ANACOR PHARMACEUTICALS

Development of Novel Broad Spectrum Antibiotics Using A Novel Target

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The Continual Battle Against Mother Nature

Antibacterial	Year FDA Approved	First Reported Resistance
Penicillin	1943	1944
Streptomycin	1947	1947
Erythromycin	1952	1955
Tetracycline	1952	1956
Methicillin	1960	1961
Nalidixic acid	1964	1966
Vancomycin	1964	1986
Ciprofloxacin	1987	1988
Linezolid	2000	2004
Daptomycin	2003	2005

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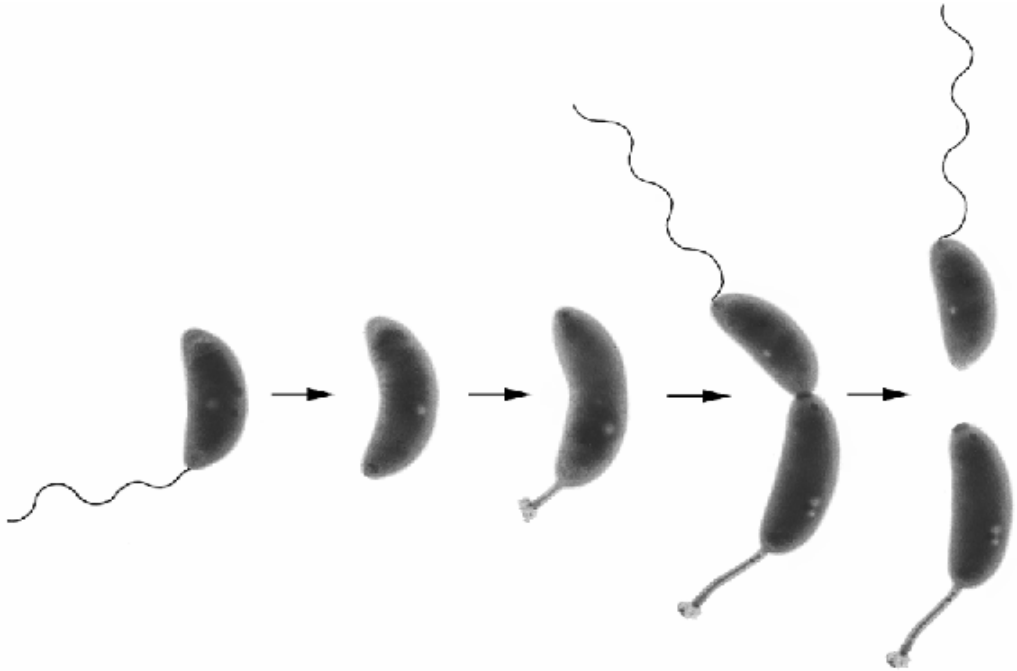
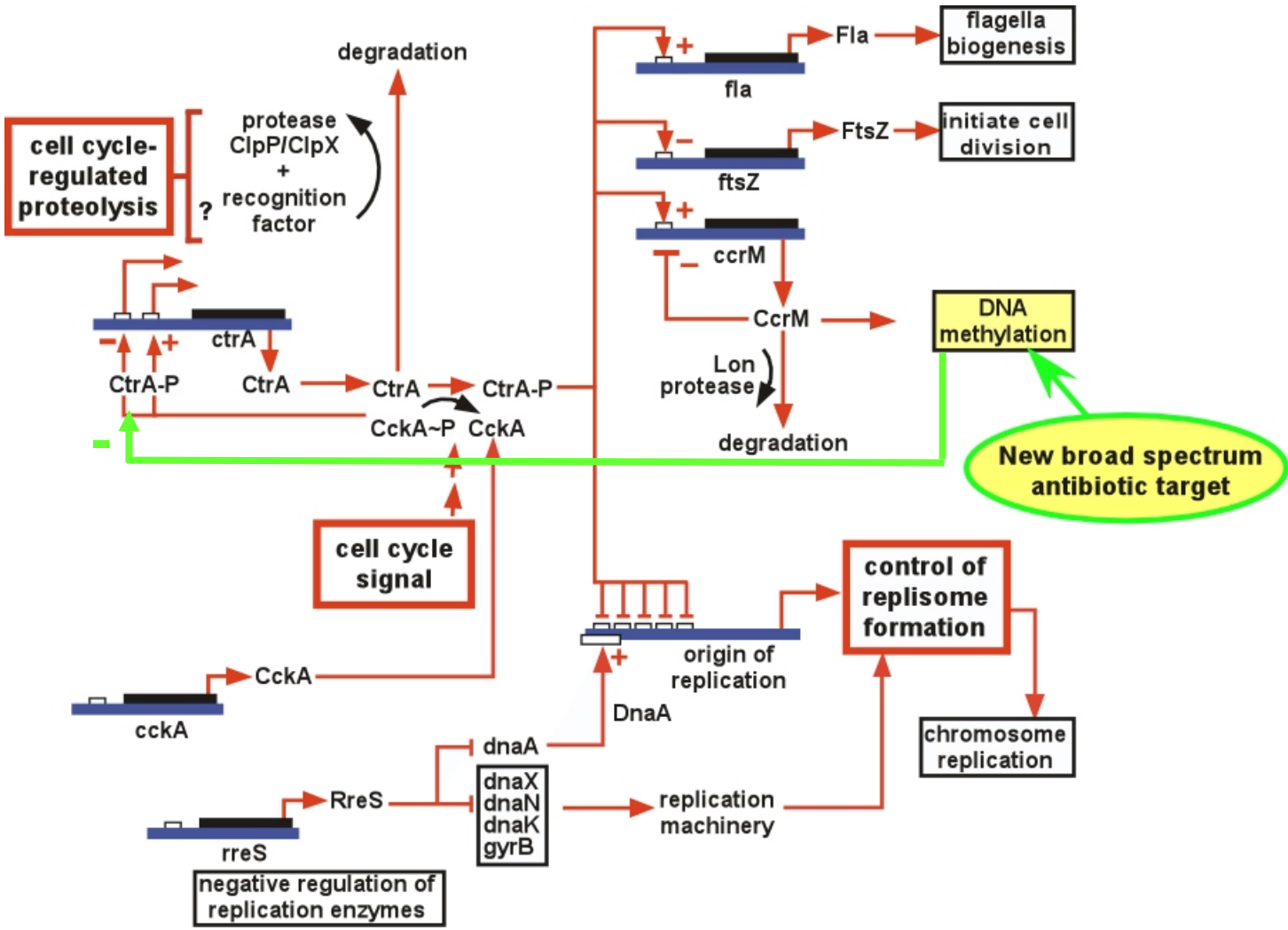
Understanding The Fundamentals In Bacteriology Yields New Targets



Caulobacter Cell Cycle

Novel Targets Identified By Research Into Bacterial Genetic Circuitry



***Caulobacter* Cell Cycle Genetic Circuit**

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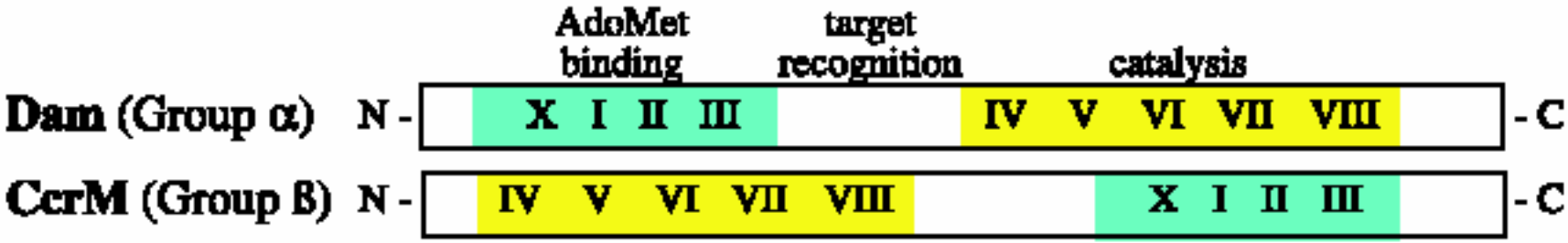
CcrM

6'methyl adenine DNA methyltransferase
Found in α-proteobacteria and ε-proteobacteria
[*Caulobacter spp.*, *Brucella spp.*, *Agrobacterium spp.*, *Rhizobium spp.*, and *Helicobacter pylori*]

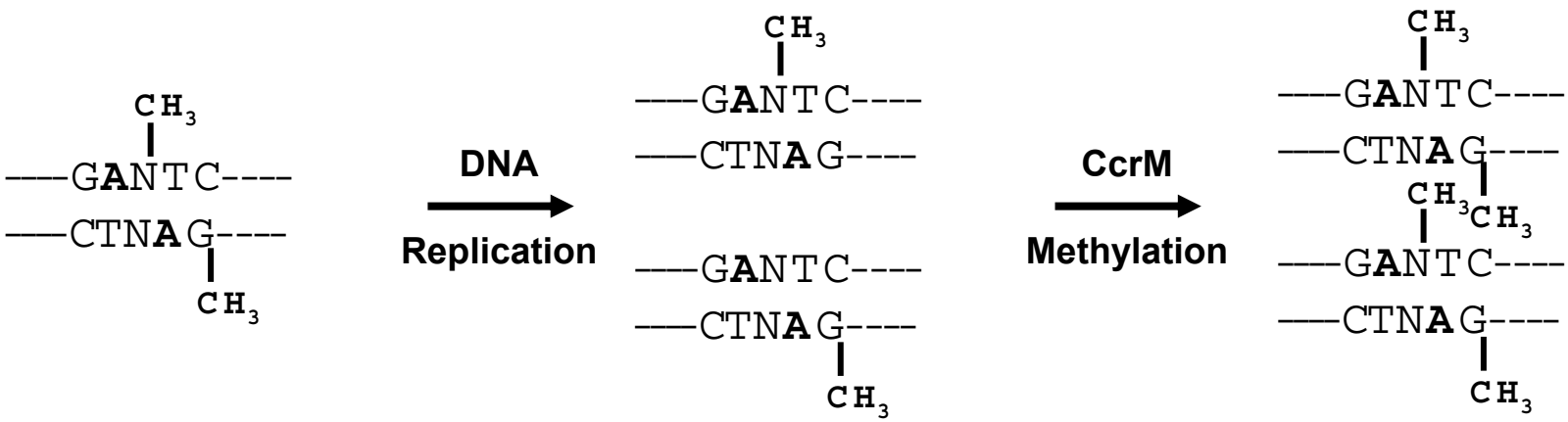
- *ccrM* is essential
- *ccrM* overexpression mutants are avirulent (*Brucella*)

CcrM inhibitors can be effective antibiotics and CcrM overexpression pathogens can serve as live attenuated vaccines

Dam And CcrM



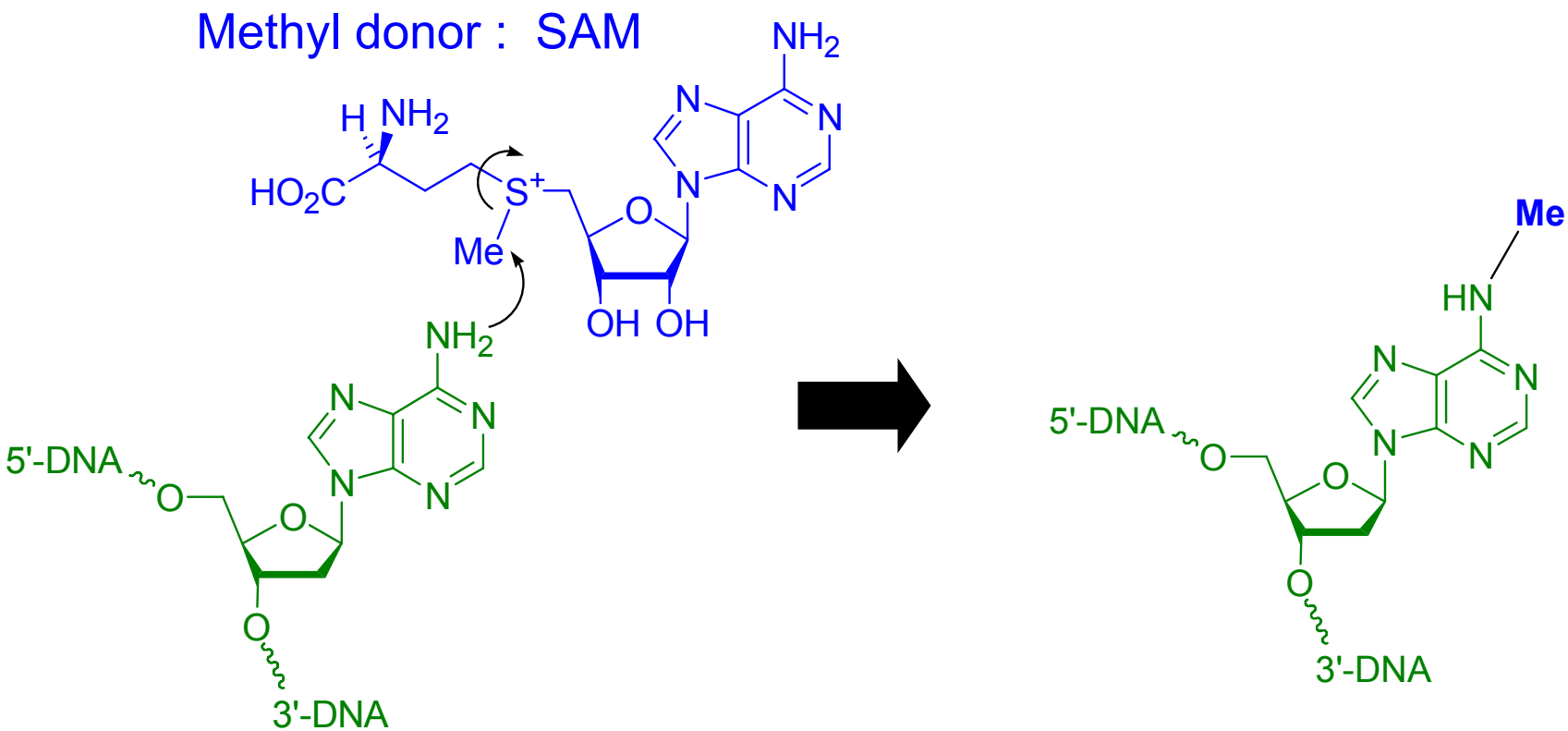
- CcrM in α-protebacteria and Dam in γ-proteobacteria (Tularemia and Plague).
- Both Dam and CcrM are 6'methyl adenine DNA methyltransferase.
- CcrM methylates G**A**NTC and Dam methylates G**A**TC.
- Both enzymes are required for viability.



Why Are Bacterial Adenine DNA Methyltransferase Good Targets?

- Required for viability.
- Active site sequence and mechanism of action differs from eukaryotic DNA methyltransferases (cytosine).
- Correct cellular levels of CcrM are critical for bacterial viability, opening two approaches for antibiotic action
  - CcrM elimination shown to kill *Caulobacter crescentus*, *Rhizobium meliloti*, *Brucella abortus, Agrobacterium tumefaciens, and Helicobacter pylori*
  - CcrM overexpression stops bacterial cell division and blocks virulence in Brucella

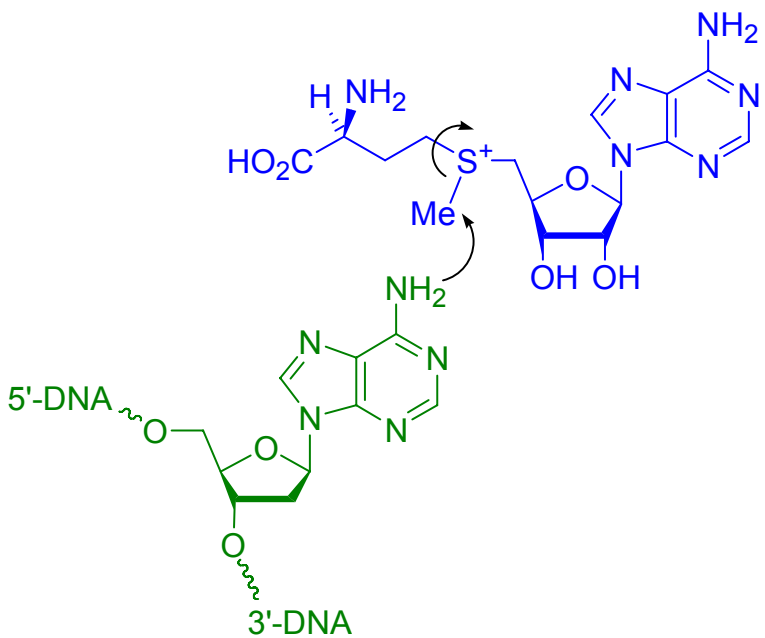
Mechanism of Action of CcrM

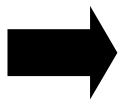


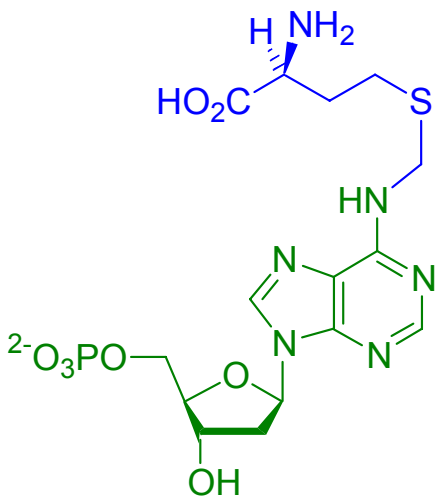
DNA Strand

Methylated product

First Inhibitor of CcrM



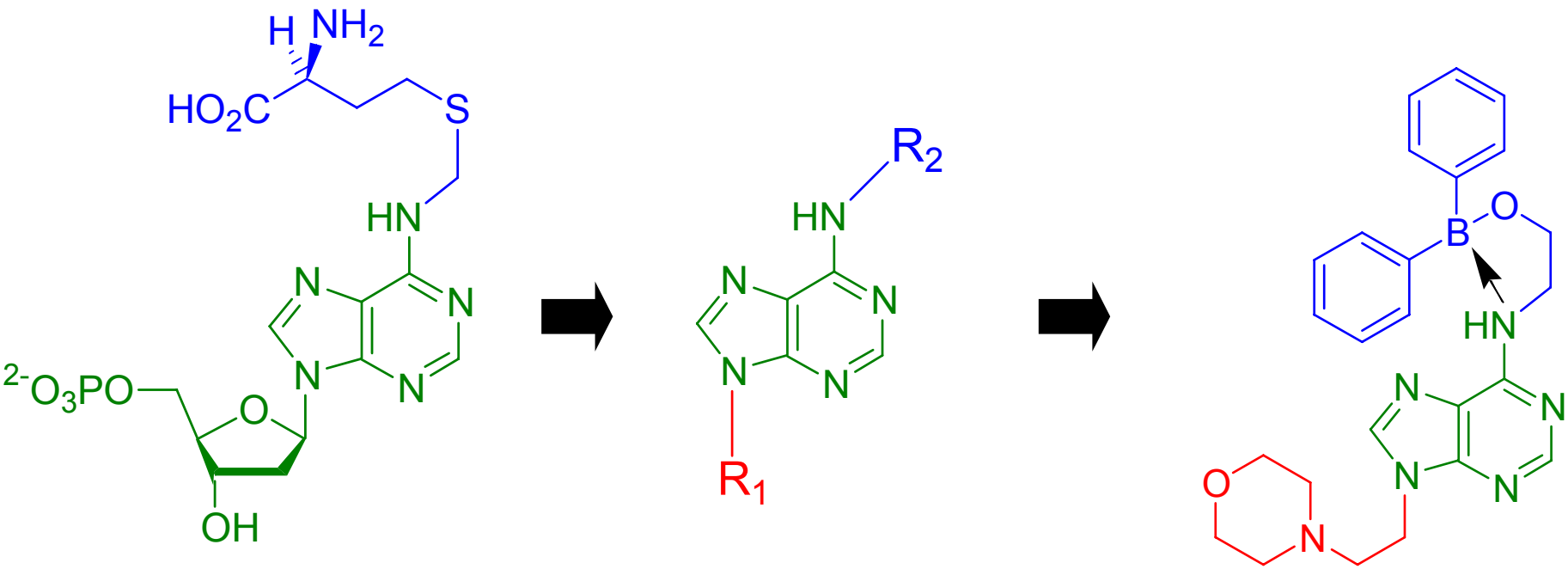




Enzyme Transition State Intermediates

Designed Multisubstrate Adduct Inhibitor

From Substrate Analogs to Borinic Esters

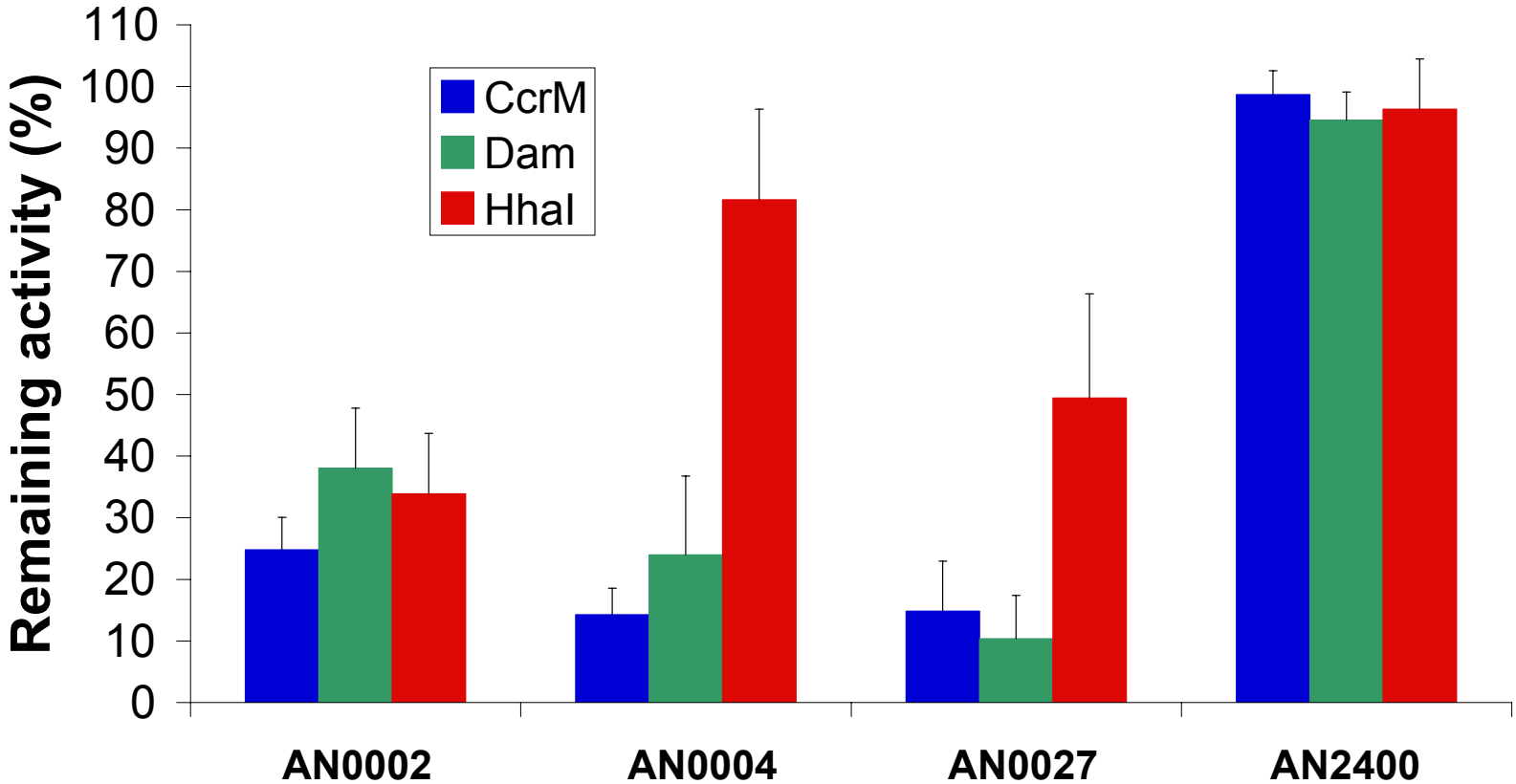


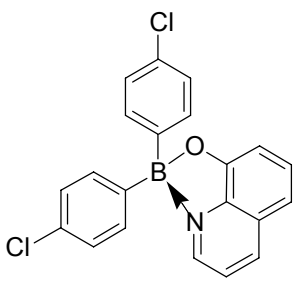
Multisubstrate Adduct Inhibitor

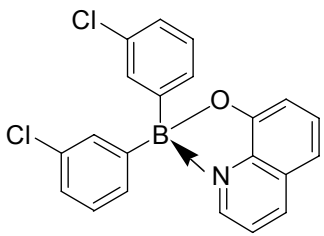
Adenine-based Library

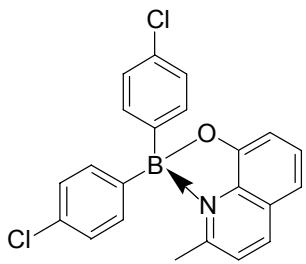
Discovery of Boron Substitution

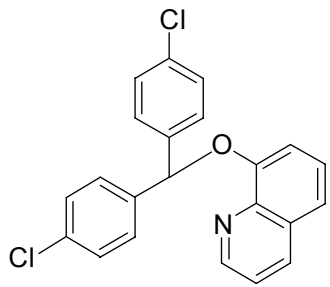
DNA Mtase Biochemical Assay





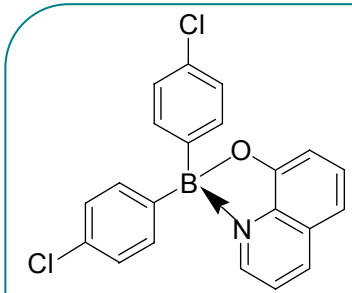




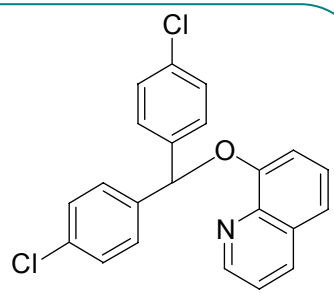


Boron Is Essential For Activity

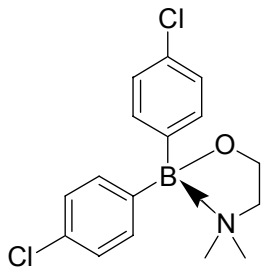
- We have replaced boron with carbon on several occasions and each time it has led to an inactive compound



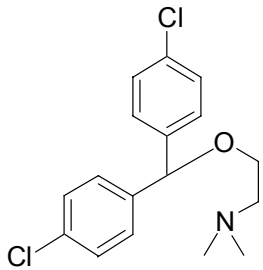
AN0002



AN2400



AN0053

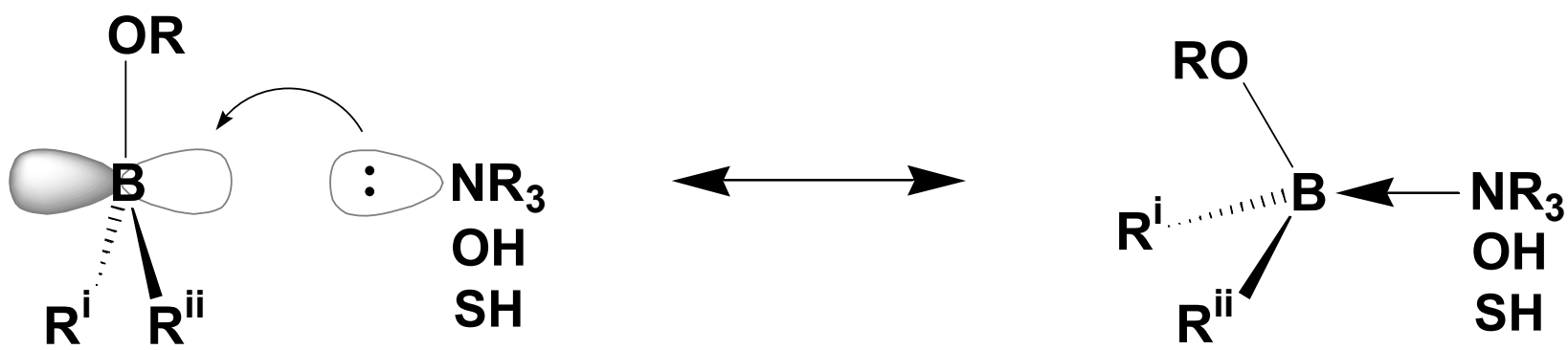


AN0192

MIC vs. *S. aureus* (μg/mL)	**2**	**>64**	**4**	**>64**

Unique Feature Of The Borinic Ester

- Boron is trivalent and trigonal planar in structure
- It has an empty P-orbital and is thus a Lewis acid
- It forms reversible coordinate (or dative) bonds with Lewis bases
- Boron is tetrahedral when part of a Lewis acid-base pair
- The trigonal-tetrahedral interconversion is not an equilibrium of both forms, it resembles a resonance hybrid
- The magnitude of the dative bond gives rise to the “tetrahedral character”[1]
- Boronic acids are inhibitors of serine/theornine proteases



Trigonal Planar

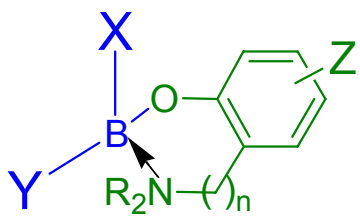
Tetrahedral

1. The tetrahedral character of the N→B bond has been studied and reviewed by H. Höpfl, J. Organomet. Chem., 1999, **581**, 129-149

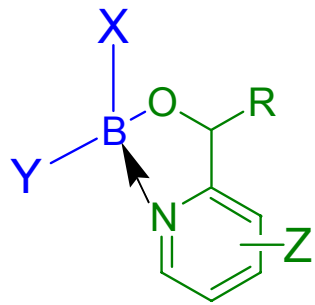
Pathogen MICs (μg/ml)

	Anthrax *B. anthracis*	Tularemia *F. tularensis*	Brucellosis *B. abortus*	Plague *Y. pestis*	MRSA *S. aureus*	PRSP **S. pneumoniae**
AN001	5.2	>0.01	41.3	4.0	2.0	1.0
AN0002	2.4	0.03	18.9	16.0	1.0	4.0
AN0004	2.4	0.03	NT	8.0	1.0	2.0
AN0005	NT	>30	NT	4.0	3.0	3.0
AN0027	2.5	6.0	3.9	NT	3.0	16.0

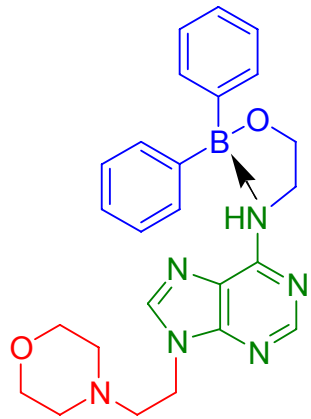
Diversifying the Scaffold Led to a Library of Over 1000 Compounds

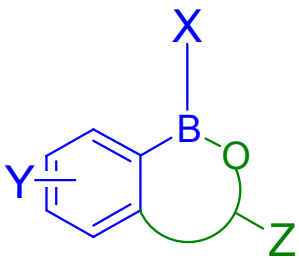


Phenol esters



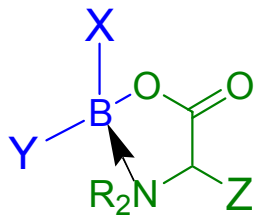
Pyridine esters





Cyclic esters

Biological activity includes,
- Antibacterial
- Antifungal
- Antiparasitic
- Anti-inflammatory



Amino acid esters

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Early Leads

- Broad spectrum activity
- Poor PK
- Poor oral bioavailability

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Need To Improve Pharmacokinetics

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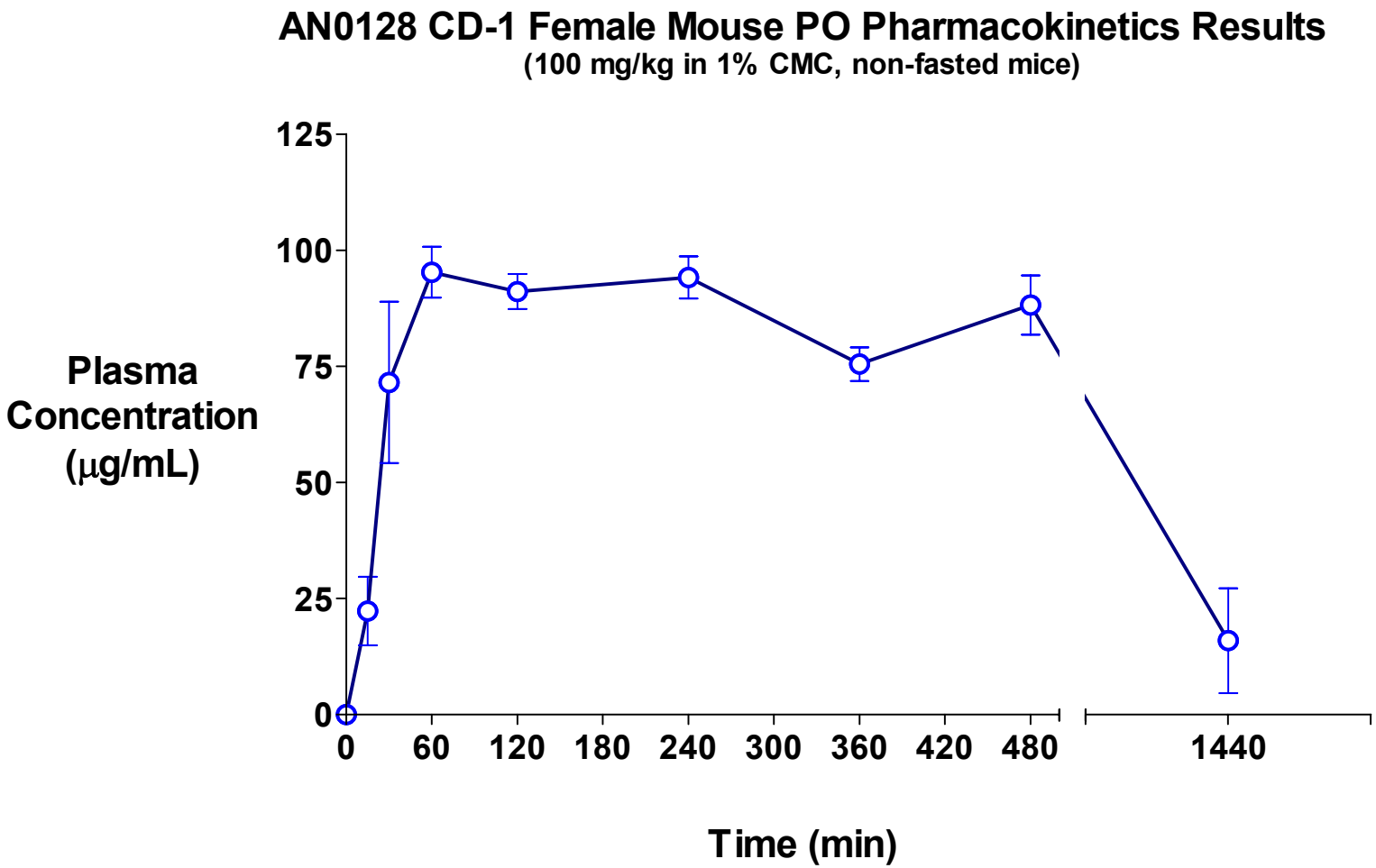
AN0128 Oral Pharmacokinetics Are Very Good



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AN0128 Is Very Safe

- 7 Day Tox Study Design
  - AN0128 dosed orally once daily for 7 days at either 30, 100, or 500 mg/kg
  - Rats were evaluated for:
    - Clinical signs
    - Mortality
    - Blood Chemistries
    - Hematology
    - Organ weights
    - Gross necropsy
    - Histopathology on Major Organs
- Results
  - No signs of acute toxicity were found at any dose
    - MTD > 500 mg/kg/day for 7 days
  - No dose-responsive changes in any blood chemistry parameters
  - No dose-responsive changes in histochemistry
  - NOAEL = 500 mg/kg/day

Antibacterial Activity Of AN0128

Bacteria			AN0128
Gram-positive	Low GC	Bacillus anthracis Ames	0.5
		Bacillus anthracis Ames ciprofloxacin R	0.5
		Bacillus cereus ATCC 1178	0.5
		Clostridium difficile ATCC 9689	1
		Enterococcus faecalis ATCC 19433	2
		Enterococcus faecalis ATCC 700802 VanR	2
		Enterococcus faecium CT-26	2
		Enterococcus faecium ATCC 700221 VanR	2
		Eubacterium nodatum ATCC 33099	0.25
		Staphylococcus aureus ATCC 29213	1
		Staphylococcus epidermidis ATCC 12228	2
		Streptococcus mutans ATCC 25175	1
		Streptococcus pneumoniae ATCC 6301	≤ 0.12
		Streptococcus pyogenes ATCC 19615	0.25
	High GC	*Mycobacterium tuberculosis* ATCC 25177	16
		Mycobacterium avium ATCC 25291	> 32
		Propionibacterium acnes ATCC 6919	2
		Propionibacterium granulosum NCTC 11865	2
Gram-negative	Bacteroides	*Bacteroides fragilis* ATCC 25285	2
		Porphyromonas gingivalis ATCC 33277	≤ 0.12
		Prevotella intermedia ATCC 25611	0.25
		Tannerella forsythensis ATCC 43037	≤ 0.12
		Escherichia coli ATCC 25922	>64
		Haemophilus influenzae ATCC 49766	>64
		Helicobacter pylori 26695	8
		Pseudomonas aeruginosa ATCC 27853	>64
		Treponema denticola ATCC 35405	8

VanR = vancomycin resistant

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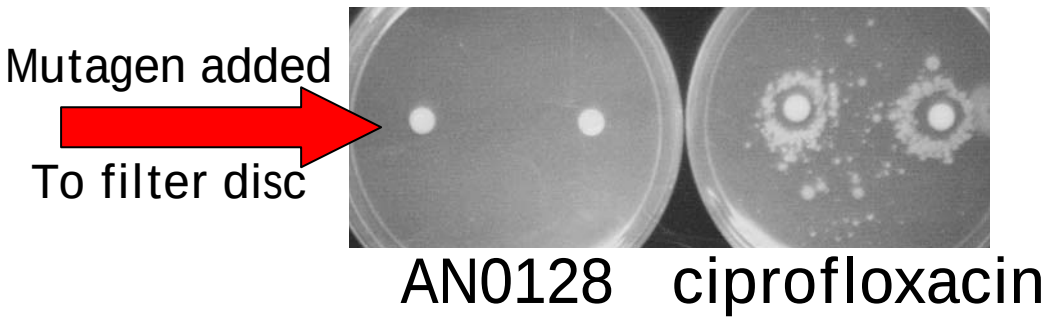
AN0128 MRSA MIC_{90} Studies

ORGANISM	ANTIBIOTIC	MIC µg/ml		
		RANGE	MIC_{50}	MIC_{90}
MRSA	**AN0128**	**0.5-1**	**1**	**1**
15 Strains Tested	Ciprofloxacin	<0.12->64	>64	>64
14 Clinical isolates	Vancomycin	<0.12-0.5	0.5	0.5
ATCC 33591	Erythromycin	<0.12->64	>64	>64
	Tetracycline	<0.12->64	<0.12	<0.12
	Amoxicillin	16->64	64	64
	Ampicillin	16->64	32	32
	Ceftazidime	32->64	>64	>64

Frequency of Resistance for AN0128 Is Extremely Low

- Frequency of resistance against *Staphylococcus aureus* is < 10^{-11}.
- Frequency of resistance against *Bacillus subtilis* is < 10^{-11}.
- No mutants isolated even when induced chemically.



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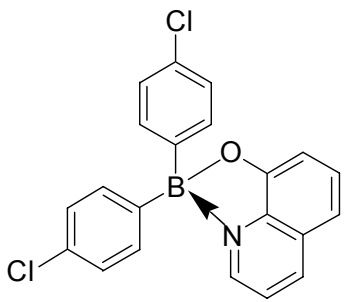
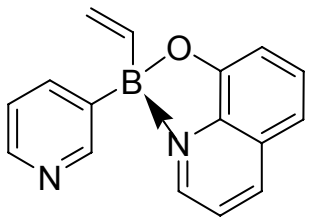
High Protein Binding Reduces Systemic Efficacy

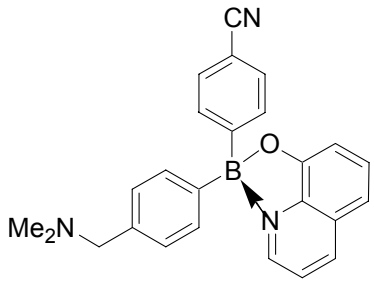
Compound	Half-life (h)	F (%)	Cmax (µg/mL)	PO Dose (mg/kg)	Protein Binding
AN0128	5.1	54	95.3	100	>98%

Protein Binding SAR

- A basic nitrogen near to boron reduces protein binding







AN0002

- No nitrogen
- PPB = >99%

AN2397

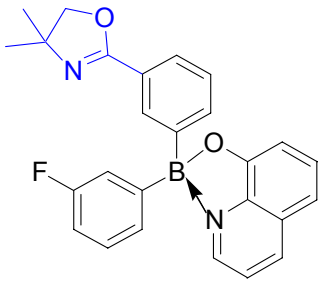
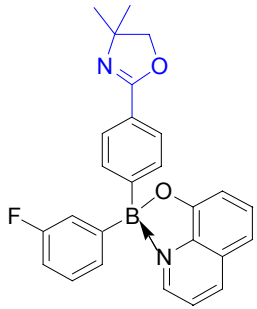
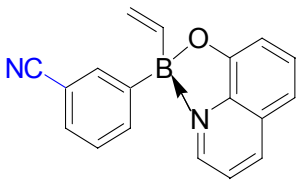
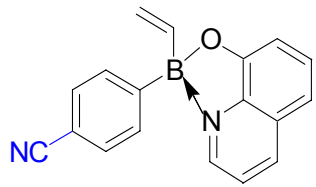
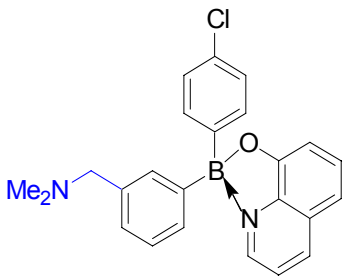
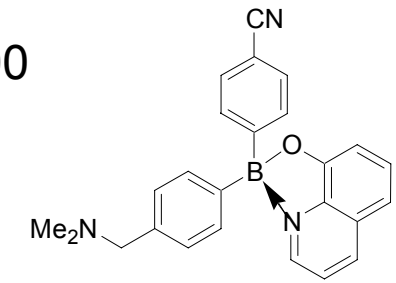
- pKa = 5
- PPB = 59%

AN2500

- pKa = 9
- PPB = 43%

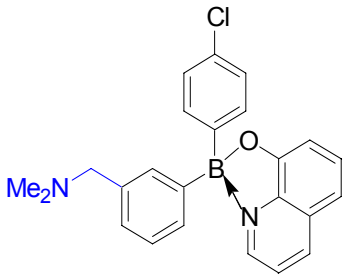
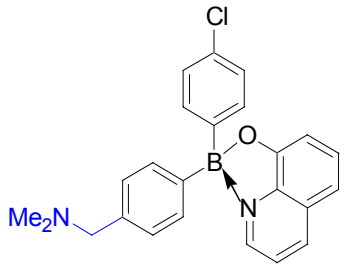
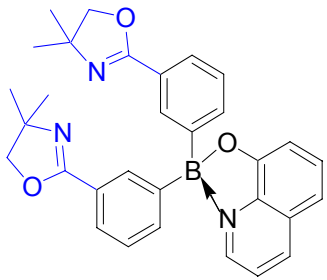
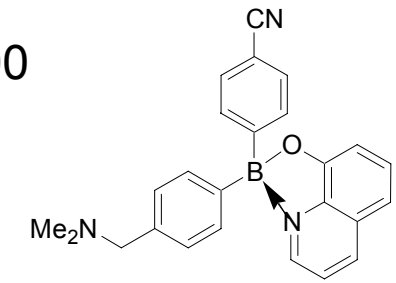
Plasma Exposure SAR

- Polar groups meta to boron give higher Cmax than para (dose = 300 mg/kg)

Meta		Cmax (μg/ml)	Para		Cmax (μg/ml)
AN1436		471	AN1443		12
AN2332		114	AN2335		70
AN2212		51.5	AN2500		56

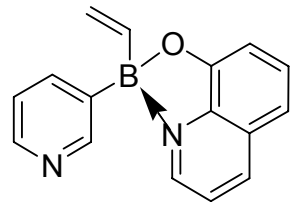
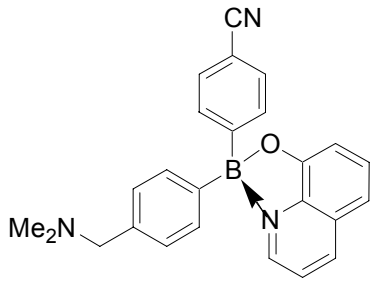
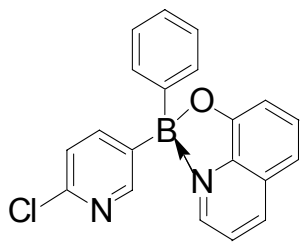
Volume of distribution SAR

- Polar groups para to boron give higher Vss than meta

	Meta	Vss (mL/kg)		Para	Vss (mL/kg)
AN2212		400	AN2211		3656
AN1288		157	AN2500		4176

Half-life SAR

- Half-life values in mice range up to 23 hr
- Half-life can be modulated through structural changes

		t ½ (hr)
AN2397		0.6
AN2500		2.7
AN1617		20

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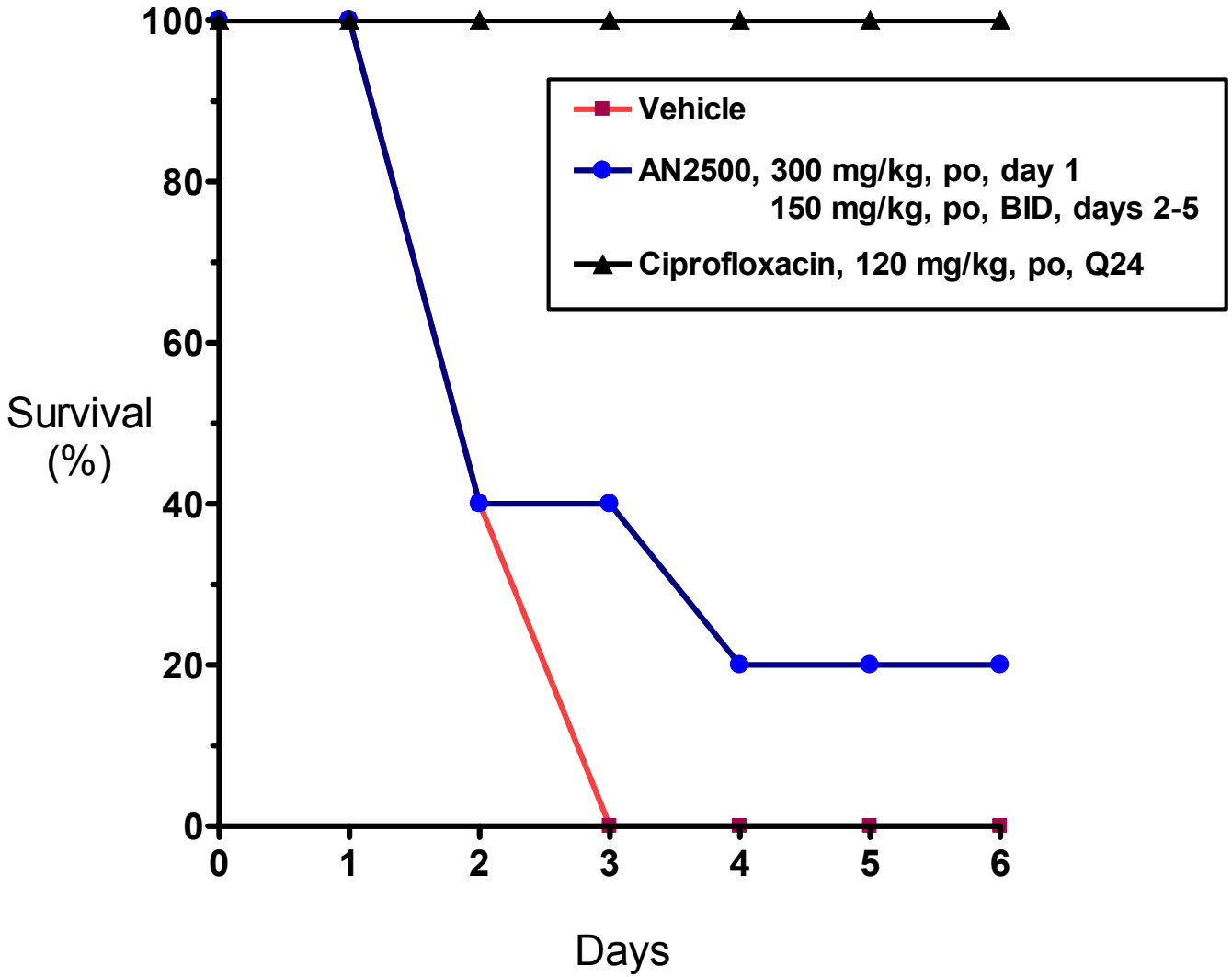
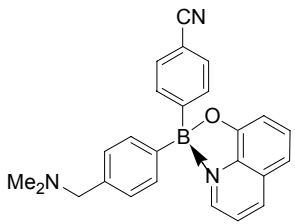
AN2500 Has Some Efficacy In A Mouse Anthrax Model



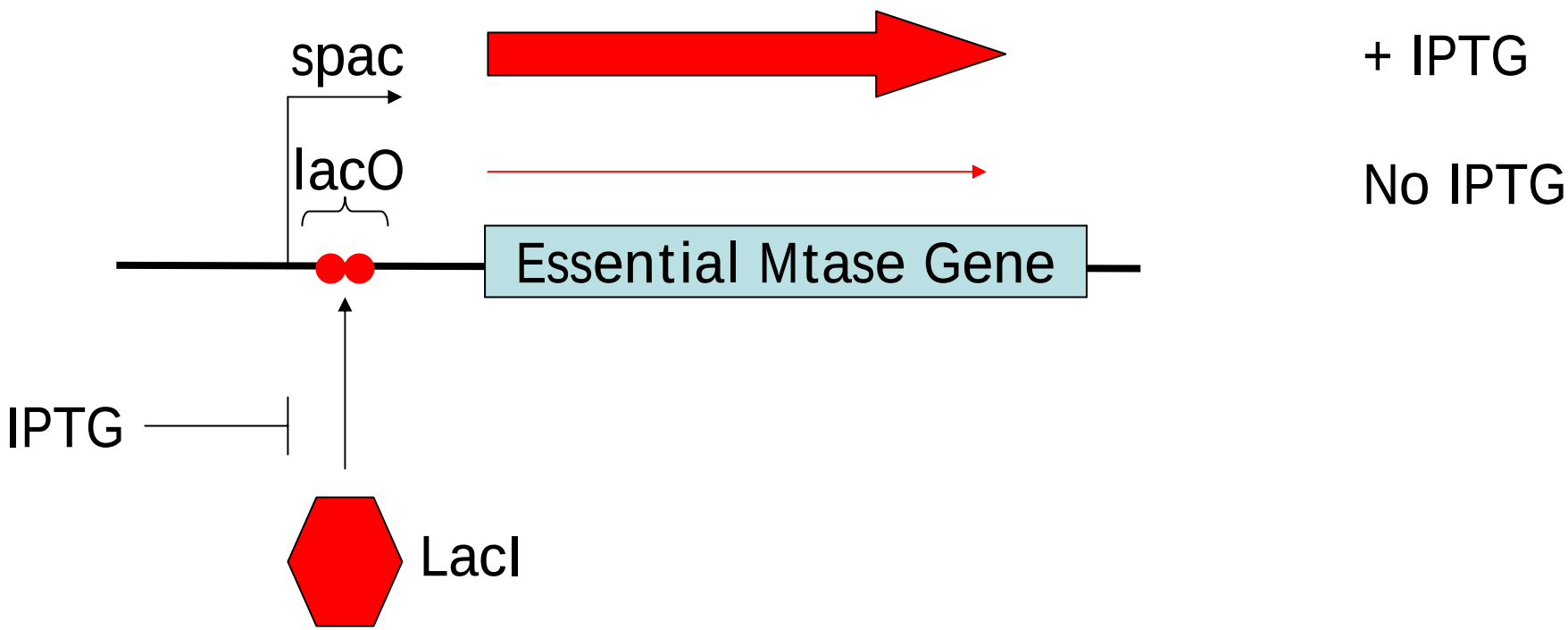
Need To Improve Potency

Compound	Structure	*B. anthracis*	*F. tularensis*	*S. aureus*	*S. aueus MRSA*	*Y. pseudotuberculosis*
AN2500		8	32	2	2	64

Identify Target in *Bacillus spp.*

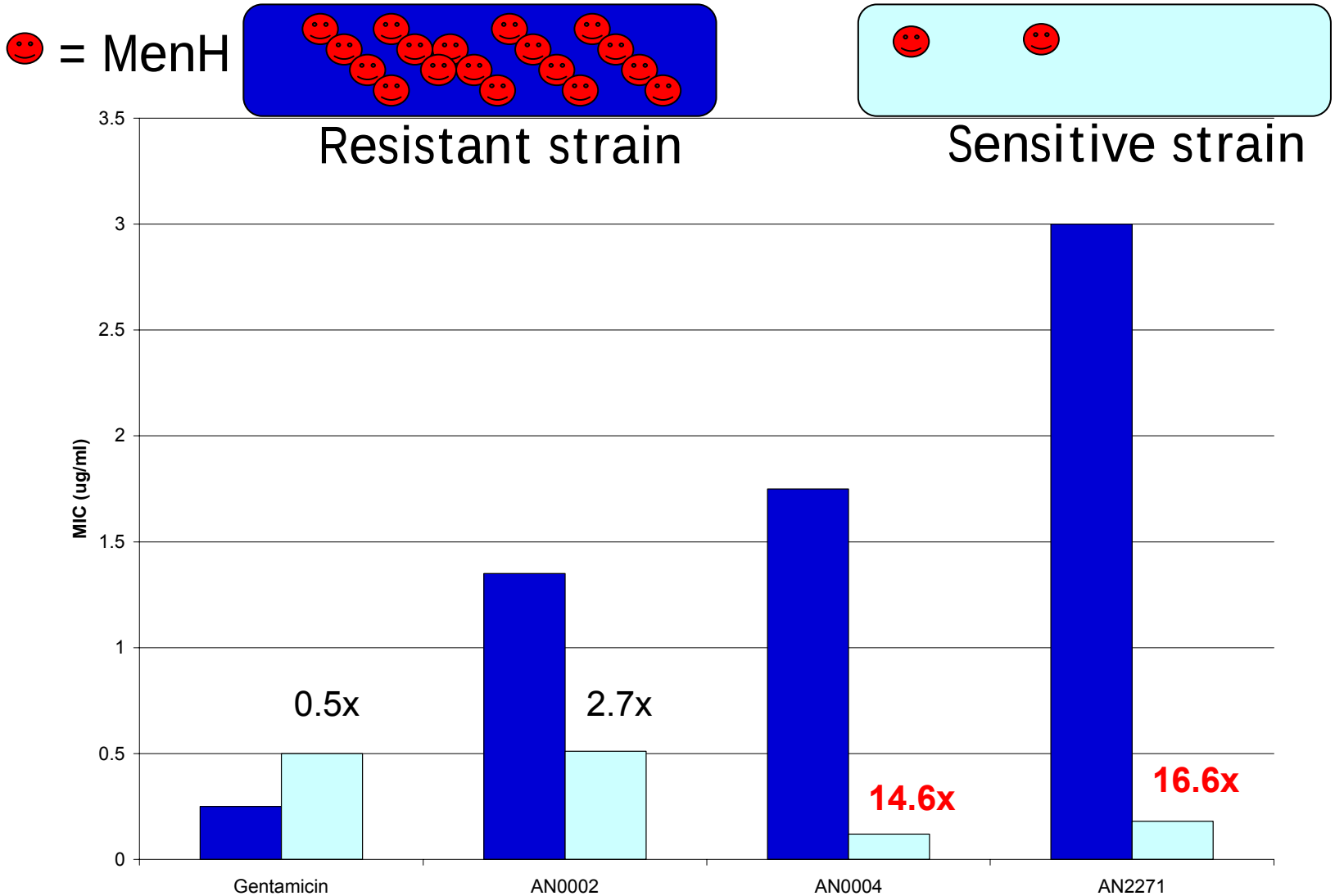
- Adenine DNA methyltransferases not present in *Bacillus anthracis*.
- Are we targeting another methyltransferase in *Bacillus spp*.

Menaquinone MTase Identified In A Mtase Screen In *Bacillus subtilis*

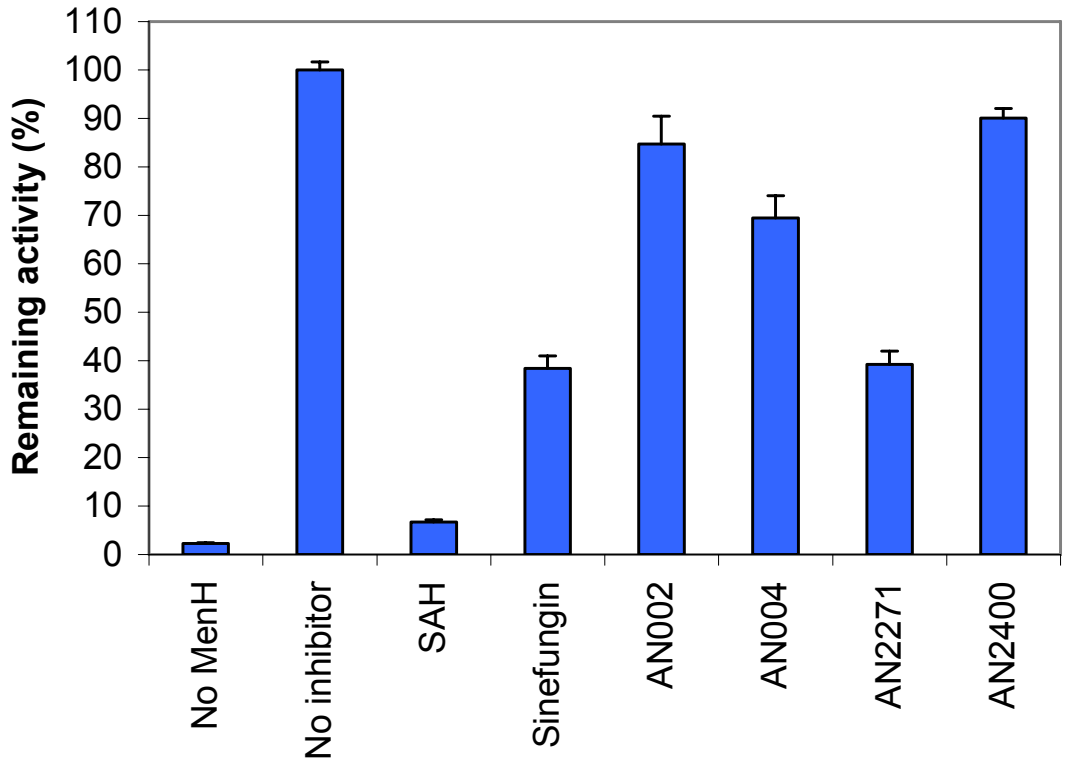


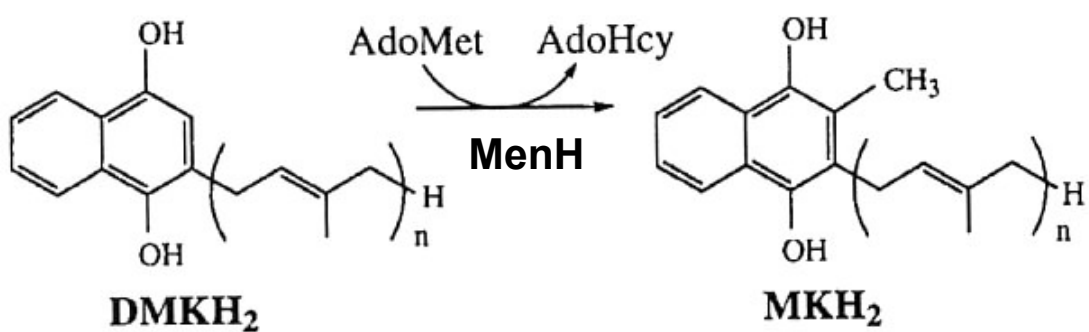
Replaced native promoters of the six essential Mtase genes in *B. subtilis* with the IPTG regulated promoter Pspac.

MenH Is A Target in *Bacillus subtilis*



Biochemical Inhibition of Menaquione Methyltransferse In Bacterial Crude Lysate





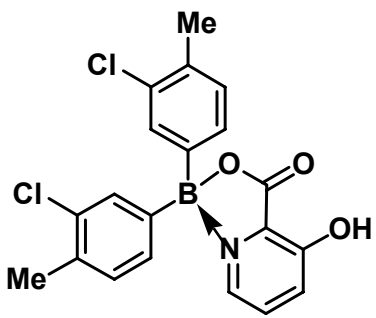
ANACOR PHARMACEUTICALS

What Is Menaquinone ?

- First electron acceptor in the electron transport chain of the oxidation of NADH using succinate dehydrogenase and fumarate reductase.
- In Gram-positives (except some streptococci) and archaea menaquinone is the quinone for both aerobic and anearobic respiration.
- In Gram-negatives is predominately used in anearobic respiration.
- Required for spore germination in *Bacillus spp.*

Anacor's Current Focus

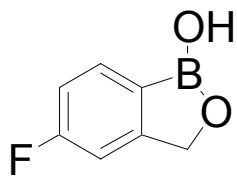
Topical Bacterial And Anti-inflammatory
For Atopic Dermatitis And Acne



AN0128

- *In vitro P. acnes* activity
  - MIC = 2 μg/mL
- Bactericidal
- Very low frequency of resistance
- Anti-inflammatory activity
- Not active systemically
- **IND Q1, 2005**

Topical Antifungal
For Onychomycosis



AN2690

- Broad antifungal activity
  - MIC = 0.06-8 μg/mL
- Exceptional nail penetration
- Preliminary 90 day clinical data shows a good response
- Novel mechanism of action
- AN2690 co-crystal with target
- **IND Q4, 2005**

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Anacor

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