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Novel Cationic Dye Based on Naphthalimide: Part 1: Synthesis, Characterization and Evaluation of Biology Efficacy as Antimicrobial Agent

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ABSTRACT

*In this paper, synthesis and antimicrobial properties of cationic dye were investigated. 4-acetylamino-N-2-aminomethylpyridine-1,8-naphthalimide was reacted with 1-bromobutane as the alkylating agent and a cationic dye was obtained. The final product was purified by column chromatography method. The chemical structure of the novel cationic dye and its intermediates was chracterisized by using FT-IR, ^{1}HNMR, ^{13}CNMR and DSC techniques. The antimicrobial efficacy of the dye was evaluated by using a minimum inhibitory cocentration (MIC) and disk difusion methods. The synthesized dye and its intermediates showed good antimicrobial activities against both Gram positive and Gram negative bacteria. Also, the results indicated that cationic dye (**3**) containing 1-bromobutane as the alkylating agent had highest antimicrobial activity against all the bacteria used. The MIC of the novel cationic dye against E. coli was 125 μg mL^{-1} and S.aureus was 62.5 μg mL^{-1}. Prog. Color Colorants Coat. 9 (2016), 261-268© Institute for Color Science and Technology.*

1. Introduction

In general, dyes and colorants are compounds whose electronic structures can absorb electromagnetic radiation in visible range (380–780 nm). Additional properties other than color can be defined as functions. Based on this definition, infrared dyes, antimicrobial dyes, laser dyes and voltage sensitive dyes fall within the category of functional dyes [1-4]. In recent years, more attention has been paid to the synthesis and applications of novel antimicrobial dyes. These dyes are needed for biological applications in different industries such as textile industry. In an attempt to prepare a functional dye, a series of antimicrobial dyes were prepared by incorporating biocidal quaternary ammonium salts (QAS) into dyes that are cationic dyes [5]. QAS inactivate microorganisms by disturbing their cytoplasmic membrane and have been widely used as surface disinfectants and antimicrobial agents [6].

In recent decades, cationic dyes with quaternary ammonium salts have been prepared in order to study

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the antimicrobial properties [5–9]. Sun et al. have reported the synthesis and characterization of two series of novel cationic dyes based on anthraquinone derivatives. The synthesized dyes exhibited good antibacterial activity against Gram positive and Gram negative bacteria [6]. Zhao et al. have synthesized an antimicrobial cationic dye by reacting amino-anthraquinone with cyanuric chloride, 3-dimethylamino-1-proponol, and lauryl chloride in sequence. The antimicrobial properties of them were evaluated against *E. coli* and *S. aureas* bacteria [8].

1,8-naphthalimide derivatives are a well known class of compounds that have applications in a number of fields for example coloration of polymers, fluorescent markers in biology, laser media, analgesics in medicine, light emitting diodes, sensors, antimicrobial agent, liquid crystal displays, etc [10-18]. The presence of 1,8-naphthalimide derivatives results in dyes having bathochromic shifts and higher color strengths (deep and intense color) because of its two electron acceptor carbonyl groups.

One group of 1,8-naphthalimide derivatives is cationic naphthalimide dyes which have antimicrobial properties because of existence of positive charge in their structure. In our previous study, the synthesis and characterization of novel azo cationic dye using 4-amino-N-2-aminomethylpyridine-1,8-naphthalimide as a diazo component and N-hydroxyethyl-N-ethylaniline as a coupler are investigated [10]. The prepared dye showed moderate antibacterial and antifungal activities against different microorganisms.

According to our knowledge, there have been few reports about the synthesis of cationic dyes with high coloration strength and antimicrobial properties. Therefore, the aim of this research is to synthesis new cationic naphthalimide dye with high potency of antimicrobial activity. To achieve this goal, a 1,8-naphthalimide derivative is connected to 1-bromobutane as a quaternary agent nitrogen atom (biocidal quaternary ammonium salt). The novel cationic dye and its intermediates were purified and characterized with thin layer chromatography (TLC) [retention values (Rf value)], differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), ^{1}H-nuclear magnetic resonance (^{1}HNMR) and ^{13}CNMR. The antibacterial ability of the novel dye and other prepared compunds was also evaluated against two Gram positive and two Gram negative bacteria in detail.

2. Experimental

2.1. Materials

Acenaphthene, 2-aminomethylpyridine and 1-bromo-butane (Merck) in pure analysis grade were used as received. All organic solvents (Merck) used in this study were of spectroscopic grades.

2.2. Instrumentation

Melting points (M.p) were determined using a Perkin Elmer Pyris 6 differential scanning calorimeter (DSC). The NMR spectra were recorded with a Bruker DRX AVANCE spectrometer, operating at 500 MHz and 125 MHz for ^{1}HNMR and ^{13}CNMR, respectively. The measurements were carried out in DMSO-d6 solution at ambient conditions. The FT-IR spectra were recorded on a Perkin Elmer Spectrum One.

2.3. Synthesis of intermediates

The synthesis methods of 5-nitroacenaphthene, 4-nitro-1,8-naphthalic anhydride and 4-nitro-N-2-amino-methylpyridine-1,8-naphthalimide are reported in the literature [10-12].

2.3.1. Synthesis of 4-amino-N-2-amino-methylpyridine-1,8-naphthalimide (1)

A mixture of 2.8 g (9 mmol) 4-nitro-N-2-amino-methylpyridine-1,8-naphthalimide, 10.15 g (45 mmol) stannous chloride and 11 mL hydrochloric acid in 45 mL methanol was refluxed for 2 hours. The reaction mixture was poured into 100 mL water and the precipitated solid was filtered off. The crude product was purified by silica gel column chromatography with acetone as eluent. ^{1}HNMR: 8.72- 8.68 (d, 1H, *J*=8.4 Hz, C-6'), 8.48- 8.25 (3H, C-5, C-7, C-2), 7.85- 7.73 (2H, C-4′, C-6), 7.31 (s, 2H, NH_2), 7.28-7.25 (d, 1H, *J*=7.9 Hz, C-3′), 7.18- 7.06 (t, 1H, *J*=6.2 Hz, C-5′), 6.66-6.62 (d, 1H, *J*=8.3 Hz , C-3), 5.21 (s, 2H, CH_2); ^{13}CNMR: 45.3, 107.8, 109, 119.4, 120.6, 122.8, 123.1, 125.0, 129.8, 130.5, 131.9, 136.1, 138.5, 148.4, 152.2, 158.1, 162.7, 163.8. FT-IR (KBr, cm^{-1}):1363 (C-N str. aromatic ring), 1583 (C=C str. aromatic ring), 1680, 1668 (C=O str. carbonyl groups), 3448, 3230 (N-H str. primary amino group); m.p: 285 °C; Yield: 78 %.

2.3.2. Synthesis of 4-acetylamino-N-2-amino-methylpyridine-1,8-naphthalimide (2)

4-amino-N-2-aminomethylpyridine-1,8-naphthalimide

(120 mg, 0.4 mmol) was suspended in a mixture of acetic acid (2 mL) and acetic anhydride (1 mL). The suspension was refluxed for 4 hours. Then it was cooled down to room temperature and neutralized with 8 mL of a 10% sodium hydroxide solution and the desired product was precipitated. The crude product was recrystallized in ethanol. ^{1}HNMR: 10.28 (s, 1H, NH), 8.88- 8.8 (d, 1H, *J*=8.5 Hz, C-6'), 8.62- 8.46 (3H, C-7, C-5, C-2), 8.38- 8.33 (t, 1H, *J*=8.2 Hz, C-6), 8.05- 7.95 (t, 1H, *J*=8 Hz, C-4'), 7.85- 7.80 (d, 1H, *J*=7.6 Hz, C-3'), 7.56- 7.51 (t, 1H, *J*=7.9 Hz, C-5'), 7.39- 7.36 (d, 1H, *J*=6.7 Hz, C-3), 5.25 (s, 2H, CH_2), 2.40 (s, 3H, CH_3), ^{13}CNMR: 25.08, 44.80, 117.96, 120.2, 121.45, 122.68, 123.15, 125.35, 126.96, 129.70, 130.58, 131.54, 133.05, 136.85, 142.60, 149.83, 156.46, 164.12, 164.74, 170.00. FT-IR (KBr, cm^{-1}): 1645 (C=O str. Acetyl amino group), 1733, 1689 (C=O str. Carbonyl groups), 3380 (N-H str. Secondary amino group); m.p: 228 °C; Yield: 68%.

2.4. Synthesis of Quaternization of 4-acetylamino-N-2-aminomethylpyridine-1,8-naphthalimide (3)

1-bromobutane (0.8 mL, 5 mmol) was added to a solution of 4-acetylamino-N-2-aminomethylpyridine-1,8-naphthalimide (0.17 g, 5 mmol) in 5 mL of acetonitryl. The mixture was heated to 90 °C for 72 h. Quaternization product was obtained by precipitation from THF. The crude product was purified by column chromatography (silica gel, eluent: acetone/ hexane =1:1). ^{1}HNMR: 10.35 (s, 1H, NH), 8.92- 8.88 (d, 1H, *J*=8.0 Hz, C-6'), 8.70- 8.55 (3H, C-7, C-5, C-2), 8.45- 8.38 (t, 1H, *J*=7.8 Hz, C-6), 8.12- 8.02 (t, 1H, *J*=8.4 Hz, C-4'), 7.96- 7.86 (d, 1H, *J*=8.0 Hz, C-3'), 7.74- 7.68 (t, 1H, *J*=7.7 Hz, C-5'), 7.65- 7.50 (d, 1H, *J*=7.0 Hz, C-3), 5.38 (s, 2H, CH_2), 3.49-3.46 (2H, (N^+-$\underline{CH_2}$-CH_2-CH_2-CH_3)), 2.84-2.78 (4H, (N^+-CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_3)), 2.25 (3H, NHCO$\underline{CH_3}$), 1.24-1.20 (3H, (N^+-CH_2-CH_2-CH_2-$\underline{CH_3}$)); ^{13}CNMR: 12.0, 13.2, 18.3, 22.4, 25.2, 45.2, 118.1, 120.2, 121.8, 123.1, 123.9, 125.0, 126.6, 128.8, 130.7, 132.0, 132.9, 136.2, 143.1, 150.1, 155.7, 163.8, 164.1, 169.6. FT-IR (KBr, cm^{-1}): 3410 (N-H str. Secondary amino group), 2970-2890 (C-H str. aliphatic), 1745, 1677 (C=O str. carbonyl groups), 1658 (C=O str. Acetyl amino group), m.p: 215°C; Yield: 55 %.

2.5. Antimicrobial assessment

The antibacterial activities of the dyes were determined against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subitilis* using the disk diffusion method and minimum inhibitory concentration (MIC) procedure. The bacterial cultures were incubated at 37 °C for 18 h. The novel dye and other synthesized dyes were dissolved (20 µg/mL) in DMSO and stored at room temperature. Mueller Hinton Agar (15 mL) that was stored at ca. 45 °C was then poured into the petri dishes and allowed to solidify. Holes of 12 mm in diameter were punched carefully using a sterile cork borer, and these were completely filled with the test solutions. The plates were incubated for 24 h at 37 °C. The mean value obtained for the two holes was used to calculate the zone of growth inhibition for each sample. DMSO was used as a control under the same conditions for the tested microorganisms. After overnight growth at 37 °C, the diameters of zones of growth inhibition of samples were measured [19].

Also, the antimicrobial properties of the synthesized dyes were evaluated using a minimum inhibitory concentration (MIC), this being the concentration at which no growth of bacteria was observed following such a procedure [20]. 1 mL of aqueous suspension containing 10^6-10^7 colony-forming units (CFU)/mL of Gram positive or Gram negative were placed in to 9 ml aqueous with different dye concentrations. After 24 h, a 100 µl aliquot of the resultant solution was serially diluted with water to 100 mL. 100 µl of the dilution were placed on to a nutrient agar plate and incubated at 37 °C for 24 h. The same procedure was applied to a distilled water solution without dye as a control. The minimal inhibitory concentration (MIC) values were determined after incubation at 37 ºC for 24 h [5].

3. Results and discussion

3.1. Synthesis of dyes and characterization

The final product was synthesized using acenaphthene (Figure 1). The structure of the synthesized dyes was verified by TLC and spectroscopic techniques such as DSC, FTIR, ^{1}HNMR and ^{13}CNMR. The reaction yields, R_f values, melting points and color of the novel synthesized dye and intermediates are shown in Table 1.

Table 1: The yield of reaction, melting point, R_f values and color of prepared compounds.

Compounds	Yield (%)	M.p. (˚C)	R_f	Color
1	78	285	0.76	Orange
2	68	228	0.81	Yellow
3	55	215	0.85	Yellow

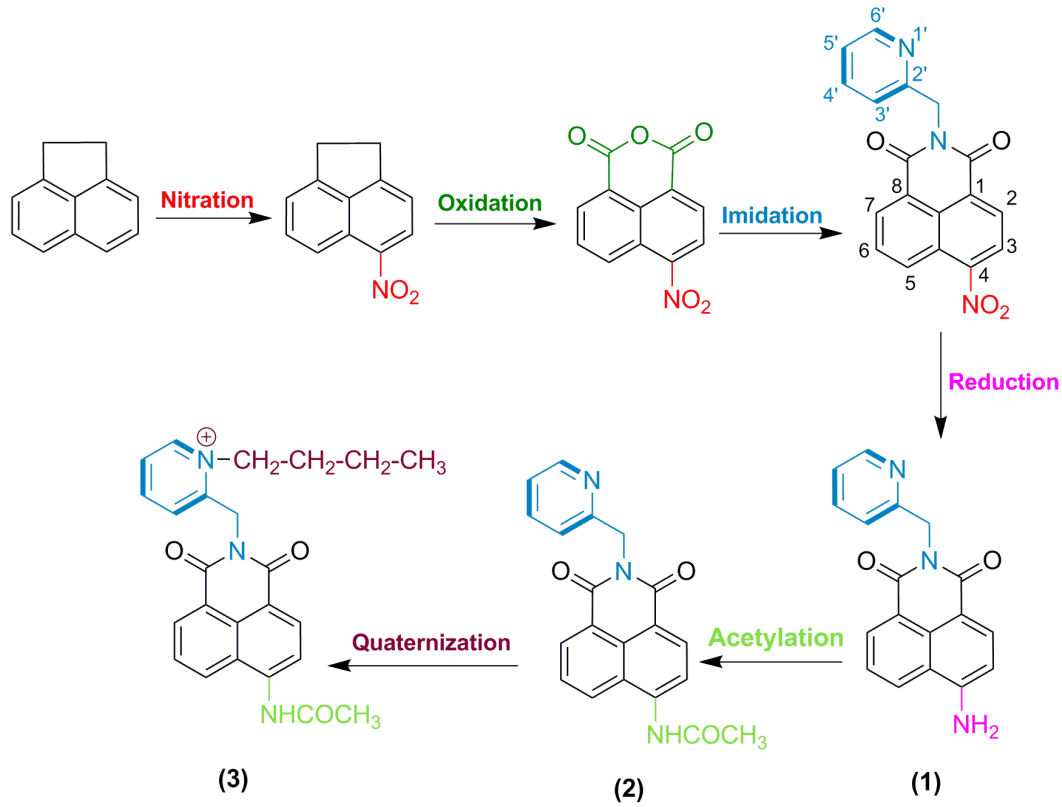

Figure 1: The synthesis route of novel cationic dye (3).

The most convenient starting material for the preparation of naphthalic anhydride is acenaphthene, which is readily available as a byproduct of processes in the coal industry. 5-nitroacenaphthene is obtained when acenaphthene reacts with nitric acid in acetic acid as a solvent at 22-25˚C [21], because of the meta directing effect of the carbonyl group in naphthalic anhydride only 3-substituted naphthalic anhydrides can be synthesized by electrophilic substitution [22]. The nitration reaction of acenaphthene gave yellow needles of 5-nitroacenaphthene with a melting point of 101-102 ˚C after being recrystallized from ethanol. 5-Nitroacenaphthene was oxidized according to the method proposed by Okasaki et al. [21], in which sodium dichromate was used as the oxidizing agent in hot glacial acetic acid as the solvent. The precipitate was recrystallized from nitric acid giving a m.p. of 229-230 ˚C. 4-nitro-1,8-naphthalic anhydride reacted with 2-aminomethylpyridine in tetrahydrofuran at room temperature. The imidation of aromatic cyclic anhydrides is a nucleophilic displacement reaction in which the reaction of the attacking amine is carried out in THF solvent. The reaction between 4-nitro-1,8-naphthalic anhydride with 2-aminomethylpyridine in tetrahydrofuran results in pale yellow compound with yield of 85%. The precipitate was recrystallized from a

mixture of ethanol and dichloromethane giving a M.p. of 191 °C. Preparation of 4-amino-1,8-naphthalimide as an intermediate plays a very important role in the naphthalimide azo dyes series. Also, the naphthalimides, which have an amino group in the 4-position, tend also to be fluorescent. However, 4-amino-N-substituted 1,8-naphthalimides were obtained by the reaction of 4-nitro-N-substituted 1,8-naphthalimides with tin(II) chloride as the reducing agent and hydrochloric acid as the solvent under reflux condition [23]. The purification of the synthesized dye was carried out by column chromatography with acetone as eluent. The FT-IR spectrum of the synthesized dye (1) showed primary amino group in 3448 and 3230 cm^{-1}. This FT-IR spectrum showed the elimination of nitro group (peaks in 1528 and 1339 cm^{-1} regions) in 4-amino-N-2-aminomethylpyridine-1,8-naphthalimide.

Also, the ^{1}HNMR spectrum of this dye showed the presence of primary amino group protons as a singlet peak in 7.31 ppm region which there is not this singlet peak in its intermediate compound. The synthesis of 4-acetylamino-N-2-aminomethylpyridine-1,8-naphthalimide was done through acetylation of 4-amino-N-2-aminomethylpyridine-1,8-naphthalimide.

4-amino-N-2-aminomethylpyridine-1,8-naphthalimide was reacted with acetic anhydride under reflux conditions for 4 hours. The precipitate was recrystallized from ethanol giving a M.p. of 228 °C. The FT-IR spectrum of the dye confirmed the presence of secondary aromatic amines in 3380 cm^{-1}. Also, the ^{1}HNMR spectrum of the synthesized dye (2) demonstrated the presence of NH proton of acetylamino ($\underline{NH}COCH_3$) in region 10.28 ppm and the CH_3 protons of the acetyl group were observed at 2.4 ppm as a singlet peak.

For quaternization of nitrogen atom in benzene ring in order to prepare cationic dye, the solution of 4-acetylamino-N-2-aminomethyl-pyridine-1,8-naphthalimide in acetonitryl was reacted with 1-bromobutane (Figure 1) for 72 h. The cationic compound was isolated as powder solid compound simply inducing precipitation by the addition of tetrahydrofuran to the reaction mixture. The purification of the synthesized dye was carried out by column chromatography (eluent: acetone/ hexane =1:1). Results cream compound with yield of 55% (Table 1). DSC of the synthesized cationic dye indicated that melting point was 215 °C. Moreover, the DSC diagram showed only one sharp peak for novel dye in the region of 215 °C, proving that the synthesized dye was pure (Figure 2).

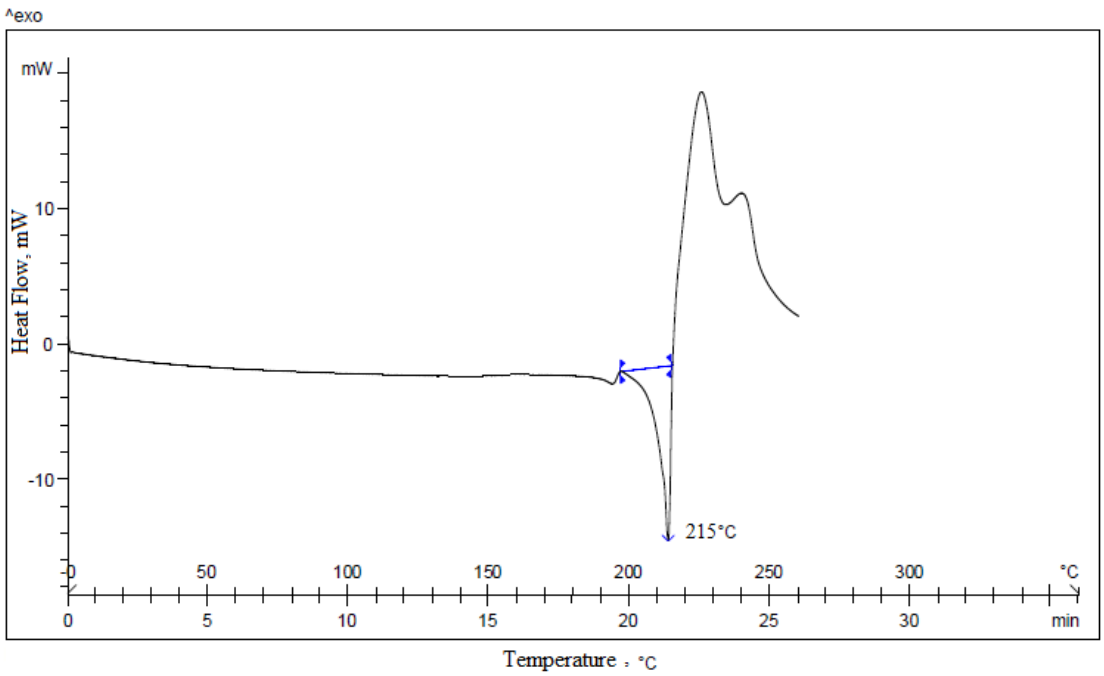

Figure 2: DSC spectrum of cationic dye (3).

The FT-IR spectrum of the dye confirmed the presence of secondary aromatic amines in 3410 cm^{-1}. The presence of alkylating agent (quaternary ammonium salt) in chemical structure of dye **3** was confirmed by ^{1}HNMR spectrum. The peak in region 3.49-3.46 ppm is assigned to the CH_2 protons of (adjacent to N^+) N^+-$\underline{CH_2}$-CH_2-CH_2-CH_3 group in dye **3**, the peaks in regions 2.84-2.78 ppm are attributed to the protons of the CH_2 (N^+-CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_3) which there are not these peaks in the dyes of **1** and **2**.

3. 2. Antimicrobial activity

Antimicrobial properties of the prepared dyes in aqueous solutions were measured by the MIC and disk diffusion procedures and the results showed in Table 2 & Figure 3. The minimum inhibitory concentration values were determined as the lowest concentration that completely prevented visible growth of the bacteria and fungi.

In zone of inhibition test, the dye solutions are placed on an agar plate where bacteria have been placed, and the plate is left to be incubated. If a solution of dyes stop the bacteria from growing or kills the bacteria, there will be an area around the dyes where the bacteria have not grown enough to be visible which is called a zone of inhibition. The results illustrated that the prepared compounds **1** and **2** as well as the novel cationic dye had antimicrobial activities against bacteria.

According to Table 2, among the synthesized compounds, cationic dye showed the most antimicrobial properties and dye **1** had lowest antimicrobial activity against both two Gram positive and Gram negative bacteria

The MIC and inhibition zone diameter of the cationic dye were 125 µg mL^{-1} and 22 mm, respectively, against *E. coli* and 62.5 µg mL^{-1} and 23 mm, respectively, against *S.aureus*. Determination of MIC of dye **3** is shown in Figure 4. Positive electrical charge of cationic dye allows dye molecules to be adsorbed more readily onto microbial surfaces, then penetrate into the cell membrane which will result in the destruction of the cell membrane [8, 24]. Simultaneously, bacterial enzyme systems are destroyed, causing the demise of the bacteria. Moreover, the results demonstrated that the inhibitory activity of synthesized dyes against the Gram positive bacteria was higher than that against the Gram negative bacteria. This is because of structural differences between the bacteria [25]. The existence of outer membrane is the main cause of low permeability of dyes into Gram negative bacteria. Passing of molecules through these barriers is affected by hydrophilic–lipophilic balance of the dye. The results of the current study suggest that changing this balance could affect antimicrobial activity of the dyes [6].

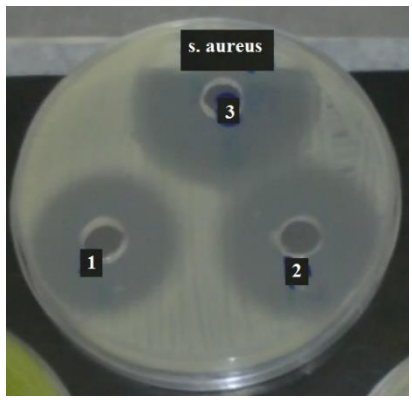

Figure 3: Zone of inhibition of compounds 1, 2 & 3 against *S. aureus* bacteria.

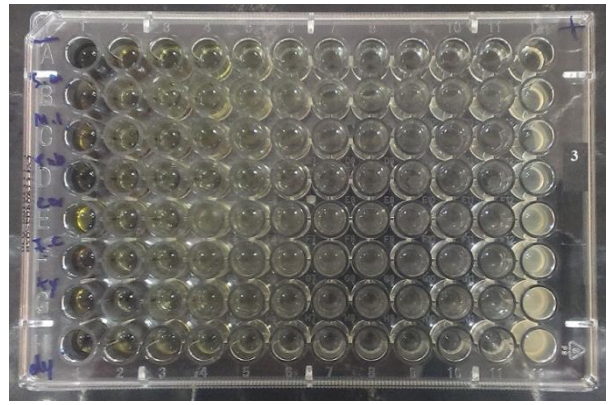

Figure 4: Determination of MIC of cationic dye (3) against bacteria.

Table 2: Minimum inhibitory concentration and zone of inhibition of the prepared compounds.

Compound	Gram positive bacteria				Gram negative bacteria			
	S. aureus		B. subitilis		E. coli		P. aeruginosa	
	MIC ($\mu g\ mL^{-1}$)	Inhibition zone diameter (128 $\mu g\ mL^{-1}$, mm)	MIC (μgmL^{-1})	Inhibition zone diameter (128 μgmL^{-1},mm)	MIC (μgmL^{-1})	Inhibition zone diameter (128 $\mu g\ mL^{-1}$, mm)	MIC (μgmL^{-1})	Inhibition zone diameter (128 $\mu g\ mL^{-1}$, mm)
1	250	14	250	14	500	14	500	13
2	250	17	250	15	250	15	250	14
3	62.5	23	125	21	125	22	250	20
DMSO	-	-	-	-	-	-	-	-

4. Conclusions

Novel antimicrobial cationic dye based on naphthalimide was synthesized using nitration, oxidation, imidation, reduction, acetylation and quaternization reactions. The chemical structure of the final product and other prepared compounds was characterized by using FT-IR, ^{1}HNMR, ^{13}CNMR and DSC techniques. The antimicrobial efficacy of the new dye and other prepared dyes was evaluated by using a minimum inhibitory concentration (MIC) and disk diffusion methods. The cationic dye (**3**) exhibited most antimicrobial efficacy against microorganisms. The antimicrobial dye provided higher antibacterial efficacy against Gram positive bacteria than Gram negative bacteria, which could be due to the structural differences between bacteria.

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