Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Tables

Title: “Pyrazolopyrimidinones, a novel class of copper-dependent bactericidal antibiotics against multi-drug resistant *S. aureus”*

Authors: Cameron L. Crawford[1], Alex G. Dalecki[1], Whitney T. Narmore[1], Jessica Hoff[1], Audra A. Hargett[2], Matthew B. Renfrow[2], Man Zhang[3], Madumali Kalubowilage[3], Stefan H. Bossmann[3], Stacy L. Queern[4,5], Suzanne E. Lapi[4,5], Robert N. Hunter III[6], Donghui Bao[6], Corinne E. Augelli-Szafran[6], Olaf Kutsch[1], and Frank Wolschendorf[1,#]

[1]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA
[2]Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA
[3] Department of Chemistry, Kansas State University, Manhattan, KS, USA
[4] Department of Radiology, University of Alabama at Birmingham, Birmingham, AL, USA
[5] Department of Chemistry, Washington University in St. Louis
[6] Department of Chemistry, Drug Discovery Division, Southern Research, Birmingham, AL, USA

Table S1: Antibiotic resistance profile of MRSA strains.

All MRSA strains were tested for antibiotic resistance by UAB Laboratory Medicine. R= resistant and S = sensitive. Amox/Cla = Amoxicillin, Amp/Sul = Ampicillin/sulbactam, Cetri = Ceftriaxone, Cipro = Ciprofloxacin, Clinda = Clindamycin, Dapto = Daptomycin, Eryth = Erythromycin, Gent = Gentamicin, Levo = Levofloxacin, Linez = Linezolid, Ox = Oxacillin, Pen = Penicillin, Quin/Dalf = Quinupristin/dalfopristin, Rif = Rifampicin, SXT = Sulfamethaxole/Trimethoprim, Tetra = Tetracycline, and Vanc = Vancomycin.

ABX	MRSA-1	MRSA-2	MRSA-3	MRSA-4	MRSA-5
Amox/Cla	R	R	R	R	R
Amp/Sul	R	R	R	R	R
Cetri	R	R	R	R	R
Cipro	S	R	S	S	R
Clinda	R	R	S	S	R
Dapto	S	S	S	S	S
Eryth	R	R	R	R	R
Gent	S	S	S	S	S
Levo	S	R	S	S	R
Linez	S	S	S	S	S
Ox	R	R	R	R	R
Pen	R	R	R	R	R
Quin/Dalf	S	S	S	S	S
SXT	S	S	S	S	S
Tetra	R	S	S	S	S
Vanc	S	S	S	S	S

Table S2: Drug-like properties of PZPs.

MW: molecular weight in g/mol; cLogP: $\log_{10}$ of Octanol:water partition coefficient; LogSW: $\log_{10}$ of intrinsic water solubility; RB: rotatable bonds; tPSA: topological polar surface area; hDon: proton donating atoms; hAcc: proton accepting atoms. All values were provided by the supplier (www.hit2lead.com).

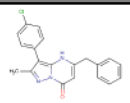
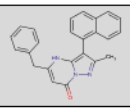
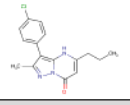
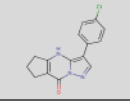
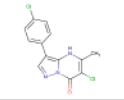
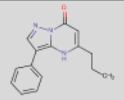
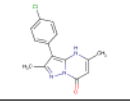
PZP	Structure	MW	Mol. Formula	cLogP	LogSW	RB	tPSA	hDon	hAcc
915		349.8	C20H16ClN3O	3.85	-5.039	3	50.16	1	3
716		365.4	C24H19N3O	4.31	-5.545	3	50.16	1	3
720		301.8	C16H16ClN3O	3.34	-4.253	1	50.16	1	3
894		285.7	C15H12ClN3O	2.82	-3.692	1	50.16	1	3
832		294.1	C13H9Cl2N3O	3.04	-3.942	1	50.16	1	3
284		253.3	C15H15N3O	2.65	-3.311	1	50.16	1	3
643		273.7	C14H12ClN3O	2.28	-3.144	1	50.16	1	3

Table S3: ADME properties of PZP 915. Half-life of 1 μM 915 or 1 μM 915 + 0.5 μM Cu was assessed in human liver microsomes. Solubility of 915 was determined in ddH_2O. Distribution coefficient (LogD) was determined experimentally.

ID	Half-life (min)	Solubility (μM)	Sol RSD%	LogD
915	55.9	38	1.9	>3.70
915+Cu	52.6	44.4	6.4	>3.53

Table S4: Binding constants for $M(PZP\ 915)_3^{2+}$ complexes. Counter anions: a) bromide, b) sulfate, c) chloride

915 metal complexes	Binding constant	Units
$Cu(PZP915)_3^{2+\ a)}$	$1.12 \times 10^8 \pm 0.02 \times 10^8$	L^3mol^{-3}
$Zn(PZP915)_3^{2+b)}$	$6.6 \times 10^8 \pm 0.3 \times 10^8$	L^3mol^{-3}
$Fe(PZP915)_3^{2+c)}$	$5.3 \times 10^8 \pm 0.3 \times 10^8$	L^3mol^{-3}
$Cu(PZP915)_2^{+\ a)}$	$2.35 \times 10^6 \pm 0.9 \times 10^5$	L^2mol^{-2}
$Cu(PZP915)^{+\ a)}$	$3.80 \times 10^5 \pm 0.4 \times 10^4$	$L\ mol^{-1}$

Table S5: Binding constants for the individual binding reactions. Binding constants were calculated based on the assumption of a stepwise addition of ligands.

915 Binding Reactions	Binding Constants	Units
K1 = Cu+PZP915	3.80×10^5	$Lmol^{-1}$
K2 = Cu(PZP915)+PZP915	6.18	$Lmol^{-1}$
K3 = $Cu(PZP915)_2$+PZP915	47.65	$Lmol^{-1}$