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Recent advances in sensitizer dyes for photodynamic therapy

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Abstract

Photodynamic therapy has been extensively studied in the last few decades as a potential clinically approved therapy for a broad range of malignant tumors. A main purpose for the treatment of cancer is the discriminating destructive process of malignant cells without damaging healthy tissues. Photodynamic therapy is a photoactivation process of a sensitizer hold on to tumor cells, which generates reactive singlet oxygen species with the ability to wipe out the targeted tumor cells. The mechanisms concerned in explaining the photodynamic therapy of cancer cells annihilation have not been completely recognized. However, recent studies have assisted to clarify the special impacts of photodynamic therapy at tissue, cellular and molecular levels. Porphyrin derivatives have been widely investigated and many other new types of sensitizers have been materialized and verified their efficiency in photodynamic therapy.

Keywords: *Dyes; Photosensitizers; Tumors; Photodynamic Therapy.*

1. Introduction

Colorants that have been designed for high technical applications have been typically called functional dyes [1-7]. Those high technical applications include chromic materials, dye sensitized solar cells, optical data storage, fluorescent and colorimetric sensors, organic semiconductors and light emitting devices, liquid crystalline displays, and electrophotography [8-15]. Functional dyes have been extensively used for a variety of (bio)medical applications, such as photodynamic therapy, diagnostic biosensors, and fluorescent bio-imaging [2, 16-22].

Photodynamic therapy has been a promising medical modality to handle the non-malignant lesion. Applying photodynamic therapy requires a photosensitizer, light, and oxygen. As a sequence of photochemical reactions of these three elements; a singlet oxygen produced from triplet state excited molecular oxygen leads to tissue damage. This effect is limited to a small area for the reason that the half-life of singlet oxygen is too low (0.6×10^{-6} sec). Photodynamic therapy can be used to wipe out the vasculature adjacent tumor cells, and turn on the immunological reactions against them. The major feature of photodynamic therapy is likely for dual selectivity coming from the favored accumulation of the used photosensitizer and focusing of light at the targeted tissues. One of the good advantages of photodynamic therapy is the ability of its application repetitively without induction of resistance [23-25].

A purified hematoporphyrin derivative called Porfimer sodium approved as an original medical photodynamic therapy photosensitizer for cancer in bladder, lung and esophagus. Porfimer sodium can be stimulated with red light at wavelength equal to 630 nm; that cannot go through tissue further than only some millimeters. This character makes Porfimer sodium only appropriate for surface tumors. However, Porfimer sodium shows a small extinction coefficient at the above wavelength demanding light from a high-energy source which usually leads to difficulties on treatment. An additional drawback of Porfimer sodium is that its is cannot be cleaned rapidly leading to additional healing of skin. Identification of the above drawbacks of Porfimer sodium has stimulated hard work to build up more efficient photosensitizers with advantageous properties including; low toxicity, low side-effects, high extinction coefficient, efficient singlet-to-triplet intersystem crossing, low quantum

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yield for photobleaching; and suitable lipophilic/hydrophilic equilibrium [26, 27].

The majority of the clinically applicable photodynamic therapy photosensitizers are cyclic tetrapyrroles such as porphyrins, chlorins, and bacteriochlorins. Cyclic tetrapyrroles are not reachable synthetically, and variations to revise their photophysical properties are also difficult. Hence, there is a significant attention to the non-porphyrin-based sensitizers that could be made more simply. Phenothiazinium-based sensitizers are a well-known class of photosensitizers. They have been easy to formulate but own low light to dark toxic ratio. Boron-dipyrromethene (BODIPY) is another category of photodynamic therapy sensitizer became known over the precedent decade. BODIPY has numerous of supreme photosensitizer properties counting high absorption coefficient, high light–to-dark toxic ratio, and stability against environment and photobleaching. This review article summarizes properties and behavior of selected categories of sensitizer in photodynamic therapy [27-30]. This review article summarizes properties and behavior of selected categories of sensitizer in photodynamic therapy. The mechanisms concerned with the photodynamic therapy of cancer cells are discussed.

2. Photodynamic therapy

Cancer treatment usually includes invasive procedures such as catheters to permit preliminary chemotherapy to reduce cancer in size, surgical treatment to then eliminate the tumor, then extra chemotherapy to execute the cancer cells that are characterized by their faster reproduction than the healthy cells. Chemotherapy has been developed over the precedent three decades leading to raise the value of life for patients with cancer. Such developments include better carriers to permit different dosing methods and novel therapeutic goals such as blood vessels fueling tumor enlargement. The success of cancer therapy is related to the talent to treat the tumor cells without disturbing healthy cells. However, managing these bolus doses rises up side effects that might require terminating therapy before wipe out the cancer cells. The progress of cancer therapy is succeeding rapidly in terms of novel sensitizers and methods of drug delivery [20, 30-33].

Single cancer cell bordered by other healthy cells in a specific tissue, can be reproduced at a rate quicker than these healthy cells to result in the creation of a tiny tumor. The healthy cells will not be capable to struggle with these tumor cells for the insufficient available nutrients. The infected cells normally will not commence apoptosis in this low nutrient situation; also, they do need standard cell construction components such as amino acids, glucose and molecular oxygen. Hence, the infected cells will continue replicating regardless to the insufficient provided nutrients however many other infected cells will die as a result of this low nutrients environment. The infected cells at the surface of the tumor have the better access to nutrients at the same time as contained core infected and non-infected cells die to produce a necrotic core contained by the tumor. The reproduction rate is equivalent to the death rate leads to steady state tumor size [34]**.**

2.1. Mechanism of photodynamic therapy

The photodynamic therapy consequences belong to group of photochemical reactions including a diversity of reactive biomolecules and more than a single chemical pathway. The photodynamic effects are mostly attributed to energy or electron transfer of the lowest excited triplet-state (T1) of a sensitizer to molecular oxygen or another organic agent. Two different quenching mechanisms can be distinguished as type I and II mechanisms [35]. For type I mechanism, the excited-state of the sensitizer produces radical species by means of electron transfer or H-abstraction. So, the electron transfer-mediated biomolecule oxidation does not absolutely require oxygen. In type II mechanism, an energy transfer takes place from the excited sensitizer to molecular oxygen to provide the ground-state of the photosensitizer in addition to the singlet oxygen [28, 29]. The oxidation of biomolecule through $^1O_2$ generation is defined as Type II major. Another reactive oxygen species (ROS) mediated process, superoxide ($O_2^{\bullet-}$) mediated biomolecule oxidation is also categorized as the Type II, minor (Figure 2).

2.1.1. Electron transfer mechanism

For a type I mechanism, the excited-state of the sensitizer produces radical species by means of electron transfer from/to an organic agent or the H-abstraction from an organic agent. These radical species subsequently interact with the ground-state molecular oxygen so that the total process is a photochemical stimulated autoxidative process [36] (Scheme 1).

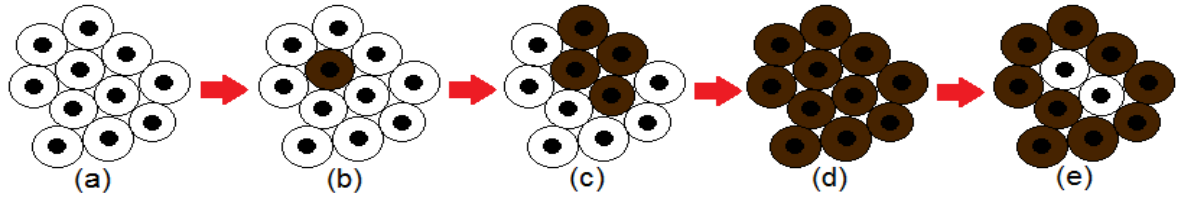


Figure 1. Tumor growth starting from a single cell to a tumor; (a) layer of normal cells, (b) one cell attains sufficient mutation to turn out to be cancerous, (c) cancerous cells split at an accelerated rate displacing normal cells, (d) healthy cells and organ functions are compromised, (e) tumor cells grow and die in a continuous process as steady-state size till the regeneration of new blood vessels.

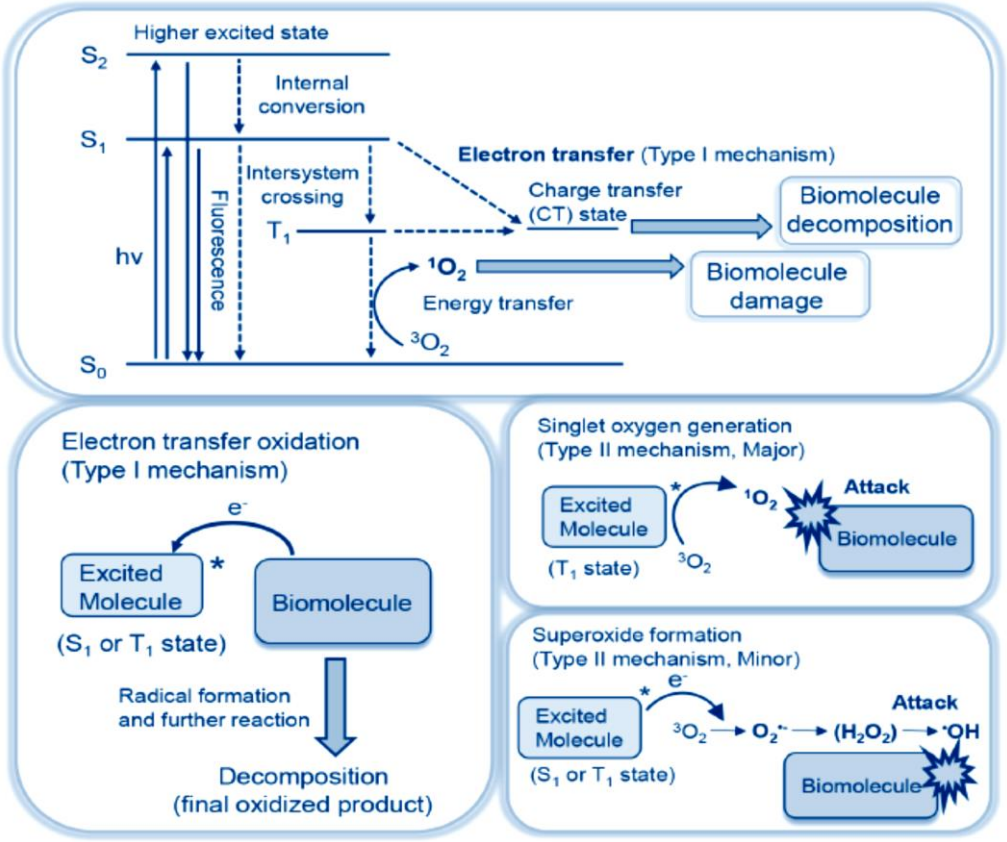
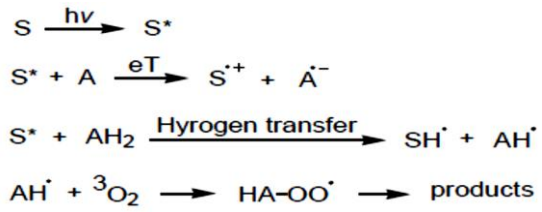
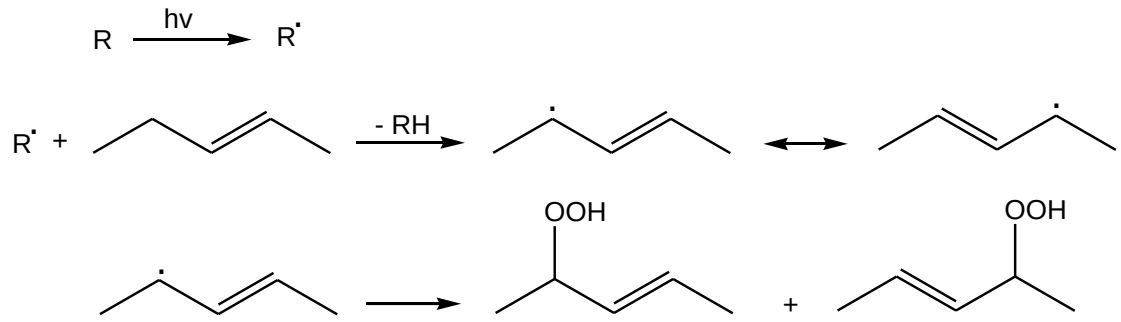


Figure 2. Relaxation process of photoexcited state of photosensitizer and the typical photosensitized biomolecule damaging mechanisms [36]. (https://www.intechopen.com/online-first/electron-transfer-supported-photodynamic-therapy)



Scheme 1. Schematic representation of a type I mechanism.

An additional propagation progression in the dark goes after the photochemical free radical initiation step. The propagation step followed by the termination step through quenching of these free radicals. A biological lipid peroxidation process can proceed through this photochemical autoxidation process producing R• that can abstract hydrogen from an allylic location of an unsaturated lipid. The produced allylic radical then reacts with triplet excited state molecular oxygen to afford a mixture of hydroperoxides and their related decomposition products, leading to a more complicated mixture than that gained from the singlet oxygen reaction [37].



The electron transfer to molecular oxygen offers the superoxide radical molecular oxygen anion $O_2^{-\bullet}$:

$$S \xrightarrow{hv} S^* \xrightarrow{^3O_2,\, eT} S^{\bullet+} + O_2^{\bullet-}$$

$$S(T_1) + A \longrightarrow S^{\bullet-} + A^{\bullet+} \xrightarrow{^3O_2} S(S_0) + O_2^{\bullet-}$$

The produced superoxide radical molecular oxygen anion is not reactive enough and consequently quickly oxidizes back to molecular oxygen. The protonated hydroperoxide radical form HOO• suffers a dismutation that takes place spontaneously but in addition is catalyzed by superoxide dismutase enzyme to provide hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) [27].

The produced H_2O_2, which is toxic for the individual, is transformed to water by glutathione peroxidase enzyme. On the other hand, when H_2O_2 produced in vivo in a photochemical reaction course in absence of peroxidase enzyme and presence of traces of transition metal, leads to the generation of hydroxyl radical ($HO^{\bullet}$) which is reactive radical to stimulate autoxidation processes:

$$O_2^{\bullet-} \xleftrightarrow{H^-} HOO^{\bullet} \xrightarrow{2x} H_2O_2 + O_2$$

$$H_2O_2 + Fe(II) \xrightarrow{\text{Fenton reaction}} HO^{\bullet} + OH + Fe(III)$$

$$AH + HO^{\bullet} \longrightarrow A^{\bullet} + H_2O \xrightarrow{^3O_2} A\text{-}OO^{-} \longrightarrow \text{products}$$

2.1.2. Energy transfer mechanism

In a type II mechanism, the electron excitation energy is transported from the photosensitizer excited-triplet T_1 produced by intersystem crossing from the excited singlet S_1; to lowest excited triplet O_2, to afford the photosensitizer in its singlet ground-state as well as a singlet molecular oxygen:

$$S(S_0) \xrightarrow{hv} S(S_1) \xrightarrow{isc} S(T_1)$$

$$S(T_1) + {}^3O_2 \longrightarrow S(S_0) + {}^1O_2$$

$$A + {}^1O_2 \longrightarrow \text{products}$$

2.2. Types of photosensitizers

In the phototherapy process, only visible or near-visible light is used for treatment. The photodynamic effect is result of light absorption by endogenous photosensitizers already exist in the individual [38]. Photodynamic therapy is not restricted to a specific structural sensitizer. However, some properties are essential. The toxicity in lack of light should be very low. This means that tumor destruction can be controlled by light dose with a specified photosensitizing drug dose. The composition should be invariable, and it is favored to use a single material with no chiral center. The preparation should be as simple and as high yield as possible. Amphiphilic sensitizers demonstrate a better selectivity for tumor and faster clearance [39, 40]. Hydroxy, sulfonic acid, and quaternary ammonium salts are the mainly used functional groups to raise the hydrophilic properties of the lipophilic macrocyclic nuclei. Delivering systems are generally necessary because many sensitizers are solid and not soluble in water. Many delivering approaches can be used such as water-miscible organic solvents, surfactant micelles, liposomes, inclusion complexes, lipoproteins, microparticulates [41]. A chromophore must be available with high molar extinction coefficient in the visible or near-visible light. The triplet energy transfer should be to some extent larger than the energy of singlet oxygen above the ground state, the triplet excited state should be

produced in high quantum yield with long enough life-time [42].

The toxic outcome originated by the mutual action of a photosensitizer and visible light was firstly revealed by Oscar Raab in 1897 at University of Munich. Raab noticed that paramecia were killed with low concentrations of acridine photosensitizer. The initial research trials were done with haematoporphyrin on paramecia, erythrocytes, mice, guinea pigs and humans [43]. In 1930, Nobel Prize was awarded to the Austrian physician, Hans Fischer, for his research on porphyrins. The haematoporphyrin derivatives are a mixture of porphyrins with extensively dissimilar characteristics led to studies for pure materials known as second generation sensitizers [44]. Winkelman established tetraphenyl porphine sulfonate and declared a better tumor localization skill than haematoporphyrin derivatives [45]. Ben-Hur and co-workers established phthalocyanines with better extinction coeficient in the near-IR region, where strong laser dyes are accessible and biological tissues is more transparent [46]. The meso-substituted porphyrins were presented by Berenbaum and co-workers [47]. The application of endogenous protoporphyrins, reported by Malik et al. in 1987, stimulated by exogenous 5-aminolevulinic acid, is a new approach of medical photodynamic therapy [48].

2.2.1. First generation photosensitizers

First generation photosensitizers are generally characterized by several advantages including easy preparation from readily accessible starting material, wide clinical practice, and first material to be given regulatory approval. However, other disadvantages were considered such as highly complicated mixture of isomers are obtained, modest reactivity in cancer photodynamic therapy, and low selectivity for cancer tissues [49]. Haematoporphyrin derivatives or the first-generation photosensitizers have two benzylic alcohol functional groups at C_3 and C_8, with resultant diastereo-isomers. Furthermore, as a result of benzylic reactivity, the naturally derived samples include up to 15 components [50].

Haematoporphyrins can be obtained in its crude form from the slaughter house blood treated with mineral acids then hydrolytic workup to get rid of proteins and iron. The commercially available Photofrin, initially reported by Dougherty in 1970, is usually based on high molecular weight fraction of haematoporphyrin which increases with the HPLC or GPC separation, however some monomers are still present **(Figure 3)**. The ratios of monomer: dimer: oligomer increases from 22:23:55 to 14:19:67 for the Photofrin. In both of dimers and oligomers the porphyrin components are connected by ester, ether and carbon-carbon bonds. The overall number of components is about [51].

Photofrin has absorption band at wavelength 630 nm with extinction coefficient ε = 3000 mol^{-1} cm^{-1} in a saline solution buffered with phosphate. In typical photodynamic therapy, Photofrin is managed by intravenous injection of drug dose (2-5 mg kg^{-1}) then irradiation with visible light at a wavelength of 630 nm with a light dose of 100-200 J/cm^2 at 1-2 days after injection. Photofrin involves a long clearance period of time of 1-2 months after injection to keep away from skin photosensitization. Photofrin has been used worldwide for medical application to cancer from bladder, esophageal, lung as well as non-malignant, malignant and early-stage cervical cancer [52].

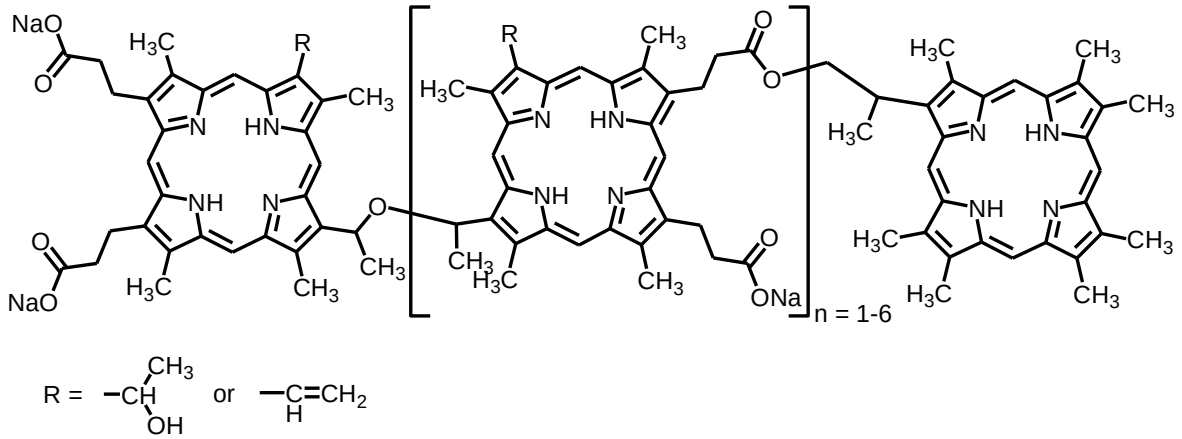


Figure 3. Commercially Photofrin, initially reported by Dougherty in 1970.

2.2.2. Porphyrin derivatives

Porphyrins are aromatic macrocyclic sensitizers with 18 π-electron system. Porphyrin unit is planar, however higher substitution at the periphery leads to structural deformation. The porphyrin inner core can complex with a broad range of metal ions and some metalloids. Porphyrin derivatives demonstrate an intense absorption band with extinction coefficient ε = 2 x 10^5 L cm^{-1} mol^{-1} at wavelength 400 nm. Porphyrin derivatives can be found in three major sources;

haemoglobin by total synthesis or via management of the biosynthetic pathway to protohaem. Haemoglobin can be isolated from blood in slaughter house by treatment using heparin to avoid coagulation, haemolysis and centrifugation to get rid of the erythrocyte ghosts, then crystallized (**Figure 4)** [53]. Demetallization of protohaemin produces protoporphyrin. Handling of protohaemin with molten resorcinol followed by demetallization in acidic circumstances affords deuteroporphyrin. Deuteroporphyrin, protoporphyrin and haematoporphyrin are the most familiar starting materials for photodynamic photosensitizers accessible from haemoglobin [54]. Total synthesis of porphyrin derivatives can be classified into three major synthetic strategies; categories A and B are stepwise approaches which permits a substitution patterns with low symmetry, whereas category C is one-pot reaction process which is inexpensive approach for symmetrical substitution patterns (**Figure 5)** [55].

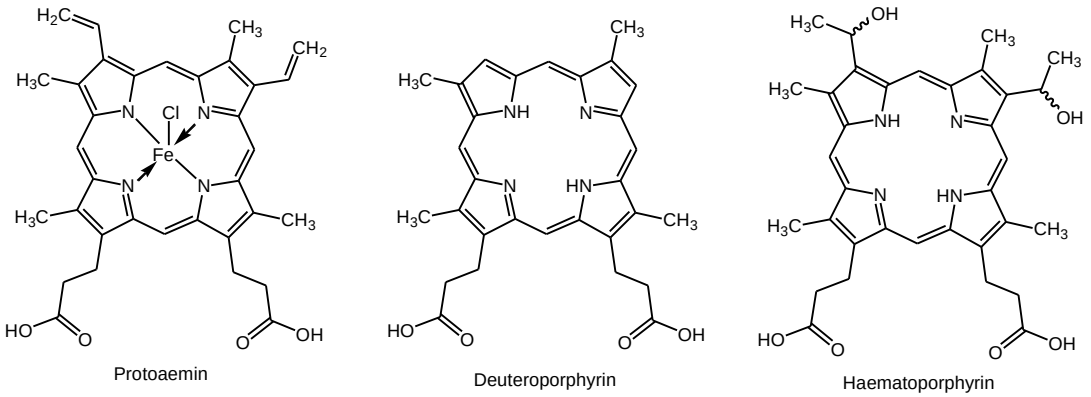


Figure 4. Porphyrins as macrocyclic aromatic sensitizers.

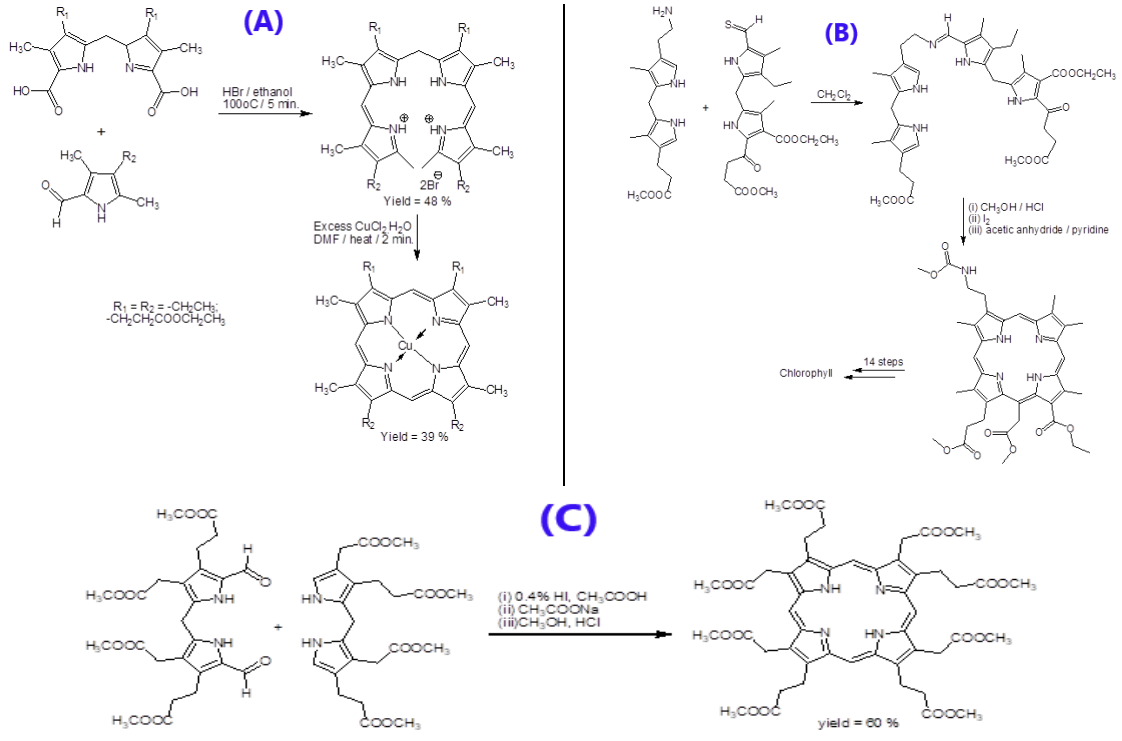


Figure 5. Category A approach is Johnson's biladiene-ac synthesis (A), Woodward's chlorophyll synthesis (B), and Category B approach is MacDonald's synthesis of uroporphyrin III octametyl ester; MacDonald's synthesis of uroporphyrin III octamethyl ester (C).

The most familiar porphyrin synthetic approach that have been carried out in the laboratory is the category C approach or Rothemund-Adler reaction, which affords meso-substituted porphyrins, such as

tetraphenylporphyrin. Meso-tetra(hydroxyphenyl)porphyrins are generally obtained via Rothemund-Adler route, typically with the protection of the phenolic function **(Figure 6)**. Porphyrin derivatives with six-membered anhydride, imide, or isoimide ring system fused across a meso-position and a neighboring *β*-position are distinguished by a retro-etio absorption spectrum, in which the most intense Q-band is band I **(Figure 6)** [25].

This type of porphyrin derivatives can be obtained from readily accessible porphyrin esters by means of intramolecular Friedel-Crafts reaction, but both the Mesoverdin and Purpuroporphyrin regioisomers are produced, which therefore have to be separated by chromatographic techniques and fractional crystallization. The most familiar and suitable starting materials are chlorophyll and bacteriochlorophyll, which are oxidized using DDQ to afford the equivalent anhydride-fused porphyrins, presently being studied as photosensitizers for photodynamic therapy [51, 56, 57].

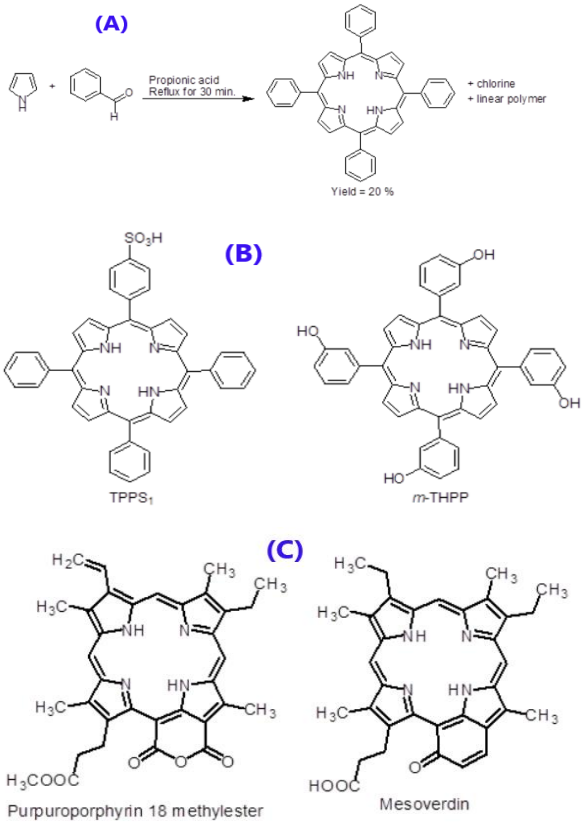


Figure 6. Rotemund-Adler reaction (A), Meso-substituted porphyrins (B), and Mesoverdin and Purpuroporphyrin regioisomers (C).

2.2.3. Endogenous porphyrins

The third method to the synthesis of porphyrins photosinstizers is the endogenous production of protoporphyrin using the body's enzymes to afford the porphyrin naturally. In this novel strategy, a non-phototoxic pro-drug, so-called 5-aminolevulinic acid or known as an endogenous non-proteinogenic amino acid, is managed to result in the endogenous synthesis of the proto-porphyrin IX photosensitizer (**Figure 7**). 5-Aminolevulinic acid is already included in the biosynthesis of heme. In the initial synthesis stage of the porphyrin-heme, 5-aminolevulinic acid is produced in mitochondria from glycine 5-aminolevulinic acid synthase enzyme and succinyl-CoA. Four enzyme catalyzed steps occur in the cytoplasm. The condensation process of two 5-aminolevulinic acid molecules affords porphobilinogen, the head to tail condensation process of four porphobilinogen molecules affords the linear hydroxylmethylbilane, which rearranges to the corresponding uroporphyrinogen III and is transformed to coproporphyrinogen by subtraction of carbon dioxide. The first three biosynthesis stages of porphyrin-heme return back to the mitochondria. Coproporphyrinogen III is oxidized initial to protoporphyrinogen, then to protoporphyrin [51, 56]. The fluorescence of porphyrin collected in tumor tissue after 5-aminolevulinic acid administration facilitates the visualization of the neoplastic districts. This procedure, usually called fluorescence diagnosis has been clinically useful in the detection of neoplasms [58].

2.2.4. Chlorins and bacteriochlorins

Chlorins and bacteriochlorins are different derivatives of *β*-dihydroporphyrin and *β*-tetrahydroporphyrin, correspondingly. Two categories of *β*-tetrahydroporphyrins are available; adjacent or isobacteriochlorin and opposite or bacteriochlorin. *β*-hexahydroporphyrin is also known (**Figure 7**)[59].

The absorption bands of chlorins are different in relative intensities of porphyrins. These hydroporphyrins can be obtained from natural resources or can be totally synthesized (Figure 8). The two major reactive functional groups of chlorophyll A are the vinyl group at C_3 that can be reduced to ethyl, giving the meso series materials, and the *β*-ketoester group that can be autoxidized and thermally can demethoxycarbonylized. Smith and Mironov have been investigated bacteriopurpurins analogues of purpurin 18 in the bacteriochlorophyll series. These extremely stable sensitizers demonstrate absorption band at wavelength $\lambda > 800$ nm [60].

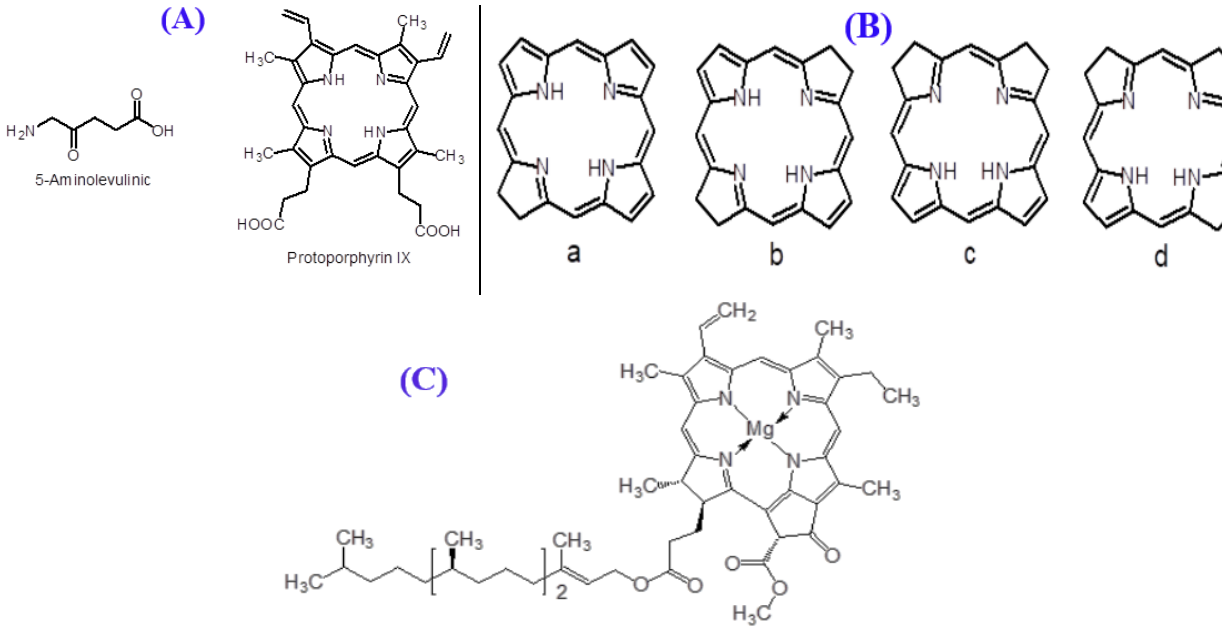


Figure 7. (A) 5-Aminolevulinic acid and protoporphyrin IX photosensitizer, (B) Macrocyclic chromophors have vital function in the living organisms such as the chromophore of chlorophyll A and chlorophyll B; chlorin (a), bacteriochlorin (b), isobacteriochlorin (c), and *β*-hexahydroxyporphyrin (d), and (C) molecular structure of Chlorophyll A.

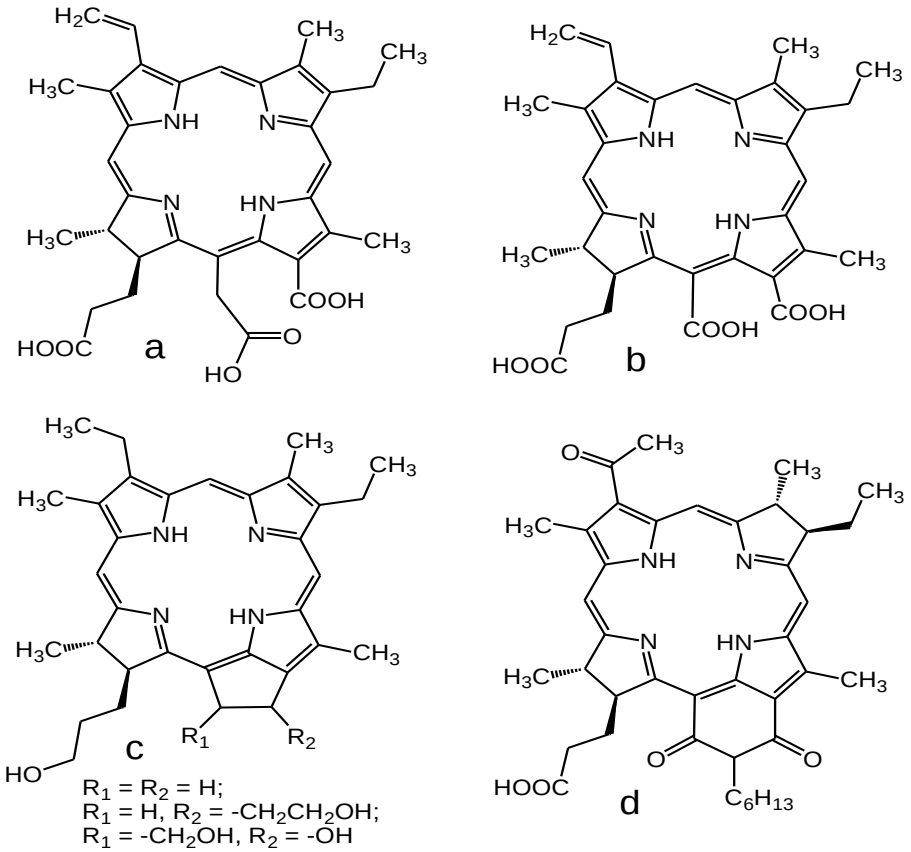


Figure 8. Different structures of Chlorins and bacteriochlorins; chlorin e_6 (a), chlorin p_6 (b), chlorin polyols (c), and bacteriopurpurin imide (d).

Commercial Laserphyrin is one of the first chlorophyll-based chlorins with established photodynamic therapy application. It is characterized by its solubility in water and has a small tumor accumulation time, thus a small drug-light gap, and fast clearance thus remaining skin photosensitivity is short. Laserphyrin is mainly obtained from chlorin tricarboxylic acid by DCC reaction with di-t-butyl aspartate. The clinical process uses relatively low drug dose and light dose. Laserphyrin has been approved for clinical exercise in Japan in 2003 for early-stage lung cancer, liver cancer, and head cancer. Another chlorophyll-based photosensitizer is the commercially available Photochlor with high lipophilicity for improved penetration of the cell membrane. Photochlor exhibits a higher level of tumor accumulation compared to Photofrin and Foscan. Clinical trials have been studied for esophageal cancer, basal cell carcinoma, Barrett's esophagus, cell lung cancer and lung cancer. Foscan, in water: PEG 400: ethanol = 5:3:2 ratio, was intravenous injected with a drug dose of 1.5 x 10^{-1} mg kg^{-1} then irradiated with at wavelength of 652 nm with a light dose of 5-20 J/cm^2 at 1-3 days after injection. Foscan efficiency is roughly 100 times higher than that of Photofrin when using similar photodynamic doses. However, Foscan necessitates long clearance time of about 4-6 weeks after injection [61]. Formulation of Nickel (II) etioporphyrin under Vilsmeier-Haack circumstances affords the meso-formyl derivative that can undergo Wittig reaction followed by demetallization to produce the meso-acrylic ester **(Figure 9).** Under soft acidic settings, the meso-acrylic ester experiences a reversible thermal cyclization to the neighboring *β*-positions. The produced Purlytin has been studied in clinical trials for Kaposi's sarcoma in patients with AIDS (acquired immunodeficiency syndrome), basal cell carcinoma, and metastatic breast adeno-carcinoma.

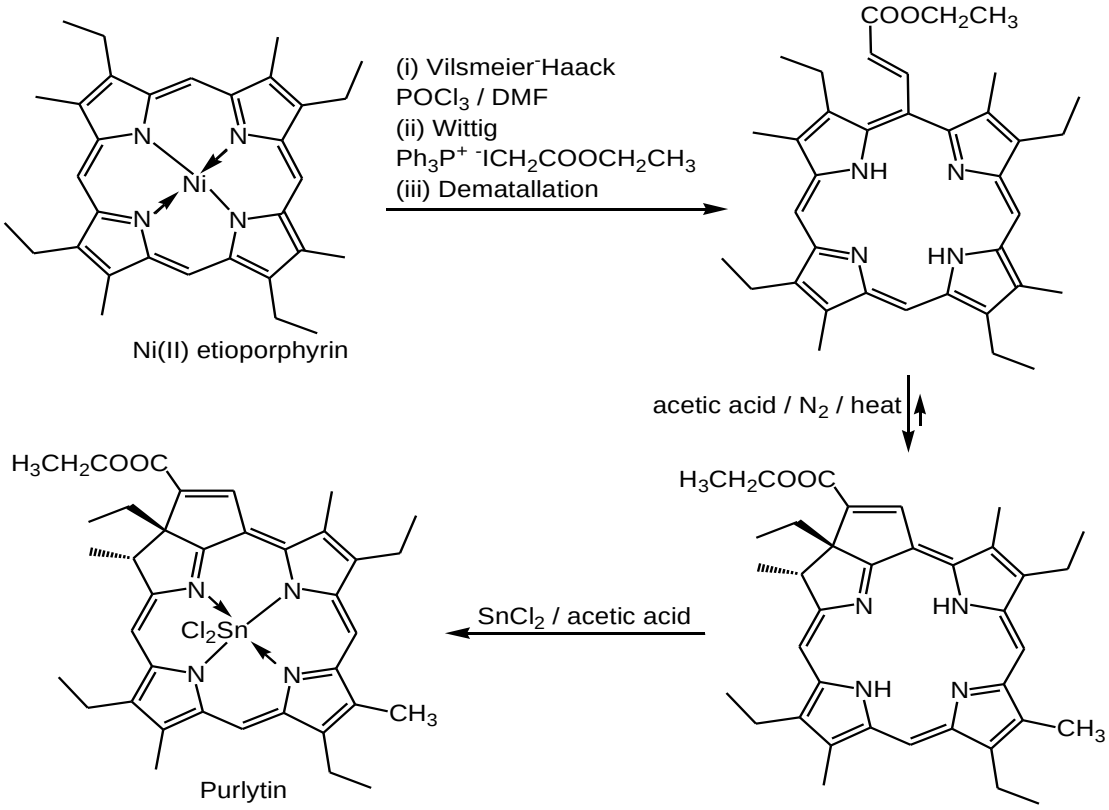


Figure 9. Formulation of Nickel (II) etioporphyrin under Vilsmeier-Haack conditions

2.2.5. Phthalocyanines and naphthalocyanines

Replacement of nitrogen for a meso-carbon-bridge in porphyrins affords azaporphyrins, while replacement of all the meso-carbon-bridges by nitrogen affords porphyrazines. These macrocyclic sensitizers produce extra stable metal complexes and turn out to be less basic with rising the meso-nitrogen substitution. Hence, the absorption spectrum of phthalocyanines changes significantly **(Figure 10)**. The absorption band of phthalocyanine derivatives is strong with high ε = 1-4 x 10^5 mol L^{-1} cm^{-1} at wavelength 670-700 nm. Metal free phthalocyanine derivatives possess two high extinction absorption maxima in the wavelength range, while the metal-containing phthalocyanine

derivatives typically possess only one high extinction absorption maxima [62].

A number of 5-azaporphyrins and benzoporphyrins have been reported as photodynamic therapy photosensitizers **(Figure 10)**. Demetallized and non-demetallized phthalocyanine derivatives can be obtained commercially in tonnage quantities and find a function as blue-green pigments and dyes. Iron-based phthalocyanine derivatives were serendipitously reported in 1928 throughout the path of manufacturing of phthalimide at Scottish Dyes. The procedure depends in passing ammonia to molten phthalic anhydride in iron-based vessels as it was established that throughout the formulation process, traces of a dark-blue material were produced in the reaction container [63].

The water solubility of the different phthalocyanine derivatives could be accomplished by employing polar functional groups to afford neutral, anionic, and cationic phthalocyanine derivatives **(Figure 11)**. The commercially available phthalocyanine photosensitizer, Photosens, composed of both di- and tri-sulphonic aluminium phthalocyanine. It absorbs at wavelength 675 nm with the highest extinction coefficient $\varepsilon = 2 \times 10^5$ mol L^{-1} cm^{-1} as a second-generation sensitizer. Photosens can be offered in a drug dose of 0.5-0.8 mg kg^{-1} and a light dose of 150 J cm^{-2} at 1-3 days after injection. It has been studied clinically for skin, breast, oropharyngeal, lung and larynx cancers. The remaining light photosensitivity of photosensitizer is a considerable difficulty [64].

2.2.6. Cyanine photosensitizers

Polymethine sensitizers contain an odd number of methine function groups bonded mutually through alternating single and double bonds **(Figure 11)**. Cyanine photosensitizers are a polymethine category [65]. Indocarbocyanine derivatives Cy3 and Cy5 are the first-known two sensitizers of the cyanines category which have been functionalized in imaging because of their notable light stability, high absorption cross-section, and better fluorescence efficiency. Merocyanine derivatives has been extensively applied in imaging and photodynamic therapy of leukemia [66].

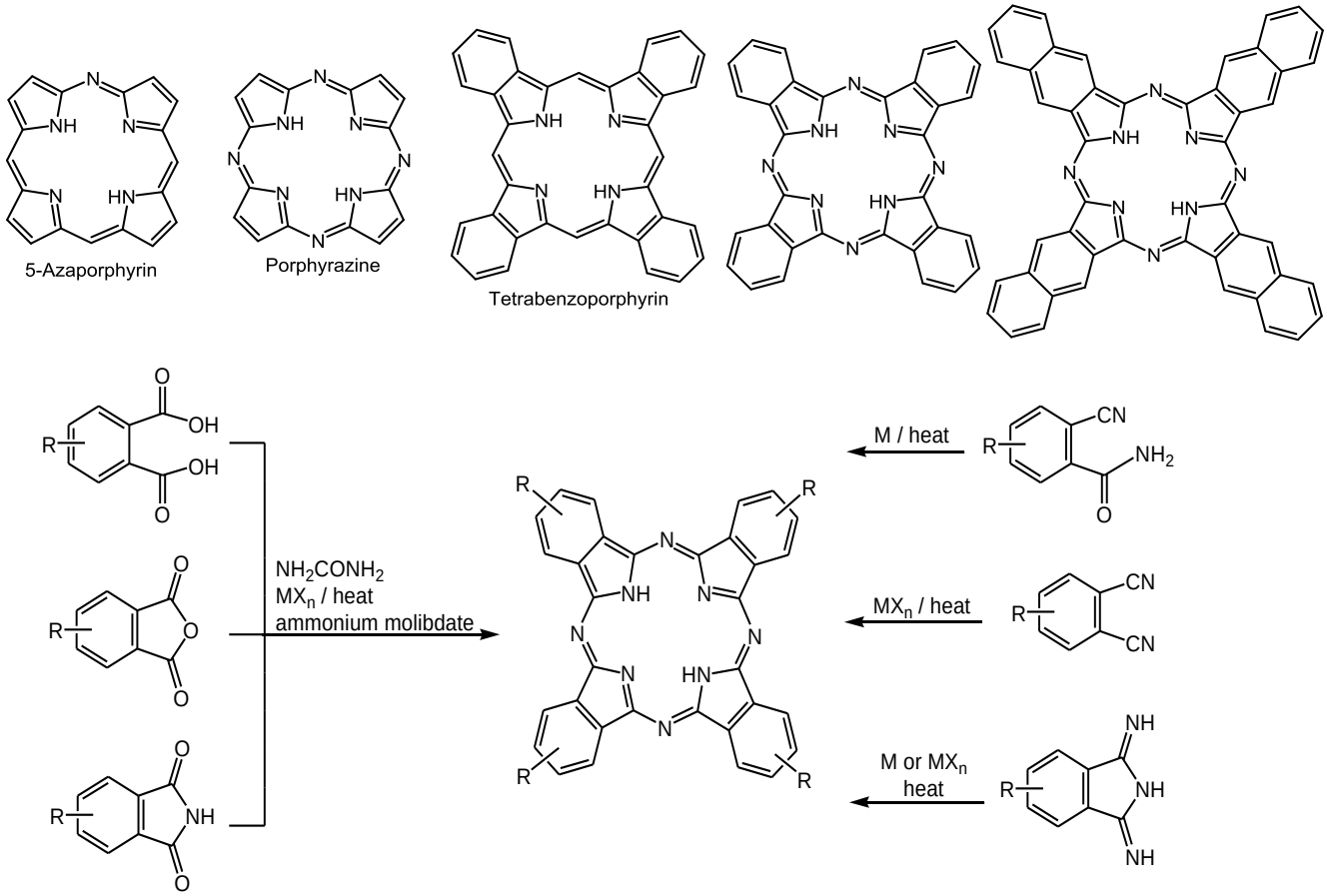


Figure 10. Metal-free phthalocyanines *(upper)* and synthetic routes to metallophthalocyanines *(lower).*

2.2.7. Hypericin photosensitizers

Hypericin sensitizers are one of the main active components of Hypericum **(Figure 11)**. This category absorbs at wavelength λ = 590 nm with absorption coefficient ε = 41600 L mol^{-1} cm^{-1}. Hypericin derivatives have been reported as a photodynamic therapy photosensitizer for viruses-124 and pancreatic cancer [67].

2.2.8. Phenothiazines photosensitizers

Phenothiazine sensitizers are cationic dyes, such as Nile blue A, toluidine blue and methylene blue **(Figure 12)** [68]. This sensitizer category demonstrates light stimulated microbicidal characteristics with reactivity in photodynamic therapy. Mellish et al. methodically reported a synthetic approach to methylene blue derivatives with a variety of alkyl chains at the amino functional groups in place of the methyl substituents to decrease the hydrophilicity [69].

2.2.9. BODIPY photosensitizers

BODIPY sensitizers are amenable to wide variations on their basic core structure; 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene **(Figure 12)**. Majority of these sensitizers comprise various perfect properties as a photosensitizer for photodynamic therapy. BODIPY photosensitizers are characterized by low dark toxicity, cellular uptake, high absorption coefficient, low quantum yield. Nearly all BODIPY photosensitizers are well excited into higher level singlet state, can launch fluorescence from this singlet state, and do not undergo inter-system-crossing to triplet excited state. The basic absorption wavelength of BODIPY sensitizers is 510–530 nm [24, 25].

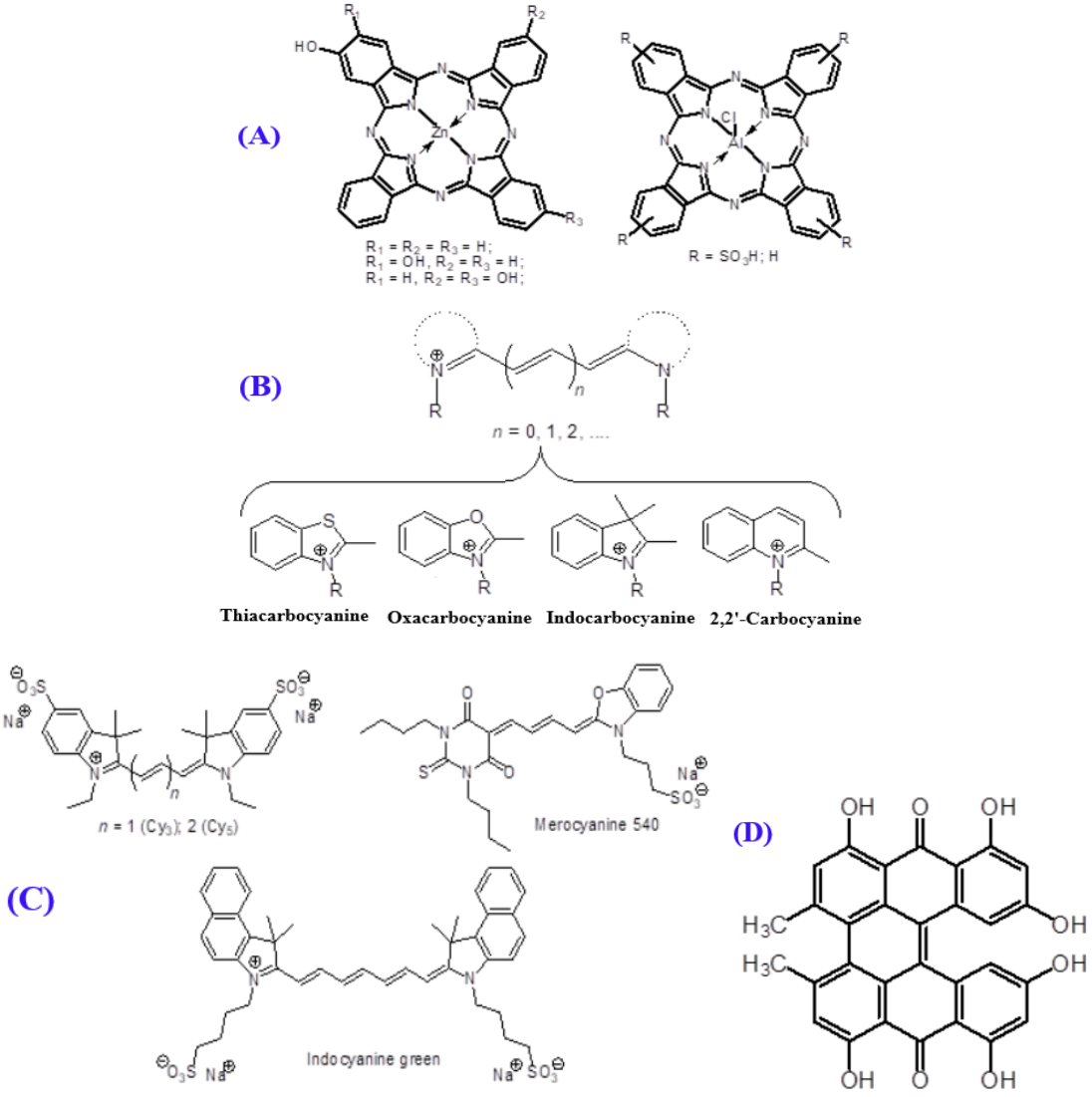
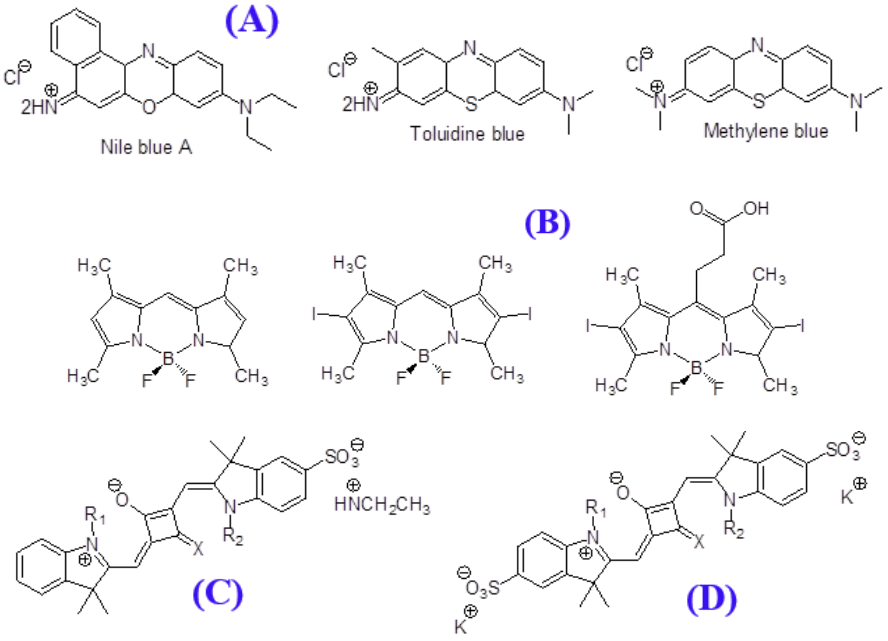


Figure 11. (A) Commercially phthalocyanine photosensitizers, (B) Chemical structure of cyanine photosensitizers, (C) Merocyanines, and Hypericin.

2.2.10. Squaraine photosensitizers

Squaraine sensitizers are model candidates for fluorescence recognition in biomedical purposes **(Figure 12)**. They have narrow intense absorption maxima, with absorption coefficient up to 3 x 10^5 L mol^{-1} cm^{-1}. They are distinguished by an extremely tiny Stoke-shift. Squaraine sensitizers have also been effectively functionalized as a second-generation photosensitizer [36].

Pagani et al. examined the photosensitizing characteristics of benzothiazole squaraines in different tumors [70]. In the dark, benzothiazole squaraines are mainly non-toxic, but when subjected to visible light, they stimulate strong photodynamic effects that lead to cell death in all examined tumor cells. Squaraine derivatives are usually not soluble in water. However, their solubility was raised by polar functionalization using sulfonates, carbohydrates, quaternary ammonium salts, the carboxylates, polyethylene glycols, hydroxylates, and phosphonates [36].



C			D	
X	**R_1**	**R_2**	**X**	**R_1**
O	CH_3	$(CH_2)_5COOH$	O	$(CH_2)_3COOH$
$C(CN)_2$	CH_3	$(CH_2)_5COOH$	$C(CN)_2$	$(CH_2)_3COOH$
S	CH_3	$(CH_2)_5COOH$	S	$(CH_2)_3COOH$
$C(CN)_2$	$(CH_2)_3COOH$	CH_2CH_3	O	$(CH_2)_3COOSu$
O	CH_3	$(CH_2)_5COOSu$	$C(CN)_2$	$(CH_2)_3COOSu$
$C(CN)_2$	CH_3	$(CH_2)_5COOSu$	S	$(CH_2)_3COOSu$
S	CH_3	$(CH_2)_5COOSu$		
$C(CN)_2$	$(CH_2)_3COOSu$	CH_2CH_3		

Figure 12. (A) Phenothiazine cationic chromophores, (B) BODIPY sensitizers, and (C & D) squaraine sensitizers.

3. Conclusion and future prospective

Photodynamic therapy has been enthusiastically investigated for various therapeutic applications in oncology and various non-anticancer applications. Oncologically, the photodynamic therapy is rising as a potential therapy for a broad diversity of malignant tumors. Furthermore, Photodynamic therapy is applied as a post-effective adjuvant in curing head, lung and neck cancers. The outcomes from preclinical investigations propose that photodynamic therapy could improve the purge of bone marrow by removing malignant cells before grafting. Clinical investigations regarding this potential application are currently in progress. The real limitation in photodynamic therapy is the tumor size that is subject for treatment. The evaluation of photosensitizers absorbing in the infrared region, fulfils an actual requirement the infrared absorption wavelength penetrate more deeply into the infected tissue and could permit treatment of large size tumor. In order to enhance the efficiency of photodynamic therapy and expand its applications, a diversity of second and third generation sensitizers are currently being assessed for their effectiveness in cancer treatment. Photosensitizers for photodynamic therapy should meet these standards; chemical and water stability, large quantum yield of singlet oxygen production, zero cytotoxicity in dark environment, tumor selectivity, fast accumulation in targeted tumor, fast clearance from subject, and high extinction coefficient at longer wavelengths in the infrared area. Photosensitizers for photodynamic therapy should meet these standards; chemical and water stability, large quantum yield of singlet oxygen production, zero cytotoxicity in dark environment, tumor selectivity, fast accumulation in targeted tumor, fast clearance from subject, and high extinction coefficient at longer wavelengths in the infrared area. Thus, various difficulties may still need to be solved in the future.

Conflict of Interests

The authors declare no conflict with interest.

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