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In vitro activity of Nacubactam, a novel dual action diazabicyclooctane, alone and with meropenem, against beta-lactamase-positive *Enterobacteriaceae*

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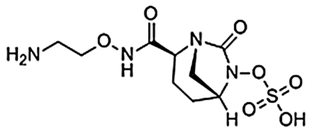
INTRODUCTION

The aim of the current study was to investigate the activity of nacubactam (OP0595, RO7079901, RG6080) in combination with meropenem against recent clinical *Enterobacteriaceae* isolates that are positive for beta-lactamase genes. Nacubactam is a novel diazabicyclooctane beta-lactamase inhibitor (Fig. 1). Other members of the class include avibactam, relebactam and zidebactam.

Nacubactam has been shown to have a dual mode of action: (i) as an inhibitor of serine beta-lactamases (classes A, C, and D), resulting in protection of the partner beta-lactam; and (ii) as an inhibitor of penicillin-binding protein 2 (PBP2) of *Enterobacteriaceae*, resulting in antibacterial activity that can additionally translate to an "enhancer" effect when partnered with beta-lactams. [1-4].

Figure 1. Structure of nacubactam



MATERIALS & METHODS

Isolates originating from US and European hospitals between 2013 and 2016 were investigated (n=1,553). Isolates were screened for genes encoding β-lactamases by PCR and full-gene DNA sequencing.

Class A (non-carbapenemase) beta-lactamase isolates only possessed β-lactamases from this class (Table 1), whereas isolates β-lactamases from other classes often contained multiple enzymes (Table 1).

MICs were determined by broth microdilution following CLSI methodology [5]. Meropenem and nacubactam were tested as a ratio (1:1 and 2:1) and also with fixed Nacubactam (2 and 4 mg/L).

Susceptibility to comparator compounds were determined using CLSI breakpoints where available [6].

RESULTS

Table 1. Isolates tested by beta-lactamase-class

Ambler Class	Species	Beta-lactamase	No.
Class A (non-carbapenemase) (N=577)	*E. aerogenes*	SHV-30	1
	E. cloacae	CTX-M-15	1
	E. coli	CTX-M-15	279
		CTX-M-27	47
		CTX-M-14	38
		CTX-M-55	7
		CTX-M-1	5
		Other[a]	27
	K. oxytoca	SHV-7	7
		SHV-12	4
		CTX-M-15	3
		SHV-12 & CTX-M-15	1
		TEM-26	1
	K. pneumoniae	CTX-M-15	69
		SHV-12	32
		CTX-M-14	4
		SHV-2A	4
		CTX-M-27	3
		Other[b]	23
	P. mirabilis	CTX-M-15	4
		CTX-M-14	2
		TEM-10	2
		TEM-155	2
		TEM-92	2
		Other[c]	5
	S. marcescens	CTX-M-3	3
		SHV-12	1
KPC (n=381)	*C. freundii*	KPC-3*	23
		KPC-2*	2
	C. koseri	KPC-3	1
	E. aerogenes	KPC-2*	1
	E. asburiae	KPC-2*	1
	E. cloacae	KPC-3*	4
		KPC-2	2
	E. coli	KPC-2*	2
		KPC-3*	9
		KPC-18	1
	K. oxytoca	KPC-2*	4
		KPC-3*	2
		KPC-12*	1
	K. pneumoniae	KPC-3*	153
		KPC-12*	108
		KPC-2*	81
		KPC-9*	2
	S. marcescens	KPC-2	2
		KPC-3	2
GES carbapenemase (n=6)	*E. cloacae*	GES-6*	1
	K. oxytoca	GES-6*	2
	K. pneumoniae	GES-20	2
	K. pneumoniae	GES-6*	1
Class B (n=123)	*C. freundii*	VIM-31*	5
		VIM-1*	4
		VIM-32*	1
	C. sedlakii	NDM-1*	1
	E. asburiae	VIM-2*	1
	E. cloacae	VIM-1*	15
		NDM-1*	14
		VIM-5*	14
	E. coli	NDM-1*	2
	K. oxytoca	VIM-1*	2
	K. pneumoniae	NDM-1*	42
		VIM-1*	4
		VIM-26*	2
		VIM-4*	2
		NDM-1 & VIM-1*	1
		VIM-2*	1
		VIM-3*	1
	P. mirabilis	NDM-1*	2
		VIM-1*	1
	P. stuartii	VIM-1*	2
	S. marcescens	NDM-1	3
		VIM-1	3
Class C (n=254)	*C. freundii*	CMY-TYPE*	15
		CMY-48*	1
	E. asburiae	ACT-TYPE	18
		MIR-TYPE	9
	E. cloacae	ACT-TYPE*	129
		MIR-TYPE	7
		DHA-1 & ACT-TYPE	1
	E. kobei	MIR-TYPE	5
	E. ludwigii	ACT-TYPE	1
	E. coli	CMY-2*	32
		CMY-TYPE*	17
		DHA-TYPE	3
	H. alvei	ACC-TYPE	1
	K. pneumoniae	CMY-6	1
		CMY-2	1
		DHA-1	1
		MIR-3	1
	M. morganii	DHA-TYPE	7
	P. mirabilis	ACC-4	1
		CMY-TYPE*	3
Class D (n=212)	*C. freundii*	OXA-48*	1
	E. aerogenes	OXA-48	2
	E. cloacae	OXA-48	6
		OXA-162	1
	E. coli	OXA-181*	17
		OXA-48*	8
	K. oxytoca	OXA-48*	6
	K. pneumoniae	OXA-48*	157
		OXA-232	5
	M. morganii	OXA-48*	1
	P. mirabilis	OXA-48	1
	R. ornithinolytica	OXA-48*	3
	R. planticola	OXA-48	3

*Isolates contain more than 1 enzyme class

[a]*E. coli* Other Class A: CTX-M-3 (4), SHV-12 (4), SHV-7 (3), CTX-M-15 & CTX-M-33 (2), CTX-M-101 (1), CTX-M-134 (1), CTX-M-14-19 (1), CTX-M-15 &CTX-M-14 (1), CTX-M-15-new variant (1), CTX-M-181 (1), CTX-M-65 (1), CTX-M-71 (1),CTX-M-9 (1), SHV-2 (1), SHV-5 (1), TEM-12 (1), TEM-28 (1) & VEB-1 (1).

[b]*K. pneumoniae* Other Class A: SHV-12 & CTX-M-15 (3), CTX-M-55 (2), SHV-2 (2), SHV-5 (2), CTX-M-1 (1), CTX-M-65 (1), SHV-12-38 (1), SHV-189 (1), SHV-2 & CTX-M-27 (1), SHV-238V (1), SHV-28 (1), SHV-2A & CTX-M-27 (1), SHV-55 (1), SHV-7 (1), SHV-7 & SHV-26 (1), SHV-new variant (1), TEM-12 & CTX-M-15 (1), SHV-non-ESBL (1).

[c]*P. mirabilis* Other Class A: CTX-M-1 (1), CTX-M-27 (1), CTX-M-65 (1), PER-1 (1), TEM-92 & PER-1 (1).

Table 2. Summary susceptibility and MIC data[a]

Drug	CLSI Breakpoints (Sus|Int|Res)	Group	Percentage			MIC (mg/L)			
			Sus	Int	Res	MIC_{50}	MIC_{90}	MIN	MAX
Meropenem	≤1 | 2 | ≥4	ALL	56.6	3	40.4	0.5	128	0.015	> 256
		Class A	99.1	0.5	0.3	0.03	0.12	0.015	64
		Class B	0.8	3.3	95.9	32	> 256	0.25	> 256
		Class C	95.3	1.2	3.5	0.12	0.5	0.015	16
		Class D	28.8	15.1	56.1	4	64	0.12	256
		KPC	0.3	0.8	99	64	256	0.06	> 256
Meropenem/ Nacubactam (1:1 ratio)	None	ALL	-	-	-	0.12	2	≤ 0.004	> 256
		Class A	-	-	-	0.03	0.06	≤ 0.004	> 256
		Class B	-	-	-	2	32	0.25	> 256
		Class C	-	-	-	0.06	0.25	≤ 0.004	> 256
		Class D	-	-	-	1	4	0.12	8
		KPC	-	-	-	1	2	0.03	> 256
Meropenem/ Nacubactam (fixed 2 mg/L)	None	ALL	-	-	-	≤ 0.004	0.5	≤ 0.004	4
		Class A	-	-	-	≤ 0.004	0.015	≤ 0.004	32
		Class B	-	-	-	0.008	64	≤ 0.004	4
		Class C	-	-	-	≤ 0.004	0.06	≤ 0.004	2
		Class D	-	-	-	0.25	8	≤ 0.004	> 256
		KPC	-	-	-	0.008	0.5	≤ 0.004	2
Meropenem/ Nacubactam (2:1 ratio)	None	ALL	-	-	-	0.25	4	≤ 0.004	64
		Class A	-	-	-	0.03	0.06	≤ 0.004	32
		Class B	-	-	-	4	32	0.25	8
		Class C	-	-	-	0.06	0.25	≤ 0.004	> 256
		Class D	-	-	-	2	8	0.12	4
		KPC	-	-	-	1	4	0.12	32
Meropenem/ Nacubactam (fixed 4 mg/L)	None	ALL	-	-	-	≤ 0.004	0.25	≤ 0.004	8
		Class A	-	-	-	≤ 0.004	0.015	≤ 0.004	2
		Class B	-	-	-	≤ 0.004	64	≤ 0.004	> 256
		Class C	-	-	-	≤ 0.004	0.015	≤ 0.004	0.5
		Class D	-	-	-	0.12	4	≤ 0.004	64
		KPC	-	-	-	≤ 0.004	0.25	≤ 0.004	2
Nacubactam	None	ALL	-	-	-	2	> 32	0.5	> 32
		Class A	-	-	-	2	> 32	1	> 32
		Class B	-	-	-	4	> 32	1	> 32
		Class C	-	-	-	2	> 32	0.5	> 32
		Class D	-	-	-	32	> 32	1	> 32
		KPC	-	-	-	4	> 32	1	> 32
Piperacillin/ Tazobactam	≤16 |32-64| ≥128	ALL	30.9	10.2	58.9	> 128	> 128	≤ 0.25	> 128
		Class A	66.6	17	16.5	8	> 128	≤ 0.25	> 128
		Class B	0	2.4	97.6	> 128	> 128	64	> 128
		Class C	35.8	18.1	46.1	64	> 128	0.5	> 128
		Class D	1.4	2.4	96.2	> 128	> 128	8	> 128
		KPC	0.3	1.6	98.2	> 128	> 128	2	> 128

[a]Sus, susceptible; Int, intermediate; Res, resistant; MIC 50/90, MIC to inhibit 50%/90% of the bacterial population; MIN, minimum MIC; MAX, maximum MIC

Figure 2. MIC distribution for nacubactam against Class B, Class D and KPC isolates.

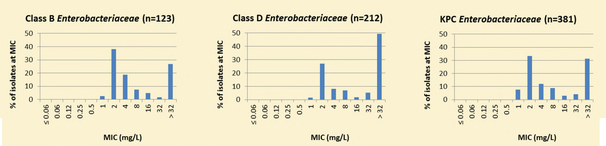
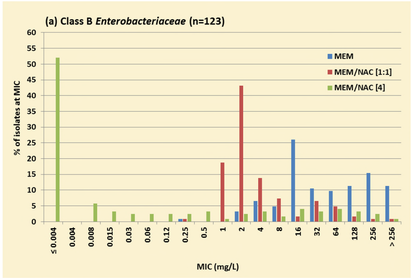
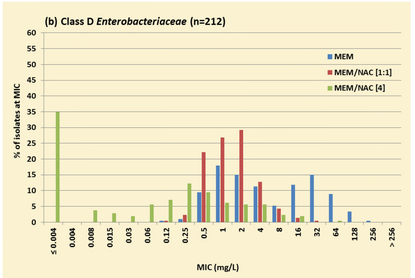
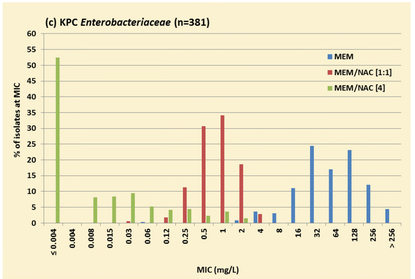


Figure 3. MIC distribution for meropenem (MEM) and meropenem/nacubactam (NAC) (1:1 ratio and fixed concentration at 4 mg/L) against (a) Class B, (b) Class D and (c) KPC isolates.







RESULTS SUMMARY

- Nacubactam showed activity alone with MIC_{50} of 2 or 4 mg/L against most isolate sub-groups but lower activity against Class D isolates (Table 2 & Figure 2).
- Meropenem/nacubactam demonstrated better activity against class B-, class D- and KPC-producing *Enterobacteriaceae* than meropenem alone (Table 2 & Figure 3).
- Meropenem/nacubactam is active against ESBL- and class C- producing isolates showing 2-fold lower MIC_{90} than meropenem alone (Table 2).
- MICs derived from a fixed ratio method are less biased towards the direct antibacterial activity of nacubactam and better reflect the contributions of both components of the combination.

CONCLUSIONS

- Nacubactam works as a beta-lactamase inhibitor and as an antibacterial agent.
- These combined activities translate into the observed excellent in vitro activity of meropenem/nacubactam against beta-lactamase-producing *Enterobacteriaceae*.
- A ratio of 1:1 is the method of choice for clinical susceptibility testing (CLSI QC ranges have been approved and will be published in January 2019).

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