

# Research progress of berberine mediated photodynamic therapy (Review)

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**Abstract.** Berberine (BBR) is a plant secondary metabolite that has been used in photodynamic therapy (PDT) in the last few decades. The present review aimed to discuss the research progress of BBR-mediated photodynamic actions. The following key words were searched in several databases: 'Berberine' combined with 'photodynamic therapy', 'sonodynamic therapy (SDT)', 'ultraviolet', 'reactive oxygen' and 'singlet oxygen'. The results demonstrated that both type I and type II reactions participated in the photodynamic progression of BBR derivatives. In addition, the photochemical characteristics of BBR derivatives were affected by the polarity, pH and O<sub>2</sub> content of solvents. DNA binding increases the lifespan of the photoexcited BBR state and generation of singlet oxygen (<sup>1</sup>O<sub>2</sub>). The chemical properties of substituents in different positions of the BBR skeleton are pivotal for its photochemical properties, particularly the methylenedioxy group at the C-2 and C-3 positions. BBR is a promising agent for mediating both PDT- and SDT-treated diseases, particularly in tumors. However, further studies are required to validate their biological effects. In addition, the molecular mechanisms underlying the antitumor effects of BBR-PDT remain unclear and warrant further investigation. The structural modification and targeted

delivery of BBR have made it possible to broaden its applications; however, experimental verification is required. Overall, BBR acts as a sensitizer for PDT and has promising development prospects.

## Contents

1. Introduction
2. Photodynamic characteristics of BBR
3. Biological effects of BBR-mediated PDT
4. Targeted delivery for BBR
5. Conclusions and prospects

## 1. Introduction

Photodynamic therapy (PDT) has significantly developed in the last 100 years and is a promising therapeutic modality for the treatment of cancer, dermatosis and bacterial infections (1-3). The main components of PDT include a photosensitizer, molecular oxygen and light irradiation (1). Following light irradiation of the photosensitizer, molecular oxygen can produce reactive oxygen species (ROS), including singlet oxygen (<sup>1</sup>O<sub>2</sub>), superoxide radical anion (O<sub>2</sub><sup>•-</sup>), hydrogen peroxide and hydroxyl radicals (<sup>•</sup>OH), to exert various roles in different biological processes, in general, <sup>1</sup>O<sub>2</sub> is involved in energy transfer and the free radicals are involved in electron transfers (4). The photosensitizer is the most important factor for PDT (2). However, cell specificity and selectivity remain insufficient for most photosensitizers (5). Notably, products from herbal plants, such as hypericin, curcumin, psoralen and berberine (BBR) have been demonstrated to act as photodynamic agents, exhibiting potential for wide applications in PDT (6-8).

BBR is an important isoquinoline alkaloid isolated from traditional medicines, such as *Berberis aristata* Sims, *Coptis chinensis* Franch and *Coptis japonica* Makino (Fig. 1). *Coptis chinensis* Franch belongs to the wild plant medicinal materials under secondary protection in China (9). BBR is produced in plants as a secondary metabolite and possesses a range of pharmacological properties (10-12). To the best of our knowledge, Philogène *et al* (13) were the first to demonstrate

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**Abbreviations:** BBR, berberine; <sup>3</sup>BBR<sup>\*</sup>: triple excited state of BBR; BBR<sup>•+</sup>, BBR radical cation; PDT, photodynamic therapy; SDT, sonodynamic therapy; ROS, reactive oxygen species; <sup>1</sup>O<sub>2</sub>, singlet oxygen; O<sub>2</sub><sup>•-</sup>, superoxide radical anion; <sup>•</sup>OH, hydroxyl radicals; DMF, 2,5-dimethyl furan; CH<sub>2</sub>Cl<sub>2</sub>, dichloromethane; EPR, electron paramagnetic resonance; D<sub>2</sub>O, aqueous; MeOH, methanol; DMSO, oxygenated dimethyl sulfoxide; Lys, lysozyme; EtOH, ethanol; EAC, ehrlich ascites carcinoma; PLGA, poly (lactic-co-glycolic acid); NPs, nanoparticles

**Key words:** berberine, photodynamic therapy, reactive oxygen species, target drug delivery

that BBR generates  $^1\text{O}_2$ , which is considered the main product of photodynamic reactions (2). Wang *et al* (14) studied the photodynamic activity of BBR on cancer cells in 1986. Our recent studies demonstrated that BBR-mediated PDT and sonodynamic therapy (SDT) significantly inhibit tumor cells *in vivo* (15,16). As a photosensitizer, BBR possesses several advantages, such as easy production, low cost and various pharmacological effects, including antitumor functions (15). However, BBR also possesses several disadvantages, including toxicity and poor water solubility (16).

The present review focuses on the photodynamic characteristics of BBR and its biological effects via PDT and SDT, as well as its molecular mechanisms, defects and potential strategies to provide novel insights for future development.

## 2. Photodynamic characteristics of BBR

BBR is sensitive to light and can react in the presence of changing energy levels, such as several other secondary metabolites, including coralyne, sauguinarine and palmatine (15,17). All plant compounds have specific biological functions, whose effects have been demonstrated to be effective in animal cells, including humans (17-23). Philogène *et al* (13) were the first to report that BBR is an  $^1\text{O}_2$  generator via 2,5-dimethyl furan (DMF) experiments. DMF is easily oxidized, thus, by measuring its decrease in absorbance at 220 nm,  $^1\text{O}_2$  generation can be indirectly measured (24). The results of this study demonstrated that the first-order rate constant ( $k$ ) of  $^1\text{O}_2$  generation induced by BBR and UV was  $2.1 \times 10^{-4}/\text{s}^1$ . Arnason *et al* (25) detected 1,270 nm near-infrared phosphorescence produced by BBR with near UV exposure, where the phosphorescence at 1,270 nm was considered as direct evidence of  $^1\text{O}_2$  generation. The results of this study demonstrated that the singlet oxygen yield ( $\Phi_\Delta$ ) of BBR was 0.25 in dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) solution. Since then, a series of studies have demonstrated that both type I and type II reactions are involved in UV light excitation for BBR, the photochemistry is influenced by polarity, oxygen content and pH of solvents, and the DNA-binding and photochemical functional group of BBR (26-28).

*Types of photodynamic reactions for BBR.* There are two types of reactions that are involved in PDT. Type I reactions involve electron transfers and result in the production of free radicals, while type II reactions involve energy transfer and result in the production of  $^1\text{O}_2$  (4,5). The 1,270 nm near-infrared phosphorescence is considered the characteristic absorption peak of  $^1\text{O}_2$  and has been used to calculate the  $\Phi_\Delta$  in most studies (29,30). The electron paramagnetic resonance (EPR) spin trapping technique is typically used to detect free radical photogeneration and triplet state, with the aid of 5,5-dimethyl-1-pyrroline N-oxide (26). EPR and 2,2,6,6-tetramethyl-4-piperidinol can also be used to detect  $^1\text{O}_2$  (27).

Both types of reactions are involved in BBR-PDT (Fig. 2). Briefly, following exposure to UV light, BBR is excited (4). There are three ways for the excited-state BBR to return to the ground state: Internal conversion, release of fluorescence and reaching the triplet excited state of BBR ( $^3\text{BBR}^*$ ) through intersystem crossing (28).  $^3\text{BBR}^*$  produces  $^1\text{O}_2$  and other oxygen free radicals by energy transfer and electron transfer, under

aerobic conditions (28). Both  $^1\text{O}_2$  and free radicals can react with biomolecules, but  $^1\text{O}_2$  is more efficient (2). Furthermore, a 1,270 nm phosphorescence can be detected in combination with  $^1\text{O}_2$  generation (29).

Inbaraj *et al* (26) reported that BBR in aqueous ( $\text{D}_2\text{O}$ ) solutions exposed to light does not generate  $^1\text{O}_2$ . However, the  $\Phi_\Delta$  in  $\text{CH}_2\text{Cl}_2$  was 0.34, which was higher than that detected in the study by Philogène *et al* (13); in non-polar environments, BBR can generate both  $^1\text{O}_2$  and radical species, such as  $\text{O}_2^{\cdot-}$ ,  $\cdot\text{OH}$  and neutral BBR radicals ( $\text{BBR}^{\cdot}$ ). Irradiation (313 nm) to BBR in frozen methanol (MeOH) produced strong triplet signals, while in  $\text{D}_2\text{O}$ , no populated triplet state of BBR was observed (13). Brezová (27) *et al* demonstrated that both  $^1\text{O}_2$  and  $\text{O}_2^{\cdot-}$  are produced following BBR irradiation in oxygenated dimethyl sulfoxide (DMSO) solvent. In addition, Jantová *et al* (31) observed the generation of superoxide anion radicals and  $^1\text{O}_2$  in aerated solutions of BBR upon photoexcitation, and demonstrated that both type I and II mechanisms are involved in the efficient activation of molecular oxygen. Cheng *et al* (28,32) reported that BBR can be activated by both 266 and 355 nm laser and produce  $^3\text{BBR}^*$ , BBR radical anions and BBR radical cations ( $\text{BBR}^{\cdot+}$ ). Furthermore, fast deprotonation of  $\text{BBR}^{\cdot+}$  can produce BBR neutral radicals, suggesting that both type I and type II reactions are activated (28). These results were verified by investigating the interaction mechanisms between BBR and lysozyme (Lys), and the results demonstrated that type I reactions compete with  $^1\text{O}_2$  (33). Shen and Ji (34) obtained the lowest triplet excitation energy of BBR and elucidated the characteristics of the triplet excited state using the time-dependent density functional theory. The results of this study proved that the direct energy transfer and electron transfer pathways are involved in the generation of  $^1\text{O}_2$  and  $\text{O}_2^{\cdot-}$ , respectively. Liu and Zhang (35) investigated the UV-induced photo-electrocatalytic degradation of the BBR chloride form and demonstrated that the ROS produced during the reaction were  $\text{O}_2^{\cdot-}$ ,  $\cdot\text{OH}$  and sulfate radicals.

*Effects of solvents on the photodynamic reaction of BBR.* It has been demonstrated that BBR can mediate photodynamic reactions. Several studies have confirmed that the photodynamic reactions of BBR are affected by several factors, such as solvent type, DNA binding and photochemical functional group (28,34,36). The photochemical reactions of BBR are influenced by solvents polarity (26). Inbaraj *et al* (26) systemically studied the photochemistry of BBR in different solvents. The results of the present study demonstrated that the  $\Phi_\Delta$  of BBR was the highest in  $\text{CH}_2\text{Cl}_2$  (0.34), followed by toluene (0.044), 1,4-dioxane (0.042) and acetonitrile (MeCN; 0.038), while exposure to light did not generate  $^1\text{O}_2$  in  $\text{D}_2\text{O}$  solutions. The order of the fluorescence quantum yield in different solvents was as follows: 1,4-dioxane (0.071) > zethanol (EtOH, 0.036) > MeCN (0.025) > propylene carbonate (0.007). Taken together, these results suggest that BBR is a weak photosensitizer in water; however, it can produce both  $^1\text{O}_2$  and radical species in nonpolar environments. Shen and Ji (34) observed the ROS photogeneration of BBR and demonstrated that both the generation of  $^1\text{O}_2$  and  $\text{O}_2^{\cdot-}$  were highly dependent on solvent polarity.  $\text{O}_2^{\cdot-}$  was only produced in EtOH; however,  $^1\text{O}_2$  was generated in both EtOH and benzene. Collectively, these results indicate that a

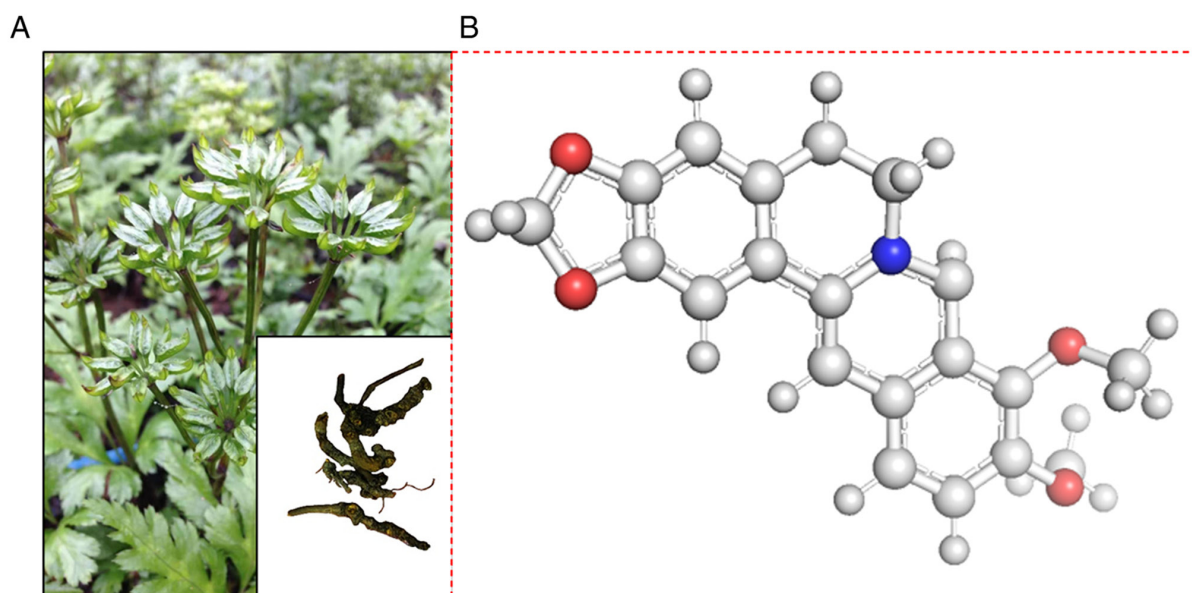


Figure 1. *Coptis chinensis* Franch and structural formula of BBR. (A) The root and stem of *Coptis chinensis* Franch. (B) Structural formula of BBR. BBR, berberine.

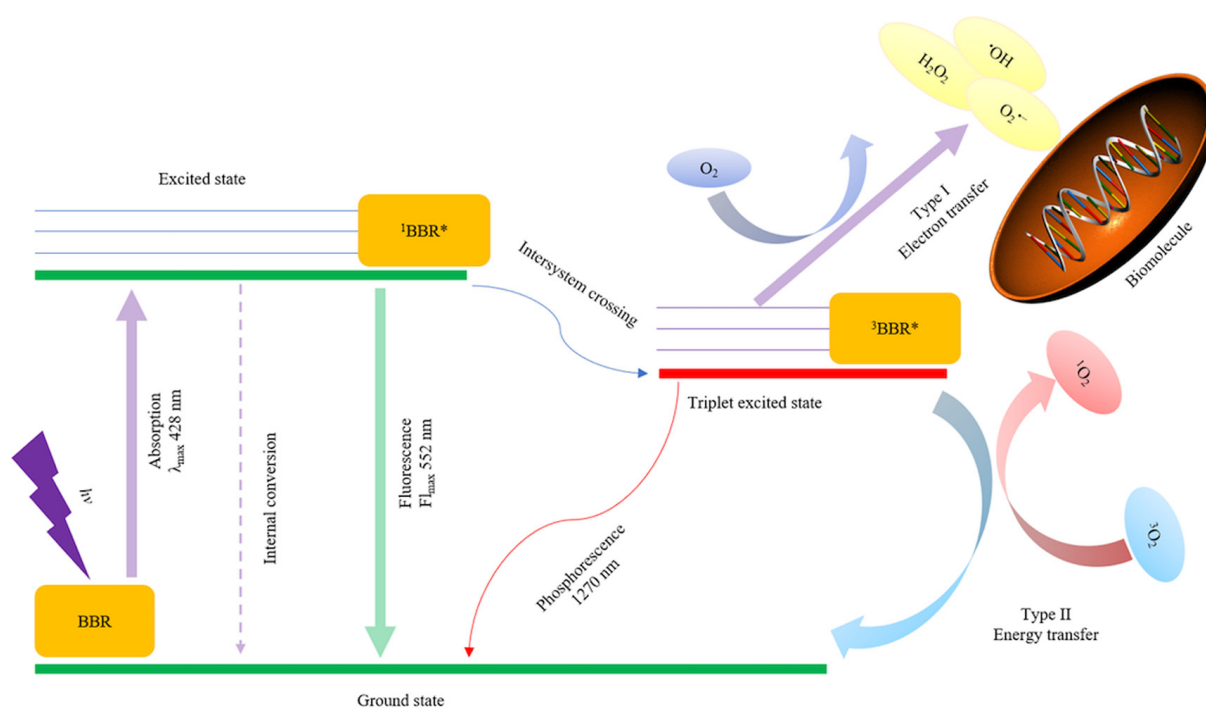


Figure 2. Graphical illustration of type I and type II photochemical reactions of BBR. The value of absorption and fluorescence was detected in MeCN. BBR, berberine;  $^1\text{BBR}^*$ , excited state of BBR;  $^3\text{BBR}^*$ , triple excited state of BBR;  $^1\text{O}_2$ , singlet oxygen;  $^3\text{O}_2$ , triplet oxygen;  $\text{O}_2^{\bullet-}$ , superoxide radical anion;  $\text{H}_2\text{O}_2$ , hydrogen peroxide;  $\cdot\text{OH}$ , hydroxyl radicals.

much higher  $\Phi_{\Delta}$  is observed in non-polar solvents compared with polar solvents. Görner *et al* (37) implied that the decrease in BBR  $\Phi_{\Delta}$  upon increasing solvent polarity was due to the dramatical acceleration of its internal conversion and reduction of the triplet rate formation yield. Notably, Cheng *et al* (28) reported that in laser flash photolysis, BBR exposure to a 266 nm laser in an aqueous solution produces hydrated electrons through a monophotonic process, and the hydrated electrons react with the steady state of BBR to form anion radicals, radical cations and neutral radicals. The

quantum yield of photoionization was determined to be 0.03. Notably, Görner *et al* (36) demonstrated that photoinduced electron ejection does not play a role in oxidation. Generally, BBR is more prone to photochemical reactions in nonpolar solvents than in polar solvents, and the ROS yield is higher in non-polar solvents (28,36,37). In addition, the pH of solvents also affects the reaction (36). Görner *et al* (36) reported that the quantum yield of BBR-sensitized oxidation in oxygenated alkaline MeOH solution significantly increased with an increase in pH, reaching a yield of 0.4 at pH 13.8.



In addition to solvent polarity, the oxygen content of solvents is also considered to play an important role in the light activation of BBR. Brezová *et al* (27) demonstrated that upon irradiation, BBR produces both  $O_2^{\cdot-}$  and  $^1O_2$  in DMSO. Furthermore, Hirakawa *et al* (38) reported that photoexcited BBR causes DNA cleavage under aerobic conditions, but no DNA photodamage was observed under anaerobic conditions. Jantová *et al* (31) revealed that EPR signals of photoexcited BBR in argon-saturated DMSO solutions are very low, while EPR signals of photoexcited BBR was higher in DMSO saturated by air or oxygen; the addition of SOD substantially decreased the formation of superoxide anion radicals, thus confirming unambiguously the photoinduced electron transfer from the photoexcited BBR to molecular oxygen; and the generation of superoxide anion adducts declined following the addition of superoxide dismutase; the aforementioned set of experiments confirmed the requirement of BBR,  $O_2$  and light for effective ROS generation.

Notably, other reactions upon laser irradiation of BBR exist besides photooxidation processes. Cheng *et al* (28,32,33) studied the photoionization and photoexcitation of BBR in  $N_2$ - and  $O_2$ -saturated systems. The results of these studies demonstrated that upon laser excitation at 355 nm, an absorption band  $\sim 420$  nm and an obvious bleach  $\sim 360$  nm of BBR was observed in  $N_2$ -saturated MeCN, and  $O_2$  significantly affected the species with 420 nm absorption. In the presence of  $O_2$ , the decay of  $^3BBR^*$  in  $N_2$ -bubbled MeCN was accelerated; the absorption value of bleaching is lower in the  $O_2$ -saturated system than in the  $N_2$ -saturated system, which indicates that another transient species may have been formed in addition to  $^3BBR^*$ , and the total yield of bleaching should include both  $BBR^{++}$  and  $^3BBR^*$  in the  $N_2$ -saturated system (32). Similar phenomena were observed upon 266 nm laser excitation. After the laser pulse, the depletion of ground states of BBR was observed as chartered absorption bleaching at 350 nm and both ionization and excitation contributed to the bleaching of BBR in the  $N_2$ -saturated system, while in the  $O_2$ -saturated system, only ionized processes were involved (28). Active radicals of BBR have also been observed, induced by laser oxidized tryptophan (Trp) of Lys via electron transfer progression in  $O_2$ -free polar solvents; however, in the presence of  $O_2$ , both electron and energy transfer mechanisms are involved in photooxidation (33). Görner *et al* (37) studied the photoreduction and ketone-sensitized reduction of alkaloids, including BBR. The results of this study demonstrated that the major photo-products of alkaloids are dihydroalkaloids and are reverted back to the corresponding alkaloids upon exposure of  $O_2$ . In addition, it was indicated that Rose Bengal, a photosensitizer, significantly accelerates BBR photooxidation in oxygenated alkaline MeOH solutions, suggesting the importance of Rose Bengal-sensitized  $^1O_2$  in the reaction (36,37).

Taken together, these findings demonstrate the complexity of the photochemical reactions of BBR and the important roles of  $O_2$ . Since  $^1O_2$  has proven efficient in destroying biomolecules, more attention should be paid on the balance between photooxidation, photoionization and photoreduction in biological applications.

**Interaction between BBR and DNA.** DNA is an important molecular target of BBR (39). DNA binding and its effects

on the photochemical properties of BBR were recently discovered. Hirakawa *et al* (38) revealed the mechanism of guanine-specific photooxidation of BBR and demonstrated that BBR easily binds to DNA and forms a strong fluorescent complex, which is necessary for  $^1O_2$  generation. In addition, BBR is unable to produce  $^1O_2$  in the absence of DNA. Furthermore, the authors discovered switches in  $^1O_2$  activity resulting from the DNA microenvironment, and that BBR can bind to DNA by electrostatic forces. This binding significantly enhances the lifespan of its photoexcited state by inhibiting quenching via electrostatic forces (40). The dynamics of  $^1O_2$  generation by DNA-binding BBR were assessed and the results demonstrated that  $^1O_2$  generation is led by energy transfer from the DNA-binding BBR complex to molecular oxygen (41). The guanine sequence decreased the  $\Phi_\Delta$  by deactivating the singlet excited state of BBR, and steric hindrance of DNA decreased the constant rate of  $^1O_2$  generation (41).

BBR also induces DNA photodamage (40). Guanine is the most easily oxidized base of DNA (42). Hirakawa *et al* (38) demonstrated that photoexcited BBR induces piperidine-sensitive calf thymus DNA damage, specifically at almost all guanine residues under aerobic conditions, but barely induces direct DNA strand breaks. Conversely, Jantová *et al* (31) reported that BBR significantly induces direct DNA strand breaks in NIH-3T3 and Ehrlich ascites carcinoma (EAC) cells. Cheng *et al* (43) demonstrated the capability of BBR to photosensitize DNA cleavage, and demonstrated that electron transfer via BBR neutral radicals to guanine neutral radicals plays an important role in DNA cleavage, and that both single- and double-stranded DNA at guanine moieties can be selectively cleaved by photoexcited BBR. The results of this study also clarified the molecular mechanisms of BBR-induced photodamage of dGMP and DNA.

DNA binding enhances the lifespan of photoexcited BBR states and generates  $^1O_2$ ; meanwhile, it is destroyed in the process (31,38). Understanding and utilizing this interaction and antinomy may provide insight to photosensitizer design based on BBR and its biological applications.

**Photochemical functional groups of BBR.** The functional groups of BBR are important for its photochemical properties (27,44,45). The structures of BBR derivatives are summarized in Fig. 3. Brezová *et al* (27) compared the photoexcited oxygen activation of BBR and its derivatives, palmatine and jatrorrhizine. The methylenedioxy group at the C-2 and C-3 positions of BBR is photolabile (27). When these groups are replaced by two methoxy groups (palmatine) or C-2 methoxyl and C-3 hydroxyl (jatrorrhizine), the photochemical generation of  $O_2^{\cdot-}$  and  $^1O_2$  significantly decreases in DMSO solution (27). The UV/Vis absorption in regions of 350 and 425 nm in DMSO and EtOH is also influenced by the substitutional changes (27). However, DNA binding and associated photochemical reactions have failed to demonstrate differences between BBR and palmatine (38,40,41). In addition, the effects of solvent polarity on the photoirradiation reactions of BBR and palmatine are similar (37). The antitumor effects of palmatine mediated PDT were proven *in vitro* using several cancer cell lines, such as MCF-7 and HT-29 cells (44,46,47).

Coralyne, a protoberberine alkaloid (45), has a C-7 methyl group replace based on palmatine, which has been

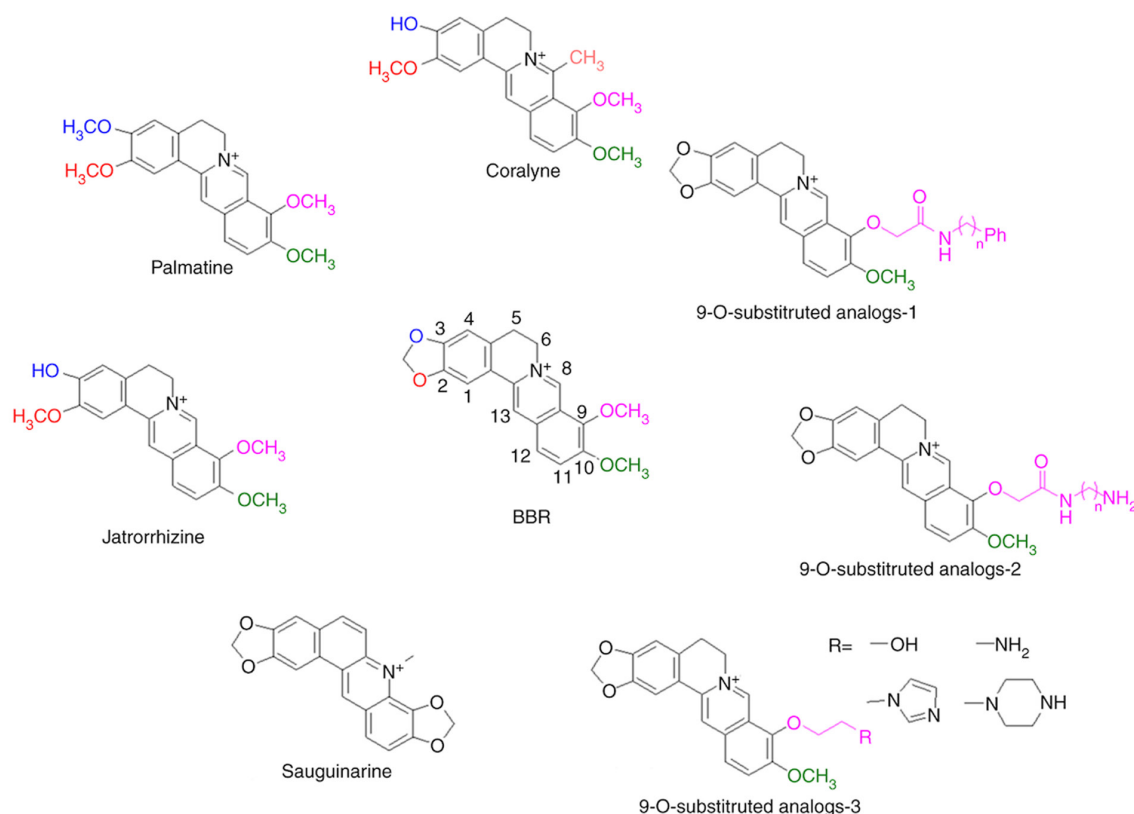


Figure 3. Effects of functional groups on the photoactivities of BBR. Chemical properties of substituents in different positions of the BBR skeleton are pivotal for its photochemical properties, particularly the methylenedioxy group at C-2 and C-3 positions, C-7 methyl group and the 9-O-position of the isoquinoline chromophore. BBR, berberine.

demonstrated to cause robust photosensitization of cancer cells (45). The  $\Phi_A$  of coralyne is independent of solvent polarity unlike BBR and palmatine (37). Patro *et al* (48) reported that coralyne induces significant nicking of plasmid DNA via an O<sub>2</sub>-independent photochemical process; however, the phenomenon has not been observed in BBR or jatrorrhizine. Ihmels and Salbach (49) demonstrated that coralyne irradiation results in significant DNA damage. The extent of the damage does not differ between aerobic and anaerobic conditions; however, the extent of the damage is significantly different to that induced by BBR.

Basu *et al* (50,51) synthesized BBR analogs with substituents at the 9-O-position of the isoquinoline chromophore and demonstrated that these analogs enhance the binding affinities of BBR to both DNA and double stranded poly(A). Liu *et al* (52) confirmed increased DNA binding of several 9-O-substituted BBR derivatives, and one of the derivatives exhibited higher cytotoxicity in HL-60 and BGC-823 cells compared with BBR. Since the photochemical functional groups were not altered, and DNA binding has significant influence on the  $\Phi_A$  of BBR, such substitutions may enhance the photochemical properties of BBR; however, further experimental verification is required. Arnason *et al* (25) determined the  $\Phi_A$  of sanguinarine, which was demonstrated to be lower than BBR (0.16 vs. 0.25). The median lethal dose of sanguinarine to mosquito larvae significantly decreased from 23.3 mg/ml to 0.096 mg/ml following near-UV exposure. Görner *et al* (36) confirmed that the absorption spectra of sanguinarine is altered upon change in pH in a 9:1 mixture of MeOH and water, whereby the main

product upon irradiation at 313 nm is 6-oxy-sanguinarine. The  $\Phi_A$  of sanguinarine is lower than BBR in several solvents (37), suggesting that the methylenedioxy group of C-9 and C-10 is not beneficial to photoactivity.

Taken together, these results suggest that changing the substituents of photochemical functional groups of BBR affect its photochemical properties, which provides insight for the molecular design of a BBR-based photosensitizer.

### 3. Biological effects of BBR-mediated PDT

Since the discovery of the photochemical properties of BBR, the applications of BBR-mediated PDT in biology have attracted great attention.

*Application of BBR-mediated-PDT in non-neoplastic diseases.* BBR-PDT has been proven to treat a variety of non-neoplastic diseases. Philogène *et al* (13) demonstrated the phototoxicity of BBR to mosquito larvae, whereby the median lethal concentration decreased from 250 to 8.8 ppm in the presence of near UV. Molero *et al* (53) reported that the growth of *Allium cepa* Linn. var. *cepa* roots is inhibited by BBR upon irradiation of violet light (420 nm). Inbaraj *et al* (26) determined the phototoxicity of BBR to HaCaT keratinocytes and demonstrated that 50  $\mu$ M BBR combined with UVA irradiation decreases the cell viability to 20%, and the DNA damage exhibited a 3-fold increase compared with UVA alone. BBR is an efficient fluorescent probe, and BBR-PDT has demonstrated potent bactericidal effects, both *in vivo* and

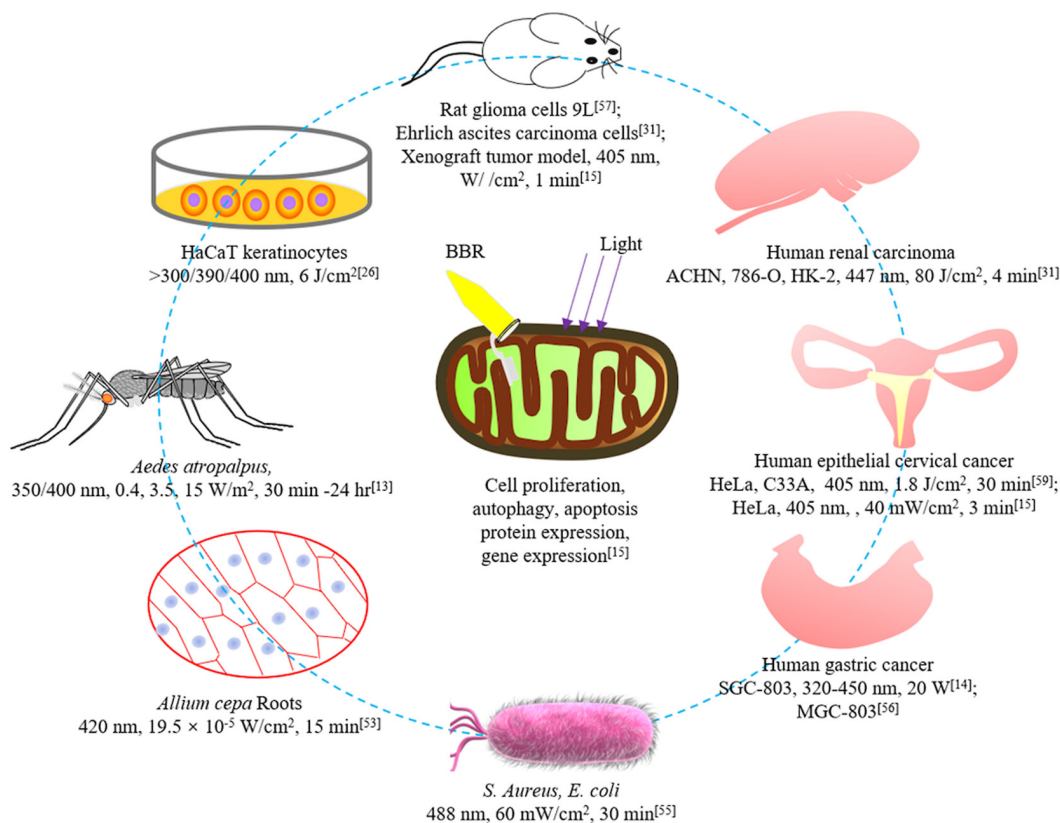


Figure 4. Biological effects of BBR-PDT. BBR-PDT exhibits destructive effects in different cells, including human tumor cells. The illumination parameters involved in relevant studies are presented. BBR, berberine; PDT, photodynamic therapy.

*in vitro* (54). Lee *et al* (55) reported that BBR successfully stains the Gram-positive bacteria, *Staphylococcus aureus* (*S. aureus*), whereas it only partly stains the Gram-negative *Escherichia coli* (*E. coli*). BBR-PDT significantly decreases the viability of *S. aureus*, and the bactericidal effects occur in a dose-dependent manner (5-10 mM BBR). BBR-PDT also exhibits bactericidal effects in *E. coli*; however, the efficiency is lower and the required concentration of BBR is higher (55).

**Antitumor effects of BBR-mediated PDT.** Recently, the antitumor effects of BBR-PDT have attracted great interest. The activity *in vitro* was first demonstrated by Wang in 1986 (14), who observed the strong inhibitory proliferation of SGC-803 gastric cancer cells combined with BBR (10 µg/ml) under illumination, using a black light lamp (320-450 nm; 20 W). Ma *et al* (56) demonstrated that BBR combined with 5-ALA-PDT significantly inhibits the proliferation of MGC-803 gastric cancer cells, induces cell apoptosis, inhibits the expression levels of survivin and Bcl-2, and upregulates the expression levels of p53 and Bax. Taken together, these results suggest that BBR has the potential to be used in combination with other photosensitizers. Chen *et al* (57) studied the effects of BBR in combination with argon ion lasers on 9L rat glioma cells, and demonstrated that the argon ion laser enhances the inhibition of BBR on DNA, RNA and protein biosynthesis, resulting in cell-killing effects. Jantová *et al* (31) compared the cytotoxic and phototoxic effects on murine fibroblast NIH-3T3 and EAC cells. The results demonstrated that EAC cells are more sensitive to photoactivated BBR

compared with non-cancer NIH-3T3 cells. BBR-PDT induces the DNA damage of EAC cells, subsequently blocking the S and G<sub>2</sub>/M phases, and decreases cell proliferation. In addition, DNA damage induces necrotic or apoptotic death of EAC cells (31). Lopes *et al* (58) demonstrated that BBR cytotoxicity increases by 20% following the combination of light. ROS production and activation of autophagy and apoptosis induced by BBR-PDT are responsible for the death of renal carcinoma cells. In addition, BBR-PDT affects the expression of anticancer drug target genes in 786-O cells (58). Warowicka *et al* (59) investigated the potential anticancer effects of a protoberberine alkaloidal fraction on HeLa and C33A cervical cancer cells. Liu *et al* (15) verified the antitumor effects of BBR-PDT in xenograft animal models, and revealed the role of the PI3K/AKT/mTOR signaling pathway.

The biological effects of BBR-PDT are summarized in Fig. 4. Among these, the mechanisms of BBR-PDT in treating tumors may involve inhibiting cell proliferation, inducing apoptosis and autophagy, and altering the expression of proteins or genes (15). However, there are a few studies currently investigating these mechanisms. BBR exhibits clear inhibitory effects on different types of cancer, including colorectal, lung, ovarian, prostate, liver and cervical cancers (60). This suggests that BBR-PDT may have more potent antitumor effects compared with other photosensitizers due to the superposition of the antitumor effects of BBR and PDT (15,61). However, only a few studies have proven the antitumor effects of BBR-PDT (15,31,59). The possible reasons are as follows (16): i) There are multiple factors influencing the photodynamic



reactions of BBR; ii) the hydrophobic properties, along with poor stability and bioavailability of BBR limits its application; iii) BBR has high toxicity, particularly when administered by injection; and iv) the light source of BBR-PDT is UV, which has several side effects, such as burn and pain and no advantages compared with other photosensitizers (2).

**BBR-mediated SDT.** SDT is a promising new option for the invasive treatment of tumors (62). SDT is similar to PDT in that it requires the combination of sensitizers, ultrasound and molecular oxygen (61). The major advantage of SDT compared with PDT is the increased penetration of ultrasound through mammalian tissues compared with light, indicating that it can be used to treat deeper and less accessible tumors (61,62). As the light source of BBR-PDT is UV, which has several side effects, such as burn and pain, BBR-SDT may be a more favorable choice for treating tumors (16). Wang *et al* (63) reported that BBR can enhance ultrasound-induced BSA molecule damage. The increase in the degree of damage is accompanied with the increase of BBR concentration and ultrasonic irradiation time. It was further revealed that BBR-SDT-mediated BSA damage is mediated by the production of ROS, particularly  $^1\text{O}_2$  (63).

Atherosclerosis (AS) is the major contributing factor of cardiovascular events. SDT-targeting AS is considered a novel promising treatment strategy for AS (64). Tian and Guo (65) investigated the effects of BBR-SDT on macrophages within atherosclerotic plaques. Kou *et al* (66) demonstrated that BBR-SDT induces autophagy and cholesterol efflux in THP-1 macrophages and derived foam cells, which may potentially involve inhibiting phosphorylation of the PI3K/AKT/mTOR signaling pathway. In addition, the effects of BBR-mediated SDT on macrophages within atherosclerotic plaques were investigated.

Only a few studies have reported the antitumor effects of BBR-SDT. For example, Liu *et al* (16) demonstrated that BBR-SDT inhibits the proliferation of HeLa cells and induces apoptosis both *in vitro* and *in vivo*, and revealed the role of the PI3K/AKT/mTOR signaling pathway in this process. However, further studies are required to validate the antitumor effects of BBR-SDT. SDT has several advantages over PDT due to its deeper penetration and fewer side effects (64). However, the chemical properties of BBR, such as its hydrophobic properties, poor stability and bioavailability, and high toxicity need to be resolved (16).

#### 4. Targeted delivery for BBR

The question remains as to how to overcome the physical shortcomings of BBR. Feasible options include chemical structure modification and targeted drug delivery (51,67). The photochemical properties of BBR are associated with the substituents at the C-2, C-3, C-7 and 9-O positions (27,45,50). Appropriate chemical structure modifications may increase its water solubility and retain or even improve its photodynamic activity; however, this requires experimental verification (68). In addition, targeted delivery may also avoid the dissolution and low absorption of BBR (69-73).

Several nanoparticle delivery systems have been used to target the delivery of BBR. For example, Sun and Ouyang (67)

prepared BBR hydrochloride, a nanoemulsion, which is a clear transparent solution with stable contents and diameter, even under high humidity and high temperatures. As a stable drug delivery system, BBR nanoemulsion has a high affinity for gastrointestinal lymphatic tissue. It can deliver larger amounts of BBR into the blood via the lymphatic system, avoid the biotransformation and absorption of the liver and intestines, and exhibit a first pass effect in the liver (67). Solid lipid nanoparticles can effectively control drug release, avoid drug degradation or leakage, and have potent targeting properties (74). Hou and Zhou (75) prepared solid lipid nanoparticles of BBR hydrochloride using a rotary-evaporated film-ultrasonication method. In addition, Wang *et al* (76) determined its entrapment efficiency.

Liposomes have similar structures to cell membranes and high affinities to tissues or cells (77). There are several approaches for the preparation of BBR liposomes, such as thin film evaporation and active loading (78). Incubation temperature and duration, the proportional weight of drugs and lipids, soybean phosphatidylcholine and cholesterol affect the encapsulation rate (79). Luo *et al* (78) prepared a long-circulating liposome of BBR hydrochloride using an ionophore A23187-mediated  $\text{ZnSO}_4$  gradient method. Using poly (lactic-co-glycolic acid) (PLGA) nanoparticles is a new strategy to promote specific targeting and elimination of drugs (80). Khan *et al* (81) developed a co-delivery system of BBR and doxorubicin using the conjugation/encapsulation strategy through PLGA nanoparticles. Co-delivery of BBR and other anticancer drugs is valuable to achieve higher anticancer effectivity (82). Guo *et al* (83) designed a liver-target nanotechnology system to facilitate BBR consisting of  $\alpha$ -tocopheryl hydrophobic core and on-site detachable polyethylene glycol-thiol shell to improve its liver deposition.

Cellular and molecular targeting can further enhance the effects of BBR and decrease its adverse reactions. Bhatnagar *et al* (84) prepared BBR chloride (BRB)-encapsulated hyaluronic acid-grafted [d(HA)-g]-PLGA nanoparticles (NPs). The BRB-d(HA)-g-PLGA NPs were used to target CD44-positive cancer cells. These NPs released BRB at a faster rate in HeLa and MCF-7 cells compared with BRB-PLGA NPs and bulk BRB, and increased its cytotoxicity. These results offer a promising and improved alternative for antitumor therapy. Song *et al* (68) obtained a novel mitochondria-targeting nano-drug self-assembled BBR derivative from a 9-O-octadecyl substitute using a simple nano-precipitation approach. These authors further used 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethyleneglycol) 2000] to modify the BBR derivative to increase its stability. Negatively charged HA was further coated to conceal positive charges and achieve tumor targeting. Grebinyk *et al* (85) formed a nanocomplex combining  $\text{C}_{60}$  fullerene and BBR, and  $\text{C}_{60}$  was demonstrated to promote BBR intracellular uptake by leukemic cells.

Collectively, these targeted delivery strategies provide solutions to the limitations of BBR, such as its hydrophobic properties, poor stability and bioavailability. Cellular and molecular targeted delivery allows the possibility of further improving its therapeutic effects. However, there is currently no evidence regarding the use of these BBR delivery systems in PDT or SDT, which is worth investigating in prospective studies. In addition, the toxicity of BBR should be concerned

in future applications with targeted delivery technology, particularly for free BBR, which may produce systemic adverse reactions.

## 5. Conclusions and prospects

BBR is a photosensitizer that can be activated by both light and ultrasound, and produce  $^1\text{O}_2$ . Both type I and type II reactions participate in the photochemical reactions of BBR. However, BBR reactions under ultrasound irradiation remain unclear. Both BBR-PDT and BBR-SDT have been proven to inhibit the proliferation and induce the apoptosis of several tumor cell lines *in vitro*. However, only a few studies have been validated in animal models, and the antitumor activities *in vivo* require further confirmation. Notably, both BBR-PDT and BBR-SDT can induce autophagy, which may be associated with the phosphorylation inhibition of the PI3K/AKT/mTOR signaling pathway. Although autophagy is generally considered to exert protective effects on cells, its role in the biological effects of BBR-PDT and BBR-SDT remain to be elucidated, particularly their antitumor effects. In addition, only a few proteins and genes involved in apoptosis have been identified to be regulated by BBR-PDT or BBR-SDT. Thus, it is important to clarify the antitumor mechanisms of BBR-PDT and BBR-SDT in prospective studies.

The photothermic characteristics of BBR are affected by polarity, pH and  $\text{O}_2$  content of solvents. Its hydrophobic properties, along with poor stability and bioavailability, and the high toxicity of BBR limits its application. It is also unclear whether the interactions between BBR and DNA is beneficial for PDT. Appropriate structural modifications at the C-2, C-3, C-7 and 9-O positions may change the water solubility and photodynamic efficiency of BBR; however, whether this is advantageous to BBR-PDT requires experimental verification. Targeting drug delivery via microbubbles may bypass the physical and chemical defects of BBR and enhance its antitumor effects via PDT and SDT. However, it is worth noting that BBR is highly toxic following intravenous injection. Although targeted delivery can theoretically only sequester BBR in the target tissues, the safety requires verification, particularly with regards to systemic toxicity.

In conclusion, BBR has a developmental prospect as a sensitizer for PDT and SDT treatment. Its simple structure, low cost and extensive pharmacological activities, such as antitumor effect, exhibit great advantage over other photosensitizers. Thus, the present study hypothesizes that BBR-PDT and BBR-SDT will be extensively used in clinical treatment in the future.

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## Availability of data and materials

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## Authors' contributions

YWA and HQL drafted the initial manuscript. YWA, HTJ and HQL confirmed the authenticity of all the raw data. HTJ, BY, JCW and CW performed the literature review and produced Figs. 1 and 4. All authors have read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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