Phage-Inspired Discovery of Small-Molecule Antibiotics

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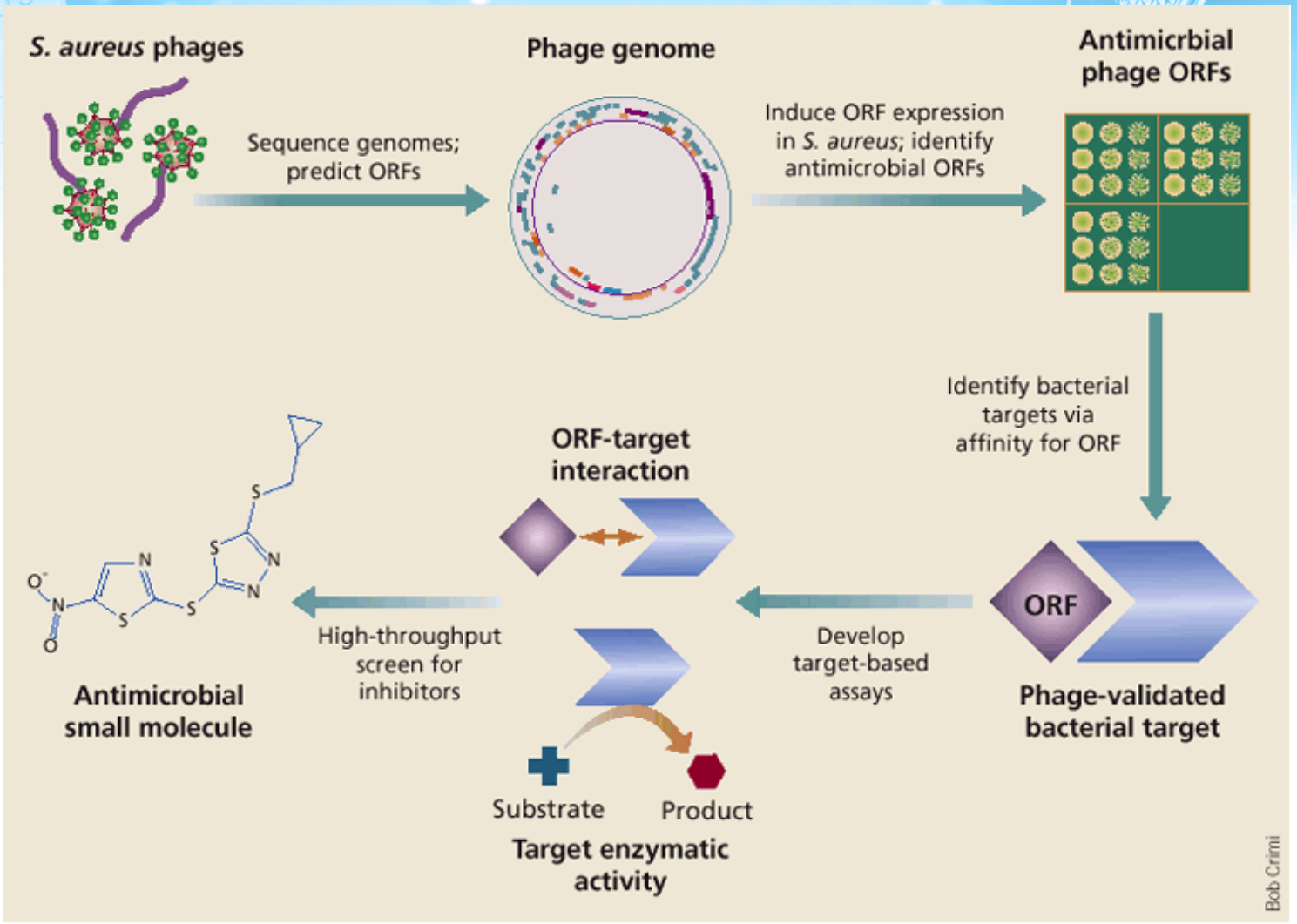
Phage-Inspired Drug Discovery Approach



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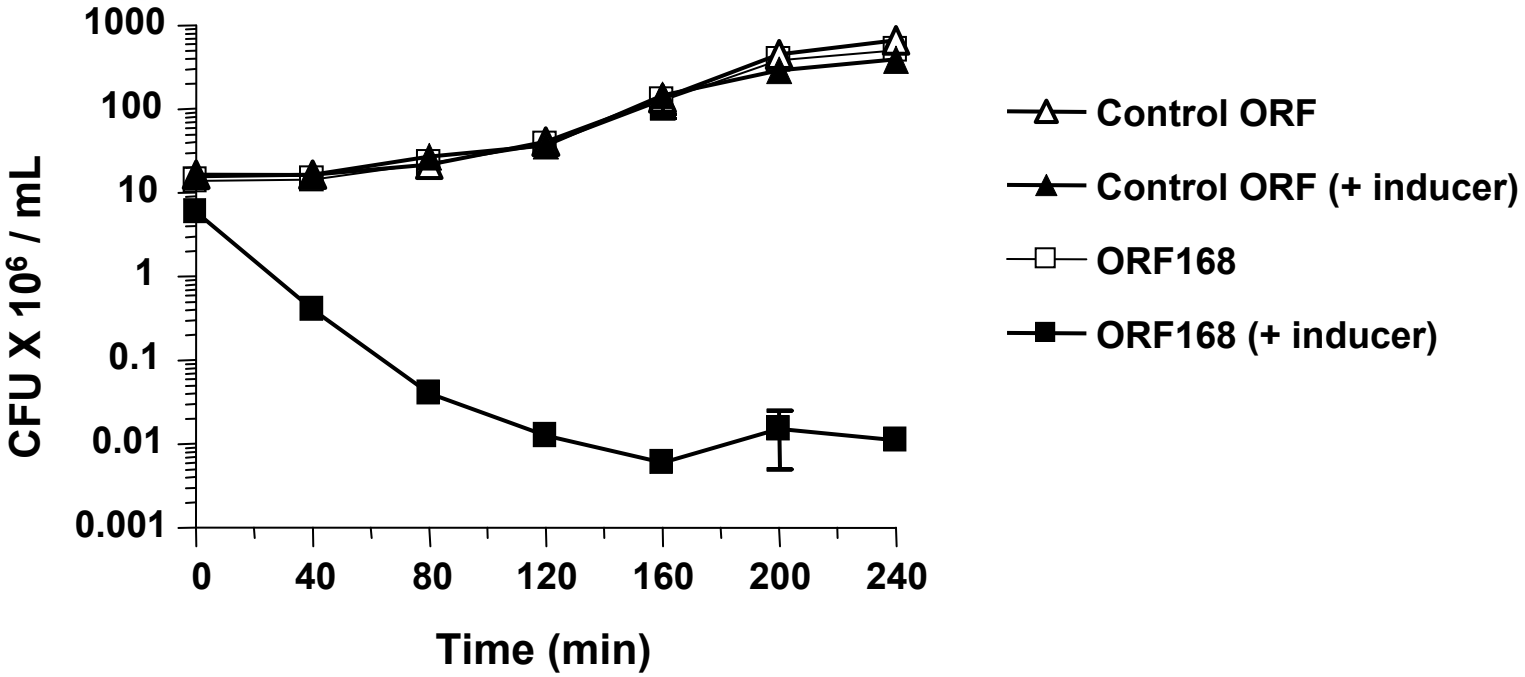
Update On Recent Data

- Target validation by phage polypeptides
- Identification of small molecule inhibitors
- Optimization of inhibitors via medicinal chemistry
- Two examples:
  - *S. aureus* DNA replication target: DNA polymerase β subunit
  - *S. aureus* transcription target: primary sigma factor

Expression of ORF168 in *S. aureus* is Bactericidal

- Dot screening → phage open reading frames that inhibit growth when expressed within *S. aureus*
- Broth assay → rapid killing kinetics of selected phage ORFs:



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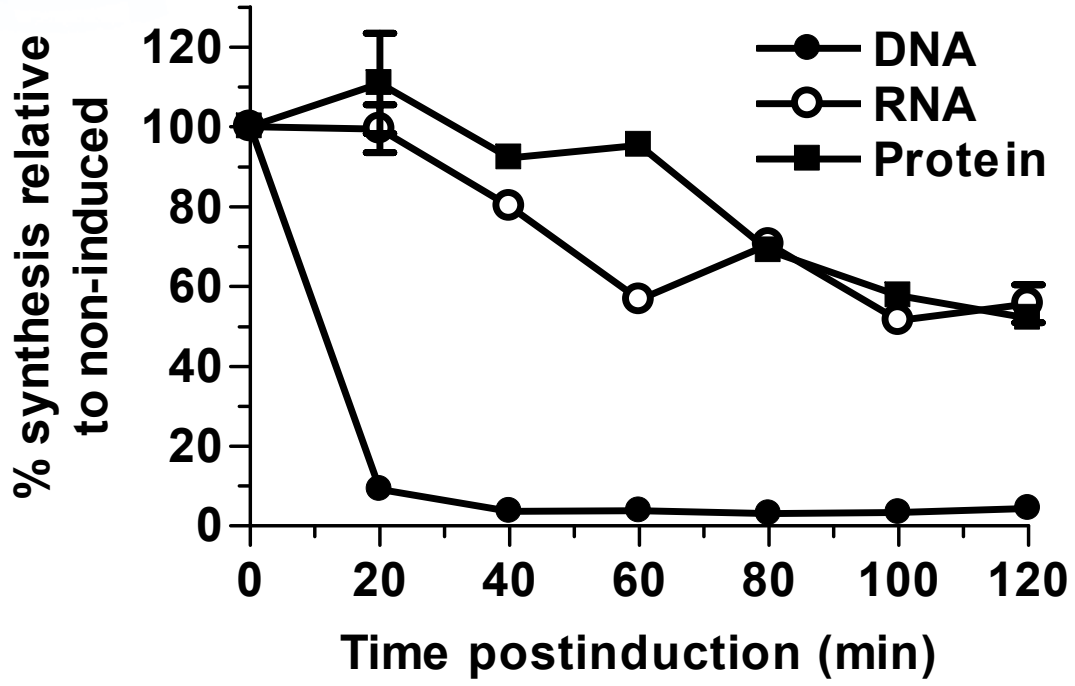
Expression of ORF168 in *S. aureus* Selectively Inhibits DNA Synthesis

- Macromolecular synthesis assay in *S. aureus* → selectivity of inhibition:



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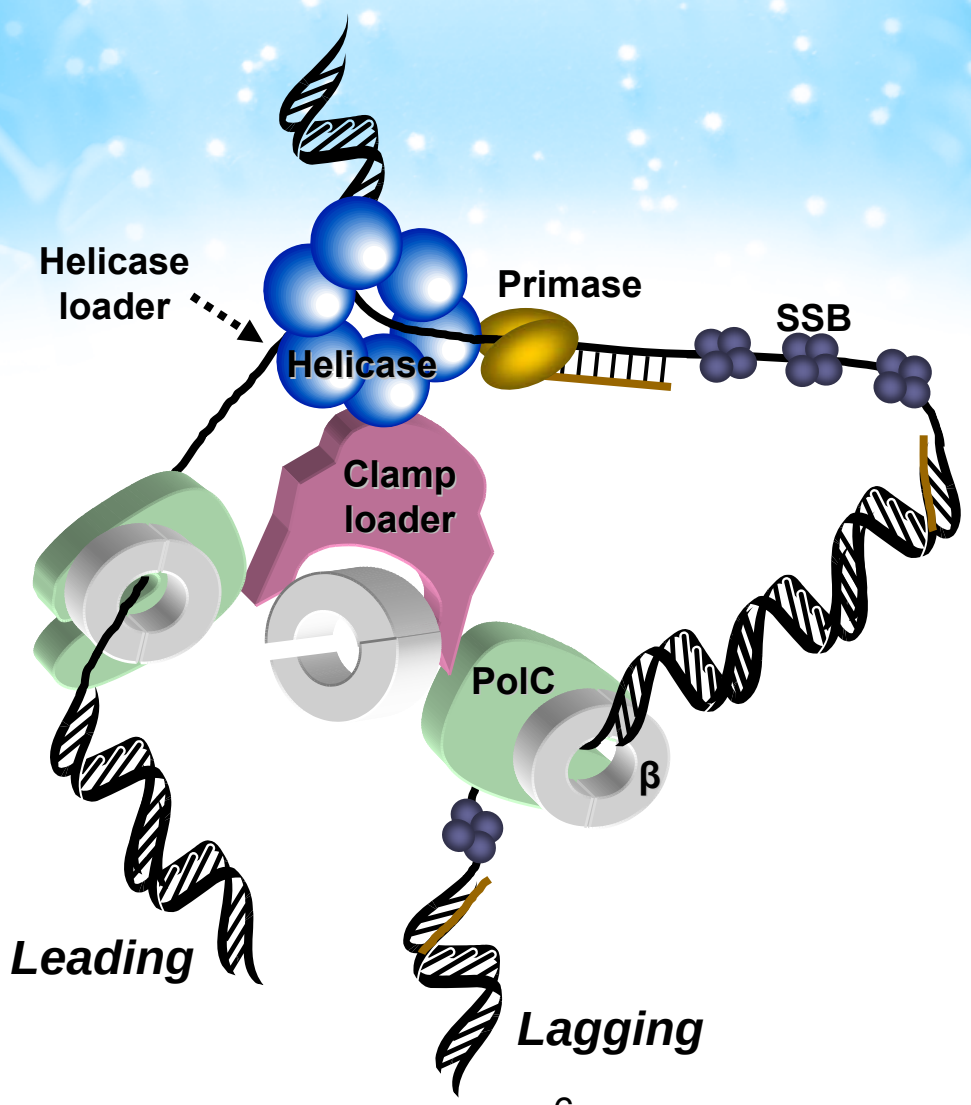
The Bacterial DNA Replication Machinery Offers Essential, Under-Exploited Targets



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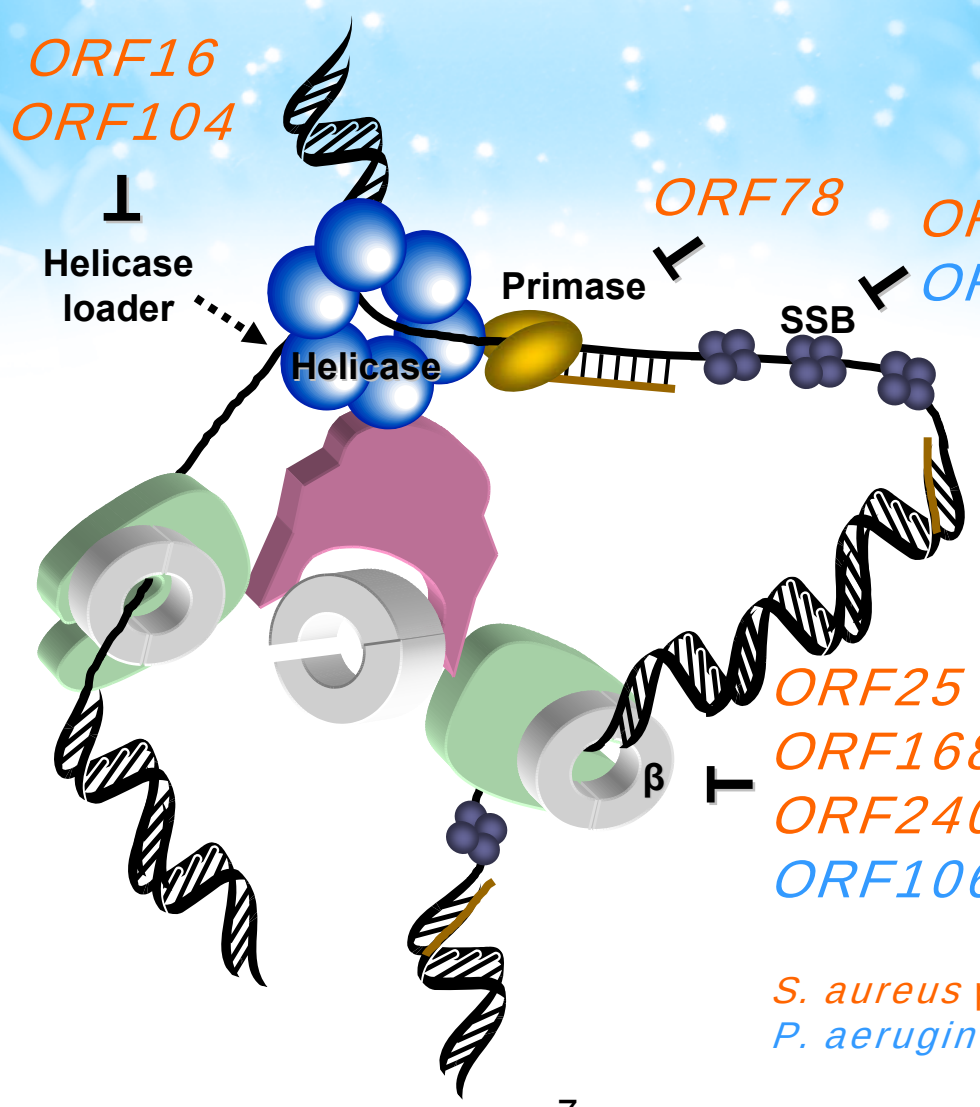
Affinity Chromatography Identifies Replication Machinery Targets for Phage Polypeptides



S. aureus phage polypeptide
P. aeruginosa phage polypeptide

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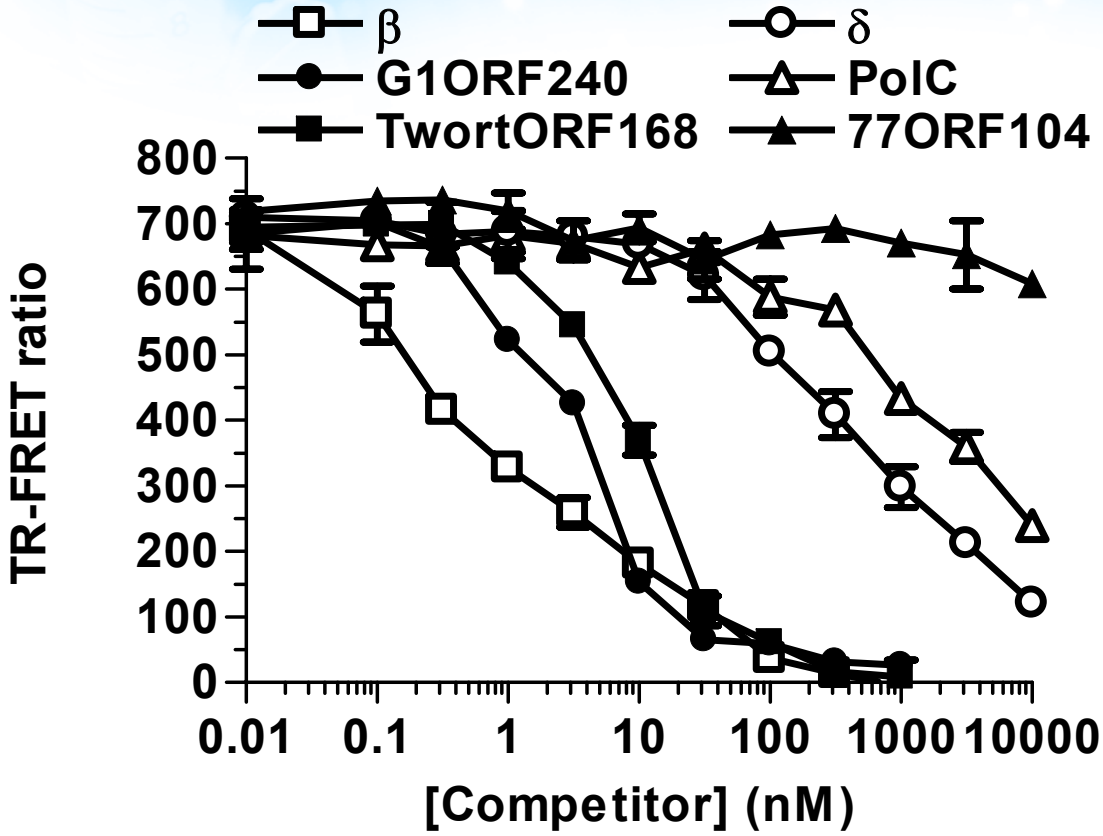
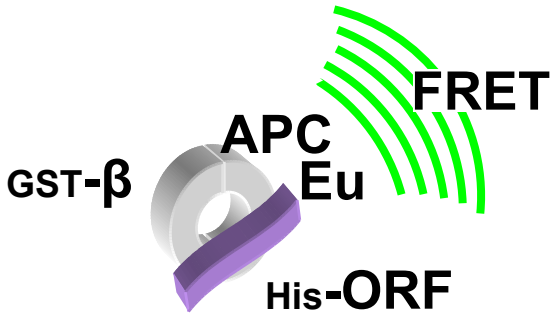
Phage Polypeptide Binding Site on β Appears to be Shared by Replicase Components

- TR-FRET fluorescence assay → study competitors of the interaction:





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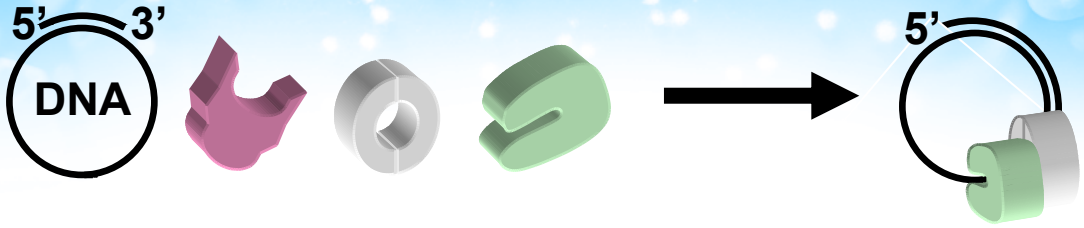
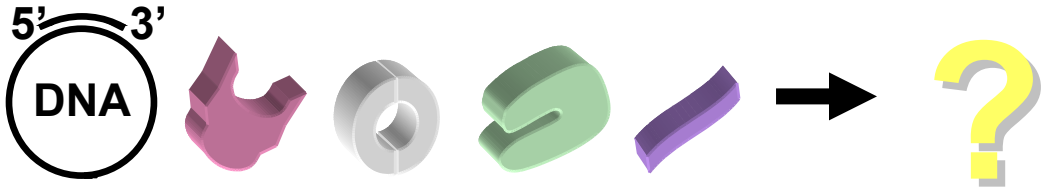
Does Phage Polypeptide Binding to β have a Functional Consequence?



Clamp loader β PolC

Processive DNA replication



Clamp loader β PolC ORF

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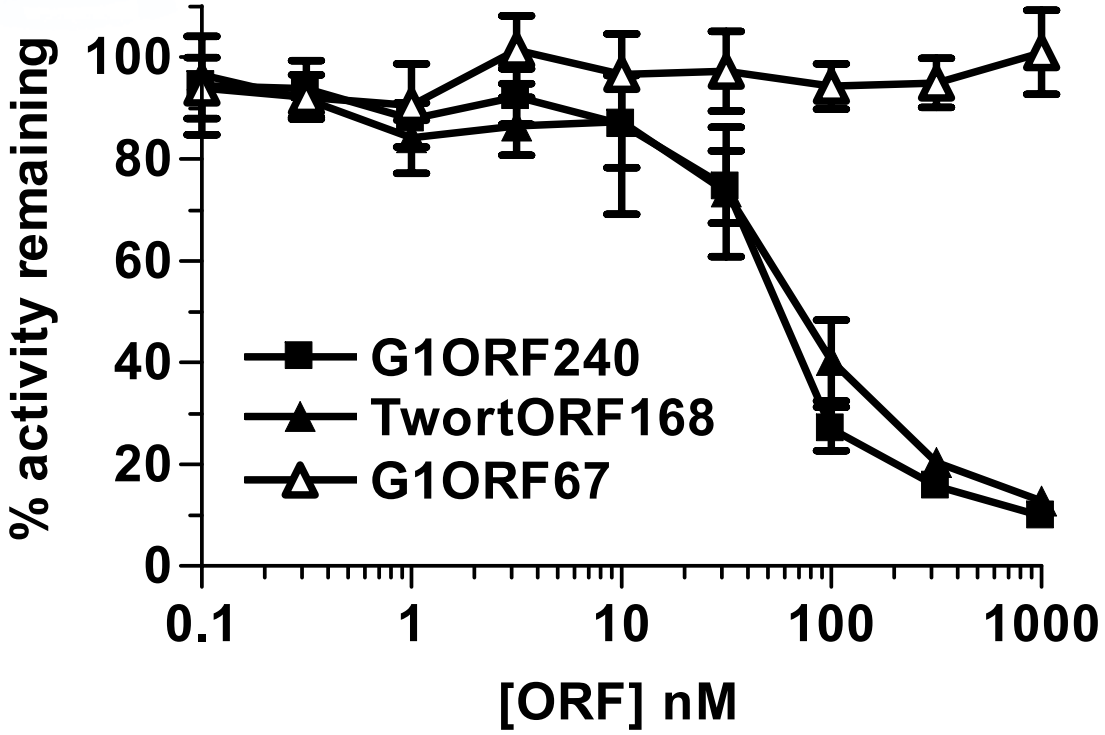
β-binding Phage Polypeptides Inhibit the *S. aureus* DNA Replicase *in vitro*

• Plate-based assay with reconstituted replicase → study effect of phage polypeptides on processive DNA synthesis *in vitro*:



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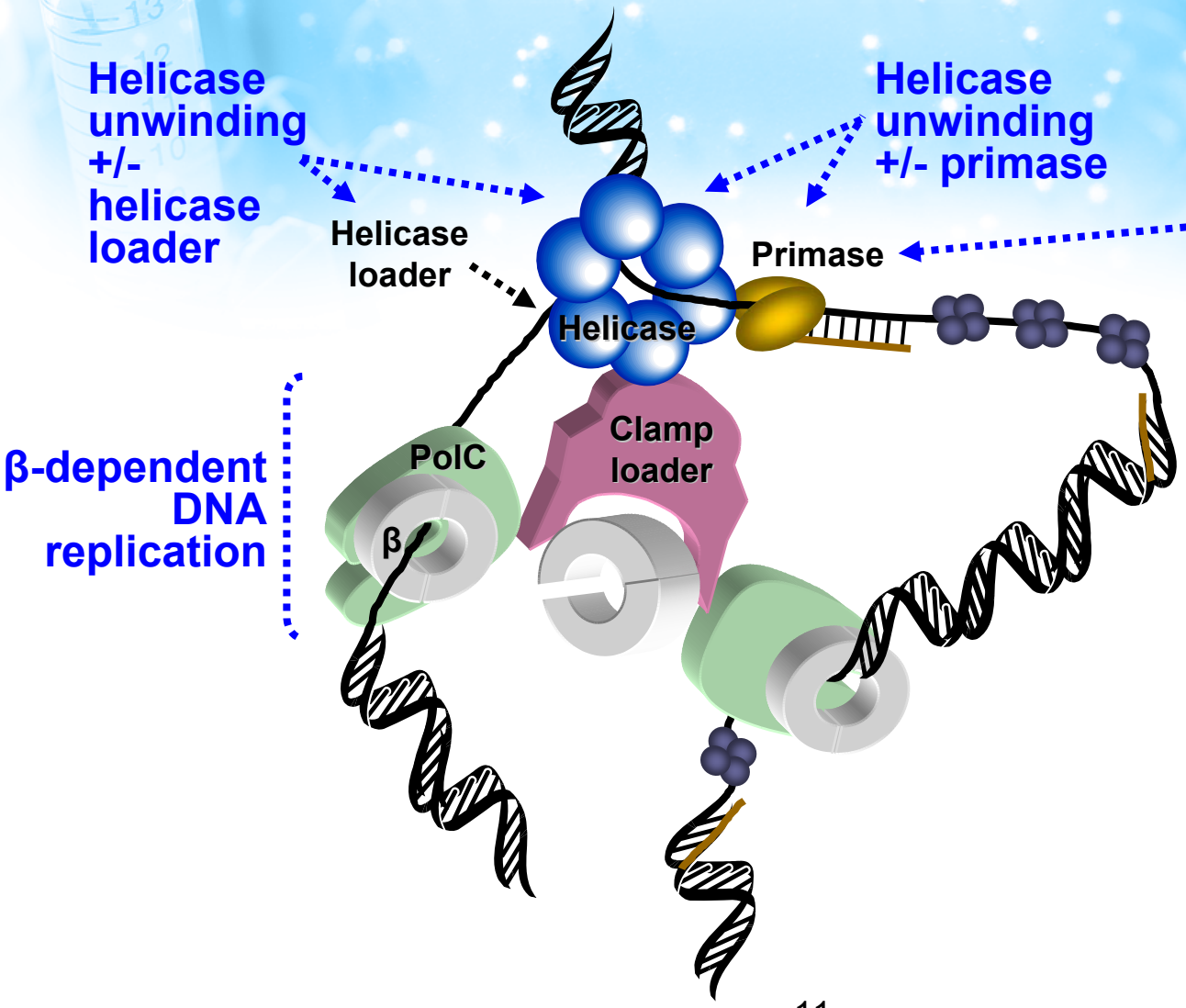
Enzyme-Based Screens for Small Molecule Inhibitors Exploit Essential Activities of the Replicase



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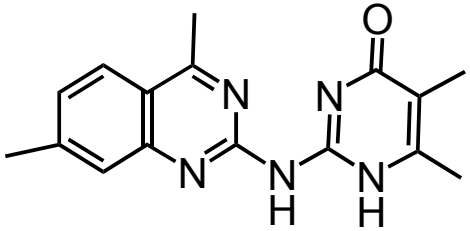
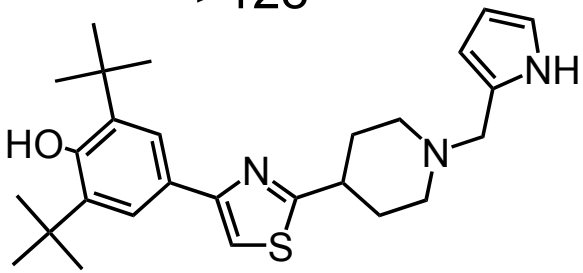
In vitro Activities of Two *S. aureus* Replicase Inhibitors

	Compound 1	Compound 2
IC_{50} (μM), *S. aureus* replicase	18 ± 7.1	7.9 ± 0.2
IC_{50} (μM), mammalian DNA replicase	>50	>50
IC_{50} (μM), DNA binding assay	>50	>50
IC_{50} (μM), HeLa cytotoxicity assay	>100	14
MIC (μg/mL)		
• *S. aureus* ATCC 13709 (MSSA)	4	8
• *S. aureus* ATCC 13709 + 4% HSA	64	64
• *M. bovis* BCG (Denmark, Phipps)	4	n.d.
• *H. influenzae* ATCC 49766	>32	>128
Structure		

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Summary

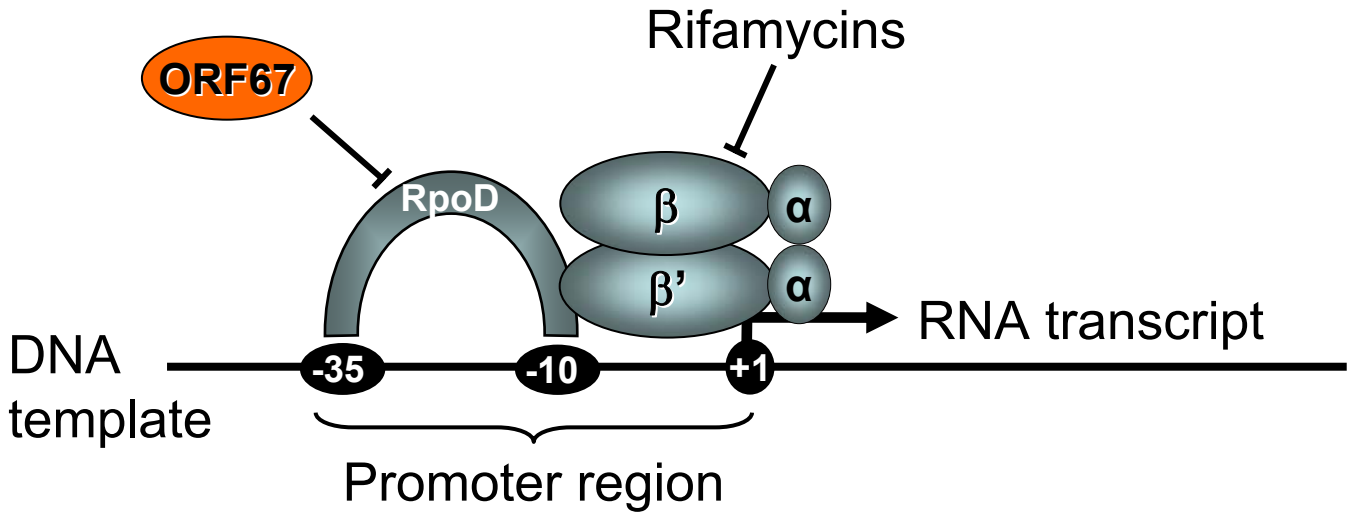
- **ORF168 and ORF240 polypeptides:**
  - inhibit DNA synthesis selectively in *S. aureus*
  - bind selectively to the *S. aureus* DNA sliding clamp *in vitro*
  - inhibit processive DNA replication *in vitro*

- **Small-molecule inhibitors from *S. aureus* replicase screen**:
  - active against G+ including MRSA, and efflux-deficient G-
  - validate the replicase for inhibitor screening
  - well-tolerated (to solubility limit) in mice: 2 x 10 mg/kg, i.v. bolus
  - suffer from serum shift
  - lack efficacy in rigorous models of *S. aureus* infection

- **Additional series are under study**

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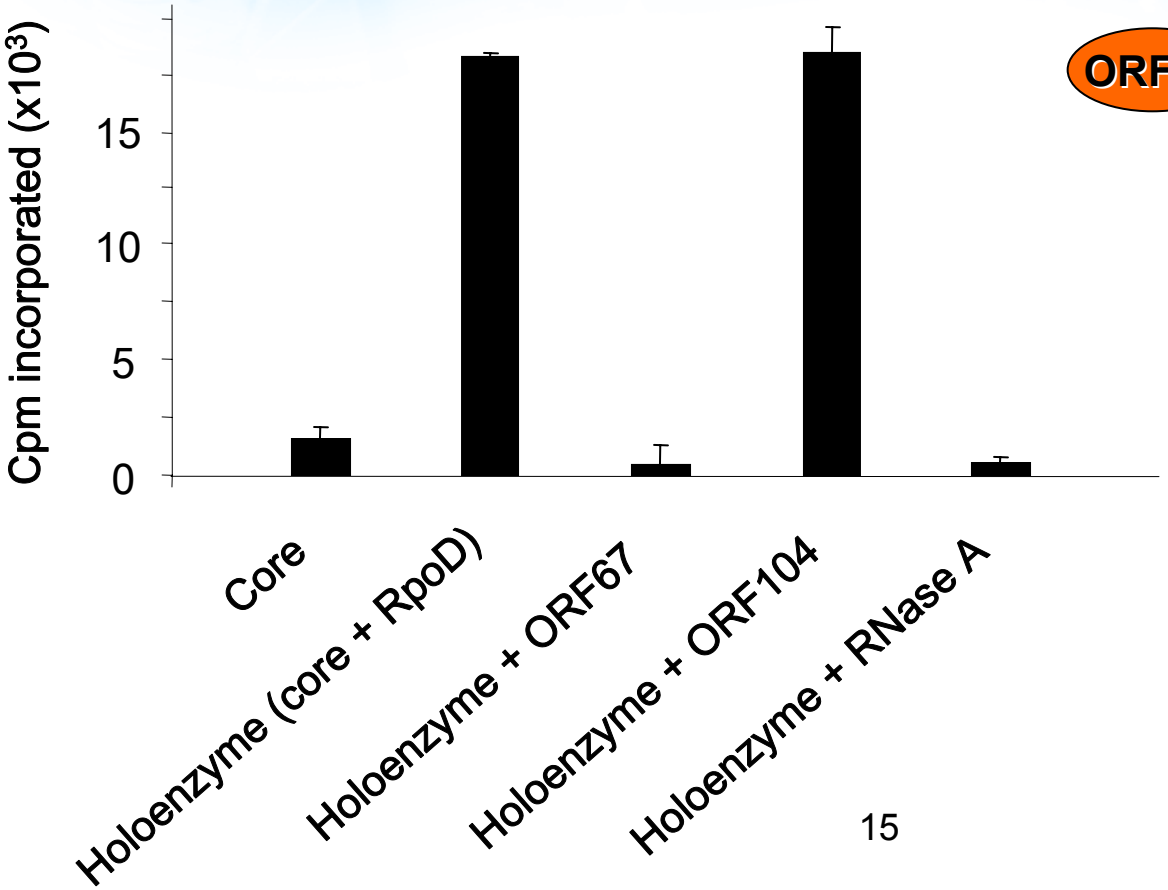
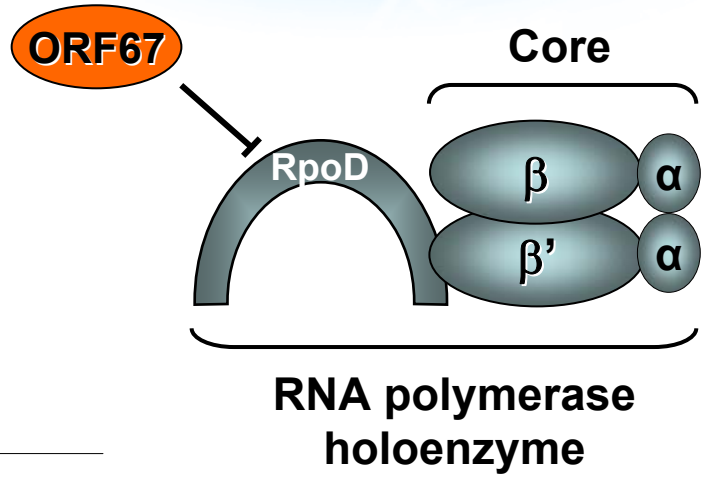
$RpoD_{Sa}$-Specific Phage Polypeptide (ORF67) Inhibits *S. aureus in vitro* Transcription

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





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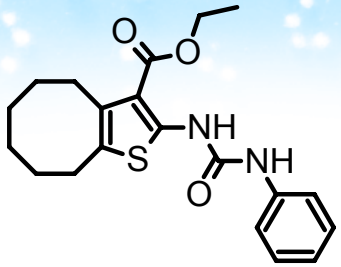
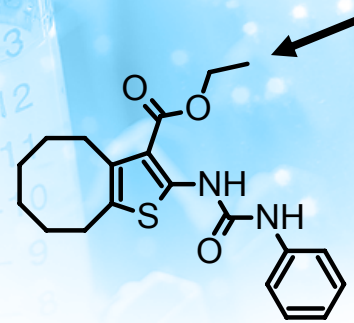
S. aureus RpoD-Dependent Transcription Screen Identifies a Novel Ureidothiophenecarboxylate Inhibitor



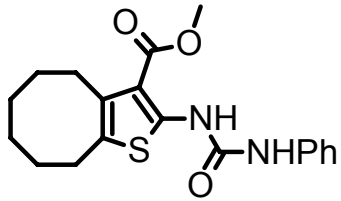
⇒ IC50 (*in vitro S. aureus* transcription assay): 730 nM
⇒ MIC (*S. aureus* Smith ATCC13709): 1-2 µg/mL
⇒ Serum binding issue (MIC >128 µg/mL in 50% serum)
⇒ Spectrum limited to select strains of Staphylococci
⇒ No *in vitro* cytotoxicity
⇒ No promiscuity or inhibition of *E. coli* or mammalian *in vitro* transcription

Ester Variations and Activity

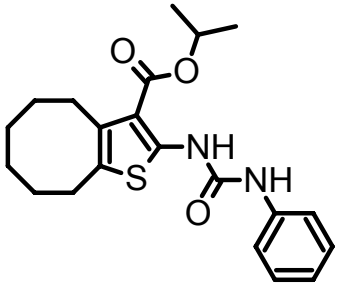


IC50 0.73 µM
MIC >128 µg/mL (RN4220)
1-2 µg/mL (Smith)

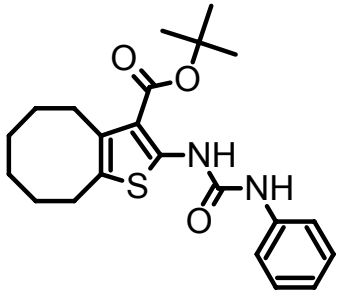
- Ester functionality is necessary
  - amides, ketones, alcohols, acid
- Polar groups are undesirable
  - small heterocycles, charged or uncharged
- **Isopropyl ester is optimum:**



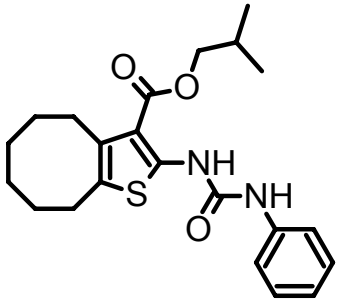
IC50 1.0 µM
MIC >128 µg/mL
(Smith and RN4220)



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



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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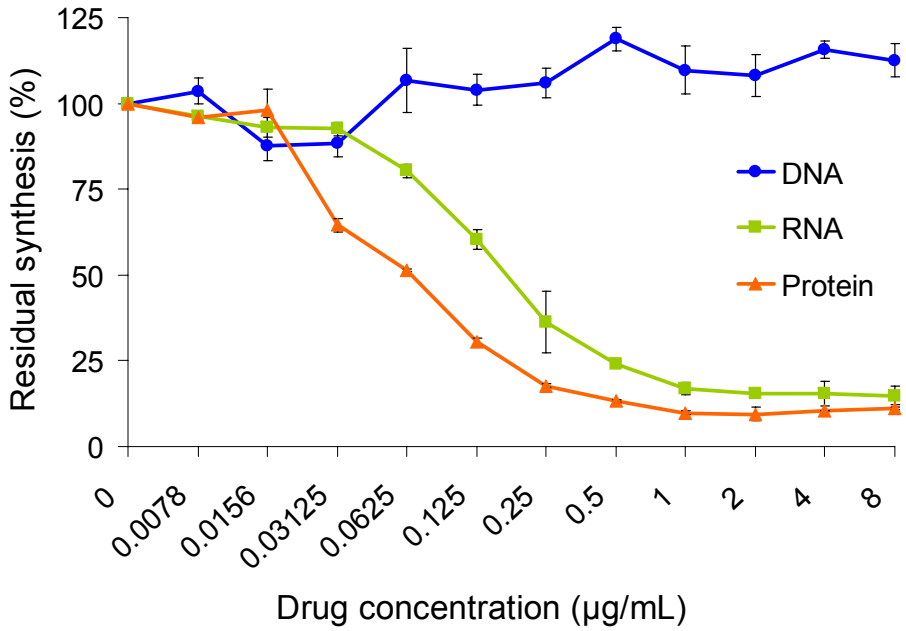
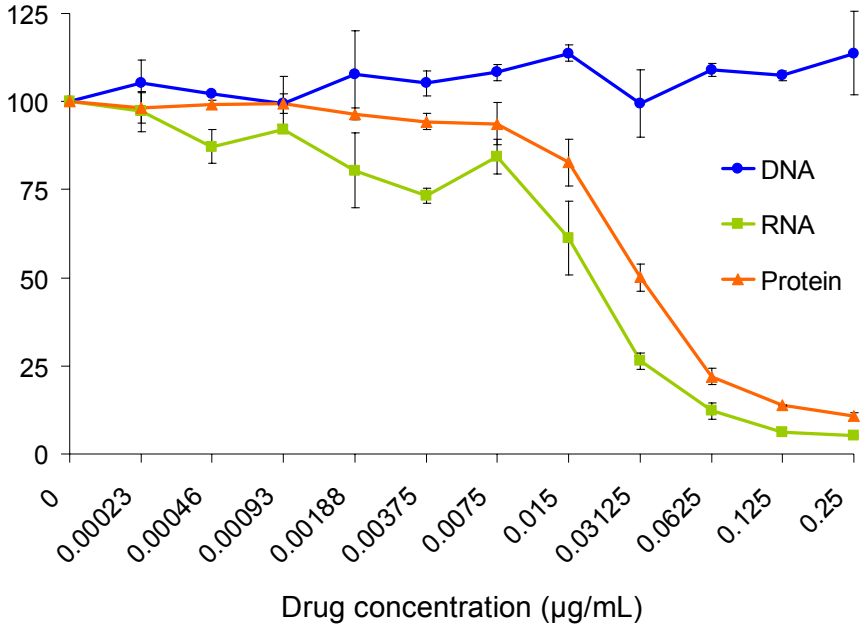
Ureidothiophenecarboxylate Inhibits Transcription in *S. aureus*

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

Ureidothiophenecarboxylate



Rifampicin



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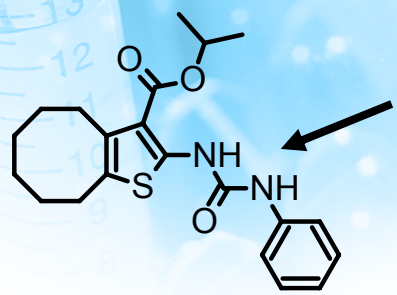
Ureidothiophenecarboxylate is Active Against Antibiotic Resistant Strains of *S. aureus*

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*

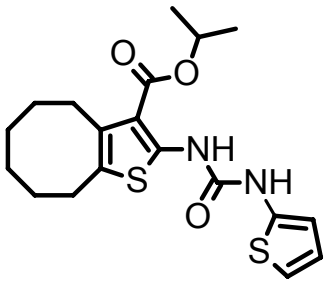
Urea Variations and Activity



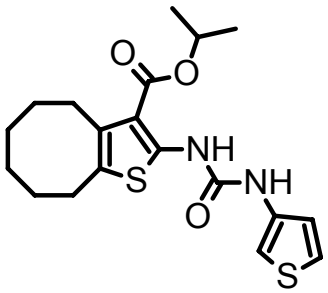
IC50 0.06 μM
MIC 0.5-1 μg/mL
(Staphylococci)

- 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

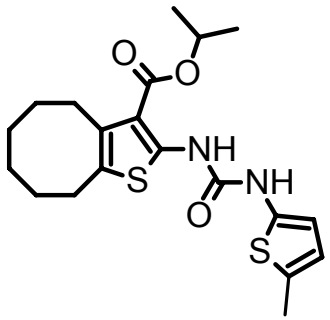
➢ Only thiophenes are tolerated as phenyl replacements:



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

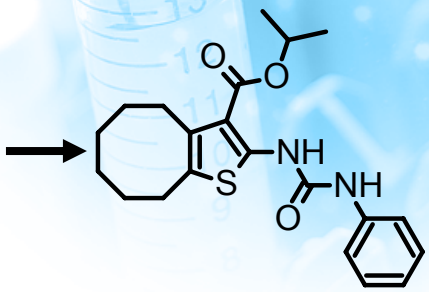


IC50 0.20 μM
MIC 0.5-1 μg/mL
(Staphylococci)



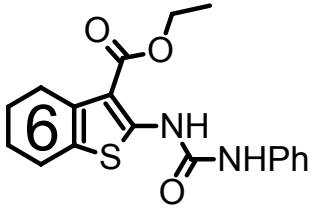
IC50 0.49 μM
MIC 1 μg/mL
(Smith)

Ring Variations and Activity

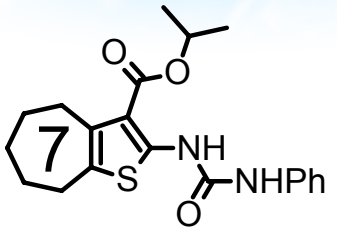


IC50 0.06 µM
MIC 0.5-1 µg/mL
(Staphylococci)

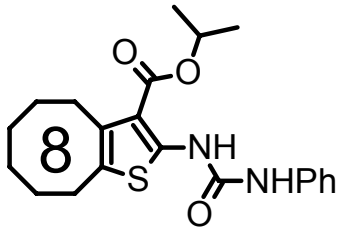
- Heteroatoms in ring abolish antibacterial activity
- Acyclic replacements are detrimental (IC50 5-10 µM)
- **Eight and nine membered rings optimum:**



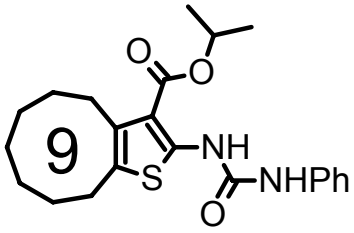
IC50: 2.4 µM
MIC >128 µg/mL
(Smith and RN4220)



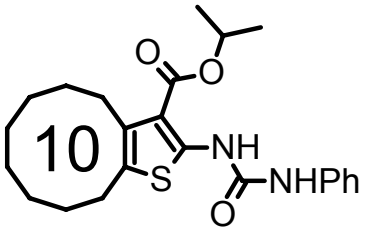
IC50: 0.1 µM
MIC >128 µg/mL (RN4220)
MIC 1 µg/ml (Smith)



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

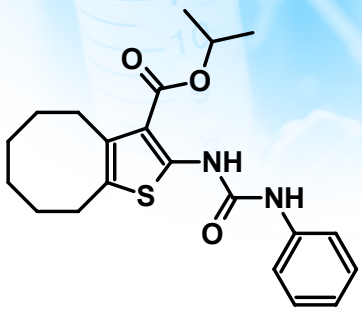


IC50: 0.05 µM
MIC 0.5 µg/mL
(Smith and RN4220)



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

Ureidothiophenecarboxylate - Summary



IC50 0.06 µM
MIC 0.5-1 µg/mL
(Staphylococci)
cLogP 5.72

- >120 compounds made in 6 month campaign
- Compound is likely only active against staphylococci and is subject to high frequencies of resistance
- 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)
- Additional SAR of hydrophobic ring required to address serum binding issue

Acknowledgements

Targanta's CSO

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Targanta Biology

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