Decisions, Decisions:

What Makes a Good Hit? A Good Lead? Why Do You Write a TPP? How Do You Write a TPP?

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Agenda

- Short talks
  - Lynn Silver: Early discovery, hit validation
  - Tim Waddell: Medicinal chemistry
  - Tom Dougherty: Case histories
  - John Tomayko: TPPs [Target Product Profile]
- Panel discussion
- Audience Q&A

Discovery Strategies

- May be directed toward TARGETS
  - Finding inhibitors of specific bacterial functions
- Or Empirical, using KILL-THE-BUG screens

- Each approach has adherents
  - Empirical screening was the source of almost all antibiotics
  - Target-based screening is/seems more rational
- *You can get "hits" pretty easily*

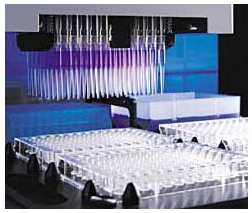
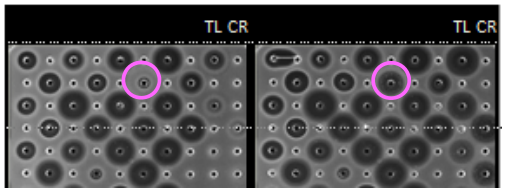
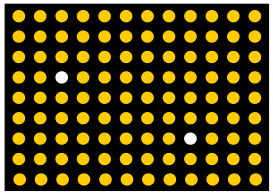
HOWEVER

- Neither an enzyme inhibitor nor a bactericidal compound is a drug
- It’s not even a lead
- Many steps to qualify a hit as a lead
- And many more to qualify an optimized lead as a clinical candidates

“Hit to lead” in discovery of small molecule antibacterial agents

- Find hits by various methods
- Each has a different path for follow-up
- *Basic questions are similar for all paths*

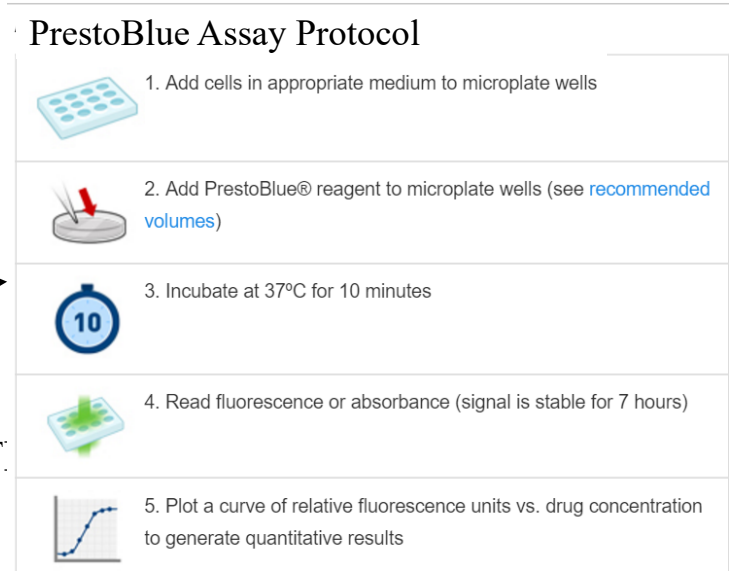
Three scenarios for hit generation from synthetic libraries

Screen for target inhibition in vitro	Whole cell directed phenotypic screen	Bacterial killing (empirical) screen
		
Chemical attractiveness and tractability	Chemical attractiveness and tractability	Chemical attractiveness and tractability
Does it have an MIC?	Counterscreens to eliminate false positives	**Toxicity**
MIC due only to inhibiting in vitro target?	**Secondary assays to confirm MOA**	Resistance
Explore MOA	Resistance	MOA
Resistance	Initial toxicity	Spectrum ± Serum
Initial toxicity	Spectrum ± Serum	Static/cidal
Spectrum ± Serum	Static/cidal	
Static/cidal		
If no MIC, why?		
Can it be optimized for entry?		

Lead for optimization

In vitro measures of toxicity

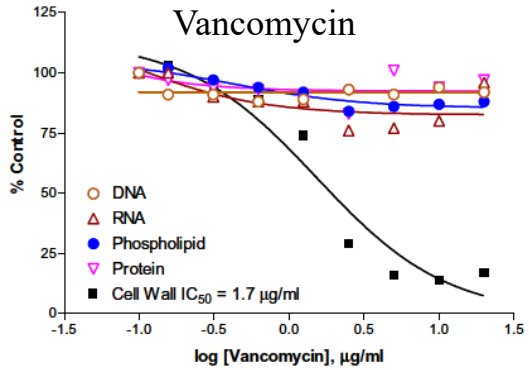
- Initial surrogate measures of toxicity
  - Lytic activity
    - RBC lysis
    - LDH cytolysis assay
  - Cytotoxicity in human cell lines
    - Use positive and negative controls
    - Measure viability/death – determine a CC_{50}
      - dye exclusion [Trypan blue]
      - redox dyes registering metabolic activity [Alomar blue, PrestoBlue®, MTT
      - LIVE/DEAD double fluorescence [SYTOX green/resazurin]



- Rough Therapeutic Index (TI) = CC_{50} /MIC in comparable amount of serum
- Aim for TI >100 – but could start at >10
- *Caveat: – high plasma protein binding (PPB) can interfere with cytotoxicity tests*

Mechanism of Action (directed screening)

- Is the MIC due to inhibition of your target?
  - *Macromolecular synthesis labeling [MMS] identifies pathway*
    - Supports specificity



  - Does overproduction of target raises MIC?
  - Does underexpression of target lowers MIC?
  - *Resistance mutations map in the target gene*

Resistance…

- Select for resistance
  - Map mutations
- Curses! At what frequency of resistance [FoR]?
- If high frequency and fit, the compound may select rapidly for resistance in the clinic
- But what is "high frequency?"
  - Related to the infectious load of the pathogen
  - If 10^{10} bacteria in an infection, then resistant mutants could be present [before challenge] at frequencies higher than $1x10^{-10}$
  - 10^{-8} is probably too high; 10^{-9}?? Need more modeling to be predictive

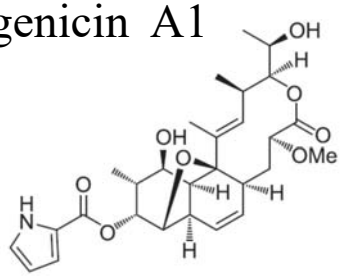
Rapid resistance is probable with single-targeted antibacterials

Nargenicin: discovered in GyrB underexpression screen

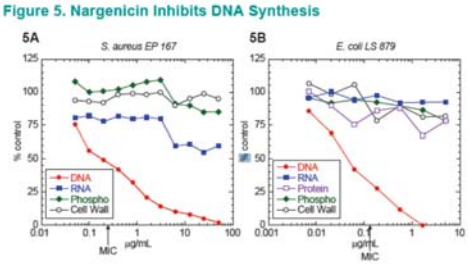
Anti-sense downregulated strain shows much larger zone of inhibition than wild type

Painter, Ronald E M., et al. (2015) Elucidation of DnaE as the Antibacterial Target of the Natural Product, Nargenicin. *Chemistry & Biology 22, 1362-1373.*

Nargenicin A1



MMS

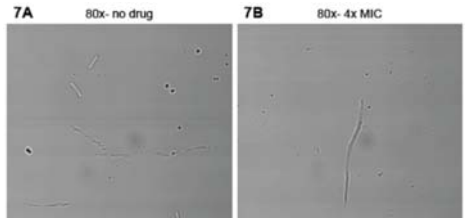


Induction of SOS

Compound	Mechanism of Action	SOS Assay	UvrA Assay	DNA binding Assay
Ciprofloxacin	Gyrase	SOS+	Not reversed	Not reversed
Mitomycin C	Alkylating agent	SOS+	Reversed	Not reversed
Griseolutein	Alkylating agent	SOS+	Reversed	Not reversed
Actinomycin D	Intercalator	SOS-	Not reversed	Reversed
Nargenicin	unknown	SOS+	Not reversed	Not reversed

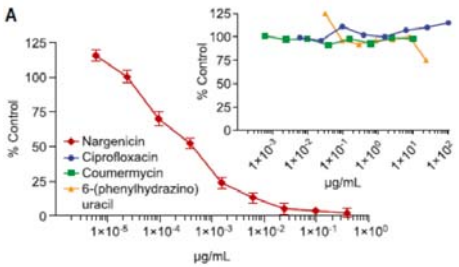
FoR 1 to 3 x 10^{-9}
Maps to DnaE (S765L)

E. Coli filamentation



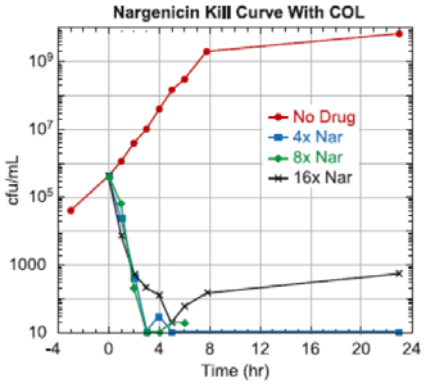
DnaE inhibition

S. aureus



Did not inhibit human
α, β, γ Polymerases at 100X MIC

Rapid killing

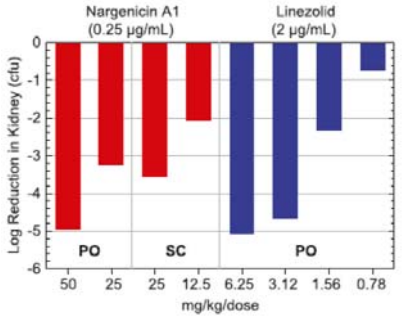


Narrow spectrum

Species	Phenotype	MIC (µg/mL)
C. albicans		>32
S. aureus Smith	macS, linS	0.25
S. aureus Smith	+ 50% serum	1
St. pneumo	(pS, qS, mS) Iso	>32
E. faecalis	VSE macR	>32
B. subtilis	+	>32
H. influenzae	ampS, quinS, macS	>32
E. coli	Mouse pathogen	>32
E. coli	Wild-type	>64
E. coli	*lpxC*	64
E. coli	*tolC*	0.25
E. coli	*lpxC, tolC*	≤0.0625
P. aeruginosa	Wild-type	>64
P. aeruginosa	Efflux del	4
P. aeruginosa	*mexXY*	>64
P. aeruginosa	*mexEF-oprN*	>64

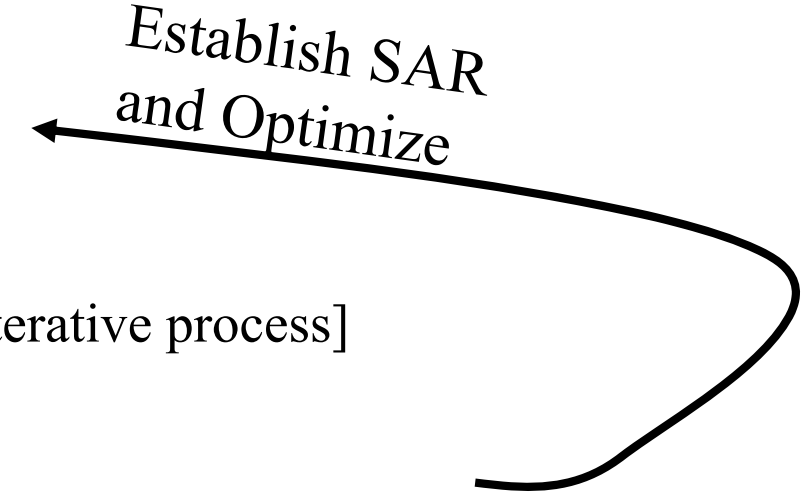
S. aureus
Gram⁻ efflux Δ
Serum raises MIC 4x

In vivo efficacy (*S. aureus*)



Is the hit worth further work?

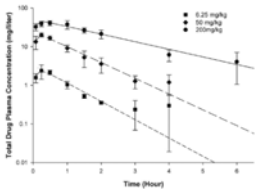
- What are pros and cons?
  - Reasonable potency
  - Low toxicity
  - Low resistance potential
  - Spectrum
  - Chemically attractive and tractable
- Now
  - Try to improve by medicinal chemistry [iterative process]
  - Evaluate pharmacology
  - In vivo efficacy
  - Consider the TPP



With a more optimized lead

- ADME - Pharmacology
  - In vitro and in vivo measures of Absorption, Distribution, Metabolism, Excretion

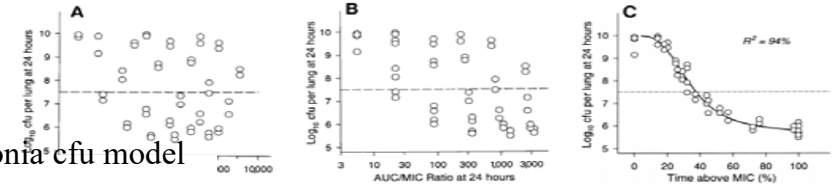
Such as in vitro measures of
- Solubilitly
- CYP inhibition
- Chemical stability
- Stability in microsomes, plasma, hepatocytes
- Plasma binding [mouse, human]
- hERG inhibition
- Caco-2 permeability



- Pharmacokinetics/Pharmacodynamics

Model	Dose (mg/kg)	V (liter/kg)	$AUC_{0-\infty}$ (mg · h/liter)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (mg/liter)
Gepotadacin SC dosing	6.25	2.055	2.788	0.636	0.249	2.319
	50	2.130	24.965	0.737	0.239	18.739
	200	3.959	111.417	1.529	0.285	44.406

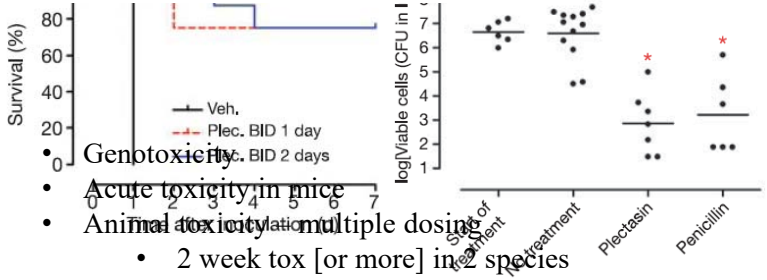
C_{max}/MIC | AUC_{0-24}/MIC | Time above MIC



ionia cfu model

Cefotaxime in *K. pneumoniae*

- Animal efficacy



- In vivo toxicity
  - Genotoxicity
  - Acute toxicity in mice
  - Animal toxicity – multiple dosing
    - 2 week tox [or more] in 2 species
    - No Observed Adverse Effect Level (NOAEL)
    - Maximum tolerated dose (MTD)

Do you have a candidate?

- Is it safe enough to dose at levels high enough to cure infections?
- Does it have a useful antibacterial spectrum?
- Is dosing route and regimen commensurate with desired indication?
- In other words, does it meet the criteria of the TPP?