ETX0462, a novel, non-β-lactam PBP inhibitor, has potent antibacterial activity against a panel of geographically diverse Gram-negative bacterial clinical isolates

John O'Donnell[1] on behalf of:

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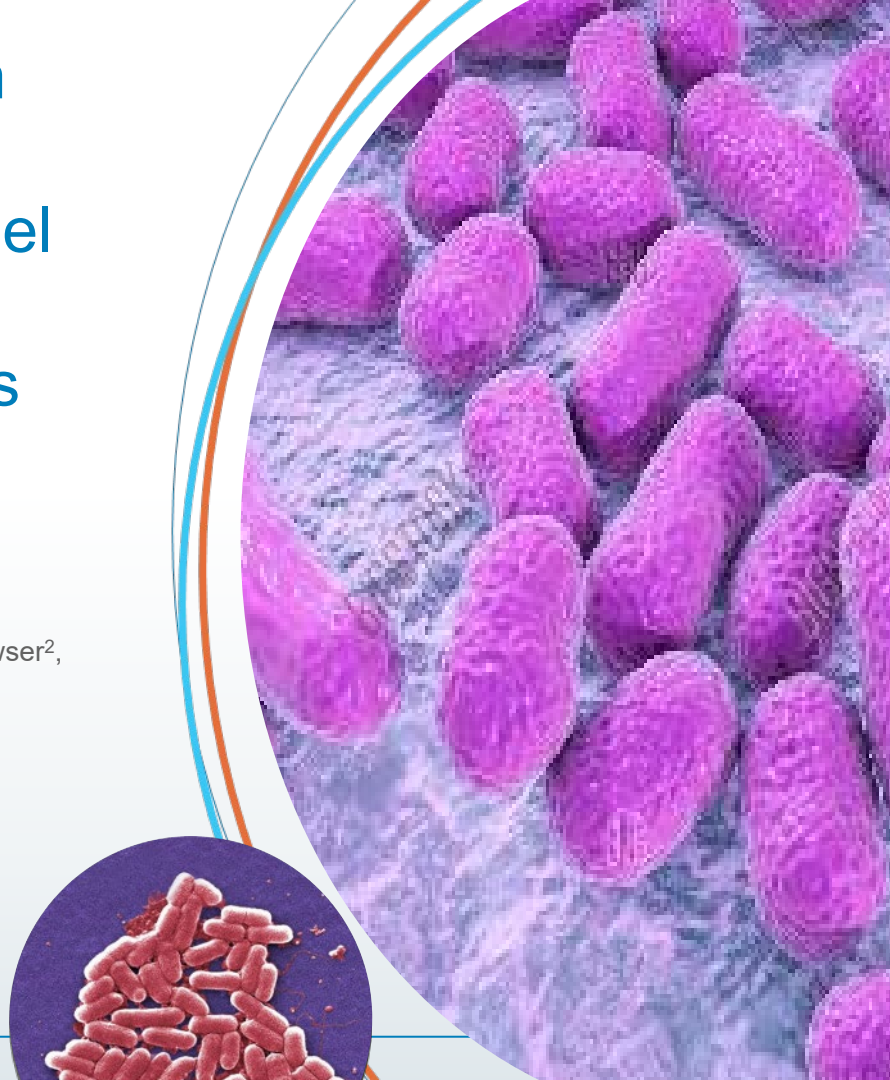
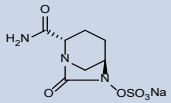
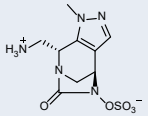
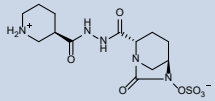
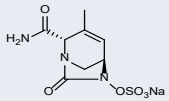
Novel Structural Advances in the Inhibition of Penicillin-binding Proteins
Salon GHI, Saturday, June 11, 2022



Rationally designed DBO inhibitors of Gram-negative PBPs

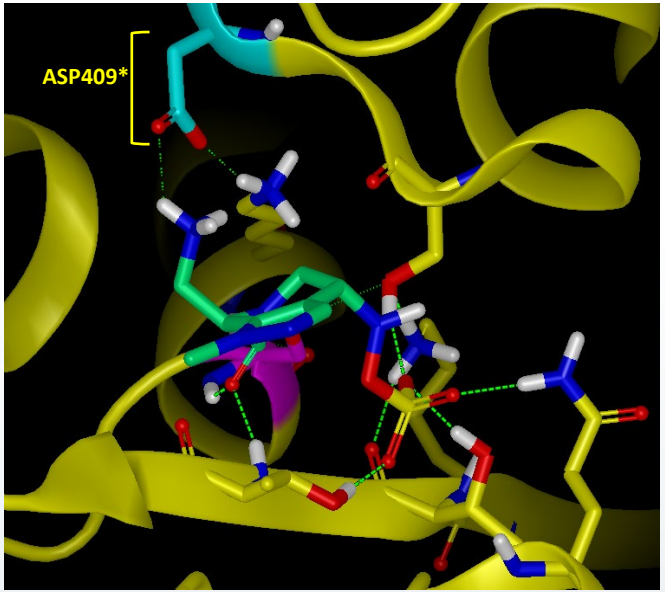
- Diazabicyclooctane (DBO) scaffolds retain activity in the presence of β-lactamases, the primary resistance mechanism associated with β-lactam therapy in Gram-negative bacteria
- However, those with intrinsic antibacterial activity driven solely by PBP2 inhibition suffer from **high frequencies of resistance** and little to **no *in vivo* efficacy** when dosed alone

Examples of DBOs	Structure	Ambler Class β-lactamase inhibitory activity	In vitro antibacterial activity
Avibactam		A, C, limited D (OXA-48)	Weak to none
NXL-105		Weak to none	*Enterobacterales, Pseudomonas* spp.
Zidebactam		A and C	*Enterobacterales, Pseudomonas* spp.
Durlobactam		A, C and broad-spectrum D	*Enterobacterales*

Durand-Reville, T.F., Miller, A.A., O'Donnell, J.P. *et al.* Rational design of a new antibiotic class for drug-resistant infections. *Nature* 597, 698–702 (2021).

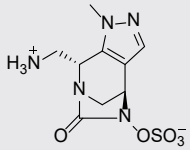
NXL-105 shows potent activity against *P. aeruginosa in vitro* but lacks *in vivo* efficacy

NXL-105 model in *P.a.* PBP2



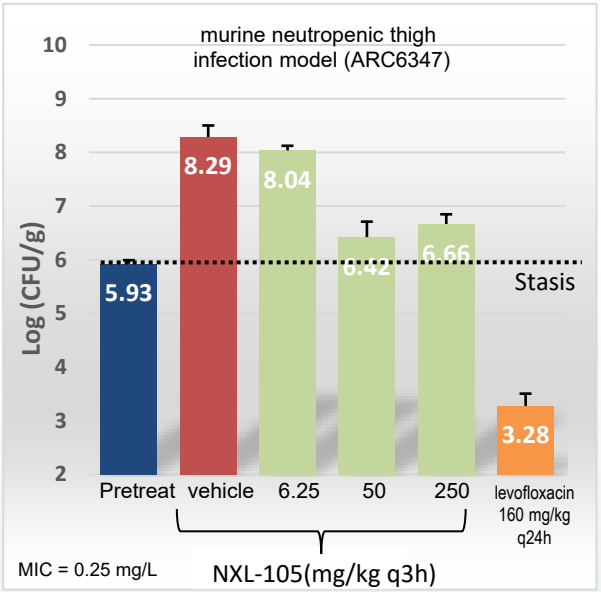
PBP2 selectivity rationalized by key salt bridge with ASP409

NXL-105 *in vitro* activity

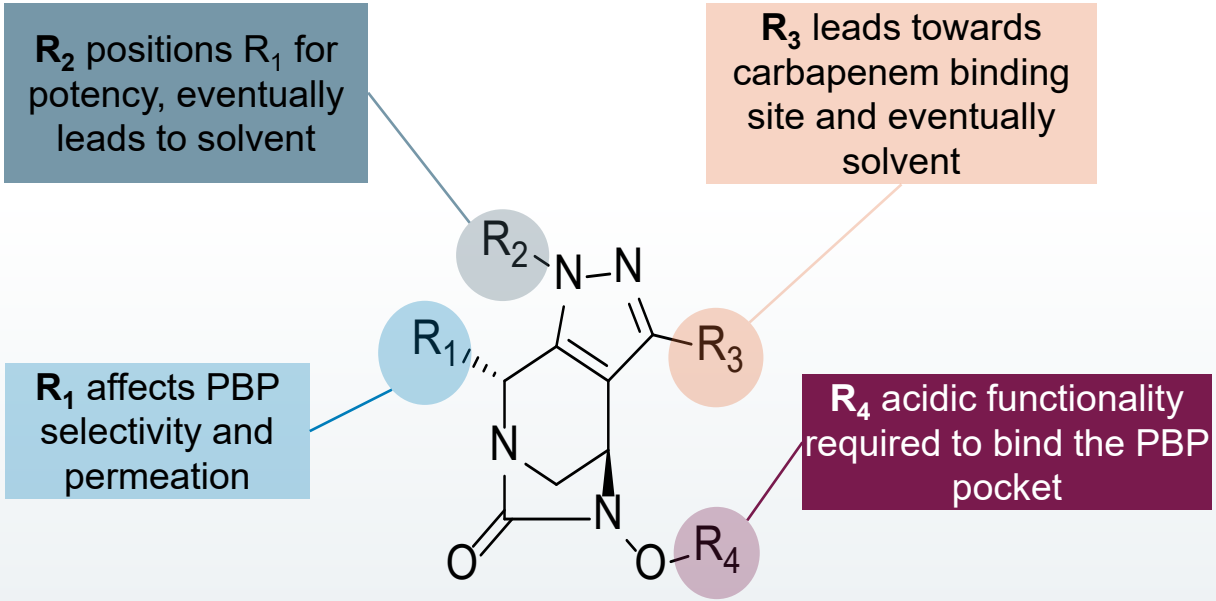
NXL-105 PBP2 Inhibitor	
P.a. PBP2 acylation rate $k_{(on)}$ ($M^{-1}.s^{-1}$)	5,200
P.a. PBP3 acylation rate $k_{(on)}$ ($M^{-1}.s^{-1}$)	11
P.a. PBP1a acylation rate $k_{(on)}$ ($M^{-1}.s^{-1}$)	2
P.a. MIC (ARC6347, OXA-486, PDC-24) (mg/L)	0.25
P.a. MIC_{90} (N=302) (mg/L)	1
Average FOR at 4X MIC	1×10^{-5}

P.a., P. aeruginosa; FOR, frequency of resistance

NXL-105 *in vivo* activity



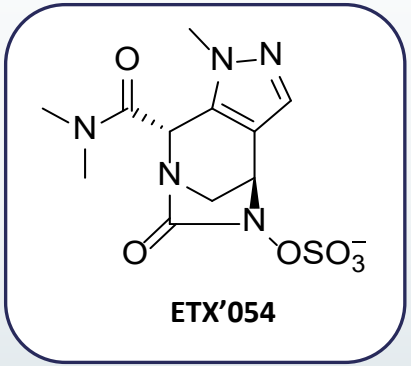
Structurally informed rational design was used to re-engineer target inhibition from PBP2 to PBP1/3



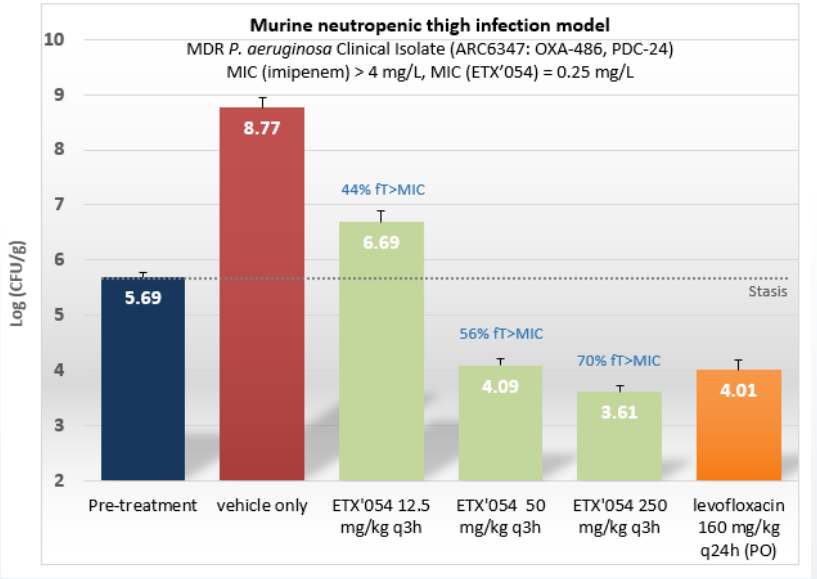
Durand-Reville, T.F., Miller, A.A., O'Donnell, J.P. *et al.* Rational design of a new antibiotic class for drug-resistant infections. *Nature* 597, 698–702 (2021).

Rationally designed DBO inhibitors of Gram-negative PBPs

- An initial lead was successfully re-engineered to inhibit PBP1/3, gaining *in vivo* efficacy and low frequency of spontaneous resistance
  - However, the features that enhanced target potency were found to preclude compound uptake, resulting in inadequate potency against a large collection of clinical isolates (MIC_{90} = 32 mg/L)

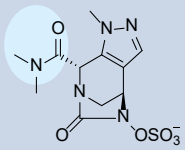
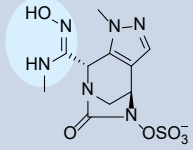


ETX'054

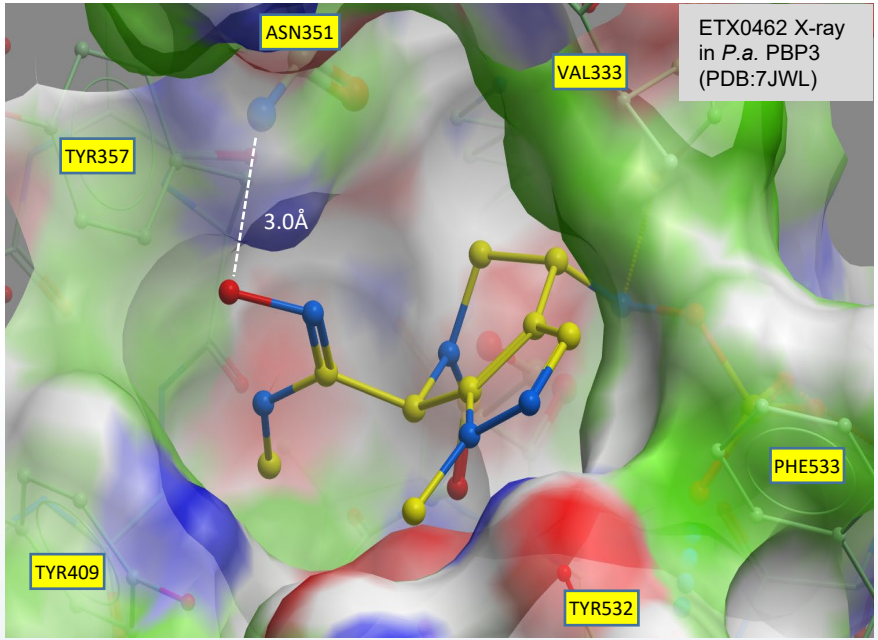


Durand-Reville, T.F., Miller, A.A., O'Donnell, J.P. *et al.* Rational design of a new antibiotic class for drug-resistant infections. *Nature* 597, 698–702 (2021).

Design of ETX0462 addressed permeation challenge

	ETX'054	ETX0462
R_1 substitution		
P.a. PBP3 acylation rate $k_{(on)}$ ($M^{-1}.s^{-1}$)	582,000	260,000
P.a. PBP2 acylation rate $k_{(on)}$ ($M^{-1}.s^{-1}$)	< 8	< 8
P.a. PBP1a acylation rate $k_{(on)}$ ($M^{-1}.s^{-1}$)	2,310	1,100
P.a. PAO1 (mg/L)	2	0.5
K.p. ATCC700603 (mg/L)	8	1
K.p. ARC1865 (mg/L)	2	0.5
E.c. ATCC25922 (mg/L)	2	1
E.c. ATCC35218 (mg/L)	1	0.5
P.a. PAO1 FOR (4x MIC)	<1 x 10^{-9}	<1.7 x 10^{-10}
P.a. PAO1 MBC/MIC ratio	1	1
Porin permeation	None	+++

P.a. = Pseudomonas aeruginosa; *K.p. = Klebsiella pneumoniae*; *E.c. = Escherichia coli*



Unique rational design – hypothesis confirmed by X-ray crystal structure

- Hydrogen bond with ASN351
- High-occupancy water in active site displaced
- Fit in TYR357 pocket
- Pyrazole methyl important to orient R_1 group

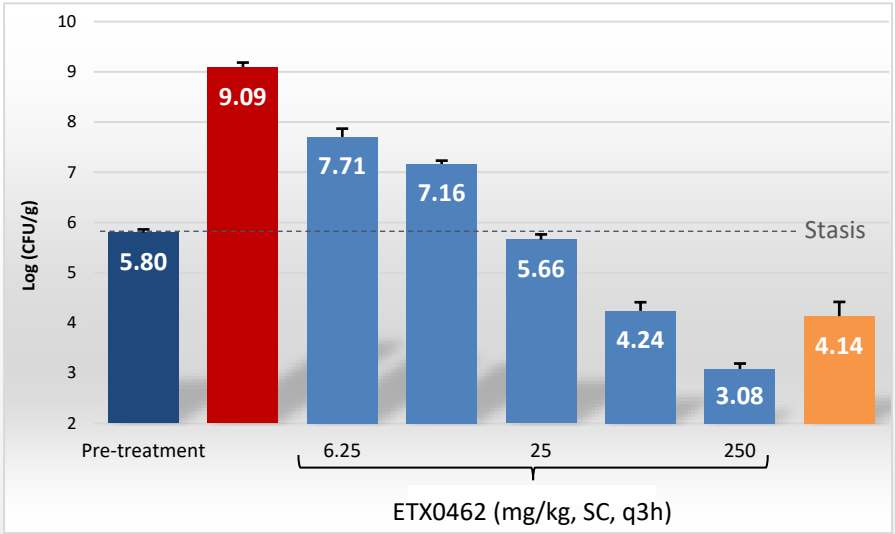
Durand-Reville, T.F., Miller, A.A., O'Donnell, J.P. *et al.* Rational design of a new antibiotic class for drug-resistant infections. *Nature* 597, 698–702 (2021).

ETX0462 demonstrates robust *in vivo* efficacy

- Robust *in vivo* activity achieved in neutropenic murine thigh (7 *P.a.* strains) and lung (3 *P.a.* strains) efficacy models
- MIC range of 0.25 to 4 mg/L used to achieve robust PK/PD analysis
- Consistent results observed *in vitro* and *in vivo* with magnitude requirement of ~60% fT>MIC for 1-log kill against *P. aeruginosa*
- *In vivo* efficacy also demonstrated against *Y. pestis* and *B. pseudomallei*

Murine neutropenic thigh infection model

MDR *P. aeruginosa* Clinical Isolate (ARC6347: OXA-486, PDC-24)
MIC (imipenem) > 4 mg/L, MIC (ETX0462) = 0.25 mg/L



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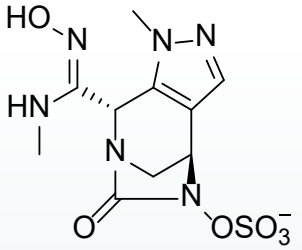
ETX0462 has broad-spectrum gram-negative potency against contemporary clinical isolates of 'KAPE' pathogens

Also active against *S. maltophilia* and biothreat pathogens, including *Burkholderia* spp.

Bacterial Species	N	ETX0462 (mg/L)		Comparator Agent (mg/L)	
		MIC Range	$MIC_{50/90}$	MIC Range	$MIC_{50/90}$
2017-2018 global clinical isolates				Imipenem	
P. aeruginosa	205	≤0.06 - 4	0.5/1	0.06 - >16	1/16
A. baumannii	200	0.25 - >16	2/4	0.06 - >16	8/>16
E. coli	204	0.12 - 16	0.25/1	0.03 - 0.25	0.12/0.12
K. pneumoniae	201	0.25 - >16	0.5/4	0.06 - >16	0.12/0.5
S. maltophilia	101	0.25 - 2	2/2	>16	>16/>16
Biothreat pathogens				Doxycycline	
F. tularensis	5	0.125 - 0.2	NA	0.5	NA
Y. pestis	4	0.25	NA	1 - 2	NA
B. anthracis	16	0.5 - 1	NA	≤0.01 - 0.06	NA
				Ceftazidime	
B. pseudomallei	13	0.25 - 2	NA	1 - 8	NA
B. mallei	6	0.25 - 0.5	NA	1 - 4	NA

NA = not applicable



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CARB-X Cross-Project susceptibility study

- CARB-X initiated a cross-project susceptibility study to enable direct comparisons of funded compounds against the same sets of global clinical isolates.
  - Isolates from 2019 were selected from different hospital sites or clinical sample types from the different countries to avoid any duplicate isolates.
  - No enrichment was performed based on any susceptibility phenotype.
  - However, the number of isolates from low- and middle-income countries were enriched to ~2:1 vs. high-income countries for most species.
  - MIC testing was conducted at IHMA-Europe.

Bacterial isolates selected for susceptibility testing of ETX0462 and comparator agents

Bacterial species	N
Acinetobacter baumannii	298
Pseudomonas aeruginosa	300
Stenotrophomonas maltophilia	150
Escherichia coli	300
Klebsiella pneumoniae	300
Enterobacter cloacae	152
Citrobacter spp.	151
Proteus spp.	152
Morganella spp.	43
Providencia spp.	61
Serratia marcescens	45

ETX0462 activity vs. *Pseudomonas aeruginosa* (N = 300)

ETX0462 vs. comparator compounds	MIC (mg/L)			%Susceptible (CLSI)
Compound	MIC_{50}	MIC_{90}	Susceptibility breakpoint (CLSI)	
Amikacin	4	64	16	85
Ceftazidime	4	>32	8	71
Ceftazidime-Avibactam	2	>16	8	87
Colistin	0.5	1	NA	NA
Gentamicin	1	>32	4	83
Levofloxacin	0.5	32	1	69
Meropenem	0.5	32	2	78
Piperacillin-Tazobactam	8	128	16	74
Tobramycin	0.5	>32	4	85
ETX0462	0.25	2	4*	100*

NA, not applicable (colistin susceptibility breakpoints are not recognized by CLSI); *preliminary breakpoint

ETX0462 activity by region and infection type

Region (N)	Africa (37)	Asia-PAC (58)	Europe (99)	Middle East (30)	North America (9)	South America (67)
MIC_{90}	2	2	2	2	4	1

Infection Type (N)	Intra-abdom'l (11)	Blood (43)	Skin/Soft Tissue (51)	Urinary Tract (37)	Respiratory Tract (158)	MBL+ (22)
MIC_{90}	1	2	1	2	2	2

ETX0462 activity vs. other Gram-negative pathogens in CARB-X panel

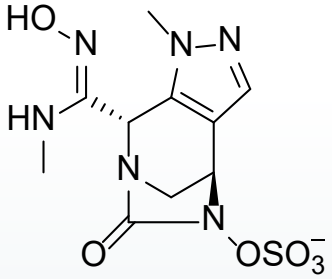
Species	N	MIC (mg/L)				%S at 4 mg/L	% MEM-S (CLSI)
		Min	Max	MIC_{50}	MIC_{90}		
Non-fermenting organisms							
A. baumannii	298	0.25	32	2	8	79	30
S. maltophilia	150	0.5	8	2	2	100	NA (77% LVX-S)
Enterobacterales							
Citrobacter spp.	151	0.125	>32	0.5	8	87	93
E. cloacae	162	0.125	16	0.5	8	82	92
E. coli	300	0.125	>32	0.25	1	96	97
K. pneumoniae	300	0.25	32	0.5	8	88	84
Proteus spp.	152	0.125	4	0.5	1	100	100
M. morganii	43	1	16	2	4	95	100
Providencia spp.	61	0.125	8	0.5	2	98	70
Serratia marcescens	45	0.5	8	1	2	98	96

NA, not applicable (*S. maltophilia* is intrinsically carbapenem-resistant); MEM-S, susceptible to meropenem based on 2021 CLSI interpretive criteria

- Susceptibility patterns were consistent across regions and infection types

ETX0462: a single antibacterial agent to treat Gram-negative infections, including MDR *P. aeruginosa*

- Engineered critical biochemical spectrum (PBP3 and PBP1a) and optimized permeation in DBO scaffold using structure-based drug design
- Excellent broad-spectrum activity against Gram-negative 'KAPE' and biothreat pathogens as a single agent (*in vitro* and *in vivo*)
- Results from CARB-X study are consistent with previous susceptibility results
- ETX0462 well tolerated in 14d rat GLP toxicology study to a limit dose of 2000 mg/kg



- If successfully developed, ETX0462 would represent the first new antibiotic class in over 25 years to treat MDR Gram-negative and biothreat infections.

Acknowledgements and disclaimer:

CARB-X
Combating Antibiotic-Resistant Bacteria

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