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The synthesis of novel cationic anthraquinone dyes with high potent antimicrobial activity

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Abstract

Quaternary ammonium salts were incorporated into anthraquinone dyes via a stable alkyl amino linkage. The structures of the antimicrobial colorants were characterized by Fourier Transform Infrared (FT-IR), Nuclear Magnetic Resonance (NMR) and UV–vis spectrometries. The dyes demonstrated excellent antimicrobial ability against both Gram-negative and Gram-positive bacteria in aqueous solution, as indicated by very low minimum inhibitory concentration (MIC). The colorants showed excellent stability in water under light, continuous heating as well in as acidic and alkaline conditions.

Keywords: Synthesis; Characterization; Quaternary ammonium salts; Antimicrobial colorants; Stability; Anthraquinone

1. Introduction

Many cationic dyes possess antimicrobial functions and have been widely applied in topical cleaning, clinical use, and preservatives for food and fishery industries since the 19th century [1–3]. The most commonly used antimicrobial colorants are derivatives of triphenylmethane dyes such as gentian violet, brilliant green and malachite green, which have poor light stability and tend to be decolorized by bacteria [4]. Also, high concentration of these dyes is needed to achieve the expected functions due to high minimum inhibition concentration (MIC) of the dyes, while high concentration of the dyes created concerns of staining. In recent decades, scores of new antimicrobial agents, especially quaternary ammonium compounds have been invented to substitute these antimicrobial dyes.

Quaternary ammonium salts (QAS) are cationic surface active compounds that can provide combined effects of disinfection, surface-activation, and antistatic properties [5]. Because they destroy microbes by physical penetration mechanism [6], QAS are relatively mild in action and effective against a broad spectrum of microorganisms such as bacteria (both Gram-positive and Gram-negative), fungi and enveloped viruses. So far, QAS have been extensively employed as disinfectants in many fields such as chemical formulations, personal care products, surface cleaning spray, and dental products [6–8]. QAS are also applied in resins, textiles and other polymers to incorporate antimicrobial functions by chemical grafting or finishing processes [9–16]. Efforts have been made to incorporate antimicrobial function to textiles using *N*-halamine structures [17,18]. Although QAS are not as effective as *N*-halamine biocides in contact time for disinfection, dyes incorporated with QAS biocidal groups still is a reasonable alternate, particular chlorine solution may bleach many colors on textiles.

The aim of this research is to synthesize new antimicrobial cationic colorants with high potency and good hydrolytic stability under light, heat, and pH conditions. To achieve this goal, anthraquinone structures that have excellent light and heat stability were chemically connected to biocidal QAS with different hydrocarbon chain lengths. QAS with the chain length of 8–18 carbons normally show good antimicrobial properties [19–21]. Previous work on colored QAS showed increased antimicrobial efficacy with the alkyl chain length increase from 4

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to 12, consistent to the literatures. However, that group of compounds exhibited low stability to hydrolysis due to an amide connection between the anthaquinone and QAS [22–24]. In this study, two series of cationic colorants with hydrocarbon chain lengths of 4, 8, 12 and 16 were prepared. The structures of the colorants were characterized using ^{1}H NMR, ^{13}C NMR, FT-IR, and UV–vis spectroscopies. Antimicrobial property and stability of the compounds were measured following different protocols and discussed.

2. Experimental

2.1. Materials and instrumentation

1-Aminoanthraqinone (97%, Aldrich, Milwaukee MI), 1,4-diaminoantraquinone (90%, Acros, Pittsburg, PA), epichlorohydrin, (98%, Aldrich), *N*,*N*-dimethylbutylamine (99%, Acros), *N*,*N*-dimethyloctylamine (97%, Acros), *N*,*N*-dimethyldodecylamine (95%, Acros), *N*,*N*-dimethylhexadecylamine (95%, Acros) were used as-received.

FT-IR spectra were taken on a Nicolet 6700 spectrometer (Thermo, USA) using KBr pellets. ^{1}H NMR, ^{13}C NMR and COSY NMR spectra were recorded on a Bruker DRX 500 spectrometer (Bruker, USA). Electronic absorption spectra were recorded on a HITACHI U-2000 spectrophotometer (Hitachi, Japan) with a concentration of 100 ppm in distilled water solution.

2.2. Synthesis

The first step of the synthesis was alkylation of amino groups on the anthraquinones. The second step was nucleophilic substitution of tertiary amines by the intermediates. The synthetic procedures are described in Scheme 1, and the synthesized compounds are listed in Table 1.

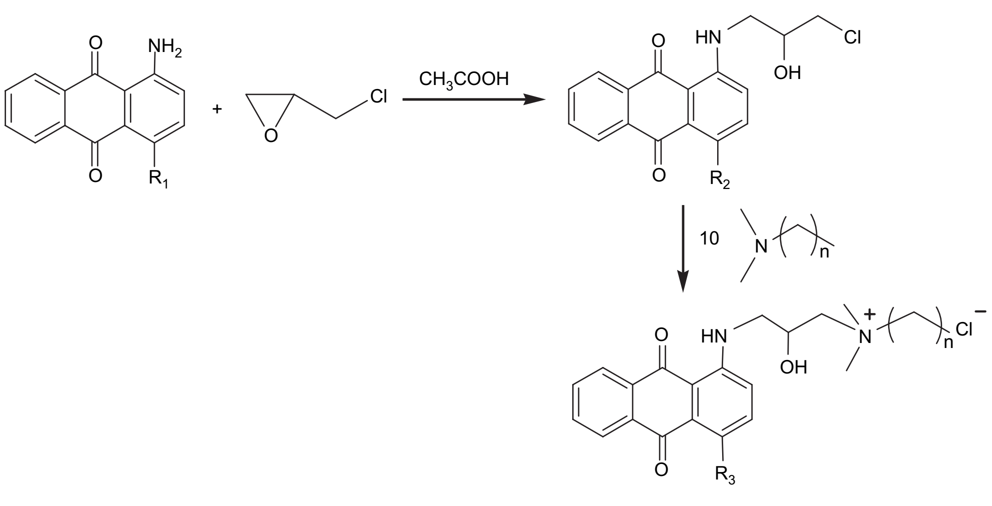
2.2.1. Synthesis of M-4

1-Aminoanthraquinone (0.01 mol) and 0.01 mol of epichlorohydrin in 20 mL acetic acid were kept for 9 h at 95 °C. During the first 6 h, additional 0.01 mol of epichlorohydrin was added to the reacting system slowly. The reaction was monitored by TLC using hexane/ethyl acetate (2.5:1 volume ratio) as the eluent. Afterward, the reaction mixture was cooled to room temperature and stirred overnight. The product was precipitated with water and purified by recrystallization from methanol with a yield of 75%. The structure of the product was confirmed by NMR.

Then, 0.01 mol of the purified product, together with 0.1 mol tertiary amine, was dissolved in 15 mL *n*-propanol. The mixture was refluxed at 115 °C for 24 h monitored by TLC in methanol. The suspension was precipitated by ethyl ether and then vacuum filtered. The crude product was recrystallized with alcohol–ether co-solvent (2:1 volume ratio). Yield: 95%. ^{1}H NMR spectra data (DMSO): δ 9.872 (s, 1H, $-\mathrm{N}H-\mathrm{CH_2}$); 8.220–8.206, 8.147–8.133, 7.935–7.907, 7.871–7.843, 7.697–7.665, 7.488–7.474, 7.393–7.375 (d, $J = 7.0$ Hz, d, $J = 7.0$ Hz, t, $J = 7.0$ Hz, t, $J = 7.0$ Hz, t, $J = 7.0$ Hz, d, $J = 8.0$ Hz, d, $J = 7.0$ Hz, 7H, protons attached to C2, C3, C4, C5, C6, C7, C8); 6.165–6.153 (d, $J = 9.0$ Hz, 1H, $\mathrm{CH}-\mathrm{O}H$); 4.375 (m, $J = 6.0$ Hz, 1H, $\mathrm{C}H-\mathrm{OH}$); 3.537–3.363 (m, 6H, $\mathrm{C}H_2-\mathrm{CH}(\mathrm{OH})-\mathrm{C}H_2-\mathrm{N^+(CH_3)_2}-\mathrm{C}H_2$); 3.137–3.125 (d, $J = 6.0$ Hz, 6H, $\mathrm{N^+(C}H_3)_2-\mathrm{CH_2-CH_2-CH_2-CH_3}$); 1.725–1.602 (m, 2H, $\mathrm{N^+(CH_3)_2-CH_2-C}H_2\mathrm{-CH_2-CH_3}$); 1.267–1.256 (m, 2H, $\mathrm{N^+(CH_3)_2-CH_2-CH_2-C}H_2\mathrm{-CH_3}$); 0.897–0.868 (t, $J =$ Hz, 3H, $\mathrm{N^+(CH_3)_2-CH_2-CH_2-CH_2-C}H_3$).

2.2.2. Synthesis of M-8

By using the same procedures of M-4 synthesis, M-8 was also prepared. Overall yield: 71%. ^{1}H NMR spectra data (DMSO): δ 9.857 (s, 1H, $-\mathrm{N}H-\mathrm{CH_2}$); 8.209–8.194, 8.136–8.121, 7.923–7.895, 7.863–7.835, 7.685–7.653, 7.478–7.464, 7.408–7.391 (d, d, t, t, t, d, d, 7H, protons attached to C2, C3, C4, C5, C6, C7, C8); 6.210–6.199 (d, 1H, $\mathrm{CH}-\mathrm{O}H$); 4.351 (m, 1H, $\mathrm{C}H-\mathrm{OH}$); 3.508–3.362 (broad m, 6H, $\mathrm{C}H_2-\mathrm{CH}(\mathrm{OH})-\mathrm{C}H_2-\mathrm{N^+(CH_3)_2}-\mathrm{C}H_2$); 3.132 (s, 6H, $\mathrm{N^+(C}H_3)_2-(\mathrm{CH_2})_7-\mathrm{CH_3}$); 1.697–1.549 (m, 2H, $\mathrm{N^+(CH_3)_2-(CH_2)_6-C}H_2\mathrm{-CH_3}$); 1.230–1.167 (m, 2H, $\mathrm{N^+(CH_3)_2-CH_2-CH_2-(C}H_2)_5\mathrm{-CH_3}$); 0.850–0.823 (t, 3H, $\mathrm{N^+(CH_3)_2-(CH_2)_7C}H_3$).



Scheme 1. Synthesis route of antimicrobial colorants ($n = 3$, 7, 11, and 15).

Table 1
List of synthesized compounds

Compound	R1	R2	R3
M-4	H	H	H
M-8	–	–	–
M-12	–	–	–
M-16	–	–	–
Di-4	$\mathrm{NH_2}$	$\mathrm{NHCH_2CH(OH)CH_2Cl}$	$\mathrm{NHCH_2(OH)CH_2N^+(CH_3)_2(CH_2)_3CH_3Cl^-}$
Di-8	–	–	$\mathrm{NHCH_2(OH)CH_2N^+(CH_3)_2(CH_2)_7CH_3Cl^-}$
Di-12	–	–	$\mathrm{NHCH_2(OH)CH_2N^+(CH_3)_2(CH_2)_{11}CH_3Cl^-}$
Di-16	–	–	$\mathrm{NHCH_2(OH)CH_2N^+(CH_3)_2(CH_2)_{15}CH_3Cl^-}$

2.2.3. Synthesis of M-12

M-12 was prepared by following the same procedures. Yield: 69%. ^{1}H NMR spectra data (DMSO): δ 9.860 (s, 1H, $-\mathrm{N}H-\mathrm{CH_2}$); 8.213–8.198, 8.141–8.127, 7.928–7.900, 7.868–7.840, 7.689–7.658, 7.483–7.469, 7.409–7.392 (d, d, t, t, t, d, d, 7H, protons attached to C2, C3, C4, C5, C6, C7, C8); 6.199–6.188 (d, 1H, $\mathrm{CH}-\mathrm{O}H$); 4.329 (m, 1H, $\mathrm{C}H-\mathrm{OH}$); 3.549–3.354 (broad m, 6H, $\mathrm{C}H_2-\mathrm{CH(OH)}-\mathrm{C}H_2-\mathrm{N^+(CH_3)_2}-\mathrm{C}H_2$); 3.135 (s, 6H, $\mathrm{N^+(C}H_3\mathrm{)_2}-\mathrm{(CH_2)_{11}}-\mathrm{CH_3}$); 1.701–1.559 (m, 2H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_{10}}-\mathrm{C}H_2-\mathrm{CH_3}$); centered at 1.194 (m, 2H, $\mathrm{N^+(CH_3)_2}-\mathrm{CH_2}-\mathrm{CH_2}-(\mathrm{C}H_2)_9-\mathrm{CH_3}$); 0.854–0.827 (t, 3H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_{11}C}H_3$).

2.2.4. Synthesis of M-16

M-16 was prepared by the same procedures. Yield: 70%. ^{1}H NMR spectra data (DMSO): δ 9.860 (s, 1H, $-\mathrm{N}H-\mathrm{CH_2}$); 8.227–8.212, 8.152–8.136, 7.923–7.893, 7.864–7.835, 7.688–7.657, 7.498–7.483, 7.403–7.386 (d, d, t, t, t, d, d, 7H, protons attached to C2, C3, C4, C5, C6, C7, C8); 6.154–6.143 (d, 1H, $\mathrm{CH}-\mathrm{O}H$); 4.382 (m, 1H, $\mathrm{C}H-\mathrm{OH}$); 3.557–3.345 (broad m, 6H, $\mathrm{C}H_2-\mathrm{CH(OH)}-\mathrm{C}H_2-\mathrm{N^+(CH_3)_2}-\mathrm{C}H_2$); 3.149 (s, 6H, $\mathrm{N^+(C}H_3\mathrm{)_2}-\mathrm{(CH_2)_{15}}-\mathrm{CH_3}$); 1.729–1.603 (m, 2H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_{14}}-\mathrm{C}H_2-\mathrm{CH_3}$); centered at 1.228 (m, 2H, $\mathrm{N^+(CH_3)_2}-\mathrm{CH_2}-\mathrm{CH_2}-(\mathrm{C}H_2)_{13}-\mathrm{CH_3}$); 0.865–0.838 (t, 3H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_{15}C}H_3$).

2.2.5. Synthesis of Di-4

1,4-Diaminoanthraquinone (0.01 mol) and 0.10 mol of epichlorohydrin in 50 mL acetic acid were heated at 75 °C for 1 h followed by precipitation with water. The crude product was purified by methanol. Yield: 78%. Quanterization of the intermediate was conducted by following the same procedures as mono-substituted series except using a molar ratio of 1/5 (intermediate/tertiary amine). A good yield of 95% was reached. ^{1}H NMR spectra data (DMSO): δ 10.923 (s, 2H, v-$\mathrm{N}H-\mathrm{CH_2}$); 8.246–8.229, 7.815–7.797, 7.619 (m, m, s, 6H, protons attached to C2, C3, C4, C5, C6, C7, C8); 6.279–6.269 (d, 2H, $\mathrm{CH}-\mathrm{O}H$); 4.349 (m, 2H, $\mathrm{C}H-\mathrm{OH}$); 3.588–3.356 (broad m, 12H, $\mathrm{C}H_2-\mathrm{CH(OH)}-\mathrm{C}H_2-\mathrm{N^+(CH_3)_2}-\mathrm{C}H_2$); 3.150–3.138 (d, 12H, $\mathrm{N^+(C}H_3\mathrm{)_2}-\mathrm{(CH_2)_3}-\mathrm{CH_3}$); 1.728–1.606 (m, 4H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_2}-\mathrm{C}H_2-\mathrm{CH_3}$); 1.265–1.252 (m, 4H, $\mathrm{N^+(CH_3)_2}-\mathrm{CH_2}-\mathrm{CH_2}-\mathrm{C}H_2-\mathrm{CH_3}$); 0.893–0.869 (t, 6H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_3}-\mathrm{C}H_3$).

2.2.6. Synthesis of Di-8

Di-8 was prepared using the same procedure for preparation of Di-4. Yield: 74%. ^{1}H NMR spectra data (DMSO): δ 10.913 (s, 2H, $-\mathrm{N}H-\mathrm{CH_2}$); 8.240, 7.806, 7.636 (m, m, s, 6H, protons attached to C2, C3, C4, C5, C6, C7, C8); 6.279 (d, 2H, $\mathrm{CH}-\mathrm{O}H$); 4.329 (m, 2H, $\mathrm{C}H-\mathrm{OH}$); 3.541–3.357 (broad m, 12H, $\mathrm{C}H_2-\mathrm{CH(OH)}-\mathrm{C}H_2-\mathrm{N^+(CH_3)_2}-\mathrm{C}H_2$); 3.135 (s, 12H, $\mathrm{N^+(C}H_3\mathrm{)_2}-\mathrm{(CH_2)_7}-\mathrm{CH_3}$); 1.702–1.563 (m, 4H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_6}-\mathrm{C}H_2-\mathrm{CH_3}$); 1.247–1.172 (m, 4H, $\mathrm{N^+(CH_3)_2}-\mathrm{CH_2}-(\mathrm{C}H_2)_5-\mathrm{CH_2}-\mathrm{CH_3}$); 0.824–0.811 (t, 6H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_7C}H_3$).

2.2.7. Synthesis of Di-12

Di-12 was also prepared following the same procedure. Yield: 73%. ^{1}H NMR spectra data (DMSO): δ 10.913 (s, 2H, $-\mathrm{N}H-\mathrm{CH_2}$); 8.249–8.231, 7.809–7.792, 7.640 (m, m, s, 6H, protons attached to C2, C3, C4, C5, C6, C7, C8); 6.279–6.269 (d, 2H, $\mathrm{CH}-\mathrm{O}H$); 4.329 (m, 2H, $\mathrm{C}H-\mathrm{OH}$); 3.549–3.354 (broad m, 12H, $\mathrm{C}H_2-\mathrm{CH(OH)}-\mathrm{C}H_2-\mathrm{N^+(CH_3)_2}-\mathrm{C}H_2$); 3.135 (s, 12H, $\mathrm{N^+(C}H_3\mathrm{)_2}-\mathrm{(CH_2)_{11}}-\mathrm{CH_3}$); 1.701–1.559 (m, 4H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_{10}}-\mathrm{C}H_2-\mathrm{CH_3}$); centered at 1.194 (m, 4H, $\mathrm{N^+(CH_3)_2}-\mathrm{CH_2}-(\mathrm{C}H_2)_9-\mathrm{CH_2}-\mathrm{CH_3}$); 0.854–0.827 (t, 6H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_{11}C}H_3$).

2.2.8. Synthesis of Di-16

Di-16 was prepared according to the same method in synthesis of Di-4. Yield: 73%. ^{1}H NMR spectra data (DMSO): δ 10.868–10.845 (t, 2H, $-\mathrm{N}H-\mathrm{CH_2}$); 8.259–8.241, 7.801–7.783, 7.627 (m, m, s, 6H, protons attached to C2, C3, C4, C5, C6, C7, C8); 6.199–6.189 (d, 2H, $\mathrm{CH}-\mathrm{O}H$); 4.359 (m, 2H, $\mathrm{C}H-\mathrm{OH}$); 3.602–3.378 (broad m, 12H, $\mathrm{C}H_2-\mathrm{CH(OH)}-\mathrm{C}H_2-\mathrm{N^+(CH_3)_2}-\mathrm{C}H_2$); 3.154 (s, 12H, $\mathrm{N^+(C}H_3\mathrm{)_2}-\mathrm{(CH_2)_{11}}-\mathrm{CH_3}$); 1.729–1.608 (m, 4H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_{10}}-\mathrm{C}H_2-\mathrm{CH_3}$); centered at 1.232 (m, 4H, $\mathrm{N^+(CH_3)_2}-\mathrm{CH_2}-(\mathrm{C}H_2)_9-\mathrm{CH_2}-\mathrm{CH_3}$); 0.866–0.839 (t, 6H, $\mathrm{N^+(CH_3)_2}-\mathrm{(CH_2)_{11}C}H_3$).

2.3. Antimicrobial test

Antimicrobial activity of the agents in aqueous solution was evaluated by a minimum inhibitory concentration (MIC) procedure [25]. MIC refers to the lowest concentration of biocides that prohibit population and reproduction of microorganisms. In this method, 1 mL of an aqueous suspension containing 10^6–10^7 colony-forming units (CFU)/mL of *Staphylococcus aureus* (*S. aureus*, ATCC #12600, Gram-positive) or *Escherichia coli* (*E. coli*, K-12, Gram-negative) were placed into 9 mL aqueous solutions containing different concentrations of the agents for a contact time of 24 h. After the contact, a 100 μL aliquot of the resultant solution was serially diluted by sterilized distilled water to 10^1, 10^2, 10^3, 10^4 and 10^5. The last four dilutions (100 μL each) were placed onto a nutrient agar plate and incubated at 37 °C for 24 h. The same procedure was applied to a distilled water solution without the antimicrobial agents as a control. In this paper, the reported MIC of the antimicrobial colorants is the minimum concentrations that can eliminate more than 4 log reductions of bacteria (1 log reduction is 90%, 2 log reduction is 99%, and so forth).

2.4. Stability study

The stability of the antimicrobial colorants was qualitatively studied by using a UV–vis spectrophotometer under different experimental conditions. The tests were performed with antimicrobial colorant solutions at the concentration of 100 ppm in flasks. The colorants were sampled and the absorbance at the maximum absorption wavelength was tested before treatment as a reference. After visible light exposure in a conditioning room for over 30 days, or heating at boiling for 4 h, or changing to different pH conditions (pH = 4 and 10) for over 24 h, the solutions were sampled, centrifuged, filtered and tested by UV. The new UV–vis spectra were taken and compared with the control.

3. Results and discussion

3.1. Structure characterization

FT-IR spectra of the mono-substituted dyes are shown in Fig. 1. The infrared absorbance bands at 3419, 3304, and 1666 cm^{-1} in 1-aminoanthrquinone (A) were ascribed to $-NH_2$ and C=O stretching bands of the aminoanthraquinone structures, which is in agreement with the literature data [26]. In the spectrum of the intermediate (B), two stretching peaks representing primary amine in 1-aminoanthrquinone disappeared, proving the substitution of epichlorohydrin occurred on $-NH_2$. The intermediate (B) and all the mono-substituted colorants show one broad band in the region of 3200–3500 cm^{-1}, which was referenced as the combination stretching of secondary amine −NH and hydroxyl group −OH. It is also worth of noting that with the increasing QAS alkyl chain length, the intensities of the alkyl absorption bands (2800–3000 cm^{-1}) rose accordingly. Similar phenomena can be observed in the FT-IR spectra of the di-substituted colorants.

The chemical structures of QAS were confirmed by ^{1}H NMR and ^{1}H ^{1}H COSY spectra (Figs. 2 and 3). Taking M-4 as an example, the peak at 9.872 ppm is attributed to amino proton (H_a), the signal at 4.375 ppm is assigned to the proton on the carbon next to −OH (H_d). The proton in −OH showed chemical shifts to around 6.165–6.153 ppm. The coupling between H_a and H_b and other ^{1}H–^{1}H coupling can be observed from Fig. 3. By comparing the ^{1}H NMR spectra of M-4, M-8 and M-12, we can see that the intensity of alkyl groups (1.1–1.3 ppm) is going up significantly with the increase of alkyl chain length. The above analysis suggests that the antimicrobial colorants follow the proposed synthesis routes.

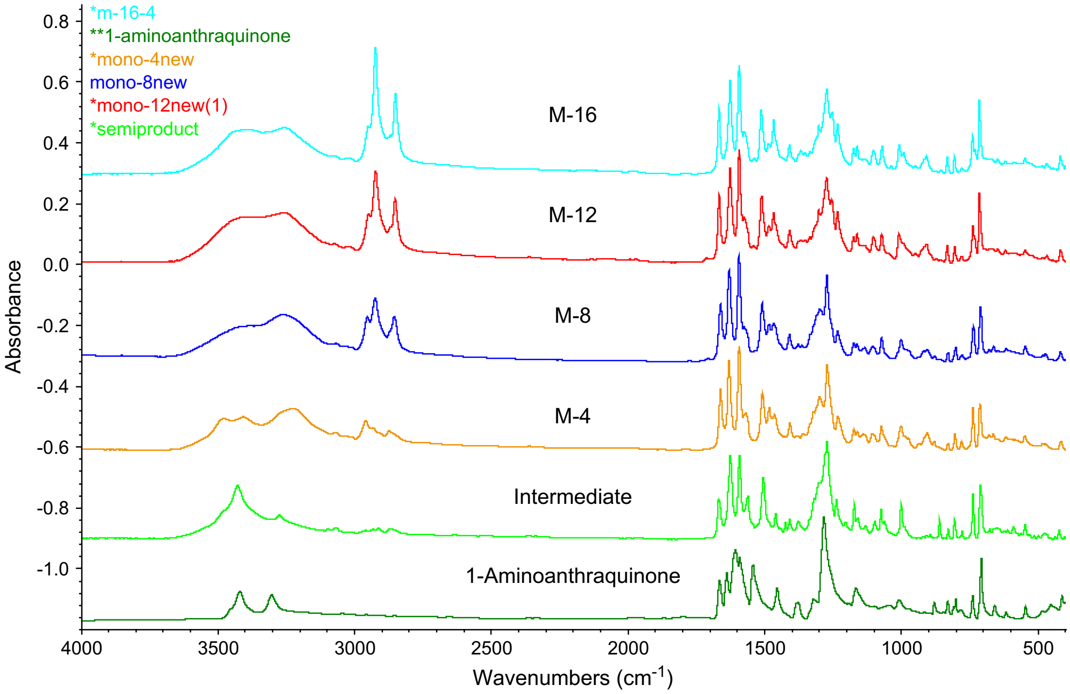


Fig. 1. FT-IR spectra of the M-series antimicrobial colorants.

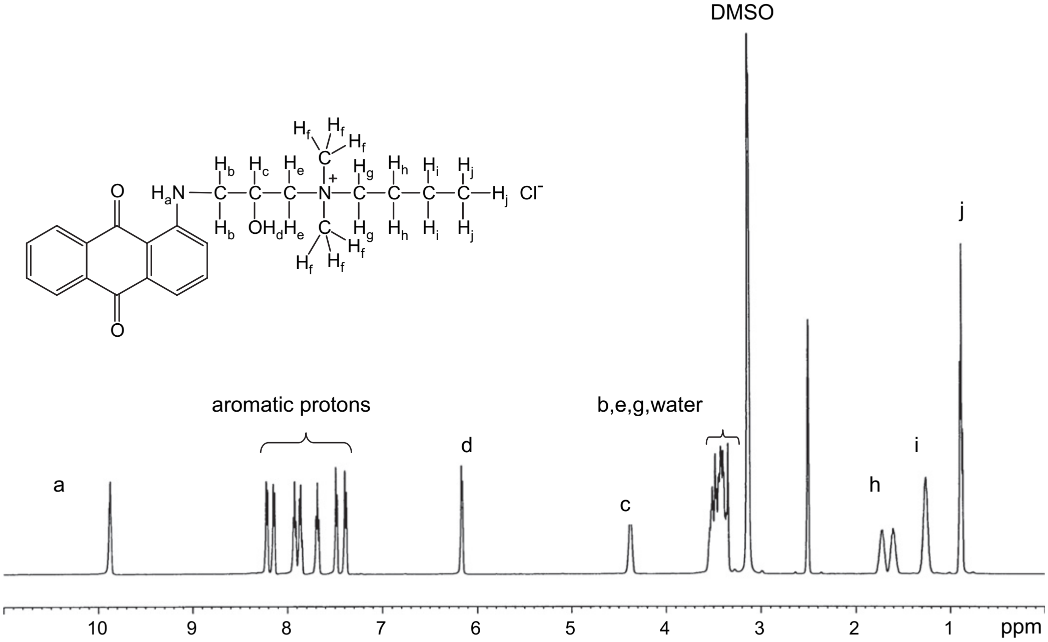


Fig. 2. ^{1}H NMR spectrum of M-4.

The UV–vis spectra of the synthesized QAS were measured to identify the absorbance–structure relationship and are revealed in Fig. 4. The di-substituted anthraquinone dyes show greater bathochromicity compared with the mono-substituted series as a result of the increasing p–π conjugation between the aromatic ring and amino groups. The additional auxochromic groups such as –NH, –OH and Cl in the di-substituted series further enhance this effect.

The mono-substituted QAS show the same maximum absorption wavelength (λ_{max}) at 503 nm, while the di-substituted series present a bathochromic shift from 628 nm to 631.5 nm. This phenomenon can be interpreted by two factors: Steric hindrance and possible intramolecular hydrogen bonding. The longer alkane chains in the di-substituted QAS render relatively greater steric hindrance effect, which induces a red shift. Possible intramolecular hydrogen bonding within the dye molecules may also leads to bathochromic shift by holding the groups in a planar configuration [22].

3.2. Antimicrobial assessment

Antimicrobial properties of the antimicrobial colorants in aqueous solutions were measured by the MIC procedure. The MIC of the antimicrobial colorants against both *E. coli* and *S. aureus* are listed in Table 2. The results indicate that the colorants can inactivate both Gram-negative and Gram-positive bacteria effectively. Generally speaking, Gram-negative bacteria are more resistant to QAS due to the thick lipopolysaccharide wall structure. However, no difference in antimicrobial efficacy

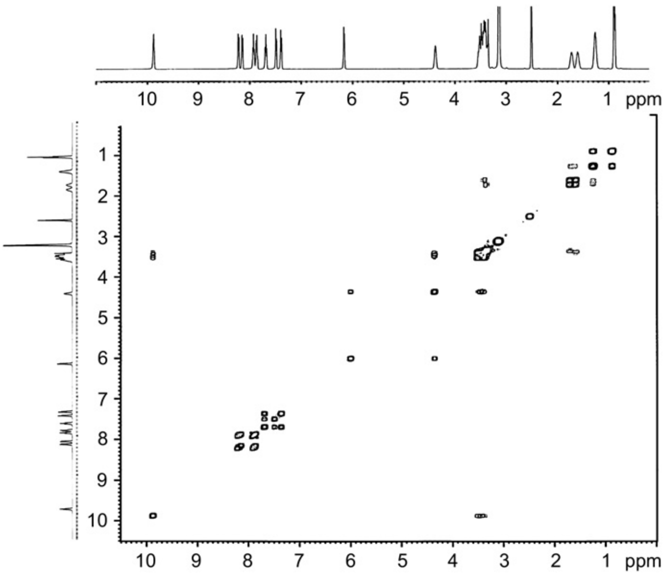


Fig. 3. ^{1}H ^{1}H COSY spectrum of M-4.

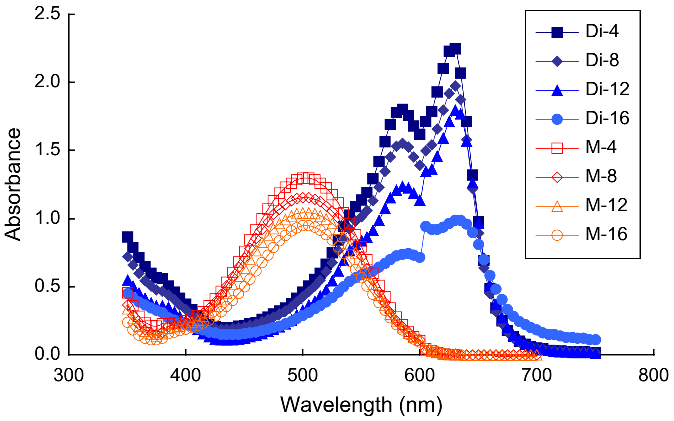


Fig. 4. UV–vis absorbance spectra (concentration = 100 ppm).

Table 2
MIC of cationic colorants (ppm)

		Hydrocarbon chain length			
		4	8	12	16
Mono	*Escherichia coli*	200	5	4	60
	Staphylococcus aureus	200	5	4	60
Di	*E. coli*	200	5	4	60
	S. aureus	200	5	4	60

Note: 10^7-10^8 CFU/ml, contact time: 24 h.

was detected on these two microorganisms from these two series of cationic colorants under this testing condition. The MIC values indicated that all eight colorants could destroy bacteria completely at quite low concentrations, depending on the hydrocarbon chain lengths of them. The colorants with dodecyl group showed the most powerful function. Not surprisingly, the antimicrobial efficacy of the colorants bearing butyl group are relatively low due to short alkyl chain length. In addition, the M-series and Di-series with the same chain length show no distinct biocidal activities using this test method even though Di-series compounds possess two QAS structures in one molecule.

3.3. Stability of cationic colorant solutions

The stability of the antimicrobial colorants is of great importance because they are mostly applied in aqueous solutions as biocides or dyes. All stability tests were conducted at a concentration of 100 ppm. UV–vis absorbance of the colorant solutions was observed to examine their stability under different conditions. The UV–vis spectra before and after light exposure showed no appreciable difference, indicating excellent stability of the colorants under visible light. Compared with triphenylmethane dyes that could degrade in half of hour under unfiltered daylight [27,28], this colorant showed outstanding stability against daylight.

In practical applications, these colorants could be used under either acidic or basic conditions, particularly in coloration of acrylics, nylon and wool. For example, acidic dye bath is preferred for dyeing wool fabrics. Thus, the stability of the colorants in low or high pH solution is more important for textile applications. In fact, the colorants prepared previously exhibited very disappointing hydrolytic stability, particularly under alkaline conditions [23]. The low stability of those colorants was caused by hydrolysis of an amide linkage between QAS and aminoanthraquinone. Thus, in the new colorants the amide bond is replaced by alkyl amino structures that are resistant to both acidic and alkaline hydrolysis. The colorant solutions were adjusted to pH 4 and pH 10 by using pH buffers at the concentration of 100 ppm for 24 h. UV–vis spectra of the solutions were compared with the solution prepared at neutral. No dramatic shift of both wavelengths and absorbance was observed, indicating that the colorants were stable under the tested pH range. The results strongly supported the expectation of the new structures.

Hydrolysis of the structures could be catalyzed by elevated temperature. So the colorant solutions should be able to stand long duration of heating in dyeing process. In this test, the colorants at the concentration of 100 ppm were heated at 100 °C for 4 h in aqueous solutions. UV–vis spectra of M-12 and Di-12 before and after heating are shown in Fig. 5, the wavelengths of the two colorants showed negligible change (within system error); the slight increase in absorbance are believed to be caused by the loss of water when heating. This is another significant improvement compared with the earlier work [23].

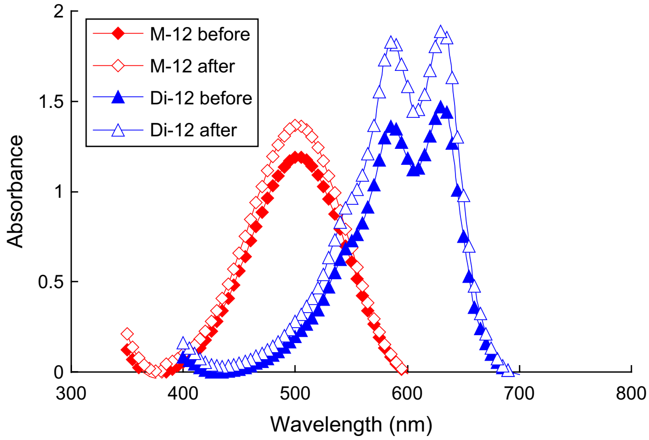


Fig. 5. UV–vis spectra of M-12 and Di-12 solutions (100 ppm concentration, 100 °C).

4. Conclusions

Two series of antimicrobial colorants with different hydrocarbon chain lengths were prepared by connection of quaternary ammonium salt groups with anthraquinone dyes. The minimum inhibition concentrations of the colorants were dependent on the hydrocarbon chain length and in an order of C12 > C8 > C16 > C4. The antibacterial activities of the Di-series compounds were similar to the M-series. All colorants exhibited different antimicrobial efficacy against both Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*) in terms of MIC. The colorants were very stable under daylight, heating and at different pH conditions in aqueous solutions.

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