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

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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 ≤0.001 to 0.004 mg/L, and the MIC₅₀, MIC₉₀ and modal MIC all ≤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*.\textsuperscript{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.\textsuperscript{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 toxicity profile.\textsuperscript{16}

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 isolates were defined as previously \textsuperscript{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 \textsuperscript{26} 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 \textsuperscript{26} 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, but very high MICs in single reference strains of 28 other bacterial species.\textsuperscript{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 toxicity profile.\textsuperscript{16}

### 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.\textsuperscript{10} The MICs (mg/L) of ceftriaxone, cefixime, azithromycin, ciprofloxacin, spectinomycin, tetracycline and ampicillin were determined using the Etest method (AB bioMérieux, Marcy l’Étoile, 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 \(\leq 0.001\)–0.004 mg/L (MIC \(\leq 0.001\) 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 \(\leq 0.001\) 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)      | \(\leq 0.001\)–0.004  | \(\leq 0.001\)     | \(\leq 0.001\)     | \(\leq 0.001\)    | ND              |
| consecutive (100)                | \(\leq 0.001\)–0.002  | \(\leq 0.001\)     | \(\leq 0.001\)     | \(\leq 0.001\)    | ND              |
| reference (34)                   | \(\leq 0.001\)–0.002  | \(\leq 0.001\)     | \(\leq 0.001\)     | \(\leq 0.001\)    | ND              |
| selected AMR (128)               | \(\leq 0.001\)–0.004  | \(\leq 0.001\)     | \(\leq 0.002\)     | \(\leq 0.001\)    | ND              |
| CRO-resistant isolates (15)      | \(\leq 0.001\)–0.002  | \(\leq 0.001\)     | \(\leq 0.002\)     | \(\leq 0.001\)    | ND              |
| AZM-resistant isolates (116)     | \(\leq 0.001\)–0.004  | \(\leq 0.001\)     | \(\leq 0.002\)     | \(\leq 0.001\)    | ND              |
| CIP-resistant isolates (139)     | \(\leq 0.001\)–0.004  | \(\leq 0.001\)     | \(\leq 0.002\)     | \(\leq 0.001\)    | ND              |
| CRO (262)                        | 0.002–4                | 0.008              | 0.064              | 0.004             | 94.3/ND/5.7    |
| CFM (262)                        | \(\leq 0.016\)–0.8    | \(\leq 0.016\)     | 0.25               | \(\leq 0.016\)    | 88.2/ND/11.8   |
| AZM (262)                        | 0.016–0.256            | 0.5                | 2                  | 1                 | 55.7/ND/44.3   |
| SPT (262)                        | 4 to 1024              | 16                 | 16                 | 16                | 98.1/ND/1.9    |
| CIP (262)                        | \(\leq 0.002\) to >32 | 2                  | \(>32\)            | >32               | 46.9/0.0/53.1  |
| AMP (262)                        | \(\leq 0.016\) 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 |

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

\(\text{MIC was determined using the agar dilution technique for DIS-73285 and Etest for the additional antimicrobials.}

\(\text{S, susceptible; I, susceptible, increased exposure; R, resistant. The EUCAST clinical breakpoints (www.eucast.org/clinical_breakpoints) were applied for all antimicrobials.}\)
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<sub>90</sub> of ≤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, 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. 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. Additional novel gonorrhea therapeutic antimicrobials are the pleuromutilin lefamulin, the triazaacenaphthylene gepotidacin and the spiropyrimidinetrione zoliflodacin, 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.

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 treatment specifically for urogenital and extra-genital gonorrhoea.

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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.

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