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ETX0462, a Novel Non β-lactam PBP Inhibitor, Is Bactericidal Against Gram Negative Pathogens with a Low Propensity for Mutant Generation

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Abstract

Background: ETX0462 is a novel, rationally designed, diazabicyclooctane (DBO) inhibitor of bacterial penicillin-binding proteins 1a and 3 with potent *in vitro* and *in vivo* activity against multidrug-resistant Gram-negative pathogens, including *Pseudomonas aeruginosa* (Pa), *Klebsiella pneumoniae* (Kp), *Acinetobacter baumannii* (Ab) and *Escherichia coli* (Ec). The potential of ETX0462 to generate resistance was determined by spontaneous frequency of resistance (FOR) and serial passage studies. Additionally, ETX0462 was characterized in time kill studies against multiple, diverse clinical Pa isolates.
Methods: The FOR of ETX0462 for clinical Pa, Ec, Ab and Kp isolates was measured after 48 hours of growth at 35°C at 4X and 8X the minimal inhibitory concentration (MIC) against a starting inoculum of ~10^9-10^{10} colony-forming units (CFU)/ml. During serial passage studies, four Pa isolates were passaged in the presence of increasing concentrations of ETX0462 over the course of ten days, compared to ceftolozane. Concentrations selected for daily passages were the highest concentration that allowed growth. The genomes of stably resistant isolates were sequenced. Kill kinetics of ETX0462 was measured against four isolates by exposing logarithmically growing cultures to 4X and 8X the MIC, according to CLSI guidelines, with the modification that the tubes were shaken. Samples were taken over a 24-hour period and CFU/mL was measured. Cidality was defined as a ≥3 $\log_{10}$ CFU reduction.
Results: The FOR of ETX0462 was <10^{-9} for all strains tested at both 4X and 8X the MIC. In serial passage studies, stably resistant isolates were raised after ≥5 days with slight decreases in susceptibility (≤4X MIC increase). In contrast, high levels of resistance (>128X) were observed after 5 days in the presence of ceftolozane. The modest decrease in ETX0462 susceptibility mapped to efflux-related genes, with no observed cross-resistance to other agents. ETX0462 was bactericidal at both 4X and 8X the MIC against four Pa clinical isolates.
Conclusions: ETX0462 was bactericidal against multiple clinical isolates of Pa at both 4X and 8X the MIC. No spontaneously resistant mutants could be isolated during FOR studies at either 4X or 8X MIC. Strains with only modest decreases in ETX0462 susceptibility were isolated from serial passage studies. Resistance mapped to efflux systems without conferring cross-resistance to other antibacterial agents. These results support the further development of ETX0462 for the treatment of multidrug-resistant Gram-negative infections.

Introduction

A new class of non-β-lactam β-lactamase inhibitors (BLIs), the diazabicyclooctanes (DBOs) was introduced with the approval of avibactam in 2015[1]. Some DBO BLIs, including avibactam, durlobactam, zidebactam and nacubactam, have intrinsic antibacterial activity due to inhibition of PBP2[2,3,4,5,6]. ETX0462 is a DBO with antibacterial activity through PBP1a and PBP3.
The goals of this study were to:
1 - Examine the frequency of spontaneous resistance and mechanism of resistance of ETX0462 against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*.
2 - Determine the rate of bacterial cell death of *P. aeruginosa* in the presence of ETX0462. Four different *P. aeruginosa* clinical isolates were selected for testing based on their varied gene content, which had been previously determined by whole genome sequencing.
3 - Serially passage four strains of *P. aeruginosa* over a period of 10 days in the presence of increasing concentrations of ETX0462 to induce stepwise resistance to ETX0462. Clinical isolates were selected for testing based on their varied gene content, previously determined by whole genome sequencing.

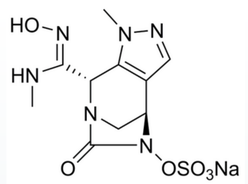
Frequency of Resistance

	Compound	Strain Description	Multiple of MIC	Agar MIC (mg/L)	Avg. Frequency of Resistance after 48 hours
P. aeruginosa	ETX0462	ATCC 27853	4X	0.5	<1.5E-10
			8X	0.5	6.0E-09
	Aztreonam	ATCC 27853	4X	4	1.6E-08
			8X	4	6.1E-09
	ETX0462	PDC-35; OXA-10; OXA-488; VEB-1; MexT [G79fs]; MexZ [G169fs]; OpdF [ΔH25-G30]; PA1025 (OprD-family porin) [G71fs]; OprD [P405fs];	4X	1	3.8E-09
			8X	1	<1.2E-10
	ETX0462	OXA-486, PDC-24; OpdR [E181K]; PA0032 (transcriptional regulator) [G2*]	4X	0.25	<1.1E-10
			8X	0.25	<1.1E-10
	Aztreonam	OXA-486, PDC-24; OpdR [E181K]; PA0032 (transcriptional regulator) [G2*]	4X	0.25	4.9E-08
			8X	0.25	4.2E-08
E. coli	ETX0462	KPC-2; TEM-1; OXA-1; AmpC	4X	1	<6.1E-08
			8X	1	<6.1E-08
K. pneumoniae	ETX0462	SHV-33; TEM-1; OXA-1; DHA-1	4X	32	5.6E-09
			8X	32	5.6E-09
	ETX0462	NDM-1; TEM-1; SHV-11; CTX-M-15; OmpK35 [K3fs]	4X	8	<4.7E-09
			8X	8	<4.7E-09
A. baumannii	ETX0462	ADC-158; OXA-98	4X	1	<3.3E-09
			8X	1	<3.3E-09
	ETX0462	ADC-99 [N379S]; OXA-259	4X	2	<4.6E-09
			8X	2	<4.6E-09

The frequency of spontaneous resistance to ETX0462 was tested in triplicate against *P. aeruginosa, E. coli, K. pneumoniae* and *A. baumannii* clinical isolates. Selection plates were incubated up to 48 hours to see if colonies emerged. Colonies were passaged on drug-free media three times and tested for susceptibility to confirm stable resistance.

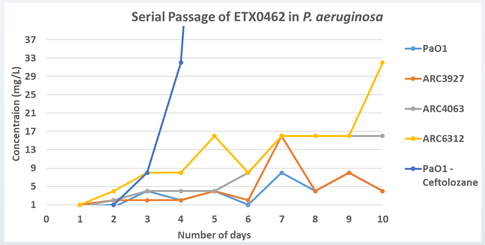
None of the isolates were found to be stably resistant to ETX0462.

ETX0462



Serial Passage

A serial passage in *P. aeruginosa* was tested over the course of 10 days. Decreases in susceptibility to ETX0462 changed over time; however, the degree of change was strain dependent and was fairly gradual. For two of the strains, the ETX0462 concentration only increased 4-fold, one strain increased by 16-fold and one strain increased 32-fold by day 10.
This is in contrast to results observed with the control agent, ceftolozane, which had larger single-step increases and reached 128 µg/mL by day 5.
For ETX0462 in strain ARC4063, there was a color change of the bacteria on day 3 from the normal 'green' color of *P. aeruginosa* to a brownish color, which remained throughout the study.



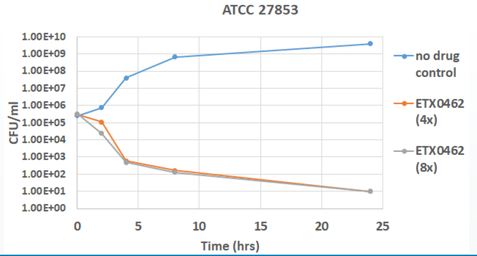
Serial Passage Day	Genotype/Genetic Changes from Parental Strain	ETX0462 MIC (mg/L)
	Parent (PaO1)	1
Day 10	AtpD (ATP synthase subunit beta) [A299E]; NalD (repressor - mexAB operon) [T11N];	4
	Parent (OXA-4; AmpC; PoxB)	2
Day 6	Glyoxykase / bleomycin resistance protein (metal binding enzyme) [T82I]; 18kb deletion within a lysogenic phage; 20kb deletion	4
	Parent (AmpC; PoxB; VEB-1a)	1
Day 6	>140kb deletion; several ~50kb deletions	8
	Parent (OXA-4, OXA-396, PER-1, PDC-8; oprD [tpn insert before K133]; MexZ [frameshift after A88])	4
Day 2	no variants found	16
Day 9	MexB (efflux transporter) [G288S]	64

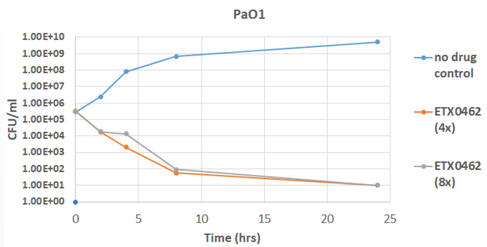
Killing Kinetics

Four *P. aeruginosa* isolates were used to evaluate the killing kinetics of ETX0462 under shaking conditions. The genotypes was confirmed using whole genome sequencing. Change in $\log_{10}$ CFU/ml values were calculated as an average of three independent experiments. There was no rebound in growth observed for any of the strains after 24 hours (Graphs for 2 strains not shown). A ≥3-log reduction in viable cells was observed in the presence of 4x and 8x MIC ETX0462. Therefore, ETX0462 is bactericidal.

$\log_{10}$ change in CFU/ml

P. aeruginosa Isolate	No Drug Control		ETX0462 4X MIC		ETX0462 8X MIC	
	8 hours	24 hours	8 hours	24 hours	8 hours	24 hours
ATCC 27853	-3.41	-4.2	3.26	4.48	3.42	4.52
PaO1	-3.36	-4.25	3.75	4.5	3.52	4.48
PDC-35; OXA-10; OXA-488; VEB-1; MexT [G79fs]; MexZ [G169fs]; OpdF [delta H25-G30]; PA1025 (OprD-family porin) [G71fs]; OprD [P405fs];	-3.14	-4.2	3.41	4.13	3.11	4.28
AmpC; PoxB; VIM-2; OXA-4; OprD [K389fs]	-3.08	-4.3	3.65	4.23	3.61	4.36





Conclusions

- **ETX0462 was found to be bactericidal against four clinical *P. aeruginosa* isolates and there was no rebound in growth observed after 24 hours.**
- **The frequency of spontaneous resistance in *P. aeruginosa, E. coli, K. pneumoniae* and *A. baumannii* at 4x and 8x ETX0462 MIC was found to be very low. These experiments failed to generate any stable resistant isolates.**
- **It was possible to force mutations in *P. aeruginosa* with ETX0462, but only in serial passage experiments with stepwise increases in ETX0462 concentration. These isolates with reduced susceptibility to ETX0462 were found to have acquired mutations in efflux systems.**

References

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