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Cyanine Dyes Containing Quinoline Moieties: History, Synthesis, Optical Properties, and Applications

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Abstract

Cyanine dyes carrying quinoline moieties are an important class of organic molecules that are of great interest for applications in many fields like medicine, pharmacology, and engineering. Despite their exceptional properties, such as stability, high molar extinction coefficients, and high pH-sensitivity, this class of dyes has been less analyzed and reviewed in the last few decades. Therefore, this review article focuses on discussing the history of quinoline compounds, various synthetic routes to prepare quinolinium salts and symmetrical and asymmetrical mono-, di-, tri-, penta- and heptamethine cyanine dyes, containing quinoline moieties, together with their optical properties and applications as photosensitizers in photodynamic therapy, probes in biomolecules for labeling of nucleic acids, as well as imaging agents.

Keywords

cyanine dyes; lepidine; NIR; quinaldine; quinoline

Introduction to Cyanine Dyes

Cyanine dyes are a class of organic functional dyes, distinguished by two nitrogen centers. The general structure of the dyes is shown in Figure 1. Cyanine dyes have conjugated systems represented by alternating single and double bonds in a chain. The chain is constituted of sp^2 -hybridized carbon atoms forming a conjugated bond. Conjugation between groups results in the displacement of π electrons and positive charge on the nitrogen atom.^[1]

The carbon chain makes cyanine dyes unique, giving them a more extensive absorption wavelength than the other known dyes. With the addition of each covalent bond in the chain, the absorption of the dye is increased by about 100 nm. As a result, cyanine dyes can display

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Conflict of interest

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absorption and fluorescence in the range of the electromagnetic spectrum from the visible to infrared (Figure 2). The strong absorption of light at various wavelengths causes solutions of the cyanine molecules to become brightly colored. The stronger the absorption, the higher influence the molecule will have towards applications in various research areas.^[2-4] These dyes also tend to aggregate, which gives rise to a narrower absorption of light.

The name of the dye is specific to every compound, from the length of the chain between the two nitrogen atoms, and the number methine groups involved. If one group is present in the chain, the dye is called a monomethine cyanine. The dyes with 2, 3, 5, and 7 methine groups are called dimethine, trimethine, pentamethine, and heptamethine cyanine dyes accordingly.^[6]

Cyanine dyes can be structurally classified based on the nature of the end groups. Compounds with two heterocycles are called closed-chain cyanines, those with one terminal heterocyclic group and a non-cyclic end group are hemicyanines, and those without heterocyclic groups are defined as streptocyanines. These three classes of cyanine dyes are presented in Figure 3.

Each nitrogen atom is an independent part of the heterocyclic moieties, taking the form of indole, benzoxazole, benzothiazole, and quinoline. A variety of commonly used heterocyclic groups is illustrated in Figure 4.

If the heterocyclic moieties on both sides of the chain have the same structure, then the dye is called symmetrical. The electron density distribution of such dyes is also symmetrical. If the heterocyclic terminal groups differ from one another, then the dye is called asymmetrical.^[7]

There is one more type of cyanines in which two nitrogen groups are directly linked and have no methine groups, these are called apocyanine dyes. The only known compounds of this type of dyes are red Erythroapocyanine and Xanthoapo-cyanine as shown in Figure 5.^[8]

Charles Hanson Greville Williams synthesized the first cyanine dye in 1856. Williams obtained quinoline by distillation of cinchonine and heated the distillate with amyl iodide and an excess of ammonia, which resulted in “a magnificent blue color” compound, called Quinoline Blue, which is illustrated in Figure 6.^[9-10]

Williams noted that quinolinium salts tend to give intense colors on heating with silver oxide. But it was useless for dyeing fabrics because of its photo instability. Later in 1873, H. W. Vogel started to use them as photosensitizers in photography.^[11] This led to the synthesis of a series of photographic sensitizers, including Ethyl Red and Sensitol Red (Pynacyanole), shown in Figure 7. Since then, the class of cyanine dyes has grown exponentially and they have been used in a wide range of applications. Another important dye, pseudoisocyanine (PIC; Figure 7), synthesized in 1936 by Jelley and Scheibe, was the first dye, possessing unique J-aggregation properties, that showed two absorption maxima.^[12]

In this review article, we focus on closed-chain cyanine dyes, where at least one heterocyclic moiety contains quinoline. The history, synthesis, optical properties, and applications of

symmetrical and asymmetrical mono-, di-, tri-, penta-, and heptamethine cyanines with quinoline moieties are discussed in detail.

Introduction to Cyanine Dyes Containing Quinoline Moieties

In the 1940s, acridine compounds were reported to have antibacterial results. Some simple aminoacridines had a wide range of applications before penicillin and became easily accessible to the armed forces in World War II. Moreover, in 1981, it was found that aminoacridines' affinity for DNA caused the poisonous effect on yeasts in culture.^[13] To understand the reasons for the acridine compounds' performance, the activity of recognizable moieties was examined. The quinoline and pyridine fractions were identified by the inspection of the acridine molecule. The cyanine fragment from the acriflavine molecule was also explored as an antibacterial agent and looked promising for in vivo studies (Figure 8).^[14]

The quinoline ring system has been widely used in medicinal and industrial chemistry.^[15–18] Quinoline has become an attractive scaffold for the development of new fluorescent reagents owing to its small molecular size and nitrogen's ability to form hydrogen bonds. A lot of drugs and natural compounds have had this moiety in their structures. For these reasons, quinoline-containing dyes are valuable and show great potential for various studies.^[19]

Quinolines (also known as benzo[*b*]pyridines, 1-azanaphthalenes, and benzazines) are heterocyclic aromatic compounds with one nitrogen atom. It consists of a benzene ring condensed with a pyridine ring at the 2,3-positions. First, quinoline was synthesized from coal tar in 1834 by a German chemist Friedlieb Ferdinand Runge. Coal tar remains the main source of commercial quinoline.^[20] The prime sources of quinoline are petroleum, coal processing, wood preservation, and shale oil.

In 1881, Skraup presented the classic quinoline synthesis by heating aniline with glycerol and with concentrated sulfuric acid with the subsequent addition of nitrobenzene (Scheme 1). The use of inexpensive starting materials in the synthesis of quinolines makes this method important despite the low yields and other practical shortcomings.^[21]

Alkylquinolines can be obtained from petroleum, coal tar, shale oil, soya bean, and green coffee.^[22] The quinoline derivatives can be found in various natural products like tecleabine, tecleoxine, quinine, ciprofloxacin, which are interesting for medicinal chemistry and biomedical uses, are obtained by the thermal degradation of natural organic compounds. For instance, furoquinoline alkaloids tecleoxine and tecleabine can be isolated from the aerial parts of the *Teclea nobilis* plant, which is used in traditional medicine in Africa as an analgesic and antipyretic and to treat gonorrhoea.^[23]

Toddaquinoline can be separated from the root bark of formosan *Toddalia asiatica*, which is used as a folk medicine in Asia.^[24] The bark of the cinchona tree was used to isolate quinine in 1820 to treat malaria and babesiosis.^[25] Quinoline-containing drugs have been used to treat malaria for a long time, and some of them are presented in Figure 9. The highly cost-effective chloroquine was widely used in antimalarial therapy during World War II.^[26]

The most frequently used quinoline compounds are the 2- and 4-methyl derivatives, called quinaldine and lepidine, respectively.

Lepidine (4-methyl quinoline) is used in medicine, synthesis of dyes, and as a food additive. Lepidine can be obtained from the highest-boiling fraction of the coal-tar quinoline bases through addition of *o*-cresol compound. It can also be formed by reaction of aniline with methyl vinyl ketone in the presence of hydrochloric acid in ethanol or ethanolic ferric chloride or with 3-oxobutanal with a catalytic amount of concentrated sulfuric acid (Scheme 2).^[27]

Quinaldine (2-methyl quinoline, Scheme 3) is a useful ingredient in antimalarial drugs, in manufacturing dyes, food colorants, pharmaceuticals, and pH indicators. Quinaldine can be produced from aniline by various synthetic methods:

- a. The condensation of aniline with acetaldehyde under the influence of hydrochloric acid (Doebner–Miller synthesis). This reaction may also be completed in the microwave within several mins;^[27]
- b. The reaction of aniline with an aldehyde and pyruvic acid (Doebner synthesis);^[28]
- c. The optimized method—an aerobic oxidative aromatization of simple aliphatic alcohols and anilines by using a catalytic system containing 2,4,6-collidine, Pd(OAc)₂, and trifluoroacetic acid (TFA) with 93 % yield.^[29]

Quinoline is a weakly basic compound, as the lone pair of electrons on nitrogen does not participate in the formation of a delocalized π molecular orbital. As a result, quinolines can undergo nucleophilic substitution reactions and form various salts. These salts are an essential step in the synthesis of cyanine dyes. A lot of different types of salts have been synthesized and various methods have been optimized.^[30–34] There are over 180 published salts synthesized by various methods from quinaldine and almost 450 salts from lepidine.^[28]

The common methods for the synthesis of some lepidine- and quinaldine-based salts available in the literature are presented in Scheme 4 and Scheme 5. The identical salts are shown to compare the conditions used for the preparation of quinoline-containing salts. Most of them are synthesized from lepidine and quinaldine directly, for example, salts **1–6** (Scheme 4) and **8–13** (Scheme 5), whereas some of them require a different initial reagent, such as diphenylamine for the synthesis of salts **7** (Scheme 4) and **14** (Scheme 5).^[30, 31, 33–48]

Winstead's group presented an alternate synthetic route for salts **1a** and **2** in 2013. This technique uses a microwave and allows the reaction time to be decreased to 5 min and production of the target quinolinium salts with no purification necessary and with higher yields above 70 %.^[42]

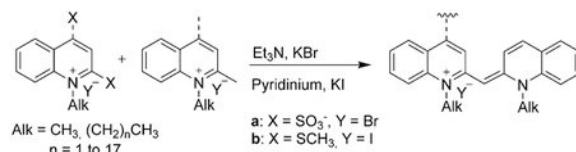
Further, the detailed synthesis of each class of cyanine dyes containing quinoline moieties, their optical properties, and applications in biomedicine were discussed.

Synthesis of Monomethine Cyanine Dyes Containing Quinoline Moieties

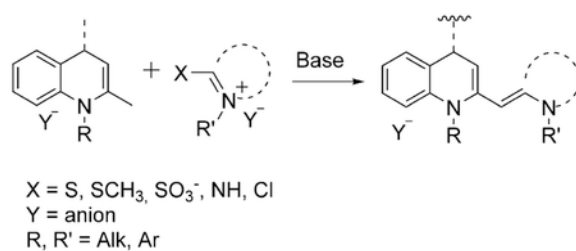
Monomethine dyes have only one methine unit in the conjugated chain between heterocycles. They absorb in the visible region at 450–520 nm. Commonly, monomethine cyanine dyes are synthesized by the condensation of two heterocyclic quaternary salts with a basic reagent.^[49, 50]

One of the methods to synthesize symmetrical monomethine cyanines is carried out by the condensation of sulfobetaines from *N*-alkylheterocyclic compounds with quaternary salts of heterocyclic 2- or 4-methyl compounds in the presence of triethylamine. Another method is performed upon heating of 2-methylthioquinolinium salt and quinaldine salts in pyridine at reflux. Both routes are shown in Equation (1).^[51]

The majority of synthesized monomethine dyes are found to be asymmetrical, so the heterocyclic moieties are usually different. One of them should have a good leaving group, and the reaction should be run in the presence of a base, such as triethylamine or pyridine. Asymmetrical monomethine cyanines have shown very high binding constants, high molar absorptivities and quantum yields, and the generation of high fluorescence signals upon binding. General synthesis of monomethine cyanine dyes containing quinoline, where X is a leaving group, and Y is an anion is shown in Equation (2).



(1)

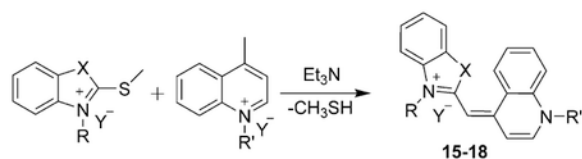


(2)

Most approaches towards the preparation of these monomethine dyes function under the principle where quaternary salts of heterocyclic compounds are catalyzed by heat in the presence of a basic compound. Two well-known asymmetrical dyes Thiazole Orange (TO) and Oxazole Yellow (YO) are synthesized by the reaction of 2-methylthiobenzothiazole (TO) or 2-methylthiobenzoxazole (YO) salts with quaternized heterocyclic salts containing an active methyl group in the presence of triethylamine.^[37, 49] The various TO and YO related monomethine dyes containing different side chains and anions **15–18** are shown in

Equation (3). The alternate method of synthesis of TO monomethine dyes uses microwave-assisted synthesis, decreasing the reaction time to 10 min and improving the yield to 90 %.^[37] But both methods have some disadvantages owing to the toxicity of released methyl thiol.

In 1995, Deligeorgiev and co-workers offered another method of TO and YO monomethine dyes synthesis, which avoided the evolution of methyl thiol.^[52] The new synthetic route is based on the condensation of 2-iminobenzothiazolines with 1-alkyl-4(or 2)-methylquinolinium salts with no solvents and are presented in Scheme 6.^[51] In 1999, he also offered an option to heat an *N*-alkylheterocyclic compound together with the quaternary salt of a heterocyclic 4-methylquinoline either in solvents or by melting of the starting compounds under vacuum at 150–220°C. Dyes **19** and **20** are examples of compounds synthesized by these methods with good yields above 60 %.^[52]

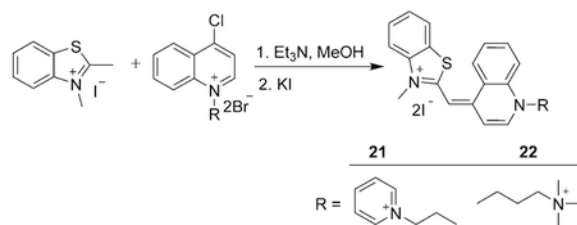


	15 TO	16 TO-PRO-1	17 YO	18 YO-PRO-1
X	S	S	O	O
Y	I	2I	I	2I
R	CH ₃	CH ₃	CH ₃	CH ₃
R'	CH ₃	(CH ₂) ₃ N ⁺ (CH ₃) ₃	CH ₃	(CH ₂) ₃ N ⁺ (CH ₃) ₃

(3)

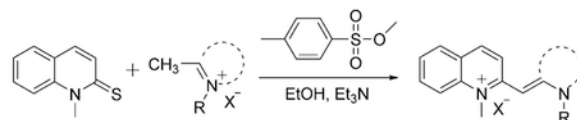
Another pathway for the production of a monomethine dye involves the reaction of *N*-alkyl-2-methylbenzothiazolium salt with 4-chloroquinolinium salt. This method also avoids the formation of toxic volatile byproducts. This synthetic route can be used for the preparation of dicationic benzothiazole cyanine dyes **21** and **22** [Eq. (4)]. The goal of increased cationic charge is to increase the water solubility of the dyes.^[52]

Fei et al. offered a new method of synthesis TO and its derivatives by using solid-phase resin support. The schematic representation of the preparation of TO and its derivatives is shown in Scheme 7. The first step is the attachment of 2-mercapto-benzothiazole to the Merrifield resin, and then the formed intermediate **23** reacts with 4-methylbenzenesulfonate to form *N*-methyl-benzothiazole salt **24**. The final step involves the condensation of the *N*-methyl-benzothiazole salt with lepidine derivatives forming cyanine dyes **15**, **25–27** (Scheme 7) in high yields above 95 % and with excitation wavelengths at around 570–600 nm.^[53]



(4)

The majority of published monomethine dyes are synthesized from lepidine salts. However, various asymmetrical monomethine dyes have been synthesized from quinaldine and thiazole salts or two quinolinium salts connected at the 2- and 4-positions. Asymmetrical compounds **28–34** in Figure 10 are synthesized by the condensation of two salts in the presence of methyl *p*-toluenesulfonate and triethylamine as shown in Equation (5).^[54]



(5)

Compounds **28–34** (Figure 10) were synthesized in yields range from 18 to 50 %. Dyes **32** and **34**, which differ only by the bridge attachment position were synthesized in 19 % and 45 % yields and showed excitation wavelengths at 522 nm and 557 nm, respectively.^[54] Dye **35** was excited at the same wavelength as dye **34**.^[55]

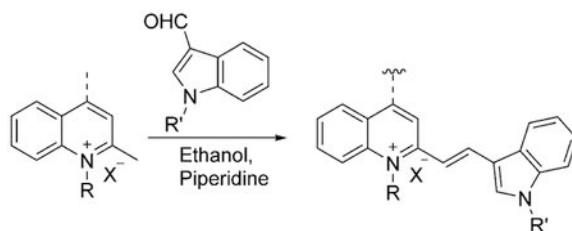
Synthesis of Dimethine Cyanine Dyes Containing Quinoline Moieties

Dimethine cyanine dyes have two methine carbons in a chain and absorb in the visible range at 450–620 nm. As well as monomethine cyanines, dimethine cyanines have been found to be mostly asymmetrical and do not differ much in terms of synthetic methods. Compared with monomethine cyanine dyes, dimethine and other polymethine cyanine dyes are more environmentally friendly to synthesize, avoiding the formation of thiol molecules.^[15]

Only two available symmetrical dimethine cyanine dyes were found, which were synthesized by Kuz'mina et al. in 2006.^[56, 57] Dyes **36** and **37** contain several cations and anions and are prepared in two steps with the formation of intermediate **35**, which is subsequently quaternized by ethyl 4-methylbenzenesulfonate or 3-bromopropylamine hydrobromide and treated by concentrated perchloric acid. The synthesis is presented in Scheme 8.

The commonly used synthesis of asymmetrical dimethine dyes includes the reaction of a heterocyclic quaternary salt with an active methyl group at the 2- or 4-position with an aromatic heterocyclic aldehyde. Ethanol with piperidine has become the most often

used conditions for making dimethine cyanine dyes [Eq. (6)], showing the highest yielding results.



(6)

The method, shown in Equation (6), has been improved by microwave irradiation with no solvent used. This approach results in shorter reaction times and simple product isolation procedures with no toxicity or harm.^[32] There are some examples of dimethine cyanine dyes **38–48** available in the literature in which they are synthesized in ethanol with piperidine (Figure 11).^[15, 32, 58–61] Compounds **42–45** were synthesized by using a microwave in yields above 90 % in less than 5 min and with absorption maxima in the presence of DNA at 483–499 nm.^[32]

Synthesis of Trimethine Cyanine Dyes Containing Quinoline Moieties

Trimethine cyanine dyes have three methine carbons in the bridge and absorb in the visible range at 450–620 nm. The first trimethine quinoline-containing cyanine dye was symmetrical and described in 1920 by Mills and Pope and by Wise, Adams, Stewart, and Lund. Symmetrical dye 1,1-diethyl-2,2-carbocyanine iodide, also known as Pinacyanol or Sensitol Red (Figure 12), was prepared from two molecules of quinaldine ethyl iodide and formaldehyde. The same method was used to synthesize another symmetrical 1,1-diethyl-4,4-carbocyanine iodide, called kryptocyanine. Another synthesis method uses ethyl orthoformate in the presence of acetic anhydride instead of formaldehyde.^[9]

One more synthetic route to synthesize symmetrical trimethine cyanines includes the reaction of lepidine salts with triethoxymethane. The intermediates **51** and **52** were obtained in 26 % and 19 % yields. The resulting cyanine iodide **51** and its tosylate **52** were treated with lithium bis(perfluorobutylsulfonyl)-imide, forming new trimethine cyanine dyes **53** and **54** (Scheme 9).^[62]

Usually, for the synthesis of asymmetrical trimethine cyanine dyes, one molecule of quaternary salt is used to form a hemicyanine intermediate, which then reacts with another salt molecule to gain the asymmetrical dye. Triethylamine and pyridine are the most used bases. Two of the most famous trimethine asymmetrical cyanines are TO-3 (**55**) based on Thiazole Orange (TO) and YO-3 (**56**) based on oxazole yellow (YO) with three carbons in a chain, and their dicationic derivatives TO-PRO-3 (**57**) and YO-PRO-3 (**58**) are presented in Figure 13.

TO analogs have attracted researchers' attention and different modifications and incorporations of TO-3 have been synthesized. Firstly, either quinoline or thiazole salt is connected to the linker. The formed hemicyanine **59** condenses with another quaternary salt and gains various alternatives of TO-3. Sun et al. synthesized the dye **61** with two positive charges, forming first the intermediate bromo-version **60** in 64 % yield. Then, the bromine atom in dye **60** is converted into a quaternary ammonium in dye **61** (53 % yield), as shown in Scheme 10.^[63]

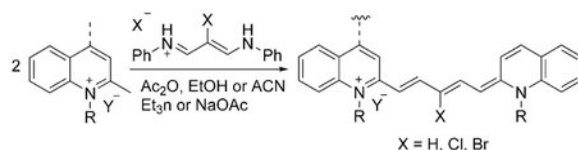
The indole moiety is often used instead of thiazole in asymmetrical cyanine dyes and may form Dimethyl Indole Red (DIR) **63**. The first step of **63** synthesis includes quaternary salt preparation by alkylation of lepidine with propanesultone, which is subsequently condensed with *N,N*-diphenylformamidine and forms hemicyanine dye **62** (40 % yield). The last step includes the formation of trimethine dye **63** (23 % yield) by condensation of the hemi-dye with methyl indoline bromide under basic conditions, as illustrated in Scheme 11.^[64]

Another approach to the synthesis of asymmetrical trimethine cyanine dyes with an indole moiety was accomplished by Fei's group (Scheme 12). Herein, the hemicyanine **64** is formed from the methyl indoline derivative, which then reacts with lepidine salt under basic conditions to form the asymmetrical cyanine dyes **65–68**.^[65]

The synthetic approach, presented in Scheme 12, has some disadvantages, such as the formation of intermediates and difficulties with the purification of dyes. To avoid these drawbacks, in 2005, Mason et al. offered some new approaches to synthesize quinoline-containing trimethine cyanine dyes, **72–76**.^[66] The synthesis of carbocyanine dye **72** is presented in Scheme 13 and involves two synthetic routes. The first approach includes the formation of hemicyanine **69**, which then is activated by sulfonylation with sulfonyl chloride polystyrene, forming **70**, which then reacts with quinolinium salt **2**. Unreacted hemicyanine remains on the resin, and the pure trimethine dye can be isolated. The second method involves the synthesis of polystyrene-bound immediate **71** from immobilized aniline, which is subsequently used to react with quinolinium salt **2** to form the dye **72** in 19 % yield.^[66] Figure 14 shows various dyes synthesized by this method; including both quinaldine (**73** and **75**) and lepidine, (**74** and **76**) based fluorophores.

Synthesis of Pentamethine Cyanine Dyes Containing Quinoline Moieties

Pentamethine cyanine dyes commonly absorb in the visible and near-infrared (NIR) region at 650–850 nm. The general synthetic procedure of symmetrical pentamethine dyes involves the use of malonaldehyde bis(phenylimine) derivatives under basic conditions and two moles of quinoline-containing salts as exemplified in Equation (7). Pentamethine fluorophores often have a precursor at the *meso*-position to improve their photophysical properties.^[67] *Meso*-substituents in the pentamethine chain usually belong to proton or halogen atoms.



(7)

The presented method in Equation 7 can be used to synthesize symmetrical pentamethine dyes **77–83** in good yields, which are illustrated in Figure 15.^[16, 67, 68]

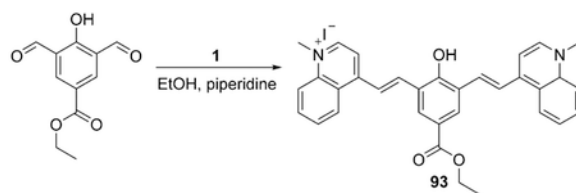
To synthesize asymmetrical pentamethine cyanine dyes, the first step is to prepare the hemicyanine intermediate, which then reacts with quinolinium salts under basic conditions. The same method as described earlier for trimethine synthesis was used by Mason et al. for the synthesis of pentamethine cyanines **86–90**, based on the use of solid-phase resin support (Scheme 14). The first step involves the reaction of malonaldehyde diacetal, forming the intermediate precursor **84**. The second step is the reaction of the received precursor with methyl indolinium salt to form hemicyanine **85**, which generates the pentamethine cyanine dye **86** in 60 % yield by subsequent reaction with quinolinium salts.^[66] The described method was used for the synthesis of dyes **87–90**, which are illustrated in Figure 16.

Synthesis of Heptamethine Cyanine Dyes Containing Quinoline Moieties

Heptamethine cyanine dyes usually absorb in the near infrared (NIR) region (750–900 nm) and contain seven carbons in a chain. Heptamethine dyes usually have substituents at different positions of the chain, as they can help to improve the photophysical and photochemical properties of the dyes. Streckowski and co-workers found that the introduction of a chloro-cyclohexenyl ring in the middle of the polymethine chain may enhance the dye's photostability.^[69] That is why the majority of synthesized heptamethine cyanines have a six-membered ring in the chain.

Chloro heptamethine cyanine dyes have traditionally been synthesized by the condensation of the quaternary ammonium salt and bisaldehyde or its equivalent with the subsequent addition of the second molar equivalent of the salt. The process usually takes place in acetic anhydride or ethanol with sodium acetate, triethylamine, piperidine, or pyridine. But sometimes a benzene/butanol mixture may be also used as the solvent (Scheme 15).^[70]

The cyclohexene moiety in the polymethine core can be replaced or modified with substituents instead of the chlorine atom with phenyls, or at any other position of the chain, as in dyes **93–97** [Eq. (8), Scheme 16]. The synthesis of symmetrical heptamethine dyes **93–97** implies the synthesis of 2 moles of quinolinium salts and 1 mole of the linker, which allows formation of the dye within one step.^[71–73] The resulting iodides **94** and **95** may be treated with sodium tetraphenylborate to form new fluorophores **96** and **97** in excellent yields (93–94 %).



(8)

The same synthetic route is used to synthesize asymmetrical cyanine dyes, but instead of 2 moles of salts, one salt differs from another one as illustrated in Schemes 17 and 18.^[17, 74] For the synthesis of fluorophores **99** and **101**, the first step is the preparation of hemicyanines **98** and **100** accordingly, which are heated at reflux with other salts under basic conditions.

The methine bridge may be modified and have substituents at different positions in the chain. Štacková et al. offered a new approach for the generation of heptamethine cyanine dyes with the introduction of substituents at the C3 position of the chain. The synthesis includes the condensation of 3-phenylpyridine and 2,4-dinitrophenyl *p*-toluenesulfonate. The subsequent reaction of the resulting semi-product **102** with 3 equivalents of salt **9** in the presence of sodium acetate and 4-bromoaniline forms the final product **103** in 47 % yield (Scheme 19).^[72]

The synthetic routes of cyanine dyes containing quinoline moieties that were published in the literature are summarized and presented in the schemes and equations outlined above. The optical properties and recent applications of some selected fluorophores are further discussed in the next section of this review article.

Optical Properties and Applications of Cyanine Dyes Containing Quinoline Moieties

The first synthesized cyanine dyes were mostly used as photosensitizers in photography. In 1936, Scheibe and Jelley discovered pseudoisocyanine (PIC; Figure 7), which showed a new type of aggregation with two absorption maxima at 490 and 525 nm and the formation of a third peak in aqueous solutions with DNA at 580 nm (Figure 17). This type of dye was named J-aggregates, which may be formed through dispersion forces, induced by the high molecular polarizability. The J-aggregates have highly emissive properties, whereas a monomeric PIC shows no emission.^[75] Pseudoisocyanine has become one of the most investigated organic compounds owing to its unique properties and has been used in photographic processes as a component of signal processing light-harvesting materials.^[76] For many years, J-aggregates of cyanines have been used in silver halide photography and as photodetectors. The majority of known fluorophores, forming J-aggregates, have been designed for binding and staining the membranes of living cells.^[77]

The major application of monomethine cyanine dyes is as fluorescent labels for DNA and imaging probes for macromolecules, because of their fluorescence enhancement and

outstanding photophysical properties. Thiazole Orange (TO) **15**, Oxazole Yellow (YO) **17** [Eq. (3)], and their derivatives are used as fluorescent polymer base substitutions, imaging probes in biology, and DNA and RNA detection.^[64, 79–80] TO is an example of a dye that has favorable physical, optical, and biological staining characteristics with absorption wavelength at 502–510 nm both in methanol and with the addition of DNA, RNA, and protein. In the absence of DNA or RNA, TO has quite low fluorescence because of the absence of a binding site. TO is very sensitive, whether it is free or bound to the nucleic acid. In general, if dyes are sensitive to the environment and have high quantum yields, they are useful for imaging of biological and other systems.^[81] This property of the dyes has led to the development of “light-up probes” where TO is attached to the end of the probe strand.^[82]

YO is like TO, having a high enhancement of quantum yield after binding to DNA. The molecular structures of TO and YO often undergo modifications to achieve higher sensitivity, photostability, or imaging of bioactivity in living cells.^[83] Both TO and YO derivatives usually have quite low fluorescence, but become strongly fluorescent with high quantum yield after binding to biomolecules.^[84]

TO-PRO-1 and YO-PRO-1 can form the dimers TOTO and YOYO (Figure 18), which excite at 490 and 460 nm and emit at 530 and 510 nm, respectively, bounded to double-strand (ds)DNA in solution.^[85] The length of the linker between two TO monomers affects the quantum yield. As a result, TOTO exhibits a nucleic acid binding affinity 100 times greater than the TO monomeric form.^[86] TOTO causes a single-strand cleavage approximately five times less efficiently than YOYO. The binding of YO and YOYO to Bacteriophage T5 has been analyzed and has proved the usefulness of dyes as spectroscopic probes for T5, despite the YOYO association to the T5 phages being much slower than to DNA, whereas YO association is quite similar. YO shows very fast association to T5 within just 1 min.^[87]

Table 1 presents the spectral properties of two similar dimethine cyanine dyes, **38** and **48** (Figure 11), differing only by the position the linker attaches to the quinoline moiety (4-position for **38** and 2-position for **48**). According to the spectral data, both absorption and fluorescence wavelengths for dye **38** are higher than for dye **48** in four different solvents. Stokes shift values increase with the increase of solvent polarity, whereas the molar extinction coefficient of dye **38** is decreasing. The analysis of spectral characteristics of dyes shows that dye **38** has very low cytotoxicity, which is helpful for applications in biomedicine as fluorescent probes and in cellular imaging. The results of cell staining of dye **38** by incubation with A172 cells demonstrate that **38** may stain cytoplasm (Figure 19).

Another dimethine cyanine SLM **40** (Figure 11) was investigated as an excellent theranostic agent for diagnosis and therapy of Alzheimer’s disease (AD). This compound has been successfully applied to perform near infrared in vivo imaging of A β species in transgenic (Tg) and wild-type (WT) mice in vivo (Figure 20). Dye **40** strongly binds to the A β species in the brain of Tg mice, and as a result, the mice can recover from Alzheimer’s disease. Moreover, SLM-treated mice have shown a considerable reduction in each of oligomeric A β content and τ proteins in their brain. These findings prove that dye **40** is a good theranostic agent with in vivo effectualness for diagnosing and treatment of AD in mouse models.^[18]

Dye SLOH **41** (Figure 11) also shows potential as a therapeutic agent for Alzheimer's disease, inhibiting the aggregation of A β peptides and oligomers.^[88]

Intensity properties for labeling living cells with low cell cytotoxicity and little photobleaching are shown by dyes **42** and **43** (Figure 11).^[58] The report says that these dyes can be applied as fluorescent probes in flow cytometry and as double staining agents for measuring sperm cell viability.^[58] Figure 21 illustrates the fluorescence intensity profile of the sperm cells stained with dyes **42** and **43**.

As previously mentioned, Sensitol Red (Figure 7), a symmetrical trimethine cyanine dye, was widely used as a photosensitizer in photography and against staphylococci and *B. coli*. Another symmetrical trimethine cyanine dye Kryptocyanine (Figure 12) after study in red blood cell (RBC) membranes and isolation of mitochondria, shows the ability to be useful for photochemotherapy in tumors, as a light-activated cytotoxic agent, and for photodestruction of leukemic cells.^[89]

Dyes **55** (TO-3) and **57** (TO-PRO-3; Figure 13) are trimethine analogs of TO dyes **15** (TO) and **16** (TO-PRO-1; Equation (3)). Compared with TO-1, the TO-3 and TO-PRO-3 contain an extended carbomethine bridge that considerably shifts the absorption and emission to the red at 642 and 661 nm, respectively. Like TO, TO-PRO-3 strongly binds to nucleic acids with a high fluorescence enhancement. Moreover, the dye can bind to human cytoplasmic, and mitochondrial A-site RNAs.^[90] TO-PRO-3 is also used in the pretreatment of samples for gel or capillary electrophoresis, determination of viability and resistance to staining in experiments with several labels.^[90]

TO-PRO-3 (**57**, Figure 13), the same as TO-PRO-1 (**16**, Equation (3)), has a dimeric analog, TOTO-3 (Figure 22). TOTO-3 has the same absorption and emission wavelengths, strongly stains the cytoplasmic and nucleolar RNAs but weakly stains the nuclear DNA.^[91] YO-PRO-3 (**58**, Figure 13) has a dimeric analog YOYO-3 (Figure 22), which has the same optical properties and applications for DNA staining.

According to the collected properties of TO and YO dicationic monomers and tetracationic dimers (Figure 23), monomers' absorption and emission wavelengths correspond to the wavelengths of their dimers. TO derivatives' wavelengths, differ from YO derivatives by 22–30 nm. Stokes shifts of all considered dyes (Table 2) lie in the range 16–19 nm. Molar extinction coefficients increase with the extension of chain length and number of cations. The dimers have bright fluorescence signals and may be used as nuclear and chromosome counterstains for multicolor fluorescence labeling experiments and for staining nucleic acids on solid supports, such as microarrays.^[92]

Another trimethine dye **63** Dimethyl Indole Red (DIR; Scheme 11), exhibits a weak visible fluorescence when an excess quantity of calf thymus (CT) DNA and artificial dsDNA is present, thereby making it potential for specific light-up probes of G-quadruplexes emitting long wavelength.^[59] The study of the conformational switch of G-quadruplex structure within the absence and presence of **63** has been explored by circular pleochroism (CD) spectroscopic analysis. The CD spectra of the parallel G-quadruplex exhibits a specific positive peak at 265 nm and a negative peak at 240 nm (Figure 24). Additionally, no elicited

CD signal peaks within the wavelength longer than 350 nm can be detected. The probe DIR (**63**) has a negligible impact on the production of G-quadruplex structures. Such a G-quadruplex probe can disclose the distribution of intracellular shaped G-quadruplexes and avoid the controversy regardless of whether the detectable G-quadruplexes are present.

For fluorophores **65–68** shown in Scheme 12, the effect of various substituent groups on the absorption and emission maxima can be seen in Figure 25. Absorption maxima of dyes **65–68** (Figure 25A) lies between 599 and 603 nm, whereas absorption of dye **68** with R=CH₃ redshifts by 9–12 nm. In addition, intensities of Cl-substituted dyes **66** and **67** are higher than that of **65** (R=H) and **68** (R=CH₃).

According to Figure 25B, each curve has two peaks with the utmost emission wavelengths of indole quinoline dyes at 650 nm. The fluorescence intensity decreases in the order **67**>**66**>**65**>**68**. This means that the utmost peaks of dyes with electron-withdrawing groups redshift compared with those dyes with electron-donating groups. The second peaks were completely different, and the fluorescence intensity of **65** was stronger than the others.

The fluorescence spectra of compounds excited at 550 nm are illustrated in Figure 25C. All fluorophores show similar fluorescent characteristics. There is only one peak in each line, and the fluorescence intensities of all compounds are higher than those excited at 480 nm. Most emission wavelengths are set between 647 nm and 657 nm. The Stokes shifts of dyes **65–68** are all around 100 nm. The longer wavelength signs and larger Stokes shifts indicate low UV interference when labeling the organism.^[65]

Three identical symmetrical dyes, differing only by the number of carbons in the chain, such as monomethine Ethyl Red (Figure 7), trimethine Kryptocyanine (Figure 12), and pentamethine Dye **79** (Figure 15), have absorption wavelengths at around 600 nm, 700 nm, and 800 nm, respectively (Figure 26). Emission wavelengths also lie in the range 600–830 nm (Figure 26). Meanwhile, absorption and emission wavelengths for identical mono-, tri-, and pentamethine cyanines, PIC (Figure 7), Sensitol Red (Figure 12), and dye **83** (Figure 15), connected to the linker at the 2-position (instead of the 4-position) are 100 nm less for each one compared with the same ones at the 4-position and lie in the ranges 500–700 nm and 550–750 nm, respectively (Figure 27). This means that lepidine-containing cyanine dyes have higher excitation and emission wavelengths owing to the presence of an extra double bond between the nitrogen atoms of the heterocyclic rings.

Four other dyes differ by the position of the chain connection to the moiety (at the 2- and 4-positions) and substitution at the *meso* carbon. Comparison of two symmetrical 4-methylquinoline based pentamethine dyes with *meso*-substitution of H (**77**) and Cl (**78**) in the polymethine bridge is illustrated in Figure 15, resulting in the 32–90 nm difference in the UV/Vis wavelength in DMSO, buffer, and buffer/DNA (Figure 28). In the presence of DNA, dye **78** shows high stabilization, in contrast to dye **77**, owing to the presence of the electron-withdrawing chlorine atom. After the addition of CT DNA to dye **78**, the absorption maximum of the dye increases to 805 nm. As a result, dye **78** shows excellent DNA photocleaving properties. Likewise, this dye was used to treat ES2 cancer cells and

exposed to an 808 nm laser diode. The results showed the increase of intracellular reactive oxygen species (ROS) levels in the cells and the decrease of their viability (Figure 29).^[16, 94]

Fluorophores connected to the linker at the 2-position with *meso*-substitution of Br (**81**) and H (**82**) show similar results with stability in DMSO. With the addition of CT DNA, the absorbance of dye **81**, the same as **78**, stays constant, whereas dye **82** noticeably decreases even before DNA inclusion (Figure 30). The electron-withdrawing bromine atom allows the dye to stay stable with no autooxidation in aqueous solutions. After showing promising photocleaving properties, dye **81** was incubated with ES 2 ovarian carcinoma cells and shows ready uptake by cells and quick localization in the intracellular cytosolic and perinuclear regions (Figure 31).^[94] Also, dye **81** shows high phototoxicity, decreasing ES 2 cells viability under 694 nm light irradiation.

As was mentioned earlier, heptamethine cyanine dyes are near-infrared (NIR) absorbing and emitting. This allows them to be applicable in photovoltaics and nonlinear optics and in vivo cell imaging. Heptamethine dyes **96** and **97** (Scheme 16) were synthesized with a chlorine atom and phenyl at the *meso* carbon, respectively. The neat thin-film absorption spectra of dyes and in CH₂Cl₂ solutions are presented in Figure 32. Interestingly, that phenyl substitution leads to the blueshift in CH₂Cl₂ compared with chloride substitution, owing to the inductive effect, whereas the neat thin film leads to a redshift.

Heptamethine cyanine dye **99** (Scheme 17) has been investigated for application in dye-sensitized solar cells (DSCs). Figure 33 presents the absorption spectra of dye **99** and AN50 electrolyte as well as the transmission spectra of the DSC. Transmittance in the visible range 400–700 nm is achievable. Dye **99** has the high potential for application in DSCs sensitization.^[74]

Fluorophore **101** (Scheme 18) was analyzed by Shimizu and co-workers after encapsulation in a lactosome and evaluated as a NIR fluorescent probe for in vivo tumor imaging.^[17] The optical properties of dye **101** and its lactosome are shown in Table 3. The dye has a maximum emission wavelength in methanol at 932 nm, whereas its lactosome shows an emission maximum at 905 nm. Interestingly, the absorption maximum of dye lactosome in water is higher than of the dye itself in organic solvents. The excitation and emission wavelengths are higher for dye **101** in methanol. As for photostability, the absorbance of lactosome is stable within 1 h under tungsten lamp irradiation.

After injection of dye **101** lactosome into a mouse, the fluorescence intensity in the tumor region becomes visible and gradually grows 6 and 24 h after injection (Figure 34). The developed dye **101** lactosome probes, possessing low toxicity, good photostability, high fluorescence in the tumor, and low background signals in liver and muscle tissue, have been suggested for application in noninvasive in vivo imaging techniques to detect tumors.^[17]

Summary and Outlook

The cyanine fluorophores containing quinoline moieties are worthy of attention because of their optical properties such as stability, high molar extinction coefficients, high sensitivity,

and low aggregation. Therefore, they are useful for biomedicine, as they possess antiseptic and photocleaving properties.

They have always had widespread applications in various fields such as pigmenting and photosensitizing, biomedical and pharmaceutical, and as probes in biomolecule labeling, detection of nucleic acids and tumors, and potential imaging agents.

This review summarizes the synthetic procedures with different initial compounds with no solvent, using solid-phase resin support, or microwave irradiation. Even though the preparation of these dyes follows traditional methodologies, the synthetic routes are very efficient to produce these dyes in high yields.

Based on the valuable optical properties and various applications of these fluorophores, as presented and discussed in this review, it is evident that these dyes have great potential for many useful applications. With further development of cyanines containing quinoline moieties, the forthcoming improvements and refinements of their structures, it would be possible to widen the practical applications of these dyes based on their excellent properties.

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Biographies



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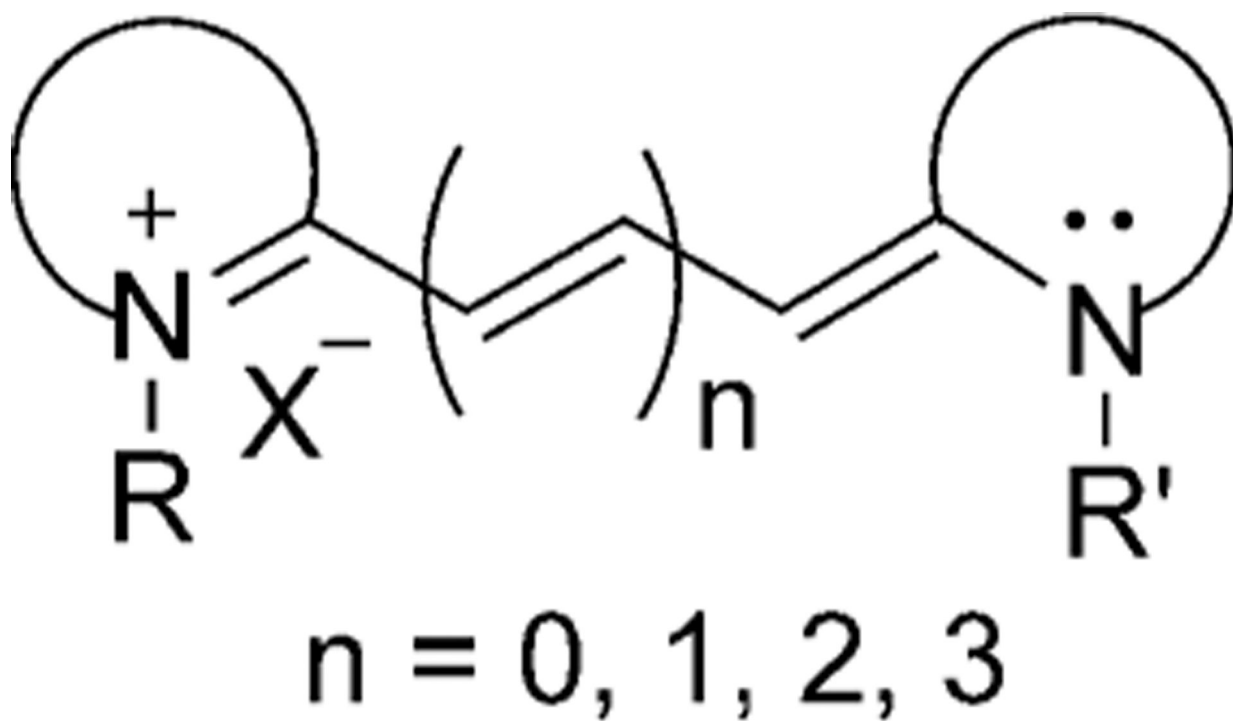


Figure 1.
General structure of cyanine dyes.

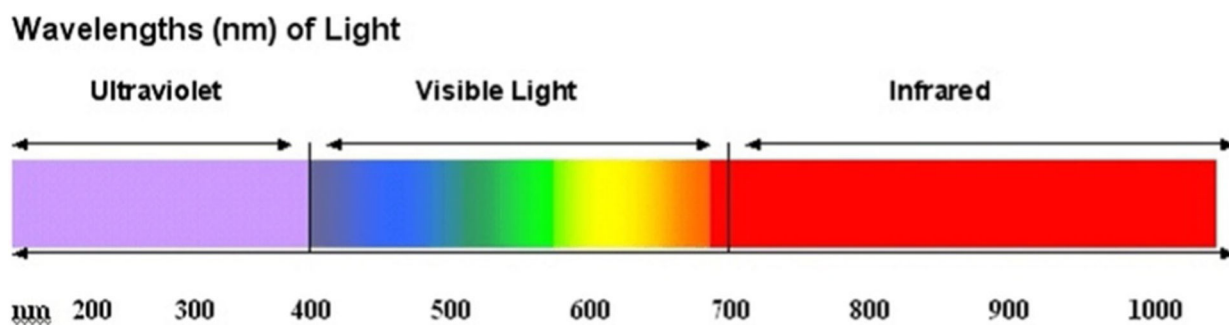


Figure 2.
Electromagnetic spectrum.^[5]

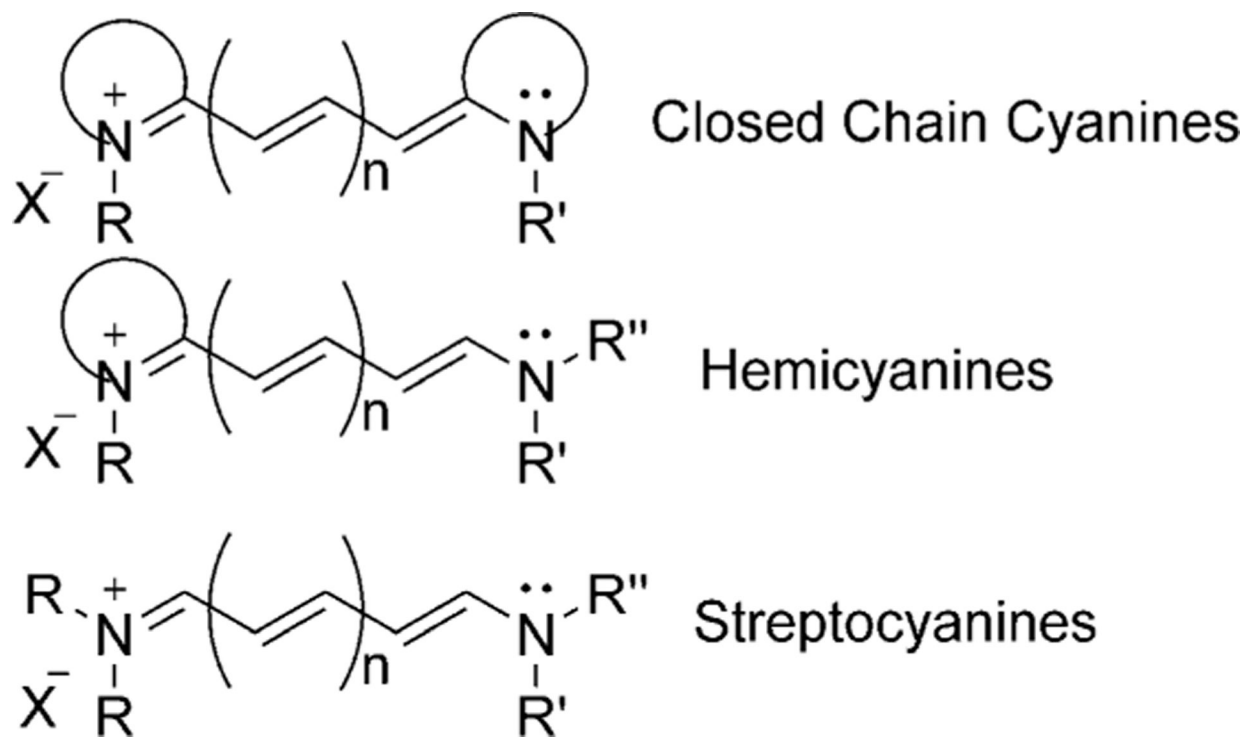


Figure 3.
Classification of cyanine dyes.

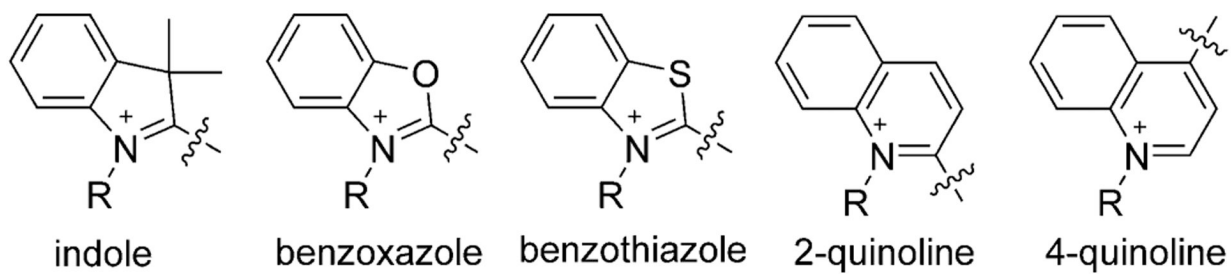
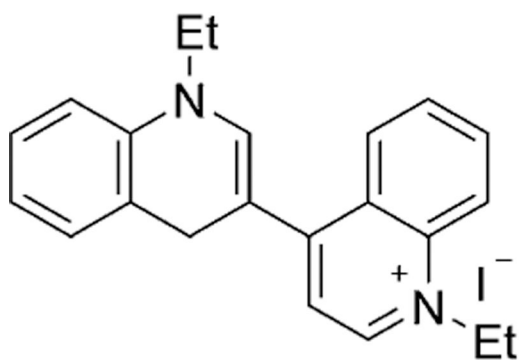
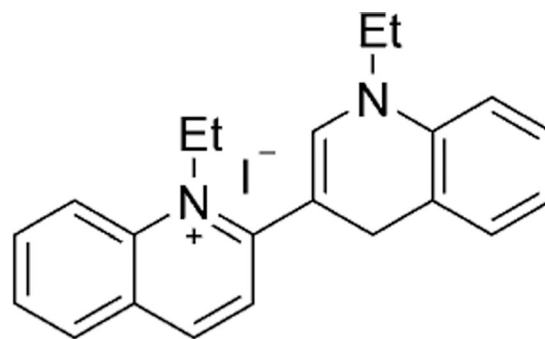


Figure 4.
Examples of heterocyclic moieties used to synthesize cyanine dyes.

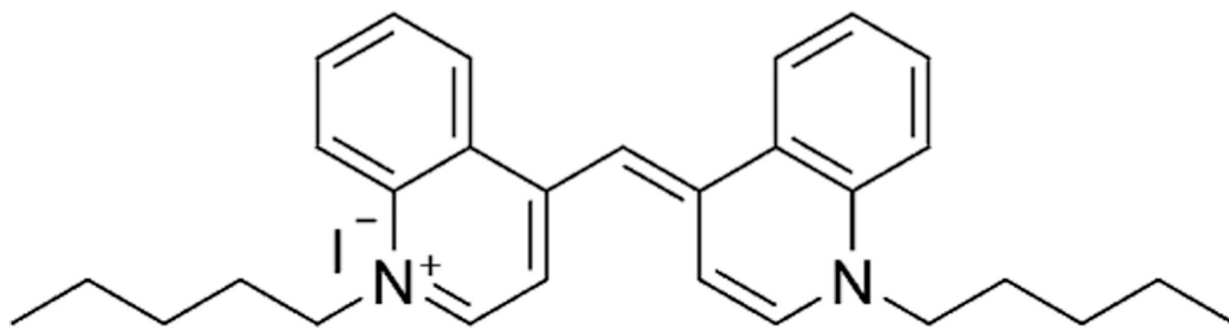


Erythroapocyanine



Xanthoapocyanine

Figure 5.
Structures of apocyanine dyes.



Quinoline Blue

Figure 6.
The structure of the first synthesized cyanine dye.

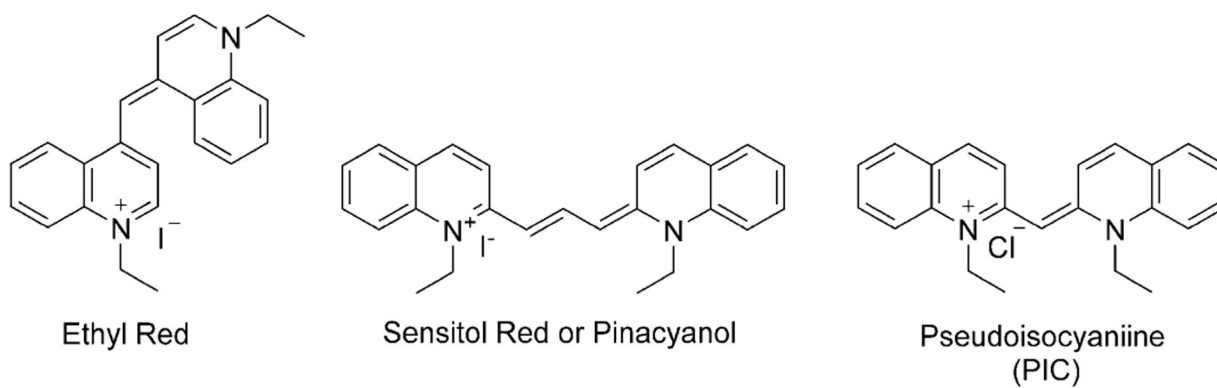


Figure 7.
Dyes used as photosensitizers in photography.

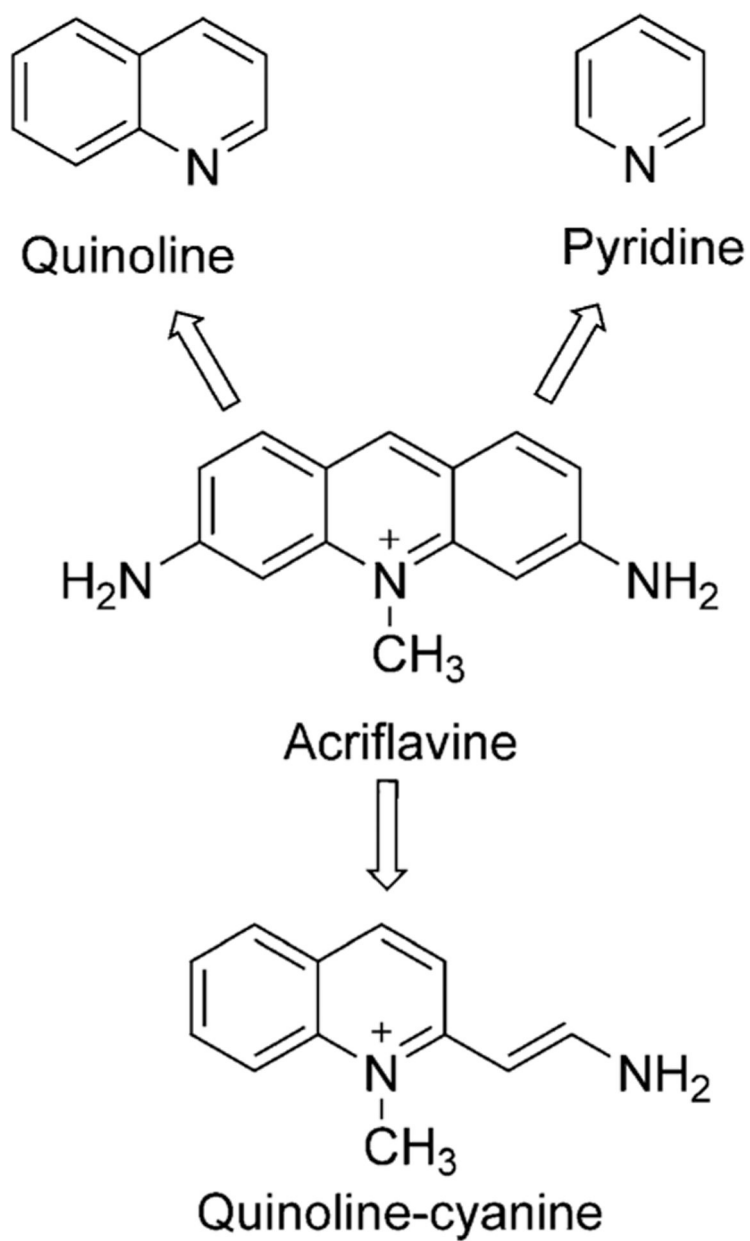


Figure 8.
Historic cyanine development from Acriflavine.

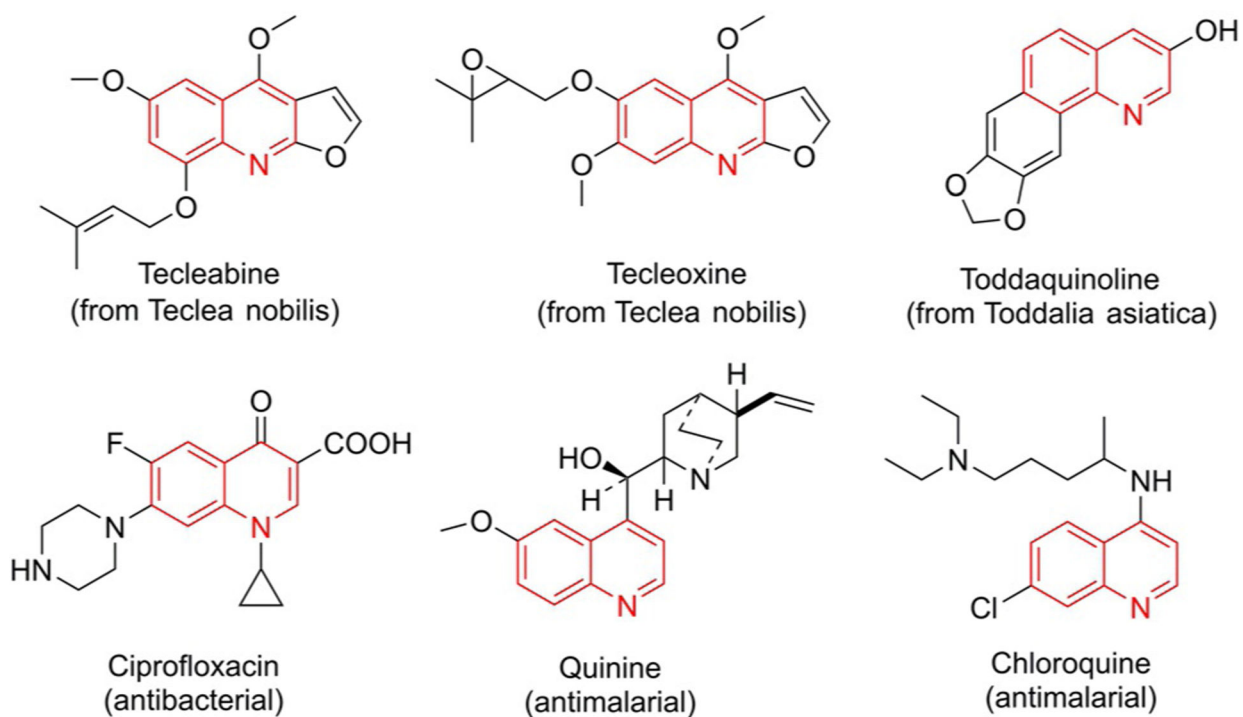
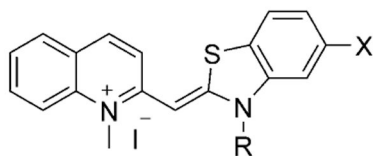


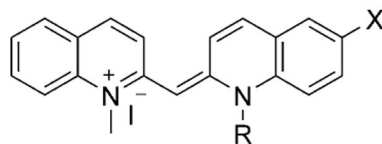
Figure 9.
Natural products containing quinoline nucleus.



15 (TO)

28: R = $(\text{CH}_2)_3\text{SO}_3^-$, X = Cl

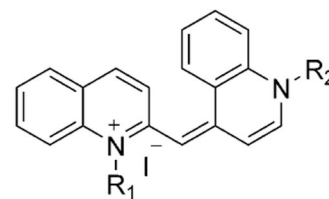
29: R = $(\text{CH}_2)_3\text{SO}_3^-$, X = CH_3



30: R = CH_3 , X = Br

31: R = CH_3 , X = OCH_3

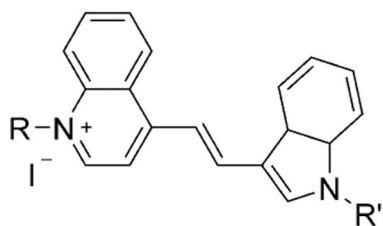
32: R = $(\text{CH}_2)_8\text{I}$, X = H



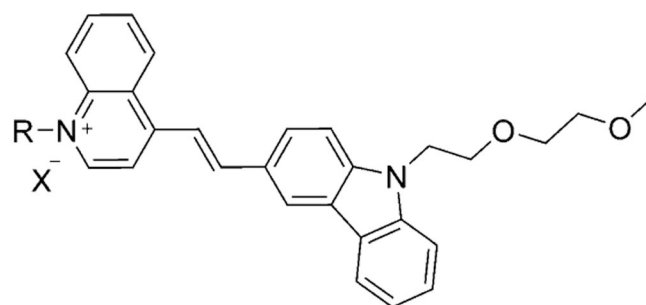
33: R₁ = CH_3 , R₂ = CH_3

34: R₁ = $(\text{CH}_2)_8\text{I}$, R₂ = CH_3

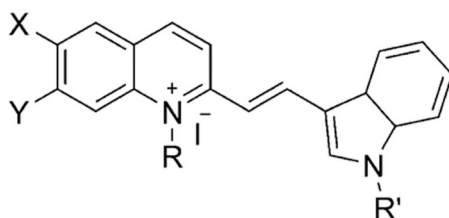
Figure 10.
Monomethine quinaldine-containing cyanine dyes.^[54, 55]



- 38:** R = CH₃, R' = CH₂Ph
39: R = (CH₂)₃N₃, R' = CH₃



- 40 (SLM):** R = CH₃, X = I
41 (SLOH): R = (CH₂)₂OH, X = Cl



- 42:** X = CH₃, Y = H, R = CH₃, R' = H
43: X = H, Y = CH₃, R = CH₃, R' = H
44: X = Br, Y = H, R = CH₃, R' = H
45: X = H, Y = Br, R = CH₃, R' = H
46: X = H, Y = H, R = (CH₂)₃OH, R' = H
47: X = H, Y = H, R = (CH₂)₃Ph, R' = H
48: X = H, Y = H, R = CH₃, R' = CH₂Ph

Figure 11. Structures of some known dimethine cyanine dyes, synthesized from lepidine and quinaldine.^[15, 32, 58–61]

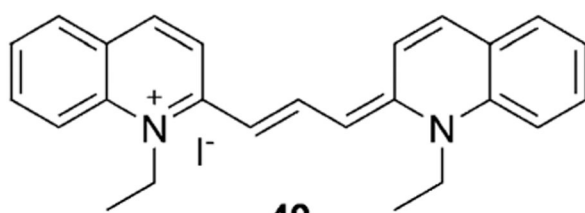
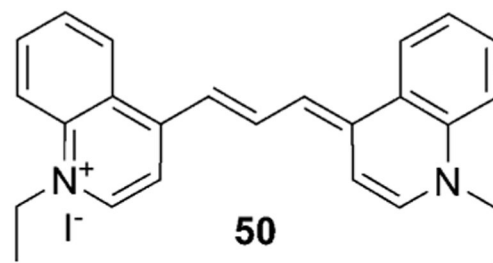
**49****Sensitol Red or Pinacyanol****50****Kryptocyanine**

Figure 12.
Structures of first symmetrical trimethine cyanine dyes containing quinoline moieties.

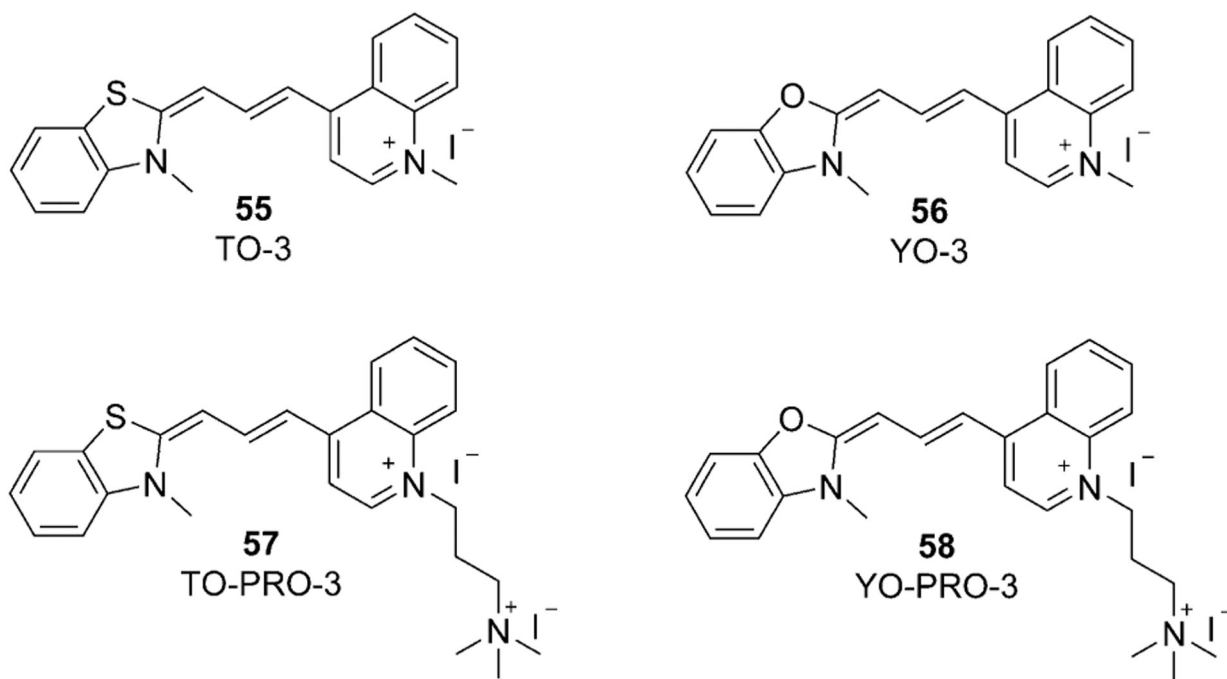


Figure 13.
Chemical structures of TO-3, YO-3, TO-PRO-3, and YO-PRO-3.

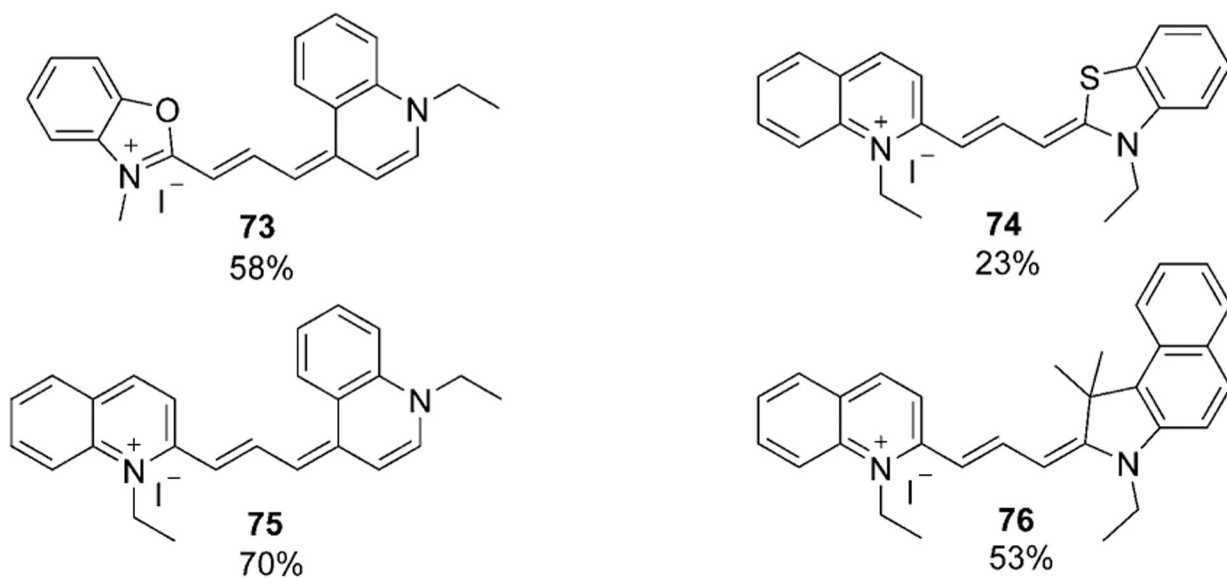
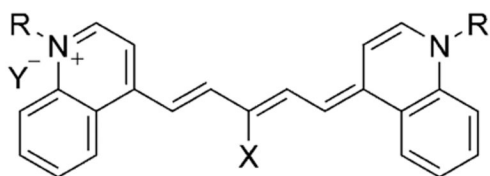
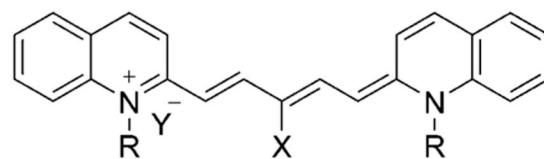


Figure 14.
Trimethine cyanine dyes synthesized by solid-phase method.



- 77:** R = CH₃, X = H, Y = I
78: R = CH₃, X = Cl, Y = I
79: R = C₂H₅, X = H, Y = I
80: R = (CH₂)₄SO₃⁻/(CH₂)₄SO₃Na, X = H



- 81:** R = CH₃, X = Br, Y = I
82: R = CH₃, X = H, Y = I
83: R = C₂H₅, X = H, Y = I

Figure 15.
Examples of symmetrical pentamethine cyanine dyes.^[16, 67, 68]

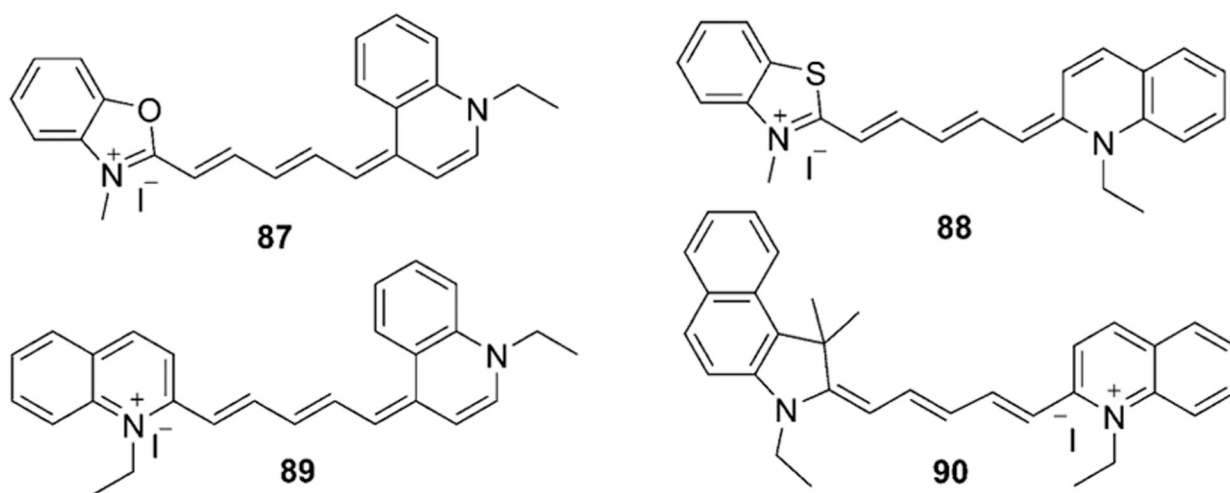


Figure 16.
Pentamethine cyanines synthesized by solid-phase method.

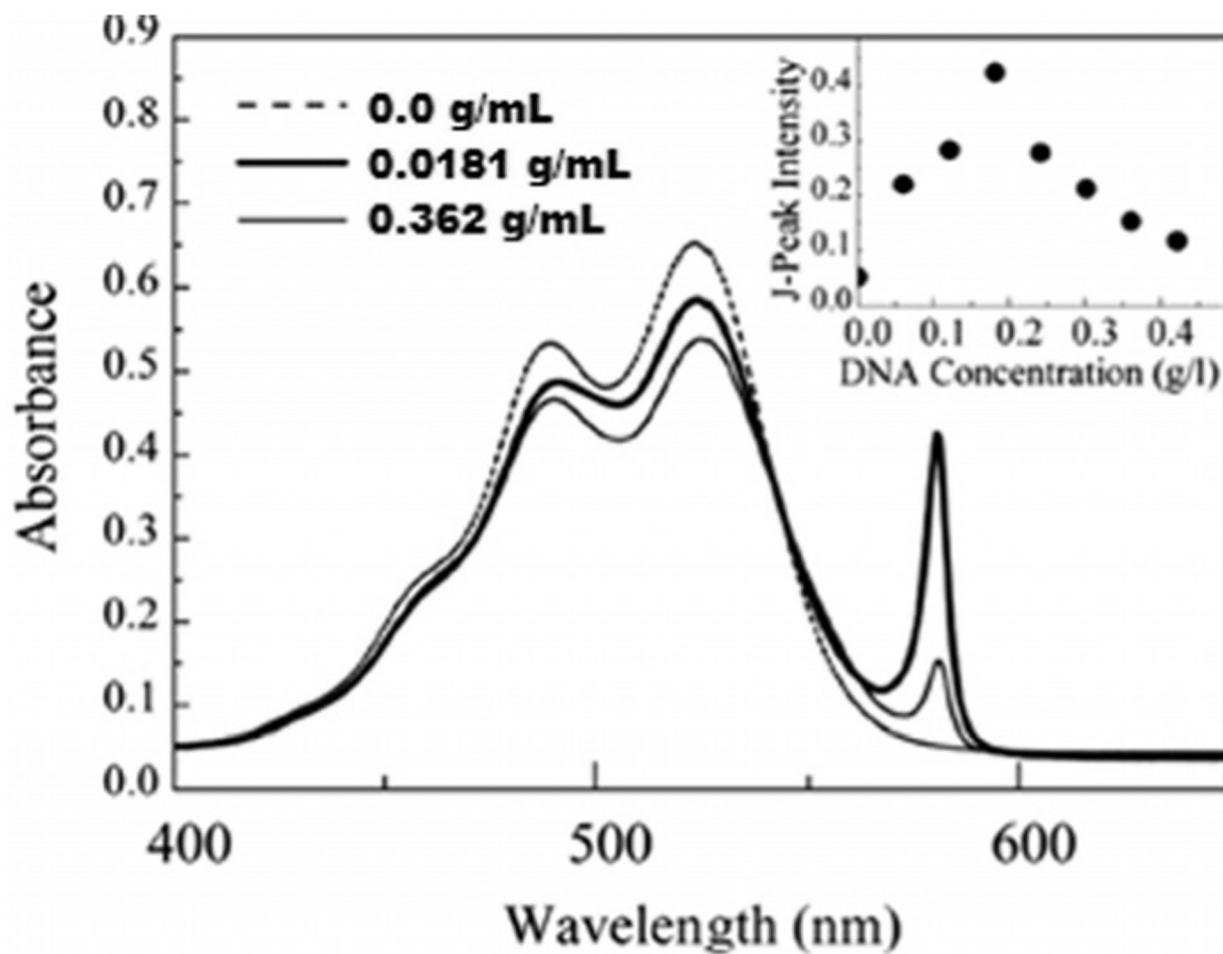


Figure 17. The absorption spectra of PIC in a mixture of water and methanol in the absence (dash) and the presence (solid) of DNA at different concentrations.^[78]

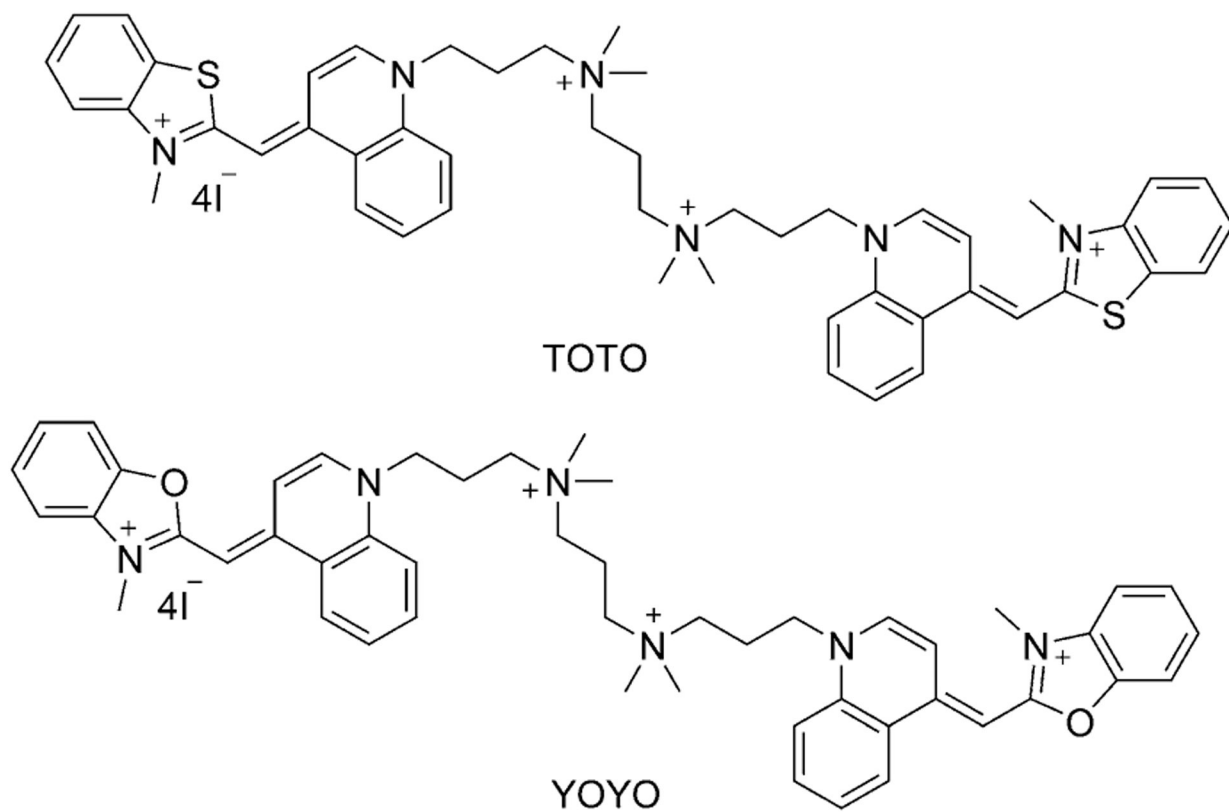


Figure 18.
Structure of TOTO and YOYO dimers.

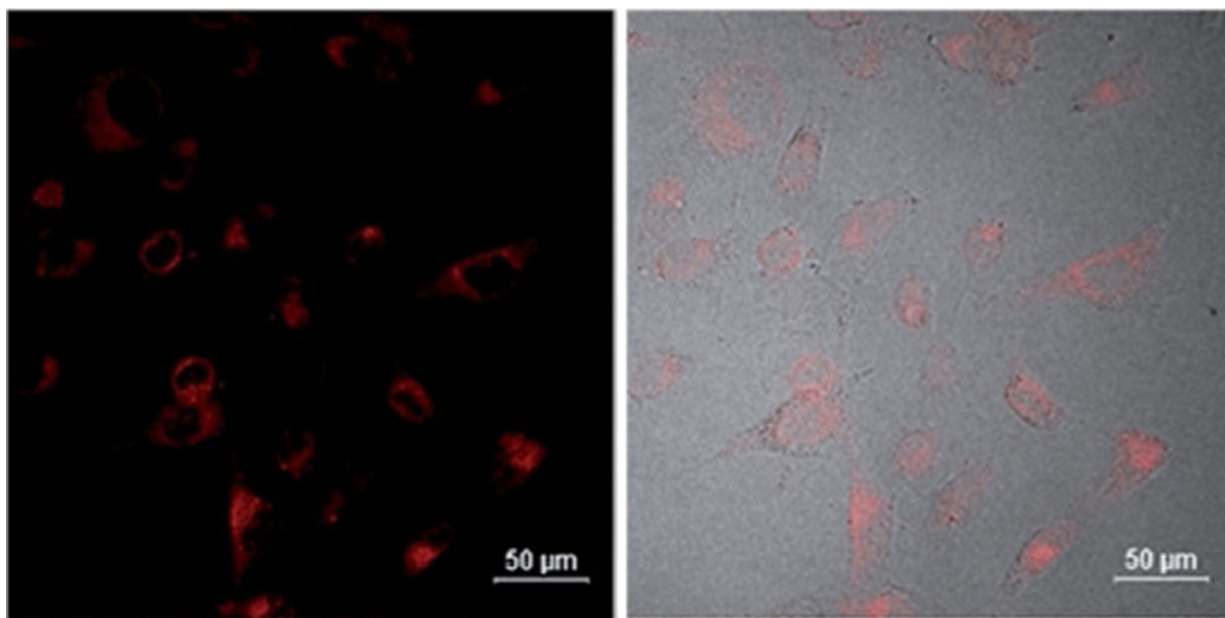


Figure 19. Confocal fluorescence microscopic images of A172 cells incubated with dye **38** at concentration $20 \mu\text{molL}^{-1}$ at 37°C for 10 min.^[15]

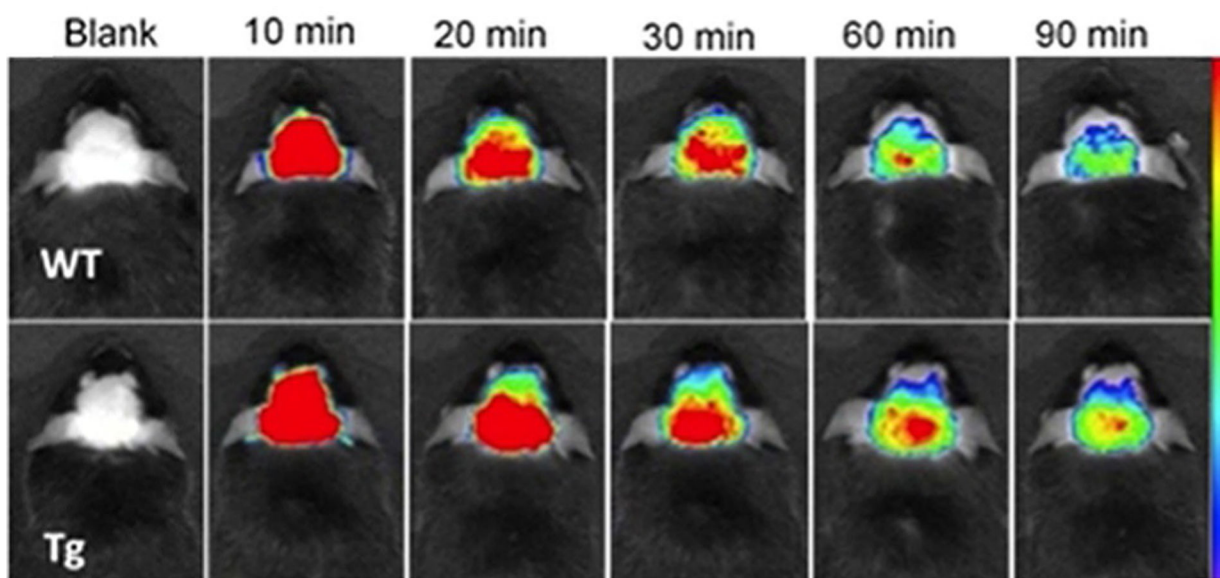


Figure 20. Fluorescence images of Tg (9 months old) and wild-type mice at different time points before (blank) and after intravenous injection of SLM **40** (dosage of 5 mgkg^{-1}) with recordings at 10, 20, 30, 60, 90 min.^[18]

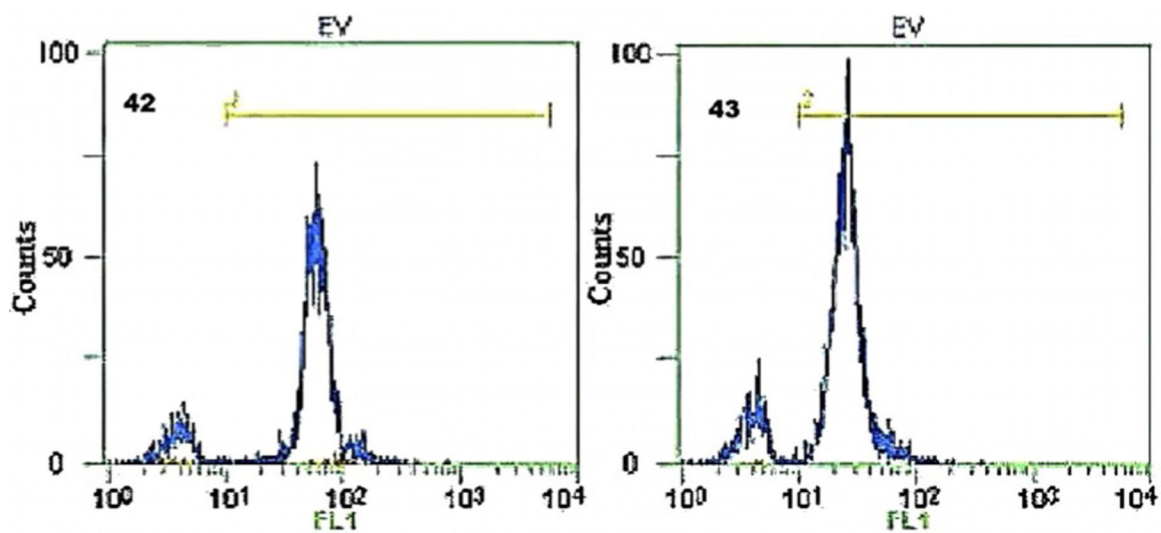


Figure 21. Flow cytometry histograms of dyes **42** and **43**. The x -axis presents the fluorescence intensity of stained sperm cells, and the y -axis shows the number of the stained sperm cells.^[58]

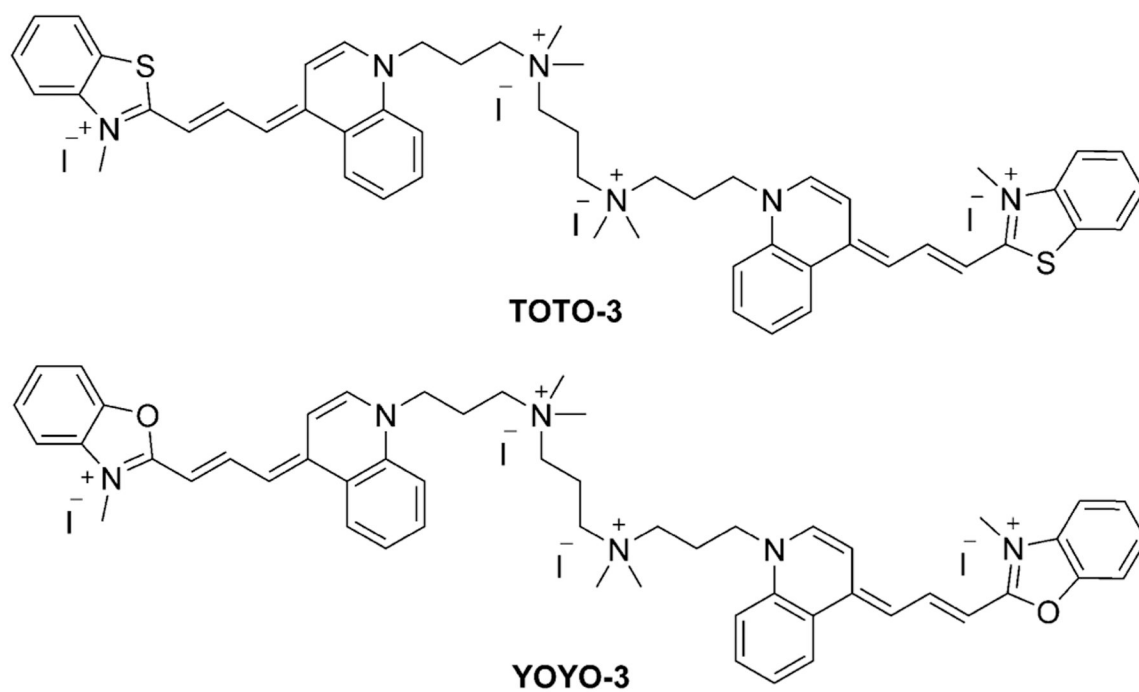


Figure 22.
Structure of the dimers TOTO-3 and YOYO-3.

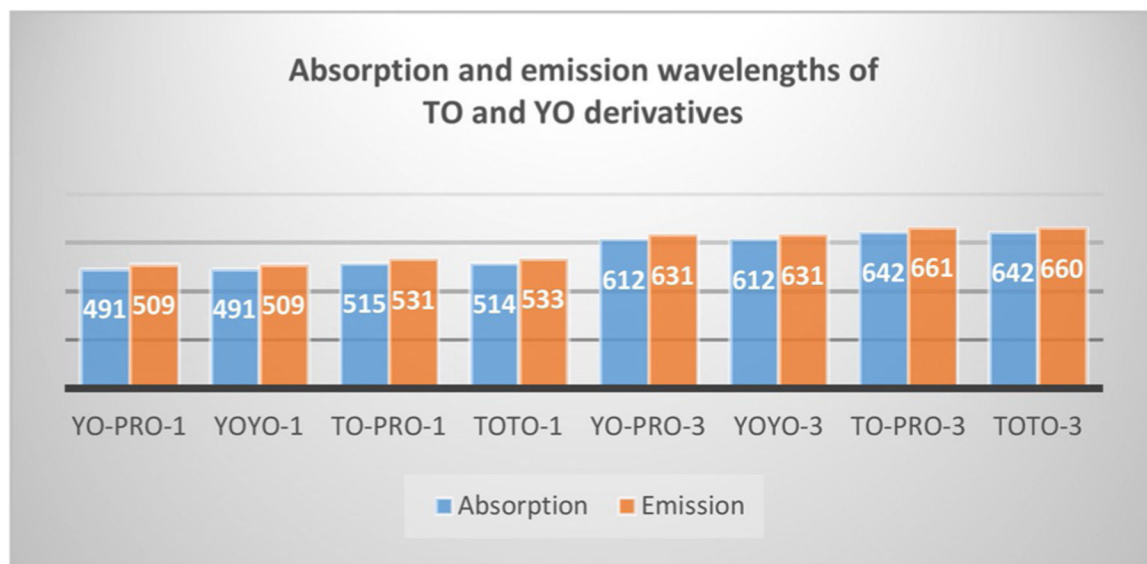


Figure 23.
Absorption and emission wavelengths of TO and YO monomers and dimers.

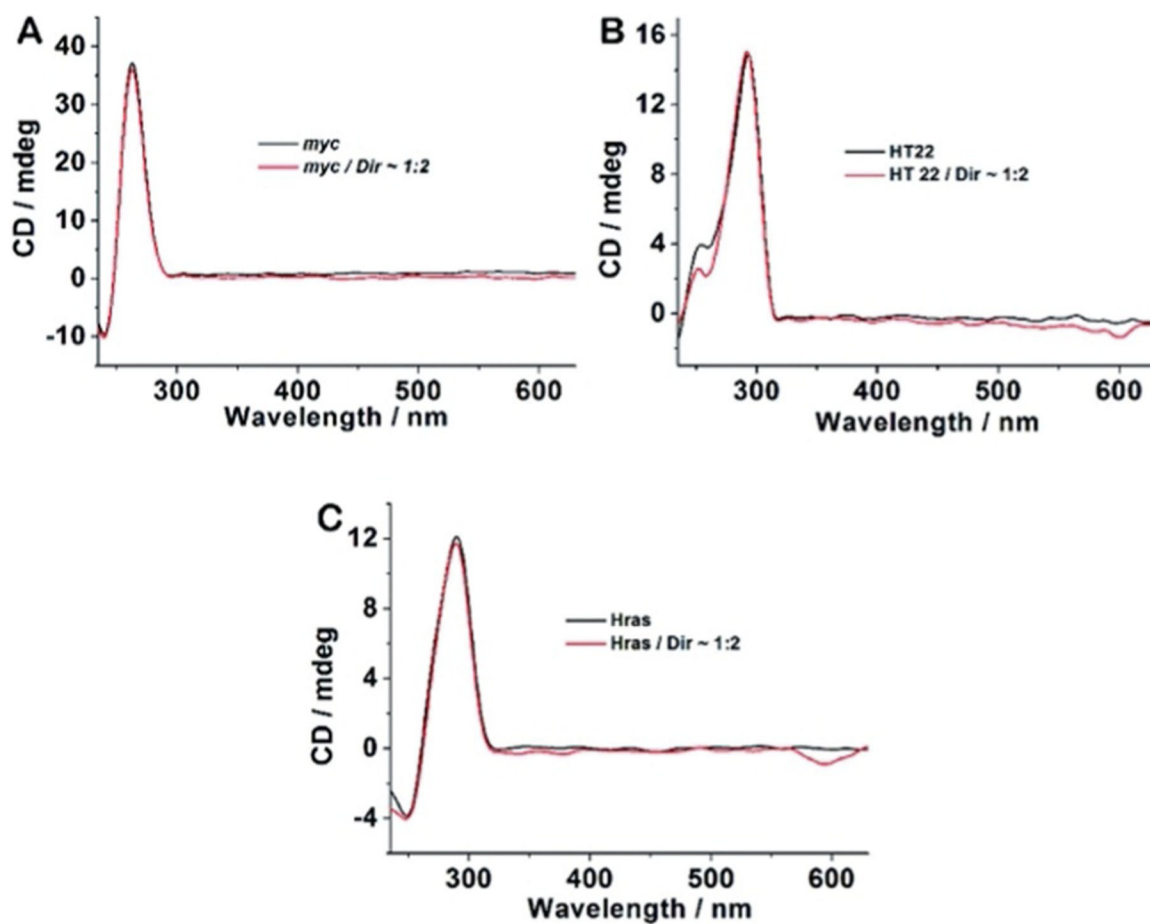


Figure 24. CD spectra of 4 μ m G-quadruplex-forming oligonucleotides c-myc (A), HT 22 (B), and Hras (C) in the absence and presence of DIR 63 (8 μ m) in 20 mM Tris-HCl buffer with 100 mM KCl, pH 7.4.^[93]

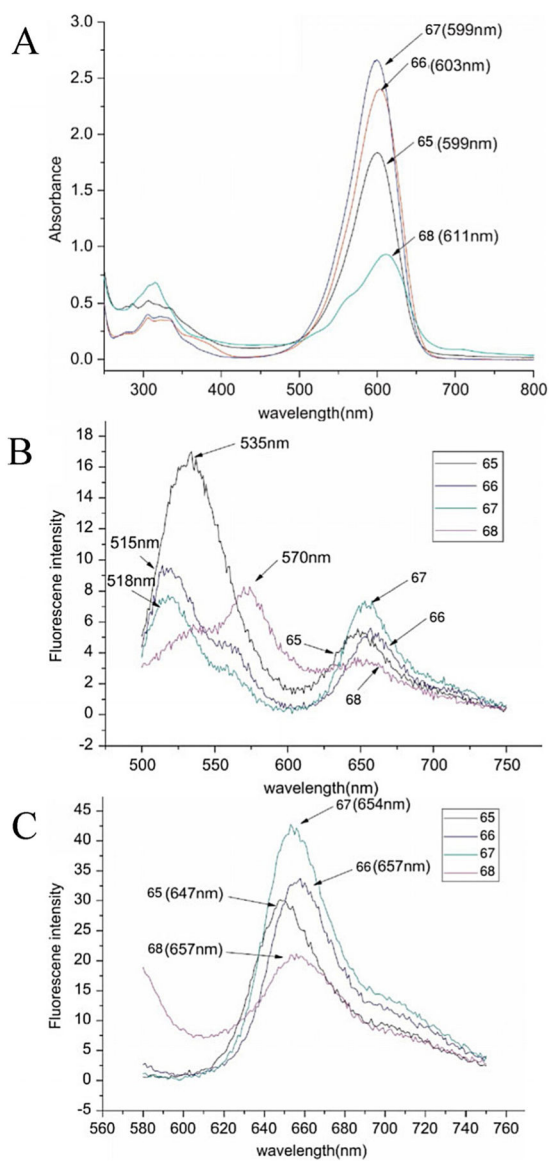


Figure 25. UV/Vis (A) and fluorescence (B,C) spectra of dyes **65–68** excited at 480 nm (B) and 550 nm (C).^[65]

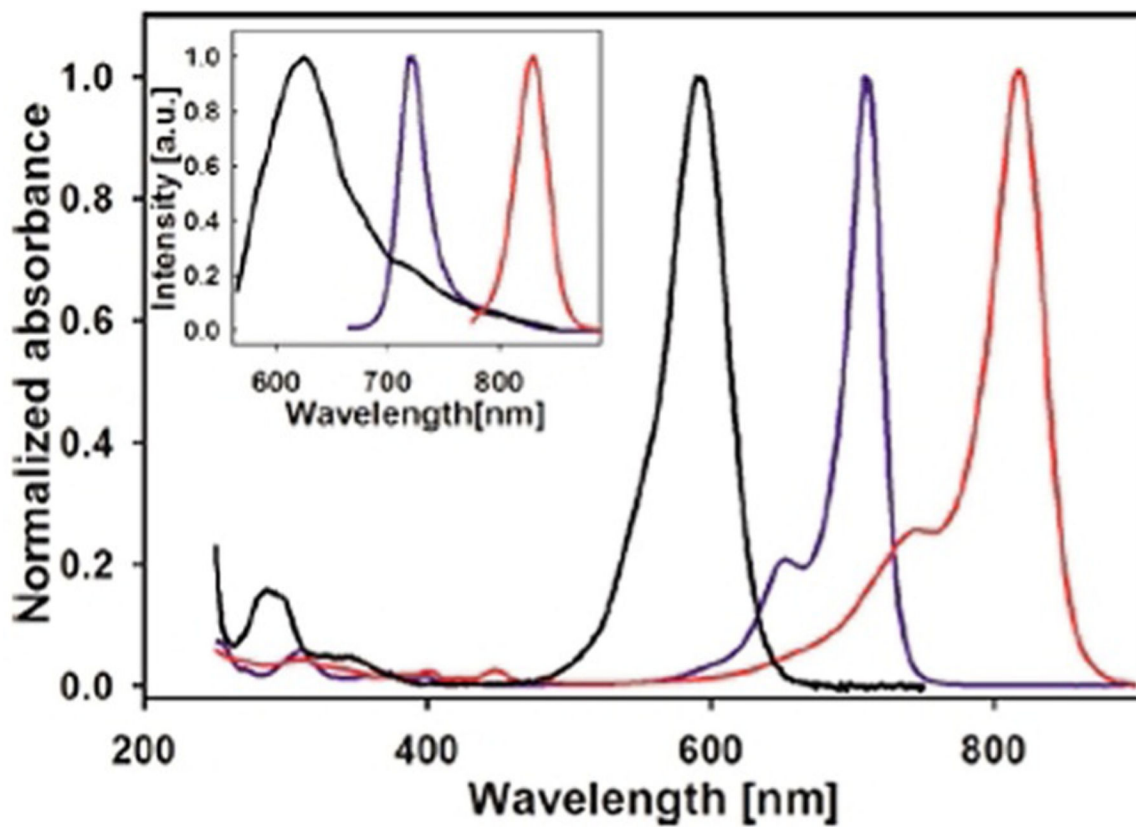


Figure 26. Absorption (main graph) and emission (inset) spectra of three symmetrical dyes: Ethyl Red (black), Kryptocyanine (blue), and **79** (red).^[68]

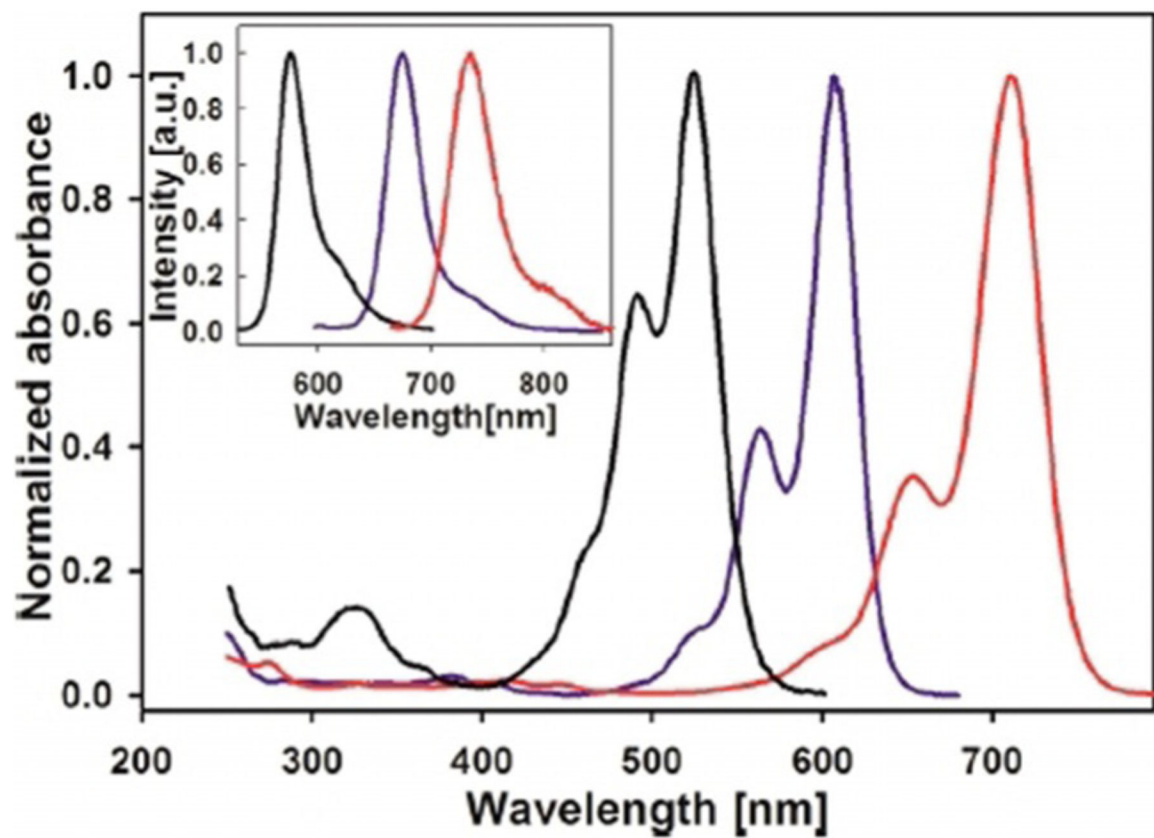


Figure 27. Absorption (main graph) and emission (inset) spectra of three symmetrical dyes: pseudoisocyanine (black), Sensitol Red (blue), and **83** (red).^[68]

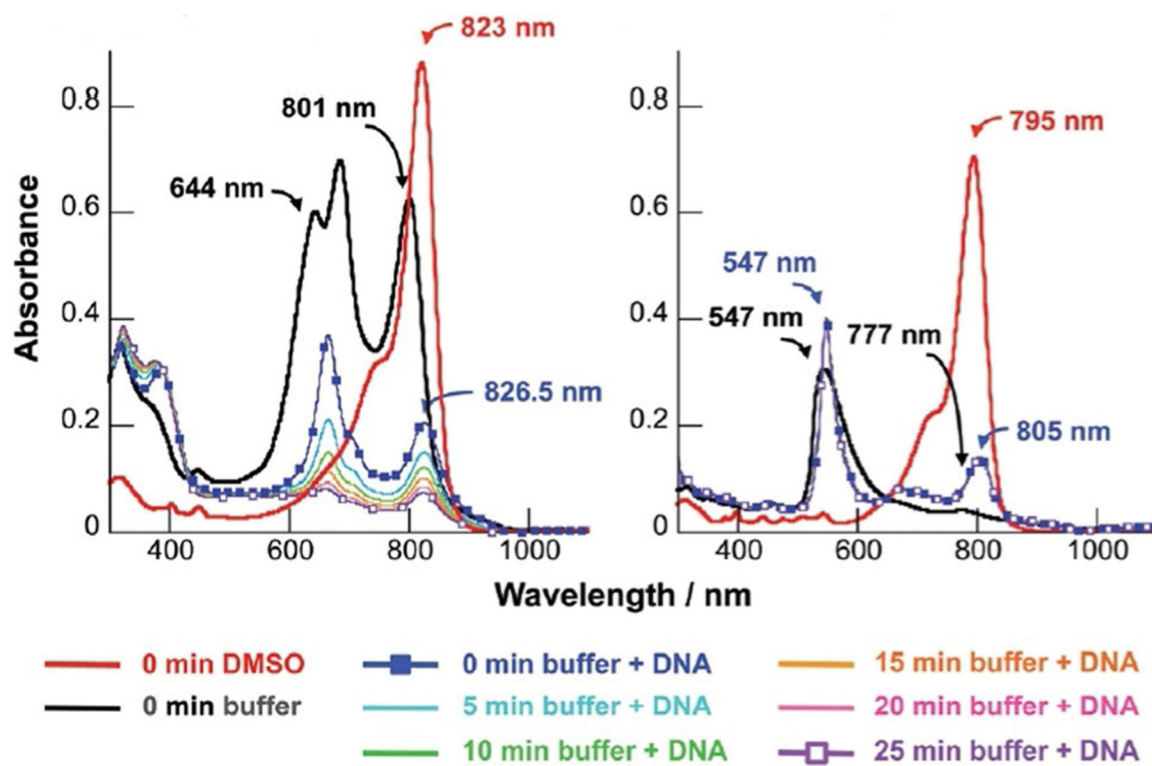


Figure 28.

UV/Vis spectra of 10 μm of dyes **77** (left) and **78** (right): in DMSO, 10 mm sodium phosphate buffer, pH 7.0, and in buffer with 150 μm bp CT DNA.^[16]

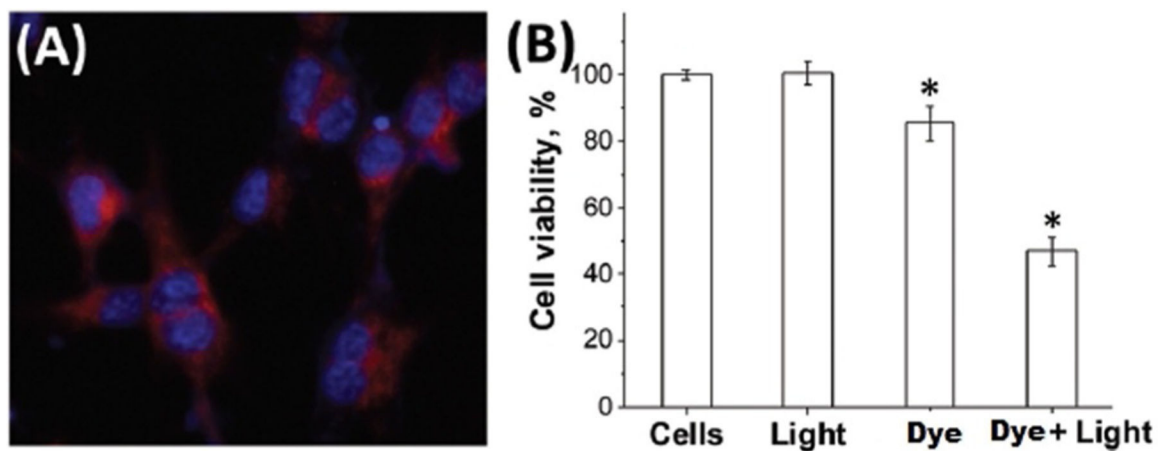


Figure 29.

(A) Superimposed fluorescence microscopy image revealing the intracellular localization of dye **78** (red) in ES2 cancer cells after 24 h incubation with the subsequent staining of nuclei Hoechst 33342 (blue). (B) ES2 cancer cell viability: with no treatment, under 808 nm laser exposure, after dye **78** incubation in the dark, after dye **78** incubation under 808 nm laser exposure.^[16]

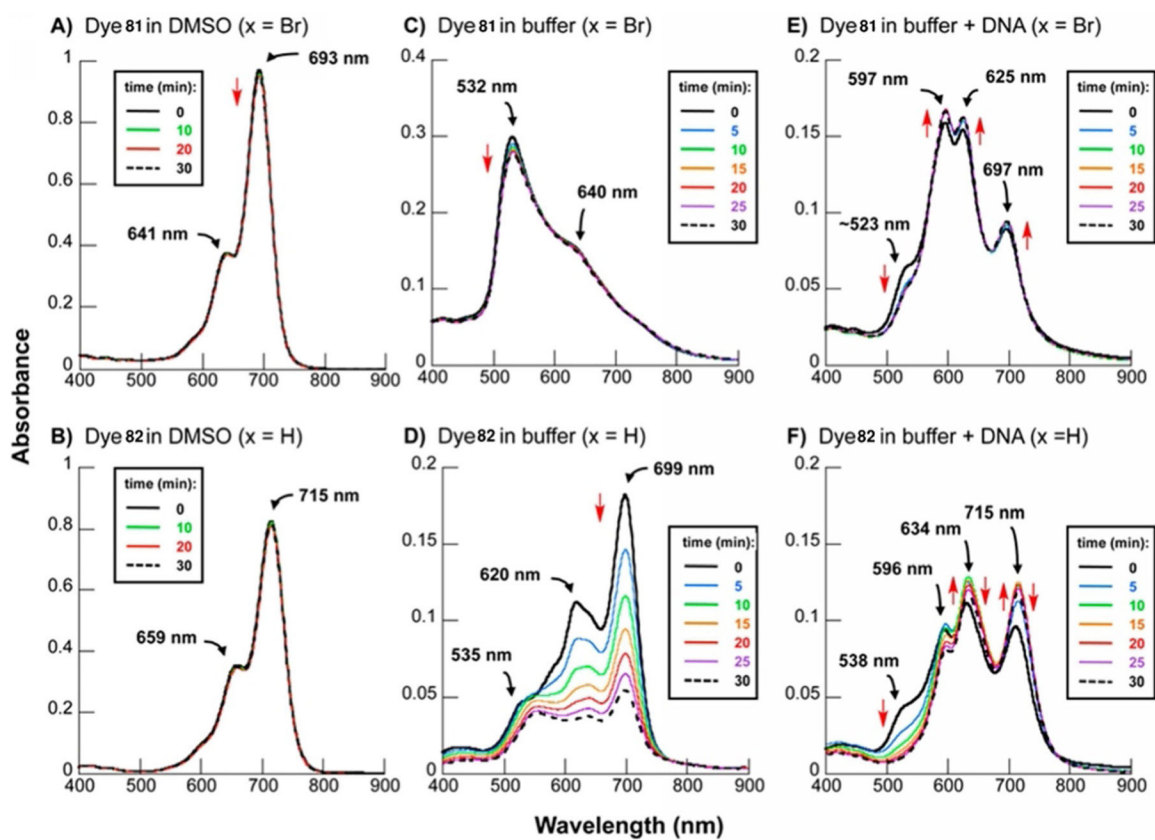


Figure 30.

UV/Vis spectra of dyes **81** (top) and **82** (bottom) in DMSO (A, B), buffer pH 7.0 (C,D), and buffer pH 7.0 with 150 μ M CT DNA (E,F).^[94]

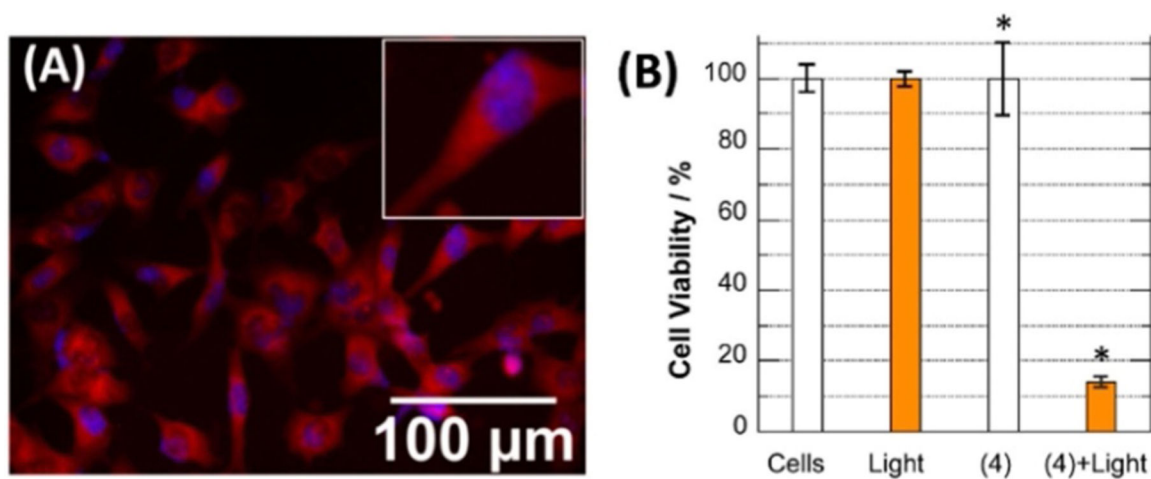


Figure 31.

(A) Superimposed fluorescence microscopy image revealing intracellular localization of dye **81** (red) in ES2 ovarian cells after 24 h incubation with the subsequent staining of nuclei Hoechst 33342 (blue). (B) ES2 cancer cell viability: with no treatment, under 694 nm laser exposure, after dye **81** incubation in the dark, after dye **81** incubation under 694 nm laser exposure.^[16]

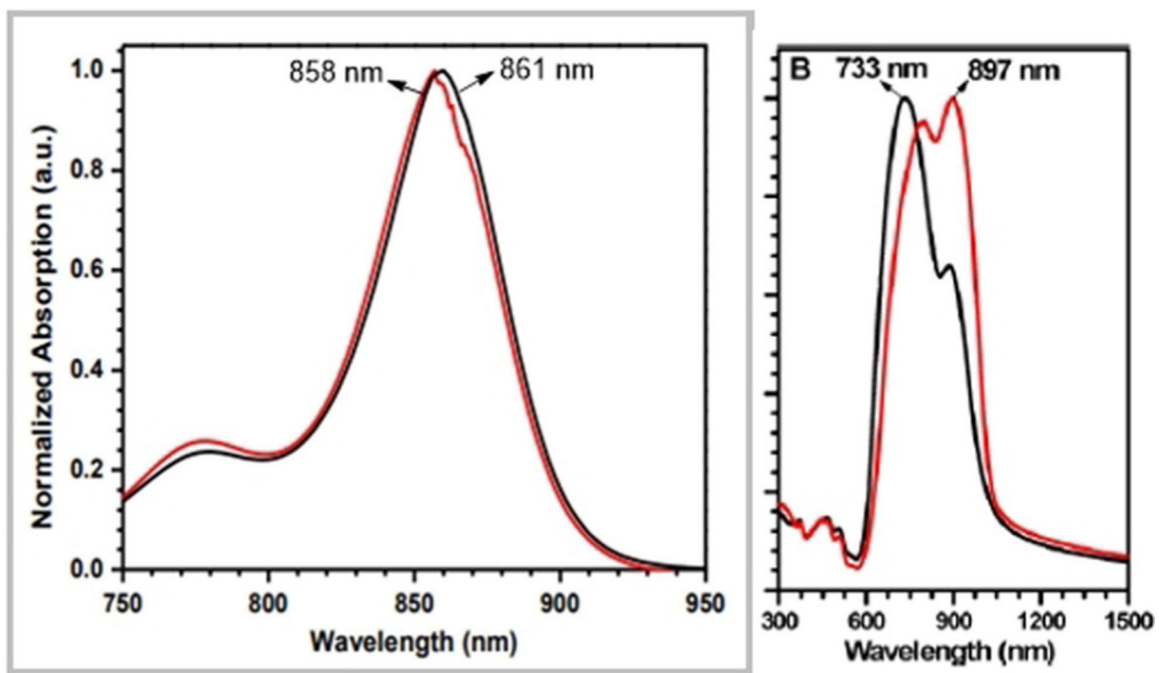


Figure 32. The absorption spectra for dyes **96** (black) and **97** (red) in CH_2Cl_2 (left) and as neat thin film (right).^[71]

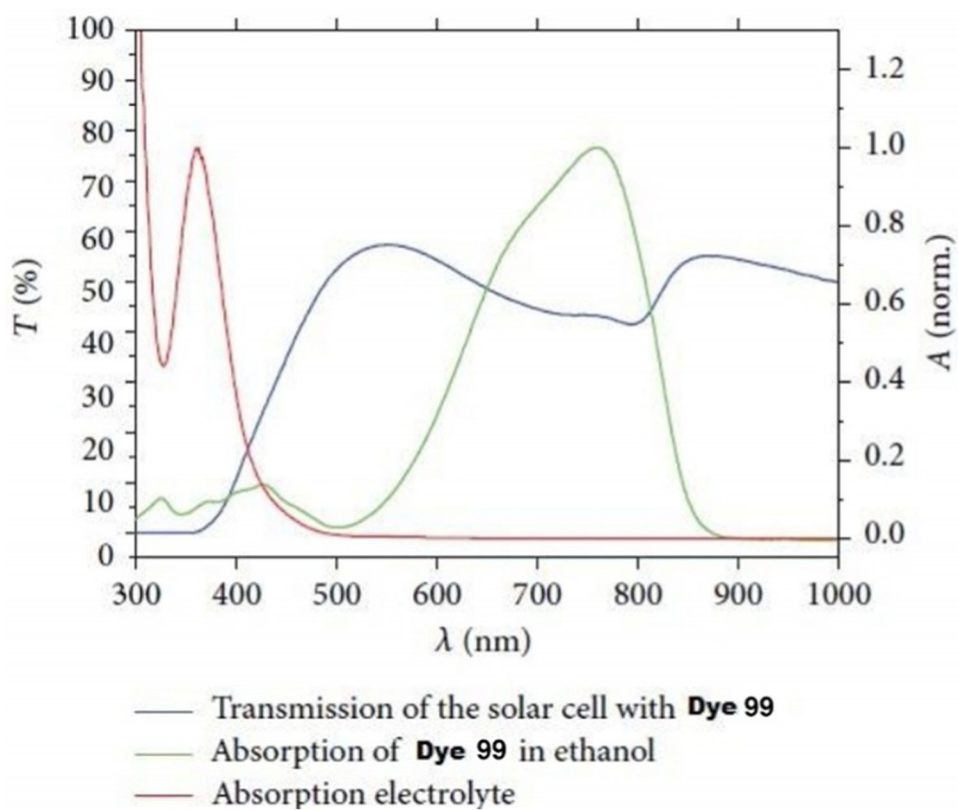


Figure 33.
Transmission of DSC with dye **99**.^[74]

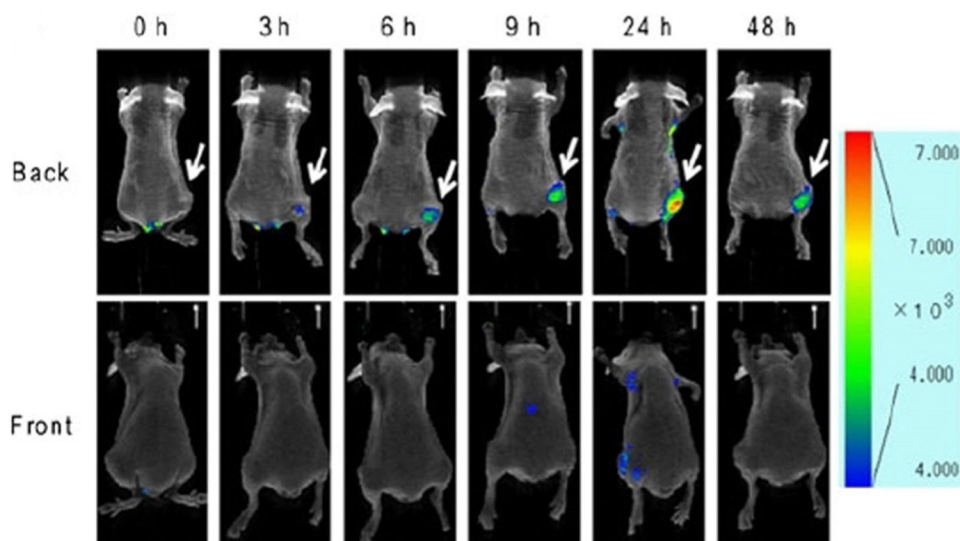
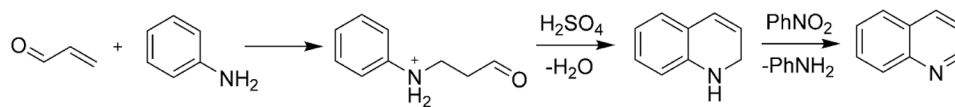
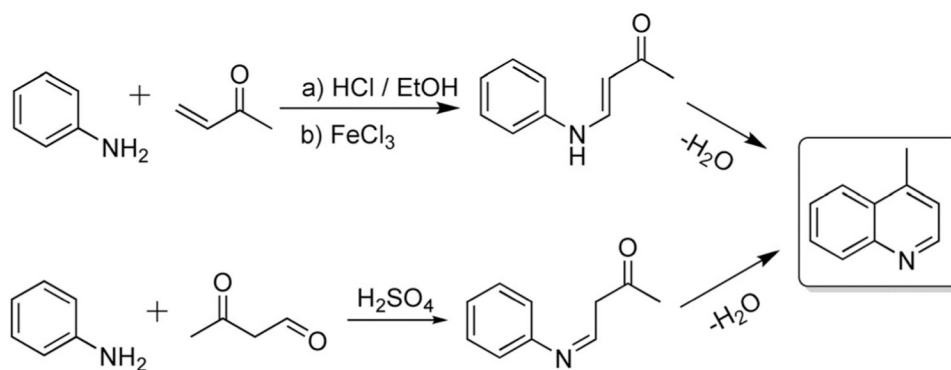


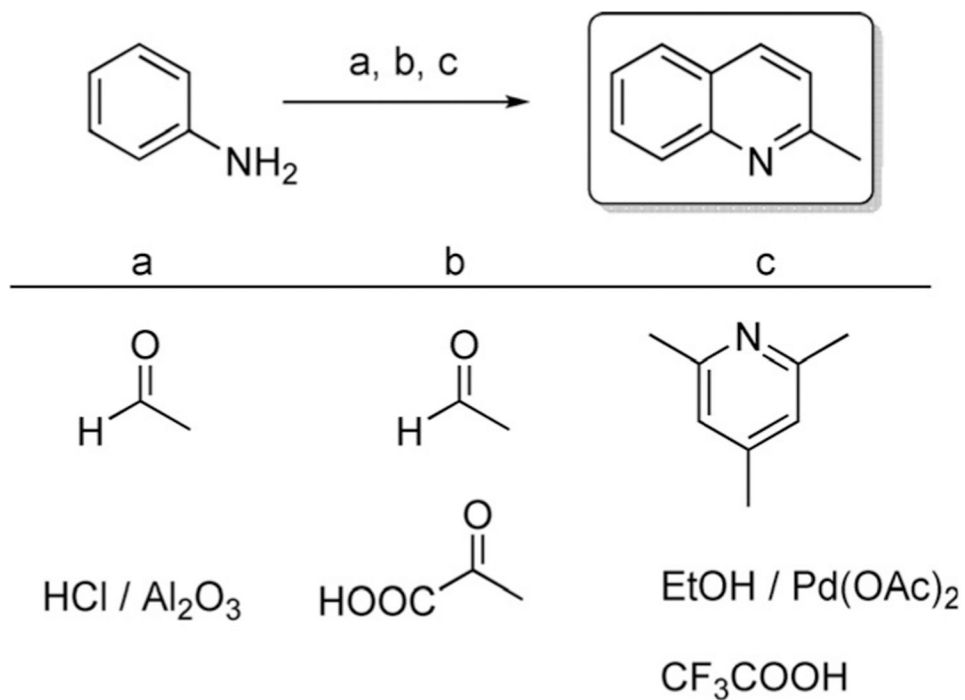
Figure 34. Back (top) and front (bottom) sides of mouse fluorescence images of FM3A cell xenografted mouse after injection of dye **101** lactosome, recorded at 0, 3, 6, 9, 24, and 48 h.^[17]



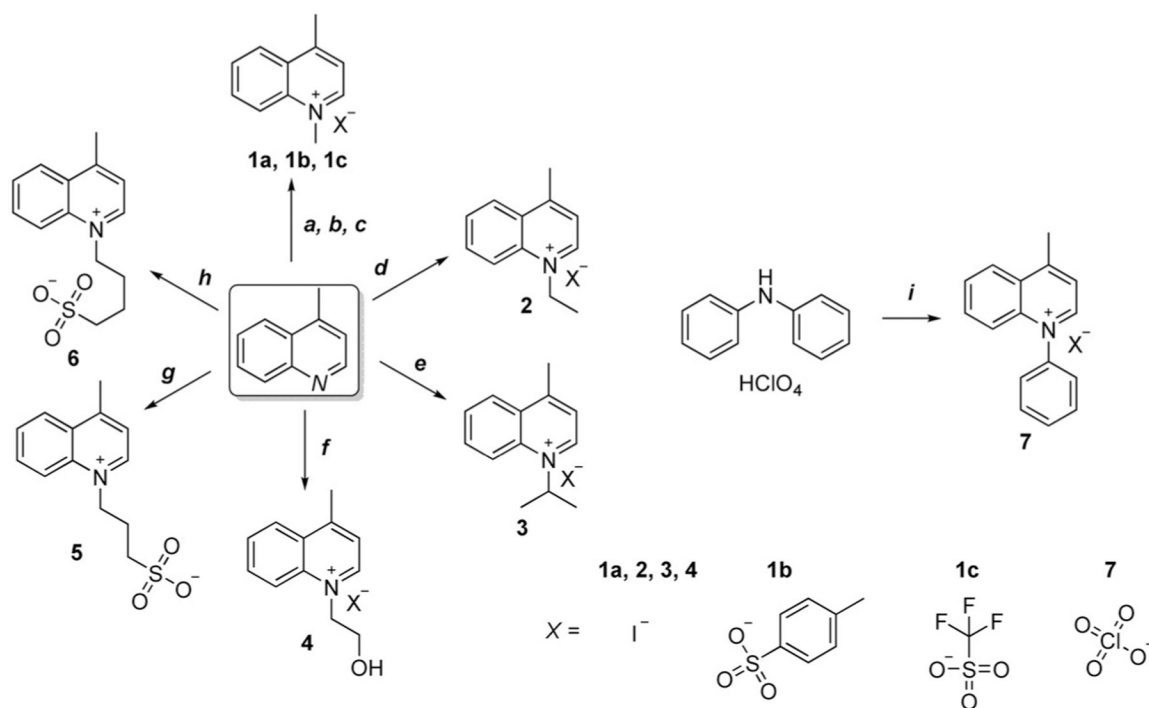
Scheme 1.
Skraup synthesis of quinolines.



Scheme 2.
Lepidine (4-methylquinoline) synthetic routes.



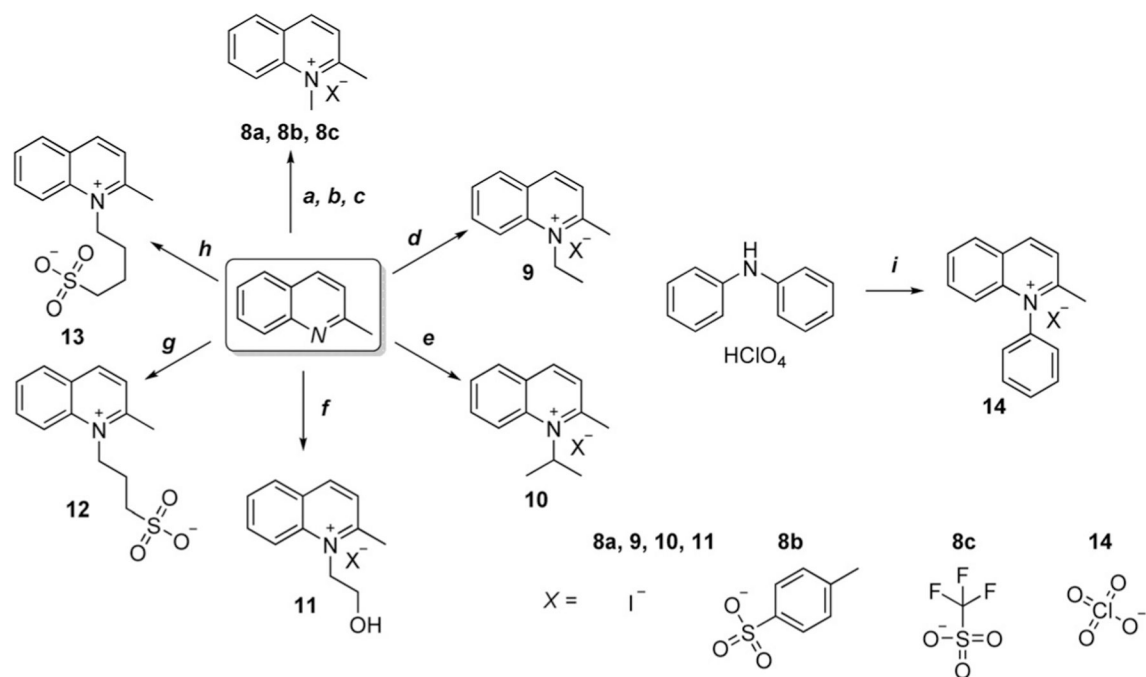
Scheme 3.
Quinaldine (2-methylquinoline) synthetic routes.



a = CH_3I , MeCN or Sulfolane or EtOH or PhMe or Et_3N ; **b** = methyl 4-methylbenzenesulfonate; **c** = methyl trifluoromethanesulfonate, DCM; **d** = C_2H_5I , PhMe or DCM; **e** = C_2H_7I , DCM; **f** = 2-iodoethanol, PhMe; **g** = 1,3-propane sultone; **h** = 1,4-butane sultone; **i** = formaldehyde, acetone, $PhNO_2$.

Scheme 4.

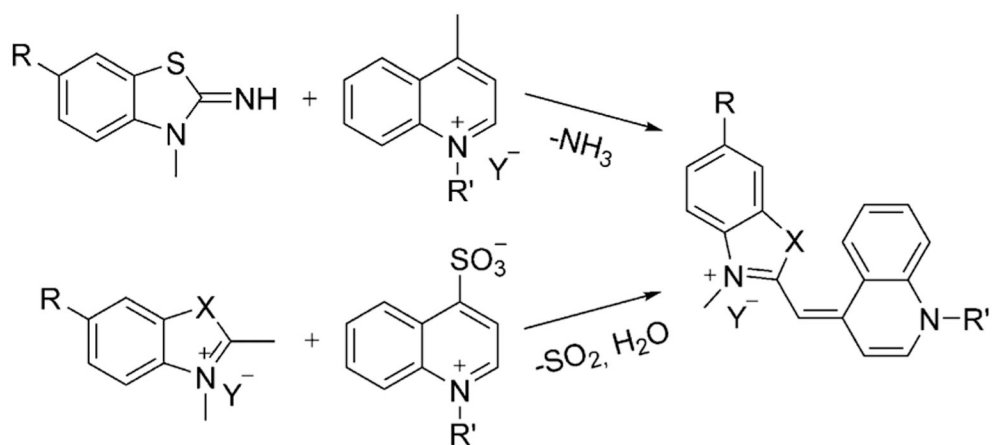
Synthetic routes of various lepidine salts.



a = CH_3I , MeCN or EtOH or PhMe; **b** = methyl 4-methylbenzenesulfonate, MeOH or $(CH_2OMe)_2$;
c = methyl trifluoromethanesulfonate, PhMe; **d** = C_2H_5I , PhMe or MeCN; **e** = C_2H_7I , MeCN;
f = 2-iodoethanol, PhMe, acetone; **g** = 1,3-propane sultone, benzene or NaH, THF, MeOH;
h = 1,4-butane sultone, PhMe; **i** = paraldehyde or acetaldehyde or butyl vinyl ether.

Scheme 5.

Synthetic routes of various quinaldine salts.

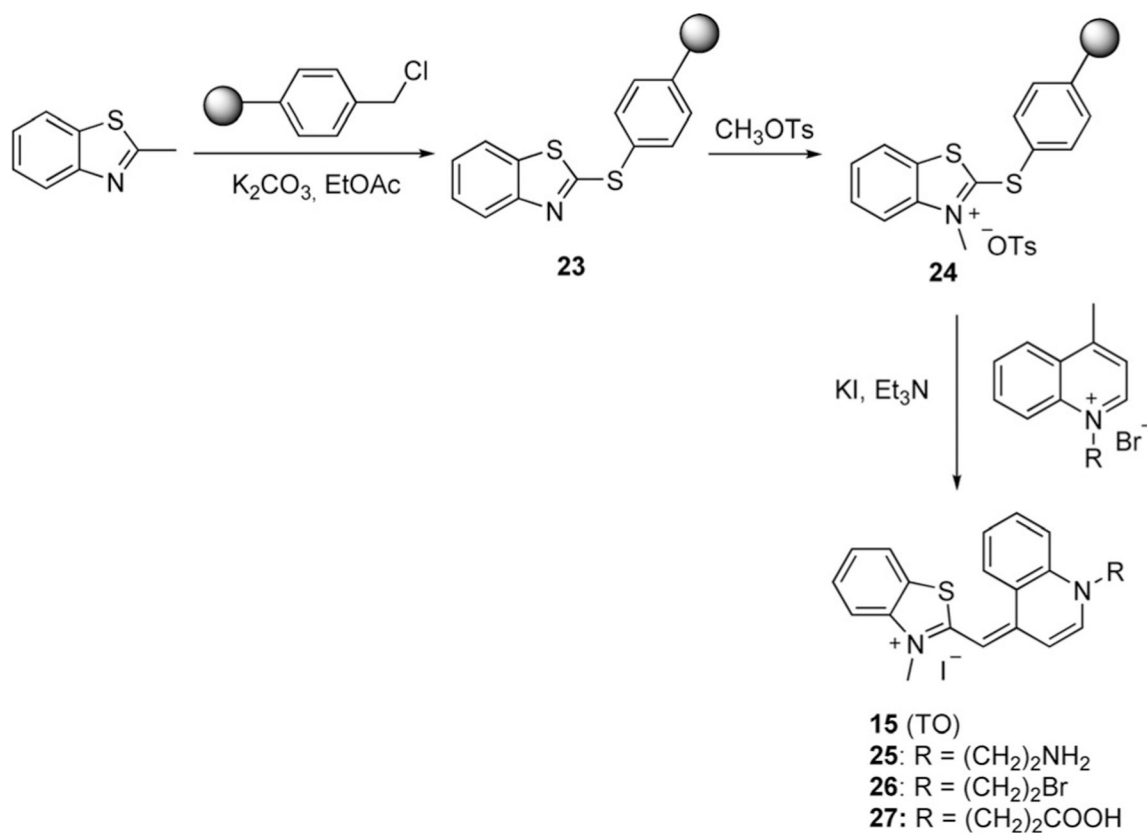


X = S or O,
 R = H, Cl, NO₂, CH₃O, CH₃CONH
 R' = CH₃, C₂H₅, C₂H₄OH

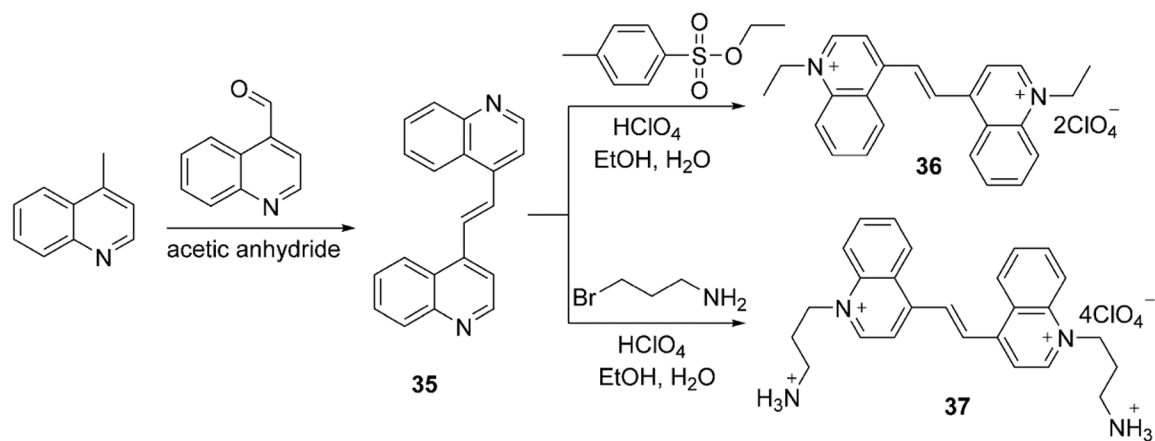
19: X = S, Y = I, R = NO₂, R' = CH₃
20: X = S, Y = Br, R = CH₃O, R' = C₂H₅

Scheme 6.

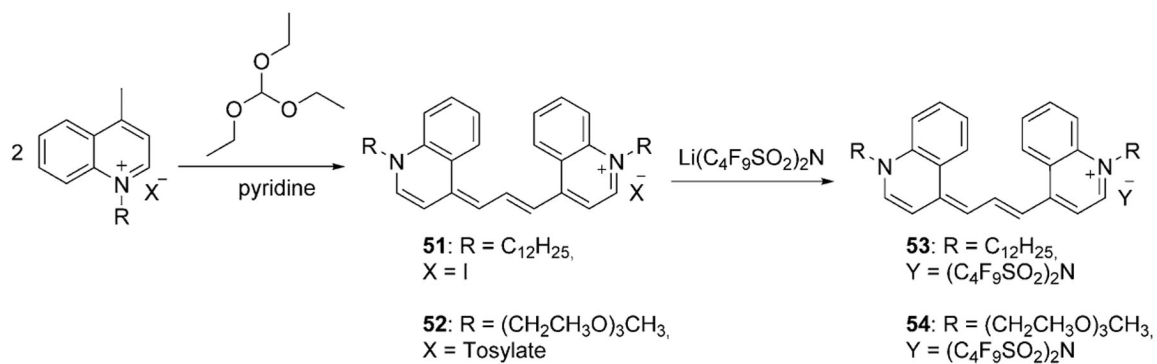
Synthesis of TO and YO monomethine cyanine dyes.



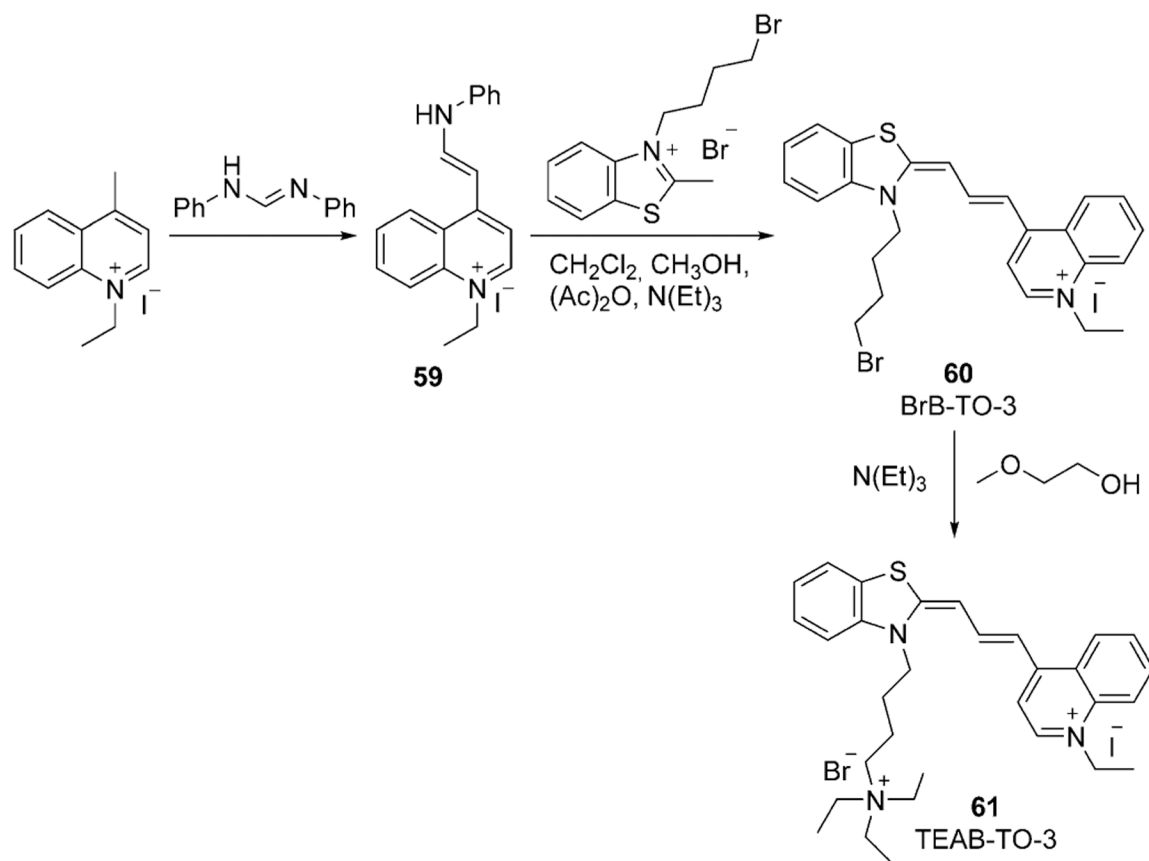
Scheme 7.
Solid-phase synthesis of monomethine cyanines.



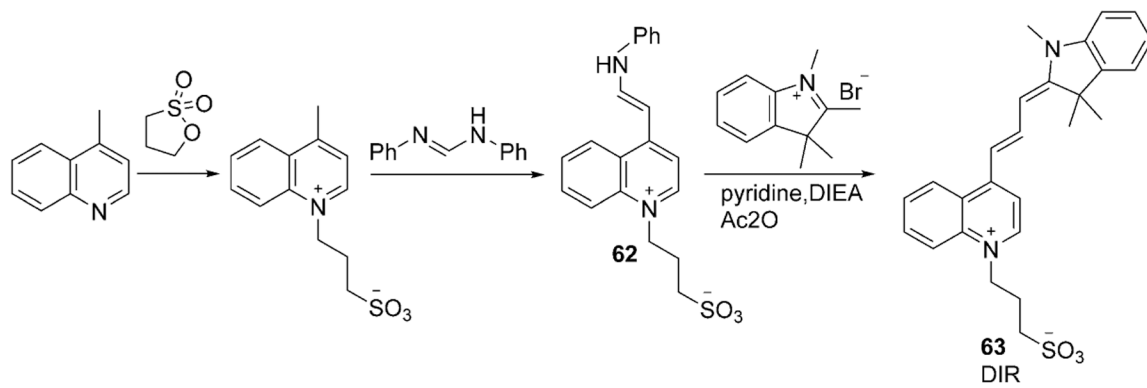
Scheme 8.
Synthesis of symmetrical dimethine cyanine dyes.^[56, 57]



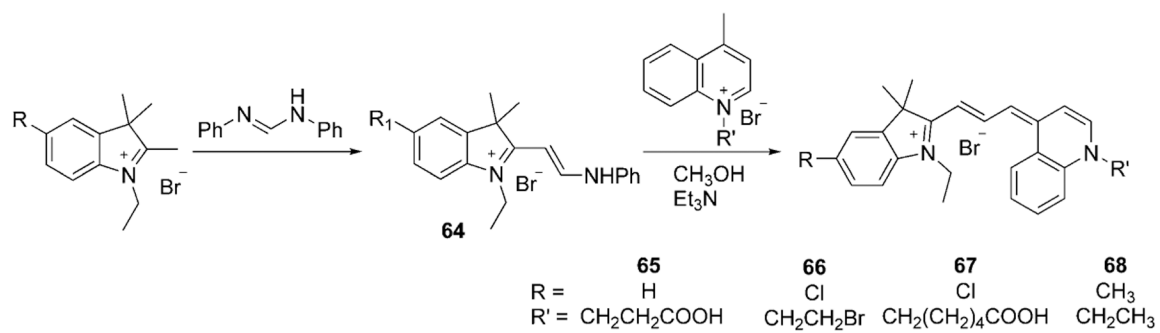
Scheme 9.
 Synthesis of symmetrical trimethine cyanine dyes.



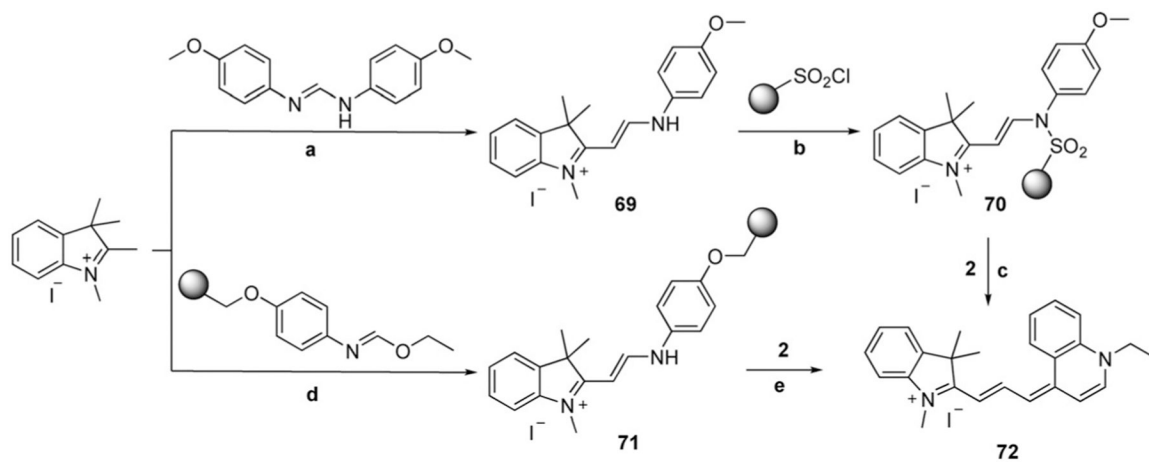
Scheme 10.
Synthesis of trimethine cyanine TEAB-TO-3.^[63]

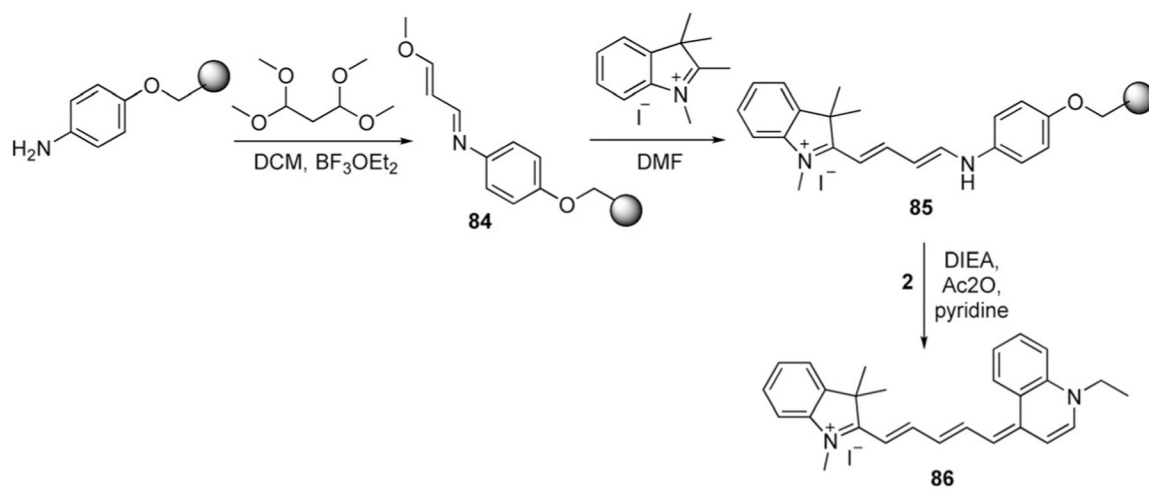


Scheme 11.
Synthesis of dimethyl indole red (DIR).^[64]

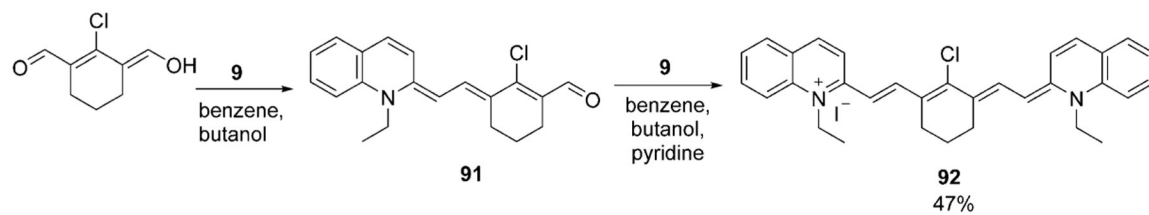


Scheme 12.
Synthesis of trimethine indoline quinoline dyes.

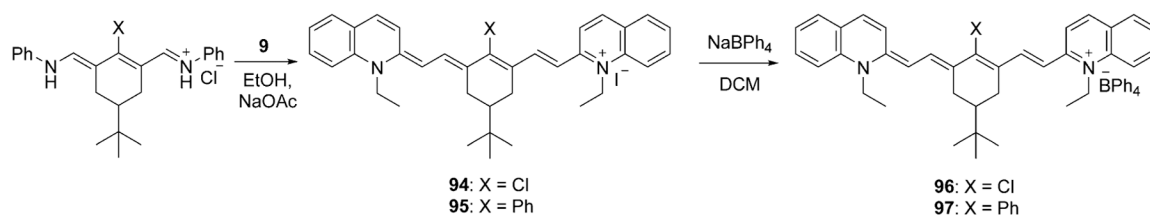
**Scheme 13.**Solid-phase synthesis of trimethine cyanine dyes.^[66]



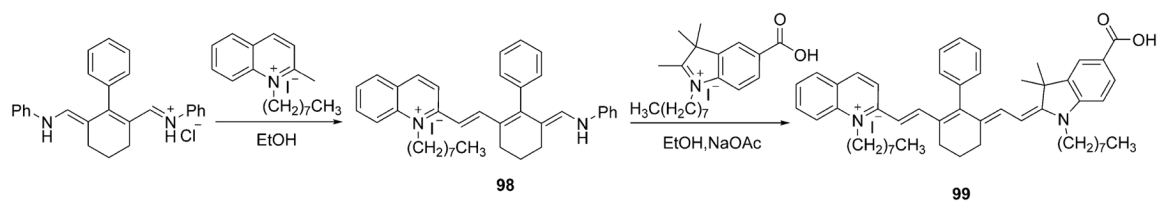
Scheme 14.
Solid-phase synthesis of pentamethine cyanine dyes.^[66]



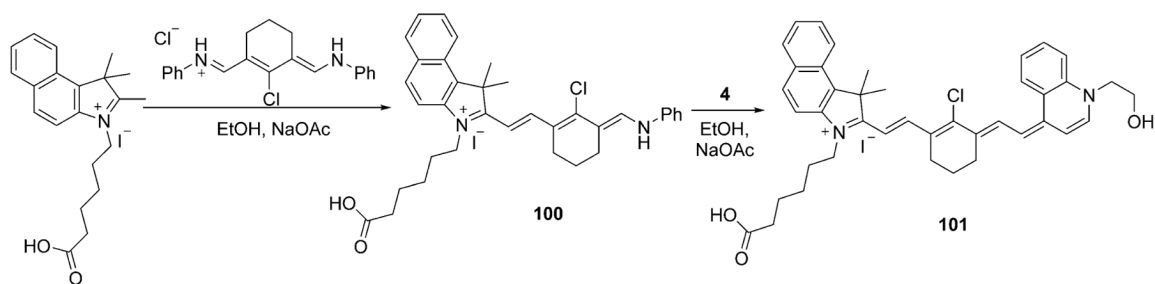
Scheme 15.
Synthesis of symmetrical heptamethine cyanine dye.^[70]

**Scheme 16.**

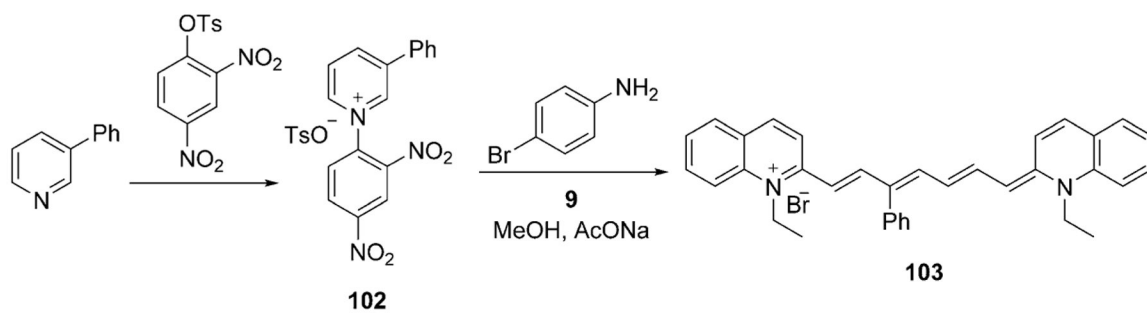
Synthesis of symmetrical heptamethine cyanine dyes with substitutions at the center of the bridge.



Scheme 17.
Synthesis of asymmetrical heptamethine cyanine dye **99**.^[17]

**Scheme 18.**

Synthesis of asymmetrical heptamethine cyanine dye **101**.^[74]

**Scheme 19.**

Synthesis of heptamethine cyanine dye **103** with the substituent at the C3 position.

Table 1.The spectral characteristics of dyes **38** and **48** in different solvents.^[15]

Solvents	Characteristics	Dye 38	Dye 48
H ₂ O	λ_{ex} [nm]	473	458
	λ_{em} [nm]	574	554
	Stokes shift [nm]	101	96
	ϵ [$\times 10^{-4}$ Lmol ⁻¹ cm ⁻¹]	1.91	3.52
DMSO	λ_{ex} [nm]	503	481
	λ_{em} [nm]	596	565
	Stokes shift [nm]	93.6	84
	ϵ [$\times 10^{-4}$ Lmol ⁻¹ cm ⁻¹]	2.09	3.21
MeOH	λ_{ex} [nm]	503	479
	λ_{em} [nm]	581	558
	Stokes shift [nm]	78.6	79.4
	ϵ [$\times 10^{-4}$ Lmol ⁻¹ cm ⁻¹]	2.28	4.13
CHCl ₃	λ_{ex} [nm]	513	503
	λ_{em} [nm]	570	568
	Stokes shift [nm]	57	65
	ϵ [$\times 10^{-4}$ Lmol ⁻¹ cm ⁻¹]	2.82	2.16

Table 2.

The spectral characteristics of monomers YO-PRO-1 (**18**, Equation (3)), TO-PRO-1 (**16**, Equation (3)), YO-PRO-3 (**58**, Figure 13), TO-PRO-3 (**57**, Figure 13), and their dimers YOYO and TOTO (Figure 18), and YOYO-3 and TOTO-3 (Figure 22).^[92]

Dye	λ_{abs} [nm]	λ_{em} [nm]	Stokes shift [nm]	ϵ [$\times 10^{-4}$ Lmol ⁻¹ cm ⁻¹]
YO-PRO-1	491	509	18	5.2
YOYO-1	491	509	18	9.9
TO-PRO-1	515	531	16	6.3
TOTO-1	514	533	19	11.7
YO-PRO-3	612	631	19	10
YOYO-3	612	631	19	16.7
TO-PRO-3	642	661	19	10.2
TOTO-3	642	660	18	15.4

Table 3.Optical properties of dye **101** and its lactosome.^[17]

Entry	Solvent	Abs max	Ex max	Em max	ϵ [$\times 10^{-4}$ Lmol ⁻¹ cm ⁻¹]
Dye 101	chloroform	781	805	897	9.2
	methanol	790	883	932	2.6
Dye 101 lactosome	water	830	844	905	6.0