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Synergy/Antagonism of Ceftibuten/VNRX-7145 in Combination With Other Commonly Used Antimicrobial Agents in Gram-Negative Aerobic Bacteria, Anaerobic Bacteria, and Yeast

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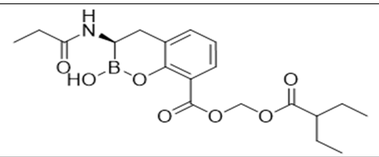
Introduction

VNRX-7145 is a novel cyclic boronate β-lactamase inhibitor (BLI) that undergoes biotransformation *in vivo* to the active BLI VNRX-5236. When tested with ceftibuten, the combination has shown potent inhibitory activity against gram-negative organisms expressing Ambler class A, C, and D enzymes including those that hydrolyze carbapenems. As combination therapies are frequently used in practice, it is important that the administration of ceftibuten/VNRX-7145 not interfere with the activity of other antimicrobial agents that a patient could be treated with concurrently[1]. Here we test ceftibuten/VNRX-5236 in checkerboard experiments with several commonly used antimicrobial agents in gram-negative aerobic bacteria, anaerobic bacteria, and yeast to assess the potential for synergy or antagonism.

Structure of VNRX-7145



Methods

Broth microdilution minimum inhibitory concentration (MIC) assays were carried out according to CLSI and/or EUCAST guidelines[2, 3, 4, 5, 6]. For each drug combination, several representative isolates were tested in the checkerboard assay: six gram-negative aerobic bacterial isolates were tested with levofloxacin, linezolid, nitrofurantoin, rifampin, and trimethoprim-sulfamethoxazole; three anaerobic bacterial isolates were tested with metronidazole; and three yeast isolates were tested with fluconazole. Two-fold dilutions of ceftibuten/VNRX-5236 occurred across the microtiter plates. The second agent was titrated down two microtiter plates. Each of the agents was tested alone and a growth control was included on each plate. For all plates the MIC was read visually as the lowest drug concentration well with no discernable growth in each column and row.

The fractional inhibitory concentration index (FICI) was calculated for the combinations using the following formula[7]:

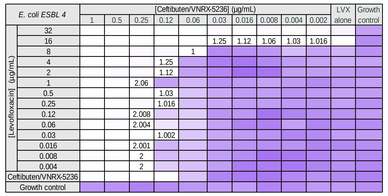
$$FICI = FIC_A + FIC_B = \frac{[A]}{MIC_A} + \frac{[B]}{MIC_B}$$

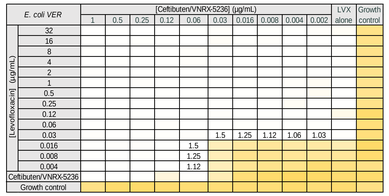
Where [A] is the MIC of ceftibuten with VNRX-5236 fixed at 4 µg/mL in combination with a second agent in a given well along the growth/no-growth interface; [B] is the MIC of the other agent in combination with ceftibuten/VNRX-5236 fixed at 4 µg/mL in a given well along the growth/no-growth interface; MIC_A is the control MIC of ceftibuten/VNRX-5236 fixed at 4 µg/mL alone; and MIC_B is the control MIC of the other agent alone.

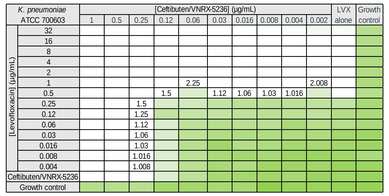
The FICI is interpreted as follows: ≤0.5 = potential synergy, >0.5 – 4 = indifference, and >4 = potential antagonism. The mean FICI was reported for each agent against each isolate tested.

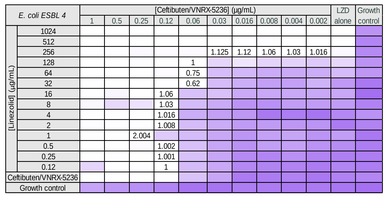
Aerobic Gram-Negative Bacterial Isolate Checkerboard Examples

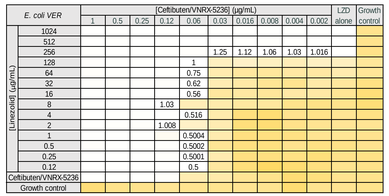
Below is a selection of checkerboard experimental plates as observed using a Biotek® Cytation™ 3 imaging reader showing indifference of the combinations. Ceftibuten/VNRX-5236 is titrated across the microtiter plates. Levofloxacin (LVX); linezolid (LZD), or nitrofurantoin (NIT) are titrated down the microtiter plates. Darker colored wells indicate denser bacterial growth. The FICI values for each well along the growth/no-growth interface are indicated. In all cases, no synergy or antagonism were observed in the three bacterial isolates shown.

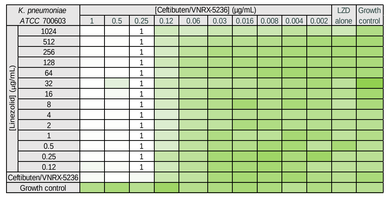


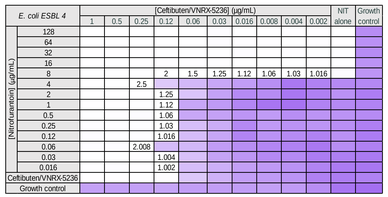


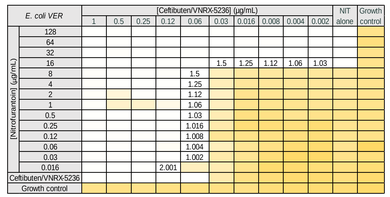


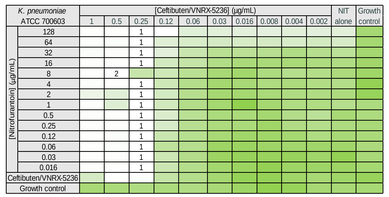












Aerobic Gram-Negative Bacterial Isolate FICI Table

Table 1. Summary of mean fractional inhibitory concentration index (FICI) scores for ceftibuten/VNRX-5236 in combination with all antibiotics tested in *β*-lactamase-producing and β-lactam-susceptible aerobic gram-negative isolates.

Species	Strain ID	Enzymes	Levofloxacin	Linezolid	Nitrofurantoin	Rifampin	Trimethoprim-sulfamethoxazole
E. cloacae	SI-ECL01	p99	0.98	0.40	0.96	0.58	1.05
E. coli	ESBL 4	CTX-M-15	1.41	1.06	1.31	0.86	1.33
E. coli	VER	OXA-48	1.23	0.81	1.20	1.12	1.47
E. coli	ATCC 25922	None	1.33	0.51	1.03	1.34	1.22
K. pneumoniae	ATCC BAA 1705	KPC-2	0.71	1.01	1.00	1.15	1.00
K. pneumoniae	ATCC 700603	SHV-18	1.29	1.00	1.15	0.74	1.03

FICI interpretation: ≤ 0.5 = potential synergy; >0.5 – 4 = indifferent; >4 = potential antagonism

Anaerobic Bacterial Isolate FICI Table

Table 2. Summary of fractional inhibitory concentration index (FICI) scores for ceftibuten/VNRX-5236 in combination with metronidazole in anaerobic gram-negative and gram-positive isolates.

Species	Strain ID	Metronidazole FICI
B. fragilis	ATCC 25285	0.55
B. thetaiotaomicron	ATCC 29741	1.00
C. difficile	ATCC 700057	1.00

FICI interpretation: ≤ 0.5 = potential synergy; >0.5 – 4 = indifferent; >4 = potential antagonism

Candida spp. Isolate FICI Table

Table 3. Summary of fractional inhibitory concentration index (FICI) scores for ceftibuten/VNRX-5236 in combination with fluconazole in *Candida* spp. isolates.

Species	Strain ID	Fluconazole FICI
Candida albicans	ATCC 90028	0.88
Candida krusei	ATCC 6258	0.75
Candida tropicalis	ATCC 750	0.85

FICI interpretation: ≤ 0.5 = potential synergy; >0.5 – 4 = indifferent; >4 = potential antagonism

Conclusions

- Ceftibuten/VNRX-7145 did not demonstrate antagonism in any of the combinations tested, and is not expected to antagonize or synergize with other agents.
- In some cases, ceftibuten/VNRX-7145 could potentially increase the permeability of the gram-negative cell wall allowing linezolid, an anti-gram-positive agent, to enter the cell and bind its ribosomal RNA target[8].

References

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