Chapter 3
Photosensitizers

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Abstract Photodynamic therapy (PDT) was discovered over 100 years ago when it was observed that certain dyes could kill microorganisms when exposed to light in the presence of oxygen. Since those early days, PDT has mainly been developed as a cancer therapy with regulatory approvals and clinical trials steadily accumulating for different types of cancer and different photosensitizer structures. A very important milestone for PDT was the introduction of 5-aminolevulinic acid (ALA), which functions as a prodrug to induce endogenous porphyrin biosynthesis that acts as an endogenous photosensitizer produced by our cells. PDT with ALA and its derivatives have become mainstays of the clinical dermatologist's practice covering everything from skin cancer, premalignant lesions, acne, and skin rejuvenation. Another milestone in PDT development was the realization that PDT may also be used as an effective antimicrobial modality and a potential treatment for localized infections. To some extent, this means that PDT has gone full circle and returned to its roots from when it was first discovered in 1900. In this chapter we discuss, in a contextualized fashion, what are the expected characteristics of an ideal photosensitizer and

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which are the main molecular frameworks used for development of synthetic, natural, and nanostructured photosensitizers.

3.1 Introduction

Photodynamic therapy (PDT) was discovered over 100 years ago when it was observed that certain dyes could kill microorganisms when exposed to light in the presence of oxygen (see Chap. 1). Since those early days, PDT has mainly been developed as a cancer therapy with regulatory approvals and clinical trials steadily accumulating for different types of cancer and different photosensitizer structures, starting with Photofrin® [1]. A very important milestone for PDT was the introduction of 5-aminolevulinic acid (ALA), which functions as a prodrug to induce endogenous porphyrin biosynthesis [2]. See Fig. 3.1 for a depiction of how ALA can induce accumulation of protoporphyrin IX (PpIX) in tissue to which it has been topically applied.

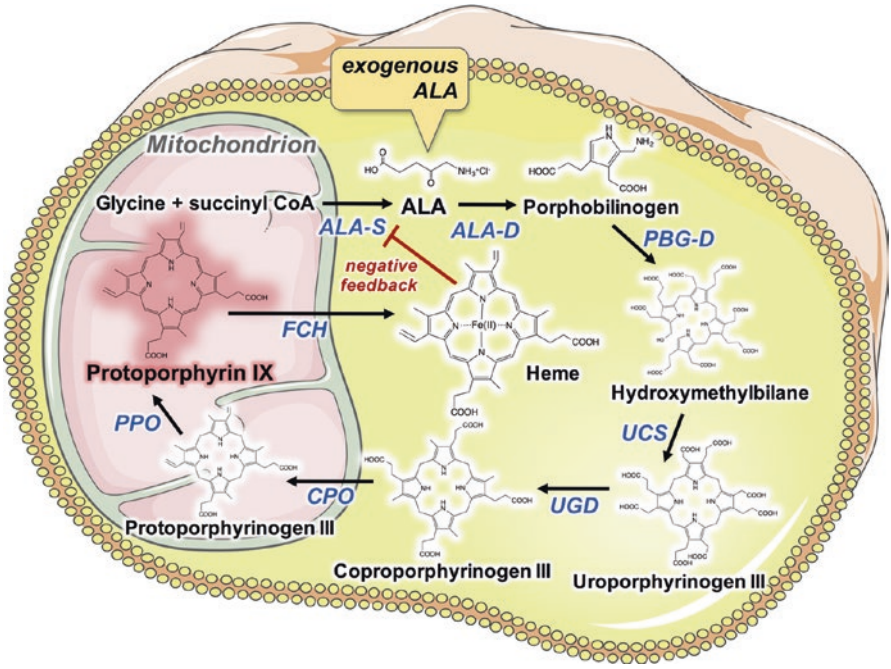


Fig. 3.1 Heme biosynthesis cycle and role of ALA-induced PPIX. *ALA-S* ALA synthase, *ALA-D* ALA dehydratase, *PBG-D* porphobilinogen deaminase, *UCS* uroporphyrinogen synthase, *UGD* uroporphyrinogen decarboxylase, *CPO* coproporphyrinogen oxidase, *PPO* protoporphyrinogen oxidase, *FCH* ferrochelatase [49]

ALA-PDT and PDT using ALA esters have become mainstays of the clinical dermatologist's practice covering everything from skin cancer, premalignant lesions, acne, and skin rejuvenation. A second major milestone in the development of PDT was its introduction as a clinical treatment for choroidal neovascularization secondary to age-related macular degeneration. The PS called Visudyne (benzoporphyrin derivative) was injected IV, and soon afterward red light was shone into the back of the eye as a way to destroy proliferating blood vessels in the retina [3]. The third milestone in PDT development was the realization that PDT may also be used as an effective antimicrobial modality and a potential treatment for localized infections [4]. To some extent, this means that PDT has gone full circle and returned to its roots from when it was first discovered in 1900. The antimicrobial applications have been enthusiastically embraced by dentists, who treat periodontitis, periimplantitis, and endodontics [5]. The fourth milestone in PDT development (which unfortunately has not been widely accepted as yet) is the realization that PDT can stimulate an adaptive and/or innate immune response against both tumors [6] and also against pathogens [7].

3.2 Important Features of Photosensitizers

The characteristics of the ideal PS can be summarized as follows. PS should have low levels of dark toxicity to both humans and animals and a low likelihood of adverse pharmacological effects upon administration such as hypotension (decreased blood pressure) or allergic reactions. PS should absorb light in the red or far-red wavelength regions in order for the tissue-damaging effect to reach as deep as possible. It is known that both absorbance and scattering of light are minimized at longer wavelengths that penetrate the tissue deeper. Absorption bands at shorter wavelengths have less tissue penetration and are more likely to lead to skin photosensitivity (the power in sunlight drops off at $\lambda > 600$ nm). Absorption bands at too high wavelengths (>800 nm) mean that the photons will not have sufficient energy to promote PS molecules to excited triplet state to allow energy transfer to the ground state oxygen molecule to excite it to the singlet state. They should have relatively high absorption bands (>20,000 $M^{-1}cm^{-1}$) to minimize the dose of PS and light needed to achieve the desired effect. Synthesis of the PS should be relatively easy and the starting materials readily available to make large-scale production feasible. The PS should be a pure compound with a constant composition and a stable shelf life and be ideally water soluble or soluble in a biocompatible drug delivery vehicle. It should not aggregate unduly in biological environments as this reduces its photochemical efficiency. The pharmacokinetic elimination from the patient should be rapid, i.e., less than 1 day to avoid the necessity for posttreatment protection from light exposure due to prolonged skin photosensitivity. A short interval between injection and illumination is desirable to facilitate outpatient treatment that is both patient-friendly and cost-effective. Pain on treatment is undesirable, as PDT procedures do not usually require anesthesia or heavy sedation.

Although high PDT activity is generally thought to be a good thing, it is possible to have excessively powerful PS that is sometimes considered to be "unforgiving." With limitations in the effectiveness of both PS and light dosimetry, highly active PS may easily permit treatment overdosage when the surrounding normal tissue is damaged as well as the target tumor forming extensive necrotic areas. It is at present uncertain whether it is better to have a PS "tailored" to a specific indication, and to have families or portfolios of PS designed for various specific diseases or patient types, or alternatively to seek a single PS that works against most diseases. For cancer treatment it has been thought that an ideal PS should selectively accumulate in tumors after intravenous injection. Although the exact mechanisms for this "tumor-localizing effect" are not completely understood, some PS can achieve a 5:1 or higher accumulation in tumors compared to the surrounding normal tissue. Lastly, a desirable feature might be to have an inbuilt method of monitoring PS dosimetry, localization, and following response to treatment by measuring fluorescence and its loss by photobleaching in real time.

Many of the early attempts to kill microorganisms with PDT employed the same photosensitizers that were used for PDT of cancer. However, it was later realized that these structures were not optimal. Because phenothiazinium dyes (such as methylene blue) that have an intrinsic cationic charge were able to photoinactivate many classes of microorganism, it was concluded that the presence of cationic charges was crucial for broad-spectrum antimicrobial effects [8]. Although neutral and anionic PS are able to kill Gram-positive bacteria, in order to kill Gram-negative bacteria, one needs positive charges on the PS to bind and/or penetrate through the outer membrane barrier composed with large amounts of negatively charged lipopolysaccharides.

3.3 Main PS Structures

3.3.1 Tetrapyrroles

The class of tetrapyrrole PS contains (by a considerable margin) the largest number of individual compounds that have been explored for PDT both in the laboratory and also approved and tested clinically. The four most commonly explored backbone structures are porphyrins, chlorins, bacteriochlorins, and phthalocyanines. Reduction of one double bond (green arrow) in a porphyrin leads to a red shift and increase in absorption intensity of the Q-bands (e.g., 600 nm → 660 nm) in the chlorin (name derived from green chlorophyll), while reduction of two double bonds (purple arrows) leads to a further red shift (660 nm → 780 nm) and a intensity increase in the peak of the bacteriochlorin (name derived from the pigment in purple photosynthetic bacteria). The more conjugated nature of the phthalocyanine macrocycle (four additional aromatic rings) leads to a very intense absorption band at 700 nm.

Hematoporphyrin derivative (which later became known as Photofrin® and Photogem®) was approved in 1990 [9] and remains today as the most widely used PS in clinical PDT. As mentioned above, the PpIX production is induced by exog-

enously administered ALA, a therapeutic strategy widely used by dermatologists [10]. A later pharmacologic strategy uses methylated ALA molecules (M-ALA) to facilitate deeper prodrug diffusion through the skin using topical administration. Other porphyrin derivatives such as hematoporphyrin monomethyl ether (Hemoporfin), 2,4-di(1-methoxyethyl)-deuteroporphyrin-IX (Dimegin), and sinoporphyrin sodium [11] have been tested for PDT of cancer and a wide range of cationic porphyrins for antimicrobial PDT.

Chlorins represent a class of PS containing several examples, which have advanced as far as clinical trials. In addition to mTHPC and BPD mentioned in Table 3.1, 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPH), mono-N-aspartyl chlorin (e6) (Npe6) or talaporfin sodium (LS11), photoditazine, and tin(IV) etiopurpurin (SnEt2) are all classified as chlorins. Bacteriochlorins are not as stable as porphyrins and chlorins, and their use has not been as widely studied despite their more advantageous absorption peaks in the infrared region. Besides soluble TOOKAD® (Table 3.1), another compound, LUZ11 (a non-metallated fluorinated bacteriochlorin sulfonamide), has been tested in clinical trials for cancer.

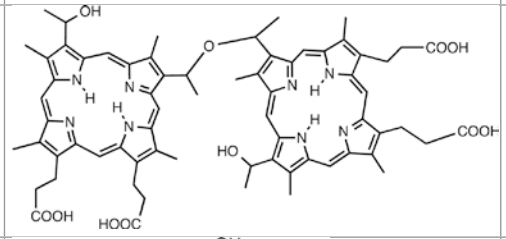
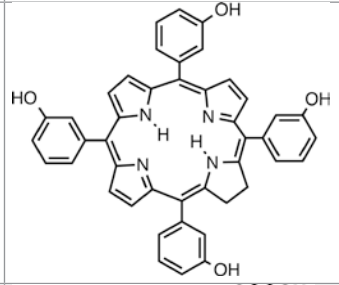
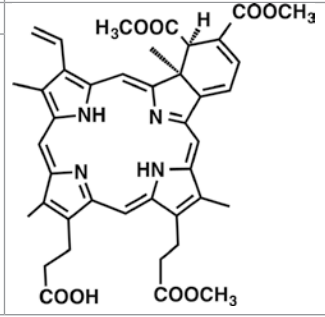
3.3.2 Synthetic Dyes

The dye industry developed a very large number of conjugated heterocyclic compounds with high absorption bands in the visible region of the spectrum (Table 3.2). The vast majority of these dyes were found (or later designed) to be photostable so that they could be used for dyeing fabrics and clothing. However, a few of these dyes that were not photostable but were found to have an appreciable quantum yield to the triplet state that meant they could be used as PS for PDT. There remain some concerns about these synthetic dye compounds on whether they may be darker toxic compared to the tetrapyrrole compounds, which are derived from natural molecules. Most dyes were designed to be water soluble (so clothing could be dyed) that makes them suitable for antimicrobial PDT applications. Moreover, many dyes are cationic and this again encourages their use against a broad spectrum of bacteria (including Gram-negative species). Interestingly, veterinarians already use triarylmethane dyes in some countries to treat local infections and decontaminate fish tank water due to their intrinsic microbicidal activity, even though most people disregard the great antimicrobial activity enhancement that can be provided by photodynamic reactions promoted by the same dyes. In recognition to this potential, we strongly recommend those users to expose the stained water or lesions to either sunlight or artificial light sources.

3.3.3 Other Structures

To illustrate the diversity of different structures that have been investigated as PS in PDT, we will describe some innovative chemical structures (Table 3.3) that have not (yet) progressed as far as those shown in Tables 3.1 and 3.2.

Table 3.1 Examples of porphyrins, chlorins, bacteriochlorins, and phthalocyanines most used against cancer

Class	Name	Structure	λmax (nm)	Ref
Porphyrin	Photofrin hematoporphyrin derivative (HpD)		630	[12]
Chlorin	Foscan m-tetrahydroxyphenylchlorin (mTHPC)		652	[13]
Chlorin	Visudyne or verteporfin Benzoporphyrin derivative (BPD)		690	[14]

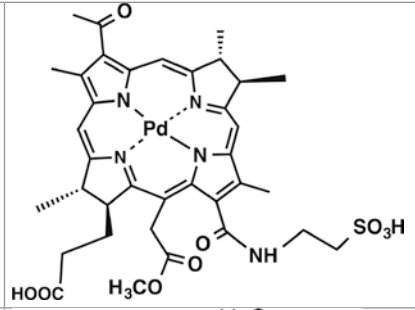
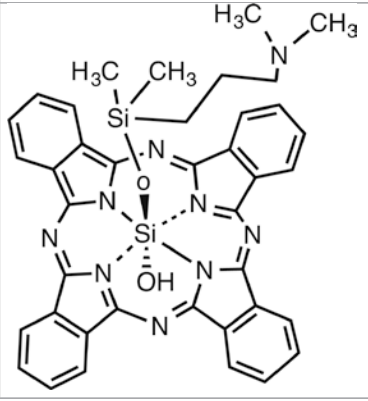
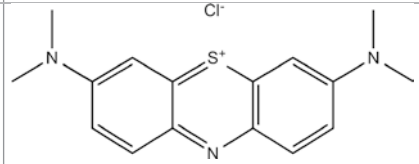
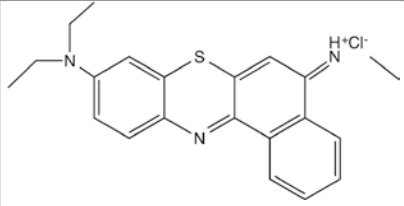
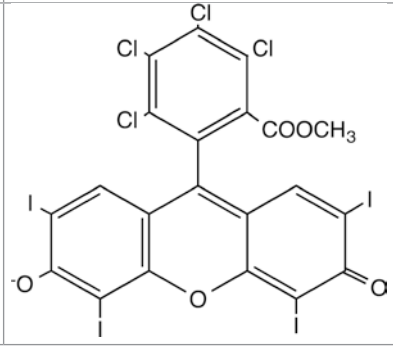
Bacteriochlorin	TOOKAD® (WST-11)		753	[15]
Phthalocyanine (Pc)	Silicon phthalocyanine (Pc4)		675	[16]

Table 3.2 Examples of synthetic dyes used as PS

Class	Name	Structure	λmax (nm)	Application	Ref
Phenothiazine	Methylene blue		660	Cancer, antimicrobial	[17]
Benzophenothiazine	EtNBS		670	Cancer, antimicrobial	[18]
Halogenated xanthene	Rose Bengal		540	Cancer, antimicrobial, tissue bonding	[19]

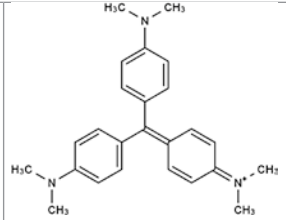
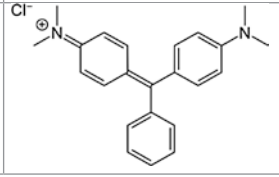
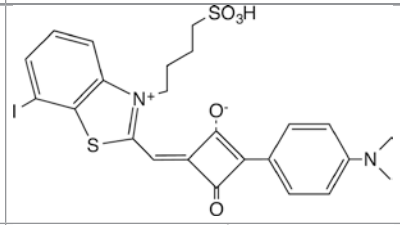
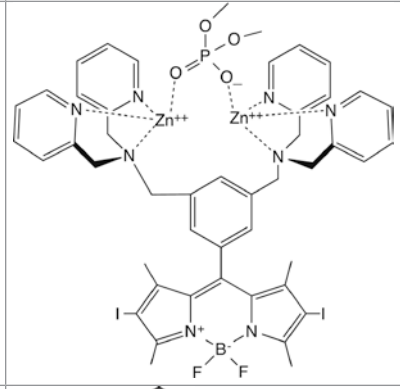
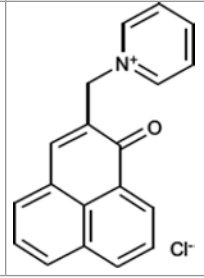
Triarylmethane	Crystal (or gentian) violet		590	Antimicrobial	[20]
Triarylmethane	Malachite green		615	Antimicrobial	[21]

Table 3.3 Examples of innovative structures used as PS

Class	Name	Structure	λmax (nm)	Ref
Squaraine	ASQI		610	[22]
BODIPY	Zinc(II)-dipicolylamine diiodo-BODIPY		540	[23]
Phenalenone	SAPYR		380 nm	[24]

3.3.4 PS Derived from Natural Products

In common with other areas of medicine, in PDT there is also a movement toward the use of natural products and away from synthetic drugs produced by "Big Pharma." The idea is that natural product-derived PS will have less overall toxicity and fewer side effects (Table 3.4). However, there may be a flaw in that argument in that since nearly all life-forms have evolved in sunlight, they will be unlikely to contain very powerful PS, as the consequent phototoxicity caused by sun exposure would have been selected against.

3.3.5 Inorganic Structures

Inorganic compounds have occasionally been reported to be able to carry out PDT. Two examples are given in Table 3.5. Titanium dioxide (TiO_2) presents great photostability and preferentially undergoes through type 1 photodynamic reactions (see Chap. 2). Because it is colorless but efficiently absorbs UV light, construction companies have been using TiO_2 to coat external walls of buildings forming a self-cleaning surface. As a surprising consequence, even the air quality has been reported to be better in the surroundings of TiO_2-coated buildings adding an environmental appeal for its large-scale application.

Transition metal complexes, such as $[Ru(bpy)_3]^{2+}$, are probably the most studied structures by photophysicists and photochemists over the past 40 years. Other metals such as cobalt and chromium are also often used for the same purposes. Due to spin-orbital coupling governed by the so-called heavy atom effect, intersystem crossing efficiency is extremely favored and can reach values above 99 %. This characteristic makes transition metal complexes particularly potent as PS since their singlet oxygen yield is in general the greatest possible.

3.3.6 Nanostructures

The use of nanotechnology in PDT can be broadly divided into different approaches, for example, when the nanoparticle (NP) is itself the PS (fullerenes, gold NP, silver NP, quantum dots), when the nanoparticle can absorb light to improve PDT efficiency (quantum dots, silver NP, gold NP, upconversion NP), and when the NP acts as a nano-drug delivery vehicle to improve solubility or targeting (dendrimers, liposomes, mesoporous silica NP) (see Chap. 14). Examples of these classes are shown in Table 3.6.

Table 3.4 Examples of natural product structures used as PS

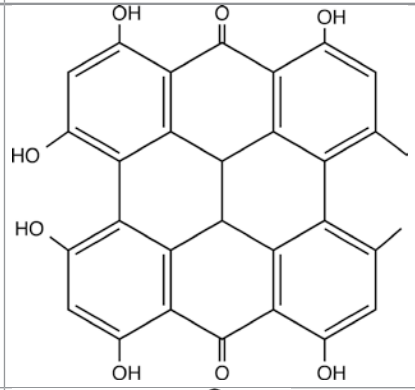
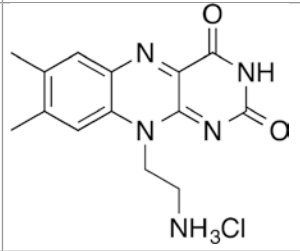
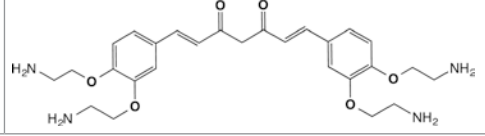
Class	Name	Structure	λmax (nm)	Application	Ref
Perylenequinone (St John's wort)	Hypericin		570	Cancer, antimicrobial	[25]
Flavin (vitamin B2)	Riboflavin		UVA or 440	Antimicrobial	[26]
Curcuminoid (turmeric spice)	SACUR-3		420	Antimicrobial	[27]

Table 3.5 Examples of inorganic structures used as PS

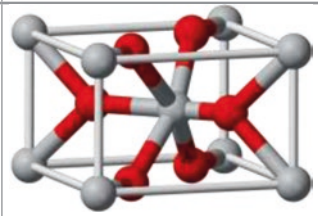
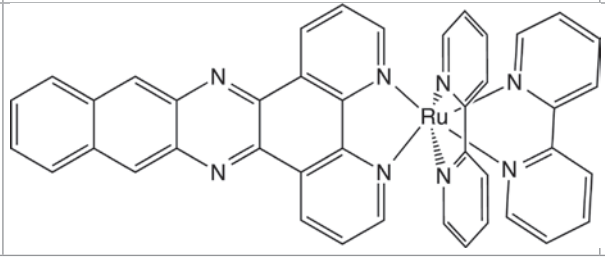
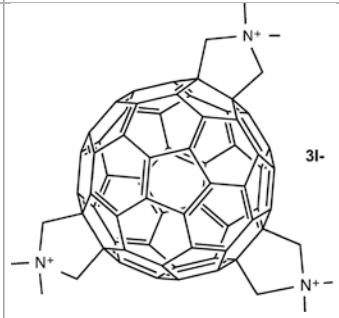
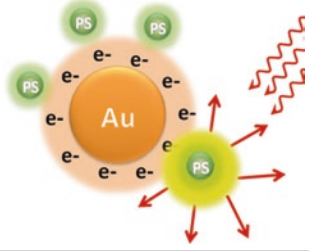
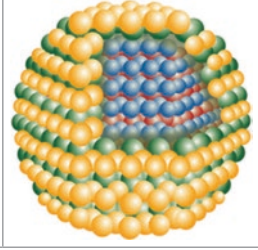
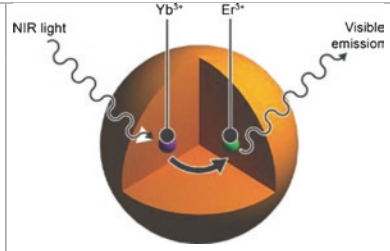
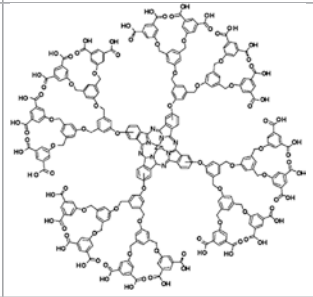
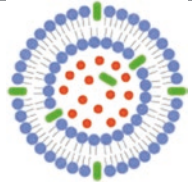
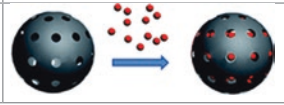
Class	Name	Structure	λmax (nm)	Application	Ref
Large band gap semiconductor	Titanium dioxide TiO_2		360	Antimicrobial, environmental	
Transition metal complex	Ruthenium bipyridyl Ru(bpy)		450	Antimicrobial, cancer	[28]

Table 3.6 Examples of nanostructures used in PDT

Class	PS	Structure	Goal	Application	Ref
Functionalized fullerenes	BB6		Photostable, type 1	Antimicrobial	
Gold or silver nanoparticles	Can increase activity of conventional PS		Plasmonic enhancement of PDT	Anticancer	[29, 30]
Quantum dots	Polymer QD + TPP carbon QD		Solubilize PS, FRET (downconversion), generate ROS	Anticancer, antimicrobial	[31, 32]

Upconversion nanoparticles	Rare-earth NP/ hypocrellin A		Excite with NIR light Emit blue	Cancer cells	[33]
Dendrimers	PAMAM-zinc phthalocyanine (ZnPc)		Prevent aggregation	Cancer atherosclerosis	[34, 35]
Liposomes	Foslip (liposomal mTHPC), Fospeg (PEGylated Foslip)		Solubilize and protect, reduce side effects	Cancer	[36]
Mesoporous silicon NP	PEI-PEG-MSN/ ZnPc		Solubilize and protect	Cancer	[37]

FRET Förster or fluorescence resonance energy transfer, *ROS* reactive oxygen species, *NIR* near infrared, *PAMAN* polyamidoamine, *PEI* polyethylenimine, *PEG* polyethylene glycol, *MSN* mesoporous silica nanoparticles

Table 3.7 Examples of targeted PS

Class	Ligand	Target	PS	Application	Ref
Monoclonal antibody	OC125	Ovarian cancer	Chlorin(e6)	Cancer	[39]
Peptide	Octreotide	Somatostatin receptor	Chlorin(e6)	Leukemia	[40]
Lipoprotein	Low-density lipoprotein (LDL)	LDL receptor	Tetra-t-butyl silicon phthalocyanine	Cancer, atherosclerosis	[41]
Serum protein	Modified albumin	Scavenger receptor	Chlorin(e6)	Atherosclerosis	[42]
Vitamin	Folic acid	Folate receptor	Pheophorbide a	Cancer	[43]
Steroid	Estradiol	Steroid receptor	Pheophorbide a	Breast cancer	[44]
Carbohydrate	Mannose	Mannose receptor	Meso-tetraphenylporphyrin	Cancer	[45]

3.3.7 PS Targeting Using Conjugates with Ligand-Receptor Recognition

Since the mechanism of preferential accumulation of various PS in tumors (or other pathological lesions) is not completely understood [38], it is rather challenging to design a PS with a high inherent ability for tumor targeting. However, there is a valid alternative approach to increase the specificity of PS for tumors. This relies on covalent conjugation of PS to various targeting ligands (either macromolecules or small molecules) that show specific molecular recognition ability with some cognate receptor that is over-expressed on tumor or other cells of interest. The advantage is that the PS can be chosen solely based on its photochemical ROS (reactive oxygen species) generation and ability to be conjugated, and the tumor-targeting component can be provided by the ligand. Examples of this ligand-receptor interaction are given in Table 3.7.

3.3.8 Nonlinear Excitation (Two-Photon, Upconversion, Second Harmonic, and Four-Wave Mixing)

The use of long wavelength light (600–1300 nm) provides deeper tissue penetration. Although some PS that absorb in the 700–800 nm range have been described, photons at wavelengths longer than 800 nm do not have enough energy to promote generation of singlet oxygen. There have been some innovative approaches developed to overcome this limitation. The most well-studied is two-photon absorption in which a very short-pulsed laser (ideally below 100 fs pulse duration) can deliver photons with such high peak power that the chances of two of them being absorbed

at the same time by the PS become non-negligible [46]. The next most studied is photon upconversion by rare-earth nanoparticles that change NIR laser (often 800 or 980 nm) to visible or ultraviolet light, providing deep tissue penetration allied to site-specific generation of short wavelength light [47]. Optical upconversion can also be accomplished by second-harmonic generation in collagen, and four-wave mixing approaches, including coherent anti-Stokes Raman scattering [48].

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