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Synthesis and antimicrobial activity of meso-substituted polymethine cyanine dyes

R.M. Abd El-Aal[a,*] and M. Younis[b]

[a] *Chemistry Department, Aswan Faculty of Science, South Valley University, Egypt*
[b] *Botany Department, Aswan Faculty of Science, South Valley University, Egypt*

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Abstract

The condensation reaction of equivalent amounts of 2-cyanomethyl benzooxazole or its derivatives with variously substituted aromatic aldehydes gave 2-cyano-styryl benzooxazole or its derivatives. The subsequent reaction of the 2-cyano-styryl benzooxazoles with 2(4)-methyl substituted heterocyclic quaternary salts afforded meso-substituted styryl-2(4)-polymethine cyanines. The condensation reaction of 2-cyanomethyl benzooxazole or its derivatives with α-nitroso-β-naphthol followed by reaction with 2(4)-methyl substituted heterocyclic quaternary salts gave meso-substituted aza-2(4)-polymethine cyanines. The reaction of 2-cyanomethyl benzooxazole or its derivatives with *N*-methyl heterocyclic quaternary salts followed by the reaction with 2-methylquinolinium methiodide afforded the corresponding meso-substituted trimethine cyanine dyes. Elemental analyses, visible absorption, IR, ^{1}H NMR spectroscopy, and mass spectra established the structures of these compounds. The relationship between the structure and properties of these dyes has been studied and the solvatochromic behavior of some selected cyanine dyes in organic solvents is discussed. Finally, the antimicrobial activity of selected novel dyes was investigated in vitro using a wide spectrum of microbial strains.

Keywords: Cyanine dyes; Solvatochromism; Antimicrobial activity

* Corresponding author. Fax: +20-96-211-279.
E-mail address: abdelal2001@yahoo.com (R.M. Abd El-Aal).

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1. Introduction

Much work has been carried out on the synthesis of an assembled heterocyclic system to prepare and study the properties of different types of polymethine cyanine dyes [1–4]. Cyanine dyes are used as spectral sensitizers in photographic emulsions [5], probes for the physical state and membrane potential of liposomes and synthetic bilayers [6], as well as potential sensitizers for photodynamic therapy [7]. They are also used as inhibitors of cell-growth and division [8], and can be used for the determination of the sensitivity of microorganisms to antibiotics [9]. Cyanine dyes are retained in vitro and vivo to a much greater extent in the mitochondria of Carcinoma and melanona cells than in normal cells [10]. Thus, cyanine dyes can be used as tumor-cell specific photosensitizers with reduced skin photoxicity and damage to normal tissue [11].

The objective of this investigation is to synthesize and study the solvatochromic behavior and antimicrobial activity of some new cyanine dyes. A correlation has been established between molecular structure and spectral behavior of the synthesized compounds. The solvatochromic behavior was investigated to determine which ones were suitable for use as photosensitizers.

2. Material and methods

Melting points (m.p.) were recorded on a Galenkanp melting point apparatus and are uncorrected. Infrared spectra were recorded in potassium bromide on a Pye-Unicam SP 3-300 Infrared spectrophotometer. ^{1}H NMR spectra were recorded in deuterated $CDCl_3$ on a Varian Gemini 200 NMR spectrometer using tetramethylsilane as an internal reference. Mass spectra were recorded on GCMS-Qp 1000EX mass spectrometer at 70 eV. Microanalyses were carried out at the Microanalytical center at Cairo University. Visible spectra (300–700 nm) were recorded on a Shimadzu UV/visible 160-A spectrophotometer at the Aswan-Faculty of Science. All reagents and solvents were obtained from Aldrich Chemical (Milwaukee, WI, USA). The syntheses of 2-cyanomethyl oxazolo[4,5b]pyridine (1b) and 2-cyanomethyl pyrazolo[3,4-d]oxazole (1c) were carried out in the same manner as the synthesis of 2-cyanomethyl benzoxazole (1a) according to [12].

2.1. Synthesis of dyes

*2.1.1. Synthesis of meso-[benzo(hetero-)-oxazolo]-substituted styryl-2(4)-dimethine cyanine dyes **3a–3g***

A mixture of compounds **1a–1c** (0.01 mol) and variously substituted aromatic aldehydes (0.01 mol) was dissolved in ethanol (30 ml) and piperidine (3–5 drops) was added. The reaction mixture was refluxed for 6–8 h, filtered hot, concentrated, and cooled. The precipitated products were crystallized from methanol to give the intermediate compounds **2a–2e**. The characterization data are summarized in Table 1. A mixture of compounds **2a–2e** (0.01 mol) and 2(4)-methyl substituted heterocyclic

Table 1
Analytical data for compounds **2a–2e**, **3a–3g**, and **4a–4c**

Compound No.	Mol. formula (mol. wt.)	Calcd. % Found %			Yield (%)	M.p. (°C)	IR (ν_{max}^{KBr}) (cm^{-1})	^1H NMR ($CDCl_3$)	
		C	H	N				δ (assignment)	M^+
2a	$C_{16}H_{10}N_2O_2$ (262)	73.28 72.97	3.82 4.09	10.69 10.49	67	120–123	3100–2995 (=CH) 3300 (OH) 2200 (CN)	6.5–7.9 (m, 9H, Ar—H + het—H + CH=), 8.5 (s, 1H, OH)	263
2b	$C_{15}H_9N_3O_2$ (263)	68.44 68.69	3.42 3.57	15.97 15.81	51	180–182		6.7–8.1 (m, 8H, Ar—H + het—H + CH=), 8.7 (s, 1H, OH)	264
2c	$C_{20}H_{14}N_4O$ (326)	73.62 73.51	4.30 4.55	17.18 16.93	63	112–114		6.7–7.9 (m, 11H, Ar—H + het—H + CH=), 1.07 (s, 3H, CH_3)	326
2d	$C_{20}H_{14}N_4O_2$ (342)	70.18 69.95	4.09 4.21	16.37 16.13	65	118	3100–2990 (=CH) 3300 (OH) 2200 (CN)		343
2e	$C_{20}H_{13}N_5O_3$ (371)	64.69 64.81	3.50 3.73	18.87 18.69	69	95–97		6.7–8.2 (m, 10H, Ar—H + het—H + CH=), 1.1 (s, 3H, CH_3)	372
3a	$C_{27}H_{22}N_3O_2I$ (547)	59.23 59.39	4.02 3.89	7.68 7.83	87	150–152	3100–2990 (=CH) 3300 (OH) 3400–3200 (NH) 2980–2940 (CH_3I)	8.6 (s, 1H, OH), 6.6–8.1 (m, 16H, Ar.—H + het.—H + CH=), 3.9 (s, 3H, CH_3—N^+), 4.9 (s, 2H, NH_2)	548
3b	$C_{26}H_{21}N_4O_2I$ (548)	56.93 57.11	3.83 4.07	10.22 10.02	66	185–187			549
3c	$C_{31}H_{26}N_5OI$ (611)	60.88 60.63	4.26 4.41	11.46 11.33	71	130–133	3300 (OH) 1680 (CHO) 1720 (C=O)	6.7–8.2 (m, 18H, Ar.—H + het.—H + CH=), 4.9 (s, 2H, NH_2), 3.9 (s,3H, CH), 1.1 (s, 3H, CH_3)	610

Table 1 (*continued*)

Compound No.	Mol. formula (mol. wt.)	Calcd. % Found %			Yield (%)	M.p. (°C)	IR (ν_{max}^{KBr}) (cm^{-1})	^{1}H NMR ($CDCl_3$)	
		C	H	N				δ (assignment)	M^+
3d	$C_{27}H_{24}N_5O_2I$ (577)	56.15 56.39	4.16 3.91	12.14 10.93	63	107–109			578
3e	$C_{31}H_{26}N_5O_2I$ (627)	59.33 59.53	4.15 4.37	11.16 11.29	68	142–145			627
3f	$C_{27}H_{24}N_5O_2I$ (577)	56.15 56.29	4.16 4.39	12.14 12.37	59	115–117	3400 (NH_2) 2970 (CH_3I) 1630 (C=N)	8.6 (s, 1H, OH), 5.1 (s, 2H, NH_2), 6.6–7.9 (m, 15H, Ar.—H + het.—H + CH=), 3.8 (s, 3H, CH_3—N^+), 1.05 (s, 3H, CH_3)	
3g	$C_{31}H_{25}N_6O_3I$ (656)	56.71 56.49	3.81 4.17	12.81 12.63	62	155–157	3400 (NH_2) 2970 (CH_3I) 1585 (CH=CH)	6.6–8.1(m, 17H, Ar—H + het—H + CH=), 5.1 (s, 2H, NH_2), 1.5 (t, 3H, CH_3), 4.2 (q, 3H, CH_3–N^+)	658
4a	$C_{19}H_{11}N_3O_2$ (313)	72.84 72.67	3.51 3.73	13.42 13.31	57	100–102	2200 (CN) 1640 (C=N)	8.5 (s, 1H, OH), 7.6–8.2 (m, 10H, Ar.—H)	312
4b	$C_{18}H_{10}N_4O_2$ (314)	68.79 68.47	3.19 3.33	17.83 17.71	45	95–97	2220 (CN) 1630 (C=N)	8.7 (s, 1H, OH), 7.3–8.1 (m, 9H, Ar.—H + het.—H)	
4c	$C_{23}H_{15}N_5O_2$ (393)	70.23 69.95	3.82 4.16	17.81 17.69	67	83–85		8.5 (s, 1H, OH), 7.2–8.1 (m, 11H, Ar.—H + het.—H), 1.1 (s, 3H, CH_3)	394

quaternary salts (α-picoline, quinaldine, and/or γ-picoline) methiodide (0.01 mol) was dissolved in ethanol (30 ml) and piperidine (3–5 drops) was added. The reaction mixture was refluxed for 10–12 h, filtered hot, concentrated, and cooled. The precipitated products were separated in dilution with water and crystallized from methanol to give **3a–3g**. The characterization data are summarized in Table 1.

*2.1.2. Synthesis of meso-[benzo(hetero-)-oxazolo]-substituted aza-2(4)-dimethine cyanine dyes **5a–5e***

A mixture of compounds **1a–1c** (0.01 mol) and α-nitroso-β-naphthol (0.01 mol) was dissolved in ethanol (20 ml) and piperidine (3–5 drops) was added. The reaction mixture was refluxed for 7–9 h, filtered hot, concentrated, and cooled. The precipitated products were crystallized from methanol to give the intermediate compounds **4a–4c**. The characterization data are summarized in Table 1. A mixture of compounds **4a–4c** (0.01 mol) and 2(4)-methyl substituted heterocyclic quaternary salts (1-methylpyridin-2-ium, 1-methylquinolin-2-ium, and/or 1-methylpyridin-4-ium) methiodide (0.01 mol) was dissolved in ethanol (30 ml) and piperidine (3–5 drops) was added. The reaction mixture was refluxed for 10–12 h, filtered hot, concentrated, and cooled. The precipitated products were separated in dilution with water and crystallized from methanol to give **5a–5e**. The characterization data are summarized in Table 2.

*2.1.3. Synthesis of meso-[benzo(hetero-)-oxazolo]-substituted trimethine cyanine dyes **7a–7e***

A mixture of compounds **1a–1c** (0.01 mol) and *N*-methylheterocyclic quaternary salts [*N*-methyl(pyridinium, quinolinium, and/or isoquinolinium)iodide] (0.01 mol) was dissolved in ethanol (25 ml) and triethylamine (3 ml) was added. The reaction mixture was refluxed for 8–10 h, filtered hot, concentrated, and cooled. The precipitated products were crystallized from methanol to give the intermediate compounds **6a–6e**. The characterization data are summarized in Table 2. A mixture of compounds **6a–6e** (0.01 mol) and 2-methylquinolinium methiodide (0.01 mol) was dissolved in ethanol (30 ml) and piperidine (3–5 drops) was added. The reaction mixture was refluxed for 10–12 h, filtered hot, concentrated, and cooled. The precipitated products were separated in dilution with water and crystallized from methanol to give **7a–7e**. The characterization data are summarized in Table 2.

2.2. Microbiological testing

Twelve Compounds [200 μg/ml (w/v) solutions in sterile DMSO] were tested using 14 microorganisms. These organisms included six Gram positive bacteria (1, *Bacillus subtilis* NRS-744; 2, *Micrococcus luteus* SW 712; 3, *Bacillus megaterium* SW 354; 4, *Staphylococcus aureus* B-767; 5, *Streptomyces sp.* SW 123; and 6, *Bacillus cereus* ATCC-9634), five Gram negative bacteria (7, *Serratia Mar.* SW 98; 8, *Pseudomonas aeruginosa* ATCC-6NA10245; 9, *Escherichia coli* B-3704; 10, *Salmonella sp* SW 476; and 11, *Pseudomonas sp* SW 653), one ascosporogenous yeast (12, *Saccharomyces cerevisiae* SW 43), and two fungi [13, *Candida albicans* IMRU3669 and 14, *Aspergillus*

Table 2
Analytical data for compounds **5a–5e**, **6a–6e**, and **7a–7e**

Compound No.	Mol. formula (mol. wt.)	Calcd. % Found %			Yield (%)	M.p. (°C)	IR (ν_{max}^{KBr}) (cm^{-1})	^1H NMR ($CDCl_3$)	
		C	H	N				δ (assignment)	M^+
5a	$C_{26}H_{21}N_4O_2I$ (548)	56.93 56.72	3.83 3.63	10.22 10.35	73	130–132	3400 (NH_2) 1640 (C=N) 2985 (CH_3 I)	8.9 (s, 1H, OH), 6.7–8.1(m, 15H, Ar.—H + het—H + CH=CH), 5.1 (s, 2H, NH_2), 3.8 (s, 3H, CH_3)	547
5b	$C_{30}H_{23}N_4O_2I$ (598)	60.20 67.91	3.85 4.41	9.37 9.21	61	176–178	3450 (NH_2) 1650 (C=N) 2970 (CH_3I)		599
5c	$C_{26}H_{21}N_4O_2I$ (548)	56.93 56.83	3.83 3.63	10.22 10.15	79	146–148	3300 (NH_2) 1640 (C=N) 2985 (CH_3 I)		646
5d	$C_{29}H_{22}N_5O_2I$ (599)	58.10 57.89	3.67 3.59	11.69 11.47	68	125–127	3350 (NH_2) 2970 (CH_3I) 1635 (C=N)	8.7 (s, 1H, OH), 6.5–8.1(m, 16H, Ar.—H + het—H + CH=CH), 5.2 (s, 2H, NH_2), 3.9 (s, 3H, CH_3)	
5e	$C_{34}H_{27}N_6O_2I$ (678)	60.18 60.25	3.98 4.13	12.39 12.13	84	164–166	3345 (NH_2) 2985 (CH_3I) 1635 (C=N)	8.5 (s, 1H, OH), 1.15 (s, 3H, CH_3), 6.6–7.9 (m, 18H, Ar.—H + het—H + CH=CH), 5.3 (s, 2H, NH_2), 4.1 (s, 3H, CH_3)	
6a	$C_{19}H_{14}N_3O$ (300)	76.00 75.85	4.67 4.51	14.00 13.89	85	112–114	1580 (C=C) 2220 (CN)	7.2-8.2 (m, 11H, Ar.—H + het.—H), 1.7 (s, 3H, CH_3)	299

6b	$C_{18}H_{13}N_4O$	71.76	4.32	18.61	65	195–197	1600 (C=C)	7.3–8.1 (m, 10H, Ar.—H + het.—H),	
	(301)	71.43	4.17	18.49			2200 (CN)	1.6 (s, 3H, CH_3)	
6c	$C_{19}H_{15}N_5O$	69.30	4.56	21.28	73	103–105	1600 (C=C)		330
	(329)	69.05	4.67	20.99			2230 (CN)		
6d	$C_{23}H_{17}N_5O$	72.82	4.49	18.47	72	116–118	1585 (C=C)	7.3–8.1 (m, 11H, Ar.—H + het.—H),	
	(379)	72.66	4.27	18.61			2200 (CN)	1.6 (s, 3H, CH_3), 1.1 (s, 3H, CH_3)	
6e	$C_{23}H_{17}N_5O$	72.82	4.49	18.47	77	85–87	1595 (C=C)		378
	(379)	72.69	4.37	18.59			2220 (CN)		
7a	$C_{30}H_{25}N_4OI$	61.64	4.78	9.59	91	162–164		6.7–7.9 (m, 17H,	583
	(584)	61.81	4.57	9.81				Ar.—H + het.—H + CH=CH—),	
								1.6 (s, 3H, CH_3), 5.2 (s, 2H, NH_2),	
								3.9 (s, 3H, CH_3)	
7b	$C_{29}H_{24}N_5OI$	59.49	4.10	11.97	71	225–227	3500 (NH_2)	6.6–7.9 (m, 16H,	
	(585)	59.63	4.33	12.11			2960(CH_3I)	Ar.—H + het.—H + CH=CH—),	
								1.6 (s, 3H, CH_3), 5.1 (s, 2H, NH_2),	
								4.1 (s, 3H, CH_3)	
7c	$C_{30}H_{27}N_6OI$	58.63	4.40	13.68	63	130–132	3400 (NH_2)		615
	(614)	58.75	4.57	13.47			2960 (CH_3I)		
7d	$C_{34}H_{29}N_6OI$	61.45	3.37	12.65	89	122–124	3450 (NH_2)	6.5–8.1 (m, 18H,	
	(664)	61.31	3.27	12.79			2970 (CH_3I)	Ar.—H + het.—H + CH=CH—),	
							1590 (C=C)	1.7 (s, 3H, CH_3), 5.3 (s, 2H, NH_2),	
								4.05 (s, 3H, CH_3), 1.1 (s, 3H, CH_3)	
7e	$C_{34}H_{29}N_6OI$	61.45	3.37	12.65	69	175–177	3500 (NH_2)		663
	(664)	61.35	3.29	12.83			2980 (CH_3I)		

flavus S-C 43 (3/3)]. The microorganisms (numbers 1, 4, 6, 8, 13, and 14) were provided by the culture collection of the US Department of Agriculture, Northern Regional Research Laboratory (Peoria, IL, USA). Other bacterial strains (identified according to the key given in Bergy's Manual of Determinative Bacteriology 8th ed. 1974 [13]) were obtained from the culture collection of the Faculty of Science, Department of Botany, Aswan, Egypt. Each bacteria was suspended in sterile nutrient broth media (5 ml), inoculated, and then incubated at 37 °C for 2 h. Each suspension (1 ml) was added to the center of a sensitivity testing plate. A sterile dry cotton wool swap was used to spread the inoculum on the media. The inocula were allowed to dry for few minutes. Ten discs of filter paper (6 mm diameter) were placed in petri dish and sterilized at 180 °C for 1 h. After cooling, 1 ml of the compound solution was added to each one of the 10 discs (20 μg concentration per one disc). The discs were dried in the incubator at 35–37 °C for 1 h. The plates were incubated overnight at 37 °C. The diameters of the non-inhibition zones were measured to the nearest 0.5 mm and compared with the control DMSO. The data obtained are expressed as the area of the inhibition zone (see Table 5). Malt-yeast extract agar and Czapek–Dox's agar were used to cultivate the yeast and the fungi, respectively. Filter paper discs (6 mm diameter) were prepared [14] at a concentration of 100 μg/disc for each compound. A disc of each compound was placed in a plate of young culture (48 h old yeast and 7-day-old fungi). The plates were kept at 5 °C for 1 h to allow diffusion of the compounds through the media. The plates of the yeast and fungi were kept at 30 °C for 4 days. At the end of the incubation period, the inhibition zones were determined. The preliminary antimicrobial activity was standardized against the antibacterial ampicillin (10 mg/ml) and the antifungal nystatin (10 mg/ml) in distilled water [15].

(1a–1c):

Z = phenyl (a);
Z = pyridine (b);
Z = 3-methyl-1-phenylpyrazole (c).

(2a–2e):

Z = phenyl, X = *p*-OH (a); Z = pyridine, X = *p*-OH (b);
Z = 3-methyl-1-phenylpyrazole, X = H (c);
Z = 3-methyl-1-phenylpyrazole, X = *p*-OH (d);
Z = 3-methyl-1-phenylpyrazole, X = *o*-NO_2 (e).

(3a–3g):

Z = phenyl, X = *p*-OH, A = 1-methylquinolin-2-ium (a);
Z = pyridine, X = *p*-OH, A = 1-methylquinolin-2-ium (b);
Z = pyrazole, X = H, A = 1-methylquinolin-2-ium (c);
Z = pyrazole, X = *p*-OH, A = 1-methylpyridin-2-ium (d);
Z = pyrazole, X = *p*-OH, A = 1-methylquinolin-2-ium (e);
Z = pyrazole, X = *p*-OH, A = 1-methylpyridin-4-ium (f);
Z = pyrazole, X = *o*-NO_2, A = 1-methylquinolin-2-ium (g).

(5a–5e):

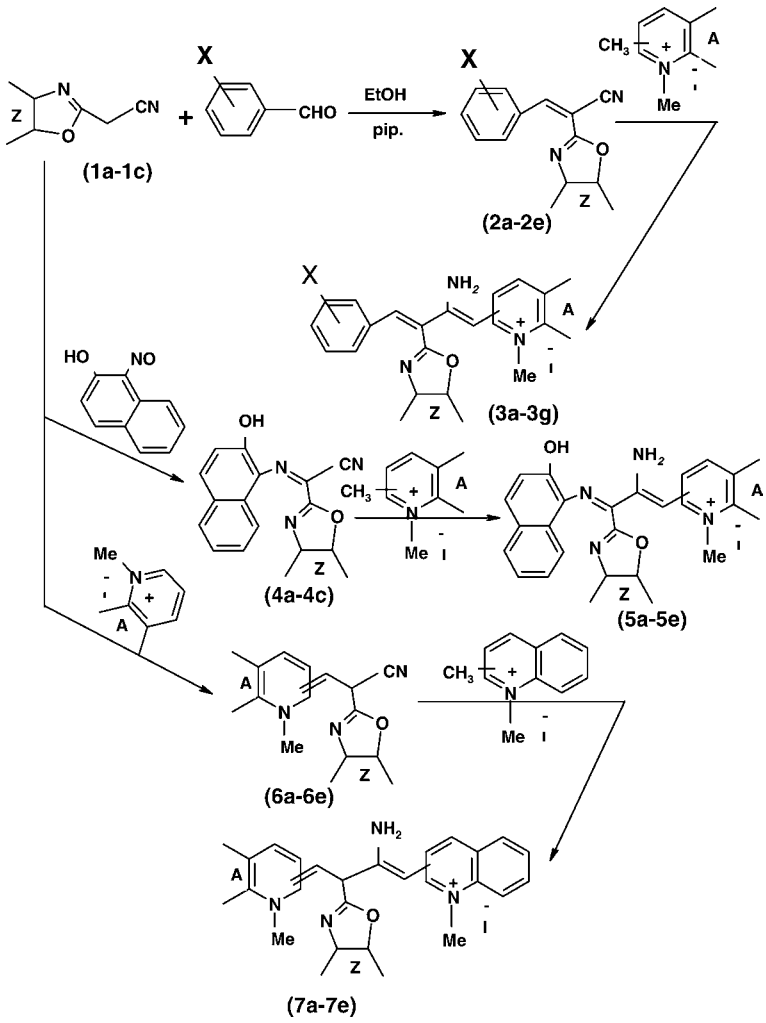
Z = phenyl, A = 1-methylpyridin-2-ium (a);
Z = phenyl, A = 1-methylquinolin-2-ium (b);

Z = phenyl, A = 1-methylpyridin-4-ium (c);
Z = pyridine, A = 1-methylquinolin-2-ium (d);
Z = pyrazole, A = 1-methylquinolin-2-ium (e).
(6a–6e) and (7a–7e):
Z = phenyl, A = *N*-methylquinolin-4-ium (a);
Z = pyridine, A = *N*-methylpyridin-4-ium (b);
Z = pyrazole, A = *N*-methylquinolin-4-ium (c);
Z = Pyrazole, A = *N*-methylquinolin-4-ium (d);
Z = pyrazole, A = *N*-methylisoquinolin-1-ium (e).

3. Results and discussion

3.1. Synthesis

Most of the meso-substituted styryl-(aza-)-dimethine and trimethine cyanine dyes in the present work were synthesized from the starting material 2-cyanomethyl benzoxazole or its derivatives **1a–1c** (Scheme 1) [12]. Condensation of equimolar ratios of **1a–1c** with variously substituted aromatic aldehydes in piperidine afforded the intermediate compounds **2a–2e** [16]. The reaction of equimolar amounts of compounds **2a–2e** with 2(4)-methyl substituted heterocyclic quaternary salts (α-picoline, quinaldine, and/or γ-picoline) methiodide in the presence of piperidine afforded the corresponding meso-[benzo(hetero)-oxazole]-substituted styryl-2(4)-dimethine cyanine dyes **3a–3g** [17]. The structures of compounds **2a–2e** and **3a–3e** were established by analytical data which are summarized in Table 1. Thus, the IR spectrum for compounds **2a** showed an absorption band for the cyano group (2200 cm^{-1}) but the IR spectrum for **3a** did not show the CN group. The ^1H NMR spectrum ($CDCl_3$) showed signals at δ 6.5–7.9, corresponding to the nine protons of the aromatic and methine groups for compound **2a** and at δ 6.6–8.1, corresponding to the 16 protons of the aromatic, heterocyclic, and methine groups for compound **2b**, and at δ 3.9, corresponding to the three protons of the methyl group and at δ 4.9, corresponding to the two protons of the amino group for compound **3a**. The reaction of equimolar amounts of compounds **1a–1c** with α-nitroso-β-naphthol using a basic catalyst gave the intermediate compounds **4a–4c** [18]. The reaction of equimolar amounts of compounds **4a–4c** with 2(4)-methyl substituted heterocyclic quaternary salts (methylpyridin-2-ium, methylquinolin-2-ium, and/or methylpyridin-4-ium) in presence of piperidine afforded the corresponding meso-[benzo(hetero)-oxazole]-substituted aza-2(4)-dimethine cyanine dyes **5a–5e** (Scheme 1). The structures of compounds **4a–4c** (Table 1) and **5a–5e** (Table 2) were confirmed by analytical and spectral data. Thus, the IR spectrum of compound **4b** showed an absorption band for the cyano group (2220 cm^{-1}) and the IR spectrum of **5d** showed an absorption band for methyl iodide (2970 cm^{-1}). The IR spectrum for **5d** does not show the CN group. The ^1H NMR spectrum ($CDCl_3$) for compound **4b** showed signals at δ 7.3–8.1 corresponding to the nine protons of the aromatic and heterocyclic groups. For **5d**, the ^1H NMR spectrum showed signals at δ 6.5–8.1, corresponding to the 16 protons of



Scheme 1.

the aromatic, heterocyclic, and methine groups, and δ 3.9, corresponding to the three protons of the methyl group.

The reaction of equimolar amounts of compounds **1a–1c** with *N*-methylheterocyclic quaternary salts [*N*-methyl(pyridinium, quinolinium, and/or isoquinolinium)iodide] using base catalyst afforded intermediate compounds, merocyanine dyes **6a–6e** [19]. The reaction of equimolar ratios of the intermediate merocyanine **6a–6e** with 2-methylquinolinium methiodide in the presence of piperidine as a base catalyst afforded the corresponding meso-[benzo(hetero)-oxazole]-substituted trimethine cyanine dyes 7**a–7e** (Scheme 1). The structures of compounds **6a–6e** and **7a–7e** were established by analytical data which are summarized in Table 2. Thus, the IR spectrum of compound **6d** showed an absorption band for the cyano group

(2200 cm^{-1}) and for compound **7d** an absorption band for the methyl iodide (2970 cm^{-1}) group but did not show the CN group for compound **7d** and reveal the presence of an amino group (3450 cm^{-1}). The ^1H NMR spectrum ($CDCl_3$) showed signals at δ 7.3–8.1, corresponding to the 11 protons of the aromatic and heterocyclic groups for compound **6d** and at δ 6.5–8.1, corresponding to the 18 protons of the aromatic, heterocyclic, and methine groups for compound **7d** and at δ 4.05, corresponding to the three protons of the methyl group and at δ 5.3, corresponding to the two protons of the amino group for compound **7d**.

3.2. Characterization of the synthesized cyanine dyes

The λ_{max} and ε_{max} values for the synthesized cyanine dyes **3a–3g**, **5a–5e**, **6a–6e**, and **7a–7e** in ethanol are presented in Table 3. The visible absorption maxima of meso-substituted styryl polymethine cyanine dyes in ethanol undergo bathochromic or hypsochromic shifts depending on the nature of the meso-substituted heterocyclic

Table 3
The visible absorption spectra of meso-substituted cyanine dyes in 95% EtOH

λ_{max} (nm)/log ε_{max} (mol^{-1} cm^{-1})						
Meso-substituted styryl dimethine cyanine dyes (***3a–3g***)						
3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**
440(4.07)	470(3.45)	407(4.58)	—	414(4.06)	—	—
—	—	510(4.58)	490(3.00)	519(4.21)	510(3.05)	510(4.62)
548(4.11)	579(3.57)	560(4.36)	—	—	—	—
585(4.01)	600(3.52)	Sh595(4.31)	—	598(4.16)	—	588(4.30)
Sh640(3.74)	690(2.97)	680(3.55)	—	690(3.62)	—	670(3.98)
Meso-substituted aza-dimethine cyanine dyes (***5a–5e***)						
5a	**5b**	**5c**	**5d**	**5e**		
411(4.30)	415(4.76)	413(3.o4)	425(4.65)	415(3.04)		
476(3.85)	510(4.38)	491(2.82)	518(4.22)	514(2.77)		
—	580(4.69)	—	587(4.33)	585(1.27)		
—	—	—	610(4.11)	—		
Merocyanine dyes (***6a–6e***)						
6a	**6b**	**6c**	**6d**	**6e**		
—	—	410(3.65)	415(3.90)	—		
480(3.91)	499(3.61)	477(3.60)	488(3.93)	465(3.48)		
—	518(3.32)	—	Sh512(3.73)	—		
—	—	—	—	—		
Meso-substituted trimethine cyanine dyes (***7a–7e***)						
7a	**7b**	**7c**	**7d**	**7e**		
440(3.99)	452(3.52)	—	445(4.35)	—		
512(3.98)	522(3.81)	510(4.40)	516(4.78)	511(4.61)		
552(4.00)	sh560(3.75)	555(4.43)	558(4.52)	—		
583(3.79)	595(3.85)	578(4.41)	590(4.46)	565(4.27)		
Sh690(3.11)	693(3.27)	688(3.93)	690(3.65)	676(3.98)		

Z, aryl moiety **R**, and the quaternary residue **A**. Thus, replacing the benzooxazole group in compound **3a** with a oxazolo[3,2b]pyridine (compound **3b**) causes a bathochromic shift of $\Delta\lambda_{max} = 30$, 31, and 50 nm at absorption maxima $\lambda_{max} = 470$, 579, and 690 nm, respectively. This shift is due to the increased accepting ability of the meso-substituted group (pyridine ring) which increases the charge transfer from the lone pair electrons of the hydroxy group of the aryl moiety to the positively charged nitrogen of the quaternary residue. Also, replacing the nitro group in compound **3g** with a hydroxyl group (compound **3e**) results in a bathochromic shift of $\Delta\lambda_{max} = 9$–20 nm, accompanied by the appearance of a new absorption band at $\lambda_{max} = 414$ nm. This is attributed to increasing the conjugation through the electron donating hydroxy group in compound **3e**. Additionally, the visible absorption maxima are influenced by the changing the quaternary residue A. Substitution of the 1-methylpridin-2-ium in compound **3d** by a 1-methylquinolin-2-ium (compound **3e**) results in a bathochromic shift of $\Delta\lambda_{max} = 29$ nm, accompanied by the appearance of three new absorption bands at $\lambda_{max} = 414$, 598, and 690 nm, respectively. This could be attributed to the more extensive π-conjugation in compound **3e**.

The visible absorption maxima of meso-substituted aza-polymethine cyanine dyes **5a–5d** are influenced by both meso-substituted heterocyclic Z and quaternary residue A. Thus, replacing the benzooxazole group in compound **5b** with an oxazolo[3,2b]pyridine group (compound **5d**) results in a bathochromic shift of $\Delta\lambda_{max} = 7$–10 nm, accompanied by the appearance of a new absorption band at $\lambda_{max} = 610$ nm. This may be due to increasing the charge transfer by the electron accepting ability of the meso-substituted oxazolo[3,2b]pyridine. Replacing 1-methylpyridin-2-ium in compound **5a** with 1-methylquinolin-2-ium (compound **5b**) causes a bathochromic shift of $\Delta\lambda_{max} = 4$–34 nm, accompanied by the appearance of a new absorption band at $\lambda_{max} = 580$ nm. This is due to the more extensive π-conjugation in the 1-methylquinolin-2-ium moiety.

The quaternary residue A and their linkage position also influence the visible absorption maxima of meso-substituted merocyanine dyes **6a–6e**. Thus, replacing *N*-methylpyridin-4-ium in compound **6c** with *N*-methylquinolin-4-ium (compound **6d**) causes a bathochromic shift of $\Delta\lambda_{max} = 5$–11 nm, accompanied by the appearance of a new shoulder absorption band at $\lambda_{max} = 512$ nm. Replacing the *N*-methy-Liosquinlin-1-ium moiety in compound **6e** with a *N*-methylquinolin-4-ium moiety (compound **6d**) results in a bathochromic shift of $\Delta\lambda_{max} = 23$ nm, accompanied by the appearance of two new absorption bands at $\lambda_{max} = 415$ and 512 nm, respectively. This could be attributed to the more extensive π-conjugation in the quinolin-4-ium moiety.

The visible absorption maxima of meso-substituted trimethine cyanine dyes **7a–7e** are influenced by both the meso-substituted heterocyclic Z and quaternary residue A. Thus, replacing *N*-methylpyridin-4-ium in compound **7c** with *N*-methylquinolin-4-ium (compound **7d**) causes a bathochromic shift of $\Delta\lambda_{max} = 5$–35 nm, at $\lambda_{max} = 445$ and 516 nm, respectively. This could be attributed to the more extensive π-conjugation in the quinolin-4-ium moiety. Also, changing the linkage position of quinoline residue from quinolin-1-ium in compound **7e** to quinlin-4-ium in compound **7d** results in a bathochromic shift of $\Delta\lambda_{max} = 5$–25 nm, accompanied by the

appearance of two new absorption bands at $\lambda_{max} = 445$ and 558 nm, respectively. This is due to the more extensive π-conjugation in the quinolin-4-ium than the quinolin-1-ium moieties.

Solvatochromic dyes generally exhibit steady bathochromic (positive solvatochromism) or hypsochromic (negative solvatochromism) shifts in solvents having different polarities. If the ground state is more stabilized than the excited state due to the solvation by solvents of increasing polarity, negative solvatochromism is exihibited. The electron absorption spectra of some selected cyanine dyes **3a, 3b, 6a, 6b**, and **7b**, in the 350–750 nm range, have been studied in different organic solvents (H_2O, DMF, EtOH, acetone, acetonitrile, $CHCl_3$, benzene, and CCl_4) [20] and the results are presented in Table 4. These dyes show negative solvatochromism with increased solvent polarity. This is due to several reasons, which depend on the structure and the type of dye. The styryl cyanine dyes **3a** and **3b** show a large change in dipole moment upon excitation due to the relative contribution of both dipolar zwitterionic benzenoid and neutral quinoid forms [21]. Thus, dyes **3a** and **3b** are found to exhibit benzenoid (Mb) and quinonoid (Mq) forms as a result of intramolecular charge transfer [22] as shown in Scheme 2. The strong hypsochromic shift of **3a** and **3b** with increasing polarity of solvent results from the interaction between the total dipole moment of the solvent molecules with the dipolar solute molecule. This also decreases the Taft, s π^*, and α (hydrogen bond donor ability) parameters and increases the energy of the ground state by decreasing the solvent cage of oriented solvent molecules around dipolar solute molecules. On the basis of valence-bond theory, an intermediate meso-polymethine (Mpm) form is proposed (as shown in Scheme 2).

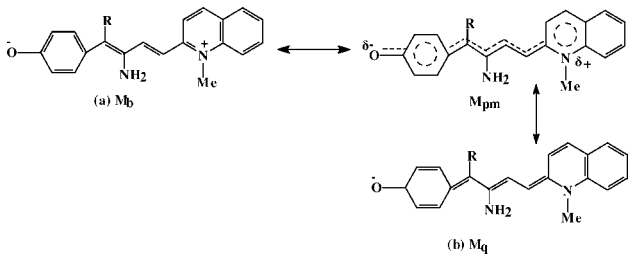
The solvatochromism of dyes **6a, 6b**, and **7b** is caused by differential solvation of the ground state and the Franck–Condon excited state [23]. This is due to the absorption of the electromagnetic radiation in the UV–Vis region. The absorption maxium of these dyes depends on the polarity and hydrogen-bonding ability of the solvents, a marked hypsochromic shift was observed with increasing polarity of solvent. The negative solvatochromism of dyes **6a, 6b**, and **7b** is caused by strong hydrogen-bonding solvents which stabilized structure **a** (Scheme 3) and thereby the electron-donating ability of the nitrogen. In addition the intramolecular charge transfer (ICT) interaction with the quinolinium moiety decreases. In the Franck–Condon excited state, hydrogen-bonding takes place between solvent and iodide ions and stabilizes **b** (Scheme 3) and prevents its conversion to **c** (Scheme 3). The energy gap between the Franck–Condon excited state and the relaxed excited state is small and increases with a decrease in the polarity of the solvents. Structure **c** is stabilized in solvents of low polarity because these solvents do not form hydrogen bonds with the iodide anions.

3.3. Antimicrobial activity of selected cyanine dyes

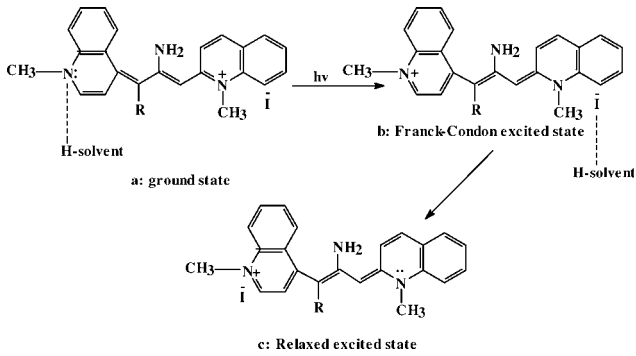
The activities of twelve cyanine dyes **(3b**, **4a**, **4b**, **5a**, **5b**, **5d**, **5e**, **6a**, **6b**, **6d**, **7a**, and **7b)** were investigated in vitro against a wide spectrum of microbial strains, including six strains of Gram positive bacteria, five strains of Gram negative bacteria, one strain of yeast, and two strains of fungi (Table 5). Several organic compounds are

Table 4
Characterization of selected cyanine dyes in different organic solvents

Compound No.	λ_{max} (nm)/log ε_{max} ($mol^{-1}\,cm^{-1}$)								$\Delta\lambda$ (DMF-CCl_4)
	Water	DMF	EtOH	$CHCl_3$	Acetone	CH_3CN	Benzene	CCl_4	
3a	406(3.89)	432(3.88)	440(4.07)	440(4.04)	435(4.03)	432(4.04)	443(4.03)	447(4.06)	8
	545(3.85)	538(3.85)	548(4.11)	545(4.18)	530(4.08)	542(4.06)	547(4.08)	568(4.11)	7
	—	—	sh640(3.74)	660(3.52)	659(3.46)	—	664(3.51)	669(3.45)	New band
3b	426(3.56)	439(3.48)	470(3.45)	561(3.42)	436(3.34)	427(3.38)	565(3.56)	568(3.51)	122
	565(3.45)	582(3.42)	579(3.57)	584(3.44)	576(3.43)	574(3.45)	587(3.43)	589(3.40)	2
	647(3.20)	689(2.66)	690(2.97)	692(2.76)	685(2.70)	681(2.73)	704(2.64)	704(2.60)	
6a	446(3.50)	458(3.48)	480(3. 91)	498(3.81)	477(3.76)	480(3.78)	488(3.76)	482(3.29)	40
6b	498(3.51)	508(3.32)	499(3.61)	512(3.34)	501(3.24)	503(3.35)	510(3.28)	506(3.30)	4
	515(3.42)	517(3.46)	518(3.32)	525(3.12)	519(3.34)	520(3.15)	527(3.11)	525(3.01)	8
7b	—	413(3.34)	452(3.52)	421(2.81)	418(3.66)	415(2.86)	420(2.87)	415(2.73)	8
	—	518(3.39)	522(3.81)	530(3.76)	526(3.72)	523(3.52)	532(3.23)	533(3.52)	12
	—	565(3.78)	560(3.71)	572(3.65)	568(3.60)	564(3.63)	573(3.11)	575(3.68)	7
	595(.34)	602(3.76)	595(3.85)	605(3.84)	596(3.75)	595(3.78)	602(3.83)	602(3.80)	0
	690(2.70)	697(3.28)	693(3.27)	699(3.18)	597(3.10)	695(3.15)	700(2.92)	700(3.02)	2



Scheme 2.



Scheme 3.

well known to have antimicrobial activity against many species of bacteria, yeast, and fungi [24–29]. These compounds are useful in treatment and/or control of human, animal, and plant diseases. The present investigation was carried out to determine the antimicrobial and antifungal activity of these 12 cyanine dyes. The data of the disc susceptibility for the tested complexes clearly showed varied responses with the test organisms depending on both the type of organism and dye. Thus, compounds **4a** and **4b** exhibit pronounced antimicrobial activity towards both Gram positive and Gram negative bacteria, yeast, and fungi. This may be due to the toxic effect of both the naphthol and cyano groups. The meso-substituted aza-2(4)-polymethine cyanine dyes **5a**, **5b**, **5d**, and **5e** showed antimicrobial activity against the tested microorganisms, where the amount of the activity is dependent on the structure of the dye. Thus, **5e** has more activity towards both Gram positive and Gram negative bacteria, yeast and fungi than do **5a**, **5b**, and **5d**. This is due to the fact that **5e** contains a pyrazole heterocyclic ring which increases the activity. Pyrazole rings are effective inhibitors of cell growth and destroy the progressive cell of microorganisms [30]. Merocyanine dyes also exhibit antimicrobial activity against the tested microorganisms, where Gram positive and negative bacteria, and yeast, and fungi were sensitive to **6a**, **6b**, and **6d**. However, **6d** has a greater effect than **6a** because **6d** contains a pyrazole ring. Meso-substituted trimethine cyanine dyes **7a** and **7b** also showed antimicrobial activity against the tested microorganisms. The

Table 5
Antibacterial properties of the tested compounds expressed as size (mm) of inhibition zone

Test organism	Compound													
	3b	**4a**	**4b**	**5a**	**5b**	**5d**	**5e**	**6a**	**6b**	**6d**	**7a**	**7b**	Ampicillin	Nystatin
Bacillus subtilis	12	22	24	9	13	15	23	12	18	14	17	16	+	–
Micrococcus luteus	17	21	25	11	12	17	21	16	12	11	15	16	+	–
Bacillus megaterium	13	17	21	12	10	12	18	13	15	17	15	17	+	–
Staphylococcus aureus	11	21	24	8	14	11	22	10	18	19	11	14	+	–
Streptomyces sp.	15	18	21	10	13	9	19	16	14	13	18	17	+	–
Bacillus cereus	18	25	23	12	15	10	22	11	16	15	10	13	+	–
Serratia Mar.	9	10	13	6	8	9	17	8	7	8	9	7	+	–
Pseudomonas aeruginosa	7	12	19	11	10	7	15	8	9	8	11	8	+	–
Escherichia coli	8	12	16	12	10	7	11	8	10	10	7	8	+	–
Salmonella sp.	6	18	16	11	8	9	15	7	6	8	7	9	+	–
Pseudomonas sp.	9	19	12	9	11	9	13	9	11	11	8	9	+	–
Saccharomyces cerevisiae	11	17	13	9	11	10	11	7	10	12	10	7	–	+
Candida albicans	10	18	16	6	9	8	12	6	11	9	11	6	–	+
Aspergillus flavus	7	18	12	8	9	8	13	6	8	9	11	7	–	+

meso-substituted trimethine dye **7a** exhibited higher activity than **6a**, which may be due to its ability to penetrate the cell membrane of microorganisms [31].

These observations show that the synthesized compounds are capable of inhibiting the growth of Gram positive bacteria, Gram negative bacteria, yeast, and fungi. Compounds **4a**, **4b**, **5e**, and **7a** possess superior antimicrobial activity against all microorganisms under investigation (Table 5). There still exists a need for the development of new antimicrobial agents having superior activity and less side effects to overcome resistant strains of microorganisms. Further studies to elucidate the mechanism of these compounds action and to determine whether their activity is lethal or inhibiting to microorganisms are underway.

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