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Synthesis, spectral behavior and biological activity of some novel 1,3,4-oxadiazine cyanine dyes

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ABSTRACT

New methine cyanine dyes covering monomethine cyanine dyes and dimethine cyanine dyes derived from benzo[(2,3-b)benzoxazine; (2,3-e)1,3,4-oxadiazine]-5,12-dione were prepared. The electronic visible absorption spectra of all the synthesized cyanine dyes were investigated in 95% ethanol solution. Biological activity for a number of selected compounds was tested and evaluated against various bacterial strains (*Bacillus subtilis*, *Escherichia coli*, *Pseudomona aeruginosa* and *Staphylococcus aureus*). Structural determination was carried out via elemental analysis, visible, mass, IR and ^{1}H NMR spectroscopic data.

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1. Introduction

Cyanine dyes are photosensitizing dyes which have been studied for over 150 years and continue to be of interest in biology and medicine [1-12]. They have been shown to possess various biological activities, including antimicrobial, anti-oxidant, macrophage activating and oxidative phosphorylation uncoupling activities. Some of cyanine dyes have been used as immune modulators to treat allergy and rheumatoid arthritis, cancer, and to promote wound healing. The demand for novel synthesis representatives of cyanine dyes with better properties is very strong. This is because the developments in biological and biomedical applications research of cyanine dyes on molecular level, in molecular biology, in bio-assay and in bio-medical analysis is growing continuously and rapidly. Certainly, this will make the present and/or the future of cyanine dyes chemistry is effective, fruitful and very bright.

On the other side, oxadiazine compounds have been demonstrated to be important heterocyclic scaffold platform with bioactive diversity, which present wide activities such as cardiovascular, antitumor, antibacterial, acricidal, insecticidal, plant-growth regulating, chitin biosynthesis inhibitors and monoamine oxidase inhibition [13-23].

Based on this concept, our main concern was to prepare some novel 1,3,4-oxadiazine cyanine dyes as new synthesis contribution, spectroscopic investigation and antimicrobial evaluation with the hope that of combination of the favorite properties of both oxadiazine and cyanine dyes may be achieved.

2. Experimental

2.1. Instrumentation

All the melting points of the prepared compounds are measured using Electrothermal 15V, 45W 1 A9100 melting point apparatus (Chemistry Department, Faculty of Science, Aswan University, Aswan, Egypt) and are uncorrected. Elemental analysis was carried out at the Microanalytical Center of Cairo University by an automatic analyzer (Vario EL III Germany). Infrared spectra were measured with a FT-IR (4100 Jasco, Japan), Cairo University. ^{1}H NMR spectra were accomplished using Varian Gemini-300 MHz NMR Spectrometer (Cairo University). Mass Spectroscopy was recorded on Mass 1: GC-2010 Shimadzu Spectrometer (Cairo University). Electronic visible absorption spectra were carried out on Shimadzu UV-Visible recording spectrophotometer (Chemistry Department, Faculty of Science, Aswan University, Aswan, Egypt). Biological activity studies were carried out at the Microanalytical Center, Microbiology division (Cairo University).

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Table 1. Characterization of the prepared compounds.

Comp. No	Nature of products			Molecular formula	Elemental analysis, %						Absorption spectra in 95% ethanol	
					Calculated			Found			λ_{max} (nm)	ε_{max} ($mol^{-1}.cm^2$)
	Color	Yield, %	M.p., °C		C	H	N	C	H	N		
3a	Brown	45	235	$C_{14}H_9N_3O_4$	59.36	3.18	14.84	59.3	3.15	14.81		
3b	Dark brown	54	125	$C_{20}H_{13}N_3O_4$	66.85	3.62	11.7	66.81	3.6	11.68		
5a	Brown	50	185	$C_8H_4N_2O_3Cl_2$	38.87	1.62	11.34	38.82	1.59	11.3		
5b	Dark brown	55	130	$C_{14}H_8N_2O_3Cl_2$	52.01	2.48	8.67	52	2.44	8.65		
6a	Brown	65	145	$C_{16}H_{14}N_3O_4I$	43.7 4	3.19	9.57	43.71	3.15	9.54		
6b	Dark brown	43	133	$C_{22}H_{18}N_3O_4I$	51.26	3.5	8.16	51.22	3.47	8.14		
7a	Red	50	155	$C_{23}H_{21}N_4O_4I$	50.74	3.86	10.29	50.7	3.83	10.23	428	5790
7b	Deep red	59	170	$C_{27}H_{23}N_4O_4I$	54.55	3.87	9.43	54.5	3.81	9.4	443	7850
7c	Deep red	61	178	$C_{27}H_{23}N_4O_4I$	54.55	3.87	9.43	54.51	3.8	9.4	431	1970
7d	Deep red	53	180	$C_{33}H_{27}N_4O_4I$	59.1	4.03	8.36	59.05	4.01	8.32	501	1980
8a	Brown	72	170	$C_{14}H_7N_3O_5$	56.57	2.36	14.14	56.51	2.33	14.11		
8b	Brown	52	180	$C_{20}H_{11}N_3O_5$	64.34	2.95	11.26	64.29	2.9	11.21		
9a	Red	43	185	$C_{22}H_{17}N_4O_4I$	50	3.22	10.61	49.98	3.19	10.57	368, 439	480, 1010
9b	Violet	57	220	$C_{26}H_{19}N_4O_4I$	53.98	3.29	9.69	53.9	3.24	9.65	560, 605	1320, 2080
9c	Deep red	57	190	$C_{22}H_{17}N_4O_4I$	50	3.22	10.61	49.97	3.18	10.56	378, 445	251, 710
9d	Deep violet	61	192	$C_{32}H_{23}N_4O_4I$	58.72	3.52	8.56	58.66	3.5	8.51	570, 615	1120, 1550

Table 2. IR, ^{1}H NMR, and Mass spectral data.

Compound No	IR (KBr, cm^{-1})	^{1}H NMR, DMSO-d_6, δ, ppm	Mass data
3a	755, 814, 860 (o-disubstituted benzene), 1122, 1199 (C-O-C cyclic), 1483 (C=N), 1599 (C=C), 1750 (C=O quinone), 3401 (NH)	3.30 (s, 3H, CH_3), 6.75-6.97 (s, 2H, 2NH), 7.00-7.80 (m, 4H, aromatic)	M+1: 284
3b	591, 699 (mono substituted benzene), 751, 857 (o-disubstituted benzene), 1071, 1181 (C-O-C cyclic), 1487 (C=N), 1590 (C=C), 1750 (C=O quinone), 3387 (NH)	3.31 (s, 3H, CH_3), 6.48-6.98 (b, 1H, NH), 7.0-7.81 (m, 10H, aromatic)	M+2: 357
5a	1072, 1198 (C-O-C cyclic), 1451 (C=N), 1611 (C=C), 1750 (C=O quinone), 3419 (NH)	3.40 (s, 3H, CH_3), 6.98 (s, 1H, NH)	M+1: 248
6a	753, 813, 862 (o-disubstituted benzene), 1035, 1084, 1185 (C-O-C cyclic), 1454 (C=N), 1597 (C=C), 1625 (C=O quinone), 2925 (quaternary salt), 3388 (NH).	0.8 (b, 3H, CH_3 of position 3), 1.2 (m, 2H, CH_2 of position 3), 3.4 (s, 3H, CH_3 of position 2), 6.923-6.982 (s, 2H, 2NH), 7.116-7.8 (m, 4H, aromatic).	M+1: 440
7b	750, 851, 883 (o-disubstituted benzene), 1022, 1116, 1190 (C-O-C cyclic), 1485 (C=N), 1547 (C=C), 1750 (C=O quinone), 2925, 2853 (quaternary salt), 3398 (NH).	0.8 (b, 3H, CH_3 of position 3), 1.2 (m, 2H, CH_2 of position 3), 1.6 (s, 3H, CH_3 of N-quinolinium), 1.9-2 (m, 2H, CH_2 of N-quinolinium), 5.561 (m, 1H, -CH=), 6.73 (b, 2H, 2NH), 7.047-7.8 (m, 10H, aromatic + heterocyclic).	
8a	753, 801, 870 (o-disubstituted benzene), 1112, 1176 (C-O-C cyclic), 1458 (C=N), 1570 (C=C), 1685 (C=O quinone), 1765 (CHO), 3433 (NH)	6.6 (s, 2H, 2NH), 7.224-7.8(m, 4H, aromatic), 10.5 (b, 1H, CHO).	M^+: 297
9b	755, 840 (o-disubstitutal benzene), 1164 (C-O-C cyclic), 1449 (C=N), 1597, 1545 (C=C), 1750 (C=O quinone), 2923, 2588 (quaternary salt), 3424 (NH)	0.8 (m, 3H, CH_3 of N-quinolinium), 1.2 (m, 2H, CH_2 of N-quinolinium), 5-5.2 (b, 2H, -CH=CH-), 6.6-6.8 (b, 2H, 2NH), 7.2-8.9 (m, 10H, aromatic + heterocyclic)	

2.2. Synthesis

2.2.1. Synthesis of2-methyl-4-H-benzo[(2,3-b)benzoxazine;(2',3'-e)1,3,4-oxadiazine]-5,12-dione (3a) and 2-methyl-4-ph-benzo[(2,3-b)benzoxazine;(2',3'-e)1,3,4-oxadiazine]-5,12-dione (3b)

Two different routes are employed to prepare these compounds:

(i) Equimolar ratio (0.01 mol) of 3,4-dichlorobenzo[*b*] benzoxazine-2,5-dione (**1**) and acetic acid azide of either hydrazine or phenyl hydrazine (**2a**,**b**) were refluxed in ethylene glycol (100 mL) for 1 h until the mixture attain a red color. Aqueous sodium bicarbonate solution (20%) (5 mL) were added to the reaction mixture which then refluxed again for 3 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, dried, collected and crystallized from ethanol (Scheme 1). The data are reported in Table 1 and 2.

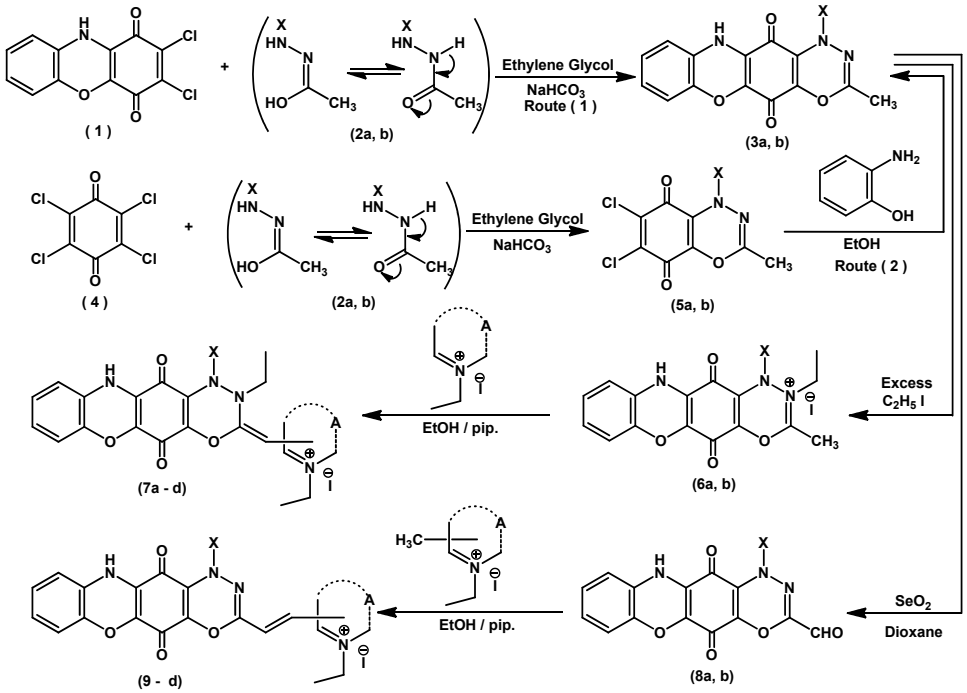
(ii) This route is carried out through the following two steps (a) and (b).

(a) Equimolar ratio (0.01 mol) of *p*-chloranil (2,3,5,6-tetra chloro-*p*-benzoquinone) (**4**) and acetic azide of either hydrazin hydrate or phenyl hydrazine (**2a**,**b**) were refluxed in ethylene glycol (100 mL) for 1 h where the mixture attain green color for compound 6,7-dichloro-2-methyl-4-*H*-benzo[e][1,3,4] oxadiazine-5,8-dione (**5a**) and reddish brown for compound 6,7-dichloro-2-methyl-4-*ph*-benzo[e][1,3,4]oxadiazine-5,8-di one (**5b**). Aqueous sodium bicarbonate (20%) (5 mL) were added to the reaction mixture, which then refluxed again for 3 h and attained deep red color for compounds **5a**,**b** at the end of refluxing. The reaction mixture is 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, dried, collected and crystallized from ethanol to give 6,7-dichloro-2-methyl-4-*H*-benzo[e] [1,3,4]oxadiazine-5,8-dione (**5a**) and 6,7-dichloro-2-methyl-4-*ph*-benzo[e][1,3,4]oxadiazine-5,8-dione (**5b**) (Scheme 1). The data are listed in Table 1 and 2.

(b) Equimolar ratio (0.01 mol) of compounds **5a** and 5**b** and *ortho*-amino phenol were refluxed in ethanol (50 mL) for 6-8 h until the mixture attain brown color. The reaction mixture are filtered off while hot to remove unreacted materials, concentrated then poured in ice water mixture with continuous shaking. The precipitated compounds were filtered, washed with cold water, dried, collected and crystallized from ethanol to give the same compounds **3a**,**b** obtained by *Route i*, characterized by melting points, mixed melting points, same IR and ^{1}H NMR spectral data. The data are summarized in Table 1 and 2.

2.2.2. Synthesis of 3-ethyl-2-methyl-4-H-benzo[(2,3-b)benzo xazine;(2',3'-e)1,3,4-oxadiazinium]-5,12-dione-iodide salt **(6a) and** *3-ethyl-2-methyl-4-ph-benzo[(2,3-b)benzoxazine;(2',3'-e)1,3,4-oxadiazinium]-5,12-dione-iodide salt* **(6b)**

A pure crystallized sample of compounds **3a**,**b** (0.04 mol) was suspended in excess of iodoethane (30 mL) and heated gently under reflux at low temperature (40-60 °C) for 1 h.



(**2a**,**b**); (**3a**,**b**), (**5a**,**b**); (**6a**,**b**); (**8a**,**b**): X = H (**a**); Ph (**b**)
(**7a-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-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**)

Scheme 1

The solvent was evaporated and the residue was collected and crystallized from ethanol (Scheme 1). See data in Table 1 and 2.

2.2.3. Synthesis of 3-ethyl-4-H(Ph)-benzo[(2,3-b)benzo xazine; (2',3'-e')1,3,4-oxadiazine]-5,12-dione-2[4(1)] mono methine cyanine dyes (7a-d)

A mixture of equimolar ratios (0.02 mol) of compounds **6a**,**b** and iodoethane quaternary salts of pyridine, quinoline or isoquinoline was refluxed in ethanol (50 mL) containing piperidine (3-5 drops) for 6-8 h. The reaction mixture, which changed from brown to red during the refluxing time, was filtered off while hot to remove any impurities, concentrated, cooled and precipitated by adding cold water. The precipitate was collected and crystallized from ethanol (Scheme 1). The relevant data are given in Table 1 and 2.

2.2.4. Synthesis of 2-formyl-4-H(ph)-benzo[(2,3-b)benzo xazine:(2',3'-e)1,3,4-oxadiazine-5,12-dione (8a,b)

A pure sample of compounds **3a**,**b** (0.01 mol) was refluxed in dioxoxae (50 mL) containing selenium dioxide (0.01 mol) for 14-16 h. The reaction mixture was filtered off while hot to remove selenium metal, concentrated, cooled and then precipitated by adding cold water. The precipitated products were filtered, dried, collected and crystallized from ethanol (Scheme 1). The data are shown in Table 1 and 2.

2.2.5. Synthesis of 4-H(ph)-benzo [(2,3-b)benzoxazine; (2', 3'-e')1,3,4-oxadiazine]-5,12-dione-2[2(4)] dimethine cyanine dyes (9a-d)

A mixture of the formyl compounds **8a**,**b** (0.01 mol) and ethyl iodide quaternary salts of α-picoline, γ-picoline, quinaldine (0.01 mol) were dissolved in ethanol (50 mL) containing piperidine (3-5 drops). The reaction mixture was heated under reflux for 6-8 h and attained brown color for compounds **9a**,**c** and violet color for compounds **9b**,**d** at the end of refluxing. It was filtered off while hot, concentrated to half its volume and cooled. The precipitated dyes were filtered, washed with water, dried and crystallized from ethanol (Scheme 1). The data are registered in Table 1 and 2.

2.3. Spectral behavior

The electronic visible absorption spectra of the prepared cyanine dyes were examined in 95% ethanol solution and recorded using 1 cm quartz cell in Shimadzu UV-Visible recording spectrophotometer. A stock solution (1×10^{-3} M) of the dyes was prepared and diluted to a suitable volume in order to obtain the desired lower concentrations [24-26]. The spectra were recorded immediately to eliminate as much as possible the effect of time.

2.4. Biological Activity

The tested compounds **3a**, **3b**, **6a**, **7a**, **7b**, **7c**, **8a**, **9a**, **9b** and **9c** were dissolved in DMSO to give a final concentration (1 mg/mL) [27,28]. Susceptible sterile discs were impregnated by the tested substance (50 µg/disc) [27,28] *via* a means of 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.

Table 3. Antibacterial activity of some of the oxadiazine and their derived cyanine dyes compounds.

Sample	Inhibition zone diameter (mm/mg sample)			
	Bacillus subtilis (G^+)	*Escherichia coli* (G^-)	*Pseudomonas aeruginosa* (G^-)	*Staphylococcus aureus* (G^+)
3a	11	12	10	14
3b	13	14	11	16
6a	15	14	11	15
7a	17	16	14	19
7b	15	15	11	16
7c	14	15	14	16
8a	12	12	12	13
9a	11	13	14	12
9b	10	10	11	13
9c	10	12	10	13
DMSO	0.0	0.0	0.0	0.0
Tetracycline	30	32	31	28
Ampicillin	20	22	17	18

3. Results and discussions

3.1. Synthesis

3,4-Dichloro benzo[*b*]benzoxazine-2,5-dione (**1**) was reacted with acetic acid azide of hydrazine and phenyl hydrazine (**2a**,**b**) in equimolar ratios in ethylene glycol as organic solvent and sodium bicarbonate as a basic catalyst and afforded 2-methyl-4-H-benzo[(2,3-b)benzoxazine;(2',3'-e) 1,3, 4-oxadiazine]-5,12-dione (3a) and 2-methyl-4-ph-benzo[(2,3-b)benzoxazine;(2',3'-e)1,3,4-oxadiazine]-5,12-dione (3b) as new oxadiazine heterocyclic compounds, Route (i), Scheme 1, Table 1 and 2.

Chemical confirmations for the compounds **3a**,**b** takes place via Route (ii), by reaction of *p*-chloranil (2,3,5,6-tetra chloro-*p*-benzoquinone) (**4**) with acetic acid azide of hydrazine and phenyl hydrazine (**2a**,**b**) in equimolar ratios in ethylene glycol containing sodium bicarbonate to achieve of 6, 7-dichloro-2-methyl-4-*H*-benzo[e][1, 3, 4]oxadiazine-5, 8-di one (**5a**) and 6,7-dichloro-2-methyl-4-*ph*-benzo[e][1,3,4] oxadiazine-5,8-dione (**5b**). Further reactions of compounds **5a**,**b** with *ortho*-amino phenol in ethanol as organic solvent, produced the same compounds **3a**,**b** obtained by Route (i), characterized by melting points, mixed melting points, same IR and ^{1}H NMR spectral data, Scheme 1, Route (ii), Table 1 and 2.

Quaternization of compounds **3a**,**b** using excess of iodoethane gives its 3-iodoethane quaternary salts (**6a**,**b**). Subsequent reactions of compounds **6a**,**b** and iodoethane quaternary salts of pyridine, quinolone and/or isoquinoline in equimolar ratios in ethanol containing few drops of piperidine resulted in the 2[4(1)]-monomethine cyanine dyes (**7a-d**), Scheme 1, Table 1 and 2.

Selenium dioxide oxidation of the starting material compounds **3a**,**b** in equimolar ratios, in dioxane as solvent afforded in the 2-carbaldehyde compounds **8a**,**b**. Farther reactions of compounds **8a**,**b** and iodoethane quaternary salts of α-picoline, quinaldine and/or γ-picoline in equimolar ratios in ethanol and the presence of few drops of piperidine achieved the 2[2(4)]-dimethine cyanine dyes (**9a-d**), Scheme 1, Table 1 and 2.

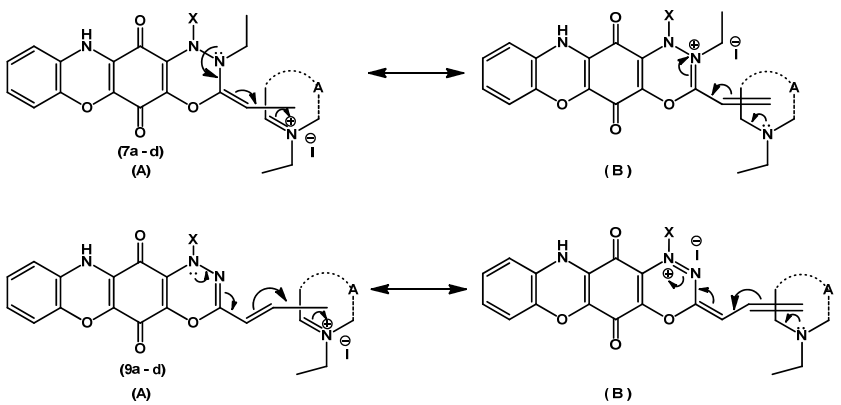
The structure of the prepared compounds was confirmed by elemental analysis (Table 1), visible (Table 1) mass spectrometer, IR [29] and ^{1}H NMR [30] spectroscopic data (Table 2).

3.2. Spectral behavior

The electronic visible absorption spectra of the monomethine cyanine dyes (**7a-d**) in 95% ethanol solution discloses absorption bands in the visible region (428-501 nm). The positions of these bands and their molar extinction coefficients are largely influenced by the nature of the heterocyclic quaternary salt residue (A), their linkage positions and by the type of the *N*-substituted (X) on the oxadiazine heterocyclic ring system. So, substituting A = 1-ethyl pyridinium-4-yl salt by A = 1-ethyl quinolinium-4-yl salt and/or by A = 2-ethyl isoquinolinium-1-yl salt, transferring from dye **7a** to dyes **7b** and/or **7c** causes bathochromic shifts for the bands by 15 nm and/or 3 nm in addition to increasing and/or decreasing the intensity of the bands, respectively. This could be attributed to increasing of π-delocalization conjugation in the later dyes **7b** and/or **7c** due to the presence of quinoline and/or isoquinoline rings compared by pyridine ring in the former dye **7a**, Scheme 1, Table 1. Changing the linkage position from 1-ethyl quinolinium 4-yl salt in dye (**7b**) to 2-ethyl isoquinolinium-1-yl salt to give dye (**7c**) resulted in a hypsochromic shift for the bands by 12 nm, accompanied by decreasing the intensity of the bands. This can be related to decreasing the length of the π-delocalization conjugation to the quaternary nitrogen atom in the isoquinoline dye **7c** compared to the quinoline dye **7b**, Scheme 1, Table 1. Replacing the type of *N*-substituted (X) in the oxadiazing heterocyclic ring system from H in dye **7a** to Ph in dye **7d**, makes strong red shifts for the bands by 58 nm. This can be explained in the light of increasing conjugation in the dye **7d** due to the presence of the additional benzene ring system, Scheme 1, Table 1.

Additionally, the electronic visible absorption spectra of the dimethine cyanine dyes (**9a-d**) discloses bands in the visible region (368-615 nm). These bands underwent displacements to give bathochromic and/or hypsochromic shifts depending upon the nature of the heterocyclic quaternary salt residue (A), their linkage position and the type of the *N*-substituents (X) in the oxadiazine heterocyclic ring system. So, substituting A = 1-ethyl pyridinium-2-yl salt in dye (**9a**) by A = 1-ethyl quinolinium-2-yl salt to obtain dye **9b**, makes strong bathochromic shifts for the absorption bands accompanied by increasing the intensity of the bands. This is due to increasing conjugation in the quinaldine dye **9b** compared to the α-picoline dye (**9a**), Scheme 1, Table 1. Changing the linkage position from 1-ethyl pyridinium-2-yl salt in dye (**9a**) by 1-ethyl pyridinium-4-yl salt to give dye (**9c**) resulted in red shifted bands. This can be attributed to increasing length of π-delocalization conjugation to the heterocyclic quaternary nitrogen atom in the γ-picolinium dye (**9c**) compared to α-picolinium dye (**9a**), Scheme 1, Table 1. Substituting X = H by X = Ph in the oxadiazine heterocyclic ring system moving from dye **9b** to dye **9d** causes bathochromic shifts for the absorption bands by 10 nm. This can be related to the conjugation of the additional phenyl ring system in the latter dye **9d**, Scheme 1, Table 1.

Generally, it is noticed that, the electronic visible absorption spectra of the dimethine cyanine dyes (**9a-d**) are strongly bathochromically shifted in addition to increasing number of the bands, if compared by the spectra of the monomethine cyanine dyes (**7a-d**). This can be illustrated in the light of increasing number of the methine units between the two heterocyclic ring systems of the cyanine dyes molecules in the former dyes, Scheme 1, Table 1.



Scheme 2

3.3. Biological activity

Structural-biological (antimicrobial) activity relationship for some selected newly synthesized oxadiazine and their derived cyanine dyes compounds **3a**, **3b**, **6a**, **7a**, **7b**, **7c**, **8a**, **9a**, **9b** and **9c** were studied and determined against some bacterial strains (*Bacillus subtilis*, *Escherichia coli*, *Pseudomona aeruginosa*, and *Staphylococcus aureus*), Table 3. According to this study, it was observed that:

Replacing *N*-hydrogen atom in compound **3a** by phenyl ring to give compound **3b** makes increasing for the antimicrobial activity for all the bacterial strains. This might be attributed to the increasing conjugation in the latter compound **3b** due to the additional conjugation of phenyl ring system, Table 3.

Comparing the biological activity of compound **3a** and its 3-iodoethane quaternary salt (**6a**), showed that, the latter compound **6a** have higher effect to destroy the bacterial strains. This may be related to increasing electron attracting character of compound **6a** due quaternization, Table 3.

The bacterial inhibition effects of compounds **3a** and **6a** have a remarkable decrease if compared by their derived monomethine cyanine dyes (**7a-c**). This could be related to the cyanine dyes structure effects of these compounds, Table 3.

Comparison between the antimicrobial activity of the monomethine cyanine dyes (**7a-c**) and the dimethine cyanine dyes (**9a-c**) showed that, the latter dyes possess lower potency as antimicrobial activity than the former ones. This might be correlated to increasing number of methine groups in the latter dyes (**9a-c**), Table 3.

Changing the quaternary salts in the monomethine cyanine dyes (**7a-c**) from 1-ethyl pyridinium-yl salt in dye (**7a**) to 1-ethyl quinolinium-4-yl salt and/or 2-ethyl isoquinolinium-4-yl salt to get dyes (**7b**) and/or (**7c**), discloses that the latter dyes have lower antimicrobial effects on the bacterial strains. This might be attributed to increasing π-delocalization conjugation in the latter dyes (**7b**), (**7c**) due to the presence of quinoline and/or isoquinoline rings in correspondence to pyridine ring in the former dye (**7a**), Table 3.

Changing the type of the quaternary heterocyclic salt residue and/or their linkage positions in the dimethine cyanine dyes (**9a-c**) from 1-ethyl pyridinium-2-yl salt in dye (**9a**) to 1-ethyl quinolinium-2-yl salt and/or 1-ethyl pyridinium-4-yl salt to obtain dyes (**9b**) and/or (**9c**) declared that the former dye (**9a**) have higher antimicrobial activity for all the bacterial strains except staphylococcus aureus. This could be related to increasing π-delocalization conjugation in the latter dyes (**9b**) and/or (9c) due to the presence of quinaldine and/or γ-picoline nucleus in correspondence to α-picoline nucleus in the former dye (**9a**), Table 3.

The antimicrobial activity of all the tested compounds (except compound **9a**) increase to give higher inhibition zone diameter in the case of *Staphylococcus aureus* compared with the other bacterial strains. This indicates that these compounds are more effective against these bacterial strains, Table 3.

Comparison between the biological activity of the monomethine cyanine dyes (**7a**) and the standard antibacterial agent Ampicillin, against *Staphylococcus aureus*, showed that, the former one have higher potency inhibition zone than the latter one. This indicates the strong effective of the monomethine dye (**7a**) against this bacterial strain, Table 3.

General comparison of the antibacterial activity for all the tested compounds declared that, the monomethine cyanine dye (**7a**) gives the highest inhibition zone diameter against all the bacterial strains and particularly against *Staphylococcus aureus*. This indicates the increased effect of the monoethine cyanine dye (**7a**) to be used as antibacterial against these strains, Table 3. In contrast the dimethine cyanine dyes (**9b**,**c**) gives the lowest inhibition action against all the bacterial strains. These reflect their deficiency to be used as biological active material against these bacterial strains Table 3.

From the above discussed results we could conclude that the antimicrobial inhibition action activity of the oxadiazine and their derived cyanine dyes compounds increase and/or decrease to give higher and/or lower bacterial inhibition zone diameter depending upon the following factors:

1. Nature of the *N*-substituted groups in the oxadiazine heterocyclic ring system (hydrogen and/or phenyl), Table 3.
2. Types of cyanine dye molecule (monomethine and/or dimethine cyanine dyes), Table 3.
3. Nature of the heterocyclic quaternary salt residue (A) (pyridinium and/or quinolinium salt residue; α-picolinium and/or quinaldinium salt residue), Table 3.
4. Linkage positions of the heterocyclic quaternary salt residue (quinolinium and/or isoquinolinium salt residue; α-picolinium and/or γ-picolinium salt residue), Table 3.
5. Kind of the bacterial strains tested (higher in the case of staphylococcus aureus compared with the other bacterial strains), Table 3.

4. Conclusion

a) The electronic visible absorption spectra of the monomethine cyanine dyes (**7a-d**), and the dimethine cyanine dyes (**9a-d**) underwent displacements to give bathochromic and/or hypsochromic shifted bands depending upon:

1. The nature of the heterocyclic quaternary salt residue (A), in the order of (i) Quinolinum dyes > pyridinium dyes (for the monomethine cyanine dyes), (ii) Quinaldinium dyes > α-picolinium dyes (for the dimethine cyanine dyes).
2. Linkage positions of the heterocyclic quaternary salt residue (A), in the order of (i) Quinolinium dyes > isoquinolinium dyes (for the monomethine cyanine dyes), (ii) γ-picolinium dyes > α-picolinium dyes (for the dimethine cyanine dyes).
3. Type of the N-substituents (X) in the oxadiazine heterocyclic ring system in the order of: Ph substituents dyes > H substituents dyes.
4. The number of methine groups between the two heterocyclic ring systems of the cyanine dyes molecules in the order of: Dimethine cyanine dyes > monomethine cyanine dyes.

b) The intensity of the colors of the monomethine cyanine dyes **7a-d** and the dimethine cyanine dyes **9a-d** can be illustrated in the light of the suggested two mesomeric structures (A) and (B), producing a delocalized positive charge over the conjugated system, Scheme 2.

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