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NOVEL CARBOCYANINE AND DICARBOCYANINE DYES: SYNTHESIS, SPECTRAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY

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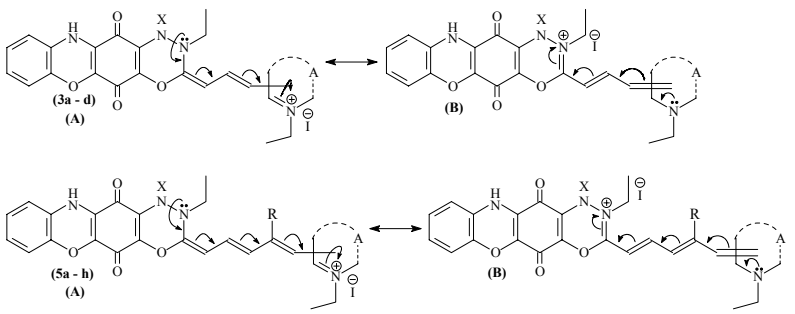
Novel carbocyanine dyes (trimethine cyanine dyes) and dicarbocyanine dyes (pentamethine cyanine dyes) having the nucleus of benzo[(2,3-b)benzoxazine;(2',3'-e)1,3,4-oxadiazine]-5,12-dione were prepared. The electronic visible absorption spectra of all the synthesized cyanine dyes were examined in 95% ethanol solution to evaluate their photosensitization properties. The dyes are thought to be better photosensitizer when they absorb light at longer wavelength bands (bathochromic shifted and/or red shifted bands).



Consequently, the photosensitization of the dyes decreases when they absorb light at shorter wavelength bands (hypsochromic shifted and/or blue shifted bands. Biological activity of some selected compounds was tested and evaluated against various bacterial strains (*Bacillus subtilis, Escherichia coli, Pseudomona aeruginosa* and *Staphylococcus aureus*) to assess their bactericidal properties. Structural confirmation was obtained out by elemental analysis, mass spectrometer, visible, IR and ^{1}H NMR spectral data.

INTRODUCTION

Cyanine dyes[1-10] have applications in a broad and numerous diverse areas: biological, medical and drug developments, imaging of bio-targets molecules, cells and organelles and conformational studied by fluorescence energy transfer. Their excellent staining properties make them useful fluorescent probes in such methods as flow cytometry, for the detection of nucleic acids in solutions, gel electrophoresis and fluorescence microscopy. In addition, cyanine dyes have a wide spectrum of biological applications, such as bactericidal, fungicidal, anti-tumor, anti-cancer and inhibitors for cell growth and division. These advantages of cyanine dyes encouraged and prompted scientists and researchers to investigate new methods for their synthesis.

On the other side oxadiazine compounds[11-22] have played an important role in medicinal chemistry. They have been studied extensively because of their ready accessibility and broad spectrum of biological activities such as inhibition of bacterial growth and antimicrobial agents. Besides, oxadiazine compounds have a superfine applications in many other important fields such as in agriculture as insecticides and in industry as adhesives.

In this point of view we prepared here a series of oxadiazine cyanine dyes as new synthesis contribution, spectroscopic investigation and antimicrobial evaluation to be used and/or applied

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in any of the wide areas of cyanine dyes, particularly as photographic sensitizers for silver halide emulsion in photographic industry and/or as chemo-therapeutic and bactericidals in pharmaceutical industry.

RESULTS AND DISCUSSION

1. Synthesis

Equimolar reaction of 3-ethyl-4H(Ph)-benzo [2,3-b)benzoxazine;(2',3'-c)1,3,4-oxadiazinium]-5,12-dione iodide salts (1a,b)[23] and triethylorthoformate in ethanol containing piperidine gave the intermediate compounds (2a,b). Subsequent reactions of the intermediate compounds (2a,b) with iodoethane quaternary salts of α-picoline, quinaldine and/or γ-picoline in ethanol as organic solvent and piperidine as a basic catalyst resulted in the 2[2(4)]-triethine cyanine dyes (3a-d), Scheme (1), Table (1).

Reactions of the intermediate compounds (2a,b) with acetaldehyde, acetone, acetophenone, p.methoxy acetophene and/or p. nitro acetophenone in equimolar ratios, in ethanol and presence of piperidine yielded the intermediate compounds (4a-f). Further reactions of the intermediate compounds (4a-f) with N-ethyl (α-picolinium, quinaldinium and/or γ-picolinium) iodide salts in equimolar ratios in ethanol containing piperidine produced the 2[2(4)]-pentamethine cyanine dyes (5a-h), Scheme (1), Table (2).

The structure of the prepared compounds were identified by elemental analysis (Tables 1, 2), visible absorption spectra (Tables 1, 2), mass spectrometer, IR[24] and ^{1}H NMR[25] spectral data (Table 3).

2. Spectral characterization

The electronic visible absorption spectra of the trimethine cyanine dyes (3a-d) in 95% ethanol solution gives bands in the visible range (495 nm-655 nm). The positions of these bands underwent displacements to give bathochromic and/or hypsochromic shifts depending on the nature of the heterocyclic quaternary salt residue (A), their linkage positions and by the type of the N-substituted (X) in the oxadiazine heterocyclic ring system. So, substituting A = 1-ethyl pyridinium-2-yl salt in dye (3a) by A = 1-ethyl quinolinium-4-yl salt to get dye (3b) resulted in a strong bathochromic shift by 125 nm, accompanied by increasing number and the intensity of the absorption bands. This may be attributed to the increasing of the π-delocalization conjugation in quinoline ring (dye 3b) compared by pyridine ring (dye 3a), Scheme (1), Table (1). Changing the linkage position from 1-ethyl pyridinium-2-yl salt (dye 3a) to 1-ethyl pyridinium-4-yl salt (dye 3c) causes red shifts for the absorption bands by 10 nm in addition to increasing the intensity of the band. This can be related to the increasing of the length of π-delocalization conjugation to the quaternary nitrogen in γ-picoline dye (3c) compared to α-picoline dye (3a), Scheme (1), Table (1). Replacing the type of the N-substituents (X) in the oxadiazine heterocyclic ring system from H in dye (3a) by Ph to give dye (3d), makes red shifted for the maximum absorption bands by 35 nm, accompanied with increasing the intensity of the band. This can be attributed to the increasing π-delocalization conjugation in the latter dye due to the presence of the additional phenyl ring, Scheme (1), Table (1).

Additionally, the electronic visible absorption spectra of the pentamethine cyanine dyes (5a-h) displays bands in the visible region (540 nm-687 nm). The positions of these bands and their molar extinction coefficients (molar absorptivity) are remarkable effected by the nature of the heterocyclic quaternary residue (A), their linkage positions, type of the triene side chain (R) and kind of the N-substituents (X) in the oxadiazine heterocyclic ring system. So, substituting A = 1-ethyl pyridinium-2-yl salt by A = 1-ethyl quinolinium-2-yl salt, transferring from dye (5a) to dye (5b) causes strong bathochromic shift by 37 nm accompanied by increasing the number and intensity of the bands. This can be illustrated according to increasing π-delocalization in the quinaldinium nucleus dye (5b) compared to α-picolinium nucleus dye (5a). Changing the linkage position from 2-yl salt in dye (5a) to 4-yl salt to obtain dye (5c) produced red shifted and intensified absorption bands. This can be explained in the light of increasing the length of the π-delocalization conjugation to the quaternary nitrogen in the γ-picolinium dye (5c) compared to α-picolinium dye (5a), Scheme (1), Table (2). Substituting R = H in the triene side chain by R = CH_3 and/or Ph moving from dye (5b) to dyes (5d) and/or (5e) resulted in bathochromic shifts by 9 nm and/or 50 nm for the bands, in addition to increasing intensity of the bands. This can be due

to electron donating character of CH_3 in the dye (5d) and/or increasing conjugation of phenyl ring in dye (5e), Scheme (1), Table (2). Substituting R = C_6H_5 in the triene side chain in dye (5e) by R = C_6H_4.p-OCH_3 and/or by R = C_6H_4.p-NO_2 to obtain dyes (5f) and/or (5g) gives red shifted and intensified bands in the case of dye (5f) and/or blue shifted and lower intensity bands in the case of dye (5g). This is because the electron pushing character of OCH_3 group in dye (5f) and/or electron pulling character of NO_2 group in dye (5g), Scheme (1), Table (1). Electron donating and/or pushing groups like CH_3, OCH_3 makes increasing for the intensity of the electronic charge transfer pathways to the heterocyclic quaternary nitrogen by pushing electrons and consequently red shift occurs. Inversely, electron attracting and/or pulling groups makes decreasing for the intensity of the electronic charge transfer pathways to the heterocyclic quaternary nitrogen by pulling electrons and consequently blue shift occurs. Replacing X = H in the N-substituents of the oxadiazine heterocyclic ring system by X =Ph moving from dye (5b) to dye (5h) resulted in strong bathochromic shifts for the absorption bands, accompanied by increasing number and intensity of the bands. This is due to increasing π-delocalization conjugation in the latter dye due to the presence of the additional phenyl ring system, Scheme (1), Table (2).

Generally, comparing the electronic visible absorption spectra of the trimethine cyanine dyes (3a-d) with those of the pentamethine cyanine dyes (5a-h) discloses strong bathochromic shifted and intensified bands accompanied by increasing the number of the absorption spectra bands for the latter pentamethine cyanine dyes (5a-h). This can be attributed to increasing the number of methine groups between the two heterocyclic ring system of the cyanine dye molecules in the latter dyes (5a-h) by two methine units, Scheme (1), Table (2).

3. Biological activity

Structural-biological (antimicrobial) activity relationship for some of the synthesized oxadiazine and their derived cyanine dyes compounds (2a, 3a, 3b, 3c, 4a, 5a, 5b, 5c, 5d, 5e, 5f, 5g) were studied and evaluated against some bacterial strains (*Bacillus subtilis, Escherichia coli, Pseudomona aeruginosa, Staphylococcus aureus*), Table (4). According to this study, it was observed that:

The biological activity of the trimethine cyanine dyes (3a-c) showed that α-picolinium and/or γ-picolinium cyanine dyes (3a) and/or (3c) possess higher potency as antimicrobial activity if compared with their analogous quinaldinium trimethine cyanine dye (9b). This could be attributed to increasing conjugation in the latter dye (9b) due to the presence of quinoline ring in correspondance to pyridine rings in the former dyes (9a) and/or (9c), Table (4).

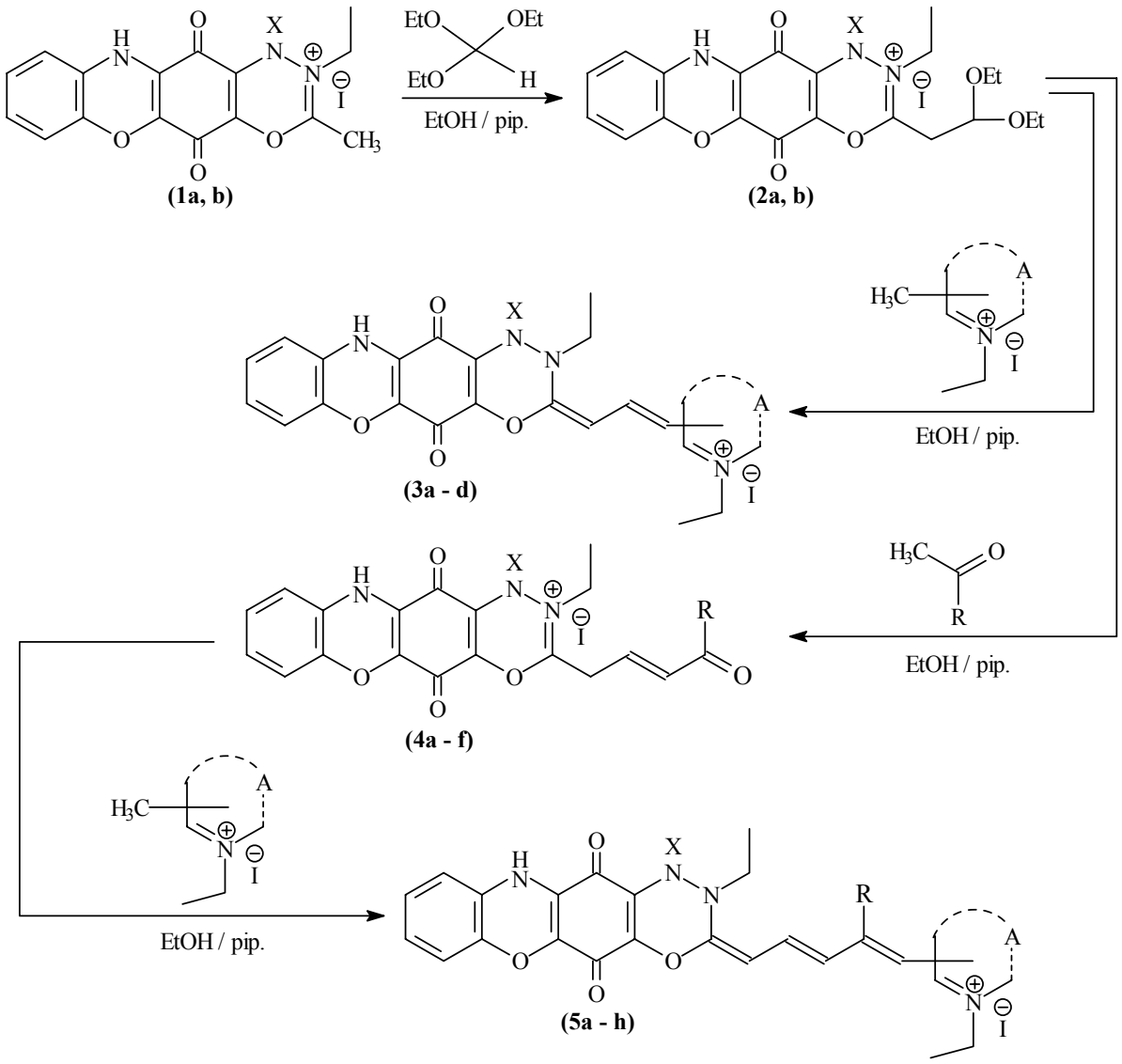
Converting the intermediate compound (2a) to its derived trimethine cyanine dyes (3a-c) makes increasing for the antimicrobial inhibition zone diameter for all the bacterial strains. This could be related to the strong electron attracting character of the diethoxy groups in compound (2a) and/or to the cyanine dyes structure effects of compounds (3a-c), Table (4).

Comparing the antimicrobial activity of the two intermediate compounds (2a), (4a), showed that, the latter one (4a) have higher potency effect for all the bacterial strains. This could be related to the replacing of the higher electron accepting character of the diethoxy groups in the former compound (2a) by the less electron accepting character of the acroleinyl group in the latter compound (4a), Table (4).

The antibacterial inhibition effects of the pentamethine cyanine dyes having quinoline ring system (5b) is lower than their analogous containing pyridine ring system (5a), (5c). This could be explained in the light of increasing conjugation in the former dye (5b) due to the presence of quinoline ring in correspondence to pyridine rings in the latter dyes (5a), (5c), Table (4).

Comparison of the biological activity of the intermediate compound (4a) with its derived α-picolinium pentamethine cyanine dyes (5a), declared that, the latter compound (5a) have higher inhibition zone diameter for *Bacillus subtilis* and *Escherichia* bacterial strains. This could be explained in the light of electron attracting character of acroleinyl group in compound (4a) and/or to the cyanine dyes structure effects of compound (5a), Table (4).

Substituting the triene side chain (R) from H and/or CH_3 in the pentamethine cyanine dyes (5b) and/or (5d) by Ph to get the pentamethine cyanine dye (5e) causes increasing for the biological activity toward *Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus* bacterial strains. This could be correlated to increasing π-delocalization conjugation in the latter dye (5e) due to the phenyl ring system, Table (4).



Scheme 1

Substituents in Scheme 1:

(1a,b); **(2a,b)**: X = H (a); Ph (b).

(3a-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);

(4a-f): X= H, R = H (a); X = H, R = CH_3 (b), X = H, R = Ph (c);
X = H, R = C_6H_4.p.OCH_3 (d); X = H, R = C_6H_4.p.NO_2 (e); X = Ph, R = H (f).

(5a-h): X = H, R = H, A = 1-ethyl pyridinium-2-yl salt (a);
X = H, R = H, A = 1-ethyl quinolinium-2-yl salt (b);
X = H, R = H, A = 1-ethyl pyridinium-4-yl salt (c);
X = H, R = CH_3, A = 1-ethyl quinolinium-2-yl salt (d);
X = H, R = Ph, A = 1-ethyl quinolinium-2-yl salt (e);
X = H, R = C_6H_4.p.OCH_3, A = 1-ethyl quinolinium-2-yl salt (f);
X = H, R = C_6H_4.p.NO_2, A = 1-ethyl quinolinium-2-yl salt (g);
X = Ph, R = H, A = 1-ethyl quinolinium-2-yl salt (h).

Table 1

Characterization of the prepared compounds (**2a, b**) and (**3a-d**)

Comp No.	Nature of products			Molecular formula (M.Wt.)	Analysis%						Absorption spectra in 95% ethanol solution	
					Calculated			Found				
	Colour	yield %	M.P. C°		C	H	N	C	H	N	λ_{max} (nm)	ε_{max} (mol^{-1} cm^2)
2a	Dark brown	75	150	$C_{21}H_{24}N_3O_6I$ (541)	46.58	4.44	7.76	46.51	4.4	7.71		
2b	Black	79	136	$C_{27}H_{28}N_3O_6I$ (617)	52.51	4.54	6.81	52.45	4.51	6.78		
3a	Reddish brown	54	160	$C_{25}H_{23}N_4O_4I$ (570)	52.63	4.04	9.82	52.6	4.01	9.75	495	3300
3b	Violet	51	190	$C_{29}H_{25}N_4O_4I$ (620)	56.13	4.03	9.03	56.08	4	9	476, 536, 579, 620	4260, 4270, 4140, 4540
3c	Brown	53	165	$C_{25}H_{23}N_4O_4I$ (570)	52.63	4.04	9.82	52.59	4.02	9.76	505	3550
3d	Violet	62	182	$C_{35}H_{29}N_4O_4I$ (696)	60.34	4.17	8.05	60.31	4.13	8.02	534,581,655	5990,5040,5710

Table 2

Characterization of the prepared compounds **(4a-f) and (5a-h)**

Comp No.	Nature of products			Formula (M.Wt.)	Analysis%						Absorption spectra in 95% ethanol solution	
					Calculated			Found			λ_{max} (nm)	ε_{max} ($mol^{-1}cm^2$)
	Colour	yield %	M.P. C°		C	H	N	C	H	N		
4a	Brown	69	115	$C_{19}H_{16}N_3O_5I$ (493)	46.25	3.25	8.52	46.2	3.19	8.48		
4b	Brown	56	145	$C_{20}H_{18}N_3O_5I$ (507)	47.33	3.55	8.28	47.27	3.51	8.22		
4c	Brown	65	170	$C_{25}H_{20}N_3O_5I$ (569)	52.72	3.51	7.38	52.66	3.48	7.33		
4d	Brown	45	210	$C_{26}H_{22}N_3O_6I$ (599)	52.09	3.67	7.01	52.05	3.62	7		
4e	Brown	54	100	$C_{25}H_{19}N_4O_7I$ (614)	48.86	3.09	9.12	48.82	3.04	9.08		
4f	Brown	69	145	$C_{25}H_{20}N_3O_5I$ (569)	52.72	3.51	7.38	52.69	3.48	7.32		
5a	Brown	56	193	$C_{27}H_{25}N_4O_4I$ (596)	54.36	4.19	9.4	54.31	4.12	9.36	540,585	1850,3300
5b	Deep violet	61	230	$C_{31}H_{27}N_4O_4I$ (646)	57.59	4.18	8.67	57.51	4.13	8.61	545,574,622	2680,6090,3560
5c	Brown	61	211	$C_{27}H_{25}N_4O_4I$ (596)	54.36	4.19	9.4	54.33	4.11	9.32	560,600	2350,3500
5d	Deep violet	54	225	$C_{32}H_{29}N_4O_4I$ (660)	58.18	4.39	8.48	58.11	4.34	8.42	582,631	4140,4510
5e	Deep violet	53	215	$C_{37}H_{31}N_4O_4I$ (722)	61.5	4.29	7.76	61.44	4.22	7.73	564,604,672	4660,4380,4930
5f	Deep violet	62	205	$C_{38}H_{33}N_4O_5I$ (752)	60.64	4.39	7.45	60.6	4.31	7.4	563,603,680	5980,5190,5714
5g	Deep violet	50	213	$C_{37}H_{30}N_5O_6I$ (767)	57.89	4.91	9.13	57.82	4.87	9.09	561,593,655	3320,3540,4790
5h	Deep violet	40	230	$C_{37}H_{31}N_4O_4I$ (722)	61.5	4.29	7.76	61.45	4.24	7.72	507,568, 608,687	4260,4270,4150,6090

Table 3

IR, ^{1}H NMR, and Mass Spectral data

Comp. No	IR (KBr, cm^{-1})	^{1}H NMR (DMSO, δ); Mass data
2a	755,915 (o-disubstituted benzene). 1013,1179 (C-O-C cyclic). 1286 (C-O ether). 1439 (C=N). 1593 (C=C). 1687 (C=O quinone). 2925,2857 (quaternary salt). 3420 (NH)..	0.8(b, 3H, CH_3 of position 3). 1.2(m, 2H, CH_2 of position 3). 1.9(m, 6H, $2CH_3$ of diethoxyethyl of position 2). 2.1(m, 4H, $2CH_2$ of diethoxyethyl of position 2). 3.38 (s, 2H, CH_2 of position 2). 4.3 (b, 1H, -CH of position 2). 6.7-6.9 (b, 2H, 2 NH). 7.1-7.9 (b, 4H, aromatic). M^+: 541
3b	754,852 (o.disubstituted benzene). 1185 (C-O-C cyclic). 1453 (C=N). 1603 (C=C). 1731,1770 (C=O quinone). 2923,2855 (quaternary salt). 3427(NH).	0.8 (b, 3H, CH_3 of position 3). 1.2 (m, 2H, CH_2 of position 3). 1.6 (b, 3H, CH_3 of N-quinolinium) 1.9-2.1(b, 2H, CH_2 of N-quinolinium). 6.2-6.6(b, 3H, 3 -CH=). 6.7-6.8(b, 2H, 2NH). 7.2-8.4(b, 10H, aromatic + heterocyclic).
4a	753,819 (o.disubstituted benzene). 1052,1182 (C-O-C cyclic). 1451 (C=N). 1603 (C=C). 1736 (C=O quinone). 1778 (CHO conjugated). 2925,2857 (quaternary salt). 3384(NH).	0.8 (b, 3H, CH_3 of position 3). 1.2 (m, 2H, CH_2 of position 3). 4-4.4 (b, 2H, CH_2 of position 2). 5-5.2(b,2H, 2 -CH= of position 2) 6.7-6.8(b, 2H, 2NH). 7.137-8. 2(m, 4H, aromatic). 11.3 (s, 1H, CHO). M^+: 494
5b	753,840 (o-disubstituted benzene). 1078,1182 (C-O-C cyclic). 1449 (C=N). 1600,1553 (C=C). 1729 (C=O quinone). 2924,2856 (quaternary salt). 3418 (NH).	0.8 (b, 3H, CH_3 of position 3). 1.3 (m, 2H, CH_2 of position 3). 1.7 (b, 3H, CH_3 of N-quinolinium). 1.9-2.1(b, 2H, CH_2 of N-quinolinium). 5-5.2(b,5H, 5-CH=) 6.6-6.9 (b, 2H, 2NH). 7-8.9(m, 10H, aromatic + heterocyclic)

Table 4

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^+)
Control: DMSO		0.0	0.0	0.0	0.0
Standard: Antibacterial	*Tetracycline*	30	32	31	28
	Ampicillin	20	22	17	18
2a		9	10	9	11
3a		13	13	11	17
3b		11	12	10	13
3c		13	16	14	16
4a		11	11	12	15
5a		12	12	12	15
5b		9	9	9	10
5c		11	12	12	14
5d		9	9	9	10
5e		11	10	9	12
5f		0.0	0.0	0.0	9
5g		0.0	0.0	0.0	9

Replacing the H atom in the p.position of the triene side chain of the pentamethine cyanine dye (5e) by OCH_3 and/or NO_2 groups to get the pentamethine cyanine dyes (5f) and/or (5g) makes complete destroying for the bacterial inhibition effect for *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas*, in addition to lowering the inhibition action for *staphylococcus aureus*. This could be attributed to the strong electron accepting character of the oxygentated methyl group (OCH_3) and/or the dioxygenated nitrogen atom (NO_2) in the latter dyes (5f) and (5g), respectively, Table (4).

The antimicrobial activity of all the tested compounds increases to give higher inhibition zone diameter in the case of *Staphylococcus aureus* compared with the other bacterial strains. This indicates that these conpounds are more effective against this bacterial strain, Table (4).

General comparison of the antibacterial activity for all the tested compounds declared that the trimethine cyanine dye (3c) gives the highest inhibition zone diameter against most of the bacterial strains. This reflects its increasing effect to be used as antimicrobial active materials against these bacterial strains Table (4). In contrast, the pentamethine cyanine dyes (5f), (5g) give the lowest inhibition against all the bacterial strains, which reflect their deficiency to be used as biologically active material against these bacterial strains, Table (4).

From the results discussed in this study we could conclude that the antibacterial inhibition action activity of the oxadiazine and their derived cyanine dyes compounds increases and/or decreases to give higher and/or lower bacterial inhibition zone diameter depending upon the following factors:

Nature of the heterocyclic quaternary salt residue (A) (α-picolinium and/or quinaldinium dyes).

Linkage positions of the heterocyclic salt residue (A) (α-picolinium and/or γ-picolinium dyes), Table (4).

Electron accepting and/or electron donating groups (diethoxy, acroleinyl, Ph, OCH_3, NO_2 and/or CH_3 groups).

Types of the triene side chain (R) in the cyanine dye molecule (H, CH_3, Ph, C_6H_4.p.OCH_3 and/or C_6H_4.p.NO_2).

Kind of bacterial strains (higher in the case of *staphylococcus aureus* compared with the other bacterial strains).

EXPERIMENTAL

1. General

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) and are uncorrected. Elemental analysis were 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 were 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). Biological activity were carried out at the Microanalytical center, Microbiology division (Cairo University).

2. Synthesis

2.1. Synthesis of 3-ethyl-4- H(ph)-benzo [(2,3-b)benzoxazine;(2',3'-e)1,3,4oxadiazinium]-5,12-dion-2(1,1'-diethoxy) ethyl-iodide salt (2a,b)

An equimolar ratios (0.06 mol) of the compounds (1a,b) and triethylorthoformate was heated under reflux in ethanol (50 mL) containing piperidine (3-5 drops) for 3-5 hrs. The dark brown mixture which attainded at the end of refluxing was filtered on hot to remove any impurities, concentrated and precipitated by cold water. The separated intermediate compounds (2a,b) were filtered, washed with water and crystallized from ethanol. The results are registered in Table 1.

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

A mixture of the intermediate compounds (2a,b) (0.02 mo1) and N-ethyl α-picolinium iodide salt, N-ethyl γ-picolimium iodide salt, or N-ethyl quinaldinium iodide salt (0.02 mol) was heated under reflux in ethanol (50 mL) and presence of piperidine (3-5 drops) for 6-8 hrs. The colour of the reaction mixture attained redish brown (for 3a), brown (for 3c) and deep violet (for 3b,d) at the end of refluxing. It was filtered off on hot, concentrated and precipitated by adding cold water. The separated cyanines were filtered, washed with cold water and crystallized from ethanol. The results are listed in Table 1.

2.3. Synthesis of 3-ethyl-4- H(ph)-benzo[(2,3-b)benzoxazine;(2',3'-e)1,3, 4-oxadiazinium]-5,12-dione-2(1-acroleinyl) ethyl-iodide salt (4a-f)

Equirnolar ratios (0.04 mol) of (2a,b) and acyl or acyl derivatives (acetaldehyde, acetone, acetophenone, p-methoxy acetophenone, or p-nitro acetophenone) were heated under reflux in ethanol (50 mL) containing piperidine (3-5 drops) for 4-6 hrs. The dark brown mixture were filtered off on hot to remove unreacted materials, concentrated and precipitated using cold water. The separated intermediates compounds (4a-f) were filtered off, washed with cold water, dried and recrystallized from ethanol. The results are summarized in Table 2.

2.4. Synthesis of 3-ethyl-4-H(ph)-benzo[(2,3-b)benzoxazine;(2',3'-e)1,3,4-oxadiazine]-5,12-dione-2[2(4)]-pentamethine cyanine dyes **(5a-h)**

Piperidine (3-5 drops) was added to a mixture of equimolar ratios (0.01 mol) of (4a-f) and N-ethyl(α-picolineium, γ-picolimiutu, quinaldinium) iodide salts dissolved in ethanol (50 mL). The reaction mixture was heated under reflux for 6-8 hrs and attained brown colour (for 5a,c) and deep violet colour (for 5b, 5d-h) at the end of refluxing. It was filtered off while hot, concentrated and cooled. The precipitated products which appear on dilution with cold water were filtered off, washed with water, dried and crystallized from ethanol. The data are given in Table 2.

3. Spectral characterization

The electronic visible absorption spectra of the prepared cyanine dyes were examined in 95% ethanol solution and recorded using 1Cm Qz cell in Shimadzu UV-Visible Recording Spectrophotometer. A stock solution (1 x 10^{-3}M) of the dyes was prepared and diluted to a suitable volume in order to obtain the desired lower concentrations. The spectra were recorded immediately to eliminate as much as possible the effect of time.

4. Biological activity

The tested compounds (2a, 3a, 3b, 3c, 4a, 5a, 5b, 5c, 5d, 5e, 5f, 5g) were dissolved in DMSO to give a final concentration (l mgm/mL). Susceptible sterile discs were impregnated by the tested substance (50 µgm/disc) 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 (4).

CONCLUSIONS

The electronic visible absorption spectra of the trimethine cyanine dyes (3a-d) and/or the pentamethine cyanine dyes (5a-h) underwents displacements to give bathochromic and/or hypsochromic shifts depending upon:

The nature of the heterocyclic quaternary salt residue (A) in the order of: quinaldinium dyes > α-picolinium dyes.

Linkage positions of the heterocyclic quaternary salt residue (A) in the order of: γ-picolinium dyes > α-picolinium dyes.

Type of the N-substituents (X) in the oxadiazine heterocyclic ring system in the order of: Ph substituents dyes > H substituents dyes.

Types of the triene side chain (R) in the order of: (i) Ph dyes > CH_3 dyes > H dyes; (ii) C_6H_4.p.OCH_3 dyes > C_6H_5 -dyes > C_6H_4.p.NO_2 dyes.

The number of methine units and/or groups between the two heterocyclic ring system of the cyanine dye molecules in the order of: pentamethine cyanine dyes > trimethine cyanine dyes.

The intensity of the colour of the trimethine cyanine dyes (3a-d) and/or the pentamethine cyanine dyes (5a-h) is illustrated according to the following two suggested mesomeric structures (A) and (B) producing a delocalized positive charge over the conjugated system, Scheme (2).

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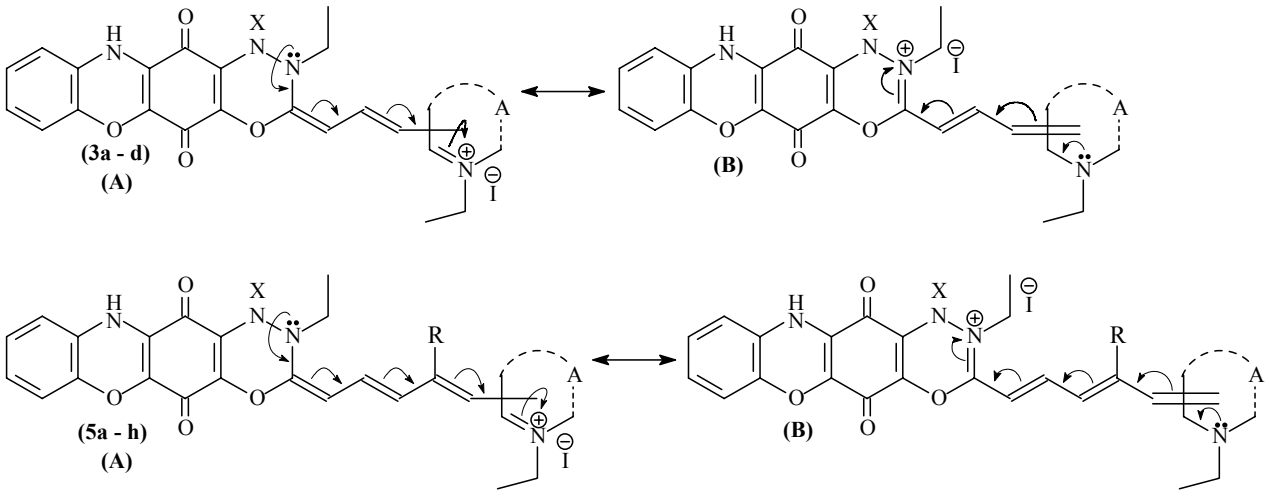

Scheme 2

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