Activity of Cefepime in Combination with the Novel β -Lactamase Inhibitor Taniborbactam (VNRX-5133) against Extended-Spectrum- β -Lactamase-Producing Isolates in *In Vitro* Checkerboard Assays

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ABSTRACT Extended-spectrum- β -lactamase (ESBL)-producing strains are increasing worldwide, limiting therapeutic options. Taniborbactam (VNRX-5133) is a newly developed β -lactamase inhibitor with a wide spectrum of activity covering both serine and metallo enzymes. We therefore evaluated cefepime-taniborbactam activity against ESBL-producing isolates and determined the concentrations to be used in MIC determinations in the clinical laboratory. The *in vitro* activity of cefepime (0.06 to 256 mg liter⁻¹) combined with taniborbactam (0.03 to 32 mg liter⁻¹) against 129 clinically and molecularly well-documented ESBL-producing isolates (42 *Escherichia coli*, 39 *Klebsiella pneumoniae*, 28 *Pseudomonas aeruginosa*, 16 *Enterobacter cloacae*, 2 *Citrobacter freundii*, and 2 *Enterobacter aerogenes*) was tested with a broth microdilution checkerboard method based on the ISO standard. The MICs of cefepime alone and in combination, together with percentage resistance at different concentrations of taniborbactam, were calculated for each species and resistance mechanism. The median (range)/MIC₉₀ of cefepime was 32 (0.125 to 256)/256 mg liter⁻¹ for all *Enterobacteriales* isolates ($n = 101$), with 72% being resistant, and 32 (8 to 256)/128 mg liter⁻¹ for the 28 *P. aeruginosa* isolates, with 86% being resistant. The median (range)/90th percentile concentration of taniborbactam required to restore *Enterobacteriales* susceptibility to cefepime (MIC ≤ 1 mg liter⁻¹) was 0.06 (≤ 0.03 to 32)/4 mg liter⁻¹ and *P. aeruginosa* susceptibility to increased exposure to cefepime (MIC ≤ 8 mg liter⁻¹) 1 (≤ 0.032 to 32)/32 mg liter⁻¹. At a fixed concentration of 4 mg liter⁻¹ of taniborbactam, cefepime median (range)/MIC₉₀ were reduced to 0.125 (0.06 to 4)/1 mg liter⁻¹ for *Enterobacteriales* with no resistant isolates found, and to 8 (2 to 64)/16 mg liter⁻¹ for *P. aeruginosa* isolates, where 36% remained resistant. The combination cefepime-taniborbactam demonstrated a potent activity against ESBL isolates, restoring susceptibility of all *Enterobacteriales* and two-thirds of *P. aeruginosa* isolates.

KEYWORDS ESBL, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, β -lactamase inhibitor, cefepime, checkerboard, combination, taniborbactam

The global spread of extended-spectrum β -lactamases (ESBLs) has been an ongoing problem since the first report appeared in 1983 (1). β -Lactamases are enzymes produced by some bacteria that confer resistance to β -lactam antibiotics. They are most commonly found in members of the *Enterobacteriales* and in nonfermenting Gram negative bacteria. ESBLs are enzymes that can hydrolyze what were supposed to be β -lactamase-resistant oxyimino- β -lactams. Currently, many third and fourth generation cephalosporins have little activity against these ESBL-producing strains (2).

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TABLE 1 Antimicrobial activity of cefepime at various concentrations of taniborbactam

Taniborbactam concn (mg liter ⁻¹)	<i>E. coli</i> (n = 42)			<i>K. pneumoniae</i> (n = 39)			<i>P. aeruginosa</i> (n = 28)			Others ^a (n = 20)		
	Median (range) MIC (mg liter ⁻¹)	MIC ₉₀	%R ^b	Median (range) MIC (mg liter ⁻¹)	MIC ₉₀	%R ^b	Median (range) MIC (mg liter ⁻¹)	MIC ₉₀	%R ^b	Median MIC (mg liter ⁻¹)	MIC ₉₀	%R ^b
0	32 (0.125–256)	256	83	32 (0.25–256)	256	72	32 (8–256)	128	86	8	32	50
0.03	4 (0.06–>256)	128	40	8 (0.06–>256)	128	51	32 (4–>256)	128	82	2	16	25
0.06	1 (0.06–>256)	32	29	2 (0.06–>256)	64	44	32 (8–>256)	128	82	1	8	10
0.125	0.5 (0.06–128)	32	14	2 (0.06–>256)	64	33	32 (8–>256)	128	86	0.5	4	10
0.25	0.25 (0.06–128)	8	14	1 (0.06–128)	32	28	16 (4–>256)	128	79	0.5	2	10
0.5	0.25 (0.06–16)	2	7	0.5 (0.06–32)	32	15	16 (4–>256)	64	71	0.5	2	5
1	0.125 (0.06–8)	1	2	0.5 (0.06–16)	4	8	16 (2–256)	64	54	0.25	1	0
2	0.125 (0.06–8)	1	2	0.25 (0.06–8)	2	5	8 (2–64)	32	36	0.25	0.5	0
4	0.125 (0.06–2)	0.5	0	0.25 (0.06–4)	1	0	8 (2–64)	16	36	0.25	0.5	0
8	0.06 (0.06–2)	0.25	0	0.125 (0.06–2)	1	0	8 (1–64)	16	25	0.25	0.5	0
16	0.06 (0.06–2)	0.25	0	0.125 (0.06–1)	1	0	8 (1–32)	16	21	0.125	0.5	0
32	0.06 (0.06–0.5)	0.125	0	0.125 (0.06–1)	0.5	0	8 (1–32)	16	25	0.125	0.5	0

^aOther isolates: 2 *Citrobacter freundii*, 2 *Enterobacter aerogenes*, and 16 *Enterobacter cloacae*.

^b%R, percentage of resistant isolates (cefepime MIC above 4 and 8 mg liter⁻¹ for *Enterobacteriales* and *P. aeruginosa*, respectively).

The development of new drugs has always played a major role in providing the solution to these resistance mechanisms. Venatorx Pharmaceuticals has developed a new β -lactamase inhibitor, called taniborbactam (formerly VNRX-5133), to overcome resistance (3). The compound has a wide spectrum of activity, including inhibition of Ambler class A extended spectrum β -lactamases (ESBLs), KPC class A enzymes, class B enzymes such as NDM and VIM, class C (AmpC) enzymes, and class D enzymes such as OXA-48 (4). Cefepime is a broad-spectrum cephalosporin often used clinically but susceptible to degradation by many of the existing β -lactamases, as are most cephalosporins. Studies *in vitro* have shown that the MICs of the cephalosporins ceftazidime and ceftolozane against resistant strains were drastically reduced in the presence of the β -lactamase inhibitors avibactam and tazobactam, respectively, restoring susceptibility (5).

We therefore determined the *in vitro* activity of the combination of cefepime with taniborbactam against ESBL-producing isolates that in most cases also expressed serine (KPC) or metallo (NDM, VIM) carbapenemases with a broth microdilution checkerboard technique. The potential clinical feasibility of the combination was assessed, and the taniborbactam concentration for MIC testing of the combination was determined. Subsequently, these data will be used for *in vivo* pharmacokinetic and pharmacodynamic studies of cefepime-taniborbactam combinations.

RESULTS

Taniborbactam alone at a concentration up to and including 32 mg liter⁻¹ did not have an inhibitory effect on *Enterobacteriales* or *P. aeruginosa* isolates (not shown). Overall, the median (range)/MIC₉₀ of cefepime alone was 32 (0.125 to 256)/256 mg liter⁻¹ for *Enterobacteriales* and 32 (8 to 256)/128 mg liter⁻¹ for *P. aeruginosa*, with 72% and 86% of isolates being resistant, respectively. The addition of increasing concentrations of taniborbactam to either *Enterobacteriales* or *P. aeruginosa* isolates resulted in decreasing MICs and % resistance for cefepime (Table 1).

The decrease in MICs was, however, more pronounced in *Enterobacteriales* than in *P. aeruginosa* isolates. To obtain susceptibility to cefepime (according to EUCAST criteria) (6) in $\geq 90\%$ of the *Enterobacteriales* isolates (MIC ≤ 1 mg liter⁻¹ cefepime), 4 mg liter⁻¹ taniborbactam was required, whereas even at the highest taniborbactam concentration (32 mg liter⁻¹), only 75% of *P. aeruginosa* isolates were susceptible to increased exposure (MIC ≤ 8 mg liter⁻¹ cefepime). At 4 mg liter⁻¹ taniborbactam, none of the *Enterobacteriales* isolates were resistant to cefepime, while 36% of *P. aeruginosa* isolates were still resistant to cefepime (Table 1).

Table 2 includes taniborbactam concentrations required to reduce cefepime MICs to different levels, including the EUCAST (7) and CLSI (8) breakpoints of cefepime for

TABLE 2 Concentration of taniborbactam required to obtain a given MIC of cefepime for the median (range) and the 90th percentile of isolates

Cefepime target MIC (mg liter ⁻¹)	Median (range)/90th percentile of taniborbactam concentrations in mg liter ⁻¹			
	<i>E. coli</i> (n = 42)	<i>K. pneumoniae</i> (n = 39)	<i>P. aeruginosa</i> (n = 28)	Others ^a (n = 20)
1	0.06 (≤0.03–32)/4	0.25 (≤0.03–32)/16	32 (8–32)/32	0.06 (≤0.03–2)/0.5
2	0.03 (≤0.03–32)/0.5	0.06 (≤0.03–32)/4	32 (1–32)/32	≤0.03 (≤0.03–1)/0.25
4	0.03 (≤0.03–32)/0.25	0.06 (≤0.03–32)/2	32 (0.03–32)/32	≤0.03 (≤0.03–1)/0.125
8	≤0.03 (≤0.03–32)/0.25	0.03 (≤0.03–32)/1	1 (≤0.03–32)/32	≤0.03 (≤0.03–0.5)/0.06
16	≤0.03 (≤0.03–0.5)/0.125	≤0.03 (≤0.03–32)/0.5	≤0.03 (≤0.03–32)/4	≤0.03 (≤0.03–0.25)/≤0.03

^aOther isolates: 2 *Citrobacter freundii*, 2 *Enterobacter aerogenes*, and 16 *Enterobacter cloacae*.

Enterobacteriales (susceptible at ≤1 mg liter⁻¹ cefepime) and *P. aeruginosa* (susceptible to increased exposure at ≤8 mg liter⁻¹ cefepime). The median (range)/90th percentile concentrations of taniborbactam required to reduce the cefepime MIC to 1 mg liter⁻¹ were 0.06 (≤0.03 to 32)/4 mg liter⁻¹ for *E. coli* isolates and 0.25 (≤0.03 to 32)/16 mg liter⁻¹ for *K. pneumoniae* isolates. For *P. aeruginosa* isolates, a median (range)/90th percentile taniborbactam concentration of 1 (≤0.03 to 32)/32 mg liter⁻¹ was required to reduce the cefepime MIC to 8 mg liter⁻¹.

In Fig. 1, the maximum decrease in cefepime MIC in number of 2-fold dilutions is shown. The addition of taniborbactam to cefepime reduced the MIC of cefepime by 8 to 12 2-fold dilutions for the majority of the *Enterobacteriales* isolates. The maximum reduction effect for 50% of the *Enterobacteriales* was reached at 4 mg liter⁻¹ taniborbactam. The maximum effect for *P. aeruginosa* isolates was less pronounced; the majority only reached 2 to 6 2-fold reductions. Although higher concentrations of taniborbactam were required to significantly potentiate cefepime activity against *P. aeruginosa*, 50% of the isolates already reached their maximum possible reduction effect at 2 mg liter⁻¹ taniborbactam. Of the 28 *P. aeruginosa* isolates, 3 did not show any change in MIC at all.

Fig. 1 also shows the groups of resistance genes harbored by the isolates in each bar of the graph. *Enterobacteriales* and *P. aeruginosa* isolates harbor different sets of resistance mechanisms, but the maximum MIC-lowering effect did not appear to be explained by the presence of certain mechanisms. The influence of specific resistance mechanisms on the MIC of cefepime is detailed in Table 3. *Enterobacteriales* isolates harboring a TEM-1, VIM, KPC, KPC-2, or CTX-M-15 gene showed the highest cefepime median MICs (>32 mg liter⁻¹) and also showed the greatest median 2-fold MIC reduction (≥7) compared to other resistance mechanisms. For *P. aeruginosa* isolates, the median 2-fold reduction of cefepime MIC was 1 and 2 for both AmpC- and VIM-producing isolates, respectively.

FIG 1 Distribution of the maximum effect of cefepime-taniborbactam combinations over the tested concentration range expressed as a 2-fold dilution in the MIC decrease of cefepime. The maximum MIC-lowering effect of the addition of taniborbactam to cefepime, compared to the MIC of cefepime alone, for percentage of isolates of both *Enterobacteriales* and *P. aeruginosa* is presented on a 2-log transformation x axis. Bars are segmented by the most common groups of resistance genes.

TABLE 3 Effect of taniborbactam on the antimicrobial activity of cefepime for the most frequently found resistance mechanisms (≥ 5 isolates per mechanism)

Resistance mechanism	<i>P. aeruginosa</i> (n = 28)				<i>Enterobacterales</i> (n = 101) ^a			
	No. isolates	Cefepime median (range)/MIC ₉₀		Median (range) 2-fold MIC reduction ^a	No. isolates	Cefepime median (range)/MIC ₉₀		Median (range) 2-fold MIC reduction ^a
		Cefepime alone	With 4 mg liter ⁻¹ TAN			Cefepime alone	With 4 mg liter ⁻¹ TAN	
TEM-1	0	-	-	-	26	32 (0.5–256)/256	0.125 (0.06–1)/1	8 (1–12)
TEM-84	0	-	-	-	7	1 (0.5–64)/32	0.06 (0.06–0.25)/0.25	5 (2–10)
SHV-1	0	-	-	-	5	256 (4–256)/256	0.5 (0.06–0.5)/0.5	5 (2–10)
SHV-12	0	-	-	-	9	4 (1–256)/256	0.125 (0.06–0.5)/0.5	5 (1–12)
OXA-1	0	-	-	-	23	32 (1–256)/256	0.25 (0.06–1)/1	5 (1–12)
VIM	9	32 (8–128)/128	8 (1–32)/32	2 (0–6)	11	256 (8–256)/256	0.25 (0.125–2)/1	8 (0–12)
KPC	0	-	-	-	8	128 (16–256)/256	0.25 (0.06–1)/1	7 (0–11)
KPC-2	0	-	-	-	13	256 (8–256)/256	0.125 (0.06–1)/1	10 (0–12)
CTX-M-1	0	-	-	-	5	32 (8–128)/128	0.06 (0.06–0.125)/0.125	9 (2–11)
CTX-M-9	0	-	-	-	11	4 (1–32)/16	0.06 (0.06–1)/0.25	5 (1–8)
CTX-M-15	0	-	-	-	23	128 (8–256)/256	0.125 (0.06–1)/0.5	9 (1–12)
AmpC	14	32 (8–128)/64	8 (2–64)/16	1 (0–4)	-	-	-	-

^aThe median (range) 2-fold MIC reduction over the entire taniborbactam range (0.03 to 32 mg liter⁻¹) tested. TAN, taniborbactam; -, not applicable.

DISCUSSION

The current study shows that addition of taniborbactam renders cefepime-resistant, ESBL-producing *Enterobacterales* and *P. aeruginosa* isolates susceptible to cefepime. The concentration of taniborbactam required to achieve this, however, is species and isolate dependent. It should be taken into account that the isolates included in the present study were selected for their cefepime resistance so as to study the concentration-effect profile of taniborbactam when combined with cefepime against β -lactamase-producing isolates, and are therefore not in any way representative of any current population of clinical isolates. The results obtained will be essential for ensuing pharmacokinetic/pharmacodynamic (PK/PD) studies.

The maximum effective taniborbactam concentration was determined for the different species. From this comparison, it can be concluded that the cefepime-taniborbactam combination has a more pronounced effect on *Enterobacterales* than on *P. aeruginosa* isolates. Seven *P. aeruginosa* isolates (25%) did not reach susceptibility according to EUCAST criteria (6), even at 32 mg liter⁻¹ taniborbactam. Of these 7 isolates, 3 harbored a VIM gene, whereas the remaining 4 harbored a combination of OprD and/or AmpC constitutive/inducible/transcript overexpressed, bla_{poxB}, or nitrocefinase activity. Of the *Enterobacterales*, all except 3 isolates reached susceptibility at 4 mg liter⁻¹ taniborbactam. Of these 3 isolates, 1 harbored a VIM gene and the other two harbored VIM-1, CMY-13, and qnrA1 genes. It seems that VIM and AmpC may reduce cefepime/taniborbactam activity for both *Enterobacterales* and *P. aeruginosa*, or else other underlying resistance mechanisms are responsible given the potent inhibition of those enzymes in engineered isolates (4).

The optimum inhibitory concentrations of the *in vitro* cefepime-taniborbactam combination were also explored, in which the ideal concentration of taniborbactam is one at which the majority of the isolates are inhibited by the combination. Of the 28 *P. aeruginosa* isolates, 86% were resistant (>8 mg liter⁻¹, EUCAST criteria) (6) for cefepime alone. Adding 4 mg liter⁻¹ taniborbactam brought the percentage of cefepime-resistant isolates down to 36%.

For *Enterobacterales* (n = 101 isolates), the presence of a KPC (n = 23 isolates), VIM (n = 24), CTX-M-1 (n = 5), CTX-M-2 (n = 3), CTX-M-3 (n = 2), or CTX-M-15 (n = 23) enzyme always rendered the isolate cefepime resistant (>4 mg liter⁻¹, EUCAST criteria). This was also the case for all except one of the isolates harboring SHV-1 (n = 5) or CTX-M-9 (n = 11). Addition of 1 mg liter⁻¹ taniborbactam reduced the percentage of cefepime-resistant isolates carrying any of these β -lactamases to 0% to 3% of all *Enterobacterales*

TABLE 4 Resistance specifications of clinical isolates included in the checkerboard assay

Species	No. of isolates	Resistance specifications ^{a,b}
<i>Pseudomonas aeruginosa</i>	28	KPC-2+, VIM, carbapenem resistant, AmpCcon, AmpCind
<i>Escherichia coli</i>	42	TEM-1, 12, 52, 84, SHV-12, OXA-1, CTX-M-1, 2, 3, 9, 14, 15, KPC-2, VIM, VIM-1, LEN, GES-1, qnrB2
<i>Klebsiella pneumoniae</i>	39	TEM-1, 84, SHV-1, 2, 2A, 5, 11, 12, 33, OXA-1, 48, CTX-M-1, 2, gr. 9, 15, 39, KPC, KPC-2, 3, VIM, VIM-1, LEN, GES-1, OmpK35red, OmpK36red, CMY-13, qnrA1
<i>Enterobacter cloacae</i>	16	TEM-1, 84, SHV-12, OXA-1, CTX-M-9, 15, 39, KPC, VIM
<i>Enterobacter aerogenes</i>	2	TEM-1, KPC-2, VIM-1
<i>Citrobacter freundii</i>	2	TEM-1, CTX-M-39, KPC-2

^aIsolates harbored one or multiple of the shown resistance mechanisms, but never all of them.

^bAmpCcon, AmpC derepressed; AmpCind, AmpC inducible.

isolates, rendering them susceptible (≤ 1 mg liter⁻¹, EUCAST criteria) or susceptible to increased exposure (2 to 4 mg liter⁻¹, EUCAST criteria). The greatest cefepime MIC reduction among *Enterobacterales* isolates was found for the most resistant isolates harboring TEM-1, VIM, KPC, KPC-2, or CTX-M-15 genes. Not all resistance could be attributed to the presence of these specific β -lactamases, however, as 4 mg liter⁻¹ taniborbactam only reduced the cefepime MIC of 90% of the *P. aeruginosa* isolates to 16 mg liter⁻¹, and to 2 mg liter⁻¹ for 90% of the *Enterobacterales* when 1 mg liter⁻¹ taniborbactam was added (Table 2).

Taniborbactam has shown significant activity in *Enterobacterales* against β -lactamases of all classes. Compared to other β -lactamase inhibitors, such as avibactam (9), taniborbactam was able to successfully inhibit isolates harboring the metallo- β -lactamase VIM, as well as KPC. This makes taniborbactam a promising inhibitor. Compared to avibactam concentrations required to restore ceftazidime's activity in 90% of the same collection of isolates, higher taniborbactam concentrations were required to restore cefepime activity against *P. aeruginosa* (8 versus 32 mg liter⁻¹), *E. coli* (2 versus 4 mg liter⁻¹), and *K. pneumoniae* (8 versus 16 mg liter⁻¹).

In conclusion, taniborbactam in combination with cefepime enhanced the overall *in vitro* activity against most *Enterobacterales* and *P. aeruginosa* isolates in this study. Four mg liter⁻¹ taniborbactam was required to reduce the cefepime MIC₉₀ of the *Enterobacterales* from 256 to 1 mg liter⁻¹, thus reversing resistance in all of them, whereas for *P. aeruginosa* isolates, the cefepime MIC₉₀ was reduced from 128 to 16 mg liter⁻¹, reducing resistance levels from 86% to 36%. Whether these concentrations of taniborbactam will prove safe and achievable *in vivo* will have to be explored in further experiments.

MATERIALS AND METHODS

Antibacterials. Cefepime (Maxipime, lot no. 143F005) and taniborbactam (VNRX5133-[HCl]₂; lot no. A/2191/31/1) were provided by Venatorx Pharmaceuticals, Inc. The drugs were reconstituted in sterile water for Maxipime or DMSO for VNRX-5133 to a stock solution of 10,240 mg liter⁻¹ and further dilutions were prepared in Mueller-Hinton broth (MHB; Becton, Dickinson and Company).

Bacterial isolates. We used 129 well-characterized ESBL-producing isolates, including 101 *Enterobacterales* (42 *Escherichia coli*, 39 *Klebsiella pneumoniae*, 16 *Enterobacter cloacae*, 2 *Enterobacter aerogenes*, and 2 *Citrobacter freundii*) and 28 *Pseudomonas aeruginosa* isolates (Table 4). Presumptive ESBLs were previously confirmed (10). Of the *Enterobacterales* isolates, 14.9% harbored a VIM gene and 20.8% harbored a KPC gene.

Susceptibility testing. MICs were determined by broth microdilution in accordance with ISO (11), CLSI (12), and EUCAST (7) methods. Working stock solutions of cefepime and taniborbactam were made in freshly prepared MHB (Becton, Dickinson and Company). For the checkerboard assay of cefepime with taniborbactam, both agents were tested in 2-fold dilutions over a range of 0.06 to 256 mg liter⁻¹ cefepime and 0.03 to 32 mg liter⁻¹ for taniborbactam. Two disposable plastic microdilution trays were required to cover all the different combinations and single compounds. Each well contained 50 μ l of 2 $\times$ the final concentrations of cefepime and taniborbactam alone and in combination. Every tray contained a negative broth and a positive growth control. Microdilution trays were stored at -80°C until use for a maximum of one month. On the day of the experiment, trays were thawed, inoculated with 50 μ l of 0.5×10^6 CFU/ml bacterial suspension prepared from a 24-h culture grown on a blood agar plate, and incubated at 37°C in ambient air. Each set of MIC determinations included three ATCC control strains, *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *K. pneumoniae* (ATCC 700603), and all showed cefepime-taniborbactam MICs within the current CLSI QC ranges (13). After 18 to 20 h, MICs were manually

read, using an angled mirror with a support stand, as the lowest concentration of the agent that competently inhibited visible growth.

Analysis. The median (range) MIC and MIC₉₀ of cefepime were calculated together with the percentage of resistant isolates (>4 mg liter⁻¹ for *Enterobacterales* and >8 mg liter⁻¹ for *P. aeruginosa*) (6) at different concentrations of taniborbactam. The median (range) and 90th percentile concentration of taniborbactam required to obtain a given MIC of cefepime were calculated for each cefepime MIC. The effect of taniborbactam on the MIC of cefepime was expressed as the number of 2-fold dilution decreases compared to the MIC of cefepime alone. The effect of taniborbactam on the activity of cefepime was graphed as cumulative percentage isolates inhibited. The susceptibility to cefepime was interpreted according to EUCAST criteria (6).

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