



Tackling Microbial Resistance: Novel Targets Accessed by Boron Antibacterials

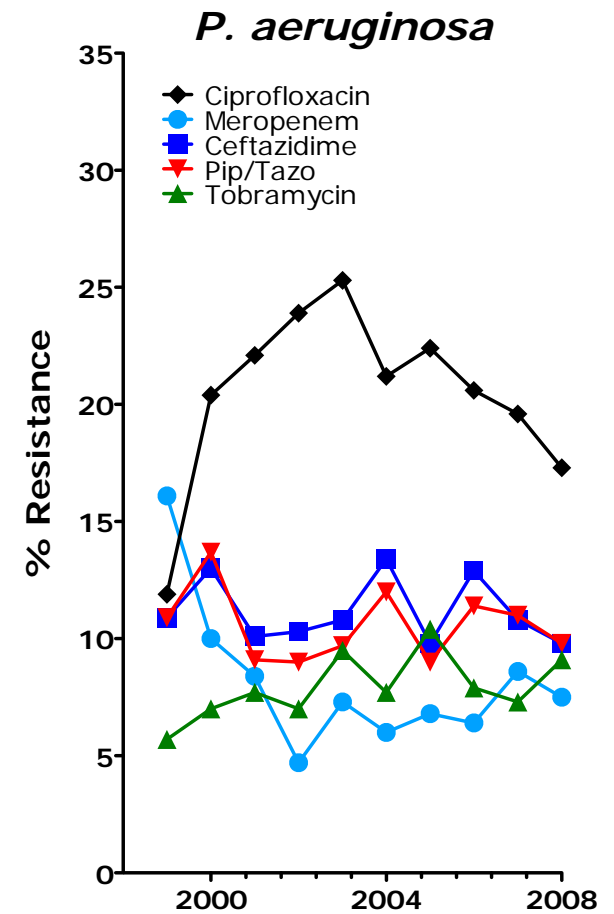
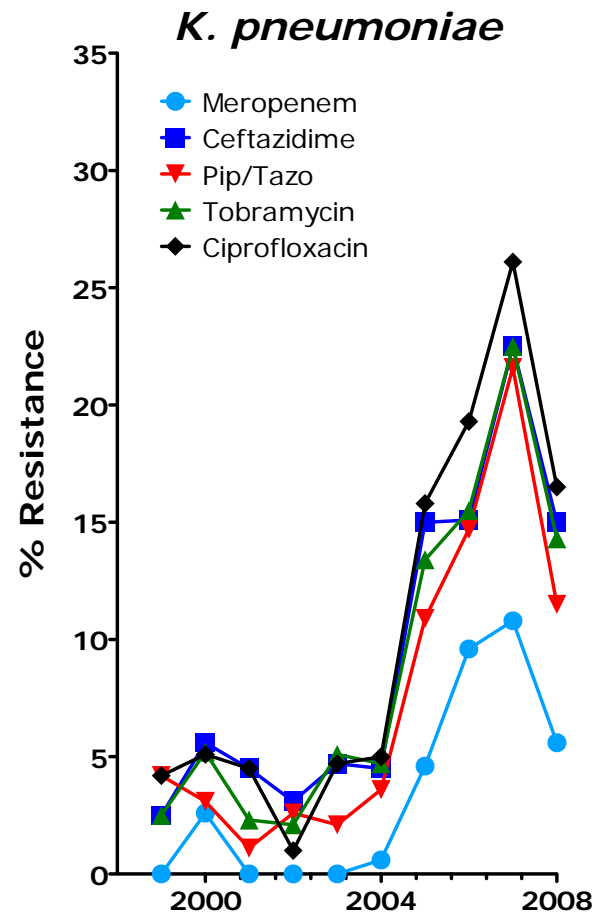
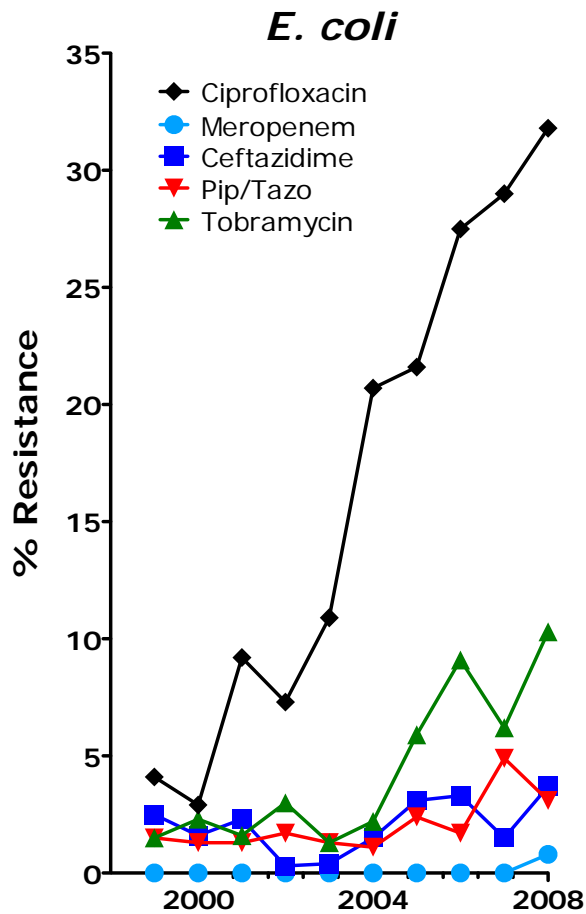
Dickon Alley, Discovery Biology, Anacor Pharmaceuticals

- The resistance problem, multidrug-resistant Gram-negative bacteria is on the rise
- Discovery of novel oxaborole-tRNA trapping (OBORT) inhibition mechanism of leucyl-tRNA synthetase in fungi
- Utilization of structure-aided design to develop novel antibacterials
 - First Gram-negative antibacterial GSK2251052 (AN3365) with a truly novel mechanism of action to reach phase II trials in over 30 years
- The boron advantage
- Novel boron-based antibacterials
 - Additional atom to help the antibacterial arsenal



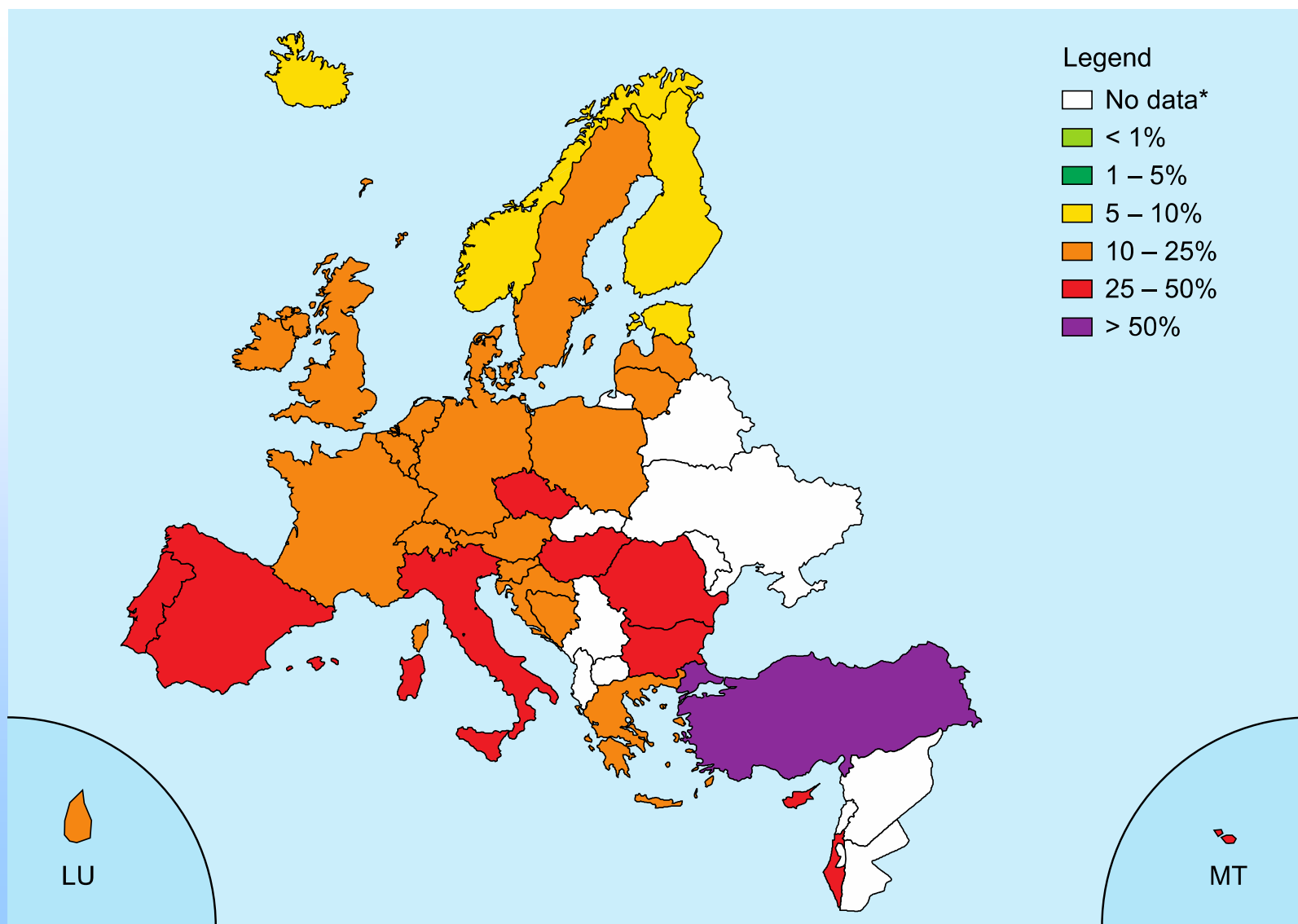
Resistance in Gram-negative Bacteria

Gram-negative Bacterial Resistance in the USA (1999-2008)



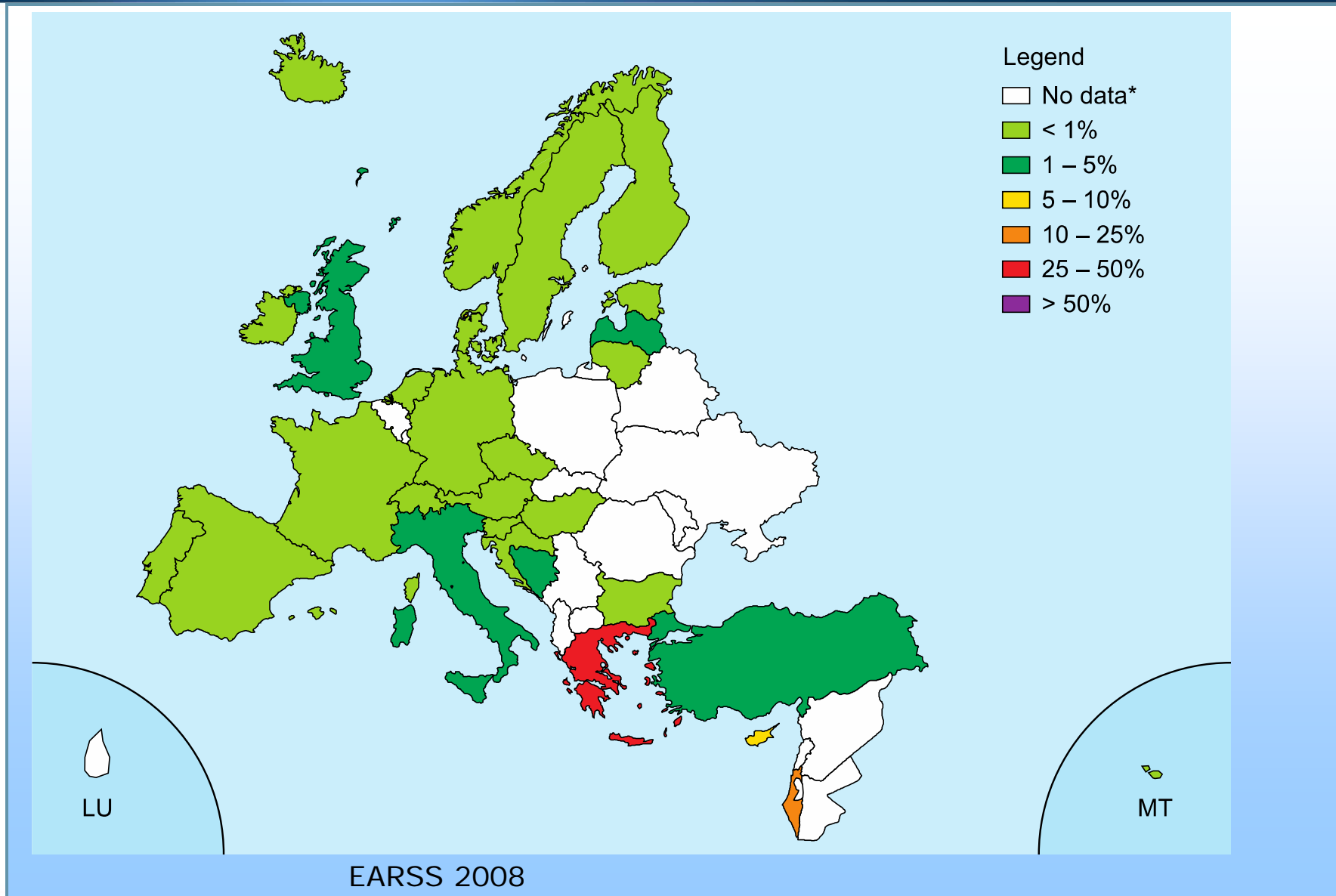
Rhomberg & Jones (2009) Diagnostic Microbiology and Infectious Disease 65: 414-426

E. coli Fluoroquinolones Resistance in Europe 2008: Oral Switch a Problem

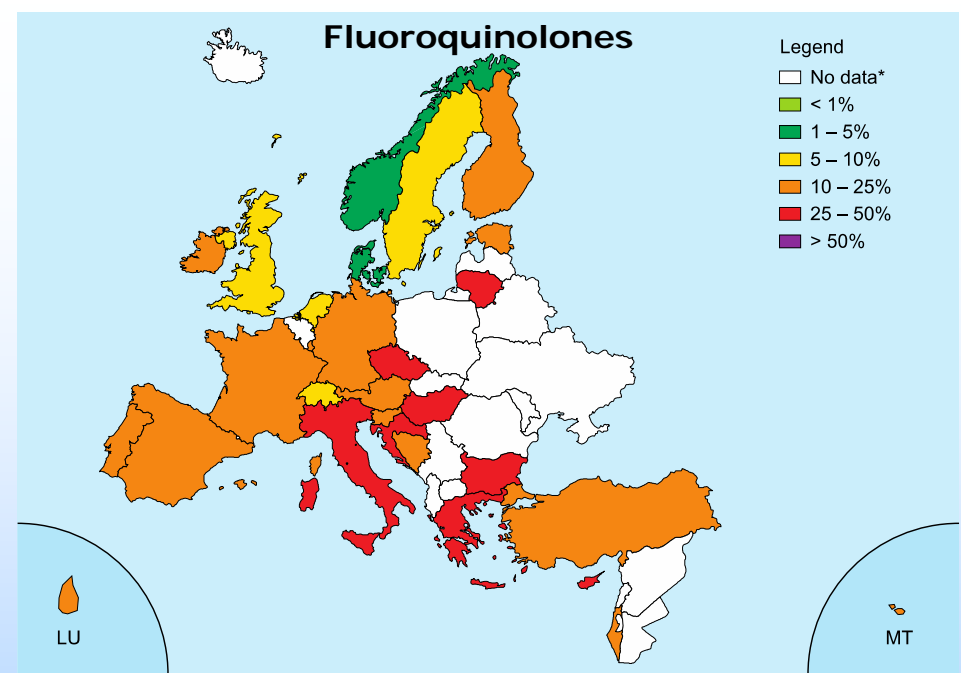
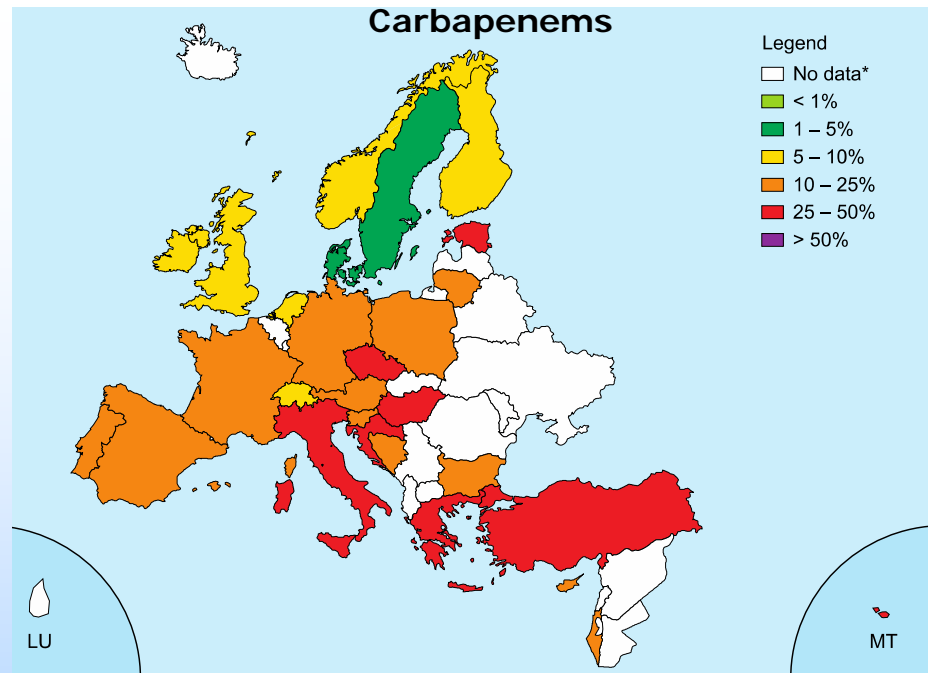


EARSS 2008

K. pneumoniae Carbapenem Resistant in Europe 2008

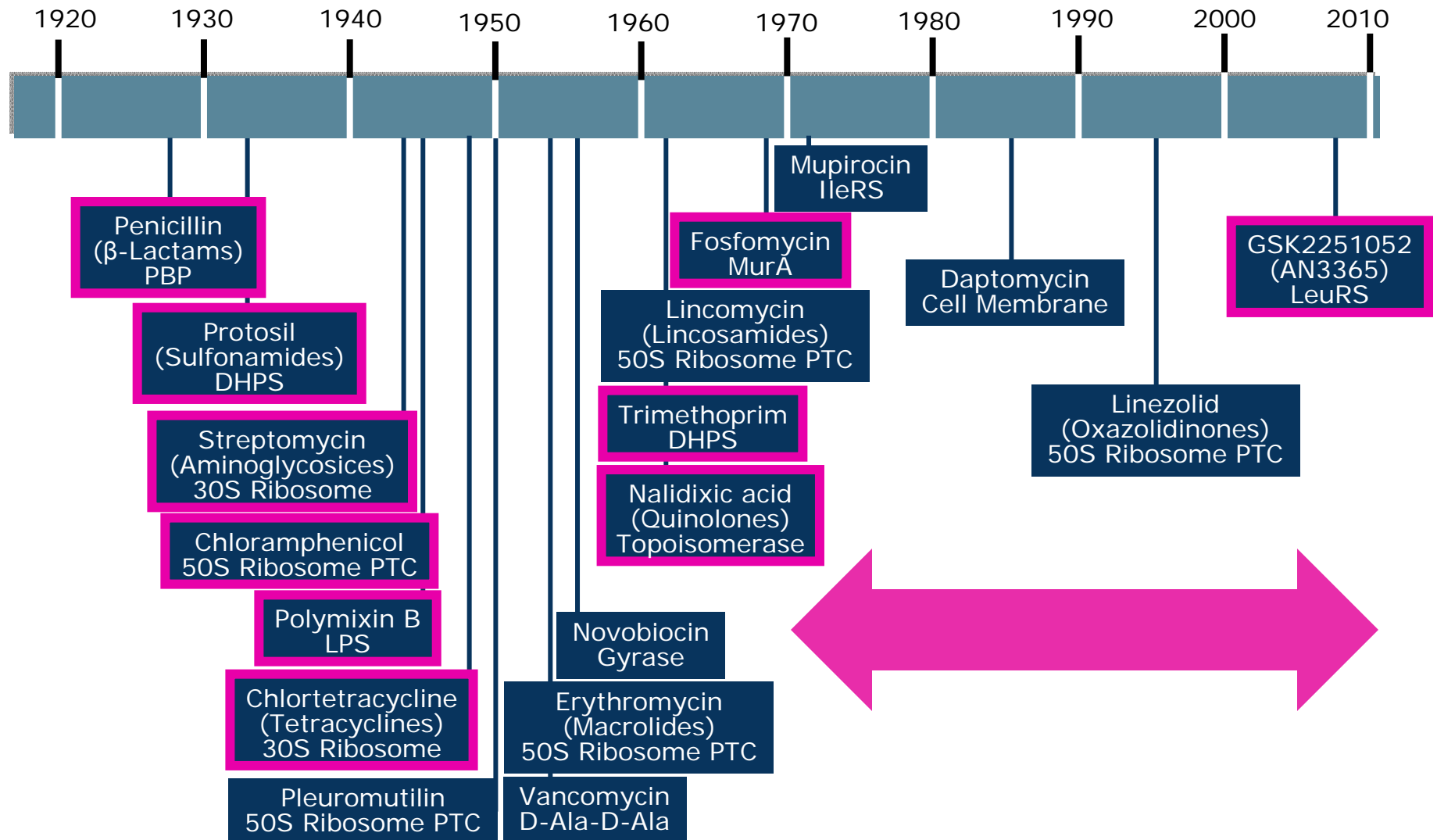


P. aeruginosa Carbapenems and Fluoroquinolones Resistant in Europe 2008



EARSS 2008

First Phase II Gram-negative Antibacterial to Inhibit a Novel Target in Over 30 Years



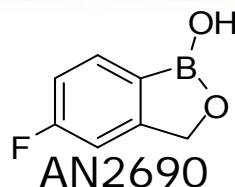
Modified from Lynn Silver (2011) Clinical Microbiology Reviews 24: 71–109



Discovery of Boron-based LeuRS Inhibitors

Addition of a New Atom to the Antimicrobial Arsenal

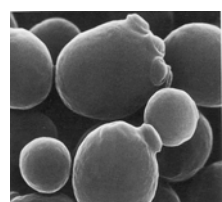
AN2690 has Broad-spectrum Antifungal Activity ($\mu\text{g}/\text{mL}$)



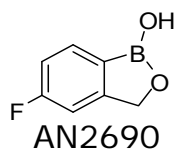
| Yeasts | AN2690 | Dermatophytes | AN2690 | Molds | AN2690 |
|--|------------|---------------------------------|--------|-------------------------------------|--------|
| <i>Saccharomyces cerevisiae</i> | 0.125 | <i>Trichophyton rubrum</i> | 1-8 | <i>Aspergillus fumigatus</i> | 0.5 |
| <i>Candida albicans</i> | 0.5 | <i>T. mentagrophytes</i> | 2-8 | <i>Rhizopus microsporus</i> | 2 |
| <i>C. albicans</i> (fluconazole resistant) | 0.5 | <i>T. tonsurans</i> | 2 | <i>Alternaria alternata</i> | 0.5 |
| <i>C. glabrata</i> | <0.5 | <i>Epidermophyton floccosum</i> | <0.5 | <i>Penicillium chrysogenum</i> | 2 |
| <i>C. krusei</i> | 1 | <i>Microsporum audouinii</i> | 2 | <i>Cladosporium cladosporioides</i> | 0.5 |
| <i>C. parapsilosis</i> | ≤ 0.5 | <i>M. canis</i> | 2 | <i>Fusarium solani</i> | <0.5 |
| <i>C. tropicalis</i> | ≤ 0.5 | <i>M. gypseum</i> | 2 | | |
| <i>Cryptococcus neoformans</i> | 4 | | | | |
| <i>Malassezia fufur</i> | 1 | | | | |
| <i>M. pachydermatis</i> | 1 | | | | |
| <i>M. sympodialis</i> | 1 | | | | |

Currently in phase III trials for the topical treatment of onychomycosis

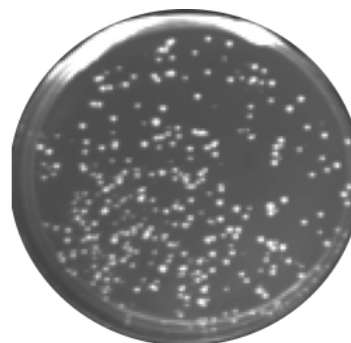
Identification of Leucyl-tRNA Synthetase (LeuRS) as Target for AN2690



+



AN2690 Inhibits
Protein Synthesis
in *S. cerevisiae*



Isolation AN2690
resistant mutants in
S. cerevisiae



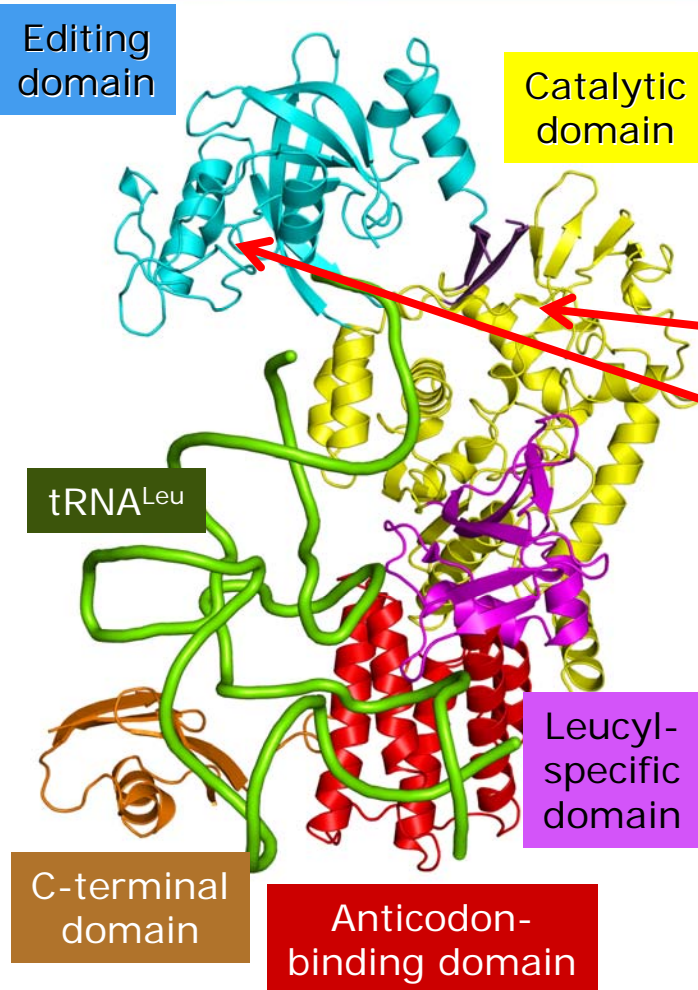
LeuRS (Cdc60p)
(Leucyl-tRNA Synthetase)



All mutations mapped
to the editing active
site

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

Leucyl-tRNA synthetase (LeuRS)

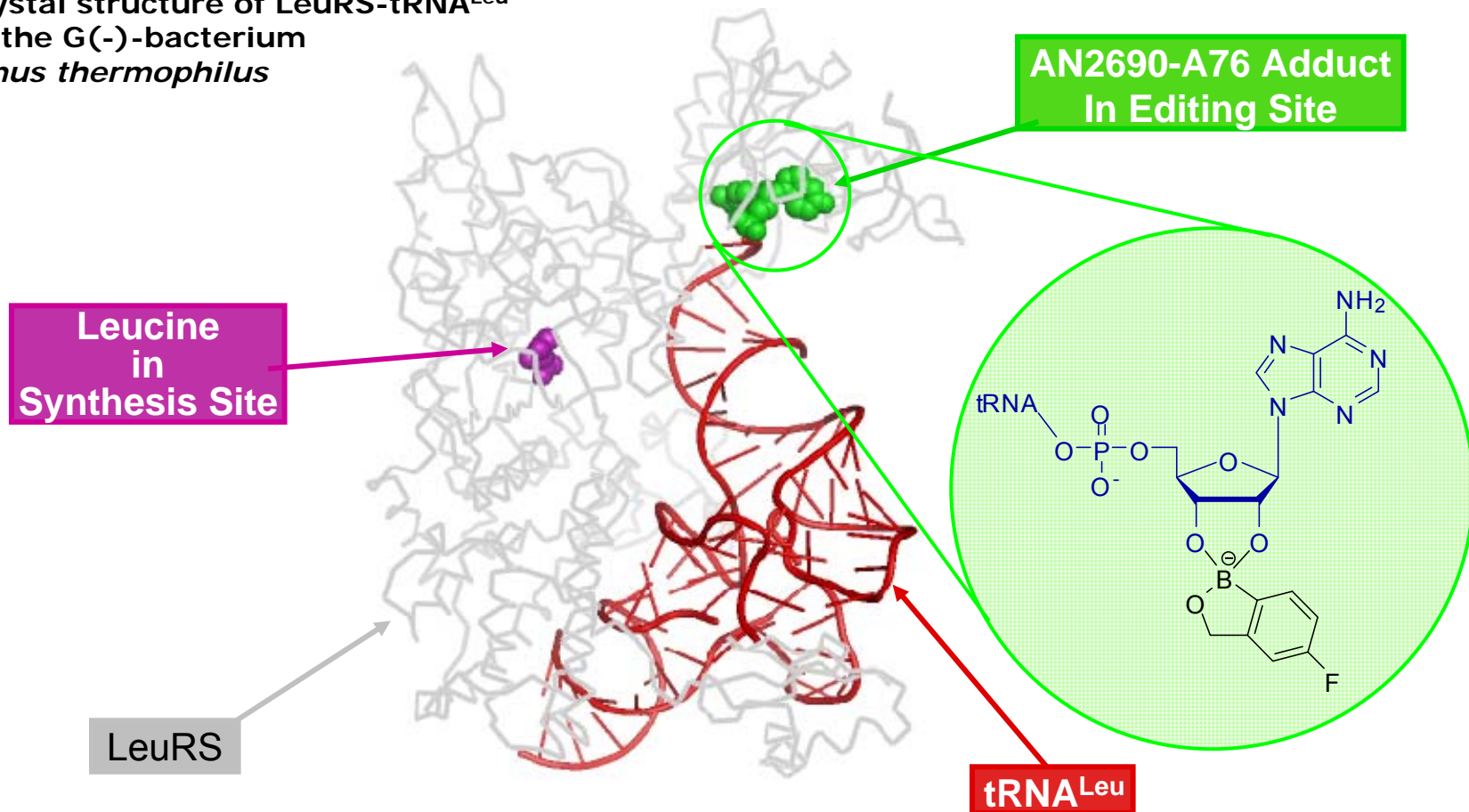


LeuRS-tRNA^{Leu} crystal structure at 2.3 Å

- The aminoacyl-tRNA synthetase LeuRS is essential for protein synthesis
 - Attaches leucine to the 3' terminal nucleotide of tRNA^{Leu} (A76)
- Two active sites separated by 38 Å
 - Synthetic active site synthesizes aminoacylated tRNA^{Leu}
 - Editing (proof-reading) active site
 - A76 moves between the two active sites
- Editing site ensures fidelity of protein synthesis
 - Synthetic site can attach isoleucine, valine, methionine, norvaline and other amino acids to tRNA^{Leu}
 - Editing site hydrolyzes all aminoacyl-tRNA^{Leu} other than leucine
 - Mischarged tRNA^{Leu} are extremely deleterious to the cell
 - Editing mutants are super-sensitive to non-cognate leucine analogues like norvaline

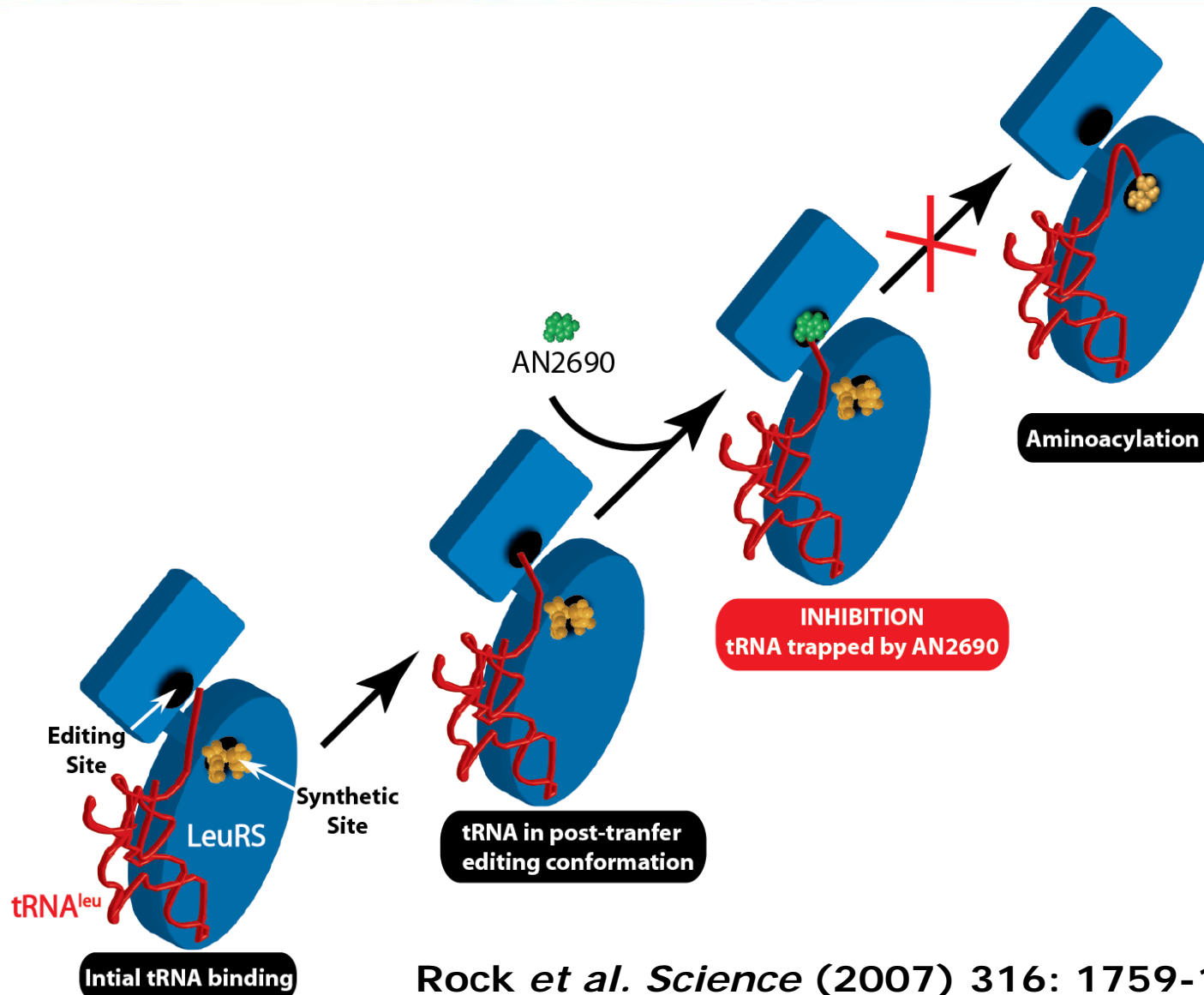
AN2690 Forms Adduct with tRNA^{Leu} in the LeuRS Editing Site

Co-crystal structure of LeuRS-tRNA^{Leu} from the G(-)-bacterium *Thermus thermophilus*



- Boron is absolutely essential
 - Carbon and other analogues are inactive
- Unlike mupirocin (IleRS inhibitor) AN2690 MICs do not increase with the addition of mM Leucine to media

Inhibition By Oxaborole-tRNA Trapping (OBORT) Mechanism



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

AN2690-Bacterial Crystal Structure Suggested We can Design LeuRS Antibacterials



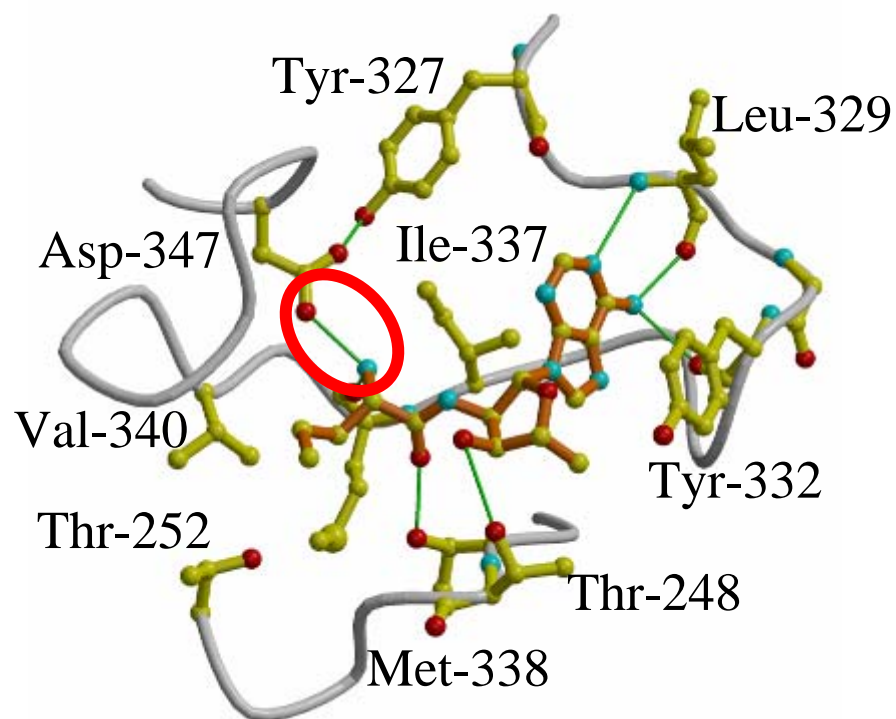
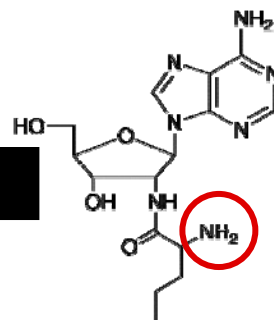
AN2690 co-crystal structure of LeuRS-tRNA^{Leu} from the Gram-negative bacterium *Thermus thermophilus*



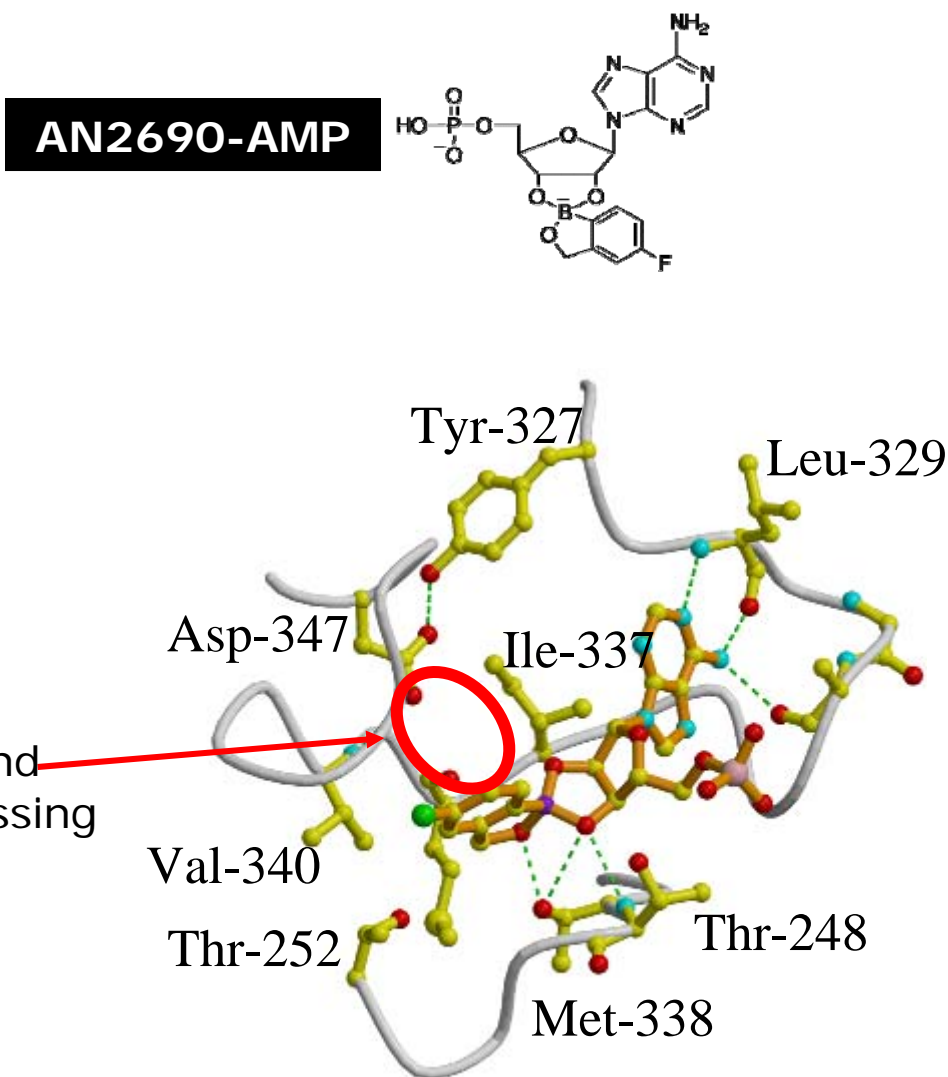
Rock, et al, *Science* (2007) 316: 1759-1761.

Key H-bond Interaction Observed in Substrate Analogue Co-crystal

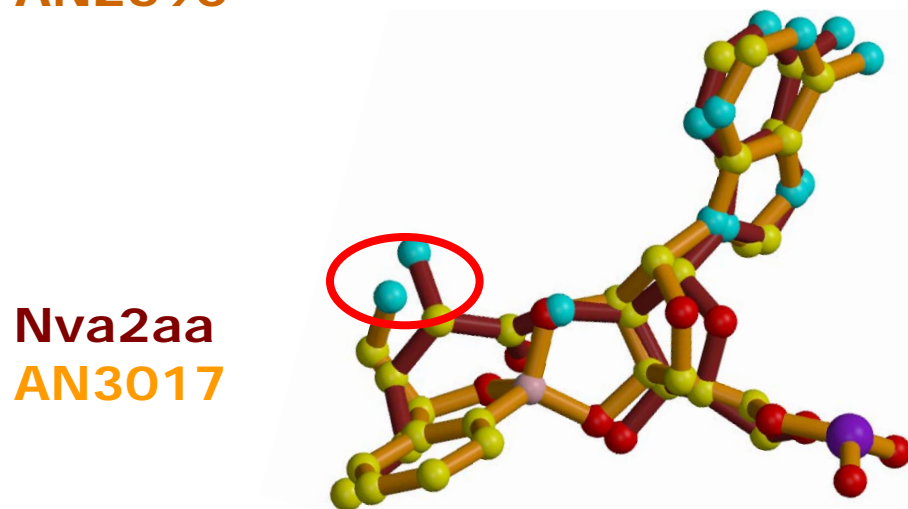
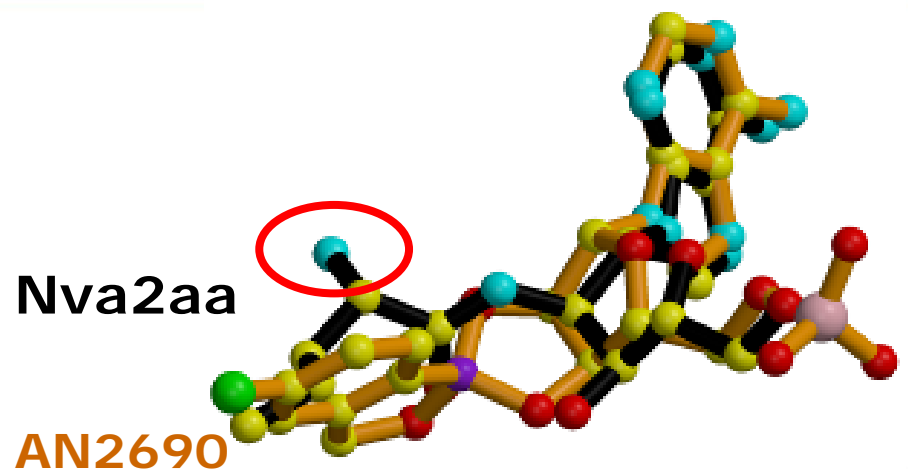
Editing Substrate Analogue



Key H-bond Interaction Not Found With AN2690 Co-crystal Structure

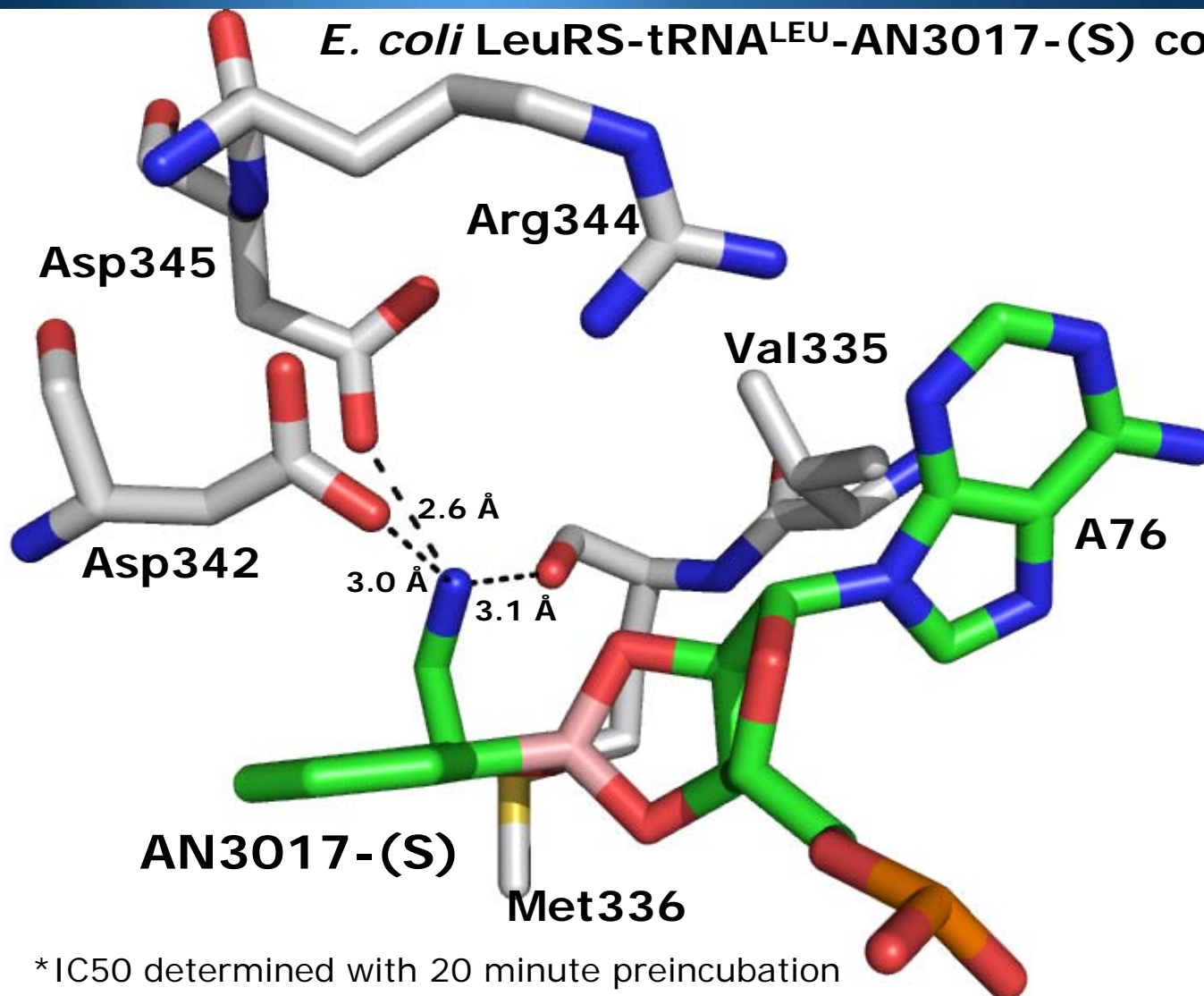


Amine Substitution was added to Gain This Key H-bond with Asp347/345

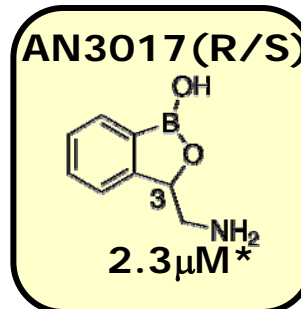


The Addition of the 3-CH₂NH₂ to the Oxaborole Nucleus Gained Key Interactions

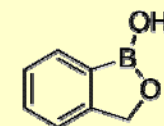
E. coli LeuRS-tRNA^{LEU}-AN3017-(S) complex



E. coli LeuRS IC₅₀

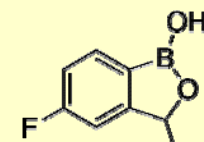


AN2679



27.5 μM*

AN2714

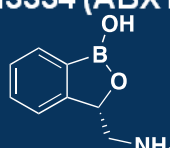


>400 μM*

- *IC₅₀ determined with 20 minute preincubation
- AN3017 inhibition is time-dependent
- Only S-enantiomer is active

ABX1 (AN3334) has Excellent *P. aeruginosa* Activity But is Poor for *A. baumannii* and *Proteus*

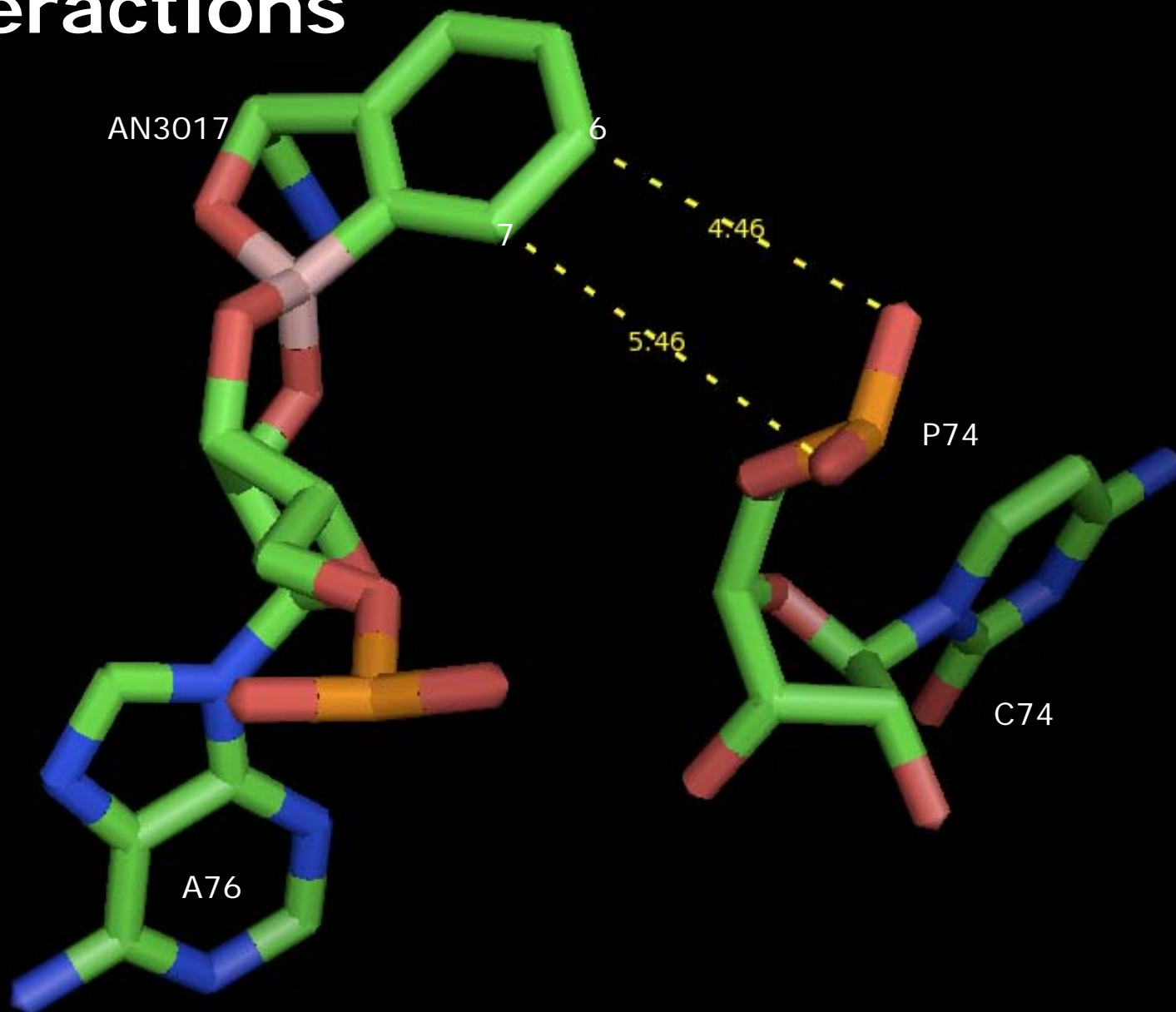


| AN3334 (ABX1)  | No. of strains | MIC ₉₀ (µg/mL) | | | | | | | |
|--|----------------|---------------------------|-------------|----------|----------|--------------|------------|-------------|-------------------------|
| | | ANACOR | Tigecycline | Imipenem | Cefepime | Levofloxacin | Gentamicin | Ceftazidime | Piperacillin/tazobactam |
| <i>Pseudomonas aeruginosa</i> (WT) | 50 | 1 | 16 | 1 | 8 | 2 | 4 | 16 | 32 |
| <i>Pseudomonas aeruginosa</i> (MbL+) | 26 | 1 | >16 | >64 | >32 | >16 | >16 | >32 | >128 |
| <i>Acinetobacter baumannii</i> (WT) | 25 | >128 | 1 | 0.25 | 8 | 4 | 2 | 16 | 8 |
| <i>Stenotrophomonas maltophilia</i> (WT) | 50 | 1 | 1 | >64 | >32 | 4 | >16 | >32 | >128 |
| <i>Burkholderia cepacia</i> | 50 | 4 | 4 | 16 | 32 | 8 | >16 | 16 | 32 |
| <i>Escherchia coli</i> (WT) | 27 | 1 | 0.25 | 0.12 | ≤1 | >16 | 2 | ≤1 | 8 |
| <i>Escherchia coli</i> (ESBL) | 25 | 2 | 0.25 | 0.25 | >32 | >16 | >16 | >32 | 128 |
| <i>Klebsiella</i> spp. (WT) | 25 | 1 | 0.5 | 0.25 | ≤1 | ≤0.5 | 1 | ≤1 | 16 |
| <i>Klebsiella</i> spp. (ESBL) | 15 | 1 | 2 | 1 | >32 | 16 | >16 | >32 | >128 |
| <i>Klebsiella</i> spp. (KPC) | 10 | 2 | 1 | >64 | >32 | >16 | 16 | >32 | >128 |
| <i>Enterobacter</i> spp. (WT) | 25 | 1 | 0.5 | 1 | ≤1 | ≤0.5 | ≤0.5 | 2 | 8 |
| <i>Enterobacter</i> spp. (AmpC) | 26 | 1 | 4 | 0.5 | 8 | >16 | >16 | >32 | >128 |
| <i>P. mirabilis</i> (WT) | 42 | 128 | 4 | 2 | ≤1 | 2 | 2 | ≤1 | 0.5 |
| <i>P. mirabilis</i> (ESBL) | 11 | >128 | 4 | 2 | >32 | >16 | >16 | >32 | 4 |
| <i>Morganella morganii</i> (WT) | 17 | 2 | 2 | 4 | ≤1 | 4 | 2 | 4 | 2 |

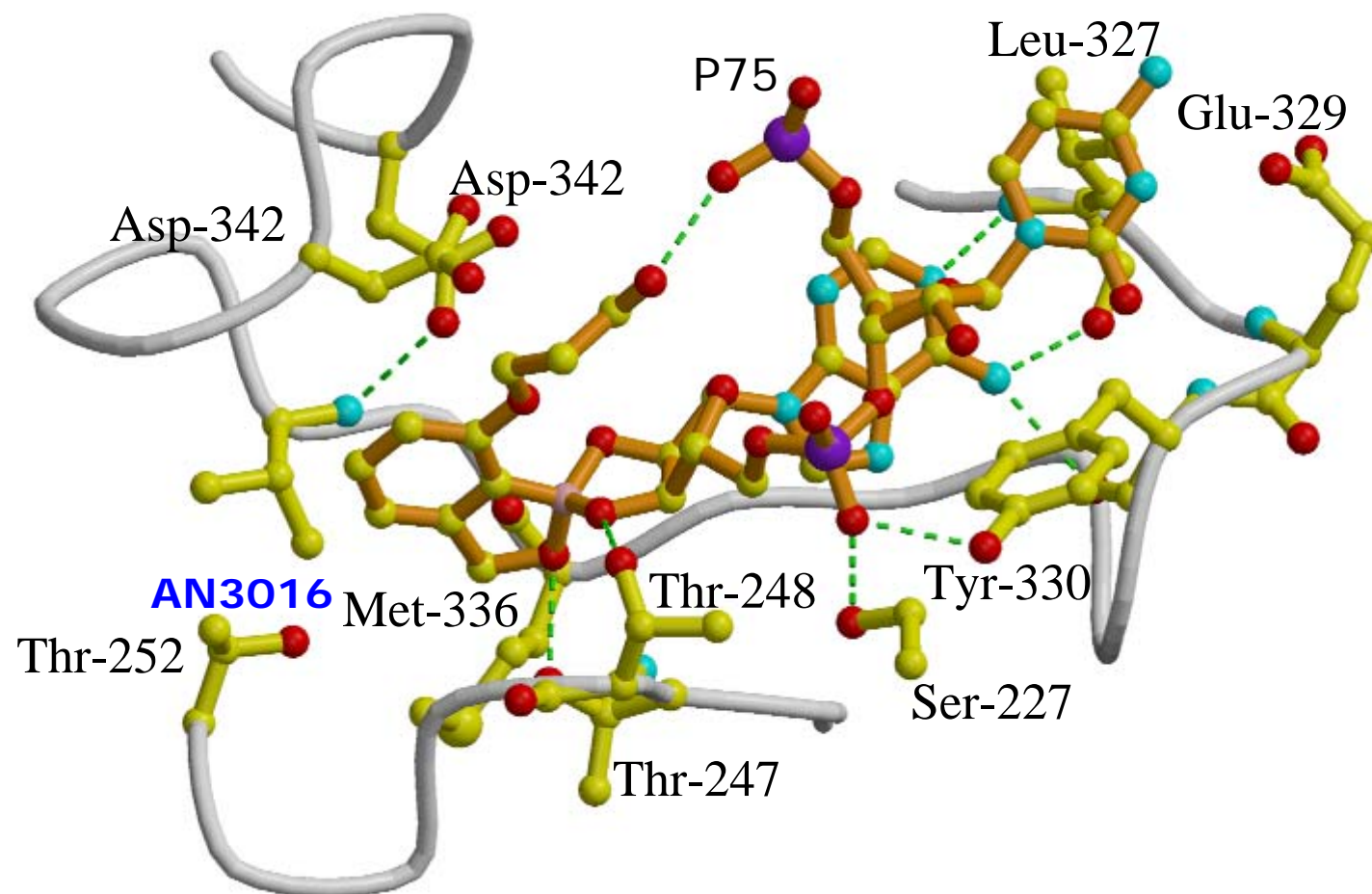


Co-crystal Structures were Used to Add Interactions to Gain Further Potency

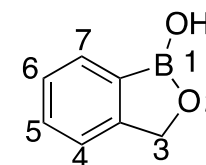
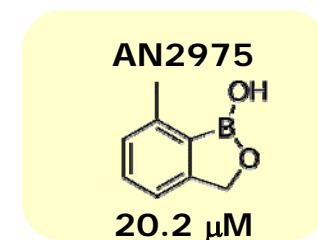
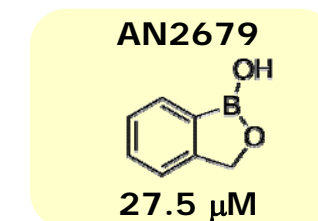
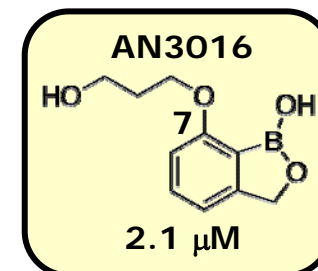
Can We Add Further tRNA Interactions



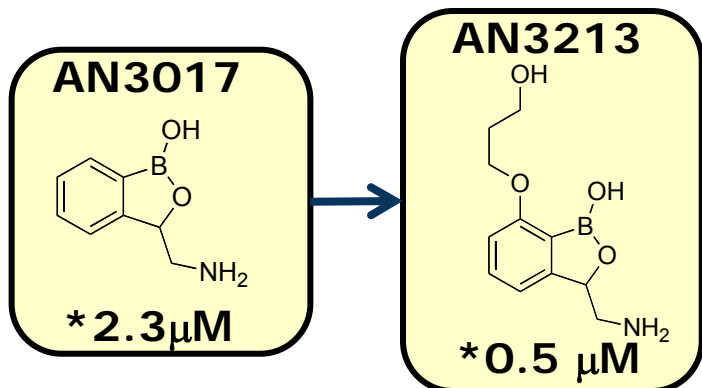
AN3016 Identified a New Interaction in C-7 position



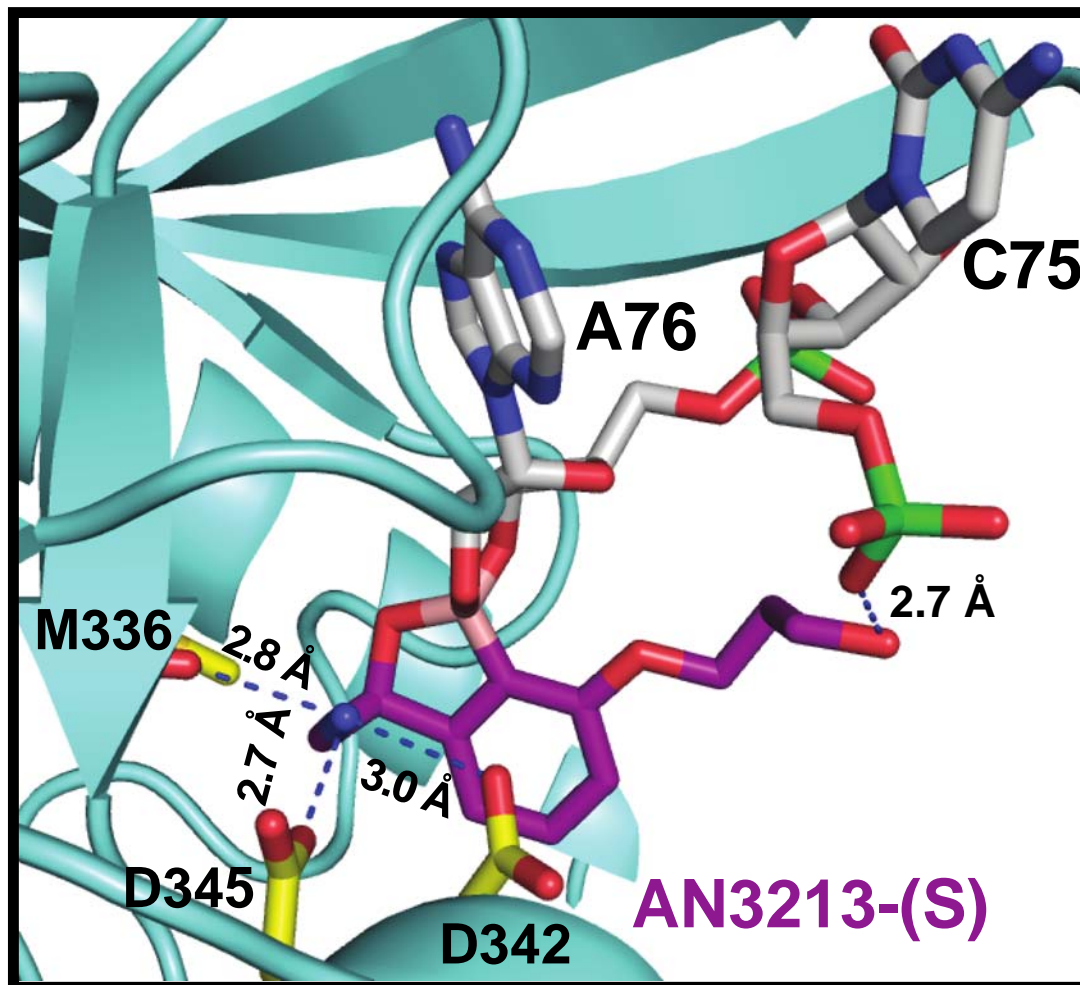
E. coli LeuRS IC₅₀



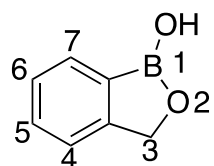
Combination of C-3 and C-7 Substitutions Improved Biochemical Potency



- AN3365 is the active S-enantiomer in AN3213 (R/S)



Crystal structure of active S-enantiomer of AN3213 with *E. coli* tRNA/LeuRS

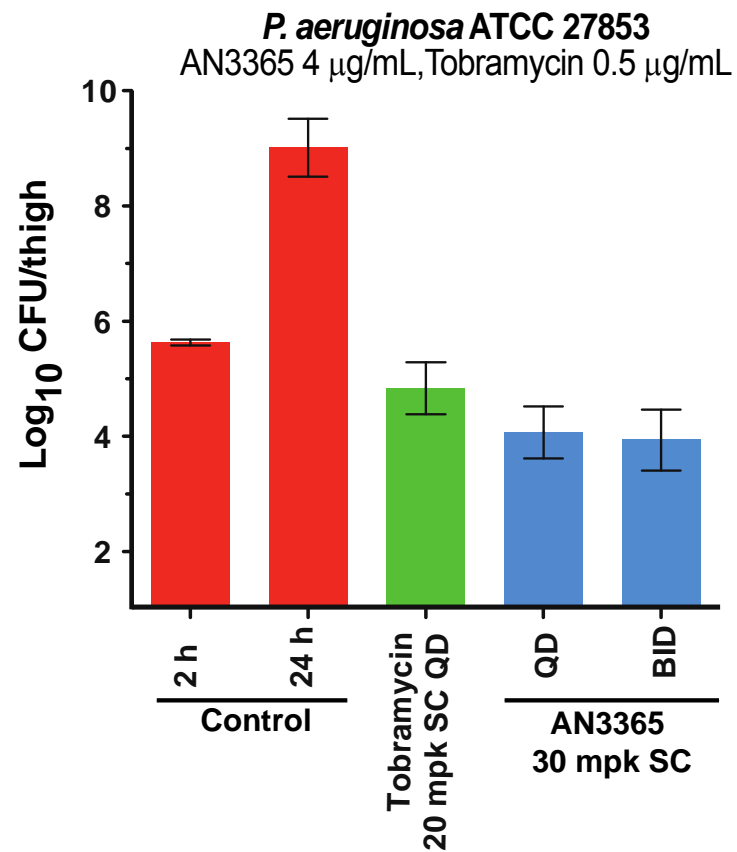
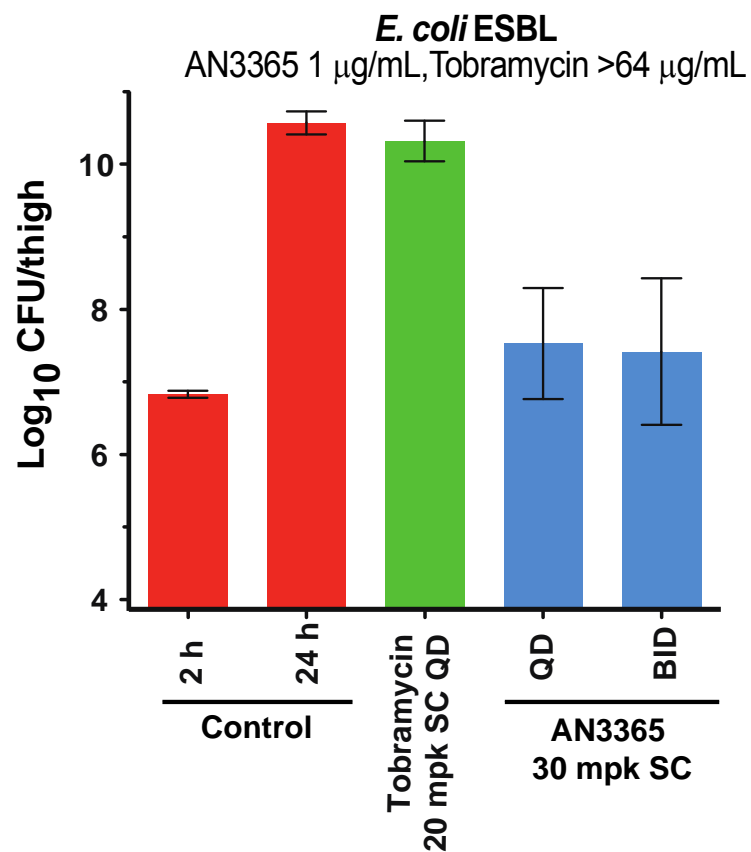


* *E. coli* LeuRS IC₅₀ after 20 minutes preincubation

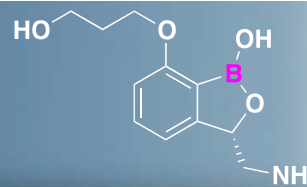
AN3365 is Efficacious in *E. coli* and *P. aeruginosa* Animal Models of Infections



Mouse Neutropenic Thigh Model



AN3365 has Good Activity against Enterobacteriaceae



- AN3365 has a very narrow MIC range (0.25-4 µg/mL)
- AN3365 has good activity against multidrug-resistant Enterobacteriaceae including isolates bearing metallo-β-lactamases
- Phase I is now complete and progressing for the treatment of cUTI, cIAI and VAP
- AN3365 was licensed to GSK and is now called GSK2251052 (GSK '052)

| Strain | No. of strains | MIC ₉₀ (µg/mL) | | | | | | | | |
|--------------------------------|----------------|---------------------------|--------------|-------------|----------|----------|--------------|------------|-------------|-------------------------|
| | | AN3365 | AN3365 Range | Tigecycline | Imipenem | Cefepime | Levofloxacin | Gentamicin | Ceftazidime | Piperacillin/tazobactam |
| Enterobacteriaceae | 2,029 | 1 | 0.25-4 | 2 | 2 | 4 | 16 | 16 | >16 | 64 |
| <i>E. coli</i> (ESBL) | 27 | 1 | 0.5 - 2 | 0.5 | 0.5 | >32 | >16 | >16 | >16 | >128 |
| <i>Klebsiella</i> spp. (ESBL) | 71 | 1 | 0.5 - 2 | 2 | 0.5 | >32 | >16 | >16 | >16 | >128 |
| <i>Klebsiella</i> spp. (KPC) | 26 | 2 | 0.25-2 | 2 | 64 | >32 | >16 | 16 | >16 | >128 |
| <i>E. cloacae</i> (AmpC) | 50 | 0.5 | 0.25-1 | 4 | 1 | >32 | 16 | >16 | >16 | >128 |
| <i>Citrobacter</i> spp. (AmpC) | 54 | 1 | 0.25-2 | 2 | 1 | 16 | 8 | >16 | >16 | >128 |
| <i>P. mirabilis</i> (ESBL) | 15 | 1 | 0.5 - 2 | 4 | 4 | >32 | >16 | >16 | 16 | 4 |
| <i>S. marcescens</i> (AmpC) | 26 | 1 | 0.25-1 | 2 | 2 | 8 | 4 | >16 | >16 | 32 |

Colour coding according to CLSI breakpoints (M100-S21) except for EUCAST were used for Tigecycline. Green=S, Yellow=I, Red=R



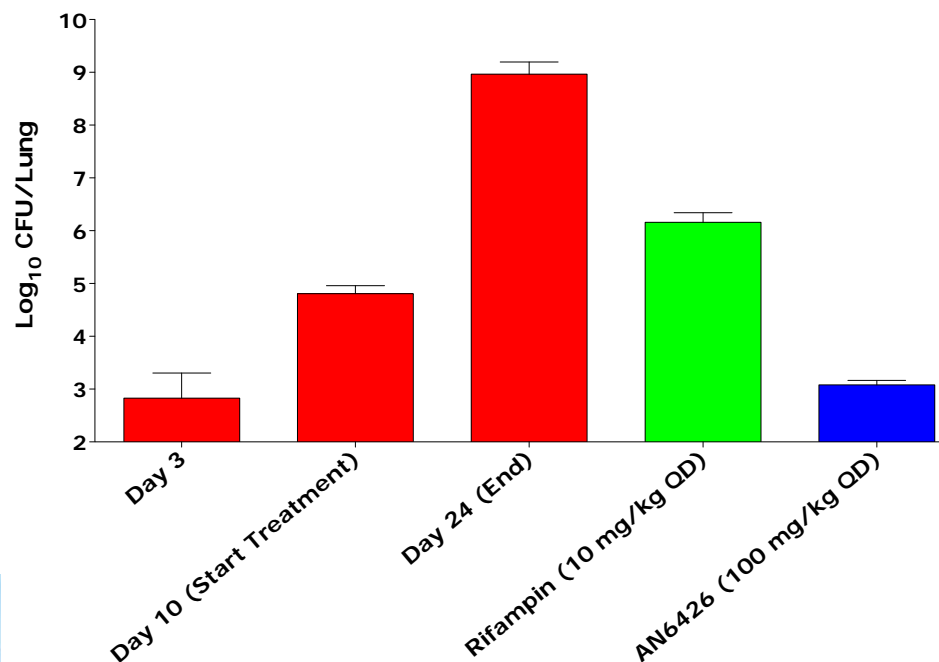
LeuRS is An Excellent Target for *M. tuberculosis*

The LeuRS Inhibitor AN6426 is an Extremely Potent and Selective for *M. tuberculosis*



| MIC (µg/mL) | AN6426 | Rifampin |
|---|--------|----------|
| <i>M. tuberculosis</i> H37Rv (MABA aerobic) | 0.0027 | 0.03 |
| <i>M. tuberculosis</i> H37Rv (LORA anaerobic) | 0.016 | 0.54 |
| <i>S. aureus</i> ATCC 29213 | 64 | 0.01 |
| <i>S. pneumoniae</i> ATCC 700673 | 32 | 0.06 |
| <i>S. pyogenes</i> ATCC 19615 | 64 | 0.03 |
| <i>E. coli</i> ATCC 25922 | 64 | 8 |

Fourteen days treatment Mtb C57BL/6 mice
GKO model



Funded By

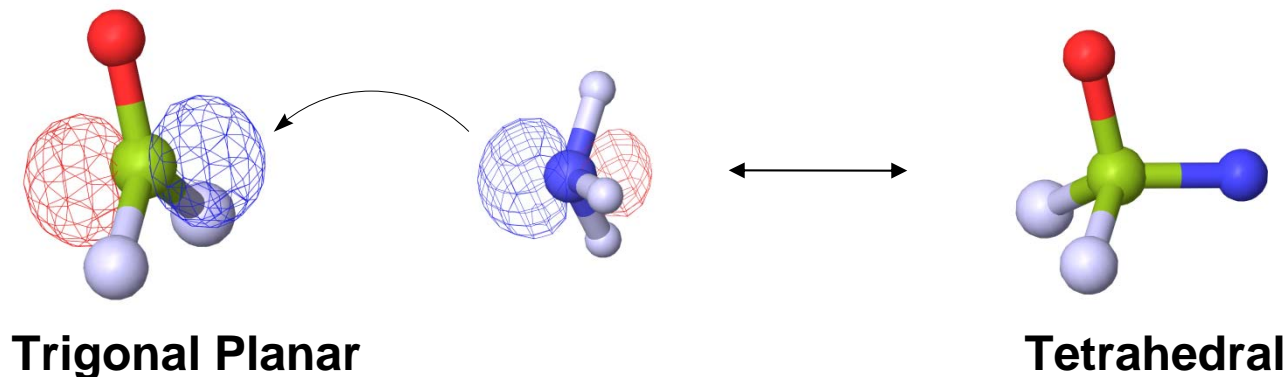


TB ALLIANCE
GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT



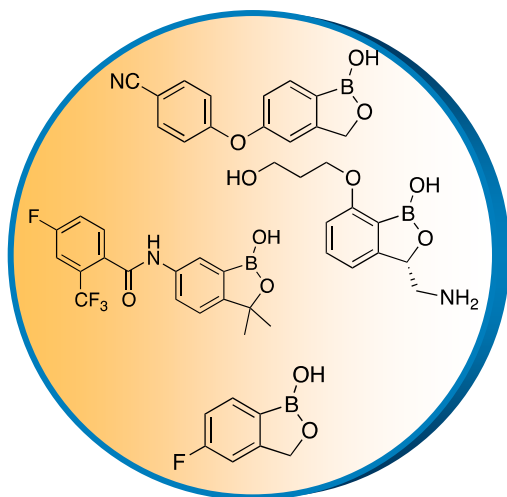
The Advantage of Boron

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



- Boron has an empty P-orbital and can form a new bond under specific conditions
- The new bond forms a tetrahedral structure
- Exploitation of P-Orbital Expands Drug Design Possibilities

Key Features of Anacor's Boron Compounds



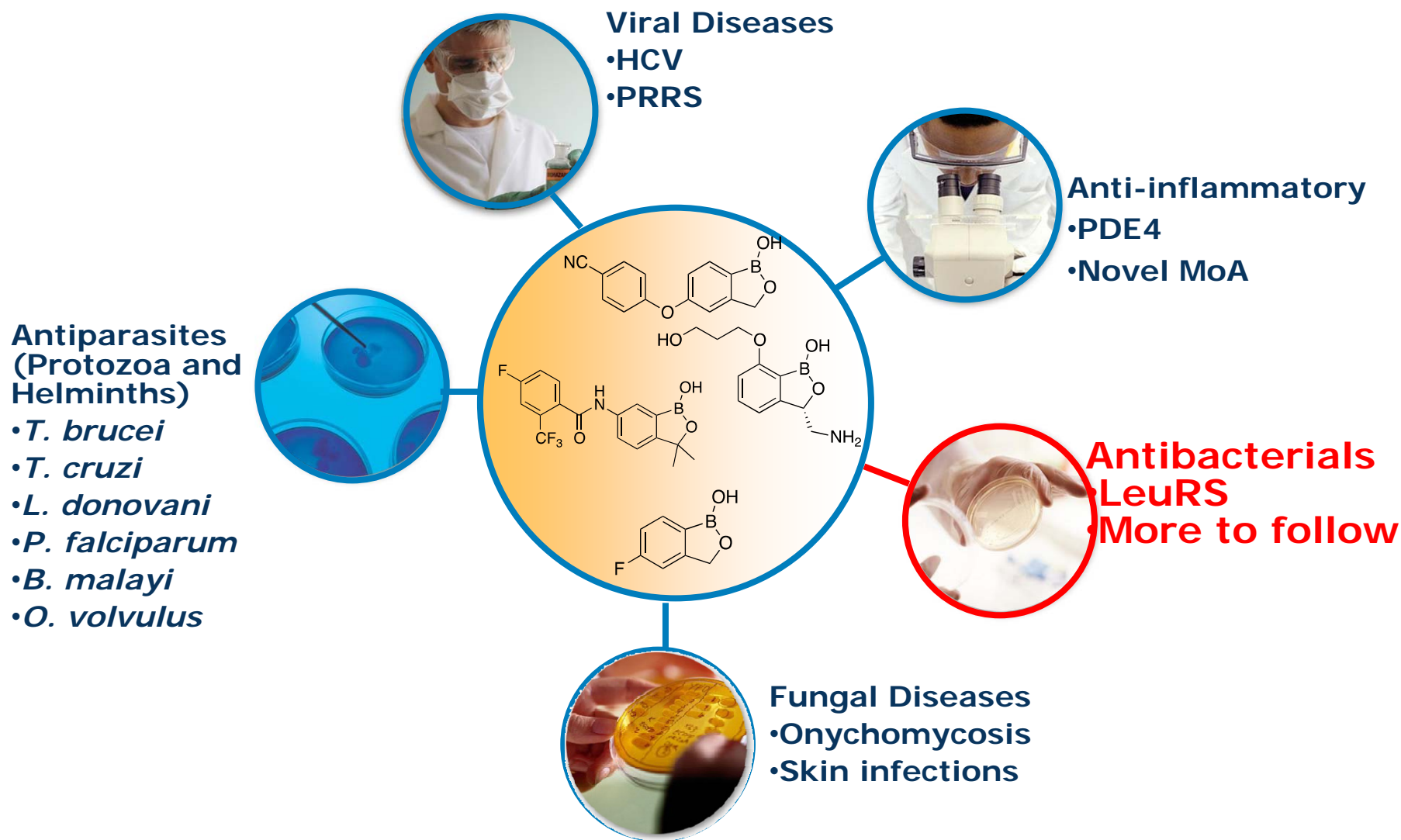
Characteristics

- Boron is part of a fused aromatic ring system
- Well-defined parameters for MW, solubility, PSA, LogP, H-bond donors/acceptors
- Reactive P-orbital

Advantages

- Can address a broad range of biochemical targets
- Good biochemical target selectivity
- Applicable to broad range of diseases
- Relatively Open IP landscape
- Intrinsic drug-like properties

Broad Application of Boron Compounds Across Multiple Therapeutic Areas

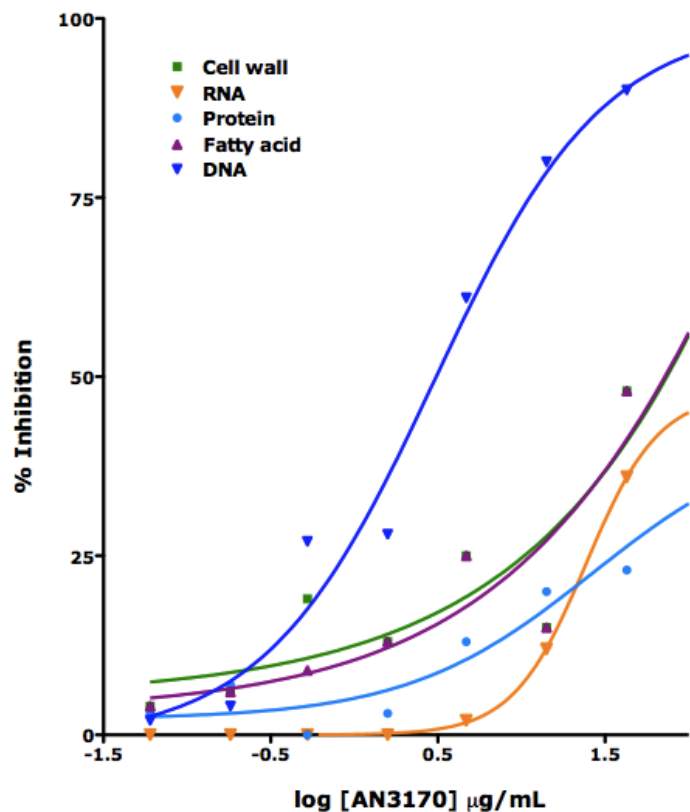




Non-topoisomerase Boron-based DNA Synthesis Inhibitors

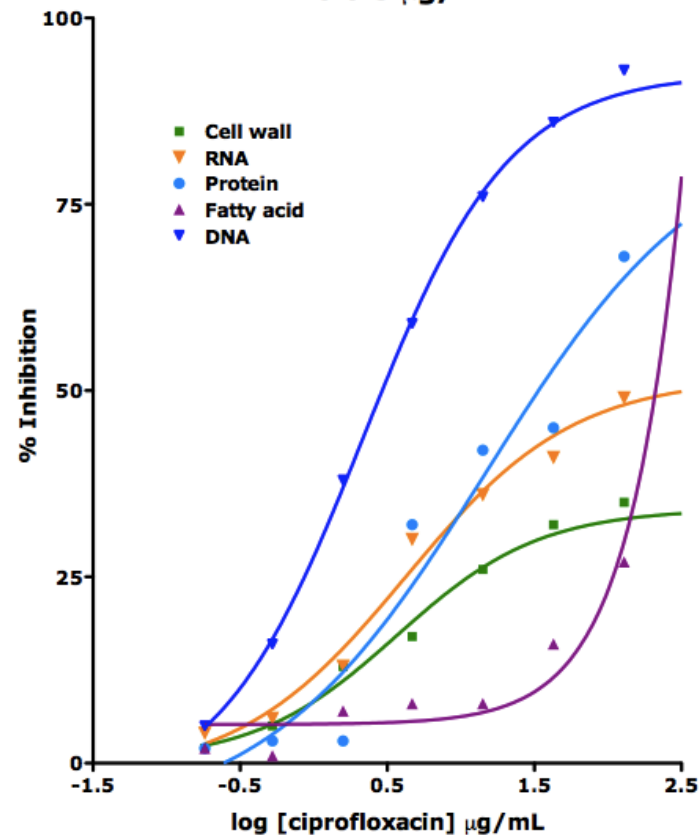
Novel Specific DNA Synthesis Inhibitors

AN3170
S. aureus ATCC 29213
MIC 8 $\mu\text{g/mL}$



| Pathway | Protein | DNA | RNA | Fatty Acid | Cell Wall |
|---------------------------------------|---------|-----|-----|------------|-----------|
| EC ₅₀ ($\mu\text{g/mL}$) | 55 | 3.3 | 42 | 32 | >64 |

Ciprofloxacin
S. aureus ATCC 29213
MIC 0.5 $\mu\text{g/mL}$



| Pathway | Protein | DNA | RNA | Fatty Acid | Cell Wall |
|---------------------------------------|---------|-----|-----|------------|-----------|
| EC ₅₀ ($\mu\text{g/mL}$) | 25 | 2.9 | >64 | >64 | >64 |

Novel DNA Synthesis Inhibitors are Unaffected by Multidrug Resistance in *S. aureus*

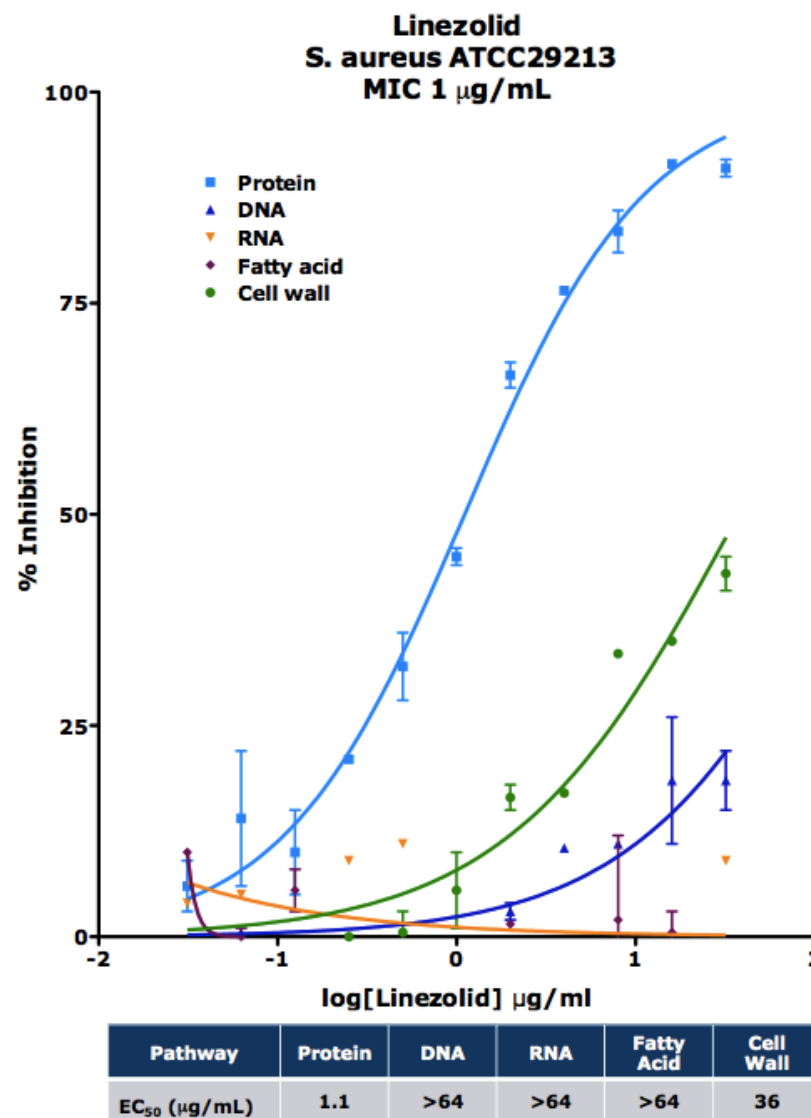
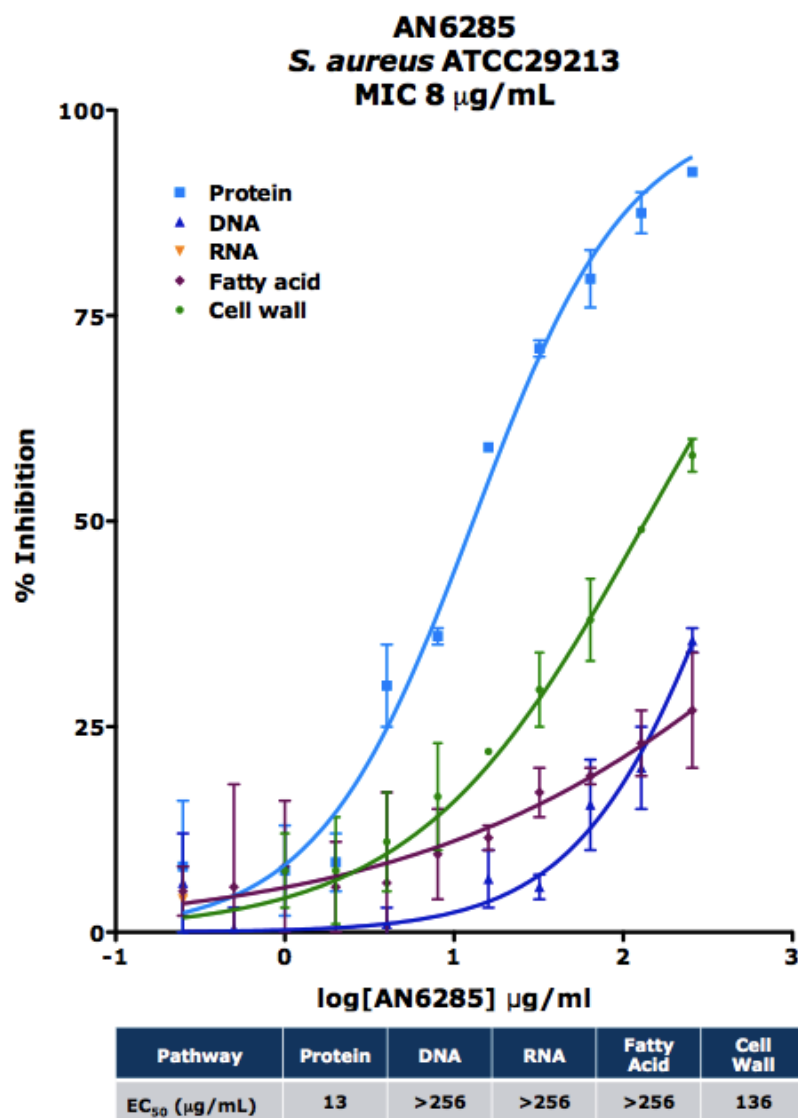


| Compound | <i>S. aureus</i> MIC ($\mu\text{g/mL}$) | | | | | | | | | | |
|---------------|---|------------|--------|---------|---------|---------|---------|-----|--------|-------|-------|
| | ATCC 29213 | ATCC 33591 | NRS 71 | NRS 120 | NRS 121 | NRS 127 | NRS 382 | cfr | VRS 10 | VRS 5 | VRS 6 |
| AN3170 | 8 | 8 | 4 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 4 |
| Ciprofloxacin | 0.5 | 0.5 | 32 | 32 | 64 | 32 | 16 | 64 | 128 | 128 | 64 |
| Clindamycin | 0.25 | 4 | 64 | 1 | 1 | 0.25 | 128 | 128 | 128 | 128 | 128 |
| Linezolid | 1 | 1 | 1 | 32 | 32 | 4 | 1 | 8 | 1 | 1 | 1 |
| Vancomycin | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 128 | 128 | 128 |

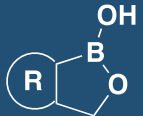

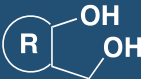


Boron-based Non-LeuRS
Protein Synthesis Inhibitors

Novel Non-LeuRS Protein Synthesis Inhibitors



Boron Essentiality and Potential Broad-spectrum Activity

| Organism |  |  |  |
|--------------------------------|--|---|---|
| <i>S. aureus</i> ATCC 33591 | 8 | >64 | >64 |

| Compound | MIC (µg/mL) | | | | | | | | | | |
|----------|-----------------------------------|------------------------------|------------------------------|--------------------------------------|-------------------------------------|------------------------------------|--------------------------------|-----------------------------------|----------------------------------|----------------------------------|---------------------------------|
| | <i>A. baumannii</i> ATCC 17978 | <i>E. coli</i> ATCC 25292 | <i>P. aeruginosa</i> PA01 | <i>P. aeruginosa</i> PA01 pump KO | <i>S. maltophilia</i> ATCC 13637 | <i>H. influenzae</i> ATCC 49766 | <i>S. aureus</i> ATCC 33591 | <i>S. pneumoniae</i> ATCC 6301 | <i>S. pyogenes</i> ATCC 19615 | <i>E. faecalis</i> ATCC 29212 | <i>E. faecium</i> ATCC 19434 |
| AN6285 | 16 | >64 | >64 | 8 | 8 | 16 | 8 | 2 | 2 | 8 | 8 |

- GSK2251052 (AN3365) has good activity against Enterobacteriaceae including MDR isolates
 - Boron is absolutely essential for its activity
- GSK2251052 (AN3365) was shown in a phase I trial to be safe and well tolerated with no serious adverse events, and is progressing for treatment of cUTI, cIAI and HAP/VAP
- The addition of boron to the medicinal chemistry “tool kit” should enable access to additional targets

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