Phage-Inspired Discovery of Small-Molecule Antibiotics

RNA Transcription

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Targanta Therapeutics

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Liu *et al*., 2004 Nature Biotechnology 22

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Bacteriophages (Phages)

- Phages are viruses that infect and kill bacteria
- Almost all bacterial species have known phages
- Over billions of years of evolution, phages have evolved highly efficient mechanisms to kill their hosts
- This information is contained in a compact genome

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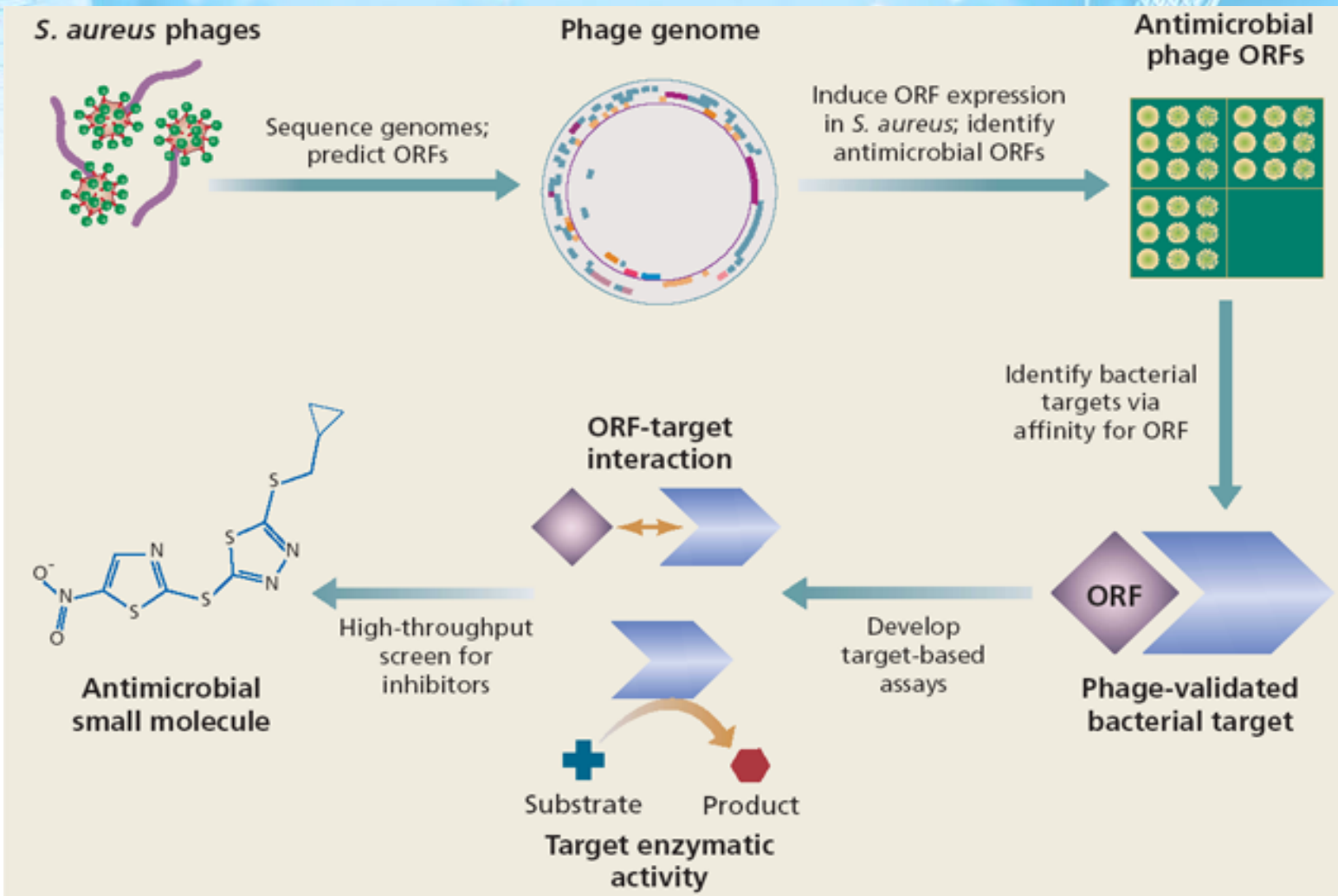
Targanta Drug Discovery Platform



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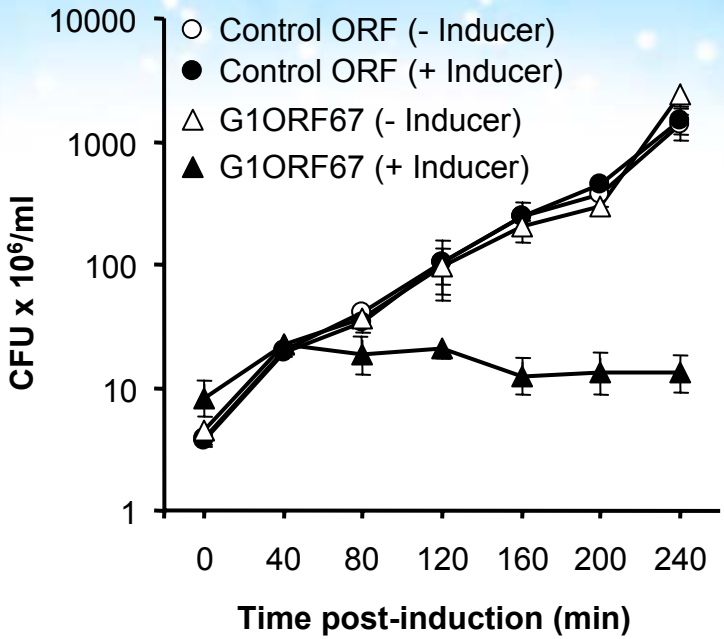
Expression of ORF67 in *S.aureus* is Bacteriostatic



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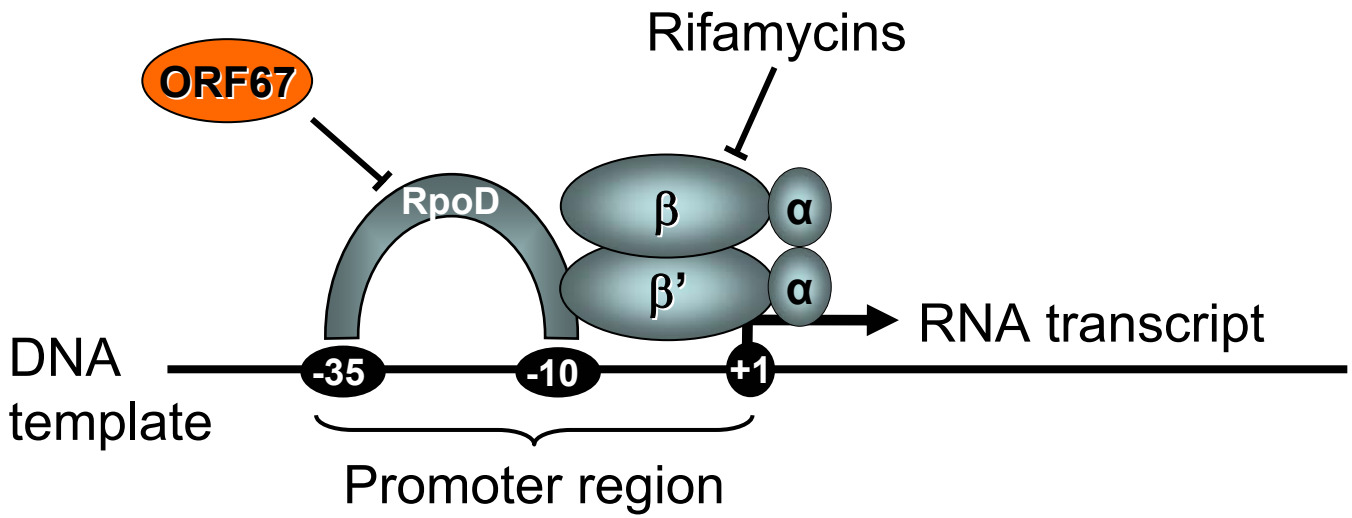
RpoD is an Essential Component of the Bacterial Transcription Machinery

- Primary σ factor RpoD directs transcription of growth, housekeeping genes
- *S. aureus* RpoD is the target of phage polypeptide ORF67
- $RpoD_{Sa}$-dependent transcription assay was developed for HTS



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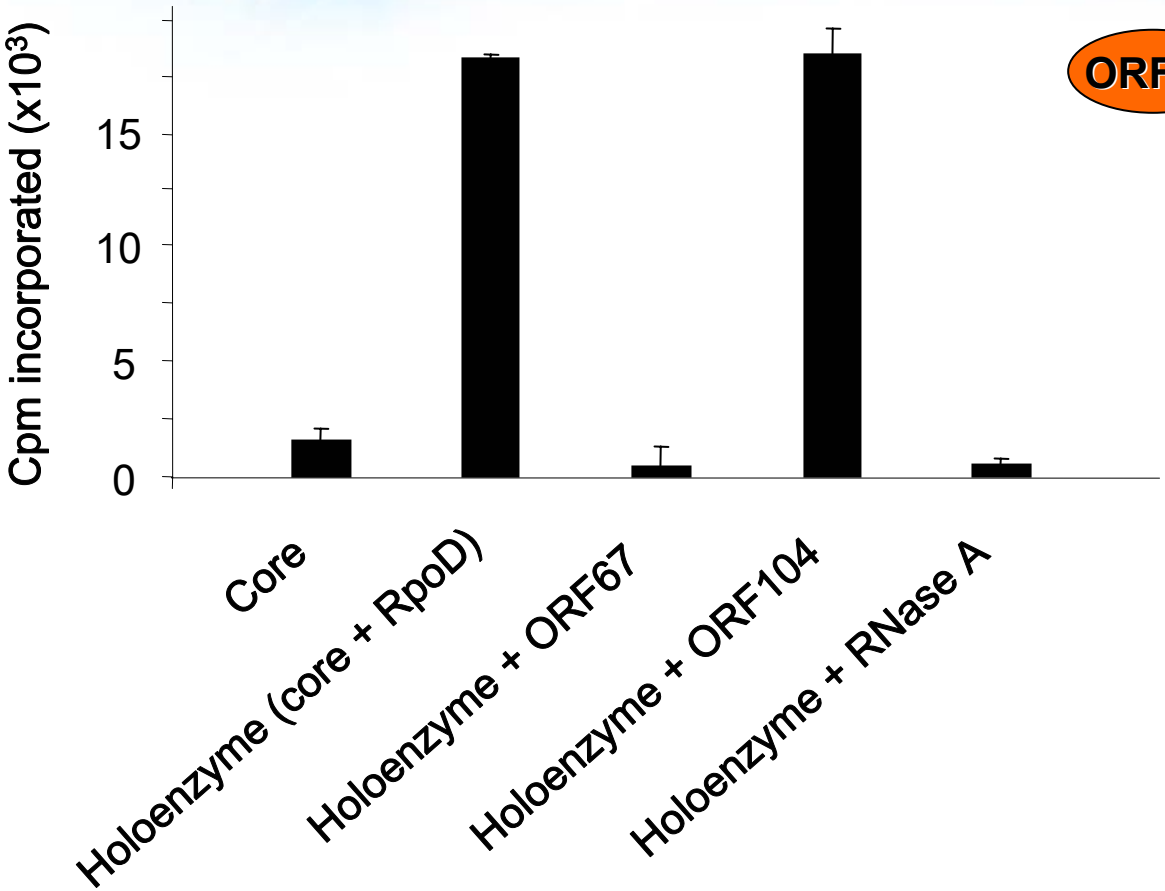
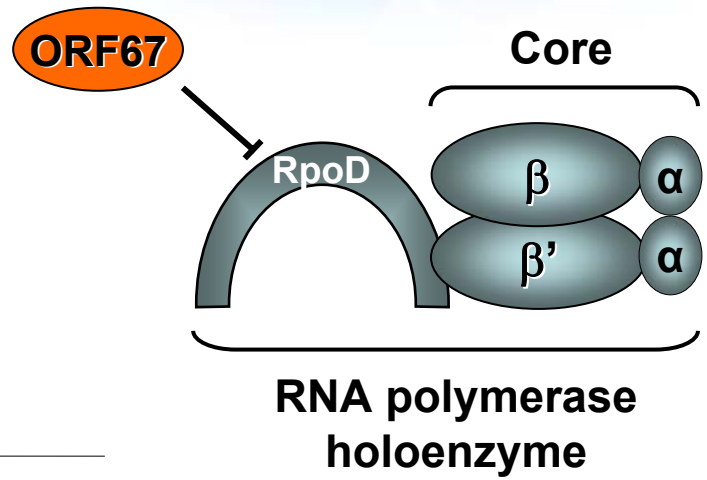
$RpoD_{Sa}$-Specific Phage Polypeptide (ORF67) Inhibits *S. aureus in vitro* Transcription

• Plate-based assay with purified *S. aureus* RNAP → study effect of phage polypeptides on RNA synthesis *in vitro*:





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$RpoD_{Sa}$-Dependent HTS *in vitro* Transcription Assay

$RpoD_{Sa}$ (12.5 nM) +
Core enzyme (12.5 nM)

↓

Cpds (20 µM in 2% DMSO) /
G1ORF67 pos. ctrl (10 µM)

↓

Template DNA (pTM with multiple promoters)
NTPs (^{32}P-UTP)

↓

TCA precipitation

↓

Washes

↓

Add LSC & read

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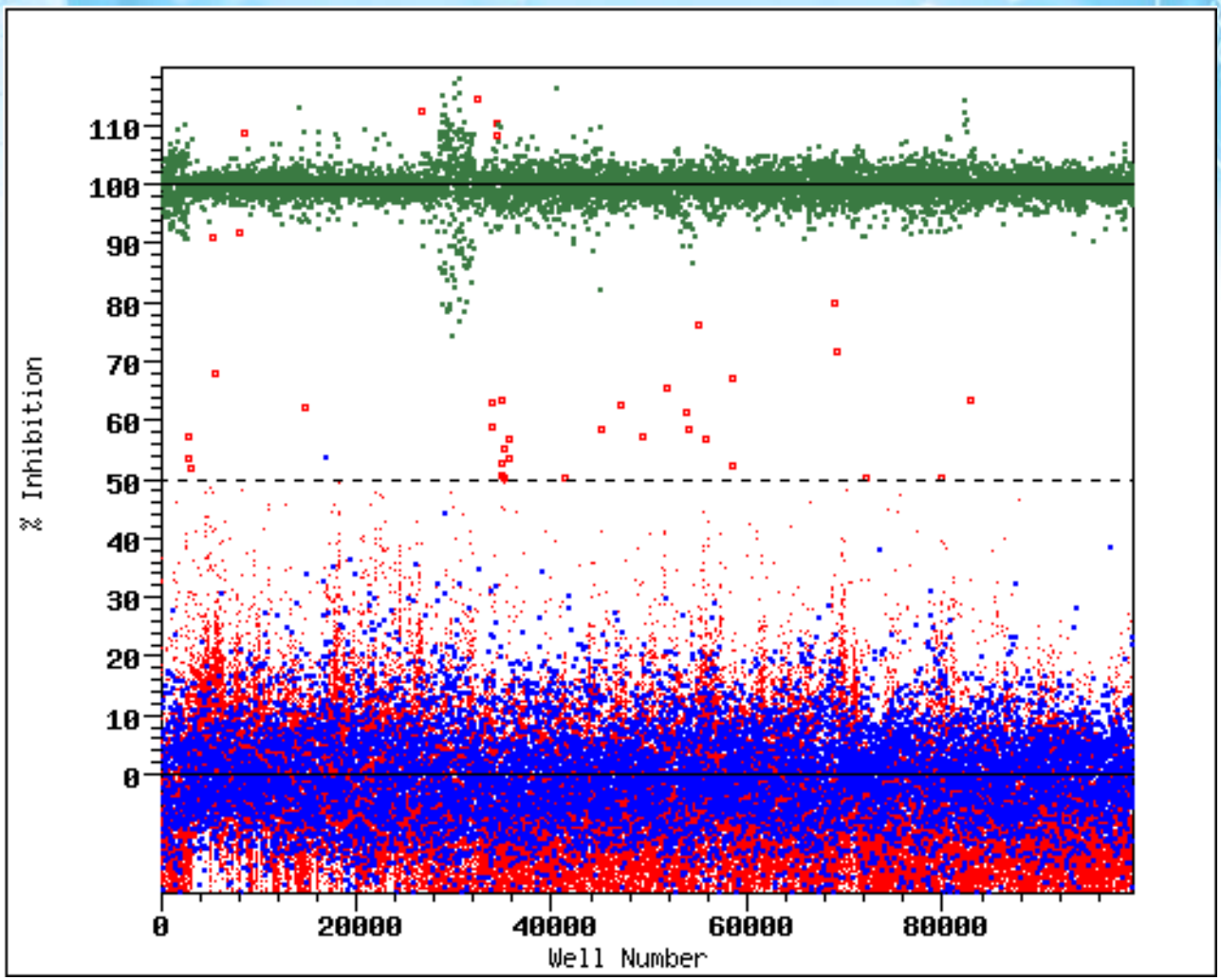
$RpoD_{Sa}$-Dependent *in vitro* Transcription Assay



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Overview of $RpoD_{Sa}$ HTS and Hit Profiling

~250, 000 cpds screened

230 confirmed actives → 79 pass chem filter → 43 hits (IC_{50}<~50 μM) → 39 chemical classes (incl. single cpds) → 5 chemical classes Investigated (Prioritized)

<u>151 confirmed actives discarded</u>
- reactive
- intractable
- prior IP

<u>36 cpds discarded</u>
- IC_{50} >~50 μM

<u>3 classes discarded</u>
- 1 class: activity lost upon re-synthesis
- 2 classes: cytotoxicity

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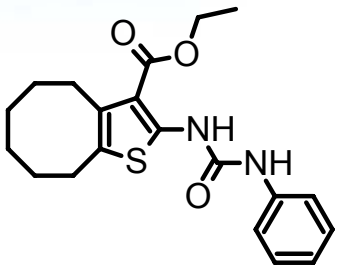
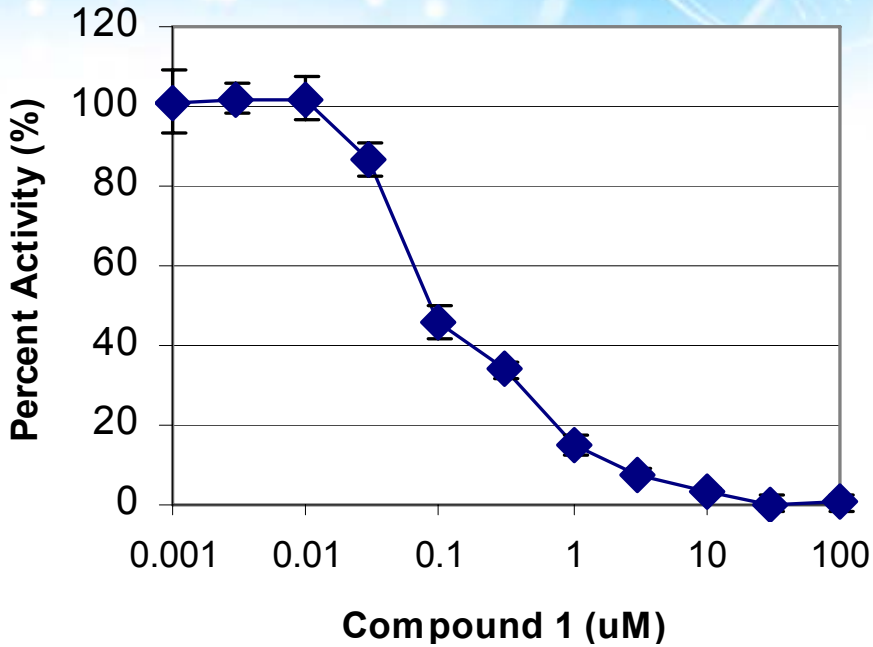
S. aureus $RpoD_{Sa}$-Dependent Transcription Screen Identifies a Novel Ureidothiophenecarboxylate Inhibitor



Compound 1



⇒ IC50 (*in vitro S. aureus* transcription assay): 730 nM
⇒ MIC (*S. aureus* ATCC 13709): 0.5-1 µg/ml

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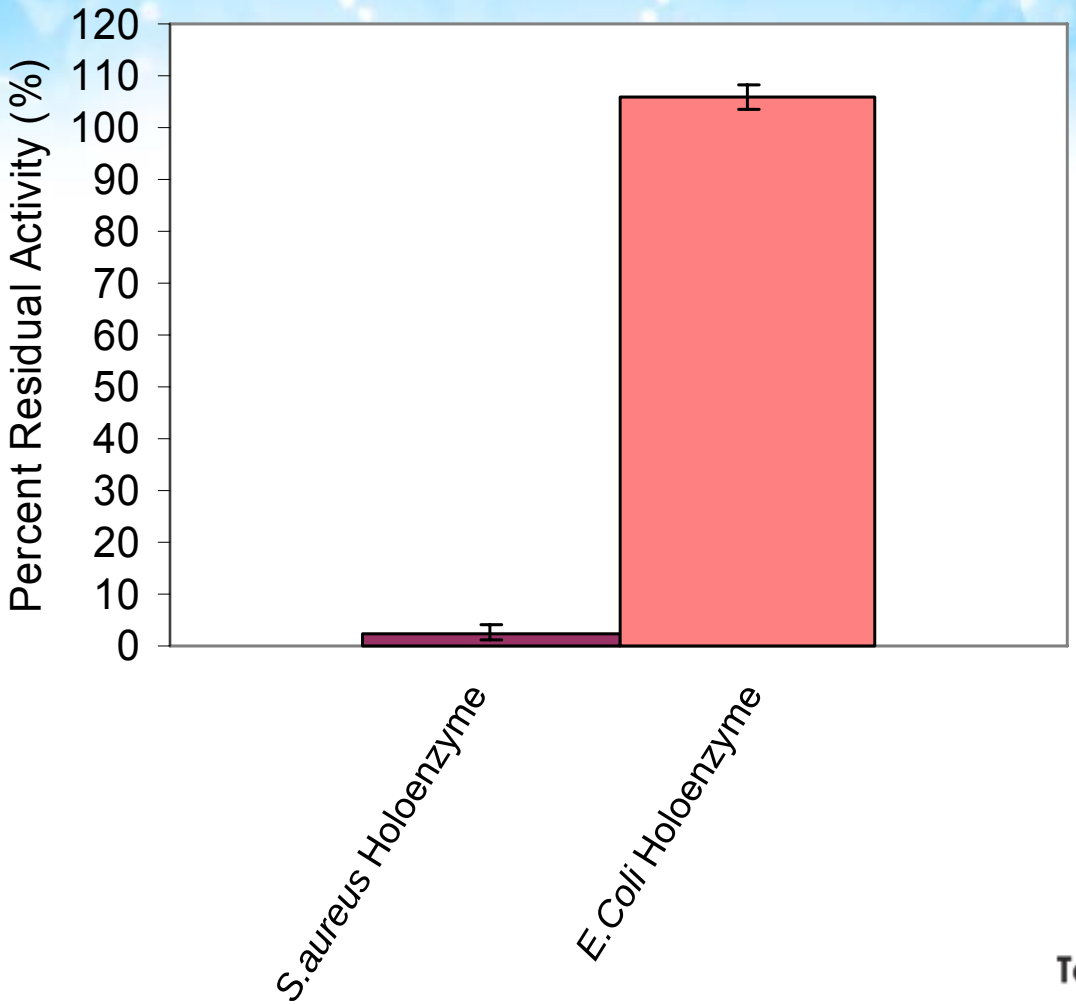
In Vitro Specificity of a Novel RNA Transcription Ureidothiophenecarboxylate Inhibitor



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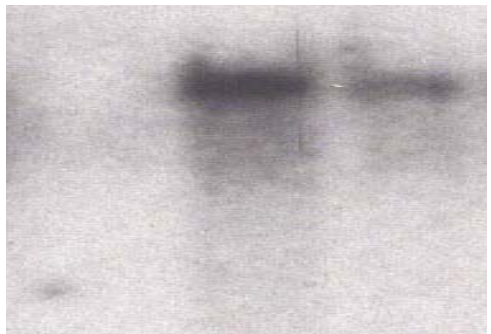
Ureidothiophenecarboxylate Activity Against Mammalian Transcription Machinery



100 μM α-Amanitin

100 μM Compound 1

NO CPD

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In Vitro Activity Summary

- 250 000 compounds screened against $RpoD_{Sa}$ HTS Assay
- Ureidothiophenecarboxylate identified as good antimicrobial candidate and demonstrates dose dependent inhibition of *S.aureus* transcription
- The activity is specific to *S.aureus* transcription machinery and shows no inhibition of either *E.coli* or mammalian transcription *in vitro* assays

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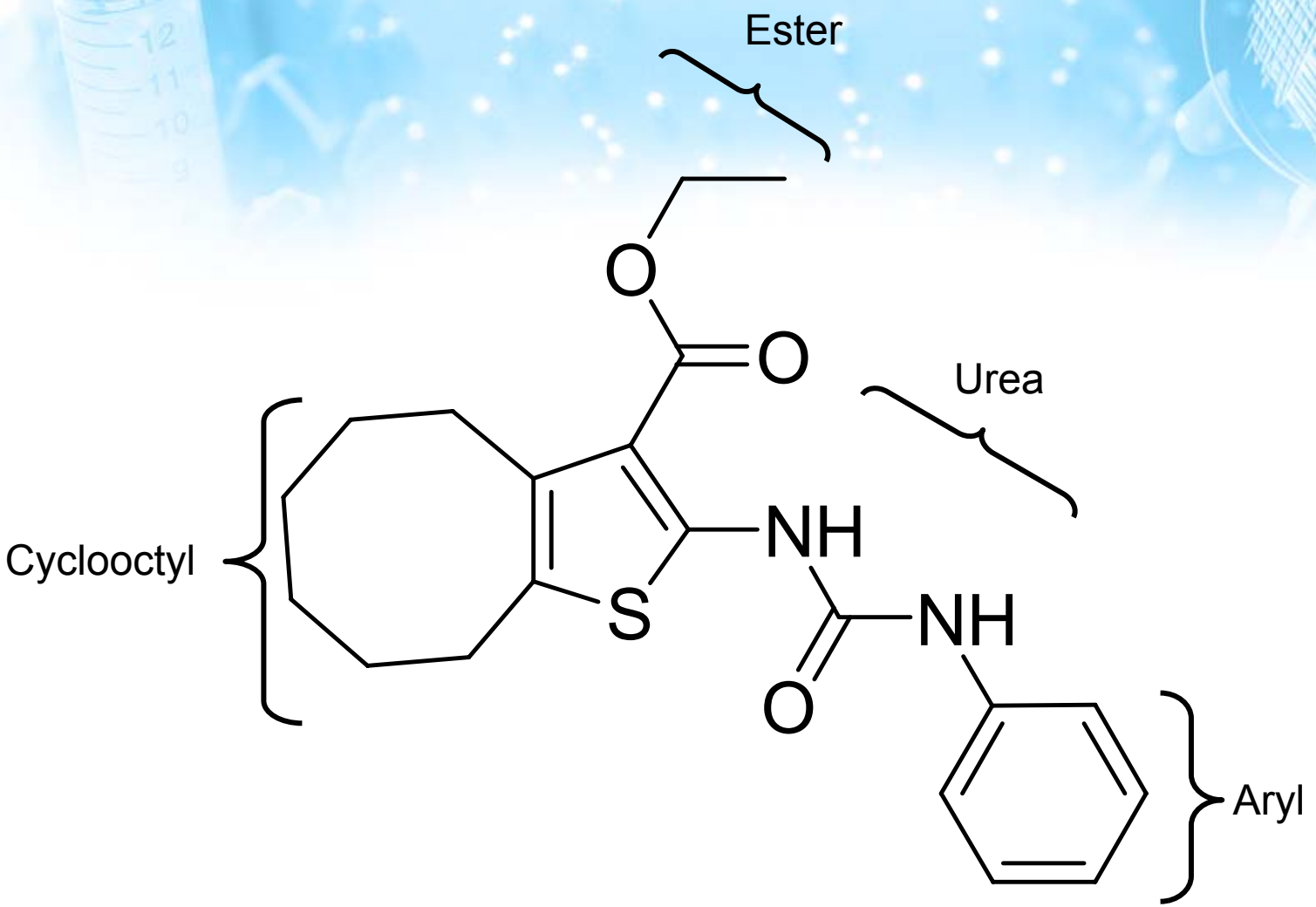
$RpoD_{Sa}$- Ureidothiophenecarboxylate SAR



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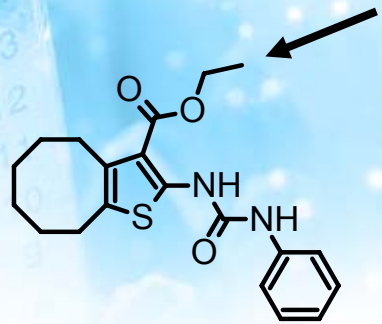
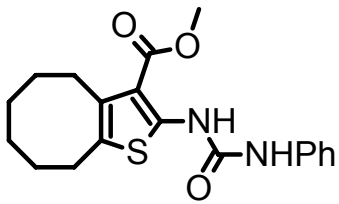
Ester Variations and Activity



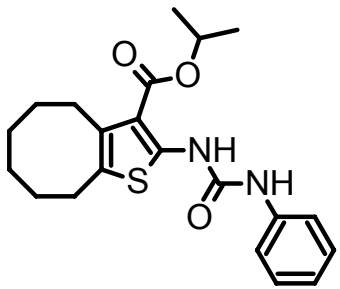
IC50 0.73 µM
MIC >128 µg/mL (RN4220)
1-2 µg/mL (Smith)

Compound 1

- Ester functionality is necessary
  - amides, ketones, alcohols, acid
- Polar groups are undesirable
  - small heterocycles, charged or uncharged
- **Isopropyl ester is optimum:**

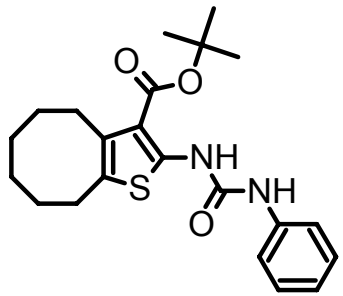


IC50 1.0 µM
MIC >128 µg/mL
(Smith and RN4220)

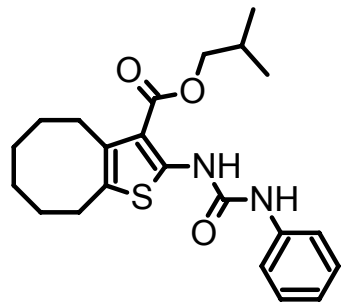


IC50 0.06 µM
MIC 0.5-1 µg/mL
(Staphylococci)

Compound 2



IC50 0.22 µM
MIC >128 µg/mL (RN4220)
0.5 µg/mL (Smith)



IC50 0.14 µM
MIC >128 µg/mL
(Smith and RN4220)

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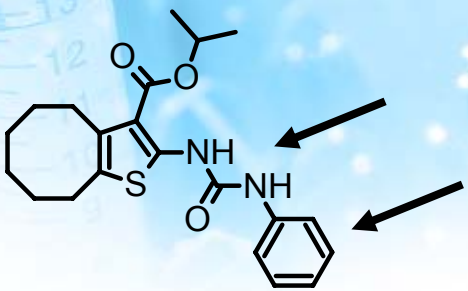
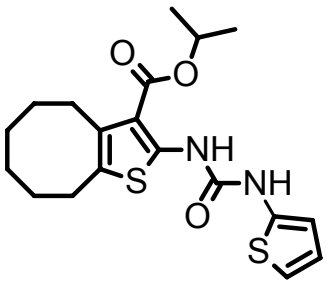
Urea Variations and Activity



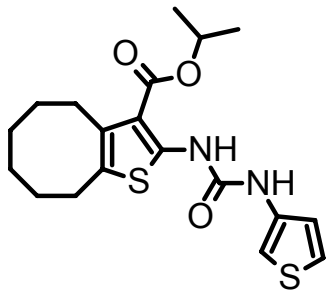
IC50 0.06 µM
MIC 0.5-1 µg/mL
(Staphylococci)

Compound 2

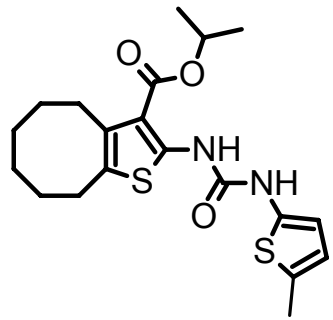
- Urea functionality is necessary; amides, carbamates, thioureas, sulfuric diamides lose inhibitory activity
- Replacement of phenyl ring with alicyclics or heterocyclics abolishes antibacterial activity
- Substituents on phenyl group abolish antibacterial activity
  - meta, para substituents retain inhibitory activity
  - ortho substituents destroy inhibitory activity
- **Only thiophenes are tolerated as phenyl replacements:**



IC50 0.06 µM
MIC 0.5-1 µg/mL
(Staphylococci)



IC50 0.20 µM
MIC 0.5-1 µg/mL
(Staphylococci)



IC50 0.49 µM
MIC 1 µg/mL
(Smith)

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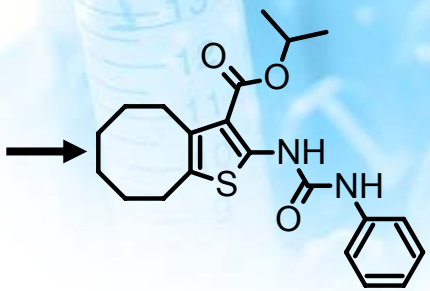
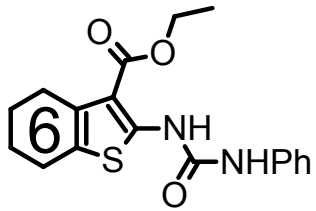
Ring Variations and Activity



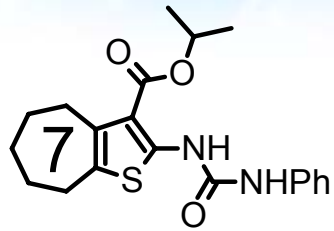
IC50 0.06 µM
MIC 0.5-1 µg/mL
(Staphylococci)

Compound 2

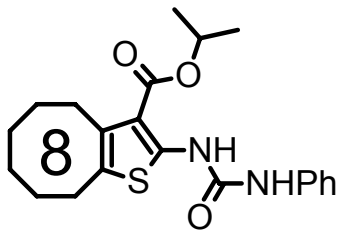
- Heteroatoms in ring abolish antibacterial activity
- **Eight and nine membered rings optimum:**



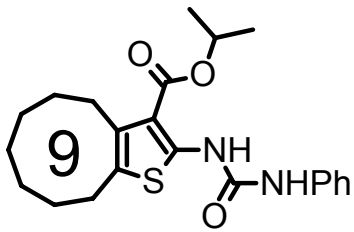
IC50: 2.4 µM
MIC >128 µg/mL
(Smith and RN4220)



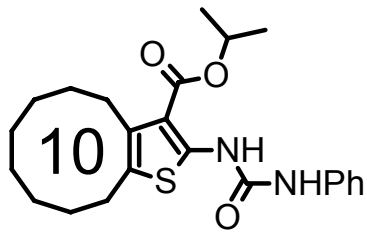
IC50: 0.1 µM
MIC >128 µg/mL (RN4220)
MIC 1 µg/ml (Smith)



IC50: 0.06 µM
MIC 0.5-1 µg/mL
(Smith and RN4220)



IC50: 0.05 µM
MIC 0.5 µg/mL
(Smith and RN4220)



IC50: 0.14 µM
MIC >128 µg/mL
(Smith and RN4220)

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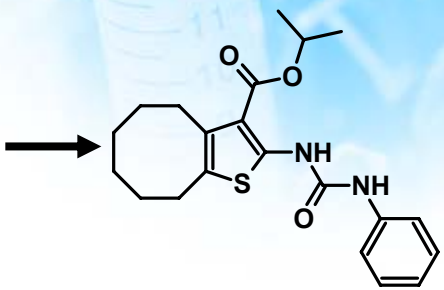
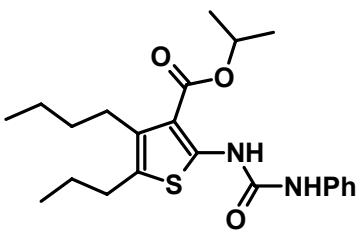
Ring Variations and Activity

- Large hydrophobic ring is important but not essential
- **Fused ring is optimum:**

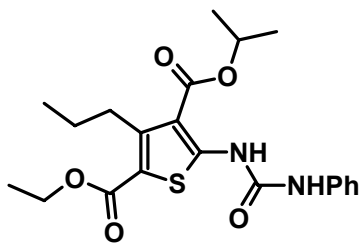


IC50 0.06 μM

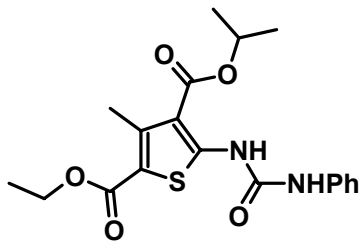
Compound 2



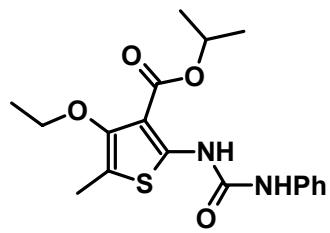
IC50 4.0 μM
MIC >128 μg/mL (all)



IC50 5.3 μM
MIC >128 μg/mL (all)



IC50 10 μM
MIC >128 μg/mL (all)



IC50 0.7 μM
MIC >128 μg/mL (all)

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Ureidothiophenecarboxylate SAR Summary

- Isopropyl ester is the optimal functional group and can not be substituted with amides, ketones, alcohols or acids

- Urea functionality is required and can not be replaced with amides, carbamates, thioureas or other groups

- Phenyl substitution is poorly tolerated

- Fused 8 or 9 membered rings are optimum and heteroatom substitution is detrimental to activity

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Biological Characterization of Ureidothiophenecarboxylates

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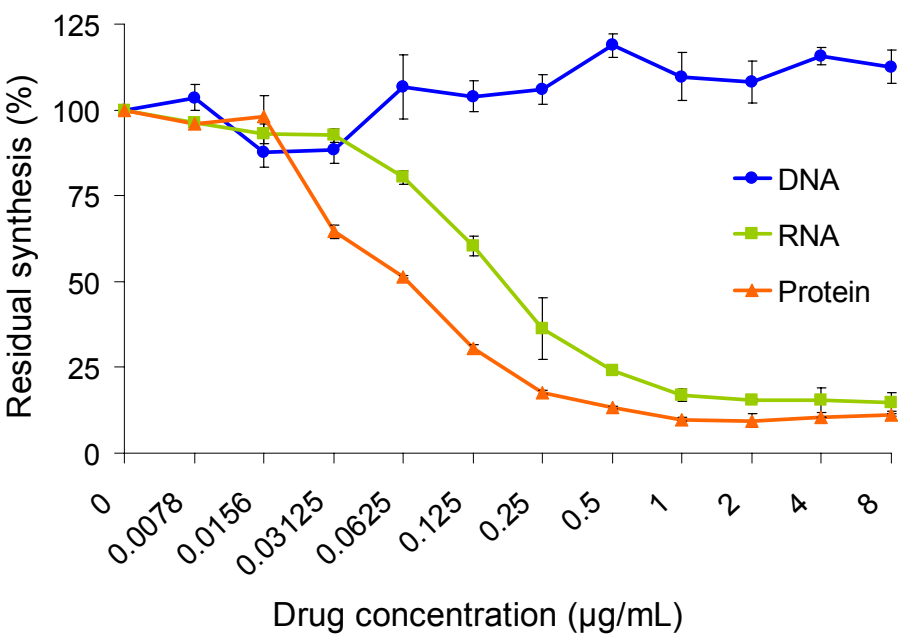
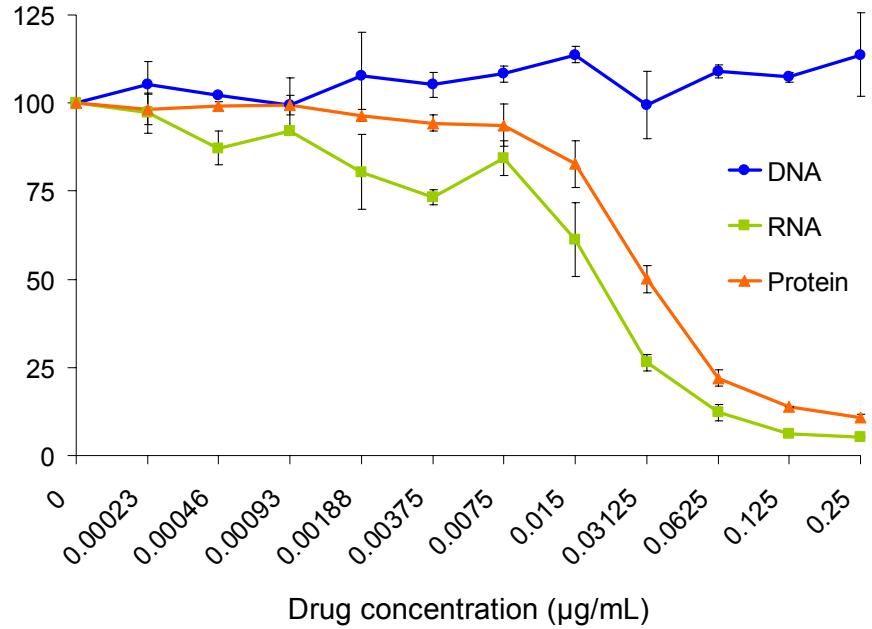
Ureidothiophenecarboxylate Inhibits Transcription in *S. aureus*

• Macromolecular synthesis assay in *S. aureus* → Ureidothiophene carboxylate inhibits RNA and protein synthesis similarly to Rifampicin:

Ureidothiophenecarboxylate



Rifampicin



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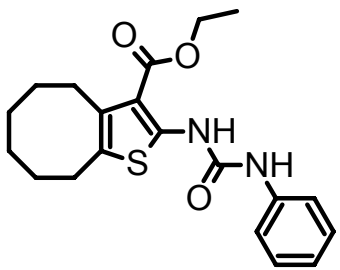
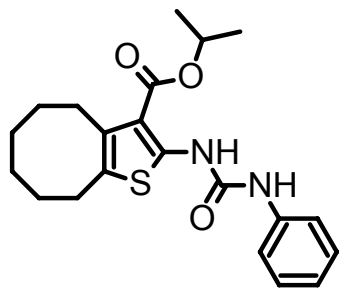
In Vitro Activity of Ureidothiophenecarboxylates

	MIC (μg/ml)			
	S.aureus ATCC 13709		*S.aureus* RN4220	
Compound	CAMHB	CAMHB + 50% Serum	CAMHB	CAMHB + 50% Serum
1	1	>128	>128	>128
2	0.5	>128	0.5	>128



Compound 1



Compound 2

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Ureidothiophenecarboxylate Bacterial Spectrum of Activity

Susceptible	Non-Susceptible
S.aureus, S.hyicus, S.carnosus, CNS *Staph, S.epidermidis*	*Pseudomonas, Escherichia, Haemophilus, Salmonella, Bacillus, Enterococcus*

- *Activity against only Staphylococcus genera.*

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Ureidothiophenecarboxylate is Active Against Antibiotic Resistant Strains of *S. aureus*

Resistant Category	***n***	**MIC or MIC range (μg/mL)**
Mupirocin-resistant	12	0.5 (11 strains) >128 (1 strain)
Rifampicin-resistant	9	< 0.125 - 1
MRSA	14	0.25 - 2
VISA ATCC 700699	1	0.25

- *Activity against Rif^{R} strains suggests distinct binding site or mechanism*

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In Vitro Resistance Frequency of *Staphylococcus aureus*

	ATCC 13709	
Compound	4xMIC (μg/ml)	Resistance frequency
Rifampicin	0.032	$1.6x10^{-8}$
Ciprofloxacin	0.5	$4.8x10^{-7}$
Mupirocin	0.5	$2.0x10^{-9}$
Ureidothiophene	2	$6.8x10^{-8}$

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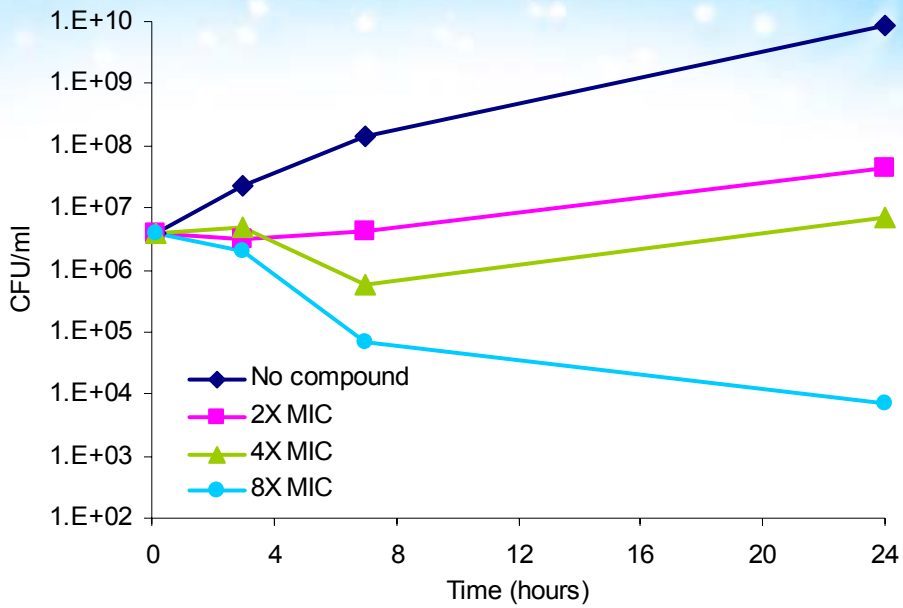
Concentration Dependent Ureidothiophenecarboxylate Activity in *S. aureus* Time-Kill Studies



• Compound 2 demonstrates a bacteriostatic effect against *S. aureus* ATCC 13709

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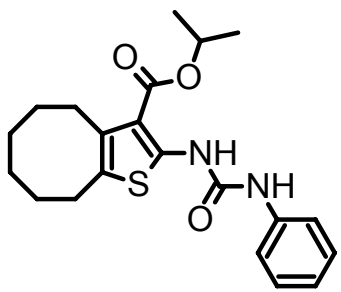
Low-Stringency Mouse Model of Infection

Lethal Sepsis Model

Group #	Compounds	Dose (mg/kg)	# Animal Survival (of 10)		
			24h	48h	72h
1	Untreated	-	0	0	0
2	Moxifloxacin	2	10	10	10
3	Compound 2	2 x 50	7	7	7



Compound 2

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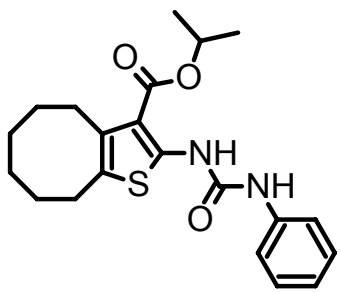
High-Stringency Mouse Model of Infection

Lethal Sepsis Model

Group #	Compounds	Route	Dose (mg/kg)	# Animal Survival (of 10)		
				24h	48h	72h
1	Untreated	IV	2x	0	0	0
2	Rifampicin	SC	1x2.5	10	10	10
3	Compound 2	IV	2x25	3	2	2



Compound 2

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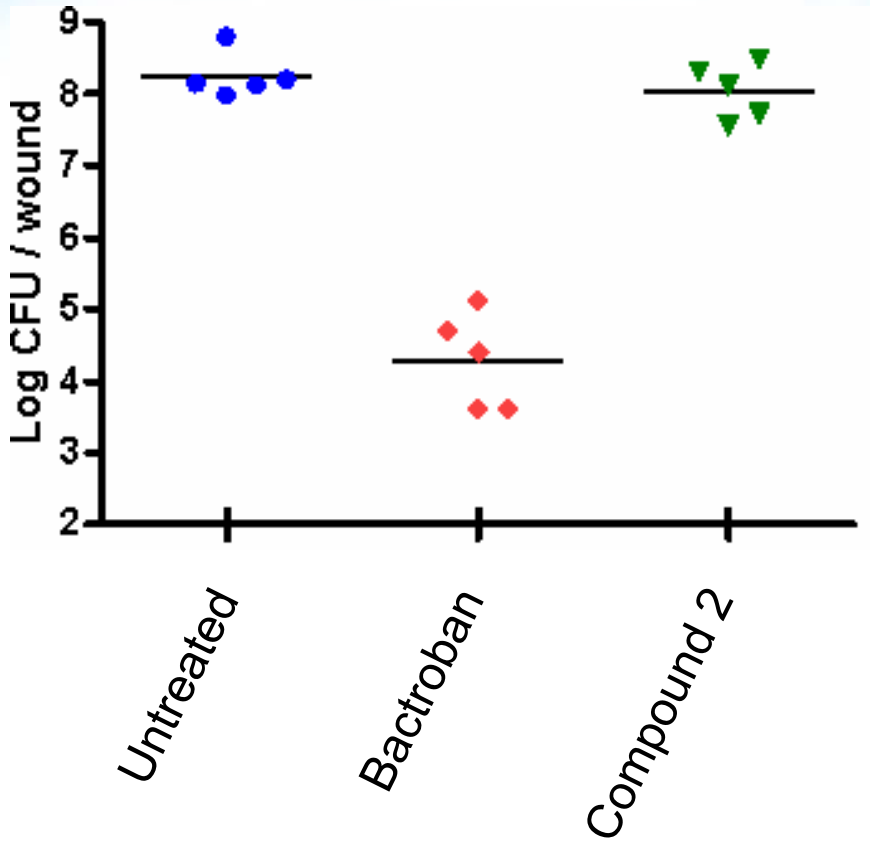
Mouse MRSA Infected Wound Model



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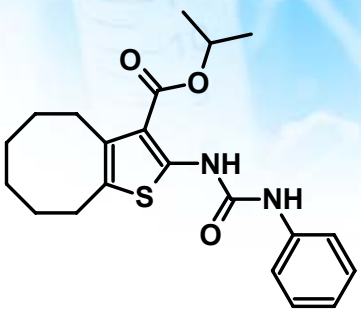
Ureidothiophenecarboxylate - Summary



Compound 1

- >160 compounds made in 6 month campaign
- Ureidothiophenecarboxylates are highly specific for *Staphylococcus* transcription holoenzyme
- Compound is likely only active against *Staphylococcus* and not related Gram-positives including *Enterococcus* or *Streptococcus*
- Well-tolerated in mice to near solubility limit of 2x25 mg/kg i.v. bolus
- Active in a low-stringency mouse model of systemic *S. aureus* infection (i.p. infection / i.p. injection) but inactive in high stringency model or topical treatment model
- Additional SAR of hydrophobic ring required to address serum binding issue

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Acknowledgements

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Targanta Biology

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Ingrid Sarmiento
Ibtihal Fadhil
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