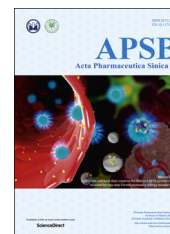Chinese Pharmaceutical Association
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REVIEW

An updated overview on the development of new photosensitizers for anticancer photodynamic therapy

CrossMark

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Abstract Photodynamic therapy (PDT), based on the photoactivation of photosensitizers (PSs), has become a well-studied therapy for cancer. Photofrin®, belonging to the first generation of PS, is still widely used for the treatment of different kinds of cancers; however, it has several drawbacks that significantly limit its general clinical use. Consequently, there has been extensive research on the design of PS molecules with optimized pharmaceutical properties, with aiming of overcoming the disadvantages of traditional PS, such as poor chemical purity, long half-life, excessive accumulation into the skin, and low attenuation coefficients. The rational design of novel PS with desirable properties has attracted considerable research in the pharmaceutical field. This review presents an overview on the classical photosensitizers and the most significant recent advances in the development of PS with regard to their potential application in oncology.

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1. Introduction

Cancer is among the leading causes of morbidity and mortality worldwide. In 2012, approximately 14 million cancer cases were newly diagnosed, and the number of cancer-related deaths was 8.2 million, which is projected to rise by about 70% over the next two decades[1]. Currently, clinical treatments for cancer include surgery, radiation therapy, chemotherapy and, more recently, immunotherapy and other small-molecule targeted therapies, along with a combination of these strategies[2]. However, these treatments present some important drawbacks. For instance, traditional chemotherapy, as it interferes in cell division, is often associated with severe systemic adverse effects, such as myelosuppression, mucositis, alopecia, and others[3]. Also, surgical resection of certain tumors cannot avoid a high recurrence rate[4], while the cumulative radiation dose extremely limits the radiotherapy[5]. Thus, although refinement of the conventional anticancer therapy is important, development of new treatment approaches that are safe, potent, and cost-effective seems especially urgent.

2. Photodynamic therapy

2.1. An accidental finding for cancer treatment

In the 1890s, Raab[6] accidentally found that the irradiation with visible light was lethal to paramecia previously exposed to acridine, and postulated that the transfer of light energy to the acridine red was the crucial event behind the cytotoxicity observed in paramecia and that this effect was related to the fluorescence of the dye[7]. Then the first clinical observation of PDT with oral eosin to treat epilepsy was reported by the neurologist Jean Prime in 1900[8]. Later, von Tappeiner and Jesionek[9] proposed the use of topical eosin with light exposure to treat skin tumors. This was the first published report on the use of PDT to treat tumors in human patients. Subsequently, von Tappeiner[10] observed that O_2 was an important component of the events found by Raab, and coined the term "photodynamic action".

The study of the anticancer potential of PDT was conducted by few researchers up to the 1950s when the interest in this field began to increase. The publication of some seminal reports on the use of porphyrins as both PSs and fluorescence diagnostic tools[11] in the 1950s and 1960s was followed by a series of works on the anticancer activity of PDT against different tumors[12]. Mainly over the last three decades, several types of PSs have been developed and applied in preclinical and clinical trials; some of these molecules reached the market and have shown to be effective against different kinds of cancers[13–15]. The main advantage of PDT over conventional anticancer therapies is the ability to limit toxic effects to the biological tissues exposed to both the PS and light, thus protecting normal tissues.

In addition, PDT has also been used successfully against non-malignant disorders in diverse fields, such as urology[16], immunology[17], ophthalmology[18] dentistry[19], dermatology[20] and others.

2.2. Photodynamic therapy mechanisms

PDT is based on the excitation of PS with light at specific wavelengths, culminating in type I and type II photochemical reactions[21]. As shown in Fig. 1, a PS can be activated from its ground state to a short-lived excited singlet state (PS_{Es}) by light. Then, either the excited PS may decay back to the ground state by
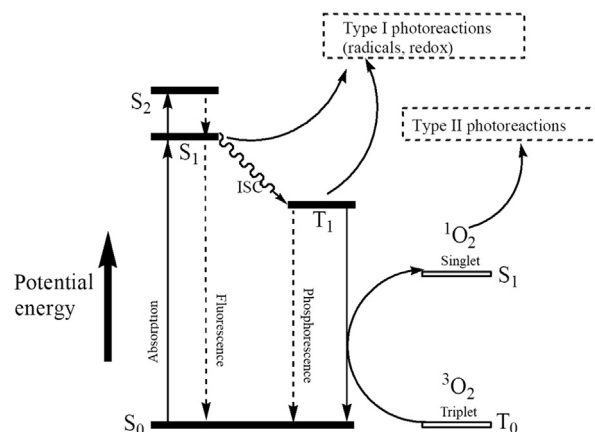
Figure 1 Jablonski diagram showing the main events leading to type I and type II photochemical reactions, which eventually may result in oxidative cell damage. S_0, ground state of the photosensitizer (PS); S_1, first excited singlet state of PS; S_2, second excited singlet state of PS; T_1, first excited triplet state of PS; ISC, intersystem crossing; $^3O_2$, triplet oxygen; $^1O_2$, singlet oxygen.

emitting fluorescence, or it can undergo intersystem crossing whereby the spin of its excited electron inverts to form a relatively long-lived triplet state (PS_{Et}). The triplet excited PS can also decay back to the ground state by emitting phosphorescence, but most importantly it can directly interact with surrounding substrates (e.g., cell membrane or other biomolecules) to form radicals, which then react with O_2 to produce reactive oxygen species (ROS), such as superoxide anion radicals ($O_2^{-\cdot}$), hydroxyl radicals ($\cdot OH$), and hydrogen peroxides (H_2O_2, type I reaction). Alternatively, the energy of the excited PS can be directly transferred to $^3O_2$ (itself a triplet in the ground state) to form $^1O_2$ (type II reaction). It is worth noting that both type I and type II reactions can occur simultaneously, and the ratio between these processes is affected by the nature of the PS, as well as by the concentrations of $^3O_2$ and other substrates. However, most of the experimental studies indicate that the photoactivated production of $^1O_2$, namely type II reaction, plays a dominant role in in vivo PDT.

In a biological medium the reactive species generated by the photodynamic process can react with a large number of biomolecules, mainly proteins, nucleic acids, and lipids. The damage to biomolecules may (i) irreversibly damage tumor cells resulting in necrosis, apoptosis, or autophagy, (ii) cause tumor ischemia following PDT-induced vascular injury, and (iii) activate the immune response against tumor antigens[22–25]. Therefore, the main downstream targets of PDT include tumor cells, as well as tumor-associated microvasculature, and, indirectly, the host immune system[26]. Moreover, the combination of PDT with other chemotherapeutic drugs may help to achieve a long-term tumor control, due to their possible synergistic effects resulting from the combination of downstream responses in PDT and the mechanisms of chemotherapeutic drugs[27].

3. The photosensitizers for anticancer PDT

3.1. First generation PSs

Hematoporphyrin (Hp), a complex mixture of porphyrinic compounds[28], was the first porphyrin used as PS. The purification and chemical modification of Hp led to the discovery of a

hematoporphyrin derivative (HpD), which was shown to be more selective for tumor tissues, inducing a less intense skin photosensitization in comparison to Hp[29]. Later, a mixture of porphyrin dimers and oligomers isolated from HpD was marketed as Photofrin[30]. Despite Photofrin® was widely used for treating different cancers, the clinical use was limited by its intrinsic drawbacks, including I) poor chemical purity with a mixture of more than 60 molecules; II) its long half-life and intense accumulation in the skin, responsible for the induction of a prolonged skin photosensitization, which sometimes persists for 2 or even 3 months after Photofrin® administration; III) its low molar attenuation coefficient (1.17×10^3 mol/L·cm); and IV) its activation wavelengths being too short for a good tissue penetration[30–32].

3.2. Second generation PSs

The disadvantages associated with first generation PSs have led to extensive investigation aimed at improving the efficacy of PS molecules *via* alteration of the peripheral functionality of the porphyrin[33], or direct modification of the porphyrin core[34], The following the seminal works on the first generation PS have resulted in the production of several new non-porphyrinoid PS molecules (Fig. 2). These have been developed over the decades, including metalloporphyrins (Lutrin® and Lutex®)[35,36], porphycenes[37,38], pheophorbides (Tookad®)[39,40], purpurins (Purlytin®)[41], phthalocyanines[42–46], chlorins (Foscan®)[47], protoporphyrin IX precursors (Hexvix®, Metvix® and Levulan®)[48,49], phenothiazines (methylene blue, and toluidine blue)[50–55], cyanines (merocyanine 540)[56,57], dipyrromethenes[58,59], hypericin[60–62], and xanthenes (Rose Bengal)[63].

3.3. Strategies for designing new generation PSs

Despite the extensive research performed to develop new and improved PS, only a few second generation PSs, such as Levulan®, MetVix®, Photochlor®[64] and NPe6[65], have been approved for the clinical treatment of cancer[66]. The rational design of novel PS with desirable properties remains a big challenge for the pharmaceutical industry. The latest review article related to the design of PSs for photodynamic therapy, authored by Garland et al.[67], dates back to 2009. As stated before, the overall success of PDT mainly depends on the $^1O_2$ yield, molecule stability, the penetration depth of absorbed light and distribution of PSs, so that:

1) The $^1O_2$ generated in type II photoreaction is a key factor for PDT, since it is considered as the major cytotoxic species in PDT[68]. Generally, the introduction of heavy atoms (such as Br, and I) into the PS molecule or inhibition of the interaction

between triplet excited PS and native free radicals can increase the $^1O_2$ production;

2) A disadvantage of many of the current PSs is their tendency to aggregate, resulting in short triplet state lifetimes and decreased $^1O_2$ yields. Therefore, a structural modification that can suppress the aggregation tendency of a PS should be considered carefully to improve the property of PS. Usually, the presence of a central metal ion and the number of charges in the molecule will have an important role for the stability of PSs;

3) The increased energy of light of longer wavelength is also a major motivation for the development of new PSs. Generally, expanding the molecular conjugate system by introducing an electron-rich donor can improve the PS absorption efficiency of red light, and enhance the penetration of light into human tissue;

4) Improving the target distribution of PS is also very important to increase its efficacy and reduce adverse effects. The ligand-mediated targeting strategy in PDT has been explored. Herein, the targeted ligands, such as biotin, folate, peptide, etc., were frequently used for delivery of PS to cancer tissues;

5) In addition, the positions and types of the substituent groups on the molecule can influence the lipophilicity of the molecule, which further influences the tissue location of PS.

4. Recent development in anticancer PSs

A literature survey indicates that there are quite a lot of review articles about PSs and PDT[2,69–71], but few of them specifically focus on the discovery and development of new PSs as anticancer agents. With the rapid development in PS research area, a number of new, more potent and tumor-specific PSs showing promising clinic potential have been investigated. Herein, this section summarizes the recently reported PSs mainly focusing on porphyrin, chlorin, phthalocyanine and BODIPY derivatives, aiming to provide a better understanding of the factors affecting the efficacy of PS molecules.

4.1. Porphyrin-type PSs

The porphyrin macrocles were mainly developed and clinically used in the last few decades. Side-chains containing functional groups, such as nitrogen atoms[72,73], carboxylic acid[74] and sugar[75], are frequently incorporated into the porphyrin skeleton. For example, a novel porphyrin-based PS (5,10,15,20-tetrakis[(5-diethylamino)pentyl] porphyrin, TDPP, 1) with four diethylaminopentyl side-chains recently reported by Li et al.[72] showed a high $^1O_2$ yield with the ability to kill human esophageal cancer cell lines (Eca-109) and significantly reduce the growth of Eca-109 xenograft tumors in BABL/c nude mice. Among a series of β-alkylaminoprophyrins reported later by Chen's group[73], derivative 2 showed higher phototoxicity than Hp monomethyl ether and the lowest toxicity in the dark. Another new porphyrin derivative 3 bearing ethylenediaminetetraacetic acid showed intense *in vitro* phototoxicity on HepG2 and BGC823 cell lines, and further inhibited the growth of BGC823 tumors in nude mice[74], Mechanism studies indicated that 3 can induce cell death *via* the mitochondrial apoptotic pathway mainly triggered by lysosomal photodamage. Costa et al.[76] recently reported a hydrophobic porphyrin derivative 4 bearing four isoquinoline moieties, which showed a high quantum yield of $^1O_2$ generation, the absence of toxicity in the dark, and significant *in vitro* phototoxicity against HT29 cells with an IC_{50} in the micromolar range.
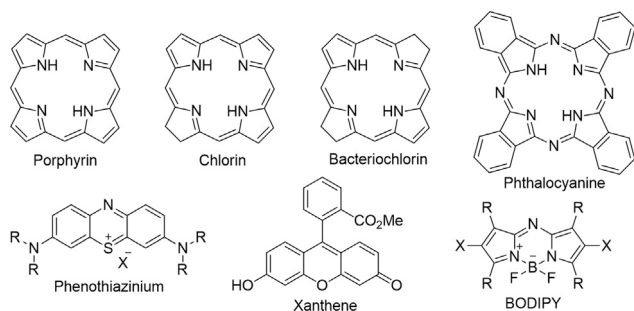
**Figure 2** Structural skeletons of several anticancer PSs.

Horiuchi and co-workers[77] studied the effect of a silyl group on the photodynamic properties of tetraphenylporphyrin derivative **5**. The results indicated that silylation could lead to an improvement in the quantum yield of $^1O_2$ sensitization for derivatives. In addition, there has been recent growth in interest in the preparation of metal-porphyrin conjugates as potential photocytotoxic agents. The porphyrins functionalized with Pt^{II} were the first metal-porphyrin derivatives developed for biomedical application[78]. Later, examples related to other metal-porphyrin conjugates have been described. For example, the glucopyranoside-conjugated porphyrin **6** bearing In^{+3} synthesized by Nakai et al.[75] exhibited strong phototoxicity, correlating with its high abilities of $^1O_2$ yield and cell permeability. Another Re-porphyrin conjugate **7**, bearing four [1,4,7]-triazacyclo-nonane units prepared by Mion et al.[79], showed remarkable phototoxicity on Hela cells with non-toxicity in the dark (Fig. 3).

4.2. Chlorin-type PSs

Compared with porphyrins, chlorin-type PSs attracted considerable attention due to their intense absorption in relatively harmless NIR region, which can penetrate deeply into biological tissues. However, the development of chlorin derivatives was significantly limited by their poor water solubility. Therefore, chlorin-type PSs have been modified by conjugation with amino acids, peptides, and sugars to improve their solubility for PDT investigations. For example, Meng et al.[80] prepared a series of chlorin P_6-based water-soluble amino acid conjugates. Among these synthetic derivatives, compound **8** showed strong absorption in the phototherapeutic

window, relatively high $^1O_2$ quantum yield, and high phototoxicity against melanoma cells with low toxicity in the dark. Also, compound **8** exhibited better *in vivo* PDT antitumor efficacy than verteporfin on mice bearing B16F10 tumors. Another photocyto-toxic chlorin e_6 bis(amino acid) conjugate **9** bearing two different amino acids, lysine at 13 and aspartate at 15 was regioselectively synthesized by Smith and co-workers[81]. A water-soluble chlorin derivative **10**, which was surrounded by four perfluorinated aromatic rings and conjugated with maltotriose (MaI_3) molecules, showed excellent biocompatibility, strong photoabsorption in the longer wavelength regions and high photocytotoxicity[82].

The use of boron-containing substances in the treatment of cancer has received a great deal of attention due to their high probability of producing particles and lithium-7 nuclei[83]. Boron-containing chlorin derivatives have also been explored in PDT applications[84]. Recently, a new chlorin derivative **11** containing phenylboronic acid moieties was synthesized by Tai and co-workers[84]. This compound could significantly inhibit tumor growth *in vivo* and showed rapid clearance from normal tissues. To improve the cell permeability of chlorin-type PSs, Gushchina et al.[85] introduced hydrophobic carbon chains into chlorin e_6 to yield a series of new amide derivatives **12**, which exhibited good photoactivity and low toxicity in the dark against P388 and K562 cancer cells. Chlorin and bacteriochlorin derivatives **13**[86] bearing chloro-5-sulfophenyl fragments showed promising phototherapeu-tic properties, such as high water solubility, high photostability, high $^1O_2$ quantum yields and negligible dark cytotoxicity. Patel et al.[87] recently reported an NIR bacteriochlorin analogue **14** to be
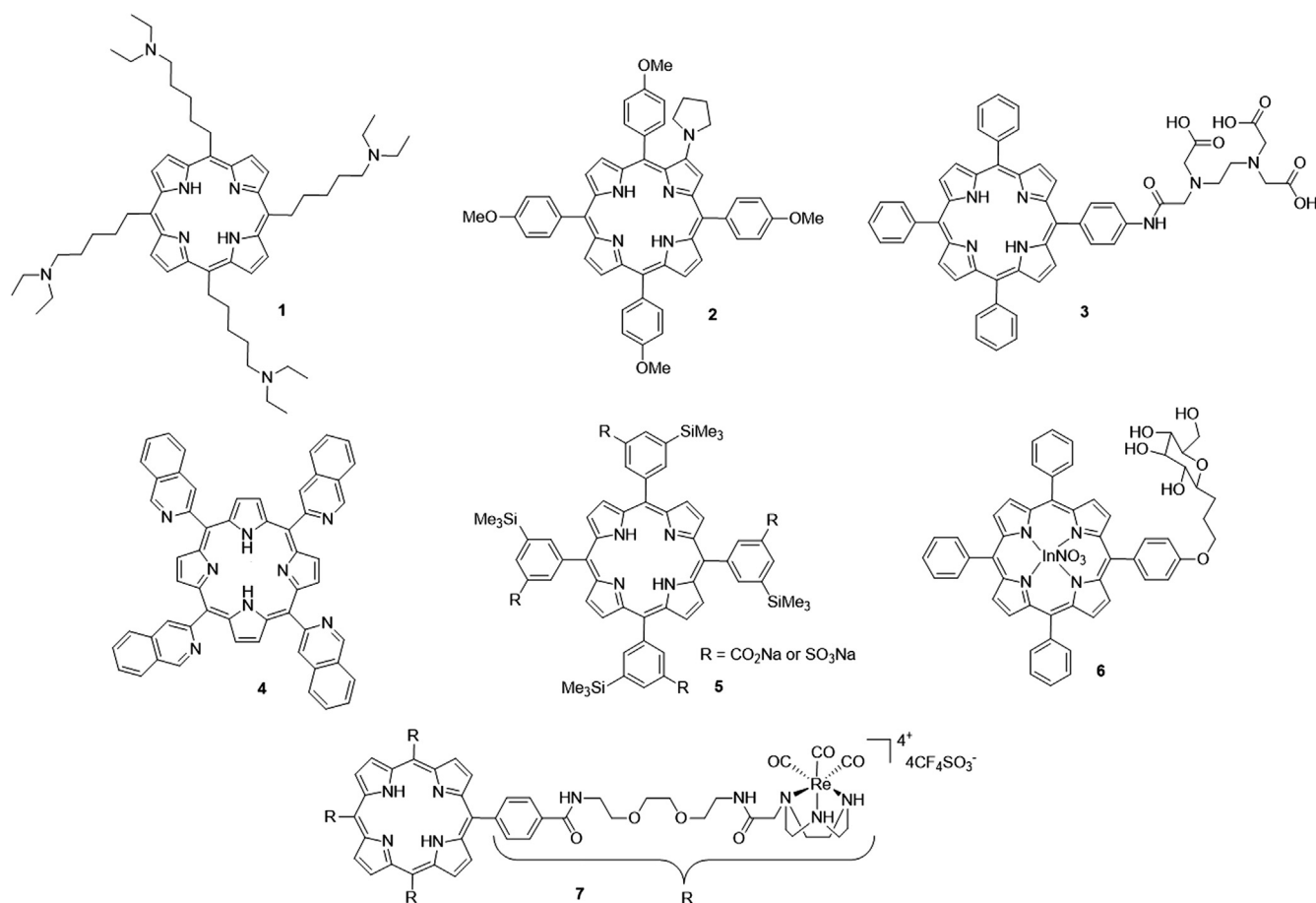
Figure 3 Chemical structures of porphyrin-type PSs **1**–**7**.

a promising dual-function agent for fluorescence-guided surgery with an option for treating cancer in PDT. This compound exhibited higher tumor uptake and long-term cure in BALB/c mice bearing Colon 26 tumors. Most of all, it showed low skin phototoxicity, which provides a significant advantage over the clinically approved HD as well as other porphyrin-based PSs (Fig. 4).

4.3. Phthalocyanine-type PSs

Phthalocyanine derivatives were shown to be most promising PSs. However, the low solubility and π–π stacking in these molecules limited their further clinical application. Strategies to overcome these disadvantages can involve incorporations of cationic[88] or anionic groups[89], peptides[90], β-cyclodextrins[91], crown ethers[92], glycerinum[93] and so on. Recently, 2-(morpholin-4-yl)ethoxy-substituted phthalocyanine 15 was synthesized by Kucinska et al.[94] Biological test results indicated that 15 showed potent cytotoxicity against PC3 and A375 under irradiation, while its cytotoxicity in the dark was very low. Another novel Mg(II)-phthalocyanine 16, bearing (2-methyl-5-nitro-1H-imidazol-1-yl)ethoxy substituent at a non-peripheral position, was found to show strong photocytotoxicity at 1 mol/L with 100% photokilling of the human oral squamous cell carcinoma cell lines, HSC-3[95].

The development of multifunctional molecules has also been considered for overcoming drug resistance and low therapeutic efficacy. For example, Zhou et al.[96] reported a derivative 17 bearing a cytostatic coumarin moiety, zinc(II) phthalocyanine and a tri(ethyleneglycol) linkage showed dual photodynamic and chemotherapeutic activities. These conjugates exhibit high photocytotoxicity against HepG2 cells (IC$_{50}$ ≈ 14–44 nmol/L), low aggregation tendency and high cellular uptake. Other similar examples are the phthalocyanine-8-hydroxyquinoline conjugates

18[97]. Ranyuk et al.[98] reported a series of water-soluble zinc phthalocyanine-peptide conjugates 19, which targeted the gastrin-releasing peptide receptor. Novel far-red-absorbing Zn(II) phthalocyanine derivative 20 bearing [(triethylammonio)ethyl]sulfanyl substituents in the peripheral or nonperipheral positions were synthesized by Machacek et al.[99] The bioassay results indicated that the Zn complex exhibited photocytotoxicity against 3T3, Hela, SK-MEL-28, and HCT116 cancer cell with IC$_{50}$ values in a submicromolar range, and low toxicity in the dark (TC$_{50}$ ≈ 1500 mol/L). Shen et al.[100] reported a series of first silicon(IV) phthalocyanine nucleoside (uridine, 5-methyluridine, cytidine, and 5-N-cytidine) conjugates. Among them, the uridine-containing complex 21 exhibited the highest photocytotoxicity against HepG2 cells (IC$_{50}$ = 6 nmol/L), with high cellular uptake and non-aggregated nature in the biological media. Bio et al.[101] developed a multifunctional prodrug 22 composed with Si phthalocyanine, a SO-labile aminoacrylate linker and the cytotoxic drug combretastatin A-4 (CA4). Once illuminated, 22 showed improved toxicity, but reduced toxicity in the dark compared with CA4 (Fig. 5).

4.4. BODIPY-type PSs

Another promising class of PS suitable for PDT is the distyryl boron dipyrromethene (BODIPY) dyes, which have proven to be a valuable family of compounds with diverse applications rivaling that of porphyrin. The BODIPY PSs are equipped with heavy halogen atoms, such as Br and I, in the organic chromophore to make compounds reach the triplet excited state by quenching the fluorescence and facilitating intersystem crossing.

Erbas-Cakmak et al.[102] designed a water-soluble pH and GSH responsive distyryl-BODIPY PS 23, which could be activated by protonation at neutral pH and reductive cleavage of the disulfide linker at elevated GSH concentration. Recently, Lo and
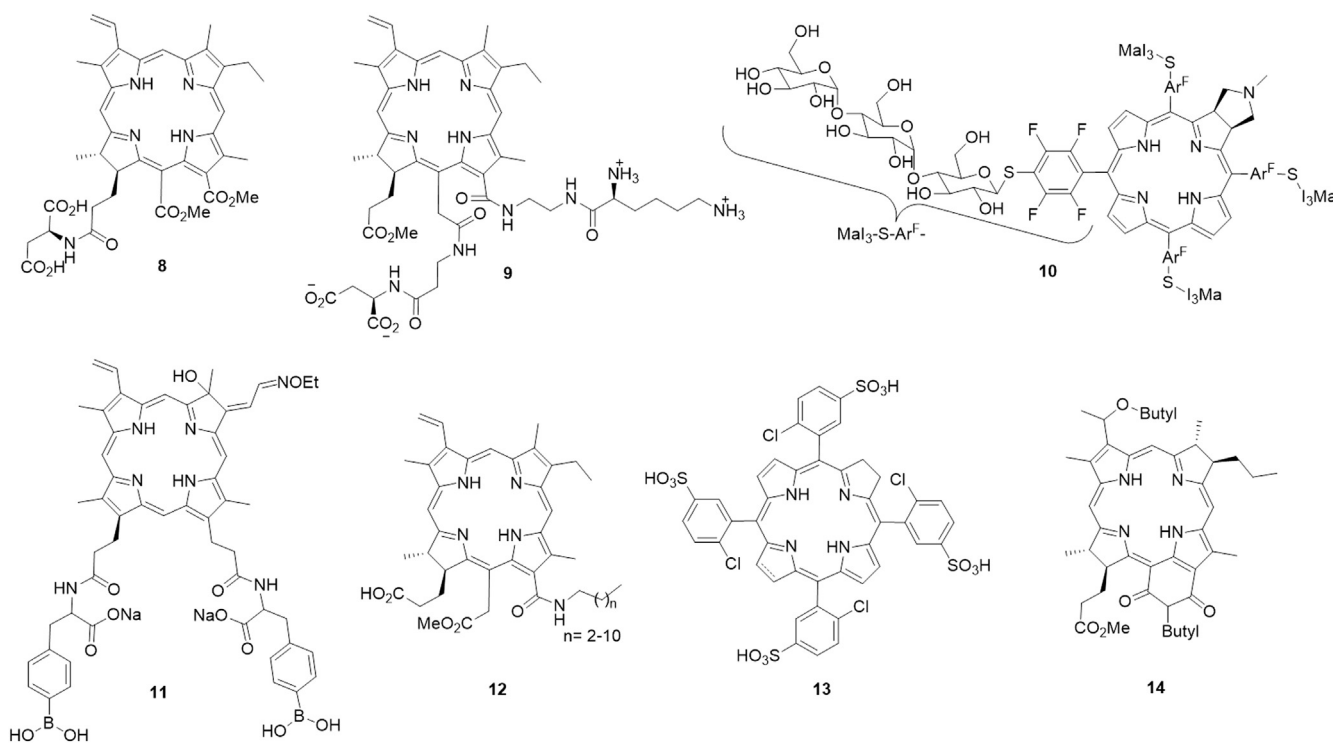

Figure 4 Chemical structures of chlorin-type PSs 8–14.

coworkers[103] reported another new class of pH/thiol responsive BODPY PSs that contained either the ketal or disulfide linker. Noteworthy, the unsymmetrical complex **24** exhibited the greatest enhancement in the $^1O_2$ generation and fluorescence intensity upon activation, which was considered to be a promising theranostic agent for targeted imaging and PDT of cancer. Platinum (II) complex **25** synthesized by Mitra et al.[104] could achieve mitochondria-targeted photocytotoxicity *via* disruption of the mitochondrial membrane potential and apoptosis. This complex generated excellent photocytotoxicity against HaCaT cells but remained non-toxic in the dark ($IC_{50} > 100$ mol/L). A novel photoactivatable bichromophoric conjugate **26** was developed by Fraix and coworkers[105]. This compound combined BODIPY and aniline derivative as nitric oxide photodonor, which had an amplified photomortality on melanoma cancer cells. Lincoln et al.[106] prepared two small meso-acetoxymethyl BODIPY dyes **27**, which showed improved photostability against singlet oxygen compared to the BODIPY PSs lacking the acetoxymethyl group. Compounds **27** can readily embed in the lipid membranes of Hela cells and efficiently induced light-dependent apoptosis at nanomolar concentration. An orthogonal BODIPY trimer **28** without halogen atom substituent was shown to have strong absorption in the visible region and high $^1O_2$ generation capability[107] (Fig. 6).

5. Conclusions

The discovery of novel PS molecules with desired pharmaceutical properties and the application of novel PS in clinical trials are challenging tasks. During the last several years, most research work is based on the modification and optimization of old-style PSs. Therefore, molecules considered as the second generation PS mainly have been derived from porphyrin and porphyrin-related structures. The most recent activity in the PS field for PDT of cancer has been considerable, and the design of non-porphyrin PSs, which possess shorter periods of photosensitization, longer activation wavelengths and higher singlet oxygen yield, still attracts more attention in the field of anticancer PDT.

Additional to the synthesis of new types of PS molecules, the association of classical PSs to different carriers has also been explored to improve their photophysical properties and/or their targeting to tumors. On one hand, antibodies, receptor ligands, and other targeting molecules have been used to actively increase the accumulation of PSs in tumors. On the other hand, different nanostructures have been used to enhance or to maintain the activity of PSs in aqueous media, and to actively and/or passively deliver these molecules to tumors. Both of these systems, and even combinations of them, have been referred to as the third generation PS, and some encouraging results have been reported in the literature regarding the use of this strategy in anticancer PDT. With the significant successes on the developments of new PSs for PDT, it is expected that PDT will gain more widespread use in clinical practice.
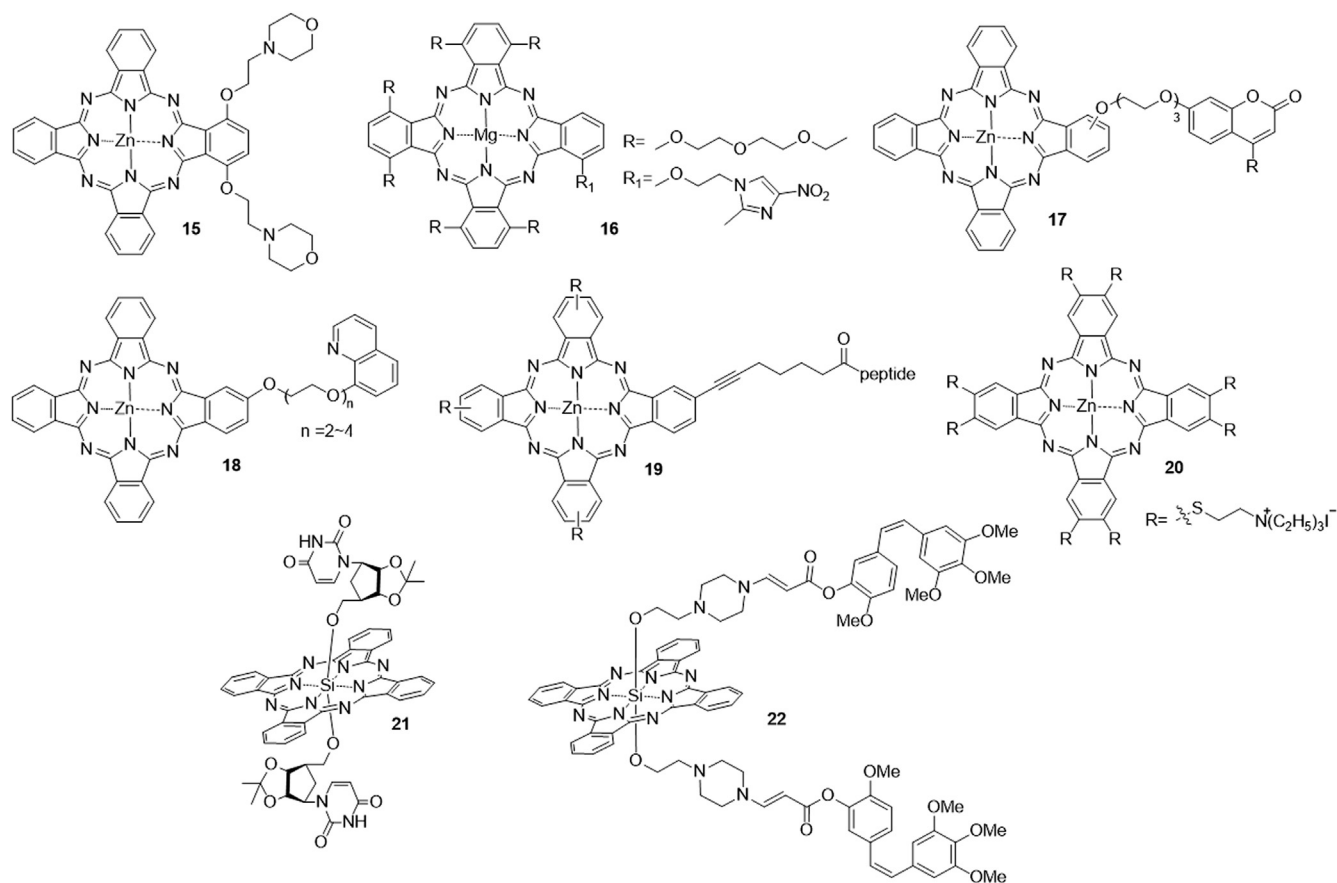
Figure 5 Chemical structures of phthalocyanine-type PSs **15–22**.
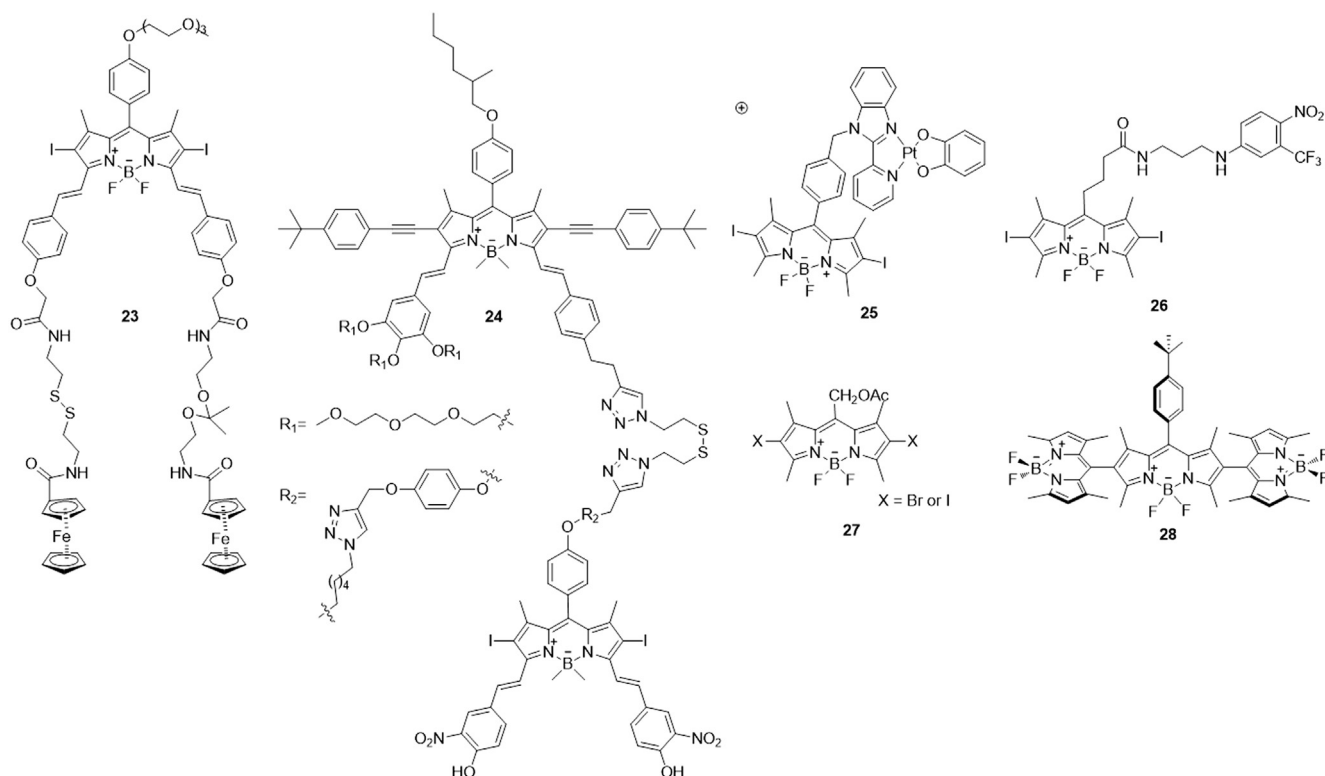
Figure 6 Chemical structures of BODIPY-type PSs **23–28**.

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