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Synthesis and evaluation of amphiphilic cationic quinine-derived for antibacterial activity against methicillin-resistant *Staphylococcus aureus*

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Abstract—Several representative amphiphilic cationic quinine-derived have been synthesized and evaluated against methicillin-resistant *Staphylococcus aureus*. This is the first reported antibacterial activity of this class of compounds. In vitro the minimal inhibitory concentration values of the best compound **Q7** ranged from 0.4 to 1.6 μg/mL (MBC < 3.2 μg/mL).

Drugs from a wide variety of pharmacological groups are cationic amphiphilic in nature, such as antiarrhythmics, local anesthetics, antimalarials, β-blockers, and tricyclic antidepressants.[1] The drugs are prone to interact with membrane phospholipids: the cationic nitrogen is attracted to the negatively charged phosphate of the phospholipid head-group, and the aromatic ring system is directed toward the hydrophobic interior of the phospholipid layer. The cationic amphiphilic nature may have impact on drug pharmacokinetics and pharmacodynamics.[2] In the past two decades, amphiphilic cationic compounds have also been known to exhibit strong antimicrobial activity.[3] They exhibit rapid activity against a broad range of microorganisms such as bacteria (both Gram-positive and Gram-negative),[4] fungi,[5] and certain viruses.[6,7]

The impact on human health of *Staphylococcus aureus* infection in community and hospital settings has led to intensive investigation of this organism over recent years. It is the causative agent of a wide range of diseases, from carbuncles and food poisoning, through more serious device and wound-related infections, to life-threatening conditions. *S. aureus* produces a plethora of virulence factors that facilitate attachment, colonization, cell–cell interactions, immune evasion, and tissue damage. The number of effective antibiotics has been reduced by the emergence of resistance to penicillin, methicillin, and, more recently, vancomycin,[8,9] a problem that has been compounded by the recent emergence of methicillin-resistant *S. aureus* (MRSA) carriage and disease in the community.[10,11]

Antimalarial drugs are currently widely used to treat patients with autoimmune dermatologic and rheumatologic diseases, and have also recently been proposed as additional therapy for patients with human immunodeficiency virus (HIV) infection.[12] Indeed, the antibacterial effect of these drugs may be especially important to these often immunocompromised patients. Since many patients who receive antimalarials for the treatment of non-infective inflammatory diseases are also immunosuppressed because of their disease or treatments, and may have concomitant bacterial infections. Another advantage of antimalarials is that they do not act directly on the invading pathogens, but rather on the host cells, so that there are few, if any, chances for the microorganisms to become resistant to their effects. Quinine has been used as an important natural antimalarial medicine. Wolf et al.[13] investigated the effect of the antimalarial drug on the growth and invasion of several bacteria, they found that the invasive ability of *E. agglomerans* (*Enterobacter agglomerans*) and *S. aureus* was significantly inhibited by 50 and 100 μM quinine sulfate (QS).

Acetophenone is used in consumer fragrances and as an industrial solvent. Recently, the interest in the study of

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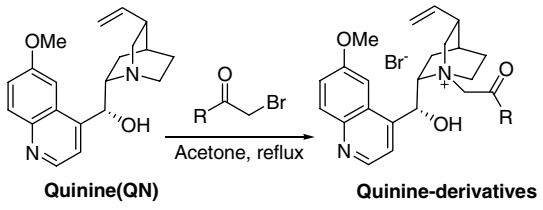
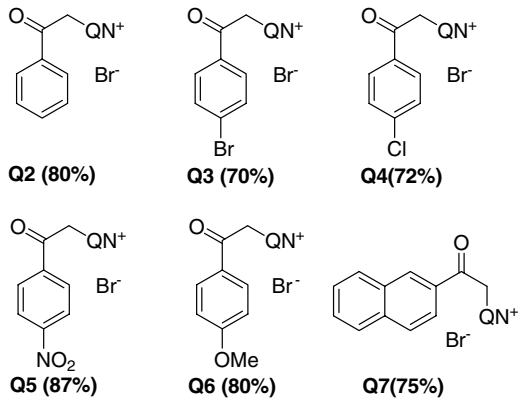
some acetophenone derivatives arises from their significant antimicrobial activity against Gram-positive bacteria and fungi,[14,15] and antimutagenic activity.[16] In the present letter, based on the biological properties as well as structural features of quinine and acetophenone, we synthesized a series of amphiphilic cationic quinine-derived compounds against *S. aureus* and methicillin-resistant *S. aureus* as a step in studying potential antibacterial activity of these chemical compounds.

The amphiphilic cationic quinine-derived compounds were prepared from the quinine and the corresponding α-bromo acetophenone derivatives (Scheme 1), which can be easily prepared by the bromination of acetophenone derivatives with cupric bromide. Quinine and α-bromo acetophenone derivatives were stirred in refluxing acetone for 2 h, to give the corresponding amphiphilic cationic quinine-derived **Q2**–**7** in 70–87% yields (Scheme 2). Both elemental and spectral (IR, ^{1}H NMR and ^{13}C NMR) analysis data[17] of all the synthesized compounds are in full agreement with the suggested molecular structures. The IR spectra of pure products **Q2**–**7** indicated the presence of strong C=O bands at 1700–1710 cm^{-1}. The signals from aromatic C=C bonds at 1640–1579 cm^{-1} were expressed. ^{1}H NMR spectra of compounds **Q2**–**7** showed characteristic signals from protons of quinoline heterocycle and aromatic ring in the range of δ 8.91–7.89 and 7.54–7.20 ppm, respectively. The ^{13}C NMR spectra of compounds **Q2**–**7** also showed the presence of C=O bands at 195.1–192.3 ppm.



Scheme 1.



Scheme 2.

Amphiphilic cationic quinine-derived compounds and quinine were tested against a panel of representative pathogenic bacteria, together with ampicillin and kanamycin as references. Various methicillin-susceptible and methicillin-resistant *S. aureus* were included in order to evaluate the activities of this novel series of amphiphilic cationic quinine-derived compounds to overcome bacterial resistance. *S. aureus* ATCC 25923 is a methicillin-susceptible strain. *S. aureus* CMAH 0430, *S. aureus* CMAH 0504, and *S. aureus* CMAH 0515 are methicillin-resistant clinical strains. Besides, all the quinine-derived and quinine were also tested against other Gram-positive and Gram-negative strains of bacteria, such as *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* CMCC(B) 28001, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. The in vitro antibacterial activities were reported as minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) that were determined by the broth microdilution method as recommended by the NCCLS (National Committee for Clinical Laboratory Standards).[18]

The results in Table 1 show the antibacterial activity of amphiphilic cationic quinine-derived compounds with the reference compounds (ampicillin and kanamycin). Ampicillin and kanamycin have good activity against methicillin-susceptible *S. aureus* but not active against the methicillin-resistant strains.

Quinine was not active against *S. aureus*. However, the amphiphilic cationic compound **Q2** exhibited improved activity compared to quinine against *S. aureus* and methicillin-resistant *S. aureus* tested, which were sensitive to the same extent (MICs = 12.5 μg/mL) (Table 1). Thus, 4′-substituted bromine amphiphilic cationic compound **Q3** had better activity against methicillin-susceptible and methicillin-resistant *S. aureus* (MICs 3.13–6.25 μg/mL). However, the presence of the other groups at the 4-position of the aromatic ring (such as –NO_2

Table 1. In vitro antibacterial activity of quinine-derived compounds (MIC/MBC, μg/mL)

Strain	Quinine	Q2	Q3	Q4	Q5	Q6	Q7	Ampicillin	Kanamycin
Staphylococcus aureus ATCC 25923	—	12.5/25	3.13/6.25	—	25/50	12.5/12.5	1.56/3.13	0.39/0.78	0.39/0.78
MRSA CMAH 0430	—	12.5/25	3.13/6.25	—	50/50	25/50	0.39/1.56	—	—
MRSA CMAH 0504	—	12.5/25	6.25/12.5	—	50/50	25/50	0.78/1.56	—	—
MRSA CMAH 0515	—	12.5/25	3.13/6.25	—	50/50	25/50	0.39/1.56	—	—
Bacillus subtilis ATCC 6633	—	25/25	3.13/6.25	—	25/50	12.5/25	3.13/6.25	0.39/0.39	6.25/6.25
Micrococcus luteus CMCC(B) 28001	—	6.3/12.5	6.25/6.25	—	25/50	12.5/12.5	3.13/3.13	0.39/0.78	0.39/0.39
Escherichia coli ATCC 25922	—	—	100/—	—	—	—	50/100	50/100	0.39/0.39
Pseudomonas aeruginosa ATCC 27853	—	—	100/—	—	—	—	100/—	—	—

or –OMe) gave similar or lowered activity against the *S. aureus*, especially a chlorine substituent at the 4-position of the aromatic ring **Q4** was not active against *S. aureus*. Interestingly, the amphiphilic cationic compound **Q7**, by replacing the phenyl group of **Q2** with the bulkier β-naphthyl moiety, showed good activity against *S. aureus* tested (MIC = 1.56μg/mL), and much better against MRSA (MIC 0.39 to 0.78 μg/mL), while ampicillin and kanamycin were inactive up to the concentration of 100 μg/mL. Furthermore, **Q3** and **Q7** had also good activity against other Gram-positive bacteria (MIC $\leqslant$ 6.25 μg/mL) and weak activity against Gram-negative bacteria, however, other amphiphilic cationic compounds were not active against Gram-negative bacteria. The MBC data for the amphiphilic cationic compounds except inactive compounds against Gram-positive bacteria are shown in Table 1. The MBC value was considered as the lowest amphiphile concentration at which there was no viable cell present. In all cases, the MBCs of the amphiphilic cationic compounds were twofold higher than the corresponding MICs against Gram-positive bacteria tested, suggesting a bactericidal activity. Moreover, the MBC value of **Q7** is only 1.56 μg/mL against MRSA.

Figure 1 shows the log(survivors) versus exposure time plots for the different concentrations of amphiphilic cationic compound **Q7** against *S. aureus* ATCC 25923 and methicillin-resistant *S. aureus* CMAH 0504.[19] Exposure of the **Q7** to bacterial cells was carried out in Mueller–Hinton broth (Difco) medium. The concentration of **Q7** was kept at 5, 10, and 15 μg/mL against *S. aureus* and at 2.5, 5, and 10 μg/mL against MRSA. When exposed to **Q7**, all bacterial cells were killed within 20–30 min for MRSA (CMAH 0504) at 10 μg/mL, whereas not until 40 min for *S. aureus* (ATCC 25923) at 15 μg/mL. It is evident that with the compound **Q7** the time required to kill MRSA is less than that for the common *S. aureus*.

Next, amphiphilic cationic compounds **Q3** and **Q7**, which proved to have good activity against *S. aureus* and MRSA, were tested for the efficacy of detecting *S. aureus* and three MRSA isolates' growth inhibition by disk-diffusion method outlined by the NCCLS with

Table 2. Inhibition zone (diameter in mm) of quinine-derived compounds and various antibiotics against *S. aureus* and three methicillin-resistant *S. aureus* (MRSA) isolates by disk diffusion method

Antibiotics 30 μg/disk	Zone of inhibition (mm)			
	S. aureus	MRSA CMAH 0430	MRSA CMAH 0504	MRSA CMAH 0515
Q3	24	23	22	22
Q7	26	26	23	25
Ampicillin	34	—	—	—
Cefazolin	30	2	—	—
Tetracycline	14	15	17	17
Kanamycin	28	—	—	—
Midecamycin	27	—	—	—
Levofloxacin	32	—	—	—
Cloramphenicol	32	27	26	27
Vncomycin	23	24	22	24

Mueller–Hinton agar (Difco) with 2% NaCl,[20] and other kinds of antibiotics, such as ampicillin, cefazolin, tetracycline, kanamycin, midecamycin, levofloxacin, chloramphenicol, vancomycin, etc., were used as reference antibacterial agents (Table 2). Although amphiphilic cationic compounds **Q3** and **Q7** were a little weaker against *S. aureus* than the other antibiotics, **Q3** and **Q7** were more active against three isolates of MRSA. As shown in Figure 2 (MRSA CMAH 0504), **Q3**, **Q7**,

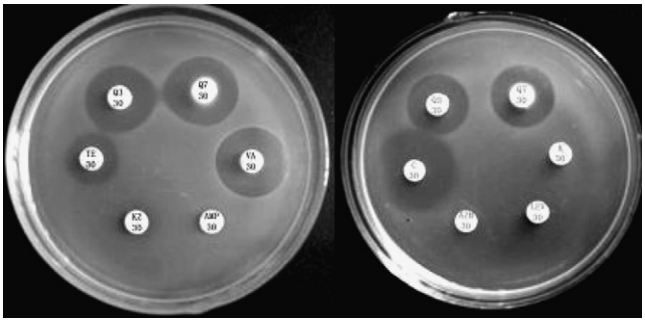


Figure 2. Comparison of activities of various antibacterial compounds with **Q3** and **Q7** against MRSA (CMAH 0504) isolate by disk-diffusion method.

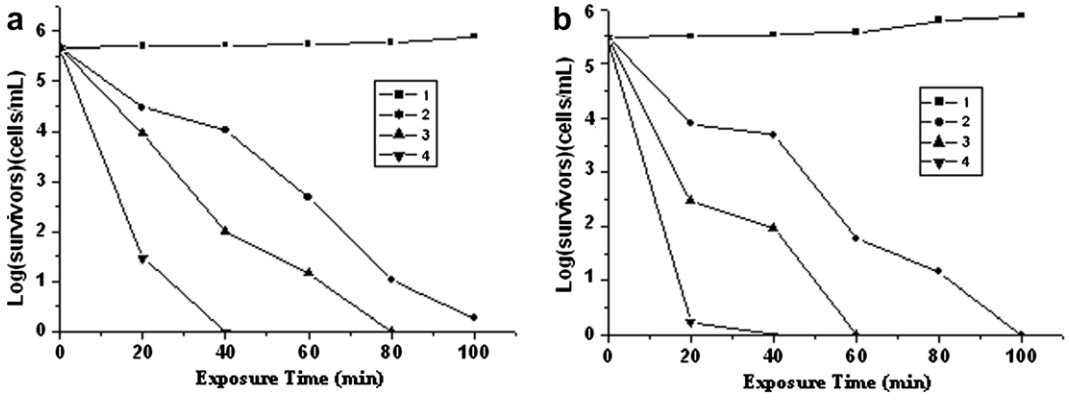


Figure 1. log(survivors) versus exposure time plots for compound **Q7** against (a) *S. aureus* (ATCC 25923): 1, control (without **Q7**); 2, 5 μg/mL; 3, 10 μg/mL; 4, 15 μg/mL; (b) MRSA (CMAH 0504): 1, control (without **Q7**); 2, 2.5 μg/mL; 3, 5 μg/mL; 4, 10 μg/mL.

Table 3. Hemolytic activity of quinine-derived compounds

Compound	Percent lysis of human red blood cells as a function of compound concentration			
	2000 μg/mL	1000 μg/mL	500 μg/mL	250 μg/mL
Quinine	0	0	0	0
Q2	5	0	0	0
Q3	25	7	0	0
Q5	0	0	0	0
Q6	0	0	0	0
Q7	55	17	9	0

and other antibiotics at 30 μg/mL diffused outward and inhibit the growth of MRSA (CMAH 0504). The bacteria on the left plate are susceptible to all but two drugs (ampicillin and kanamycin), whereas on the right is resistant to three drug (cefazolin, midecamycin, levofloxacin). The compound **Q7** was less active against MRSA (CMAH 0504) than chloramphenicol, better than vancomycin and tetracycline. **Q3** also has the same activity as vancomycin.

Since a number of the amphiphilic cationic compounds exhibited strong antibacterial activity against MRSA, they had potential to be developed as antibacterial therapeutics. But because of the high affinity of the amphiphilic cationic compounds toward biological membranes, it was thought that they might have a simple lytic mode of action and be broadly cytotoxic against human cells. We therefore determined the hemolytic activity of these compounds as a measure of their cytotoxicity and an initial test of their suitability for further development.

Compounds Q5 and **Q6** were the only compounds other than quinine itself that did not lyse the erythrocytes at any of the concentrations studied (Table 3).[21] For the strongly antibacterial **Q3** at concentrations well above the MICs, for example, 1000 μg/mL, hemolysis is only 7%, while for **Q7**, hemolysis is 17% at this concentration, but drops to 9% at 500 μg/mL, which is still well above the MICs. It is clear that these quinine-derived ammonium salts have surprisingly low lytic properties, although they are also amphiphilic cationic compounds. Compounds **Q3** and **Q7** show no hemolytic activity at concentrations 100-fold greater than the MICs.

In conclusion, a series of new amphiphilic cationic quinine-derived compounds were synthesized and characterized. It appears that **Q3** and **Q7** may be able to maintain good antibacterial activity against most pathogenic bacteria. Interestingly, the two amphiphilic cationic quinine-derived compounds have good activity against methicillin-susceptible and methicillin-resistant *S. aureus* (from clinical isolates), which may be to hold promise against drug-resistance bacteria. Though **Q3** and **Q7** are amphiphilic cationic compounds, they have surprisingly low lytic properties, and thus may be suitable lead candidates for drug development.

Acknowledgments

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17. All new compounds gave satisfactory analytical and spectral data.
Compound **Q2**: yield: 80%. Mp 176–178 °C. IR (KBr) ν (cm^{-1}): 3445, 2953, 2883, 1702, 1640, 1601, 1559, 1450, 1024, 917, 832. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): δ 1.21–0.96 (m, 2H), 1.59–1.56 (m, 1H), 1.80–1.78 (m, 1H), 2.01–1.99 (m, 2H), 2.86–2.79 (m, 2H), 3.83–3.71 (m, 2H), 3.98 (s, 3H), 4.27–4.23 (m, 1H), 4.43–4.38 (m, 1H), 4.60–4.58 (m, 1H), 5.17 (d, J = 10.5 Hz, 1H), 5.35 (d,

J = 17.4 Hz, 1H), 5.91–5.80 (m, 1H), 6.38 (d, J = 7.5 Hz, 1H), 7.20 (s, 1H), 7.54–7.46 (m, 5H), 7.89 (d, J = 15.6 Hz, 2H), 8.04–8.00 (m, 2H), 8.91 (d, J = 4.5 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 194.1, 158.5, 148.1, 144.4, 144.0, 139.0, 135.8, 134.8, 132.3, 129.7, 129.3, 126.0, 125.6, 121.1, 116.3, 101.5, 66.0, 63.6, 60.8, 57.8, 56.7, 37.3, 26.0, 25.5, 22.1. MS (ESI): 443 $[M-Br]^+$. Calcd for $C_{28}H_{31}BrN_2O_3$: C, 75.82; H, 7.04; N, 6.32%. Found: C, 75.80; H, 7.01; N, 6.35%.

Compound **Q3**: yield: 70%. Mp 180–182 °C. IR (KBr) ν (cm^{-1}): 3414, 3147, 2939, 1701, 1637, 1619, 1586, 1507, 1469, 1397, 1239, 1024, 997, 860, 837. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): δ 1.18–0.96 (m, 1H), 2.11–1.92 (m, 4H), 2.87 (s, 1H), 3.32 (d, J = 8.8 Hz, 2H), 3.85–3.72 (m, 2H), 4.07 (s, 3H), 4.21–4.18 (m, 1H), 4.59 (d, J = 10.0 Hz, 2H), 5.50–5.41 (dd, J_1 = 10.0, J_2 = 10.4 Hz, 1H), 5.14–5.36 (dd, J_1 = 17.6, J_2 = 10 Hz, 1H), 5.81–5.67 (m, 3H), 7.11 (s, 1H), 7.42 (d, J = 9.2 Hz, 1H), 7.71 (d, J = 4.0 Hz, 1H), 7.96–7.87 (m, 3H), 8.10 (d, J = 8.4 Hz, 2H), 8.76 (d, J = 4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 193.2, 158.4, 148.1, 144.4, 144.1, 140.8, 139.0, 133.6, 131.3, 129.8, 126.0, 122.7, 121.1, 116.2, 101.6, 65.9, 63.5, 60.7, 57.8, 56.8, 37.3, 31.4, 26.0, 25.5, 22.1. MS (ESI): 478 $[M-Br]^+$. Calcd for $C_{28}H_{30}Br_2N_2O_3$: C, 55.83; H, 5.02; N, 4.65%. Found: C, 55.85; H, 5.01; N, 4.60%.

Compound **Q4**: yield: 72%. Mp 174–176 °C. IR (KBr) ν (cm^{-1}): 3414, 3125, 2960, 1703, 1638, 1584, 1463, 1400, 1262, 1084, 871, 800. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): δ 1.08–1.06 (m, 1H), 2.12–2.05 (m, 4H), 2.87 (s, 1H), 3.11 (s, 2H), 3.85–3.74 (m, 2H), 4.07 (s, 3H), 4.22–4.19 (m, 1H), 4.63–4.58 (m, 2H), 5.01 (d, J = 10.4 Hz, 1H), 5.21 (d, J = 17.2 Hz,1H), 5.81–5.64 (m, 3H), 7.12 (s, 1H), 7.44–7.41 (m, 1H), 7.75–7.71 (m, 3H), 7.95 (d, J = 9.2 Hz, 1H), 8.19 (d, J = 8.4 Hz, 2H), 8.76 (d, J = 4.4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 193.2, 158.4, 148.1, 144.4, 144.1, 140.8, 139.0, 133.6, 132.2, 131.3, 129.8, 126.0, 122.7, 121.1, 116.2, 101.6, 65.9, 63.5, 60.7, 57.8, 56.8, 37.3, 31.4, 26.0, 25.5, 22.1. MS (ESI): 521 $[M-Br]^+$. Calcd for $C_{28}H_{30}BrN_2O_3$: C, 60.28; H, 5.42; N, 5.02%. Found: C, 60.24; H, 5.40; N, 5.00%.

Compound **Q5**: yield: 87%. Mp 199–201 °C. IR (KBr) ν (cm^{-1}): 3345, 3171, 2953, 2883, 1705, 1620, 1687, 1509, 1451, 1342, 1228, 1185, 1024, 917, 832. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): δ 1.00–0.97 (m, 1H),1.23–1.19 (m, 1H), 1.60–1.57 (m, 1H), 1.83 (br, 1H), 2.88–2.82 (m, 2H), 3.35 (s, 4H), 3.84–3.81 (m, 2H), 4.00 (s, 3H), 4.36 (d, J = 13.5 Hz, 1H), 4.56 (d, J = 13.5 Hz, 1H), 4.69–4.65 (m, 1H), 5.18 (d, J = 10.8 Hz, 1H), 5.93–5.57 (m, 1H), 6.42 (d, J = 7.5 Hz, 1H), 7.23 (d, J = 2.1 Hz, 1H), 7.51–7.47 (dd, J_1 = 2.4, J_2 = 1.2 Hz, 1H), 8.06–8.02 (m, 2H), 8.19 (d, J = 8.7 Hz, 2H), 8.37 (d, J = 8.7 Hz, 2H), 8.93 (d, J = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 192.7, 151.1, 150.1, 148.2, 145.7, 142.0, 138.8, 130.9, 130.7, 130.5, 128.5, 125.2, 124.2, 123.7, 119.8, 117.2, 65.6, 63.2, 61.2, 56.9, 53.7, 37.9, 37.5, 26.7, 25.0, 22.3. MS (ESI): 488 $[M-Br]^+$. Calcd for $C_{29}H_{30}BrIN_3O_5$: C, 59.16; H, 5.32; N, 7.39%. Found: C, 59.13; H, 5.33; N, 7.38%.

Compound **Q6**: yield: 80%. Mp 195–197 °C. IR (KBr) ν (cm^{-1}): 3324, 3171, 2975, 2888, 1700, 1621, 1579, 1520, 1509, 1475, 1459, 1343, 1231, 1085, 992, 931, 855. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): δ 1.23–0.93 (m, 2H), 1.56 (d, J = 9.2 Hz, 1H), 2.11–1.79 (m, 3H), 2.89–2.78 (m, 2H), 3.35 (s, 3H), 3.84–3.81 (m, 3H), 3.97 (s, 3H), 4.18–4.07 (m. 1H), 4.37 (d, J = 13.2 Hz, 1H), 4.74–4.58 (m, 2H), 5.13 (d, J = 10.8 Hz, 1H), 5.34 (d, J = 17.2, 1H), 5.91–5.83 (m, 1H), 6.42 (d, J = 7.2 Hz, 1H), 7.43–8.18 (m, 5H), 8.40 (d, J = 9.2 Hz, 1H), 8.44 (d, J = 8.4 Hz, 1H), 8.89 (d, J = 4.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 193.2, 159.0, 148.4, 148.2, 144.1, 140.9, 138.8, 132.3, 130.9, 128.5, 126.3, 124.2, 123.0, 119.9, 116.8, 101.5, 65.8, 62.8, 61.0, 57.9, 57.2, 56.8, 53.8, 37.6, 26.2, 24.9, 22.3. MS (ESI): 473 $[M-Br]^+$. Calcd for $C_{28}H_{33}BrN_2O_4$: C, 62.93; H, 6.01; N, 5.06%. Found: C, 62.90; H, 6.04; N, 5.03%.

Compound **Q7**: yield: 75%. Mp 170–172 °C. IR (KBr) ν (cm^{-1}): 3345, 3113, 2946, 2888, 1710, 1678, 1620, 1588, 1508, 1473, 1433, 1357, 1262, 1239, 1120, 1042, 1228, 1085, 1024, 917, 832. MS (ESI): 488.1.^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.09–0.99 (s, 1H), 1.91–2.12 (m, 3H), 2.46 (s, 2H), 2.88 (s, br, 1H), 3.35 (s, 1H), 3.81–3.89 (m, 2H), 4.11 (s, 3H), 4.32–4.30 (m, 1H), 4.40–4.65 (m, 2H), 5.02 (d, J = 10.4 Hz, 1H), 5.24 (d, J = 17.2 Hz, 1H), 5.83–5.73 (m, 3H), 7.14 (s, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.40–7.43 (m, 3H), 7.94 (d. J = 8.8 Hz, 1H), 8.19–8.04 (m, 4H), 8.76 (s, 1H), 9.02 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 193.2, 159.0, 148.4, 148.2, 144.1, 140.9, 138.8, 132.3, 130.9, 128.5, 126.3, 124.2, 123.0, 119.9, 116.8, 101.5, 65.8, 62.8, 61.0, 57.9, 57.2, 56.8, 53.8, 37.6, 26.2, 24.9, 22.3. MS-ESI: m/z: Positive polarity: 493 $[M-Br]^+$. Calcd for $C_{32}H_{33}BrN_2O_3$: C, 67.01; H, 5.80; N, 4.88%. Found: C, 66.99; H, 5.78; N, 4.84%.

18. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Fifth Edition; Approved standard M7-A5. Wayne, PA: NCCLS; 2001.
19. The killing time studies were performed with compound **Q7** at the final concentrations higher than MBC values against MRSA CMAH 0504 and *S. aureus* ATCC 25923, respectively. The mid-logarithmic phase cultures were appropriately diluted with Mueller–Hinton broth medium to achieve a final inoculum of 3–5 × 10^5cell/mL. The same inoculum was added to drug-free medium as a growth control. The test tubes were incubated in a water bath at 30 °C. Next, 100 μL of samples was taken from each tube at known time intervals, centrifuged, and washed three times with fresh Mueller–Hinton broth. After vortexed vigorously for 10 s, the suspension was suitably diluted and spread onto Mueller–Hinton agar plates. The plates were incubated at 30 °C for 24 h and colonies were counted. Each number of viable cells was determined from three-independent experiments performed in triplicate.
20. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests. Eighth Edition; Approved Standard M2-A8. Wayne, PA: NCCLS; 2003.
21. Human blood from healthy volunteers was collected in 10 mL Vacationer tubes containing sodium heparin as anticoagulant. The cells were washed three times with calcium- and magnesium-free phosphate-buffered saline (PBS) and centrifuged at 2000*g* for 10 min. The third supernatant liquid was clear and colorless. Then 0.1 mL erythrocyte suspension diluted with PBS (erythrocyte concentration around 1.0 × 10^9 cells/mL) was mixed with 0.1 mL of test substances at a series of concentrations (1–2000 μg/mL). The mixtures were incubated at 37 °C for 1 h. After incubation, tubes were centrifuged at 2000*g* for 10 min. The supernants were transferred into 96-well polystyrene plates (Costar 3590, incorporated) and the optical density was measured at 540 nm using MTP120 microplate reader (Colona Electric, Japan). The values for 0% and 100% lysis were determined by incubating erythrocytes with PBS, and 0.1% (v/v) Triton X-100 (Amresco 0694), respectively. Assays were carried out in triplicate and the results were confirmed in three-independent experiments.