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Recent Photosensitizer Developments, Delivery Strategies and Combination-based Approaches for Photodynamic Therapy†

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ABSTRACT

Photodynamic therapy of cancer (PDT) is a therapeutic technique, minimally invasive, which is currently used to treat cancerous lesions and tumors that have been in the spotlight for its potential over the recent decades. Nonetheless, PDT still needs further development to become a first-option treatment for patients. This review compiles recent progress in several aspects of the current research in the constantly growing area of PDT to overcome the main challenges as an attempt to serve as a guide and reference for newcomers into this research area. This review has been prepared to highlight the use of chemical modifications on photosensitizers to improve their solubility, photostability, selectivity and phototoxicity. Additionally, the use of liposomes and cavitands as drug delivery systems to aid in the biodistribution and bioaccumulation of photosensitizers is presented. Also, the combination of PDT with chemotherapy or immunotherapy as an option to boost and improve treatment outcomes is discussed. Finally, the inhibition of antioxidant enzymes as a strategy for a synergistic effect to ameliorate the performance of the photosensitizers in PDT is presented as an alternative for future researchers.

INTRODUCTION

Photodynamic therapy (PDT) is a currently approved, minimally invasive therapeutic strategy, for the treatment of cancer. PDT utilizes a photoactive drug denoted as photosensitizer (PS), which is allowed to accumulate in the cancerous lesion or tumor and later illuminated with light of an adequate wavelength to generate reactive oxygen species (ROS). These ROS ablate cancer cells by oxidizing biomacromolecules, specifically proteins, nucleic acids and/or lipids, therefore increasing the oxidative stress within the cell and inducing cell death through apoptosis, necrosis, or autophagy (1–3).

The first literature report on using photosensitizers to treat human tumors is from 1903 by Von Tappeiner *et al*. (4) using Eosin along with light irradiation to treat skin tumor. Since then, several photosensitizers have been used over the years such as Hematoporphyrin derivative (HpD) and others denoted as first-generation photosensitizers (5), which are characterized for being natural mixtures of photoactive oligomers (6). These photosensitizers were relatively effective in treating several types of cancers, but presented problems such as low purity, skin photosensitization and short absorption wavelengths, which translated into poor photoactivation with the therapeutic light source, that is, absorbed mostly by the skin and not by the photosensitizer (7).

To overcome those problems, second-generation photosensitizers were developed such as *N*-aspartyl chlorin e6 and Meso-tetrakis(4-sulfonatophenyl porphyrin) (TPPS) (5) or methylene blue (MB) derivatives. Second-generation photosensitizers were characterized as being pure, of synthetic origin, with known properties, high ROS generation and long wavelengths of absorption (8,9).

Third-generation photosensitizers are conjugates of well-known photosensitizers with other chemical moieties that enhance biodistribution, bioaccumulation, target selectivity, cell uptake and other properties that emerge from therapeutic aspects of the technique (10). For third-generation photosensitizers, chemical modifications and encapsulation within carriers

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(proteins, macrocycles, micelles and liposomes among others) have become very useful strategies to modulate photosensitizer properties and increase the therapeutic outcome of PDT (11).

PDT like many other cancer treatments has advantages and disadvantages as a therapy. One of the main advantages that could be mentioned for PDT is that it is multitarget, uses a single injection, and it has not been reported to induce resistance. Moreover, it should be highlighted that PDT can be used in combination with other conventional treatments for cancer. On the downside, disadvantages such as poor solubility of photosensitizers, tendency to aggregate, which decreases photoactivity and enzymatic degradation are well recognized. Also, some PS have poor biodistribution and bioaccumulation profiles, a problem that needs to be overcome for PDT to improve as a therapeutic alternative (1,12).

Depending on the nature of the photochemical process by which the PS generates ROS, one could devise different approaches to improve their performance. These photochemical processes need to be fully understood and well characterized as a starting point for PDT. The mechanistic nature of these processes has been well characterized and guidelines to identify them have been recently summarized in the literature (13).

Another relevant aspect of research involving PDT is knowing the biological targets for the ROS generated by the PS of our choice. Having an all-around understanding of these photosensitization reactions, their targets and the possible photooxidation products is a key component of designing PDT experiments. These aspects have also been reviewed and well discussed recently in the literature (14).

The are several recent reviews on PDT research that focus mainly on fundamental aspects of PDT, photosensitizers, natural products as sources of photosensitizers, applications and future directions (15–17). Also, there is one recent review that has dissected how many reviews have been published recently from different perspectives classifying them by topics such as overviews, light sources, dosimetry, PDT mechanism, type of cancers and even the meta-analyses of photosensitizers (18).

Although, a lot of attention has been given to PDT in the last few years, there is still much to be done to overcome some of the limitations previously mentioned. We have been working for several years on different aspects of fundamental and applied photochemistry and photobiology, which have influenced the selection of topics for this review. We are presenting herein a review of the recent literature that has utilized different approaches to increase the selectivity, modified the photochemical and photophysical properties and improved the phototoxicity of photosensitizers. Besides the aforementioned, another strategy that we are presenting in this work is the use of drug delivery systems (DDS). First, we describe the use of liposomes due to their useful properties to overcome some major drawbacks of some PS commonly used in PDT. Secondly, we pay special attention to cavitands, particularly macrocycles due to their increased popularity in recent years for the development of applications for PDT. Then, we highlight some interesting reports of the combination of PDT with chemotherapy or immunotherapy to enlighten future researchers in this area. Finally, we highlight some interesting works that have been published where they combined the inhibition of antioxidant enzymes together with PDT to accomplish a synergistic effect to improve the overall performance.

PHOTOSENSITIZERS

The key component for the development of PDT as an alternative treatment for cancer are the photoactive drugs or photosensitizers. Therefore, we start by presenting some of the most relevant aspects that should be considered if you are a new researcher in this area. In this section, we consider the chemical modifications introduced to improve the photophysical properties and enhance the overall performance of photosensitizers. We also present some cutting-edge approaches in synthesis to improve the selectivity and phototoxicity of diverse photosensitizers.

The effectiveness of PDT depends on many factors such as biodistribution, bioaccumulation, dark toxicity of the photosensitizer and the photophysical properties. The development of new photosensitizers for PDT applications has been widely studied over the last several decades to enhance these relevant properties (7). The main mechanisms of action of photodynamic therapy of cancer are based on photophysical processes that in most cases can be summarized according to Fig. 1 as the following:

Light of a specific wavelength is absorbed by a photosensitizer and is excited to a high-energy singlet state S_1. From this state, the molecule can decay back to the ground state by emitting a photon (fluorescence), decaying through nonradiative ways or can go through intersystem crossing (ISC), resulting in generation of a triplet excited state T_1 (longer-lived excited state). From this state, the photosensitizer can then react with other molecules. The most important reactions that are relevant to PDT are the ones where the photosensitizer interacts with: (1) biomolecules or (2) molecular oxygen. In the first case called a type I mechanism, the reaction proceeds mainly via electron transfer to relevant substrates in the presence of oxygen-generating reactive oxygen species (ROS) such as superoxide radical anions ($O_2^{\bullet -}$), hydrogen peroxides (H_2O_2) and hydroxyl radical ($^{\bullet}OH$). Alternatively, in type II mechanisms the triplet excited state photosensitizers can interact directly with molecular oxygen via energy transfer to generate singlet oxygen ($^1O_2$), a highly reactive form of oxygen (19,20).

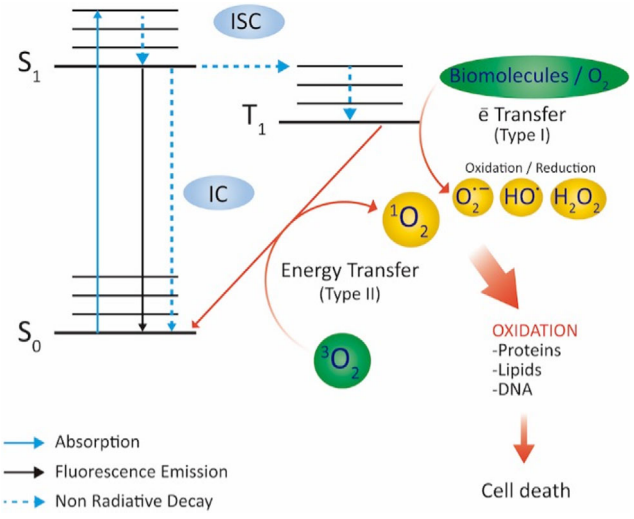

Figure 1. Jablonski diagram for photophysical processes relevant to photodynamic therapy. Reproduced from (19).

Chemical modifications to enhance photophysical properties

The design and development of new photosensitizers have been necessary to overcome the limitations of traditional PS commonly used for PDT, but this remains a problem for the scientific community. Considering all the above, it is evident that over the years, many endeavors have been made to augment the photophysical properties of the photosensitizers to improve the overall effectiveness of this therapeutic treatment and the chemical modification of photosensitizers has been one of the main strategies used. This section will review the main photophysical conditions necessary to have an efficient photosensitizer such as correct light absorption properties, high photostability and high ROS generation and how these conditions can be reached through chemical modification of photosensitizers.

Reviewing the literature, it can be deduced that high photostability, high ROS generation and long-wavelength absorption are desirable properties for photosensitizers and with chemical modification of the molecules these can be achieved. The implications of those photophysical properties for PDT are:

1 Photostability: Photodegradation of photosensitizers is a common problem in PDT, *e.g.* one of the main photodegradation pathways of protoporphyrin IX is auto-oxidation by the singlet oxygen molecules generated (21). Photosensitizers with high photostability are desired because they can provide efficient and consistent $^1O_2$ production without degrading in secondary oxidation products with unknown properties that could be deleterious. A photostable photosensitizer can increase the effectiveness of PDT without the need for high concentrations, which can cause side effects such as skin photosensitization (22).
2 Generation of ROS: When photosensitizers are present together with low light doses, one of the accepted mechanisms of action against cancer cells of PDT is the oxidation of important structures such as mitochondria, an event that leads to the release of signaling proteins that induce apoptosis (20,23). Without considering location, PDT effectiveness is related directly to ROS generation. Due to this fact, the capability to develop a long-lived triplet excited states and high singlet oxygen quantum yields leads to an enhanced photodynamic killing of cancer cells.
3 Long absorption wavelength: In an ideal therapeutic situation, the photosensitizer enters the organism by injection and then accumulates selectively in tumor cells. When the tumor is not at a superficial level (namely skin cancer) and is a deep-seated tumor inside the organism, there must be a light penetration depth to activate the photosensitizer. For this reason, and to overcome the strong absorption of melanin, proteins, vitamins and other molecules present in the superficial layers of skin, the phototherapeutic window used for external beam PDT usually needs to be within 600–800 nm for the absorption wavelength of photosensitizers (24). It is for this reason that developing photosensitizers that absorb at long wavelengths with a high extinction coefficient, is one of the main goals for the overall effectiveness of PDT. Although, interstitial light delivery using optic fibers is recognized as a promising option to treat internal tumors in several locations of the human body (25,26).

Most relevant examples in the literature of the last few years on chemical modification of photosensitizers and their effect on photophysical properties are summarized below.

Castro *et al.* (27) synthesized chlorin derivatives using 1,3-dipolar cycloadditions on 5,10,15-tris(pentafluorophenyl)-20-(4-pyridyl) porphyrin, resulting in enhanced singlet oxygen generation (φ_Δ values from 0.65 to 0.81) with good photostability and with relatively strong absorption band in 650 nm. The authors reported that the reduction of one of the β-pyrrolic because of the introduction of a pyrrolidine group is responsible for the intense absorption in 650 nm.

Mangalath *et al.* (28) reported BODIPY-graphene oxide quantum dots nanoconjugates (GQD-BDPA) with high triplet excited state and singlet oxygen quantum yields, plus excellent water solubility. The GQD-BDPA showed a high singlet oxygen generation quantum yield of 90% vs 85% of free BDPA, redshift from 530 nm to 550 nm and an increase in the triplet state lifetime from 0.49 μs of free BDPA to 0.55 μs for GQD-BDPA.

Mai *et al.* (29) improved several photophysical properties of heptamethine cyanine IR780, a NIR absorbing photosensitizer, by introducing different chain lengths of tethered morpholine groups in the structure. The photostability of IR780 derivatives was increased compared to IR780 without chemical derivatization. The authors reported that the photostability is increased due to a dispersal of the pull-push π-conjugation system in the structure of IR780 because of a direct N atom substitution of the meso-chlorine atom.

Li *et al.* (30) synthesized a series of new fluorinated hematoporphyrin derivatives with low HOMO–LUMO energy gaps which presented long absorption wavelengths. The singlet oxygen generation rates were six times higher for this new derivative compared to Hematoporphyrin. The same authors reported the synthesis of new pyropheophorbide derivatives with methyl 5-aminolevulinate. The derivatives showed a more efficient generation of singlet oxygen than pyropheophorbide without derivatization together with a redshift of the Q band. Also, they reported that the introduction of 5-aminolevulinic acid chains and the modification of ethylene groups of the pyropheophorbide molecules could inhibit self-aggregation processes and as a result of that enhances singlet oxygen generation (31).

Szurko *et al.* (32) synthesized novel chlorin e6 derivatives with polyol amines acting as nonionic hydrophilic center. The derivatives showed in the red region of the spectra a high molar absorption coefficient with values higher than 1×10^4 mol^{-1} cm^{-1} at 665 nm, triplet lifetimes of 0.23–0.27 μs and negligible photodegradation under physiological conditions.

Babu *et al.* (33) synthesized chlorins substituted with thien-2-yl and meso-tetra(5-bromothien-2-yl) moieties. These derivatives possess redshifts on their Q bands from 650 nm to 660 and 664 nm, respectively; long triplet excited state lifetimes reaching 89.3 μs and high photostability up to 95% under LED irradiation. The authors interpreted that the derivatization with sulfur and bromine-containing moieties acting as heavy atoms facilitates the ISC to triplet state by spin-orbit coupling, therefore populating the triplet state.

Xiao *et al.* (34) developed AQPO and AQPI, two phenanthro [9,10-d]imidazole derivatives, with ultra-low singlet-triplet splitting energy (ΔE_{st} of 0.09 and 0.12 eV) which causes an enhanced ISC process, promoting efficient triplet exciton formation and therefore incrementing ROS generation. The authors demonstrated that the derivatives achieved a three-fold increase in ROS generation via Type I mechanism.

Chemical modifications to enhance selectivity and phototoxicity

As previously mentioned, the first generation of PS included hematoporphyrin derivatives such as Photofrin, which is currently approved by the FDA for treating esophageal and endobronchial cancer. Many porphyrin and nonporphyrin derivatives with chemical modification of the core (second-generation PS) were communicated in the literature to overcome major drawbacks like poor aqueous solubility, low selectivity, broad biodistribution, *etc*. (35). Herein, we summarize the current reports on the development of new PS, consisting of porphyrin and nonporphyrin derivatives, and the synthetic approaches to improve their solubility, selectivity, and phototoxicity.

The family of porphyrin derivatives includes PS such as TMPyP (36), chlorins (37), protoporphyrin IX (38) and phthalocyanines (39). Several recent reports on the literature have emphasized the necessity to increase their solubility to improve their PDT performance. Introducing polar substituents like hydroxyl groups on porphyrin-based PS show higher hydrophilicity and more efficient PDT effects compared to nonpolar substituents (40). Feng *et al*. (41) developed two isomers of porphyrin derivatives using their carboxylic acid groups and the formation of an ether bond with the porphyrin core. These derivatives show advantages such as high-water solubility, their absorption bands were red shifted when compared to the precursor and high ROS generation. These properties were related to an increase in their phototoxicity, a decrease in the PS doses and more selectivity toward cancer cells. In addition, Hou *et al*. (42) synthesized halogenated porphyrin derivatives using metallic and nonmetallic complexes with zinc and copper. These derivatives showed an increase in solubility, higher singlet oxygen quantum yield and phototoxicity, lower photobleaching of the ligands than metal complexes, cancer cell selectivity and significant cell death by apoptosis.

Desgranges *et al*. (43) synthesized amphiphilic derivatives of protoporphyrin IX with improved properties like increased water solubility and high photodynamic effect. On the other hand, the group of Faustino has synthesized derivatives of TMPyP and showed higher singlet oxygen generation than TMPyP, high phototoxicity effect through apoptosis and autophagic mechanisms of cancer cells (44). In addition, the derivatization of chlorine with maleimide, cysteine and glutathione was reported by Guo *et al*. These results confirmed a higher phototoxicity effect and ROS generation for the maleimide derivative than the glutathione derivative due to the reaction of maleimide group with the intracellular glutathione enhancing the oxidative stress within the cells (45). Novel phthalocyanine derivatives with iodomethane choline developed by Zhao *et al*. (46) have shown nonaggregation effects, high fluorescence, high singlet oxygen generation and good photostability which are promising theranostic agents for cancerous cells. Huang *et al*. (47) synthesized a phthalocyanine derivative with indomethacin which reduced the aggregation of the PS, enhanced the fluorescence and selectivity targeting the tumor cells therefore improving the photodynamic therapy.

Nonporphyrin PS such as distyryl boron dipyrromethene (BODIPY), methylene blue, toluidine blue, cyanine, fluorescein and rhodamine, among others, have also been derivatized (48). A wide range of BODIPYs have been reported in the literature for PDT applications. Ucar *et al*. (49) developed BODIPY derivatives using triphenylphosphonium (TPP) that increased their amphiphilic character and selectivity for mitochondria in HeLa cells, showing an increasing PDT effect. The group of Kim synthesized derivatives of lactose-functionalized BODIPY nanoparticles with heavy atoms; these derivatives showed an increase in their solubility and good imagining properties, therefore showing the capabilities to be used as theranostic agent and remarkable selectivity for asialoglycoprotein in cancer cells (50).

The group of Qian has derivatized a photosensitizer based in cyanine and rhodamine; this PS is a FRET system that showed enhanced singlet oxygen quantum yield, mitochondria targeting ability and high phototoxicity effect (51). Furthermore, Zhao *et al*. (52) synthesized two derivatives of cyanine with substituents in *meso* position to improve singlet oxygen generation, high excited-state lifetime, hydrophilicity, biocompatibility, mitochondrial selectivity and phototoxicity in cancer cells. On the other hand, chloro-hemicyanine derivatized with cysteine showed a synergetic effect of both photodynamic and photothermal activities with selectivity toward cancer cells due to high level of cysteine (53).

Liu *et al*. (54) developed theranostic fluorescein derivatives with applications for PDT. These derivatives showed lysosomal targeting ability, singlet oxygen generation and phototoxicity under hypoxia conditions.

The group of Zhang developed a toluidine blue derivative conjugated with biotin and encapsulated within cucurbit[8]uril to increase selective accumulation in the tumor and theranostic capabilities (55). Our group, Robinson-Duggon *et al*. (56), synthesized toluidine blue derivatives conjugated with fatty acids and studied the interaction with drug delivery systems such as cucurbit[7]uril and Human Serum Albumin; these fatty acids-derivatives showed enhanced interactions with Human Serum Albumin, phototoxicity and incorporation within cancer cell. In the same manner, our group, Mariño-Ocampo *et al*. (57), synthesized two derivatives of toluidine blue containing thiol groups that were covalently bound with Human Serum Albumin, which showed changes in its photophysical properties such as enhanced photostability and cell uptake.

Thomas and Greer's groups have prepared over the years several lipophilic pterins derivatives to improve membrane interactions and singlet oxygen generation capabilities of these photosensitizers. They utilized a synthetic approach where they added a decyl chain to several pterins derivatives through a nucleophilic substitution (SN_2) reaction. These photosensitizers are an excellent addition to the current PS because there is a need for lipophilic photosensitizers. First, they reported lipophilic decyl chain–pterin conjugates that demonstrated more singlet oxygen generation than the parent molecule and photooxidative capabilities amenable for applications in PDT (58). Later, a derivative denoted as CapC, a decyl pterin-6-carboxyl ester, which maintain the pterin amide group with the capability of being a membrane intercalator, as well as a fluorophore and $^1O_2$ sensitizer. Therefore, this compound was able to be a "self-monitoring" fluorescent probe and photodamaging agent for membranes (59). Recently, they reported a *mono-* and *bis*-decylated lumazines that showed a somewhat increase in the fluorescence quantum yield as well as an increase in organic solvent solubility, although less photostability when compared to the parent molecule (60).

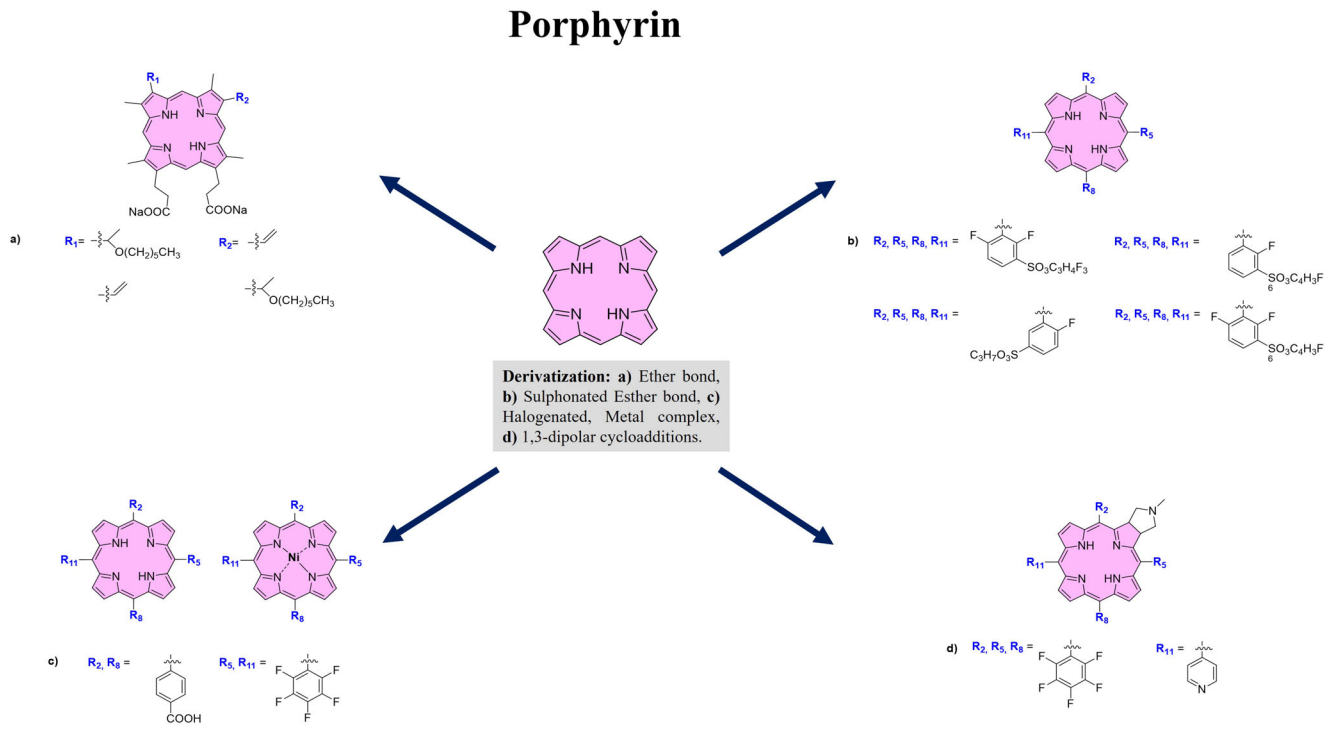

Figure 2. Chemical structures of Porphyrin derivatives. Summarized for references (27,41,42).

Figures 2–4 summarize the chemical modifications of the above-mentioned PS in both sections of this review. Porphyrin and nonporphyrin PS have been optimized for solubility purposes and enhanced photophysical properties. PS modifications with sugars, biotin and other reactive molecules have been used to increase their selectivity toward cancer cell receptors, proteins, *etc*. Different synthetic approaches to increase photostability, modify photochemical properties of PS, enhance the selectivity and phototoxicity were presented in this section 3. Nevertheless, some PS still need more assistance to improve the PDT treatment outcome. Therefore, in the next section, the use of drug delivery systems to boost the overall performance of PS used in PDT are summarized.

USE OF DRUG DELIVERY SYSTEMS FOR PHOTOSENSITIZERS

In this section, we present relevant system for the use of drug delivery systems (DDS) to improve biodistribution, bioaccumulation and overall performance of PS used for PDT applications. Herein, the focus has been put on two commonly used DDS, that is, liposomes and the emerging use of cavitands.

Liposomes for PDT

Liposomes, also known as lipid vesicles, have been considered as a good model for biological membranes, were used first in 1965 (61,62) and selected as a simple system of DDS in 1970 (63). Specifically, they are formed by a phospholipid bilayer that is self-enclosed in an aqueous medium, leaving an aqueous solution inside the membrane, known as an internal aqueous core, separated from the external medium by the bilayer. In particular, liposomes can be classified as small, large and giant unilamellar vesicles according to its size (SUV, 50–100 nm, LUV, 100–250 nm, GUV, 1–200 μm). In balanced osmotic conditions, liposomes have a spherical shape, with the hydrophilic segment of the lipid (polar head) located both outside and inside the liposome, in contact with the aqueous phase (64). Besides, the hydrophobic segment (apolar tails) forms the inner section of the lipid bilayer. The aqueous phase found inside the liposome (internal pool) is initially composed of the same solution with which the liposome was made, these properties of liposomes have allowed for many years to enhance PDT treatment effectively with minimal damage in a patient (65). On the other hand, several formulations of liposomes have been accepted through the U.S. Food and Drug Administration (FDA) (66,67). Liposomes have shown great potential in the encapsulation of different PS, chemotherapeutic drugs and other therapeutically activated molecules in lipophilic/hydrophobic and hydrophilic sites (68), respectively, for improving cellular barriers that make the drug delivery toward targeted side difficult and for an effective tumor PDT.

In these contexts, it has been shown that the use of the liposomes as DDS has significantly impacted pharmacology improving the biodistribution, accumulation and efficacy of PS or drugs, for effective tumor photodynamic therapy (69). In particular, the incorporation of the PS or drugs in liposomes has been evaluated through encapsulation into a hydrophobic medium or hydrophilic medium depending on the properties of the PS or Drugs (Fig. 5). First, we present different studies that use liposome types with PS or drugs incorporated in the hydrophobic medium.

In recent years, Wang *et al*. (70) reported the incorporation of 7-dehydrocholesterol and meso-tetraphenylporphyrin (TPP) in liposomes which can efficiently enhance the anticancer activity due to an increase in the selectivity toward tumoral cells. In particular, the cytotoxicity and phototoxicity of the different

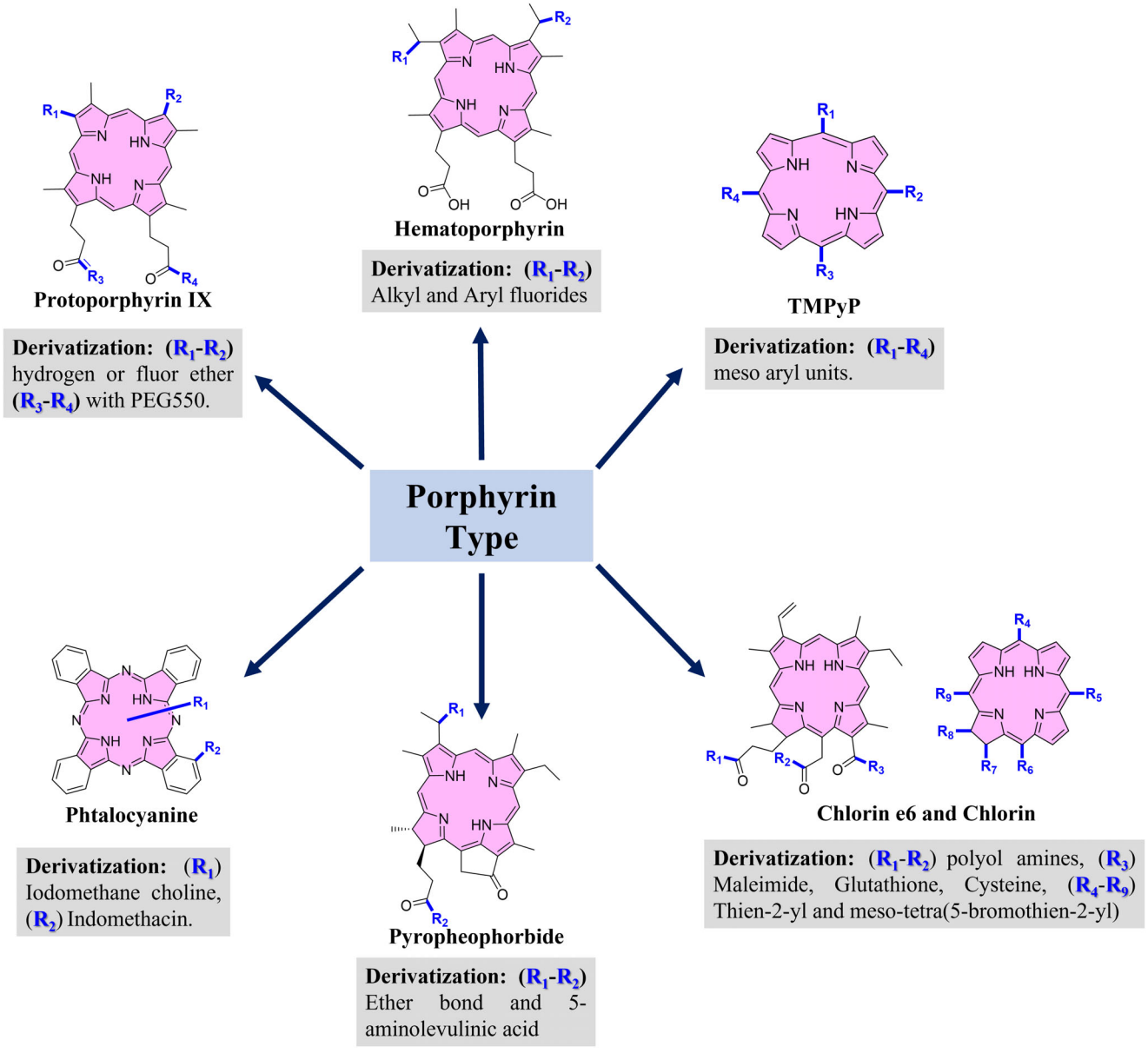

Figure 3. Chemical structures of Porphyrin-type derivatives. Summarized for references (30–33,43–47).

liposomes used for human ovarian cancer (SKOV-3 cells) evaluated by MTT assay, showed low and high toxicity in dark and under irradiation respectively, indicating the effective use of liposomes for the increases the PDT efficacy.

Besides, the modification designed to carry liposomes has permitted the control of their surface properties ensuring their accumulation and selectivity by tissue. Przybylo *et al.* reported that the electrostatic potential modification and surface topology of liposomes affected the internalization in single cells. Protoporphyrin IX was encapsulated into liposomes and examined on HeLa cells. The results showed that liposomes are an effective DDS for hydrophobic photosensitizers, favoring delivery to HeLa cells followed by their accumulation throughout the endomembrane system. Finally, they also demonstrated that the properties of the liposome affected not only the bioaccumulation of the PS but also the distribution within the cells (71).

The development of new PEGylated cationic liposomes containing hydrophobic PS such as zinc phthalocyanine in combination with acriflavine (ACF) that increased cell death in human epidermoid carcinoma (A431) cells (72). Broekgaarden *et al.* (72) reported that PEGylation is necessary to avoid the consumption of the liposomes in no cancer cells and reduce the effects *in vivo* produced by the cationic lipids.

Furthermore, the authors evaluated that the inhibition of the hypoxia-inducible factor 1 (HIF-1) together with ACF improved the cell death under hypoxic conditions and decreasing the production of HIF-1 favoring the PDT efficacy.

Vetha *et al.* (73) reported the use of natural curcuminoids (CUR). They presented high anticancer and anti-inflammatory activities of CUR, but their low solubility and bioavailability restrict their therapeutic effects and also restrict the use of CUR as a photosensitizer. For this reason, the design of liposomal

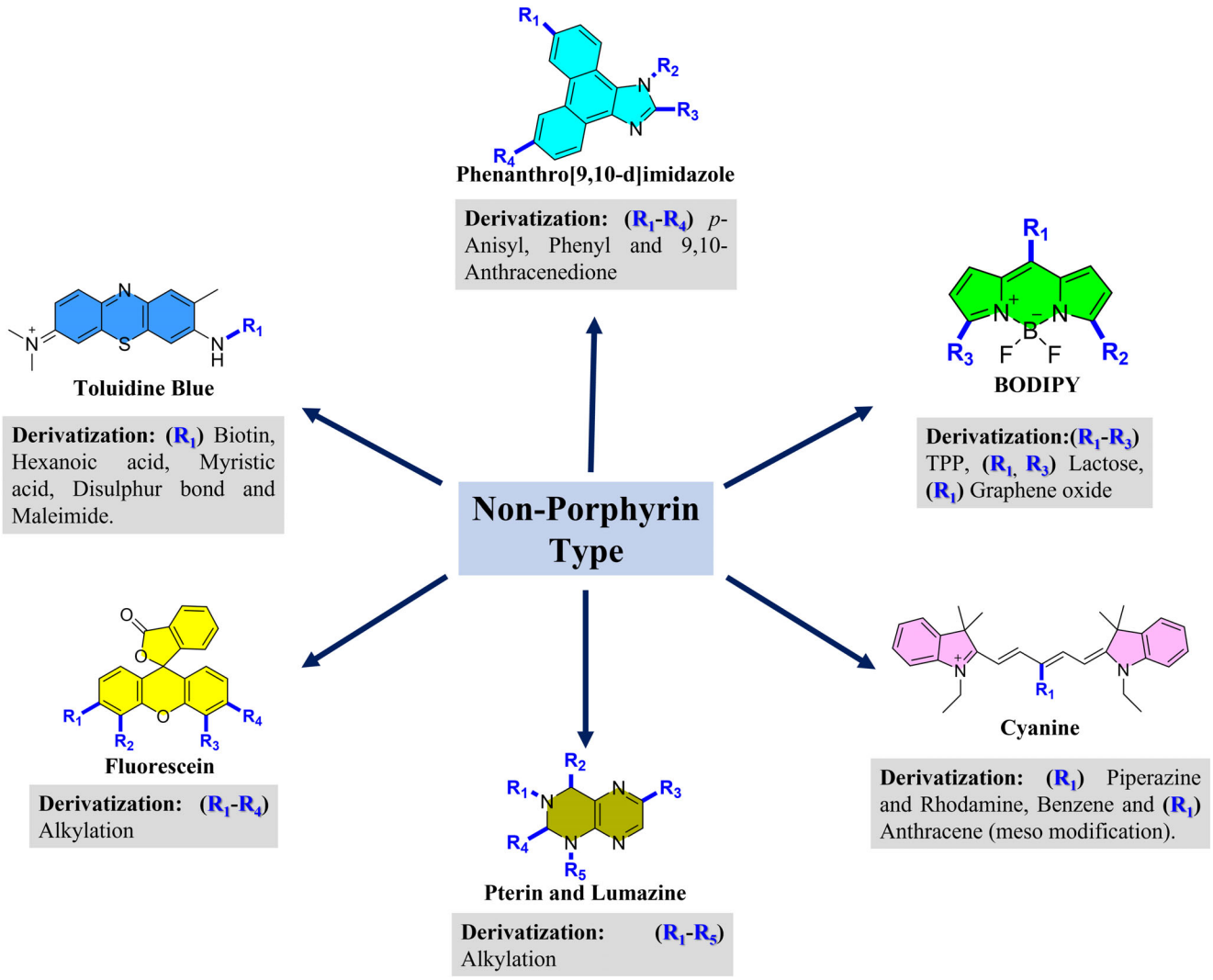

Figure 4. Chemical structures of Nonporphyrin derivatives. Summarized for references (28,29,34,48–60).

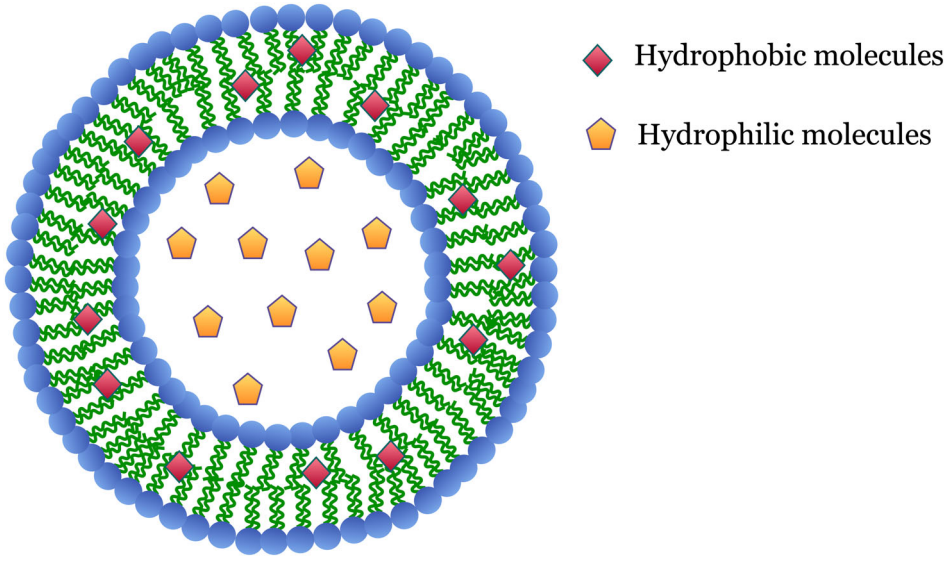

Figure 5. Schematic representation of the incorporation of hydrophobic and hydrophilic PS into liposomes.

nanocarriers for encapsulating CUR into the lipid bilayer (LIP-CUR) and under blue light irradiation produced by blue light-emitting diode-induced photodynamic therapy (BLED-PDT), enhanced the production of intracellular ROS levels increasing the apoptotic cell death in A549 cells.

Barras *et al*. (74) reported that the use of combination of two PS (Protoporphyrin IX, (PpIX) and Hypericin, (Hy)) encapsulated inside liposomes (LNCs) favored PDT. The spectroscopic studies showed that the encapsulation of both PS in the lipid core increases their solubilization and photophysical properties; besides, the encapsulation of both Ps into a liposome (PpIX–Hy-loaded LNC25) incremented the phototoxicity in HeLa and MDA-MB-232 cells line and provided a synergistic effect.

Also, Zhang *et al*. (75) have reported the use of liposomes through a phototheranostics methodology that involves PDT with photothermal therapy (PTT). Specifically, the use of hydrophobic cyanine (Cy) which is an excellent target for mitochondria, but its hydrophobic structure increases the aggregation, and its low solubility resulted in a poor biocompatibility and limited therapeutic efficacy. For this reason, the encapsulation into liposomes bilayers favored the photophysical properties, blood circulation, bioaccumulation and specificity of the PS-enhanced mitochondria tumor targeting. Furthermore, the NIR irradiation of Cy induces an increasing temperature that favored the PPT and it can also be a good tool for the diagnostic by fluorescence imaging in cancer treatment.

Guan *et al*. (76) reported a multifunctional nanoparticle system (NPs-Lip@PTX/CyA/Ce6), where bovine serum albumins (BSA), drug paclitaxel (PTX) and P-gp inhibitor cyclosporin A (CyA) were incorporated into aqueous pool of liposome, and the PS Chlorin e6 (Ce6) was incorporated into liposomal bilayer. This multifunctional system showed perfect monodispersity, morphology and high stability in an aqueous solution. Besides, irradiation produces abundant ROS that can be incorporated by 4T1 cells effectively. This multifunctional DDS would be a great strategy combined with chemo-photodynamic treatment.

On the other hand, many photosensitizers clinically approved can accumulated in normal tissues, for instance, skin, which would produce the phototoxicity under irradiation of conventional light, and for this reason, the patients have to be in rooms without light for a few weeks after PDT treatments. In this context, Yang *et al*. (77) proposed an aggregation-induced emission PS (AIE-PS) system incorporated into liposomes for controlling the photosensitization. Their results showed low phototoxicity and ROS productivity of AIE-PS incorporated in hydrophobic phase, while significantly increased when AIE-PS@liposomes targeted tumors and even degraded there. The use of these strategies can control the photosensitivity of AIE-PS and would be triggered and turned-on when liposome is degraded inducing the cytotoxicity in the cells.

Other studies have utilized liposomes as strategies for incorporating hydrophilic PS or drugs into the aqueous pool. These allow the release of the PS or drugs in specific sites of the cell increasing the selectivity and bioaccumulation in the tumor cell and have shown an elevated photodynamic efficiency. In particular, Yen *et al*. (78) described the use of liposomes to include hydrophilic active substance (chlorin e6 di-N-methylglucaminate) in comparison with the commercially available aqueous solution, this result showed a significant enhancement of the level and selectivity of biodistribution of the photosensitizer in tumor tissue compared with normal tissue through the use of liposomes, consequently resulting in a significant enhancement of photodynamic efficiency in comparison to the original commercially produced drug form (aqueous solution) of Fotoditazin.

In order to increase the activity of photosensitizers, Roh *et al*. (79) compared the PDT efficiency of chlorin e6 (Ce6) and cationic PS encapsulated into polymeric nanoparticle (PS-pNP), formulated of Ce6, PEG and polyethyleneimine. They proved that the use of PS-pNP liposome enhanced the bioaccumulation of the PS in all cell lines improving its synergic effect in comparison with nonencapsulated PS.

The vast applications of liposomes in PDT have allowed them to be a great strategy for improvements in biodistribution, bioaccumulation, as well as, in the efficacy of photosensitizers, promising to continue being a good strategy in cancer treatments (69,80,81).

Host–guest complexes of photosensitizers

Supramolecular chemistry, defined as "the chemistry beyond the molecule," focuses on the study of molecular structures generated by the association of two or more chemical species. These species are held together through noncovalent, intermolecular forces (82). The later includes, beside others, electrostatic interactions, hydrophobic effects, π-effects and van der Waals forces. Noncovalent forces play a relevant role in biological systems (*i.e*. 3D-structure of proteins and nucleic acids, lipid bilayer in membranes, *etc*.). In recent years, supramolecular self-assembly has found a space in medicine (83). Benefitting of the nature of supramolecular interactions, supramolecular assembled complexes are being used as tools to revolutionize some biomedical technics, including PDT.

Chemical procedures related to the manipulation of chemical species or supramolecular building blocks may be applied for the fabrication of supramolecular aggregates with controlled size and shape. Noncovalent interactions are dynamic in nature, causing supramolecular arrays to disassemble and reconstruct themselves with low energy spending (83). This intrinsic dynamism causes those supramolecular arrays to respond quickly to different kinds of external perturbations. External stimuli may be of different nature: biological (biological molecules like enzymes or nucleic acids), chemical (ion concentration, redox agents); or physical (ultrasound, light, temperature) (84). This response may be used to engineer supramolecular arrays with controlled properties (fluorescence and ROS emission) as a response of an external perturbation; or construction of devices capable of probing and responding to ambient stimuli.

Intermolecular interactions with PS molecules produced by supramolecular self-assembly can alter ROS production. The rational design of supramolecular photosensitizers may permit to manage the generation of ROS, either to increase, decrease or control the activation of this process. An important issue of PDT are the aftereffects, caused by the "always-on" state of ROS generation of photosensitizers. Control of ROS generation represents a promising approach to make PDT specific, efficient and potentially personalized (85).

More than half of the new chemical drugs, which are promising candidates for PDT, display low water solubility (86,87). Inclusion of PS into the cavity of a water-soluble host molecule can improve its solubility in aqueous media. Drugs can be protected from chemical/thermal/photochemical degradation or reactions by including them inside the cavity of a host molecule, limiting its interaction with the surrounding media. Controlled

release of the drug can be achieved by manipulating the external stimuli (thermal changes, pH variations, using a chemical specie acting as a competitor for the cavity, among others) (88).

Another big challenge is the aggregation tendency of PS which results in a lower production of singlet oxygen and an unsatisfactory efficiency in PDT (89). Despite the high singlet oxygen quantum yield of some PS, its biological application in PDT is restricted by reason of low water solubility and quenching of its photodynamic capacity due to self-aggregation in solution (1,90,91). Some approaches have tried to directly conjugate PS with water-soluble functional groups, like PEG, to increase its solubility, but this strategy is time consuming and must be accompanied by purification methods. Due to these inconveniences, host–guest chemistry stands out to overcome these difficulties in the photodynamic therapy of cancer.

Host–guest chemistry corresponds to a special field of supramolecular chemistry. A *guest* molecule (generally a small molecule) is encapsulated inside the cavity of a *host* molecule (generally the larger molecule). This inclusion process is dynamic and reversible in nature (92). Among host molecules, most used and studied are natural cyclodextrins (CDs); and synthetic hosts such as cucurbit[n]urils (CBs), calix[n]arenes (CXs) and pillar[n]arenes (PA). Cyclodextrins comprise a family of cyclic saccharides. α, β and γ-cyclodextrins, the three main cyclodextrins, are crystalline, homogeneous, nonhygroscopic substances made up from glucose units (6,7 and 8 molecules, respectively) bonded together by α-1,4-glycosidic bond, forming a ring with torus-like shape (93). Cucurbit[n]uril are cyclic oligomers which resembles to a pumpkin, made up of methylene-bridged glycoluril units (94). Calix[n]arenes are cyclic molecules made up of phenolic rings bonded by methylene groups which form a cup-like structure (95). Pillar[n]arenes are cyclic molecules made up of 1,4-dihydroxybenzene molecules bonded by methylene bridges at *para* positions (96). Pillar[n]arenes, as cucurbit[n]urils, possess a highly symmetrical cylindrical structure (92).

Hereafter, we present different host–guest strategies, with the above-mentioned cavitands and different photosensitizers separated by classes: (1) porphyrins; (2) phthalocyanines and (3) phenothiazines/acridines.

Porphyrins. One of the first attempts of using host–guest systems with porphyrins was made by Venema *et al*. (97). They encapsulated water-soluble anionic porphyrins inside the cavity of β-CD dimers with different lengths of the linkers holding together the CDs. CD dimers showed an increase in the association constant with the porphyrins compared to the monomer.

Sortino *et al*. (98) constructed a carrier system made of nanoparticles consisting of a host–guest complex of heptakis(2-ω-amino-*O*-oligo(ethylene oxide)-6-hexylthio)-β-CD (SC6CDNH2, a cationic amphiphilic cyclodextrin) and 5,10,15,20-tetrakis-(4-sulfonatephenyl)-21H,23H-porphyrin (TTPS, an anionic porphyrin) varying the ratio of both components. By changing the molar ratio of SC6CDNH2:TTPS (between 1:10 and 1:50) they were able to retain the singlet oxygen quantum yields of the PS in the complex, comparable to free TTPS. Furthermore, they have shown that this system can be taken up by HeLa cells, reaching a maximum at a ratio of 1:10. This ratio also showed the best balance between dark toxicity and cell death.

Using the same amphiphilic CD (SC6CDNH2) as Sortino, Ferro *et al*. (99) constructed nanoparticles using the monocationic meso-substituted porphyrin 5-[4-(1-dodecanoylpyridinium)]-10,15,20-triphenyl-porphine (TDPyP). The system showed a high singlet oxygen quantum yield (φ_Δ = 0.90) and was effective in the inactivation of Gram-positive methicillin-resistant bacterium *Staphilococcus aereus* (MRSA) and Gram-negative bacterial pathogens as *Escherichia coli*.

Kralová *et al*. (100) reported a system where a fluorinated porphyrin was linked to one, two and four β-cyclodextrins and two γ-cyclodextrins to incorporate PDT and chemotherapy. They included doxorubicin and paclitaxel, as chemotherapy drugs, inside the cavity of the cyclodextrin. *In vitro* studies with 4T1 cells (mouse mammary carcinoma cells) or K562 cells (human chronic myelogenous leukemia cells) showed that derivatives with two or three cyclodextrins were more efficient in killing cancer cells than the application of chemotherapeutic drugs or PDT alone. *In vivo* studies showed that *bis* derivatives formed a complex at physiological conditions which exhibited PDT efficacy and active release properties in cancer cells, producing a more noticeable anti-tumor effect.

Zhang *et al*. (101) constructed water-soluble porphyrinic nanospheres (NSs) by linking per-O-methylated β-cyclodextrins (two or four) to tetraphenylporphyrin. This linkage allowed the formation of self-inclusion complexes which disrupted the aggregation of the porphyrin and enhanced its fluorescence and singlet oxygen generation capabilities.

Zhang *et al*. (102) extended this approach by combining PDT and chemotherapy. They constructed self-assembled nanoparticles using host–guest complexes of β-cyclodextrin dimer linked through a platinum(IV) prodrug (host) and a porphyrin derivative containing an adamantyl group which would act as the guest moiety. *In vitro* studies in A549 cells (adenocarcinoma epithelial cells) showed that nanoparticles caused a higher cellular uptake of platinum compared to cisplatin. Furthermore, irradiation with visible light showed an increase in the cellular ROS.

Liu *et al*. (103) created a supramolecular delivery system by host–guest assembly of the supramolecular amphiphiles poly (ethylene glycol)-modified β-cyclodextrin and a porphyrin derivative containing an adamantyl group and a disulfide bond. The amphiphiles self-organize into micelles with spherical shape in water. The release of the porphyrin can be modulated by the reduction of the disulfide bond with GSH. *In vitro* studies in MCF-7 cells exhibited increased uptake of the system compared to the free porphyrin. Cytotoxicity studies showed that the micelles displayed negligible dark toxicity and after irradiation, micelles exhibited significant phototoxicity.

Tong *et al*. (104) developed a PS supramolecular nanocarrier with dual targeting ability for enhanced PDT. They conjugated chlorin e6 (Ce6) with β-cyclodextrin though an amide bond (β-CD-Ce6) and synthesized an adamantane terminated peptide which bears different recognition motives, one of which can be cleaved by cathepsin B (overexpressed in cancer cells). The two compounds were self-assembled into micelles with the peptide acting as the hydrophilic shell and Ce6 acted as the hydrophobic core. The structures showed an increased uptake by MCF-7 cells, with negligible dark toxicity and a dose-dependent photodynamic phototoxicity.

Xu *et al*. (105) fabricated polypseudorotaxane nanoparticles by self-assembly of methoxy polyethyleneglycol (mPEG) conjugated protoporphyrin IX with α-cyclodextrin via host–guest complexation. Nanoparticles were loaded with the

chemotherapeutical drug doxorubicin (DOX). Nanoparticles loaded with DOX displayed significant cellular uptake, high phototoxicity and low IC_{50} in HepG2 and L929 cell lines.

Deng *et al*. (106) developed a supramolecular nanocarrier containing nitric oxide-releasing properties through GSH reduction. They used two conjugated α-cyclodextrins. One cyclodextrin was conjugated to chlorin e6; the other cyclodextrin to a GSH-sensitive NO conveyer. Nanoparticles were prepared by self-assembly of these two modified cyclodextrins with PEG. *In vitro* studies in MCF-7 cells displayed that the NPs could be efficiently internalized with NO release caused by intracellular GSH, causing depletion of this specie. Depletion of intracellular GSH caused an increase in overall ROS concentration, improving PDT efficacy. The GSH-freed NO was able to reduce hypoxia inside tumor tissues, increasing blood flow. Also, they detected the generation of peroxynitrite anions (ONOO-), a major reactive nitrogen species (RNS), by the reaction of GSH-released NO and light-generated ROS, which are even more lethal than ROS, producing cell damage by oxidation.

Khurana *et al*. (107) reported the development of supramolecular nanorods of 5,10,15,20-tetrakis(4-N-methylpyridyl) porphyrin (TMPyP) with sulfobutylether-β-cyclodextrin (captisol) through multiple host–guest interactions. The availability of four cationic N-methylpyridyl moieties in TMPyP produces sequential binding interactions with captisol, building a continued supramolecular structure. Complexation of TMPyP produced an increase in the singlet state quantum yield and fluorescence lifetime, an enhancement in the singlet oxygen quantum yield (from 0.73 to 0.95) and improved photostability of the PS.

The nanorods showed improved antibacterial activity toward *Escherichia coli*. Furthermore, the system exhibited lower dark toxicity and higher cytotoxicity under irradiation toward A549 cells (lung carcinoma) compared to TMPyP alone.

Dai *et al*. (108) have constructed cyclodextrin-prodrug supramolecular NPs made through orthogonal host–guest self-assembly of permethyl-β-cyclodextrin linked through a disulfide bond to camptothecin (PMCD-SS-CPT), an anionic porphyrin photosensitizer functionalized with an adamantane moiety and hyaluronic acid modified with triphenylphosphine and β-cyclodextrin (TPP-HACD). *In vitro* studies in A549 cell line exhibited a high uptake of the NPs by the mitochondria, compared to 293T normal cells. The high intramolecular concentration of GSH produced *in situ* cleavage of the disulfide bond, releasing camptothecin, the anticancer drug. Under light irradiation, the porphyrin can produce singlet oxygen causing mitochondrial dysfunction and increasing death rate depending on the increasing micromolar concentration of the NPs.

Xu *et al*. (109) have designed and constructed a nanosystem, based on host–guest assembly and electrostatic complexing, composed of biocompatible adamantane-functionalized hydroxyethyl starch (HES), low-toxic β-cyclodextrin linked ethanolamine-functionalized poly(glycidyl methacrylate) (CD-PGEA) and combined plasmid pKR-p53. *In vitro* studies showed that the system without the plasmid exhibited little cytotoxicity, enhanced uptake by cells and improved gene transfection. The combined plasmid pKR-p53 can express apoptosis-related protein p53 and KillerRed proteins, which are photosensitive, simultaneously in the same cell. Also, studies in the 4T1 tumor model showed that the system containing the combined plasmid showed improved antitumor effects under irradiation compared to the dark conditions.

Zhang *et al*. (110) have obtained nanovesicles made by self-assembly of a chemically modified amphiphilic and cationic single-isomer β-cyclodextrin (CD-IL) with chlorin e6 (Ce6) via multiple noncovalent interactions. Nanovesicles could overcome self-aggregation and low water solubility of the PS. *In vitro* studies in three different human hepatoma cell lines (Hep3B, HepG2 and SMMC-7721) exhibited higher uptake of the vesicles compared to normal liver cells (L02). Vesicles showed greater cellular uptake, increased ROS formation, cell viability and apoptosis compared to free Ce6.

Koc *et al*. (111) reported the conjugation of a trimannosylated-tetraphenylporphyrin (TTP) with monopropargyloxy-functionalized-cucurbit[7]uril (CB[7]). The resulting structure dissolves in DMSO (10 mg mL^{-1}), in water (0.2 mg mL^{-1}) and in water/DMSO mixture (4:1, v/v, 2 mg mL^{-1}). The singlet oxygen quantum yield for the conjugate was around 0.8 in DMF, higher than the reported value of unfunctionalized TTP of 0.44 in DMF. An increase in the singlet oxygen quantum yield could be caused by disruption of the π–π interactions between the porphyrins due to the bulky mannosyl and CB[7] functional groups. This supramolecular assembly has the ability to be used in combined chemo and photodynamic therapies: the porphyrin core acting as a photosensitizer and the cavity of CB[7] can host and carry anticancer drugs or antibiotics.

Özkan *et al*. (112) have reported the conjugation of cucurbit[7]uril with a triglycosylated tetraphenyl porphyrin. The assembly can act as a photosensitizer for PDT due to the porphyrin core and as a drug carrier. This PS shows low dark toxicity against both gram-negative and gram-positive bacteria as well as MCF-7 cancer cells even at high doses. Under low doses of white light, the PS becomes highly toxic, killing almost the totality of bacteria and cells proved.

Liu *et al*. (113) have reported supramolecular organic frameworks (SOFs) constructed via cucurbit[8]uril encapsulation of tetra cationic monomers. These SOFs have pores (2.1-nm porosity) which can adsorb (micromolar concentrations) and retain different photosensitizers (Photofrin, HiPorfin and Talaporfin) and suppress skin phototoxicity. *In vitro* and *in vivo studies* showed that SOFs are highly biocompatible. Studies with mouse models (healthy and tumor-bearing) demonstrated that the administration of the PS at doses comparable to clinical doses, and posterior treatment with SOF, suppressed skin phototoxicity caused by the PS. The PDT outcome of mice treated with SOF post-PDT remained unchanged.

Yan *et al*. (114) have introduced supramolecular nanoparticles (SNPs) made by self-assembly of a tetrameric porphyrin functionalized with four calix[4]arene rings and biviologen derivatives with different lengths. *In vitro* studies have shown SNPs can be internalized in HeLa cells by endocytosis. Also, SNPs show good biocompatibility and low dark toxicity. Under light irradiation, SNPs showed higher photooxidation properties compared to the porphyrin derivative not forming nanoparticles at the same concentration.

Chen *et al*. (115) have prepared spherical micelles by self-assembly of supramolecular amphiphiles. The micelles were prepared by host–guest interactions between pillar[5]arene modified with PEG (PEG-P[5]A) and a porphyrin derivative, functionalized with a pyridinium terminal group and the linker bearing a disulfide bond (TPPC6-SS-Py). Micelles release porphyrin photosensitizer in reducing environment (intramolecular GSH). *In vitro* studies in A549 cells showed that micelles displayed improved

cellular uptake and antitumor properties compared to the porphyrin alone.

Rui *et al*. (116) have developed a supramolecular photosensitizer delivery system fabricated by host–guest interactions of tetraphenylporphyrin functionalized with quaternary ammonium salts (TPP-QASs), docecyltrimethylammonium bromide (DTAB) and pillar[5]arene (WP5) in water. *In vitro* studies in A549 cells showed that fluorescence and PDT activity of the nanoparticles was suppressed in physiological environment, but release and activation of TTP-QASs can be achieved in an acidic environment, leading to a selective accumulation in mitochondria from cancer cells. Light irradiation generates singlet oxygen which produces oxidative damage to the mitochondria which was corroborated by the loss of the membrane potential.

Wu *et al*. (89) have designed a supramolecular complex of pyrophaeophorbide A-pyridinium (PPhA-Py) and a water-soluble pillar[5]arene (P[5]A) by host–guest interactions with improved water solubility and disaggregation properties. The complex self-assembled into stable vesicles which presented increased fluorescence and singlet oxygen generation in aqueous media. The surface of the vesicles was functionalized with a biotin-pyridinium moiety (Bt-Py) to increase cellular uptake of the PS. *In vitro* studies in HeLa cells showed no dark toxicity for the vesicles after 24 h of incubation, compared to PPhA alone which showed a cell viability of 81%. Also, biotinylated vesicles showed the highest phototoxicity compared to biotin-free vesicles, demonstrating the enhanced targeting ability and its improved singlet oxygen generation of the vesicles.

Min *et al*. (117) have reported a water-soluble aggregation-induced emission (AIE) photosensitizer (WAPS). WAPS contains an electron donor, an electron acceptor and a recognition moiety. Host–guest interaction of pillar[5]arene with WAPS in neutral aqueous solution results in a donor–acceptor complex with weak photodynamic activity. Under acidic environment (pH 5.2), the binding properties of the system change, increasing singlet oxygen generation and photodynamic activity. *In vitro* studies in MCF-7 and CT26 cells showed high phototoxicity for the system at pH 5.2 but negligible phototoxicity at pH 7.2, while negligible dark toxicity was observed at both pHs. *In vivo* studies in tumor-bearing mice showed the complex can inhibit tumor growth, without systemic phototoxicity, negligible morphological differences in observed major organs.

Finally, protoporphyrin IX prodrug 5-aminolevulinic acid (ALA) has also been encapsulated. Tong *et al*. (118) have investigated ALA polypseudorotaxane micelles with pH-responsive capabilities. ALA was covalently linked to α-cyclodextrin through an acid-cleavable hydrazone bond. Host–guest assembly of the prodrug-α-cyclodextrin with a cell-penetrating peptide linked to PEG formed the polypseudorotaxanes which self-assembled into micelles. *In vitro* studies in HepG2 cells showed that the micelles can be taken up by cells in a weakly acidic tumor environment. Micelles showed negligible dark toxicity and a dose-dependent phototoxicity.

Phthalocyanines. Zheng *et al*. (119) have constructed pH-sensitive nanoparticles by self-assembly of an acetylated β-cyclodextrin (Ac-CD) and doxorubicin (DOX). The nanoparticles were coated with PEGylated amphiphilic zinc phthalocyanines ($ZnPc\text{-}(PEG)_5$). Animal studies showed NPs presented improved tumor-targeting abilities as well as a synergistic antitumor effect due to an increase in ROS production and cell apoptosis ratio. Furthermore, NPs exhibited low tissue toxicity.

Xue *et al*. (120) prepared an activatable supramolecular nanosystem by self-assembly of β-cyclodextrin functionalized with a phthalocyanine photosensitizer (Pc-CD) and a PEGylated ferrocene (Fc-PEG) in aqueous media. The system (Pc-CD@Fc-PEG) fluorescence and ROS generation capabilities were low due to self-quenching and the ferrocene moiety which acts as a quencher. Displacement of the ferrocene guest was achieved in water and in EGFR-positive HT29 and A431 cells by the addition of adamantane modified covalently with EGFR-targeting peptide (Ad-QRH*), causing the disassembly of the particles and restoring the photoactivity of the system. Activation of the system in an Ad-QRH*-treated HT29 tumor in nude mice led to an increase in the fluorescence intensity inside the tumor. Under light irradiation, effective elimination of the tumor was achieved.

Bolfarini *et al*. (121) have prepared magnetoliposomes (MLs) loaded with a host–guest complex of zinc phthalocyanine with cucurbit[7]uril (CB[7]:ZnPc-MLs) and magnetic biocompatible fluid (citrate-coated maghemite nanoparticles) for dual photodynamic and hyperthermia effects. *In vitro* studies in B16-F10 melanoma cells incubated with CB[7]:ZnPc-MLs showed a synergistic effect in the reduction of the cell viability after application of both therapies (laser light and AC magnetic field) compared to the both stimuli applied individually.

Two years later, the same group (122) prepared liposomes of phosphatidylcholine, cholesterol and sterylamine and loaded with a 1:1 inclusion complex of ZnPc and CB[7] (CB[7]:ZnPc-Lp). *In vitro* studies in B16-F10 melanoma cells showed, for the complex not loaded into liposomes, a phototoxicity of about 50%, which was independent of the light-dose applied. Photodynamic activity of CB[7]:ZnPc-Lp was dependent of the light-dose applied, ranging from 70% (0.5 J cm^{-2}) to 9% (5 J cm^{-2}).

Di Bari *et al*. (123) have reported micellar-like nanoassemblies of amphiphilic calix[4]arene functionalized with choline which can co-encapsulate a nitric oxide photodonor (NOPD, hydrophobic) along with either singlet oxygen second-generation photosensitizers temoporfin (hydrophobic) or zinc phthalocyanine tetrasulfonate (hydrophilic). Both, the PS and NOPD retain their individual properties when they are co-assembled in the container, allowing a dual-photo responsive behavior under light irradiation, even if the components are close together, permitting the delivery of both cytotoxic singlet oxygen and NO.

Phenothiazines and Acridines. Wang *et al*. (55) have reported an activatable photosensitizer prepared by host–guest recognition of cucurbit[8]uril (CB[8]) and a toluidine blue derivative functionalized with biotin (TB-B) to form 1:2 inclusion complexes (2TB-B@CB[8]). Fluorescence intensity and singlet oxygen production were suppressed by the formation of the complex. FGG tripeptide was used to displace the encapsulated dye, recovering fluorescence and singlet oxygen generation. As this peptide is present in cells, it can trigger the activation of this system. *In vitro* studies in SCC-7 (squamous cell carcinoma) and COS7 (African green monkey kidney fibroblast cells) cells showed greater incorporation of the system to cells compared to unbiotinylated TB complex. Minimal dark toxicity was reported for TB-B and 2TB-B@CB[8] systems, but phototoxicity was improved by complex formation. *In vivo* studies in SCC7-tumor-bearing nude mice model showed that the complex enhanced the

stability of the PS for intravenous injection, presenting longer retention time inside the body, improved accumulation in tumor and cancer ablation ability.

Our group, Robinson-Duggon *et al.* (124), have reported a reversible switch for singlet oxygen generation of toluidine blue O (TBO^+) using cucurbir[7]uril, cucurbir[8]uril and memantine (Mem) as a displacement agent. Binding affinities for $2TBO^+$@CB[8] and TBO^+@CB[7] were investigated showing that binding affinities are higher for CB[8] compared to CB[7]. $2TBO^+$@CB[8] complex shows a lower singlet oxygen quantum yield compared to TBO^+@CB[7]. To turn the switch on, the addition of memantine produces the formation of the Mem@CB[8] complex, by displacement of TBO^+ molecules. TBO^+ can form a host–guest complex with CB[7] which recovers singlet oxygen production. To turn the switch off, the addition of a slight excess of CB[8] is sufficient to displace the PS from CB[7] to CB[8].

Recently, Robinson-Duggon *et al.* (56) have reported host–guest complexes of toluidine blue O (TBO) and two fatty acid derivatives with different chain lengths (six and fourteen carbons, TBOC6 and TBOC14, respectively) of the PS with cucurbit[7]uril (CB[7]) and human serum albumin (HSA). *In vitro* studies in HeLa cells showed lower phototoxicity for TBOC14 derivative compared to TBO. TBOC14 derivative shows stronger binding to HSA, which could lead to enhanced quenching of ROS caused by the protein, diminishing its phototoxicity. The formation of inclusion complexes with CB[7] did not show any significant improvement in the cellular uptake of the PSs or in their phototoxicity. The combination of both supramolecular carriers showed that phototoxicity was similar to HSA complexes without CB[7] and the cellular uptake was reduced. CB[7] had an enhancer role: lower cellular uptake was compensated with an enhanced photodynamic effect in the presence of HSA.

Narayanan *et al.* (125) have reported a nanotheranostic probe in which an inclusion compound of methylene blue (MB) is placed in between two gold nanorods (GNRs), generating linear assemblies with sensitive plasmonics. Functionalization of the system by targeting anti-Her2 monoclonal antibodies allows localized photothermal and photodynamic therapies simultaneously on Her2-positive SKBR3 cells. *In vitro* studies showed negligible dark toxicity for the system, which increases rapidly upon laser irradiation.

Yang *et al.* (126) have designed a host–guest complex between the water-soluble pillar[6]arene (WP6) and methylene blue (MB). Results show that the complex can significantly diminish the dark toxicity of free MB. *In vitro* studies in MCF-7, HeLa, HepG2, HL7702 and 293T cells exhibited that the complex can reduce photobleaching of MB, extending the period for ROS generation, compared to uncomplexed MB.

Hu *et al.* (127) have prepared a supramolecular photosensitive nanocarrier system with photodynamic (PDT) and chemodynamic (CDT) properties. The group synthesized a zeolitic organic framework (ZOF) containing Cu^{+2} (Cu/ZIF-8@ZIF-8) which can adsorb O_2 and engage in a Fenton reaction with Cu^{+2}. Then, this nanocarrier was decorated with inclusion complexes of methylene blue (MB) and a water-soluble carboxylated pillar[6]arene (WP6) (OCZWM). O_2 loading ability of OCZWM was tested at different pH conditions measuring the O_2 content in the OCZWM solution. At acidic pH 6.5, an increase in O_2 concentration was observed over time. At neutral pH, pH 7.4, O_2 concentration remained without significant change. These results indicate good O_2 loading and pH responsiveness by the OCZWM system. *In vitro* studies in HepG2 cells showed good cellular uptake and efficient release of MB. Cell cytotoxicity analysis showed that at low O_2 concentrations, OCZWM exhibited high cytotoxicity. *In vitro* evaluation of the chemodynamic properties of the system in HepG2 cells showed that OCZWM can react with GSH (ROS scavenger) and enhance PDT outcome compared to WP-MB.

Zhou *et al.* (128) have reported a host–guest complex of methylene blue (MB) and pillar[5]arene covalently functionalized with salicylic hydrazide (P5SB) through an imide bond (Schiff base) which self-assembled into pH-responsive vesicles (P5SB-MB). These vesicles, then, were loaded with doxorubicin (DOX). Since imide bonds can be hydrolyzed under acidic conditions, these vesicles disassembled at pH 6.0, releasing DOX rapidly. *In vitro* studies in HeLa cells showed that DOX-loaded P5SB-MB vesicles exhibited improved cell inhibition rate compared to control groups, showing synergistic effects.

Our group, Solis-Egaña *et al.* (129), have reported host–guest inclusion complexes of acridine orange (AO) and oxaliplatin (OxPt) with cucurbit[8]uril. *In vitro* studies in HeLa cells showed good cellular uptake for AO@CB[8] complex and high phototoxicity. OXPt@CB[8] complex was toxic at 24 h of incubation time. Preincubation with OXPt@CB[8] for 24 h and posterior treatment with AO@CB[8] complex showed no photodynamic activity. Co-incubation of the complexes for 90 min exhibited that combined cytotoxicity/phototoxicity was enhanced (by 30%) compared to the individual treatments, displaying a cooperative effect.

In summary, we have presented the use of several cavitands as a strategy to control the release of the PS through different stimuli, as well as, for the codelivery of both PS and chemotherapy drugs to potentiate the PDT effect. Cavitands were shown as an excellent tool to reduce the enzymatic degradation of PS and avoid aggregation and photodegradation which are desirable traits to improve the outcome of PDT. Interestingly, the use of cavitands provides the opportunity to control the generation of singlet oxygen and in some cases to increase it by several folds boosting the performance of PS.

COMBINATION OF PDT WITH OTHER THERAPIES

PDT acts as a more localized, efficient, less invasive, controllable and effective therapeutic procedure than other cancer treatments. Particularly, the treatment of solid tumors continues to be a challenge for PDT (130). Lately, it has been increasingly used combined with other traditional or nontraditional cancer therapies that have synergies and show promising results, making the therapy more effective and with less side effects (15). The following therapies are known to improve the therapeutic effect of PDT: chemotherapy (131), radiotherapy (132,133), immunotherapy (134) and iRNA (135). In this section, we will focus on chemotherapy and immunotherapy combined with PDT. These combinations can help to decrease the amount of chemo drugs as well as PS, overcome the multidrug resistance (MDR) for tumor cells, target primary tumors to inhibit drug resistance, extend survival periods with better life quality and preventing tumor recurrence (131,134,136).

PDT and chemotherapy

Chemotherapy is the more traditional and main treatment used in early or lately stages of cancer to decrease surgical procedures, shrink tumors and decrease re-incidence (137). Traditionally, the

chemotherapy drugs are cytotoxic and their main function is to inhibit the proliferation of cancer cells. These anti-tumor drugs bind to the DNA and prevent the cell division processes, leading to cell death (138). These drugs have a tendency to increase drug resistance and reduce low selectivity toward regular cells with high rate of division affecting gonads, bone marrow, gastrointestinal tract and hair, among others (139). To overcome drug resistance, side effects, and to improve the outcome of the treatment, the combination of chemotherapy with PDT has been tested effectively (131,136).

Chemophototherapy, as it has been termed, is a promising treatment option for solid tumors, due to its synergistic effects (131,140). In particular, the synergy demonstrated between chemotherapy and PDT is proposed to prevent re-incidence of tumors and avoid resistance (141). The application of PDT alone can directly induce tumoral cell death and vascular damage, leading to its regression; however, if viable cells are present the possibility of tumor recurrence is often high (12,20,142–144). If chemotherapeutic drugs are also administered, they can induce further cell death through oxidative stress or other mechanisms and since the tumoral tissue has already been sensitized by PDT, a more effective tumor inhibition can be achieved (131,141). This synergistic concept has been demonstrated to be effective in cultured cells and in mice (145–147), therefore it is expected that further clinical trials can prove its effectiveness in patients.

Oxaliplatin has been used in combination with Verteporfin against a metastatic pancreatic cancer cell line with improved effectiveness (147). Recently, oxaliplatin-cucurbit[8]uril complex in combination with acridine orange has shown enhanced tumoral cell killing *in vitro* (129). 5-fluorouracil has also been tested in combination with 5-aminolevulinic acid to treat squamous cell carcinoma cell lines (146). Other combinations include the use of the drugs doxorubicin, mytomycin, cisplatin, methotrexate and gemcitabidine with the photosensitizers photofrin, 5-aminolevulinic acid or aluminum phthalocyanine (131).

Porphyrin-phospholipid (PoP)-based liposomal formulations have been demonstrated to be great candidates for the controlled release of cargo upon irradiation with red light (148). Several chemotherapeutic molecules have been encapsulated in PoP liposomes, which combined with the photoactivation of the porphyrin have been used for chemophototherapy. For example, cabazitaxel (149), doxorubicin (150) and irinotecan (151).

The *in vivo* evaluation of combined chemotherapy/PDT has been done through two main models: (1) In the first model, mice are subcutaneously injected with different tumoral cells, mimicking diverse solid tumors in the liver, lung, prostate, breast, colon, bladder and pancreas. (2) The second model uses patient-derived xenografts (PDX) from numerous varieties of cancer (shown in Table 1). Chemophototherapy in these models has shown many advantages over the monotherapy strategy; it has a great synergism (152,153,161), it produces a specific drug delivery efficiency (153), a pH-regulated drug release (154,155,157), it induces apoptosis at superior cancer cells and it improves antitumoral effectiveness (154,156). The mutual assistance of both therapies carries antitumor activity inducing vascular permeabilization (156), and good biocompatibility for either solids or superficial tumors (158,161).

To this end, parameters such as drug amount, light dose and illumination periods are critical. In fact, modifying drug-light interval can enhance significantly the results of the therapy. Lovell *et al*. (160) have shown that two short irradiations would be a better strategy than one long irradiation: the first laser treatment lets the deposition of the carrier into the tumor and the drug release, and the second laser could destroy the tumor enhancing the efficacy of chemophototherapy.

Other interesting system for chemophototherapy are platinum nanoparticles (nano-Pt), which have a potent cytotoxicity as a chemotherapeutic agent through the leaching of Pt ions (162). Because nano-Pt particles display catalase-like activity they release oxygen to the media. For example, the combination of Pt-nanoparticles with PDT agent verteporfin, inhibits the growth of aggressive tumors, metastasis and prolongs animal survival with low cytotoxicity (159).

Other systems that have been tried are black phosphorus quantum dots with MnO_2 nanoparticles loaded with doxorubicin (163); nanocomposites of amphiphilic gelatin containing doxorubicin and copper sulfide-loaded dendrimer (PRDCuS@AG) for chemophototherapy (164); gold nanoclusters with protoporphyrin IX and doxorubicin (165); micellar nanoprobes with protoporphyrin IX and anti-angiogenic simvastatin (166); metallo-responsive hydrogels containing doxorubicin and indocyanine green (167); CuS nanoparticles loaded with docetaxel (168); nanoerythocytes carrying Pt(II) complex, BSA and indocyanine green (169) and nanoparticles of poly lactide-co-glycolide with indocyanine green and docetaxel for brain cancer (170).

Complex theranostic systems have also been used for chemophototherapy (171). DOX-loaded UCNPs@SnO_2-BSA combined PDT, hyperthermia and chemotherapy with great efficiency. Additionally, tin was able to act as an X-ray contrasting agent for easy detection *in vivo*.

Polymeric multifunctional micelles are also a versatile vehicle for the combination of chemotherapy and phototherapies (172–174).

Single injection of extracellular vesicles loaded with indocyanine green and paclitaxel, where a significant tumor inhibition of proliferation was achieved *in vivo*, with great clinical translational potential (175).

Nanocomposites based on graphene oxide and magnetite, containing chloring e6 photosensitizer and conjugated aptamers showed enhanced and selective cytotoxicity of MCF-7 cells (176).

Nanoerythrosomes containing camptothecin and indocyanine green showed enhanced efficiency in mice (177).

A multicomponent nanoformulation for drug-resistant hepatocellular carcinoma was developed, containing doxorubicin, chlorin e6 and Mn ions with synergistic chemophototherapy effects *in vivo* (178).

Nanotubes assembled from IR780-conjugated peptides have been used for the chemophototherapy of malignant glioma (179).

Cisplatin-cyclodextrin complex has also been combined with IR780 in nanoparticles to target mitochondrial dysfunction of cancer cells and induce potent chemophototherapy (180).

Peng *et al*. (181) reported PEGylated liposomes containing Chlorin Ce6 and cisplatin, where the chemotherapeutic drug was located in the aqueous portion, while the photosensitizer was bound to the lipid bilayer. Studies in BALB/c mice bearing C26 colon tumors showed that these liposomes completely eradicate over 90% of the tumors in mice along with minimal toxicity and extender survival.

In summary, PDT combined with chemotherapy emerges as a potent strategy to reduce the dose and nonspecific cytotoxic effects of chemotherapeutic drugs, as well as enhance the

Table 1. Selected examples of combinations of PDT with chemotherapy.

Carrier	Photosensitizer (PS)/dose	Chemo/dose	Irradiation dose	Tumor model (*in vitro*/*in vivo*)	Effect	References
HA/SN-38/GO Graphene oxide (GO)	Hypocrellin A (HA)	7-ethyl-10-hydroxycampto-thecin (SN-38)/ 5 mM	470 nm LED (25 mW) for 5 min	A549 cells/NA	The synergistic combination of PDT and chemotherapy for the treatment of cancer	(152)
DOX- NPs (nanoparticule) and Ce6-MBs (micro-bubbles)	Chlorin e6 (Ce6)/ 0.1 mg kg^{-1}	DOX (doxorubicin) 5 mg kg^{-1}	US (2.0 W cm^{-2}, 50% duty cycle, 1 min $mouse^{-1}$) and 670 nm laser (100 J cm^{-2}, 200 mW)	MIA-paca-2 human pancreatic cancer cells/ mice were inoculated with MIA-paca-2 cells	The dual-loaded NPs/MBs complex irradiated with ultrasound and laser shown the synergism and improvement of tumor-specific drug delivery efficiency	(153)
$MoSe_2$@PEG-Dox	($MoSe_2$) 2D layered transition metal dichalcogenides	DOX (doxorubicin)/ 60 μg mL^{-1} $MoSe_2$@PEG-Dox	808 nm laser (2 W cm^{-2}) for 20 min	HepG2 and HeLa cells/U14 cells were implanted in the left armpit of each female Kunming mouse	The carrier leads to an acid/photothermal triggered drug release, and the synergistic effect induces apoptosis at the superior cancer cell	(154)
In situ hydrogel formed (TNP^{gel})	zinc phthalocyanine (ZnPC)/17.8 g mL^{-1}	DOX (doxorubicin)/ 20 g mL^{-1}	660 nm laser (100 J cm^{-2}) for 90 s	5637 cells/nude mice bearing 5637 cells xenograft (bladder tumor)	Thermosensitive thermal-responsive hydrogel could be formed in situ-matrix for DOX- and ZnPC-based photo/chemo combination treatment for bladder cancer therapy	(155)
LC-Dox-PoP liposomes	porphyrin-phospholipid (PoP)/0.59 mg kg^{-1}	DOX (doxorubicin)/ 4 mg kg^{-1}	665 nm laser light (200 J cm^{-2}) for 16.7 min	SCID mice bearing patient-derived pancreatic cancer xenografts (PDX)	A semi-mechanistic model for quantitative analysis results on the PDT-induced vascular permeabilization and improve in anti-tumor efficacy	(156)
mPEG-lys(Ce6)-PGA-AIM, micelles	Chlorine e6 (Ce6)	cisplatin/0.69 g, 2.05 mmol	660 nm (100 mW cm^{-2}) for 10 min	SKOV3 cells/prostate cancer subcutaneous injection of SKOV3 cells	The micelles exhibited remarkable stability, pH regulated drug release, good biocompatibility and effective tumor penetration	(157)
microneedle (MN) patches	IR820/10 μg mL^{-1}	cisplatin/2 μg mL^{-1}	808-nm laser exposure at 100 mW cm^{-2} for 5 min	4T1 cells/female Balb mice were subcutaneously injected of 4T1 cells	The MN shown an effective, rapid, secure and humanized therapy of superficial malignant tumor for breast cancer	(158)
nano-Pt/VP@MLipo	verteporfin (VP) 0.025 mg mL^{-1}	Platinum nanoparticles 50 μg mL^{-1}	690 nm laser (100 mW cm^{-2}) for 1 min	Mouse 4T1 breast cancer cell line/Orthotopic 4T1 tumor model.	A single injection of nano-Pt/VP@MLipo and light irradiation inhibits the growth of aggressive tumors, their lung metastasis and prolongs animal survival without over toxicity.	(159)
LC-Dox-PoP liposomes	porphyrin-phospholipid (PoP)/3 mg kg^{-1}	DOX (doxorubicin)/ 15 mg kg^{-1}	150 J cm^{-2} laser treatment 1 h after drug administration. 50 J cm^{-2} 2^{nd} laser treatment 8 h after drug administration	Two human pancreatic cancer subcutaneous mouse tumor models; MIA PaCa-2 or PDX	A second laser treatment could influence and potentially enhance chemophototherapy. The deposition of the LC-Dox-Pop to the tumor due to the first laser treatment and the drug release and tumoral destruction by PDT were followed after the second laser	(160)
Cisplatin and pheophorbide-loaded PLGA nanoparticles	Pheophorbide (PBR) 1 μg mL^{-1}	cisplatin 0.001−10 μg mL^{-1}	670 nm (0.3 W and 0.75 J) for 120 s	The nasopharyngeal carcinoma cell lines (CNE-1 and HNE-1)/ xenograft model generation, cells CNE-1 were subcutaneously injected into mice.	The combination of therapeutics, combination of treatment modalities and active targeting approach paved way for the higher antitumor activity in nasopharyngeal carcinoma model	(161)

effectiveness of PDT. The study of the complex interplay between both therapies that leads to synergistic effects will take PDT further into more efficient treatments.

PDT and immunotherapy

The monotherapy PDT increases ROS production (182), which triggers immunogenic cell death (ICD). These lead to relocation and immune effector cell activation, cytokines expression and the transformation of memory T lymphocytes to maturated cells (134,183). These effects promote an immune response to ablate cancer, but at the same time, there are many studies reporting that excessive inflammation could induce immunosuppression by PDT (184). Ironically, the production of ROS could cause hypoxia (182) which is responsible for metabolic switching, leading to the massive production of lactate in the tumor microenvironment. This metabolite activates a series of immunosuppressive pathways (185). This is one of the Achilles heels of PDT, so the combination with immunotherapy is an excellent alternative to decrease immunosuppression. That's why, immunotherapy is used to enhance immune system response to eliminate cancerous cells through different mechanisms such as checkpoint inhibitors, cytokines, agonistic antibodies, chimeric antigen receptor T, or cancer vaccines (186). Taking this into account, the use of drug delivery systems such as nanoparticles or even T cells to deliver various drugs could reduce nonspecific effects and improve the overall efficiency of the therapy (134,186). PDT is mainly combined with checkpoint inhibitors like programmed cell death-1 (PD-1)/PD- ligand 1 (PD-L1), which play a dominant role in the suppression of T cell responses, or Cytotoxic T-lymphocyte-associated protein 4 (CTLA4), an inhibitory receptor that affects T cell in the priming phase of the immune response *in vivo* (187,188), meaning that the blockage of these molecules leads to decrease the immunosuppression tumor microenvironment (ITM).

An action mechanism to battle tumors has been reported due to PDT combined with checkpoint inhibitors (Fig. 6). PDT acts first on the tumor triggering immune responses, which is later combined with an immune checkpoint inhibitor (CTLA4 or PD-L1) inducing a long-lasting prevention against tumor recurrence (189–195). This immune memory is achieved due to PDT increasing apoptosis and necrosis, which induce tumor-associated antigens (196). Then, the maturation of dendritic cells occurs and it increases the presence of cytokines such as IL-12, and IL-18 (189,190,194,195), which activates the production of cytotoxic T leukocyte (CTL), $CD8^+$ and $CD4^+$. The evaluation of tumor-infiltrating $CD8^+$ cells has shown that it directly kills tumor cells, while also enhances cytokines secreting INF-γ and TNF-α, leading to more infiltration and expansion of myeloid-derived suppressor cells (MDSCs) (190,194,195). Consequently, this leads to killing/clearance of tumor cells (Table 1).

Indoleamine 2,3-dioxygenase (IDO) transforms tryptophan (Trp) to kynurenine (Kyn) and is an immune checkpoint also involved in ITM. IDO is frequently upregulated in both cancer cells and host APCs. IDO overexpression lowers the levels of Trp, which is essential for survival and function of effector T cells, causing G1 cycle arrest and apoptosis (197,198). Lately, NLG919, which is a potent inhibitor of IDO, has been used combined with PDT in different tumor models. It has shown a synergistically enhancement of PDT (Fig. 6), reducing immune suppression restoring the Trp/Kyn balance, and increasing CTL activity. This leads to enhanced antitumor immunity, a low

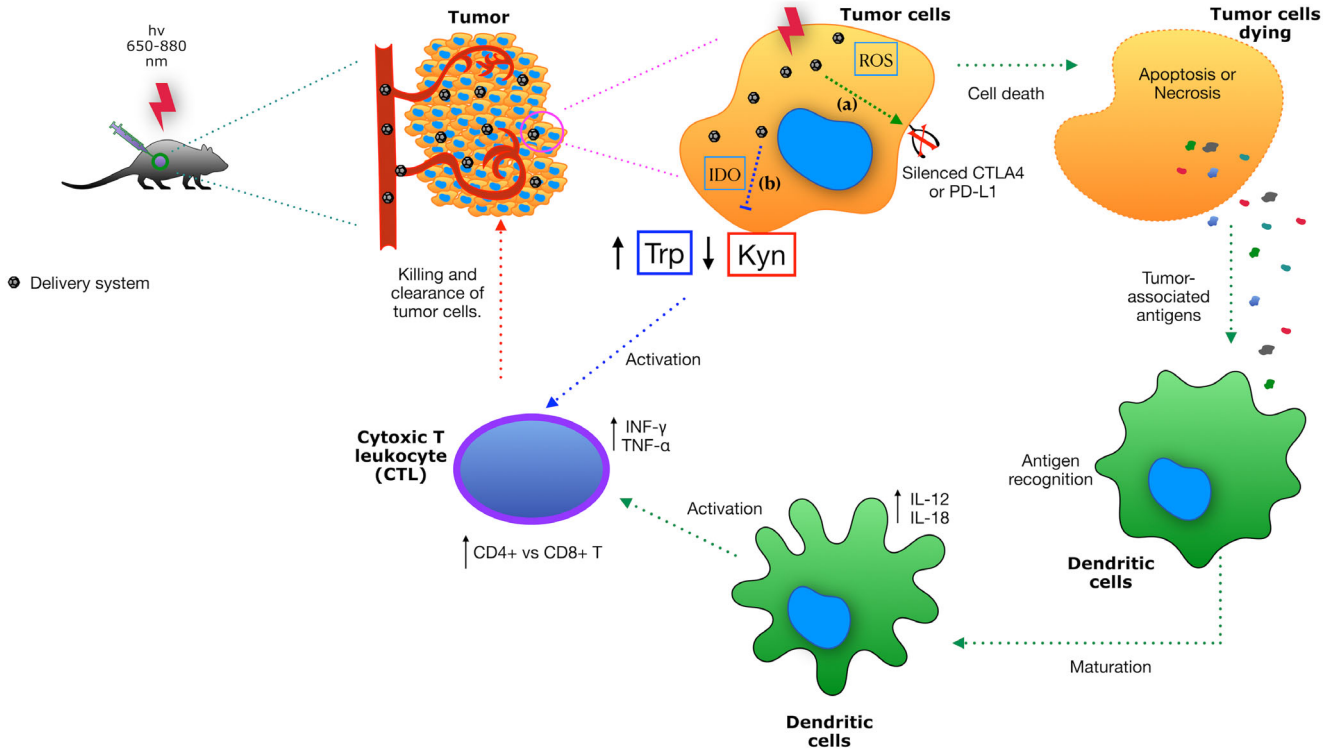

Figure 6. Schematic illustration showing the mechanisms behind checkpoint inhibitors of immunotherapy associated with diverse delivery system *in vivo*. (a) Green arrows follow the pathway of CTLA4 or PD-L1 blockage. (b) Purple arrows follow the inhibition of IDO.

system toxicity, and it inhibits the tumor growth, and tumor relapses (199–202). Particularly, this combination results in simultaneously inhibiting both primary and distant metastatic tumors (200,203).

A very interesting example of the combination of PDT and immunotherapy was reported by Liu *et al*. (204) where they constructed a nanovesicle platform (Lip-SC) that combined PDT and immunotherapy therapy in A375 cells. The importance of the use of liposome in this work permitted the encapsulation of Ce6 into the lipid bilayer due to a low solubility increasing the photophysical properties and generation of ROS under 660 nm irradiation, also increasing the accumulation in tumor areas together with inducing the apoptosis in cancer cells. Therefore, these studies have demonstrated that PDT activates NK cells, which may be attributed to the induction of NKG2DLs in tumor tissues boosting the sensitivity of cancer cells to NK cells. The importance of the nanoliposomes combined with immunotherapy and PDT is a promising alternative for melanoma.

In summary, combining adjuvant nanoparticle-based PDT with checkpoint-blockade immunotherapy is a great therapeutic strategy that eliminates primary tumors, inhibits metastases, overcomes adaptive immune resistance, improves the inflammatory response and prevents tumor recurrence. This will shed light on the advancement of self-delivery nanomedicine for clinical transformation in tumor precision therapy in turn allowing clinical benefits for cancer treatment.

ENZYMES OF IMPORTANCE FOR PDT

Cells depend for their survival on maintaining the redox homeostasis of the organism. When the cells are not able to maintain the redox homeostasis within the cells the reactive oxygen species may induce stress, therefore leading to a modification of functions. This condition characterized by the increase in ROS generation and the cellular defense mechanisms' disability to detoxify itself is collectively called oxidative stress. To ensure that the cells can elude or abate this situation, cells contain superoxide dismutase (SOD) and catalase (CAT). These enzymes are known as antioxidant enzymes and have the biological function of guaranteeing the adequate balance of ROS in the cells. Additionally, these enzymes aid in maintaining the redox homeostasis in the cells by antioxidants molecules (carotenoids and vitamins E and C) (205).

SOD and CAT are considered as first-line antioxidants, due to the fact that glutathione is not required for their function; meanwhile, glutathione peroxidases and glutathione-S-transferases are considered secondary antioxidants because they depend on its disponibility (206). Several studies have reported the overexpression of these antioxidant enzymes as a mechanism of cytoprotection of tumor cells that have undergone PDT (1,207–211).

Superoxide dismutase and its role in PDT

There is a family of enzymes known as Superoxide Dismutase EC 1.15.1.1 (SOD) (212) with a fundamental role in aerobic metabolism with the biological function of breaking down the superoxide anion radical, ($O_2^{\bullet -}$) (213–215), thus they are an important part of the antioxidant enzyme protection system, to ensure that damage by reactive oxygen species that may be generated during aerobic metabolic processes is minimized and thus prevent oxidative stress from occurring (215). SOD catalytic activity is in charge of converting its substrate superoxide anion radical into two products molecular oxygen and hydrogen peroxide (20).

Three isoforms for the SOD enzyme have been described that are distributed in different locations at the cellular level. The manganese (Mn)-containing SOD2 is located in the mitochondrial matrix, copper-zinc(Cu,Zn)-containing SOD1 is expressed in the cytosol and EC-SOD is found in the extracellular milieu (216). The catalytic activity of SOD has been reported to be present in different pathologies involving oxidative stress (216). When PDT is used in *in vitro* studies, SOD2 (207,217) expression has been observed due to increased generation of the superoxide anion radical and has a protective role in tumor cells that have been subjected to PDT (1,207).

Catalase and its role in PDT

Catalase E.C. 1.11.1.6 (CAT) catalyzes the transformation of hydrogen peroxide (H_2O_2) into molecular oxygen and water and together with glutathione peroxidase plays a fundamental role in the decomposition of hydrogen peroxide (218).

Catalase is the main enzyme for removing H_2O_2 when high intracellular concentrations of (H_2O_2) hydrogen peroxide are encountered (218,219). It is one of the enzymes known to have high activity (220). Mammalian catalase also exhibits limited peroxidase activity (219). Catalase is present in several organelles, like endoplasmic reticulum and mitochondria, but most of the enzyme is expressed in peroxisomes (219). Several studies have been reported suggesting that catalase may play a cytoprotective role in cells that have undergone PDT, but this theory has not yet been fully elucidated (208–211).

Secondary antioxidants and their role in PDT

The enzyme glutathione peroxidase (GPX) labeled EC 1.11.1.9 has the biological function of catalyzing the decomposition of H_2O_2 together with the conversion of reduced glutathione (GSH) to glutathione disulfide (GSSG) (220). Besides catalase, glutathione peroxidase has an important role in H_2O_2 scavenging. It has been reported that its affinity toward H_2O_2 is even higher than catalase by several folds (219). Another role reported for GPX is its ability to decomposed organic hydroperoxides (ROOH) into water or alcohol and additionally oxidizing GSH (220).

There are two forms reported for glutathione peroxidase, one that is dependent on selenium denoted as glutathione peroxidase (GPX), and the other independent of selenium denoted as glutathione-S-transferase (GST) (220). A total of five isoforms have been reported for GPX. Among these isoforms there is one named GPX1 located in the cytosol and mitochondria that catalyzes the reduction of H_2O_2 and fatty acid hydroperoxides. Also, there is the cytosolic GPX2 and the extracellular GPX3 which are not well characterized. The reductions of hydroperoxides from fatty acid, cholesterol and phospholipids is catalyzed by GPX4, which has been reported in both membrane fraction as well as the cytosol. Lastly, GPX5, a selenium-independent isoform and up to now, it has been reported only in murine epididymis (221). Several studies have been reported suggesting that both GPX and GST may play a cytoprotective role in cells that have undergone PDT (20,203).

Nitric oxide synthase and its role in PDT

The enzymes nitric oxide synthase (NOS) with an EC 1.14.13.39 is responsible for the biological production of nitric oxide (NO), which originates from oxygen and L-arginine (222). The bioactive free radical Nitric oxide (·NO or NO) has some biologically interesting characteristics such as the ability to diffuse freely, a lifetime between 1 and 2 s reported in H_2O, and ability to partition into hydrophobic milieu (*i.e.* cell membranes) (223–226). It has been reported that these enzymes possess different biochemical properties depending on the tissue where they are located (222). Three isoforms of these enzymes are known and denoted accordingly to the tissue where they are more abundant: endothelial type (eNOS or NOS3), inducible/immune type (iNOS or NOS2) and neuronal type (nNOS or NOS1) (227,228). It is important to mention that it has been reported that a low-level endogenous NO has the ability to promote in some cases an expansion of many tumors, angiogenesis and even survival, as well as resistance to various therapies (229).

Extensive studies have been conducted by Girotti *et al*. (229) since 2002 to elucidate the mechanistic role that NO has in preventing the cytotoxic effect of PDT on tumor cells. They used ALA as a photosensitizer and experimental conditions where PpIX was located either in the mitochondria (230) or plasma membrane (231). Therefore, they could replicate the conditions of existing photosensitizers that could localize in a different intracellular compartment (1,232). They began their experiments evaluating low-level NO effects generated from exogenous donors and their effect on cell resistance to cells subjected to the photodynamic effect of PDT. Later on, they concentrated their efforts to evaluate endogenous NO effects generated from a single or several NOS donors (229).

Inhibition of enzyme activities of relevance for a synergistic effect in PDT

In the following section, we will highlight several studies that have reported the evaluation of the synergetic effect of antioxidant enzyme inhibition combined with PDT to boost the performance of the PS, and therefore the outcome of the therapy.

Golab *et al*. (207) reported that the inhibition of SOD enzymes with the preincubation with 2-methoxyestradiol (2-ME) generated a synergistic effect upon the combination with Photofrin generated PDT in different tumor cell lines (five human and three murine). Also, they observed the effectiveness of the combination *in vivo* since they reported tumor growth retardation and a lengthening of the mice survival bearing the tumor. Therefore, they concluded that the inhibition of MnSOD with 2-ME has the potential to generate a synergism in tumor cells to improve the overall performance in PDT.

Soares *et al*. (233) combined the addition of ascorbate (vitamin C), a known antioxidant and singlet oxygen quencher, together with the inhibition of superoxide dismutase with 3 μM 2-ME and reported that they were able to potentiate redaporfin PDT effect against A549 cells, which have low SOD activity. When they used 10 mM 3-amino-1,2,4-triazole (3-AT) for the inhibition of the catalytic activity of catalase they were able to potentiate once more the PDT effect mediated by redaporfin in A549 cells. Also, upon the addition of ascorbate to cells subjected to PDT that were incubated with redaporfin together with 5 mM 3-AT an increase in the PDT effect of redaporfin toward A549 cells was observed again.

Kimani *et al*. (234) used MCF-7 cancer cells and 5μg mL^{-1} $AlPcS_2$ to investigate the combined effect of both PDT and several antioxidant inhibitors. They used 2-methoxyestradiol (2-ME) and diethyldithiocarbamate (DDC) inhibitors of Mn-SOD and Cu/Zn-SOD, respectively. Also, they used the well-known inhibitor of GSH synthesis L-buthionine sulfoximine (BSO) and 3-amino-1,2,4-triazole (3-AT) to inhibit catalase catalytic activity. They evaluated the antioxidant inhibitors themselves or in combination to see if they could increase cell death through the generation of ROS in PDT. They focused on understanding the cell death induced by PDT with the inhibition of a specific antioxidant pathway and evaluated if there was any correlation between them. Also, they studied the relationship between the level of ROS generated within the cell in the presence of antioxidant inhibitors and the cell death. They observed that BSO alone or in combination with other antioxidant inhibitors was successful in potentiating the cytotoxicity due to PDT by increasing the generation of ROS and apoptosis. The best result was achieved when the cells were subjected to a 24-h preincubation with 3-AT (10 mM and 300 μM) or 2-ME (300 μM and 1 μM) or when all four inhibitors BSO, 3-AT, 2-ME and DDC were combined in the following concentrations 300 μM, 10 mM, 1 μM and 10 μM, respectively. They concluded that MCF-7 cancer cells that were subjected to a pretreatment with antioxidant inhibitors before preforming PDT, specifically the inhibitors of hydroperoxide degradation, the generated ROS will accumulate within the cell and therefore induce the cell death because of the enhancement of the PDT cytotoxicity.

Miller and Henderson (235) reported that the use of BSO combined with PDT was able to boost dihematoporphyrin ether (DHE) performance in 4 cellular lines (Chinese hamster ovary cell (CHO), Chinese hamster V-79 cells, mouse-derived EMT-6 cells and mouse-derived RIF cells). The observed effect depended on the BSO dose and the illumination times. They concluded that the PDT effect was dependent on the exposure to BSO prior to the PDT therefore enhancing the overall effect of DHE.

In the same manner, Jiang *et al*. (236) reported that BSO was able to increase the PDT effect of Photofrin *in vitro* on human U87 and U251n glioma cells, as well as, *in vivo* on human U87 glioma cells, but in a BSO and light dose-dependent manner.

Regarding the evidence reported in the literature for the response of GPXs within the cells after the treatment with PDT, it can be mentioned that when HpD was used as a photosensitizer which was accompanied by a depletion of selenium it was reported a significantly increased in cytotoxicity (237). Also, it was reported that after the PDT treatment with Hypericin a decrease in the enzymatic activity of GPX was observed, together with a substantial decrease intracellularly of the levels of the reduced glutathione (210). Furthermore, it has been reported the protection of GPX4 to cells subjected to PDT, which was attributed to the decrease in lipids hydroperoxides inside the cells (231,238). In the same manner, it was reported that an expression of GST to protect cells from damage was induced by the PDT treatment. Nevertheless, the cytoprotective mechanism needs to be fully elucidated. It could be due to GST sequestering the photosensitizer, but it needs yet to be proven (239).

A novel approach was designed by Sun *et al*. (240) where they reported NIR photothermal liposome nanoantagonists (PLNA) that upon the release of antagonists showed the capabilities for enhancing cancer PDT. This PLNA was composed of both BSO and indocyanine green (ICG) and encapsulated in the aqueous region of the liposome. ICG is a PS approved by FDA and very used in PDT and PTT. The liposome under NIR irradiation increases the transition temperature of the liposome producing changes noticeable in the transition from gel phase into the fluid phase, this leads to the demand for BSO to be release, decreasing the levels of glutathione in the tumor microenvironment and amplifying the generation of ROS by NIR irradiation of ICG. This work provides a novel strategy for the enhancement of the efficiency of PDT.

It should be noted that regarding the NOS activity, scarce studies so far have been reported that explore the possibility of the combination of both the inhibition of NOS, and PDT may potentiate the performance of the photosensitizers (241). Nevertheless, enough evidence has been reported in the literature that these inhibitors might have the potential to be used alone or in combination with other existing therapies such as radiotherapy as well as chemotherapy to explore the possibility of synergistic effects (229,242). Therefore, it is plausible that the efficiency of PDT to several tumors could be potentiated it by combining it with iNOS inhibitors as a coadjutant of the therapy. It has been reported that L-NIL and GW274150, which are two inhibitors, have been tried out in clinical trials for other diseases that are not cancer, and have shown no undesirable side effect, and some works have been cut out to develop future application in PDT (243,244).

Fahey *et al*. (245,246) have reported that cells subjected to the photosensitizer ALA and later irradiation presented an iNOS transcription stimulation by the bromodomain protein Brd4 upon the binding to p65-acK310. They latter reported (246) the use of JQ1, which is a known inhibitor of Brd4 and other BET proteins, once it bounds to the BET domains, impedes that acK groups can interact either with histones or transcription factors (247,248), therefore diminishing the iNOS upregulation after irradiation, and thus preventing the proliferation of survival cells together with a more aggressive invasion.

The use of photosensitizers that can combine both photochemical processes previously mentioned together with inhibiting antioxidant enzymes could have the ability to potentiate the PDT effect. Also, the inhibition of NOS to avoid resistance in PDT-treated cells could potentiate the overall performance of the available and future photosensitizers. Therefore, researchers should explore the reported enzyme activities of the cellular lines to generate data that could be later used for the experimental design of *in vivo* experiment. These results could offer the possibility of better clinical trials in the PDT community.

SUMMARY AND OUTLOOK

Chemical modifications of photosensitizers can be tailored to specific needs depending on their objectives, different researchers could modify the core of the molecule to improve photostability, selectivity, lipophilicity or phototoxicity. Several examples were presented in this review, some of them currently approved, other under clinical trials and other in preclinical states (see Appendix A) where researchers depending on their specific research need have improved one or more of the above-mentioned properties of the PS. Although a lot of work in this area is conducted throughout the scientific community, a novel total synthesis is always welcome.

The use of DDS to obtain better biodistribution and bioaccumulation is a trending topic in the PDT community. Liposomes have demonstrated over the years to be excellent DDS for photosensitizer and a lot of evidence have been reported in the literature of the incorporation of hydrophobic and hydrophilic PS, including their use to control the release of drugs, for targeted delivery, as well as, for image-guided therapy and even for the combination with other therapies. Nevertheless, cavitands offer increases in solubility, can be modified to make them more specific and increase selectivity and cellular uptake. The host–guest chemistry approach avoids aggregations and, in most cases, increases singlet oxygen generation without the need to chemically modify the photosensitizer. It is noteworthy, that the release of photosensitizers from these complexes is highly controllable unlike in liposomes. Moreover, using macrocycles that deactivate photosensitizers can prevent undesired photosensitizations.

Combining PDT with other therapies, carried out through diverse strategies, shows cooperative effects and provides new opportunities for solid tumor treatments. These combinations offer a significant advantage such as reducing the side effects, and with this comes an increase in survival benefits. Also, there is an increase in the anti-tumor effects and thus this can enhance the quality of life.

Additionally, the inhibition of antioxidant enzymes could be combined with the above-mentioned strategies to boost the performance of the PS in PDT. A synergistic effect could be achieved under the right conditions which could be extrapolated to future clinical trials to achieve a better outcome in the PDT treatment.

Several other approaches can be used to improve PDT efficacy, which are not highlighted within this review, such as light delivery techniques, dosimetry techniques, among others. For other approaches to improve the overall performance of PDT we invite new researchers in the field to review the current literature to revise the most updated reports.

We finish by encouraging new researchers in PDT to carefully decide the right approach for them depending on the PS and the desired outcome.

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Nory Mariño-Ocampo received her degree in Chemistry in 2016 from the Universidad del Valle, Cali, Colombia. Later, she received her Ph.D. in Chemistry from the Pontificia Universidad Católica de Chile in 2022, where she worked in the research group of Professor Denis Fuentealba. Her Ph.D. research focuses on biosupramolecular chemistry, especially on the derivatization of photosensitizers and the use of drug delivery systems like human serum albumin and cucurbit[7]uril. She is interested in the development of new photosensitizers, drug delivery systems involving proteins and its applications for photodynamic therapy.

Dr. Luciano Dibona-Villanueva is a highly motivated Ph.D. researcher who, in 2017, enters the Pontificia Universidad Catolica de Chile's Chemistry Ph.D. program under the supervision of Professor Denis Fuentealba in the Supramolecular Chemistry and Photobiology Lab. During his studies, he worked in Antimicrobial Photodynamic Inactivation on Penicillium digitatum *in vitro*. He is currently conducting postdoctoral work as a researcher in Institut Químic de Sarrià characterizing photophysics on persistent luminescence particles used for the control of lung infection bacteria under Light4Lungs European project in Barcelona. His main research areas are Antimicrobial Photodynamic Inactivation, Photophysics and Photochemistry.

Elizabeth Escobar-Álvarez is a Chemist with a Master in Chemistry Sciences from Universidad de Antioquia, Colombia and Ph.D. degree in Chemistry from Pontificia Universidad Católica de Chile, Santiago, Chile. Her Master's and Bachelor's project were both focused on the innate immune responses of mononuclear phagocytes against the *M. tuberculosis* infection using *in vitro* models. Her Ph.D. work was focused on understanding the chemistry of irreversible oxidation in proteins (previously expressed and purified from E. coli) mediated by peroxyl radicals.

Daniel Guerra-Díaz received his degree in Chemistry in 2012 and his Ph.D degree in Chemistry in 2018 from the University of Chile, Santiago, Chile. His Ph.D research focused on the design of host–guest supramolecular architectures for their use in metal-enhanced fluorescence. In 2019, he was awarded a postdoctoral research project where he is been studying the changes in the photophysical and photochemical properties of host–guest complexes of photosensitizers used in photodynamic therapy at the Biosupramolecular chemistry group of Dr. Denis Fuentealba. His main research interests are host–guest chemistry of cyclodextrins and cucurbit[n]urils, nanotechnology, drug delivery systems and photochemistry.

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Denis Fuentealba has a Chemistry degree from Universidad de Tarapacá and Doctorate degree in Chemistry from Pontificia Universidad Católica de Chile, with specialization in Photobiology and medical applications. Later he specialized in Supramolecular Chemistry at the University of Victoria, BC in Canada. Since 2012, he is the director of the Supramolecular Chemistry and Photobiology Lab at Pontificia Universidad Católica de Chile where he is an Associate Professor. His research interests include the use of supramolecular host–guest systems based on cucurbiturils and related molecular containers for the encapsulation and release of photoactive drugs with applications in Anticancer and Antimicrobial Photodynamic Therapies.

José Robinson-Duggon received his degree in Chemistry from Universidad de Panamá (2007), a M.S. in Chemistry from the University of Connecticut (2011) and his Ph.D. in Chemistry at Pontificia Universidad Católica de Chile under the advisement of Professor Denis Fuentealba in 2018. He is currently a Professor of Biochemistry at the Universidad de Panamá and a National Researcher I at the Sistema Nacional de Investigación of Panamá. His research interests include supramolecular systems for drug delivery applications in photodynamic therapy and the synergistic effect of antioxidant enzyme inhibition and supramolecular encapsulation of photosensitizers in its overall performance for photodynamic therapy.

APPENDIX A

PS	Country	Clinical uses	References
Approved for clinical use			
Verteporfin (VP, visudyne®)	FDA	The treatment of choroidal neovascularization caused by wet age-related macular degeneration (AMD)	(249)
5-Aminolevulinic acid (ALA)	Worldwide	Skin, bladder, brain, esophagus	(1)
Photofrin®	Worldwide	Lung, esophagus, bile duct, bladder, brain, ovarian	(1)
N-aspartyl chlorin e6 (Talaporfin, Laserphyrin®)	Japan	Lung cancer, colorectal neoplasms, Liver metastasis	(35,250)
Under clinical trial			
Chlorin e6 (Ce6)	USA, South Korea, Brazil	Health Care Associated Infection, Benign Prostatic Hyperplasia, Lower Urinary Tract Symptoms, Squamous Cell Lung Cancer, Lung Cancer, Benign Prostatic Hyperplasia	(251–255)
Redaporfin	Portugal	Advanced Head and Neck cancer	(256)
Hypericin	United States	Cutaneous T-cell Lymphoma	(257,258)
Dihematoporphyrin ether (DHE)	United States	Brain and Central Nervous System tumors	(259)
Indocyanine green (ICG)			(260)
	United States FDA	Choroidal Melanoma Approved as a contrasting agent	
Preclinical studies			
Porphyrin-fluor cyclodextrin derivative	Czech Republic	*In vitro* studies with mouse mammary carcinoma cells (4T1) and human chronic myelogenous leukemia cells (K562). *In vivo* studies with BALB/c mice bearing subcutaneously growing mammary carcinoma (4T1) and nude mouse model with human amelanotic melanoma C32.	(100)
TPyP (porphyrin)-adamantyl derivative	China	*In vitro* studies in adenocarcinoma epithelial cells (A549)	(102)
TPP (porphyrin)-adamantyl derivative bearing a disulfide bond	China	*In vitro* studies in MCF-7 cells	(103)
Chlorin e6 β-cyclodextrin derivative	China	*In vitro* studies in MCF-7 cells	(104)
mPEG-protoporphyrin IX (PpIX)	China	*In vitro* studies in HepG2 and L929 cell lines	(105)
Chlorin e6 α-cyclodextrin derivative	China	*In vitro* studies in MCF-7 cells and *In vivo* studies in tumor-bearing mice	(106)
Nanorods of 5,10,15,20-tetrakis(4-N-methylpyridyl) porphyrin (TMPyP) with sulfobutylether-β-cyclodextrin (captisol)	India	*In vitro* studies in E. coli and lung carcinoma A549 cells	(107)
Tetraphenylporphyrin/trimannosyl CB[7] derivative	Turkey	*In vitro* studies in E. coli, B. subtilis and MCF-7 cells	(111,112)
Tetrameric porphyrin/calix[4]arene conjugate	Singapore	*In vitro* studies in HeLa cells	(114)
Porphyrin/pyridinium derivative bearing a disulfide bond	China	*In vitro* studies in A549 cells	(115)
Tetraphenylporphyrin/quaternary ammonium salt derivative	China	*In vitro* studies in A549 cells	(116)
Pyrophaeophorbide A-pyridinium	China	*In vitro* studies in HeLa cells	(117)
PEGylated Zinc phthalocyanine	China	*In vitro* studies in HepG2 cells and *in vivo* studies in H22 tumor-bearing mice models	(119)
Zinc phthalocyanine/β-cyclodextrin conjugate	China	*In vitro* studies in EGFR-positive HT29 and A431 cells and *in vivo* studies in HT29 tumor in nude mice	(120)
Porphyrin-phospholipid (PoP)	USA	*In vitro* studies in SCID mice bearing patient-derived pancreatic cancer xenografts (PDX)	(156)
IR820	China	*In vitro* studies in 4T1 cells/*in vivo* studies with female Balb mice were subcutaneously injected of 4T1 cells	(158)
Pheophorbide (PBR)	China	*In vivo* studies in nasopharyngeal carcinoma cell lines (CNE-1 and HNE-1)/*in vivo* studies with xenograft model generation, cells CNE-1 were subcutaneously injected into mice.	(161)
Aluminum Phthalocyanine (tetrasulphonate)	South Africa, Brazil	*In vitro* studies in A549 lung cancer cell line. *In vitro* studies in MCF-7 breast cancer cell line.	(261)
Meso-tetrakis(4-sulfonatophenyl porphyrin)	China	*In vitro* studies with HeLa cells, Tumor imaging in mice	(262)
Methylene blue	Mexico, Brazil, Egypt, USA	Basal cell carcinoma, *In vitro* studies with MCF-7, MCF-10A and MDA-MB-231 cells, *In vitro* studies with SKBR-3 cells, Tumor imaging	(263–265)
Phorphyrin – Ether derivative	China	*In vitro* studies with MCF-7, 4T1 murine and *in vivo* studies with MDA-MB-231 cells,	(41)
Phorphyrin – Halogen derivative	China	*In vitro* studies with HepG2, H-1975 and A549 cells	(42)

(continued)

Appendix A (continued)

PS	Country	Clinical uses	References
Protoporphyrin IX – amino-PEG550 derivative	France, Norway, China	*In vitro* studies with 4T1, WiDr and scc-U8 cells	(43)
TMPyP – *meso-a*ryl derivatives	Portugal, Belgium	*In vitro* studies with MCF-7 cells	(44)
Chlorine – maleimide derivatives	China	*In vitro* studies with Hep-G2 cells	(45)
Phthalocyanine – choline derivative	China	*In vitro* studies with Nthy-ori3-1 cells	(46)
Phthalocyanine – Indomethacin derivative	China	*In vitro* studies with Hep-G2, and HELF cells	(47)
BODIPY – TPP derivative	Turkey, Korea, China	*In vitro* studies with HeLa cells	(49)
BODIPY – Lactose derivative	Korea, Philippines	*In vitro* studies with Huh-7, and HeLa cells	(50)
Cyanine – Piperazine and Rhodamine derivative	China	*In vitro* studies with A-549 cells	(51)
Cyanine – anthracene derivative	China, Singapore	*In vitro* studies with 4T1 cells, and in vivo studies	(52)
Chloro-hemicyanine	Turkey	*In vitro* studies with HeLa, A-549 and L-929 cells	(53)
Fluorescein – Alkylation	China	*In vitro* studies with HeLa cells, and *in vivo* studies	(54)
Toluidine Blue – Biotin derivative	China	*In vitro* studies with SCC-7, and COS7 cells; *in vivo* studies	(55)
Toluidine Blue – Fatty acids derivatives	Chile, Panama, Canada	*In vitro* studies with HeLa cells	(56)
Toluidine Blue – disulfur and maleimide derivative	Chile	Development of synthetic derivatives for future *in vitro* studies	(57)
Pterins – Lipophilic and ester derivatives	Argentina, USA	Development of synthetic derivatives for future *in vitro* studies	(58,59)
Lumazines – decylated derivatives	Argentina, USA	Development of synthetic derivatives for future *in vitro* studies	(60)
5,10,15-tris(pentafluorophenyl)-20-(4-pyridyl)porphyrin chlorin derivatives	Brazil, Portugal	*In vitro* studies with B16F10 cells	(27)
Graphene oxide quantum dot (GQD)–BODIPY nanoconjugates	India	*In vitro* studies with MDA-MB-231 cancer cell line	(28)
Heptamethine cyanine IR780 derivatives	China	*In vitro* studies with B16F10 tumor model in C57 mice	(29)
Hematoporphyrin ether derivatives	China	*In vitro* studies with A549 lung tumor cells	(30)
Pyropheophorbide A derivatives	Ireland, China	*In vitro* studies with A549 cells	(31)
Polyol amide chlorin e6 derivatives	Germany, Poland	*In vitro* studies with HCT116 cells	(32)
Tetraphenylporphyrin/Liposome	China	*In vitro* studies with SKOV-3 cells	(72)
Protoporphyrin IX/Liposome (DOTAP:PS)	Poland	*In vitro* studies with HeLa cells	(73)
Zinc phthalocyanine/PEGylated cationic liposomes	Netherlands, Hungary	*In vitro* studies with human epidermoid carcinoma (A431) cells	(74)
Curcuminoids/liposome	South Korea	*In vitro* studies with A549 human cancer cells	(75)
Protoporphyrin IX/Hypericin/liposomes (LNCs)	France	*In vitro* studies with HeLa and MDA-MB-232 cells	(76)
Cyanine-liposome (LPs, LPB)	China	*In vitro* studies with breast cancer 4T1 and MCF-7 cell lines. *In vivo* studies with 4T1 tumor-bearing mice	(77)
Chlorin e6/multifunctional nanoparticle system (NPs-Lip@PTX/CyA)	China	*In vitro* studies with 4T1, MCF-7 and MCF-7/ADR cell lines. *In vivo* studies with 4T1 tumor-bearing mice	(78)
Bis(pyrene)/liposome	China	*In vitro* studies with MCF-7 cell line. *In vivo* studies with BALB/c female nude mice.	(79)
Chlorin e6 di-N-methylglucaminate/liposome	United States, Russia	*In vitro* studies with BALB/c mice bearing Ehrlich (ELD) tumor intramuscularly	(80)
Chlorin e6/polymeric nanoparticle liposome	Korea	*In vitro* studies with AsPC-1, MIA PaCa-2, and MIA PaCa-2/ABCG2 cell lines. *In vivo* studies with BALB/c nude mice	(81)