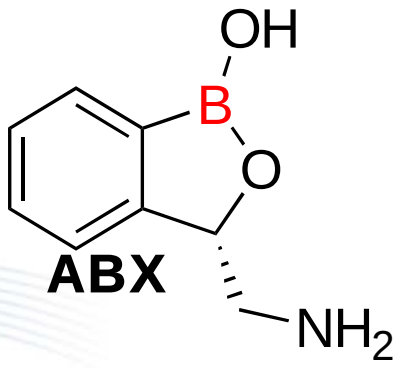

Structure-Guided Discovery of (S)-3-(aminomethyl)benzo[c][1,2]oxaborol-1(3H)-ol hydrochloride (ABX): A First in Class Gram-negative Antibacterial

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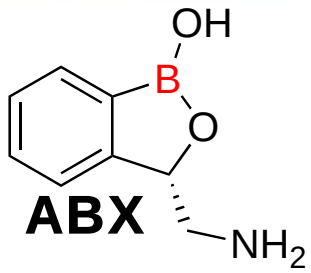
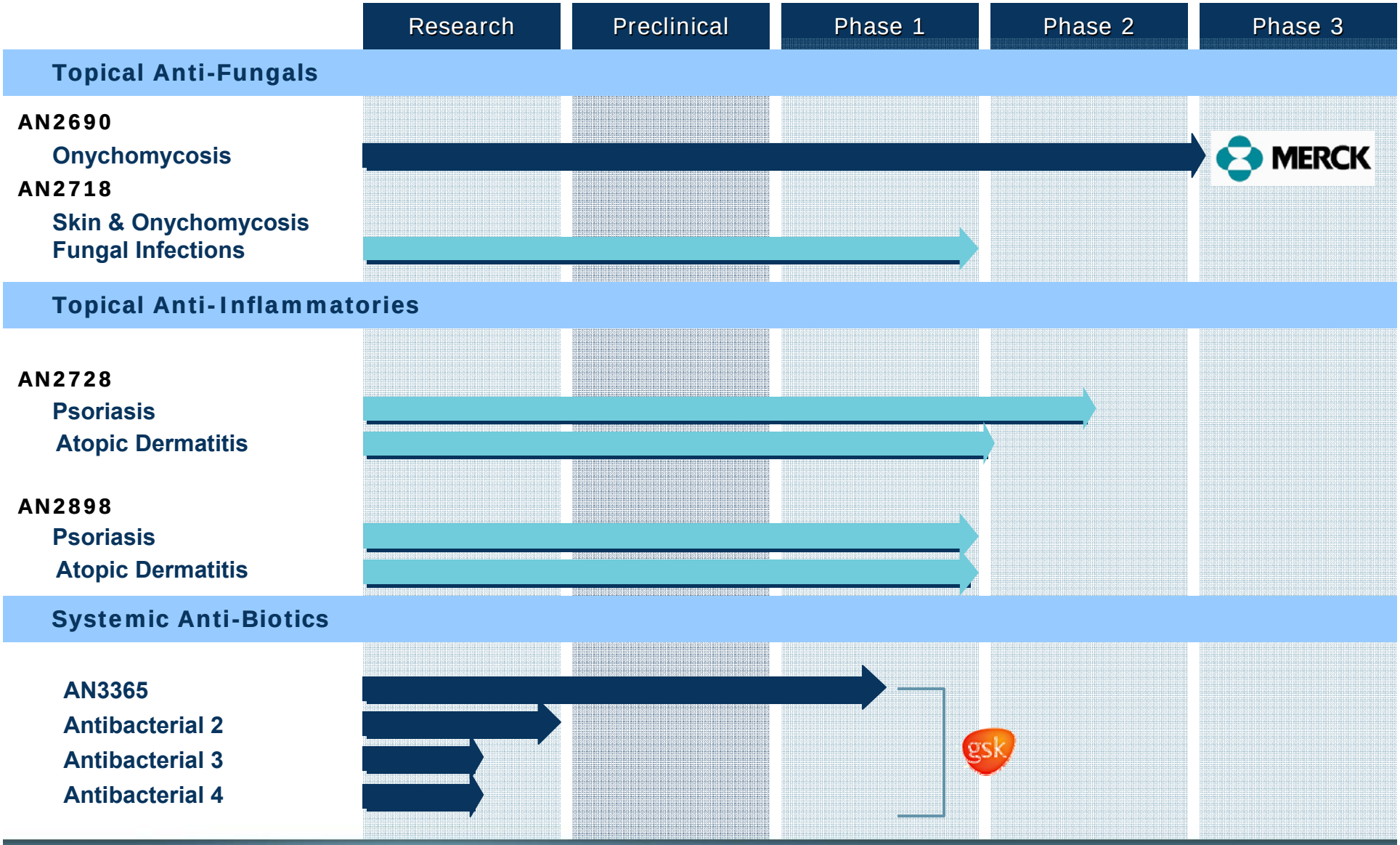
Talk Summary



ABX

- Anacor Pharmaceuticals has a rich pipeline of boron-containing therapeutics in the clinic
- Boron is underexploited in medicinal chemistry and has tremendous potential in drug discovery
- ABX inhibits bacterial Leucyl tRNA synthetase and represents a new class of Gram-negative antibacterial agents
- This novel mechanism of action means ABX is not affected by existing modes of bacterial resistance
- ABX is efficacious *in vivo* against *E. coli* and *Pseudomonas* in mouse models of infection
- AN3365 has advanced to Phase I clinical development for the treatment of Gram-negative bacterial infections

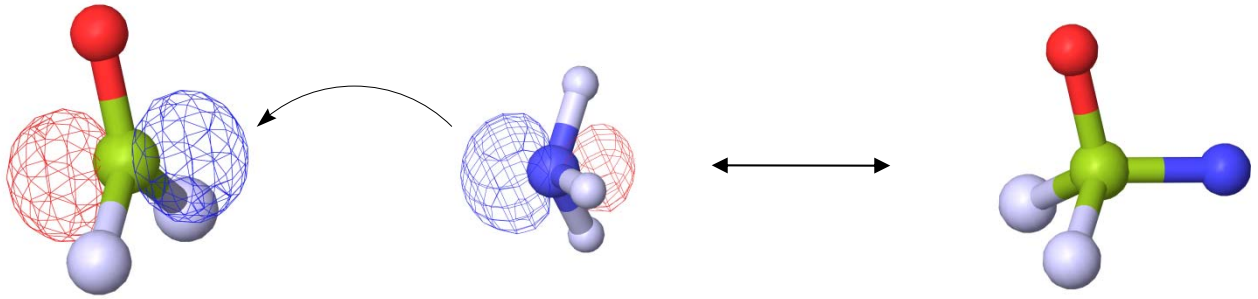
Anacor Has a Large Pipeline of Novel Boron-containing Candidates



Boron is Commonly Found in Our Environment

- In nature, boron is present as boric acid
- Boric acid is the main ingredient of Goop
  - Children's brightly colored toy, that they squeeze through their fingers
- Boric acid is used as a preservative in eye wash and vaginal creams
- Boric acid has an LD_{50} similar to regular table salt (~3000 mg/kg)
- Boron is an essential plant nutrient
- We consume up to 4 mg of boron a day, primarily from fruits, vegetables and nuts
- At Anacor, we found background levels of 200 ng/mL of boron in mouse plasma

Boron has a Unique Bonding Orbital Configuration: An Empty P-Orbital



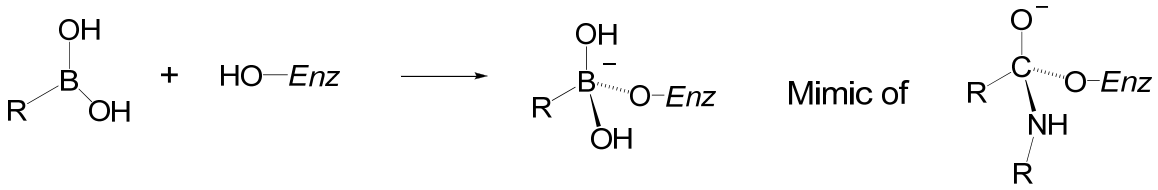
Trigonal Planar

Tetrahedral

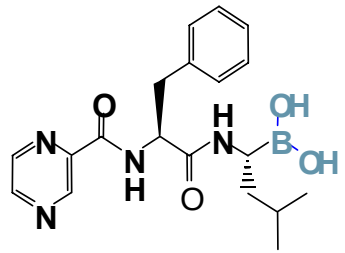
- Boron has an empty *p*-orbital & can form a dative bond under specific conditions
- The dative bond forms a tetrahedral structure
- Exploitation of *p*-orbital expands drug design possibilities

History and Overview of Organo-boron Drug Discovery Efforts

- Design of boronic acid protease inhibitors initiated in 1990s



- Multiple disease targets have been pursued
  - Thrombin
  - Factor Xa
  - Bacterial β-lactamases
  - HCV protease
  - DPP4
  - Arginase



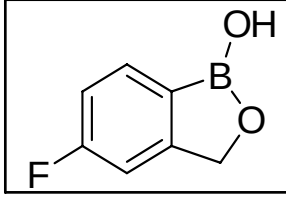
Velcade

- Only Velcade has reached FDA approval
- Lack of success despite substantial efforts attributed to poor drug-like properties of boronic acids

Baker *et al.* (2009) Future Medicinal Chemistry, 1(7), 1275-1288

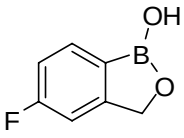
AN2690 was Found to be a Broad Spectrum Antifungal Agent

Minimum Inhibitory Concentration Against Fungal Pathogens (µg/mL)

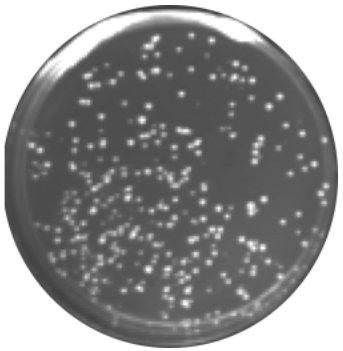
AN2690	***T. rubrum***	***T. mentagrophytes***	***C. albicans***	***C. neoformans***	***A. fumigatus***
	**1**	**1**	**0.5**	**0.25**	**0.25**

- AN2690 has shown good efficacy, safety and tolerability in Phase I and II clinical trials
- AN2690 is scheduled to begin Phase III

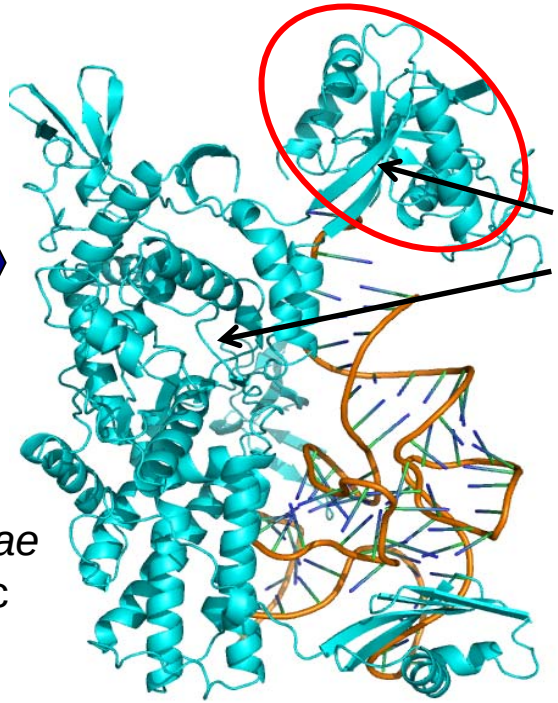
Antifungal Validated the LeuRS Editing Site as a Novel Drug Target



AN2690



Genetic study in *Saccharomyces cerevisiae* identified the cytoplasmic LeuRS gene (*CDC60*)



LeuRS has two active sites

- Editing site
- Synthetic site

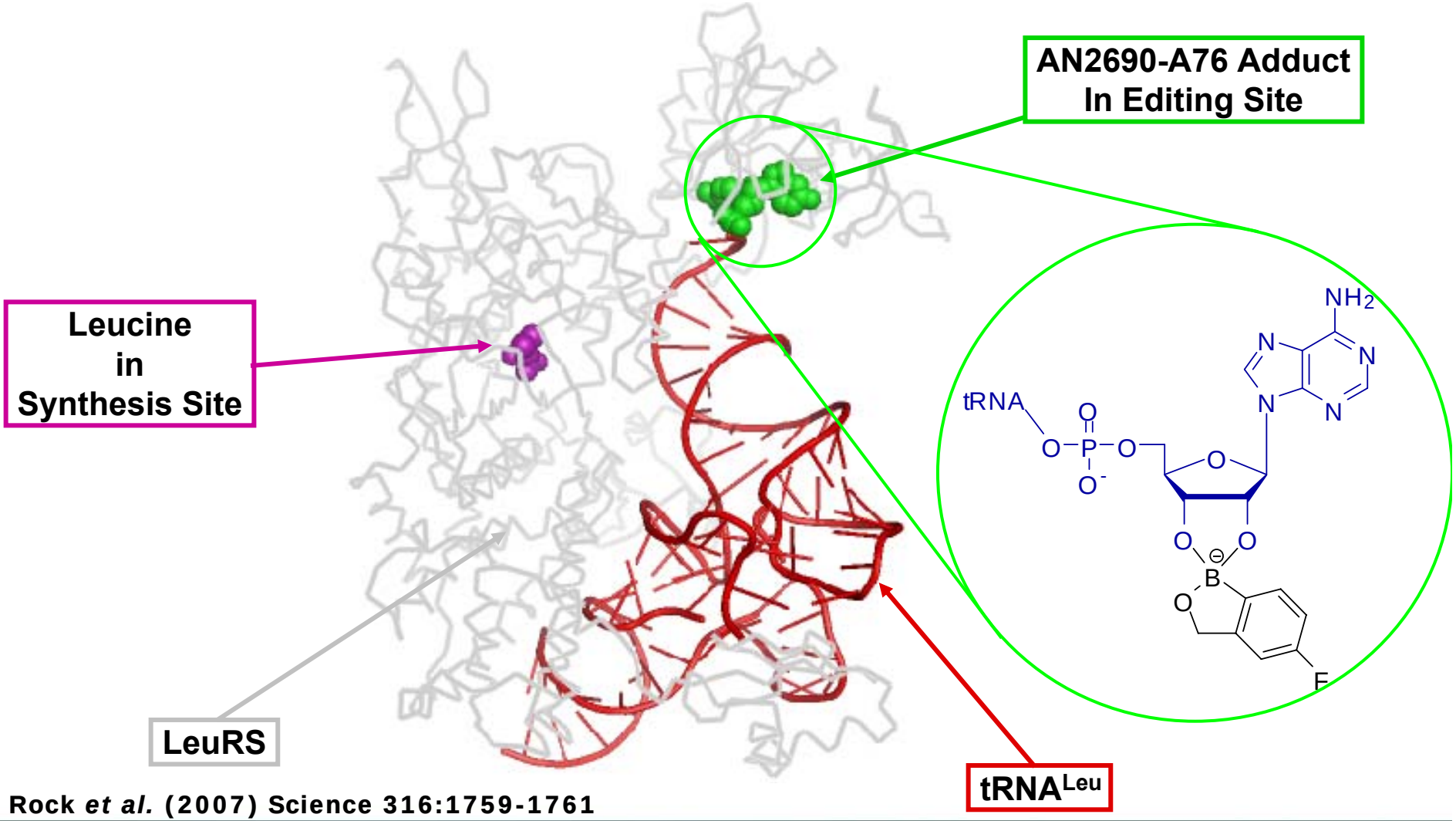
All mutations mapped to the editing domain

Rock *et al.* (2007) Science 316:1759-1761

The Target: Leucyl-tRNA Synthetase (LeuRS)

- Aminoacyl-tRNA synthetase
  - Structurally related to isoleucyl-tRNA synthetase and valyl-tRNA synthetase
- Leucyl-tRNA synthetase attaches leucine to the 3' end of $tRNA^{Leu}$
- Essential enzyme in protein synthesis
- Enzyme has two active sites
  - Aminoacylation active site
  - Editing active site (proofreading)
- Editing activity ensures fidelity of protein synthesis
  - Editing mutants are supersensitive to leucine analogues, like norvaline

X-ray Structure Revealed A $tRNA^{Leu}$ Adduct in the Editing Site of Leucyl tRNA Synthetase

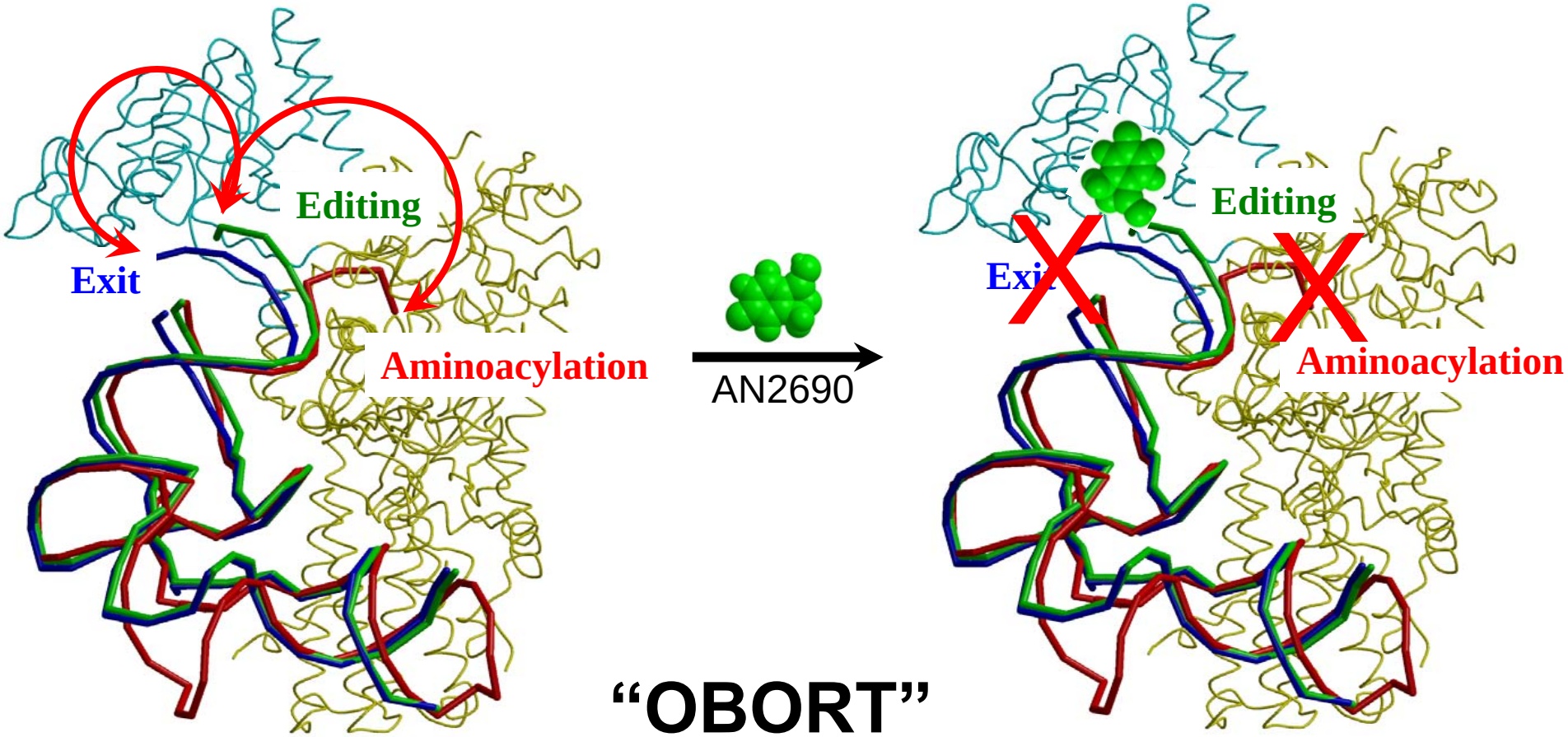


Rock *et al.* (2007) Science 316:1759-1761

AN2690 Traps $tRNA^{Leu}$ In The Editing Site Thus Inhibiting Aminoacylation And Editing

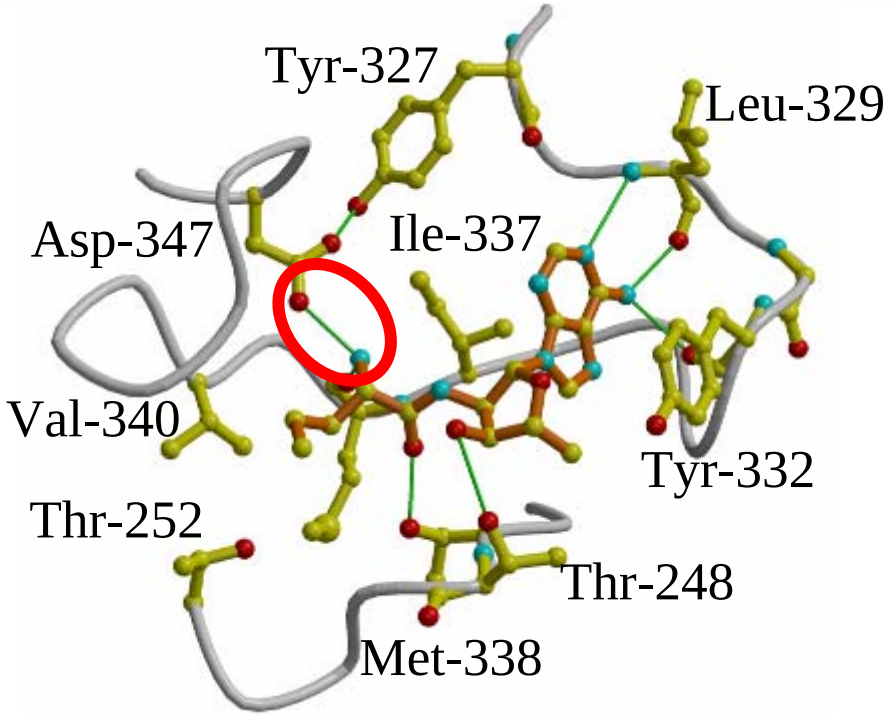
Under normal conditions tRNA is free to move through domains

Oxa**bo**role t**R**NA **t**rapping in editing domain

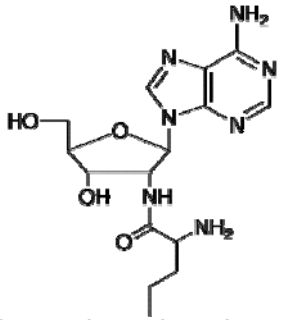


"OBORT"

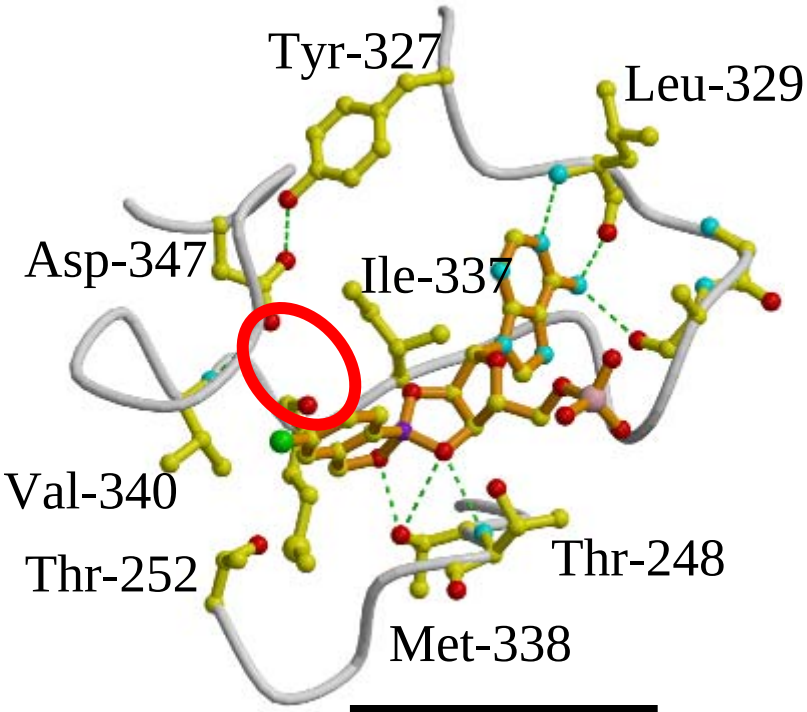
X-ray Structure of AN2690 in LeuRS Revealed a Key Binding Site was not Utilized



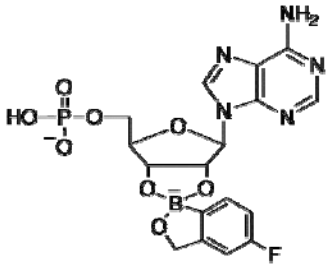
Norvaline post-transfer substrate analogue



Lincecum et al. (2003) Molecular Cell **11:** 951-963



AN2690-AMP

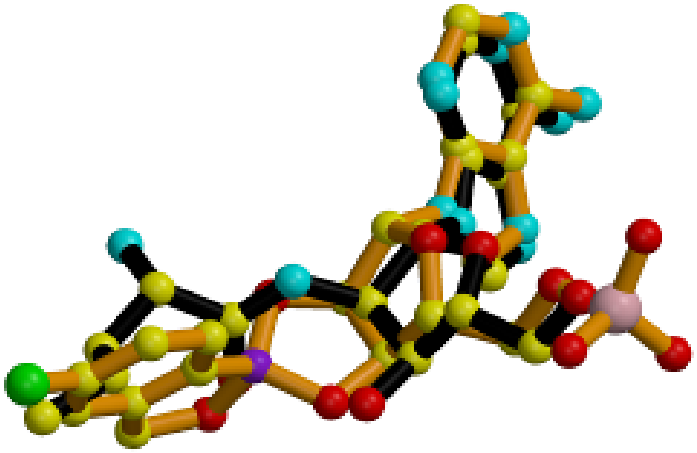


Rock *et al.* (2007) Science 316:1759-1761

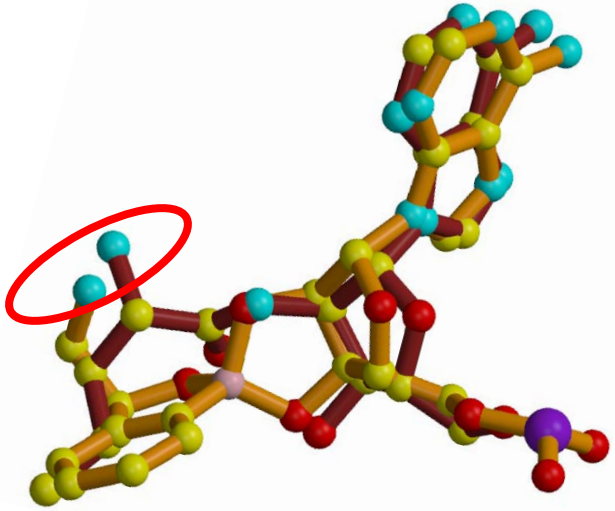
3-Aminomethyl Substitution was Added to Gain These Key H-bonds

Nva2aa

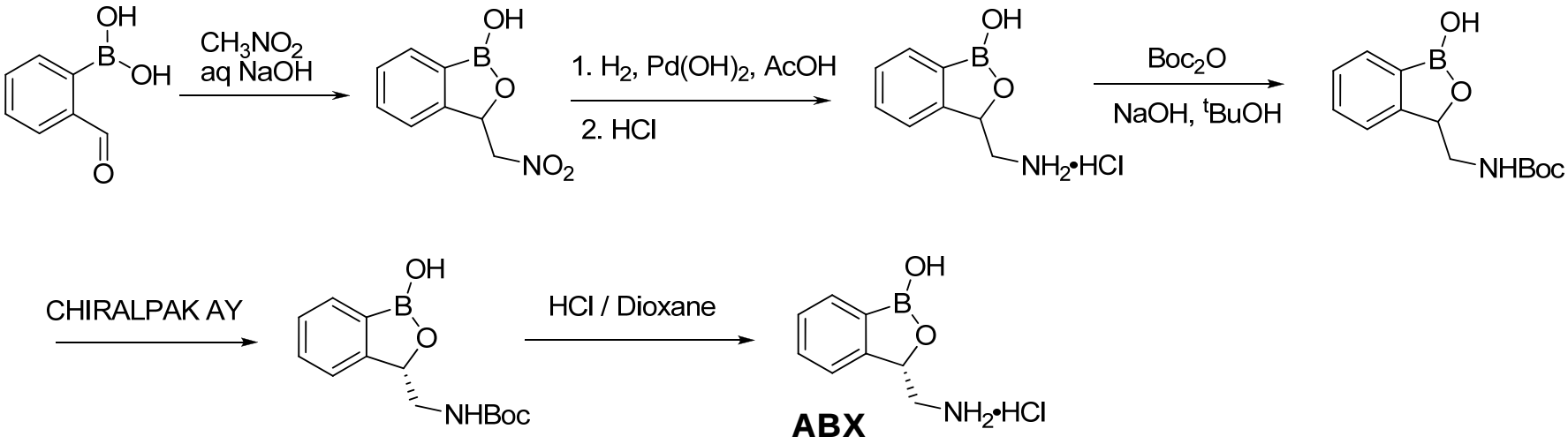
AN2690



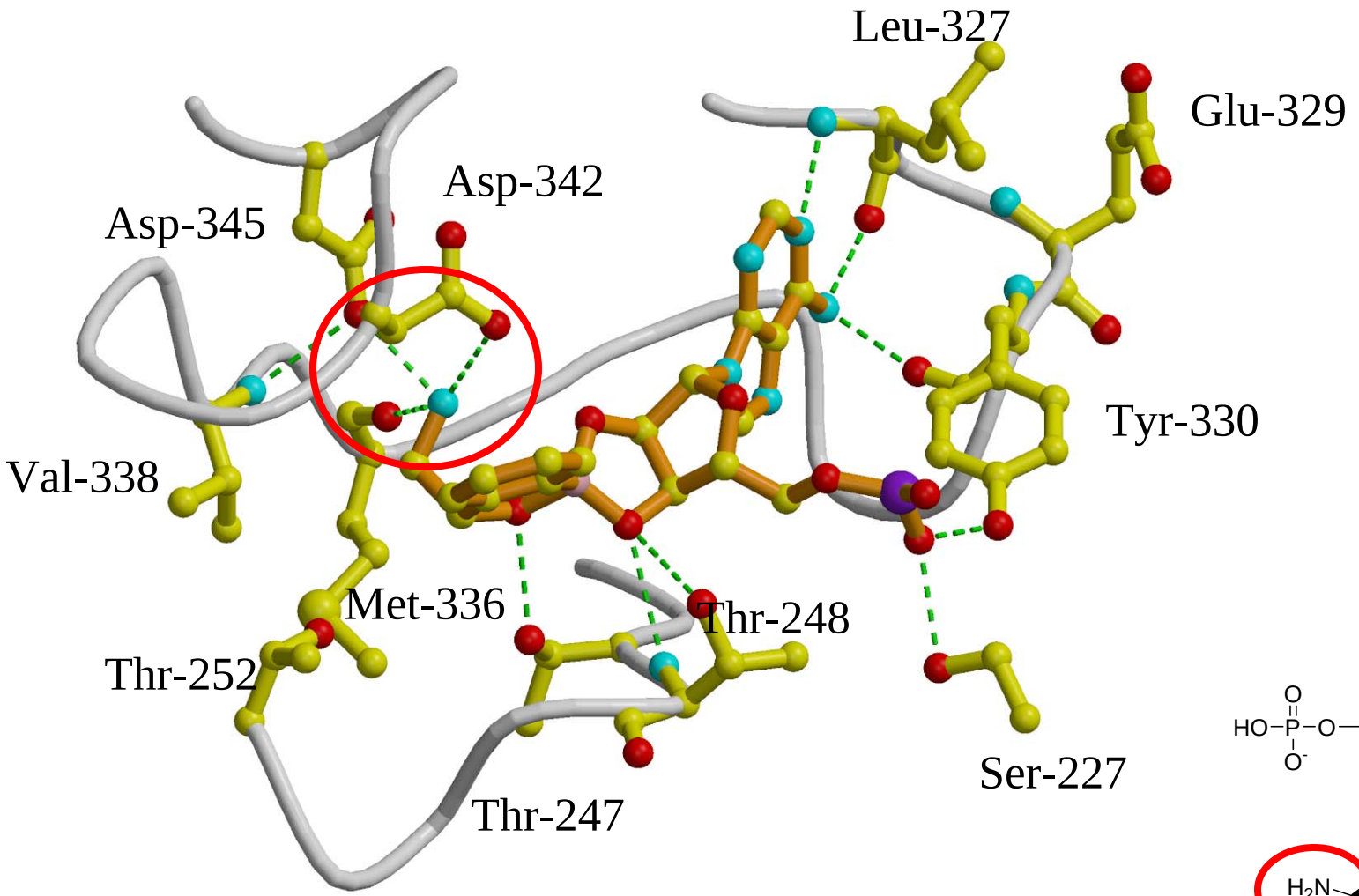
Nva2aa
ABX

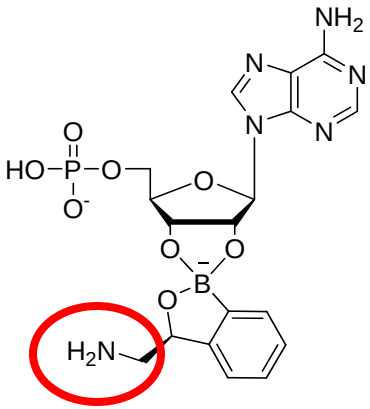


Synthesis of ABX

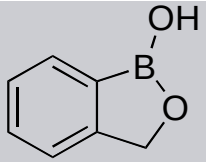
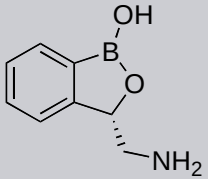
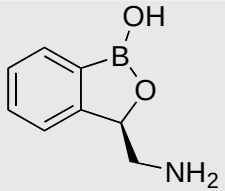


Aminomethyl Group of ABX Makes Three Hydrogen Bonds with LeuRS



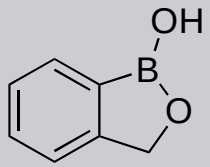
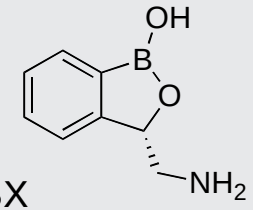


Addition of Aminomethyl Group Provided Greatly Improved Inhibition For the S-Isomer

Compound	IC_{50}* (μM)		MIC (μg/mL)		
	E. coli	*P. aeruginosa*	*E. coli* K12	*E. coli* K12 *tolC*	*P. aeruginosa* ATCC 27853
	27.5	22.3	16	16	>64
 ABX	1.0	2.8	2	2	1
	48.0	>100	16	32	16

* IC_{50} determined after 20 minutes pre-incubation with enzyme and tRNA

Enzyme Kinetics Shows Slow Tight Binding Inhibition and Slow Off-Rate

Compound	IC_{50} (20 mins)	IC_{50} (60 mins)	Enzyme Recovery ($t_{½}$, hr)
	27.5 µM	26.3 µM	0.2
 ABX	1.0 µM	0.4 µM	5.5

Gram-negative MIC_{90} Panel Demonstrates Broad-spectrum Activity

Strain	No. of strains	ABX	Tigeccyline	Imipenem	Cefepime	Levofloxacin	Gentamycin	Ceftazidime	Piperacillin/ tazobactam	Amoxycillin/ clavulanate	Ampicillin
P. aeruginosa (WT)	50	1	16	1	8	2	4	16	32	>64	>64
P. aeruginosa (MbL-)	25	1	>16	32	>32	>16	>16	>32	>128	>64	>64
P. aeruginosa (MbL+)	26	1	>16	>64	>32	>16	>16	>32	>128	>64	>64
A. baumannii (WT)	25	>128	1	0.25	8	4	2	16	8	32	32
Acinetobacter spp. (MDR)	26	>128	8	64	>32	>16	>16	>32	>128	>64	>64
S. maltophilia (WT)	50	1	1	>64	>32	4	>16	>32	>128	>64	>64
B. cepacia	50	4	4	16	32	8	>16	16	32	>64	>64
E. coli (WT)	*27*	1	0.25	0.12	≤1	>16	2	≤1	8	32	>64
E. coli (ESBL)	25	2	0.25	0.25	>32	>16	>16	>32	128	64	>64
Klebsiella spp. (WT)	25	1	0.5	0.25	≤1	≤0.5	1	≤1	16	8	>64
Klebsiella spp. (ESBL)	15	1	2	1	>32	16	>16	>32	>128	64	>64
Klebsiella spp. (KPC)	10	2	1	>64	>32	>16	16	>32	>128	>64	>64
Enterobacter spp. (WT)	25	1	0.5	1	≤1	≤0.5	≤0.5	2	8	>64	>64
Enterobacter spp. (AmpC)	26	1	4	0.5	8	>16	>16	>32	>128	>64	>64
Citrobacter spp. (WT)	36	1	0.5	1	≤1	1	>16	2	16	>64	>64
Citrobacter spp. (AmpC)	16	0.5	0.5	1	2	16	2	>32	128	>64	>64
P. mirabilis (WT)	42	128	4	2	≤1	2	2	≤1	0.5	8	>64
P. mirabilis (ESBL)	11	>128	4	2	>32	>16	>16	>32	4	64	>64
P. vulgaris (WT)	20	>128	2	2	≤1	≤0.5	1	≤1	0.5	16	>64
M. morganii (WT)	17	2	2	4	≤1	4	2	4	2	>64	>64
Indole positive *Proteae*	14	16	2	2	≤1	16	4	≤1	4	>64	>64
S. marcenscens (WT)	38	0.5	1	1	≤1	1	1	2	32	>64	>64
S. marcenscens (AmpC)	16	0.5	2	1	4	4	>16	>32	64	>64	>64

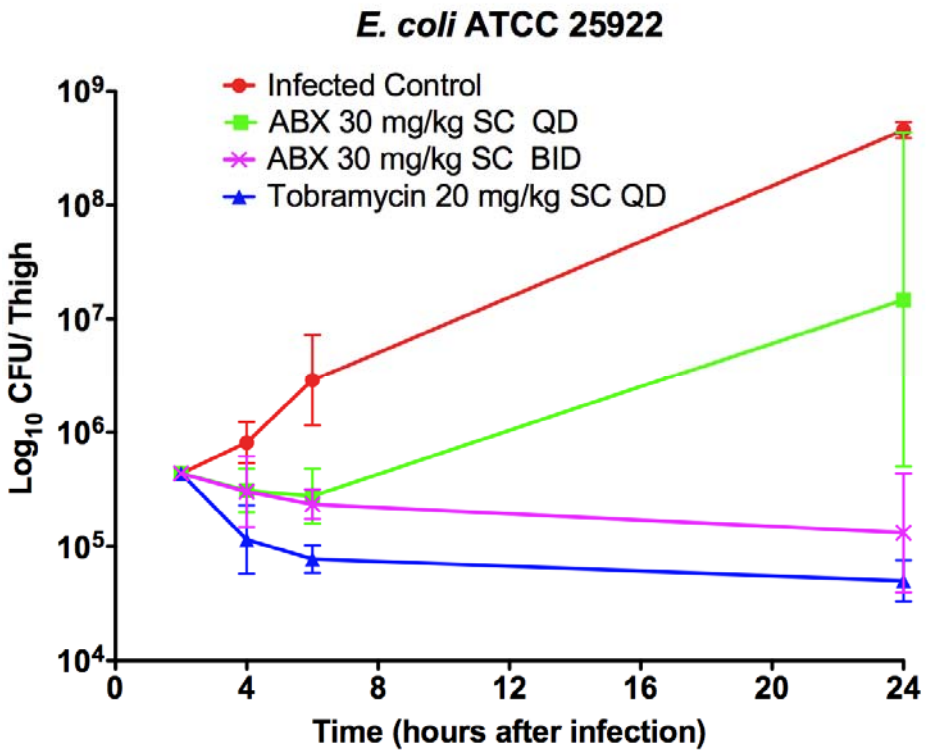
ABX is Active Against Problematic Multi-drug Resistant (MDR) Enterobacteriaceae

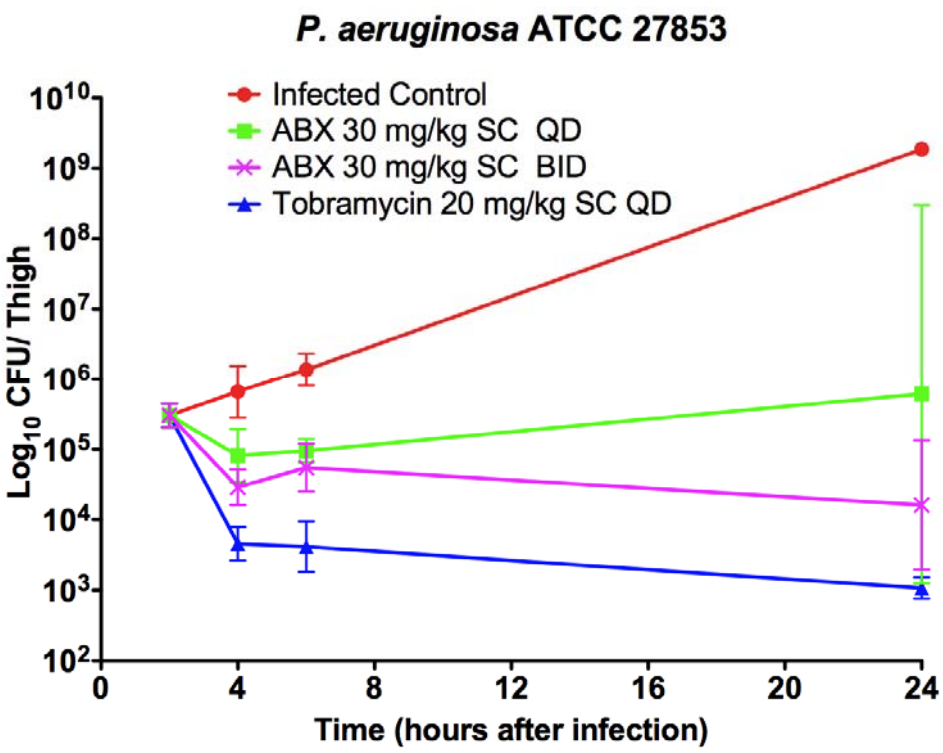
	ABX	Aztreonam	Cefotaxime	Ceftazidime	Piperacillin/ Tazobactam	Imipenem	Meropenem	Ciprofloxacin	Tobramycin	Amikacin	Gentamicin	Colistin
MIC90	4	>128	>256	>256	>128	128	128	128	>32	>128	>128	1
MIC50	2	128	256	>256	>128	16	32	8	16	4	2	<=0.5

- Panel of 94 strains of MDR Enterobacteriaceae were tested by the Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency Centre for Infections
- ABX is not affected by existing modes of bacterial resistance

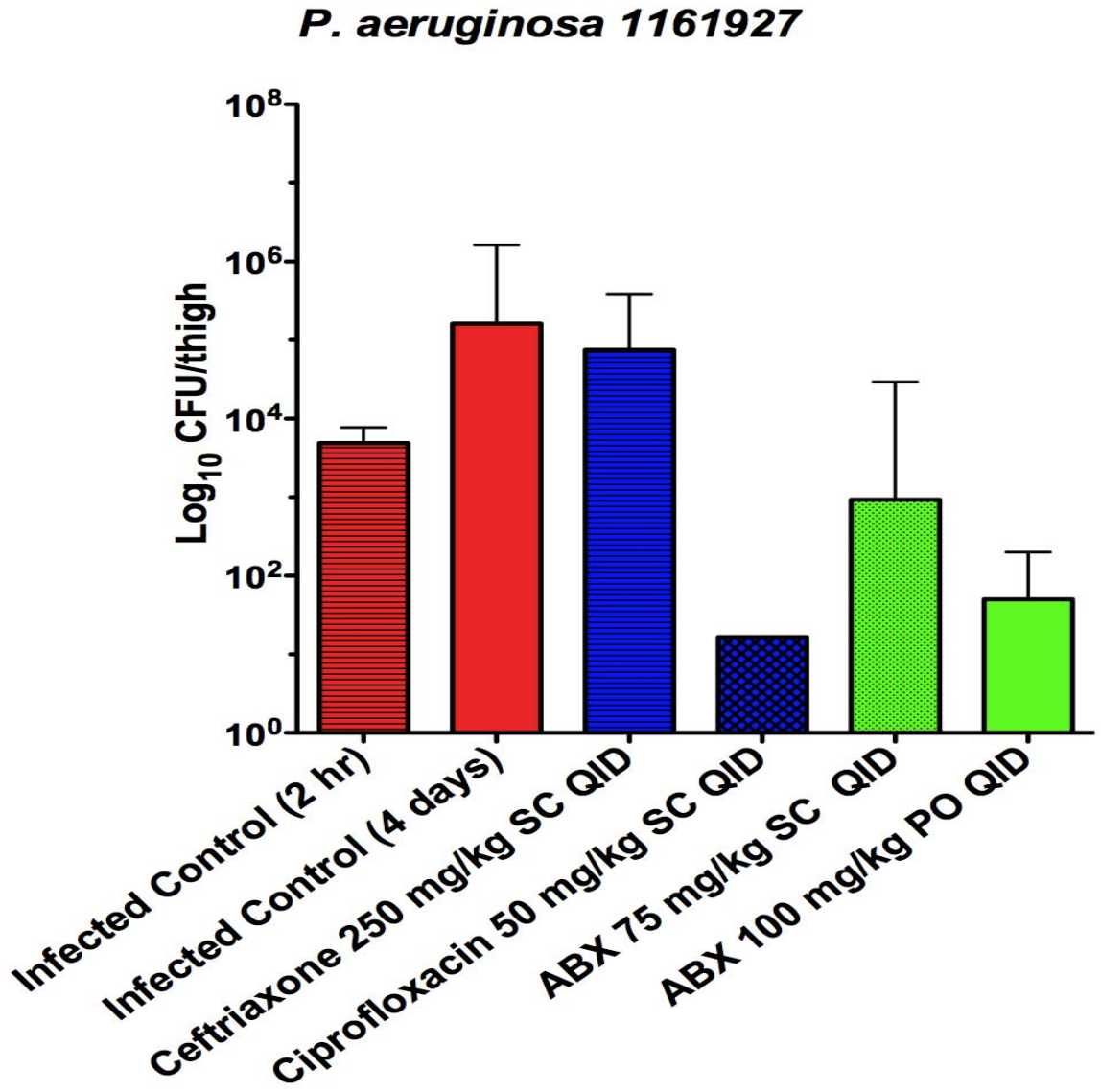
David Livermore Unpublished Data

ABX Efficacy in a Neutropenic Mouse Thigh Infection model of *E. coli* and *P. aeruginosa*





Oral Efficacy in an Immuno-competent Mouse Thigh Infection Model



Interspecies Pharmacokinetics of ABX Scales Well from Mouse to Monkey

Species	IV parameters					
	Dose (mg/kg)	Cmax (µg/mL) @ 5 min	CL (mL/hr/kg)	Vss (mL/kg)	Mean Retention Time (hr)	AUC (µg/mL*hr)
Mouse	10	4.52	3510	2711	0.77	2.85
Rat	10	3.15	3500	5130	1.4	3.68
Dog	10	13	327	1499	4.6	31
Monkey	15	15	339	2975	8.9	47

- ABX is stable to incubation with liver microsomes and simulated gastric fluid
- ABX does not inhibit CYP450 enzymes
- Plasma protein binding range 4-13%
- Good oral bioavailability
  - 29% Rat
  - 100% Dog
  - 79% Monkey

Toxicology of ABX

- *In vivo* rat micronucleus study was negative
- *In vitro* mouse lymphoma was negative up to 2000 μg/mL
- ABX showed IC_{50} ≥ 10 μM for all receptors except $5HT_7$
  - IC_{50} of $5HT_7$ is 1 μM, is neither an agonist or antagonist
- hERG IC_{50} > 100 μM
- Not hemolytic at concentrations up to 45 mg/mL in rat RBC
- In the 7-day rat safety study no significant toxicity was observed up to 600 mg/Kg/d, IV

ABX Represents a Novel Drug Class with Promising Activity against Gram Negative Bacteria

- ABX is the first member of a new class of boron-containing anti-bacterial agents
- ABX is a selective inhibitor of Leucyl tRNA synthetase and has broad spectrum activity against Gram Negative bacteria
- MIC_{90} of ABX is 1-4 µg/mL against a panel of *Enterobacteriaceae* (276)
  - With the exception of Proteae (MIC_{90} >64 µg/mL)
- ABX is not affected by pre-existing clinical resistance to established drug classes
- ABX is efficacious *in vivo* and is orally bioavailable
- ABX has good interspecies scaling from mouse to monkey
- ABX has a good safety profile and proved to be safe in repeat dose studies in rats at up to and including 600 mg/kg/d, IV
- AN3365 has advanced to Phase I clinical development

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