Supporting Information

Copper Influences the Antibacterial Outcomes of a β-Lactamase-Activated Prochelator against Drug-Resistant Bacteria

Jacqueline M. Zaengle-Barone,[1] Abigail C. Jackson,[1] David M. Besse,[1] Bradford Becken,[2] Mehreen Arshad,[2] Patrick Seed,[3] Katherine J. Franz[1]*

[1] Department of Chemistry, Duke University, Durham, North Carolina 27708, United States

[2] Department of Pediatrics, Duke University, Durham, North Carolina 27708, United States

[3] Ann and Robert H. Lurie Children's Hospital and Stanley Manne Children's Research Institute, Chicago, Illinois 60611, United States

[4] Northwestern University, Chicago, Illinois 60611, United States

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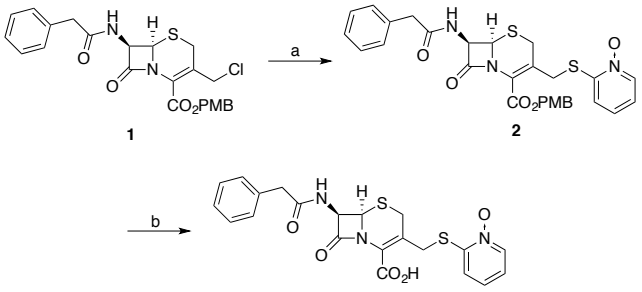
Table S1. Minimum inhibitory concentrations (MICs) in μg/mL of PcephPT, PT, cephalothin, and ceftriaxone against *E. coli* MG1655 and *E. coli* UTI89 expressing different β-lactamases.

Strain	β-lactamase	PcephPT (μg/mL)			Pyrithione (μg/mL)			Cephalothin (μg/mL)	Ceftriaxone (μg/mL)
		compound only	+ 10 μM $CuCl_2$	+ 2 mM BCS	compound only	+ 10 μM $CuCl_2$	+ 2 mM BCS	compound only	compound only
MG1655	null	32	32	16	2-4	2-4	16-32	8-16	<1
	OXA-1	32	32	32	4-8	2-4	>16	16-32	<1
	TEM-1	16	32	>64	4-8	2-4	16-32	>32	<1
	CTX-M-1	8	4	>64	2-4	1-2	>16	>32	>64
	CMY-2	8	4	>64	2-4	1-2	>16	>32	>64
	NDM-1	8	8	>64	2-4	2-4	>16	>32	32-64
UTI89	null	16	16	16	4-8	2-4	>16	8-16	<1
	OXA-1	16	32	32	4-8	4-8	>16	8-16	<1
	TEM-1	32	64	>64	4-8	4-8	>16	>32	<1
	CTX-M-1	8	8	>64	4-8	4-8	>16	>32	>64
	CMY-2	8	8	>64	2-4	2-4	>16	>32	>64
	NDM-1	8	8	>64	4-8	4-8	>16	>32	>64

Table S2. Minimum inhibitory concentrations (MICs) in μg/mL of PcephPT, PT, cephalothin, and ceftriaxone against *E. coli* MG1655 *copA* knockout, LEM8, expressing different β-lactamases.

Strain	β-lactamase	PcephPT (μg/mL)			Pyrithione (μg/mL)			Cephalothin (μg/mL)	Ceftriaxone (μg/mL)
		compound only	+ 10 μM $CuCl_2$	+ 2 mM BCS	compound only	+ 10 μM $CuCl_2$	+ 2 mM BCS	compound only	compound only
MG1655 LEM8 copAΔ	null	16	16	32	2-4	0.5-1	>16	16-32	<1
	TEM-1	16	4	>64	2-4	0.5-1	>16	>32	<1
	CTX-M-1	4	1	>64	2-4	0.5-1	>16	>32	>64
	CMY-2	8	2	>64	2-4	0.5-1	>16	>32	>64
	NDM-1	8	2	>64	2-4	0.5-1	>16	>32	32-64



Scheme S1. Synthesis of PcephPT. (a) 2-mercaptopyridine N-oxide, $KOSiMe_3$, MeCN, rt (b) TFA, PhOH, 45 - 50 °C.

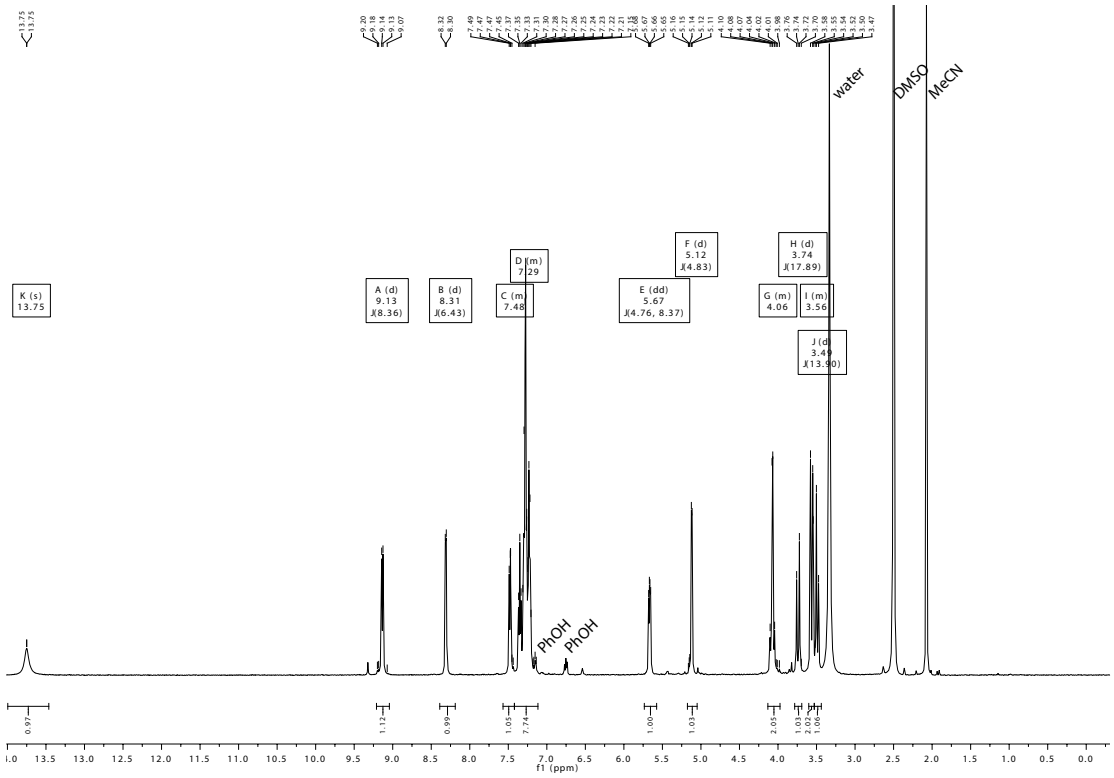


Figure S1. ^{1}H NMR Spectrum of PcephPT in DMSO-d$_6$.

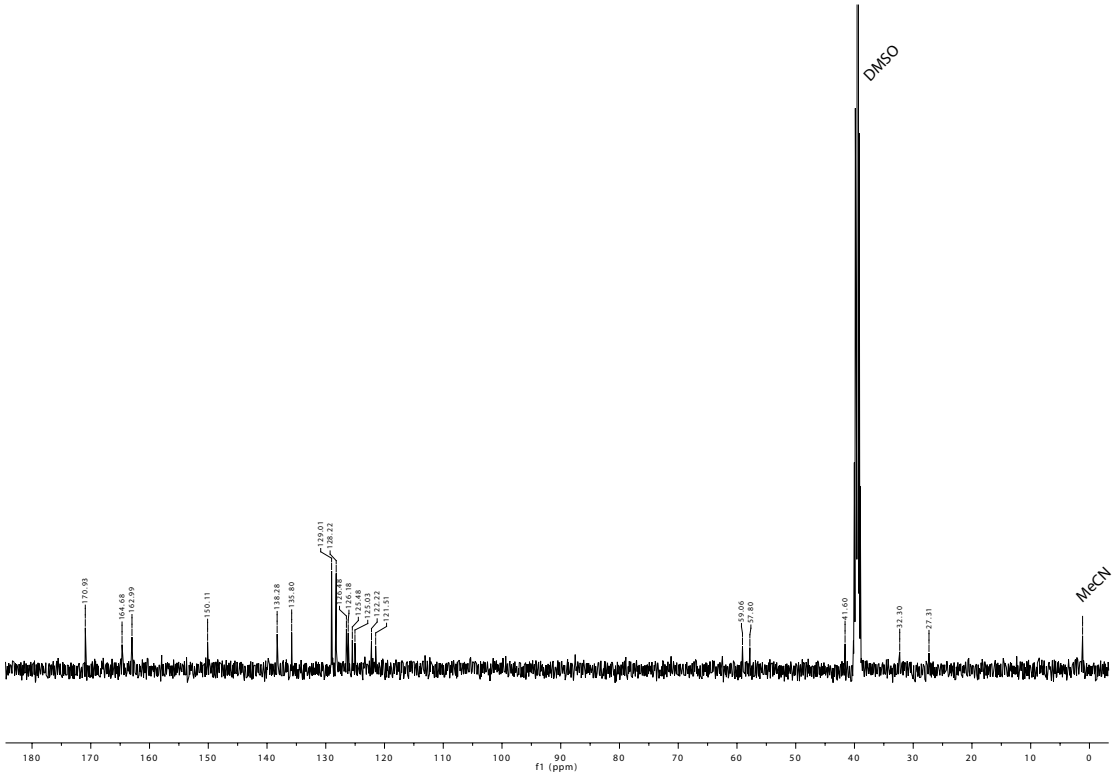


Figure S2. ^{13}C NMR Spectrum of PcephPT in DMSO-d$_6$.

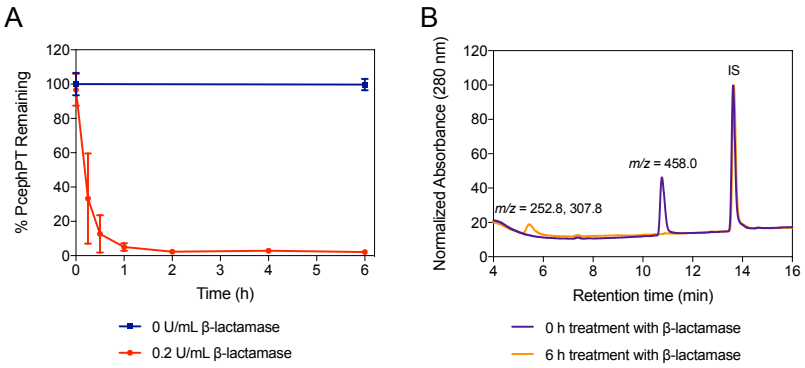


Figure S3. LC-MS detection of turnover of PcephPT by β-lactamase. (A) Timecourse of PcephPT cleavage, determined by integration of UV chromatogram peaks at 280 nm relative to internal standard (IS). (B) Sample chromatograms for PcephPT treated with β-lactamase (U/mL based on benzylpenicillin hydrolysis). Ion masses detected by ESI-MS are shown. PcephPT: calcd. for $[C_{21}H_{19}N_3O_5S_2+H]^+$ 458.1; PT_2 (disulfide) calcd. for $C_{10}H_8N_2O_2S_2+H]^+$ 253.0; $FePT_2$ calcd. for $C_{10}H_8FeN_2O_2S_2]^+$ 307.9. the 2:1 complex of PT with Fe(III) results from extracting trace Fe during LC analysis. The other expected product of enzymatic cleavage was not detected above background noise in the UV or total ion chromatograms. We suspect the initial hydrolysis product is unstable, perhaps degrading into species that elute with the solvent front, where sensitivity is low. The appearance of PT is not easily quantified due to its tendency to tautomerize, become oxidized, or chelate metals in the course of LC-MS analysis.

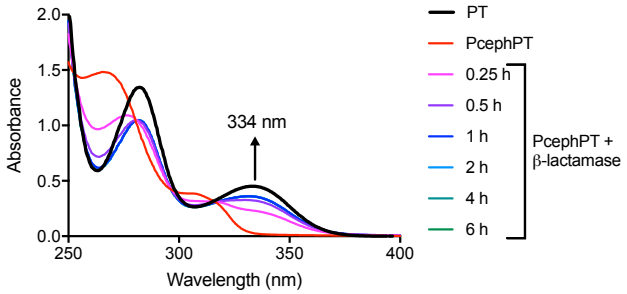


Figure S4. UV/Vis detection of turnover of PcephPT by β-lactamase. The UV/Vis spectrum of PcephPT (100 μM) in the presence of β-lactamase (0.2 U/mL) becomes similar to that of pyrithione (100 μM, black line) over time. The spectrum of PcephPT alone is also shown (100 μM, red line). The increase in absorbance at 334 nm relative to the absorbance of PT at this wavelength was used to determine that 75 μM of detectable PT was released. A separate experiment using an acetate-releasing analog of PcephPT confirmed that there is no change in absorbance in this region due to other products of the enzymatic cleavage.

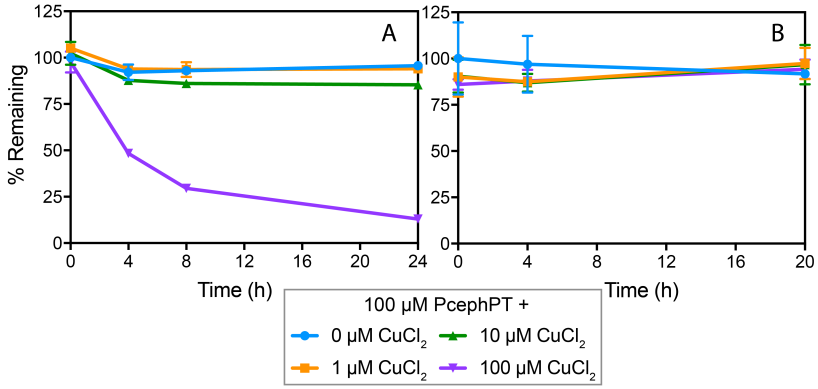


Figure S5. Stability of PcephPT determined by LC-MS. (A) PcephPT degrades in the presence of $CuCl_2$ in unbuffered water. **(B)** PcephPT is stable even at high concentrations of $CuCl_2$ in LB at 37 °C.

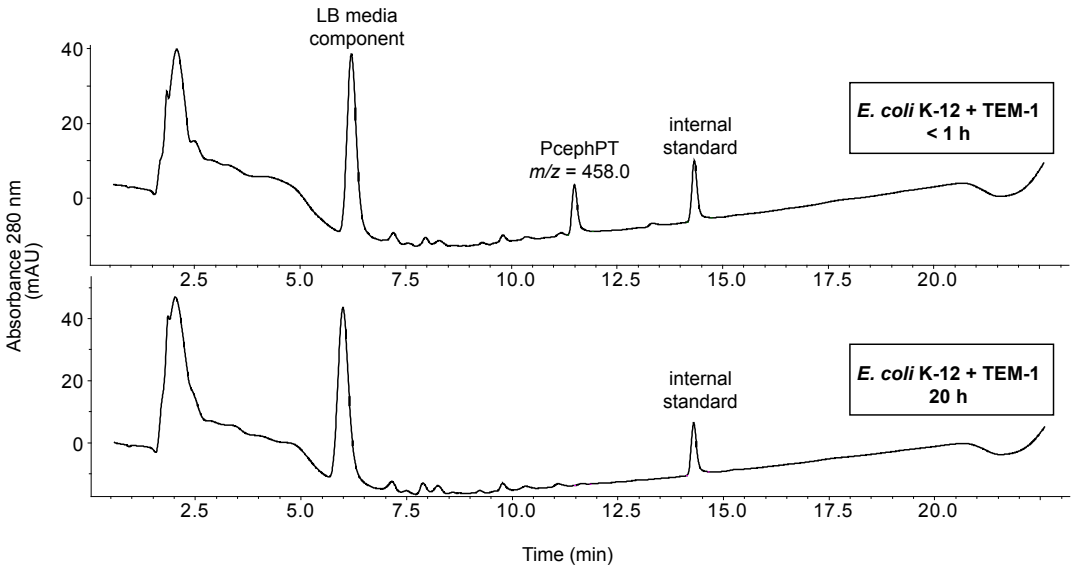


Figure S6. Representative UV chromatograms of PcephPT turnover in bacterial culture. A liquid culture of *E. coli* K-12 MG1655 expressing TEM-1 was treated with 50 μM PcephPT for < 1 h (top) and 20 h (bottom) before extraction and addition of internal standard (Rofecoxib). PT cannot be detected in chromatograms due to overlap with LB media components.

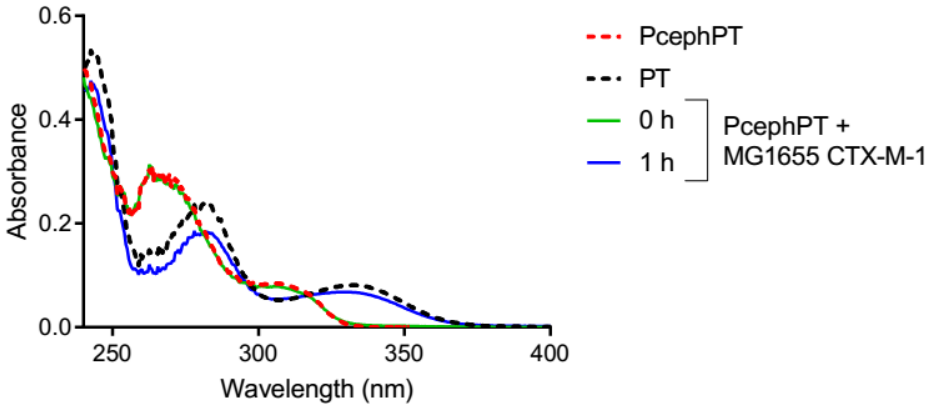


Figure S7. Example of UV/Vis detection of turnover of PcephPT by MG1655 CTX-M-1. The UV/Vis spectrum of PcephPT (20 μM) in the presence of CTX-M-1-expressing MG1655 *E. coli*. PcephPT + MG1655 CTX-M-1 becomes similar to that of pyrithione (20 μM, black dashed line) within an hour. The spectrum of PcephPT alone is also shown (20 μM, red dashed line). Overnight cultures of bacteria were diluted 1:1000 in LB, treated with 200 μM PcephPT, incubated at 37 °C for 1 h, then diluted 1:10 in water prior to acquisition of UV/Vis spectra.

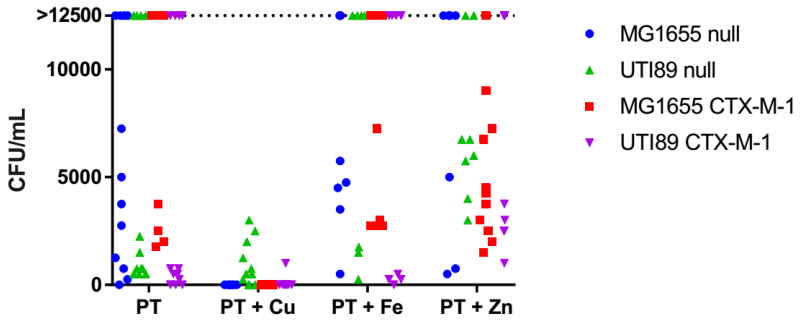


Figure S8. Co-treatment with PT and Cu decreased colony regrowth compared to PT alone, with Fe, or with Zn. Bacteria treated with growth-inhibiting concentrations of PT with and without supplemental $CuCl_2$, $FeCl_3$, or $ZnCl_2$ (10 μM) in liquid medium for 20 h were plated on fresh agar and incubated at 37 °C for 24 h prior to enumeration of colonies. Each point on the graph is an individual replicate. 0 h CFU/mL: values: MG1655 null = 5900; UTI89 null = 97000; MG1655 CTX-M-1 = 35000; UTI89 CTX-M-1 = 40000. CFUs above 12500 were not enumerated and are grouped collectively on the plot as >12500.

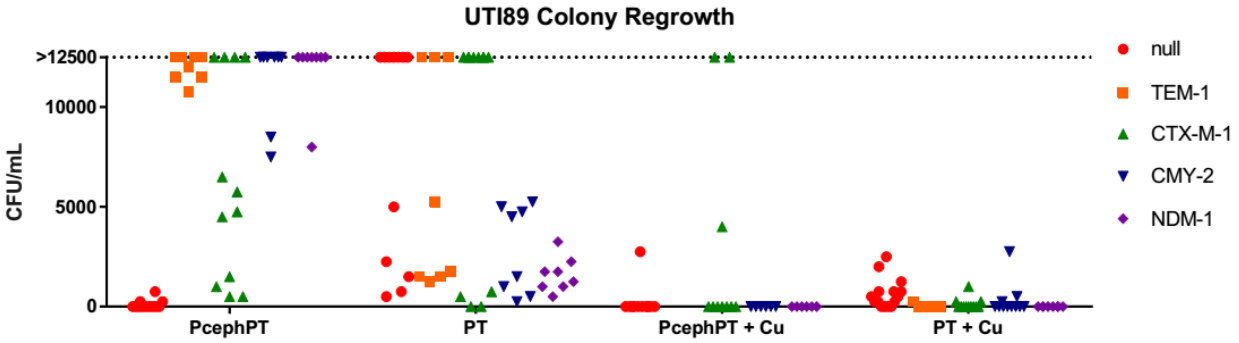


Figure S9. Ability of PcephPT to kill UTI89 bacteria depends on both the presence of β-lactamase and availability of Cu. Bacteria treated with growth-inhibiting concentrations of PcephPT or PT with and without supplemental $CuCl_2$ (10 μM) in liquid medium for 20 h were plated on fresh agar and incubated at 37 °C for 24 h prior to enumeration of colonies. Each point on the graph is an individual replicate. Conditions with MIC > 70 μM were not plotted (TEM-1 PcephPT + Cu). 0 h CFU/mL values: null = 97000, TEM-1 = 39000, CTX-M-1 = 40000, CMY-2 = 170000, NDM-1 = 210000. CFUs above 12,500 were not enumerated and are grouped collectively on the plot as >12500.

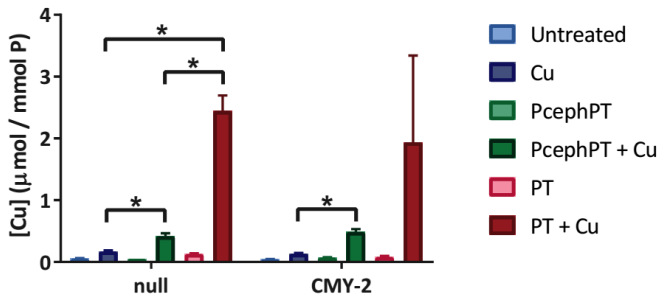


Figure S10. Cell-associated Cu in MG1655 null and CMY-2 strains after 2 h treatment determined by ICP-MS. Cu concentration was normalized to phosphorous content for each sample. Treatment conditions: 4 µM PcephPT, 4 µM PT, 10 µM $CuCl_2$. * indicates $p < 0.05$ using one-tailed Student's t-test.

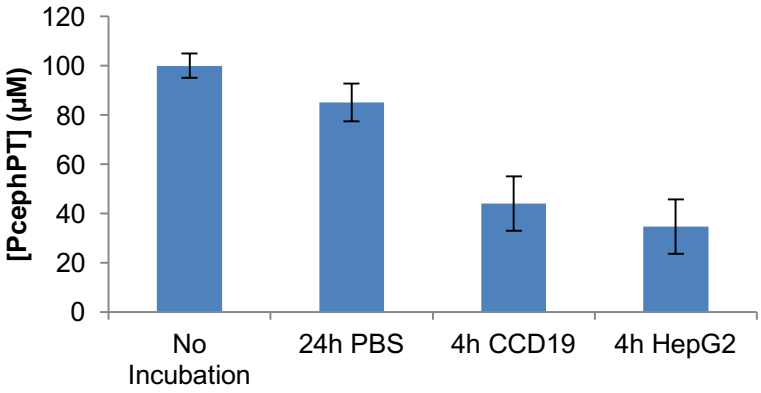


Figure S11. Concentration of PcephPT after incubation with mammalian cells. The integrated area under the LC-MS UV absorbance peak that corresponds to the prochelator was compared to a calibration curve of PcephPT to calculate the concentration of PcephPT present after incubating 100 µM in PBS with CCD-19Lu or HepG2 cells for 4 h.