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Polynuclear Heterocyclic Monomethine and Trimethine Cyanine Dyes: Synthesis and Various Absorption Spectra Studies

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Abstract

New polynuclear heterocyclic compound namely 4-methyl-2-phenyl-benzo[(2,3-b)benzoxazine; (2', 3'-b')furo(3,2-d)pyrazole]-5,12-dione was designed, prepared and employed as starting material in the synthesis of new methine cyanine dyes, covering monomethine cyanine dyes (simple cyanine dyes) and trimethine cyanine dyes (carbocyanine dyes). The electronic visible absorption spectra of all the synthesized cyanine dyes were investigated in 95% ethanol solution to evaluate their spectral sensitization properties. The electronic visible absorption spectra for some selected dyes were examined in pure solvents having different polarities [Water (78.54), Dimethylformamide (36.70), Ethanol (24.3), Chloroform (4.806), Carbontetrachloride (2.238) and Dioxane (2.209)] and/or in aqueous universal buffer solutions owing varied pH values (1.99, 2.99, 4.30, 6.87, 7.96, 8.91, 10.55 and 12.04 units) to evaluate their solvatochromic and/or halochromic properties, respectively. Structural determination was carried out via elemental analysis, visible, mass, IR and ¹HNMR spectroscopic data.

Keywords: cyanine dyes, methine cyanine dyes, synthesis, absorption spectra, solvent effects, acid/base properties.

1. Introduction

In the recent years, a considerable attention have been given to the chemistry of cyanine dyes, dealing with their synthesis, characterizations and applications (Shindy, 2017; Shindy, 2018; Shindy et al., 2019; Arjona et al., 2016; Ashitate et al., 2016; Hyun et al., 2015; Soriano et al., 2015; Sato et al., 2019; Schwechheimer et al., 2018; Rodríguez-Pérez et al., 2017). Essentially, this can be related to the excellent photophysical and photochemical properties of these dyes which makes them easily applicable in a diverse and a broad area of science, technology, engineering, pharmacology and medicine. Cyanine dyes possess two nitrogen containing heterocyclic groups that are connected by a conjugated methine bridge as shown in Figure 1. The delocalization of electrons across this chain causes them to be highly fluorescent and exhibit long wavelength absorption that span from the visible to the near infrared regions (Wyler, 1969; Wyler, 1969a; Musso, 1979; Reichardt, 1995). In past, with the beginning of the 1800s, cyanine dyes were used in photographic emulsions and chemotherapy (Hamer, 1964), and the great commercial value of the cyanine dyes at this time was associated only with their power of conferring extra sensitiveness on silver halide photographic plates. Ordinarily, such plates are sensitive to the violet and blue regions of the spectrum, but adding suitable cyanine dyes to the liquid emulsion or by bathing the dried emulsion film in the dye solution, the plate may be rendered remarkably sensitive to green, yellow, orange, red, and even to the invisible infra-red portions of the spectrum (Dach, Daehne 1997). But

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more recently cyanine dyes have been used as functional dyes in high technique fields such as in laser printing (Dähne et al., 1998), pH sensors (Xu et al., 2007), fluorescence *in vivo* imaging (Choi et al., 2011; Choi et al., 2013; Licha et al., 2000; Achilefu, et al., 2000), data storage (Nakazumi, 2008), and as labels for nucleic acid detection (Warner et al., 1996; Haugland et al., 1969; Deligeorgiev et al., 1998).

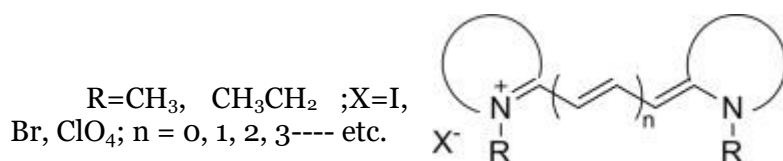


Fig. 1. General structure of cyanine dyes

In this research paper we prepared new polynuclear heterocyclic monomethine and trimethine cyanine dyes as new synthesis contribution and spectroscopic investigation in the field, and to may be used and/or applied in any of the wide range applications of cyanine dyes, and particularly (according to this study) as photographic sensitizers in photographic material industry (due to their spectral sensitization properties), as probes for determining solvent polarity in solution chemistry (due to their solvatochromic properties) and/or as indicators in operations of acid/base titration in analytical chemistry (due to their halochromic properties).

2. Results and discussion

2.1.Synthesis:

An equimolar ratios of 3,4-dichloro-benzo[b]-phenoxazine-2,5-dione (1) and 3-methyl-1-phenyl-5-pyrazolone (2) were reacted in pyridine and achieved 4-methyl-2-phenyl-benzo[(2,3-b)benzoxazine; (2', 3'-b')furo(3,2-d)pyrazole]-5,12-dione (3) as new polyheterocyclic starting material compound, Scheme (1).

Quaternization of (3) using an excess of iodoethane led to the formation of 3-ethyl-4-methyl-5,12-dione-2-phenyl-benzo[(2,3-b)benzoxazine;(2',3'-b')furo(3,2-d)pyrazolium]iodide quaternary salt compound (4), Scheme (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 3-ethyl-5,12-dione-2-phenyl-benzo[(2,3-b)benzoxazine;(2',3'-b')furo(3,2-d)pyrazole]-4[4(1)]-monomethine cyanine dyes (5a-c), Scheme (1).

Additionally, the quaternized compound (4) was reacted with a unimolar ratios of triethylorthoformate in presence of acetic anhydride and led to the formation of the intermediate compound 3-ethyl-4(1,1'-diethoxyethyl)-5,12-dione-2-phenyl -benzo[(2,3-b)benzoxazine;(2',3'-b')furo(3,2-d)pyrazolium] iodide quaternary salt (6), Scheme (1).

The intermediate compound (6) was further reacted with equimolar ratios of N-ethyl (2-picolinium, quinaldinium, 4-picolinium) iodide quaternary salts in ethanol containing piperidine as a basic catalyst to give 3-ethyl-5,12-dione-2-phenyl-benzo[(2,3-b)benzoxazine;(2',3'-b')furo(3,2-d)pyrazole]-4[2(4)]-trimethine cyanine dyes (7a-c), Scheme (1).

The structure of the prepared compounds were characterized and identified by elemental analysis, Table 1, Visible spectra, Table 1, mass spectrometer, IR (Wade, 1999) and ¹H-NMR (Wade, 1999a) spectroscopic data, Table 3.

2.2. Absorption spectra studies in 95 % ethanol solution:

This study was carried out to evaluate the spectral sensitization properties of the synthesized cyanine dyes to may used and/or applied as photographic sensitizers in photosensitive material industry.

The electronic visible absorption spectra of the monomethine cyanine dyes (5a-c) in 95 % ethanol solution discloses bands in the visible region 410-460 nm. The positions of these bands and their molar extinction coefficient (molar absorptivity) are largely influenced by the nature of the heterocyclic quaternary residue (A) and their linkage positions. So, substituting A=1-ethyl pyridinium-4-yl salt in the monomethine cyanine dye 5a by A=1-ethyl quinolinium-4-yl salt to get the monomethine cyanine dye 5b causes strong bathochromic shift by 20 nm, accompanied by increasing intensity of the absorption bands Scheme (1), Table 1. This can be attributed to

increasing π -delocalization conjugation in the latter dye due to the presence of quinoline ring system in correspondance to the pyridine ring system in the former dye.

Changing the linkage positions from 1-ethyl quinolinium-4-yl salt to 2-ethyl isoquinolinium-1-yl salt passing from the monomethine cyanine dye 5b to the monomethine cyanine dye 5c resulted in a remarkable blue shift by 10 nm, 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 salt dye 5c compared to the former 1-ethyl quinolinium-4-yl salt dye 5b.

Additionally, the electronic visible absorption spectra of the trimethine cyanine dyes (7a-c) in 95 % ethanol solution discloses bands in the visible region 410-650 nm. The positions of these bands and their molar extinction coefficient are largely influenced by the nature of the heterocyclic quaternary residue (A) and their linkage positions. So, substituting A=1-ethyl pyridinium-2-yl salt in the trimethine cyanine dye 7a by A=1-ethyl quinolinium-2-yl salt to get the trimethine cyanine dye 7b causes strong bathochromic shift by 80 nm, Scheme (1), Table 1. This can be attributed to increasing π -delocalization conjugation in the latter dye due to the presence of quinoline ring system in correspondance to the pyridine ring system in the former dye.

Changing the linkage positions from 2-yl salt to 4-yl salt passing from the trimethine cyanine dye 7a to the trimethine cyanine dye 7c resulted in a remarkable red shifts by 10 nm accompanied by increasing the intensity of the absorption bands, Scheme (1), Table 1. This can be explained in the light of increasing the length of the π -delocalization conjugation in the latter 4-yl salt dye 7c due to the presence of the γ -picolinium structure system compared to the former 2-yl salt dye 7a which contain the α -picolinium structure system.

Comparison the electronic visible absorption spectra of the monomethine cyanine dye (5a-c) with those of the trimethine cyanine dyes (7a-c) reveals that the later trimethine cyanine dyes (7a-c) have strong bathochromic shifted bands accompanied by increasing number of the absorption bands compared with the former monomethine cyanine dyes (5a-c). 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), Table 1.

2.3-Absorption spectra studies in pure solvents having different polarities:

This study was carried out to select the best solvents to use of these cyanine dyes as photosensitizers when there are applied in photographic material industry. The other important purpose of this study is to evaluate the solvatochromic properties of these cyanine dyes to may be used and/or applied as probes for determining solvent polarity, in physical, physical organic, inorganic and/or in solution chemistry.

So, the electronic visible absorption spectra of the monomethine cyanine dye (5b) and trimethine cyanine dye (7b) in pure solvents of different polarities (different dielectric constant) namely water (78.54), dimethylformamide (DMF) (36.70), ethanol (24.3), chloroform (4.806), carbontetrachloride (2.238) and dioxane (2.209) (Shindy, et al., 2014; Shindy, et al., 2014a) are recorded. The λ_{max} (wavelength) and ϵ_{max} (molar extinction coefficient) values of the absorption bands due different electronic transitions within the solute molecule in these solvents are represented in Table 3.

From Table 3, it is clear that the electronic visible absorption spectra of the cyanine dyes (5b) and (7b) in the ethanolic medium are characterized by the presence of two essential absorption bands (for the dye 5b) and three essential absorption bands (for the dye 7b). These bands can be assigned to intermolecular charge transfer transition (Shindy, et al., 2014; Shindy, et al., 2014a). These charge transfer is due to transfer of lone pair of electrons from the N-ethyl pyrazole nitrogen atoms (the basic and / or the electron pushing center of the dyes) to the positively charged quaternary nitrogen atoms of the quinolinium salts residue, (the acidic and / or the electron pulling center of the dyes) and vice versa, Scheme (2).

The data given in Table (3) show that the charge transfer band exhibits a hypsochromic shift in ethanol relative to DMF, dioxane, chloroform and carbontetrachloride. This effect may be related to the following factors:

a- The bathochromic shifts in DMF relative to ethanol is a result of the increase in solvent polarity due to the increasing of dielectric constant of DMF relative to ethanol.

b- The hypsochromic shift occurs in ethanol relative to dioxane, chloroform and carbontetrachloride is a result of the solute solvent interaction through intermolecular hydrogen

bond formation between ethanol and the lone pair of electrons of the N-ethyl pyrazole nitrogen atoms, Scheme (3) (A). This decreases slightly the electron density on the N-ethyl pyrazole nitrogen atoms and consequently decreases to some extent the moving and mobility of the attached π -electrons over the conjugated pathway to the positively charged quaternary nitrogen atom of the quinolinium salt residue, and consequently a hypsochromic shift occurs.

Also, from the data given in Table 3 it is observed that occurrence of unexpected hypsochromic shifts in water relative to ethanol and the other solvents. This can be mainly ascribed to the possible interaction of water molecules with the lone pair of electrons of the N-ethyl pyrazole nitrogen atoms, Scheme (3) (B). This makes difficult the transfer of electronic charge from the N-ethyl pyrazole nitrogen atoms to the quaternary nitrogen atoms of the heterocyclic quinolinium salt residue, and accordingly there is observed a hypsochromic shift in water relative to ethanol and the other solvents.

2.4-Absorption spectra studies in aqueous universal buffer solutions having varied pH values:

The solutions of the monomethine (5b) and trimethine (7b) cyanine dyes behaves as halochromic compounds where, their ethanolic solutions gives changeable colours in acid/base media being yellow or colourless on acidification and getting back (restore) their original permanent intense colour on basification. This encouraged us to study their spectral behaviour in different buffer solutions to select a suitable pH for use of these cyanine dyes as photosensitizers. The other purpose of this study is to evaluate the halochromic properties of these cyanine dyes in order to identify the possibility of their uses and/or applications as indicators in operations of acid/base titrations in analytical chemistry. The acid dissociation or protonation constants of these dyes have been determined. The effect of the compounds as photosensitizers increase when there are present in the ionic form, which has higher planarity (Shindy, et al., 2014; Shindy, et al., 2014a) and therefore more conjugation.

The electronic visible absorption spectra of the dyes (5b) and (7b) in aqueous universal buffer solutions of varying pH values (1.99, 2.99, 4.30, 6.87, 7.96, 8.91, 10.55 and 12.04 units). showed bathochromic shifts with intensification of their absorption bands at high pH (alkaline media) and hypsochromic shifts with reduction in the intensity of the bands at low pH (acidic media), Table 4.

Therefore the mentioned dyes which have free lone pair of electrons on the N-ethyl pyrazole nitrogen atom undergo protonation in acidic media. This generates positive charge on the N-ethyl pyrazole nitrogen atom, and consequently the electronic charge transfer pathways from the N-ethyl pyrazole nitrogen atom to the heterocyclic quaternary nitrogen atom of the quinolinium salt residue will be greatly affected and difficult resulting in a hypsochromic shift, protonated structures (colourless), Scheme (4) (A).

On increasing the pH of the media, the absorption bands are intensified and bathochromically shifted as a result of deprotonation of the N-ethyl pyrazole nitrogen atom, and accordingly the electronic charge transfer pathways to the quaternary heterocyclic nitrogen atom of the quinolinium salt residue will be easier, facilitated and more favoured resulting in a bathochromic shift, deprotonated structures (coloured), Schemes (4) (B).

Several methods have been developed for the spectrophotometric determination of the dissociation or protonation constants of weak acids. The variation of absorbance with pH can be utilised. On plotting the absorbance at fixed λ_{max} vs pH, S-shaped curves are obtained. On all of the S-shaped curves obtained, the horizontal portion to the left corresponds to the acidic form of the indicator, while the upper portion to the right corresponds to the basic form, since the pK_a is defined as the pH value for which one half of the indicator is in the basic form and the other half in the acidic form. This point is determined by intersection of the curve with a horizontal line midway between the left and right segments (Shindy et al., 2014; Shindy et al., 2014a). The acid dissociation or protonation constants values of the dyes (5b) and (7b) are listed in Table 5.

3. Conclusion

From the previous discussed results we could conclude that:

1. The electronic visible absorption spectra of the monomethine (5a-c) and trimethine (7a-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 cyanine dyes).
 - ii) Quinaldinium dyes > α -picolinium dyes (in the trimethine cyanine dyes).
- (B) Linkage position of the heterocyclic quaternary salt residue in the order of:
- i) quinolinium dyes > isoquinolinium dyes (in the monomethine cyanine dyes).
 - ii) γ -picolinium dyes > α -picolinium dyes (in the 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: trimethine cyanine dyes > monomethine cyanine dyes.

2. The intensity of the colours of the monomethine cyanine dyes, and 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 electronic visible absorption spectra of the examined cyanine dyes (5b) and (7b) in pure solvents having different polarities (solvatochromism) underwent displacements to give positive solvatochromism (occurrence of a bathochromic shift with increasing solvent polarity) and/or negative solvatochromism (occurrence of a hypsochromic shift with increasing solvent polarity) depending upon the following factors:

a. Increasing and/or decreasing the polarity (dielectric constant) of the solvent (General solvent effect).

b. Hydrogen bond and/or molecular complex formation between the solute (dyes molecules) and the solvent used (specific solvent effect).

4. The electronic visible absorption spectra of the monomethine (5b) and trimethine (7b) cyanine dyes in aqueous universal buffer solutions having varied pH values (halochromism) underwent displacements to give hypsochromic shifted and lower intensity bands in the lower pH media (acidic media) due to the protonated and/or colourless structures of the dyes in this media. Inversely, the bands of these dyes are intensified and bathochromically shifted in high pH media (basic media) due to the deprotonated and/or coloured structures of the dyes in this media.

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, Faculty of Science, Aswan University, Aswan, Egypt) and are uncorrected. Elemental analysis was carried out at the Microanalytical Center of Cairo University by an automatic analyzer (Vario EL III Germany). Infrared spectra were measured with a FT-IR (4100 Jasco, Japan), Cairo University. ¹H NMR spectra were accomplished using Varian Gemini-300 MHz NMR Spectrometer (Cairo University). Mass Spectroscopy was recorded on Mass 1: GC2010 Shimadzu Spectrometer (Cairo University). Electronic visible absorption spectra were carried out on visible spectrophotometer spectra 24 RS Labomed, INC (Chemistry Department, Faculty of Science, Aswan University, Aswan, Egypt).

4.2-Synthesis:

4.2-1-Synthesis of 4-methyl-2-phenyl-benzo[(2,3-b)benzoxazine; (2', 3'-b')furo(3,2-d)pyrazole]-5,12-dione (3).

Equimolar ratios of 3,4-dichloro-benzo[b]-phenoxazine-2,5-dione (1) (0.01 mol, 2.8 gm) and 3-methyl-1-phenyl-5-pyrazolone (2) (0.01 mol, 1.7 gm) were dissolved in pyridine (50 ml). The reaction mixture was heated under reflux for (6-8 hrs) until the mixture attained a permanent 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 3-ethyl-4-methyl-5,12-dione-2-phenyl-benzo[(2,3-b)benzoxazine;(2',3'-b')furo(3,2-d)pyrazolium]iodide quaternary salt (4).

A pure crystallized sample of (3) (0.04 mol, 1.5 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 3-ethyl-5,12-dione-2-phenyl-benzo[(2,3-b)benzoxazine;(2',3'-b')furo(3,2-d)pyrazole]-4[4(1)]-monomethine cyanine dyes (5a-c).

A mixture of compound (4) (0.01 mol, 0.5 gm) and iodoethane quaternary salts (0.01 mol) of pyridine (0.2 gm), quinoline (0.3 gm), or isoquinoline (0.3 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 colour (for 5a), and/or deep red colour (for 5b, c) 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 3-ethyl-4(1,1'-diethoxyethyl)-5,12-dione-2-phenyl - benzo[(2,3-b)benzoxazine; (2',3'-b')furo(3,2-d)pyrazolium] iodide quaternary salt as intermediate compound (6).

This intermediate compound (6) was synthesized by refluxing of the quaternary salt compound (4) (0.04 mol, 2.4 gm) with triethylorthoformate (0.04 mol, 0.8 ml) in acetic anhydride (50 ml) 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 1.

4.2-5. Synthesis of 3-ethyl-5,12-dione-2-phenyl-benzo[(2,3-b)benzoxazine;(2',3'-b')furo(3,2-d)pyrazole]-4[2(4)]-trimethine cyanine dyes (7a-c).

A mixture of the intermediate compounds (6) (0.01 mol, 0.6 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 violet (for 7a), deep violet (for 7b) and violet (for 7c) 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 1.

4.3. Absorption spectral behavior in 95 % ethanol:

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, spectra 24 RS Labomed, INC. A stock solution (1×10^{-3} M) of the dyes was prepared and diluted to a suitable volume in order to obtain the desired lower concentrations. The spectra were recorded immediately to eliminate as much as possible the effect of time.

4.4. Absorption spectral behavior in pure solvents and/or in aqueous universal buffer solutions:

The electronic visible absorption spectra of some selected synthesized cyanine dyes were investigated in pure organic solvents of spectroscopic grade (Shindy et al., 2014; Shindy et al., 2014a) and different polarities and/or in aqueous universal buffer solutions of varying pH values and recorded using 1cm quartz cell in Vis spectrophotometer spectra 24 RS Labomed, INC. A stock solution (1×10^{-3} M) of the dyes was prepared and diluted to a suitable volume using the suitable solvent and/or the buffer solution to obtain the required lower concentrations. The spectra were recorded immediately to eliminate as much as possible the effect of time.

5. Conflict of interest

There is no conflict of interest.

6. Acknowledgement

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References

- Achilefu et al., 2000 – Achilefu S., Dorshow R.B., Bugaj J.E., Rajagopalan R. (2000). Novel receptor-targeted fluorescent contrast agents for in vivo tumor imaging. *Invest Radiol*, 35 (8), pp. 479-485.
- Arjona et al., 2016 – Argona A., Stolte M., Wilerthner F. (2016). Conformational switching of π -conjugated junctions from merocyanine to cyanine states by solvent polarity. *Angewandte Chemie*, 55(7), 2470-2473.

Ashitate et al, 2016 – Ashitate Y., Levitz A., Park M.H., Hyun H., Venugopal V., Park G., El Fakhri G., Henary M., Gioux S., Frangioni J. V. et al. (2016). Endocrine-specific NIR fluorophores for adrenal gland targeting. *Chem. Commun.*, 52, 10305–10308.

Choi et al., 2013 – Choi H.S., Gibbs S.L., Lee J.H., Kim S.H., Ashitate Y., Liu F.B. et al. (2013). Targeted zwitterionic near-infrared fluorophores for improved optical imaging. *Nat Biotechnol*, 31 (2), pp. 148-153.

Choi, et al., 2011 – Choi H.S., Nasr K., Alyabyev S., Feith D., Lee J.H., Kim S.H. et al. (2011). Synthesis and in vivo fate of zwitterionic near-infrared fluorophores. *Angew Chem Int Ed*, 50 (28), pp. 6258-6263.

Dach, Daehne 1997 – Dach G., Daehne S. (1997). Second supplements to the 2nd edition of rodd's chemistry of carbon compounds, Vol. IV B. Elsevier Science B.V., Chapter 15, Cyanine dyes and related compounds (edited by Sainsbury, M.).

Dähne et al. 1998 – Dahne S., Resch-Genger U., Wolfbeis O.S. (1998). North Atlantic Treaty Organization. Scientific Affairs Division. Near-infrared dyes for high technology applications. Kluwer, Dordrecht; Boston.

Deligeorgiev et al., 1998 – Deligeorgiev T.G., Zaneva D.A., Kim S.H., Sabnis R.W. (1998). Preparation of monomethine cyanine dyes for nucleic acid detection. *Dyes Pigments*, 37 (3), pp. 205-211.

Hamer, 1964 – Hamer F.M. (1964). The cyanine dyes and related compounds. Interscience Publishers, New York.

Haugland et al., 1969 – Haugland R.P., Spence M.T.Z., Johnson I.D. (1969). Handbook of fluorescent probes and research chemicals. (6th ed.), Molecular Probes, Eugene, OR, USA (4849 Pitchford Ave., Eugene 97402).

Hyun et al., 2015 – Hyun H., Owens E.A., Wada H., Levitz A., Park G., Park M.H., Frangioni J.V., Henary M., Choi H.S. (2015). Cartilage-specific near-infrared fluorophores for biomedical imaging. *Angew. Chem. Int. Ed. Engl.*, 54, 8648-8652.

Licha et al., 2000 – Licha K., Riefke B., Ntziachristos V., Becker A., Chance B., Semmler W. (2000). Hydrophilic cyanine dyes as contrast agents for near-infrared tumor imaging: synthesis, photophysical properties and spectroscopic in vivo characterization. *Photochem Photobiol*, 72(3), pp. 392-398.

Musso, 1979 – Musso H. (1979). The pigments of fly agaric, *Amanita muscaria*. *Tetrahedron*, 35 (24), pp. 2843-2853.

Nakazumi, 2008 – Nakazumi H. (2008). Organic colorants for laser disc optical data storage. *J Soc Dye Colour*, 104(3), pp. 121-125.

Reichardt, 1995 – Reichardt C. (1995). Chiral polymethine dyes: a remarkable but forgotten conjugated pi system. *J Phys Org Chem*, 8 (12), pp. 761-773.

Rodríguez-Pérez et al., 2017 – Rodríguez-Pérez L., Villegas C., Herranz M.A., Delgado J.L., Martín, N. (2017). Heptamethine Cyanine Dyes in the Design of Photoactive Carbon Nanomaterials. *ACS Omega*, 2(12): 9164–9170.

Sato et al., 2019 – Sato Y., Yajima S., Taguchi A., Baba K., Nakagomi M., Aiba, Y., Nishizawa, S. (2019). Trimethine cyanine dyes as deep-red fluorescent indicators with high selectivity to the internal loop of the bacterial A-site RNA. *Chem. Commun.*, 55, 3183-3186.

Schwechheimer et al., 2018 – Schwechheimer C., Ronicke F., Schepersb, U., Wagenknecht, H. (2018). A new structure–activity relationship for cyanine dyes to improve photostability and fluorescence properties for live cell imaging. *Chem. Sci.*, 9, 6557.

Shindy et al., 2014 – Shindy H.A., El-Maghraby M.A., Eissa F.M. (2014). Effects of Chemical structure, solvent and solution pH on the visible spectra of some new methine cyanine dyes. *European Journal of Chemistry*, 5(3), 451-456.

Shindy et al., 2014a – Shindy H.A., El-Maghraby M.A., Eissa F.M. (2014). Synthesis and Spectral Properties of Novel Hemicyanine dyes. *Izv. AN. Ser. Khim*, 63(3), 707-715.

Shindy et al., 2019 – Shindy H.A., El-Maghraby M.A., Goma M.M., Harb N.A. (2019). Novel styryl and aza-styryl cyanine dyes: synthesis and spectral sensitization evaluation. *Chemistry International*, 5(2), 117-125.

Shindy, 2017 – Shindy H.A. (2017). Fundamentals in the Chemistry of Cyanine Dyes: A Review. *Dyes and Pigments*, 145, 505-513.

Shindy, 2018 – Shindy H.A. (2018). Structure and solvent effects on the electronic transitions of some novel furo/pyrazole cyanine dyes. *Dyes and Pigments*, 149, 783-788.

[Soriano et al., 2015](#) – Soriano E., Holder C., Levitz A., Henary M. (2015). Benz[c,d]indolium-containing monomethine cyanine dyes: Synthesis and photophysical properties. *Molecules*, 21.

[Wade, 1999](#) – Wade Jr.L.G. Organic. Chemistry. 4th Edn., Pearson Educ. (Prentice Hall, Upper Saddle River, New Jersey 07458, USA), 500-538.

[Wade, 1999a](#) – Wade Jr.L.G. Organic. Chemistry. 4th Edn., Pearson Educ. (Prentice Hall, Upper Saddle River, New Jersey 07458, USA), 544-604.

[Warner et al., 1996](#) – Warner I.M., Soper S.A., McGown L.B. (1996). Molecular fluorescence, phosphorescence, and chemiluminescence spectrometry. *Anal Chem*, 68 (12), pp. R73-R91.

[Wyler, 1969](#) – Wyler H. (1969). Die Betalaine. *Chemie unserer Zeit*, 3 (5), pp. 146-151.

[Wyler, 1969a](#) – Wyler H. (1969). Das Experiment: Papierelektrophorese. *Chemie unserer Zeit*, 3 (4), pp. 111-115.

[Xu et al., 2007](#) – Xu Y.F., Liu Y., Qian, X.H. (2007). Novel cyanine dyes as fluorescent pH sensors: PET, ICT mechanism or resonance effect? *J Photochem Photobiol A*, 190(1), pp. 1-8.

Appendix

Table 1. Characterization of the prepared compounds 3, 4, (5a-c), 6 and (7a-c)

Comp No	Nature of products			Molecular formula (M.Wt)	Analysis%						Absorption spectra in 95% ethanol	
	Colour	yield %	MP C°		Calculated			Found			$\lambda_{max}(nm)$	$\epsilon_{max} (mol^{-1}.cm^2)$
					C	H	N	C	H	N		
3	Brown crystals	70	150	C ₂₂ H ₁₃ N ₃ O ₄ (383)	68.93	3.39	10.97	68.93	3.12	10.88
4	Dark brown crystal	64	145	C ₂₄ H ₁₈ N ₃ O ₄ (539)	50.7	3.17	7.39	50.56	3.11	7.24
5a	Red	60	144	C ₃₁ H ₂₅ N ₄ O ₄ (644)	57.76	3.88	8.7	57.66	3.76	8.59	410, 440	12360, 13390
5b	Deep red	64	155	C ₃₅ H ₂₇ N ₄ O ₄ (694)	60.52	3.89	8.07	60.45	3.77	8.02	440, 460	14760, 14990
5c	Deep red	62	165	C ₃₅ H ₂₇ N ₄ O ₄ (694)	60.52	3.89	8.07	60.49	3.86	8.01	420, 450	16180, 14100
6	Dark brown crystal	59	160	C ₂₉ H ₂₈ N ₃ O ₆ (641)	51.94	4.18	6.27	51.44	4.13	6.22
7a	Violet	66	163	C ₃₃ H ₂₇ N ₄ O ₄ (670)	59.1	4.03	8.36	59.05	4.01	8.33	410, 440, 570	10590, 11590, 6400
7b	Deep violet	69	186	C ₃₇ H ₂₉ N ₄ O ₄ (720)	61.67	4.03	7.78	61.63	4.02	7.72	460, 590, 650	15390, 8780, 5000
7c	Violet	67	178	C ₃₃ H ₂₇ N ₄ O ₄ (670)	59.1	4.03	8.36	59.02	4.01	8.31	420, 450, 580	13080, 14090, 7350

Table 2. IR and ¹H NMR (Mass) Spectral Data of the Prepared Compounds (3), (4), (5b), (6) and (7b)

Comp. No.	IR Spectrum (KBr, Cm ⁻¹)	¹ H NMR Spectrum (DMSO, δ); & (Mass data).
3	689, 755 (monosubstituted phenyl). 870 (o.disubstituted phenyl). 1485 (C=N). 1597 (C=C). 1712 (C=O quinone). 3423 (NH).	2.1 (m, 3H, CH ₃ of position 4). 3.5 (b, 1H, NH). 6.8-9.25 (m, 9H, aromatic). M ⁺ : 383.88
4	619, 686 (monosubstituted phenyl). 1116 (C—O—C cyclic). 1363 (C—N). 1489 (C=N). 1594 (C=C). 1712 (C=O quinone). 2924 (quaternary salt). 3428 (NH).	1.2 (m, 3H, CH ₃ of position 3). 1.6 (s, 3H, CH ₃ of position 4). 2.2 (m, 2H, CH ₂ of position 3). 3.5 (b, 1H, NH). 6.8-9.3 (m, 9H, aromatic). M ⁺ : 539.90
5b	618, 688 (monosubstituted phenyl). 755 (o.disubstituted phenyl). 1115 (C—O—C cyclic). 1381 (C—N). 1490 (C=N). 1627 (C=C). 2924 (quaternary salt). 3441 (NH).	1.3 (m, 3H, CH ₃ of position 3). 1.6 (m, 3H, CH ₃ of N-quinolinium). 2.1 (b, 4H, 2CH ₂ of position 3 and N-quinolinium). 3.4 (m, 1H, NH). 5.15 (s, 1H, —CH=). 7.1-9.6 (m, 15H, aromatic + heterocyclic).
6	688, 755 (monosubstituted phenyl). 838 (o.disubstituted phenyl). 1366 (C—N). 1491 (C=N). 1616 (C=C). 1711 (C=O quinone). 2925 (quaternary salt). 3437 (NH).	0.9 (b, 3H, CH ₃ of position 3) 1.1-1.6 (m, 7H, 2CH ₃ of diethoxyethyl + 1H, —CH $\begin{matrix} \diagup \\ \diagdown \end{matrix}$ of diethoxyethyl). 0.9-2.2 (m, 8H, CH ₂ of position 3 + 3CH ₂ of diethoxyethyl). 3.35 (b, 1H, NH). 7-9.2 (m, 9H, aromatic). M ⁺ : 641.15
7b	617, 688 (monosubstituted phenyl). 755 (o.disubstituted phenyl). 1116, 1157 (C—O—C cyclic). 1363 (C—N). 1494 (C=N). 1626, 1599 (C=C). 1711 (C=O quinone). 2922, 2845 (quaternary salt). 3437 (NH).	0.9-1.4 (m, 3H, CH ₃ of position 3). 1.5-1.8 (m, 3H, CH ₃ of N-quinolinium). 1.9-2.4 (b, 4H, 2CH ₂ of position 3 and N-quinolinium). 3.4 (s, 1H, NH). 6.8-8.5 (m, 18H, aromatic + heterocyclic + 3 —CH=).

Table 3. Visible absorption spectra of the dyes (5b and 7b) in pure solvents having different polarities

Solvent Dye No.	H ₂ O		EtOH		DMF		CHCl ₃		CCl ₄		Dioxane	
	λ_{\max} (nm)	ϵ_{\max} (mole ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mole ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mole ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mole ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mole ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mole ⁻¹ cm ²)
5b	420	14040	440	14760	480	21000	440	15950	460	11860	470	17000
	450	13000	460	14990	500	21500	470	15870	480	12330	490	16680
7b	440	14160	460	15390	490	20040	470	14820	480	17900	480	18630
	480	14620	590	8780	520	19590	600	10300	510	17210	500	18360
	580	7290	650	5000	620	11910	660	6100	610	10160	610	10160
	640	4530			690	8100			670	7011	680	4510

Table 4. Visible absorption spectra of the dyes (5b and 7b) in aqueous universal buffer solutions

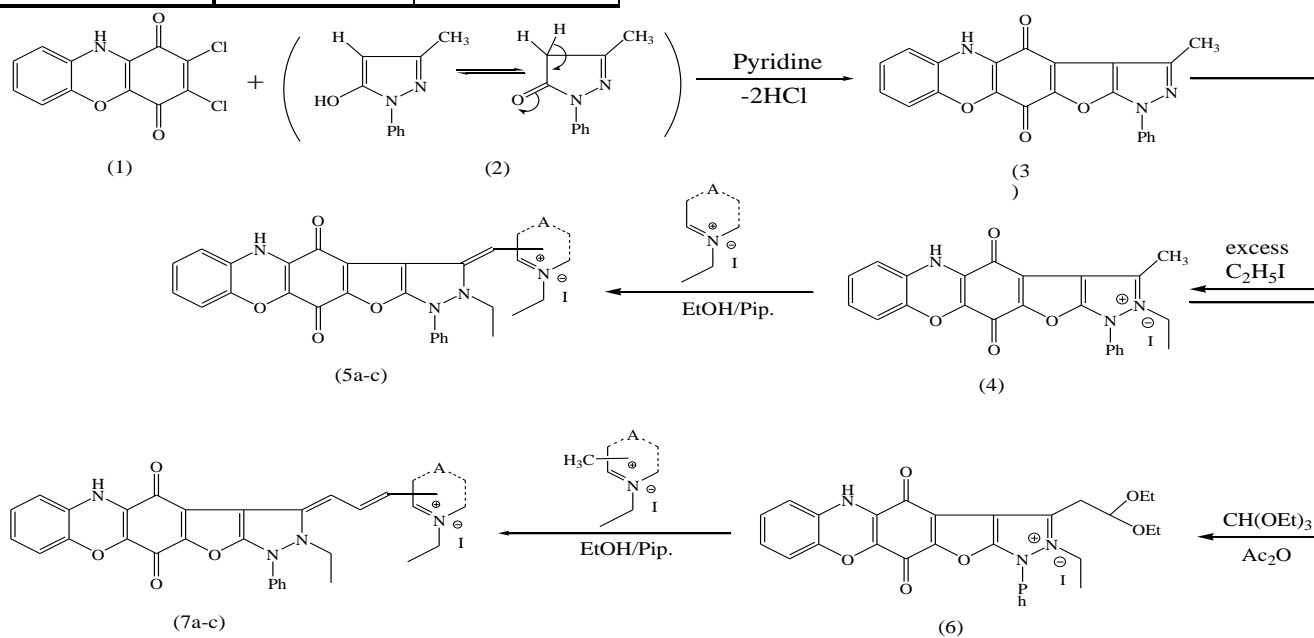
Comp. No.	Universal Buffers							
	1.99		2.99		4.30		6.87	
	λ_{\max} (nm)	ϵ_{\max} (mol ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mol ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mol ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mol ⁻¹ cm ²)
5b	415	9200	416	9400	417	9500	418	9700
	440	11280	450	10780	452	10900	454	10950
	578	6310	580	6320	582	6500	583	7300
7b	420	9180	430	9800	422	9181	425	9730
	450	9960	470	8690	453	9964	440	11720
	560	6570	570	6690	580	6890	580	6891
	620	3360	630	3380	640	3380	642	3590

Table 4. Continue. Visible absorption spectra of the dyes (5b and 7b) in aqueous universal buffer solutions

Comp. No.	Universal Buffers							
	7.96		8.91		10.55		12.04	
	λ_{\max} (nm)	ϵ_{\max} (mol ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mol ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mol ⁻¹ cm ²)	λ_{\max} (nm)	ϵ_{\max} (mol ⁻¹ cm ²)
5b	419	10000	420	13270	422	13500	424	13270
	457	10780	440	13100	445	13300	450	13750
	585	8500	586	9500	587	9900	589	11900
7b	430	9800	440	11720	430	12140	430	12142
	460	10390	460	11760	450	12610	460	13250
	580	6890	590	7360	591	7380	593	84200
	648	4000	650	4200	652	4400	660	5000

Table 5. The variation of absorbance with pH at fixed λ for the dyes (5b and 7b) in aqueous universal buffer solutions

pH	Compound Number	
	Absorbance at fixed λ	
	5b $\lambda=580$ (nm)	7b $\lambda=650$ (nm)
1.99	0.63	0.273
2.99	0.7	0.224
4.30	0.75	0.226
6.87	0.8	0.295
7.96	0.9	0.299
8.91	1	0.37
10.55	1.05	0.43
12.04	1.2	0.48
Pka	8.7	8.9 6.1



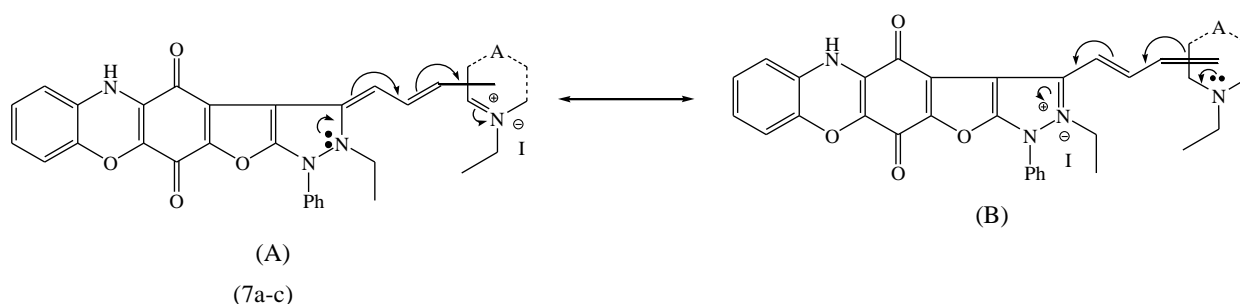
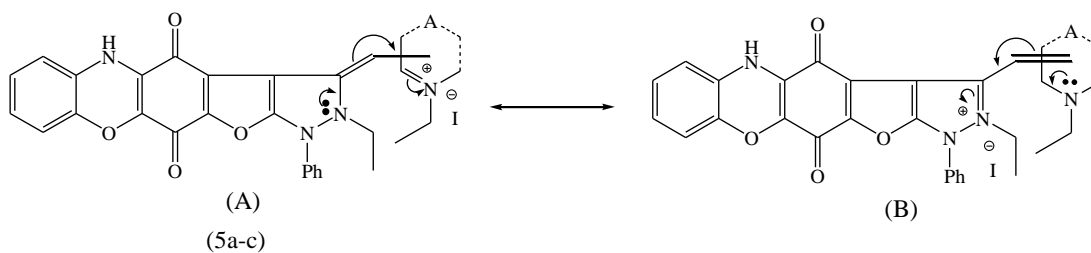
Scheme (1)

Synthesis Strategy of the prepared compounds (3), (4), (5a-c), (6) and (7a-c).

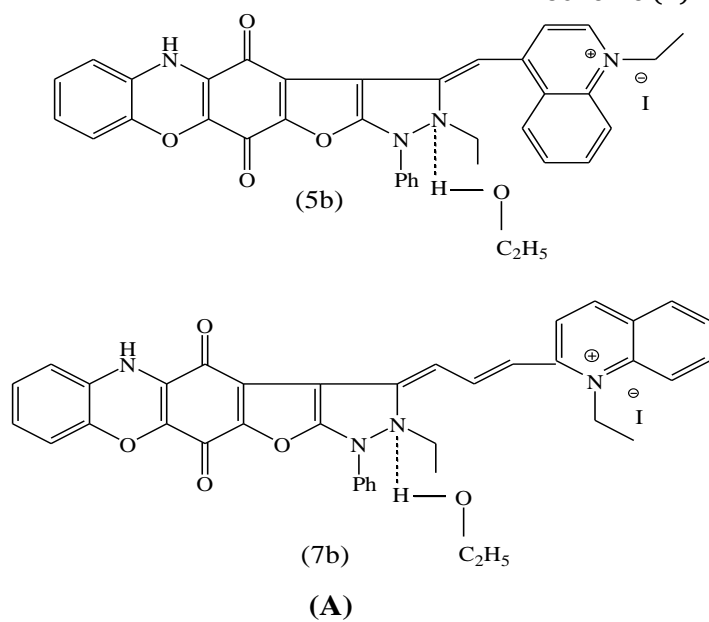
Substituents in scheme (1):

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

(7a-c): A = 1-ethyl pyridinium-2-yl salt (a), 1-ethyl quinolinium-2-yl salt (b), 1-ethyl pyridinium-4-yl salt (c).

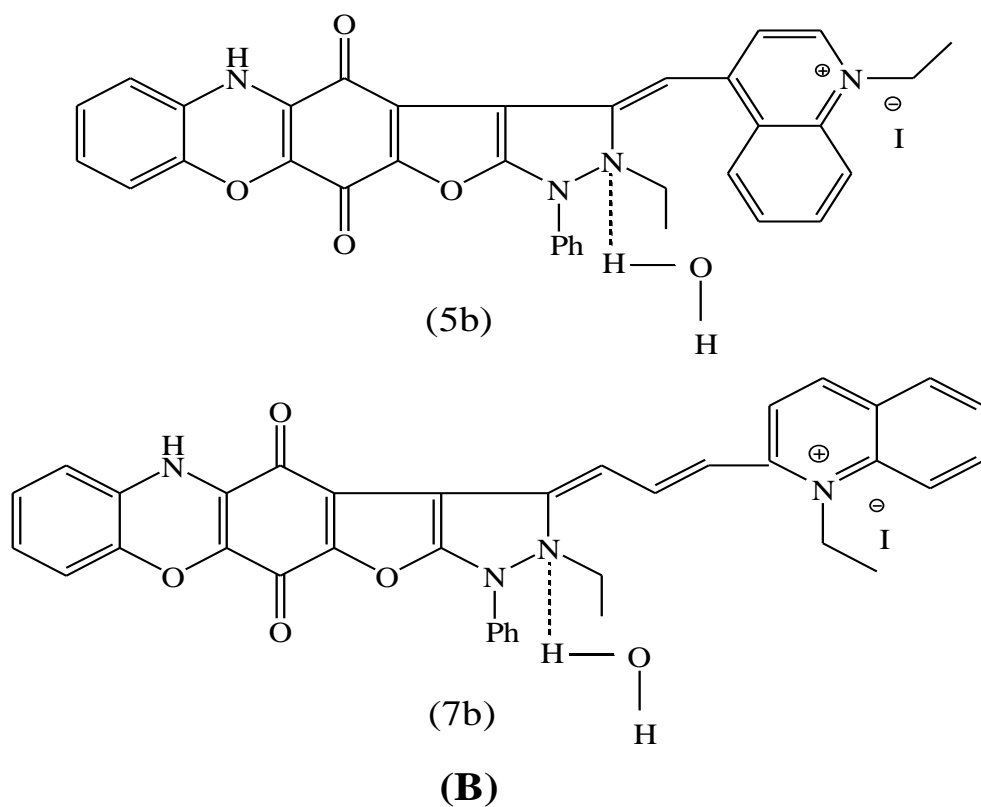


Scheme (2)



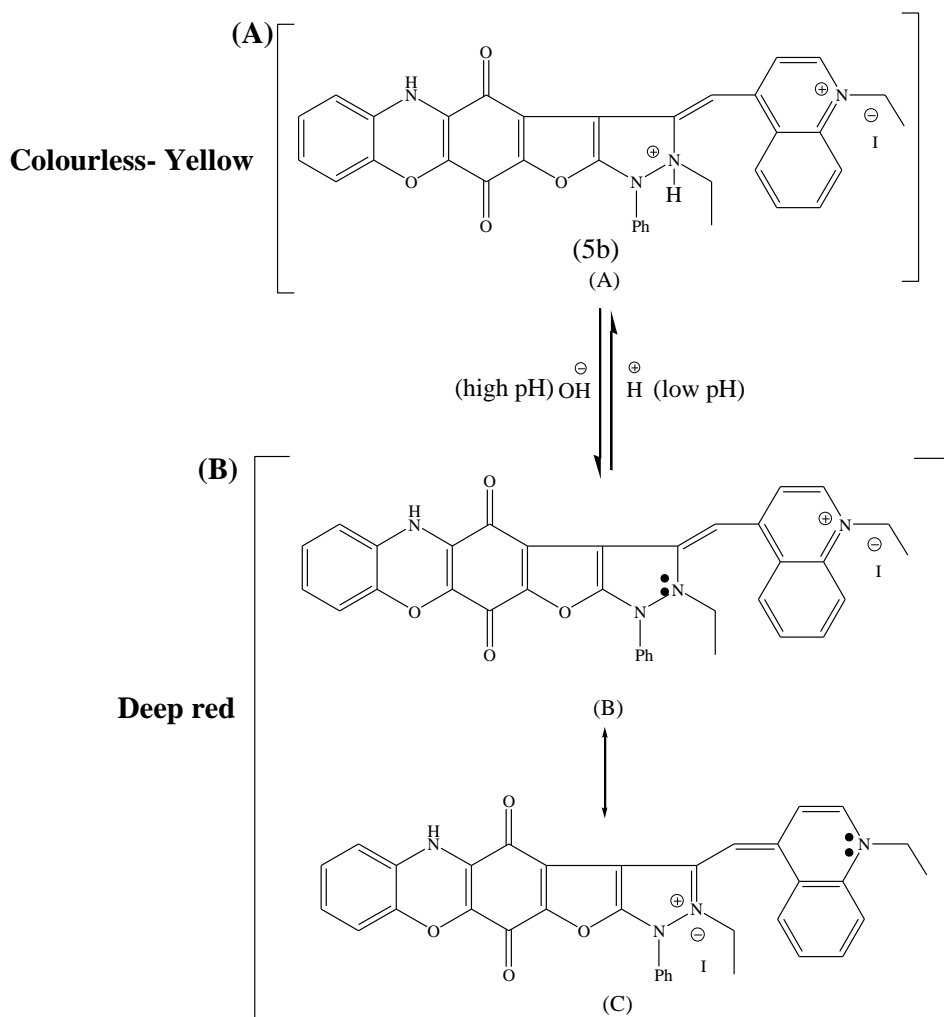
Scheme (3)

Colour intensity and the electronic charge transfer pathways illustration of the synthesized monomethine cyanine dyes (5a-c) and trimethine cyanine dyes (7a-c). Hydrogen bond formation between the monomethine cyanine dye (5b), trimethine cyanine dye (7b) and ethanol molecules (specific solvent effect).



Scheme (3)

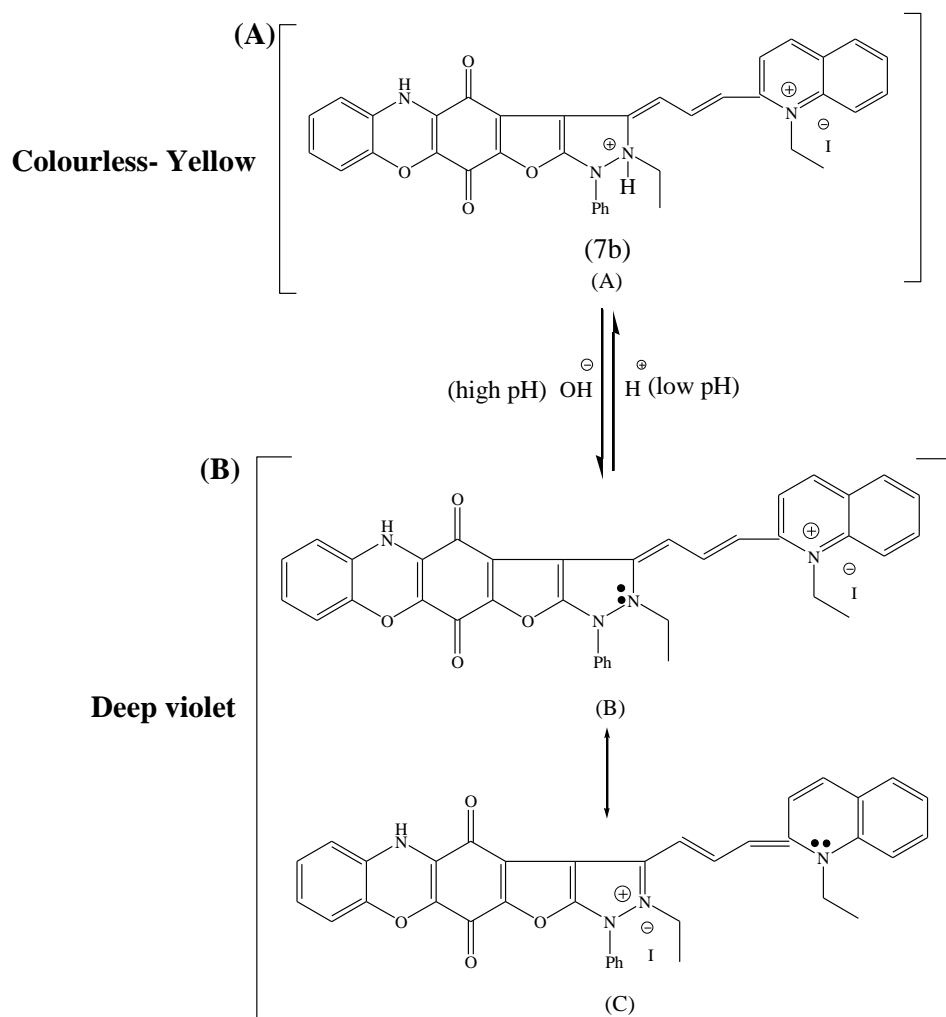
Hydrogen bond formation between the monomethine cyanine dye (5b), trimethine cyanine dye (7b) and water molecules (specific solvent effect).



Effects of pH media on the colour change of the monomethine cyanine dye (5b)

Decolourization (protonation) and colourization (deprotonation) of the monomethine cyanine dye (5b) in acid and base media, respectively (acido-basic equilibrium).

Scheme (4)



Effects of pH media on the colour change of the trimethine cyanine dye (7b)

Decolourization (protonation) and colourization (deprotonation) of the trimethine cyanine dye (7b) in acid and base media, respectively (acido-basic equilibrium).

Scheme (4) Continue