Chapter

Phenazines and Photoactive Formulations: Promising Photodrugs for Photodynamic Therapy

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Abstract

Photodynamic Therapy (PDT) is a therapeutic modality that can be applied with many photosensitizing compounds (PS). Photosensitization has shown promising results in damage against abnormal cell growth as cancer and inactivating a broad spectrum of microorganisms with no reported microbial resistance. Photodynamic processes occur by the light action at the appropriate wavelength in the presence of a PS that will be excited by the energy absorbed from the light source, where the interaction with the oxygen present in the cell will generate reactive oxygen species (ROS). The potential of phenazines as a photosensitizer is reviewed in this chapter as a practical guide to the future development of formulations that are effective for cancer treatment and microorganism control. Here we mainly summarize articles about phenazines from 2005 to 2021 when we performed a systematic search in the Science Direct, PubMed, Google Scholar, Web of Science, and Scopus databases. The carrier systems formed by micellar copolymers type Pluronic® have demonstrated effectiveness in incorporating several PS, ensuring its monomeric form for PDT applications. The fundamentals of the photosensitization mechanism are discussed. Studies have shown the beneficial impact of an appropriate incorporation technique to enhance the cellular uptake of phenazines compounds.

Keywords: phenazines, photodynamic therapy, photosensitizing agents, nanoplatform, micelles

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1. Introduction

Success in treating diseases such as cancer or microbial diseases directly depends on the therapeutic modality applied. Given the side effects presented and the limitation in the efficiency of traditional procedures (surgery, chemotherapy, and radiotherapy), other alternatives are constantly being proposed in oncology [1]. Among the most promising modalities, Photodynamic Therapy (PDT) stands out as it does not present serious side effects and does not have limited efficiency [2]. PDT and antimicrobial Photodynamic Therapy (aPDT) are medical modality that has high specificity and selectivity in the treatment of infections caused by a virus, bacteria, protozoa, and fungi, as well as several cardiovascular, dermatological, and other diseases related to abnormal cell growth as cancer [2–5].

The PDT efficiency directly depends on a photosensitizing compound (PS) with ideal properties for the photophysical and photochemical processes that leads to the formation of singlet oxygen ($^1O_2$) and/or reactive oxygen species (ROS) that cause cell damage [2, 6]. PDT aims at the localized damage of living tissue with abnormal cell growth through its necrosis or infeasibility [7]. Likely targets of PDT are mitochondria, plasma membrane and other cell organelles, tumor cell nucleus, and blood vessels [8, 9]. This selectivity occurs due to the high concentration of lipoprotein receptors in neoplastic cells, where PS accumulates preferentially in diseased tissues, forming intravascular complexes with low-density proteins (LDL) [10].

The $^1O_2$ is a free radical produced during PDT and other related therapies. It is made when light activates the photosensitive compound administered to the patient. The $^1O_2$ action mechanism is based on its ability to cause damage to DNA, proteins, and other cellular molecules, leading to cell death [2]. Lipid peroxidation occurs when $^1O_2$ reacts with lipids in cell membranes, causing the formation of secondary free radicals and damaging the cell membrane structure. DNA damage can arise when $^1O_2$ reacts with DNA nucleotides, causing damage and interfering with replication and gene expression. The $^1O_2$ stops energy production when reacting with the enzymes of the respiratory cell chain and leads to cell death. The $^1O_2$ can react with cellular ribosomes and interrupt protein synthesis, causing cell death. The $^1O_2$ is an essential agent in photodynamic therapy and other radiation therapies, as these action mechanisms lead to the destruction of cancer cells and a reduction in the size of tumors [2].

Among the main characteristics of adequate PS is their low toxicity in the dark, light absorption between 400 and 850 nm (therapeutic window), high molar absorptivity values, and considerable formation of ROS inherent in the technique [11].

The development of promising drugs requires science to improve investigations based on the action of a compound or the joint effort of two or more drugs, thus synergy [12]. The first generation of phototherapeutic agents used in PDT is based on mixtures of porphyrin derivatives [7]. The search for PS with better optical and pharmacokinetic characteristics gave rise to the second generation of PS, similar to porphyrin molecules such as benzoporphyrins, chlorins, texapyrins, phthalocyanines, and naphthalocyanines [13]. Some of those PS compounds are already approved by the Food and Drug Administration (FDA/USA) for clinical applications using PDT. In addition, some countries are already approved, for example, Photofrin®, Levulan Kerasticks®, and Visudyne® (Verteporfin) [14].

Due to advances in research, studies, and treatments based on PDT, there is a great demand for new naturally occurring or synthetic PS that are biocompatible and have adequate properties [15, 16]. Over time, hematoporphyrin derivatives have been replaced by various PS compounds [17]. The third generation of PS is based on
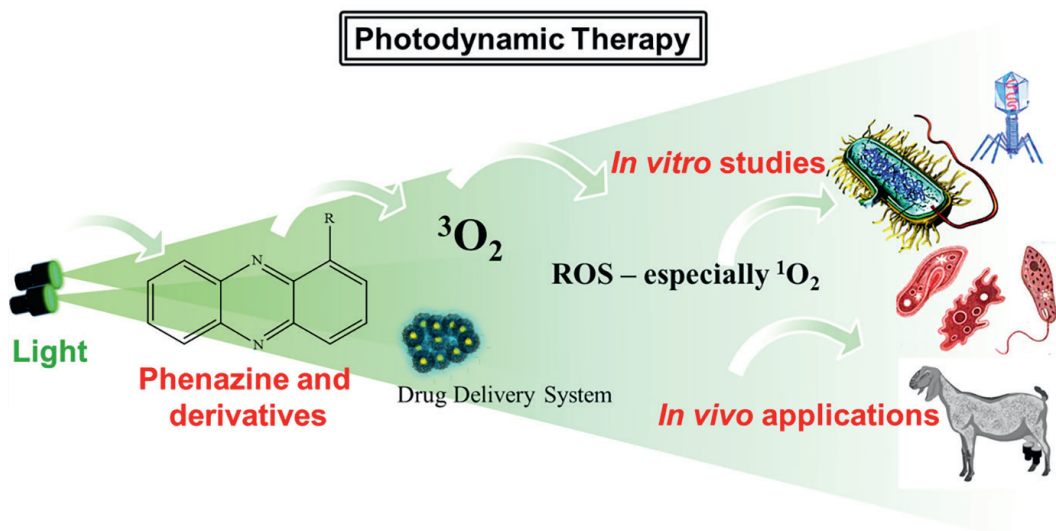
Figure 1.
Phenazines compounds incorporated into drug delivery system have great potential for photodynamic therapy applications.

formulations with different drug delivery systems (DDS) that provide better solubility in physiological media, more favorable pharmacokinetics, and allow the use of light sources with more remarkable penetration power into tissues.

Due to economic and environmental considerations, PS compounds obtained from abundant raw materials attract more interest than those prepared by complex chemical routes [15, 18]. Studies involving naphthodianthrones (hypericin), phenothiazines (methylene blue and toluidine blue), phthalocyanines, chlorins (chlorophyll A), xanthenes (rose bengal), and curcuminoids (curcumin) stand out in the literature [14]. These PS compounds are extensively studied *in vitro*, *in vivo*, and in photodiagnostic aspects [19–21].

This particular chapter presents explicitly an overview of compounds of the phenazine class (**Figure 1**), in which promising photoactive drugs such as neutral red (NR) [22], phenosafranin (PhS) [23], and safranine-O (Sf) [24], still poor explored for PDT applications.

2. Search strategy

The strategy we performed a systematic literature search in Science Direct, PubMed, Google Scholar, Web of Science, and Scopus databases using the combinations of the term "phenazines" with the following: "photodynamic therapy," "aPDT or antimicrobial Photodynamic Therapy, or PACT or photodynamic inactivation or PDI," "Animal studies or *in vitro* studies involving phenazines". Peer-reviewed articles published in English from 2005 to 2021 were included to compile this chapter. We have also scanned references for relevant articles.

3. Photosensitization mechanism

The combination of a PS compound with molecular oxygen ($^3O_2$) and visible light of the adequate wavelength generates ROS that causes cell components to oxidize and

lead to death [2, 25]. In addition, PS reacts with neighboring molecules by electron or hydrogen transfer leading to the production of free radicals or by energy transfer to oxygen, inducing the production of $^1O_2$ [2, 26].

The photochemical processes originate from the interaction of light with matter which, by absorbing energy with adequate wavelength, allows the promotion of an electron from the ground state, called HOMO (Highest Occupied Molecular Orbital), to the excited state LUMO (Lowest Unoccupied Molecular Orbital), of higher energy [27]. The excited state is unstable, and in it, the molecule can suffer chemical processes (rearrangements or fragmentation of the molecule) or physical processes (deexcitation) [27]. According to the Molecular Orbital Theory, oxygen in the ground state and excited can assume different forms of occupation of molecular orbitals antibinders, as shown in **Figure 2**.

In the ground state, molecular oxygen has two unpaired electrons in the doubly degenerate antibonding orbitals, π^*_x, and π^*_y. These electrons have the same spin, resulting in a maximum multiplicity and, thus, the lowest state oxygen energy. Therefore, the ground state of molecular oxygen is a triplet, which has the spectroscopic term $^3\sum_g$. The excited state of oxygen that has all valence electrons paired is singlet oxygen. Singlet oxygen has two forms with distinct symmetries, one of smaller energy $^1\Delta_g$, doubly degenerate ($^1\Delta_x$ and $^1\Delta_y$; 92.4 kJ mol^{-1}), and another one with higher energy ($^1\Sigma$; 159.6 kJ mol^{-1}). The second excited state of oxygen has a short lifetime since the transition to the $^1\Delta_g$ state is allowed by spin. The different symmetry of the $^1\Delta_g$ species with respect to the ground state and the spin prohibition of the $^1\Delta_g - {}^3\sum_g$ transition ensures that the $^1\Delta_g$ species has a long enough lifetime to allow oxidation of organic molecules [7].

The photochemical processes that occur in the excited state of photoactive molecules can be represented by the Jablonski diagram (**Figure 3**) [27, 28]. Among these processes, internal conversion (IC), fluorescence, intersystem crossing (ISC), and phosphorescence stand out [27, 29].
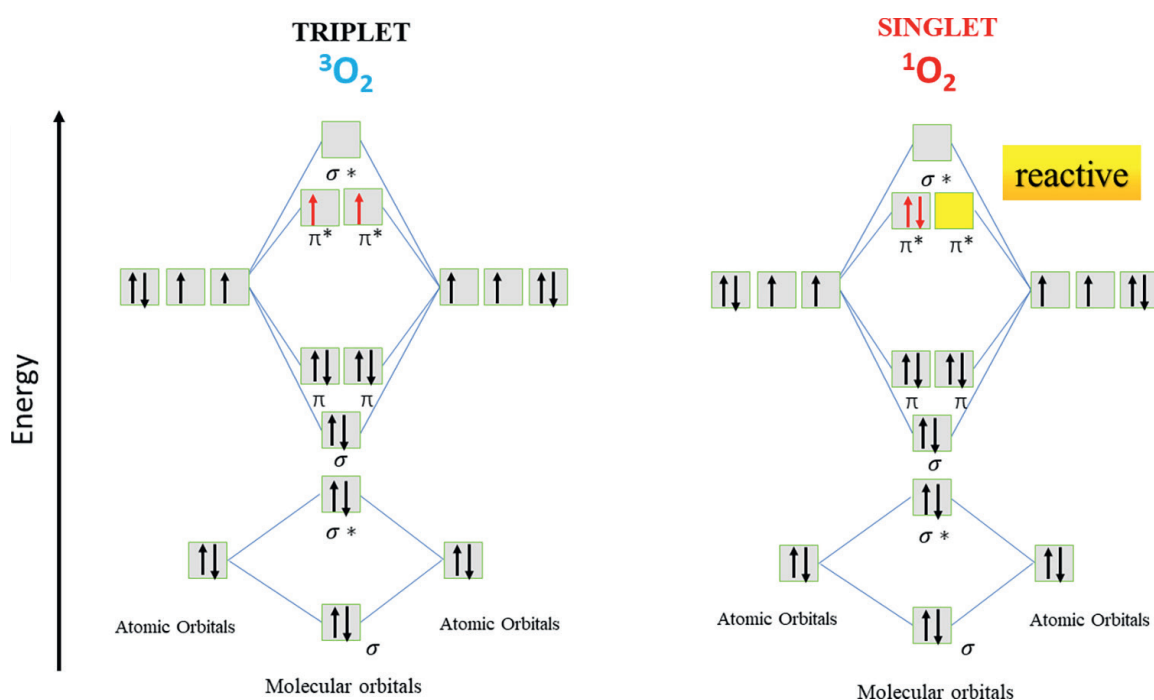

Figure 2.
Electronic distribution in the antibonding molecular orbitals for the oxygen electronic states. It was adapted from Lakowicz [27].
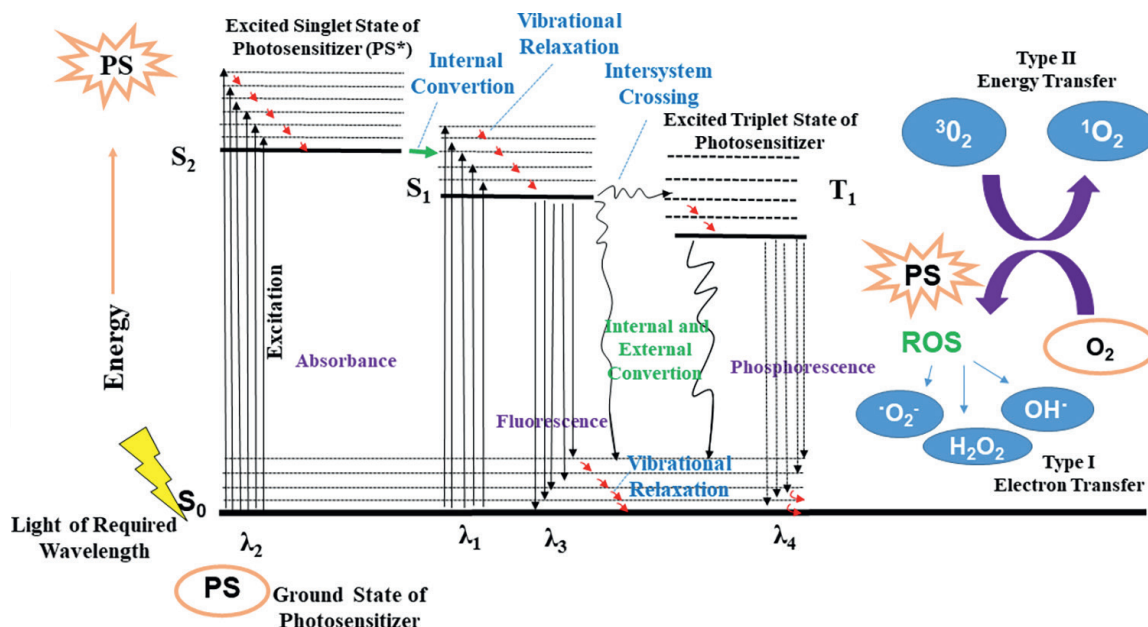
Figure 3.
Jablonski diagram: Electron transfer scheme by type I and type II mechanisms with singlet oxygen, superoxide anions, and hydroxyls production. It was adapted from Lakowicz [27].

After molecule excitation to the second excited state, the relaxation process for the first excited state can occur by IC (where the internal energy is lost by collision) and then return to the ground state via fluorescence emission. Deactivation can also arise by ISC between vibrational levels of the same energy and electronic states of different multiplicities. The decay from the triplet state to the singlet fundamental can then occur by phosphorescence emission or collisions [27, 29]. For PDT, it is interesting that the PS is preferably in the excited triplet state, as it will have the same spin multiplicity of $^3O_2$ favoring the photochemical processes of producing $^1O_2$ and ROS. The interaction with biological substrates in PDT can occur by two mechanisms: Type I, where photo-oxidation occurs through the transfer of electrons between the triplet state of PS and the substrate forming radical ions that react with oxygen in the ground state resulting in ROS. The Type II mechanism involves the energy transfer from the triplet excited state of PS to molecular oxygen generating $^1O_2$ [2, 28].

The literature reports the use of PDT in treating primary carcinomas or metastases in the head and neck regions, such as the oral cavity, pharynx, and larynx [30]. However, after tumor recession, secondary diseases such as those caused by bacteria may manifest. Photodynamic treatment circumvents this problem as PDT is not restricted to microorganisms.

With the increasing cases of acquired resistance by bacteria against antibiotics, the search for the control of microorganisms via PDT also attracts interest from the scientific community [31]. As a result, PDT has been widely applied in the microbiological area, standing out as a promising technique in microorganism inactivation [32]. Furthermore, PDT offers advantages over the usual antimicrobial agents, triggering rapid cell death and unlikely development of resistance by the microorganism [33].

4. Photosensitizer formulation

Several PS can be used for different applications because of their particular properties. Therefore, it is necessary to know the essential characteristics of each PS

to use its therapeutic properties better. The most cited PS compounds in the literature present, for the most part, structures containing aromatic rings or conjugated systems, which give them high hydrophobicity. In this sense, efforts in developing strategies in the formulation of PS administration stand out. However, even PS with a small structure presents a self-aggregation problem, making it challenging to apply in aqueous media.

Incorporating PS compounds into nanostructured systems aims to minimize the effects of self-aggregation in aqueous media, protect PS against degradation and elimination by the organism, and facilitate the biotransport. In some cases, the adequately incorporated PS assists the vectorization of the nanostructured system, increasing the bioavailability of the photoactive drug at the application site (third generation), promoting a controlled release in the regions (tissues and organs) to be treated [34]. In addition, they decrease side effects (toxicity) and microbial resistance. In this sense, several carrier systems are generally constituted by colloidal dispersion systems, such as copolymeric micelles [35], liposomal vesicles [36], dendrimers [37], cyclodextrins [38, 39], polymer-DNA complexes (polyplexes) [40], nanogels [41], nanotubes [42], nanosuspensions [43], nanocrystals [44], solid lipid nanoparticles [45], metallic or ceramic nanoparticles [46], among other nanoscale materials for medical use are being widely studied [47–49]. **Figure 4** illustrates the most commonly found nanoparticulate carrier systems in the literature for carrying PS [50].
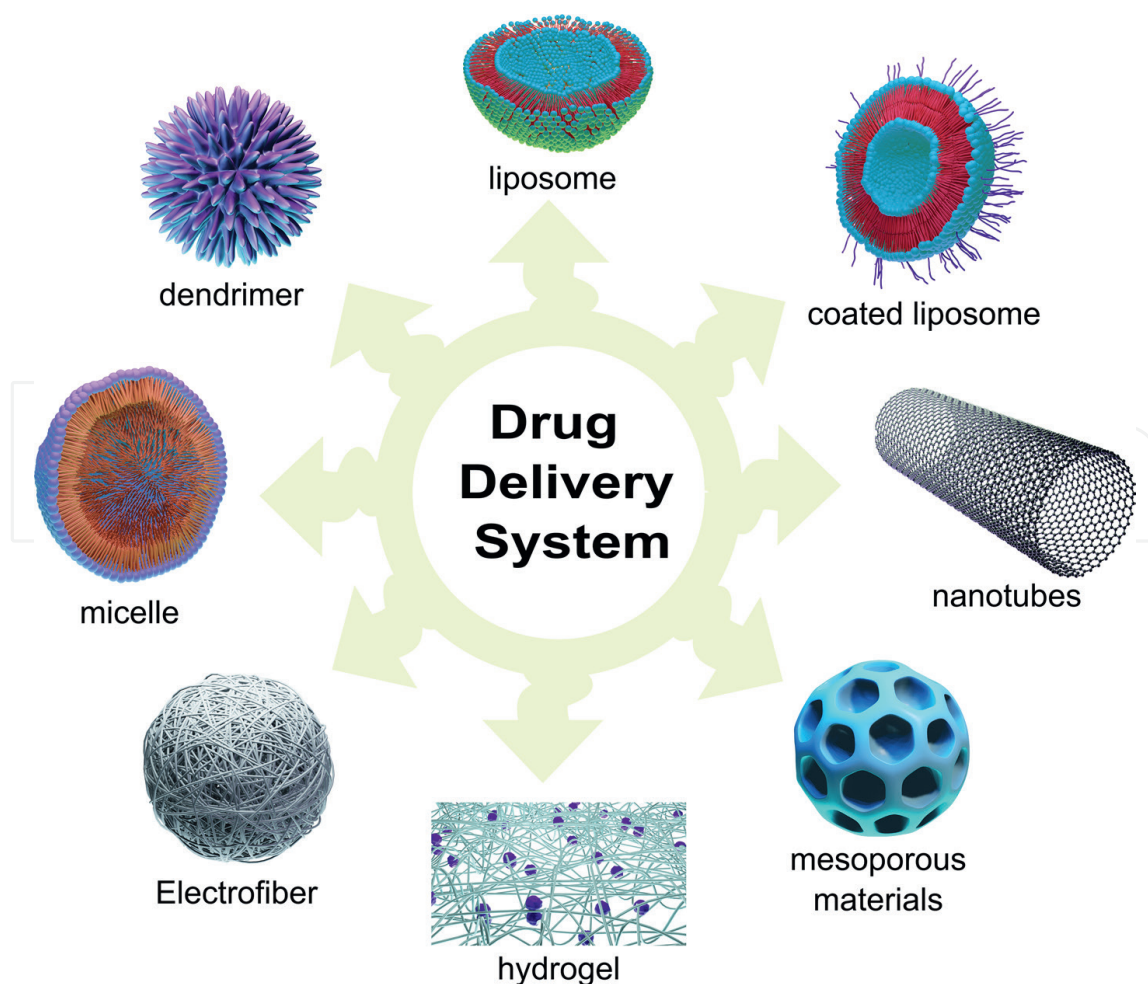

Figure 4.
Exemplification of some carrier systems currently used. I was adapted from Senapati et al. [50].

All these carrier systems have the advantage of protecting PS as a common characteristic. In addition, they contribute to keeping the PS monomeric form ideal for application in PDT [48, 51]. The leading site of photodynamic action is the cell membranes; therefore, it is of great importance that the carrier system is similar or presents a specific interaction with it. The PS efficiency in PDT applications depends on the microenvironment in which the PS is located so that there are no changes in its physicochemical and photophysical properties and in its interaction with living biological systems [36]. Parameters such as bioaccessibility, passive transport, and permeation of the drug into the membrane are of great importance in developing new drugs for PDT applications [11, 52]. Micellar and liposomal environments, for example, are reasonable models of cellular environments and provide useful information about PS molecules against organized systems and biomimetics [5, 41, 52, 53].

Since the cell membrane is a complex system composed of a lipid bilayer consisting of several types of phospholipids, cholesterol, glycolipids, and proteins, the copolymer micelles are good carrier systems for biomimetic the cell membrane [54]. Different micellar microdomains, called hydrophobic and hydrophilic, allow for estimating the drug partition tendency according to the region where it was solubilized. Compared to membrane models, polymeric micelles have the following advantages: minimal toxicity, narrow size distribution, longer residence time in the circulatory system, improved bioavailability, and more excellent stability of the incorporated photosensitizer [54].

5. Polymeric nanoparticles: copolymeric micellar systems in drug formulation

Most dyes, including phenazines, are characterized by forming aggregates in an aqueous solution harming their application. For this case, using DDS systems such as copolymeric micelles contributes to stabilization and solubilization, which maintains the PS characteristics inherent in PDT application [55]. In addition, these compounds are readily adsorbed by anionic micelles due to their distinct and amphiphilic character [56]. Therefore, studying the interaction of dyes with micellar systems is essential for biological applications since PS-associated biopolymers provide high drug efficacy with reduced toxicity [57].

Polymeric nanoparticles have been extensively applied in the pharmaceutical industry for drug formulation [58–60]. Currently, research is directed toward studying the association of photoactive molecules with nanostructured systems aiming for a specific carrier for controlled release in the regions (tissues and organs) to be treated [58]. Colloidal copolymers stand out, forming micelles (triblock systems) and effectively stabilizing hydrophobic molecules in aqueous media, such as PS, that remain in the form of monomers [16].

Polymeric colloidal systems are excellent for drug delivery and have been widely explained in several studies [48, 53]. These carrier systems can be classified as anionic, cationic, zwitterionic, and nonionic (according to their state of charge) [6]. Micelles are amphiphilic structures composed of a hydrophobic and a hydrophilic region (**Figure 5A**) [52]. In the aqueous phase, the micelles keep the hydrophobic portion facing the inside of the structure (lipophilic core) [61].

Micelles simulate the interface of a biomembrane and provide a biomimetic environment for the study of specific interactions, as well as the penetration and location of PS in the intracellular domain [4, 5]. Furthermore, studies have shown that PS
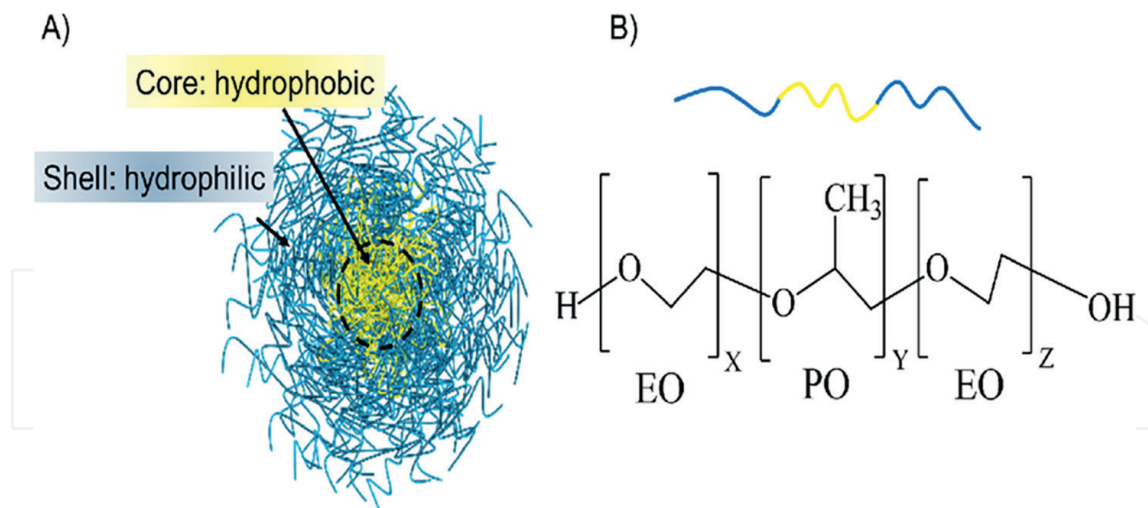
Figure 5.
(A) General monomer structure of polymeric micelle, and (B) general structure of the copolymeric monomer (Pluronic® class).

incorporation into micellar systems can reduce toxicity, target PS to specific sites and improve permeation and bioavailability in topical applications [11, 41]. Given these particularities, the biotransport and delivery of the drug in the target tissue become a particular challenge in PDT [4].

Several studies point to the use of surfactants of the Pluronic® class (or Poloxamers) [62]. The copolymer unimers (**Figure 5B**) consist of triblock molecules of repeating units of oxyethylene (EO – hydrophilic) and oxypropylene (PO – hydrophobic) with the following configuration: $(EO)_X(PO)_Y(EO)_X$ [63]. This group of copolymers is non-toxic, biocompatible, and has binding sites suitable for the solubilization of hydrophobic drugs [63]. In addition, triblock copolymers have a low critical micellar concentration (CMC, $10^{-6} – 10^{-7}$ mol L^{-1}) and the micelles formed to have high thermodynamic and kinetic stability, which guarantees a slow destructuring for unimers when they are exposed to an environment where the concentration is below the CMC [64, 65]. Another factor to be considered for studies with polymeric surfactants is the temperature. The micellization process will only occur above a specific critical micellar temperature (CMT), which, in turn, is a function of the surfactant concentration [64, 65]. In addition, there is evidence that temperature increases also cause an increase in the aggregation number (N_{ag}) of the copolymers [64]. Therefore, to be effective, the polymer used to obtain the micelle must present CMT compatible with the conditions necessary for the application [65].

Due to their favorable properties, the triblock copolymers of the Pluronic® class are potentially useful for drug delivery and controlled release systems [4, 53]. Furthermore, compared to other membrane models, copolymeric micelles have the following advantages: minimal toxicity, narrow size distribution, longer residence time at the application site, improved bioavailability, and more excellent stability of the incorporated PS [54]. These characteristics allow us to describe the micellar copolymer system as promising for clinical applications [66, 67].

6. Phenazines compound as photosensitizers or photoactive drugs

Phenazines ($C_{12}H_8N_2$) are heterocyclic aromatic nitrogenous compounds and the most important backbone is a pyrazine ring (1,4-diazobenzene) with two annulated benzenes (**Figure 6**) [68].
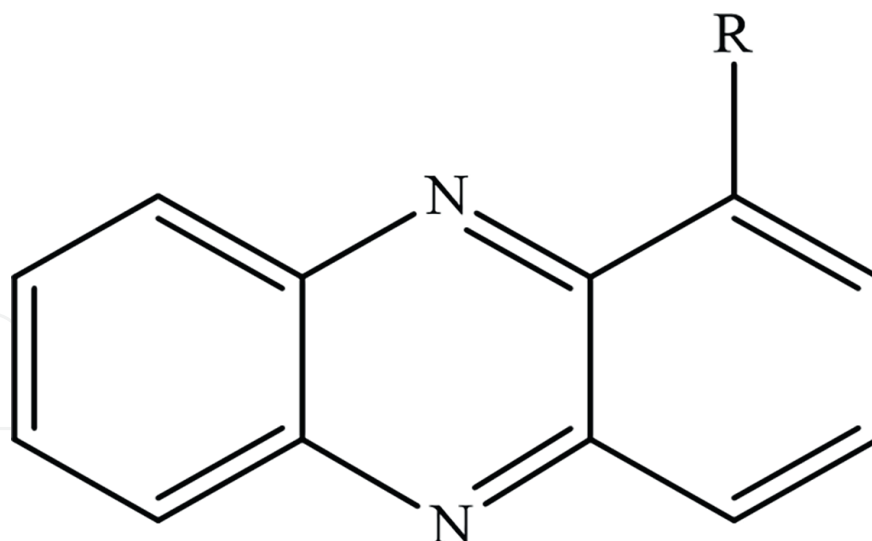
Figure 6.
General chemical structure of phenazines compounds.

This compound class is classified as redox-active secondary metabolites and pigmented. They are produced by fluorescent *Pseudomonas* species and have antimicrobial, antiparasitic, neuroprotective, insecticidal, anti-inflammatory, and anticancer activity [69].

Phenazines can undergo redox cycling in the presence of various reducing agents and molecular oxygen, which leads to the accumulation of superoxide (O_2^-) and hydrogen peroxide (H_2O_2), causing oxidative cell injury or death [68]. This property can be better explored considering the photochemical/photophysical potential of phenazines in PDT applications.

Natural phenazines are isolated from marine and terrestrial microorganisms, including (*Pseudomonas ssp.*, *Streptomyces ssp.*, and *Actinomycete ssp.*), which generates an infinity of synthetic derivatives [69]. *Pseudomonas aeruginosa* is a Gram-negative bacterium most studied for its ability to produce phenazine-active pigments, such as pyocyanin, phenazine-1 carboxyamide, and pyorubrins [68]. Furthermore, *P. aeruginosa* can survive in varied environments such as soil and water and colonize plant and animal tissues [70].

The literature describes the antioxidant, anti-inflammatory, and non-cytotoxic properties of the red and yellow phenazynic pigments produced by *P. aeruginosa*. These pigments confer great potential for application in the pharmaceutical and cosmetic industry [71, 72]. Phenazine derivatives differ in their chemical and physical properties based on the type of functional group position present, and they can also be used against the proliferation of various cancer cell lines [73–76]. There are more than 100 different compounds of natural origin and over 6000 synthetic compounds. Many of these compounds have been investigated as potential anticancer agents and microorganism control [77]. Some *in vitro* studies using phenazine derivatives are shown in **Table 1**.

Most studies involving compounds of the phenazines class have been reported promisingly in the dark [23]; however, the photodynamic potential cannot be disregarded. The literature presents scattered articles concerning different studies of phenazines derivative compounds performed *in vitro*, but without continuity or emphasis on the photophysical potential of phenazines, as well as *in vitro* and *in vivo* studies. Further, this group of compounds has also been used to develop color-emitting materials [91] or fluorescent biosensors [92].

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Phenazines derivatives	Obtaining	Applications	Reference
2-chloroacetylamino-7(8)-nitrophenazine N5,N10-dioxide 2-amino-7(8)-(1,3-dioxol-2-yl)phenazine N5,N10-dioxide(2),2-chloroacetylamino-7(8)-(1,3-dioxol-2-yl)phenazine N5,N10-dioxide 2-amino-7(8)-methoxyphenazine N5,N10-dioxide	Cytotoxins of 2-amino-or2-hydroxy phenazine5,10-dioxide derivatives	*in vitro* antitumoral effect against Caco-2 cells	[78]
phenazine 5,10-dioxide derivatives	Derivative from N-oxides containing heterocycles	*in vitro* growth inhibitors of *T. cruzi*	[79]
phenazine-1-carboxamide	Obtained from the *Pseudomona aeruginosa*	antibacterial activity of a *Pseudomonas aeruginosa*-derived compound against methicillin-resistant *S. aureus* (MRSA) strains	[80]
phenazin-1-ol phenazine-1-carboxylic acid 2-heptyl-3-hydroxyl-4(1H)-quinolone phenazine-1-carboxamide	Producing from *Pseudomona aeruginosa* fluorescent	Medically important fungi: *Aspergillus flavus* MTCC 183, *Candida albicans* MTCC 277, *Candida tropicalis* MTCC 184, *Cryptococcus gastricus* MTCC 1715, and *Trichophyton rubrum* MTCC 296. Agriculturally important fungi: *Fusarium oxysporum* MTCC 284, *Rhizoctonia solani* MTCC 4634, and *Penicillium expansum* MTCC 2006	[81]
phenazine 1-carboxamide	Produced by *Pseudomonas* strain MCC2142	fungi such as *Candida albicans*, *Candida glabrata*, *Cryptococcus neoformans*, *Fusarium oxysporum*, *Aspergillus fumigatus*, *Aspergillus niger* and *Benjaminiella poitrasii*	[82]
Pontemazines A and B	Isolated from the culture broth of *Streptomyces sp.* UT1123	neuronal cell protective effect on glutamate-induced mouse hippocampal HT-22 cell damage	[83]
A series of 2,3,7-trisubstituted phenazines	Introduction of carboxylic or carboxamide group in 2,3-dialkoxy-phenazine	*in vitro* on human pancreatic (MiaPaCa-2) cell lines	[74]
imidazo[4,5-b]phenazine-2-thione methylthio ethyl 1-aryl-3H-[1, 2, 4]triazolo[2,3-a]imidazo[4,5-b] phenazines ethyl (2Z)-3-aminophenazin-2-yl)amino] (phenylhydrazono) ethanoate pyrazino[2,3-b]phenazine [1, 4]diazepino[2,3-b]phenazine 2,3-dibenzoylaminophenazine 1H-Imidazo[4,5-b]phenazine 4-[(E)-(3-amino phenazin-2-yl)diazenyl]	2,3-Diaminophenazine was used as a precursor	all compounds were tested as inhibitors of the proliferation of human lung carcinoma and colorectal cancer cell lines through inhibition of Tyrosine Kinases	[73]

Phenazines derivatives	Obtaining	Applications	Reference
benzo[a]phenazin derivatives	Treatment of 2-fluoro-nitrobenzene or 2-chloro-3-nitropyridine	four human cancer cell lines (HL-60, K-562, HeLa, and A549)	[84]
16-(4-ethoxyphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 16-(4-Ethoxyphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 16-(2,5-Dimethylphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 16-(Benzo[d][1, 3]dioxol-5-yl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 3,3-Dimethyl-16-(o-tolyl)-2,3,4,16-tetrahydro-1H-benzo-[a]chromeno[2,3-c]phenazin-1-one 16-(3-Bromophenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 16-(2-Bromophenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 16-(3-Hydroxyphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-]phenazin-1-one 16-(3-Methoxyphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-]phenazin-1-one 4-(3,3-Dimethyl-1-oxo-2,3,4,16-tetrahydro-1H-benzo-[a]chromeno[2,3-c]phenazin-16-yl)benzonitrile 16-(4-Fluorophenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 16-(2-Methoxyphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-]phenazin-1-one 16-(1H-Indol-3-yl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 3,3-Dimethyl-16-(3,4,5-trimethoxyphenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 3,3-Dimethyl-16-(thiophen-2-yl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 3,3-Dimethyl-16-(3-nitrophenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 16-(3-Fluorophenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 3,3-Dimethyl-16-(4-nitrophenyl)-2,3,4,16-tetrahydro-1Hbenzo[a]chromeno[2,3-c]phenazin-1-one 16-(4-(Dimethylamino)phenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one 16-(4-Ethoxyphenyl)-2,3,4,16-tetrahydro-1H-benzo[a]-chromeno[2,3-c]phenazin-1-one16-(4-Isopropylphenyl)-2,3,4,16-tetrahydro-1H-benzo-[a]chromeno[2,3-c]phenazin-1-one 16-(2-Chlorophenyl)-2,3,4,16-tetrahydro-1H-benzo[a]-chromeno[2,3-c]phenazin-1-one 16-(2-Methoxyphenyl)-2,3,4,16-tetrahydro-1H-benzo-[a]chromeno[2,3-c]phenazin-1-one 16-(3-Methoxyphenyl)-2,3,4,16-tetrahydro-1H-benzo-[a]chromeno[2,3-c]phenazin-1-one 16-(3-Chlorophenyl)-2,3,4,16-tetrahydro-1H-benzo-[a]chromeno[2,3-c]phenazin-1-one 16-(3-Fluorophenyl)-2,3,4,16-tetrahydro-1H-benzo-[a]chromeno[2,3-c]phenazin-1-one	Synthesis by condensation reaction of benzo[a]phenazine-5-ol	antioxidant and anticancer activities against HeLa and SK-BR-3 cell lines	[75]
Phenazine-1-carboxamide	Isolated from *Pseudomonas sp.* strain PUP6	cytotoxic activity against lung (A549) and breast (MDA-MB-231) cancer cell lines	[85]

Phenazines derivatives	Obtaining	Applications	Reference
phenazine-1,6-dicarboxylic acid phenazine-1-carboxylic acid	Produced by *Lactococcus* BSN307 strain	antifungal activity against *Aspergillus niger*, *Penicillium chrysogenum* as well as *Fusarium oxysporum* cytotoxicity against cancer cell lines HeLa and MCF-7 and normal H9c2 cells	[86]
phenazine-1-carboxamide	Isolated from the bacterium *Pantoea agglomerans* naturally present in soil	cytotoxicity on the cancer cell lines A549, HeLa, and SW480	[87]
Pyrano[3,2-a]phenazine derivatives: 3-amino-10-((1-aryl-1H-1,2,3-triazol-5-yl) methyl)-20-oxospiro[benzo[a] pyrano[2,3-] phenazine-1,30-indoline]-2-carbonitrile	Synthesized from 2-amino-3-hydroxyphenazine	cytotoxicity against HCT116, MCF7, HepG2 and A549 cancer cell lines *in vitro*	[76]
Riminophenazine	Synthesized from clofazimine and TMP-phenazines	*in vitro* antiplasmodial, cytotoxic, and oxidative activities against HeLa cells	[88]
N-((tert-Butyldimethylsilyl) oxy)-9-chlorophenazine-1- carboxamide 9-Chloro-N-hydroxyphenazine-1-carboxamide 9-Chlorophenazine-1-carboxamide 9-Chloro-N-cyanophenazine-1-carboxamide 9-Chloro-N-(1H-tetrazol-5-yl) phenazine-1-carboxamide 9-Chlorophenazine-1-carbonitrile 2-((2,5-Dichlorophenyl)amino)-3-nitrobenzoic acid 6,9-Dichlorophenazine-1-carboxylic acid Methyl 6,9-dichlorophenazine-1-carboxylate 6,9-Dichlorophenazine-1-carboxamide 6,9-Dichloro-N-(methylsulfonyl) phenazine-1-carboxamide 9-Bromo-6-methoxyphenazine-1-carboxylic acid 7-Bromo-9-methoxyphenazine-1-carboxylic acid 6,9-Dimethoxyphenazine-1-carboxylic acid 9-Bromo-6-methoxy-N-(methylsulfonyl) phenazine-1-carboxamide 9-Bromo-N-cyano-6-methoxyphenazine-1-carboxamide Methyl 7-bromo-9-methoxyphenazine-1-carboxylate 9-Bromo-7-methoxyphenazine-1-carboxylic acid (25) and 7-methoxyphenazine-1-carboxylic acid Methyl 9-bromo-7-methoxyphenazine-1-carboxylate Methyl 7-methoxyphenazine-1-carboxylate N-(Methylsulfonyl)phenazine-1-carboxamide N-Methyl-N-(methylsulfonyl) phenazine-1-carboxamide N-Cyano-9-fluorophenazine-1-carboxamide 9-Fluoro-N-(methylsulfonyl) phenazine-1-carboxamide 9-Methyl-N-(methylsulfonyl)phenazine-1-carboxamide Methyl 9-methoxyphenazine-1-carboxylate 9-Chloro-N-methyl-N-(methylsulfonyl) phenazine-1-carboxamide	Chloro-substituted phenazines containing acid bioisosteres	*in vitro* antimicrobial activity against Gram-positive (methicillin-resistant *Staphylococcus aureus*, MRSA) and Gram-negative (*Escherichia coli*) bacteria	[89]

Phenazines derivatives	Obtaining	Applications	Reference
phenazine analogue (CPUL1)	Synthesized from 2-amino-3-hydroxyphenazine	antitumor activities in initial stage of Hep G2 cells	[90]

Table 1.
Studies of phenazines derivatives compounds.

Udumula *et al*. [93] described the synthesis and biological evaluation of two phenazines natural products and a series of phenazines that show promising activities against methicillin-resistant *Staphylococcus aureus* associated with the CA-MRSA community with low minimum inhibitory concentration (MIC) values in the micromolar range [93]. The most active compound also showed good IC_{50} values against Human Keratinocyte Cell (HaCat) [93]. In work proposed by Krishnaiah *et al*. [89], the authors synthesized a series of phenazines, and the *in vitro* antimicrobial activity was evaluated against Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus*, MRSA) and Gram-negative bacteria (*Escherichia coli*) [89]. These studies have indicated that the molecules do not disrupt bacterial membranes, and activity is not directly linked to the reactive oxygen species generation [89]. Therefore, despite a large number of studies on this compound class, this review highlights the great photodynamic potential of compounds of this class that can be used in the control of microorganisms and cancer treatment [94].

Neutral Red (NR) cationic dyes have great potential as photosensitizers. The exciting characteristic of NR as a PS is its water solubility compared with the majority of photosensitive and absorption bands located in the range of 550–700 nm [22]. Concerning with few phenazines studies as photodrugs explored in the literature (**Table 2**), for instance, the NR phenazines compound, when incorporated into Gold Nanoparticles, contributed to the *in vitro* tumor cell lines reduction [22], or when in aqueous medium significantly reduced *in vitro S. aureus* colonies in photoinactivation assays [98, 103].

Phenosafranin (PhS), another phenazines class compound, has the potential for use as a PS in PDT. It is well known that PhS absorbs strongly at 503–530 nm. Additionally, this dye presents a good photodynamic effect, namely low toxicity in the dark and higher toxicity under optical excitation [23]. PhS was incorporated into single-wall carbon nanotubes and showed satisfactory photodynamic effects against the BHK-21 cell line [42]. Furthermore, when encapsulated in liposomes (DMPC), PhS exhibited excellent *in vitro* activity against Human Cervical Carcinoma (HeLa) [23]. Another application for PhS found in the literature is *in vitro* photodynamic inactivation of *S. aureus* and *E. coli*. The bacterial cells were eradicated by ROS produced upon irradiation [102].

Sf, phenazine compound can also be used as a sensitizer in electron transfer reactions in a homogeneous medium [104–106], semiconductors [106], polymeric medium [104] and as probes in the reverse micellar system [107, 108] due to its absorbs band in 500–550 nm [56, 109]. In addition, the antimicrobial photodynamic ability of Sf is exploited for the inactivation of microorganisms, such as *Staphylococcus aureus* and *Escherichia coli* [24, 43], *Shigella flexneri*, *Bacillus subtilis* [24], periodontopathogenic bacteria (biofilms in periodontal treatment) [97], and mitochondrial oxidation [95].

Among the compounds of this class, the most cited for PDT applications is Sf, which presents some photophysical studies with properties already defined. Sf has properties that allow its use as a PS in PDT, such as considerable singlet oxygen quantum yield ($\Phi_\Delta {}^1O_2$) [33], an amphiphilic character that confers more significant interaction with biological substrates, does not present toxicity to healthy cells and generates reactive oxygen species (ROS), which inactivate microorganisms [101].

Although Sf presents several favorable properties to be applied in PDT, it has been poorly explored for this purpose. For example, spectroscopic studies on the interaction of Sf in liposomes L-egg lecithin phosphatidylcholine (PC) showed interaction. Still, the photodynamic effects of this formulation were not explored [57].

Sf incorporated into a silica matrix as a heterogeneous delivery system indicated that Sf has the potential for oxidation with singlet oxygen production [110]. Recent studies report Sf is incorporated into copolymer micelles [101]. This formulation ensured the Sf monomerization and preserved its physicochemical and photodynamic properties [33]. The use of triblock copolymers in the Sf incorporation guaranteed better results in PDT applications when compared to aqueous solutions [33].

Some authors evaluated the bactericidal effect of a serum combined with the action of Sf [24]. The authors did not use carrier systems, considering only the implications observed for the PS, not taking into account the self-aggregation processes characteristic of a PS in an aqueous medium. The photoefficacy of Sf in aqueous media was evaluated as an acaricide against female *Hyalomma dromedarii* ticks using *in vitro* immersion bioassays [99].

Similarly, Sasnauskiene *et al*. [95] evaluated the stimulated production of ROS by Sf in the inner space of mitochondria. The authors do not consider the self-aggregating effects resulting from the gradual release of PS in an aqueous medium [95].

Li *et al*. [96] explored the damage of bovine serum albumin (BSA) caused by Sf under ultrasonic irradiation [96]. The authors used Sf in an aqueous medium, a condition in which Sf tends to form self-aggregates that drastically reduce the $^1O_2$ formation. Therefore, we propose the use of low-cost biocarrier systems, Pluronic® class. This triblock copolymer was used to solubilize Sf and maintain its photophysical properties [33]. Such attributes favored the prevention and treatment of mastitis via PDT [33, 100, 101].

Table 2 presents some photochemotherapeutic antimicrobial studies of phenazines found in the literature.

Compound	Delivery system	Application	Illumination condition	Effect	Reference
Sf	Aqueous solution (1 mMdm^{-3})	Bactericidal effect of serum combined with the action of PS	White non mutagenic light (5Wcm^{-2})/90 min	The strains are sensitive to the photodynamic action	[24]
Sf	Aqueous solution (in PBS 0.7 μg mL^{-1})	Stimulated production of ROS in the inner space of mitochondria	LED at λ = 509 ± 5 nm (29 Wm^{-2})/0.5–15min	Damage induced apoptosis	[95]
PhS	Single-wall carbon nanotubes modified	BHK-21 cell line (from mouse fibroblasts)	Visible light irradiation (Hg lamp)	Low toxicity in the dark and higher toxicity in the presence of light	[42]
Sf	Bovine Serum Albumin pH 7.4 (in Tris–HCl-NaOH buffer solution)	Study of the damage caused by ROS in bovine serum albumin	Controllable Serial-Ultrasonic apparatus (frequency 59 kHz and power 50 W	Damage of Bovine Serum Albumin under ultrasonic irradiation in the presence of Sf.	[96]
Sf	Aqueous solution (PBS containing 7% ethanol)	Oral-pathogenic species (Gram-positive and Gram-negative)	Laser Light (20 J cm^{-2})	Significant antibacterial impact on different oral pathogenic species	[97]

Compound	Delivery system	Application	Illumination condition	Effect	Reference
Sf	Gold nanoparticles incorporated into a copolymer emulsion	*Staphylococcus aureus* and *Escherichia coli*,	28 W white light source	The bacterial count reduced (>4 ± 0.3 log kill)	[43]
NR Monob rominated NR	Aqueous solution (in PBS pH 7.4)	*Staphylococcus aureus*	Light dose 7.6–30.2 J cm^{-2}	Photoantimicrobial effect (>3 log$_{10}$) killing	[98]
Sf	Aqueous solution	*Hyalomma dromedarii*	Spot white-light source (power 100 W)	Reduction of ovipositing, eggs per female, tickets laying viable eggs and hatched eggs	[99]
NR	Gold Nanoparticles and Sodium thioglycolate	NIH-3 T3 fibroblast (noncancerous) and 4 T1 tumor cell lines	Twin Flex Laser LED MM optics λ = 440 nm; 220 mW	Reduction of cell viability	[22]
PhS-Chlorambucil conjugate	Encapsulated liposomes (DMPC)	HeLa (human, cervical carcinoma)		Excellent cell contrast facilitating its use as a theranostic anti-cancer drug	[23]
Sf	Pluronic® F127 and P123 4% (w/V)	*In vitro*: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae*, *Corynebacterium bovis* *In vivo*: prevent mastitis in Dutch dairy cows.	*In vitro* studies: Green LED λ = 520 nm (7.2 mW cm^{-2}) *In vivo* studies: Green LED λ = 520 nm (12.7 mW cm^{-2})	*In vitro* studies of the Sf-F127 and Sf-P123 systems proved to be efficient in inactivating the bacteria that cause bovine mastitis. *In vivo* studies prevent bovine mastitis	[33]
NR Mono brominated NR	Aqueous solution (in PBS pH 7.4)	*Staphylococcus aureus*	Parathom lamp (OSRAM-5 W) 8.4 mW cm^{-2} (15–30 min)	2–3 log of killing	[98]
Sf	Stimuli-responsive hydrogel by F127 Pluronic® and Carbopol (C934P)	Mastitis treatment	*In vitro* studies: Green LED λ = 520 nm (7.2 mW cm^{-2}) *In vivo* studies: Green LED λ = 520 (12.7 mW cm^{-2})	*In vitro*: efficiency in the inactivation of pathogens that cause mastitis. I*n vivo*: Sf is highly efficient for mastitis treatment.	[100]
Sf	Pluronic® F127 4% (w/V)	*In vitro*: *Staphylococcus aureus*, *Escherichia coli*	Green LED λ = 520 (7.2 mWcm^{-2})	The bacteria were sensitive to the photodynamic action of Sf	[101]
PhS polyhedral oligomeric silsesquioxane	Aqueous solution (in PBS pH 7.4)	*Staphylococcus aureus* and *Escherichia coli*	LED-based light source λ = 522 nm (10.6 mW cm^{-2})	Bacterial cells were eradicated by ROS produced upon irradiation	[102]

Table 2.
Studies of phenazine class compounds in photodynamic applications.

Table 2 shows a few amounts of work on this class of compounds and the way performed majority *in vitro*, which the scientific community can still better explore for applications in PDT. Combining the biocompatibility of the copolymer micelles and the non-toxicity of phenazines compounds, the continuity of pre-clinical studies for developing new photoactive-based phenazine formulations has been motivated for applications in different species of animals. These formulations can treat a wide range of diseases associated with different types of pathogenic microorganisms such as bacteria, fungi, viruses, and parasites. In this way, recent research has still been developed for the first time *in vivo*. It is in the submission phase of (unpublished) promising results concerning the prevention and treatment of mastitis. This review seeks to address the lack of literature on the approach of phenazines as a potential PS for PDT application. It is hoped that this work could offer some valuable information in developing new types of DDS systems.

7. Conclusion

Several products are used to control microorganisms and treat diseases like cancer; however, they present disadvantages such as cost and lack of effectiveness against resistant microorganisms. Photosensitization has recently gained attention benefiting from the use of a wide range of PS associated with a light source. The oxygen species produced by photoexcitation of a PS attack cancer cells or microorganisms non-selectively. Phenazine compounds showed promising phototoxicity, and their applicability has still been poorly investigated in the prevention and treatment of diseases caused by microorganisms and diseases related to abnormal cell growth, such as cancer. Furthermore, incorporating PS into polymeric micelles produced efficient, biocompatible formulations with better stability than aqueous systems.

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

PDT	Photodynamic Therapy
aPDT	antimicrobial Photodynamic Therapy
PS	photosensitizing compounds
ROS	reactive oxygen species
$^3O_2$	molecular oxygen
$^1O_2$	singlet oxygen
O_2^-	superoxide
H_2O_2	hydrogen peroxide
$\Phi_\Delta {}^1O_2$	singlet oxygen quantum yield
LDL	low-density proteins
FDA	Food and Drug Administration
DDS	drug delivery systems
IC	internal conversion
ISC	intersystem crossing

CMC	critical micellar concentration
CMT	critical micellar temperature
HOMO	Highest Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital

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