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Lead Development of PolC Polymerase Inhibitors against Resistant Gram-positive Pathogens

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ABSTRACT

Background: There is an urgent and growing public health need to discover and develop new anti-infectives as a countermeasure to the emergence of resistance and the possibility of biothreats and bioterrorism. Our promising small-molecule lead compounds derive from a series of thiadiazole urea compounds (TZUs) that exhibit potent inhibition of PolC, the catalytic subunit of the replicative DNA polymerase in Gram-positive bacteria.
Materials/methods: MIC testing of clinical isolates was performed according to CLSI guidelines. Protein binding, *in vitro* time-kill kinetic studies and spontaneous resistance development assays followed standard protocols. ADME (Eurofins), PK (Absorption Systems) and tolerability/efficacy (Neosome) was assessed for several TZU compounds.
Results: Advanced lead compounds of the TZU PolC inhibitor series demonstrated broad-spectrum Gram-positive activity against clinically important pathogens and biothreat organisms, with MIC_{90}=1-2 µg/mL against methicillin-resistant *Staphylococcus aureus* (MRSA), MIC_{90}=1-2 µg/mL against penicillin-resistant *Streptococcus pneumoniae* (PRSP), MIC_{90}=0.5-1 µg/mL against macrolide-resistant *Streptococcus pyogenes* (Group A strep), and MIC_{90}=2-4 µg/mL against vancomycin-resistant Enterococci (VRE). The TZU compounds were highly active against *Bacillus anthracis* and *Listeria monocytogenes*, with MIC values of 0.25-0.5 µg/mL.
TZU compounds demonstrate bactericidal activity, moderate protein binding (80-90%), a low rate of spontaneous resistance (<10^{-9} at 4x MIC), oral bioavailability, and favorable PK properties and initial (non-GLP) safety profiles.
In vivo efficacy of TZU lead compounds with oral dosing BID was demonstrated in mouse MRSA thigh infection models, MRSA septicemia and pneumococcal pneumonia models, as well as in anthrax post-exposure prophylaxis and treatment models.
Conclusions: TZU compounds show great potential as a novel class of oral Gram-positive antibacterial drugs. No preexisting resistance was found, as expected for a novel agent against a novel target. The observed *in vivo* efficacy in a number of different murine models of infection with oral administration is encouraging toward the selection of a strong development candidate with potentially great clinical utility.

Figure 1. Thiadiazole Urea (TZU) Pharmacophore

TZU Compounds are guanosine mimics. Base-pairing with a cytosine residue during replication leads to inhibition of the bacterial PolC DNA polymerase.

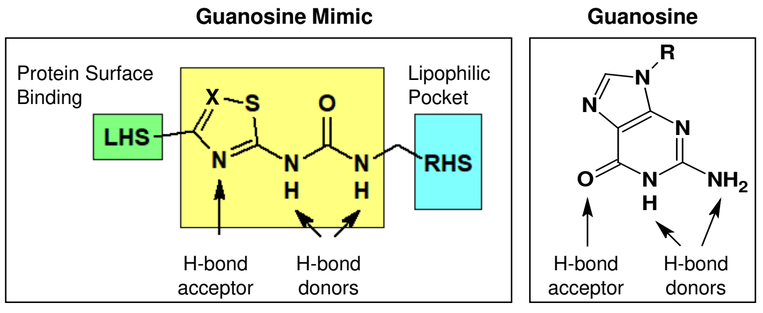
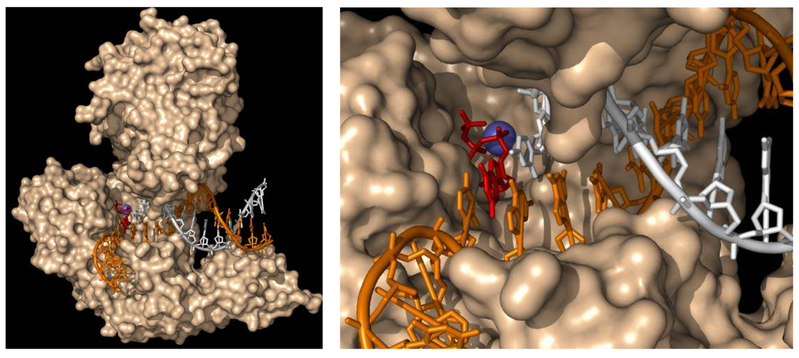


Figure 2. Co-crystal Structures of PolC, DNA, and TZU

Mechanism of action unambiguously confirmed: Direct inhibition of PolC enzyme activity. Bound structures for 18 compounds at 2.3 – 2.8 Å resolution can guide lead optimization. Shown below are structures of GkaPolC–DNA–dGTP complexes.



SUMMARY AND CONCLUSIONS

Novel Oral Agent for Gram-positive Infections

- Brand new antibiotic class
- Highly active *in vitro* and *in vivo*, bactericidal
- Consistently high oral bioavailability
- Proprietary crystal structures available for lead optimization
- Active against MRSA, VRE, PRSP, Group A Strep, anthrax
- Goal: IND candidate
- Substantial market opportunity based on recent precedents

Target Product Profile of PolC Inhibitor Series

- Broad Gram-positive spectrum
  - ✓ Staphylococci, Streptococci, Enterococci
  - ✓ *B. anthracis, L. monocytogenes*
  - ✓ MRSA MIC_{90} = 1 µg/mL
  - ✓ No cross-resistance with other antibiotics
- Favorable PK/ADME properties for oral dosing
  - ✓ High oral bioavailability in mice, with sustained high drug serum levels at >4 hours
  - ✓ Good metabolic stability
  - ✓ Moderate serum binding (~80%)
  - ✓ Demonstrated efficacy at 100 mg/kg in murine thigh infection model with MRSA
- Low propensity for resistance development
  - ✓ Spontaneous resistance rates of 10^{-9}
- Bactericidal mode of action
  - ✓ *In vitro* time-kill kinetic profile shows >99.9% reduction of viable cells at 3-6 hours
- Favorable safety profile
  - ✓ Successful 5-day repeat dose tolerability study (300 mg/kg BID) in mice (studies in other species ongoing)
  - ✓ Clean profile in receptor panel toxicology screen

Table 1. Activity of TZU Compounds Against NIAID Category A, B, and C Priority Pathogens

Strain	CRS_ID	370540	371016	371032	371360	CEF	AZI	LEVO	TET	GENT	LZD	VANC	IMI
S. aureus NRS384 (CA-MRSA, PVL+)		1	0.5	0.5	0.5	>16	>16	1	>16	≤0.25	2	0.5	8
S. aureus NRS119 (Levo-R, Lzd-R)		1	0.5	0.5	≤0.25	>16	1	>16	>16	>16	>16	1	>16
S. epidermidis NRS8 (MRSE)		2	1	2	1	>16	>16	16	0.5	>16	1	8	0.5
S. pyogenes F758883 (Azi-R)		≤0.25	≤0.25	≤0.25	≤0.25	1	1	0.5	>16	4	1	≤0.25	≤0.25
S. pneumoniae MDR-1 (MDR)		2	1	2	2	>16	>16	1	>16	>16	0.5	≤0.25	1
S. pneumoniae F1064366 (Levo-R)		1	1	1	1	2	16	16	>16	16	1	≤0.25	≤0.25
E. faecalis F1111404 (VRE)		1	1	1	1	>16	>16	>16	0.5	>16	2	>16	4
E. faecium F1111434 (VRE)		1	≤0.25	0.5	0.5	>16	>16	>16	>16	>16	2	16	>16
L. monocytogenes ATCC 15313		1	0.5	0.5	NT	NT	NT	NT	NT	NT	NT	NT	NT
B. anthracis Ames		0.5	0.25	0.25	NT	NT	NT	NT	NT	NT	NT	NT	NT

Figure 3. Biochemical *vs.* Antimicrobial Potency and Effect of Protein Binding

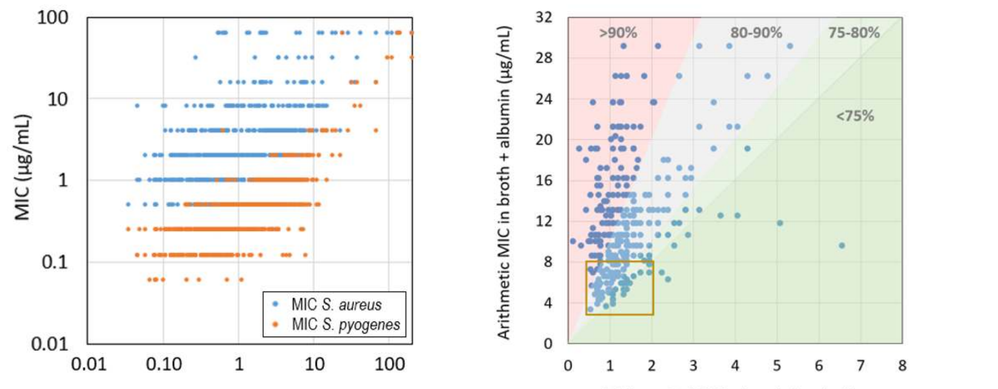


Table 2. *In vitro* Activity of Advanced TZU Compounds against 350 Strains of Gram-positive Staphylococci, Pneumococci, and Enterococci ($MIC_{50/90}$, MIC Range, Geometric Mean MIC)

Organism	*S. aureus* (n=54) Clinical Isolates[1]			*S. aureus* (n=50) Challenge Set (NARSA)[2]			Staphylococci (n=59) Coagulase-negative[3]			Enterococci (n=50) Clinical Isolates[4]			*S. pneumoniae* (n=48) Clinical Isolates[5]			*S. pyogenes* (n=51) Clinical Isolates[6]			Other Streptococci (n=38) Clinical Isolates[7]		
Compound	**$MIC_{50/90}$**	**Range**	**Mean**	**$MIC_{50/90}$**	**Range**	**Mean**	**$MIC_{50/90}$**	**Range**	**Mean**	**$MIC_{50/90}$**	**Range**	**Mean**	**$MIC_{50/90}$**	**Range**	**Mean**	**$MIC_{50/90}$**	**Range**	**Mean**	**$MIC_{50/90}$**	**Range**	**Mean**
CRS370540	2 / 2	1 - 4	1.76	2 / 4	0.5 - 8	1.74	2 / 4	0.5 - 8	2.44	2 / 2	0.5 - 8	1.58	1 / 2	0.12 - 2	0.77	0.5 / 1	0.12 -2	0.41	4 / 4	0.5 - 2	1.22
CRS370683	1 / 2	1 - 2	1.26	1 / 2	0.5 - 8	1.41	2 / 4	0.5 - 4	1.60	1 / 2	0.25 - 4	0.84	0.5 / 1	0.12 - 2	0.48	0.25 / 0.5	0.06 - 1	0.19	2 / 2	0.25 -1	0.61
CRS370815	1 / 2	0.5 - 2	1.07	1 / 2	0.5 - 4	1.10	2 / 4	0.5 - 4	1.86	1 / 2	0.25 - 4	1.15	1 / 2	0.12 - 2	0.73	0.25 / 0.5	0.12 - 1	0.34	2 / 4	0.5 -2	1.02
CRS370886	1 / 2	0.5 - 2	1.21	1 / 2	0.5 - 4	1.23	2 / 4	0.5 - 8	2.17	2 / 2	0.5 - 8	1.47	1 / 2	0.12 - 2	0.82	0.25 / 1	0.12 - 1	0.34	2 / 4	0.25 - 2	1.22
CRS371016	1 / 2	0.5 - 2	1.08	1 / 2	0.5 - 8	1.12	2 / 4	0.25 - 4	1.84	1 / 2	0.25 - 4	0.95	1 / 2	0.12 - 2	0.70	0.25 / 0.5	0.06 - 1	0.31	2 / 4	0.25 - 2	0.93
CRS371032	1 / 2	0.5 - 4	1.20	1 / 2	0.5 - 4	1.30	2 / 4	0.5 - 8	2.07	1 / 2	0.25 - 8	1.38	1 / 2	0.12 - 2	0.97	0.25 / 0.5	0.06 - 1	0.34	2 / 4	1 - 4	2.31
CRS371204	2 / 2	1 - 4	1.69	2 / 4	1 - 4	1.89	2 / 4	0.5 - 8	2.53	1 / 2	0.25 - 4	1.30	1 / 2	0.25 - 2	0.96	0.25 / 1	0.06 - 1	0.34	4 / 4	0.5 -2	1.28
CRS371214	2 / 2	1 - 8	1.59	2 / 2	1 - 8	1.62	2 / 4	0.25 - 8	2.41	1 / 2	0.25 - 4	1.27	1 / 2	0.12 - 4	1.06	0.5 / 1	0.12 - 2	0.50	4 / 4	0.5 -4	1.53
CRS371360	1 / 1	0.5 - 2	0.90	1 / 2	0.5 - 2	0.91	2 / 4	0.25 - 4	1.60	1 / 2	0.25 - 4	1.12	1 / 2	0.12 - 4	0.89	0.25 / 0.5	0.12 - 2	0.36	2 / 4	0.5 -2	1.15
Linezolid	2 / 2	1 - 2	1.51	1 / 4	0.5 - >16	n/a	1 / 2	0.5 - 2	1.06	1 / 2	0.5 - 2	1.34	0.5 / 1	0.06 - 1	0.42	1 / 1	0.5 - 1	0.82	1 / 1	0.5 -2	0.95
Ciprofloxacin	0.5 / >4	0.25 - >4	n/a	0.5 / >4	0.12 - >4	n/a	1 / >4	0.25 - >4	n/a	>8 / >8	0.5 - >8	n/a	NT	NT	NT	NT	NT	NT	NT	NT	NT
Levofloxacin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	1 / >8	0.5 - >8	n/a	0.5 / 1	0.5 - 4	n/a	1 / 2	0.5 - >8	n/a
Vancomycin	1 / 1	0.5 - 2	0.73	1 / 4	0.25 - 4	1.01	NT	NT	NT	2 / >16	0.5 - >16	n/a	0.25/0.25	0.06 - 0.5	n/a	NT	NT	NT	NT	NT	NT
Oxacillin	>4 / >4	0.12 - >4	n/a	>4 / >4	0.25 - >4	n/a	2 / >4	0.5 - >4	n/a	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Ampicillin	NT	NT	NT	NT	NT	NT	NT	NT	NT	1 / >16	0.12 - >16	n/a	NT	NT	NT	NT	NT	NT	NT	NT	NT
Penicillin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.12 / 2	0.06 - 4	0.25	NT	NT	NT	0.25 / >2	0.03 - >2	n/a
Doxycycline	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.25 / >2	0.12 - >2	n/a	NT	NT	NT
Azithromycin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.06 / 1	0.06 - >4	n/a	1 / >4	0.06 - >4	n/a

[1] *S. aureus* clinical isolates were predominantly from wound infections, collected 2003-2004. 54% were MRSA.
[2] Strains from the National Repository of *S. aureus* (NARSA), including linezolid-resistant and vancomycin-intermediate strains from wound/skin/soft tissue, bloodstream or respiratory infections, or isolated from peritoneal fluid or sputum.
[3] *S. epidermidis* (n=26) , *S. hemolyticus* (n=15) , *S. saprophyticus* (n=12), *S. hominis* (n=3), *S. lugdunensis* (n=3).
[4] *E. faecalis* (n=29) and *E. faecium* (n=21); 46% were VRE.
[5] *S. pneumoniae* Pen-R (n=10) , Pen-I (n=19), and Pen-S (n=19) strains.
[6] *S. pyogenes* included 17 (33%) macrolide-resistant strains.
[7] *S. agalactiae* (n=12), Group C/G Strep (n=13), and *S. viridans* (n=13).

Figure 4. TZU MIC Distributions in Drug-resistant vs. –susceptible Strains

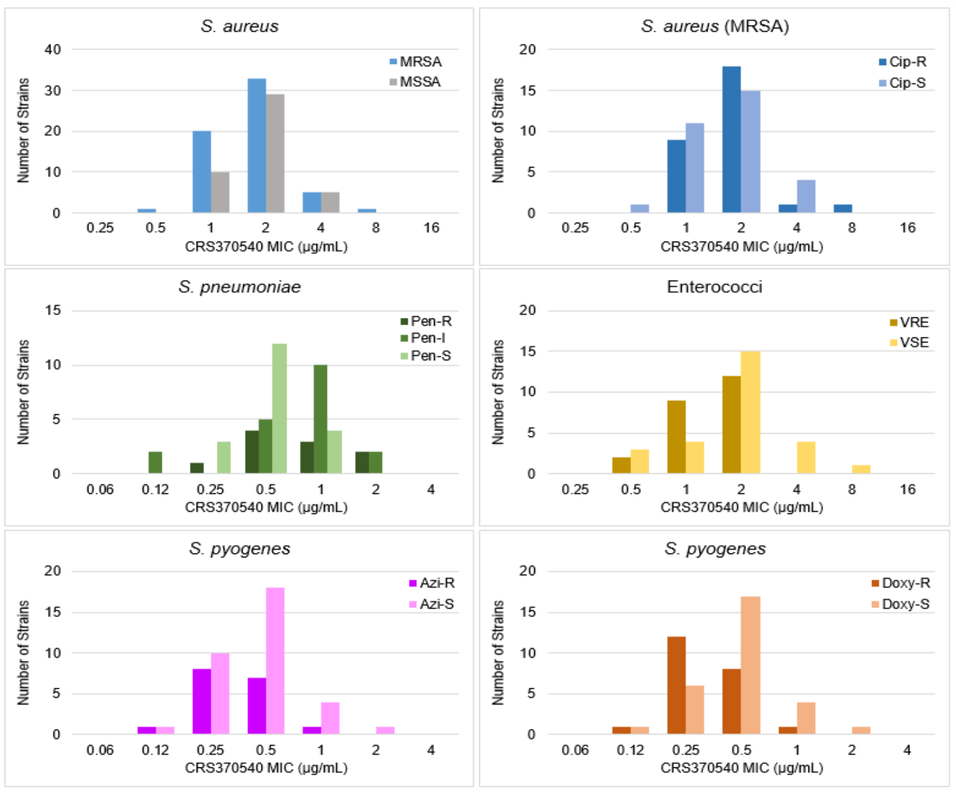
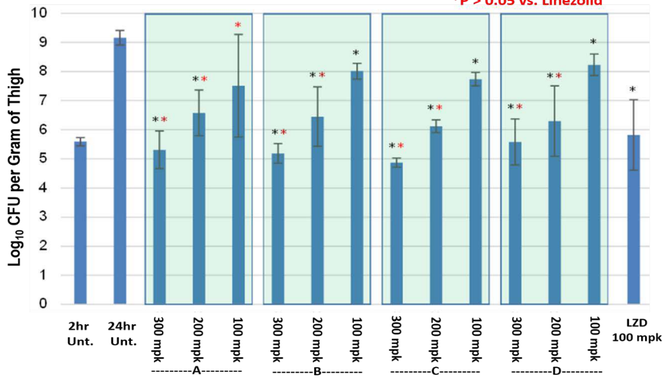


Figure 5. Efficacy of Orally Dosed TZU Compounds in Mouse Models of Infection with Gram-positive Pathogens

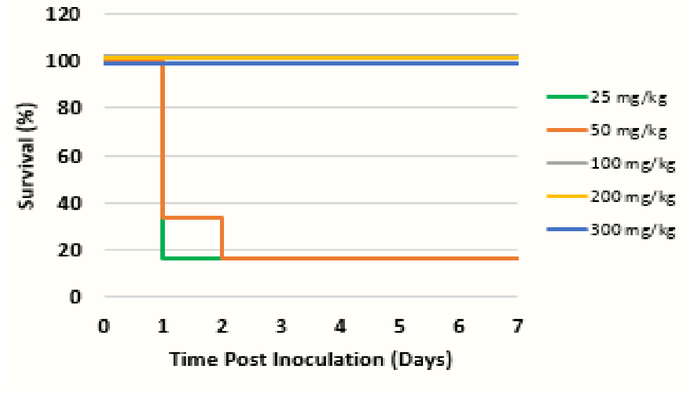
A. MRSA Thigh Infection

BID dosing of TZU compounds A-D at 2 h and 14 h post-infection



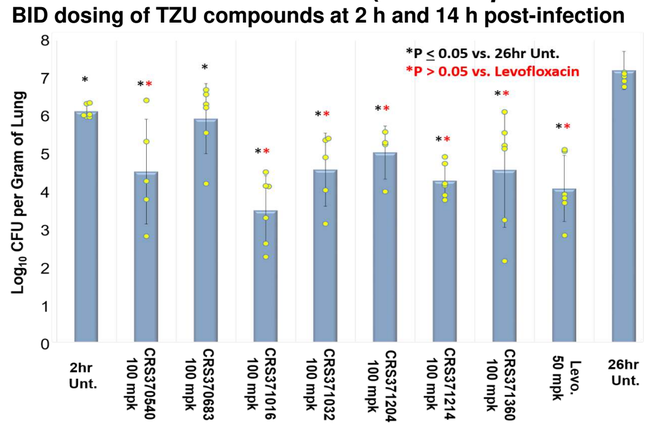
B. MRSA Septicemia

BID dosing of CRS370540 at 1 h and 5 h post-infection



C. Pneumococcal Pneumonia (Azi-R *S. pneumoniae*)

BID dosing of TZU compounds at 2 h and 14 h post-infection



D. Inhalation Anthrax

BID dosing of CRS370540 24 h post-challenge, for 5 days