Elucidating the Antimicrobial Activity, Virulence, and Resistance Mechanisms of Pentabromophenol on *Staphylococcus aureus*.

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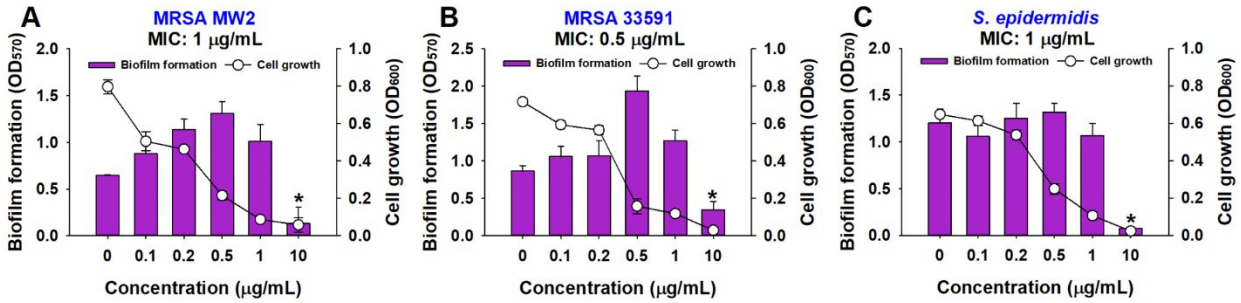
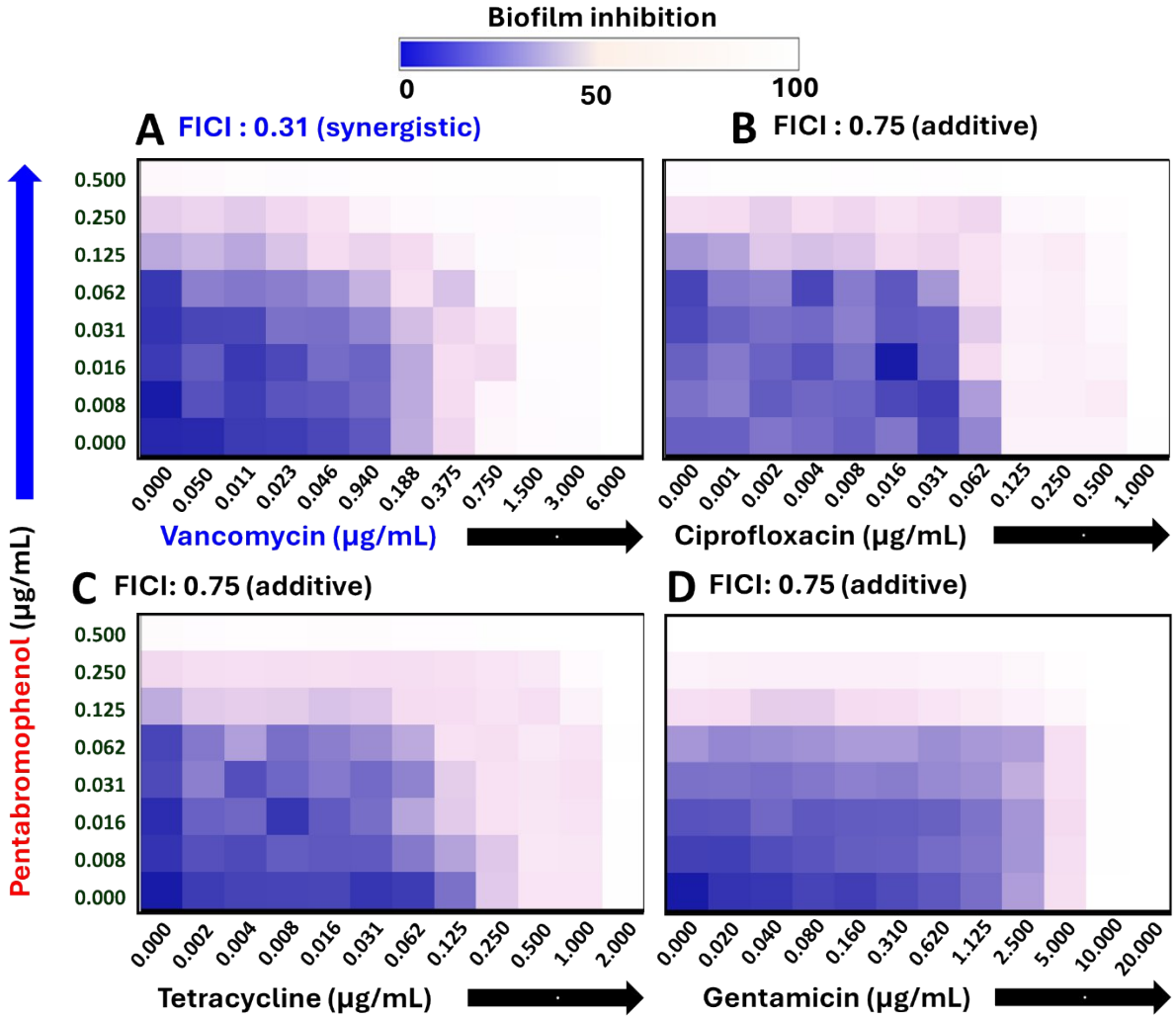
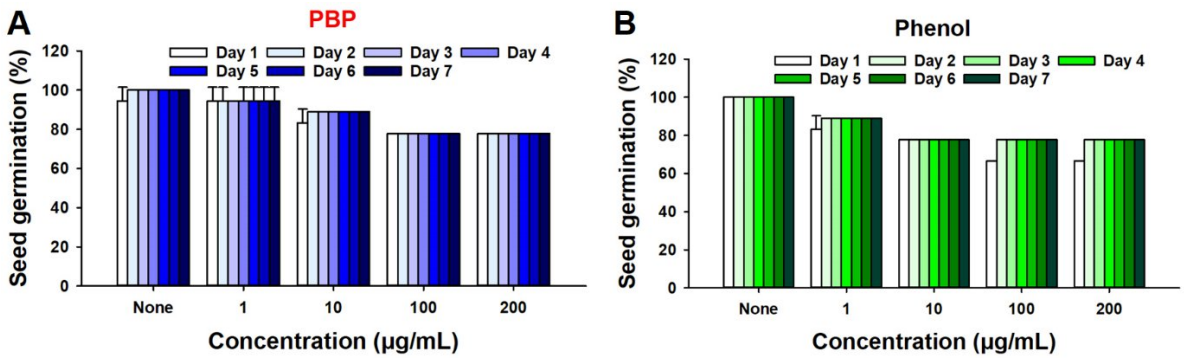
Keywords: antimicrobial; antibiofilm; halogenation; pentabromophenol; *Staphylococcus aureus*



Supplementary Figure S1: The antibiofilm activity and planktonic cell growth inhibition of pentabromophenol (PBP) at (0.1, 0.2, 0.5, 1, or 10 μg/mL) against other clinical strains of *S. aureus*, MRSA MW2 (a), MRSA 33591 (b), and *S. epidermidis*(c).



Supplementary Figure S2: Checkerboard assay heatmaps showing various interactions of pentabromophenol (PBP) with vancomycin (a), ciprofloxacin (b), tetracycline (c), and gentamicin (d) against *S. aureus* ATCC 6538. The color scale indicates OD600-based growth inhibition transitioning from deep blue (lowest inhibition) to white (highest inhibition). Results reflect two-fold MIC shifts and combinatorial efficacy, quantified by the fractional inhibitory concentration index (FICI).



Supplementary Figure S3: Seed germination experiment showing germination percentage of radish plant (*Raphanus sativus*) treated with pentabromophenol (PBP) (a) and phenol (b) at 1, 10, 100, and 200 µg/mL for 7 days.

Supplementary Table S1: Different combination strategies of pentabromophenol (PBP) with selected antibiotics: vancomycin (VAN), ciprofloxacin CIP), tetracycline (TET), and gentamicin (GEN) against *S. aureus* quantified by fractional inhibitory concentration index (FICI) and the degree of potentiation (Fold MIC Reduction).

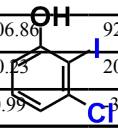
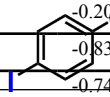
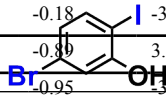
Combinations (Biofilm Formation)	MIC Drug A/ Drug B (µg/mL)	MIC Drug Combination (µg/mL)	FICI	Interaction	Degree of Potentiation (Fold MIC Reduction)	
					PBP	Antibiotics
PBP-VAN	0.5/6	0.031/1.5	0.312	Synergistic	16	4
PBP-CIP	0.5/1	0.125/0.5	0.750	additive	4	2
PBP-TET	0.5/2	0.125/1	0.75	additive	4	2
PBP-GEN	0.5/20	0.25/5	0.75	additive	2	4
Combinations (Planktonic Cell Growth)	**MIC Drug A/ Drug B (µg/mL)**	**MIC Drug Combination (µg/mL)**	**FICI**	**Interaction**	**Degree of Potentiation (Fold MIC Reduction)**	
					PBP	**Antibiotics**
PBP-VAN	0.5/6	0.031/1.5	0.312	Synergistic	16	4
PBP-CIP	0.5/1	0.125/0.5	0.750	additive	4	2
PBP-TET	0.5/2	0.125/1	0.750	additive	4	2
PBP-GEN	0.5/20	0.125/10	0.75	additive	4	2

Supplementary Table S2: The primer sequences of various *S. aureus* genes used for qRT-PCR experiment.

Gene	Name	Primer
16S rRNA	A component of ribosomes	Forward 5'-TGT TTG ACG ATG TTT GAG CA-3' Reverse 5'-CCT TCC TCC AGT TCA GAT GC -3'
agrA	Quorum-sensing regulator A	Forward 5'-TGA TAA TCC TTA TGA GGT GCT T-3' Reverse 5'-CAC TGT GAC TCG TAA CGA AAA-3'
arlR	Response regulator	Forward 5'-TTA CGG TGC AGG CGA TTA TAT AG-3' Reverse 5' TAC CGT TGA CAT CGA TAA TAT CC 3'
arlS	Histidine-protein kinase	Forward 5'-TGG AAT ACC AAT TCC ATC ATC T-3' Reverse 5'-TGC AAT CAA ATA TGA TGT GAA GAA-3'
hla	α Hemolysin Cytotoxin	Forward 5' CGG CAC ATT TGC ACC AAT AAG GC 3' Reverse 5'-GGT TTA GCC TGG CCT TCA GC-3'
sarA	Transcriptional regulator	Forward 5'-GAG TTG TTA TCA ATG GTC-3' Reverse 5'-GTT TGC TTC AGT GAT TCG-3'
nuc1	Thermonuclease (Immune evasion)	Forward 5'-CAC CTG AAA CAA AGC ATC CTA A-3' Reverse 5'-TAT ACG CTA AGC CAC GTC CAT-3'
psmα	Phenol-soluble modulins α	Forward 5'-ACC CAT GTG AAA GAC CTC CTT TGT-3' Reverse 5'- ATG GGT ATC ATC GCT GGC ATC-3'
sigB	RNA Polymerase sigma factor	Forward 5'-AAC TGA TTC GTA AGG ACG TCT-3' Reverse 5'-TCG ATA ACT ATA ACC AAA GCC T-3'

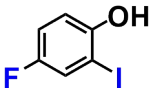
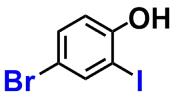
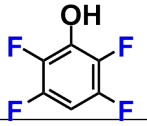
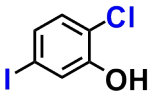
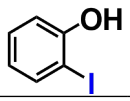
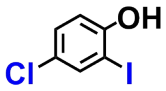
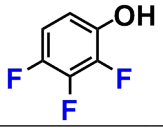
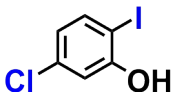
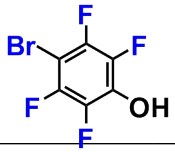
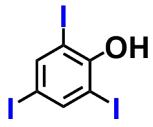
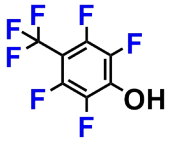
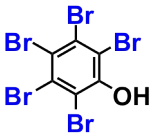
Properties	PBP	PCP	PPP	PHP	2,4,6-TIP	TePP	2,4,6-TBNP	TrFP	Phenol
Lipinski rule of five	Suitable	Suitable	Suitable	Suitable	Suitable	Suitable	Suitable	Suitable	Suitable
Lipinski rule of five violations	1	1	0	1	0	0	0	0	0
Ghose violation	2	1	2	2	1	3	1	3	3
Veber (GSK) violation	0	0	0	0	0	0	0	0	0
Muegge (Bayer) violation	2	0	1	2	1	2	1	2	2
Bioavailability Score	0.55	0.55	0.55	0.17	0.17	0.55	0.55	0.55	1.4
Lipophilicity ($Log P_{o/w}$)	5.2	4.82	3.19	4.76	0.6	2.69	2.75	2.38	0.5
P-glycoprotein inhibition (Pgp_inhibition)	Inhibitor	Inhibitor	Non	Inhibitor	Inhibitor	Non	Inhibitor	Non	Non
Plasma protein binding	100	100	100	100	44.6	100	87.04	-1	89.7
Buffer_solubility (mg/L)	8933000	399147000	309579	2654280	533.1	328105	1801	5803	6123.6
Blood-brain barrier permeability LogBB Score	Yes (12.5)	Yes (11.52)	Yes (4.19)	Yes (10.4)	Yes (6.6)	Yes (3.57)	Yes (0.91)	Yes (1.5)	Yes (1.7)
Skin permeability	-0.8	-1.1	-2.1	-1.27	-1.8	-2.08	-1.5	-1.9	-2.4
Human intestinal absorption (HIA)	High (100)	High (100)	High (100)	High (97.63)	High (99.8)	High (100)	High (96.1)	High (100)	High (100)
Lead_like_Rule_Violations	2	1	0	0	2	0	2	0	1

Caco2	49.5	46.07	46.07	50.12	53.9	31.80	7.5	22.3	21.7
Binding to Tox target: Prostaglandin synthase PGH1,	No binding to PGH1	No binding to PGH1	No binding to PGH1	No binding to PGH1	No binding to PGH1	No binding to PGH1	No binding to PGH1	No binding to PGH1	Most probable binding to PGH1
Acute algae toxicity	0.004	0.006	0.03	0.007	0.02	0.04	0.02	0.06	0.1
Acute fish toxicity (medaka)	0.0004	0.0003	0.10	0.0000003	0.0001	0.15	0.005	0.22	0.7
Acute fish toxicity (minnow)	0.0001	0.00006	0.008	0.000002	0.0003	0.016	0.002	0.03	0.180
In vitro hERG inhibition	low_risk	low_risk	low_risk	low_risk	low_risk	low_risk	low_risk	low_risk	medium_risk
miLogP	5.48	4.82	2.25	6.84	4.82	2.16	3.89	2.06	1.46
Molecular volume	181.49	159.74	116.72	212.01	164.03	111.79	169.05	106.86	92.06
TPSA	20.23	20.23	20.23	20.23	20.23	20.23	66.05	20.23	20.23
GPCR ligand	-1.01	-0.87	-0.82	-0.71	-1.14	-0.88	-1.19	-0.99	-3.47
Ion channel modulator	-0.53	-0.63	-0.18	-0.20	-0.47	-0.24	-0.68	-0.18	-3.16
Kinase inhibitor	-0.89	-1.07	-0.80	-0.83	-1.16	-0.85	-1.02	-0.89	3.51
Nuclear receptor ligand	-1.15	-0.80	-0.72	-0.74	-1.10	-0.83	-1.23	-0.95	-3.25
Protease inhibitor	-1.34	-1.43	-0.78	1.06	-1.59	-0.78	-1.49	-1.01	-3.56
Enzyme inhibitor	-0.42	-0.44	-0.30	-0.32	-0.53	-0.37	-0.57	-0.38	-3.26
Rat IP LD50 classification	Class 5 out AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 3 in AD
Rat IV LD50 classification	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 3 in AD
Rat oral LD50 classification	Class 4 in AD	Class 3 in AD	Class 4 in AD	Class 5 in AD	Class 5 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 3 in AD
Rat SC LD50 classification	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD	Class 3 in AD	Class 4 in AD	Class 3 in AD

No.	Compound	MIC (µg/mL)	Structure	No.	Compound	MIC (µg/mL)	Structure
1	Phenol	> 1000		10	3-chloro-2-iodophenol	100	
2	4-iodophenol	500		11	5-bromo-2-iodophenol	50	

Supplementary Table S3: Predicted Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of PBP, selected halogenated derivatives, and phenol scaffold. Note: PCP: Pentachlorophenol, PFP: Pentafluorophenol, PIP: Pentaiodophenol, 2,4,6- TIP: Triiodophenol, TeFP: Tetrafluorophenol, 2,4,6TBNP: Tribromo-nitrophenol, TrFP: Trifluorophenol.

Supplementary Table S4: Shows the experimentally determined MICs of the selected 17 halogenated compounds with PBP and phenol scaffold used for 3D QSAR analysis.

3	4-fluoro-2-iodophenol	500		12	4-bromo-2-iodophenol	50	
4	2,3,5,6-Tetrafluorophenol	500		13	2-chloro-5-iodophenol	50	
5	2 -iodophenol	400		14	4-chloro-2-iodophenol	50	
6	2,3,4-Trifluorophenol	200		15	5-chloro-2-iodophenol	50	
7	4-Bromotetra -fluorophenol	100		16	2,4,6-Triiodophenol	5	
8	2,3,5,6-Tetrafluoro-4-(trifluoromethyl) phenol	100		17	Pentabromophenol (PBP)	0.5	
9	2,4,6-Tribromo-3-nitrophenol	100