An Orally Active Antibiotic that Targets the Cell Division Protein FtsZ

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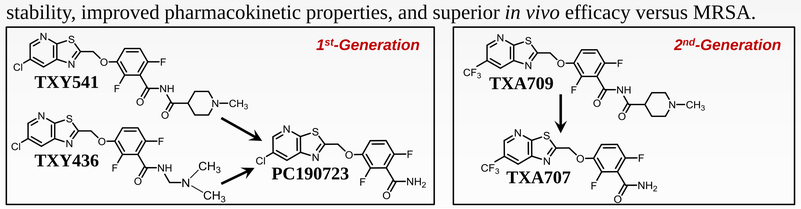
INTRODUCTION

Current standard-of-care (SOC) drugs for the treatment of MRSA infections are limited to a few drugs, which include vancomycin, daptomycin, and linezolid (1). The essential cell division protein FtsZ has been identified as an appealing new target for the development of antibiotics that can be used to treat infections caused by multidrug-resistant (MDR) bacterial pathogens (2). FtsZ self-polymerizes in a GTP-dependent manner to form a ring-like structure (the Z-ring) at midcell that serves as a scaffold for the recruitment and organization of other critical components for proteoglycan synthesis, septum formation, and cell division (3). The substituted benzamide derivative PC190723 is associated with potent bactericidal activity against *Staphylococcus* sp., including MRSA (4). However, the clinical development of PC190723 has been hindered by poor pharmaceutical and pharmacokinetic properties. We have previously reported the design and characterization of 1st-generation prodrugs of PC190723 (TXY436 and TXY541) with physico-chemical properties that significantly enhance the ease of formulation in vehicles suitable for *in vivo* administration (5,6). Here we describe a 2nd-generation prodrug (TXA709) of an FtsZ-targeting benzamide compound (TXA707) with enhanced metabolic stability, improved pharmacokinetic properties, and superior *in vivo* efficacy versus MRSA.



Oral *In Vivo* Efficacy of the 1st-Generation Prodrug TXY541 vs. MSSA and MRSA

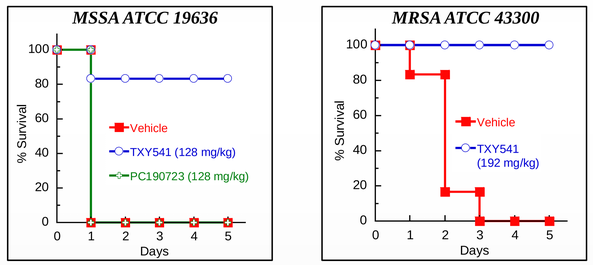


Figure 1. Antistaphylococcal efficacy was assessed in a mouse peritonitis model of systemic infection. All compound and vehicle administrations were by gavage. The vehicle was 10 mM citrate in all experiments.

•Although efficacious orally, doses of TXY541 required for efficacy are high (128 mg/kg vs. MSSA and 192 mg/kg vs. MRSA).

ADME/PK Results for TXY541 and Its Hydrolysis Product PC190723

Pharmacokinetic Parameters of PC190723 Following a Single Intravenous (i.v.) or Peroral (p.o.) Administration of TXY541 to Male BALB/c Mice

Route	Dose (mg/kg)	t_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h•ng/mL)	$t_{½}$ (h)	CL (mL/min/kg)	V_d (L/kg)	%F
i.v.	24	0.25	6646	7216	**0.56**	**55.11**	2.18	NA
p.o.	32	0.25	2263	2848	3016	NA	NA	**30**

• PC190723 is eliminated rapidly (low $t_{½}$, high CL), and is associated with a modest oral bioavailability (%F).

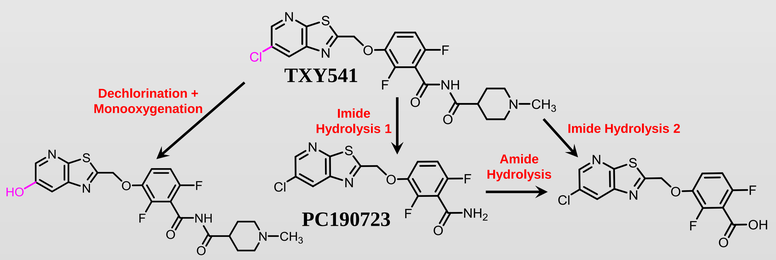


Figure 2. Structures of the prodrug TXY541 and its metabolites observed in the presence of mouse and human hepatocytes.

•The Cl group on the pyridyl ring is susceptible to metabolism.

In Vivo Efficacy of the 2nd-Generation Prodrug TXA709 vs. MSSA and MRSA

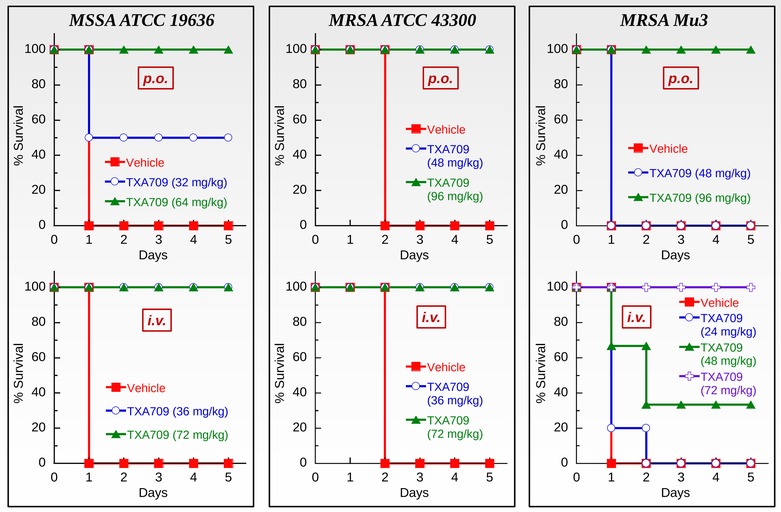


Figure 3. Antistaphylococcal efficacy was assessed in a mouse peritonitis model of systemic infection. All oral (p.o.) and intravenous (i.v.) administrations were by gavage and tail vein injection, respectively. The vehicle was 10 mM citrate in all experiments.

•TXA709 is associated with enhanced (2- to 4-fold greater) *in vivo* efficacy vs. both MSSA and MRSA compared to TXY541.

PK Results for TXA709 and Its Hydrolysis Product TXA707

Pharmacokinetic Parameters of TXA707 Following a Single Intravenous (i.v.) or Peroral (p.o.) Administration of TXA709 to Male BALB/c Mice

Route	Dose (mg/kg)	t_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h•ng/mL)	$t_{½}$ (h)	CL (mL/min/kg)	V_d (L/kg)	%F
i.v.	24	0.50	13794	42299	**3.65**	**9.40**	**2.02**	NA
p.o.	32	1.00	9850	53679	2.66	NA	NA	**95**

• TXA707 is eliminated ~6-times less rapidly and is associated with superior oral bioavailability relative to PC190723.

• The volume of distribution (V_d) of TXA707 is ~3-times greater than that of normal body water in the mouse (0.7 L/kg), indicating that the compound distributes well into the tissue.

Bactericidal Activity vs. Clinical Isolates of MRSA, VISA, VRSA, DNSSA, and LNSSA

Activities of TXA707 and TXA709 vs. Clinical MRSA, VISA, VRSA, DNSSA, and LNSSA Isolates

Agent	*MRSA (n = 20)*		*VISA (n = 20)*		*VRSA (n = 13)*		*DNSSA (n = 7)*		*LNSSA (n = 6)*	
	MIC Range (µg/mL)	MBC Range (µg/mL)	MIC Range (µg/mL)	MBC Range (µg/mL)	MIC Range (µg/mL)	MBC Range (µg/mL)	MIC Range (µg/mL)	MBC Range (µg/mL)	MIC Range (µg/mL)	MBC Range (µg/mL)
TXA707	1	1	0.5-2	0.5-2	0.5-1	1	0.5-1	1-2	1	1-2
TXA709	2	2	2-4	2-4	2	2	2-4	2-4	2	2-4
Vancomycin	0.5-1	1	4-8	4-8	32->64	64->64	1-2	2	1-2	1-2
Daptomycin	0.5-1	0.5	1-8	1-16	0.25-1	0.25-1	4	4-8	0.5-1	0.5-1
Linezolid	2-4	2	0.5-2	2->8	0.5-4	8->8	1-2	2->8	16-64	32->64

VISA = Vancomycin Intermediate-resistant *S. aureus*; VRSA = Vancomycin Resistant *S. aureus*; DNSSA = Daptomycin Non-Sensitive *S. aureus*; LNSSA = Linezolid Non-Sensitive *S. aureus*.

•TXA707 maintains potent bactericidal activity vs. *S. aureus* isolates resistant to current SOC drugs.

Validation of the Cell Division Protein FtsZ as the Bactericidal Target of TXA707

TXA707 Disrupts the Polymerization Dynamics of *S. aureus* FtsZ

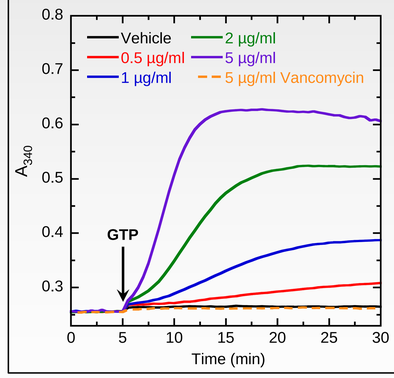


Figure 4. Concentration dependence of the impact of TXA707 on the polymerization of *S. aureus* FtsZ, as determined by monitoring time-dependent changes in absorbance at 340 nm (A_{340}) at 25 °C. Polymerization profiles were acquired in the presence of DMSO vehicle or the indicated concentrations of TXA707. Vancomycin was included as a negative (non-FtsZ-targeting) control. Polymerization reactions were initiated by addition of GTP at the time indicated by the arrow.

•TXA707 induces the overstimulation of FtsZ polymerization, with the extent of this stimulation being dependent on TXA707 concentration.

TXA707 Disrupts FtsZ Z-Ring Formation in Bacteria

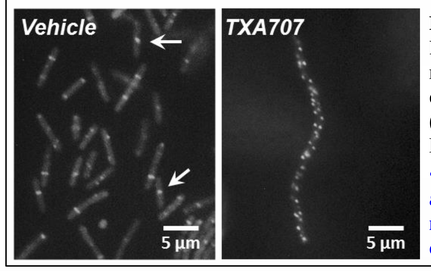


Figure 5. Fluorescence micrographs of *B. subtilis* FG347 bacteria that express a GFP-tagged, FtsZ Z-ring marker protein (ZapA). The bacteria were cultured for 2 hours in the presence of DMSO vehicle (left) or 4 µg/ml TXA707 (right). The arrows in in the left panel highlight FtsZ Z-rings at midcell.

•TXA707 induces a filamentous phenotype, as well as mislocalization of FtsZ from the septal Z-ring at midcell to multiple punctate sites throughout the cell.

TXA707 Disrupts Septum Formation, Inhibits Cell Division, and Induces Cell Death in *S. aureus*

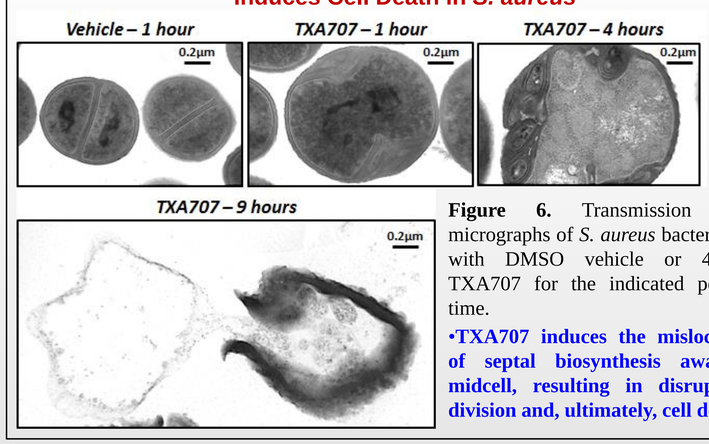


Figure 6. Transmission electron micrographs of *S. aureus* bacteria treated with DMSO vehicle or 4 µg/mL TXA707 for the indicated periods of time.

•TXA707 induces the mislocalization of septal biosynthesis away from midcell, resulting in disrupted cell division and, ultimately, cell death.

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