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# Synthesis, Electronic Transitions and Antimicrobial Activity Evaluation of Novel Monomethine and Trimethine Cyanine Dyes

H.A. Shindy [a, *], M.A. El-Maghraby [a], M.M. Goma [a], N.A. Harb [a]

[a] Department of Chemistry, Faculty of Science, Aswan University, Aswan 81528, Egypt

**Abstract**

New polyheterocyclic starting material compound namely 3, 5-dimethyl-7-phenyl-furo [(3,2-d) pyrazole;(3',2'-d) oxazole] was prepared and oriented for the synthesis of novel monomethine cyanine dyes (simple cyanine dyes), bis monomethine cyanine dyes (bis simple cyanine dyes), trimethine cyanine dyes (carbocyanine dyes) and bis trimethine cyanine dyes (bis carbocyanine dyes). The electronic transitions of all the synthesized cyanine dyes were evaluated and determined through investigating their visible absorption spectra in 95 % ethanol solution. The dyes were thought to be better electronic transitions when they absorb light at higher wavelength bands (bathochromic shifted and/or red shifted dyes). Consequently, the electronic transitions of the dyes decreases when they absorb light at lower wavelength bands (hypsochromic shifted and/or blue shifted dyes). The antimicrobial activity evaluation for a number of 10 (ten) selected compounds was tested against 4 (four) various bacterial strains (Bacillus subtilis, Escherichia coli, Pseudomona aeruginosa and Staphylococcus aureus). The antimicrobial activity evaluation can be made through measuring the inhibition zone diameter of the tested compounds against a number of bacterial and/or fungi strains. The compounds were thought to be better antimicrobial active when they give higher inhibition zone diameter against the tested bacterial and/or the fungi strains. Consequently, the antimicrobial activity of the compounds decrease when they give lower inhibition zone diameter against the tested bacterial and/or the fungi strains. Structural characterization and determination was carried out via elemental analysis, visible, mass, IR and $^1$HNMR spectroscopic data.

**Keywords:** cyanine dyes, methine cyanine dyes, synthesis, electronic transitions, antimicrobial activity.

## 1. Introduction

Cyanine dyes (Shindy, 2012; Shindy, 2016; Shindy, 2017; Shindy, 2018) are important class of functional dyes and possesses an excellent photochemical and photophysical properties, such as high molar extinction coefficients (molar absorptivity), tunable fluorescence intensities, narrow absorption bands, moderate quantum yields and absorb light mainly in the visible region, but also include (cover) UV and NIR regions (larger than any other class of dye system). Therefore, an extensive number of cyanine dyes have been synthesized and developed for numerous applications in photographic processes and more recently as fluorescent probes for bio-molecular labeling and imaging (Mishra et al., 2000; Pisoni et al., 2014; Wada et al., 2015; Hyun et al., 2014; Hyun et al., 2015; Njiojob et al., 2015; Hyun et al., 2015; El-Shishtawy et al., 2010; Henary, Levitz, 2013). On the other side, pyrazole derivatives have great interest in agrochemical, pharmaceutical,

* Corresponding author
E-mail addresses: hashindy2@hotmail.com (H.A. Shindy)

and chemical industries (Keter, Darkwa, 2012; Fustero et al., 2011). In addition, pyrazole derivatives possess a wide range of bioactivities (Fustero et al., 2011; Ouyang et al., 2008), including anti-inflammatory (Gokhan-Kelekci et al., 2007), anticonvulsant (Kaushik et al., 2010), anticancer (Balbia et al., 2011), and antifungal (Vicentini, 2007) behavior. Besides, Oxazole compounds play a fundamental role in the synthesis of numerous biologically active drugs such as analgesics, antiinflammatory, antimicrobial, anticancer, antidepressants, antidiabetic and antiobesity. Spirocyclopropyl oxazolones is the novel class of inhibitor of herpes protease. Phenacyl oxazolone involves the intermolecular Diels-Alder reaction, ensuing in synthesis of anti-cancer drugs, pancratistantin and a phenanthrene alkaloid (Sweta et al., 2017; Turchi, 1986). Based on this concepts, In this research paper we prepared new pyrazolo/oxazole cyanine dyes as new synthesis contribution, spectroscopic investigation and antimicrobial evaluation in the field and with the hope that a combination of the favourable properties of both pyrazole, oxazole and cyanine dyes may be achieved.

## 2. Results and discussion

### 2.1. Synthesis

An equimolar ratios of 4-bromo-3-methyl-1-phenyl-5-pyrazolone (1) and 2-methyl-oxazole-5-one (2) were reacted in pyridine and achieved 3, 5-dimethyl-7-phenyl-furo[(3,2-d)pyrazole; (3',2'-d) oxazole] (3) as new polyheterocyclic starting material compound, Scheme (1).

Quaternization of (3) using an excess of iodoethane led to the formation of 4,6-diethyl-3,5-dimethyl-7-phenyl-furo[(3,2-d) pyrazolium;(3',2'-d)oxazolium] diiodide quaternary salt compound (4), Scheme (1), Table 1.

Reaction of the quaternary salt compound (4) with an iodoethane quaternary salts of either pyridine, quinoline or isoquinoline in equimolar ratios and in ethanol containing few drops of piperidine gave the 4,6-diethyl-5-methyl-7-phenyl-furo[(3,2-d)pyra- zolium; (3', 2'-d)oxazole]-3[4(1)]-monomethine cyanine dyes (5a-c), Scheme (1), Table 1.

Reaction of unimolar ratios of compound (4) with bimolar ratios of iodoethane quaternary salts of pyridine, quinoline or isoquinoline in ethanol containing piperidine yielded the 4,6-diethyl-7-phenyl-furo[(3,2-d)pyrazole;(3',2'-d)oxazole]-3,5[4(1)]-bis monomethine cyanine dyes (6a-c), Route (1), Scheme (1), Table 1.

Chemical confirmations for compounds (6a-c) took place via reactions of the previously prepared monomethine cyanine dyes (5a-c) with equimolar ratios of iodoethane quaternary salts of either pyridine, quinoline or isoquinoline in ethanol catalyzed by piperidine through Route (2) to achieve the same bis monomethine cyanine dyes (6a-c) obtained through route 1, characterized by melting points, mixed melting points, same visible, IR and $^1$H-NMR spectral data, Scheme (1), Route (2), Table 1.

Reaction of the bis quaternized compound (4) with a bimolar ratios of triethylorthoformate in ethanol containing piperidine and led to the formation of the intermediate compound 4,6-diethyl-7-phenyl-furo[(3,2-d) pyrazolium; (3',2'-d) oxazolium]-3,5-bis(1,1'-diethoxy)ethyl-diiodide quaternary salt (7), Scheme (1), Table 2.

This intermediate compound (7) was then reacted with equimolar or bimolar ratios of N-ethyl (2-picolinium, quinaldinium, 4-picolinium) iodide quaternary salts in ethanol containing piperidine as a basic catalyst to give the 4,6-diethyl-5(1,1'-diethoxy)ethyl-7-phenyl-furo[(3,2-d)pyrazolium;(3',2'-d)oxazole]-3[2(4)]-trimethine cyanine dyes (8a-c) or 4,6-diethyl-7-phenyl-furo[(3,2-d) pyrazole;(3',2'-d)oxazole]-3,5[2(4)]-bis trimethine cyanine dyes (9a-c) through, Route (1), Scheme (1), Table 2, respectively.

Chemical confirmations for the bis trimethine cyanine dyes (9a-c) took place through the reaction of the previously prepared trimethine cyanine dyes (8a-c) with equimolar ratios of N-ethyl (2-picolinium, quinaldinium, 4-picolinium) iodide quaternary salts in ethanol catalyzed by piperidine through, Route (2), to achieve the same bis trimethine cyanine dyes (9a-c) obtained through Route (1), characterized by the same melting points, mixed melting points, the same visible, IR and $^1$H-NMR spectra, Scheme (1), Route (2), Table 2.

The structure of the prepared compound was characterized and identified by elemental analysis Tables 1 and 2, visible spectra Tables 1 and 2, Mass spectrometer, IR (Wade, 1999) and $^1$H-NMR (Wade, 1999a) spectroscopic data, Table 3.

### 2.2. Electronic transitions evaluation

Visible electronic transitions for all the synthesized cyanine dyes was determined through investigating their electronic visible absorption spectra in 95 % ethanol solution. The dyes were thought to be better electronic transitions when they absorb light at higher wavelength bands (bathochromic shifted and/or red shifted dyes). Consequently, the electronic transitions of the dyes decreases when they absorb light at lower wavelength bands (hypsochromic shifted and/or blue shifted dyes). So, we may say that the electronic transitions of one dye is higher than the other one if the wavelength of the maximum absorption spectrum of the former one is longer than that of the latter one. In contrary, we may say that the electronic transitions of one dye is lower than the other one if the wavelength of the maximum absorption spectrum of the former one is shorter than that of the latter one (Shindy, 2018).

The visible electronic transitions absorption spectra of the monomethine cyanine dyes (5a-c) and the bis monomethine cyanine dyes (6a-c) in 95 % ethanol solution discloses bands in the visible region 385-540 nm and 390-550 nm, respectively. The positions of these bands and their molar extinction coefficient (molar absorptivity) are largely influenced by the nature of the heterocyclic quaternary residue (A), their linkage positions and by the number of the electronic charge transfer pathways inside the dyes molecules.

So, substituting A=1-ethyl pyridinium-4-yl salts in the monomethine cyanine dye 5a and in the bis monomethine cyanine dye 6a by A=1-ethyl quinolinium-4-yl salts to get the monomethine cyanine dye 5b and the bis monomethine cyanine dye 6b causes strong bathochromic shifts by 20 nm, accompanied by increasing the number and intensity of the absorption bands in the case of the bis monomethine cyanine dye 6b, Scheme (1), Table 1. This can be attributed to increasing π-delocalization conjugation in the latter dyes due to the presence of quinoline ring system in correspondance to the pyridine ring system in the former dyes.

Changing the linkage positions from 1-ethyl quinolinium-4-yl salts to 2-ethyl isoquinolinium-1-yl salts passing from the monomethine cyanine dye 5b and the bis monomethine cyanine dye 6b to the monomethine cyanine dye 5c and the bis monomethine cyanine dye 6c resulted in a remarkable blue shifts by 10 nm accompanied by decreasing the number and intensity of the absorption bands, Scheme (1), Table 1. This can be explained in the light of decreasing the length of the π-delocalization conjugation in the latter 2-ethyl isoquinolinium-1-yl salts dyes 5c and 6c compared to the former 1-ethyl quinolinium-4-yl salts dyes 5b and 6b.

Comparing the electronic visible absorption spectra of monomethine cyanine dyes (5a-c) with those of the bis monomethine cyanine dyes (6a-c) declared that the latter dyes have bathochromically shifted bands related to the former ones. This can be attributed to the presence of two factors. The first factor is the presence of two electronic charge transfer pathways inside the latter dyes molecules in correspondance to one electronic charge transfer pathways inside the former dyes molecules, Scheme (2). The second factor is increasing conjugation due to increasing the number of methine units in bis monomethine cyanine dyes (6a-c) related to the former monomethine cyanine dyes (5a-c) by one methine unit. Scheme (1), Table 1.

Additionally, the visible electronic transitions absorption spectra of the trimethine cyanine dyes (8a-c) and the bis trimethine cyanine dyes (9a-c) in 95 % ethanol solution discloses bands in the visible region 440-650 nm and 440-660 nm, respectively. The positions of these bands and their molar extinction coefficient are largely influenced by the nature of the heterocyclic quaternary residue (A), their linkage positions and by the number of the electronic charge transfer pathways inside the dyes molecules.

So, substituting A=1-ethyl pyridinium-2-yl salts in the trimethine cyanine dye 8a and in the bis trimethine cyanine dye 9a by A=1-ethyl quinolinium-2-yl salts to get the trimethine cyanine dye 8b and the bis trimethine cyanine dye 9b causes strong bathochromic shifts by 20 nm, accompanied by increasing the number and intensity of the absorption bands in the case of the bis trimethine cyanine dyes 9b, Scheme (1), Table 2. This can be attributed to increasing π-delocalization conjugation in the latter dyes due to the presence of quinaldinium structure system ring system in correspondance to the presence of α-picolinium structure system in the former dyes.

Changing the linkage positions from 2-yl salts to 4-yl salts passing from the trimethine cyanine dye 8a and the bis trimethine cyanine dye 9a to the trimethine cyanine dye 8c and the bis trimethine cyanine dye 9c resulted in a remarkable red shifts by 10 nm accompanied by increasing the number and intensity of the absorption bands, Scheme (1), Table 2. This can be explained in

the light of increasing the length of the π-delocalization conjugation in the latter 4-yl salts dyes 8c and 9c due to the presence of the γ-picolinium structure system compared to the former 2-yl salts dyes 8a and 9a which contain the α-picolinium structure system.

Comparing the visible electronic transitions absorption spectra of trimethine cyanine dyes (8a-c) with those of the bis trimethine cyanine dyes (9a-c) declared that the latter dyes have bathochromically shifted bands related to the former ones. This can be attributed to the presence of two factors. The first factor is related to the presence of two electronic charge transfer pathways inside the latter dyes molecules in correspondance to one electronic charge transfer pathways inside the former dyes molecules, Scheme (2). The second factor is attributed to increasing conjugation due to increasing the number of methine groups in the later dyes than that of the former dyes by three methine units, Scheme (1), Table 2.

Comparison the electronic visible absorption spectra of the monomethine cyanine dye (5a-c) with those of the trimethine cyanine dyes (8a-c) reveals that the later dyes have bathochromic shifted and intensified bands than that of the former dyes. This can be related to increasing conjugation due to increasing the number of methine groups between the basic center (nitrogen atom) and the acidic center (quaternary salt) in latter dyes by two methine units, Scheme (1), Tables 1 and 2.

Comparison the electronic visible absorption spectra of the bis trimethine cyanine dyes (9a-c) with those of the bis monomethine cyanine dyes (6a-c) showed that the fomer bis trimethine cyanine dyes (9a-c) have red shifted and intensified absorption bands in comparison to the latter bis monomethine cyanine dyes (6a-c), Tables 1 and 2. This can be attributed to increasing conjugation due to increasing the number of methine units in the former bis trimethine cyanine dyes (9a-c) by four methine units, Scheme (1), Tables 1 and 2.

### 2.3. Antimicrobial activity evaluation

Antimicrobial activity evaluation of cyanine dyes can be made through measuring their inhibition zone diameter against a number of bacterial and/or fungi strains. The cyanine dyes were thought to be better antimicrobial active when they give higher inhibition zone diameter against the tested bacterial and/or the fungi strains. Consequently, the antimicrobial activity of the cyanine dyes decrease when they give lower inhibition zone diameter against the tested bacterial and/or the fungi strains. So, we may say that the antimicrobial activity of one cyanine dye is stronger than the other one if the inhibition zone diameter against the tested bacterial and/or the fungi strains of the former one is higher than that of the latter one. In contrary, we may say that the antimicrobial activity of one cyanine dye is weaker than the other one if the inhibition zone diameter against the tested bacterial and/or the fungi strains of the former one is lower than that of the latter one.

Studying the antimicrobial activity evaluation against a number of bacterial and/or fungi strains bears to have a great practical value and very important in the case of cyanine dyes because the extensive uses and applications of these dyes as bactericidal (anti-bacterial strains) and/or as fungicidal (anti-fungi strains) in pharmaceutical (pharmacological) industry and/or in pharmacochemistry.

So, in this study, the antimicrobial activity evaluation for a number of 10 (ten) selected newly synthesized compounds (3, 4, 5b, 6a, 6b, 6c, 7, 9a, 9b, 9c) were studied and determined against a number of 4 (four) bacterial strains (Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus), Table 4. According to this study, it was observed that:

Comparing the antimicrobial activity of compound (3) and its iodoethane quaternary salt (4), showed that, the latter compound (4) have higher effect to destroy the bacterial strains Table (4). This may be related to increasing the electron attracting character of compound (4) due to quaternization, Scheme (1).

The bacterial inhibition effects of the compounds (3) and (4) have a remarkable decrease if compared by their derived bis monomethine cyanine dyes (6a-c), Table 4. This could be related to the cyanine dyes structure effects in these compounds, Scheme (1).

Comparison between the antimicrobial activity of the bis monomethine cyanine dyes (6a-c) and the bis trimethine cyanine dyes (9a-c) showed that, the latter dyes possess lower potency as antimicrobial activity than the former ones Table 4. This might be correlated to increasing number of methine groups in the latter dyes (9a-c) by four methine units, Scheme (1).

Changing the quaternary salts in the bis monomethine cyanine dyes (6a-c) from 1-ethyl pyridinnium-4-yl salt in dye (6a) to 1-ethyl quinolinium-4-yl salt and/or 2-ethyl isoquinolinium-1-

yl salt to get dyes (6b) and / or (6c), discloses that the latter dyes have lower antimicrobial effects on the bacterial strains, Table 4. This might be attributed to increasing π-delocallization conjugation in the latter dyes (6b), (6c) due to the presence of quinoline and / or isoquinoline ring system in correspondance to pyridine ring system in the former dye (6a), Scheme (1).

Changing the type of the quaternary heterocyclic residue and / or their linkage position in the bis trimethine 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, Table (4). This could be related to increasing π-delocalization conjugation in the latter dyes (9b) and / or (9c) due to the presence of quinaldinium and / or γ-picolinium nucleus in correspondance to α-picolinium nucleus in the former dye (9a), Scheme (1).

Comparison the antibacterial activity of the monomethine cyanine dye (5b) with the bis monomethine cyanine dye (6b) showed that the latter one have higher inhibition zone diameter against Staphylococcus aureus and Escherichia coli bacterial strains, Table (4). This reflects its increased ability to may be used and / or applied as antimicrobial active compound against these two bacterial strains. This effect may be attributed to increasing either of the number of methine units in the bis monomethine cyanine dye (6b) by one methine unit compared with the monomethine cyanine dye (5b), Scheme (1) and / or increasing the number of the electronic charge transfer pathways inside the dye (6b) which contain two electronic charge transfer pathways compared with dye (5b) which contain one electronic charge transfer pathways in its structure, Scheme (2).

Comparing the antimicrobial activity of the bis monomethine cyanine dye (6b) with their analogous (6c) declared that the latter dye (6c) possesses higher potency effect against all the bacterial strains, Table (4). This may be related to the presence of the isoquinoline nucleus in the latter dye (6c) in correspondance to quinoline nucleus in the former dye (6b), Scheme (1).

The antimicrobial activity of the bis trimethine cyanine dye (9b) showed higher inhibition zome diameter against Staphylococcus aureus compaired with their analogous bis trimethine cyanine dye (9c) Table (4). This reflect its increased ability to may be used and / or applied as antimicrobial active against this bacterial strain. This effect may be related to the presence of quinaldinium ring system in dye (9b) compared to γ-picolinium ring system in dye (9c), Scheme (1).

General comparison the antimicrobial activity of the bis trimethine cyanine dyes (9a-c) declared that these dyes possesses higher potency effect against the Escherichia coli and Pseudomonas aeruginosa bacterial strains compared with Bacillus subtilis and Staphylococcus aureus bacterial strains, Table 4. This reflects their increased activity to may be used and / or applied as antimicrobial active compounds against the former bacterial strains.

Comparison the antimicrobial activity of the compounds (3) and / or (4) with their derived bis trimethine cyanine dyes (9a-c) displayed that the latter dyes compounds (9a-c) have higher potency effects for most of the bacterial strains, Table 4. This could be attributed to the cyanine dyes structure effects in the latter dyes (9a-c), Scheme (1).

Substituting the 3 and 5 di methyl groups in compound (4) by 3 and 5 di $CH_2CH(OEt)_2$ groups to get compound (7) make decreasing for the inhibition effects for all the bacterial strains, Table 4. This could be attributed to the strong electron pulling character of the diethoxy group in compound (7) in correspondance to the electron pushing character of $CH_3$ in compound (4), Scheme (1).

Converting the intermediate compounds (7) to its derived bis trimethine cyanine dyes (9a-c) makes increasing for the antimicrobial inhibition zone diameter for all the bacterial strains, Table (4). This could be related to the cyanine dyes structure effects in these compounds, Scheme (1).

General comparison of the antimicrobial activity for all the tested compounds disclosed that, the bis monomethine cyanine dyes (6a) gives the highest inhibition zone diameter against all the bacterial strains, Table 4. This reflect its strong and increased effect to may used and/or applied as antimicrobial active against these bacterial strains.

General comparison of the antimicrobial activity for all the tested compounds revealed that, the intermediate compound (7) gives the lowest inhibition zone diameter against all the bacterial strains, Table 4. This indicate its negative (zero) effects and/or its non availability to may used and/or applied as antimicrobial active against these bacterial strains.

## 3. Conclusion

From the above discussed results we could conclude that:

1. The electronic visible absorption spectra of the monomethine (5a-c), bis monomethine (6a-c), trimethine (8a-c) and bis trimethine (9a-c) cyanine dyes in 95 % ethanol solution underwent displacements to give bathochromic and/or hypsochromic shifted bands depending upon the following factors:

A) The nature of the heterocyclic quaternary salt residue in the order of:

i) Quinolinium dyes > pyridinium dyes (in the monomethine and bis monomethine cyanine dyes).

ii) Quinaldinium dyes > α-picolinium dyes (in the trimethine and bis trimethine cyanine dyes).

B) Linkage position of the heterocyclic quaternary salt residue in the order of:

i) quinolinium dyes > isoquinolinium dyes (in the monomethine and bis monomethine cyanine dyes).

ii) γ-picolinium dyes > α-picolinium dyes (in the trimethine and bis trimethine cyanine dyes).

C) The number of the methine units and / or groups between the two heterocyclic ring system of the cyanine dyes molecules in the order of:

i) trimethine cyanine dyes > monomethine cyanine dyes.

ii) bis trimethine cyanine dyes > bis monomethine cyanine dyes.

D) The number of the electronic charge transfer pathways inside the dyes molecules in the order of: two electronic charge transfer pathways dyes > one electronic charge transfer pathways dyes (bis monomethine cyanine dyes > monomethine cyanine dyes; bis trimethine cyanine dyes > trimethine cyanine dyes).

2. The intensity of the colours of the monomethine cyanine dyes, bis monomethine cyanine dyes, trimethine cyanine dyes and bis trimethine cyanine dyes are illustrated according to the following suggested two mesomeric electronic transitions structures (A) and (B) producing a delocalized positive charges over the conjugated chromophoric group system of the dyes, Scheme (2).

3. The antimicrobial inhibition action activity of the synthesized compounds (3, 4, 5b, 6a, 6b, 6c, 7, 9a, 9b, 9c) increase and/or decrease to give higher and/or lower bacterial inhibition zone diameter depending upon the following factors:

a – Types of cyanine dyes molecules (monomethine, bis monomethine, bis trimethine cyanine dyes).

b – Nature of the heterocyclic quaternary salt residue (A). (pyridinium and/or quinolininium salt residue; α-picolinium and/or quinaldinium salt residue).

c – Linkage positions of the heterocyclic quaternary salt residue (A) (quinolinium and/or isoquinolinium salt residue; α-picolinium and/or γ-picolinium salt residue).

f – Kind of the bacterial strains: higher in the case Escherichia coli and Pseudomonas aeruginosa bacterial strains and lower in the case Bacillus subtilis and Staphylococcus aureus bacterial strains (in the case of the bis trimethine cyanine dyes 9a-c).

## 4. 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 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 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 screening were carried out at the Microanalytical center, Microbiology division (Cairo University).

**4.2. Synthesis**

**4.2-1. Synthesis of 3, 5-dimethyl-7-phenyl-furo[(3,2-d)pyrazole;(3',2'-d)oxazole] (3)**

Equimolar ratios of 4-bromo-3-methyl-1-phenyl-5-pyrazolone (1) (0.01 mol, 2.5 gm) and 2-methyl-oxazole-5-one (2) (0.01 mol, 1 gm) were dissolved in pyridine (50 ml). The reaction mixture was heated under reflux for (6-8 hrs) until the mixture attained brown colour. It was filtered off while hot to remove any impurities, concentrated, then poured in ice water mixture with continuous shaking. The precipitated compound was filtered, washed with cold water, air dried, collected and crystallized from ethanol. The data are reported in Table 1.

**4.2-2. Synthesis of 4,6-diethyl-3,5-dimethyl-7-phenyl-furo[(3,2-d)pyrazolium; (3',2'-d)oxazolium]diiodide quaternary salt (4)**

Apure crystallized sample of (3) (0.04 mol, 1.2 gm) was suspended in excess of iodoethane (30 ml) and heated gently under reflux at low temperature (40-60°C) for 1hr. The solvent was evaporated and the residue was collected and crystallized from ethanol. See data in Table 1.

**4.2-3. Synthesis of 4,6-diethyl-5-methyl-7-phenyl-furo[(3,2-d)pyrazolium;(3',2'-d)oxazole]-3[4(1)]-monomethine cyanine dyes (5a-c)**

A mixture of compound (4) (0.01 mol, 0.8 gm) and iodoethane quaternary salts (0.01 mol) of pyridine (0.35 gm), quinoline (0.4 gm), or isoquinoline (0.4 gm) was refluxed in ethanol (50 ml) containing piperidine (3-5 drops) for 6-8 hrs. 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 precipitated products were collected and crystallized from ethanol. The relevant data are given in Table 1.

**4.2-4. Synthesis of 4,6-diethyl-7-phenyl-furo[(3,2-d)pyrazole;(3',2'-d)oxazole]-3,5[4(1)]-bis monomethine cyanine dyes (6a-c)**

Two different routes are employed to prepare these cyanine dyes:

**Route (1):** Piperidine (3-5 drops) was added to an ethanolic solution (50 ml) of (4) (0.01 mol, 0.8 gm) and iodoethane quaternary salts (0.02 mol) of pyridine (0.7 gm), quinoline (0.8 gm), isoquinoline (0.8 gm). The mixture was heated under reflux for 6-8 hrs, where its colour changed from brown to red during the refluxing time. It was filtered off while hot, concentrated to half its volume and cooled. The precipitated dyes were filtered, washed with water, air dried and crystallized from ethanol. The data are given in Table 1.

**Route (2)**: The previously prepared monomethine cyanine dyes (5a-c) (0.01 mol, 0.7 gm for 5a, 0.7 gm for 5b, 0.7 gm for 5c) and iodoethane quaternary salts (0.01 mol) of pyridine (0.2 gm), quinoline (0.3 gm), isoquinoline (0.3 gm) were dissolved in ethanol (50 ml), to which piperidine (3-5 drops) was added. The reaction mixture was heated under reflux for 3-5 hrs and attained reddish brown colours at the end of the refluxing time. 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 to give the same dyes obtained by route (1), characterized by melting points, mixed melting points, same visible, IR and $^1$H-NMR spectral data, Table 1.

**4.2-5. Synthesis of 4,6-diethyl-7-phenyl-furo[(3,2-d)pyrazolium; (3',2'-d)oxazolium]-3,5-bis(1,1'-diethoxy)ethyl-diiodide quaternary salt (7) as intermediate compound**

This intermediate compound (7) was synthesized by refluxing of the quaternary salt compound (4) (0.04 mol, 2.4 gm) with triethylorthoformate (0.08 mol, 1.6 ml) in ethanol (50 ml) and presence of piperidine (3-5 drops) for 3-5 hrs. The dark brown mixture was filtered on hot to remove any impurities, concentrated and precipitated by cold water. The separated intermediate compound was filtered, washed with water and crystallized from ethanol. The results are registered in Table 2.

**4.2-6. Synthesis of 4,6-diethyl-5(1,1'-diethoxy)ethyl-7-phenyl-furo[(3,2-d)pyrazolium; (3',2'-d)oxazole]-3[2(4)]-trimethine cyanine dyes (8a-c):**

A mixture of the intermediate compounds (7) (0.01 mol, 0.8 gm) and N-ethyl α-picolinium iodide quaternary salt (0.01 mol, 0.25 gm), N-ethyl quinaldinium iodide quaternary salt (0.01 mol, 0.3 gm) or N-ethyl γ-picolinium iodide quaternary salt (0.01 mol, 0.25 gm) were heated under reflux in ethanol (50 ml) containing piperidine (3-5 drops) for 6-8 hrs. The colour of the reaction mixture attained red (for 8a), violet (for 8b) and deep red (for 8c) at the end of the refluxing time. 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 2.

**4.2-7. Synthesis of 4,6-diethyl-7-phenyl-furo[(3,2-d) pyrazole;(3',2'-d)oxazole]-3,5[2(4)]-bis trimethine cyanine dyes (9a-c)**

Two different routes are employed to prepare these cyanine dyes:

**Route (1):** was carried out by adding piperidine (3-5 drops) to a mixture of an ethanolic solution (50 ml) of the intermediate compound (7) (0.01 mol, 0.8 gm) and N-ethyl α-picolinium iodide quaternary salt (0.02 mol, 0.5 gm), N-ethyl quinaldinium iodide quaternary salt (0.02 mol, 0.6 gm) or N-ethyl γ-picolinium iodide quaternary salt (0.02 mol, 0.5 gm). The reaction mixture was heated under reflux for 6-8 hrs. The colour of the reaction mixture attained deep red (for 9a), deep violet (for 9b) and deep red (for 9c) at the end of the refluxing time. It was filtered off while hot, concentrated and precipitated by adding cold water. The separated cyanines were filtered, washed with cold water, air dried and crystallized from ethanol. The results are listed in Table 2.

**Route (2)**: was accomplished through the reaction between equimolar ratios of the previously prepared trimethine cyanine dyes (8a-c) (0.01 mol) (8a, 0.8 gm), (8b, 0.9 gm), (8c, 0.8gm) and iodoethane quaternary salts (0.01 mol) of α-picoline (0.25 gm), quinaldine (0.03 gm) and γ-picoline (0.25 gm) in ethanol (50 ml) and presence of piperidine (3-5 drops). The reacting materials were refluxed for 6-8 hrs, wherever, it attained a reddish brown with α-picoline and γ-picoline and deep violet colour with quinaldine at the end of the reflux. They were filtered, while hot, concentrated, cooled and precipitated by adding cold water. The precipitates were collected and crystallized from ethanol to give the same dyes obtained by route (1), characterized by melting points, mixed melting points, same visible, IR and $^1$H-NMR spectral data, Table 2.

**4.3. Spectral Behavior**

The electronic visible absorption spectra of the prepared cyanine dyes were examined in 95 % ethanol solution and recorded using 1Cm Qz cell in visible spectrophotometer 24 RS Labomed, INC. A stock solution ($1x10^{-3}$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.

**4.4. Antimicrobial Activity evaluation**

The tested compounds (3, 4, 5b, 6a, 6b, 6c, 7, 9a, 9b, 9c) were dissolved in DMSO to give a final concentration (1 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 antimicrobial effect are shown in Table 4.

**5. Conflict of interest**

There is no conflict of interest.

**6. Acknowledgements**

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# Appendix

Table 1:Characterization of the prepared compounds 3, 4, (5a-c) and (6a-c)

| Comp No. | Nature of products | | | Molecular formula (M.Wt) | Analysis% | | | | | | Absorption spectra in 95%ethanol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Colour | yield % | MP C° | | Calculated | | | Found | | | $\lambda_{max}$(nm) | $\varepsilon_{max}$ ($mol^{-1}.cm^2$) |
| | | | | | C | H | N | C | H | N | | |
| 3 | Brown crystals | 40 | 230 | $C_{14}H_{11}N_3O_2$(253) | 66.4 | 4.35 | 16.6 | 66.22 | 4.28 | 16.55 | ..... | ..... |
| 4 | Dark brown crystal | 57 | 160 | $C_{18}H_{21}N_3O_2I_2$(565) | 38.2 | 3.72 | 7.43 | 38.18 | 3.7 | 7.41 | ..... | ..... |
| 5a | Red | 50 | 155 | $C_{25}H_{28}N_4O_2I_2$(670) | 44.78 | 4.18 | 8.36 | 44.75 | 4.15 | 8.32 | 385, 520 | 12820, 5750 |
| 5b | Red | 55 | 165 | $C_{29}H_{30}N_4O_2I_2$(720) | 48.33 | 4.17 | 7.78 | 48.3 | 4.15 | 7.77 | 410, 540 | 11030, 3690 |
| 5c | Red | 65 | 175 | $C_{29}H_{30}N_4O_2I_2$(720) | 48.33 | 4.17 | 7.78 | 48.29 | 4.13 | 7.75 | 395, 530 | 10670, 5840 |
| 6a | Deep red | 55 | 160 | $C_{32}H_{35}N_5O_2I_2$(775) | 49.55 | 4.52 | 9.03 | 49.52 | 4.5 | 9.01 | 390, 530 | 13510, 6360 |
| 6b | Deep red | 57 | 170 | $C_{40}H_{39}N_5O_2I_2$(875) | 54.86 | 4.46 | 8 | 54.84 | 4.43 | 7.98 | 420, 550 | 12400, 6380 |
| 6c | Deep red | 59 | 162 | $C_{40}H_{39}N_5O_2I_2$(875) | 54.86 | 4.46 | 8 | 54.83 | 4.41 | 7.96 | 400, 540 | 14600, 6350 |

Table 2:Characterization of the prepared compounds 7, (8a-c) and (9a-c).

| Comp No. | Nature of products | | | Molecular formula (M.Wt) | Analysis% | | | | | | Absorption spectra in 95% ethanol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Colour | yield % | MP C° | | Calculated | | | Found | | | $\lambda_{max}$(nm) | $\varepsilon_{max}$ ($mol^{-1}.cm^2$) |
| | | | | | C | H | N | C | H | N | | |
| 7 | Deep brown | 75 | 200 | $C_{28}H_{41}N_3O_6I_2$(769) | 43.69 | 5.33 | 5.46 | 43.65 | 5.31 | 5.43 | ..... | ..... |
| 8a | Red | 45 | 175 | $C_{32}H_{40}N_4O_4I_2$(798) | 48.12 | 5.01 | 7.02 | 48.9 | 5 | 7.01 | 440, 570, 630 | 13770, 7140, 2940 |
| 8b | Violet | 57 | 185 | $C_{36}H_{42}N_4O_4I_2$(848) | 50.94 | 4.95 | 6.6 | 50.91 | 4.91 | 6.57 | 450, 520, 650 | 15730, 19470, 2400 |
| 8c | Deep red | 50 | 180 | $C_{32}H_{40}N_4O_4I_2$(798) | 48.12 | 5.01 | 7.02 | 48.9 | 5 | 7 | 450, 490, 580, 640 | 12720, 12740, 7070, 3140 |
| 9a | Deep red | 49 | 177 | $C_{36}H_{39}N_5O_2I_2$(827) | 52.24 | 4.72 | 8.47 | 52.21 | 4.69 | 8.43 | 440, 490, 580, 640 | 14280, 15040, 7330, 3630 |
| 9b | Deep violet | 61 | 195 | $C_{44}H_{43}N_5O_2I_2$(927) | 56.96 | 4.64 | 7.55 | 56.91 | 4.61 | 7.52 | 470, 500, 520, 560, 660 | 20860, 22150, 22600, 23600, 6000 |
| 9c | Deep red | 52 | 185 | $C_{36}H_{39}N_5O_2I_2$(827) | 52.24 | 4.72 | 8.47 | 52.22 | 4.69 | 8.43 | 450, 490, 590, 650 | 13830, 12120, 7060, 4000 |

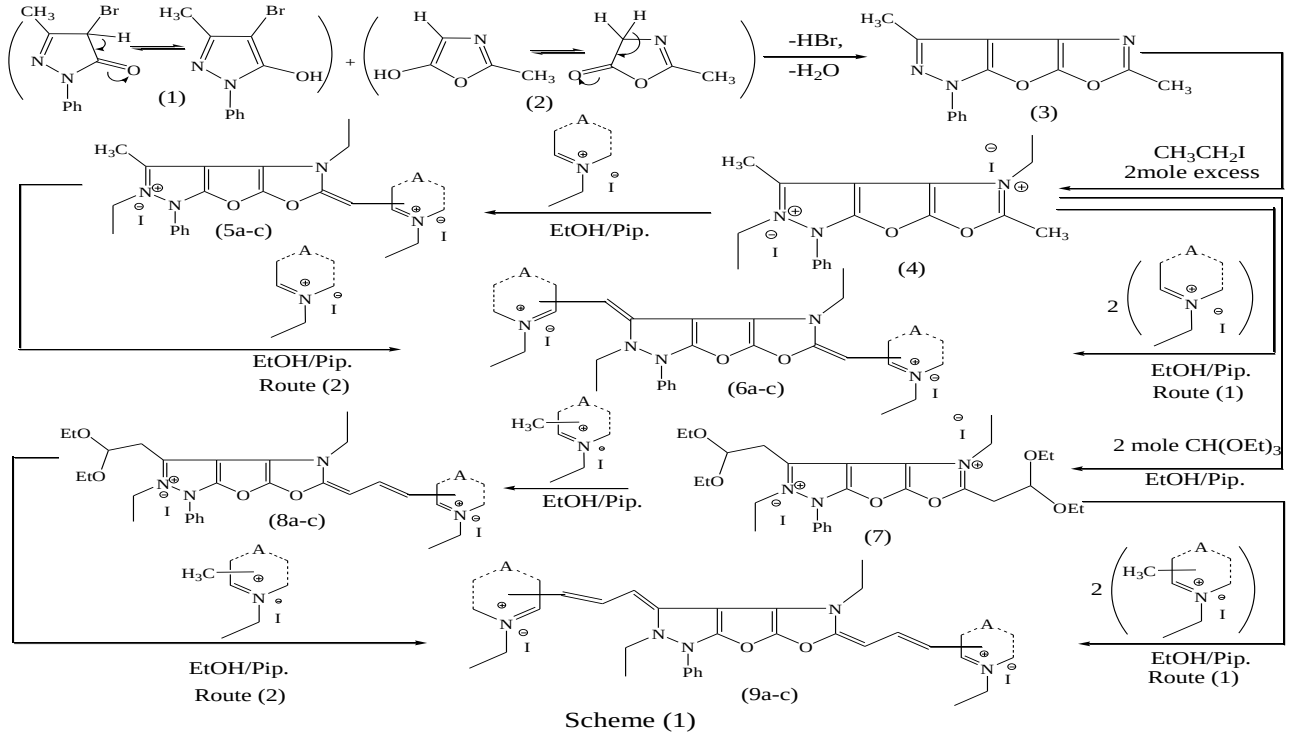
**Table 3.** IR and $^1$H NMR (Mass) Spectral Data of the Prepared Compounds

| Comp. No. | IR Spectrum (KBr, $Cm^{-1}$) | $^1$H NMR Spectrum (DMSO, δ); & (Mass data). |
|---|---|---|
| 3 | 695, 753 (monosubstituted phenyl).<br>1030, 1116 (C—O—C cyclic).<br>1303, 1365 (C—N).<br>1493, 1404 (C═N).<br>1603 (C═C). | 2.2-2.4 (m, 3H, $CH_3$ of position 5).<br>3.4 (b, 3H, $CH_3$ of position 3).<br>7.2-8 (m, 5H, aromatic).<br>$M^+$: 253 |
| 4 | 693, 753 (monosubstituted phenyl).<br>1116 (C—O—C cyclic).<br>1308 (C—N).<br>1495, 1405 (C═N).<br>1604 (C═C).<br>2917 (quaternary salt). | 1.2-1.6 (m, 6H, $2CH_3$ of positions 4, 6).<br>1.8-2.1 (m, 4H, $2CH_2$ of positions 4, 6).<br>2.2-2.4 (m, 3H, $CH_3$ of position 5).<br>3.28 (s, 3H, $CH_3$ of position 3).<br>7.2-8 (m, 5H, aromatic).<br>$M^+$: 565 |

| | | |
|---|---|---|
| 5b | 691, 756 (monosubstituted phenyl).<br>813, 904 (o.disubstituted phenyl).<br>1123 (C—O—C cyclic).<br>1323, 1363 (C—N).<br>1495 (C═N).<br>1597 (C═C).<br>2924 (quaternary salt). | 1.2-1.7 (m, 6H, $2CH_3$ of positions 4, 6).<br>1.8-2.1 (m, 4H, $2CH_2$ of positions 4, 6).<br>2.2-2.4 (m, 3H, $CH_3$ of position 5).<br>2.7-3.1 (m, 3H, $CH_3$ of N-quinolinium).<br>3.4-3.8 (m, 2H, $CH_2$ of N-quinolinium).<br>4.8-5.2 (m, 1H, —CH═).<br>6.7-8.7 (m, 11H, aromatic + heterocyclic). |
| 6b | 692, 757 (monosubstituted phenyl).<br>815, 905 (o.disubstituted phenyl).<br>1122 (C—O—C cyclic).<br>1324, 1364 (C—N).<br>1496 (C═N).<br>1598 (C═C).<br>2922, 2858 (quaternary salt). | 1.2-1.6 (m, 6H, $2CH_3$ of positions 4, 6).<br>1.8-2.3 (m, 4H, $2CH_2$ of positions 4, 6).<br>3.0 (b, 3H, $CH_3$ of N-quinolinium).<br>3.3 (b, 2H, $CH_2$ of N-quinolinium).<br>4.8-5.2 (m, 2H, 2 —CH═).<br>6.7-8.7 (m, 17H, aromatic + heterocyclic). |
| 7 | 698, 750 (monosubstituted phenyl).<br>1115, 1173 (C—O—C cyclic).<br>1366, 1305 (C—N).<br>1494, 1405 (C═N).<br>1602 (C═C).<br>2861 (quaternary salt). | 1.2-1.9 (m, 8H, $2CH_3$ of position 4, 6 + 2—CH of diethoxyethyl).<br>2-2.2 (m, 4H, $2CH_2$ of position 4, 6).<br>2.3-2.4 (m, 12H, $4CH_3$ of diethoxyethyl).<br>3.4 (b, 12H, $6CH_2$ of diethoxyethyl).<br>7.2-8 (m, 5H, aromatic).<br>$M^{+2}$: 771 |
| 8b | 614, 687 (monosubstituted phenyl).<br>755 (o.disubstitute phenyl).<br>1156 (C—O—C cyclic).<br>1263 (C—O ether).<br>1317, 1373 (C—N).<br>1492 (C═N).<br>1631 (C═C).<br>2923 (quaternary salt). | 1.2-1.7 (m, 7H, $2CH_3$ of positions 4, 6 + 1—CH of diethoxyethyl).<br>1.8-2.2 (m, 4H, $2CH_2$ of positions 4, 6).<br>2.3-2.4 (m, 6H, $2CH_3$ of diethoxyethyl).<br>2.6 (m, 6H, $3CH_2$ of diethoxyethyl).<br>3.0 (b, 3H, $CH_3$ of N-quinolinium).<br>3.3 (b, 2H, $CH_2$ of N-quinolinium).<br>4.4-5 (m, 3H, 3—CH=).<br>7-8.4 (m, 11H, aromatic + heterocyclic). |
| 9b | 622, 688 (monosubstituted phenyl).<br>754, 999 (o.disubstituted phenyl).<br>1157 (C—O—C cyclic).<br>1317, 1373 (C—N).<br>1486 (C═N).<br>1636 (C═C).<br>2919 (quaternary salt). | 1.2-1.7 (m, 6H, $2CH_3$ of positions 4, 6).<br>1.9-2.3 (m, 4H, $2CH_2$ of positions 4, 6).<br>2.7-3 (m, 6H, $2CH_3$ of N-quinolinium).<br>3.2-3.4 (m, 4H, $2CH_2$ of N-quinolinium).<br>4.4-5 (m, 6H, 6—CH═).<br>7-8.7 (m, 17H, aromatic + heterocyclic). |

**Table 4.** Antimicrobial activity of some selected prepared 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 Agent** | **Tetracycline** | 30 | 32 | 31 | 28 |
| | **Ampicillin** | 20 | 22 | 17 | 18 |
| **3** | | 0.0 | 9 | 0.0 | 9 |
| **4** | | 0.0 | 9 | 9 | 10 |
| **5b** | | 0.0 | 0.0 | 9 | 0.0 |
| **6a** | | 13 | 16 | 17 | 13 |
| **6b** | | 0.0 | 9 | 9 | 11 |
| **6c** | | 9 | 11 | 11 | 11 |
| 7 | | 0.0 | 0.0 | 0.0 | 0.0 |
| **9a** | | 11 | 16 | 15 | 12 |
| **9b** | | 0.0 | 12 | 12 | 10 |
| **9c** | | 0.0 | 12 | 12 | 9 |



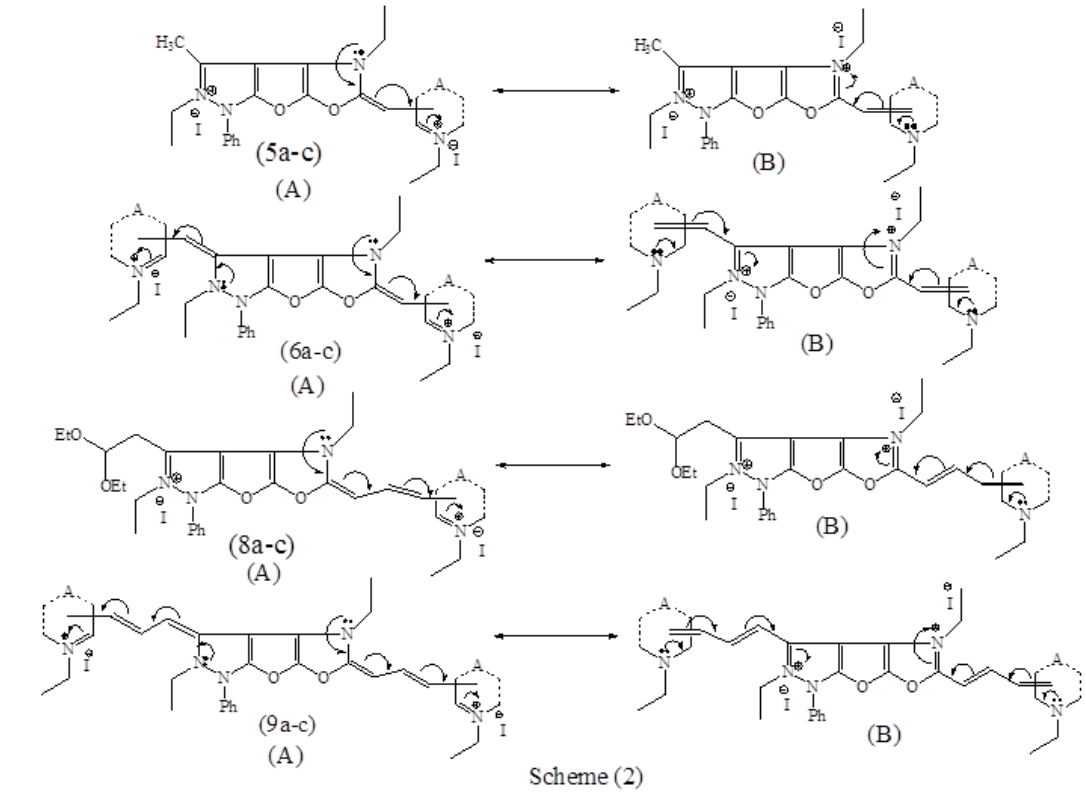

Scheme (1)

Synthesis Strategy of the prepared compounds (5a-c), (6a-c), (8a-c), and (9a-c).

## Substituents in scheme (1):

**(5a-c), (6a-c):** A = 1-ethyl pyridinium-4-yl salt (a), 1-ethyl quinolinium-4-yl salt (b), 2-ethyl isoquinolinium-1-yl salt (c).

**(8a-c), (9a-c):** A = 1-ethyl pyridinium-2-yl salt (a), 1-ethyl quinolinium-2-yl salt (b), 1-ethyl pyridinium-4-yl salt (c).




Scheme (2)

Colour intensity and / or the electronic charge transfer pathways illustration of the synthesized cyanine dyes (5a-c), (6a-c), (8a-c), and (9a-c).