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Synthesis, spectroscopic and antimicrobial studies of some novel cyanine dyes based on *bis*-coumarin heterocycles derivatives

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ABSTRACT

Novel symmetrical and unsymmetrical cyanine dyes, incorporating merocyanine monomethine like, pentamethine cyanine, monomethine-meso-substituted-pentamethine and mono-5[2(4)]-methine cyanine dye have been prepared through the synthesis of new starting compound derivatives named as 1,3-*bis*-(2-oxo-2*H*-chromen-3-yl) propane-1,3-dione and (3-oxo1,3-*bis*(2-oxo-2*H*-chromen-3-yl)prop-1-enyloxy) copper, cobalt and nickel chloride salt complexes. Structure determination of the new compounds has been characterized on the basis of elemental analysis, IR, ^{1}H NMR and MS spectra. Structure photosensitization relationship of new dyes have been discussed on the basis of their spectral behavior as criteria of photosensitizing effect through the UV visible-absorption spectra of all synthesized dyes which investigated in 95% ethanol. Antimicrobial properties of some selected cyanine dyes have been investigated against *Streptococcus sp*, *Staphylococcus sp*, *Salmonella sp*. and *Shigella sp*.

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1. Introduction

Coumarin derivatives constitute an important group of organic luminophores and laser dyes that efficiently generate radiation in the region of 400-560 nm [1-3]. Coumarin polymethines have efficient fluorescence that makes them promising probes in biochemistry and medicine [4-7]. Psoralens (furocoumarins) became important for photobiology and photo chemotherapy [8-10]. Currently, cyanine dyes that form strongly fluorescenting complexes with nucleic acids and proteins have found broad application in chemistry and biochemistry for identification of bio macromolecules [11,12]. Fluorescent characteristics of coumarin photosensitizers exhibit excellent performance as sensitizer [13-15]. Fluorescent coumarin derivatives were used in vivo imaging of zebra fish retinal cell [16]. Coumarins and related compounds have been shown to possess many biological activities such as antithrombotic [17], antimicrobials [18,19], chemotherapeutics [20], anti-inflammatory [21,22], anti-proliferative [23], antibacterial [24], anti-HIV [25], antifungal [26] and many of coumarins are used as antioxidants [27-29]. An extension of our work in the field of cyanine dyes, in this manuscript, some new cyanine dyes have been prepared from coumarin derivatives of high-sensitivity and high-wavelength with wide-field applications.

2. Experimental

2.1. Instrumentations

All melting points are uncorrected. Elemental analysis was carried out at the Micro Analytical Center (Cairo University). The IR spectra (KBr) were determined with Perkin Elmer Infrared 127 spectrophotometer (Cairo University). ^{1}H NMR spectra were recorded with Bruker AMX-500 spectrometer, using tetra methyl silane (TMS) as internal reference. Chemical shifts are reported as δ in parts per million (ppm). MS spectra were recorded on an HpMs 6988 spectrometer (Cairo University). The electronic absorption spectra were recorded within the wavelength range 350-800 nm on 6405 UV/Visible recording spectrophotometer, Faculty of Science, Aswan.

2.2. Synthesis

2.2.1. Synthesis of 3-acetylchromen-2-one (1)

The compound **1** was carried out according to reference [30].

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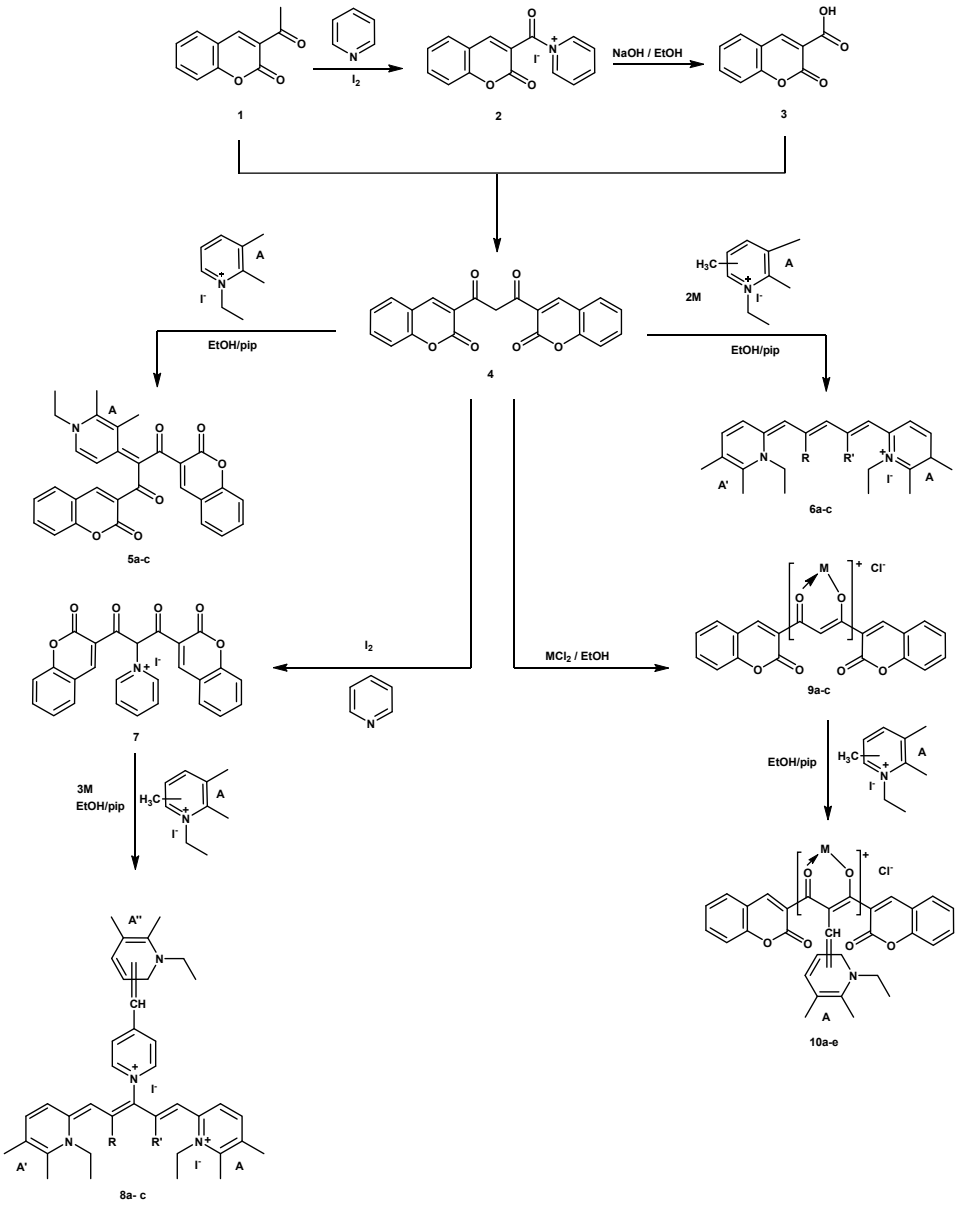

5a-c: *N*-Ethyl pyridine (a), *N*-ethylquinoline (b), *N*-ethyl isoquinoline (c); **6a-c:** A= Pyridin-2-ium ethyl iodide**,** A'= *N*-ethyl pyridine, R= R'= 2-oxo-2*H*-chromen-3-yl (a) A= Quinolin-2-ium ethyl iodide, A'= *N*-ethylquinoline, R= R'= 2-oxo-2*H*-chromen-3-yl (b) A= Pyridine-4-ium ethyl iodide, A'= *N*-ethyl pyridine, R= R'= 2-oxo-2*H*-chromen-3-yl (c); **8a-c:** A= Pyridin-2-ium ethyl iodide, A'=A''= *N*-ethyl pyridine, R= R'= 2-oxo-2*H*-chromen-3-yl (a) A= Quinolin-2-ium ethyl iodide**,** A'=A''= *N*-ethylquinoline, R= R'= 2-oxo-2*H*-chromen-3-yl (b) A= Pyridine-4-ium ethyl iodide**,** A'= A''=*N*-ethyl pyridine, R= R'= 2-oxo-2*H*-chromen-3-yl (c); **9a-c:** M= Cu^{2+} (a), M= Co^{2+} (b), M= Ni^{2+} (c); **10a-e:** M= Cu^{2+}, A= *N*-ethyl pyridine (a), M= Cu^{2+}, A= *N*-ethylquinoline (b) M= Cu^{2+}, A= *N*-ethyl pyridine (c), M= Co^{2+}, A= *N*-ethylquinoline (d) M= Ni^{2+}, A= *N*-ethylquinoline (e)

Scheme 1

2.2.2. Synthesis of 1-(2-oxo-2H-chromene-3-carbonyl) pyridinium iodide (2)

The compound **2** was carried out according to reference [31]. A mixture of compound **1** (0.01 mol), iodine (0.01 mol) and pyridine (50 mL) was refluxed for 1 h, on a water bath, filtered hot, concentrated and cooled. The precipitated products after dilution with 50 mL water were collected and crystallized from aqueous ethanol to give the corresponding compound **2** (Scheme 1). Color: White. Yield: 83%. M.p.: 196-198 °C. Anal. calcd. For $C_{15}H_{10}O_3NI$: C, 47.52; H, 2.66; N, 3.69. Found: C, 47.53; H, 2.75; N, 3.71%.

2.2.3. Synthesis of 2-oxo-2H-chromene-3-carboxylic acid (3)

The compound **2** (0.01 mol) was dissolved in ethanol 30 mL in presence of sodium hydroxide (0.5 mL of 10%). Reaction mixture was refluxed for 3 h, filtered hot, cooled and neutralized with conc. hydrochloric acid 0.5 mL. The precipitated product after dilution with water were collected and crystallized from ethanol to give the corresponding compound **3** (Scheme 1). Color: Green colored powder. Yield: 65%. M.p.: 195-197 °C. FT-IR (KBr, ν, cm^{-1}): 3300 (ν OH), 2920 (ν CH), 1719 (Lactone), 1680 (ν CO). Anal. calcd. for $C_{10}H_6O_4$: C, 63.16; H, 3.18. Found: C, 63.01; H, 3.30%.

2.2.4. Synthesis of 1,3-bis(2-oxo-2H-chromen-3-yl)propane-1,3-dione (4)

Mixture of compound **3** (0.01 mol) with compound **1** (0.01 mol) and piperidine 0.7 mL was fused for 1 h, on water bath. The reaction mixture was dissolved in 30 mL ethanol and refluxed for 3 h, cooled to room temperature and poured into 50 mL ice-water. The precipitated solid were collected and crystallized from ethanol to give the corresponding compound **4** (Scheme 1). Color: Deep green colored powder. Yield: 70%. M.p.: 145-147 °C. FT-IR (KBr, ν, cm^{-1}): 2922 (C-H), 1722 (Lactone), 1608 (CO). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.30-8.85 (m, 8H, Ar H), 8.39 (s, 2H, =CH of coumarin), 4.56 (s, 2H, CH_2). MS-EI (*m/z* (%)): 359 $[M-1]^+$, 187 $[M-C_{10}H_5O_3]^+$, 145 $[M-C_{12}H_7O_4]^+$. Anal. calcd. for $C_{21}H_{12}O_6$: C, 70.00; H, 3.36. Found: C, 70.12; H, 3.48%.

2.2.5. Synthesis of compounds 5a-c

Mixture of compound **4** (0.01 mol) and *N*-heterocyclic quaternary salts (pyridinium, quinolinium and isoquinolinium) ethyl iodide (0.01 mol) were dissolved in ethanol 20 mL and piperidine 0.7 mL were added .The reaction mixture was refluxed for 10-12 h, filtered hot, cooled, neutralized by 0.3 mL acetic acid and poured into 50 mL ice-water. The precipitated solid were collected and crystallized from ethanol to give the corresponding dyes **5a-c** (Scheme 1).

2-(1-Ethylpyridin-4(1H)-ylidene)-1,3-bis(2-oxo-2H-chromen-3-yl)propane-1,3-dione (**5a**): Color: Yellow powder. Yield: 60%. M.p.: 140-142 °C. IR (KBr, ν, cm^{-1}): 2923 (C-H), 1714 (Lactone), 1605 (CO). Anal. calcd. for $C_{28}H_{19}NO_6$: C, 72.25; H, 4.11; N, 3.01. Found: C, 72.35; H, 4.18; N, 3.11%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 420 (5.33).

2-(1-Ethylquinolin-4(1H)-ylidene)-1, 3-bis(2-oxo-2H-chromen-3-yl)propane-1,3-dione (**5b**): Color: Deep red powder. Yield: 60%. M.p.: 165-167 °C. IR (KBr, ν, cm^{-1}): 2921 (C-H), 1714 (lactone), 1606 (CO), 2856 (N-ethyl). Anal. calcd. For $C_{32}H_{21}NO_6$: C, 74.56; H, 4.11; N, 2.72. Found: C, 74.42; H, 4.10; N, 2.68%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 470 (3.28).

2-(2-Ethylisoquinoin-1(2H)-ylidene)-1,3-bis(2-oxo-2H-chromen-3-yl)propane-1,3-dione (**5c**): Color: Pale yellow powder. Yield: 55%. M.p.: 145-147 °C. IR (KBr, ν, cm^{-1}): 2925 (C-H), 1715 (lactone), 1606 (CO), 1853 (N-ethyl). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.03 (t, 3H, CH_3 of N-ethyl), 3.45 (q, 2H, CH_2 of N-ethyl), 5.95 (d, 1H, isoquinoline), 6.60-7.80 (m, 12H (Ar-H) + 1H (isoquinoline), 8.55 (s, 2H, =CH of coumarin). Anal. calcd. For $C_{32}H_{21}NO_6$: C, 74.56; H, 4.11; N, 2.72. Found: C, 74.46; H, 4.14; N, 2.70%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 410 (1.82).

2.2.6. Synthesis of compounds 6a-c

Mixture of compound **4** (0.01 mol) and (0.02 mol) 2(4)-*N*-heterocyclic quaternary salts [α-(γ) picoline, and quinaldine] ethyl iodide were dissolved in 20 mL ethanol and 0.7 mL piperidine, reaction mixture was refluxed for 8-10 h, filtered hot, cooled, neutralized by 0.3 mL acetic acid and poured into 50 mL ice-water. The precipitated solid were collected and crystallized from ethanol to give the corresponding dyes **6a-c** (Scheme 1).

1-Ethyl-2-((1E, 3E, 5Z)-5-(1-ethylpyridin-2(1H)-ylidene)-2,4-bis(2-oxo-2H-chromen-3-yl)penta-1,3-dienyl)pyridinium iodide (**6a**): Color: Green powder. Yield: 70%. M.p.: 200- 202 °C. IR (KBr, ν, cm^{-1}): 2921 (C-H), 1725 (lactone), 2851- 2922 (N-ethyl and N-ethyl iodide). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 6.20-8.89 (m, 21H, (10 H for coumarin, 8H for heterocyclic and 3H of CH olefinic)), 4.10 (q, 2H, CH_2 of N-ethyl iodide), 3.50 (q, 2H, CH_2 of N-ethyl), 1.29 (t, 3H, CH_3 of N-ethyl iodide), 1.03 (t, 3H, CH_3 of N-ethyl). Anal. calcd. for $C_{37}H_{31}N_2O_4I$: C, 63.98; H, 4.50; N, 4.03. Found: C, 63.85; H, 4.70; N, 4.12%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 350 (3.12), 455 (6.86), 415 (9.16).

1-Ethyl-2-((1E,3E,5Z)-5-(1-ethylquinolin-2(1H)-ylidene)-2,4-bis(2-oxo-2H-chromen-3-yl)-penta-1, 3-dienyl) quinolinium iodide (**6b**): Color: Deep red powder. Yield: 75%. M.p.: 210-212 °C. Anal. calcd. for $C_{45}H_{35}N_2O_4I$: C, 68.01; H, 4.44; N, 3.53. Found: C, 68.10; H, 4.49; N, 3.55%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 555 (4.01), 510 (4.43), 485 (4.07), 435 (2.84).

1-Ethyl-4-((1Z, 3E)-5-(1-ethylpyridin-4(1H)-ylidene)-2, 4-bis (2-oxo-2H-chromen-3-yl)penta-1, 3-dienyl)pyridinium iodide (**6c**): Color: Dark green powder. Yield: 65%. M.p.: 180-182 °C. Anal. calcd. for $C_{37}H_{31}N_2O_4I$: C, 63.98; H, 4.50; N, 4.03. Found: C, 63.90; H, 4.82; N, 4.11%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 545 (1.92), 475 (1.34), 425 (1.09).

2.2.7. Synthesis of 1-(1,3-dioxo-1,3-bis(2-oxo-2H-chromen-3-yl)propan-2-yl)pyridin-1-ium iodide (7)

The compound **7** was carried out according to [31]. Mixture of compound **4** (0.01 mol), iodine (0.01 mol) was dissolved in 50 mL pyridine. The reaction mixture was refluxed for 1 h, on a water bath, filtered hot, concentrated and cooled. The precipitated products after dilution with 50 mL water were collected and crystallized from aqueous ethanol to give the corresponding compound **7** (Scheme 1). Color: Brown powder. Yield: 80%. M.p.: 185-187 °C. IR (KBr, ν, cm^{-1}): 2920 (C-H), 1721 (lactone), 1610 (carbonyl). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.35-8.45 (m, 13H, Ar-H (8H) + pyridinium iodide (5H)), 8.55 (s, 2H, =CH of coumarin), 5.45 (s, 1H, aliphatic proton). Anal. calcd. for $C_{26}H_{16}O_6NI$: C, 55.24; H, 2.85; N, 2.48. Found: C, 55.28; H, 2.78; N, 2.52%.

2.2.8. Synthesis of compounds 8a-c

Mixture of compound **4** (0.01 mol) with (0.03 mol) 2(4)-*N*-heterocyclic quaternary salts [α-(γ) picoline, and quinaldine] ethyl iodide were dissolved in 20 mL ethanol and 0.7 mL piperidine, reaction mixture was refluxed for 8-10 h , filtered hot, cooled, neutralized by 0.3 mL acetic acid and poured into 50 mL ice-water. The precipitated solid were collected and crystallized from ethanol to give the corresponding dyes **8a-c** (Scheme 1).

1-Ethyl -2-((1E,3Z,5E)-5-(1-ethylpyridin-2(1H)-ylidene)-3-(4-((Z) -(1-ethylpyridin-2 (1H)-ylidene)methyl)pyridinium-1-yl)-2, 4-bis(2-oxo-2H-chromen-3-yl)penta-1, 3-dienyl)pyridinium iodide (**8a**): Color: Green powder. Yield: 75%. M.p.: 205- 207 °C. Anal. calcd. for $C_{50}H_{44}N_4O_4\ I_2$: C, 58.95; H, 4.35; N, 5.50. Found: C, 58.90; H, 4.28; N, 5.41%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 425(1.74).

1-Ethyl-2-((1E, 3Z, 5E)-5-(1-ethylquinolin-2(1H)-ylidene)-3-(4-((Z)-(1-ethylquinolin-2(1H)-ylidene)methyl)pyridinium-1-yl)-2, 4-bis(2-oxo-2H-chromen-3-yl)penta-1, 3-dienyl)quinolinium iodide (**8b**): Color: Deep red powder. Yield: 70%. M.p.: 215;-217 °C. IR (KBr, ν, cm^{-1}): 2920 (C-H), 1721 (lactone), 2853-2923 (N-ethyl and N-ethyl iodide), 2920 (C-H), 1721 (lactone), 2853- 2923 (N-ethyl and N-ethyl iodide). Anal. calcd. for $C_{62}H_{50}N_4O_4I_2$: C, 63.71; H, 4.31; N, 4.79. Found: C, 63.79; H, 4.22; N, 4.88%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 695 (1.50), 520 (1.74), 480 (1.56), 415 (1.37).

1-Ethyl-4-((1E,3Z)-5-(1-ethylpyridin-4(1H)-ylidene)-3-(4-(1-ethylpyridin-4(1H)-ylidene)methyl)pyridinium-1-yl)-2, 4-bis(2-oxo-2H-chromen-3-yl)penta-1,3-dienyl)pyridinium iodide (**8c**): Color: Dark green powder. Yield: 60%. M.p.: 220- 222 °C. Anal. calcd. for $C_{50}H_{44}N_4O_4I_2$: C, 58.94; H, 4.35; N, 5.50. Found: C, 58.99; H, 4.38; N, 5.55%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 450 (2.06).

2.2.9. Synthesis of complexes 9a-c

The compounds **9a-c** were carried out according to [31-35]. A mixture of compound **4** (0.01 mol) and metal dichloride (copper, cobalt and nickel) (0.01 mol) were dissolved in absolute ethanol 30 mL. The reaction mixture was refluxed for

1-3 h, filtered hot and concentrated. The precipitated products were isolated and recrystallized from ethanol to give the corresponding compounds **9a-c** (Scheme 1).

(Z)-(3-Oxo-1, 3-bis(2-oxo-2H-chromen-3-yl)prop-1-enyloxy) copper chloride salt (**9a**): Color: Green powder. Yield: 70%. M.p.: 220- 222 °C. Anal. calcd. for $C_{21}H_{11}O_6CuCl$: C, 55.03; H, 2.42. Found: C, 55.13; H, 4.48%.

(Z)-(3-Oxo-1, 3-bis(2-oxo-2H-chromen-3-yl)prop-1-enyloxy) cobalt chloride salt (**9b**): Color: Brown powder. Yield: 45%. M.p.: 235- 237 °C. IR (KBr, ν, cm^{-1}): 515 (M-O), 2923 (heterocyclic metal chelate), 1704 (CO). Anal. calcd. for $C_{21}H_{11}O_6CoCl$: C, 55.59; H, 2.44. Found: C, 55.64; H, 2.48%.

(Z)-(3-Oxo-1, 3-bis(2-oxo-2H-chromen-3-yl)prop-1-enyloxy) nickel chloride salt (**9c**): Color: Dark green powder. Yield: 40%. M.p.: 245-247 °C. Anal. calcd. for $C_{21}H_{11}O_6NiCl$: C, 55.62; H, 2.45. Found: C, 55.60; H, 2.40%.

2.2.10. Synthesis of *dyes 10a-e*

Mixture of compounds **9a-c** (0.01 mol), [α-(γ) picoline, and quinaldine]ethyl iodide (0.01 mol) and 0.7 mL piperidine was added. The reaction mixture was heated to 30 min. on a sand bath, cooled, triturated with 30 mL ethanol and refluxed for 1 h, filtered hot, concentrated and cooled, neutralized by 0.3 mL acetic acid and poured into 50 mL ice-water. The precipitated solid were collected and crystallized from ethanol to give the corresponding dyes **10a-e** (Scheme 1).

((Z)-2-((E)-(1-Ethylpyridin-2(1H)ylidene)methyl)-3-oxo-1,3-bis(2-oxo-2H-chromen-3-yl)-prop-1-enyloxy)copper chloride salt, mono-5[2]-methine cyanine dye (**10a**): Color: Green powder. Yield: 60%. M.p.: 190-192 °C. Anal. calcd. for $C_{29}H_{20}NO_6CuCl$: C, 60.32; H, 3.49; N, 2.43. Found: C, 60.39; H, 3.41; N, 2.48%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 570 (1.15), 410 (1.93).

((Z)-2-((E)-(1-Ethylquinolin-2(1H) ylidene) methyl)-3-oxo-1,3-bis(2-oxo-2H-chromen-3-yl)-prop-1-enyloxy)copper chloride salt, mono-5[2]-methine cyanine dye (**10b**): Color: Deep red powder. Yield: 70%. M.p.: 195-197 °C. Anal. calcd. for $C_{33}H_{22}NO_6CuCl$: C, 63.16; H, 3.53; N, 2.23. Found: C, 63.11; H, 3.48; N, 2.28%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 580 (2.19), 550 (1.60), 520 (1.23), 400 (1.63).

(Z)-(2-((1-ethylpyridin-4(1H) ylidene) methyl)-3-oxo-1, 3-bis (2-oxo-2H-chromen-3-yl)-prop-1-enyloxy)copper chloride salt, mono-5[4]-methine cyanine dye (**10c**): Color: Dark green powder. Yield: 65%. M.p.:165-167 °C. Anal. calcd. for $C_{29}H_{20}NO_6CuCl$: C, 60.32; H, 3.49; N, 2.43. Found: C, 60.38; H, 3.52; N, 2.51%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 500 (2.29), 470 (2.17), 440 (2.36), 420 (2.22).

((Z)-2-((E)-(1-ethylquinolin-2(1H) ylidene) methyl)-3-oxo-1, 3-bis(2-oxo-2H-chromen-3-yl)prop-1-enyloxy)cobalt chloride salt, mono-5[2]-methine cyanine dye (**10d**): Color: Deep reddish violet powder. Yield: 75%. M.p.: 220-222 °C. IR (KBr, ν, cm^{-1}): 525-535 (O-M-O), 2923 (N-ethyl), 1720 (CO). Anal. calcd. for $C_{33}H_{22}NO_6CoCl$: C, 63.63; H, 3.56; N, 2.25. Found: C, 63.69; H, 3.60; N, 2.31%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 690 (5.55), 580 (1.83), 560 (1.61), 480 (1.58).

((Z)-2-((E)-(1-ethylquinolin-2(1H) ylidene) methyl)-3-oxo-1, 3-bis(2-oxo-2H-chromen-3-yl)prop-1-enyloxy)nickel chloride salt, mono-5[2]-methine cyanine dye (**10e**): Color: Deep reddish violet powder. Yield: 70%. M.p.: 180-182 °C. Anal. calcd. for $C_{33}H_{22}NO_6NiCl$: C, 63.65; H, 3.56; N, 2.25. Found: C, 63.75; H, 3.62; N, 2.31%. UV/Vis (EtOH, λ_{max}, nm, (ε)): 670 (4.36), 580 (1.62), 510 (1.11).

2.3. *Antimicrobial activity*

Different concentrations (50, 100, 150 and 200 µg/mL) of $CdCl_2$ and $CoCl_2$ were added to flasks that contain nutrient media and 20 ml saline solution (20%). All flasks were sterilized in Autoclave. After sterilization the media were poured in Petri dishes. Small halls were made in the solid media by using corckoporer. Each microorganism species (clinical isolated) was inoculated separately in saline solution. 20 mL from each solution that contain organisms was added to the hall in solid media plates. All plates were incubated at 37 °C for days. All procedure was repeated with the synthetic organic compounds and antibiotic (Tetracycline and ampicillin) as positive control. Growth was measured (mm) under the treatment with different concentrations of heavy metals compared to control.

3. Results and discussion

3.1. Chemistry

Reaction of a ratio of 1 mol of 3-acetylchromen-2-one (**1**) [30] in presence of iodine with the heterocyclic nitrogen base (pyridine) in excess amount afforded the corresponding 1-(2-oxo-2*H*-chromene-3-carbonyl)pyridinium iodide (**2**) [30]. Thermal basic hydrolysis of the key intermediate **2** using aqueous ethanolic solution of sodium hydroxide gave the corresponding sodium salt of coumarin-3-sodium carboxylate which on triturating with concentrated HCl, the free 2-oxo-2*H*-chromene-3-carboxylic acid (**3**) is formed. Reaction of equimolar ratio of compound 3-acetylchromen-2-one (**1**) with 2-oxo-2*H*-chromene-3-carboxylic acid (**3**), under thermal piperidine catalysis conditions gave the corresponding starting compound **4** namely as 1,3-*bis*-(2-oxo-2*H*-chromen-3-yl) propane-1,3-dione. Reaction of equimolar ratios of compound **4** with *N*-ethyl heterocyclic quaternary salt (pyridine, quinoline and isoquinoline) iodide in the presence of piperidine as basic catalyst afforded symmetrical 2-(1-ethylpyridin-4(1*H*)-ylidene)-1, 3-*bis*(2-oxo-2*H*-chromen-3-yl) propane-1,3-dionemero cyanine monomethine like 2[4] dye **5a**, and the other derivatives **5b**, and **5c**. Treatment of such resulted compounds **5a-c** with concentrated H_2SO_4, solubility take place with no liberating iodine vapor on warming. This a criteria that the reaction is suggested to proceed through oxidative elimination reaction between active hydrogen of heterocyclic quaternary salt and active methylene group of started compound **4** followed by dehydrohalogenation -HI processes afforded the desired dyes **5a-c**. Also, reaction of equimolar ratios of compound **4** with bi molar ratios of 2(4)-methyl heterocyclic quaternary salt [α-(γ) picoline, and quinaldine]ethyl iodide under basic catalyst conditions afforded the corresponding symmetrical 1-ethyl-2-((1*E*,3*E*,5*Z*)-5-(1-ethylpyidin-2(1*H*)ylidene)-2,4-*bis*-(2-oxo-2*H*-chromen-3-yl)penta-1,3-dienyl)pyrdinium iodide pentamethine cyanine dye **6a**, and other derivatives **6b** and **6c**.

Reaction of a ratio of 1 mol of compound **4** in presence of iodine with the heterocyclic nitrogen base (pyridine) in excess amount afforded the corresponding *N*-substituted hetero cyclidinium ylide iodide **7** [31]. Further reaction of equimolar ratios of compound **7** with 3 molar ratios of 2(4)-*N*-methyl heterocyclic quaternary salt [α-(γ) picoline, and quinaldine] ethyl iodide under basic catalyst conditions afforded the corresponding 1-ethyl-2-((1*E*,3*Z*,5*E*)-5-(1-ethylpyridin-2(1*H*) ylidene)-3-(4-((*Z*)-(1-ethylpyridin-2(1*H*-lidene)methyl)pyridinium-1-yl)-2,4-*bis*(2-oxo-2*H*-chromen-3-yl)penta-1,3-dienyl) pyridinium iodide pentamethine cyanine dye **8a**, and other derivatives **8b** and **8c**.

On the other hand, reaction of equimolar ratios of compound **4** with metal dichloride (copper, cobalt and nickel) in absolute ethanol gave the corresponding complexes, namely as (*Z*)-(3-oxo-1,3-*bis*(2-oxo-2*H*-chromen-3-yl)prop-1-enyloxy) copper(cobalt and/or nickel) chloride salt complexes **9a-c** [32-38] which consider as a key intermediate compounds in the synthesis of metal complex cyanine dyes. Thus, reaction of equimolar ratio of compounds **9a-c** with 2(4)-*N*-methyl heterocyclic quaternary salt [α-(γ) picoline, and quinaldine] ethyl iodide under thermal condition in the presence of piperidine as basic catalyst afforded the corresponding

Table 1. The electronic absorption spectra of new synthesized cyanine dyes **5a-c**, **6a-c**, **8a-c** and **10a-e** in 95% ethanol.

Compound	UV-Vis spectra, λ_{max} (nm) *	ε_{max} (mol^{-1} cm^{-1})
5a	420,	5333
5b	470	3277
5c	410	1817
6a	530, 455, 415	3120, 6860, 9160
6b	555, 510, 485, 435	4012, 4427, 4065, 2837
6c	545, 475, 425	1920, 1335, 1085
8a	425	1738
8b	695, 520, 480, 415	15000, 1738, 1556, 1373
8c	450	2062
10a	570, 410	1151, 1926
10b	580, 550s, 520s, 400	2191, 1602, 1215, 1634
10c	500, 470, 440, 420s	2285, 2169, 2361, 2222
10d	690, 580, 560s, 480s	555, 1832, 1614, 1575
10e	670, 580, 510	436, 1623, 1105

* s = shoulder.

((*Z*)-2-((*E*)-(1-ethylpyridin-2(1*H*)ylidene)methyl)-3-oxo-1,3-*bis*(2-oxo-2*H*-chromen-3-yl)-prop-1-enyloxy)copper, chloride salt, mono-5[2]-methine cyanine dye **10a**, and other derivatives **10b**, **10c**, **10d** and **10e** (Scheme 1). These compounds **10a-e** on triturating with concentrated H_2SO_4 acid soluble in it with no liberating iodine vapor on warming. This indicates that these dyes **10a-e** liberated HI during the reaction. This a criteria that the reaction is suggested to proceed through oxidative elimination reaction between active hydrogen of methyl group of heterocyclic quaternary salt and active hydrogen of site 5 of 2-metalo-1,3-dioxinium metal chelate ring followed by dehydrohalogenation -HI to give the above cyanine dyes **10a-e** [31].

3.2. UV-Vis absorption spectra

The experimental UV-Vis spectra in 95% ethanol for the newly synthesized cyanine dyes **5a-c**, **6a-c**, **8a-c** and **10a-e** are shown in Figures 1-3 and their spectral data are summarized in Table 1. The results show intense, broad absorption bands in the visible wavelength region with high extinction coefficients (ε_{max}) and accompanied with strong fluorescence especially for monomethine cyanine dyes of metal complex **10a-e**.

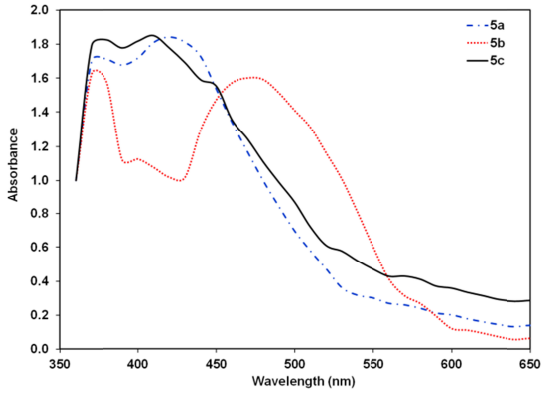

Figure 1. Absorption spectra for cyanine dyes **5a-c** in 95% ethanol solution.

The visible absorption maxima of the newly synthesized cyanine dyes in ethanol undergo bathochromic or hypsochromic shift depending on the nature of heterocyclic quaternary residue A, their linkage position and metal divalent moieties M. Thus, substitution of A = *N*-ethylpyridine in compound **5a** by A = *N*-ethylquinoline in compound **5b** resulted in a bathochromic shift of $\Delta\lambda_{max}$ = 50 nm for the increasing of π-conjugation and more extensive π delocalization in quinoline moiety. Analogously, changing the linkage position of quinoline residue from 1- in compound **5c** to 4- in compound **5b** resulted in a bathochromic shift of $\Delta\lambda_{max}$ = 60 nm. This could be attributed to the more extensive π-conjugation within position 4- of quinoline ring rather than position 1- of isoquinoline moieties. Also, the visible absorption spectra of pentamethine cyanine dyes **6a-c** are influenced by heterocyclic quaternary salts residue. Thus, substitution of A = *N*-ethylpyridin-2-ium in compound **6a** by A = *N*-ethylquinolin-2-ium in compound **6b** resulted in a bathochromic shift of $\Delta\lambda_{max}$ = 15 nm with the appearance new absorption bands at 485 and 510 nm, respectively. This is attributed to the more extensive π-conjugation in dye **6b**. Changing the linkage position of pyridine residue from pyridin-2 ium in dye **6a** to pyridin-4 ium in dye **6c** resulted in bathochromic shift of the wavelength of $\Delta\lambda_{max}$ = 10 nm. Additionally, the visible absorption spectra of monomethine-meso-substituted-pentamethine cyanine dyes **8a-c** are influenced by heterocyclic quaternary salts residue A. So, substitution of A = *N*-ethylpyridin-2-ium in compound **8a** by A = *N*-ethylquinolin-2-ium in compound **8b** resulted in a bathochromic shift of $\Delta\lambda_{max}$ = 55 nm with the appearance of new absorption bands at 520 and 695 nm, respectively. This is attributed to the more extensive π-conjugation in quinolin-2-ium moiety. Changing the linkage position of pyridine residue from pyridin-2-ium in dye **8a** to pyridin-4-ium in dye **8c** resulted in bathochromic shift of the wavelength of $\Delta\lambda_{max}$ = 25 nm. This is due to the more extensive π-conjugation in pyridine-4-ium moiety.

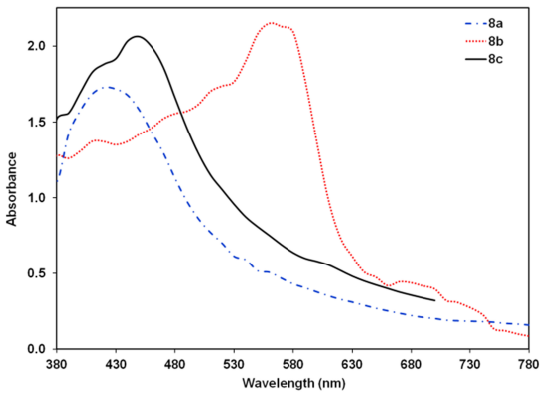

Figure 2. Absorption spectra for cyanine dyes **8a-c** in 95% ethanol solution.

The visible absorption spectra of monomethine cyanine dyes of metal complex **10a-e** depend on the nature of quaternary heterocyclic residue A and metal divalent moieties. Thus, substitution of A = *N*-ethylpyridin-2-ium in compound **10a** by A = *N*-ethylquinolin-2-ium in compound **10b** resulted in a bathochromic shift of $\Delta\lambda_{max}$ = 10 nm of red and blue shift bands with the appearance new shoulders of absorption bands at 520 and 550 nm, respectively.

Table 2. The effect of some selected synthesized compounds **4**, **5a**, **5c** and **6b** at different concentration on the growth of some bacterial strains.

Strains	Growth (mm)																
	Positive control	4 (mg/L)				6b (mg/L)				5a (mg/L)				5c (mg/L)			
		50	100	150	200	50	100	150	200	50	100	150	200	50	100	150	200
Streptococcus spp.	5	5	5	5	5	5.2	5.8	7.3	8.6	6.7	9.1	12	17	5	5.5	6.2	6.8
Staphylococcus spp	5	5	5	5	5	5.6	6.4	7.8	9.1	7.2	11	13	19	5	5.7	6.4	7.2
Salmonella spp	5	5	5	5	5	6.2	7.1	8.6	9.3	7.7	13	17	22	5.6	6.2	6.5	7.4
Shigella spp	5	5	5	5	5	6.5	7.5	8.9	9.5	8.3	14	18	23	5.8	6.3	7.2	8.1

This is attributed to the more extensive π-conjugation in dye **10b**. Changing the linkage position of pyridine residue from pyridin-2-ium in dye **10a** to pyridin-4-ium in dye **10c** resulted in bathochromic shift of the wavelength of $\Delta\lambda_{max}$ = 10 nm of the blue shift absorption band with the appearance absorption bands at 470 and 440 nm, respectively. On the other hand, dyes having Co^{2+} show more bathochromic shift than those included Ni^{2+} and/or Cu^{2+}. This is due to increases of both size and effective charge of metal cation and increases of positive charge of metal cation (Ni and Cu) causing hypsochromic shift which corresponds to a more intense cation complex of ion pair of oxygen atom in the metalo-dioxine ring similar to the effects of protonation.

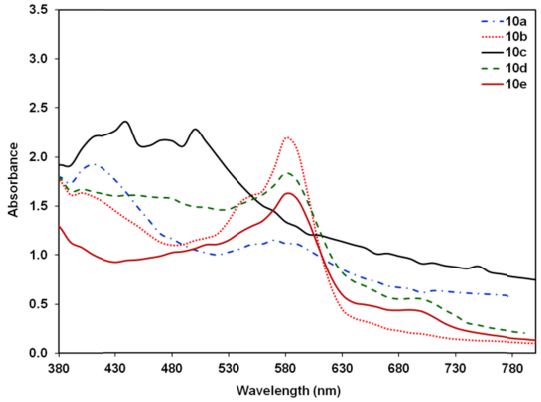

Figure 3. Absorption spectra for cyanine dyes **10a-e** in 95% ethanol solution.

3.3. *Antimicrobial activity of some selected cyanine dyes*

Newly synthesized cyanine dyes **5a**, **5c** and **6b** were chosen to study the biological activity and its relation the chemical structure. The *in-vitro* susceptibilities of the synthetic compounds were tested against *Streptococcus sp*, *Staphylococcus sp*, *Salmonella sp*. and *Shigella sp*. by using corckoporer method. Antimicrobial sensibility of the tested synthetic chemical compounds proved to be more effective than the antibiotics in inhibiting the growth of the pathogenic bacterial strains. The growth inhibitions of the selected bacterial strains by different concentrations of the tested synthetic compounds were recorded and the data were represented in Table 2. The dye **5a** has strong effect on the growth of the tested strains, dyes **5c** and **6b** showed slight inhibition effect while the starting compound **4** has no effect. This may be due to the presence of *N*-ethyl pyridine ring in dye **5a** comparing with the dyes **5c** and **6b** which containing isoquinoline and quinaldine rings connected with the starting material at positions 1 and 2, respectively [39].

4. Conclusions

In this work, new cyanine dyes **5a-c**, **6a-c**, **8a-c** and **10a-e** with potential biologically active were synthesized. Their structures were confirmed by IR, ^{1}H NMR and elemental analysis techniques. The absorption spectra of titled compounds in ethanol solution were studied. The results showed that the absorption spectra depend on the nature of heterocyclic quaternary residue A, their linkage position and metal divalent moieties (copper, cobalt and nickel). Compound **5a** is potent compound that can use in the future as good antibiotic for the tested pathogenic strains and our vision and dream to future is to try using such compounds in medicine if proved safe, there use will reduce the costs of treatment and conserve the hard currency of the country used import the row materials of the relatively more costly antibiotics.

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