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Synergistic Effect of Novel Gyrase Inhibitor Agents (SPR750 and SPR751) in Combination with a Polymyxin Derivative (SPR741) against Recent *Acinetobacter baumannii* and *Enterobacteriaceae*

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Abstract

Background: *Enterobacteriaceae* cause numerous infections in community and hospitals settings with a great proportion of these pathogens being refractory to potent antimicrobial agents. The activity of new gyrase inhibitory agents combined with SPR741 was assessed against *A. baumannii* and *Enterobacteriaceae*.

Methods: A total of 319 *Enterobacteriaceae* (10 species) and 82 *A. baumannii* isolates were selected. Isolates were tested for susceptibility by CLSI methods. SPR750 and SPR751 were tested alone and in combination with SPR741 at a fixed concentration of 2 (F2) and 8 (F8) µg/mL. CLSI/EUCAST criteria were used to interpret MICs for comparator agents.

Results: Investigational drugs tested alone had MICs of ≥4 µg/mL ($MIC_{50/90}$, >16/>16 µg/mL) against *A. baumannii*. SPR750 ($MIC_{50/90}$, >4/>4 µg/mL) and SPR751 ($MIC_{50/90}$, 4/>4 µg/mL) tested with SPR741 at F2 were less active than SPR750-SPR741 ($MIC_{50/90}$, 4/>4 µg/mL) and SPR751-SPR741 ($MIC_{50/90}$, 1/>4 µg/mL) at F8 against *A. baumannii*. SPR750, SPR751, and SPR741 showed MICs of ≥4 µg/mL ($MIC_{50/90}$, >16/>16 µg/mL) when tested against all *Enterobacteriaceae*. SPR750 (MIC_{50}, 0.5 µg/mL) and SPR751 (MIC_{50}, 0.12–0.25 µg/mL) had the lowest MICs when tested with SPR741 at F8 against non-ESBL- and ESBL-producing *Enterobacteriaceae*. SPR750-SPR741 ($MIC_{50/90}$, 0.5/1 µg/mL) and SPR751-SPR741 ($MIC_{50/90}$, 0.12/0.25 µg/mL) at F8 inhibited 98.7 and 100.0% of *E. coli* (19.5% ESBL), respectively, at ≤1 µg/mL. SPR750-SPR741 ($MIC_{50/90}$, 0.5/2 µg/mL) and SPR751-SPR741 ($MIC_{50/90}$, 0.25/1 µg/mL) at F8 inhibited 92.5 and 96.2% of *Klebsiella* spp. (26.3% ESBL), respectively, at ≤4 µg/mL. The combinations ($MIC_{50/90}$, >4/>4 µg/mL) tested here were less active against *Proteus* spp., *Providencia* spp., and *S. marcescens*. When these species were excluded, 96.6% of other *Enterobacteriaceae* species (*E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp.;13.7% ESBL and 3.1% carbapenem resistant) were inhibited by SPR750-SPR741 and SPR751-SPR741 at F8 at ≤4 and ≤1 µg/mL, respectively. Also, SPR751-SPR741 at F8 ($MIC_{50/90}$, 0.25/1 µg/mL) had MICs 2-fold lower than SPR750-SPR741 at F8 ($MIC_{50/90}$, 0.5/2 µg/mL) against these 4 genera.

Conclusions: Tested agents did not show direct *in vitro* activity against these pathogens. However, potent synergistic effect was observed for SPR750 and SPR751 combined with SPR741 at F8, except against *Proteus* spp., *Providencia* spp., *S. marcescens*, and *A. baumannii*. These *in vitro* data indicate that the combinations/therapy strategies in this study may have potential for further clinical development.

Introduction

- *Enterobacteriaceae* are a common cause of community-acquired and health care–acquired infections, with *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp. among the most common organisms
- During the late 1990s, carbapenem-resistant *Enterobacteriaceae* (CRE) began to emerge, and in 2012, 4.6% of acute-care hospitals reported at least 1 clinical infection caused by CRE
- The proportion of *Enterobacteriaceae* that were CRE increased from 1.2% in 2001 to 4.2% in 2011 in both the National Nosocomial Infection Surveillance system (NNIS) and the National Healthcare Safety Network (NHSN) and from 0% in 2001 to 1.4% in 2010 in the Surveillance Network–USA (TSN)
- Overall, the majority of CRE isolates in the US harbor a KPC serine carbapenemase-encoding gene; bla_{KPC} genes are mostly detected in *K. pneumoniae*, but have been observed in numerous *Enterobacteriaceae* species
- More recently, a plasmid-borne colistin resistance gene, *mcr*-1, has been documented primarily in *E. coli*, but has also been detected on every continent in other *Enterobacteriaceae* species from human, animal, food, and environmental samples
- SPR750 and SPR751 are new gyrase inhibitory antimicrobial agents that target the bacterial gyrase (GyrB) and topoisomerase IV (ParE) (Figure 1)
- SPR741 is a novel polymyxin-like compound that targets the gram-negative bacterial outer membrane and adversely affects the integrity of the lipopolysaccharide
- The activity of SPR750 and SPR751 combined with SPR741 was assessed against a recent collection of *A. baumannii* and *Enterobacteriaceae* clinical isolates

Materials and Methods

Organism collection

- This study used geographically diverse *Enterobacteriaceae* (319) and *A. baumannii* (82) clinical isolates collected from patients worldwide with documented infections
- Isolates originated from 19 countries/regions in Europe and the US. These isolates were responsible for pneumonia in hospitalized patients (31.2%), followed by urinary tract infections (26.7%), bloodstream infections (18.0%), skin and skin structure infections (17.2%), intra-abdominal infections (5.5%), and other less common infections (1.4%)

Susceptibility testing

- Isolates were tested for susceptibility by broth microdilution following the Clinical and Laboratory Standards Institute (CLSI) M07-A10 document
- SPR750 and SPR751 was tested in combination with SPR741 at a fixed concentration of 2 and 8 µg/mL
- Bacterial inoculum density was monitored by colony counts to assure adequate number of cells for each testing event
- MIC values were validated by concurrently testing CLSI-recommended quality control (QC) reference strains

Results

- SPR750, SPR751, and SPR741 showed MIC values of ≥4 µg/mL ($MIC_{50/90}$, >16/>16 µg/mL for all) when tested alone against *A. baumannii* (Table 1)
- The SPR750 and SPR751 MIC_{50} values decreased to 4 and 1 µg/mL, respectively, when tested in combination with SPR741 at fixed concentrations of 8 µg/mL (Table 1)
- Both investigational compounds ($MIC_{50/90}$, >16/>16 µg/mL for both) exhibited elevated MIC values when tested alone against the entire collection of *Enterobacteriaceae* (Table 2)
- The MIC_{50} values for SPR750 and SPR751 decreased to 1 and 0.25 µg/mL, respectively, when tested in combination with SPR741 at fixed concentrations of 8 µg/mL against *Enterobacteriaceae* (Table 2)
- Synergistic effects were observed for SPR750 (MIC_{50}, 0.5 µg/mL) and SPR751 (MIC_{50}, 0.25 µg/mL) when tested in combination with SPR741 at fixed concentrations of 8 µg/mL against ESBL-producing *Enterobacteriaceae* (Table 3)
- SPR750 ($MIC_{50/90}$, 0.5/1 µg/mL) and SPR751 ($MIC_{50/90}$, 0.12/0.25 µg/mL) tested with SPR741 at fixed concentrations of 8 µg/mL inhibited 98.7 and 100.0% of *E. coli* (19.5% ESBL), respectively, at ≤1 µg/mL (Table 4)
- SPR751 ($MIC_{50/90}$, 0.25/1 µg/mL) displayed MIC results 2-fold lower than SPR750 ($MIC_{50/90}$, 0.5/2 µg/mL) when tested with SPR741 at fixed concentration of 8 µg/mL against *K. pneumoniae* (26.3% ESBL) (Table 4)
- Both combinations of SPR750 and SPR751 with SPR741 tested at fixed concentration of 8 µg/mL inhibited 72.5% of *Enterobacter* spp. and 96.7% of *Citrobacter freundii* at ≤1 µg/mL (Table 4)
- The combinations ($MIC_{50/90}$, >4/>4 µg/mL) tested here were less active against *Proteus* spp., *Providencia* spp., and *Serratia marcescens* (Table 4)
- A total of 96.6% of all *E. coli*, *K. pneumoniae*, *Enterobacter* spp. and *Citrobacter* spp. (13.7% ESBL and 3.1% carbapenem resistant) were inhibited by SPR750 and SPR751 at ≤4 and ≤1 µg/mL, respectively, when tested with SPR741 at fixed concentration of 8 µg/mL (Figure 2)

Table 1 Cumulative frequency distribution of MIC results for SPR750 and SPR751 tested alone and in combinations with SPR741 against 82 *A. baumannii*

Antimicrobial combination	Number (cumulative percentage) of isolates inhibited by each MIC (µg/mL):										MIC (µg/mL)	
	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	50%	90%
SPR750									3 (3.7%)	79 (100.0%)	>16	>16
SPR751 (2 µg/mL)					1 (1.2%)	2 (3.7%)	5 (9.8%)	74 (100.0%)[a]			>4	>4
SPR741 (8 µg/mL)		1 (1.2%)	0 (1.2%)	5 (7.3%)	15 (25.6%)	15 (43.9%)	14 (61.0%)	32 (100.0%)[a]			4	>4
SPR751						6 (7.3%)	13 (23.2%)	13 (39.0%)	50 (100.0%)		>16	>16
SPR742 (2 µg/mL)				1 (1.2%)	1 (2.4%)	6 (9.8%)	18 (31.7%)	17 (52.4%)	39 (100.0%)[a]		4	>4
SPR741 (8 µg/mL)	1 (1.2%)	0 (1.2%)	7 (9.8%)	19 (32.9%)	19 (56.1%)	15 (74.4%)	6 (81.7%)	15 (100.0%)[a]			1	>4
SPR741								1 (1.2%)	1 (2.4%)	80 (100.0%)	>16	>16

[a]Represents MIC of >8 µg/mL for combinations tested

Table 2 Cumulative frequency distribution of MIC results for SPR750 and SPR751 tested alone and in combinations with SPR741 against 319 *Enterobacteriaceae*[a]

Antimicrobial combination	Number (cumulative percentage) of isolates inhibited by each MIC (µg/mL):											MIC (µg/mL)	
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	50%	90%
SPR750												319 (100.0%)	>16	>16
SPR741 (2 µg/mL)						2 (0.6%)	4 (1.9%)	9 (4.7%)	13 (8.8%)	291 (100.0%)[b]			>4	>4
SPR741 (8 µg/mL)		1 (0.3%)	2 (0.9%)	11 (4.4%)	62 (23.8%)	78 (48.3%)	65 (68.7%)	24 (76.2%)	10 (79.3%)	66 (100.0%)[b]			1	>4
SPR751									1 (0.3%)	5 (1.9%)	13 (6.0%)	300 (100.0%)	>16	>16
SPR742 (2 µg/mL)					2 (0.6%)	9 (3.4%)	27 (11.9%)	28 (20.7%)	15 (25.4%)	238 (100.0%)[b]			>4	>4
SPR741 (8 µg/mL)	2 (0.6%)	3 (1.6%)	33 (11.9%)	86 (38.9%)	68 (60.2%)	41 (73.0%)	20 (79.3%)	5 (80.9%)	5 (82.4%)	56 (100.0%)[b]			0.25	>4
SPR741											19 (6.0%)	300 (100.0%)	>16	>16

[a]Includes *Citrobacter freundii* (14), *Enterobacter aerogenes* (14), *Enterobacter cloacae* (77), *Escherichia coli* (77), *Klebsiella pneumoniae* (80), *Proteus mirabilis* (13), *Proteus vulgaris* (14), *Providencia rettgeri* (8), *Providencia stuartii* (8), *Serratia marcescens* (14)
[b]Represents MIC of >8 µg/mL for combinations tested

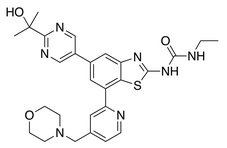
Table 3 Cumulative frequency distribution of MIC results for SPR750 and SPR751 tested in combinations with SPR741 against 37 ESBL-producing *Enterobacteriaceae*[a]

Combinations	Number (cumulative percentage) of isolates inhibited by each MIC (µg/mL):									MIC (µg/mL)	
	0.03	0.06	0.12	0.25	0.5	1	2	4	>4	50%	90%
SPR750-SPR741 (2 µg/mL)						1 (2.7%)	3 (10.8%)	2 (16.2%)	31 (100.0%)	>4	>4
SPR750-SPR741 (8 µg/mL)			1 (2.7%)	9 (27.0%)	12 (59.5%)	9 (83.8%)	3 (91.9%)	0 (91.9%)	3 (100.0%)	0.5	2
SPR751-SPR741 (2 µg/mL)					3 (8.1%)	6 (24.3%)	4 (35.1%)	1 (37.8%)	23 (100.0%)	>4	>4
SPR751-SPR741 (8 µg/mL)		3 (8.1%)	12 (40.5%)	15 (81.1%)	4 (91.9%)	0 (91.9%)	0 (91.9%)	2 (97.3%)	1 (100.0%)	0.25	0.5

[a]Includes *E. coli* (15), *K. pneumoniae* (21), *P. mirabilis* (1)

Figure 1 (a) Structure of SPR750 and (b) SPR751

A. Structure of SPR750



B. Structure of SPR751

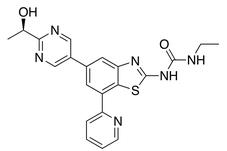
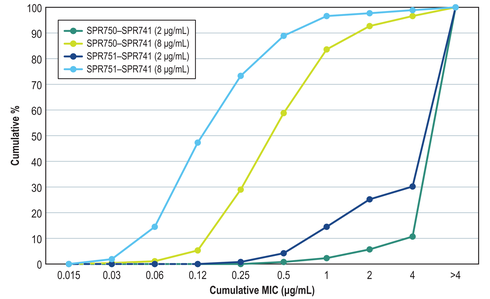


Table 4 Cumulative frequency distribution of MIC results for SPR750 and SPR751 tested in combinations with SPR741 against *Enterobacteriaceae* species

Organism combinations	Number (cumulative percentage) of isolates inhibited by each MIC (µg/mL)										MIC (µg/mL)	
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	>4	50%	90%
E. coli (77)												
SPR750-SPR741 (2 µg/mL)							2 (2.6%)	4 (7.8%)	9 (19.5%)	11 (33.8%)	>4	>4
SPR750-SPR741 (8 µg/mL)					8 (10.4%)	29 (48.1%)	29 (85.7%)	10 (98.7%)	0 (98.7%)	1 (100.0%)	0.5	1
SPR751-SPR741 (2 µg/mL)						2 (2.6%)	9 (14.3%)	22 (42.9%)	20 (68.8%)	18 (76.6%)	2	>4
SPR751-SPR741 (8 µg/mL)				12 (15.6%)	37 (63.6%)	22 (92.2%)	5 (98.7%)	1 (100.0%)			0.12	0.25
K. pneumoniae (80)												
SPR750-SPR741 (2 µg/mL)										80 (100.0%)	>4	>4
SPR750-SPR741 (8 µg/mL)					14 (17.5%)	28 (52.5%)	21 (78.8%)	9 (90.0%)	2 (92.5%)	6 (100.0%)	0.5	2
SPR751-SPR741 (2 µg/mL)							3 (3.8%)	7 (12.5%)	4 (17.5%)	66 (100.0%)	>4	>4
SPR751-SPR741 (8 µg/mL)				10 (12.5%)	28 (47.5%)	22 (75.0%)	11 (88.8%)	3 (92.5%)	1 (93.8%)	2 (96.2%)	0.25	1
Enterobacter spp. (91)												
SPR750-SPR741 (2 µg/mL)									2 (2.2%)	89 (100.0%)	>4	>4
SPR750-SPR741 (8 µg/mL)			1 (1.1%)	0 (1.1%)	1 (2.2%)	13 (16.5%)	18 (36.3%)	33 (72.5%)	15 (89.0%)	8 (97.8%)	2 (100.0%)	1	4
SPR751-SPR741 (2 µg/mL)							2 (2.2%)	1 (3.3%)	3 (6.6%)	85 (100.0%)	>4	>4
SPR751-SPR741 (8 µg/mL)		1 (1.1%)	1 (2.2%)	7 (9.9%)	15 (26.4%)	23 (51.6%)	25 (79.1%)	16 (96.7%)	2 (98.9%)	1 (100.0%)		0.25	1
C. freundii (14)												
SPR750-SPR741 (2 µg/mL)										14 (100.0%)	>4	>4
SPR750-SPR741 (8 µg/mL)				2 (14.3%)	2 (28.6%)	6 (71.4%)	3 (92.9%)	1 (100.0%)				0.25	0.5
SPR751-SPR741 (2 µg/mL)										14 (100.0%)	>4	>4
SPR751-SPR741 (8 µg/mL)		1 (7.1%)	2 (21.4%)	4 (50.0%)	6 (92.9%)	1 (100.0%)						0.06	0.12
Other species (57)[a]												
SPR750-SPR741 (2 µg/mL)										57 (100.0%)	>4	>4
SPR750-SPR741 (8 µg/mL)										57 (100.0%)	>4	>4
SPR751-SPR741 (2 µg/mL)									2 (3.5%)	55 (100.0%)	>4	>4
SPR751-SPR741 (8 µg/mL)									2 (3.5%)	53 (100.0%)	>4	>4

[a]Includes *P. mirabilis* (13), *P. vulgaris* (14), *P. rettgeri* (8), *P. stuartii* (8), *S. marcescens* (14)

Figure 2 Cumulative MIC distribution of SPR750 and SPR751 tested in combinations with SPR741 at fixed concentrations of 2 and 8 µg/mL against *C. freundii* (14 isolates), *E. aerogenes* (14), *E. cloacae* (77), *E. coli* (77), and *K. pneumoniae* (80)



Conclusions

- The investigational compounds SPR750, SPR751, and SPR741 did not show direct *in vitro* activity against *A. baumannii* and *Enterobacteriaceae*
- Potent synergistic effects were observed for SPR750 and SPR751 when combined with SPR741 at fixed concentration of 8 µg/mL against most common *Enterobacteriaceae* clinical isolates, such as *E. coli*, *K. pneumoniae* and *Enterobacter* spp.
- The investigational combinations were less active against *A. baumannii*, *Proteus* spp., *Providencia* spp., and *S. marcescens*
- These *in vitro* data indicate that the combinations/therapy strategies presented here may have potential for further clinical development

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