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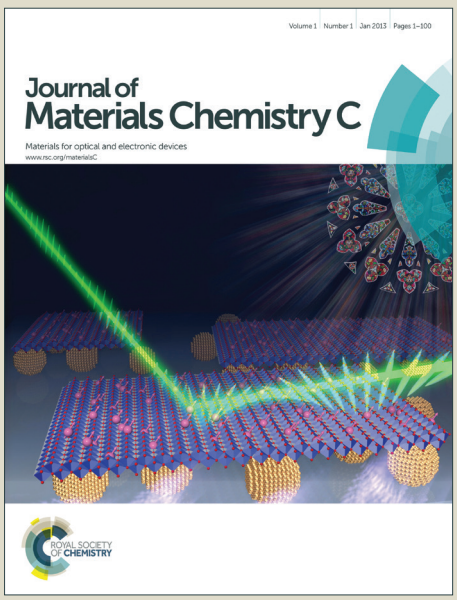



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FEATURE ARTICLE

# Activatable triplet photosensitizers: magic bullets for targeted photodynamic therapy

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Photo-dynamic therapy (PDT) is a promising minimally-invasive therapeutic approach to activate oxidative photodamage and subsequent cell death of targeted tumor. The classical non-targeted photosensitizers lack sufficient tumor selectivity and are taken up in the neighboring normal tissues, resulting in undesirable adverse effects. To overcome this obstacle, diverse tumor-targeting approaches have been developed, such as the targeted photodynamic therapy (TPDT). In the present review we have discussed the recently emerged strategies in the designing of targeted photosensitizers for TPDT including target the tumor specific enzyme, photodynamic molecular beacons, the PDT reagents target the acidic microenvironment and target the overexpressed folic acid receptors on the cancer cell surfaces. The approaches used in TPDT, such as passive or active and/or activatable were discussed. The molecular structure assembly and structure function relationship in chemistry as well as biology point of approach were also highlighted.

## 1. Introduction

Photodynamic therapy (PDT), a combination of multi components, i.e. a photosensitizer (PS), light and molecular oxygen, is promising minimally-invasive therapeutic approach for the treatment of malignant as well as non-malignant chaos. Moreover, PDT fulfil the elementary theoretical requirements for successful cancer therapy, such as selective destruction of neoplastic tissue through direct cellular damage, vascular shutdown and activation of an immune response against targeted cells followed by preserved minimal toxicity towards normal healthy tissue.[1-4]

In PDT, a non-toxic photosensitizer is introduced into the target cells followed by photoirradiation in the therapeutic window (600–900 nm) so as to excite the photosensitizer from its low energy ground state ($S_0$) to a short-lived higher energy first excited state ($S_1$) and then by intersystem crossing (ISC), triplet excited state is populated. However, $S_1$ state is uable to sensitize singlet oxygen ($^1O_2$) due to two reasons. First, the short life-time ($10^{-9}$ s) of the singlet excited state prevent it from been quenched via intermolecular manner. The reason is that the diffusion-controlled bimolecular collision frequency ($k_0$) in fluid solution is ca. $10^{10}$ $s^{-1}$ $M^{-1}$. As a result the Stern-Volmer quenching constant ($K_{SV} = k_q \times \tau_0$, where $k_q$ is the bimolecular quenching contant and always be smaller than $k_0$, $\tau_0$ is the lifetime of the excited state of the photosensitizer) will be on the scale of 10 $M^{-1}$, which is too small to induce any efficient *intermolecular* energy trasnfer or electron transfer; Second, singlet-triplet energy transfer is a forbidden process due to the spin conservation rule (the ground state of dioxygen, $O_2$, is at triplet state). Hence, it is crucial for the photosensitizer to undergo rapid ISC to produce the triplet energy state ($T_1$). $S_0 \leftarrow T_1$ transition is a forbidden process, thus

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the excited triplet state tends to have a longer lifetime (in μs to ms), as compared with excited singlet state (in ns). Intermolecular energy transfer and electron transfer is possible with triplet excited state. Photosensitizing $^1O_2$ with triplet excited state of photosensitizer is a spin-allowed process. Photosensitizers with long living $T_1$ sate are often used to stimulate biologically relevant photochemistry in two competing pathways, the Type-I and Type-II reactions. The Type-I reaction involves electron/proton transfer directly from the PS to cellular organic substrates (lipids, proteins, nucleic acids, etc.), yielding free radicals or radical ions that interact with molecular oxygen ($O_2$) to produce reactive oxygen species (ROS), such as hydrogen peroxide ($H_2O_2$), superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet}$) (Fig. 1). On the other hand, the Type-II reaction involves energy transfer from the triplet state of PS to the ground-state $O_2$, forming non-radical but highly reactive singlet oxygen, $^1O_2$.[5] Both types of reactions cause oxidation of various cellular molecules and can induce cell death *via* apoptosis, necrosis and/or autophagy (Fig. 1). In particular, $^1O_2$ produced through Type-II reaction is thought to be responsible for the cell death induced by PDT.

$^1O_2$ has a very short lifetime in biological systems (<0.04 μs) and a limited radius of action (<0.02 μm), controlled by the diffusion.[6] Therefore, the primary site of photodynamic damage is highly proximal to the area of its production and is dependent upon the subcellular localization of the PS.[7] Thus targeted PDT reagents, which are selectively distributed in the tumor tissue or cancer cells, are intrincally advantagous as compared to the first generation of the PDT reagents, which are normally non targeted reagents, which induce side effect (normal tissue or cells can be killed upon photoirradiation).

The PS is the key component of PDT. An ideal photosensitizer should exhibit the following properties: (i) light absorption at wavelengths which is able to penetrate deeply into biological tissue (600–950 nm), (ii) a high quantum yield for $^1O_2$ generation ($\Phi_\Delta$) and minimal dark toxicity, (iii) selective uptake into the diseased tissues, (iv) ability to dissolve in blood and pass through the lipid cell membrane, and (v) localisation in regions of the cell which are vulnerable to $^1O_2$ damage.[8,9] Solubility of photosensitizers in water is not an essential requirement, since many delivery vehicles, such as liposomes, dendrimers, bioconjugates and nanocarriers have been developed.[10,11]

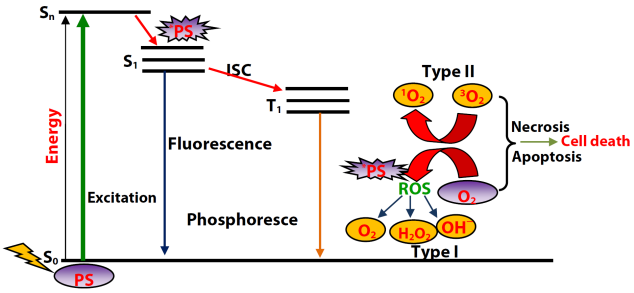
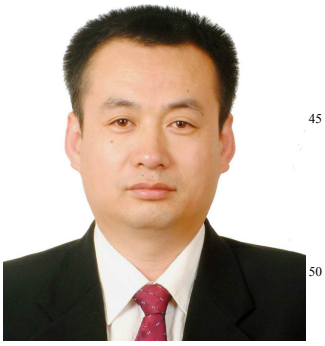



**Fig. 1.** Jablonski energy level diagram for photodynamic therapy(PDT). PDT requires three elements: a photosensitizers (PS), light and $O_2$. In the presence of molecular ground (triplet) state oxygen ($^3O_2$), the excited state PS transfers energy or electrons to produce reactive oxygen species (ROS) and/or singlet state oxygen ($^1O_2$).

The most commonly used PSs are porphyrin based molecules. A chronology in the historical development of PDT is depicted in (Fig. 2). In early 1900s,Tappeiner was[12] the first to use topical eosin and sunlight to treat skin tumours. In 1950s, Figge *et al.*[13] discovered that haematoporphyrin (HP) had tumour localising properties, and later Lipson *et al.*[14] initiated PDT in clinical applications in 1960s at the Mayo Clinic in the USA. The first PDT sensitizer used was haematoporphyrin (HP) and its derivative HPD (Photofrins), which consists of a mixture of mono, di and oligomers of porphyrins, with improved photosensitising properties.[5,15,16,17] At present, Photofrins has been approved by health organization in the United States, Canada, Europe, and Japan, for the treatment of various types of cancers.[5] However, this clinically approved first-generation PDT sensitizer, Photofrin®, exhibited prolonged patient photosensitivity (poor clearance), reduced capacity for tumour targeting. Moreover, these compounds show weak absorption in visible region,[18] restricted it to be an adequate photosensitizers in PDT. Regardless of these disadvantages, first generation photosensitizers have been modified to form new improved, second generation photosensitizers such as benzoporphyrin (Visudyne®), chlorin (Temoporfin®) and porphycene (ATMPn), which exhibited a more intense absorption at longer wavelength,[19,20] along with their metallated derivatives (Al, $AlPcS_4$; Si, SiNC (NC-naphthalocyanine); and Sn, $SnEt_2$. In spite of these modifications, no consistent correlation between metallation and augmented photodynamic activity was observed,[20] thus origin of third-generation photosensitizers came into existence highlighting the need for increased selectivity of photosensitizers for tumour tissue over healthy tissue.

Third-generation photosensitizers have been developed, by improving the existing photosensitizers, adding specific moieties and using delivery vehicles to specifically target tumor tissue or cancer cells.[21] Currently, this targeting strategies for drug delivery in PDT is termed as targeted photodynamic therapy (TPDT) which aims to increases specificity of the photosensitizer on accumulation at the malignant tissue (target) or biological pathways. Moreover, these targeted PDT reagents usually cause negligible damage to the normal tissues, when activated upon near IR photoirradiation.



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The specific accumulation of photosensitizers at the targeted site is generally based on two mechanisms, i.e. the passive and active targeting. In *passive* targeting, photosensitizer utilizes the physicochemical factors of drug carrier and pathophysiological factors of the organism, such as tumor microenvironment as well as its enhanced permeability and retention effect. The *active* targeting drug delivery to the specific target sites is due to molecular recognition, such as the targeting of the folic acid receptor which is often overexpressed on the surface of cancer cells.[22]

| | |
|---|---|
| **1900** | Acridine exhibits phototoxicity on *Paramecia*[23,24] |
| **1903** | Trappeiner applied eosin against skin cancer in presence of light[12] |
| **1913** | Meyer-Betz tested hematoporphyrin for PDT on his skin[25] |
| **1924** | Poliard found porphyrin enhanced tissue exhibits red fluorescence upon UV irradiation[26] |
| **1942** | Auler and Banzer showed tumor photonecrosis[27] |
| **1948** | Figge *et al.* diagnosised hematoporphyrin and its Zn complexes localised in mouse tumor[13] |
| **1955 to 1961** | Schwartz and Lipson developed hematoporphyrin derivative (HpD) and investigated its accumulation in mice and rats.[14] |
| **1966** | Beginning of PDT as a cancer therapy for the successful treatment of Breast cancer.[28] |
| **1978** | Beginning of Clinical trials utilizing various HpD preparations[29] |
| **1987 to 1995** | QLT PhotoTherapeutics and American Cyanamid launched the clinically approved First generation Photosensitizer drug Photofrin® for the treatment of various cancer cells. [30,31] Disadvantages: poor chemical homogeneity, weak absorbance in the therapeutic window ( $\lambda_{max}$ = ~630 nm, $\varepsilon$ = 3500 $M^{-1}$ $cm^{-1}$) and prolonged skin retention |
| **1995 -Now** | Development of Second generation Photosensitizers: Zn phthalocyanine ($\lambda_{max}$ = ~675 nm, $\varepsilon$ = 150000 $M^{-1}$ $cm^{-1}$), Al phthalocyanine-tetrasulfonic acid ($\lambda_{max}$ = ~675 nm, $\varepsilon$ = 105000 $M^{-1}$ $cm^{-1}$) , Zn naphthalocyanine ( $\lambda_{max}$ = ~764 nm, $\varepsilon$ = 160000 $M^{-1}$ $cm^{-1}$), Benzoporhyrin ($\lambda_{max}$ = ~685 nm, $\varepsilon$ = 118000 $M^{-1}$ $cm^{-1}$), Bacteriochlorin ( $\lambda_{max}$ = ~78 5nm, $\varepsilon$ = 150000 $M^{-1}$ $cm^{-1}$), Zn etiopurin ( $\lambda_{max}$ = ~ 690 nm, $\varepsilon$ = 70000 $M^{-1}$ $cm^{-1}$), Porphycene ($\lambda_{max}$ = ~630 nm, $\varepsilon$ = 52000 $M^{-1}$ $cm^{-1}$);[1] Advantages: long wavelength absorption with large extinction coefficient, greater selective accumulation in tumor tissue and rapid excretion from the body. Development of Third generation Photosensitizers ( Selective accumulation in the tumor tissue): Developed, by improving the existing photosensitizers, adding specific moieties and using delivery vehicles to specifically target these compounds e.g. monoclonal antibodies bind selectively to an antigen on cancer cells.[1] Advantages: Photosensitizers are activated by the tumor specific microenvironment or tumor associated enzymes and become photodynamically active specifically at the site of the tumor cells thus reduces the damage to nearby healthy cells, e.g Photodynamic Molecular Beacons (PMB), Matrix metalloproteinases (MMPs), pH activatable PS |
| **1999** | Weissleder applied molecular beacons in vivo for bioimaging[32] |
| **2004** | Zheng designed protease triggered photosensitizing beacon[33] |
| **2005** | O'Shea developed pH-activatable photosensitizers [34] Design, synthesis, and biological evaluation of folic acid targeted tetraphenylporphyrin as novel photosensitizers for selective photodynamic therapy[35] |
| Clinically approved photosensitizers: Photofrin®, Photosan®, Photogem®, Photohem®, Foscan®, Levulan®, Metvix®, Visudyne®.[36] | |

**Fig. 2** Chronology in the historical development of PDT.

Recently, much attentions have been paid to the third strategy, by which the targeted photosensitizers are specifically activated by the tumor microenvironment, or tumor associated enzymes and become photodynamically active specifically at the site of the tumor cells. This kind of PSs over the site of tumor cell are photodynamically inactive, hence known as activatable photosensitizer.

In view of the above importance of the activatable photosensitizers in targeted photodynamic therapy, we will highlight the recent developments in the area of targeted photodynamic therapy.

## 2. Activatable photosensitizers

In recent years, TPDT with activatable photosensitizers has become an attractive controlled therapeutic tool to kill targeted malignant cells without affecting the remaining parts of the body. Conventional PDT dependent on light delivery and photosensitizer delivery to oxygenated tissue displayed inequitable specificity for the diseased cells. Currently, passive photosensitizers are used to target tumor tissues. The passive photosensitizers are restricted to a particular type of cancer and unable to prevent photosensitizers from accumulation in normal cells. On the contrary, activatable photosensitizers are only effective in the presence of specific trigger, such as the tumor-related enzyme. The sensitizers will be activated by the diseased cell on local photoirradiation, generates $^1O_2$ and destroy the targeted cells. Activatable PS can distinguish the diseased cells from healthy cells, thus reducing damage to nearby healthy cells that otherwise might be destroyed during PDT with conventional photosensitizers. Thus, activatable photosensitizers are magic bullet that are turned on by a variety of molecular stimuli to increas cytotoxic singlet oxygen generation at the targeted diseased site. This review aims to summarize the recent emerged strategies to design activatable targeted photosensitizers highlighting their molecular structural assembly and structural functional relationship in chemistry as well as biology point of view.

### 2.1 Enzyme activatable Photosensitizers

All the cellular functions are catalyzed by enzymes. Thus, enzymes are excellent target for activatable photosensitizers. Photosensitizer activation depends on the respective enzyme overexpression in the specific diseased cell site whereas remain inactive in the tissue not expressing the specific enzyme, hence is restricted to the location of the active enzyme target.

#### 2.1.1 Photodynamic Molecular Beacons (PMB)

The tumor specific enzymes such as proteases was targeted for activatable photosensitizer. Fluorescence resonance energy transfer (FRET) as a tool for designing activatable probes for imaging retroviral proteases have been used since 1990,[37] for example, in protease-activated near-infrared (NIR) fluorescent probes for cancer imaging.[32] High tumor-to-background ratios was attained, since the probe is nonfluorescent in the native state.

In FRET, a chromophore (energy donor) in its excited state non-radiatively transfers its energy to another chromophore (energy acceptor) in the ground state through long range dipole–dipole interaction, resulting in quenching of the fluorescence of donor and/or appearance of the characteristic fluorescence of the acceptor. The linker between the energy donor and acceptor was cleaveable by tumor-related enzyme, as a result, the fluorescence of the energy donor will be switched on only in tumor cells.[38]

By combining the mechanisms of protease targeting and FRET-based activation, a probe with an extremely high level of target specificity, peptide-based molecular beacons (MBs), is created. Zheng et al.[33,39-41] have postulated that Type-II photosensitization and molecular beacons (MB) are required to

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design a photosensitizing beacon (PS-beacon), commonly known as photodynamic molecular beacons (PMB). These photosensitizers are based on a disease-specific linker, a PS, and a $^1O_2$ quencher/scavenger, such that no photosensitization will occur until the linker was cleaved by a tumor-specific target molecule, such as a specific enzyme. $^1O_2$ generation is efficiently inhibited by the quencher moiety in the beacon through PS triplet-state energy transfer. In the presence of a targeted protease (tumor-specific enzyme), the substrate sequence (the linker) will be cleaved, hence the PS (donor) and quencher (acceptor) will be separated, FRET will terminate, resulting in the photo-activation of the PS (donor), so that $^1O_2$ can be produced upon photoexcitation. The PMB selectivity to cancer cells are optimized in two ways: (1) increase the protease specificity to targeted cells, (2) minimize the phototoxicity of intact (uncleaved) PMBs in non-targeted (normal) cells. Among the linkers described in the literature, the highest efficiency of PMB are demonstrated with cleavable linkers.

#### 2.1.1.1 PMB Based On Cleavable Activation Mechanism

In cleavable linkers, the natural function of target is to cleave the chemical bonds and realeases the photosensitizer and quencher on recognition by the biomarker (Fig. 3). Without cleavage, the production of $^1O_2$ is inhibited, due to the quchening of the triplet excited state of PS by the intramolecular quencher (Q). Cleavage of the linker by specific enzyme will release the free PS, thus $^1O_2$ can be produced upon photoirradiation. Linkers are usually peptides which are cleavable by endoproteases,[42] or oligonucleotides which are cleavable by DNAses or RNAses,[43,44] or phospholipases-cleavable phospholipids.[45]

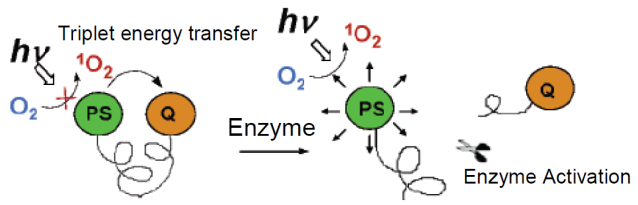


**Fig. 3** Concept of protease-controlled photodynamic molecular beacon. Reproduced with permission from ref. 33.

Zheng *et al*.[33,39] synthesized a CAR-based PMB(PPC) (**1**) (Fig. 4) with a caspase-3 cleavable peptide G*DEVD*GSGK linker, CAR was used as the quencher, and a chlorophyll analogue pyropheophorbide *a* (Pyro) as the PS unit. Carotenoids (CARs) are known to be very efficient antioxidants in animals. It is a potent scavenger of reactive oxygen species i.e photoprotective agents in the photosynthetic system of plants,[46] as well as potentially act as quencher for the excited triplet state of chlorophyll (or other porphyrin-based molecules). Thus production of harmful $^1O_2$ can be inhibited.[47] A number of carotenoporphyrins are reported since CAR transfer excitation energy ($\lambda_{abs}$ = 500 nm) to chlorophyll as part of light harvesting, and quench radical species that could potentially react with various biomolecules.[48] With caspase-3 cleavable PPC beacon, it has been shown that CAR, as a quencher molecule in PMB, turned off the $^1O_2$ production of Pyro by both quenching the PS excited states and directly scavenging $^1O_2$. Thus PDT potency of PPC toward targeted cells is compromised to some extent, however, as a trade off has lead to a very high level of protection for non-targeted cells. In *vitro* PDT study showed PS without CAR (control) remains highly potent, while CAR completely shuts (30 fold higher dose) off the photodynamic effect in non-targeted HepG2 cells.[33,39]

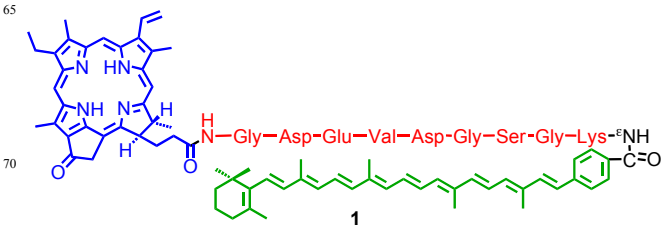


**Fig. 4** Chemical Structure of caspase-3 activatable PPC beacon: Pyro-GDEVDGSGK-CAR (PPC). Pyropheophorbide-*a* is shown in blue, the caspase-3 active peptide linker sequence is shown in red, and the carotenoid quencher is shown in green

Zheng *et al*.[49] prepared a photodynamic molecular beacon (FAP-PPB) **2** (Fig. 5) targeting a tumor-associated protease, fibroblast activation protein (FAP), a cell-surface serine protease which is highly expressed on cancer-associated fibroblasts (CAFs) of human epithelial carcinomas but not on normal fibroblasts, or normal tissues, and cancer cells.[49] FAP functions as an endopeptidase to cleave the Proline-Asparagine bond of $\alpha_2$-antiplasmin and peptide substrates.[50,51] The reported FAP-PPB **2** contains pyropheophorbide a (Pyro) ($\lambda_{abs}$ = 665 nm and $\lambda_{em}$ = 675 and 720 nm) as fluorescent photosensitizer and a black hole

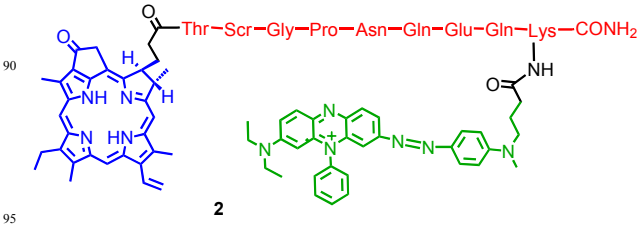


**Fig. 5** Chemical structure of fibroblast activation protein photodynamic molecular beacon, FAP-PPB. Pyropheophorbide-*a* is shown in blue, the FAP activated peptide linker sequence is shown in red, and the quencher BHQ3 is shown in green.[49]

quencher BHQ3, linked by a peptide sequence (TSGPNQEQK), which is specific to FAP. The peptide linker was effectively cleaved by both human FAP and murine FAP. In vitro and in vivo studies in cancer cells and mouse xenografts against HEK293 transfected cells (HEK-mFAP, FAP+; HEK-vector, FAP-) confirmed the PS could be activated specifically by the FAP enzyme in FAP-expressing cancer cells with a remarkable enhanced fluorescence. This PDT agents is inactive in FAP-negative cells. Moreover, FAP-PPB showed FAP-specific photocytotoxicity toward HEK-mFAP cells whereas it was non-cytotoxic toward HEK-Vector cells.

#### 2.1.1.2 PMB Based On openable Activation Mechanism

In openable activation mechanism, the target of PMB interacts strongly with a linker or a carrier, which holds the PS and quencher in a close proximity, hence they can interact with each

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other and the production of $^1O_2$ is inhibited. Once interact with the target, the PS and quencher are forced apart and PDT efficacy of PMB is activated. Nucleic acids were usually used as openable linkers, due to robust synthesis and well characterized base pairing. This method permits reliable and precise control of photosensitizer activation. Most of the diseases are due to gene mutations or altered gene expression. Nucleic acid activatable PS could form the basis of PDT capable of removing unwanted cells expressing specific genes and discriminating even single-base mismatches.

A functionalized photosensitizer design is required to realize the benefits of nucleic acid sequence-specific targeting to activate the photosensitizers. Zheng *et al.*[52,53] developed a nucleic acid-based *c-raf-1* mRNA-triggered PMB (mRNA-PMB) **3** (Fig. 6), taking the advantage of hybridization of mRNA to its complementary antisence oligonucleotides (AS-ONs). This 30 bases-mRNA-PMB, Pyro-30mer-CAR (P30C) consists of a *c-raf-1* mRNA a single-stranded oligonucleotide as a linker (target molecule). The probe forms a stem-loop structure (hairpin) using Pyro and CAR as PS-quencher pair with the middle 20 bases sequence as loop and 5 bases hybridised on each end as two complementary arm sequences. In native state, the stem-loop constructed linker induced proximity of PS (here Pyro) and quencher (CAR), making the mRNA-PMB photodynamic silent. On addition of the tumor specific mRNA, the loop sequence hybridizes with the mRNA, disrupting the hydrogen bonds of the stem and making the linker opened, followed by the removal of the quencher from the immediate vicinity of the PS, thus $^1O_2$ production of PS is restored (Fig. 7).

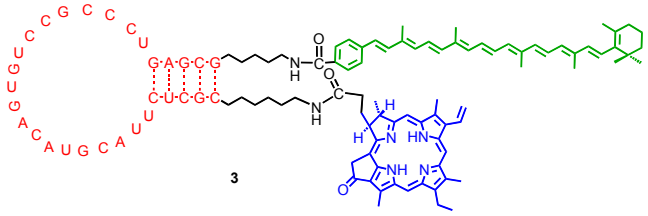


**Fig. 6** Chemical structure of *c-raf-1* mRNA-triggered PMB (P30C). Pyropheophorbide-*a* is shown in blue, the *c-raf-1* mRNA linker sequence is shown in red, and the carotenoid quencher is shown in green.[52,53]

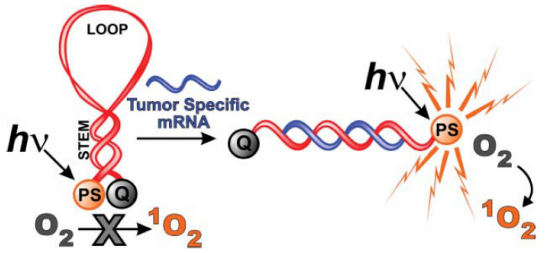


**Fig. 7** The activation mechanism of mRNA-triggered openable PMB (mRNA-PMB). Reproduced with permission from ref. 53

Based on reverse hybridization strategy, Gothelf *et al.*[54] linked the photosensitizer pyropheophorbide-*a* to an oligonucleotide sequence sharing the same sequence as the target (Fig. 8). Upon addition of a black hole quencher 3 (BHQ3)-conjugated

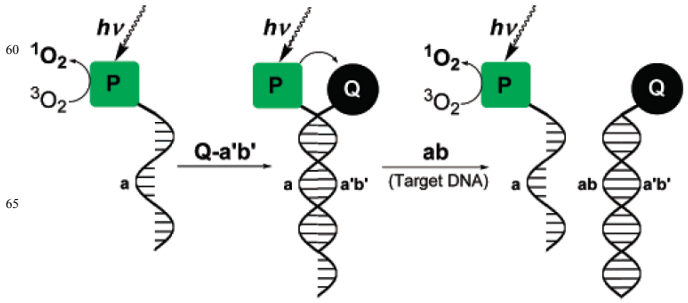


**Fig. 8** DNA sequence-specific control of $^1O_2$ generation. P and Q represents photosensitizer and quencher, respectively. Reproduced with permission from ref. 54

complementary oligonucleotide, the two strands hybridize. Hence the photosensitizer and quencher come into close contact and allowed to quench the singlet state of photosensitizer via FRET, resulting in decrease of $^1O_2$ production (Fig. 8). This quenched hybrid is the activateable PS. Once on interaction with the target nucleic acid (a third DNA sequence), displacement and release of the photosensitizer-DNA linked strand will occur, which results in fluorescence enhancement and turn on the $^1O_2$ generation (Fig. 8).

Aptamers are oligonucleic acid or peptide molecules that bind to a specific target molecule with high affinity and specificity. Aptamers can be combined with ribozymes to self-cleave in the presence of their target molecule. There are mainly two types, DNA/RNA or XNA aptamers, consisting of (usually short) strands of oligonucleotides. Another type is protein aptamer, containing a short variable peptide domain, attached at both ends to a protein scaffold. These rich targeted linkers to cancer-associated molecules have been developed for delivery of PDT agents. Tan *et al.*[55] have reported an activatable photosesitizer AP-SWNT by linking covalently bonded Ce(6)-aptamer ssDNA and non covalently with single-walled carbon nanotubes (SWNTs) by $\pi$-stacking between nucleotide bases and SWNT side walls for controlled $^1O_2$ generation on photoirradiation (Fig. 9). 98% $^1O_2$ quenching was observed on binding the Ce(6)-aptamer ssDNA to SWNTs due to the energy transfer between Ce6 and SWNT.

However, on addition of thrombin (a trypsin-like serine protease) to the activated AP-SWNT, a 20 fold fluorescence was enhanced and $^1O_2$ production was restored due to the dissociation of aptamer from the SWNTs.

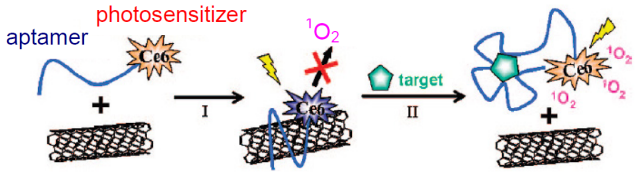


**Fig. 9** Schematic of aptamer-photosensitizer-SWNT complex and the regulation of $^1O_2$ upon target binding: (I) AP and SWNTs were mixed together to form AP-SWNT complex. The ssDNA aptamer is wrapped on the surface of SWNTs, which brings the photosensitizer close to the SWNTs to quench SOG. (II) Target binding with aptamers can disturb the interaction between AP and SWNTs, resulting in the restoration of $^1O_2$. Aptamer photosensitizer (AP) used is GGTTGGTGTGGTTGG-Ce(6). Reproduced with permission from ref. 55.

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### 2.1.2 Matrix metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases, plays a key role in normal tissue remodelling, cancer invasion and metastasis through the degradation of the basement membrane and collagen-rich extracellular matrix.[56] The PDT beacons comprising a photosensitizer and a quencher moiety, linked by a MMP-cleavable peptide, and are termed as (PMB MMP). Activatable matrix metalloproteinase-7 (MMP7) particularly demonstrate selective targeted PDT efficiency, because of its epithelial origin and its over expression in pancreatic, colon, breast, and nonsmall-cell lung cancer.[57-62]

Zheng *et al.* reported a MMP7-targeted PMB ($PP_{MMP\text{-}7}B$) **4** (Fig. 10).[63] **4** exhibits specific activation and selective PDT efficiency. Targeted $PP_{MMP\text{-}7}B$, consists of Pyro as the PS, black hole quencher 3 (BHQ3) as a dual fluorescence and $^1O_2$ quencher and a short peptide sequence, G*PLG*LARK, as the MMP7-cleavable linker, with the cleavage site between G and L are recognition site, as indicated by italics. The MMP7-positive cells effectively cleaved the peptide linker of activated PMB ($PP_{MMP\text{-}7}B$) removing the Pyro from the vicinity of BHQ3, restoring 17 fold increased fluorescence and 18 fold increased $^1O_2$ production, while leaving normal cells undetectable and unaltered. Photocytotoxicity was observed for MMP7-overexpressing cells in contrast to MMP7-negative cells.[63]

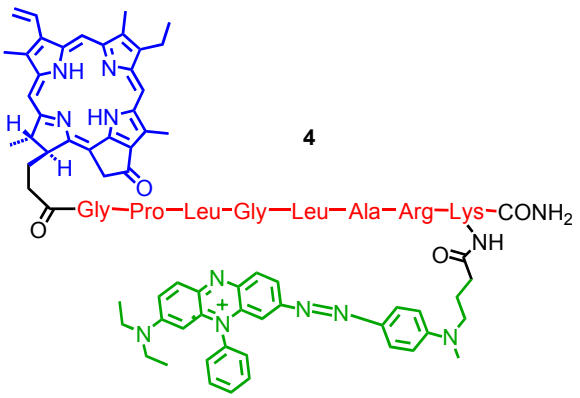


**Fig. 10** Chemical structure of $PP_{MMP\text{-}7}B$ **4**. Pyropheophorbide-*a* is shown in blue, peptide linker sequence is shown in red, and the BHQ3 quencher is shown in green.[63]

Zheng *et al*. also demonstrated the specific activation of $PP_{MMP\text{-}7}B$ by MMP-expressing MT-1 breast cancer cells using clinically relevant metastatic model.[64] Enhancement of fluorescence due to the cleavage of the linker, establishes the specific activation of $PP_{MMP\text{-}7}B$ by vertebral metastases versus normal tissue (i.e., spinal cord).

Although new activatable MBs biomarkers targeting cancer are emerging, a challenge still lies in ensuring that target protease specifically cleaved linked peptide substrates. The specificity of MBs decreases if it is taken up into the lysosomes or endosomes where a variety of strong enzymes reside, increasing the possibility of cleavage of the linker by other proteases, thereby contributing to background signal and reduced contrast. Therefore, it is necessary for peptide linker to withstand non-target cleavage by other enzymes. Even though both the activated photosensitizers FAP PMB[50] and MMP-7 PMB[63] used the same Pyro-1 and BHQ-3 quencher pair, improved fluorescence after the cleavage of the peptide linker was observed with FAP compared with that of MMP-7 activated photosensitizers. The differences in the fluorescence results are due to the differences in the peptide linkers secondary structures and chemical characteristics. As a result quenching efficiency also varies. Thus, the total dependence of the fluorescence and PDT quenching upon the random folding of the peptide linker before protease cleavage limits the sequences to ones with natural conformations that bring the quencher and PS in close proximity, in order for effective silencing to occur. Along with this, the non-specific and passive nature of the PS's delivery to target cells after protease cleavage is suboptimal.[65] Thus to renovate higher fluorescence, activatable cell-penetrating peptides (ACPP) were developed to address the passive delivery of activatable PMBs.

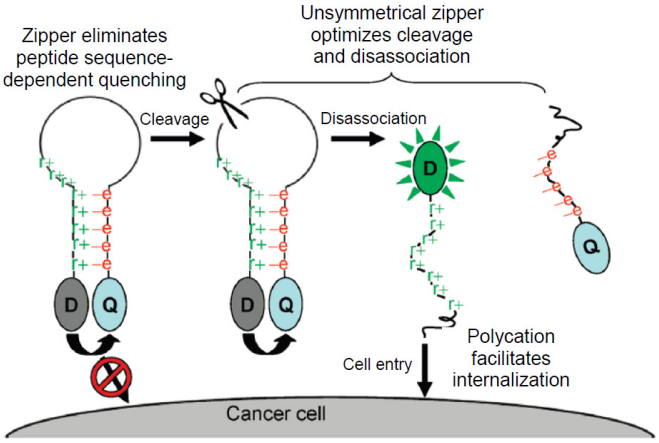


**Fig. 11** Universal Zipper Molecular Beacon Design**.** The zipper is composed of a pair of polycation and polyanion arms holding the dye (D) and quencher (Q) in close proximity due to electrostatic attraction. This results in silenced dye activity independent of peptide linker variations. Upon specific enzymatic cleavage of the linker, the dye and quencher dissociate, resulting in dye photoactivity and unleashing the polycation, which increases cellular uptake. Reproduced with permission from ref. 66

ACPP are based on the electrostatic formation of a polycation/polyanion zipper, whose peptide linker is selectively cleaved by a target protease to locally unleash the delivery function of cell-penetrating peptides (CPP). Since 'zipper' mechanism is integrated into PMBs, such activated sensitizers are termed as zip PMBs (ZMBs. Fig. 11). ZMB is designed with four functional modules: (1) a target protease cleavable peptide linker; (2) zipper structure formed due to electrostatic attraction of a polycation and a polyanion connected to ends of the linker; (3) fluorescent dye-Pyropheophorbide (D) and (4) a quencher – Black Hole Quencher 3 (Q), conjugated to the end of the polycation and polyanion chains, respectively. The zipper mechanism provides several advantages: (1) polycation/polyanion zipper through electrostatic attraction, bring the photosensitizer and quencher into closer proximity, resulting in high quenching efficiency of beacon; (2) a hairpin conformation of zipper substrate sequence can accelerate the rate of the enzyme-specific linker cleavage; (3) the polyanionic and polycationic arms of the zipper carryout opposite functions. Anionic arm prevents the probe from entering cells, by blocking the cell-penetrating function of the polycation, while the cationic arm increases cellular uptake of the dye after linker cleavage, and


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(4) the zipper is exclusively responsible for the dormant state and quenching does not depend on the natural folding of the peptide linker. In the presence of a target protease, the peptide linker is first specifically cleaved, causing the quencher-conjugated polyanion to dissociate from the photosensitizer-attached polycation, becoming photoactive, and unleashes the polycation, which enhances the delivery of the activated dye locally into the target cells.

Zheng *et al.* reported an asymmetrical zipper arm ZMB composed of eight consecutive arginines and five consecutive glutamates to achieve the high quenching efficiency and ideal activation rate with enhanced PS internalization.[66] The ZMB concept is a general approach to improve the functionality of a wide range of activated photosensitizers through a simple switching of substrate sequences. The increased selectivity, fluorescent production and targeted uptake of a ZMB could lead to more selective tumor destruction while protecting non-target ed cells.

### 2.2 Environment activatable PS

Transportation and delivery of the drug certainly depends upon the interference of the targeted cellular environmental conditions. The intracellular and/or extracellular pH of the targeted cell has played a vital role in the drug delivery to specific target. Due to the acidic pH of the growing malignant tumors (pH 6.5−6.8) as compared with the normal tissue (pH 7.4), the introduction of a photosensitizer that produces $^1O_2$ in tumor cell at acidic pH but is deactivated at physiological pH, provides the therapeutic selectivity in cancer treatment.[67,68] The acidic extracellular environment induced by glycolysis under hypoxic conditions (produces lactic acid causes for acidic conditions) is perhaps the most pervasive tumor microenvironments, regardless of the tumor types or the developmental stages.[69,70]

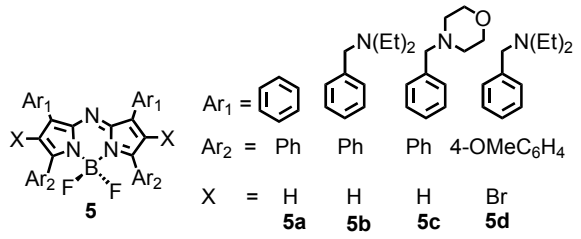


**Fig. 12** Chemical structure of $BF_2$-Chelated azadipyromethene photosensitizers **5**.[34]

O'Shea *et al.* introduced a pH-activated reversible switching off/on of $^1O_2$ generation to achieve selective PDT with a supramolecular photonic therapeutic agent (SPTA) containing an amine functional group (Fig. 12).[34] The substrate required to activate the SPTA would be a proton source of sufficient strength. On irradiation of photosensitizer (Fig. 12), $^1O_2$ production has been shut down due to the rapid quenching of the the photosensitizer excited state by a photoinduced electron transfer (PET) mechanism (**Path A**, Fig. 13). The PET process was switched off through the protonation of the amine PET donor **5b-5d**, hence enhanced the ISC efficiency and the rate of $^1O_2$ generation (**Path B**. Fig. 13). Thus this supramolecular therapeutic agent could produce a cytotoxic agent ($^1O_2$) in response to one exogenous stimulus (light) and one endogenous stimulus (microenvironment pH) for therapeutic selectivity in cancer cells.

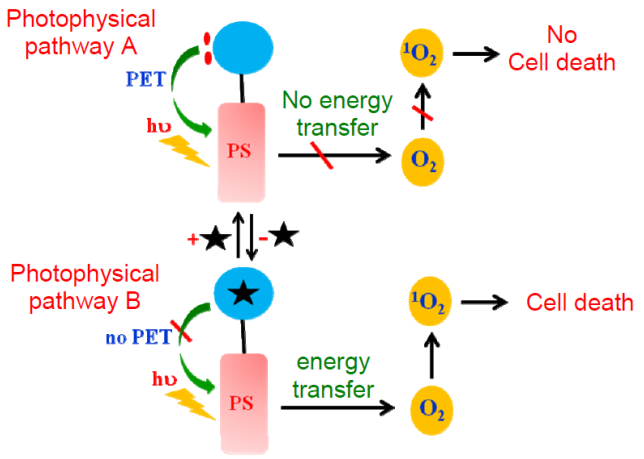


**Fig. 13** Design and function of an SPTA. Blue circle, Red rectangle and Black cross represents substrate-specific receptor, photosensitizer and substrate, respectively. Adapted with permission from ref. 34.

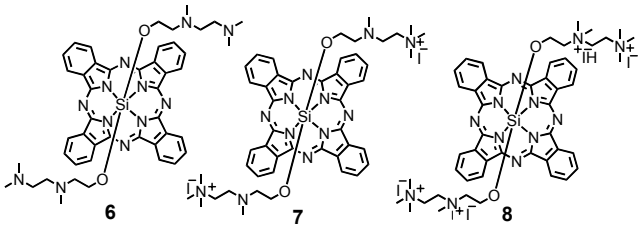


**Fig. 14** Chemical structure of photosensitizers silicon(IV) phthalocyanine **6-8.**

Ng. *et al.* also found enhanced fluorescence emission and $^1O_2$ generation efficiency for tetraamino silicon(IV) phthalocyanine **6-8**, on irradiation at lower pH in the range of ca. 5–7 (Fig. 14),[71] thus making it a promising pH-controlled and tumour-selective fluorescence probe and photosensitiser for photodynamic therapy. Compound **6** exhibited improved intracellular photosensitising property in human colon adenocarcinoma HT29 cells as compared with compounds **7** and **8**, for which the cellular uptake process was hindered by cationic groups.

5,10,15,20-Tetrakis(*N*-(2-(1*H*-imidazol-4-yl)ethyl)benzamide) -porphyrin (TIEBAP) produced twice as many singlet oxygen ($^1O_2$) molecules at pH 5.0 due to protonation of the imidazole ring *N* atoms (singlet oxygen quantum yield $\Phi_\Delta = 0.53 \pm 0.01$) **9** than deprotonated imidazole **9'** at pH 7.4 (Fig. 15), which causes photosensitizer aggregation, or owing to an inefficient formation and potential quenching of the triplet state.[72] The rate of the $^1O_2$ quenching was reduced by a factor of 2.5 at a pH change from 7.4 to 5.0, resulting in increase of therapeutic function. In photosensitizer of cationic porphyrin TIEBAP, imidazole scaffolds were separated from the porphyrin ring by ethylbenzamide chain spacers to prevent the delocalization of the positive charges onto a porphyrin ring system through direct coupling. This feature can stimulate negative effect to reduce the triplet state and $^1O_2$ yields.

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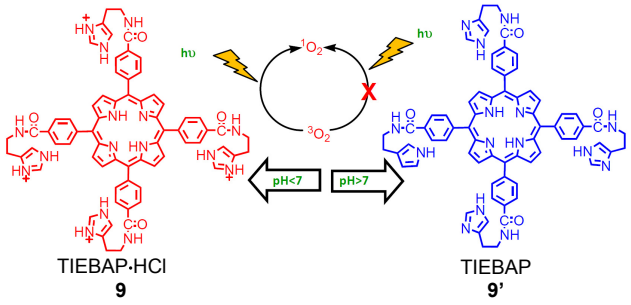


**Fig. 15.** Design and function of pH-responsive TIEBAP **9** for $^1O_2$ production.[72]

It is interesting to note that when an acid-sensitive unit is linked to the activatable fluorophore of photosensitizers, its fluorescent property can be modulated on altering the pH of the environment, for targeted photodynamic therapy (PDT).[4a,73-75]

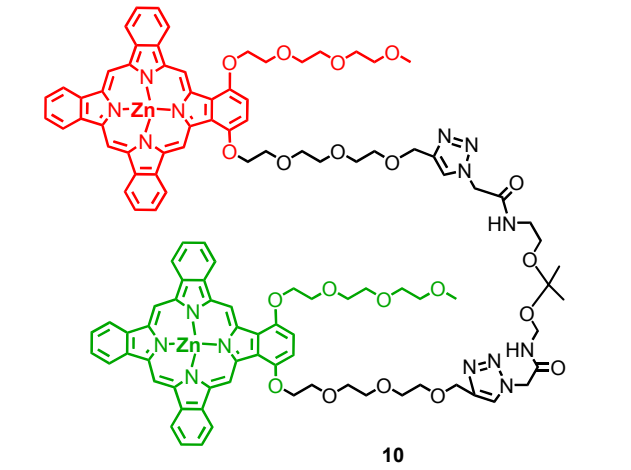


**Fig. 16** Chemical structure of phthalocyanine dimers **10**.[76]

Lo *et al.* have prepared a aggregation-induced self-quenching zinc(II) phthalocyanine dimer **10**, linked with an acid sensitive ketal unit (Fig. 16).[76] This photosensitizer can be activated by the cleavage of the ketal linker in an acidic environment (pH = 5.0–6.5). As a result, the phthalocyanine units were separated from each other, resulting in enhanced fluorescence emission and $^1O_2$ production. Thus this dimer serves as a potential tumour-selective pH sensitive fluorescent probe and photosensitiser for targeted PDT. All the drugs **5-10** exhibited negligible dark cytotoxicity and enhanced photocytotoxicity.

Yu and Ju *et al.*[77] prepared a selenium-rubyrin photosensitizer **11** with dimethylaminophenyl moiety at meso position of rubyrin to produce an acidic pH-activatable FA-selenium-rubyrin ($NMe_2Se_4N_2$)-loaded nanoparticle targeted photosensitizer **11** (Fig. 17).[77] This photosensitizers can specifically recognize cancer cells via the FA-FA receptor binding and were selectively taken up by cancer cells via receptor-mediated endocytosis to enter lysosomes, where the $NMe_2Se_4N_2$ was activated to produce $^1O_2$ ($\Phi_\Delta$ = 0.69 at pH 5.0 at 635 nm) in cancer cells to induce cell death whereas deactivated at physiological pH ($\Phi_\Delta$ = 0.06 at pH 7.4 at 635 nm) thus preventing the damage to normal cells.

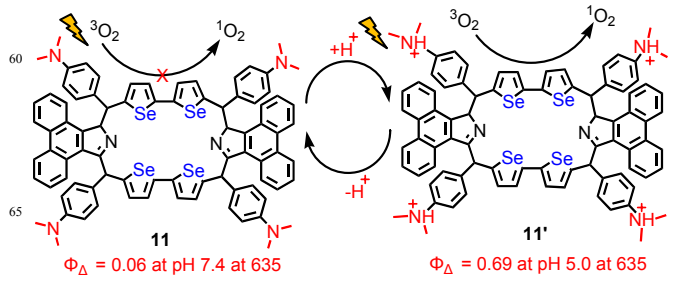


**Fig. 17** Structure of pH-activatable FA-selenium-rubyrin ($NMe_2Se_4N_2$)-loaded nanoparticle targeted photosensitizer **11**.[77]

Most of the photosensitizing agents suffers from poor solubility in water, hence these compounds aggregate in aqueous solutions, which leads to loss of photochemical activity and cell penetrating properties.[78-80]To overcome this issue, nanoparticles are currently being explored for targeted drug delivery. Quantum dots (QDs) are semiconducting nanoparticles with size dimensions in the lower nanometer size range with optical properties superior to organic fluorophores in regard to their absorption cross section, chemical and optical stability, and tunability of the emission wavelength, as well as the easy surface modifcation.[81] Therefore, QDs are potentially interesting candidates as photosensitizers for PDT. To increase rate of $^1O_2$ production, fast energy transfer from the QDs to the photosensitizer is required. The distance between the acceptor and donor has to be minimal (since the rate of resonance energy transfer scales inversely with the sixth power of the distance) and the overlap between electron clouds should be maximal. Phtotosensitizer should be adsorbed directly onto the QD surface or by a short linker for faster energy transfer.

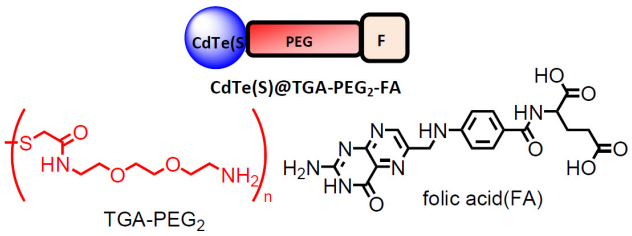


**Fig. 18** QD/spacer conjugates CdTe(S)@ TGA-PEG$_2$-FA, structure of TGA-PEG$_2$ and folic acid(FA).

Barberi-Heyob *et al.*[82] reported hydrophilic near infrared emitting thioglycolic acid(TGA)-capped CdTe(S)-type QDs ( particles diameter ca. 2-10 nm, exhibits large absorption spectra with $\varepsilon = 5 \times 10^5$ $M^{-1}$ $cm^{-1}$, narrow and symmetric emission bands),[83] to conjugate with folic acid to promote photodynamic efficiency using α,ω-poly(ethylene glycol) spacers, the 2,2'-(ethylenedioxy)-bisethylamine $PEG_2$ (Fig. 18). The relatively large hydrodynamic diameters of QDs make it to give low efficiency in triplet energy transfer with surrounding $^3O_2$ molecules to produce $^1O_2$. In case of QD-photosensitizer conjugates the excited singlet and triplet states of the photosensitizer are indirectly generated by nonradiative energy transfer, FRET (Förster Resonance Energy Transfer), from photoactivated QDs.[84-90] Thus, ROS generated by CdSe or


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CdTe-core QDs upon photoactivation due to the interaction of the QD conduction-band electron with the surrounding $O_2$ or water molecules cause irreversible damages and cell death.[91,92] Initially QDs are found to be non-cytotoxic,[86] however recent studies suggest cytotoxicity and photocytoxicity.[93-95] This causes the targeted cellular damages.[96-98] In case of CdTe(S)-type QDs the cytotoxicity is caused by the leakage of $Cd^{2+}$ ions, due to the oxidation of core Cd atoms by molecular oxygen and this ion on binding with sulfohydryl groups of mitochondria proteins, leads to cellular poisoning.[99]

QD-photosensitizer exhibited negligible cytotoxic effect for both cell lines KB cells (FR+) and HT-29 cells (FR-) in absence of light. Significantly improved efficient photocytotoxicity using PEG2 coated QD 4 photosensitizer was observed for KB cells suggesting folic acid-linked drugs in a FR-targeting strategy act as an efficient tool to improve selectivity of anti-cancer treatment for FR+ cancer cells. In contrary, previously reported CdTe quantum dots-methylene blue hybrid photosensitizer by Rakovich *et al* displayed weak phototoxicity due to the lack of folate receptors.[100]

Normally, folic acid receptor is overexpressed on the cell membrane. As a result, folic acid receptor is often used as the target of the PDT reagents. The straightforward method is to link a folate moeity to the triplet photosensitizer. Recently, Ng and Lo *et al*. prepared a styryl Bodipy-folic acid conjugate **11** (Fig. 19), which shows strong absorption at 662 nm, good solubility, and phototoxicity toward KB cells.[101]

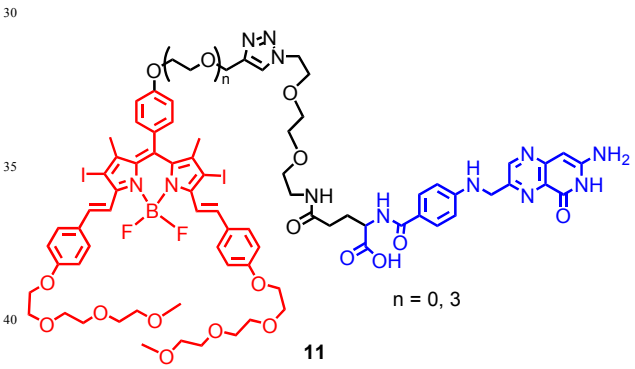


**Fig. 19** Chemical structure of folate-conjugated distyryl BODIPY photosensitizer **11**.[101]

### 2.3. New triplet photosensitizers as potential photodynamic reagents

Until now most of the photodynamic reagents are limited to the porphyrin derivatives, which are difficult to synthesis and purify. It should be pointed out that the absorption of these compounds in visible/near IR spectral region is actually weak. During recent years, some alternative chromophores have been developed which are promising to be used as PDT reagents. These molecules are usually with small moleuclar weight, strong absorption of visible light, and readily derivatizable molecular structures. For example, the Boron dipyrromethane (Bodipy) derivatives, with iodo- or bromo substitution at the 2,6-position.

Nagano *et al.* reported **12** (Fig. 20) can be used for intracellular PDT.[102] The results are promising, but the excitation wavelength of the compound is in the green region, far out of the desired near IR region. Attachment of the iodo-atoms on the π-core of the Bodipy chromophore is essential for efficient ISC.

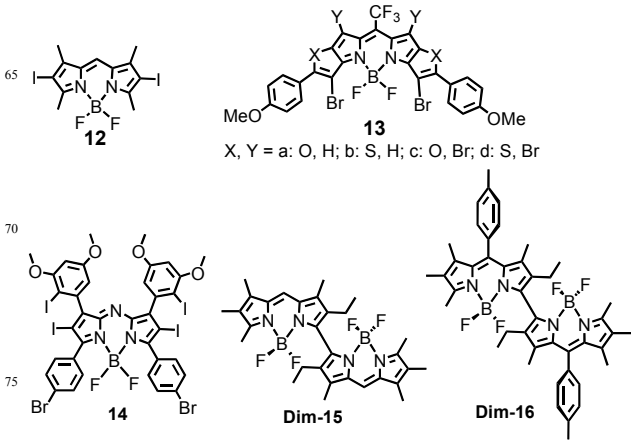


**Fig. 20**. Chemical structure of BODIPY photosensitizers **12-16.**

You *et al.*[103] reported the Bodipy analogues **13** with fused ring structure. The absorption of the compounds are in the 720 – 760 nm range, and the bromo derivative **13c** and **13d** exhibited efficient production of $^1O_2$ (Fig. 20).

Ramaiah *et al*. reported the iodo-aza Bodipy **14** (Fig. 20) as near IR absorption triplet photosensitizers (666 nm. The molar absorption coefficient is up to 69900 $M^{-1}$ $cm^{-1}$).[104,105] **14** shows a high $^1O_2$ quantum yield of 0.78 and triplet state lifetime of 1.6 μs.

Transition metal complexes are interesting candidates for PDT study because these compounds show efficient ISC, and it is well known that there is interaction between the metal and some biomolecules, such as DNA and proteins.[106] Unfortunately, most of the conventional transition metal complexes, such as those of Ru(II), Pt(II), Ir(III) or Re(I), show weak absorption in visible spectral region, and the triplet excited state lifetimes are short.[107] These features are the clear drawbacks for the complexes to be used as PDT reagents. The microenvironment of the intracellular or cancer tissue is hypoxia, thus only those 'sensitive' reagents, i.e. those with longer triplet excited state lifetime, will be effective in PDT. Recently, Thummel and McFarland *et al.* confirmed that the Ru(II) complexes with long-lived triplet excited state are capable sensitive as PDT reagents.[108]

In order to address these challenges, our group made continuing efforts during the last several years. We proposed that the $S_0 \rightarrow {}^1$MLCT transition of the conventional transition metal complexes can be switched to $S_0 \rightarrow {}^1$IL (IL intraligand) transition by selection of proper ligands. $S_0 \rightarrow {}^1$MLCT transition is usually a weakly allowed transition due to the charge transfer feature. Instead, the $S_0 \rightarrow {}^1$IL transtion is strongly allowed due to the π-π* feature of the transition. As a result, the absorption of visible light can be substantially enhanced. On the other hand, the $^3$IL state will give much longer triplet excited state lifetime than the $^3$MLCT state, due to the less involvment of metal in the $^3$IL state.

Following these lines, we have prepared a series of Pt(II), Ir(III), Ru(III) and Re(I) complexes that show strong absorption of visible light and long-lived triplet excited state.[4e,109,110]

The new triplet photosensitizers mentioned above still share a

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disadvantage, that is, heavy atoms such as bromo, iodo, or Pt(II), Ru(II), Ir(III) etc. are present in the molecules. This may cause dark toxicity for the reagents, which is detrimental for PDT reagents. Therefore, heavy atom-free organic triplet photosensitizers are highly desired.[4b,4e] From a point of view of photochemistry, it is still a challenge to design heavy atom-free organic triplet photosensitizers because the ISC property of a heavy atom-free orgnaic chromophore is almost unpredictable.

In order to overcome this challenge, a few methods have been developed. First, exciton coupling effect was employed to produce triplet excited state in chromophore dimers (Bodipy dimer dim **15** and dim **16**. Fig. 20).[111,112] Akkaya *et al.* developed a similar approach to produce triplet excited state.[113] However, these compounds show absorption in the visible region (green or yellow) and it is difficult to extend the absorption wavelength to the near IR region by following this approach.[114]

Recently, we proposed a method to design heavy atom-free organic triplet photosensitizers based on concept of intramolecular 'spin converter'.[115-117] This unit is expected to be promote efficient ISC, but it does not necessarily show strong absorption of visible light. In this case attaching of a visible light-harvesting organic chromophore with appropriate energy level will lead to RET to the spin converter (the singlet energy acceptor). Thus triplet excited state can be produced by the ISC effect of the spin converter.

Concerning this aspect, $C_{60}$ is an ideal spin converter.[105] Attaching of visible light-harvesting organic chromophores will give heavy atom free triplet photosensitizers showing strong absorption of visible light and long-lived triplet excited states. We prepared series of $C_{60}$-organic chromophore hybrid triplet photosensitizers **17** to **21** (Fig. 21).[115] These compounds show strong absorption of visible light and long-lived triplet excited states and used successfully for triplet-triplet annihilation upconversion (TTA UC) and photoredox catalytic organic transformations.[115-117,118]

The advantage of this strategy is the visible light-harvesting antenna can be feasibly changed to tune the absorption wavelength and other properties, as long as the $S_1$ state energy level of the antenna is higher than that of $C_{60}$ (ca. 1.72 eV).[119,120] In reality, any antenna giving absorption/emission wavelength shorter than 700 nm can be used for $C_{60}$ dyad based triplet photosensitizer. However, one has to be taken into account of the intramolecular electron transfer in the designing of these dyads, which may compromise the triplet state yields.[121]

We also proposed a strategy to prepare broadband visible light-absorbing triplet photosensitizers based on RET effect and spin converter. Conventional triplet photosensitizers contain only a single visible light-absorbing chromophore, as a result, there is only one major absorption band in visible spectral region.[4e] Based on dyads or triads showing RET effect and singlet energy acceptor as the spin converter, broadband visible light-absorbing triplet photosensitizers **20** to **30** can be designed (Fig. 22-24).[117,121]

Finally, it should be pointed out that most of these new triplet photosensitizers have not been used for target PDT studies. But recently Thummel and McFarland *et al.*[108] show that the Ru(II) complex with long-lived triplet excited state are highly active PDT agents. This invention is encouraging to evaluate the new PSs as targeted PDT agents. Since, these molecules have novel properties required for efficient phototoxicity such as strong absorption of visible light, long-lived triplet excited states, and readily derivatizable molecular structures. Thus, it is worthwhile to study of the applications of these new PSs in target PDT.

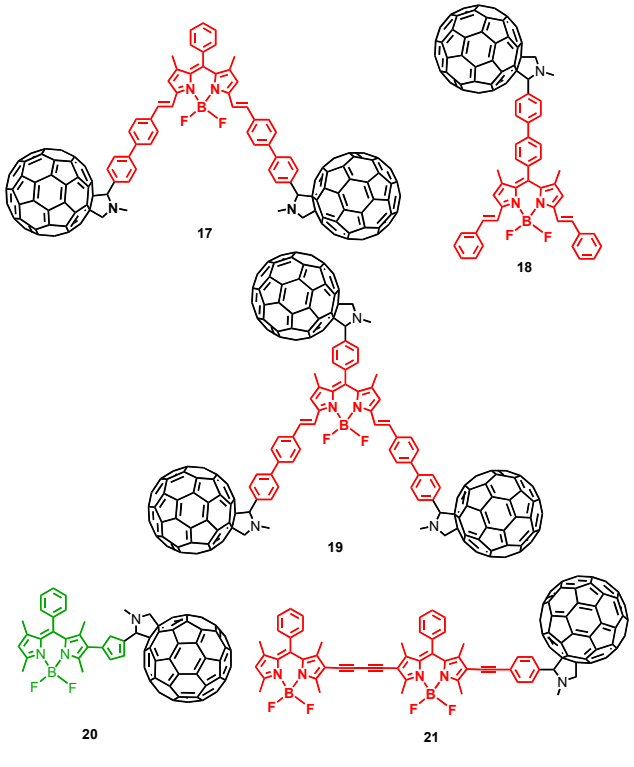


**Fig. 21** Structure of $C_{60}$-organic chromophore hybrid as heavy atom-free triplet photosensitizers with predictable ISC.

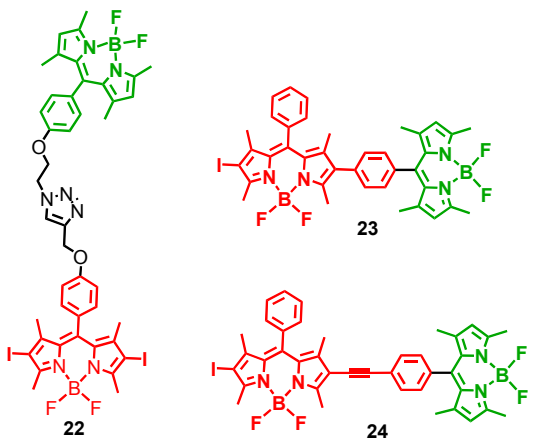


**Fig. 22.** Structure of broadband visible light-absorbing triplet photosensitizers **22-24**.[121a]


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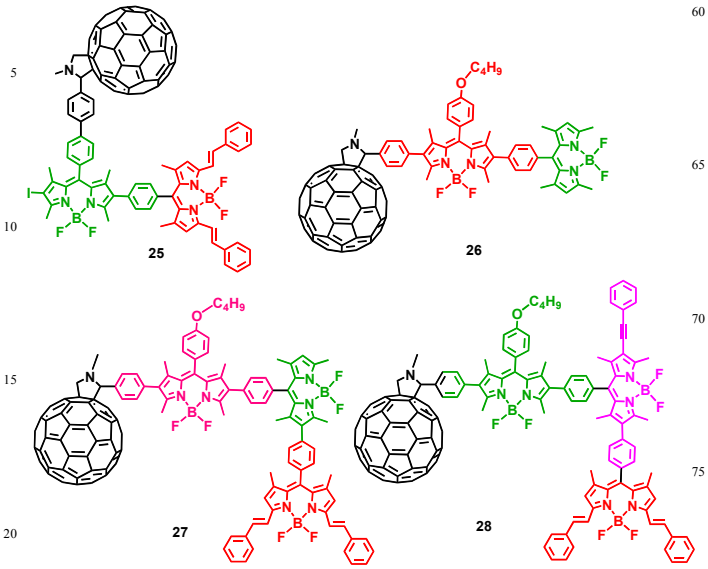


**Fig. 23.** Structure of the broadband visible light-absorbing triplet photosensitizers **25-28**.[117b]

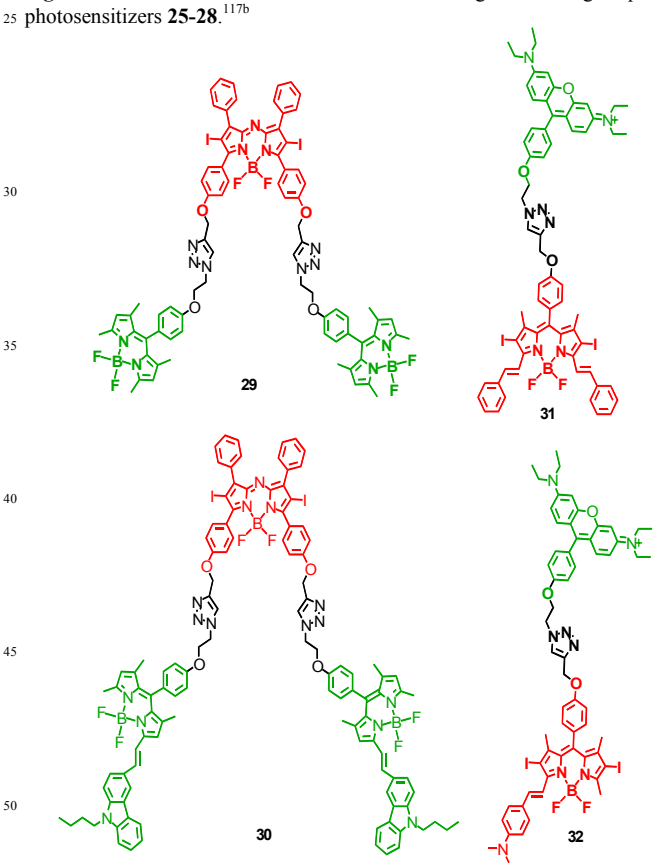


**Fig. 24.** Structure of the broadband visible light-absorbing triplet photosensitizers **29-32**.[121b,c]

## 3. Miscellaneous

Although PDT has emerged as a therapeutic agent for treating tumors. The requirement of an external light source limits its effective application in far deeper tissue penetration due to the absorption and scattering by biological tissues. Thus Wang *et al.*[122] for the first time developed a new bioluminescence resonance energy transfer (BRET) based method to target tumor cells. The photosensitizers are stimulated by chemical molecules instead of external light irradiation. Hydrogen peroxide, and horseradish peroxidase (HRP) as bioluminescent molecules were used in this method, and a cationic oligo (p-phenylene vinylene) (OPV) **31** as the photosensitizer (Fig. 25). The bioluminescence of luminol was absorbed through BRET process as the donor-acceptor pair. The BRET produced due to the electrostatic interactions between the negatively charged luminol oxidation product (dianion) and cationic OPV. At this stage the sensitization of oxygen molecules in the surroundings, takes place by the excited OPV (attached to negatively charged cells) and produce ROS to kill the targeted cancer cells.

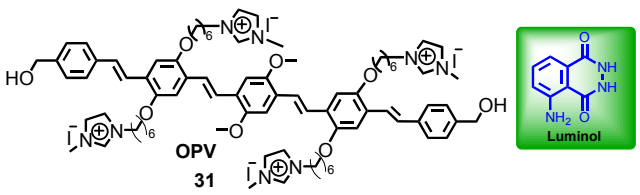


**Fig. 25** Chemical structure of OPV photosensitizer **31** and bioluminescent molecule luminal.[122]

Recently, Wang *et al.*[123] integrated nanoplatform for targeted PDT and imaging of cancer cells using folic acid and horseradish peroxidase (HRP)-bifunctionalized semiconducting polymer dots (FH-Pdots). In the FH-Pdots, meta-tetra(hydroxyphenyl)-chlorin (*m*-THPC) was used as photosensitizer to produce ROS. Fluorescent semiconducting polymer poly[2-methoxy-5-((2-ethylhexyl)oxy)-pphenylenevinyl-ene] was used as light antenna and hydrophobic matrix for incorporating m-THPC. An amphiphilic Janus dendrimer was used as a surface functionalization agent to conjugate HRP and aminated folic acid onto the surface of FH-Pdots. The doped m-THPC found to be simultaneously excited with on-site luminol−$H_2O_2$−HRP either through direct chemiluminescence resonance energy transfer (CRET) and indirect CRET-to-FRET and to produce ROS with high efficiency.

You *et al.*[124] introduced the photo-unclick chemistry of aminoacrylates as photo-labile linker which could be cleaved to release parent drugs on oxidation by $^1O_2$. Recently the same authors used the photo-unclick chemistry to prepare a anticancer far-red-light-activatable prodrug of combretastatin **A-4** (CA4) and CMP−L−$CA_4$. Where CMP is dithiaporphyrin, a photosensitizer, and L is an aminoacrylate linker (Fig. 27). The aminoacrylate linker of the prodrug was cleaved upon photoirradiation ($\lambda_{ex}$ = 690 nm) and rapidly releasing anticancer drug, CA4 (>80% in 10 min) (Fig. 26). The $IC_{50}$ of CMP−L−CA4 was found to be 6-fold increased in MCF-7 on photoirradiation due to the release of CA4 and also had better antitumor efficacy in vivo as compared to its noncleavable (NC) analog, CMP−NCL−CA4. The increase in the fluorescence intensity upon irradiation due to the release of fluorescent rhodamine dye confirmed the oxidative cleavage of the

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aminoacrylate linker of the minimally fluorescent FRET optical probe (Fig. 28), CMP−L−Rh in mouse tissue by using an in vivo optical imaging.

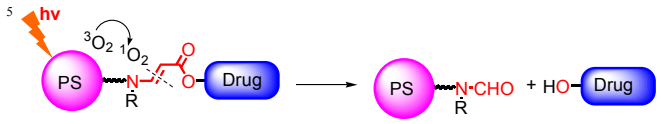


**Fig. 26** Release of a drug from tissue-penetrable-light-activatable prodrug via photo-unclick chemistry.[124]

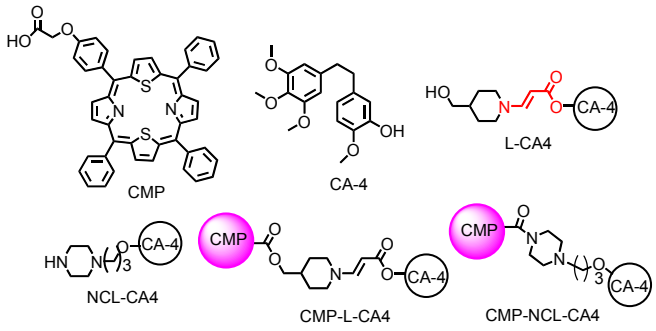


**Fig. 27** Structure of CMP = core-modified porphyrin, combretastatin A4 (CA-4), L-CA4, NCL-CA4, CMP-LCA4 and CMP-NCL-CA4 (CMP is dithiaporphyrin, a photosensitizer, and L is an aminoacrylate linker).[124]

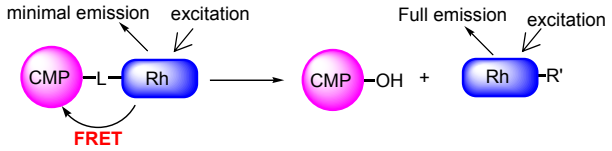


**Fig. 28** Schematic representation of CMP−L−Rh (CMP = core-modified, porphyrin, L = aminoacrylate linker, Rh = Rhodamine).[124]

## 4. Conclusion

The challenges of cell resistance towards conventional chemotherapeutic drugs and continuous toxicity on healthy tissues give impetus to the development of new therapeutic reagents. In this direction, the TPDT (targeted photodynamic therapy) has emerged as more reliable and acceptable method during this decade in curing the tumor malignant.

In this review, we summarized the recent developments on the targeted photodynamic therapeutical agents and some of ongoing efforts in the designing of efficient photosensitizers. The main focus of the present review on TPDT is tumor-specific enzyme activatable PDT agents. The following targeting strategies are introduced: (1) tumor-specific enzyme targeted photodynamic molecular beacons – a FRET based target-activatable probes, (2) the PDT reagents target the acidic microenvironment, i. e. the PDT reagents that are sensitive to pH of cell environments and activatable at acidic conditions; (3) those target the overexpressed folic acid receptors on the cancer cell surfaces. Hence, activatable photosensitizers can selectively destroy cancer cells without any damage to normal cells. Thus, activatable photosensitizers are assumed to show minimal/negligible dark cytotoxicity.

Despite these developents, it is noteworthy to step-forward towards the development of triplet photosensitizer, as one of the less developed key components of the targeting PDT agents. Recently a series of transition metal complexes that show strong absorption of visible light and long-lived triplet excited states have been reported. PDT reagents with long-lived triplet excited state is crucial because the microenvironment of tumor tissue is usually hypoxia. Under this condition only triplet photosensitizers with long-living triplet excited state can sensitizing $^1O_2$ efficiently. Some organic triplet photosensitizers include heavy atom-free organic triplet photosensitizers have also been reported. Still much room is left for application of these new triplet photosensitizers in targeted PDT studies. Our group has been continuing interests to develop efficient triplet photosensitizers.

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## Notes and references

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