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The Novel, Non-β-lactam PBP Inhibitor, ETX0462, Retains Potent Antibacterial Activity Across Isogenic Panels of Gram-negative Resistant Determinants

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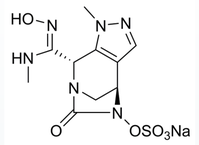
Background

Effective antimicrobial therapies for multi-drug resistant (MDR) Gram-negative infections are desperately needed. ETX0462 is a novel, rationally designed, non-β-lactam diazabicyclooctane (DBO) covalent inhibitor of Gram-negative penicillin binding proteins (PBPs). ETX0462 exhibits potent antibacterial activity, low frequency of resistance and excellent *in vivo* efficacy in preclinical infection models. We evaluated the *in vitro* antibacterial activity of ETX0462 against numerous isogenic strain panels to determine the effect that the most common β-lactam resistance mechanisms (permeation, efflux, and β-lactamases (BLAs)) in Gram-negative pathogens would have on the antibacterial activity of ETX0462.



Methods

Isogenic panels of mutants with deletions in genes encoding porins or efflux systems were created in the following parent strains: BW27783 (K-12) and ATCC 25922 (*Escherichia coli* (*Ec*)), NVT101 and KPNIH1(*Klebsiella pneumoniae* (*Kp*)) and PAO1(*Pseudomonas aeruginosa* (*Pa*)). The isogenic panels representing all four Ambler classes of BLAs were made in either *Ec* (ARC523) or *Pa* (PAO1Δ*ampC*Δ*poxB*) parent strains transformed with expression vectors that constitutively overexpressed an individual BLA gene of interest. Susceptibility to ETX0462 and comparator agents was measured by broth microdilution following Clinical and Laboratory Standards Institute (CLSI) guidelines.

Activity of ETX0462 vs. comparator agents against *P. aeruginosa* isogenic panels

strain ID	ARC545	ARC5464	ARC5998
genotype	PAO1 wildtype	PAO1 Δ5efflux*	PAO1 Δ5porin**
Meropenem	0.5	0.5	4
Ceftazidime	2	1	2
Aztreonam	8	0.5	8
Ciprofloxacin	0.13	≤0.03	0.13
Cefepime	2	0.25	4
Amikacin	4	1	4
ETX0462	1	0.25	1

*Δ*mexAB-oprM*;Δ*mexEF-oprN*;Δ*mexXY*;Δ*mexCD-oprJ*;Δ*mexJKL*
**Δ*oprD*Δ*opdP*Δ*opdT*Δ*odpB*Δ*opdC*

	MIC (mg/L)				
P. aeruginosa strain	Piperacillin	Meropenem	Aztreonam	Ceftazidime	ETX0462
PAO1 Δ*ampC*Δ*poxB* parent	2	0.5	2	1	0.5
Class A-overexpressing					
CTX-M-15	>64	0.5	64	64	0.5
GES-11	16	1	16	>64	1
KPC-2	>64	64	>64	64	2
KPC-3	>64	32	>64	>64	1
PER-1	32	1	>64	>64	0.5
SHV2a	>64	1	64	64	1
TEM-1	>64	0.5	4	2	0.5
Class B-overexpressing					
NDM-1	>64	>64	4	>64	0.5
VIM-1	>64	>64	2	>64	0.5
Class C-overexpressing					
AmpC	>64	1	16	32	2
Class D-overexpressing					
OXA-1	>64	1	8	2	1
OXA-23	>64	32	4	2	1
OXA-40	>64	>64	4	2	0.5
OXA-58	>64	8	4	2	2

Activity of ETX0462 vs. comparator agents against *E. coli* and *K. pneumoniae* isogenic panels

		MIC (mg/L)		
	Strain	Aztreonam	Ceftazidime	ETX0462
K. pneumoniae	NVT1001 parent	0.03	0.25	1
	NVT1001 ΔompK35	0.125	0.5	1
	NVT1001 ΔompK36	0.0625	0.25	1
	NVT1001 ΔompK35ΔompK36	0.25	1	1
	NVT1001ΔramR (efflux-UP)	0.125	1	1
	NVT1001ΔompK35 ΔompK36ΔramR	0.25	1	2
	ARC6715 parent	0.5	2	2
	ARC6715ΔacrB	0.25	0.5	1
E. coli	K-12 parent	0.25	1	4
	K-12 ΔompFΔompC ΔompA	8	8	2
	ARC4 parent	0.125	0.25	1
	ΔtolC	0.125	0.25	0.25

	MIC (mg/L)		
E. coli strain	Piperacillin	Ceftazidime	ETX0462
ARC523	4	0.5	1
Class A-overexpressing			
CTX-M-15	>64	16	1
GES-11	8	32	1
KPC-3	64	16	1
PER-1	8	64	1
SHV2a	>64	16	2
TEM-1	>64	1	2
Class B-overexpressing			
NDM-1	>64	>64	2
VIM-1	>64	>64	1
Class C-overexpressing			
AmpC	64	8	2
Class D-overexpressing			
OXA-1	>64	1	2
OXA-23	>64	2	1
OXA-40	>64	2	1
OXA-58	>64	2	1

Results

The MIC of ETX0462 did not change in isogenic mutants lacking all major porins relative to the *Ec*, *Kp* or *Pa* parent strains. No change in MIC was observed in KPNIH1Δ*acrB* or NVT101Δ*ramR* efflux mutants compared to parent *Kp* strains, and the MIC only decreased by two-fold in ATCC 25922 Δ*tolC* or PAO1 Δ*mexAB-oprM*Δ*mexCD-oprJ*Δ*mexJK*Δ*mexXY*Δ*mexEF-oprN* mutant strains. Against the 17 member BLA-overexpressing isogenic panels, the ETX0462 MICs ranged between 0.5 – 2 mg/L (vs. the parent *Pa* and *Ec* MICs of 0.5 and 1 mg/L, respectively).

Conclusions

These results show that ETX0462:

- does not rely solely on any major porin for cellular permeation in *Ec*, *Kp* or *Pa*
- is unaffected by efflux in *Kp* and is a weak substrate for *Ec* and *Pa* efflux systems, and
- is resistant to degradation by BLAs from any Ambler class.

Taken together, these results suggest that ETX0462 is a promising new therapy for the treatment of drug-resistant Gram-negative infections.

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