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

Xue Bai, Chungeng Dong, Xinle Shao, Faiz-Ur Rahman, Huifang Hao, Yongmin Zhang

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

Xue Bai [a], Chungeng Dong [a], Xinle Shao [a], Faiz-Ur Rahman [a], Huifang Hao [a], Yongmin Zhang [a, b, c *]

[a] Inner Mongolia University Research Center for Glycochemistry of Characteristic Medicinal Resources, School of Chemistry and Chemical Engineering, Inner Mongolia University, Hohhot, 010021, China.

[b] Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire, UMR 8232, 4 Place Jussieu, 75005 Paris, France.

[c] Key Laboratory of Tropical Medicinal Resource Chemistry of Ministry of Education, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou, 571158, China.

*Corresponding Authors E-mail: yongmin.zhang@upmc.fr (Y. Zhang)

Abstract

In contemporary studies, the predominant utilization of C_{60} 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].

C_{60}, the most common fullerene, consists 60 of a balanced distribution of carbon atoms in a unique football-like cage structure, hence the name "football alkene"[12]. C_{60} 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 ($^1O_2$) [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 C_{60} 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 a 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].

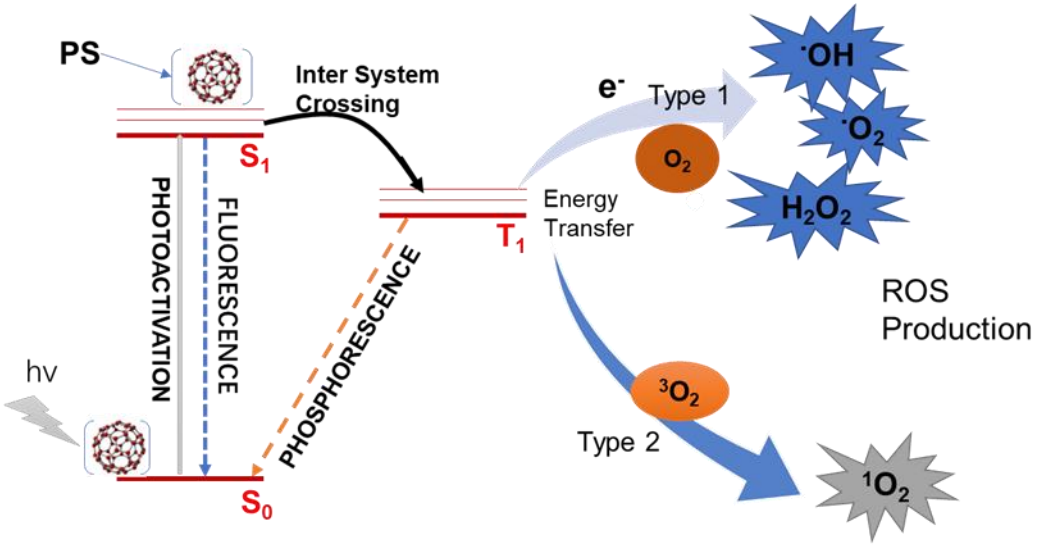

Fig. 1 Diagram of the mechanism of action of photosensitizers in photodynamic therapy

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) [5] [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

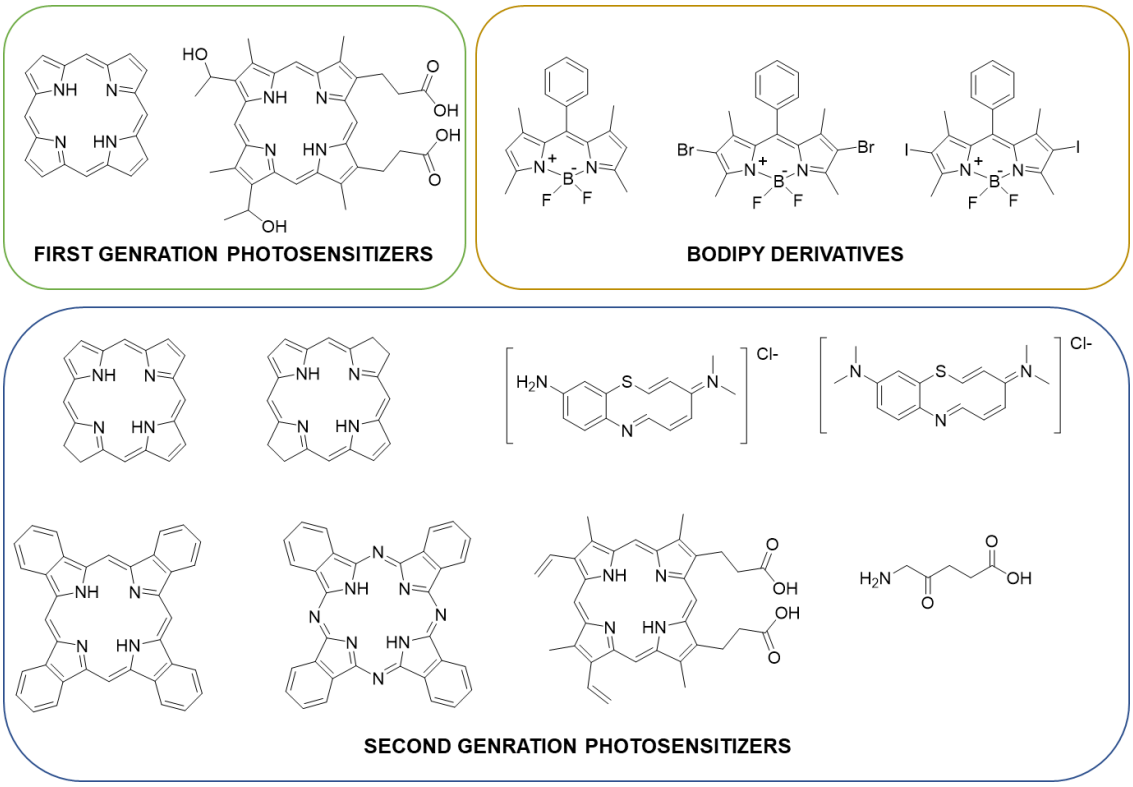

Fig .2 Representative drugs in traditional photosensitizers

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 C_{60}. 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].

C_{60} 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 C_{60} 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]. C_{60} undergoes a transformation from a ground state (S_0) to an excited singlet state (S_1) when exposed to light, and then quickly transitions to a lower energy triplet state (T_1) with a long lifetime of 40 ± 4 μs [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 ($^1O_2$) upon irradiation [74]. It has been reported that photoirradiation of fullerenes in aqueous systems leads to the generation of radical anions (C_{60}•), followed by electron transfer to produce superoxide anion radicals ($O_2^{\bullet-}$) 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.

C_{60} 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 C_{60} 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).

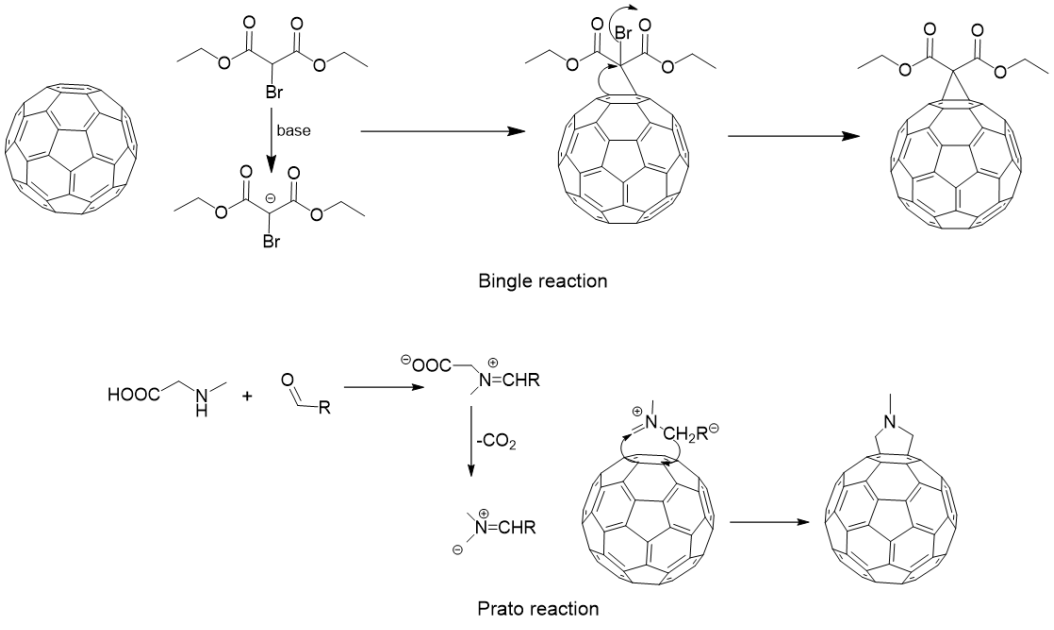

Fig. 3 Bingle reaction and Prato reaction mechanism diagram

3.2 Currently available fullerene photosensitizers

Fullerol

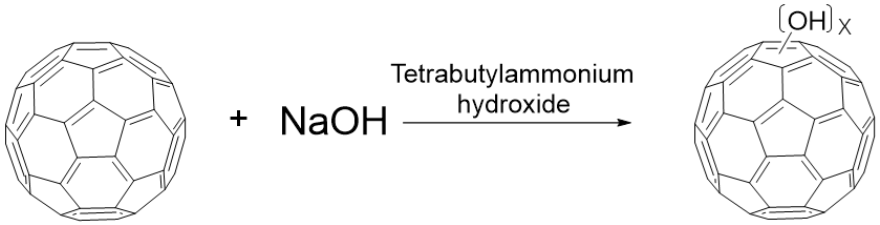

Fig. 4 Synthesis of Fullerol

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 biometals 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.

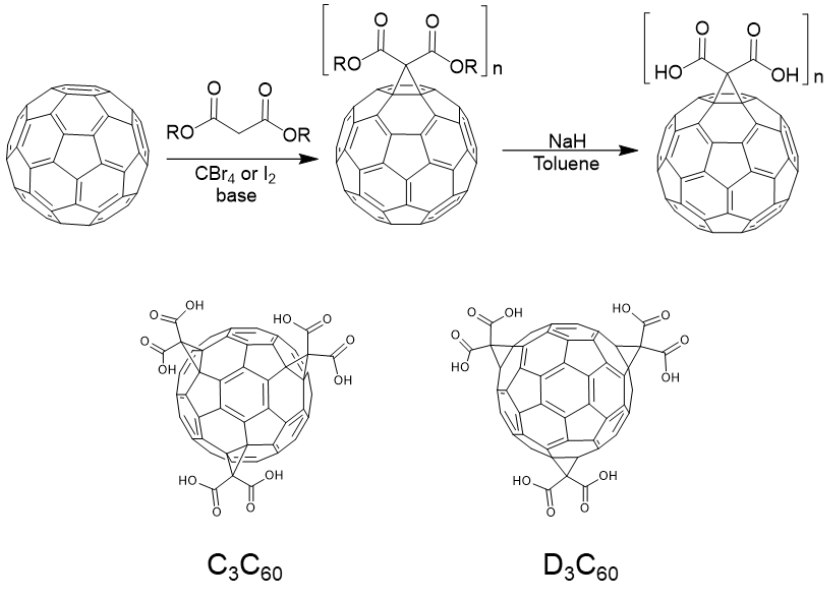

Fig .5 Synthesis process and iconic products of fullerene carboxylic acid derivatives

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}/HSA 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 μM) 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 C_{60} 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/cm^2 (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/cm^2, 4-6 orders of magnitude of Gram-negative bacteria *E. coli*, which have a less permeable outer membrane, died. At 16 J/cm^2, 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].

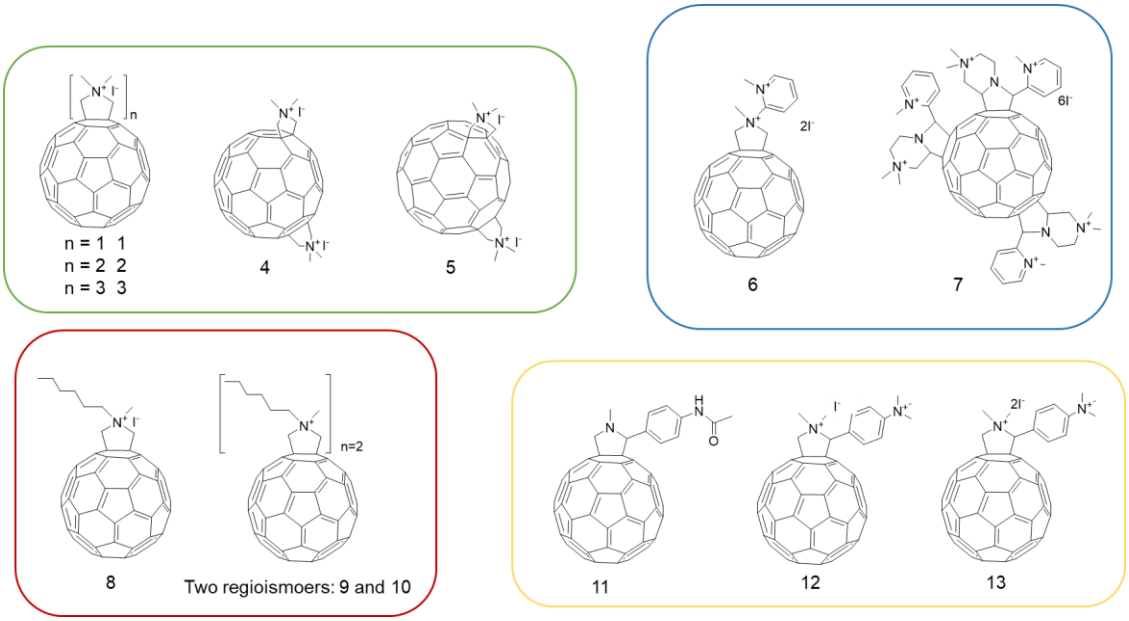

Fig. 6 Fullerene Quaternary Ammonium Derivatives

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., Mg^{2+}, Ca^{2+}), which then attach to the

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

Fullerene carbohydrate derivatives

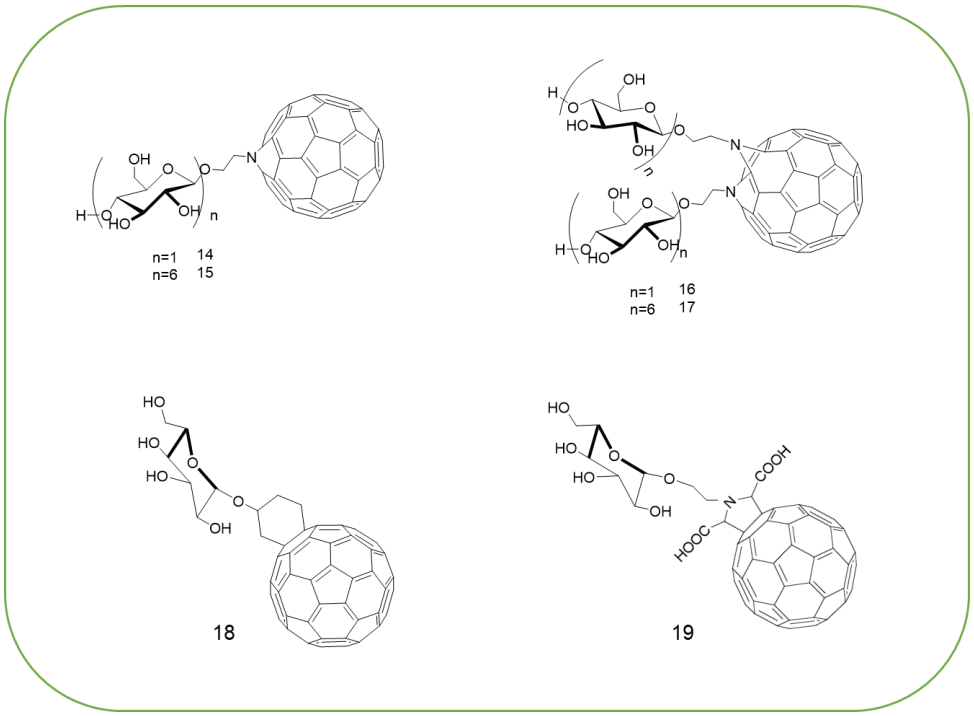

Fig .7 Fullerene carbohydrate derivatives

Mikata and Yano et al. reported that sugar-appended C_{60} derivatives, such as compounds 14 and 16 (Fig. 7), could directly observe $^1O_2$ emission in DMSO when irradiated with light at 1270 nm. Has the ability to generate $^1O_2$ [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 C_{60} derivatives, such as compound 16 did not exhibit photocytotoxicity. Sugar-side chain fullerene C_{60} derivatives 14 and 16 inhibit human plasma lipid peroxidation by scavenging free radicals [108].

ROS generated by C_{60} sugar derivatives can inhibit the growth of HeLa cells and degrade HIV aspartate protease. C_{60} monosaccharide derivatives produce more $^1O_2$ than C_{60} disaccharide derivatives. Thus, under UV irradiation, C_{60}-monosaccharides inhibit HeLa cells more than C_{60}-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 IC_{50} in the absence of light was higher at 15.1 μM than the IC_{50} 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].

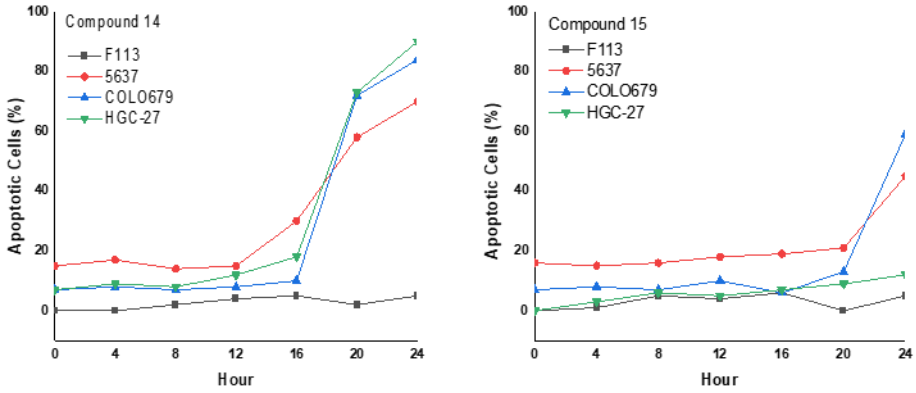

Fig. 8 Biological Activity Study Map of Compound 14 and Compound 15

Fullerene cyclodextrin derivatives

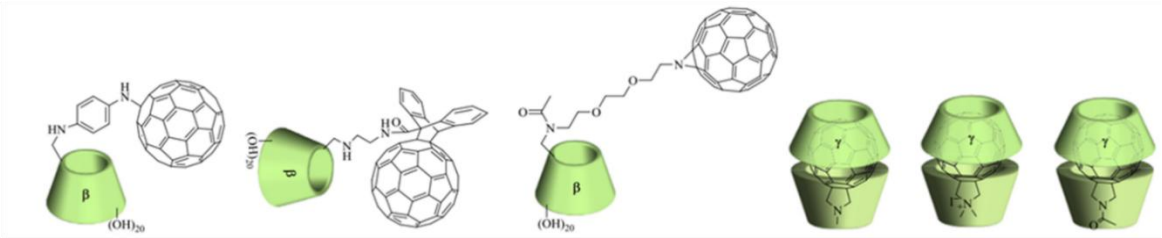

Fig .9 Fullerene cyclodextrin derivatives

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, $^3C_{60}$* and $^3O_2$ 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 $O^{2\cdot-}$ and ·OH generated through type I electron transfer play an important role. Although 33 μmol/L^{-1} of compound 22 completely fragmented DNA into small pieces, the photodynamic ability of cells was weak, and 400 μmol/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 $^1O_2$, whereas the C_{60}/HP-β-CD colloidal solution produced $^1O_2$, O_2^- and ·OH. Thus, the colloidal solution of C_{60}/HP-β-CD (40μM) 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 $^1O_2$, and cellular uptake. In bioactivity studies of human skin keratin-forming cells (HaCaT) irradiated with UVA (15 J/cm^2) the quantum yield of $^1O_2$ generated by the C_{60}/γ-CD complex was 0.78 much higher than that of $C_{60}(OH)_{24}$ in D_2O (0.08)[93]. The C_{60}/γ-CD complex (IC_{50}=0.25 μM) exhibited a higher photodynamic capacity than $C_{60}(OH)_{24}$ (IC_{50}=15 μM). Aggregates in the C_{60}/γ-CD solution increased upon heating and did not produce $^1O_2$ 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 nC_{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 nC_{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 $C_{60}(OH)_{24}$ 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 C_{60} derivatives produce $^1O_2$. The photodynamic efficacy of the derivatives for HeLa cells follows this order: 24/γ-CD complex (IC_{50} = 0.47 μM) > 25/γ-CD complex (IC_{50} = 0.95 μM) > 23/γ-CD complex ≈ C_{60}/γ-CD complex. The IC_{50} 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 $^1O_2$ quencher L-histidine, while the addition of D-mannitol ($^1O_2$ 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 $^3C_{60}$* is quenched before it is generated by energy transfer from $^1O_2$. 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.

Acknowledgements

The authors acknowledge the “JUN-MA” High-level Talents Program of Inner Mongolia University (No. 21300-5195112, No. 21300-5205107, No. 10000-21311201/110), the funding from the Science & Technology Department of Inner Mongolia Autonomous Region (No. 2021CG0029, No. 15000021T000000020229), and the funding from the Agriculture & Animal Husbandry Department of Inner Mongolia Autonomous Region (No. 21300-5223323).

References

[1] M. Lindblad, E. Tano, C. Lindahl, F. Huss, Ultraviolet-C decontamination of a hospital room: Amount of UV light needed, Burns 46(4) (2020) 842-849.

[2] M. Misovic, D. Milenkovic, T. Martinovic, D. Ciric, V. Bumbasirevic, T. Kravic-Stevovic, Short-term exposure to UV-A, UV-B, and UV-C irradiation induces alteration in cytoskeleton and autophagy in human keratinocytes, Ultrastruct Pathol 37(4) (2013) 241-8.

[3] M. Taha, H.M. Saleh, G. Fathy, M.A. Hamed, Evaluation of fluorescence microscopy using acridine orange stain versus hematoxylin and eosin stain in the diagnosis of onychomycosis, Journal of the Egyptian Women’s Dermatologic Society 14(1) (2017) 66-70.

[4] R.M. Szeimies, Geschichte der Photodynamischen Therapie, Aktuelle Dermatologie 31(5) (2005) 193-197.

[5] M. Ochsner, Photophysical and photobiological processes in the photodynamic therapy of tumours, Journal of Photochemistry and Photobiology B: Biology 39(1) (1997) 1-18.

[6] B. Chai, X. Liao, F. Song, H. Zhou, Fullerene modified C3N4 composites with enhanced photocatalytic activity under visible light irradiation, Dalton Trans 43(3) (2014) 982-9.

[7] J.W. Arbogast, A.P. Darmanyan, C.S. Foote, F.N. Diederich, R.L. Whetten, Y. Rubin, M.M. Alvarez, S.J. Anz, Photophysical properties of sixty atom carbon molecule (C60), The Journal of Physical Chemistry 95(1) (2002) 11-12.

[8] T.J. Dougherty, Hematoporphyrin derivative for detection and treatment of cancer, J Surg Oncol 15(3) (1980) 209-10.

[9] i.R.V.B. J.D. Spikes, G. Jori, E.J. Land, T.H. Truscott primary Photoprocesses in Biology and Medicine, Plenum Press (1985) 209-227.

[10] G.B. Kharkwal, S.K. Sharma, Y.Y. Huang, T. Dai, M.R. Hamblin, Photodynamic therapy for infections: clinical applications, Lasers Surg Med 43(7) (2011) 755-67.

[11] K. Wang, B. Yu, J.L. Pathak, An update in clinical utilization of photodynamic therapy for lung cancer, J Cancer 12(4) (2021) 1154-1160.

[12] R.C. Haddon, L.E. Brus, K. Raghavachari, Electronic structure and bonding in icosahedral C60, Chemical Physics Letters 125(5-6) (1986) 459-464.

[13] R. Tycko, R.C. Haddon, G. Dabbagh, S.H. Glarum, D.C. Douglass, A.M. Mujsce, Solid-state magnetic resonance spectroscopy of fullerenes, The Journal of Physical Chemistry 95(2) (2002) 518-

520.
[14] M. Junaid, E.A. Almuqri, J. Liu, H. Zhang, Analyses of the Binding between Water Soluble C60 Derivatives and Potential Drug Targets through a Molecular Docking Approach, PLoS One 11(2) (2016) e0147761.
[15] M.D. Tzirakis, M. Orfanopoulos, Radical reactions of fullerenes: from synthetic organic chemistry to materials science and biology, Chem Rev 113(7) (2013) 5262-321.
[16] Y. Quo, N. Karasawa, W.A. Goddard, Prediction of fullerene packing in C60 and C70 crystals, Nature 351(6326) (1991) 464-467.
[17] Y. Pan, X. Liu, W. Zhang, Z. Liu, G. Zeng, B. Shao, Q. Liang, Q. He, X. Yuan, D. Huang, M. Chen, Advances in photocatalysis based on fullerene C60 and its derivatives: Properties, mechanism, synthesis, and applications, Applied Catalysis B: Environmental 265 (2020).
[18] Q. Fu, S. Xiao, X. Tang, Y. Chen, T. Hu, Amphiphilic Fullerenes Employed to Improve the Quality of Perovskite Films and the Stability of Perovskite Solar Cells, ACS Appl Mater Interfaces 11(27) (2019) 24782-24788.
[19] I.M. Belousova, V.P. Belousov, O.B. Danilov, A.V. Ermakov, V.M. Kiselev, I.M. Kislyakov, E.N. Sosnov, Generation of singlet oxygen in fullerene-containing media: 1. Photodesorption of singlet oxygen from fullerene-containing surfaces, Quantum Electronics 38(3) (2008) 280-285.
[20] O.B. Danilov, I.M. Belousova, A.A. Mak, V.P. Belousov, A.S. Grenishin, V.M. Kiselev, A.V. Kris'ko, A.N. Ponomarev, E.N. Sosnov, Generation of singlet oxygen with the use of optically excited fullerenes and fullerene-like nanoparticles, Optics and Spectroscopy 95(6) (2003) 833-842.
[21] I.V. Bagrov, I.M. Belousova, A.S. Grenishin, V.M. Kiselev, I.M. Kislyakov, E.N. Sosnov, A jet-type singlet oxygen generator based on porous fullerene-containing structures, Optics and Spectroscopy 112(6) (2012) 935-942.
[22] J. Hirayama, H. Abe, N. Kamo, T. Shinbo, Y. Ohnishi-Yamada, S. Kurosawa, K. Ikebuchi, S. Sekiguchi, Photoinactivation of vesicular stomatitis virus with fullerene conjugated with methoxy polyethylene glycol amine, Biol Pharm Bull 22(10) (1999) 1106-9.
[23] V.M. Kiselev, I.V. Bagrov, Spectral properties of singlet-oxygen luminescence in the IR Region at the $1\Delta\ g \rightarrow 3\Sigma\ g$ transition in the presence of fullerene as a photosensitizer, Optics and Spectroscopy 123(4) (2017) 559-568.
[24] Y. Murata, K. Komatsu, Photochemical Reaction of the Open-Cage Fullerene Derivative with Singlet Oxygen, Chemistry Letters 30(9) (2001) 896-897.
[25] X. Kong, H. Chen, F. Li, F. Zhang, Y. Jiang, J. Song, Y. Sun, B. Zhao, J. Shi, Three-dimension chitosan hydrogel loading melanin composite nanoparticles for wound healing by anti-bacteria, immune activation and macrophage autophagy promotion, International Journal of Biological Macromolecules 237 (2023).
[26] W.B. Ko, J.Y. Heo, J.H. Nam, K.B. Lee, Synthesis of a water-soluble fullerene [C60] under ultrasonication, Ultrasonics 41(9) (2004) 727-30.
[27] X. Zhang, H. Cong, B. Yu, Q. Chen, Recent Advances of Water-Soluble Fullerene Derivatives in Biomedical Applications, Mini-Reviews in Organic Chemistry 16(1) (2018) 92-99.
[28] L.S. Litvinova, V.G. Ivanov, M.V. Mokeev, V.N. Zgonnik, Water-soluble [60]fullerene compositions with carbohydrates, Mendeleev Communications 11(5) (2001) 193-194.
[29] S. Yamago, H. Tokuyama, E. Nakamura, K. Kikuchi, S. Kananishi, K. Sueki, H. Nakahara, S. Enomoto, F. Ambe, In vivo biological behavior of a water-miscible fullerene: 14C labeling, absorption, distribution, excretion and acute toxicity, Chemistry & Biology 2(6) (1995) 385-389.

[30] V. Bilobrov, V. Sokolova, S. Prylutska, R. Panchuk, O. Litsis, V. Osetskyi, M. Evstigneev, Y. Prylutskyy, M. Epple, U. Ritter, J. Rohr, A Novel Nanoconjugate of Landomycin A with C60 Fullerene for Cancer Targeted Therapy: In Vitro Studies, Cell Mol Bioeng 12(1) (2019) 41-51.
[31] Z. Lu, W. Jia, R. Deng, Y. Zhou, X. Li, T. Yu, M. Zhen, C. Wang, Light-assisted gadofullerene nanoparticles disrupt tumor vasculatures for potent melanoma treatment, J Mater Chem B 8(12) (2020) 2508-2518.
[32] T. Ohata, K. Ishihara, Y. Iwasaki, A. Sangsuwan, S. Fujii, K. Sakurai, Y. Ohara, S.-i. Yusa, Water-soluble complex formation of fullerenes with a biocompatible polymer, Polymer Journal 48(10) (2016) 999-1005.
[33] Y. Yan, K. Zhang, H. Wang, W. Liu, Z. Zhang, J. Liu, J. Shi, A fullerene based hybrid nanoparticle facilitates enhanced photodynamic therapy via changing light source and oxygen consumption, Colloids Surf B Biointerfaces 186 (2020) 110700.
[34] M. Guan, J. Ge, J. Wu, G. Zhang, D. Chen, W. Zhang, Y. Zhang, T. Zou, M. Zhen, C. Wang, T. Chu, X. Hao, C. Shu, Fullerene/photosensitizer nanovesicles as highly efficient and clearable phototheranostics with enhanced tumor accumulation for cancer therapy, Biomaterials 103 (2016) 75-85.
[35] P. Zhang, H. Huang, S. Banerjee, G.J. Clarkson, C. Ge, C. Imberti, P.J. Sadler, Nucleus-Targeted Organoiridium-Albumin Conjugate for Photodynamic Cancer Therapy, Angew Chem Int Ed Engl 58(8) (2019) 2350-2354.
[36] A. Juzeniene, Q. Peng, J. Moan, Milestones in the development of photodynamic therapy and fluorescence diagnosis, Photochem Photobiol Sci 6(12) (2007) 1234-45.
[37] C.A. Robertson, D.H. Evans, H. Abrahamse, Photodynamic therapy (PDT): a short review on cellular mechanisms and cancer research applications for PDT, J Photochem Photobiol B 96(1) (2009) 1-8.
[38] H. Ali, J.E. van Lier, Metal complexes as photo- and radiosensitizers, Chem Rev 99(9) (1999) 2379-450.
[39] D. Kharroubi Lakouas, D. Huglo, S. Mordon, M. Vermandel, Nuclear medicine for photodynamic therapy in cancer: Planning, monitoring and nuclear PDT, Photodiagnosis Photodyn Ther 18 (2017) 236-243.
[40] A. Srivatsan, J.R. Missert, S.K. Upadhyay, R.K. Pandey, Porphyrin-based photosensitizers and the corresponding multifunctional nanoplatforms for cancer-imaging and phototherapy, Journal of Porphyrins and Phthalocyanines 19(01-03) (2015) 109-134.
[41] G. Jin, R. He, Q. Liu, Y. Dong, M. Lin, W. Li, F. Xu, Theranostics of Triple-Negative Breast Cancer Based on Conjugated Polymer Nanoparticles, ACS Appl Mater Interfaces 10(13) (2018) 10634-10646.
[42] X. Zhu, M. Sollogoub, Y. Zhang, Biological applications of hydrophilic C60 derivatives (hC60s)-a structural perspective, Eur J Med Chem 115 (2016) 438-52.
[43] T.D. Ros, M. Prato, Medicinal chemistry with fullerenes and fullerene derivatives, Chemical Communications (8) (1999) 663-669.
[44] Y. Iwamoto, Y. Yamakoshi, A highly water-soluble C60-NVP copolymer: a potential material for photodynamic therapy, Chem Commun (Camb) (46) (2006) 4805-7.
[45] E. Nakamura, H. Tokuyama, S. Yamago, T. Shiraki, Y. Sugiura, Biological Activity of Water-Soluble Fullerenes. Structural Dependence of DNA Cleavage, Cytotoxicity, and Enzyme Inhibitory Activities Including HIV-Protease Inhibition, Bulletin of the Chemical Society of Japan 69(8) (1996)

2143-2151.
[46] N. Higashi, T. Inoue, M. Niwa, Immobilization and cleavage of DNA at cationic, self-assembled monolayers containing C60 on gold, Chemical Communications (16) (1997) 1507-1508.
[47] A.S. Boutorine, M. Takasugi, C. Hélène, H. Tokuyama, H. Isobe, E. Nakamura, Fullerene–Oligonucleotide Conjugates: Photoinduced Sequence-Specific DNA Cleavage, Angewandte Chemie International Edition in English 33(2324) (1995) 2462-2465.
[48] M. Di Sante, A. Kaltenbrunner, M. Lombardo, A. Danielli, P.E. Costantini, M. Di Giosia, M. Calvaresi, Putting a "C60 Ball" and Chain to Chlorin e6 Improves Its Cellular Uptake and Photodynamic Performances, Pharmaceuticals 16(9) (2023).
[49] C.S. Foote, Definition of type I and type II photosensitized oxidation, Photochem Photobiol 54(5) (1991) 659.
[50] R. Reddavid, S. Sofia, P. Chiaro, F. Colli, R. Trapani, L. Esposito, M. Solej, M. Degiuli, Neoadjuvant chemotherapy for gastric cancer. Is it a must or a fake?, World J Gastroenterol 24(2) (2018) 274-289.
[51] M. Fujii, M. Kochi, T. Takayama, Recent advances in chemotherapy for advanced gastric cancer in Japan, Surg Today 40(4) (2010) 295-300.
[52] E. Gianotti, G. Razzini, M. Bini, C. Crivellaro, A. Righi, S. Darecchio, S. Lui, M.L. Basirico, S. Cocconi, K. Cagossi, A. Ferrari, F. Artioli, Scalp Cooling in Daily Clinical Practice for Breast Cancer Patients Undergoing Curative Chemotherapy: A Multicenter Interventional Study, Asia Pac J Oncol Nurs 6(3) (2019) 277-282.
[53] T. Watanabe, H. Yagata, M. Saito, H. Okada, T. Yajima, N. Tamai, Y. Yoshida, T. Takayama, H. Imai, K. Nozawa, T. Sangai, A. Yoshimura, Y. Hasegawa, T. Yamaguchi, K. Shimozuma, Y. Ohashi, A multicenter survey of temporal changes in chemotherapy-induced hair loss in breast cancer patients, PLoS One 14(1) (2019) e0208118.
[54] S. Jenni, A. Sour, Molecular Theranostic Agents for Photodynamic Therapy (PDT) and Magnetic Resonance Imaging (MRI), Inorganics 7(1) (2019).
[55] A. Juarranz, P. Jaen, F. Sanz-Rodriguez, J. Cuevas, S. Gonzalez, Photodynamic therapy of cancer. Basic principles and applications, Clin Transl Oncol 10(3) (2008) 148-54.
[56] U. Chilakamarthi, L. Giribabu, Photodynamic Therapy: Past, Present and Future, Chem Rec 17(8) (2017) 775-802.
[57] T. Theodossiou, J.S. Hothersall, E.A. Woods, K. Okkenhaug, J. Jacobson, A.J. MacRobert, Firefly luciferin-activated rose bengal: in vitro photodynamic therapy by intracellular chemiluminescence in transgenic NIH 3T3 cells, Cancer Res 63(8) (2003) 1818-21.
[58] M. Iranifam, Analytical applications of chemiluminescence methods for cancer detection and therapy, TrAC Trends in Analytical Chemistry 59 (2014) 156-183.
[59] P. Huang, J. Lin, S. Wang, Z. Zhou, Z. Li, Z. Wang, C. Zhang, X. Yue, G. Niu, M. Yang, D. Cui, X. Chen, Photosensitizer-conjugated silica-coated gold nanoclusters for fluorescence imaging-guided photodynamic therapy, Biomaterials 34(19) (2013) 4643-54.
[60] R. Laptev, M. Nisnevitch, G. Siboni, Z. Malik, M.A. Firer, Intracellular chemiluminescence activates targeted photodynamic destruction of leukaemic cells, Br J Cancer 95(2) (2006) 189-96.
[61] R. Chen, L. Zhang, J. Gao, W. Wu, Y. Hu, X. Jiang, Chemiluminescent nanomicelles for imaging hydrogen peroxide and self-therapy in photodynamic therapy, J Biomed Biotechnol 2011 (2011) 679492.
[62] M.O. Senge, mTHPC--a drug on its way from second to third generation photosensitizer?,

Photodiagnosis Photodyn Ther 9(2) (2012) 170-9.
[63] R.R. Allison, Photodynamic therapy: oncologic horizons, Future Oncol 10(1) (2014) 123-4.
[64] Y.N. Konan, R. Gurny, E. Allemann, State of the art in the delivery of photosensitizers for photodynamic therapy, J Photochem Photobiol B 66(2) (2002) 89-106.
[65] E. Temizel, T. Sagir, E. Ayan, S. Isik, R. Ozturk, Delivery of lipophilic porphyrin by liposome vehicles: preparation and photodynamic therapy activity against cancer cell lines, Photodiagnosis Photodyn Ther 11(4) (2014) 537-45.
[66] K.I. Ogawara, K. Higaki, Nanoparticle-Based Photodynamic Therapy: Current Status and Future Application to Improve Outcomes of Cancer Treatment, Chem Pharm Bull (Tokyo) 65(7) (2017) 637-641.
[67] G.M. Calixto, J. Bernegossi, L.M. de Freitas, C.R. Fontana, M. Chorilli, Nanotechnology-Based Drug Delivery Systems for Photodynamic Therapy of Cancer: A Review, Molecules 21(3) (2016) 342.
[68] J.M. Hawkins, A. Meyer, T.A. Lewis, S. Loren, F.J. Hollander, Crystal structure of osmylated c60: confirmation of the soccer ball framework, Science 252(5003) (1991) 312-3.
[69] D.V. Afanas'ev, A.A. Bogdanov, G.A. Dyuzhev, V.I. Karataev, A.A. Kruglikov, D. Deininger, Production of fullerenes in an arc discharge in the presence of hydrogen and oxygen, Technical Physics 44(12) (1999) 1440-1443.
[70] L. Tanzi, M. Terreni, Y. Zhang, Synthesis and biological application of glyco- and peptide derivatives of fullerene C60, Eur J Med Chem 230 (2022) 114104.
[71] F. Liao, Y. Saitoh, N. Miwa, Anticancer effects of fullerene [C60] included in polyethylene glycol combined with visible light irradiation through ROS generation and DNA fragmentation on fibrosarcoma cells with scarce cytotoxicity to normal fibroblasts, Oncol Res 19(5) (2011) 203-16.
[72] Z. Meng, W. Oh, Photodegradation of Organic Dye by CoS2 and Carbon(C60, Graphene, CNT)/TiO2 Composite Sensitizer, Chinese Journal of Catalysis 33(9-10) (2012) 1495-1501.
[73] X. Xiao, X. Zhao, X. Chen, J. Zhao, Heavy Atom-Free Triplet Photosensitizers: Molecular Structure Design, Photophysical Properties and Application in Photodynamic Therapy, Molecules 28(5) (2023).
[74] M. Galletero, x, S. a, Garcı, x, H. a, J.L. Bourdelande, Dramatic persistence (minutes) of the triplet excited state and efficient singlet oxygen generation for C60 incorporated in Y zeolite and MCM-41 silicate, Chemical Physics Letters 370(5-6) (2003) 829-833.
[75] G.D. Panagiotou, M.D. Tzirakis, J. Vakros, L. Loukatzikou, M. Orfanopoulos, C. Kordulis, A. Lycourghiotis, Development of [60] fullerene supported on silica catalysts for the photo-oxidation of alkenes, Applied Catalysis A: General 372(1) (2010) 16-25.
[76] J.W. Arbogast, C.S. Foote, Photophysical properties of C70, Journal of the American Chemical Society 113(23) (2002) 8886-8889.
[77] P.J. Krusic, E. Wasserman, B.A. Parkinson, B. Malone, E.R. Holler, P.N. Keizer, J.R. Morton, K.F. Preston, Electron spin resonance study of the radical reactivity of C60, Journal of the American Chemical Society 113(16) (2002) 6274-6275.
[78] M.J. Saadh, I.I. Alsaedi, M.A. Abbood, A. Yadav, H.A. Alsailawi, M. Mudhafar, A.J.H. Al-Athari, Y. Elmasry, A.H. Alawadi, Therapeutic potential of CX (X = 48, 60, and 70) fullerenes as drug delivery carriers for ifosfamide anti-cancer drug, Diamond and Related Materials 140 (2023).
[79] M.D. Nešić, T. Dučić, X. Liang, M. Algarra, L. Mi, L. Korićanac, J. Žakula, T.J. Kop, M.S. Bjelaković, A. Mitrović, G.D. Gojgić Cvijović, M. Stepić, M. Petković, SR-FTIR spectro-microscopic interaction study of biochemical changes in HeLa cells induced by Levan-C60, Pullulan-C60, and their

cholesterol-derivatives, International Journal of Biological Macromolecules 165 (2020) 2541-2549.
[80] P.N. Samanta, K.K. Das, Noncovalent interaction assisted fullerene for the transportation of some brain anticancer drugs: A theoretical study, Journal of Molecular Graphics and Modelling 72 (2017) 187-200.
[81] N. Zhang, Y. Wu, W. Xu, Z. Li, L. Wang, Synergic fabrication of multifunctional liposomes nanocomposites for improved radiofrequency ablation combination for liver metastasis cancer therapy, Drug Delivery 29(1) (2022) 506-518.
[82] M. Kumar, K. Raza, C60-fullerenes as Drug Delivery Carriers for Anticancer Agents: Promises and Hurdles, Pharmaceutical Nanotechnology 5(3) (2018).
[83] M.R. Hamblin, Fullerenes as photosensitizers in photodynamic therapy: pros and cons, Photochem Photobiol Sci 17(11) (2018) 1515-1533.
[84] A. Cantelli, M. Malferrari, E.J. Mattioli, A. Marconi, G. Mirra, A. Soldà, T.D. Marforio, F. Zerbetto, S. Rapino, M. Di Giosia, M. Calvaresi, Enhanced Uptake and Phototoxicity of C60@albumin Hybrids by Folate Bioconjugation, Nanomaterials 12(19) (2022).
[85] G. Martínez, M.A. Gómez, R. Gómez, J.L. Segura, Synthesis of a [60]fullerene-functionalized poly(vinyl chloride) derivative by stereospecific chemical modification of PVC, Journal of Polymer Science Part A: Polymer Chemistry 45(23) (2007) 5408-5419.
[86] N.B. Fernandes, R.U.K. Shenoy, M.K. Kajampady, C.E.M. Dcruz, R.K. Shirodkar, L. Kumar, R. Verma, Fullerenes for the treatment of cancer: an emerging tool, Environmental Science and Pollution Research 29(39) (2022) 58607-58627.
[87] Y. Xia, L. Hong, J. Zheng, Z. Lu, Q. Zhang, S. Chen, Z. Pang, L. Li, S. Qiao, Q. Wang, Y. Zhou, C. Yang, Ulcerative colitis alleviation of colon-specific delivered rhamnolipid/fullerene nanocomposites via dual modulation in oxidative stress and intestinal microbiome, Journal of Materials Chemistry B 11(25) (2023) 5882-5897.
[88] A.Y. Belik, A.Y. Rybkin, N.S. Goryachev, A.P. Sadkov, N.V. Filatova, A.G. Buyanovskaya, V.N. Talanova, Z.S. Klemenkova, V.S. Romanova, M.O. Koifman, A.A. Terentiev, A.I. Kotelnikov, Nanoparticles of water-soluble dyads based on amino acid fullerene C60 derivatives and pyropheophorbide: Synthesis, photophysical properties, and photodynamic activity, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 260 (2021).
[89] S. Goodarzi, T. Da Ros, J. Conde, F. Sefat, M. Mozafari, Fullerene: biomedical engineers get to revisit an old friend, Materials Today 20(8) (2017) 460-480.
[90] J. Fan, G. Fang, F. Zeng, X. Wang, S. Wu, Water-dispersible fullerene aggregates as a targeted anticancer prodrug with both chemo- and photodynamic therapeutic actions, Small 9(4) (2013) 613-21.
[91] H.M. Huang, H.C. Ou, S.J. Hsieh, L.Y. Chiang, Blockage of amyloid β peptide-induced cytosolic free calcium by fullerenol-1, carboxylate C60 in PC12 cells, Life Sci 66(16) (2000) 1525-1533.
[92] R. Injac, M. Perse, M. Cerne, N. Potocnik, N. Radic, B. Govedarica, A. Djordjevic, A. Cerar, B. Strukelj, Protective effects of fullerenol C60(OH)24 against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats with colorectal cancer, Biomaterials 30(6) (2009) 1184-1196.
[93] B. Zhao, Y.Y. He, P.J. Bilski, C.F. Chignell, Pristine (C60) and hydroxylated [C60(OH)24] fullerene phototoxicity towards HaCaT keratinocytes: type I vs type II mechanisms, Chem Res Toxicol 21(5) (2008) 1056-63.
[94] H. Jin, W.Q. Chen, X.W. Tang, L.Y. Chiang, C.Y. Yang, J.V. Schloss, J.Y. Wu, Polyhydroxylated C60, fullerenols, as glutamate receptor antagonists and neuroprotective agents, Journal of Neuroscience Research 62(4) (2000) 600-607.

[95] S. Oriana, S. Aroua, J.O. Sollner, X.J. Ma, Y. Iwamoto, Y. Yamakoshi, Water-soluble C60- and C70-PVP polymers for biomaterials with efficient (1)O2 generation, Chem Commun (Camb) 49(81) (2013) 9302-4.
[96] W.S. Kuo, C.Y. Chang, J.C. Liu, J.H. Chen, E.C. So, P.C. Wu, Two-Photon Photoexcited Photodynamic Therapy with Water-Soluble Fullerenol Serving as the Highly Effective Two-Photon Photosensitizer Against Multidrug-Resistant Bacteria, Int J Nanomedicine 15 (2020) 6813-6825.
[97] I.C. Wang, L.A. Tai, D.D. Lee, P.P. Kanakamma, C.K. Shen, T.Y. Luh, C.H. Cheng, K.C. Hwang, C(60) and water-soluble fullerene derivatives as antioxidants against radical-initiated lipid peroxidation, J Med Chem 42(22) (1999) 4614-20.
[98] Y.L. Huang, C.K. Shen, T.Y. Luh, H.C. Yang, K.C. Hwang, C.K. Chou, Blockage of apoptotic signaling of transforming growth factor-beta in human hepatoma cells by carboxyfullerene, Eur J Biochem 254(1) (1998) 38-43.
[99] T. Komatsu, A. Nakagawa, X. Qu, Structural and mutagenic approach to create human serum albumin-based oxygen carrier and photosensitizer, Drug Metab Pharmacokinet 24(4) (2009) 287-99.
[100] T. Mashino, N. Usui, K. Okuda, T. Hirota, M. Mochizuki, Respiratory chain inhibition by fullerene derivatives: hydrogen peroxide production caused by fullerene derivatives and a respiratory chain system, Bioorganic & Medicinal Chemistry 11(7) (2003) 1433-1438.
[101] M. Maggini, G. Scorrano, M. Prato, Addition of azomethine ylides to C60: synthesis, characterization, and functionalization of fullerene pyrrolidines, Journal of the American Chemical Society 115(21) (2002) 9798-9799.
[102] G.P. Tegos, T.N. Demidova, D. Arcila-Lopez, H. Lee, T. Wharton, H. Gali, M.R. Hamblin, Cationic fullerenes are effective and selective antimicrobial photosensitizers, Chem Biol 12(10) (2005) 1127-35.
[103] L. Huang, M. Terakawa, T. Zhiyentayev, Y.Y. Huang, Y. Sawayama, A. Jahnke, G.P. Tegos, T. Wharton, M.R. Hamblin, Innovative cationic fullerenes as broad-spectrum light-activated antimicrobials, Nanomedicine 6(3) (2010) 442-52.
[104] M.B. Spesia, M.E. Milanesio, E.N. Durantini, Synthesis, properties and photodynamic inactivation of Escherichia coli by novel cationic fullerene C60 derivatives, Eur J Med Chem 43(4) (2008) 853-61.
[105] S.A.G. Lambrechts, M.C.G. Aalders, D.H. Langeveld-Klerks, Y. Khayali, J.W.M. Lagerberg, Effect of monovalent and divalent cations on the photoinactivation of bacteria with meso-substituted cationic porphyrins, Photochemistry and Photobiology 79(3) (2007) 297-302.
[106] S. Kato, H. Aoshima, Y. Saitoh, N. Miwa, Fullerene-C60/liposome complex: Defensive effects against UVA-induced damages in skin structure, nucleus and collagen type I/IV fibrils, and the permeability into human skin tissue, J Photochem Photobiol B 98(1) (2010) 99-105.
[107] H.X. C. Du, H. Ji, Q. Liu, H. Xiao, Z. Yang, The antiviral effect of fullerene liposome complex against influenza virus (H1N1) in vivo, Sci. Res. Essays 7 (2012) 705-711.
[108] L. Xiao, H. Takada, K. Maeda, M. Haramoto, N. Miwa, Antioxidant effects of water-soluble fullerene derivatives against ultraviolet ray or peroxylipid through their action of scavenging the reactive oxygen species in human skin keratinocytes, Biomed Pharmacother 59(7) (2005) 351-8.
[109] Y. Mikata, S. Takagi, M. Tanahashi, S. Ishii, M. Obata, Y. Miyamoto, K. Wakita, T. Nishisaka, T. Hirano, T. Ito, M. Hoshino, C. Ohtsuki, M. Tanihara, S. Yano, Detection of 1270 nm emission from singlet oxygen and photocytotoxic property of sugar-Pendant [60] fullerenes, Bioorganic & Medicinal Chemistry Letters 13(19) (2003) 3289-3292.

[110] S. Tanimoto, S. Sakai, S. Matsumura, D. Takahashi, K. Toshima, Target-selective photo-degradation of HIV-1 protease by a fullerene-sugar hybrid, Chem Commun (Camb) (44) (2008) 5767-9.
[111] S. Tanimoto, S. Sakai, E. Kudo, S. Okada, S. Matsumura, D. Takahashi, K. Toshima, Target-selective photodegradation of HIV-1 protease and inhibition of HIV-1 replication in living cells by designed fullerene-sugar hybrids, Chem Asian J 7(5) (2012) 911-4.
[112] E. Otake, S. Sakuma, K. Torii, A. Maeda, H. Ohi, S. Yano, A. Morita, Effect and mechanism of a new photodynamic therapy with glycoconjugated fullerene, Photochem Photobiol 86(6) (2010) 1356-63.
[113] E. Baranska, O. Wiechec-Cudak, M. Rak, A. Bienia, A. Mrozek-Wilczkiewicz, M. Krzykawska-Serda, M. Serda, Interactions of a Water-Soluble Glycofullerene with Glucose Transporter 1. Analysis of the Cellular Effects on a Pancreatic Tumor Model, Nanomaterials (Basel) 11(2) (2021).
[114] L. Tanzi, M. Terreni, Y. Zhang, Synthesis and biological application of glyco- and peptide derivatives of fullerene C60, European Journal of Medicinal Chemistry 230 (2022).
[115] K.E.G. S. Samal, Cyclodextrinefullerenes: a new class of watersoluble fullerenes, Chem. Commun 13 (2000) 1101-1102.
[116] Y. Liu, Y.-L. Zhao, Y. Chen, P. Liang, L. Li, A water-soluble β-cyclodextrin derivative possessing a fullerene tether as an efficient photodriven DNA-cleavage reagent, Tetrahedron Letters 46(14) (2005) 2507-2511.
[117] G.W. T. Andersson, O. Wennerström, M. Sundahl, NMR and UV-vis investigation of water-soluble fullerene-60-g-cyclodextrin complex, Chem. Soc. Perkin Trans 25 (1994) 1097-1101.
[118] B. Zhao, Y.Y. He, C.F. Chignell, J.J. Yin, U. Andley, J.E. Roberts, Difference in phototoxicity of cyclodextrin complexed fullerene [(gamma-CyD)2/C60] and its aggregated derivatives toward human lens epithelial cells, Chem Res Toxicol 22(4) (2009) 660-7.
[119] K. Nobusawa, M. Akiyama, A. Ikeda, M. Naito, pH responsive smart carrier of [60] fullerene with 6-amino-cyclodextrin inclusion complex for photodynamic therapy, Journal of Materials Chemistry 22(42) (2012).
[120] A. Ikeda, T. Iizuka, N. Maekubo, R. Aono, J. Kikuchi, M. Akiyama, T. Konishi, T. Ogawa, N. Ishida-Kitagawa, H. Tatebe, K. Shiozaki, Cyclodextrin complexed [60]fullerene derivatives with high levels of photodynamic activity by long wavelength excitation, ACS Med Chem Lett 4(8) (2013) 752-6.
[121] W. Guo, C. Sun, G. Jiang, Y. Xin, Recent Developments of Nanoparticles in the Treatment of Photodynamic Therapy for Cervical Cancer, Anticancer Agents Med Chem 19(15) (2019) 1809-1819.
[122] Y. Liu, D. Zhu, X. Zhu, G. Cai, J. Wu, M. Chen, P. Du, Y. Chen, W. Liu, S. Yang, Enhancing the photodynamic therapy efficacy of black phosphorus nanosheets by covalently grafting fullerene C60, Chem Sci 11(42) (2020) 11435-11442.
[123] X. Li, Y. Liu, F. Fu, M. Cheng, Y. Liu, L. Yu, W. Wang, Y. Wan, Z. Yuan, Single NIR Laser-Activated Multifunctional Nanoparticles for Cascaded Photothermal and Oxygen-Independent Photodynamic Therapy, Nanomicro Lett 11(1) (2019) 68.
[124] Q. Zhang, S. Sun, Z. Wang, J. Li, Y. Xie, L. Shi, L. Sun, Dandelion-Inspired Hierarchical Upconversion Nanoplatform for Synergistic Chemo-Photodynamic Therapy In Vitro, ACS Appl Bio Mater 3(9) (2020) 6015-6024.
[125] J.-H. Yan, Y.-S. Ji, M.-L. Yang, J. Fu, H. Shan, L.L. Wang, L. Zhang, J.-Y. Xiao, X.-Y. Kong, J.-S. Shi, C60-Decorated Melanin Nanoparticles Conjugated with Hyaluronic Acid for Synergistic Theranostic and Immunotherapy of Tumors under near-Infrared Excitation, ACS Applied Nano Materials 3(9) (2020) 8817-8828.

[126] P. Vallecorsa, G. Di Venosa, M.B. Ballatore, D. Ferreyra, L. Mamone, D. Saenz, G. Calvo, E. Durantini, A. Casas, Novel meso-substituted porphyrin derivatives and its potential use in photodynamic therapy of cancer, BMC Cancer 21(1) (2021) 547.