Discovery of a Novel Series of Penicillin-binding Protein 3 Inhibitors to Treat *Pseudomonas aeruginosa* Infections: Rational Design of Biochemical Potency and Bacterial Permeation

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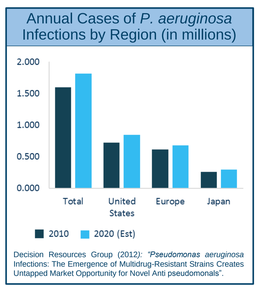
ENTASIS THERAPEUTICS
Innovative Antibacterials

Introduction

Approaching the Post-antibiotic Era?

- Multidrug-resistant (MDR) Gram-negative infections are of great concern due to high mortality rates and limited treatment options.[1,2]
- Current Gram-negative MDR clinical isolates harbor multiple resistance mechanisms:
  - β-lactamase expression
  - Porin (membrane transport protein) mutation/deletion
  - Efflux pump overexpression
- *P. aeruginosa* (*P.a.*) infections continue to be a significant and costly unmet medical need.



➡ Currently 20% MDR rate, expected to rise to ~30% by 2040 based on current worldwide trend

➡ Critical Priority 1 (WHO Priority Pathogen List, 2017 Report)

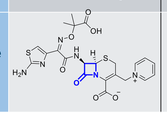
➡ Serious Threat (CDC Antimicrobial Resistance Threats, 2013 Report)

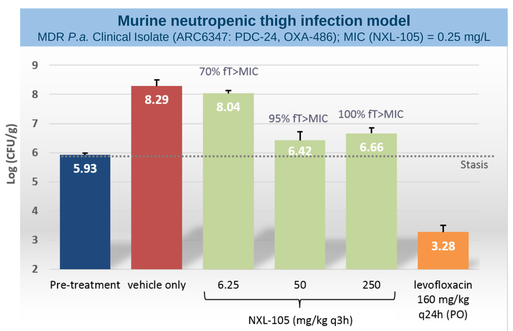
Project Goal

- Discover novel class of pathogen-targeted antibiotics to specifically address resistance mechanisms
  - MOA through inhibition of penicillin-binding proteins (PBPs), validated targets of the β-lactams)
  - Covalent binding to active site serine
  - β-lactamase-resistant non-β-lactam scaffold
  - Intravenous therapy to treat infections caused by *P. aeruginosa*, including MDR strains

Background

- β-lactam antibiotics are hydrolyzed and deactivated by β-lactamase enzymes.
- New diazabicyclooctanone (DBO) scaffold mimics β-lactam ring but is not hydrolyzed by β-lactamases.
  - De-risked safety (well tolerated preclinically and clinically)
  - Well-understood DMPK and physicochemical properties
  - Extensive in-house experience in design and synthesis
- NXL-105 (Novexel) is a DBO-based PBP2 inhibitor targeting *P. aeruginosa.*

		Biochemical Potency Acylation rate constant ($M^{-1}.s^{-1}$)			Cellular Potency wt *P.a.* MIC (mg/L)			*In vivo* Efficacy
	Structure	*P.a.* PBP2 k_{inact}/K_i	*P.a.* PBP3 k_{inact}/K_i	*P.a.* PBP1a k_{inact}/K_i	PAO1 (PDC-1, OXA-50)	MDR ARC6347 (PDC-24, OXA-486)	MDR ARC3506 (PDC-35, VEB-1, OXA-10, OXA-488, OprD-fs, OpdF-del)	Murine thigh infection model (*P.a.* ARC6347)
NXL-105 (DBO)		5,200	11	2	0.125	0.25	0.25	Not efficacious
Ceftazidime (β-lactam)		< 5	84,000	3,800	1	4	> 64	> 2 log(CFU/g) reduction



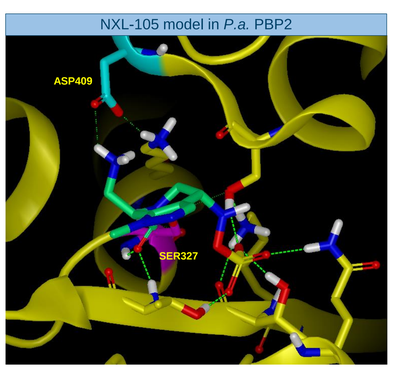
- Despite excellent *P.a.* MICs *in vitro* and exposures of up to 100% *f*T/MIC, NXL-105 is devoid of *in vivo* efficacy in a murine infection model.

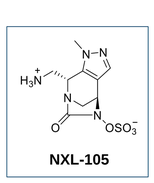
➡ **Hypothesis:**
Lack of *in vivo* efficacy is driven by PBP spectrum and can be solved by switching selectivity from PBP2 to PBP3/1a.

Optimizing Biochemical Potency to Address PBP Spectrum

Approach

- Design out PBP2 selectivity of NXL-105 and build in inhibition of PBP3/1a (similar to β-lactams' spectrum) on novel DBO analog.
- *P.a.* PBP2, PBP3 and PBP1a present over 90% sequence similarity in binding pocket.



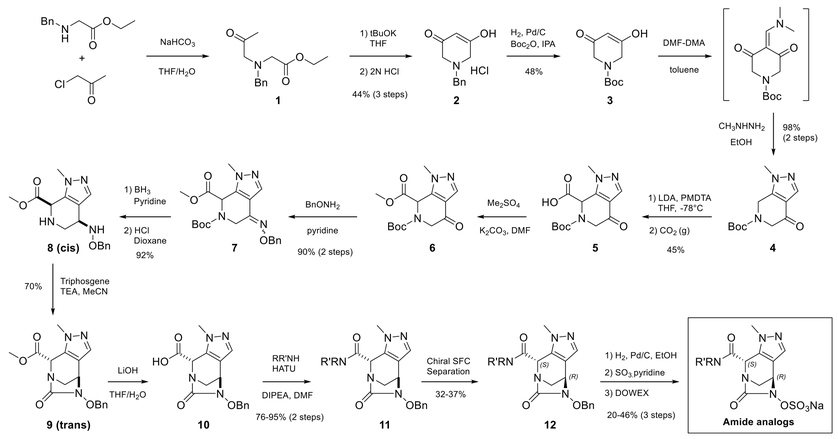


➡ NXL-105's selectivity for PBP2 can be rationalized by key salt bridge with ASP409.

➡ ASP is ASN in PBP3/1a.

➡ **Design Strategy:** Reduce nitrogen basicity and engineer H-bond with ASN351 in PBP3/1a

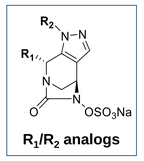
Synthesis – Medicinal Chemistry Route



- 16 linear steps
- Racemic route - chiral SFC separation required to obtain optically active compounds
- Low yields to Intermediate **3** and for carboxylation step (**4** to **5**)
- Difficult end game (polarity, stability)

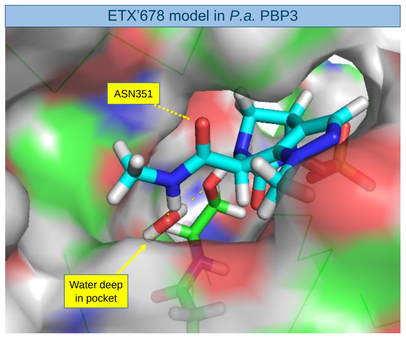
Early SAR – Striking PBP Spectrum Differences

	NXL-105	ETX'991	ETX'678	ETX'054	ETX'036
R_1 group					
R_2 group	Me	Me	Me	Me	H
P.a. PBP2 acylation rate constant k_{inact}/K_i ($M^{-1}.s^{-1}$)	5,200	131	110	< 8	< 8
P.a. PBP3 acylation rate constant k_{inact}/K_i ($M^{-1}.s^{-1}$)	11	230	610	582,000	4,060

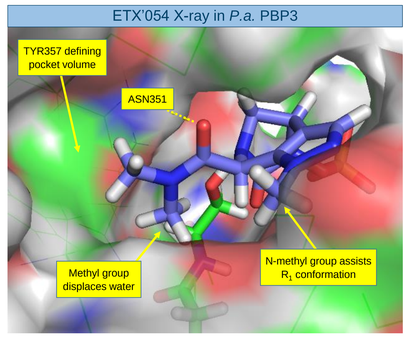


- Design hypothesis for PBP3 inhibition confirmed
  - Reduction of R_1 group basicity correlates with weaker PBP2 acylation
  - R_1 amide increases PBP3 acylation
  - Exquisite PBP3 selectivity achieved with R_1 dimethylamide

ETX'678 model in *P.a.* PBP3

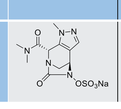


ETX'054 X-ray in *P.a.* PBP3



➡ **Key *P.a.* PBP3 Medchem design principles:**
- ~90° R_1 conformation from DBO core
- Hydrogen bond with ASN351
- Water molecule displaced
- Good fit in TYR357 pocket

ETX'054 Lead Compound – *In Vitro* Data

		Biochemical potency Acylation rate constant ($M^{-1}.s^{-1}$)			Cellular Potency wt *P.a.* MIC (mg/L)			
	Structure	*P.a.* PBP2 k_{inact}/K_i	*P.a.* PBP3 k_{inact}/K_i	*P.a.* PBP1a k_{inact}/K_i	PAO1 (PDC-1, OXA-50)	MDR ARC6347 (PDC-24, OXA-486)	MDR ARC3506 (PDC-35, VEB-1, OXA-10, OXA-488, OprD-fs, OpdF-del)	MIC_{90} (N=599 contemporary clinical isolates)
ETX'054 (DBO)		< 8	582,000	3,800	2	0.5	8	32

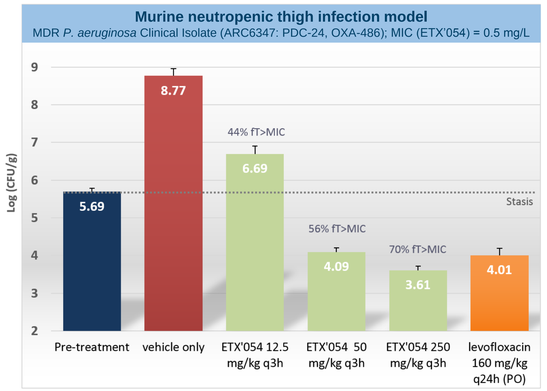
- Excellent selectivity for *P.a.* PBP3/1a over PBP2
- Good cellular potency, even in presence of multiple β-lactamases
- Limited activity when porin- and efflux pump-mediated resistance mechanisms present
- High MIC_{90} observed against large panel of *P.a.* contemporary isolates

		MIC (mg/L) against *P.a.* isogenic strains expressing individual β-lactamases													
	parent	Class A				Class B			Class C		Class D				
Compound	PAO1	CTX-M-15	KPC-2	SHV-2a	TEM-1	NDM-1	VIM-1	VIM-2	AmpC	P99	OXA-10	OXA-23	OXA-40	OXA-48	OXA-58
Piperacillin (β-lactam)	4	>256	>256	>256	>256	128	>256	128	256	128	>256	256	256	256	>256
Ceftazidime (β-lactam)	2	64	>64	32	4	>64	>64	>64	32	64	4	16	2	2	2
ETX'054 (DBO)	4	4	8	4	4	8	4	4	8	8	8	4	4	8	4

- Unlike the β-lactams which lose activity in the presence of β-lactamases, ETX'054 (DBO series) maintains activity against all 4 classes of β-lactamases tested.

ETX'054 Lead Compound – *In Vivo* Data

- PK profile similar to β-lactams and other DBOs (rat, 50mpk, IV: CL 37.2 mL/min/kg, Vd_{SS} 0.47 L/kg, $T_{1/2}$ 0.44 h)
- Plasma protein binding: fu = 0.90 in rats and dogs, 0.82 in humans



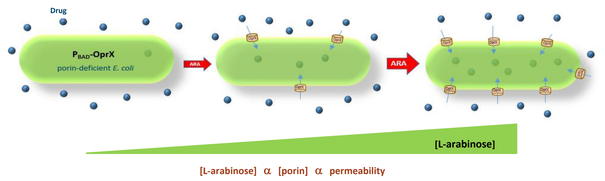
- ETX'054 achieves robust *in vivo* activity in neutropenic murine thigh efficacy model.

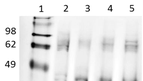
➡ But high MIC_{90} against recent *P.a.* clinical isolates requires optimization of bacterial uptake.

Optimizing Bacterial Permeation to Address MIC_{90}

Uptake Assay – An Unprecedented Approach

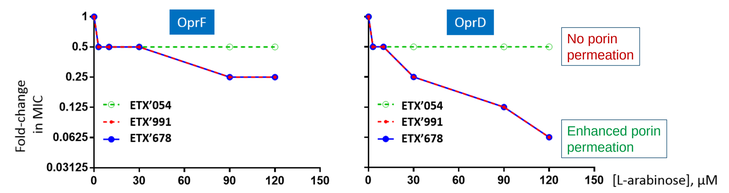
Optimized cell-based porin over-expression assay[3]
- Increasing porin concentration in the outer membrane (OM) ↔ Increasing OM permeability
- Tuning OM permeability with selective inducer (L-arabinose) in a controlled fashion
- Read out using fold-change in MIC, independent of activity level
- Heterologous porin expression (10 *P. aeruginosa*, 1 *A. baumannii* and 2 *K. pneumoniae* porins)

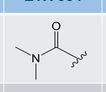
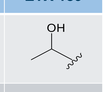


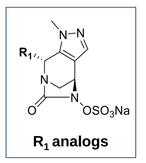


➡ **Goals:** - Build Structure-Porin Permeation Relationships (SPPR) to improve cellular potency
- Engineer multi-porin permeation early in optimization phase

Generating Structure-Porin Permeation Relationships



	NXL-105	ETX'054	ETX'678	ETX'991	ETX'189
R_1 group					
Porin permeation	+++, multiple	none	++, multiple	+++, multiple	+++, multiple
P.a. PBP3 acylation rate constant k_{inact}/K_i ($M^{-1}.s^{-1}$)	11	582,000	610	230	3,840



Potent PBP3 inhibition but effluxed, no porin-mediated permeation: Poor activity vs. MDR strains

Modest PBP3 inhibition but less effluxed, permeates through multiple porins: Better relative activity vs. MDR strains

- Striking differences in uptake with simple structural modifications
- Hydrogen-bond donor appears to be required at R_1 to effectively permeate through several porins
- Using steered MD simulation to model porin permeation at the atomic level

Conclusions and Future Directions

- Entasis is redefining antibacterial design by incorporating definition of the molecular drivers of compound uptake early in Lead Optimization.
  - Unique multidisciplinary approach combining med chem, *in vitro*/*in vivo* biology and *in silico* tools
- We discovered a novel class of non-β-lactam PBP3 inhibitors.
  - Lead compounds maintain activity in the presence of all 4 classes of β-lactamases tested
  - Using structure-based drug design, selectivity shifted from PBP2 to PBP3/PBP1a
  - Translated into robust *in vivo* activity in murine infection model
  - Uptake and efflux identified as features to increase potency against recent MDR *P.a.* clinical isolates
- The team is continuing optimization of both multi-porin permeation and target activity in parallel
  - Candidate to be selected at end of 2019

References

1. O'Neill J., London: Review on Antimicrobial Resistance, May 2016.
2. Spellberg B., Bartlett J.G., Gilbert D.N., N. Engl. J. Med. (2013) 368, 299.
3. Iyer R. *et al.*, ACS Infect. Dis. (2017) 3, 310.

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