutic antimicrobials was observed (data not shown).

Table 1. MIC range, MIC_{50}, MIC_{90} and modal MIC values for DIS-73285 and therapeutic antimicrobials currently or previously recommended for *N. gonorrhoeae* isolates

Antimicrobial and isolate group (*n*)	MIC[a] range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Modal MIC (mg/L)	S/I/R[b] (%)
DIS-73285 (*N* = 262 isolates)	≤0.001–0.004	≤0.001	≤0.001	≤0.001	ND
consecutive (100)	≤0.001–0.002	≤0.001	≤0.001	≤0.001	ND
reference (34)	≤0.001–0.002	≤0.001	≤0.001	≤0.001	ND
selected AMR (128)	≤0.001–0.004	≤0.001	0.002	≤0.001	ND
CRO-resistant isolates (15)	≤0.001–0.002	≤0.001	0.002	≤0.001	ND
AZM-resistant isolates (116)	≤0.001–0.004	≤0.001	0.002	≤0.001	ND
CIP-resistant isolates (139)	≤0.001–0.004	≤0.001	0.002	≤0.001	ND
CRO (262)	<0.002–4	0.008	0.064	0.004	94.3/ND/5.7
CFM (262)	<0.016–8	<0.016	0.25	<0.016	88.2/ND/11.8
AZM (262)	0.016–>256	0.5	2	1	55.7/ND/44.3
SPT (262)	4 to >1024	16	16	16	98.1/ND/1.9
CIP (262)	<0.002 to >32	2	>32	>32	46.9/0.0/53.1
AMP (262)	<0.016 to >256	0.5	4	1	13.7/61.1/25.2
TET (262)	0.125–256	2	16	4	24.8/21.8/53.4

CRO, ceftriaxone; AZM, azithromycin; CIP, ciprofloxacin; CFM, cefixime; SPT, spectinomycin; AMP, ampicillin; TET, tetracycline; ND, not determined due to lack of interpretative criteria.

[a]MIC was determined using the agar dilution technique for DIS-73285 and Etest for the additional antimicrobials.

[b]S, susceptible; I, susceptible, increased exposure; R, resistant. The EUCAST clinical breakpoints (www.eucast.org/clinical_breakpoints) were applied for all antimicrobials.

Discussion

This is the first extensive evaluation of the *in vitro* activities of the novel small-molecule antimicrobial DIS-73285 against a large geographically, temporally and genetically diverse collection of clinical *N. gonorrhoeae* isolates and international reference strains, including various types of high-level resistant, MDR and XDR gonococcal isolates. DIS-73285 was shown to be substantially more potent, with an MIC_{90} of $\leq$0.001 mg/L, than any of the additional antimicrobials tested, i.e. ceftriaxone, cefixime, azithromycin, spectinomycin, ciprofloxacin, ampicillin and tetracycline.

As previously mentioned, as a consequence of the widespread AMR and cross-resistance between antimicrobials in *N. gonorrhoeae*, new antimicrobials with novel targets and mechanism of action, such as DIS-73285,[26] are urgently needed and novel approaches like Summit's proprietary transposon and bioinformatics-based Discuva platform are very important. Briefly, this platform enables the identification of novel antibacterial chemical classes that are distinct from current antibiotic classes and with novel targets and mechanism of action lacking overlap with currently used antimicrobial classes.[24,25] Other promising antimicrobials in the pre-clinical and/or clinical pipeline for treatment of gonorrhoea include SMT-571 (belonging to the DDS-01 series), which is another small-molecule antimicrobial revealed by the same Discuva platform.[31,32] Additional novel gonorrhoea therapeutic antimicrobials are the pleuromutilin lefamulin,[33,34] the triazaacenaphthylene gepotidacin[35,36] and the spiropyrimidinetrione zoliflodacin,[37–39] which has recently entered a Phase 3 randomized controlled clinical trial (RCT). Although both gepotidacin and zoliflodacin performed relatively well regarding safety, tolerability and in eradicating urogenital gonorrhoea, rare treatment failures were reported in their Phase 2 RCTs.[35,39]

In conclusion, the novel small-molecule antimicrobial, DIS-73285, displays a novel target and mechanism of action, with high *in vitro* potency against *N. gonorrhoeae*. Further *in vitro* and *in vivo* studies, evaluating efficacy, resistance emergence, pharmacokinetic/pharmacodynamic parameters, toxicity and safety, are required to evaluate if DIS-73285 can be an effective and safe therapy specifically for urogenital and extra-genital gonorrhoea.

Acknowledgements

We are grateful to Summit Therapeutics, Cambridge, UK, for providing DIS-73285.

The work was performed at the WHO Collaborating Centre for Gonorrhoea and Other Sexually Transmitted Infections, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, SE-701 85 Örebro, Sweden.

Funding

This work was supported by grants from the Örebro County Council Research Committee, Örebro, Sweden; the Foundation for Medical Research at Örebro University Hospital, Örebro, Sweden; and Summit Therapeutics, Cambridge, UK.

Transparency declarations

C.M., N.K. and P.M. are employed by and hold shares in Summit Therapeutics, Cambridge, UK. S.J. and M.U.: none to declare.

References

1 Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. *Clin Microbiol Rev* 2014; **27**: 587–613.

2 Wi T, Lahra MM, Ndowa F *et al.* Antimicrobial resistance in *Neisseria gonorrhoeae*: global surveillance and a call for international collaborative action. *PLoS Med* 2017; **14**: e1002344.

3 Unemo M, Lahra MM, Cole M *et al.* World Health Organization Global Gonococcal Antimicrobial Surveillance Program (WHO GASP): review of new data and evidence to inform international collaborative actions and research efforts. *Sex Health* 2019; **16**: 412–25.

4 World Health Organization (WHO). WHO Guidelines for the Treatment of *Neisseria gonorrhoeae*. WHO; 2016. http://www.who.int/reproductivehealth/publications/rtis/gonorrhoea-treatment-guidelines/en/.

5 Workowski KA, Bolan GA; Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recomm Rep* 2015; **64**: 1–137.

6 Bignell C, Unemo M; European STI Guidelines Editorial Board. European guideline on the diagnosis and treatment of gonorrhoea in adults. *Int J STD AIDS* 2012; **24**: 85–92.

7 Romanowski B, Robinson J, Wong T. Gonococcal infections chapter. In: Canadian Guidelines on Sexually Transmitted Infections. 2013. www.phac-aspc.gc.ca/std-mts/sti-its/cgsti-ldcits/assets/pdf/section-5-6-eng.pdf.

8 Australasian Sexual Health Alliance (ASHA). Gonorrhoea. In: Australian STI Management Guidelines for Use in Primary Care. 2016. http://www.sti.guidelines.org.au/sexually-transmissible-infections/gonorrhoea.

9 Fifer H, Saunders J, Soni S *et al.* 2018 UK national guideline for the management of infection with *Neisseria gonorrhoeae*. *Int J STD AIDS* 2020; **31**: 4–15.

10 Boiko I, Golparian D, Krynytska I *et al.* Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates and treatment of gonorrhoea patients in Ternopil and Dnipropetrovsk regions of Ukraine, 2013-2018. *APMIS* 2019; **127**: 503–9.

11 Japanese Society for Sexually Transmitted Infections. Gonococcal infection. Sexually transmitted infections, diagnosis and treatment guidelines 2011 [in Japanese]. *Jpn J Sex Transm Dis* 2011; **22** Suppl 1: 52–9.

12 Fifer H, Natarajan U, Jones L *et al.* Failure of dual antimicrobial therapy in treatment of gonorrhea. *N Engl J Med* 2016; **374**: 2504–6.

13 Nakayama S-I, Shimuta K, Furubayashi K-I *et al.* New ceftriaxone and multidrug-resistant *Neisseria gonorrhoeae* strain with a novel mosaic *penA* gene isolated in Japan. *Antimicrob Agents Chemother* 2016; **60**: 4339–41.

14 Lahra MM, Martin I, Demczuk W *et al.* Cooperative recognition of internationally disseminated ceftriaxone-resistant *Neisseria gonorrhoeae* strain. *Emerg Infect Dis* 2018; **24**: 735–40.

15 Lefebvre B, Martin I, Demczuk W *et al.* Ceftriaxone-resistant *Neisseria gonorrhoeae*, Canada, 2017. *Emerg Infect Dis* 2018; **24**: 381–3.

16 Terkelsen D, Tolstrup J, Johnsen CH *et al.* Multidrug-resistant *Neisseria gonorrhoeae* infection with ceftriaxone resistance and intermediate resistance to azithromycin, Denmark, 2017. *Euro Surveill* 2017; **22**: pii=17-00659.

17 Poncin T, Fouere S, Braille A *et al.* Multidrug-resistant *Neisseria gonorrhoeae* failing treatment with ceftriaxone and doxycycline in France, November 2017. *Euro Surveill* 2018; **23**: pii=1800264.

18 Golparian D, Rose L, Lynam A *et al.* Multidrug-resistant *Neisseria gonorrhoeae* isolate, belonging to the internationally spreading Japanese FC428

clone, with ceftriaxone resistance and intermediate resistance to azithromycin in Ireland, August 2018. *Euro Surveill* 2018; **23**: pii=1800617.

19 Eyre DW, Town K, Street T *et al*. Detection in the United Kingdom of the *Neisseria gonorrhoeae* FC428 clone, with ceftriaxone resistance and intermediate resistance to azithromycin, October to December 2018. *Euro Surveill* 2019; **24**: pii=1900147.

20 Eyre DW, Sanderson ND, Lord E *et al*. Gonorrhoea treatment failure caused by a *Neisseria gonorrhoeae* strain with combined ceftriaxone and high-level azithromycin resistance, England, February 2018. *Euro Surveill* 2018; **23**: pii=1800323.

21 Whiley DM, Jennison A, Pearson J *et al*. Genetic characterization of *Neisseria gonorrhoeae* resistant to both ceftriaxone and azithromycin. *Lancet Infect Dis* 2018; **18**: 717–18.

22 Jennison AV, Whiley D, Lahra MM *et al*. Genetic relatedness of ceftriaxone-resistant and high-level azithromycin resistant *Neisseria gonorrhoeae* cases, United Kingdom and Australia, February to April 2018. *Euro Surveill* 2019; **24**: pii=1900118.

23 World Health Organization. Global Priority List of Antibiotic-resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. 2017. https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1.

24 Mason C, Meo P, Avis T *et al*. High density transposon mutant profiling to enable discovery and development of novel antimicrobials. In: *Abstracts of the ASM Microbe 2018, Atlanta, USA, 2018*. Abstract 662. https://www.summitplc.com/publications/.

25 Breidenstein EBM, Avis T, Coward C *et al*. High density transposon mutant profiling enables the discovery and development of novel antimicrobials. In: *Abstracts of the ESCMID/ASM 2018, Lisbon, Portugal, 2018*. Abstract 55. https://www.summitplc.com/publications/.

26 Avis T, Breidenstein EBM, Coward C *et al*. DDS-03: a highly potent and selective novel small molecule series to treat multi-drug resistant *Neisseria gonorrhoeae*. In: *Abstracts of the ASM Microbe 2018, Atlanta, USA, 2018*. Abstract 648. https://www.summitplc.com/publications/.

27 Unemo M, Fasth O, Fredlund H *et al*. Phenotypic and genetic characterization of the 2008 WHO *Neisseria gonorrhoeae* reference strain panel intended for global quality assurance and quality control of gonococcal antimicrobial resistance surveillance for public health purposes. *J Antimicrob Chemother* 2009; **63**: 1142–51.

28 Unemo M, Golparian D, Sánchez-Busó L *et al*. The novel 2016 WHO *Neisseria gonorrhoeae* reference strains for global quality assurance of laboratory investigations: phenotypic, genetic and reference genome characterization. *J Antimicrob Chemother* 2016; **71**: 3096–108.

29 Gianecini RA, Golparian D, Zittermann S *et al*. Genome-based epidemiology and antimicrobial resistance determinants of *Neisseria gonorrhoeae* isolates with decreased susceptibility and resistance to extended-spectrum cephalosporins in Argentina in 2011-16. *J Antimicrob Chemother* 2019; **74**: 1551–9.

30 CLSI. M07 *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eleventh Edition: M07*. 2018.

31 Jacobsson S, Unemo M, Khan N *et al*. *In-vitro* activity of SMT-571 and comparators against clinical isolates and reference strains of *Neisseria gonorrhoeae*. In: *Abstracts of the ASM Microbe 2019, San Francisco, USA, 2019*. Abstract 758. https://www.summitplc.com/publications/.

32 Jacobsson S, Mason C, Khan N *et al*. *In vitro* activity of the novel oral antimicrobial SMT-571, with a new mechanism of action, against MDR and XDR *Neisseria gonorrhoeae*—future treatment option for gonorrhoea? *J Antimicrob Chemother* 2019; **74**: 1591–4.

33 Paukner S, Gruss A, Jensen JS. *In vitro* activity of lefamulin against sexually transmitted bacterial pathogens. *Antimicrob Agents Chemother* 2018; **62**: e02380–17.

34 Jacobsson S, Paukner S, Golparian D *et al*. *In vitro* activity of the novel pleuromutilin lefamulin (BC-3781) and effect of efflux pump inactivation on multidrug-resistant and extensively drug-resistant *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 2017; **61**: e01497–17.

35 Taylor SN, Morris DH, Avery AK *et al*. Gepotidacin for the treatment of uncomplicated urogenital gonorrhea: a Phase 2, randomized, dose-ranging, single-oral dose evaluation. *Clin Infect Dis* 2018; **67**: 504–12.

36 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.

37 Unemo M, Ringlander J, Wiggins C *et al*. High in vitro susceptibility to the novel spiropyrimidinetrione ETX0914 (AZD0914) among 873 contemporary clinical *Neisseria gonorrhoeae* isolates from 21 European countries from 2012 to 2014. *Antimicrob Agents Chemother* 2015; **59**: 5220–5.

38 Basarab GS, Kern GH, McNulty J *et al*. Responding to the challenge of untreatable gonorrhea: ETX0914, a first-in-class agent with a distinct mechanism-of-action against bacterial Type II topoisomerases. *Sci Rep* 2015; **5**: 11827.

39 Taylor SN, Marrazzo J, Batteiger BE *et al*. Single-dose zoliflodacin (ETX0914) for treatment of urogenital gonorrhea. *N Engl J Med* 2018; **379**: 1835–45.