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Gepotidacin (GSK2140944) *In Vitro* Activity Against *Neisseria gonorrhoeae* (MIC/MBC, Kill Kinetics, Checkerboard, PAE/SME Tests)

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Abstract

Background: Gepotidacin (GEP) is a novel triazaacenaphthylene antibiotic, which inhibits bacterial DNA replication and has *in vitro* activity against susceptible and drug-resistant pathogens associated with a range of conventional and biothreat infections, including *Neisseria gonorrhoeae* (GC).

Methods: Broth microdilution using fastidious broth was used to evaluate the MIC/MBC activity of GEP and comparator agents against 25 GC strains (including five ciprofloxacin [CIP] non-susceptible [NS] strains). GEP *in vitro* activity was also evaluated against three GC strains (including ATCC 49226 and two tetracycline [TET]- and azithromycin [AZI]-NS strains) using time-kill kinetics and checkerboard methods, and against two GC strains for the investigation of post-antibiotic (PAE) and sub-inhibitory (PAE-SME) effects.

Results: The MIC_{50} and MIC_{90} for GEP against 25 GC isolates were 0.12 and 0.25 µg/mL, respectively. The highest GEP MIC value was 0.25 µg/mL. The MBC_{50} and MBC_{90} for GEP were 0.25 and 0.5 µg/mL, respectively, and the highest MBC value was 1 µg/mL (two isolates). GEP was bactericidal when tested against GC with 25/25 of isolates exhibiting a MBC/MIC ratio of ≤4 (≤2 for 20/25 of the isolates). GEP demonstrated bactericidal activity in time-kill curves against the three GC strains tested. For all the combinations of GEP and comparators tested against GC using checkerboard methods, there were no instances where antagonism occurred and only one instance where synergy occurred (with moxifloxacin; FIC index, 0.375), but this was not confirmed by *in vitro* time-kill studies. An extended PAE for GEP against the wild-type GC strain (0.5->2.5 hours) and an extended PAE-SME (>2.5 hours) occurred. The GEP PAE (0.7 hours at all exposures) and PAE-SME (1.2-2.7 hours) were shorter with the TET- and AZI-NS GC strain.

Conclusions: Using MBC and time-kill kinetics, GEP demonstrated bactericidal activity against the *N. gonorrhoeae* isolates tested. *In vitro* checkerboard studies showed that interactions were generally additive/indifferent and no antagonism was seen. The PAE for GEP was generally of short to modest duration, with an extended PAE-observed in the presence of sub-MIC concentrations. These *in vitro* data indicate that further study of GEP is warranted for potential use in treating infections caused by GC.

Introduction

Gepotidacin (formerly GSK2140944) is a novel, first in class triazaacenaphthylene antibacterial which selectively inhibits bacterial DNA gyrase and topoisomerase IV by a unique mechanism, not utilized by any currently approved human therapeutic agent. Structural data with a type IIA topoisomerase enzyme, DNA gyrase, has revealed the novel binding mode of the triazaacenaphthylene class and has distinguished it from the binding mode of other antibacterials, including quinolones. As a consequence of its novel mode of action, gepotidacin is active *in vitro* against target pathogens carrying resistance determinants to established antibacterials and has demonstrated *in vitro* activity against key pathogens, including drug-resistant strains of *Neisseria gonorrhoeae*, associated with a range of conventional and biothreat infections.

In this study, reference *in vitro* methods were used to evaluate the MIC/MBC activity of gepotidacin and comparator agents against *N. gonorrhoeae.* Gepotidacin *in vitro* activity was also evaluated against *N. gonorrhoeae* using time-kill kinetics, broth microdilution checkerboard methods for synergy testing and for post-antibiotic effects.

Methods

- For all *in vitro* assays described in this study, testing was performed using Fastidious Broth (FB) [Takei et. al., 2005].
- Reference *in vitro* broth microdilution methods were used to evaluate the MIC/MBC activity of gepotidacin and ceftriaxone (comparator agent) against 25 isolates of *N. gonorrhoeae* (including 5 ciprofloxacin [CIP] non-susceptible [NS], 5 penicillin [PEN]-NS, 5 PEN- and CIP-NS, and 5 azithromycin or tetracycline-NS isolates). A drug was considered to exhibit bactericidal activity against a particular isolate when the MBC/MIC ratio was ≤4.
- QC strains were tested daily for the broth microdilution method, and inoculum density was monitored by colony counts during each batch test run. ATCC QC strains were selected as appropriate for the conditions being tested and included *N. gonorrhoeae* ATCC 49226.
- For time kill kinetics, gepotidacin and comparator agents were tested at concentrations of 1/4x, 1/2x, 1x, 2x, 4x and 10x the MIC for each isolate and sampled at time 0 hours (T_0), T_2, T_4, T_8 and T_{24}. Time-kill curves were done in duplicate. Three strains were used in these studies, including ATCC 49226 and fluoroquinolone-, tetracycline- and azithromycin-non-susceptible isolates. Bactericidal activity was defined as a ≥3-log reduction in bacterial counts (log10 CFU/mL).
- Checkerboard synergy testing was performed by broth microdilution. Gepotidacin was tested alone and in combination with moxifloxacin, levofloxacin, azithromycin, tetracycline and ceftriaxone. The fractional inhibitory concentration index (∑FIC) was determined and interpreted as follows: synergy, ≤0.5; indifference, >0.5-≤4.0; antagonism, >4.0.
- Gepotidacin was tested against isolates using time kill methods to determine post-antibiotic effect (PAE) and PAE-sub-MIC effects (SME). In addition to gepotidacin, levofloxacin and ceftriaxone were tested as comparators. The organisms were exposed for one hour to gepotidacin or the comparator agent at 1x, 5x, and 10x the MIC concentration as determined from the broth microdilution testing for each agent. For the PAE-SME, only the initial 5x exposure was used, followed by ¼x or ½x MIC re-exposure. Antibiotics were removed by centrifugation and re-suspension. Colony counts were performed at T_0 (pre-antimicrobial exposure) and T_1 (after antimicrobial exposure). Colony counts were taken immediately after washing and re-suspending the organisms, and at each subsequent hour until visible turbidity was observed or up to nine hours.

Results

Quality control:
- The quality control range for gepotidacin using the reference agar dilution method is 0.25-1 µg/mL. Currently there are no published quality control criteria for gepotidacin when tested in fastidious broth against *N. gonorrhoeae* ATCC 49226.

MIC/MBC studies:
- The $MIC_{50/90}$ values for gepotidacin against 25 *N. gonorrhoeae* isolates selected for this study was 0.12/0.25 µg/mL. The highest gepotidacin MIC value was 0.25 µg/mL. The ceftriaxone MIC_{50} and MIC_{90} for the *N. gonorrhoeae* isolates was 0.002 and 0.008 µg/mL (**Table 1**).
- The $MBC_{50/90}$ for gepotidacin against 25 *N. gonorrhoeae* isolates selected for this study was 0.25/0.5 µg/mL. The highest gepotidacin MBC value was 1 µg/mL. The ceftriaxone $MBC_{50/90}$ for the *N. gonorrhoeae* isolates was 0.002/0.008 µg/mL (**Table 1**).
- Gepotidacin was bactericidal when tested against *N. gonorrhoeae* with a total of 100% (25/25) of isolates exhibiting a MBC/MIC ratio of ≤4. For 80% (20/25) of the isolates tested, the MBC/MIC ratio was ≤2. Ceftriaxone was also bactericidal against *N. gonorrhoeae* with a total of 100% (25/25) of the *N. gonorrhoeae* isolates tested exhibiting a MBC/MIC ratio of ≤2.

Time-kill studies:
- **Figure 1** shows the *in vitro* time-kill activity of gepotidacin against an isolate of *N. gonorrhoeae* (b#12584) that was non-susceptible to azithromycin, ciprofloxacin, and tetracycline. Against this isolate, gepotidacin was bactericidal at 4x MIC at the 8 hour time point for one replicate and at 24 hours for the other replicate (data not shown). At the 10x MIC concentration, gepotidacin was bactericidal at the 4 hour time point for one replicate and at 8 hours for the second replicate. **Figure 2** shows the *in vitro* time-kill activity of gepotidacin against a second isolate of *N. gonorrhoeae* (b#12588) that was also non-susceptible to azithromycin, ciprofloxacin, and tetracycline. Gepotidacin was bactericidal against this isolate at the 24 hour time point at both the 4x and 10x MIC concentrations (in both replicates). No regrowth for gepotidacin was seen for either isolate. **Figure 3** shows the *in vitro* time-kill activity of gepotidacin against a wild-type isolate of *N. gonorrhoeae,* ATCC 49226. Gepotidacin was bactericidal against this isolate at 24 hours at 2x, 4x, and 10x MIC concentrations. No regrowth for gepotidacin was noted.
- No colonies grew on the gepotidacin 4x MIC agar screen plates inoculated from the 24 hour time point time-kill curve tubes containing either 2x, 4x or 10x MIC gepotidacin concentrations for any of the three *N. gonorrhoeae* isolates tested.

Determination of interactions (checkerboard) evaluation:
- For all the combinations of gepotidacin and comparators tested *in vitro* against *N. gonorrhoeae*, there were no occurrences of antagonism and only one occurrence of synergy (**Table 2**).
- The one occurrence of synergy (FIC index of 0.375) was for gepotidacin tested in combination with moxifloxacin against *N. gonorrhoeae* strain 12588 (**Table 2**). To further confirm this synergy, an *in vitro* time-kill study was performed with varying concentrations of gepotidacin and moxifloxacin. At 1x MIC GSK2140944 there were 6.5 log_{10} viable bacteria at 24 hours, 7.3 log_{10} viable bacteria at 24 hours with 1x MIC moxifloxacin and 7.1 log_{10} viable bacteria with the combination of 1/4x MIC GSK2140944 and 1/4x MIC moxifloxacin (**Figure 4**). This *in vitro* time-kill study did not confirm the potential synergy noted in the *in vitro* checkerboard testing.
- Indifference was noted for 80.0% of *in vitro* checkerboard results and 13.3% of results were not determinable due to offscale MICs for the comparator agent (**Table 2**).

Determination of post-antibiotic and sub-inhibitory effects:
- Against the wild-type strain (ATCC 49226), the PAE at 1x MIC was 0.5 hours and there was an extended PAE for gepotidacin at 10x MIC (>2.5 hours; **Table 3**).
- Against the wild-type strain (ATCC 49226), the PAE-SME for gepotidacin was >2.5 hours at ¼x MIC and ½x MIC. The PAE-SME for ceftriaxone was 0.5 hours at ¼x MIC and 0.1 hours at ½x MIC (**Table 4**).
- Against the ciprofloxacin, tetracycline and azithromycin non-susceptible strain (12584), the PAE-SME for gepotidacin was 1.2 hours at ¼x MIC and 2.7 hours at ½x MIC. The PAE-SME for ceftriaxone was 1.3 hours at ¼x MIC and 0.0 hours at ½x MIC (**Table 4**).

Table 1. Summary of MIC_{50}/MBC_{50} and MIC_{90}/MBC_{90} values in µg/mL for gepotidacin and ceftriaxone against 25 *N. gonorrhoeae.*

Summary MIC/MBC values	Gepotidacin	Ceftriaxone
MIC_{50}	0.12	0.002
MIC_{90}	0.25	0.008
MBC_{50}	0.25	0.002
MBC_{90}	0.5	0.008

Table 2. Summary of ∑FIC interpretations for gepotidacin tested in combination with selected comparators against 3 strains of *N. gonorrhoeae.*

Combination drug	∑FIC Interpretative Category			
	Synergy	Indifferent	ND[a]	Antagonism
Azithromycin	0	3	0	0
Ceftriaxone	0	3	0	0
Levofloxacin	0	2	1	0
Moxifloxacin	1	1	1	0
Tetracycline	0	3	0	0

a. Some results were not able to be calculated due to off-scale MIC results and were labeled as not determinable (ND)

Table 3. Summary of PAE results observed from time-kill curves for gepotidacin and ceftriaxone after 1 hour exposure at 1x-, 5x-, and 10x- baseline MIC values.

Organism/ Isolate #	Gepotidacin				Ceftriaxone			
	Baseline MIC (µg/mL)	PAE (hrs.) at MIC concentration of:			Baseline MIC (µg/mL)	PAE (hrs.) at MIC concentration of:		
		1x	5x	10x		1x	5x	10x
N. gonorrhoeae								
ATCC 49226	0.25	0.5	1.0	>2.5	0.004	0.3	0.0	0.0
12584*	0.12	0.7	0.7	0.7	0.004	0.7	0.2	0.0

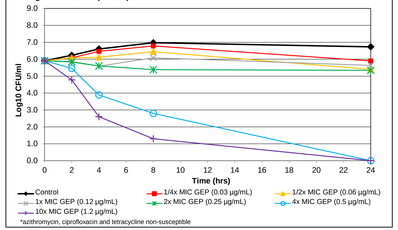
* azithromycin, ciprofloxacin and tetracycline non-susceptible

Table 4. Summary of PAE-SME results observed from time-kill curves for gepotidacin and comparator agents after 1 hour exposure at 5x MIC and addition of ¼x MIC or ½x MIC concentration of the antimicrobial agent.

Organism/ Isolate #	Gepotidacin			Levofloxacin			Ceftriaxone		
	5x MIC (µg/mL)	PAE-SME (hrs.) at MIC concentration of:		5x MIC (µg/mL)	PAE-SME (hrs.) at MIC concentration of:		5x MIC (µg/mL)	PAE-SME (hrs.) at MIC concentration of:	
		5x + ¼ MIC	5x + ½ MIC		5x + ¼ MIC	5x + ½ MIC		5x + ¼ MIC	5x + ½ MIC
N. gonorrhoeae									
ATCC 49226	1.25	>2.5	>2.5	0.02	0.5	0.1	0.02	0.5	0.1
12584*	0.625	1.2	2.7	0.02	1.3	0.0	0.02	1.3	0.0

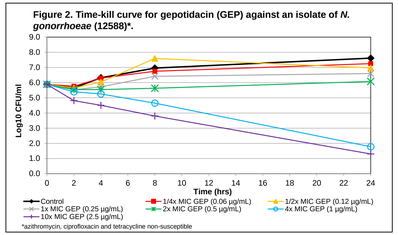
* azithromycin, ciprofloxacin and tetracycline non-susceptible

Figure 1. Time-kill curve for gepotidacin (GEP) against an isolate of *N. gonorrhoeae* (12584)*.



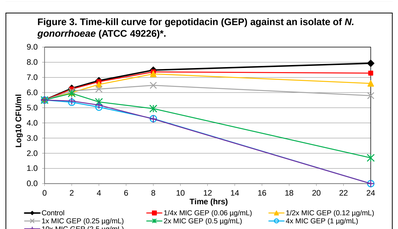
*azithromycin, ciprofloxacin and tetracycline non-susceptible

Figure 2. Time-kill curve for gepotidacin (GEP) against an isolate of *N. gonorrhoeae* (12588)*.



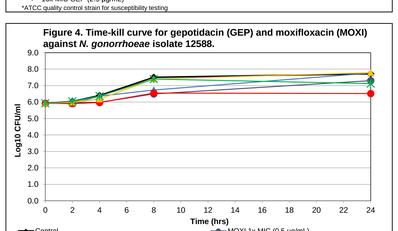
*azithromycin, ciprofloxacin and tetracycline non-susceptible

Figure 3. Time-kill curve for gepotidacin (GEP) against an isolate of *N. gonorrhoeae* (ATCC 49226)*.



*ATCC quality control strain for susceptibility testing

Figure 4. Time-kill curve for gepotidacin (GEP) and moxifloxacin (MOXI) against *N. gonorrhoeae* isolate 12588.



Conclusions

- Gepotidacin was active *in vitro* against the isolates of *N. gonorrhoeae* tested, exhibiting $MIC_{50/90}$ values of 0.12/0.25 µg/mL. Gepotidacin was bactericidal against *N. gonorrhoeae* demonstrating MBC/MIC ratios of ≤4 against 100% of the 25 isolates tested.
- Gepotidacin demonstrated bactericidal activity in *in vitro* time-kill curves against *N. gonorrhoeae* including isolates that were non-susceptible to azithromycin, ciprofloxacin, and tetracycline.
- *In vitro* checkerboard experiments showed no occurrences of antagonism when testing gepotidacin in combination with a variety of currently used antimicrobial agents against *N. gonorrhoeae.*
- The PAE for gepotidacin that occurred when testing *N. gonorrhoeae* ranged from short to modest, with an extended PAE-SME observed for both isolates tested.

Acknowledgements

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