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Synthesis, photosensitization and antimicrobial activity evaluation of some novel Merocyanine dyes

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Novel acyclic and cyclic merocyanine dyes derived from the nucleu of furo [(3,2-d) pyrazole; ($^{3,2-d}$)imidazole]were prepared. The electronic visible absorption spectra of all the synthesized new cyanine dyes were examined in 95% ethanol solution to evaluate their photosensitization properties. Antibacterial and antifungal activities for some selected dyes were tested against various bacterial and fungal strains (*Escherichia coli*, *Staphylococcus aureus*, *Aspergillus flavus* and *Candida albicans*) to evaluate their antimicrobial activity. Structural identification was carried out via elemental analysis, visible spectra, IR and ^{1}H NMR spectroscopic data.

Capsule Summary: Acyclic and cyclic merocyanine dyes were synthesized and characterized. As-synthesized dyes showed good photosensitization properties as well as antimicrobial activities.

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INTRODUCTION

Merocyanine dyes represent an important class of cyanine dyes. These dyes have strong fluorescence and absorption capacity and can be used as fluorescent labels for biomolecules. They can distinguish some certain cells and selectively enter into cancer cells, then kill it as photosensitizers directly using for PDT (photodynamic therapy) or as radiation sensitizers for the treatment of solid tumors, where the affinity between cyanine dyes and tumor cells is much higher than that between cyanine dyes and normal cells. Merocyanine dyes are also used as antitumor drugs and combining PDT with drug therapy has become a tendency and will certainly promote the treatment of tumors. In addition merocyanine dyes are used as optical sensors, spectral sensitizers for silver halide emulsion photography and recording medium in optical disks (Deligeorgiev et al., 2007; Shindy et al., 2012; Kawakami et al., 1998; Liu et al., 2011; Pudhom et al., 2008; Renikuntia et al., 2004; Sun et al., 1994; Takasu et al., 2002, 2004, 2006; Zhang et al., 2008).

Based on this concept were prepared here new acyclic and cyclic merocyanine dyes as new synthesis contribution, photosensitization and antimicrobial evaluation to may be used and/or applied as photosensitizer in photographic industry, as antibacterial in pharmaceutical industry and/or as anti-tumor, anti-cancer in medicine.

EXPERIMENTAL

General

Table 1: Characterization of the prepared compounds (2a-e), (3a,b) and (4).

Comp. No.	Nature of products			Molecular formula (M.Wt.)	Analysis%						Absorption spectra in 95% ethanol	
					Calculated			Found				
	Colour	yield %	M.P. C°		C	H	N	C	H	N	λ_{max} (nm)	ε_{max} ($mol^{-1}cm^2$)
2a	Pail brown	61	130	$C_{15}H_{12}N_4O_2$ (280)	64.28	4.28	20	64.21	4.22	19.95	420, 523	20000, 16400
2b	Brown	50	120	$C_{16}H_{14}N_4O_2$ (294)	65.30	4.76	19.04	65.13	452	19.00	425, 530	80000, 20000
2c	Deep brown	45	145	$C_{21}H_{16}N_4O_2$ (356)	70.78	4.49	15.73	70.66	4.37	15.69	430, 535	45000, 21000
2d	Deep brown	55	100	$C_{22}H_{18}N_4O_4$ (370)	71.35	4.86	15.03	71.22	4.77	15.00	440, 540	20000, 10000
2e	Deep brown	55	125	$C_{21}H_{15}N_5O_4$ (401)	62.84	3.74	17.45	62.71	3.65	17.33	415, 520	30000, 18000
3a	Brown	54	130	$C_{19}H_{18}N_4O_4$ (366)	62.29	4.91	15.30	62.11	4.85	15.20	410, 473	80000, 3500
3b	Brown	51	145	$C_{18}H_{16}N_4O_3$ (336)	64.28	4.76	16.66	64.19	4.68	16.54	420, 480	31000, 20000
4	Brown	45	150	$C_{16}H_{12}N_6O_3$ (336)	57.14	3.57	25.00	57.10	3.43	24.82	425, 490	40000, 30000

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 Mas 1: GC-2010 Shimadzu Spectrometer (Cairo University). Electronic visible absorption spectra were carried out on Visible Spectrophotometer, Spectro 24 RS Labomed, INC, Chemistry department, Faculty of Science (Aswan University). Antimicrobial activity was carried out at the Microanalytical center, Microbiology division (Cairo University).

Synthesis

Synthesis of 4-methyl-2-phenyl-furo[(3,2-d)pyrazole, ($\bar{3},\bar{2}-d$)imidazole]-6(1)-acyclic merocyanine dyes (2a-e):

A mixture of equimolar ratios (0.01 mol) of acetaldehyde, acetone, acetophenone, p-methoxyacetophenone, or p-nitroacetophenone and compound (1) was heated under reflux for 8 hrs in ethanol (30 ml) containing piperidine (1-2 ml). The reaction mixture, which changed from reddish colour to deep brown colour at the end of refluxing, was filtered while hot to remove unreacted materials, concentrated, cooled and precipitated by adding ice water mixture to give the acyclic merocyanine dyes (2a-e) which was crystallized from ethanol. The data were given in Table (1).

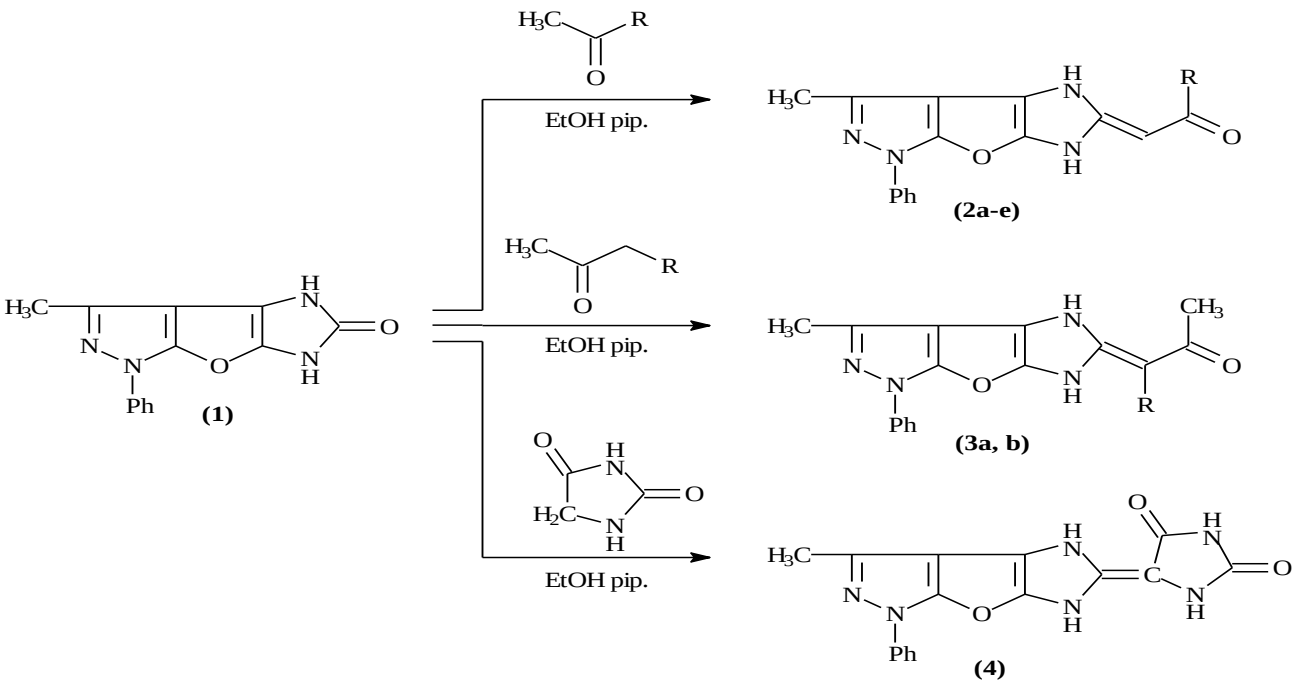
Synthesis of 4-methyl-2-phenyl-furo[(3,2-d)pyrazole, ($\bar{3},\bar{2}-d$)imidazole]-6[2(3)-acyclic merocyanine dyes (3a,b):

An equimolar ratio (0.01 mol) of ethylacetoacetate or acetyl acetone and compound (1) was heated under reflux for 8 hrs in ethanol (30 ml) containing piperidine (1-2 mL). The reaction mixture, which changed from red colour to deep brown colour at the end of refluxing, was filtered while hot to remove any impurities, concentrated, cooled and precipitated by adding cold water. The precipitates were filtered off, dried and crystallized from ethanol to give the acyclic merocyanine dyes (3a,b). The data were listed in Table (1).

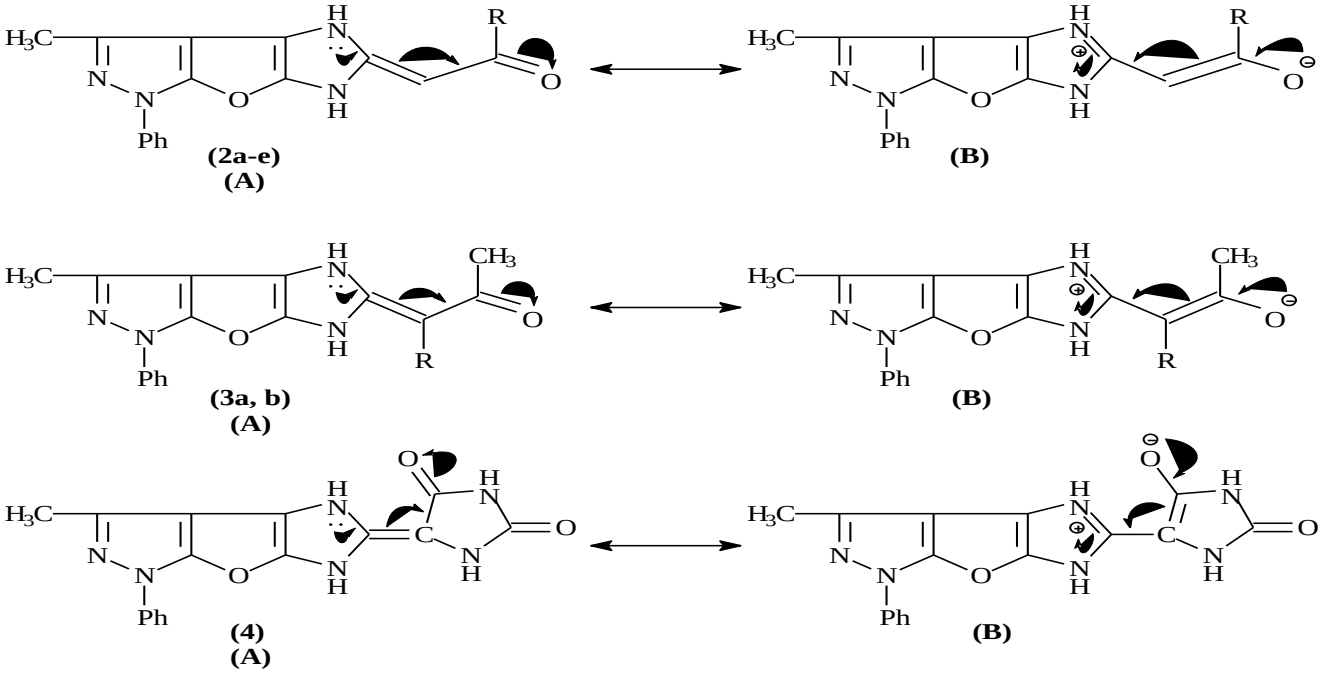
Synthesis of 4-methyl-2-phenyl-furo[(3,2-d)pyrazole, ($\bar{3},\bar{2}-d$)imidazole]-6(4)-cyclic merocyanine dye (4):

Equimolar ratios (0.01 mol) of hydantoin and compound (1) were heated under reflux in ethanol (30 ml) and presence of piperidine (1-2 ml) for 6 hrs and attained deep brown colour at the end of refluxing.. The reaction mixture was filtered off on hot, concentrated, cooled and precipitated by adding ice-water mixture. The separated cyclic merocyanine dye (4) were filtered off, washed with water, dried and crystallized from ethanol. The data were recorded in Table (1).

Photosensitization evaluation



Scheme 1: Synthesis route: Substituent in Scheme 1: (2a-e): R=H (a); CH_3 (b); Ph (c), C_6H_4.p.OCH_3 (d); C_6H_4.p.NO_2 (e). (3a, b) R=COOEt (a), $COCH_3$ (b).



Scheme 2: Colors intensity illustration of the synthesized merocyanine dyes

The electronic visible absorption spectra of the prepared cyanine dyes were examined in 95% ethanol solution and recorded using 1 cm Q_z cell in Visible Spectrophotometer, Spectro 24 RS Labomed, INC. 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.

Antimicrobial evaluation

The tested compounds (2a, 2b, 2c, 2d, 2e, 3a, 3b, 4) were dissolved in DMSO to give a final concentration (l mgm/ml).

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

Comp.No	IR Spectrum (KBr, Cm^{-1})	^{1}H NMR Spectrum (DMSO, δ)
2a	649, 692(monosubstituted phenyl).	2.1(m, 3H, CH_3 of position 4).
	1025, 1141(C-O-C cyclic).	7.1(b, 2H, 2NH).
	1498, 1451 (C=N).	7.2-8.2(m, 7H, aromatic+2 =CH).
	1598 (C=C).	
	1725 (C=O).	
	3431 (NH).	
3b	692, 648(monosubstituted phenyl).	0.8-1.6 (m, 6H, $2CH_3$ of $2COOCH_3$)
	1028, 1067, 11191, 1163 (C-O-C cyclic).	2.1(m, 3H, CH_3 of position 4).
	1497, 1450 (C=N).	6.9(b, 2H, 2NH).
	1597 (C=C).	7.2-8.2(m, 5H, aromatic).
	1712 (C=O).	
	3421 (NH).	
4	649, 693(monosubstituted phenyl).	2.2 (m, 3H, CH_3 of position 4).
	1027, 1064, 1162(C-O-C cyclic).	7.1(b, 4H, 4NH).
	1555, 1496 (C=N).	7.2-8.2(m, 5H, aromatic).
	1597 (C=C).	
	1720 (C=O).	
	3430 (NH).	

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).

RESULTS AND DISCUSSION

Synthesis

Reaction of 3-methyl-6-oxo-2-phenyl-furo [(3,2-d) pyrazole; ($\bar{3},\bar{2}-d$)imidazole] **(1)** with acyl and/or acyl derivatives (acetaldehyde, acetone, acetophenone, p.methoxy acetophenone, p.nitro acetophenone) in equimolar ratios in ethanol as organic solvents and piperidine as a basic catalysts resulted the acyclic merocyanine dyes **(2a-e)**, Scheme (1), Table (1).

Equimolar ratios of (1) and acetylacetate, ethylacetoacetate were reacted in ethanol containing few mL of piperidine and produced the acyclic merocyanine dyes **(3a, b)**, Scheme (1), Table (1).

Reaction of **(1)** and hydantoin (imidazolid-2,4-dione) in equimolar ratios in ethanol catalyzed by piperidine achieved the 4-cyclic merocyanine dye **(4)**, Scheme (1), Table (1).

The structure of the prepared compounds was confirmed by elemental analysis, Tables (1) and (2), visible spectra, Tables (1) and (2),, IR (Wade, 1999) and ^{1}H NMR (Wade, 1999) spectroscopic data, Table (3).

Photosensitization evaluation

Photosensitization evaluations for all the synthesized acyclic and cyclic merocyanine dyes were carried out through measuring their electronic visible absorption spectra in 95% ethanol solution. The dyes are thought to be better photosensitizer when they absorb light at longer wavelength bands (bathochromic shifted and/or red shifted bands). Inversely, the photosensitization of the dyes decreased when they absorb light at shorter wavelength bands (hypsochromic shifted and/or blue shifted bands).

The electronic visible absorption spectra of the acyclic merocyanine dyes (2a-e) in 95% ethanol solution revealed bands in the visible region 415-540 nm. The positions of these bands underwent displacements to give bathochromic shifts and/or hypsochromic shifts accompanied by increasing and/or decreasing the intensity of the bands depending upon the type of the side chain substituted (R), Scheme (1), Table (1).

So, substituting R=H in the acyclic merocyanine dye (2a) by R=CH_3 to give dye (2b) caused bathochromic shifts for the absorption bands by 7 nm in addition to increasing the intensity of the bands, Scheme (1), Table (1). This can be attributed to the electron donating character of the CH_3 group in the latter dye (2b) which facilitate and increase the strength and intensity of the electric change transfer to the positive center of the carbonyl group and consequently red shifts occurs in correspondence to the H atom in the former dye (2a).

Additionally, substituting R=H by R=Ph moving from dye (2a) to dye (2c) resulted in a red shifts by 12 nm accompanied with increasing the intensity of the absorption bands, Scheme (1), Table (1). This can be related to increasing π-delocalize conjugation in the latter dye (2c) due to the presence of additionally phenyl ring system.

Furthermore, substituting R = Ph in the dye (2c) by R=C_6H_4-p.OCH_3 and/or C_6H_4-p.NO_2 to give dyes (2d) and/or (2e) makes bathochromic and/or hypsochromic shifts for the absorption bands by 5 nm and/or 15 nm, accompanied by quenching the intensity of the bands, respectively, Scheme (1), Table (1). This can be related to the electron releasing character of the methyl group in dye (2d) and/or the electron attracting character of the NO_2 group in the dye (2e). Electron releasing groups increase the strength of the intensity of electronic charge transfer from the basic center of the dye (nitrogen atom) to the acidic center of the dye (polarized carbonyl group) and consequently red shifts occurs. Electron attracting groups decreases the strength of the

Table 3: The antimicrobial activity of the compounds (2a-e), (3a,b) and (4)

Sample	Inhibition zone diameter (mm/mg sample)			
	E. coli (G^-)	*S. aureus* (G^+)	*A. flavus*	*C. albicans*
Control DMSO	0.0	0.0	0.0	0.0
2a	10	10	0.0	0.0
2b	9	9	0.0	0.0
2c	10	9	0.0	0.0
2d	10	10	0.0	0.0
2e	10	9	0.0	0.0
3a	9	11	0.0	0.0
3b	0.0	0.0	0.0	0.0
4	9	9	0.0	0.0

intensity of electronic charge transfer pathways from the basic center of the dye (nitrogen atom) to the polarized acidic center of the dye (carbonyl group), and accordingly blue shift occurs.

Additionally, the electronic visible absorption spectra of the acyclic merocyanine dyes (3a, b) and cyclic merocyanine dye (4) discloses bands in the visible region 410-490 nm. The positions of these bands and their molar extinction coefficients are influenced by the kind of R substituted in the dyes (3a, b) molecules and by the cyclic ring system in dye (4), Scheme (1), Table (1). So, substituting R=COOEt by R=$COCH_3$ transfering from dye (3a) to dye (3b) makes a remarkable bathochromic shifts for the absorption bands by 7 nm. This can be related to the strong powerful electron pulling character of the ethoxy group in the former dye (3a) in correspondence to the strong electron pushing character of the methyl group in the latter dye (3b).

Furthermore, comparing the electronic visible absorption spectra of the acyclic merocyanine dyes (3a, b) with those of the cyclic merocyanine dye (4) showed that the latter cyclic merocyanine dye (4) reveals bathochromic shifted bands by 10 nm and 17 nm in addition to increasing the intensity of the bands, Scheme (1), Table (1). This may be attributed to the presence of two basic centers (two nitrogen atoms) in the cyclic ring system of the latter dye (4), which facilitate and increase the intensity of electronic charge transfer pathways to the acidic center of the dye (positively polarized carbonyl group).

Antimicrobial evaluation

The antimicrobial activity of the acyclic merocyanine dyes (2a-e) showed higher and/or lower biological action against the bacterial and fungal strains (Escherichia coli, *Staphylococcus aureus*, *Aspergillus flavus* and *Candida albicans*) depending upon the nature of the acyl group in their structures, Table (3).

So substituting R=H by R=CH_3 and/or Ph, transferring from dye (2a) to dyes (2b) and/or (2c), respectively, caused lowering for the biological inhibition against the bacterial strains, Table (3). This may be related to the electron pushing character of the CH_3 group in the dye (2b) and/or to the increasing conjugation due to the presence of phenyl ring system in the dye (2c).

Comparison the antibacterial inhibition actions of the acyclic merocyanine dye (2c), R = Ph by their analogous (2d), R = C_6H_4.p.OCH_3 showed that, the latter dye (2d) have higher potency effect against *staphylococcus aureus* bacterial strains, Table (3). This may be attributed to the electron releasing character of the OCH_3 group in the latter dye.

Also, it is noticed that the inhibition zone diameter of the acyclic merocyanine dye (2d), R = C_6H_4. p.OCH_3) is higher than its analogues (2e), R = C_6H_4.p.NO_2 against staphylococcus aureus, Table (3). This may be attributed to the electron pushing character of the OCH_3 group in the former dye (2d) and electron pulling character of the NO_2 group in the latter dye (2e).

Comparison of the antibacterial inhibition of the acyclic merocyanine dyes (2a-e) declared that the dyes (2a, R = H), (2d, R = C_6H_4.p.OCH_3) gives the highest inhibition potency against the bacterial strains. This reflects their increased ability to be used as antibacterial against these bacterial strains. In contrast, the merocyanine dye (2b, R = CH_3) gives the lowest inhibition zone diameter against the bacterial strains, Table (3). This reflects its deficiency to be used as antibacterial against these bacterial strains.

Comparing of the antimicrobial inhibition effect of the acyclic merocyanine dyes (2a-e) showed that these dyes more effective to be used as antibacterial against Escherichia coli strain compared to *staphylococcus aureus* strains, Table (3). This reflects their increased ability to may be used and/or applied as antibacterial active against these bacterial strains.

The antimicrobial inhibition effects of all the acyclic merocyanin dyes (2a-e) gives zero inhibition potency diameter against the fungal strains, Table (3). These reflect their negative effects and their valueless to be used as antimicrobial against these fungal strains.

In addition, the antimicrobial action activity of the acyclic merocyanine dyes (3a,b) showed higher and/or lower bacterial inhibition effects depending upon the kind of the side chain substituted (R), Table (3).

So, substituting R = COOEt by R = $COCH_3$ moving from dye (3a) to dye (3b) makes complete destroying for the inhibition potency diameter against the bacterial strains, Table (3). This may be attributed to the strong electron attracting character of the ethoxy group in the former dye (3a) and the strong electron donating characters of the methyl group in later dye (3b).

Comparing the antibacterial activity of the acyclic merocyanine dye (3a) and the cyclic merocyanine dye (4) declared that the dye (3a) have slight higher potency effects toward staphylococcus aureus bacterial strain, Table (3). This may be attributed to the electron attracting character of the ethoxy group in the former dye (3a).

All the acyclic merocyanine dyes (3a,b) and the cyclic merocyanine dye (4) gives zero inhibition potency diameter

against the fungal strains, Table (3). This reflects their complete negative and deficiency to be used as antimicrobial against these fungal strains.

General comparison the antimicrobial effects of the tested compounds showed that, the merocyanine dyes (2a), (2d) and (3a) gives the highest inhibitor zone diameter against the bacterial strains, Table (3). This reflects their increased effects and/or their higher availability to may be used and/or applied as biological active compounds against these bacterial strains. In contrast the merocyanine dye (3b) gives the lowest inhibition zone diameter against the bacterial strains, Table (3). This indicates its negative effects and/or its non availability to may be used and/or applied as antimicrobial against these bacterial strains.

From the above discussed results we could conclude that, the antimicrobial inhibition zone diameter of the tested merocyanine dyes underwent to give higher and/or lower inhibition potency diameters depending upon:

1- Nature of the acyl group in the acyclic merocyanine dyes (2a-e) in the order of: a-H dye > Ph dye > CH_3 dye.
b-C_6H_4-p.OCH_3 dye > C_6H_4-p.NO_2 dye > CH_3 dye.
2- Nature of the side chain substituted (R) in the acyclic merocyanine dyes (3a, b) in the order of: acyclic COOEt merocyanine dye > acyclic $COCH_3$ merocyanine dye.
3- Class nature of the merocyanine dyes cyclic and/or acyclic in the order of:

a-acyclic COOEt merocyanine dye > cyclic hydantoin merocyanine dye.
b-cyclic hydantoin merocyanine dye > acyclic $COCH_3$ merocyanine dye.

4- Kind of the bacterial strains in the order of: Higher in the case of Escherichia coli compared to *staphylococcus aureus*.
5- Bacterial and/or fungal strains in the order of: Most samples have antibacterial activity but all of them do not have antifungal activity.

CONCLUSION

The electronic visible absorption spectra of the synthesized acyclic and cyclic merocyanine dyes in 95% ethanol solution underwent displacements to give bathochromic shifted (red shifted) and/or hypsochromic shifted (blue shifted) bands accompanied by increasing and/or decreasing the intensity of the absorption bands depending upon the following factors:

a) Presence of electron donating and/or electron attracting groups in the dyes molecules in the order of: electron donating group dyes > electron attracting group dyes.
b) Increasing π-delocalization conjugations the dye molecule in order of: Ph dyes > H dyes.
c) Increasing the number of the basic centers inside the dye molecule, dye (4).

The intensity of the colors of the synthesized acyclic and cyclic merocyanine dyes can be attributed to two suggested mesomeric structures (A) and (B) producing a delocalized positive charge over the conjugated system (Scheme 2).

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