

# Novel Hemicyanine and Aza-Hemicyanine Dyes : Synthesis, Spectral Investigation and Antimicrobial Evaluation

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# Article History

Accepted : 01 March 2012 Published : 30 March 2012 **ABSTRACT** : Novel hemicyanine dyes and aza-hemicyanine dyes having the nucleus of furo[(3,2-d)pyrazolium; ()imidazol] iodide salt were prepared. The electronic visible absorption spectra of all the synthesized hemicyanine dyes and aza-hemicyanine dyes were investigated and in 95% ethanol solution to evaluate their spectral characterization. The antimicrobial effects of some selected dyes were tested against various bacterial and fungal strains (Escherichia coli, Staphylococcus aureus, Aspergillus flavus and Candida albicans) to assess their antimicrobial (bactericidal and fungicidal) properties. The results discussed in this study revealed that both the spectral characterization and the antimicrobial properties of the examined dyes is highly effected by the type of the X substituted in the phenyl ring system for the hemicyanine dyes and by the type of the phenyl and/or the naphthyl ring system for the aza-hemicyanine dyes. Structural confirmations were identified by elemental analysis, visible spectra, IR and 1H NMR spectroscopic data.

**Keywords:** Cyanine Dyes, Hemicyanine Dyes, Synthesis, Absorption Spectra, Antimicrobial Activity, Aza-Hemicyanine Dyes.

# I. INTRODUCTION

Hemicyanine dyes (1-13) are one of the most widely used and important class of functional dyes. They are used as sensitizers and other additives in the photographic industry, chemosensors, indicator dyes, in optical recording media in laser disks, as flexible dyes, in textile industry, laser dyes, as optical sensitizers and in various other fields, for example dye-sensitized solar cells and dyes with non-linear optical properties. The most important applications for these dyes are in bio-labeling and in medicinal analysis. In addition, hemicyanine dyes (14-25) is common fluorescence probe for electrical membrane potential in biochemistry and biophysical area. It is also a very important fluorescence dyes applied in lasers, molecular electronics and nonlinear optical photolimiting devices. Besides, hemicyanine dyes (26-36) have a number of good properties, such as ease of synthesis, they are fluorescent, have higher photostability than the classical cyanine dyes and they can cover the spectrum from the UV to near infrared (NIR) region. So, in this manuscript we prepared different series of hemicyanine and aza-hemicyanine dyes as new synthesis contribution, spectroscopic investigation and antimicriobial evaluation in this field to may be used and/or applied in any of the wide distributed multidisciplinary uses and application of cyanine dyes, and particularly as sensitizers in

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manufacturing technology of photosensitive material industry and/or as batericidals in pharmacology and pharmaceutical industry.

# II. RESULTS AND DISCUSSION

#### 2.1-Synthesis:

Reaction of 3-ethyl-4-methyl-6-oxo-2-phenyl-furo[(3,2-d) pyrazolium, (3, 2–d) imidazole] iodide salts (1) [37] with aromatic aldehyde (benzaldehyde, p.OH benzaldehyde, p.OCH<sub>3</sub> benzaldehyde, p.N(CH<sub>3</sub>)<sub>2</sub> benzaldehyde, p.NO<sub>2</sub> benzaldehyde, p.Cl. benzaldehyde) in equimolar ratios, in ethanol as organic solvent and piperidine as a basic catalyst achieved the 4(1)-hemicyanine dyes (2a-f), Scheme (1), Table (1).

Equimolar reaction of (1) and the nitroso compounds (p-nitroso-phenol,  $\alpha$ -nitroso- $\beta$ -phenyl,  $\beta$ -nitroso- $\alpha$ -naphthol) in ethanol containing few mls of piperidine resulted the 4[2(1)]-aza-hemicyanine dyes (3a-c), Scheme (1), Table (1).

The structure of the prepared compounds was confirmed by elemental analysis, Table (1), visible spectra, Table (1), IR [38] and <sup>1</sup>H NMR [39] spectroscopic data, Table (2).

# 2.2-Spectral investigation:

The electronic visible absorption spectral of the hemicyanine dyes (2a-f) in 95% ethanol solution gives bands in visible region 400-430 nm. These bands underwent displacements to give bathochromic shifts (red shifts) and/or hypsochromic shifts (blue shifts) in addition to increasing and/or decreasing the intensity of the absorption bands depending upon the type of the X substituents in the phenyl ring system, Scheme (1), Table (1).

So, substituting X = H in dye (2a) by X = OH, OCH<sub>3</sub> and/or N(CH<sub>3</sub>)<sub>2</sub> to obtain dyes (2b), (2c) and/or (2d) makes bathochromic shifts for the absorption bands by 5 nm, 10 nm, 15 nm, in addition to increasing for the intensity of the bands, respectively, Scheme (1), Table (1). This can be attributed to the electron pushing characters of the OH, OCH<sub>3</sub> and/or N(CH<sub>3</sub>)<sub>2</sub> groups in the latter dyes, which increase and/or facilitate the intensity of the electronic charge transfer to the quaternary nitrogen atom of the pyrazolium iodide salt (acidic center of the dye) and consequently red shifts occurs for the bands of these latter dyes (2b), (2c), (2d) in correspondence to the former parent dye (2a). Substituting X = H by X= NO<sub>2</sub> and/or Cl moving from dye (2a) to dyes (2e) and/or (2f) causes blue shifts for the absorption bands by 5 nm and/or 15, accompanied by quenching the intensity of these bands, respectively, Scheme (1), Table (1). This can be related to the strong electron pulling characters of the NO<sub>2</sub> group and/or the Cl atom in latter dyes (2e) and/or (2f) which make decreasing for intensity of electronic charge transfer to the quaternary nitrogen of the pyrazolium salt residue (acidic center of the dye), and accordingly hypsochromic shifts occurs in the spectra of the latter dyes (2e) and/or (2f) in correspondence to the parent dye (2a).

Additionally, the electronic visible absorption spectra of the aza-hemicyanine dyes (3a-c) in 95% ethanol solution reveals bands in the visible region 405-420 nm. The positions of these bands and their molar extinction coefficients are largely effected by the type of the phenyl and/or the naphthyl ring system in the dyes molecules, Scheme (1), Table (1).

So, substituting the benzene ring system in dye (3a) by naphthyl ring system to give dyes (3b) and/or (3c) resulted in a noticeable bathochromic shifts for the absorption bands by 9 nm and/or 15 nm in addition to

increasing the intensity of the bands, respectively. This can be attributed to increasing  $\pi$ -delocalization conjugation the latter dyes (3b), (3c) due to the presence of naphthyl ring systems in correspondence to phenyl ring system in the former dye (3a).

It is also, interested to notice that, substituted by X = 2.OH, 5, 6-benz by X = 2.OH, 3,4-benz transferring form dye (3b) to dye (3c) cause bathochromic shifts for the absorption bands by 5 nm, Scheme (1) Table (1). This may be related to the higher planarity of the dye (3c) in correspondence to the lower planarity of the dye (3b).

#### 2.3-Antimicrobial evaluation:

Structural antimicrobial activity relationship for the hemicyanine dyes (2a-f) and the aza-hemicyanine dyes (3a-c) were studied, determined and evaluated against some bacterial and fungal strains (Escherichia coli, Staphylococcus aureus, Aspergillus flavus and Candida albicans), Table (3). From this study it was observed that:

The antimicrobial activity of the hemicyanine dyes (2a-e) undergo to give higher and/or lower inhibition zone diameter against the bacterial strains depending upon the type of the X substituents in the benzene ring of the aromatic aldehyde consisting the dyes structures, Table (3).

So, substituting X = H in dye (2a) by X = p.OH,  $p.OCH_3$ ,  $p.N(CH_3)_2$  and  $p.NO_2$  to get dyes (2b), (2c), (2d), and (2e) makes lowering for the bacterial inhibition zone diameter against the bacterial strains, Table (3). This may be attributed to the presence of electron donating groups (p.OH, p.OCH<sub>3</sub>, p.N(CH<sub>3</sub>) and/or the presence of electron attracting group (p.NO<sub>2</sub>) the latter dyes, respectively, Table (3).

Comparison the antibacterial activity of the dye (2f) X= Cl by their analogous dyes (2b, X = p.OH), (2d, X = p.N(CH<sub>3</sub>)<sub>2</sub>, (2e, X = p.NO<sub>2</sub>) showed that the former dye (2f) have higher biological activity against all the bacterial strains, Table (3). This may be related to the strong electron attracting character of the chlorine atom in the former dye (2f).

Also, it is noticed that, the antibacterial activity of the hemicyanine dye (2c,  $X = p.OCH_3$ ) have higher antimicrobial activity if compared with their analogous dyes (2d,  $X = p.N(CH_3)_2$ ) and (2e,  $X = p.NO_2$ ), Table (3), This may be attributed to the oxygenated methyl group in the former dye (2c).

Comparison the antibacterial activity of the hemicyanine dyes (2a-f) showed that the dye (2a, X = H) gives the highest inhibition zone diameter against all the bacterial strains, Table (3). This reflects its increased ability to may used and/or applied as antimicrobial against these bacterial strains.

The comparison of the antimicrobial activity of the hemicyanine dyes (2a-f) declared that, the dye (2d,  $X = p.N(CH_3)_2$ ) gives the lowest inhibition effect against all the bacterial strains, Table (3). This reflects its deficiency to may be used and/or applied as antibacterial active against these bacterial strains.

Comparing the antimicrobial activity of all the hemicyanine dyes (2a-f) declared that these compounds possesses higher inhibition effect against Escherichia coli bacterial strain compared with staphylococcus aureus bacterial strain, Table (3). This reflects their increased ability to may be used and/or applied as antibacterial against the former bacterial strain.

All the hemicyanine dyes (2a-f) do not have antifungal activity on the tested microorganisms (Aspergillus flavus and Candida albicans) where they give zero inhibition zone diameter potency against these fungal strains, Table (3). This reflects their complete deficiency and their negative effect to may be used and/or applied as antifungal against these fungal strains.

In addition, the antibacterial activity of the aza-hemicyanine dyes (3a-c) undergo to give higher and/or lower inhibition zone diameter against the bacterial strains depending upon the types of the nitroso compounds

consisting the dyes structure. So, substituting X = 4.0H in the dye (3a) by X = 2.0H, 3,4-benz and/or 2.0H, 5,6-benz to get dyes (3b) and/or (3c) caused increasing for the antibacterial action against the bacterial strains, Table (3). This may be attributed to increasing conjugation in the latter (3b) and (3c) dyes due to the presence of the naphthyl ring system in corresponding to phenyl ring system in the former dye (3a).

Comparison the antimicrobial activity of the aza-hemicyanine dye (3b) with their analogues dye (3c) showed that the latter dye (3c) have higher potency diameter towards staphylococcus aureas bacterial strain than the former dye (3b), Table (3). This reflect its increased ability to be used and/or applied as antibacterial against this bacterial strains. This effect may be related to the higher planarity of this dye (3c) compared to the lower planarity of dye (3c).

The antimicrobial activity action of all the aza-hemicyanine dyes (3a-c) showed zero inhibition diameters against the fungal strains, Table (3). This reflects their negative effects and their complete deficiency to be used and/or applied as antimicrobial against these fungal strains.

Replacing the dimethine group (CH=CH) in the hemicyanine dye (2a) by the azamethane group (CH=N) to get the aza-hemicyanine dye (3a) makes increasing for the antimicrobial activity against the staphylococcus aureas bacterial strain, Table (3). This may be related to the effect of the azamethane group (CH=N) in the latter dye (3a).

Comparison the antimicrobial activity of the hemicyanine dyes (2a-f) and the aza-hemicyanine dyes (3a-c) declared that the latter aza-hemicyanine dyes (3a-c) are higher biological active compounds than the former hemicyanine dyes (2a-f) against the bacterial strains, Table (3). This may be related to the presence of the azamethane group (-CH=N-) in the latter aza-hemicyanine dyes (3a-c) in correspondence to the dimethine group (-CH=CH-) in the former hemicyanine dyes (3a-f).

General cmparison the antimicrobial effects of the tested compounds showed that the aza-hemicyanine dye (3c) gives the highest inhibitor zone diameter against the bacterial strains, Table (3). This reflects its increased effects and/or its higher availability to may be used and/or applied as antimicrobial against these bacterial strains. In contrast the hemicyanine dye (2d) gives the the lowest inhibition zone diameter against the bacterial strains, Table (3). This indicates its decreased effects and/or its lower availability to may be used and/or applied as antimicrobial against these bacterial strains, Table (3). This indicates its decreased effects and/or its lower availability to may be used and/or applied as antimicrobial against these bacterial strains.

#### III. CONCLUSION

Following are major conclusions were drawn from this study:

1-The electronic visible absorption spectra of hemicyanine dyes and/or the aza-hemicyanine dyes in 95% ethanol solution underwent displacements to give bathochromic and/or hypsochromic band shifts in addition to increasing and/or decreasing the intensity of the absorption bands depending upon the following factors:

a-Presence of electron releasing and/or attracting groups in the dyes molecules in the order of: electron pushing group dyes > electron pulling group dyes (for the hemicyanine dye).

b-Presence of phenyl and/or naphthyl ring system in the order of: naphthyl dyes > phenyl dyes (for azahemicyanine dyes).

c-Planarity of the dyes in the order of: higher planarity dyes > lower planarity dyes (for aza-hemicyanine dyes). 2-The intensity of the colours of the hemicyanine dyes and/or the aza-hemicyanine dyes can be related to the two suggested mesomeric structures (A) and (B) producing a delocalized positive charge over the conjugated system, Scheme (2).

3-The antimicrobial inhibition zone diameters of the tested hemicyanine dyes (2a-f) and the aza-hemicyanine dyes (3a-c) underwent to give higher and/or lower inhibition potency depending upon the following factors: a-Type of the X substituted in the aromatic aldehyde consisting the dyes structures for the hemicyanine dyes (2a-f) in the order of:

 $i) \quad p.H \; dye > p.OH \; dye > p.N(CH_3)_2 \; dye.$ 

 $ii) \quad p.OCH_3 \ dye > p.NO_2 \ dye > p.OH \ dye > p.N(CH_3)_2 \ dye.$ 

iii)  $p.Cl dye > p.NO_2 dye > p.OH dye > p.N(CH_3)_2 dye.$ 

b-Presence of phenyl and/or naphthyl ring system for aza-hemicyanine dyes in the order of : naphthyl dyes > phenyl dyes (2.OH, 5,6-benz dye > 2.OH, 3,4-benz dye > 4.OH dye)

c-Planarity of the dyes for aza-hemicyanine dyes in the order of: higher planarity dyes > lower planarity dyes (2.OH, 5,6-benz dye > 2.OH, 3,4-benz dye).

d-Kinds of the bacterial strains in the order of: higher in the case of Escherichia coloi bacterial strain compared to the staphylococcus aureas bacterial strain.

e-Bacterial and/or fungal strains in the order of: most samples have antibacterial activity, but all of them do not have any antifungal activity.

# IV. EXPERIMENTAL

#### 4.1- 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. <sup>1</sup>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 were carried out at the Microanalytical center, Microbiology division (Cairo University).

# 4.2- Synthesis:

# 4.2.1-Synthesis of 3-ethyl-6-oxo-2-phenyl-furo [3,2-d) ptrazole, (3,2-d) imidazole]-4-(1)] hemicyanine dyes (2a-f).

Quaternized compound (1) (0.01 mol) and equimolar ratios of (benzaldehyde-4-hydroxy benzaldehyde, 4methoxy benzaldehyde, p.N,N-dimethylaminobenzaldehyde, 4-nitrobenzaldehyde or 4-chlorobenzaldehyde) were refluxed in ethanol (20-30 ml) as solvent containing piperidine (1-2 ml) as catalyst for about 6 hrs. The reaction mixture changed from reddish color to dark brown at the end of the refluxing. It was filtered while hot to remove unreacted materials, cooled and precipitated in ice-water mixture. The hemicyanine dyes (2a-f) were collected, washed with water several times, dried and crystallized from ethanol, Table (1).

4.2.2-Synthesis of 3-ethyl-6-oxo-2-phenyl-furo [3,2-d) ptrazole, (3,2-d) imidazole]-4-(1)] aza-hemicyanine dyes (3a-c).

The quaternized compound (8) (0.01 mol) and equimolar ratios of either 4-nitroso phenol, 1-nitroso-3-naphthol; or 2-nitroso-1-naphthol in ethanol (30 ml)containing piperidine (1-2 ml) were heated under refluxed for 6 hrs. The reaction mixture which attained a deep permanent colour at the end of refluxing was filtered on hot to remove any impurities precipitated using ice-water mixture and dried. The aza-hemicyanine dyes (3a-c) were collected and crystallized using ethanol. See the data given in Table (1).

#### 4.3-Spectral investigation:

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<sup>-3</sup>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.

# 4.4-Antimicrobial evaluation:

The tested compounds (2a, 2b, 2c, 2d, 2e,2f 3a, 3b, 3c) were dissolved in DMSO to give a final concentration (l mgm/ml). Susceptible sterile discs were impregnated by the tested substance (50  $\mu$ gm/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).

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Analysis% Absorption spectra in 95% ethanol Nature of products Comp Molecular Calculated Found Colour vield M.P. formula (M.Wt.) С С Ν . No. н Ν Η λ<sub>max</sub> (nm) (mol<sup>-1</sup>c m<sup>2</sup>) 96 C°° 2a Brown 61 130 53.01 11.24 52.95 11.12 415 17000 C22H18N4O2I 3.81 3.72 (498) 10.89 420 50 120 C22H19N4O3I 51.36 3.69 51.21 3.60 10.77 35000 2ъ Deep brown (514) 3.97 52.16 425 2c45 145 C23H21N4O3I 52.27 10.60 3.80 10.53 24000 Deep brown (528)2d 43 95 C24H24N5O2I 12.93 53.20 430 23000 Deep brown 53.23 4.43 4.32 12.79 (541) 2e Brown 54 135 C22H18N3O4I 48.61 3.31 12.89 48.50 3.12 12.70 410 21000 (543) 2f 35000 Brown 40 120 C22H18N4O2C1I 49.57 3.38 10.51 49.44 3.29 10.46 400 (533) 3a Pale brown 55 100 CatHusNaOaI 48.93 3.49 13.59 48.87 3.38 13.45 405 25000 (515) C25H20N5O3I 3Ъ 55 125 53.09 12.38 53.00 3.42 12.13 414 57700 Deep brown 3.53 (565) 40000 3c 60 155 C25H20N5O3I 53.09 3.53 12.38 52.88 3.41 12.31 420 Deep brown (565)

Table 1: Characterization of the prepared compounds (2a-f), (3a-c)

Comp. No	IR Spectrum (KBr, Cm <sup>-1</sup> )	<sup>1</sup> H NMR Spectrum (DMSO, δ)
2a	691, 648(monosubstituted phenyl). 1025, 1065, 1118, 1164(C-O-C cyclic). 1552, 1496 (C=N). 1597 (C=C). 1715 (C=O). 2924, 2854 (quaternary salt). 3417 (NH).	1.2-1.6 (m, 3H, CH <sub>3</sub> of position 3). 3.3 (b, 2H, CH <sub>2</sub> of position 3). 7.1 (b, 2H, 2NH). 7.3-8.2(m, 12H, aromatic + 2 -CH=).
2b	642, 692(monosubstituted phenyl). 759, 888 (p.disubstituted phenyl). 1152 (C-O-C cyclic). 1496 (C=N). 1594 (C=C). 1712 (C=O). 2939, 2856 (quaternary salt). 3414 (NH).	1.2-1.7 (m, 3H, CH <sub>3</sub> of position 3). 3.4 (b, 2H, CH <sub>2</sub> of position 3). 6.9 (s, 1H, OH). 7.3 (b, 2H, 2NH). 7.4-8.2(m, 11H, aromatic + 2 -CH=).
3a	644, 692 (monosubstituted phenyl). 757, 832 (p.disubstituted phenyl). 1065, 1123, 1163(C-O-C cyclic). 1496, 1546 (C=N). 1597 (C=C). 1714 (C=O). 2933, 2855 (quaternary salt). 3059 (OH). 3420 (NH).	1.3-1.8 (m, 3H, CH <sub>3</sub> of position 3). 3.4 (b, 2H, CH <sub>2</sub> of position 3). 6.9 (b, 1H, OH). 7.2 (b, 2H, 2NH). 7.3-8.4(m, 10H, aromatic+ -CH=).

Table 2. IR and 1H NMR (	mace)	spectral d	lata of	the r	renared	compour	de
Table 2. In and III Mun	(mass)	spectral	lata OI	une p	nepareu	compoun	us

Table 3	The antim	icrobial	activity	of com	spuinds	2a-f 3	3a-c
Table J.		ICIUDIAI	activity	or comp	pounds	Za-1, i	Ja-C

	Inhibition zone diameter (mm/mg sample)							
Sample	Escherichia coli (G <sup>-</sup> )	Staphylococcus aureus (G <sup>+</sup> )	Aspergillus flavus (Fungus)	Candida albicans (Fungus)				
Control DMSO	0.0	0.0	0.0	0.0				
2a	12	11	0.0	0.0				
2b	10	0.0	0.0	0.0				
2c	12	10	0.0	0.0				
2d	9	0.0	0.0	0.0				
2e	10	9	0.0	0.0				
2f	12	10	0.0	0.0				
3a	10	11	0.0	0.0				
3b	15	15	0.0	0.0				
3c	15	17	0.0	0.0				