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Gepotidacin (GSK2140944) *In Vitro* Activity Against Gram-positive and Gram-negative Bacteria (MBC/MIC, Kill Kinetics, Checkerboard, PAE/SME Tests)

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Amended 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.

Methods: Reference *in vitro* methods were used to evaluate the MIC/MBC activity of GEP and comparators against *Staphylococcus. aureus* (SA), *Streptococcus pneumoniae* (SPN) and *Escherichia coli* (EC). GEP *in vitro* activity was also evaluated using time-kill kinetics (KK), broth microdilution checkerboard methods (CM), and for post-antibiotic (PAE) and sub-inhibitory (PAE-SME) effects.

Results: MIC_{90} values for GEP against 50 SA (including MRSA) and 50 SPN (including penicillin-intermediate and -resistant) isolates were 0.5 µg/mL and for EC (n=25) was 4 µg/mL. GEP was bactericidal against the tested strains of SA, SPN, and EC. GEP had MBC/MIC ratios of ≤4 against 98, 98, and 88% of isolates tested, respectively. KK indicated that bactericidal activity for GEP was generally observed at 4 or 10x MIC concentrations at 24 hours. In a few instances, regrowth was observed in the presence of GEP and in the presence of levofloxacin. CM experiments demonstrated no occurrences of antagonism when testing GEP in combination with a variety of currently used antimicrobial agents including aztreonam, ceftriaxone, tetracycline and trimethoprim-sulfamethoxazole. The most common interaction when testing GEP was indifference (82.7% for Gram-positive and 82.0% for Gram-negative). The PAE for GEP against SA was short (≤0.6 hours against MRSA and MSSA) and the PAE-SME was extended in length (>8 hours; 3 isolates at ½x MIC). Against the levofloxacin-susceptible SA isolate tested, the PAE for levofloxacin was modest (0.1-2.4 hours) and an extended PAE-SME was observed (>9 hours at ½x MIC).

Conclusions: GEP demonstrated bactericidal activity against the majority of Gram-positive and Gram-negative isolates tested. For the isolate and drug combinations tested, *in vitro* checkerboard studies showed that interactions were generally indifferent and no antagonism was seen. The PAE for GEP was 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.

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 *Staphylococcus aureus, Streptococcus pneumoniae* and *Escherichia coli*, 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 *S. aureus, S. pneumoniae* and *E. coli.* Gepotidacin *in vitro* activity was also evaluated using time-kill kinetics, broth microdilution checkerboard methods for synergy testing, and for post-antibiotic and sub-inhibitory effects.

Methods

- Reference *in vitro* broth microdilution methods were used to evaluate the MIC/MBC activity of gepotidacin and comparator agents against *S. aureus* (n=50), *S. pneumoniae* (n=50), and *E. coli* (n=25). A drug was considered to exhibit bactericidal activity against a particular isolate when the MBC/MIC ratio was ≤4. For all *in vitro* assays described in this study, *S. aureus* and *E. coli* were tested in cation-adjusted Mueller-Hinton broth and *S. pneumoniae* was tested in cation-adjusted Mueller-Hinton broth supplemented with lysed horse blood.
- For time-kill kinetics, gepotidacin and comparator agents were tested against 3 strains each of *S. aureus, S. pneumoniae* and *E. coli* at concentrations of 1/4x, 1/2x, 1x, 2x, 4x and 10x the MIC of 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. 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 aztreonam, linezolid, vancomycin, gentamicin, moxifloxacin, levofloxacin, azithromycin, tetracycline, ceftriaxone and trimethoprim-sulfamethoxazole for *S. aureus* (n=10), *S. pneumoniae* (n=10), and *S. pyogenes* (n=2); gentamicin, aztreonam, moxifloxacin, levofloxacin, tetracycline, and trimethoprim-sulfamethoxazole for *E. coli* (n=5); and gentamicin, aztreonam, moxifloxacin, levofloxacin, azithromycin, tetracycline, ceftriaxone and trimethoprim-sulfamethoxazole for *H. influenzae* (n=2). The interpretation of ∑FIC was applied as follows: synergy, ≤0.5; indifference, >0.5-≤4.0; antagonism, >4.0.
- Gepotidacin was tested using time-kill methods to determine post-antibiotic effect (PAE) and sub-inhibitory effects (PAE-SME). In addition to gepotidacin, the following comparators were tested against *S. aureus* (n=3)*:* linezolid and levofloxacin; *S. pneumoniae* (n=2)*:* levofloxacin; and *E. coli* (n=2)*:* levofloxacin. The organisms were exposed for one hour to gepotidacin or the comparator agent at 1x, 5x, and 10x the MIC concentration as determined by broth microdilution testing. Only the initial 5x exposure was used for the PAE-SME, 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 9 hours.

Results

- The $MIC_{50/90}$ values for gepotidacin were 0.25/0.5 µg/mL for staphylococci and streptococci and 2/4 µg/mL for *E. coli.* MBC_{90} values for staphylococci and streptococci were identical to the MIC_{90} values of 0.5 µg/mL. Gepotidacin was bactericidal against 88% (22/25) of *E. coli* isolates exhibiting a MBC/MIC ratio of ≤2. Three isolates exhibited a MBC/MIC ratio >16 (**Table 1**).
- Static *in vitro* time-kill experiments indicated that gepotidacin was bactericidal against twelve bacterial isolates including *S. aureus* (MRSA/MSSA), penicillin-intermediate and -resistant isolates of *S. pneumoniae,* and *E. coli* including ESBL-phenotype isolates (see **Figures 1-3** for representative examples). Bactericidal activity was observed at 4x or 10x concentrations at 24 hours for all isolates.
- For all six *S. aureus* isolates tested, colonies from aliquots taken from time-kill curve tubes containing ¼x to 2x MIC of gepotidacin grew on an agar screen plate containing gepotidacin concentrations of 4x MIC. When growth from these concentrations, and also growth from 24 hour time-kill curve tubes containing 4x or 10x MIC concentrations of gepotidacin, were selected and MICs determined, the resulting gepotidacin MICs ranged from ≤8- to 16-fold higher than baseline gepotidacin MICs. One selected colony had a 32-fold increase and two selected colonies had an increase of 128-fold from baseline.
- An agar screen at 4x MIC for gepotidacin showed that for *E. coli* and *S. pneumoniae,* very few colonies with elevated gepotidacin MICs relative to baseline were recovered at 24 hours from time-kill curve tubes containing varying concentrations of gepotidacin.
- A total of 29 Gram-positive and -negative bacterial isolates were evaluated by checkerboard synergy studies. There were no instances of antagonism that occurred when testing gepotidacin *in vitro* in combination with the other antimicrobials. The most common interaction noted when testing gepotidacin *in vitro* in combination was indifference (82.7%; **Table 2**).
- The *in vitro* PAE for gepotidacin against *S. aureus* was short (0-0.6 hours against MRSA and MSSA; **Table 3**) and the *in vitro* PAE-SME was extended in length (>8 hours for all 3 isolates at ½x MIC; **Table 4**).
- The *in vitro* PAE for gepotidacin against *S. pneumoniae* was modest in length (0.7-1.6 hours) with an extended *in vitro* PAE-SME (>5.5 hours at ½x MIC) (**Tables 3** and **4**). The *in vitro* PAE for levofloxacin was also modest in length (0.2-1.6 hours) with an extended *in vitro* PAE-SME (5.0->5.5 hours at ½x MIC) (**Tables 3** and **4**).
- The *in vitro* PAE for gepotidacin against *E. coli* was modest (1.2-2.2 hours) with an extended *in vitro* PAE-SME (>4.3 hours at ½x MIC for wild-type and ESBL-phenotype isolates; **Tables 3** and **4**).

Table 1. Summary of MIC/MBC_{50} and MIC/MBC_{90} values in µg/mL for gepotidacin and comparators.

	S. aureus (50)			MRSA (25)			MSSA (25)			*S. pneumoniae* (50)			*S. pneumoniae* Pen-R (20)		*E. coli* (25)	
	Gepotidacin	LEV	LZD	Gepotidacin	LEV	LZD	Gepotidacin	LEV	LZD	Gepotidacin	LEV	LZD	Gepotidacin	LEV	Gepotidacin	LEV
MIC_{50}	0.25	0.25	1	0.25	8	1	0.25	0.25	1	0.25	1	0.25	1	2	0.5	
MIC_{90}	0.5	32	2	0.5	>32	2	0.5	4	2	0.5	1	0.5	1	4	16	
MBC_{50}	0.5	0.5	>32	0.5	8	>32	0.5	0.25	>32	0.25	1	0.25	1	2	0.5	
MBC_{90}	0.5	32	>32	1	>32	>32	1	8	>32	0.5	2	0.5	2	> 32	16	

LEV, levofloxacin; LZD, linezolid

Table 2. Summary of ∑FIC interpretations for gepotidacin tested in combination with selected comparators against Gram-positive and -negative pathogens.

Organism group (# of strains)/ combination drug	∑FIC Interpretative Category			
	Synergy	Indifferent	ND[a]	Antagonism
***S. aureus* (10)**				
Azithromycin	0	5	5	0
Aztreonam	0	0	10	0
Ceftriaxone	0	8	2	0
Gentamicin	0	10	0	0
Levofloxacin	0	7	3	0
Linezolid	0	10	0	0
Moxifloxacin	0	10	0	0
Tetracycline	0	10	0	0
Trimethoprim-sulfamethoxazole	0	9	1	0
Vancomycin	0	10	0	0
***S. pneumoniae* (10)**				
Azithromycin	0	4	6	0
Aztreonam	0	2	8	0
Ceftriaxone	0	10	0	0
Gentamicin	0	10	0	0
Levofloxacin	0	8	2	0
Linezolid	0	10	0	0
Moxifloxacin	0	10	0	0
Tetracycline	0	9	1	0
Trimethoprim-sulfamethoxazole	0	10	0	0
Vancomycin	0	10	0	0
***S. pyogenes* (2)**				
Azithromycin	0	2	0	0
Aztreonam	0	2	0	0
Ceftriaxone	0	2	0	0
Gentamicin	0	2	0	0
Levofloxacin	0	2	0	0
Linezolid	0	2	0	0
Moxifloxacin	0	2	0	0
Tetracycline	0	2	0	0
Trimethoprim-sulfamethoxazole	0	2	0	0
Vancomycin	0	2	0	0
***E. coli* (5)**				
Aztreonam	0	5	0	0
Gentamicin	0	5	0	0
Levofloxacin	0	4	1	0
Moxifloxacin	0	3	2	0
Tetracycline	0	3	2	0
Trimethoprim-sulfamethoxazole	0	2	3	0
***H. influenzae* (2)**				
Azithromycin	0	2	0	0
Aztreonam	0	2	0	0
Ceftriaxone	0	2	0	0
Gentamicin	0	2	0	0
Levofloxacin	0	2	0	0
Moxifloxacin	0	2	0	0
Tetracycline	0	2	0	0
Trimethoprim-sulfamethoxazole	0	2	0	0
All (%)	0 (0.0)	220 (82.7)	46 (17.3)	0 (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 comparator agents after 1 hour exposure at 1x, 5x, and 10x baseline MIC values.

Organism/ Isolate #	Gepotidacin				Levofloxacin				Linezolid			
	Baseline MIC (µg/mL)	PAE (hrs.) at MIC concentration of:			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		1x	5x	10x
S. aureus												
ATCC 29213	0.25	0.3	0.6	0.6	0.25	0.1	1.1	2.4	2	0.1	0.5	0.5
1263	0.25	0.0	0.5	0.5	8	0.0	0.6	NT	1	0.1	0.7	0.7
12605	0.5	0.0	0.5	0.5	16	0.1	0.1	NT	1	0.2	0.3	0.7
S. pneumoniae												
35841	0.5	0.7	1.6	1.4	1	0.7	1.1	1.6	NT	NT	NT	NT
7114	2	0.7	1.1	1.0	32	0.2	0.7	NT	NT	NT	NT	NT
E. coli												
ATCC 25922	2	1.3	2.2	2.2	0.03	0.4	ND	ND	NT	NT	NT	NT
3904	4	1.2	1.8	2.0	16	0.6	1.1	NT	NT	NT	NT	NT

NT = not tested; ND = not able to be determined

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			Linezolid		
	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
S. aureus									
ATCC 29213	1.25	2.2	>9	1.25	2.5	>9	10	1.5	9.0
1263	1.25	1.4	>8	40	0.9	>8	5	1.9	3.7
12605	2.5	8.0	>8	80	0.4	1.2	5	1.0	4.5
S. pneumoniae									
35841	2.5	6.4	>6.4	5	2.3	5.0	NT	NT	NT
7114	10	5.4	>5.5	160	2.3	>5.5	NT	NT	NT
E. coli									
ATCC 25922	10	3.0	>4.3	0.15	ND	ND	NT	NT	NT
3904	20	6.6	>6.6	80	1.9	4.1	NT	NT	NT

NT = not tested; ND = not able to be determined

Figure 1. Time-kill curve for gepotidacin against *S. aureus* (isolate 139)

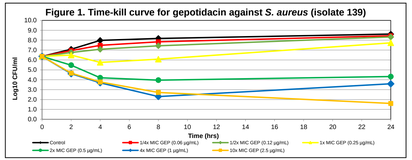


Figure 2. Time-kill curve for gepotidacin against *E. coli* (isolate 3773)

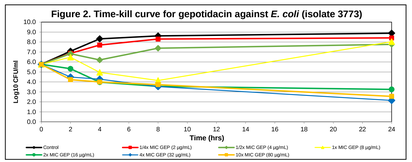
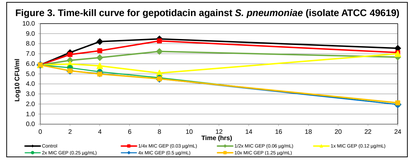


Figure 3. Time-kill curve for gepotidacin against *S. pneumoniae* (isolate ATCC 49619)



Conclusions

- Gepotidacin was active *in vitro* against staphylococci and streptococci exhibiting $MIC_{50/90}$ values of 0.25/0.5 µg/mL and 2/4 µg/mL for *E. coli*.
- Gepotidacin demonstrated bactericidal activity in static *in vitro* time-kill curves against *S. aureus* including MRSA, penicillin-intermediate and -resistant isolates of *S. pneumoniae,* and *E. coli* including ESBL-phenotype isolates.
- *In vitro* checkerboard experiments showed no occurrences of antagonism when testing gepotidacin in combination with a variety of currently used antimicrobial agents against Gram-positive and -negative organisms.
- The *in vitro* PAE for gepotidacin that occurred when testing *S. aureus, S. pneumoniae* and *E. coli* ranged from short to modest, with an extended *in vitro* PAE-SME observed for all isolates tested.
- Overall, *in vitro,* gepotidacin was active and bactericidal against the tested contemporary isolates of *S. aureus* (including MRSA), *S. pneumoniae* (including penicillin-resistant isolates) and *E. coli* (including ESBL-producing isolates). The PAE exhibited by bacterial strains exposed to gepotidacin was short to modest with a long PAE-SME and checkerboard synergy studies with multiple comparator agents indicated no antagonism.

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