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Research progress of fullerenes and their derivatives in the field of PDT

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Abstract

In contemporary studies, the predominant utilization of C₆₀ derivatives pertains to their role as photosensitizers or agents that scavenge free radicals. The intriguing coexistence of these divergent functionalities has prompted extensive investigation into water-soluble fullerenes. The photodynamic properties of these compounds find practical applications in DNA cleavage, antitumor interventions, and antibacterial endeavors. Consequently, photodynamic therapy is progressively emerging as a pivotal therapeutic modality within the biomedical domain, owing to its notable levels of safety and efficacy. The essential components of photodynamic therapy encompass light of the suitable wavelength, oxygen, and a photosensitizer, wherein the reactive oxygen species generated by the photosensitizer play a pivotal role in the therapeutic mechanism. The remarkable ability of fullerenes to generate singlet oxygen has garnered significant attention from scholars worldwide. Nevertheless, the limited permeability of fullerenes across cell membranes owing to their low water solubility necessitates their modification to enhance their efficacy and utilization. This paper reviews the applications of fullerene derivatives as photosensitizers in antitumor and antibacterial fields for the recent years.

Keywords : fullerene derivatives; photosensitizer; photodynamic therapy; ROS generation; cell death

Introduction

In history, our ancestors were already using light to treat various diseases, including psoriasis, leukoplakia, and even skin cancer. In the late 19th century, Niels Finsen demonstrated the use of concentrated ultraviolet (UV) light beams to treat patients with lupus erythematosus using light therapy^[1]. Finsen was awarded the Nobel Prize in Physiology and Medicine in 1903 for pioneering modern light therapy^[2]. Subsequently, photosensitizers such as eosin and acridine were developed and used to treat *Clostridium difficile* and skin cancer^[3]. In 1907, von Tappeiner and Jesionek proposed the concept of photodynamic therapy (PDT)^[4]. In 1913, Friedrich Meyer-Betz conducted the first study of human PDT in which he tested the effect of hematoporphyrin on his own skin^[5-7]. However, the next significant breakthrough did not occur until the 1970s. In 1975, Thomas Dougherty and his colleagues reported that a hematoporphyrin derivative could completely eliminate mammary tumors in mice when exposed to red light irradiation^[8]. In the same year, skin cancer was successfully treated with PDT for the first time. After decades of development, PDT has emerged as a promising clinical approach for the treatment of cancer and microbial infections. About 20 photosensitizers have been approved for clinical use or are currently undergoing clinical trials^[9-11].

C₆₀, the most common fullerene, consists of a balanced distribution of carbon atoms in a unique football-like cage structure, hence the name "football alkene"^[12]. C₆₀ is a three-dimensional aromatic compound, and its formation mechanism is very similar to that of graphite. In the sp² hybrid orbital, seemingly isolated carbon atoms will be connected to each other under the action of chemical bonds to form tightly connected spherical bodies^[13]. The football-shaped cage structure of this molecule is highly stable and exhibits a high degree of symmetry. Fullerenes are of interest as potential new photosensitizers because of their unique structural features, which provide both electron-accepting and electron-donating capabilities^[14-16].

Early experiments have shown that fullerenes are capable of interacting with solutes in the environment and biological systems, and they have significant effects on the metabolism, transport, and reactivity of organisms^[17, 18]. On the other hand, fullerenes are expected to be utilized as effective photosensitizers for photodynamic therapy because of their high capacity to produce singlet oxygen (¹O₂)^[19-25]. However, because it is insoluble in water, its penetration into the cell membrane is not strong^[26-28]. The ground-state absorption spectrum of C₆₀ is characterized by a strong absorption in the ultraviolet region and a weaker band extending to 700 nm in the entire visible spectral range^[29-32]. It is necessary to find suitable methods to enhance the production of reactive oxygen species. Hence, we examine the research applications of fullerene derivatives as photosensitizers for photodynamic therapy in the areas of antitumor and antibacterial treatments by scholars from both domestic and international institutions.

1. Principles, advantages and disadvantages of PDT treatment

Photodynamic therapy is an intriguing and dynamic treatment modality that has gradually become an important approach in the biomedical field^[33, 34]. Under light conditions, photosensitizers (PS) generate reactive oxygen species (ROS) that can kill target cells^[35-38]. PDT offers the advantages of spatiotemporal selectivity, low systemic toxicity, minimal invasiveness, negligible drug resistance, and reduced long-term morbidity, ultimately enhancing the quality of life of patients. The clinical use of PDT has significantly increased over the past few decades, coinciding with the approval of a wide range of photosensitizers (PSs) in clinical trials^[39, 40]. The three key elements of photodynamic therapy are photosensitizers, light of an appropriate wavelength, and oxygen. Photosensitizers play a crucial role in photodynamic therapy. The photosensitizers are effectively excited by light of specific wavelengths, transferring energy to the ground state of oxygen and generating reactive oxygen species such as singlet oxygen or superoxide ions. These can selectively damage proteins, DNA, and membranes, thereby inducing apoptosis of diseased cells^[41-47].

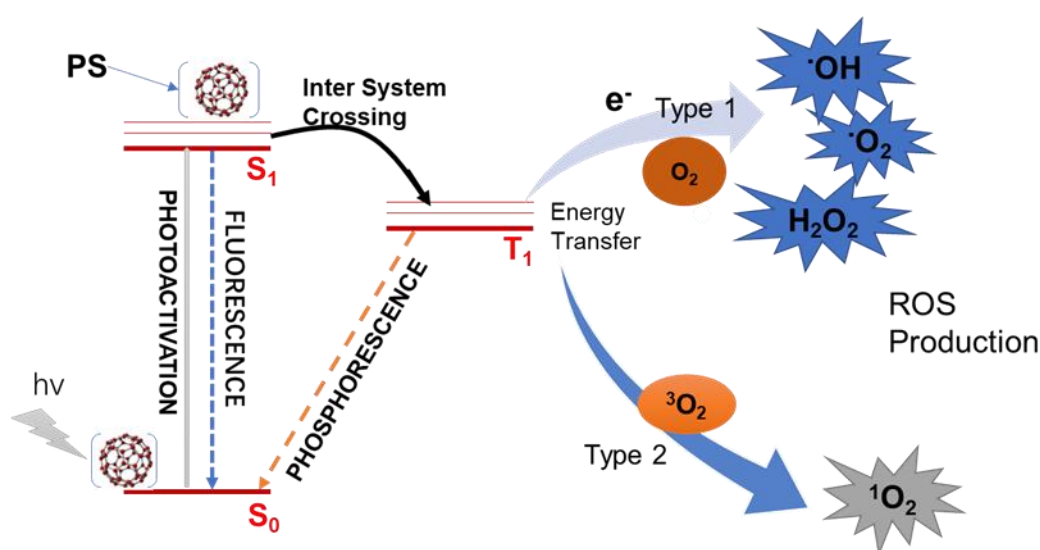


Fig. 1 Diagram of the mechanism of action of photosensitizers in photodynamic therapy

Photodynamic therapy has **two mechanisms**^[48], (1) Type I reaction involves the photosensitizer molecule absorbing light and transitioning from the ground state to the short-lived singly excited state, then moving to the long-lived triple excited state and undergoing a hydrogen pumping or electron transfer reaction with the substrate to produce oxygen radicals or radical ions; (2) Type II reaction involves the photosensitizer molecule in the triple excited state interacting with oxygen to produce singlet oxygen (Fig. 1)^{[51][49]}.

The treatment can be used to address tumors, dermatitis, psoriasis, and other conditions. In traditional cancer treatment, surgery, radiotherapy, and chemotherapy are commonly utilized. Tumor cells are typically removed during surgery, but the recurrence rate is high^[50]. The side effects of radiotherapy are significant, for instance, the treatment of breast cancer often results in damage to the heart^[51, 52]. Chemotherapy is the most widely used treatment method. In addition to suppressing tumor cells, radiotherapy can also damage normal cells and tissues, and is associated with hair loss^[53]. Photodynamic therapy does not have these issues. The photosensitizer is only activated in the presence of light, which gives PDT several advantages over other conventional cancer treatments^[54]. These advantages include low systemic toxicity, the ability to selectively

destroy tumor cells, the potential for use in combination with other treatment modalities, relatively low treatment cost, outpatient usability, and the ability for multiple applications with minimal scarring after healing ^[55, 56]. On the other hand, PDT has a number of disadvantages. Most of the PS used in PDT are hydrophobic molecules, which results in poor cell specificity. Additionally, the light penetration through the tissue is minimal, limiting PDT treatment to superficial tumors such as skin, nasopharyngeal, and oral cancers ^[57-61].

2. Traditional photosensitizers

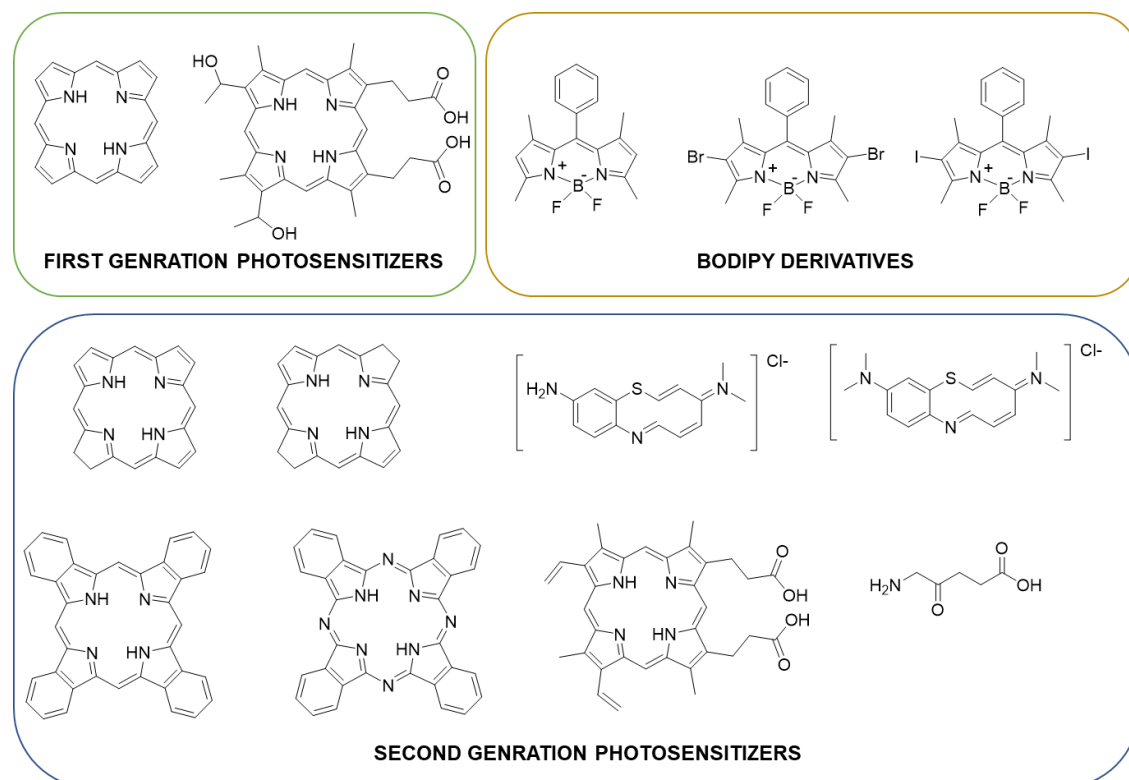


Fig .2 Representative drugs in traditional photosensitizers

The first generation of photosensitizers for PDT in clinical use are porphyrin derivatives (HpD) ^[62, 63], Although porphyrin derivatives have excellent PDT efficacy, their poor photostability, limited solubility in water, severe skin photosensitization, and sometimes undesired biodistribution specificity limit their efficacy and use ^[64-66]. Therefore, efforts have been made to develop novel photosensitizers. Researchers have been working on developing new photosensitizers, mainly includes rhodopsin derivatives, sulfur-containing cationic dyes, square acid derivatives, BODIPY derivatives, and their analogs (Fig. 2) ^[67]. Despite the advantages of these compounds in terms of water solubility, the PDT efficacy of these emerging nonporphyrin photosensitizers is still insufficient for clinical use. Limitations of photodynamic therapy (PDT) include photosensitivity after treatment, the requirement for optimal photoactivation of the tumor for therapeutic efficacy, and the critical need for oxygen presence during PDT. Another significant drawback is the current lack of photosensitizers for treating metastatic cancer.

3. Fullerene derivative photosensitizers

In 1985, the accidental discovery of fullerenes marked the introduction of carbon structures into the realm of symmetrical nanomaterials. This concept was inspired by the work of the architectural master Buckminster Fuller, specifically the United States pavilion at the Montreal World Exposition, which featured a large grid-like dome. The research team named this type of carbon molecule "fullerene" in honor of Buckminster Fuller^[68]. In 1990, Wolfgang Krätschmer of the Max Planck Institute for Nuclear Physics and Donald Huffman of the University of Arizona, along with their students, achieved mass production of fullerenes^[69]. Finally, in 1996, Kroto, Smalley, and Curl were awarded the Nobel Prize in Chemistry for the discovery of C₆₀. In recent years, the potential applications of fullerenes and their derivatives have expanded, particularly in the fields of biology and medicine. They can be utilized as DNA photocleavage agents for PDT, anti-HIV protease inhibitors, antibacterial agents, and photosensitizers, extensive attention^[70, 71].

C₆₀ is a stable, large π -conjugated system composed of 60 sp²-hybridized carbon atoms. Its shape is that of an icosahedron, with 12 pentagons separated by 20 hexagons, forming a highly symmetric cage. The diameter of the C₆₀ molecule is 0.710 ± 0.007 nm. The outer and inner diameters of the π electron cloud are estimated to be 0.340 nm and 0.350 nm respectively, with a bond energy per atom of 7.40 eV^[13]. Its distinctive architecture gives it unique physicochemical properties. It has not only electron-accepting but also electron-donating capabilities^[17]. C₆₀ undergoes a transformation from a ground state (S₀) to an excited singlet state (S₁) when exposed to light, and then quickly transitions to a lower energy triplet state (T₁) with a long lifetime of 40 ± 4 μ s^[72, 73]. This extended lifetime is crucial for its function as a photosensitizer.

Experimental studies have shown that various water-soluble fullerenes can transfer energy from the excited trilinear state of the fullerene to oxygen, effectively producing singlet oxygen (¹O₂) upon irradiation^[74]. It has been reported that photoirradiation of fullerenes in aqueous systems leads to the generation of radical anions (C₆₀^{•-}), followed by electron transfer to produce superoxide anion radicals (O₂^{•-}) and hydroxyl radicals ([•]OH). These photochemical mechanisms occur during photodynamic therapy in the presence of electron donors such as NADH or amines, demonstrating a significant pathway for the phototoxicity of fullerenes^[7, 75-77]. Fullerenes are also a promising nanostructure for drug delivery due to their strong structural properties^[78, 79]. Due to their ability to carry multiple drug payloads and enable targeted delivery, fullerenes can help mitigate various side effects of chemotherapy^[80, 81]. For example, spherical nanostructured buckyballs composed of amphiphilic fullerenes and hydrophobic regions have been used to conceal the hydrophilic surface of paclitaxel. Studies have shown that this complex can effectively enhance drug absorption, thereby improving the therapeutic effect of cancer^[82]. Therefore, due to their unique physical and chemical properties, fullerenes can be used in the preparation of a variety of drugs, such as anti-tumor and anti-inflammatory medications.

C₆₀ has considerable advantages over conventional photosensitizers used in photodynamic therapy^[83]:

(i) Fullerenes exhibit high photostability and less photobleaching compared to the conventional dyes used in PDT.

(ii) Fullerenes follow two photophysical mechanisms, while conventional dyes mainly exhibit type II mechanisms.

(iii) The yield of oxygen in the singlet state is close to 1^[84].

However, fullerenes strongly absorb in the ultraviolet region of the spectrum and moderately in the visible region. Consequently, numerous scholars have conducted extensive research to enhance the absorption intensity of fullerenes in the visible light region, achieving some success.

3.1 Modification of fullerenes and synthesis of their derivatives

Pure fullerene is a black crystal and powder that is insoluble in water and solvents that accept protons. It is soluble in halogen and alkyl-substituted benzene (1,2-dichlorobenzene 27 mg/ml)^[85]. Any class of organic compound can be covalently bonded to a fullerene nucleus through a combination of nucleophilic or electrophilic addition, cycloaddition, and radical addition. The structural and electronic properties of C₆₀ offer the potential to undergo various chemical transformations, leading to a wide variety of biologically active water-soluble derivatives. Fullerene derivatives play a crucial role in the advancement of products associated with nanobiotechnology and nanomedicine^[86-88]. Currently, there are two main approaches for modifying fullerenes^[89]: (1) using partial solubilizers to cover the fullerene surface to enhance water solubility, and (2) chemically modifying fullerenes through covalent functionalization based on their structural and physicochemical properties.

Two main modification methods are used in the application of fullerene derivatives in PDT, and they are described in detail. Fullerenes are chemically modified using the Bingle and Prato reactions to synthesize target products, including fullerene carboxylic acid derivatives, fullerene quaternary amine derivatives, fullerene peptide derivatives, and fullerene sugar derivatives^[90]. The following diagram illustrates the mechanism of the Bingle and Prato reactions (Fig. 3).

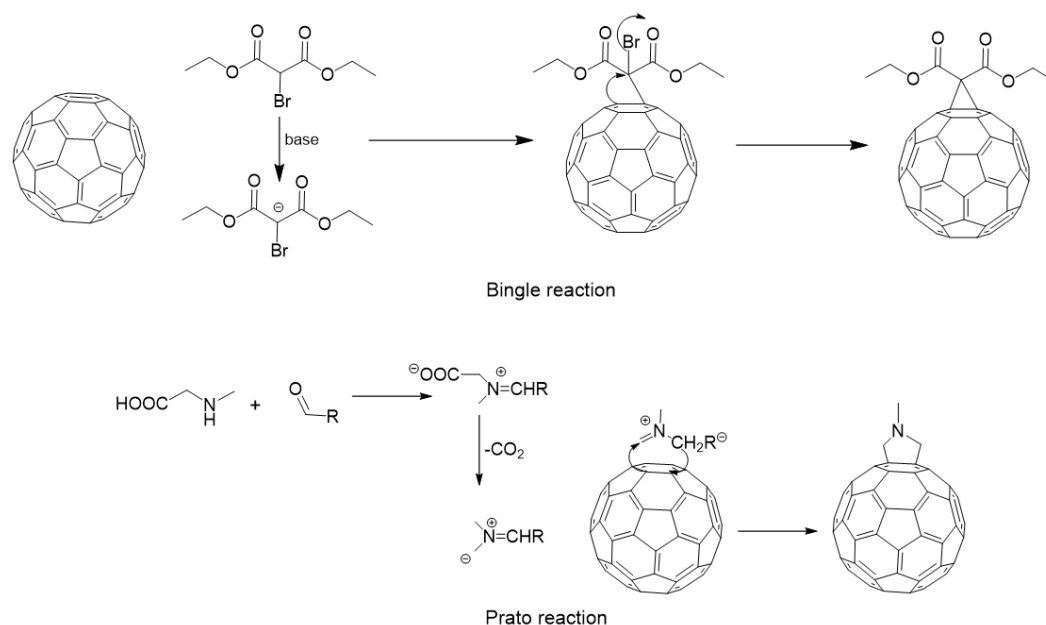


Fig. 3 Bingle reaction and Prato reaction mechanism diagram

3.2 Currently available fullerene photosensitizers

Fullerol

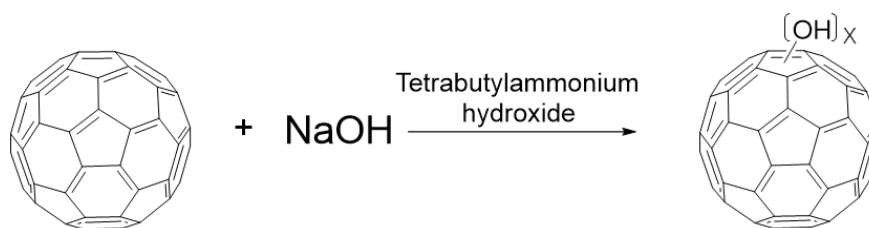


Fig. 4 Synthesis of Fullerol

Chemical modification of fullerene C_{60} molecules by attaching hydroxyl groups is a simple and straightforward method for synthesizing water-soluble fullerenes, known as fullerols or fullerols ($C_{60}(OH)_n$, $n=2-72$). The reagents commonly used for the synthesis of fullerols include $H_2SO_4:SO_3$, HNO_3 , O_2 , and H_2O_2 ^[91]. The fullerol $C_{60}(OH)_{24}$ has a diameter of approximately 1 nm and features a symmetrically arranged hydroxyl group on the C_{60} sphere. Fullerol is a dark brown amorphous substance that is soluble in water and DMSO ^[92, 93]. Fullerol is soluble in water at concentrations ranging from 10^{-3} mol/L to 5×10^{-3} mol/L and at pH levels ranging from 3.5 to 9.8. Fullerol reacts rapidly and irreversibly with various metal salts in aqueous conditions to produce insoluble metal-hydroxyl fullerene crosspolymers (M-fullerol). The interaction of fullerol with biomaterials is also crucial for the development of fullerene-based pharmaceuticals.

Several of the chemical properties of fullerols may significantly impact their biological activity. Similar to non-hydroxylated water-soluble fullerene derivatives, the most significant characteristics of fullerol in its bioactive mechanism are photosensitivity and free radical scavenging activity. $C_{60}(OH)_{18-20}$ was Several of the chemical properties of fullerols may significantly impact their biological activity. Similar to non-hydroxylated water-soluble fullerene derivatives, the most significant characteristics of fullerol in its bioactive mechanism are photosensitivity and free radical scavenging activity O^{2-} ^[94]. As research progressed, fullerol was also utilized in photodynamic therapy, with folic acid (FA) being reported as an effective target for HeLa cells. The compound DOX-hydrazone- $C_{60}(OH)_{21}$ -FA is well dispersed in water and forms aggregates when exposed to light at a wavelength of 135 nm. The optimal drug range is 100 nm to 200 nm, primarily because of the strong osmotic retention (EPR) effect of the nanoparticles ^[95]. After irradiation with 460-485 nm light, hydrazone- $C_{60}(OH)_{21}$ -FA was able to reduce the viability of HeLa cells from 100% to 60%. The single linear oxygen yield of hydrazone- $C_{60}(OH)_{21}$ -FA was tested at 0.4. Photodynamic therapy enhanced the inhibitory effect of DOX on HeLa cells. In addition, DOX- $C_{60}(OH)_{21}$ -FA was less toxic to HeLa cells than single DOX in the absence of light. This phenomenon was explained by the free radical scavenging effect of $C_{60}(OH)_n$ ^[95]. A study on $C_{60}(OH)_{30}$ against multidrug-resistant bacteria was just published in March 2022 ^[96].

Fullerene carboxylic acid derivatives

C_{60} malonic acid derivatives are primarily synthesized through the Bingle reaction, as illustrated in Figure 5. In the Bingle cycloaddition reaction, the fullerene carboxylate is catalyzed by fullerene and diethyl malonate. $C_{60}[C(COOH)_2]_3$ possesses inherent free radical scavenging ability and can be utilized for protease detection. Like fullerenols, fullerene carboxylic acids also exhibit antioxidant effects. C_3C_{60} and D_3C_{60} are regioisomers of $C_{60}[C(COOH)_2]_3$. C_3C_{60} is more potent than D_3C_{60} in providing antioxidant protection. There are two reasons for this: (1) C_3C_{60} interacts more strongly with the membrane; (2) due to the dipole structure, the C_{60} cage adjacent to the malonate group is electron deficient, which gives this region a strong ability to attract O_2 [97, 98]. In contrast, the electron density is evenly distributed throughout the D_3C_{60} cage because of the symmetrical distribution of the malonate group.

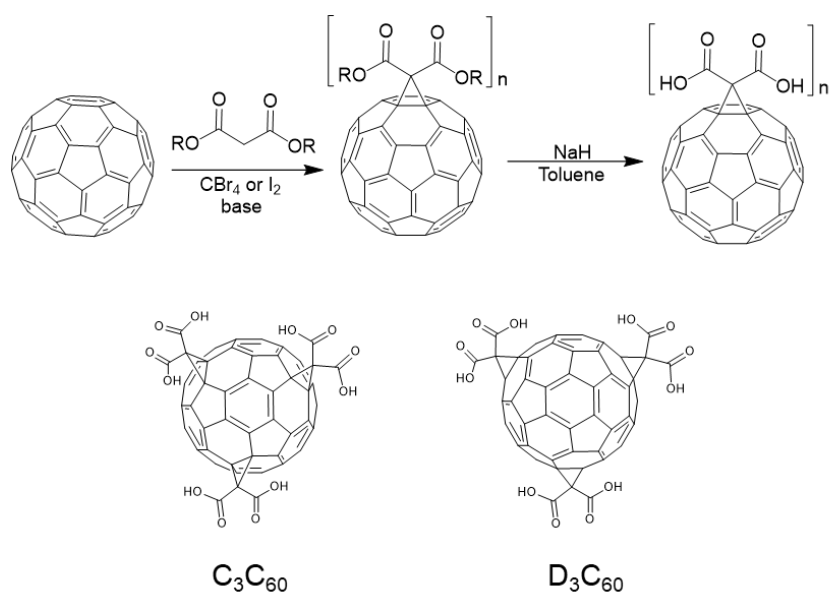


Fig .5 Synthesis process and iconic products of fullerene carboxylic acid derivatives

Malonic acid C_{60s} act as reactive oxygen species (ROS) generators when they are dispersed by human serum albumin (HSA) and PEG-modified poly(amidoamine) (PAMAM) dendrimers. Both the C_3C_{60} /HAS complex and the malonate C_{60s} /PEG-PAMAM were non-toxic in the absence of light. The 1O_2 sub-yield of the C_3C_{60} /HAS complex is 0.46, which is comparable to the singlet oxygen yield (0.48) of the monomeric C_3C_{60} . The C_3C_{60} /HAS complex (20 μ M) was able to induce 57% cell death in LY80 tumor cells after exposure to light at 350–600 nm [99]. Monomalonate C_{60} (MC_{60}) and dimalonic acid (DC_{60}) were encapsulated by PEG-PAMAM through hydrophobic and electrostatic interactions, involving the tertiary amine of PAMAM and the $-COOH$ group of malonic acid C_{60} [100]. At physiological pH, MC_{60} /PEG-PAMAM is more stable than DC_{60} /PEG-PAMAM. MC_{60} is released in an acidic environment, and PEG-PAMAM/ MC_{60} accumulates in tumor cells due to the enhanced permeability and retention (EPR) effect and the relatively acidic tumor microenvironment, leading to a reduction in the survival rate of HeLa cells under laser irradiation from 80% to 30%.

Fullerene quaternary ammonium derivatives

These cationic fullerene derivatives are produced through methylation after the 1,3-dipolar

cycloaddition of C₆₀ and azomethimine. They are synthesized from amino acids, aldehydes, or aziridines (Fig 6) ^[101]. Various isomers were obtained through the Prato reaction, but they exhibited similar activities ^[20]. Therefore, mixtures of isomers are used in additional biological applications. These cationic water-soluble derivatives of fullerene act as ROS producers. Under visible light at 2 J/cm² (400-700 nm), compound 2 at a concentration of 1 μM killed *Staphylococcus aureus*, reducing the population of Gram-positive bacteria by 4-5 logs ^[102]. After treatment with compound 2 (10 μM) at 2J/cm², 4-6 orders of magnitude of Gram-negative bacteria *E. coli*, which have a less permeable outer membrane, died. At 16 J/cm², the production by compound 2 resulted in the death of 3-5 orders of magnitude of the Gram-negative bacterium *Pseudomonas aeruginosa*, which was highly resistant. Both compounds 2 and 3 were more effective than compound 1, which was attributed to the cationic charge. The higher cationic charge facilitated the binding of the microbial membrane to the negatively charged site at position ^[103]. Further studies confirmed this result: compound 7 (with 6 cations) > compound 6 (with 2 cations), the two regional isomers 9 and 10 (with 2 cations) > compound 8 (with 1 cation); compound 13 (with 2 cations) > compound 12 (with 1 cation) > compound 11 (without cation) ^[103, 104].

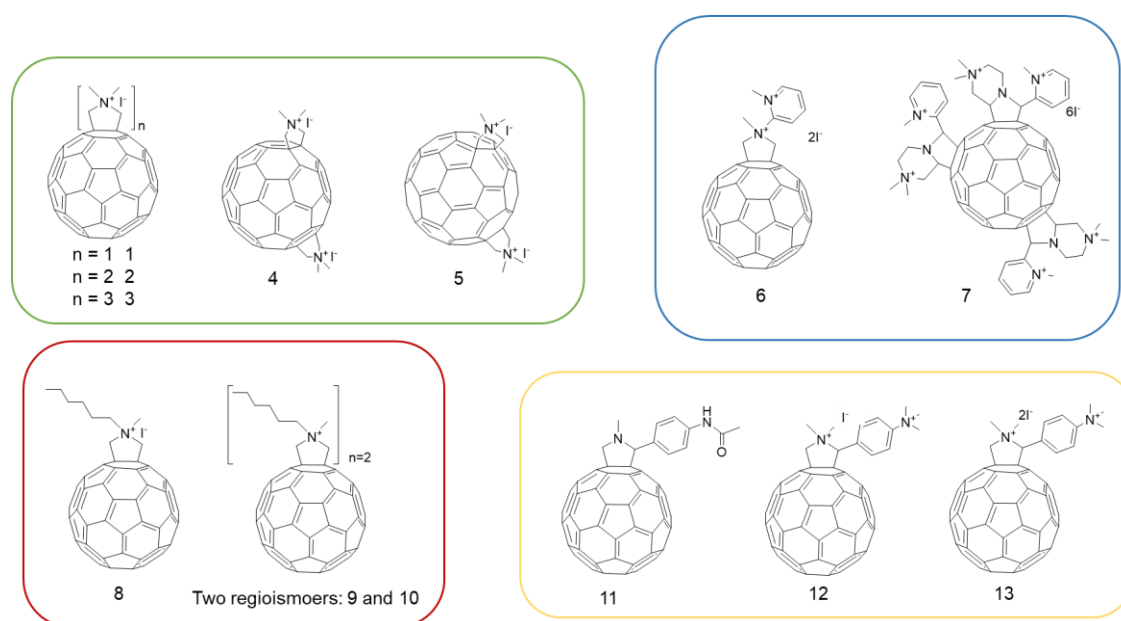


Fig. 6 Fullerene Quaternary Ammonium Derivatives

Although compound 3, with 3 cations, inhibited *S. aureus* more strongly than compound 2, which has 2 cations, compounds 2 and 3 showed similar efficacy against *E. coli* and *P. aeruginosa*. This similarity was attributed to relatively poor cellular uptake ^[103]. Additionally, the Gram-positive bacteria *S. aureus* absorbed compounds 2 and 3 more readily than the Gram-negative *E. coli* and *P. aeruginosa*. Gram-negative bacteria have different components compared to Gram-positive bacteria. The outer layer of Gram-positive bacteria is composed of peptidoglycan and lipoteichoic acid or β-glucan, allowing cationic water-soluble fullerene derivatives to easily penetrate the bacteria's cytoplasm. Gram-negative bacteria, however, have a double-membrane structure that acts as a diffusion barrier to prevent the diffusion of drugs. The entry of drugs into Gram-negative bacteria is facilitated by "self-promoted uptake," wherein cationic water-soluble fullerene derivatives are displaced by essential ions (e.g., Mg²⁺, Ca²⁺), which then attach to the

lipopolysaccharide of the outer membrane and penetrate into the cell^[105].

Fullerene carbohydrate derivatives

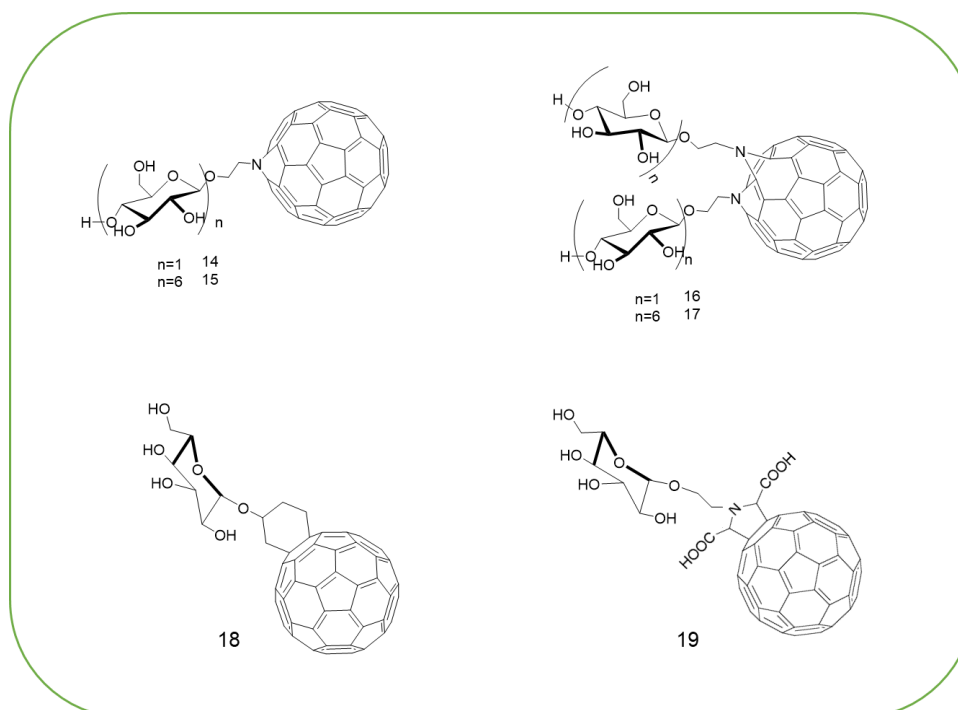


Fig .7 Fullerene carbohydrate derivatives

Mikata and Yano et al. reported that sugar-appended C₆₀ derivatives, such as compounds 14 and 16 (Fig. 7), could directly observe ¹O₂ emission in DMSO when irradiated with light at 1270 nm. Has the ability to generate ¹O₂^[106]. The use of fullerenes alone as photosensitizers resulted in significant cytotoxicity. In contrast, photodynamic therapies using glycoconjugated fullerenes showed no apparent cytotoxicity to normal fibroblasts. This suggests that compounds with glycoconjugates have the ability to target cancer cells^[107]. Disaccharide-pituitary C₆₀ derivatives, such as compound 16 did not exhibit photocytotoxicity. Sugar-side chain fullerene C₆₀ derivatives 14 and 16 inhibit human plasma lipid peroxidation by scavenging free radicals^[108].

ROS generated by C₆₀ sugar derivatives can inhibit the growth of HeLa cells and degrade HIV aspartate protease. C₆₀ monosaccharide derivatives produce more ¹O₂ than C₆₀ disaccharide derivatives. Thus, under UV irradiation, C₆₀-monosaccharides inhibit HeLa cells more than C₆₀-bisaccharides^[109]. Compounds 18 and 19 can ROS when exposed to both UV and visible light (Fig. 7), and ROS contribute to the degradation of HIV aspartate protease. Compounds 18 (1.5 μM) and 19 (15 μM) led to the complete degradation of HIV aspartate protease. Compound 19 exhibited no inhibition of HIV reverse transcriptase in comparison to compound 18. Compound 19 has an inherent ability to resist HIV aspartate protease. However, the IC₅₀ in the absence of light was higher at 15.1 μM than the IC₅₀ of 2.25 μM in the presence of light. Furthermore, compound 19 demonstrated inhibition of HIV replication in human leukemia Molt4T cells and peripheral blood mononuclear cells. Compound 19 (10 μM) significantly reduced the amount of p24 (a marker of HIV replication)^[110, 111].

As cancer cells endocytose glucose more efficiently than normal cells, the outstanding properties of fullerenes as photosensitizers can be enhanced by binding them to glucose. The cytotoxicity of PDT was subsequently studied in several cancer cell lines treated with compound 14 and compound 15, followed by irradiation with UVA1. The bioactivity plot below (Fig. 8) displays the effect of a light parameter of 10 J/cm^2 and a drug light interval of 4 hours. The results indicated that higher doses were more effective in inhibiting tumor growth compared to lower doses^[112]. In recent years, fullerene sugar derivatives have also been widely used in other biology fields^{[70] [113, 114]}.

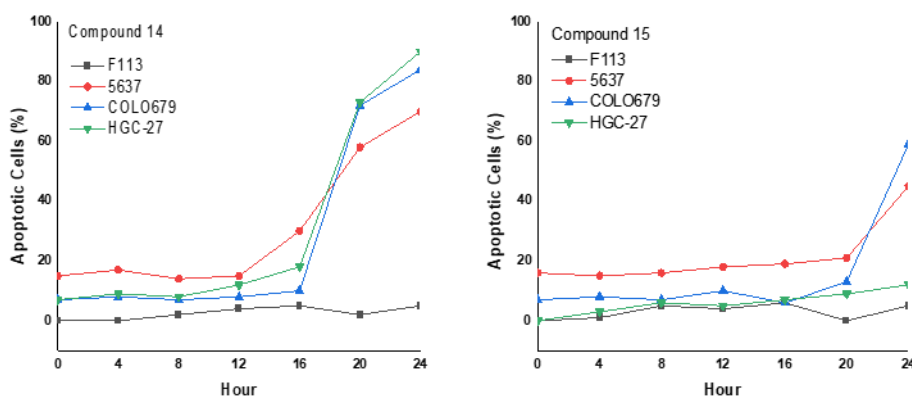


Fig. 8 Biological Activity Study Map of Compound 14 and Compound 15

Fullerene cyclodextrin derivatives

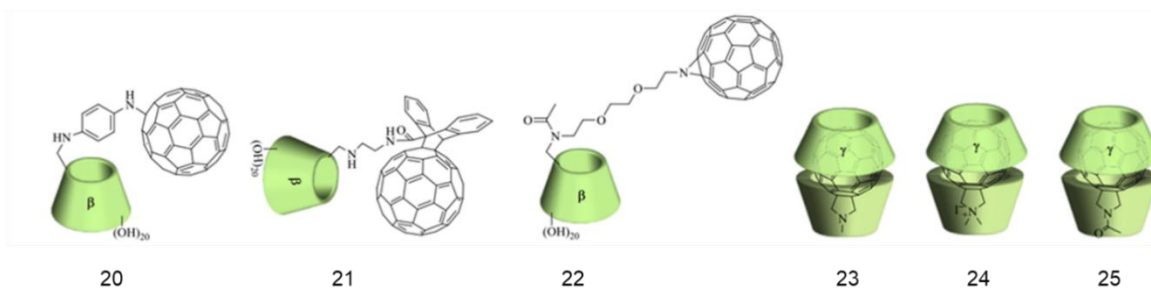


Fig. 9 Fullerene cyclodextrin derivatives

CDs with inherent hydrophilicity and spacious cavities are effective for increasing the hydrophilicity of C_{60} . Common derivatives include C_{60} - β -CD adducts, C_{60} - β -CD micelles, and C_{60} - γ -CD complexes (Fig. 9). α -CD was not utilized because of the relatively small size of the α -CD cavity. The compounds in Fig 9 are all free radical generators and act as DNA cleavers and cytostatic agents. Although the C_{60} - β -CD adducts aggregate in aqueous solution, they produce ROS under light conditions, which further damages DNA.

The mechanism of DNA cleavage by C_{60} is as follows: (1) Oxidation of guanosine to 8-oxoguanine (8G) by 1O_2 generated through type II energy transfer; (2) if the DNA strand contains

a guanosine stack that is susceptible to oxidation, $^3\text{C}_{60}^*$ and $^3\text{O}_2$ will sequentially accept electrons from guanosine (type I electron transfer) to obtain 8G. Further oxidation of 8G will produce alkali-labile sites, leading to DNA cleavage^[70]. Compound 20 is the first reported rip DNA conjugate in which the characteristic peak at 343 nm in the UV absorption spectrum of C_{60} is diminished during cleavage^[115]. Compound 21 is an example of a compound that produces singlet oxygen, which disrupts DNA^[116]. Another C_{60} - β -CD conjugate 22 demonstrated that NADH is essential for pBR322 DNA cleavage. This implies that O^{2-} and $\cdot\text{OH}$ generated through type I electron transfer play an important role. Although $33 \mu\text{mol/L}^{-1}$ of compound 22 completely fragmented DNA into small pieces, the photodynamic ability of cells was weak, and $400 \mu\text{mol/L}^{-1}$ of compound 22 killed less than 40% of SH-SY5Y cancer cells^[117].

C_{60} /HP- β -CD nanoparticles, measuring 90 nm were produced through the co-milling of C_{60} and HP- β -CD particles under reduced pressure for 3 hours. These nanoparticles were found to generate ROS and effectively eliminate HeLa cells under light exposure. It has been shown that small C_{60} aggregates can decrease the self-surge of free radicals and enhance photodynamic capabilities. However, large C_{60} aggregates reduce the surface area exposed to light, which may decrease the efficiency of ROS production. C_{60} /HP- β -CD colloidal solutions are highly stable, and the size of the aggregates remains almost constant over a period of 28 days. The level of ROS production was higher when compared to the ROS production from the C_{60} solution alone at the same concentration (40 μM). The aggregates in the C_{60} solution alone measured 427 nm. The C_{60} solution alone produced almost no $^1\text{O}_2$, whereas the C_{60} /HP- β -CD colloidal solution produced $^1\text{O}_2$, O_2^- and $\cdot\text{OH}$. Thus, the colloidal solution of C_{60} /HP- β -CD (40 μM) resulted in a 75% mortality rate of cancer cells when exposed to visible light irradiation, whereas the C_{60} solution alone had no inhibitory effect. Both the C_{60} /HP- β -CD colloidal solution and the C_{60} solution alone were found to be non-toxic in the dark.

γ -CD with large cavities can encapsulate C_{60} and prevent aggregation. Cellular inhibition depends on the capacity for ROS production, especially $^1\text{O}_2$, and cellular uptake. In bioactivity studies of human skin keratin-forming cells (HaCaT) irradiated with UVA (15 J/cm²) the quantum yield of $^1\text{O}_2$ generated by the C_{60} / γ -CD complex was 0.78 much higher than that of $\text{C}_{60}(\text{OH})_{24}$ in D_2O (0.08)^[93]. The C_{60} / γ -CD complex ($\text{IC}_{50}=0.25 \mu\text{M}$) exhibited a higher photodynamic capacity than $\text{C}_{60}(\text{OH})_{24}$ ($\text{IC}_{50}=15 \mu\text{M}$). Aggregates in the C_{60} / γ -CD solution increased upon heating and did not produce $^1\text{O}_2$ after 150 minutes. Thus, a 2 μM C_{60} / γ -CD complex resulted in approximately 95% human lens epithelial cell death, while a 30 μM n C_{60} / γ -CD aqueous solution (prepared by heating the aqueous C_{60} / γ -CD complex for 150 minutes) had minimal effect. The aggregate size of n C_{60} / γ -CD in aqueous solution is 136.6 nm^[118]. Although the C_{60} / γ -CD complex is taken up by cells much more slowly than $\text{C}_{60}(\text{OH})_{24}$ and C_{60} / γ -CD, it exhibits the most potent inhibitory effect. However, the C_{60} / γ -CD complex did not exhibit a strong inhibitory effect on HeLa cells. 10 μM C_{60} / γ -CD had little effect on HeLa cells in the absence of light or under 400-500 nm radiation. C_{60} /6-aminog- γ -CD (10 μM) resulted in over 60% cell death in HeLa cells upon light irradiation. A more intriguing phenomenon is that C_{60} /6-aminog- γ -CD can displace C_{60} when the pH decreases from 7.4 to 6.4. Despite the presence of colloidal aggregates in solution at pH 6.4, the inhibition was stronger at pH 6.4 than at pH 7.4. The size of the colloidal aggregates is small, at only 20 nm. Smaller C_{60} aggregates may be taken up by HeLa cells more rapidly than C_{60} /6-aminog- γ -CD and C_{60} / γ -CD^[119].

The original C_{60} does not absorb light at long wavelengths (610-740 nm). However, in this

irradiation range, the functionalized C₆₀ derivatives produce ¹O₂. The photodynamic efficacy of the derivatives for HeLa cells follows this order: 24/γ-CD complex (IC₅₀ = 0.47 μM) > 25/γ-CD complex (IC₅₀ = 0.95 μM) > 23/γ-CD complex ≈ C₆₀/γ-CD complex. The IC₅₀ for the clinical photosensitizer Photofrin in HeLa cells is 2μM. The photodynamic activity of the fullerene cyclodextrin derivatives was diminished when co-treated with the ¹O₂ quencher L-histidine, while the addition of D-mannitol (¹O₂ quencher) did not affect cell viability. The 23/γ-CD complex is a weak photosensitizer, attributed to electron transfer bursts caused by long pairs of electrons on amines. The 23/γ-CD complex of ³C₆₀* is quenched before it is generated by energy transfer from ¹O₂. The cationic 24/γ-CD complex exhibits the most effective inhibition due to electrostatic interactions with the anionic surface of HeLa cell [120].

4. Conclusion and Outlook

Over the past three decades, fullerenes have emerged as important molecules in a variety of health-related disciplines because of their extraordinary properties. The fullerene photosensitizers presented here represent a potential application for this group of carbon compounds. As nanomaterials become more widely used, the demand for various forms of fullerenes will increase. For example, in recent research, fullerenes have been used in combination with metal nanoparticles^[33, 121-123], hyaluronic acid^[124, 125], light-collecting antennas^[126], and other biological applications. Future work on functionalizing fullerenes is expected to significantly impact biological applications, thanks to the collaborative efforts of scientists in the fields of chemistry and medicine. Effective modification of carbon cages facilitates successful diagnostic and therapeutic outcomes in various biomedical applications. Here, we presented a comprehensive review of the growing body of research on fullerenes for biomedical engineering applications. This research has primarily focused on *in vitro* and *in vivo* studies and has not yet progressed to the final stages of clinical trials. It is expected that this trend may change in the coming years due to rapid technological advancements in this field, which will allow the unique properties of fullerenes to be utilized in clinical applications. Although there are still major challenges to overcome, fullerenes are poised to have a promising future in biomedical engineering.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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