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# Synthesis and characterization of cationic monoazo dyes incorporating quaternary ammonium salts

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## Abstract

Nine (three series) antimicrobial cationic monoazo dyes were prepared by a diazotization−coupling reaction involving two aromatic amino compounds (featuring different chain length alkyl quaternary ammonium salts) as diazo components, and *N*,*N*-dimethyl-benzeneamine and 1-phenyl-3-methyl-5-pyrazolone as coupling components. Structures of the nine dyes were determined by FTIR, $^1$H NMR, $^{13}$C NMR, and ESI-MS analysis, and have not been previously published. Wavelengths of maximum absorption ($\lambda_{max}$) and absorbance of three series dyes were tested by UV/vis spectrometer. It was found that alkyl chain length did not significantly affect $\lambda_{max}$, but did affect absorbance. Minimum inhibitory concentration (MIC) data showed the efficacy of these dyes against both *Staphylococcus aureus* and *Escherichia coli*. Nine dyes were especially sensitive to *S. aureus*. The aqueous solution of dye series **1** with *N*,*N*-dimethyl-benzeneamine as the coupling component had better antimicrobial efficacy than did the aqueous solution of dye series **2** and **3** with 1-phenyl-3-methyl-5-pyrazolone as coupling component. Antimicrobial effect increased as hydrocarbon chain length increased, with other factors held constant.

*Keywords:* Antimicrobial; Cationic dye; Minimum inhibitory concentration; Quaternary ammonium salts; Monoazo dye; Diazotization−coupling

## 1. Introduction

Textile surfaces are porous and surface areas typically are quite large, allowing prolific bacterial growth under favorable temperature and humidity conditions. Pathogenic bacteria are the source of many human diseases. Antimicrobial finishing is an important step in textile finishing, particularly for textiles that will come in contact with children, doctors, nurses and soldiers. Textile finishing increases the utility of textiles, but requires more energy resources and uses greater amounts of water. Producing functional dyes with special finishing capabilities currently is an area of active research [1]. Many functional dyes, such as water-repellent dyes [2,3], anti-UV radiation dyes [4−6], and antimicrobial anthraquinone dyes [7−10], have been reported.

Azo dyes account for more than 50% of currently available dyes. Azo dyes are available in a wide color selection and are easy to produce. Cationic surfactants involving quaternary ammonium salts have broad-spectrum antimicrobial activity. In this paper, nine antimicrobial cationic monoazo dyes having dyeing and antimicrobial characteristic were synthesized by a diazotization−coupling reaction. No previous reports have indicated synthesis of these dyes. The structures of the nine dyes are given in Fig. 1.

The antimicrobial efficacy of aqueous dye solutions was tested by a minimum inhibitory concentration (MIC) method.

## 2. Experimental

### 2.1. Materials and instrumentation

1,2-Dibromoethane, *p*-nitrophenol, *n*-propylamine, *n*-octylamine, *p*-nitrobenzyl bromide, 1-phenyl-3-methyl-5-pyrazolone,

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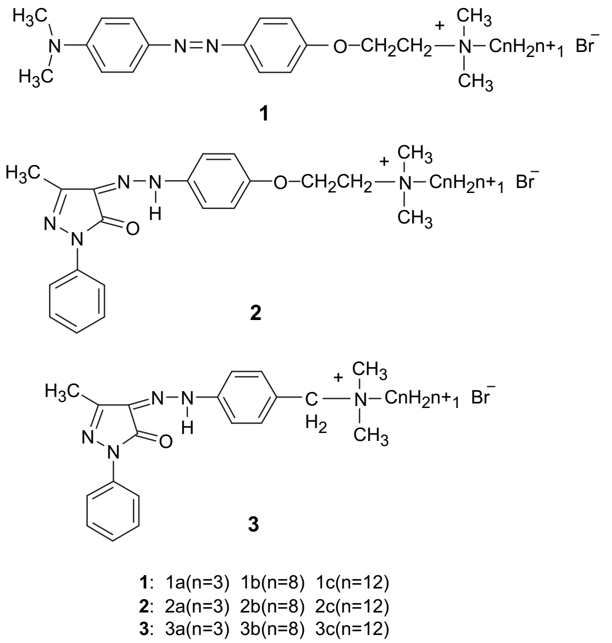

Fig. 1. Structures of nine antimicrobial cationic monoazo dyes.

stannous chloride, sodium nitrite and *N*,*N*-dimethyl-benzeneamine were of analytical purity. *N*,*N*-Dimethyl-dodecylamine (≥98%) was an industrial-grade product (Shanghai Jingwei Chemical Corporation). Formic acid (≥88%), formaldehyde (37–40%) and hydrochloric acid (36%) were also used. All reagents were not subjected to additional purification.

IR spectra were determined on a Perkin–Elmer 983 infrared spectrometer. $^1$H NMR and $^{13}$C NMR spectra were measured on a Mercury 300 (Varian) NMR spectrometer. Mass spectrum data were obtained on a Quattro Micro API (Waters) mass spectrometer. Electronic absorption spectra were determined on a UV-1601 PC (Shimadzu) spectrometer. Melting points were determined by capillary method with an uncalibrated thermometer.

### *2.2. Synthesis*

The route of dye synthesis is shown in Figs. 2 and 3.

#### *2.2.1. Synthesis of compound **4***

Synthesis was done according to Ref. [11].

Yield: 79%
Mp: 64 °C (lit. 64 °C).

#### *2.2.2. Synthesis of compound **5a***

Synthesis was done according to Ref. [12].

Yield: 80%
Bp: 63–64 °C (lit. 65.5 °C).

#### *2.2.3. Synthesis of compound **5b***

Synthesis was done according to Ref. [13].

Yield: 92%
Bp: 188–190 °C (lit. 191 °C).

#### *2.2.4. Synthesis of compound **6***

A mixture of compound **4** (10 mmol) and compound **5a** (or **5b** or *N*,*N*-dimethyl-dodecylamine) (500 mmol) was added to a three-neck flask with a mechanical stirrer and reflux condenser. The mixture was stirred well at 30–70 °C for 6–8 h. Excess tertiary amine was removed and the product was obtained.

##### *2.2.4.1. Compound **6a***

Yield: 92%

Viscous compound; IR (KBr, cm$^{-1}$): 2960, 2880 ($CH_3CH_2CH_2$–); 1510, 1340 (–$NO_2$); 1250, 1050 (Ar–O–$CH_3$); 860 (C–$NO_2$); $^1$H NMR (300 MHz, $CDCl_3$,

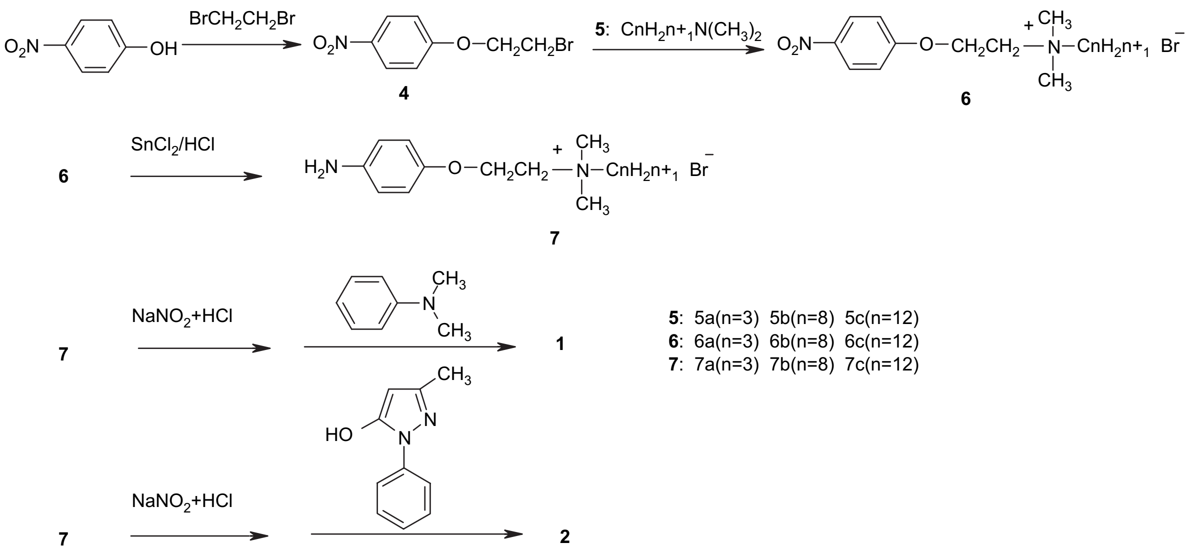

Fig. 2. Synthesis route of dyes series **1** and **2**.

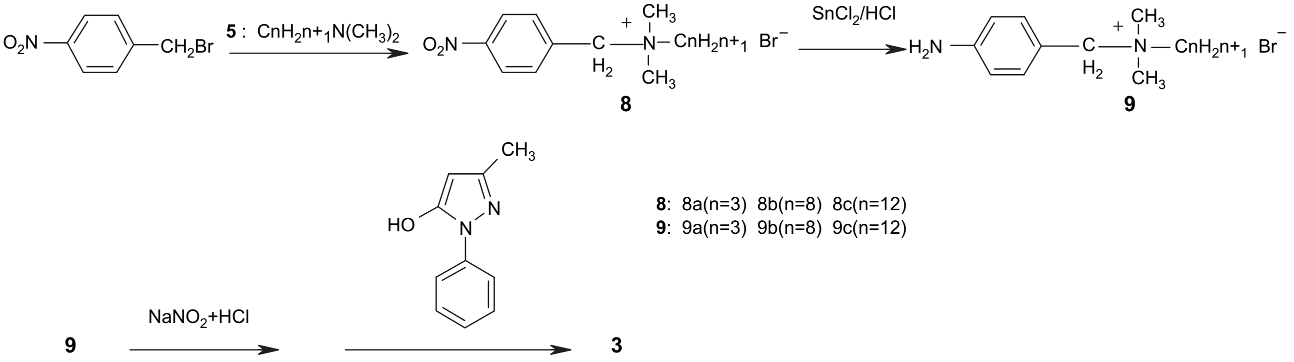

Fig. 3. Synthesis route of dye series **3**.

$\delta$): 1.030–1.054 (t, 3H), 2.073 (br s, 2H), 3.325 (s, 6H), 3.739 (br s, 2H), 4.246 (br s, 2H), 4.900 (br s, 2H), 7.332–7.364 (d, 2H), 8.228–8.251 (d, 2H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 254.2.

#### 2.2.4.2. *Compound* ***6b***

Yield: 94%

Mp: 139–140 °C; IR (KBr, $cm^{-1}$): 2960, 2860 ($CH_3(CH_2)_6CH_2$–); 1520, 1350 (–$NO_2$); 1220, 1020 (Ar–O–$CH_3$); 850 (C–$NO_2$); $^1H$ NMR (300 MHz, $CDCl_3$, $\delta$): 0.851–0.874 (t, 3H), 1.264 (br s, 8H), 1.374 (br s, 2H), 1.847 (br s, 2H), 3.647 (s, 6H), 3.663 (br s, 2H), 4.308 (br s, 2H), 4.745 (br s, 2H), 7.083–7.114 (d, 2H), 8.157–8.186 (d, 2H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 324.4.

#### 2.2.4.3. *Compound* ***6c***

Yield: 96%

Mp: 105–106 °C; IR (KBr, $cm^{-1}$): 2920, 2840 ($CH_3(CH_2)_{10}CH_2$–); 1510, 1340 (–$NO_2$); 1250, 1050 (Ar–O–$CH_3$); 860 (C–$NO_2$); $^1H$ NMR (300 MHz, $CDCl_3$, $\delta$): 0.857–0.902 (t, 3H), 1.255 (br s, 16H), 1.384 (br s, 2H), 1.842 (br s, 2H), 3.524 (s, 6H), 3.624 (br s, 2H), 4.328 (br s, 2H), 4.742 (br s, 2H), 7.078–7.105 (d, 2H), 8.192–8.219 (d, 2H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 380.4.

### 2.2.5. *Synthesis of compound* ***7***

A mixture of compound **6** (80 mmol) and ethanol (300–400 ml) was added to a four-neck flask with a mechanical stirrer and reflux condenser, and a mixture of $SnCl_2 \cdot 2H_2O$ (280 mmol) and concentrated hydrochloric acid (36%, 2500 mmol) was dropped to the flask at room temperature and then the whole mixture was allowed to react at 30–50 °C for 6–12 h. The pH was adjusted to 8–10 with sodium hydroxide aqueous solution (10%), filtrated and the residue was washed with ethanol. Ethanol and water were evaporated under vacuum. Crude product was dissolved with ethanol, and the indiscerptible salt was filtrated. Then ethanol was removed under vacuum and the product was obtained.

#### 2.2.5.1. *Compound* ***7a***

Yield: 87%

Viscous compound; IR (KBr, $cm^{-1}$): 3300, 3220, 1680 (–$NH_2$); 2960, 2880 ($CH_3CH_2CH_2$–); 1230, 1060 (Ar–O–$CH_3$); $^1H$ NMR (300 MHz, $CDCl_3$, $\delta$): 1.055–1.079 (t, 3H), 1.893 (br s, 2H), 3.130 (s, 6H), 3.757 (br s, 2H), 4.202 (br s, 2H), 4.363 (br s, 2H), 6.469–6.566 (m, 4H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 224.2.

#### 2.2.5.2. *Compound* ***7b***

Yield: 85%

Mp: 118–120 °C; IR (KBr, $cm^{-1}$): 3320, 3200, 1640 (–$NH_2$); 2920, 2840 ($CH_3(CH_2)_6CH_2$–); 1230, 1070 (Ar–O–$CH_3$); $^1H$ NMR (300 MHz, $CDCl_3$, $\delta$): 0.850–0.894 (t, 3H), 1.256 (br s, 8H), 1.340 (br s, 2H), 1.774 (br s, 2H), 3.423 (s, 6H), 3.598 (br s, 2H), 4.077 (br s, 2H), 4.309 (br s, 2H), 6.654–6.719 (m, 4H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 294.3.

#### 2.2.5.3. *Compound* ***7c***

Yield: 84%

Mp: 79–82 °C; IR (KBr, $cm^{-1}$): 3320, 3200, 1590 (–$NH_2$); 2920, 2830 ($CH_3(CH_2)_{10}CH_2$–); 1230, 1060 (Ar–O–$CH_3$); $^1H$ NMR (300 MHz, $CDCl_3$, $\delta$): 0.856–0.900 (t, 3H), 1.248 (br s, 16H), 1.332 (br s, 2H), 1.793 (br s, 2H), 3.434 (s, 6H), 3.544 (br s, 2H), 4.202 (br s, 2H), 4.324 (br s, 2H), 6.635–6.679 (m, 4H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 350.3.

### 2.2.6. *Synthesis of compound* ***1***

Compound **7** (3.52 mmol), concentrated hydrochloric acid (36%, 96.5 mmol) and water (5–10 ml) were stirred to dissolve at 0–2 °C and sodium nitrite aqueous solution (30%, 3.52 mmol) was dropped. Diazotate was obtained over 2 h. Concentrated hydrochloric acid (36%) was dropped to the mixture of *N*,*N*-dimethyl-benzeneamine (3.52 mmol) and water (10–20 ml) under stirring until they get dissolved and diazotate was dropped to the coupling bath. The pH was adjusted to 6–8 by dropwise addition of aqueous sodium carbonate and reaction was carried out at 5–15 °C for 3–12 h. The mixture was salted out with sodium chloride, filtrated and dried. The residue obtained was dissolved with ethanol, the indiscerptible salt was filtrated, ethanol was removed under vacuum and finally the product was obtained.

#### 2.2.6.1. *Compound* ***1a***

Yield: 87%

Mp: >250 °C; UV/vis ($H_2O$): $\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$): 453 (7704); IR (KBr, cm$^{-1}$): 2920, 2880 ($CH_3CH_2CH_2$–); 1400 (N=N); 1360 (–$N(CH_3)_2$); 1240, 1060 (Ar–O–$CH_3$); $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 1.057–1.104 (t, 3H), 1.903 (s, 2H), 3.091 (s, 6H), 3.518 (s, 6H), 3.553 (br s, 2H), 4.316 (br s, 2H), 4.557 (br s, 2H), 6.739–6.770 (d, 2H), 6.969–7.000 (d, 2H), 7.833–7.863 (m, 4H); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$): 10.654, 16.423, 40.294, 51.893, 62.485, 62.562, 67.659, 111.524, 114.469, 124.069, 124.741, 143.499, 148.474, 152.290, 157.723; ESI-MS (*m*/*z*, $M^+ - Br + H$): 356.3.

#### 2.2.6.2. Compound **1b**

Yield: 84%

Mp: >250 °C; UV/vis ($H_2O$): $\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$): 453 (13482); IR (KBr, cm$^{-1}$): 2920, 2850 ($CH_3(CH_2)_6CH_2$–); 1400 (N=N); 1360 (–$N(CH_3)_2$); 1240, 1060 (Ar–O–$CH_3$); $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 0.852–0.891 (t, 3H), 1.261 (br s, 8H), 1.348 (br s, 2H), 1.797 (br s, 2H), 3.079 (s, 6H), 3.495 (s, 6H), 3.616 (br s, 2H), 4.241 (br s, 2H), 4.552 (br s, 2H), 6.731–6.762 (d, 2H), 6.975–7.004 (d, 2H), 7.821–7.846 (m, 4H); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$): 13.768, 22.238, 22.574, 25.932, 28.755, 28.847, 31.304, 39.958, 51.786, 61.768, 62.165, 65.523, 111.188, 114.515, 123.596, 124.375, 142.995, 147.757, 151.893, 157.769; ESI-MS (*m*/*z*, $M^+ - Br + H$): 426.5.

#### 2.2.6.3. Compound **1c**

Yield: 83%

Mp: >250 °C; UV/vis ($H_2O$): $\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$): 452 (22540); IR (KBr, cm$^{-1}$): 2920, 2840 ($CH_3(CH_2)_{10}CH_2$–); 1400 (N=N); 1360 (–$N(CH_3)_2$); 1240, 1060 (Ar–O–$CH_3$); $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 0.855–0.900 (t, 3H),1.252 (br s, 16H), 1.360 (br s, 2H), 1.816 (br s, 2H), 3.093 (s, 6H), 3.494 (s, 6H), 3.592 (br s, 2H), 4.266 (br s, 2H), 4.489 (br s, 2H), 6.723–6.745 (d, 2H), 6.951–6.980 (d, 2H), 7.727–7.812 (m, 4H); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$): 13.920, 22.467, 22.727, 26.100, 29.030, 29.106, 29.213, 29.274, 29.381, 31.686, 40.095, 51.908, 61.905, 62.241, 65.568, 111.325, 114.622, 123.764, 124.512, 143.271, 148.119, 152.365, 157.896; ESI-MS (*m*/*z*, $M^+ - Br + H$): 482.4.

### 2.2.7. Synthesis of compound **2**

Compound **7** (3.52 mmol), concentrated hydrochloric acid (36%, 96.5 mmol) and water (5–10 ml) were stirred until dissolving at 0–2 °C and sodium nitrite aqueous solution (30%, 3.52 mmol) was dropped. Diazotate was obtained over 2 h. 1-Phenyl-3-methyl-5-pyrazolone (3.52 mmol), sodium carbonate (1.8 mmol) and water (10–20 ml) were stirred until dissolving and diazotate was dropped to the coupling bath. The pH was adjusted to 5–6 by dropwise addition of aqueous sodium carbonate and the reaction was carried out at 5–15 °C for 3–12 h. The mixture was salted out with sodium chloride, filtrated and dried. The residue obtained was dissolved with ethanol, the indiscerptible salt was filtrated, ethanol was removed under vacuum and finally the product was obtained.

#### 2.2.7.1. Compound **2a**

Yield: 90%

Mp: >250 °C; UV/vis ($H_2O$): $\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$): 409 (24124); IR (KBr, cm$^{-1}$): 3400 (–NH–); 2930, 2880 ($CH_3CH_2CH_2$–); 1610 (–C=O); 1240, 1010 (Ar–O–$CH_3$); $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 1.038–1.087 (t, 3H), 1.684 (s, 2H), 2.366 (s, 3H), 3.496 (s, 6H), 3.551 (s, 2H), 4.380 (br s, 2H), 4.530 (br s, 2H), 6.953–6.980 (d, 2H), 7.187–7.208 (d, 2H), 7.401 (s, 1H), 7.425–7.454 (d, 2H), 7.934–7.961 (d, 2H), 13.609 (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$): 10.738, 11.768, 16.500, 52.160, 62.730, 62.814, 67.530, 115.622, 117.323, 118.476, 125.092, 128.076, 128.885, 136.020, 138.019, 148.352, 155.014, 157.784; ESI-MS (*m*/*z*, $M^+ - Br + H$): 409.4.

#### 2.2.7.2. Compound **2b**

Yield: 88%

Mp: >250 °C; UV/vis ($H_2O$): $\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$): 409 (16562); IR (KBr, cm$^{-1}$): 3440(–NH–); 2930, 2860 ($CH_3(CH_2)_6CH_2$–); 1620 (–C=O); 1240, 1000 (Ar–O–$CH_3$); $^1$H NMR (300 MHz, $CDCl_3 + (CF_3CO)_2O$, $\delta$): 0.867–0.910 (t, 3H),1.291 (br s, 8H), 1.385 (br s, 2H), 1.843 (br s, 2H), 2.612 (s, 3H), 3.241 (br s, 6H), 3.406 (br s, 2H), 3.840 (br s, 2H), 4.518 (br s, 2H), 7.032–7.063 (d, 2H), 7.458–7.527 (m, 5H), 7.690–7.719 (d, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$): 11.295, 13.600, 22.070, 22.742, 26.069, 28.664, 28.832, 31.182, 52.809, 62.470, 63.004, 65.721, 115.522, 116.759, 117.751, 124.466, 127.152, 128.358, 135.104, 137.516, 147.680, 154.793, 157.052; ESI-MS (*m*/*z*, $M^+ - Br + H$): 479.5.

#### 2.2.7.3. Compound **2c**

Yield: 87%

Mp: >250 °C; UV/vis ($H_2O$): $\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$): 406 (11382); IR (KBr, cm$^{-1}$): 3440 (–NH–); 2920, 2850 ($CH_3(CH_2)_{10}CH_2$–); 1610 (–C=O); 1240, 1000 (Ar–O–$CH_3$); $^1$H NMR (300 MHz, $CDCl_3 + (CF_3CO)_2O$, $\delta$): 0.853–0.898 (t, 3H), 1.262 (br s, 16H), 1.340 (d, 2H), 1.773 (br s, 2H), 2.303 (s, 3H), 3.203 (s, 6H), 3.384 (br s, 2H), 3.875 (br s, 2H), 4.477 (br s, 2H), 6.988–7.014 (d, 2H), 7.130–7.179 (d, 2H), 7.297 (s, 1H), 7.324–7.348 (d, 2H), 7.607–7.631 (d, 2H); $^{13}$C NMR (75 MHz, $CDCl_3 + (CF_3CO)_2O$, $\delta$): 12.747, 14.277, 22.985, 23.088, 26.472, 29.391, 29.650, 29.753, 29.895, 29.918, 32.234, 52.151, 62.369, 63.201, 66.746, 115.292, 119.577, 126.167, 128.834, 129.185, 137.701, 150.277, 158.294; ESI-MS (*m*/*z*, $M^+ - Br + H$): 535.6.

### 2.2.8. Synthesis of **8**

A mixture of *p*-nitrobenzyl bromide (23.26 mmol) and compound **5a** (or **5b** or *N*,*N*-dimethyl-dodecylamine) (117 mmol) was added to a three-neck flask with a mechanical stirrer and reflux condenser. The mixture was stirred well at 30–70 °C for 3–6 h. Excess tertiary amine was removed and the product was obtained.

#### 2.2.8.1. Compound **8a**

Yield: 93%

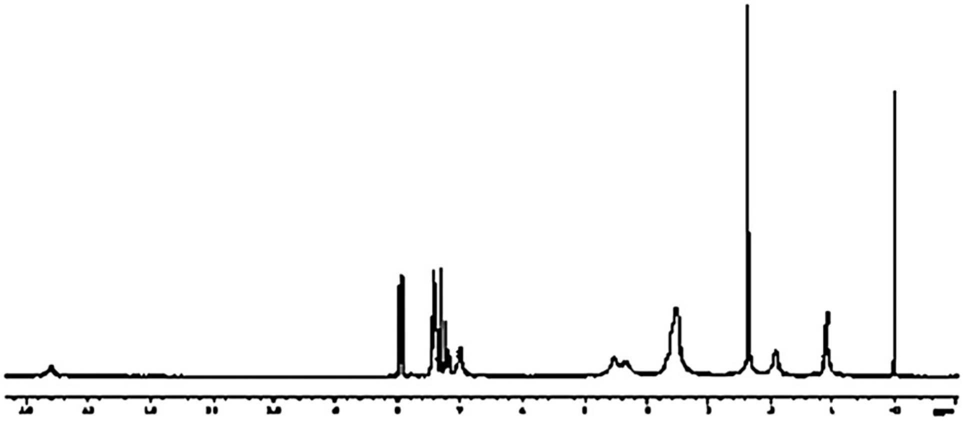

Fig. 4. The $^1$H NMR spectrum of dye **2a**.

Viscous compound; IR (KBr, cm$^{-1}$): 2920, 2860 ($CH_3CH_3CH_2-$); 1520, 1340 ($-NO_2$); 860 ($C-NO_2$); $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 1.030–1.053 (t, 3H), 1.963 (br s, 2H), 3.325 (s, 6H), 3.612 (br s, 2H), 5.420 (br s, 2H), 8.085–8.095 (d, 2H), 8.285–8.299 (d, 2H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 224.1.

### 2.2.8.2. Compound **8b**

Yield: 97%

Mp: 100–101 °C; IR (KBr, cm$^{-1}$): 2920, 2860 ($CH_3(CH_2)_6CH_2-$); 1520, 1350 ($-NO_2$); 860 ($C-NO_2$); $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 0.861–0.882 (t, 3H), 1.274 (br s, 8H), 1.342 (br s, 2H), 1.843 (br s, 2H), 3.356 (s, 6H), 3.636 (br s, 2H), 5.455 (s, 2H), 8.089–8.115 (d, 2H), 8.247–8.273 (d, 2H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 294.3.

### 2.2.8.3. Compound **8c**

Yield: 98%

Mp: 124–125 °C; IR (KBr, cm$^{-1}$): 2920, 2840 ($CH_3(CH_2)_{10}CH_2-$);1510, 1340 ($-NO_2$); 850 ($C-NO_2$); $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 0.856–0.900 (t, 3H), 1.236 (br s, 16H), 1.331 (br s, 2H), 1.826 (br s, 2H), 3.353 (s, 6H), 3.590 (br s, 2H), 5.488 (s, 2H), 8.048–8.078 (d, 2H), 8.255–8.284 (d, 2H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 350.4.

### 2.2.9. Synthesis of compound **9**

Synthesis was done according to compound **7**.

### 2.2.9.1. Compound **9a**

Yield: 80%

Viscous compound; IR (KBr, cm$^{-1}$): 3300, 3280, 1610 ($-NH_2$); 2920, 2840 ($CH_3CH_3CH_2-$); $^1$H NMR (300 MHz, $D_2O$, $\delta$): 1.018–1.048 (t, 3H), 1.775 (br s, 2H), 2.979 (s, 6H), 3.553 (br s, 2H), 4.412 (br s, 2H), 6.779–6.796 (d, 2H), 7.346–7.401 (d, 2H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 194.3.

### 2.2.9.2. Compound **9b**

Yield: 89%

Mp: 80–82 °C; IR (KBr, cm$^{-1}$): 3330, 3250, 1615 ($-NH_2$); 2920, 2840 ($CH_3(CH_2)_6CH_2-$); $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 0.854–0.892 (t, 3H), 1.259 (br s, 8H), 1.329 (br s, 2H), 1.864 (br s, 2H), 2.203–2.252 (s, 6H + br s, 2H), 4.382 (s, 2H), 6.613–6.642 (d, 2H), 7.103–7.133 (d, 2H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 264.4.

### 2.2.9.3. Compound **9c**

Yield: 88%

Mp: 160–163 °C; IR (KBr, cm$^{-1}$): 3350, 3220, 1600 ($-NH_2$); 2920, 2840 ($CH_3(CH_2)_{10}$ $CH_2-$); $^1$H NMR (300 MHz, $CDCl_3$,

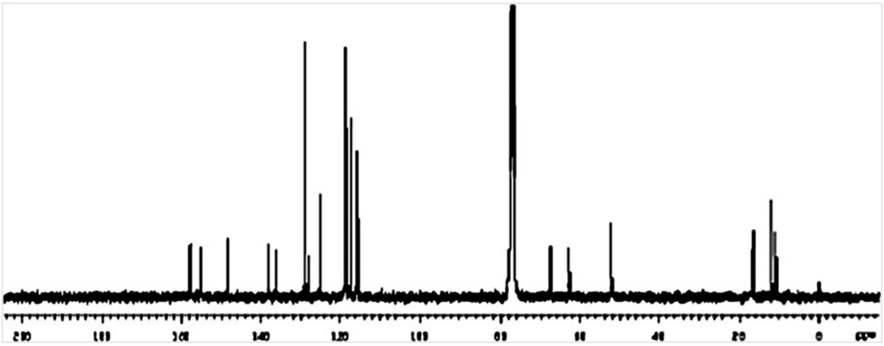

Fig. 5. The $^{13}$C NMR spectrum of dye **2a**.

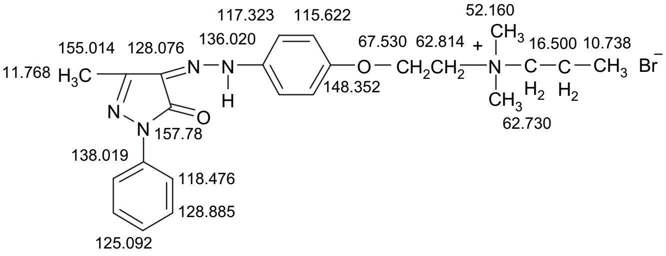

Fig. 6. The $^{13}$C NMR spectrum peak identification of dye **2a**.

δ): 0.855–0.900 (t, 3H), 1.220–1.227 (br s, 18H), 1.415 (br s, 2H), 2.203–2.252 (s, 6H + br s, 2H), 4.382 (s, 2H), 6.613–6.642 (d, 2H), 7.103–7.133 (d, 2H); ESI-MS (*m*/*z*, $M^+ - Br + H$): 320.3.

### 2.2.10. *Synthesis of compound* ***3***

Synthesis was done according to compound **2**.

#### 2.2.10.1. *Compound* ***3a***

Yield: 76%

Mp: >250 °C; UV/vis ($H_2O$): $\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$): 393 (2758); IR (KBr, cm$^{-1}$): 3350 (–NH–); 2920, 2830 ($CH_3CH_3CH_2$–); 1660 (–C=O); $^1$H NMR (300 MHz, $CDCl_3$, δ): 0.920–0.963 (t, 3H), 1.590 (s, 2H), 2.317 (s, 3H), 2.478 (s, 6H), 3.412 (s, 2H), 4.213 (br s, 2H),7.134–7.163 (d, 2H), 7.187–7.216 (d, 2H), 7.350 (s, 1H), 7.489–7.605 (d, 2H), 7.924–7.951 (d, 2H), 13.596 (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$, δ): 10.691, 11.761, 14.092, 46.170, 59.265, 65.542, 116.270, 118.564, 119.495, 125.226, 128.496, 128.911, 133.263, 137.920, 148.592, 157.674; ESI-MS (*m*/*z*, $M^+ - Br + H$): 379.3.

#### 2.2.10.2. *Compound* ***3b***

Yield: 83%

Mp: >250 °C; UV/vis ($H_2O$): $\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$): 393 (2694); IR (KBr, cm$^{-1}$): 3360 (–NH–); 2920, 2830 ($CH_3(CH_2)_6CH_2$–); 1660 (–C=O); $^1$H NMR (300 MHz, $CDCl_3$, δ): 0.858–0.881 (t, 3H), 1.253 (br s, 8H), 1.350 (br s, 2H), 1.836 (br s, 2H), 2.362 (s, 3H), 3.490 (s, 6H), 3.745 (br s, 2H), 5.254 (br s, 2H), 7.121–7.148 (d, 2H), 7.230–7.265 (d, 2H), 7.348 (s, 1H), 7.428–7.454 (d, 2H), 7.918–7.946 (d, 2H), 13.585 (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$, δ): 11.579, 13.871, 22.360, 22.450, 26.370, 29.122, 29.354, 31.428, 50.202, 64.055, 67.610, 116.029, 118.206, 118.257, 125.097128.459, 128.690, 135.015, 137.540, 148.282, 157.200, 157.402; ESI-MS (*m*/*z*, $M^+ - Br + H$): 449.3.

#### 2.2.10.3. *Compound* ***3c***

Yield: 81%

Mp: >250 °C; UV/vis ($H_2O$): $\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$): 393 (854); IR (KBr, cm$^{-1}$): 3360 (–NH–); 2920, 2830 ($CH_3(CH_2)_{10}CH_2$–); 1660 (–C=O); $^1$H NMR (300 MHz, $CDCl_3$, δ): 0.851–0.895 (t, 3H), 1.241 (br s, 18H), 1.754 (br s, 2H), 2.362 (s, 3H), 3.238 (s, 6H), 3.415 (br s, 2H), 5.228 (br s, 2H), 7.109–7.132 (d, 2H), 7.208–7.233 (d, 2H), 7.338 (s, 1H), 7.424–7.450 (d, 2H),7.918–7.943 (d, 2H), 13.581 (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$, δ): 11.692, 14.141, 22.396, 22.730, 26.140, 28.997, 29.045, 29.885, 30.025, 31.596, 51.933, 64.069, 67.564, 115.671, 118.380, 118.415, 125.012, 128.297, 128.766, 136.314, 137.958, 148.428, 153.679, 157.647; ESI-MS (*m*/*z*, $M^+ - Br + H$): 505.7.

### 2.3. *Antimicrobial activity (minimum inhibitory concentration method)*

*Tested bacterial species*: *Staphylococcus aureus* and *Escherichia coli.*

*Process*: 1 ml of an aqueous suspension containing $10^{-6}$–$10^{-7}$ colony-forming units per milliliter (CFU/ml) of bacteria was placed into a 9-ml aqueous solution with different dye concentrations. After 24 h, a 100-μl aliquot of the microbial

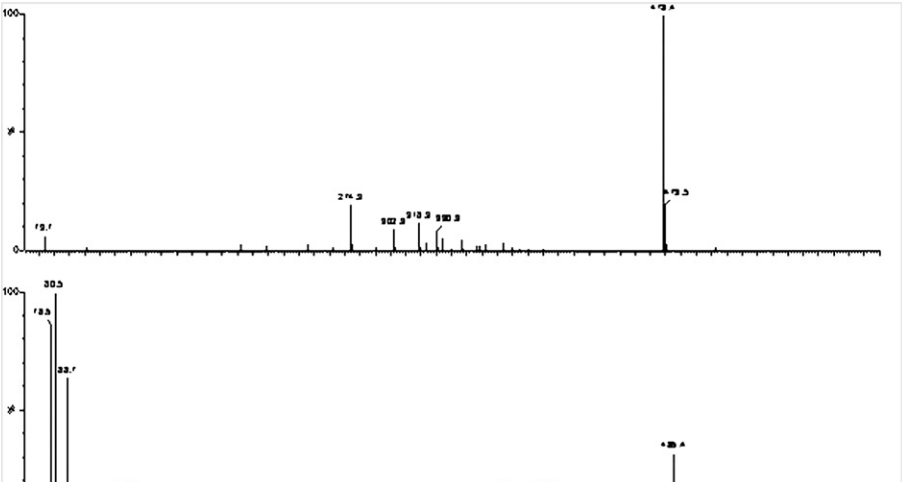

Fig. 7. ESI-MS of dye **2b** (ES+: positive ion mode; ES−: negative ion mode).

suspension was serially diluted with sterilized water, 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 dyes that served as a control.

*Assessment criterion*: lack of bacterial growth.

## 3. Results and discussion

### 3.1. Structure and electron absorption spectra

In intermediates **6** and **8**, a nitryl ($-NO_2$) group showed asymmetric and symmetric stretching vibration absorption bands at 1510–1520 $cm^{-1}$ and 1340–1350 $cm^{-1}$, respectively. The C–N bond showed stretching vibration absorption bands at 850–860 $cm^{-1}$.

In intermediates **7** and **9**, the amidogen ($-NH_2$) group showed stretching vibration absorption bands at 3300–3350 $cm^{-1}$ and 3200–3280 $cm^{-1}$. The N–H bond showed bending vibration absorption bands at 1590–1680 $cm^{-1}$.

The aromatic ether (Ar–O) bond showed asymmetric and symmetric stretching vibration absorption bands at 1230–1240 $cm^{-1}$ and 1000–1030 $cm^{-1}$, respectively.

The *para*-benzene substituent showed absorption bands at 875 $cm^{-1}$.

Different length alkyl chains showed asymmetric and symmetric stretching vibration absorption bands at 2920–2960 $cm^{-1}$ and 2830–2880 $cm^{-1}$, respectively. The longer alkyl chains showed more intense absorption bands.

Dye series **2** and **3** are pyrazolone derivatives with an azo-hydrazone tautomeric structure [15]. In the hydrazone structure, the H of the imine and the C═O bond forms a hydrogen bond. Commonly the C═O bond shows absorption bands at 1650–1775 $cm^{-1}$. But hydrogen bond forming would shift the C═O absorption bands to a low frequency domain (about 1600 $cm^{-1}$) with split absorption bands.

Azo-hydrazone tautomer is evident in the $^1H$ NMR spectrum [16–18]. For example, dye **2a** has a hydrazone structure with the H peak of the imine group characteristically appearing at 13.609 ppm (Fig. 4).

See Figs. 5 and 6 for the $^{13}C$ NMR spectrum of dye **2a** and spectrum peak identification.

Compound molecular weights were tested by ESI-MS. The spectral peak of dye **2b** is shown in Fig. 7.

The 479.5 *m*/*z* value is not the molecular ion peak of dye **2b**, but rather is equal to the molecular weights of dye **2b** − Br + H (positive ion mode). In the negative ion mode, the two *m*/*z* peaks of 78.5 and 80.5 are quite close, thus indicating the presence of element Br in the dye.

The UV/vis spectrum of three dye series was tested in the aqueous solution. (Adding a little dimethyl sulfoxide for improving the water solubility of dye series **3**.) It was found that alkyl chain length did not significantly affect the $\lambda_{max}$ in all of the dyes. But absorption of dye series **2** and **3** decreased as alkyl chain length increased. Steric hindrance may be an important factor. The structure of dye series **1** is small, providing little steric hindrance, and thus absorption did not decrease with alkyl chain length increase.

Table 1
Minimum inhibitory concentration (MIC)

| MIC (ppm) | **1a** | **1b** | **1c** | **2a** | **2b** | **2c** | **3a**[a] | **3b**[a] | **3c**[a] |
|---|---|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | 200 | 60 | 20 | 300 | 100 | 80 | >1000 | 180 | 150 |
| *Escherichia coli* | 300 | 60 | 20 | 400 | 150 | 100 | >1000 | 300 | 200 |

[a] Adding 10 μl dimethyl sulfoxide improved the water solubility of dye series **3** (A little dimethyl sulfoxide did not affect dye antimicrobial efficacy [19].).

### 3.2. Antimicrobial efficacy of aqueous dye solutions

*S. aureus* and *E. coli* are representative deleterious bacteria. Antimicrobial data are shown in Table 1.

All nine dyes have antimicrobial efficacies because of the presence of quaternary ammonium salts in their molecular structures. Positive electrical charges allow dye molecules to adsorb readily onto microbial surfaces, and then penetrate the cell membrane, followed by destruction of cell membranes and leakage of cell inclusion body. Simultaneously, bacterial enzyme systems are destroyed, causing bacteria death. The cell wall of *E. coli* (Gram-negative) is more intricate than the cell wall of *S. aureus* (Gram-positive), thus antimicrobial dyes adsorb readily onto the surfaces of the *S. aureus*. Thus, these nine dyes showed better efficacy against the *S. aureus* than *E. coli*.

The data in Table 1 show that antimicrobial effect increases as alkyl chain length increases. There are quaternary ammonium salts and an azo chromophore in the dye molecule structure. Quaternary ammonium salts synthesized in the paper have industrial uses as antimicrobial agents under the trade names Bromo Geraminum and Domiphen Bromide. Their structures and antimicrobial data are given in Fig. 8 and Table 2.

Thus, antimicrobial dyes have lower antimicrobial efficacies than industrial antimicrobial agents.

Different dye series have different antimicrobial efficacy. Relative antimicrobial efficacy of different dye series within a given alkyl chain length is as follows: dye series **1** > dye series **2** > dye series **3**. Quaternary ammonium salts are effective antimicrobial groups and the weight proportion in the dye structures determines the degree of antimicrobial effect. Dye series **2**, with an ether bond, may form hydrogen bond with water molecules more readily than dye series **3**, and the relatively high solubility enables dye series **2** to more readily form positive ions in aqueous solution, thereby inhibiting bacterial growth by adsorbing onto bacterial surfaces.

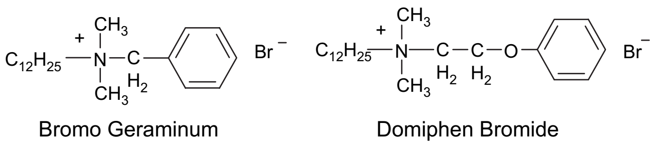

Fig. 8. The structure of Bromo Geraminum and Domiphen Bromide.

Table 2
Antimicrobial activities of Bromo Geraminum and Domiphen Bromide [20]

| MIC (ppm) | *Staphylococcus aureus*, sterilization/microbial inhibition | *Escherichia coli*, sterilization/microbial inhibition |
|---|---|---|
| Bromo Geraminum | 5/1 | 1/1 |
| Domiphen Bromide | 2.5/1 | 1/1 |

## 4. Conclusions

Nine yellow antimicrobial monoazo dyes, involving quaternary ammonium salts, were synthesized. It was found that alkyl chain length did not significantly affect $\lambda_{max}$ among the nine dyes. Within dye series **2** and **3**, absorption decreased as alkyl chain length increased, but dye series **1** showed the opposite trend. Alkyl chains of differing length placed in the dye structure provided antimicrobial efficacy against *S. aureus* and *E. coli*. The nine dyes had higher antimicrobial efficacy against *S. aureus* than against *E. coli* and longer hydrocarbon chains had better antimicrobial effect with all other factors held constant. The antimicrobial efficacy in aqueous solution of the dye series decreased in the following order: $\mathbf{1} > \mathbf{2} > \mathbf{3}$.

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