Supporting Information

A New Class of Multi-Armed Antibiotics with Low Risk of Resistance

Yuexiao Jia,[1,4] Wenwen Chen,[2,4] Rongbing Tang,[1,4] Ruihua Dong,[1] Xiaoyan Liu,[1] Fupin Hu[3] and Xingyu Jiang[1]*

[1] Department of Biomedical Engineering, Southern University of Science and Technology, No. 1088 Xueyuan Rd, Nanshan District, Shenzhen, Guangdong 518055, P. R. China

[2] Guangdong Key Laboratory for Biomedical Measurements and Ultrasound Imaging, School of Biomedical Engineering, Shenzhen University Health Science Center, Shenzhen 518055, China

[3] Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai 200040, China

[4] These authors contributed equally to this work

*e-mail: jiang@sustech.edu.cn

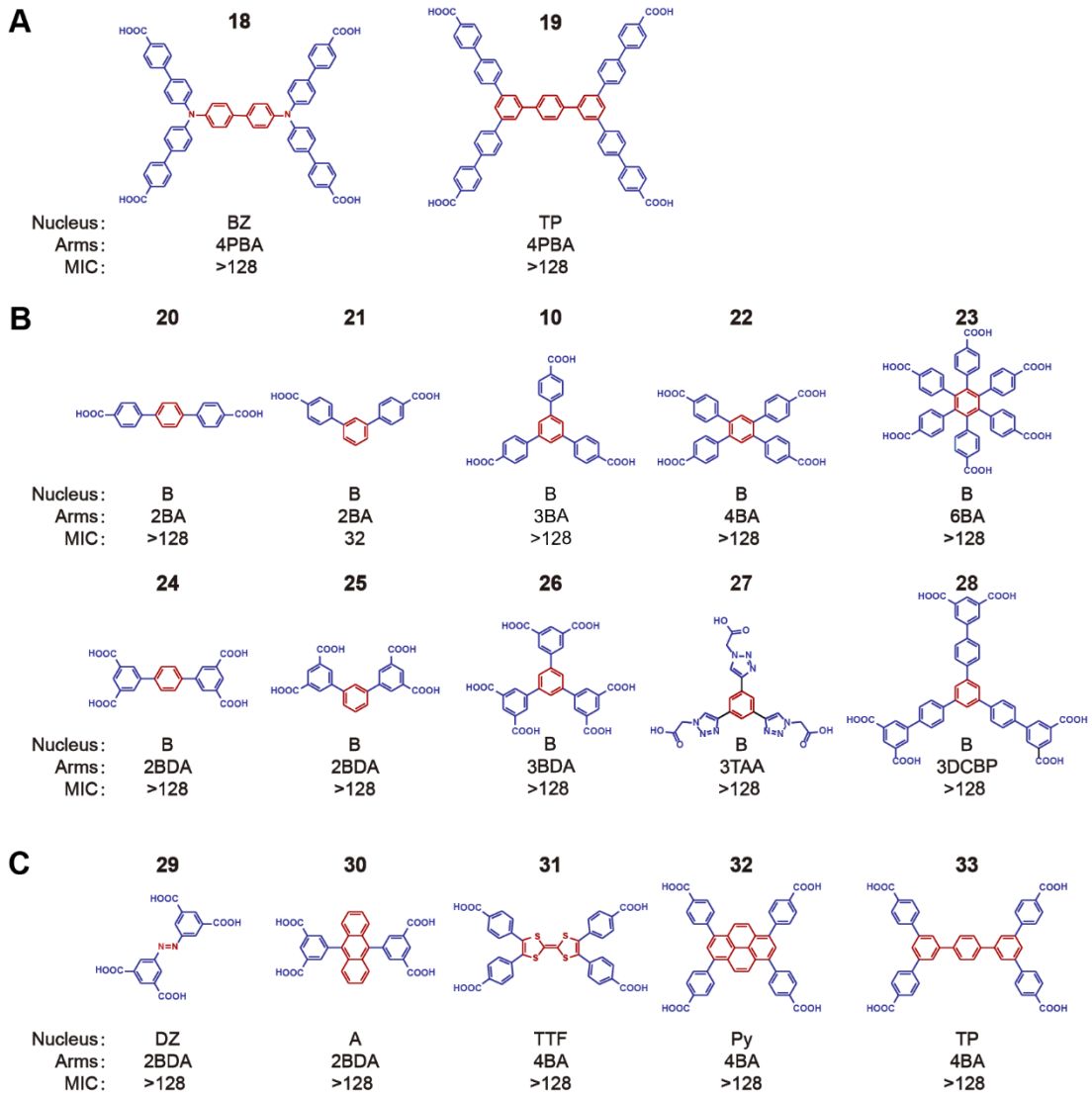

Figure S1 MAMs with different nucleus and arms. **A** MAMs with benzidine (BZ) and p-terphenyl (TP) nucleus and PBA (**18, 19**) arms. **B** MAMs with B nucleus, benzoic acid (BA, **20**-**23**) and m-benzenedicarboxylic acid (BDA, **24**-**26**), 1H-1,2,3-triazol-1-ylacetic acid (TAA, **27**), 1,3-Di(4-carboxyphenyl)benzene (DCPB, **28**) arms, **C** MAMs with diazene (DZ), anthracene (A) nucleus and BDA arms (**29**, **30**), MAMs with tetrathiafulvalene (TTF), pyrene (Py), p-terphenyl (TP) nucleus and BA (**31**-**33**) arms. MIC, minimum inhibitory concentration (μg/mL).

Table S1 Structure analysis, physicochemical properties and antibacterial activities of MAMs.

MAMs	MICs (μg/mL)	Nucleus	Arms	ClogD$_{7.4}$	MW
1	0.06	E	4PBA	-0.07	812.86
2	0.125	C	4PBA	-0.33	800.85
3	0.5	B	4PBA	1.31	862.92
4	>128	P	4PBA	3.79	1097.17
5	0.008	N	3PBA	-0.11	605.63
6	0.06	B	3PBA	1.48	666.72
7	0.25	T	3PBA	1.21	669.68
8	>128	B	6PBA	0.98	1255.32
9	>128	B	3CA	-8.6	210.14
10	>128	B	3BA	-3.47	438.43
11	>128	B	3TBA	6.42	895
12	>128	B	3ABP	9.37	579.73
13	>128	B	3PP	10.94	582.69
14	>128	B	3ABP	11.68	669.68
15	>128	B	3OBA	-3.45	486.43
16	>128	B	3ABA	-2.79	483.47
17	0.06	B	3MBA	-1.77	510.49
18	>128	BZ	4PBA	3.05	969.04
19	>128	TP	4PBA	4.61	1015.11
20	>128	B	2BA	-1.65	318.32
21	32	B	2BA	-1.65	318.32
22	>128	B	4BA	-5.32	558.53
23	>128	B	6BA	-9.04	798.74
24	>128	B	2BDA	-8.87	406.34
25	>128	B	3DCB	-9.11	453.37
26	>128	B	2BDA	-8.87	406.34
27	>128	B	3DCBP	-9.35	798.74
28	>128	B	3BDA	-14.29	570.46
29	>128	DZ	2BDA	-8.37	358.26
30	>128	A	2BDA	-6.9	506.46
31	>128	TTF	4BA	-5.65	684.78
32	>128	Py	4BA	-3.01	682.67
33	>128	TP	4BA	-1.99	710.73

MAM: multi-armed molecule, MW, molecular weight, ClogD$_{7.4}$, the predicted octanol/water distribution coefficient at pH 7.4. MIC, minimum inhibitory concentration (μg/mL) tested via microbroth dilution method using *S. aureus* as a model strain.

Table S2 Antibacterial activities of nucleus and arms of MAAs.

Molecules	Nucleus	Arms	Antibacterial activity
34	E	/	No
35	C	/	No
36	B	/	No
37	N	/	No
38	T	/	No
39	/	PBA	No
40	/	MBA	No

Table S3 Drug susceptibilities of the pathogenic microorganisms.

Organism	Antibiotics MIC (μg/mL)				
	Ampicillin	Oxacillin	cefotaxime	Vancomycin	Linezolid
S. aureus	0.25	8	4	2	1
S. aureus (MRSA)[1]	>128	>128	>128	2	2
S. aureus (MRSA)[2]	>128	>128	>128	1	2
S. epidermidis	0.5	2	4	2	1
S. epidermidis (MRSE)	>128	>128	>128	2	2
S. haemolyticus	0.5	1	4	2	1
S. haemolyticus (MDR)	>128	>128	>128	4	0.5
B. subtilis	1	1	0.5	2	1
Streptococcus pneumoniae	1	1	4	1	1
E. faecium	2	4	4	1	1
E. faecium (MDR)	4	8	>128	1	1
E. faecium (VRE)	>128	>128	>128	>128	1

S. aureus, *Staphylococcus aureus*, MRSA, methicillin-resistant *S. aureus*, MRSA[1] and MRSA[2] are two strains of MRSA, MRSE, methicillin-resistant *S. epidermidis*, MDR, multi-drug resistant, VRE, vancomycin-resistant *Enterococcus*. MIC, minimum inhibitory concentration (μg/mL).

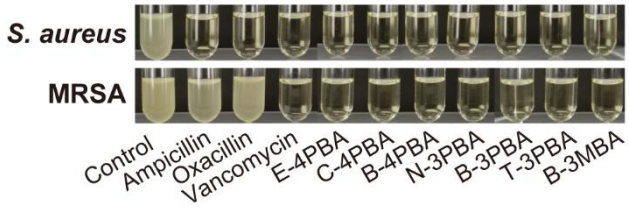

Figure S2 *S. aureus* and MRSA suspensions after the treatment of ampicillin (0.25 μg/mL for *S. aureus* and 32 μg/mL for MRSA), oxacillin (8 μg/mL for *S. aureus* and 32 μg/mL for MRSA), vancomycin (2 μg/mL) and MAAs.

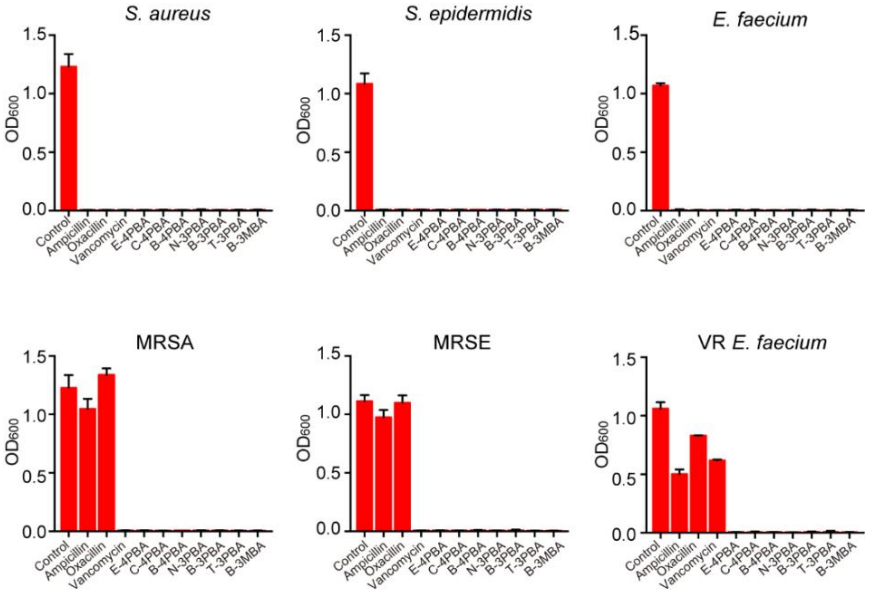

Figure S3 Inhibition effects of MAAs and clinical antibiotics including ampicillin (32 μg/mL), oxacillin (32 μg/mL) and vancomycin (2 μg/mL) on the growth of *S. aureus*, *S. epidermidis*, *E. faecium*, MRSA, MRSE and VR *E. faecium*. MAAs are used at their MICs. The groups without antibiotics are set as control.

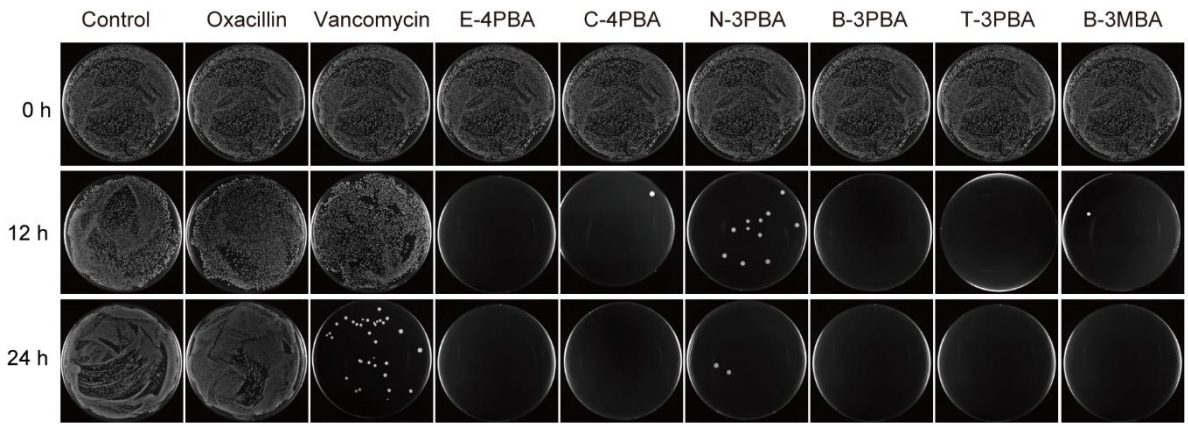

Figure S4 Time-dependent killing of MRSA at early exponential phase (~10^6 CFU/mL) by by oxacillin (Oxa, 32 μg/mL), Vancomycin (Van, 32 μg/mL) and MAAs (2 μg/mL). The bacterial suspensions are diluted for 10 times and 100 μL diluted suspensions were plated on LB plates and incubated at 37 ºC. Bacterial colonies are recorded after 12 and 24 h. The group without antibiotic is set as control.

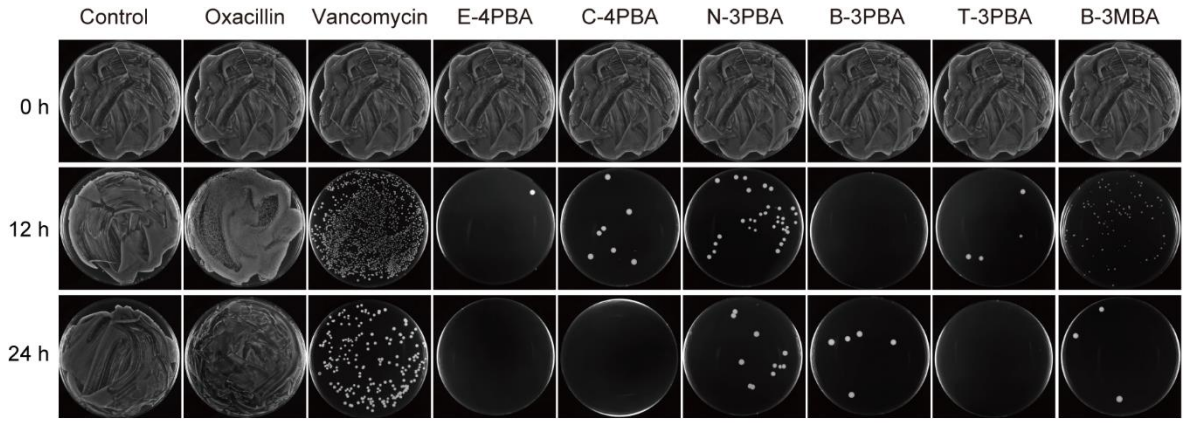

Figure S5 Time-dependent killing of MRSA at late exponential phase (~10^9 CFU/mL) by by oxacillin (Oxa, 32 μg/mL), Vancomycin (Van, 32 μg/mL) and MAAs (4 μg/mL). The bacterial suspensions are diluted for 1000 times and 100 μL diluted suspensions were plated on LB plates and incubated at 37 ºC. Bacterial colonies are recorded after 12 and 24 h. The group without antibiotic is set as control.

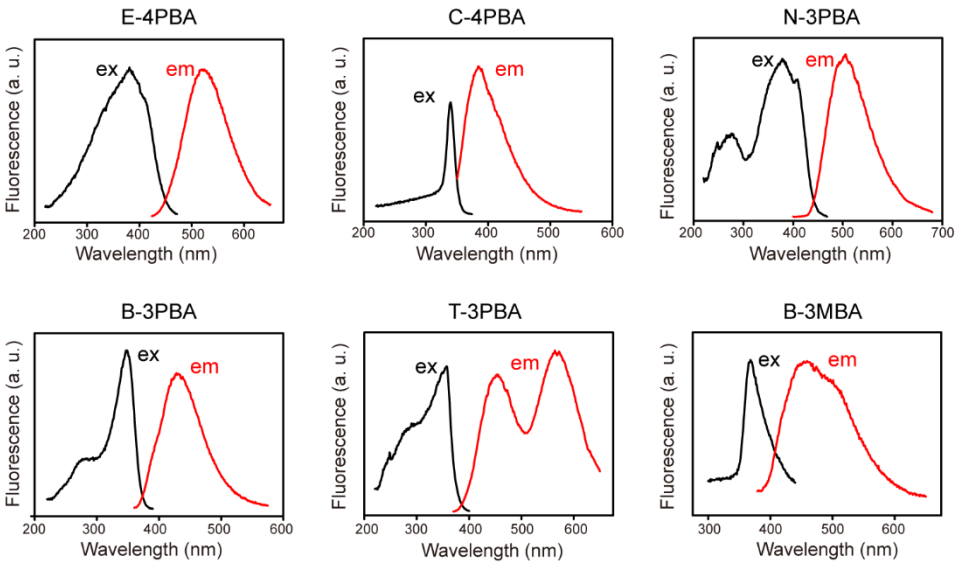

Figure S6 Excitation (ex) and emission (em) spectra of MAAs.

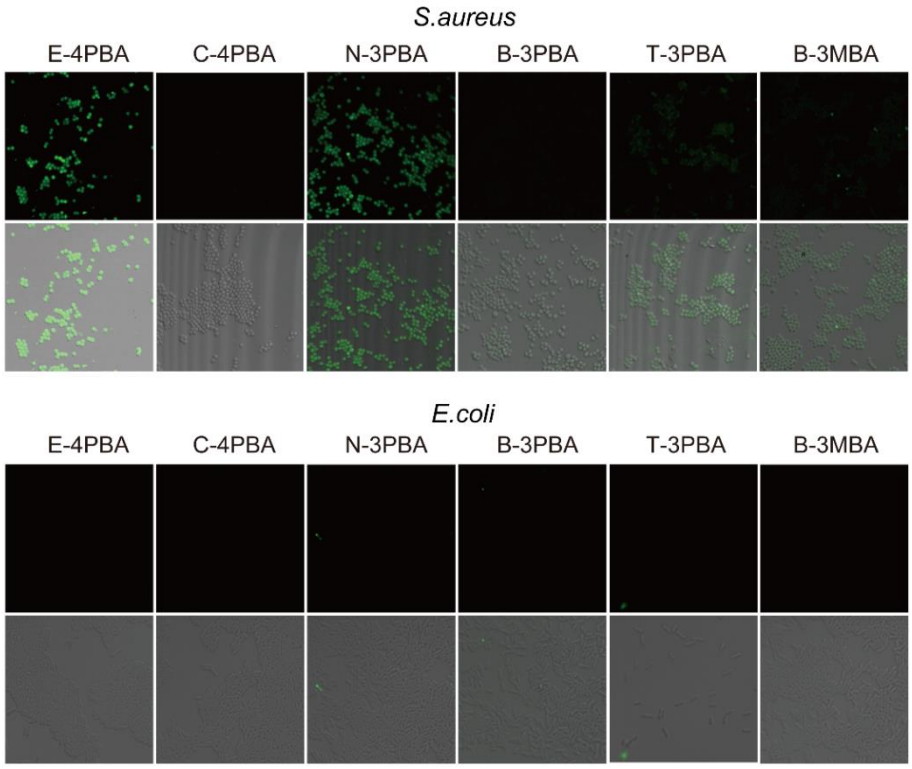

Figure S7 Fluorescent images of *S. aureus* and *E. coli* treated with MAAs.

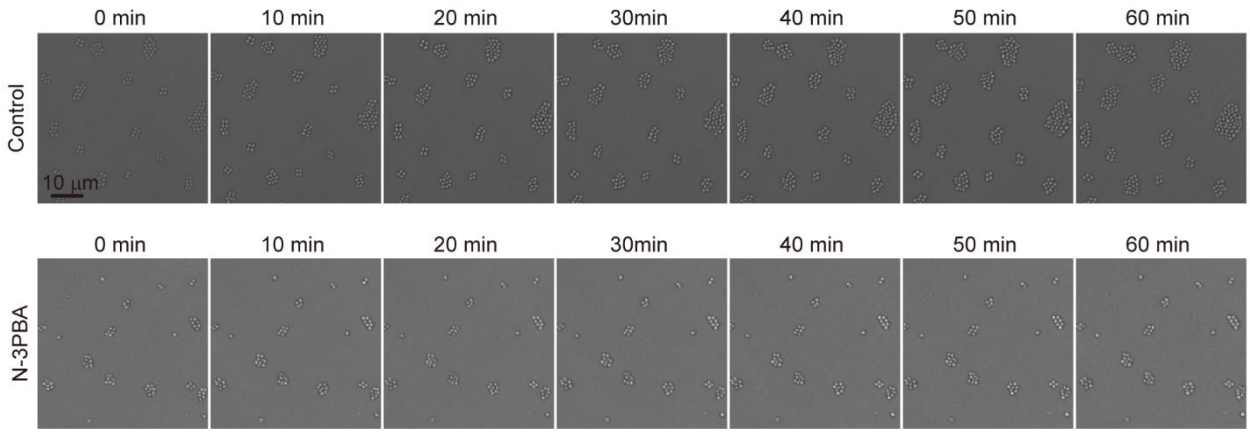

Figure S8 Monitoring of bacterial division treated with N-3PBA. The group without antibiotic is set as control.

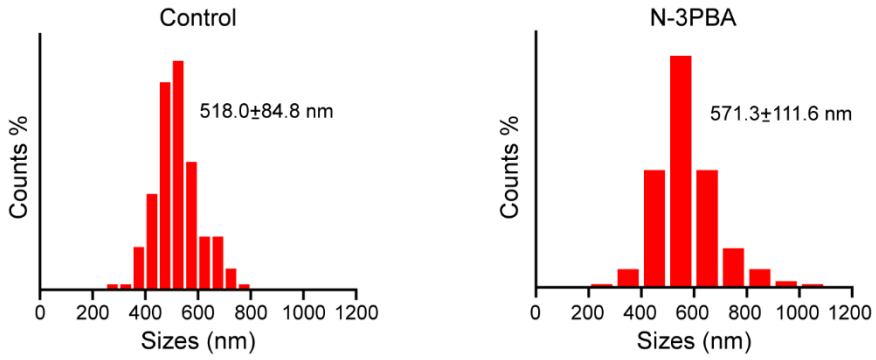

Figure S9 Cell sizes of *S. aureus* and N-3PBA-treated *S. aureus* in SEM images calculated by ImageJ.

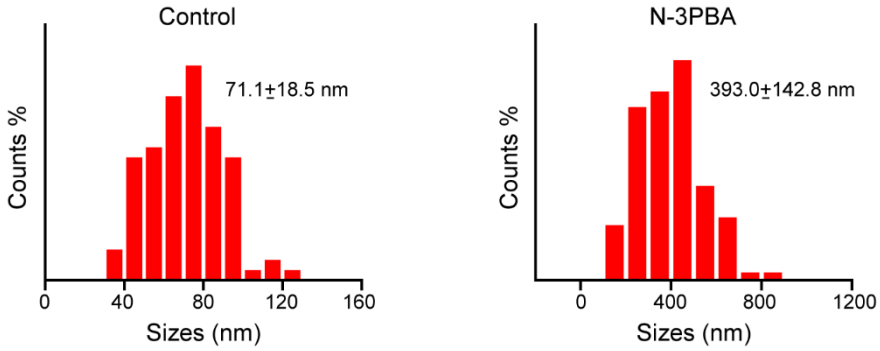

Figure S10 Septa thickness of *S. aureus* and N-3PBA-treated *S. aureus* in TEM images calculated by ImageJ.

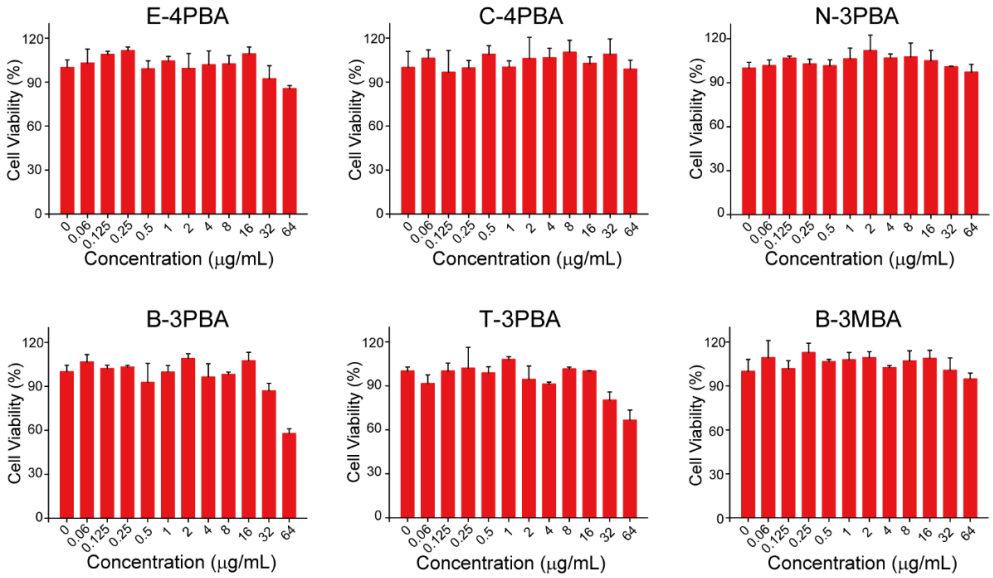

Figure S11 Cell viability of HUVECs tested by CCK-8 kit after incubation with different concentration (0-64 μg/mL) of MAAs.

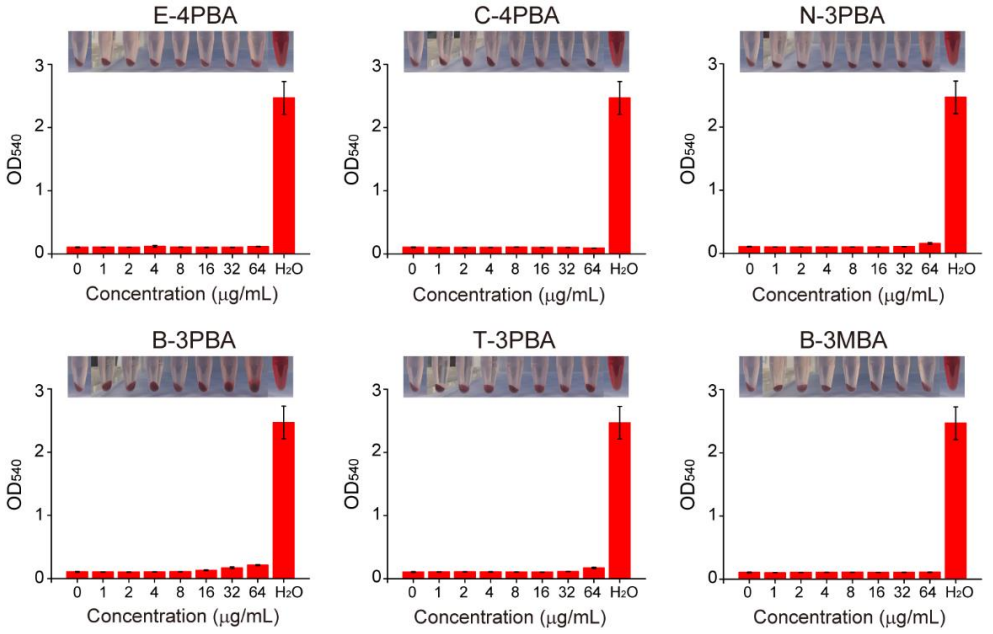

Figure S12 Hemolysis of MAAs on rat red blood cells.

Table S4 *In vivo* toxicity of MAAs.

MAAs	Survival rate (%)		
	0.5 mg/ kg	1 mg/kg	2 mg/ kg
E-4PBA	100	100	100
C-4PBA	100	100	100
N-3PBA	100	100	100
B-3PBA	100	100	100
T-3PBA	100	100	100
B-3PBA	100	100	100