ETX0462, a Novel Non-β-Lactam PBP Inhibitor, Does Not Induce AmpC Expression

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Abstract

Background:
AmpC β-lactamases are a diverse and clinically important family of Ambler Class C bacterial enzymes encoded on the chromosomes of some members of the Enterobacterales order as well as other clinically relevant organisms such as *Pseudomonas aeruginosa*. In many of these organisms, AmpC is produced at low levels but exposure to certain cell-wall targeting drugs can cause hyper-induction. AmpC induction upon exposure to antibiotic therapy is an important consideration for physicians, as it can lead to resistance even in infections caused by initially susceptible isolates. ETX0462 is a novel, rationally designed unsaturated diazabicyclooctane inhibitor of cell wall synthesis that targets penicillin binding proteins. The goal of this study was to determine whether ETX0462 induces *ampC* expression in *P. aeruginosa* and a representative *Enterobacterales* species, *Enterobacter cloacae*, as is seen with other cell wall targeting antibiotics.

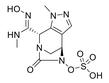
Methods:
Susceptibility to ETX0462 and comparators was measured by broth microdilution following the Clinical and Laboratory Standards Institute guidelines. Three clinical isolates each of *P. aeruginosa* and *E. cloacae* were grown to mid-log (A_{600} 0.5-0.6) at 35°C with aeration and exposed to either ETX0462 at 0.25X MIC or cefoxitin at 50 mg/L (positive control) for 30 minutes. RNA was extracted and RT-PCR was performed to quantify *ampC* and reference RNA transcripts. Fold-change of *ampC* expression upon drug treatment was determined using the comparative CT method.

Results:
Consistent with previous studies, cefoxitin induced *E. cloacae ampC* transcript levels 68- to 576-fold higher compared to the no-drug control. ETX0462, on the other hand, did not induce *ampC* expression in the 3 clinical isolates of *E. cloacae*, with no change in *ampC* RNA transcript levels compared to the no-drug control. For *P. aeruginosa*, cefoxitin robustly induced *ampC* expression, with all three strains showing greater than 1000-fold induction after drug treatment. ETX0462 showed minimal induction of *P. aeruginosa ampC*, with only 2.2- to 2.6-fold increases across the 3 strains tested.

Conclusions:
ETX0462 does not substantively induce AmpC expression in representative clinical isolates of *E. cloacae* and *P. aeruginosa*, and therefore ETX0462 exposure is not expected to drive resistance through this clinically important mechanism of β-lactam resistance.

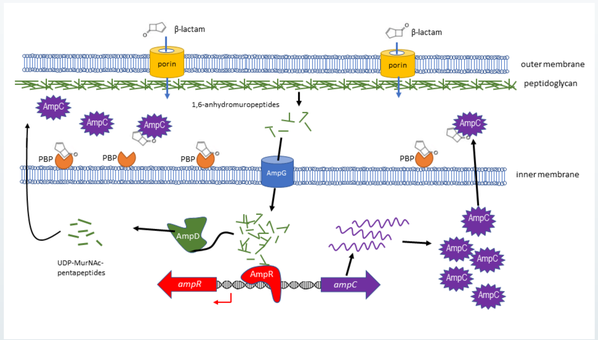
Introduction

ETX0462 is a rationally designed, non β-lactam inhibitor of PBP1a and PBP3 with potent, broad-spectrum Gram-negative activity in vitro and in vivo[1].



The AmpC family of β-lactamases drive resistance to many β-lactam antibiotic classes. In many organisms, AmpC is expressed at low levels but is inducible by exposure to certain β-lactam classes[2].
Regulation of *ampC* induction is complex and can vary in different organisms. In general, disruption of murein biosynthesis due to inhibition of penicillin binding proteins by a β-lactam compound leads to an accumulation of cell wall oligopeptides (Figure 1). These oligopeptides bind to, and induce a conformational change in AmpR, a transcriptional regulator, leading to *ampC* induction. Some organisms, like *E. coli* lack an *ampR* gene and hence *ampC* is inducible by other mechanisms[3]. The potential risk of AmpC induction upon exposure to β-lactam therapy is an important consideration for infectious disease physicians, as it can lead to resistance, even in infections caused by initially susceptible isolates[4]. In fact, many specialists discourage the use of expanded spectrum (i.e, third generation) cephalosporins alone for the treatment of organisms posing the greatest risk of *ampC* induction (for example *E. cloacae* infections)[4]. The goal of this study was therefore to evaluate whether exposure to ETX0462 had any effect on *ampC* expression in *E. cloacae* or *P. aeruginosa* as compared to a positive control compound, cefoxitin[5,6].

Figure 1. Induction of *ampC* in *P. aeruginosa*[7]



Fold-change in *ampC* RNA transcripts upon drug treatment

Strain ID	Species (ETX0462 MIC, µg/mL)	Fold-change over no drug treatment	
		ETX0462	Cefoxitin
ARC3962	*E. cloacae* (4)	-1.0 ± 0.2	576.8
ARC3963	*E. cloacae* (2)	-1.0 ± 0.1	471.4
ARC3974	*E. cloacae* (1)	-1.2 ± 0.1	68.3
ATCC 27853	*P. aeruginosa* (1)	2.2 ± 0.8	1404.3
PAO1	*P. aeruginosa* (0.5)	2.4 ± 1.2	2384.9
ARC3506	*P. aeruginosa* (2)	2.6 ± 1.0	1587.1

ETX0462 has Potent Antibacterial Activity Against a Panel of Geographically Diverse Gram-negative Clinical Isolates from 2019

Species	N	Antibiotic	MIC (µg/mL)			%S
			Range	MIC_{50}	MIC_{90}	
A. baumannii	**298**	MEM	≤0.06 - >64	64	>64	30
		ETX0462	0.25 - 32	2	8	79
P. aeruginosa	**300**	MEM	≤0.06 - >16	2	>16	78
		ETX0462	≤0.06 - 16	0.25	2	99
S. maltophilia	**150**	LVX	0.5 - >32	4	16	77
		ETX0462	0.5 - 8	2	2	100
***Citrobacter* spp.**	**151**	MEM	≤0.06 - >64	≤0.06	≤0.06	93
		ETX0462	0.12 - >32	0.5	8	87
E. cloacae	**162**	MEM	≤0.06 - >64	≤0.06	0.25	93
		ETX0462	0.12 - 16	0.5	8	82
E. coli	**300**	MEM	≤0.06 - 128	≤0.06	≤0.06	97
		ETX0462	0.12 - >32	0.25	1	96
K. pneumoniae	**300**	MEM	≤0.06 - >64	≤0.06	32	85
		ETX0462	0.25 - 32	0.5	8	88
***Proteus* spp.**	**152**	MEM	≤0.06 – 0.25	≤0.06	0.125	100
		ETX0462	0.12 - 4	0.5	1	100
M. morganii	**43**	MEM	≤0.06 - >64	0.125	0.125	100
		ETX0462	1 - 16	2	4	95
***Providencia* spp.**	**61**	MEM	≤0.06 - >64	≤0.06	64	44
		ETX0462	0.12 - 8	0.5	2	98
***Serratia* spp.**	**45**	MEM	≤0.06 - >64	≤0.06	0.125	96
		ETX0462	0.5 8	1	2	98

Study conducted by IHMA-Europe for CARB-X cross-project susceptibility study. MEM, meropenem; LVX, levofloxacin. %S, percent susceptible according to 2021 CLSI breakpoints except for ETX0462, which is based on a preliminary breakpoint of 4 µg/mL.

Conclusions

- Exposure to ETX0462 did not result in upregulation of *ampC* expression in three representative isolates of *E. cloacae* or *P. aeruginosa,* suggesting AmpC upregulation may not be a source of clinical resistance.
- The in vitro activity of ETX0462 and comparators was tested against a panel of diverse MDR Gram-negative isolates collected from around the world in 2019. In this study, ETX0462 had potent antibacterial activity versus *Enterobacterales* and non-fermenter species and generally performed better than comparator agents.
- The MIC_{90} values for ETX0462 versus *Enterobacterales* species ranged from 1 - 8 µg/mL while the meropenem MIC_{90} values ranged from ≤0.06 to 64 µg/mL.
- ETX0462 MIC_{90} values against the non-fermenters ranged from 2 - 8 µg/mL while meropenem had a range of 16 - >64 µg/mL for the same set of isolates.
- Results from this study highlight the potential for ETX0462 to be a promising broad-spectrum therapeutic with a novel mechanism of action to treat infections caused by contemporary drug-resistant Gram-negative bacteria.

References

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7. Adapted from Figure 2 of Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol Rev*. 2009;22(4):582-610.

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