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A MINI REVIEW ON THE MOLECULAR STRUCTURE, SPECTRAL CHARACTERISTICS, SOLVENT-FREE SYNTHESIS, AND MULTIDISCIPLINARY APPLICATIONS OF CYANINE DYES

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Abstract

Cyanine dyes, a subclass of multifunctional organic compounds, have attracted considerable scientific attention owing to their distinctive molecular structures, exceptional spectral attributes, and diverse applications spanning various scientific domains. This comprehensive review elucidates the molecular architectures, electronic configurations, and spectral properties of cyanine dyes, underscoring their unique absorption and emission profiles that render them invaluable tools in the fields of medicine, pharmacology, and engineering. Despite possessing remarkable traits such as stability, high molar extinction coefficients, and heightened pH sensitivity, this dye class has received comparatively limited scrutiny and evaluation in recent decades. Consequently, this review concentrates on their structural and spectral attributes, which showcase their distinctive qualities. Moreover, the review delves into the manifold applications of cyanine dyes, along with their solvent-free synthesis facilitated by microwave irradiation as an efficient methodology for minimizing chemical waste and reaction time. These techniques enable environmentally sustainable synthesis practices, not only for novel product development but also for the eco-friendly synthesis of pre-existing chemicals, achieved through the complete elimination of solvent usage.

Keywords: Cyanine Dyes, Spectral Behaviour, Classification, Microwave-assisted synthesis; Applications.

1. Introduction

Cyanine dyes represent a fascinating class of organic compounds that have garnered significant attention for their versatile applications in various fields, ranging from chemistry and biology to materials science [1-4]. These dyes are characterized by their unique spectral properties, particularly their absorption and emission in the near-infrared region, making them invaluable tools in fluorescence-based imaging and detection techniques [5-9]. Originally developed for use in photographic emulsions, cyanine dyes have evolved into a diverse family with distinct subclasses, each tailored for specific applications [10-12].

The vibrant coloration of cyanine dyes, ranging from blue-green to near-infrared, arises from their extended conjugated π-electron system. This structural feature not only contributes to their striking optical properties but also influences their electronic behavior [13,14]. Researchers and scientists have harnessed the tunability of cyanine dyes to design probes for bioimaging, fluorescent sensors for detecting biomolecules, and contrast agents for medical diagnostics [15-18].

In this review, we go into the fascinating world of cyanine dyes, exploring their historical development, fundamental chemical characteristics, and the wide array of contemporary applications that continue to make them indispensable in cutting-edge scientific endeavors. As we navigate through the intricacies of cyanine dyes, it becomes evident that their multifaceted nature holds great promise for advancing fields such as medicine, materials science, and beyond.

1.1. Introduction to Cyanine Dyes

Cyanine dyes as a class of organic compounds are interested not only for the chemists, but also for numerous other scientists working in the field of biology, medical science, technology and physics, for their special properties. In general, cyanine dye’s structure involves two heterocyclic nuclei one of them is donor (D) and the other acceptor (A) groups that are linked by a conjugated polymethine chains of various lengths carbons [19], as illustrated in **Figure 1**.

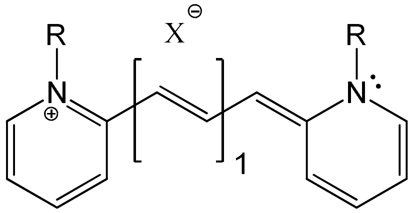


Fig. 1. General structure of cyanine dyes. Where **(**n) is the No. of conjugated methine groups.

1.2. Spectral Behaviour of Cyanine Dyes

Cyanine dyes are a class of organic compounds widely used as fluorescent probes and markers in various scientific and industrial applications. Their spectral behaviour, or the way they interact with light across different wavelengths, is a crucial aspect of their utility. Understanding the spectral behaviour of cyanine dyes is essential for optimizing their performance in applications such as fluorescence imaging, biological staining, and sensing. The unique spectral properties of cyanine dyes make them valuable tools in various applications, including bioimaging, flow cytometry, and fluorescence resonance energy transfer (FRET) studies. Researchers can choose dyes with specific spectral characteristics to match the requirements of their experiments.

1.2.1 Absorption Spectra

Cyanine dyes typically exhibit absorption maxima in the visible or near-infrared regions of the electromagnetic spectrum. The position and intensity of these absorption bands depend on the chemical structure of the dye, specifically the length of the conjugated system and the nature of substituents. The absorption spectra provide information about the wavelengths at which the dye efficiently absorbs light [20-25].

1.2.2. Emission Spectra

The emission spectra of cyanine dyes reveal the wavelengths of light they emit after being excited. This property is fundamental to their application in fluorescence. The Stokes shift, the difference between the absorption and emission maxima, is an important parameter influencing the sensitivity and spectral overlap in multiplexed imaging experiments [23-29].

1.2.3. Solvatochromism (Solvent Effect)

Solvatochromism in cyanine dyes refers to the phenomenon where the absorption spectral characteristics of these dyes undergo changes in response to variations in their surrounding solvent environment. This alteration encompasses shifts in the position, intensity, and shape of absorption bands. The manifestation of solvatochromism in cyanine dyes is intricately tied to the equilibrium between ground and excited states, where the ground state experiences better stabilization through solvation. The chemical structure of the cyanine molecules, the solute dipole moment, and the physical properties of the solvents all play pivotal roles in influencing solvatochromic behaviour [30-32].

There are two distinct categories of solvatochromism observed in cyanine dyes. Positive solvatochromism is characterized by a bathochromic shift, signifying a shift towards longer wavelengths, as the polarity of the solvent increases. This shift indicates a more effective stabilization of the molecule in the excited state compared to the ground state, accompanied by an increase in the solute dipole moment [33-36]. Conversely, negative solvatochromism in cyanine dyes is marked by a hypsochromic shift, leading to a shift towards shorter wavelengths in UV, visible, and NIR absorption bands as the polarity of the solvent rises. In this case, the molecule experiences superior stabilization by solvation in the ground state compared to the excited state, and this is concurrent with a decrease in the solute dipole moment. Understanding solvatochromism in cyanine dyes provides valuable insights into the intricate interplay between molecular structure, solvent characteristics, and the optical properties of these dyes in various environments [36, 37].

1.2.4. pH-Sensitivity

The pH sensitivity of cyanine dyes can be influenced by factors such as solvent composition, temperature, and the presence of specific ions. Understanding these factors is essential for precise control and interpretation of pH-dependent fluorescence responses. This property is particularly useful in applications where pH variations need to be monitored, such as in biological systems [38, 39].

1.2.5. Aggregation and Molecular Interactions

The tendency of cyanine dyes to aggregate in solution can significantly alter their spectral behaviour. Aggregation-induced changes in absorption and emission spectra must be considered, especially in biological applications where the dyes interact with cellular components [40, 41].

1.3. Cyanine dyes Classification:

Cyanine dyes are a class of synthetic organic dyes that are widely used in various applications, including fluorescence imaging, molecular biology, and materials science. These dyes are known for their intense and tunable absorption and emission properties, making them valuable tools in various scientific and industrial fields. Cyanine dyes typically consist of a polymethine chain with a nitrogen-containing heterocycle at each end. The number of methine groups in the chain and the nature of the heterocycles determine the specific properties of the dye. Cyanine dyes are commonly classified based on the length of their polymethine chain, which is often indicated by a numerical value. The most common subclasses include:

1.3.1. Methine cyanines

In the context of methine cyanine dyes, the term "methine" pertains to the central carbon-carbon double bond (C=C) within the molecular structure of the dye. The classification of cyanine dyes is based on the quantity of methine groups present in the conjugated chain connecting the two rings, as well as the specific ring system within the molecule. This classification is as illustrated in **Figure 1** as follows: for n=0, it corresponds to Monomethine (referred to as simple cyanine, e.g., ethyl red) [42]; for n=1, it represents Trimethine (designated as Carbocyanine, e.g., pinacyanol), as shown in **Figure 2** [43]; for n=2, it signifies Pentamethine (di-Carbocyanine); and for n=3, it denotes Heptamethine (tri-Carbocyanine), as shown in **Figure 2**.

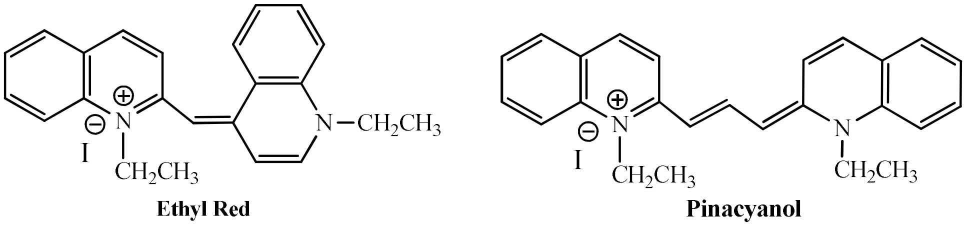
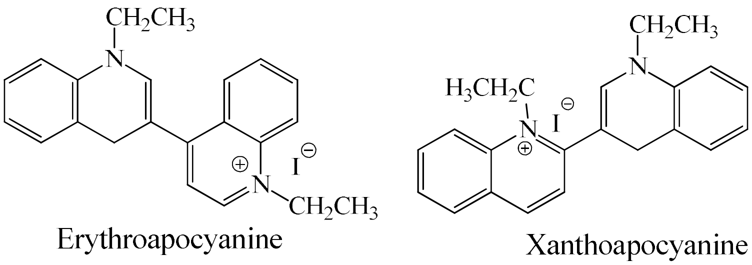


Fig. 2. Chemical structures of ethyl red and pinacyanol dyes

1.3.2. Apocyanine (Zeromethine)

Apocyanine dyes represent a distinct category of chromophores acknowledged for their capacity to absorb electromagnetic radiation within the near-infrared (NIR) domain of the electromagnetic spectrum. These dyes exhibit noteworthy optical characteristics, rendering them advantageous in diverse applications, notably within the realm of biomedical imaging. Specifically, this subset of cyanine dyes is typified by direct linkage between the two nuclei and the absence of methine cyanine, exemplified by Erythro-apocyanine and Xanthoapocyanine [44], as shown in **Figure 3**.



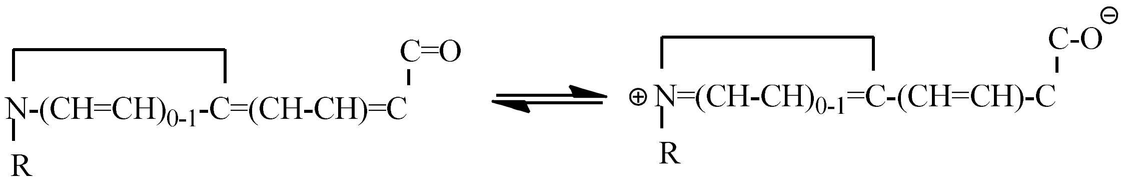
Erythroapocyanine

Xanthoapocyanine

Fig. 3. Chemical structures of apocyanine dyes

1.3.3. Merocyanine dyes

Mrocyanine dyes exhibit a structural configuration typically characterized by a conjugated system featuring alternating single and double bonds. The central core of these dyes often comprises a heterocyclic ring, such as pyridine or thiophene, flanked by electron-donating and electron-withdrawing groups. This specific molecular arrangement results in an extensive π-electron delocalization system, thereby contributing to the vivid hues observed in these dyes. This class of dyes shares notable similarities with cyanine dyes, which are distinguished by the presence of cyclic or acyclic carbonyl or thiocarbonyl groups [45, 46]. The chemical entities of these dyes are non-ionic and adhere to the general structures depicted in **Figure 4.**

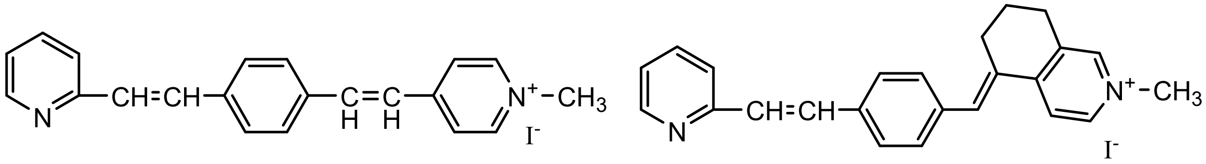


$$\underset{R}{N}\text{-}(CH{=}CH)_{0\text{-}1}\text{-}C{=}(CH\text{-}CH){=}\overset{C=O}{C} \rightleftharpoons \oplus\underset{R}{N}{=}(CH\text{-}CH)_{0\text{-}1}{=}C\text{-}(CH{=}CH)\text{-}\overset{C\text{-}O^{\ominus}}{C}$$

Fig. 4. General structure of merocyanine dyes. Where, n=0 simple merocyanine, n=1 di methine merocyanine, n=2 tetramethine merocyanine, n=3 Heptamethine merocyanine

1.3.4. Styryl cyanine

Styryl cyanine dyes are a class of fluorescent dyes that are widely used in various scientific and medical applications. These dyes are characterized by a styryl backbone, which consists of a conjugated system of alternating double bonds, and a cyanine moiety, which typically contains a positively charged nitrogen atom. On other words, this type of compound resembles cyanine in having two nitrogen atoms connected by a chain of conjugated double bonds but differ from them in that one nitrogen atom is not a part of a heterocyclic nucleus [47, 48]. The combination of these structural features imparts unique optical properties to styryl cyanine dyes, making them valuable tools in fluorescence imaging and related fields. Compound **1**, and **2** showed atypical examples of styryl cyanine dyes [49], as illustrated in **Figure 5**.



1 **2**

Fig. 5. Atypical examples of styryl cyanine dyes

1.3.5. Natural Sources of Cyanine Dyes

Although cyanine dyes are mainly created in laboratories, their inspiration frequently originates from natural sources. Gaining insight into these natural sources of cyanine-like compounds is crucial for refining and advancing dye development. Several natural sources share similarities with cyanine dyes, including a) Anthocyanins, which are pigments responsible for the red, purple, and blue hues in numerous fruits, vegetables, and flowers such as berries, grapes, red cabbage, and specific flowers. These sources serve as rich reservoirs of anthocyanins [50, 51], b) Betalains, red and yellow pigments present in certain plants like beets and particular cacti [52-54], c) Indigo, extracted from plants like Indigofera and Isatis tinctoria, serves as a blue dye historically employed in textile coloring [55-57], d) Flavonoids in citrus fruits, such as oranges and lemons, contain pigments that contribute to their vibrant coloration [58-60], e) Chlorophyll in green plants, responsible for their green colour, possesses a porphyrin structure with a conjugated system [61], f) Carotenoids, such as beta-carotene, contribute to the orange and red hues in carrots and tomatoes [62, 63]. While cyanine dyes are typically synthesized for specific applications, studying natural sources empowers researchers to design dyes with enhanced properties, such as improved stability, solubility, and biocompatibility. The diverse array of natural pigments serves as a foundation for crafting novel cyanine dyes with tailored characteristics, catering to a broad spectrum of applications in science and technology.

1.4. Green synthesis of Cyanine Dyes

1.4.1. Background and Theory of Microwave Reactions

The microwave-assisted synthesis technique employed to produce cyanine dyes represents an efficacious methodology contingent upon the utilization of dry media reactions, thereby circumventing the use of organic solvents throughout the synthetic processes in organic chemistry. This approach contributes to the realization of a clean, efficient, and economically viable technology, aligning with the principles of green chemistry. The heightened interest in employing environmentally benign reagents and methodologies is evident. Essentially, the absence of solvents, coupled with the attainment of elevated yields and abbreviated reaction durations commonly associated with such procedures, renders them highly appealing for synthetic purposes. Microwaves, in this context, are characterized as a form of electromagnetic energy situated at the lower extremity of the electromagnetic spectrum. This energy is quantified in terms of frequency, spanning from 300 to 300,000 Megahertz, corresponding to wavelengths ranging from 1 cm to 1 m [64]. The microwave region occupies the intermediate position between infrared and radio frequencies [65, 66], as illustrated in **Figure 6**. Microwave dielectric heating uses the ability of some liquids and solids to transform electromagnetic radiation into heat to drive chemical reactions. However, the advantages of using microwave dielectric heating for performing organic transformations have only emerged since the mid-1980s. This technology opens new opportunities to the synthetic chemist, in the form of new reactions that are not possible using conventional heating. Developments in this field have suggested that microwave-assisted chemistry could be used in most reactions that require heating.

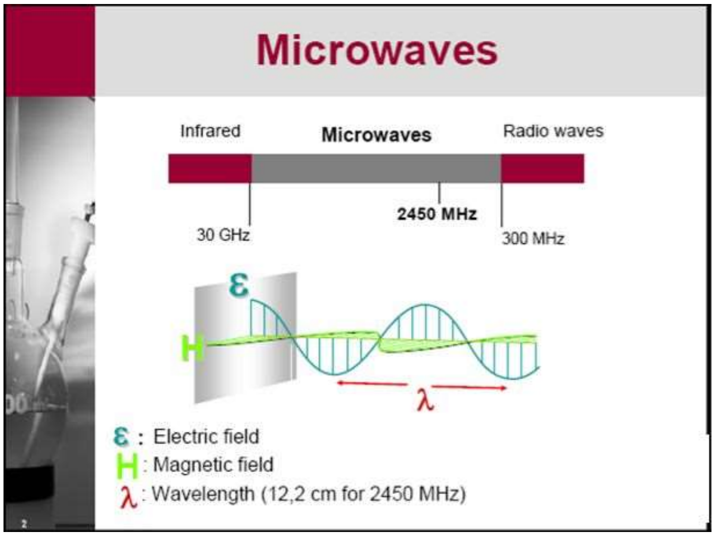


Fig. 6. Range of frequencies of microwave electromagnetic radiation

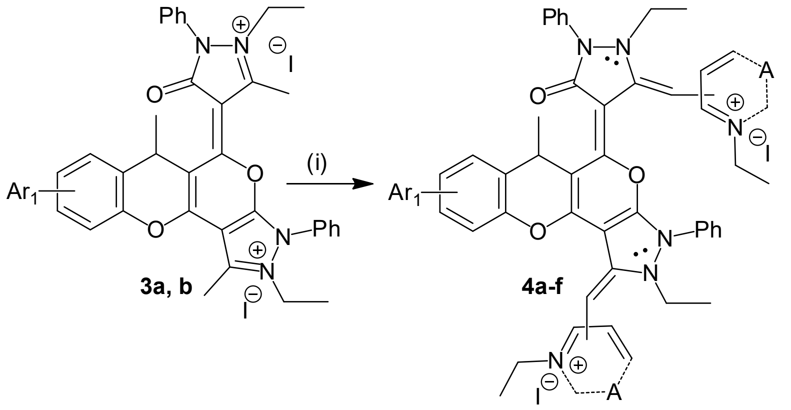
Traditionally, chemists operated under the assumption that chemical compounds engaged in reactions primarily within the liquid state or when dissolved [67]. Consequently, solvents became integral to chemical synthesis; however, the environmental impact of numerous solvent compounds proved to be undesirable. The challenge of environmentally unfriendly solvent waste disposal has been mitigated through the execution of reactions without the use of solvents under microwave irradiation (MWI) [64]. The introduction of microwave ovens into the domain of chemistry laboratories in recent years has facilitated the execution of numerous chemical transformations with heightened efficiency and simplified workup procedures [68-71]. The future prospects for the utilization of microwave technology in chemical processes appear promising due to its efficacy and potential to contribute to the generation of environmentally clean products.

1.4.2. Advantages of Microwave Assisted Synthesis

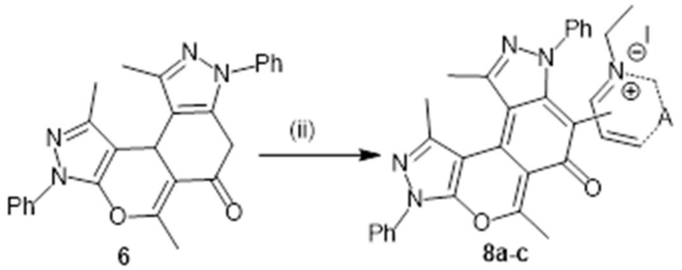
Microwave-assisted chemistry was predominantly employed as an alternative for executing specific reactions when alternative methods proved ineffective, or when prolonged reaction durations or elevated temperatures were imperative for reaction completion. However, a paradigm shift is underway as the prevalence of microwave reactors in laboratories increases, facilitating the application of microwave heating in routine synthetic transformations. Microwave heating offers several advantages over conventional methods, including uniform heating across the entire material, acceleration of process speed, high efficiency in heating, mitigation of undesired side reactions, attainment of purity in the final product, prevention of environmental heat loss, reduction in wastage of heating reaction vessels, lower operating costs, and Adoption of green technologies and promotion of dry media reactions [64, 66, 70].

1.4.3. Synthesis of Cyanine dyes *Via* Microwave

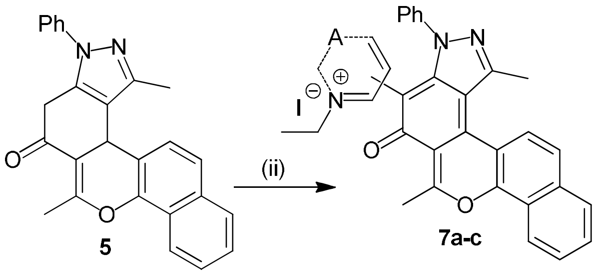
Bis monomethine cyanine dyes based on benzochromeno[2',3':4,5]pyrano[2,3-c]pyrazole ***(4a-f)***. were prepared through dehydrogenation reaction of quaternized salts **3a, b** in microwave oven by the reaction with 2 mole of active hydrogen quaternary salt such as pyridin[quinolin]-4(1)-ium-ethiodide salts to afford Bis monomethine cyanine dyes (**4a-f**), as illustrated in **Scheme 1** [36]**.**



Scheme 1: Synthetic routes of dyes **4a-f.** Where**,** Ar$_1$=2, 3-C_4H_4**,** A=1-ethyl pyridin-4-ium salt **(a)**; Ar$_1$=2, 3-C_4H_4, A=1-ethyl-quinolin-4-ium salt **(b)**; Ar$_1$=2, 3-C_4H_4, A=2-ethyl-quinolin-1-ium salt **(c)**;Ar$_1$=3, 4-C_4H_4**,** A=1-ethyl pyridin-4-ium salt **(d)**; Ar$_1$=3, 4-C_4H_4,A=1-ethyl-quinolin-4-ium salt **(e)**; Ar$_1$=3, 4-C_4H_4,A=2-ethyl-quinolin-1-ium salt **(f)**; (i)Ethyl iodide (2mole), MW., 119 watts, 4 mins. (ii)=(pyridin[quinolin]-4(1)-ium-ethiodide salts), MW., 119 watts, 5mins. and Piperidine.

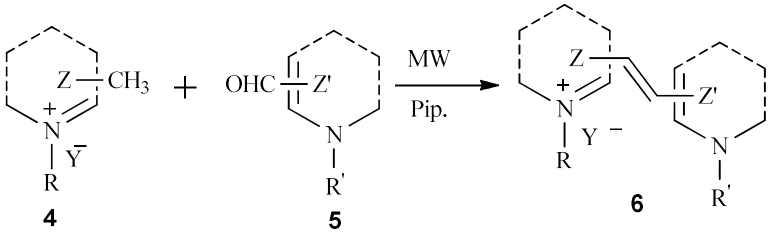


In addition, zero methine cyanine dyes based on benzo[7,8]chromeno[4,3-e]indazole **(7a-c)** and pyrazolo[4',3' :5,6] pyrano[4,3-e]indazole **(8a-c)** were synthesized through the reaction of 5,13-dimethyl-3-phenyl-3,5b-dihydrobenzo[7,8]chromeno[4,3-e]indazol-1(2H)-one **5** and 1,5,10-trimethyl-3,8-diphenyl-8,10b-dihydro-3H-pyrazolo[4',3':5,6] pyrano[4,3-e]indazol-6(7H)-one **6** respectively with active hydrogen compounds such as pyridin[quinolin]-4(1)-ium-ethiodide salts with active hydrogen quaternary salts such as pyridine [quinolin]-4(1)-ium-ethiodide salts under piperidine catalysis in microwave oven at appropriate time at 200 watt under solvent-free conditions afforded the target cyanine dyes **(7a-c)** and **(8a-c)** respectively, **Scheme 2** [36]**.**



Scheme 2: Synthetic routes for dyes **7a-c** and **8a-c**. Where, A = 1-ethyl pyridin-4-ium salt (**a**); 1-ethyl-quinolin-4-ium salt (**b**); 2-ethyl-quinolin-1-ium salt **(c)**; (ii) (pyridin[quinolin]-4(1)-ium-ethiodide salts), MW, 10 mins. and Piperidine.

On the other hand, a series of dimethine cyanine dyes (**6**), used as fluorescent probes, were synthesized under microwave irradiation and using piperidine as a catalyst by the reaction of aromatic aldehyde (**5**) with active methyl quaternary salt (**4**). The properties of the dyes as fluorescent probes for living cells imaging and flow cytometry were investigated [72], as illustrated in **Scheme 3** and **Figures 7-9**.



Scheme 3: Synthesis of dimethine cyanine dyes utilizing microwave irradiation

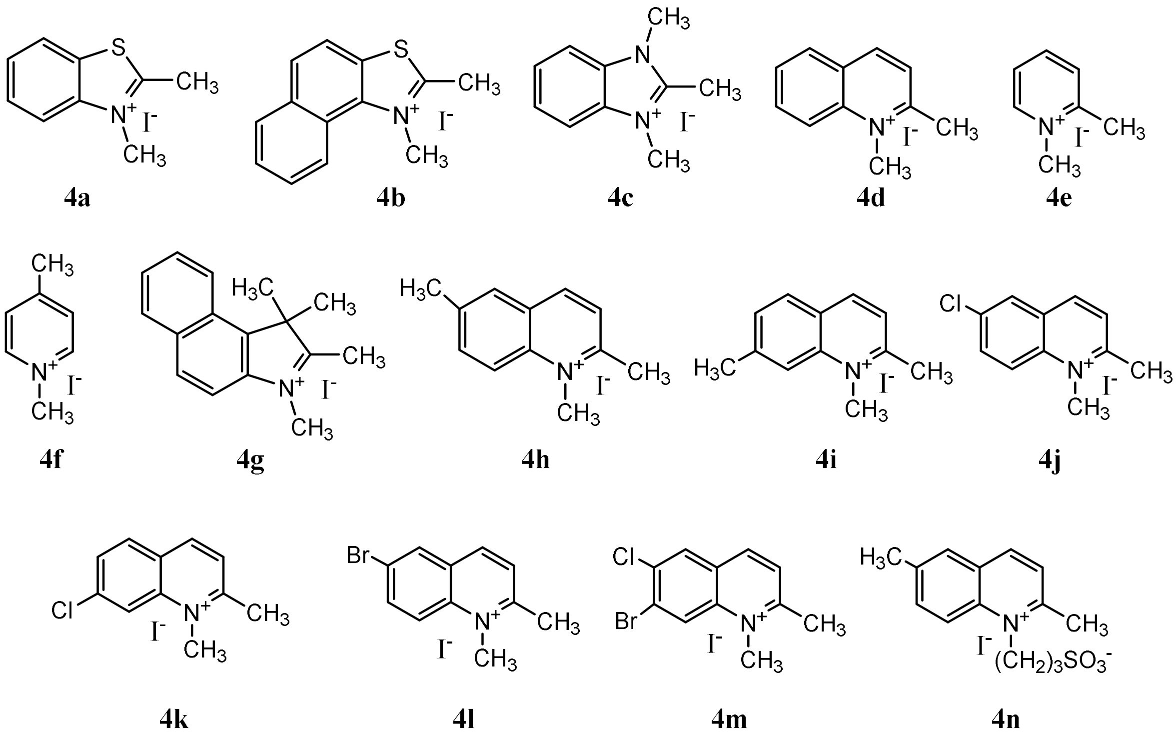


Fig. 7. Chemical structures of the precursor molecules **(4a-n)**

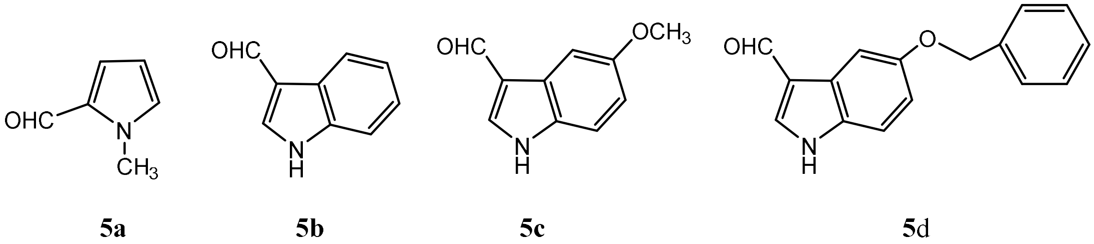
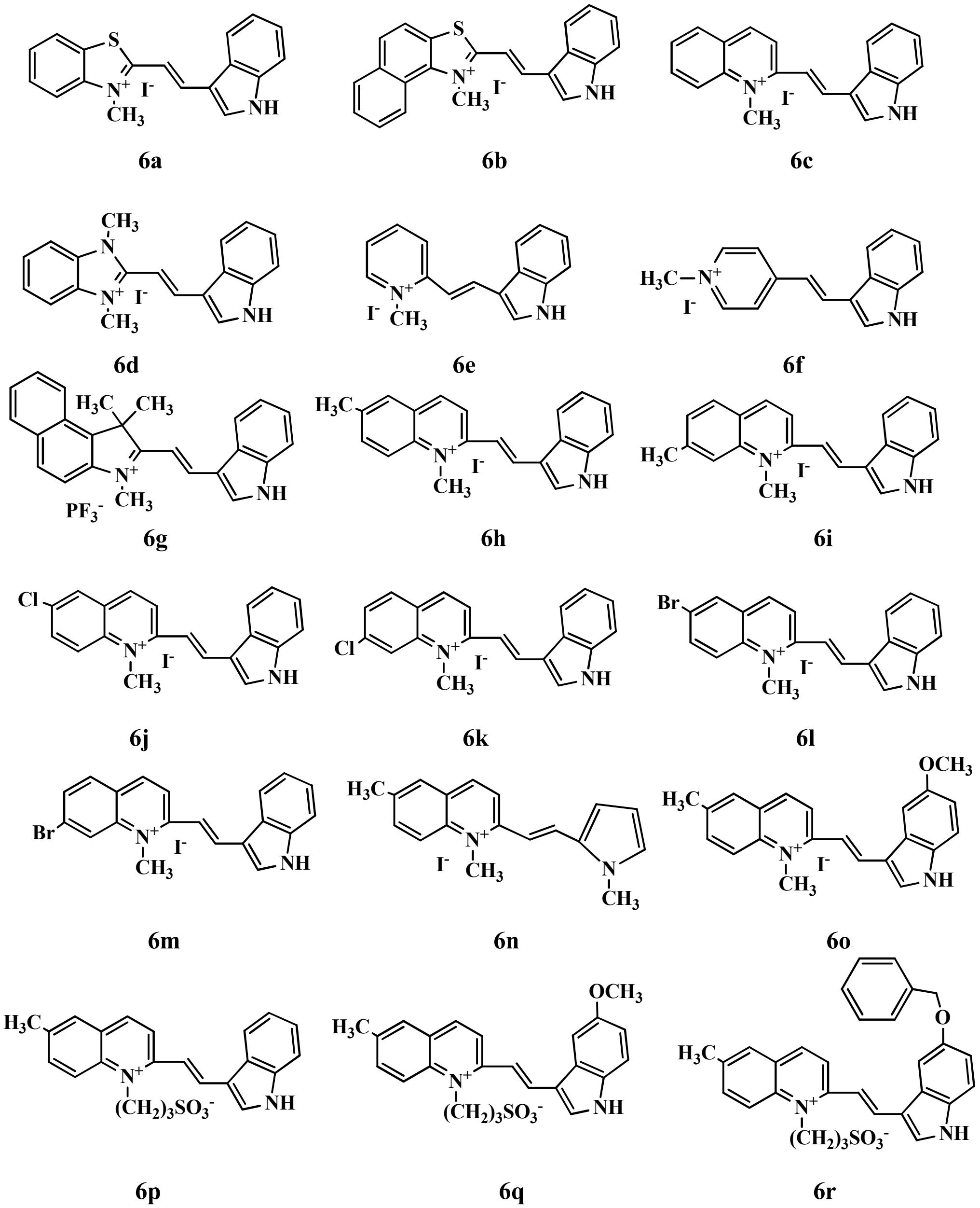


Fig. 8. Chemical structures of the precursor molecules **(5a-d)**



6a 6b 6c

6d 6e 6f

6g 6h 6i

6j 6k 6l

6m 6n 6o

6p 6q 6r

Fig. 9. Chemical structures of the target cyanine dye **(6a-r)**

1.5. Applications of Cyanine Dyes

Cyanine dyes are a versatile class of fluorescent dyes with a wide range of applications in various fields. Their unique optical properties, such as high molar absorptivity, strong fluorescence, and photostability, make them valuable tools in research, industry, and medical fields. Here are some of the diverse applications of cyanine dyes.

1.5.1. Fluorescence Imaging

Cyanine dyes are widely used as fluorescent probes in cellular imaging. They can label specific cellular structures, proteins, or nucleic acids, allowing researchers to visualize and study the dynamics of living cells under a fluorescence microscope. On the other hand, cyanine dyes are employed for in vivo imaging. They can be used to track and visualize biological processes, such as tumour growth, by labelling specific targets [73].

1.5.2. Molecular Biology

Cyanine dyes, such as SYBR Green and SYTOX series, are commonly used for nucleic acid (DNA and RNA) staining in molecular biology. They are employed in techniques like gel electrophoresis, real-time PCR, and fluorescence in situ hybridization (FISH). In addition, cyanine dyes can be used to label proteins for visualization and quantification in techniques like Western blotting and 2D gel electrophoresis [74].

1.5.3. Flow Cytometry

Cyanine dyes are employed in flow cytometry for cell sorting based on their fluorescent properties. This is crucial in various biological and medical applications, including cancer research and immunology [75].

1.5.4. Material Science

Cyanine dyes can be used to label nanoparticles, facilitating their tracking and analysis in material science research. This is particularly useful in studying the behaviour of nanomaterials in biological systems [76].

1.5.5. Photodynamic Therapy

Some cyanine dyes exhibit photosensitizing properties, making them suitable for use in photodynamic therapy (PDT) for cancer treatment. These dyes can generate reactive oxygen species upon exposure to light, leading to targeted destruction of cancer cells [77,78-83].

1.5.6. Chemical and Environmental Sensing

Cyanine dyes can act as pH indicators, changing their fluorescence properties in response to variations in pH. This makes them valuable tools in pH sensing applications. Additionally, certain cyanine dyes can be utilized for detecting specific ions, such as metal ions, making them applicable in environmental monitoring and chemical analysis [38, 39].

1.5.7. Optical Materials and Data Storage

Some cyanine dyes are used as laser dyes in the development of lasers for various applications, including telecommunications and scientific research [19]. In addition, cyanine dyes have been explored for use in optical data storage systems due to their ability to absorb and emit light at specific wavelengths.

1.5.8. Textile Dyeing

cyanine dyes play a crucial role in the textile industry by offering a combination of vibrant colours, stability, and versatility. These qualities enable manufacturers to produce textiles that meet aesthetic expectations while ensuring durability and longevity, contributing to the overall quality of dyed fabrics in the market [57].

2. Conclusion

Finally, this review goes into the world of cyanine dyes and their numerous applications, providing a thorough overview of cyanine classifications and presenting solvent-free synthesis methodologies inspired by cutting-edge publications. The most recent advances in research have considerably increased our fundamental understanding and practical application of cyanine dyes in a variety of sectors, including the textile industry, molecular biology, material chemistry, and bioimaging. This review advocates for simple, efficient, high-yield, and practical one-pot procedures by opening the way for the microwave irradiation synthesis of novel heterocyclic moieties and associated cyanine dyes. Notably, the environmentally responsible decision to avoid using harmful organic solvents throughout the dye synthesis process using microwave irradiation emphasizes the green component of this approach, which aligns with eco-friendly concepts.

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Competing interests

The authors declare that there are no competing interests.

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