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Synthesis, photosensitization and antimicrobial activity of certain oxadiazine cyanine dyes

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Abstract

New heterocyclic compounds having 1,3,4-oxadiazine nuclei were prepared and employed for the synthesis of some new photosensitizers cyanine dyes. The electronic visible absorption spectra of all the synthesized cyanines were investigated in 95% ethanol. Antimicrobial activity of selected compounds against some bacterial strains was tested. Structural identification was carried out via elemental and spectroscopic analyses.

Keywords: Synthesis; Photosensitizers; Visible spectra; Cyanine dyes; Oxadiazine; Antimicrobial activity

1. Introduction

Heterocyclic cyanine dyes play a vital role in many biological processes as inhibitors for cell growth and division [1–3], as antitumor [4] and anticancer [5] agents, in addition to their wide use as photographic sensitizers for various silver halide emulsions [6].

On the other side, oxadiazine compounds have a diversity of biological effects [7] and have been shown to significantly inhibit the growth in several bacterial strains [8]. In addition, they are useful intermediates in the synthesis of tenidap prodrugs or B-lactam antibiotics, in particular in the synthesis of carbapenems and penems [9,10]. Also, they are exhibiting cardiovascular antibacterial, plant growth regulating, miticidal and nematocidal, acricidal, insecticidal and anticonvulsive activities [11,12]. In this point of view, new heterocyclic oxadiazine cyanine dyes were synthesized with the hope that a combination of the favourable properties of both oxadiazine and cyanine dyes may be achieved.

2. Results and discussion

2.1. Synthesis

p-Chloranil (**1**) was reacted with Acetic acid azide of hydrazine and phenylhydrazine (**2a**, **b**) in 1:2 molar ratios in ethylene glycol as organic solvent and sodium bicarbonate as basic catalyst and afforded 4,9-dihydro (diphenyl)-2,7-dimethyl-benzo[2,3-*e*; 2′,3′-*e*′]bis-1,3,4-oxadiazine-5,10-dione (**3a**, **b**) (Scheme 1, Table 1).

Quaternization of (**3a**, **b**) using excess of iodoethane produced the 3,8-diquaternized compounds (**4a**, **b**) (Scheme 1, Table 1). Further reaction of the 3,8-diquaternized compounds (**4a**, **b**) with bimolar ratios of *N*-ethyl(pyridinium, quinolinium, or isoquinolinium) iodide salts in ethanol and presence of piperidine as catalyst achieved the 2,7[4(1)]-bismonomethine cyanine dyes (**5a–d**) (Scheme 1, Table 1).

On the other side, oxidation of the starting compounds (**3a**, **b**) using selenium dioxide in 1:2 molar ratios and dioxane as solvent yielded the 2,7-diformyl compounds (**6a**, **b**) (Scheme 1, Table 1). Subsequent reaction of the bisoxidized compounds (**6a**, **b**) with bimolar ratios of *N*-ethyl(α-picolinium, quinaldinium, or γ-Picolinium)

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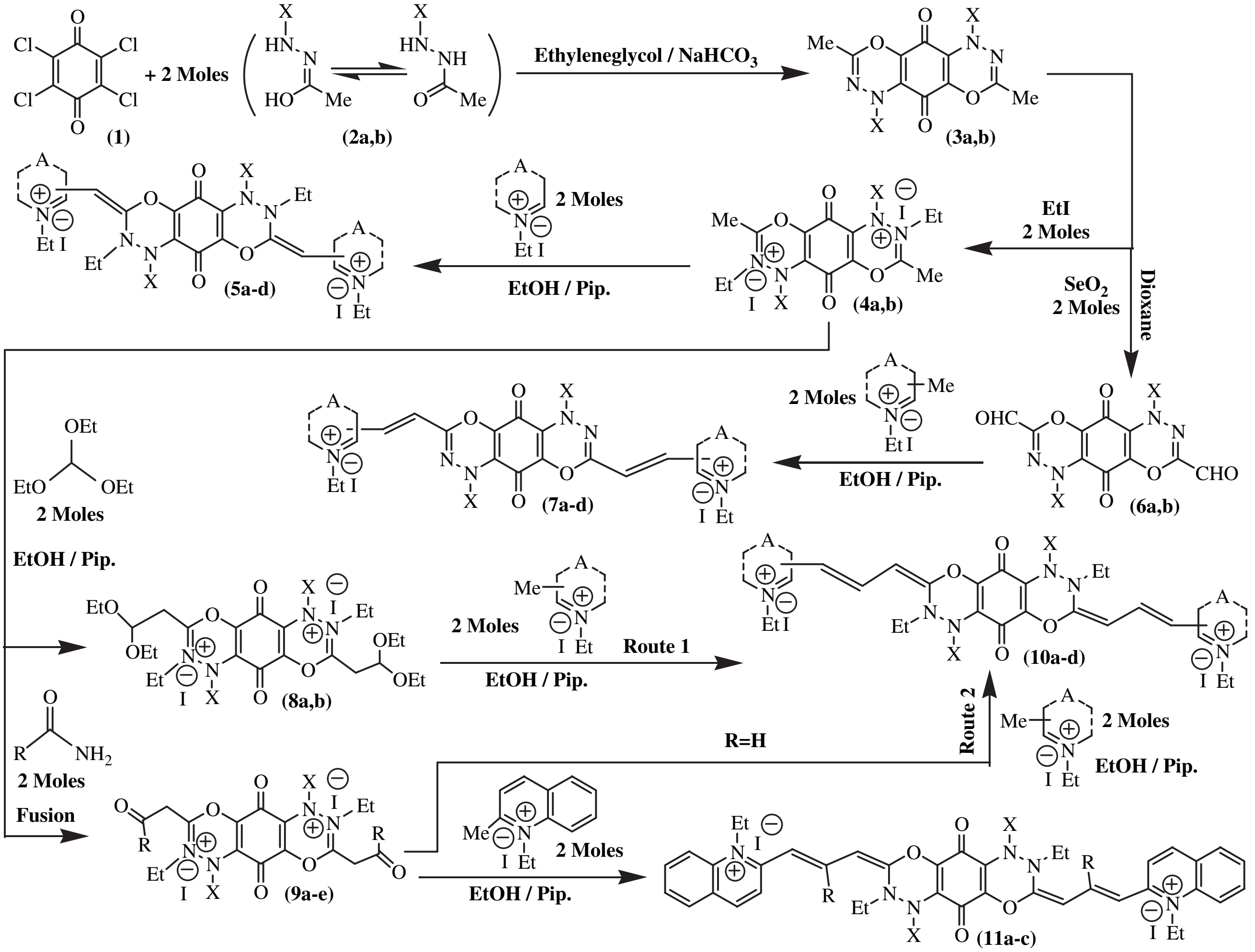

(2a,b); (3a,b); (4a,b); (6a,b) & (8a,b): X=H (a), X=Ph (b).

(5a-d): X=H, A= 1-ethyl pyridinium-4-yl salt (a),
X=H, A= 1-ethyl quinolinium-4-yl salt (b),
X=H, A= 2-ethyl isoquinolinium-1-yl salt (c),
X=Ph, A= 1-ethyl quinolinium-4-yl salt (d),

(9a-e): X=H, R= (H) (a),
X=Ph, R= H (b),
X=H, R= $-CH_3$ (c),
X=H, R= $-C_6H_5$ (d)
X=Ph, R= $-CH_3$ (e).

(7a-d), (10a-d): X=H, A= 1-ethyl pyridinium-2-yl salt (a),
X=H, A= 1-ethyl quinolinium-2-yl salt (b),
X=H, A= 1-ethyl pyridinium-4-yl salt (c),
X=Ph, A= 1-ethyl quinolinium-2-yl salt (d),

(11a-c): X=H, R= $-CH_3$ (a),
X=H, R= $-C_6H_5$ (b),
X=Ph, R= $-CH_3$ (c).

Scheme 1.

Table 1
Characterization of the prepared compounds

Compound no.	Molecular formula (M. wt.)	M.P. (°C)	Yield (%)	Analysis calculated/found C	H	N	λ_{max} (nm)	ε_{max} ($mol^{-1}\,cm^2$)
3a	$C_{10}H_8N_4O_4$ (248.2)	181	61	48.39/48.42	3.25/3.21	22.57/22.60	–	–
3b	$C_{22}H_{16}N_4O_4$ (400.39)	162	34	66.00/66.03	4.03/4.02	13.99/14.00	–	–
4a	$C_{14}H_{18}I_2N_4O_4$ (560.13)	195	71	30.02/30.00	3.24/3.27	10.00/9.98	–	–
4b	$C_{26}H_{26}I_2N_4O_4$ (712.32)	167	31	43.84/43.79	3.68/3.64	7.87/7.90	–	–
5a	$C_{28}H_{32}I_2N_6O_4$ (770.4)	201	48	43.65/43.66	4.19/4.22	10.91/10.88	476	17,130
5b	$C_{36}H_{36}I_2N_6O_4$ (870.52)	207	69	49.67/49.70	4.17/4.21	9.65/9.67	414, 499	20,370, 16,980
5c	$C_{36}H_{36}I_2N_6O_4$ (870.52)	193	40	49.67/49.65	4.17/4.15	9.65/9.61	481	17,320
5d	$C_{48}H_{44}I_2N_6O_4$ (1022.71)	188	76	56.37/56.41	4.34/4.37	8.22/8.20	420, 465 Sh, 506	21,330, 13,730, 18,110
6a	$C_{10}H_4N_4O_6$ (276.16)	174	53	61.69/61.65	1.46/1.50	20.29/20.33	–	–
6b	$C_{22}H_{12}N_4O_6$ (428.35)	162	42	61.69/61.72	2.82/2.88	13.08/13.12	–	–
7a	$C_{26}H_{24}I_2N_6O_4$ (738.32)	203	67	42.30/42.29	3.28/3.26	11.38/11.40	541	20,010
7b	$C_{34}H_{28}I_2N_6O_4$ (838.43)	207	43	48.71/48.75	3.37/3.41	10.02/10.00	440, 555	23,940, 23,110
7c	$C_{26}H_{24}I_2N_6O_4$ (738.32)	200	39	42.30/42.29	3.28/3.26	11.38/11.40	547	21,090
7d	$C_{46}H_{36}I_2N_6O_4$ (990.62)	179	58	55.77/55.80	3.66/3.68	8.48/8.53	455, 567	25,610, 23,780
8a	$C_{24}H_{38}I_2N_4O_8$ (764.39)	209	38	37.71/37.68	5.01/4.97	7.33/7.30	–	–
8b	$C_{36}H_{46}I_2N_4O_8$ (916.58)	179	48	47.17/47.20	5.06/5.00	6.11/6.16	–	–
9a	$C_{16}H_{18}I_2N_4O_6$ (616.15)	203	37	31.19/31.17	2.94/2.90	9.09/9.14	–	–
9b	$C_{28}H_{26}I_2N_4O_6$ (768.34)	202	41	43.77/43.71	3.41/3.39	7.29/7.32	–	–
9c	$C_{18}H_{22}I_2N_4O_6$ (644.20)	212	50	33.56/33.53	3.44/3.51	8.70/8.65	–	–
9d	$C_{28}H_{26}I_2N_4O_6$ (768.34)	206	43	43.77/43.75	3.41/3.38	7.29/7.37	–	–
9e	$C_{30}H_{30}I_2N_4O_6$ (796.39)	211	53	45.24/45.31	3.80/3.78	7.04/6.99	–	–
10a	$C_{32}H_{36}I_2N_6O_4$ (822.47)	211	42	46.73/46.79	4.41/4.40	10.22/10.18	559	27,040
10b	$C_{40}H_{40}I_2N_6O_4$ (922.59)	216	70	52.07/52.00	4.37/4.39	9.11/9.13	460, 585	34,000, 29,130
10c	$C_{32}H_{36}I_2N_6O_4$ (822.48)	210	73	46.73/46.75	4.41/4.38	10.22/10.20	561	27,130
10d	$C_{52}H_{48}I_2N_6O_4$ (1074.78)	191	63	58.11/58.15	4.50/4.53	7.82/7.90	475, 600	34,950, 29,780
11a	$C_{42}H_{44}I_2N_6O_4$ (950.65)	213	30	53.06/53.09	4.67/4.71	8.84/8.80	480, 530 Sh, 609	31,660, 20,370, 26,310
11b	$C_{52}H_{48}I_2N_6O_4$ (1074.78)	221	29	58.11/58.09	4.50/4.47	7.82/7.76	483, 585 Sh, 630	32,360, 15,170, 24,280
11c	$C_{54}H_{52}I_2N_6O_4$ (1102.84)	191	29	58.81/58.83	4.75/4.78	7.62/7.65	488, 525 Sh, 621	34,000, 25,310, 29,100

iodide salts in ethanol catalyzed by piperidine gave the 2,7[2(4)]-bisdimethine cyanine dyes (**7a**–**d**) (Scheme 1, Table 1). Additionally, interaction of equimolar ratios of the 3,8-diquaternized compounds (**4a**, **b**) with bimolar ratio of triethylorthoformate in ethanol containing few milliliters of piperidine as a basic catalyst achieved the 2,7-di(2-diethoxy-ethane) compounds as intermediates (**8a**, **b**) (Scheme 1, Table 1). Otherwise, fusion of the 3,8-diquaternized compounds (**4a**, **b**) with bimolar ratios of (formamide, acetamide, or benzamide) at about 100 °C produced 2,7-di(2-R-ethan-2-one) compounds as other intermediates (**9a**–**e**) (Scheme 1, Table 1). Further reaction of the intermediate compounds (**8a**, **b**) with bimolar ratios of *N*-ethyl(2-picolinium, quinaldinium, or 4-picolinium)iodide salts in ethanol and presence of piperidine resulted the 2,7[2(4)]-bistrimethine cyanine dyes (**10a**–**d**) (route 1, Scheme 1, Table 1). Chemical confirmation took place, through route 2 in Scheme 1, via interaction of the intermediate compounds (**9a**, **b**) with *N*-ethyl(2-picolinium, quinaldinium, or 4-picolinium) iodide salts in 1:2 molar ratios and in ethanol containing piperidine to achieve the same 2,7[2(4)]-bistrimethine cyanine dyes (**10a**–**d**), obtained through route 1, characterized by the same melting points, mixed melting points, same IR and ^{1}H NMR spectral data (Scheme 1, Table 1).

Furthermore, interaction of unimolar ratios of the intermediate compounds (**9c**–**e**) with bimolar ratios of *N*-ethyl-quinaldinium iodide salt in ethanol containing few milliliters of piperidine gave the 2,7(2)-bismeso-substituted trimethine cyanine dyes (**11a**–**c**) (Scheme 1, Table 1).

The structures of the prepared compounds were identified by elemental analysis, (Table 1), IR [13], ^{1}H NMR [14], and mass spectroscopic spectra (Table 2).

2.2. Photosensitization activity

Photosensitization activity of the newly synthesized cyanine dyes was determined by studying their electronic visible absorption spectral behaviour in 95% ethanol. The dyes were thought to be better photosensitizers when they absorb the visible light to initiate the electronic transitions at higher wavelength (bathochromic and/or red shifted dyes), consequently, the photosensitization of the dyes decreases when they absorb light at shorter wavelength (hypsochromic and/or blue shifted dyes).

The electronic visible absorption spectra of the bismonomethine cyanine dyes (**5a**–**d**) in 95% ethanol showed bands in the visible region (476 nm–506 nm) which their positions and molar extinction coefficients

Table 2
IR, ^{1}H NMR, and mass spectral data of the prepared compounds

Compound no.	IR (ν_{max}^{KBr}) cm^{-1}	^{1}H NMR (DMSO) δ assignment {mass spectra *m/z*}
3a	1357 (C–N); 1584, 1723 (C=O quinone); 3431 (NH cyclic)	3.30 (s, 6H, 2CH$_3$ at C$_2$ & C$_7$); 5.73 (s, 2H, 2NH of Oxadiazine ring). {*m/z*: 248}
3b	633, 687 (–Ph); 1300, 1482 (C–N); 1594, 1732 (C=O quinine)	3.21 (s, 6H, 2CH$_3$ at C$_2$ & C$_7$); 7.13–8.57 (m, 10H, aromatic). {*m/z*: 400}
4a	1356 (C–N); 1594, 1752 (C=O quinone); 2925 (Et–N$^+$); 3651 (NH cyclic)	1.12–1.29 (t, 6H, 2CH$_3$ of Et–N$^+$); 3.19 (s, 6H, 2CH$_3$ at C$_2$ & C$_7$); 3.69–3.98 (q, 4H, 2CH$_2$ of Et–N$^+$); 6.83 (s, 2H, 2NH); {*m/z*: 561}
5b	1369 (C–N); 1583, 1723 (C=O quinone); 2882 (Et–N$^+$); 3313 (NH cyclic)	1.10–1.35 (t, 6H, 2CH$_3$ of Et–N); 1.37–1.51 (t, 6H, 2CH$_3$ of Et–N$^+$); 3.52–3.751 (q, 4H, 2CH$_2$ of Et–N); 3.75–3.91 (q, 4H, 2CH$_2$ of Et–N$^+$) 6.61–7.9 (m, 16H, 2NH, 2CH= & aromatic)
6a	1397 (C–N); 1581 (C=O quinone); 1717 (CHO); 3200 (NH cyclic)	6.67 (s, 2H, 2NH); 9.40 (s, 2H, 2CHO at C$_2$ & C$_7$). {*m/z*: 276}
7b	1412 (C–N); 1600, 1723 (C=O quinone); 2915 (Et–N$^+$); 3438 (NH cyclic)	1.24–1.45 (t, 6H, 2CH$_3$ of Et–N$^+$); 3.65–3.89 (q, 4H, 2CH$_2$ of Et–N$^+$); 6.31–8.12 (m, 18H, 2NH, 4CH= & aromatic)
8a	1379 (C–N), 1593, 1723 (C=O quinone); 2900 (Et–N$^+$); 3261 (NH cyclic)	1.10–1.23 (t, 6H, 2CH$_3$ of Et–N$^+$); 1.29–1.51 (t, 12H, 4CH$_3$ of Et–O); 2.93–3.12 (d, 4H, 2CH$_2$ side chain); 3.66–3.80 (q, 4H, 2CH$_2$ of Et–N$^+$); 3.82–3.99 (q, 8H, 4CH$_2$ of Et–O); 4.1–4.28 (t, 2H, 2CH side chain); 6.92 (s, 2H, 2NH). {*m/z*: 764}
9c	1375 (C–N); 1589, 1732 (C=O quinone); 2906 (Et–N$^+$); 3400 (NH cyclic)	1.19–1.41 (t, 6H, 2CH$_3$ of Et–N$^+$); 2.96 (s, 4H, 2CH$_2$ side chain); 3.47 (s, 6H, 2CH$_3$ of COCH$_3$); 6.96 (s, 2H, 2NH). {*m/z*: 644}
10b	1318 (C–N); 1600, 1723 (C=O quinone); 3000 (Et–N$^+$); 3481 (NH cyclic)	1.23–1.31 (t, 6H, 2CH$_3$ of Et–N); 1.34–1.51 (t, 6H, 2CH$_3$ of Et–N$^+$); 3.62–3.82 (q, 4H, 2CH$_2$ of Et–N); 3.83–3.99 (q, 4H, 2CH$_2$ of Et–N$^+$) 6.92 (s, 2H, 2NH); 6.74–7.98 (m, 18H, 6CH= & aromatic)
11a	1364 (C–N); 1583, 1733 (C=O quinone); 2937 (Et–N$^+$); 3381 (NH cyclic)	1.05–1.19 (t, 6H, 2CH$_3$ of Et–N); 1.32–1.57 (t, 6H, 2CH$_3$ of Et–N$^+$); 3.68 (s, 6H, 2CH$_3$ on side chain); 3.67–3.93 (q, 4H, 2CH$_2$ of Et–N); 416–4.45 (q, 4H, 2CH$_2$ of Et–N$^+$); 6.47–7.99 (m, 18H, 2NH, 4CH= & aromatic)

are largely influenced by the nature of the heterocyclic quaternary residue (*A*), their linkage position and the type of *N*-substituent (X) on the oxadiazine ring of the heterocyclic ring system. So, substituting *A* = 1-ethyl pyridinium-4-yl salt in dye **5a** by *A* = 1-ethyl quinolinium-4-yl salt and/or *A* = 2-ethyl isoquinolinium-1-yl salt to obtain dyes **5b**, **5c** caused bathochromic shifts by 23 nm and 5 nm, respectively (Table 1). This can be attributed to increasing π–delocalization conjugation in the later dyes due to the presence of quinoline and isoquenoline rings (Scheme 1). Otherwise, changing the linkage position from 1-ethylquinolinium-4-yl salt in dye **5b** to 2-ethylisoquinolinium-1-yl salt to give dye **5c** resulted in a hypsochromic shifts for the absorption bands by 18 nm (Table 1). This can be related to decreasing length of the π–delocalization conjugation to the quaternary nitrogen atom in the latter dye (Scheme 1). In the other side, substituting X = H by X = Ph transferring from dye **5b** to dye **5d** generated a bathochromic shift for the absorption band by 7 nm accompanied by increasing intensity of the band (Table 1). This is due to increasing conjugation in the latter dye due to the presence of additional conjugation of the phenyl ring (Scheme 1).

Similarly, the electronic absorption spectra of bisdimethine cyanine dyes **7a–d** and bistrimethine cyanine dyes (**10a–d**) showed bands in the visible region 541 nm–567 nm and 559 nm–600 nm, respectively, which underwent bathochromic and/or hypsochromic shifts depending on the nature of the heterocyclic quaternary residue (*A*), their linkage position and the type of *N*-substituent (X) on the oxadiazine ring of the heterocyclic ring system. So, substituting *A* = 1-ethyl pyridinium-2-yl salt in dyes **7a**, **10a** by *A* = 1-ethyl quinolinium-2-yl salt to obtain dyes **7b**, **10b** caused strong bathochromic shifts by 14 nm and 26 nm, respectively, in addition to increasing the band intensity (Table 1). This is due to increasing conjugation in quinoline rings in dyes **7b**, **10b** comparing with pyridine rings in dyes **7a**, **10a** (Scheme 1). Otherwise, changing the linkage position from -2-yl salt in dyes **7a** and **10a** to -4-yl salt in dyes **7c** and **10c** resulted in bathochromic shifts by 6 nm and 2 nm, respectively, in addition to increasing intensities of the bands (Table 1). This can be attributed to increase in the lengths of the π–delocalization conjugation to the quaternary nitrogen atom in the later dyes (Scheme 1). On the other side, substituting X = H by X = Ph transferring from dyes **7b** and **10b** to dyes **7d** and **10d** generated bathochromic shifts for the positions of the bands by 12 nm and 15 nm, respectively, with increasing intensities of the bands (Table 1). This is can be related to increase in the π–delocalization in the latter dyes due to the presence of additional conjugation of the benzene ring (Scheme 1).

Additionally, the electronic visible absorption spectra of the bismeso-substituted trimethine cyanine dyes **11a–c** showed bands in the visible region (609 nm–630 nm) which underwent bathochromic and/or hypsochromic shifts depending upon the nature of meso-substituted group (R), and the kind of *N*-substituent (X) on the oxadiazine ring of the heterocyclic ring system (Table 1). So substituting R = Me in dye **11a** by R = Ph to obtain dye **11b** produced strong bathochromic shift by 21 nm (Table 1). This is mainly due to the increased π-delocalization excreted along the methine chain by the phenyl group (Scheme 1). Otherwise, substituting X = H by X = Ph transferring from dye **11a** to dye **11c** generated bathochromic shift for the absorption band by 12 nm in addition to intensification of the absorption bands (Table 1). This is attributed to the same reason cited before in the bismonomethine, bisdimethine, and bistrimethine cyanine dyes (Scheme 1).

Generally, comparing the electronic visible absorption spectra of the bistrimethine cyanine dye **10b** with the electronic visible absorption spectra of the bismeso-substituted trimethine cyanine dyes **11a** and **11b** declared that the latter dyes had a higher photosensitization character than the former one since they absorb light at higher wave lengths (Table 1). This is due to the hyper-conjugation donation character of the methyl group in dye **11a** and increasing extent of conjugation due to the benzene ring in dye **11b** (Scheme 1). It is also interesting to report that the bistrimethine cyanine dyes (**10a–d**) gave bathochromic shifted absorption spectra with intensified absorption spectra bands if compared with the absorption spectra of both the bismonomethine cyanine dyes **5a–d** and bisdimethine cyanine dyes **7a–d**. Consequently, the bisdimethine cyanine dyes **7a–d** are characterized by red shifted and intensified absorption bands relative to those of the bismonomethine cyanine dyes **5a–d** (Table 1). This largely supported that increasing the number of methine units in the methine chain increased the wavelength at which the dye absorbed as well as the intensity of the resulted absorption band (Scheme 1).

2.3. Antimicrobial activity

Structure–antimicrobial (biological) activity relationship for some selected newly synthesized oxadiazine compounds {**3a**, **3b**, **4a**, **5b**, **6a**, **7b**, **8a**, **9c**, **10b** and **11a**} were studied and determined against some bacterial strains {*Pseudomonas*, *Serratia*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus citrus*, *Bacillus* 1 from air (Gram −ve) and *Bacillus* 2 from air (gram negative)} (Table 3).

According to this study, it was observed that replacing *N*-hydrogen in compound **3a** by phenyl group to give compound **3b** caused decrease in biological activity. This might be attributed to the electron attracting character of the phenyl ring in the latter compound (Scheme 1, Table 3).

Also, it was noticed that quaternization of compound **3a** by iodoethane to give compound **4a** made inhibition for the biological activity against the selected strains. This could be related to increase in the electron accepting character of compound **4a** due to quaternization (Scheme 1, Table 3).

Additionally, substituting the 3,8-dimethy groups in compound **4a** by 3,8-di{$CH_2CH(OEt)_2$} groups to give compound **8a**, completely destroyed the antimicrobial activity against all bacterial strains. This could be attributed to the strong electron accepting character of the diethoxy groups in compound **8a** (Scheme 1, Table 3).

On the other hand, the comparison between the antimicrobial activity of the monomethine cyanine dye **5b** and the dimethine cyanine dye **7b** showed that the later dye possessed higher potency as antibacterial activity than the former one. This might be correlated to increasing number of the methine units in dye **7b** (Scheme 1, Table 3).

Also, it was noticeable that the meso-substituted bistrimethine cyanine dye **11a** has higher biological activity if compared with its analogous bistrimethine cyanine dye **10b**. This could be due to the electron donating character of the methyl group in the former dye (Scheme 1, Table 3).

Furthermore, it was observed that the intermediate compound (**9c**) gave higher antimicrobial activity if compared with the intermediate compound (**8a**). This could be related to the electron releasing character of the methyl group in compound (**9c**) and the electron attracting character of the diethoxy groups in compound (**8a**) (Scheme 1, Table 3).

On the other hand, it was noticed that the monomethine cyanine dye **5b**, the dimethine cyanine dye **7b**

Table 3
Biological activity of some newly synthesized compounds

Bacterial strain	Tested compound									
	3a (mm)	**3b** (mm)	**4a** (mm)	**5b** (mm)	**6a** (mm)	**7b** (mm)	**8a** (mm)	**9c** (mm)	**10b** (mm)	**11a** (mm)
Pseudomonas	–	–	–	–	–	–	–	–	–	–
Serratia	–	–	–	–	–	–	–	–	–	–
Bacillus cereus	9	8	–	–	10	8	–	8	–	9
Escherichia coli	–	–	–	–	–	–	–	–	–	–
Staphylococcus citrus	–	–	7	7	12	12	–	9	8	12
Bacillus 1	15	12	7	–	8	9	–	7	7	15
Bacillus 2	10	–	7	7	9	9	–	–	–	8

Bacillus 1 and *Bacillus* 2 are Gram −ve unknown bacillus bacterial strains taken from air.

and the trimethine cyanine dye **10b** possessed lower biological activity towards the selected bacterial strains than the starting material **3a**. This might be ascribed as a result of increasing electron attracting character of the former dyes due to their attached heterocyclic quaternary salts (Scheme 1, Table 3).

Also, it was observable that replacement of the 3,8-dimethyl groups in compound **3a** by the 3,8-diformyl to give compound **6a** caused increases and/or decreases of the antimicrobial activity against *B. cereus*, *S. citrus* and/or {*Bacillus* 1 from air (gram negative)}, {*Bacillus* 2 from air (gram negative)}, respectively (Scheme 1, Table 3).

From the above discussed results, we could conclude that the antibacterial activity of the oxadiazine compounds depended on the nature of the *N*-substituted group (hydrogen or phenyl), quaternization, electron donating and/or electron attracting groups inside the entire molecule and the type of the cyanine dye under testing.

3. Experimental

3.1. General

Melting points were recorded on a Galenkanp melting point apparatus and are uncorrected. Elemental analyses were carried out at the micro analytical center at Cairo University. Infrared spectra were measured with a 1650 FT-IR instrument. ^{1}H NMR spectra were carried out on an EM-390 90 MHz NMR Spectrometer, and mass spectroscopy was recorded on GCMS-QP-1000 EX spectrometer at Cairo University.

The organic solvents were of spectroscopic grades and the prepared dyes were investigated and purified using TLC and column chromatographic procedures.

3.2. Synthesis

3.2.1. 4,9-dihydro(diphenyl)-2,7-dimethyl-benzo[2,3-e; 2′,3′-e′]bis-1,3,4-oxadiazine-5,10-dione (*3a, b*)

p-Chloranil (**1**) (0.01 mol) and bimolar ratios (0.02 mol) of acetic acid azide of hydrazine and/or phenyl hydrazine (**2a**, **b**) were refluxed in ethylene glycol for 1 h until the mixture attain a red color. Five milliliters of 20% aqueous sodium bicarbonate solution was added to the reaction mixture which then refluxed again for extra 4 h. The reaction mixture was filtered off while hot to remove unreacted materials, poured in ice-water mixture with continuous shaking. The precipitated compounds were filtered, washed with cold water and dried. The dry compounds were collected and recrystallized from ethanol. The data are reported in Table 1.

3.2.2. 3,8-diethyl-4,9-dihydro(diphenyl)-2,7-dimethyl-benzo[2,3-e; 2′,3′-e′]bis-1,3,4-oxadiazinium-5,10-dione iodide salts (*4a, b*)

A pure crystallized sample of (**3a**, **b**) (0.01 mol) was suspended in excess of iodoethane (10 mL) and heated gently under reflux at low temperature (40–60 °C) for 1 h. The solvent was evaporated and the residue (**4a**, **b**) was collected and recrystallized from ethanol (see data in Table 1).

3.2.3. 3,8-diethyl-4,9-dihydro(diphenyl)-benzo[2,3-e; 2′,3′-e′]bis-1,3,4-oxadiazine-5,10-dione-2,7[4(1)]-bismonomethine cyanine dyes (*5a–d*)

Unimolar ratios of the 3,8-diquaternized compounds (**4a**, **b**) (0.01 mol) and bimolar ratios (0.02 mol) of *N*-ethyl(pyridinium, quinolinium, or isoquinolinium) iodide salts were refluxed in ethanol containing piperidine (1 mL) for 6 h. The reaction mixture was filtered off while hot to remove unreacted materials, cooled, poured in ice-water mixture with continuous shacking till complete precipitation and neutralized with acetic acid. The precipitates were filtered, washed with water, dried and recrystallized from ethanol. Relevant data are given in Table 1.

3.2.4. 4,9-dihydro(diphenyl)-2,7-diformyl-benzo[2,3-e; 2′,3′-e′]bis-1,3,4-oxadiazine-5,10-dione (*6a, b*)

A pure sample of (**3a**, **b**) (0.01 mol) was refluxed in 50 mL dioxane containing bimolar ratio (0.02 mol) of selenium dioxide for 16 h. The reaction mixtures were filtered off while hot to remove selenium metal, concentrated, cooled, and precipitated by adding cold water. The precipitated products (**6a**, **b**) were filtered, dried, collected, and recrystallized from ethanol. The data are shown in Table 1.

3.2.5. 4,9-dihydro(diphenyl)-benzo[2,3-e; 2′,3′-e′]bis-1,3,4-oxadiazine-5,10-dione-2,7[2(4)]-bisdimethine cyanine dyes (*7a–d*)

Molar ratios (1:2) of the 2,7-diformyl compounds (**6a**, **b**) and *N*-ethyl(2-methyl-pyridinium, 2-methyl-quinolinium, or 4-methyl-pyridinium)iodide salts were refluxed in ethanol containing piperidine (1 mL) for 8 h. It was filtered off while hot to remove unreacted materials, cooled, neutralized with acetic acid and precipitated by adding cold water. The precipitates were filtered, washed with water, dried and recrystallized from ethanol. See given data in Table 1.

3.2.6. 3,8-diethyl-4,9-dihydro(diphenyl)-2,7-di(2-diethoxy-ethane)-benzo[2,3-e; 2′,3′-e′]-bis-1,3,4-oxadiazinium-5,10-dione iodide salts as intermediate compounds (*8a, b*)

A mixture of the 3,8-diquaternary compounds (**4a**, **b**) (0.01 mol) and bimolar ratios (0.02 mol) of triethylorthoformate was dissolved in ethanol (50 mL) containing piperidine (1 mL) and refluxed for 4 h, filtered while hot to remove unreacted materials, concentrated to one half its initial volume, cooled, acidified with acetic acid, and precipitated by cold water. The precipitates ware filtered, washed with water, dried and recrystallized from ethanol. See relevant data in Table 1.

3.2.7. *3,8-diethyl-4,9-dihydro(diphenyl)-2,7-di(2-R-ethan-2-one)-benzo[2,3-e; 2′,3′-e′]-bis-1,3,4-oxadiazinium-5,10-dione iodide salts (**9a–e**)*

The 3,8-diquaternized compounds (**4a**, **b**) (0.01 mol) and bimolar ratios (0.02 mol) of formamide, acetamide, or benzamide were heated under fusion for 1 h till complete evolution of ammonia gas. Then, it was extracted via dissolving in ethanol, filtered off, and precipitated in ice-water mixture. The precipitates were dried and recrystallized from ethanol. See reported data in Table 1.

3.2.8. *3,8-diethyl-4,9-dihydro(diphenyl)-benzo[2,3-e; 2′,3′-e′]bis-1,3,4-oxadiazine-5,10-dione-2,7[2(4)]-bistrimethine cyanine dyes (**10a–d**)*

This synthesis was accomplished via two different routes:

Route 1: Unimolar ratios of the intermediate compounds (**8a**, **b**) (0.01 mol) and bimolar ratios (0.02 mol) of *N*-ethyl(2-picolinium, quinaldinium, or 4-picolinium) iodide salts were dissolved in ethanol (50 mL) containing piperidine (1 mL), heated under reflux for 8 h and attained a highly colored violet mixture. The mixture was filtered off while hot, poured in ice-water mixture with continuous shacking till complete precipitation and neutralized with acetic acid. The precipitates were filtered, washed with water, dried and recrystallized from ethanol. See recorded data in Table 1.

Route 2: Piperidine (1 mL) was added to a mixture of the intermediate compounds (**9a**, **b**) (0.01 mol) and iodoethane quaternary salts of α-picoline, quinaldine, and/or γ-picoline (0.02 mol) dissolved in ethanol (50 mL). The reaction mixture was refluxed for about 3–5 h until attained a permanent dark violet color. It was filtered off while hot, concentrated, cooled and acidified by glacial acetic acid. The precipitated products, which appeared on dilution with cold water were filtered off, washed several times with water, dried, recrystallized from ethanol, and gave the same 2,7[2(4)]-bistrimethine cyanine dyes (**10a–d**) obtained via route 1, characterized by the same melting points, mixed melting points, same IR and ^{1}H NMR spectral data (Scheme 1). See recorded data in Table 1.

3.2.9. *3,8-diethyl-4,9-dihydro(diphenyl)-benzo[2,3-e; 2′,3′-e′]bis-1,3,4-oxadiazine-5,10-dione-2,7(2)-bismeso-substituted trimethine cyanine dyes (**11a–c**)*

A mixture of the intermediate compounds (**9c–e**) (0.01 mol) and bimolar ratios (0.02 mol) of *N*-ethyl-quinaldinium iodide salt were heated under reflux in ethanol containing piperidine (1 mL) for 8 h and attained a highly colored dark violet mixture at the end of refluxing. The mixture was filtered off while hot to remove unreacted materials, poured in ice-water mixture with continuous shacking, lifted aside overnight till complete precipitation, and neutralized with acetic acid. The precipitates were filtered, washed with water, dried and recrystallized from ethanol. See relevant data in Table 1.

3.3. *Absorption spectroscopy*

The electronic visible absorption spectra of the prepared cyanine dyes were examined in absolute ethanol and recorded on a Shimadzu UV/visible 160-A spectrophotometer. The stock solution was about 1×10^{-3}, the lower molarities were obtained by accurate dilution.

3.4. *Biological activity*

The tested compounds (**3a**, **3b**, **4a**, **5b**, **6a**, **7b**, **8a**, **9c**, **10b**, and **11a**) were dissolved in 95% ethanol to give a final concentration (1 mgm/mL). Susceptible sterile discs were impregnated by the tested substance (50 μgm/disc) via micropipette. The biological activity for each substance was tested on surface-seeded nutrient agar medium with the prepared susceptible discs. Bacterial strains and the biological effect are shown in Table 3.

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