Recent advances in theranostic agents based on natural products for photodynamic and sonodynamic therapy

Jiasheng Wu, Jie Sha, Chuangli Zhang, Weimin Liu, Xiuli Zheng, Pengfei Wang

1 Key Laboratory of Photochemical Conversion and Optoelectronic Materials and CityU-CAS Joint Laboratory of Functional Materials and Devices, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, P.R. China
2 School of Future Technology, University of Chinese Academy of Sciences, Beijing, P.R. China

Correspondence
Pengfei Wang, Key Laboratory of Photochemical Conversion and Optoelectronic Materials and CityU-CAS Joint Laboratory of Functional Materials and Devices, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, P.R. China.
Email: wangpf@mail.ipc.ac.cn

Funding information
External Cooperation Program of Chinese Academy of Sciences, Grant/Award Number: GJHZ1723; the Beijing Municipal Science & Technology Commission, Grant/Award Number: Z19110004819004; the Instrument Developing Project of the Chinese Academy of Sciences, Grant/Award Number: YJKYYQ20170015; the Strategic Priority Research Program of the Chinese Academy of Sciences, Grant/Award Number: XDB17000000; National Natural Science Foundation of China, Grant/Award Numbers: 21873110, 21673265, 61720106014

Abstract
The integration of diagnosis and therapy based on natural products has been receiving considerable attention in recent years because nature can contribute many fantastic functional molecules with good biocompatibility and low toxicity. Diagnostic and therapeutic agents combined with the technique of photodynamic therapy (PDT) and sonodynamic therapy (SDT) have been extensively developed thanks to the advantages of PDT and SDT, such as good selectivity, low toxicity, and noninvasive treatment for cancers and other diseases compared with traditional treatments. In this review, we summarize the recent advances in theranostic agents for natural products categorized as porphyrins, perylenequinone, curcumin, and others. Some representative examples of disease diagnosis in fluorescence/photoacoustic imaging and disease treatment in PDT/SDT were introduced. Potential limitations and future perspectives of these natural products for theranostic agents were also discussed.

KEYWORDS
hypocrellin, natural product, photodynamic therapy (PDT), sonodynamic therapy (SDT), theranostic agent

1 INTRODUCTION OF THERANOSTIC AGENTS

The integration of diagnosis and therapy, raised by John Funkhouser in 1998, is a new biomedical technology in which all functions are combined in a synergistic system by virtue of diagnostic and therapeutic tools such as light, electron, magnetism, and sound. With the help of theranostic agents, it is expected to realize early diagnosis, accurate localization, in-situ treatment, and real-time monitoring of diseases. In this regard, theranostic agents based on photodynamic therapy (PDT) and sonodynamic therapy (SDT) provide an opportunity for patients to receive good treatment. As for disease diagnosis,
fluorescence imaging and photoacoustic imaging are two common diagnostic methods. For instance, fluorescence imaging has advantages of easy operation, high sensitivity, and rapid response, whereas it still has some defects in terms of spatial resolution and penetration depth, which limit its application in vivo imaging. Photoacoustic imaging can achieve deep penetration and good spatial resolution by detecting phonons produced by thermal radiation, but it is expensive and less sensitive. Moreover, multimodality imaging has become an important trend to combine two or more imaging modes, which is helpful to observe the tumor mechanism at the cellular and molecular levels and to realize the early diagnosis, staging, and evaluation of therapeutic effect.

As for the disease treatment, PDT and SDT have been receiving considerable attention in recent years, and photosensitizers and sonosensitizers are used for theranostic agents of PDT and SDT, respectively. As noninvasive treatment models for cancer, infection, and inflammation, PDT and SDT have the advantages of less invasiveness, good selectivity, low toxic and side effects, and repeatable treatment. A clinically approved form of phototherapy is PDT, in which the excited photosensitizer can oxidize key cellular macromolecules, such as nucleic acids and proteins, leading to tumor cell ablation, through a photo-induced generation of reactive oxygen species (ROS), that is, singlet oxygen ($^1$O$_2$). However, most photosensitizers are dependent on surrounding oxygen levels, and the cure rates are severely limited by an anoxic tumor microenvironment. The excited photosensitizers can also transform photoenergy to heat (c, PTT) from hyperthermia to ablate tumors without relying on intracellular oxygen levels. Thus, PDT efficacy can be enhanced by the photothermal effect. However, phototherapy can only treat localized and superficial tumors due to the limitation of laser-penetration depth. Meanwhile, SDT can effectively treat deep-seated tumors because the ultrasound wave can reach deep-seated tissues. In the case of SDT, ultrasound can trigger singlet oxygen produced by a sonosensitizer enriched in deep-seated tumors to effectively kill tumor cells. As a kind of mechanical wave, the ultrasonic wave with suitable intensity and frequency almost does not damage normal cells and tissues. Therefore, the theranostic agents based on PDT and SDT have considerable advantages over traditional treatments for diseases.

The action mechanisms photosensitizer can be demonstrated in Figure 1A. Under light irradiation, the photosensitizer absorbs photons and is excited to the excited state (i.e., $S_1$), and the exciton in $S_1$ reaches the triplet excited state (i.e., $T_1$) by an intersystem crossing. The exciton in the $T_1$ state interacts with oxygen to form reactive oxygen species (ROS) through either electron transfer (Type I, free radical mechanism that produces hydroxyl or superoxide radicals) or energy transfer (Type II mechanism that...
produces singlet oxygen). ROS are highly oxidized and can interact with nucleic acids and proteins in cells to induce apoptosis. During the sensitization of photosensitizers, Type I and Type II processes occur simultaneously and compete with each other, but the singlet oxygen plays a more important role in PDT.\(^\text{12}\) Compared with PDT, the mechanism of SDT is currently still unclear. Ultrasound is generally believed to interact with water in the environment and cause an ultrasonic cavitation (Figure 1B). During this process, tiny cavities nucleate, grow, and collapse, resulting in high pressure and temperature. The released energy may be transferred to a sonosensitizer through a mechanism of sonoluminescence or pyrolysis, whereas sonoluminescence excites a sonosensitizer to produce electron–hole (\(e^-\text{−}h^+\)) pairs and generates ROS in water.\(^\text{11}\) The mechanism of pyrolysis involves the sonosensitizer that generates free radicals, which further react with the endogenous substrate to generate ROS. Although evidence to prove the mechanism of SDT is not sufficient, ROS generation is an important factor in SDT.

Over the past few decades, with the boom in nanomedicine, various theranostic agents including carbon dots,\(^\text{13−15}\) polymer dots,\(^\text{16}\) organic molecules,\(^\text{17}\) AIEgens,\(^\text{18}\) and inorganic and organic nanoparticles,\(^\text{1,2,6}\) have been developed and applied in PDT and SDT.\(^\text{19}\) For examples, Jia et al. introduced carbon dots-based nanotheranostics for imaging and PDT\(^\text{13}\); Guo et al. reviewed \(\pi\)-conjugated organic polymer dots as theranostic agents\(^\text{16}\); Lan et al. summarized organic molecule-based photosensitizers and their nanoparticles\(^\text{17}\); Son et al. also reviewed various multifunctional sonosensitizers in sonodynamic cancer therapy.\(^\text{19}\) These inorganic or organic materials have the following advantages as diagnostic and therapeutic agents: (i) nanostructures with controlled size and morphology for different requirements, (ii) easy superficial modification with various functional groups to achieve specific tissue targeting, and (iii) the EPR effect that can be enriched effectively in tumor sites. These theranostic agents have shown enhanced photodynamic and/or sonodynamic efficiency to realize the early diagnosis, staging, and evaluation of therapeutic effect. However, these materials are unnatural, and their inherent toxicity and long-term safety limit their further clinical uses. Therefore, diagnostic and therapeutic agents based on natural products are necessary to study for practical applications.

2 | THERANOSTIC AGENTS BASED ON NATURAL PRODUCTS

For thousands of years, natural products have received widespread attention due to their diverse chemical structures and unique pharmacological activity.\(^\text{20,21}\) More than half of the drugs are derived directly or indirectly from natural products.\(^\text{22−24}\) Thus far, natural products are still an important source for screening drug molecules because no complicated chemical syntheses are needed to obtain effective drug molecules. Moreover, natural products usually have good biocompatibility and low toxicity in living organisms. To date, many natural products are effective for photosensitizers\(^\text{25}\) and sonosensitizers,\(^\text{19,26−28}\) in which several nature-original theranostic agents, such as porphyrins, perylenequinone, curcumin, and others, have been widely applied in PDT and SDT. Among most theranostic agents, some natural products, such as porphyrin and its derivatives, due to low toxicity and good biocompatibility in vivo, have been approved for clinical uses.\(^\text{29,30}\) Among of these, hematoporphyrin derivative (HpD), as the first generation of photosensitizer approved for clinical PDT, is isolated from hemoglobin.\(^\text{31}\)

Therefore, natural products have a good clinical prospect as theranostic agents of cancers in PDT and SDT.

Currently, photo- and sonotheranostic agents based on natural products can be primarily classified as porphyrins, perylenequinone, curcumin, and others. Porphyrin-based theranostic agents have been extensively studied in the past few decades, and several derivatives have been developed for clinic uses as photodynamic drugs. These references have been well reviewed in the literatures. For examples, Rajora et al. reviewed porphyrin-based supramolecules used for biomedical imaging and PDT.\(^\text{29}\) Almeida-Marrero et al. summarized the porphyrinoid biohybrid materials for phototheranostics.\(^\text{30}\) Perylenequinone-based theranostic agents have also been studied for many years, but they are still in the basic research stage and no clinical drugs have been developed yet. To date, it has not been well summarized except for a small section included in photodynamic\(^\text{25}\) and sonodynamic\(^\text{28}\) reviews. Curcumin and its derivatives have a weak absorbance band in the phototherapeutic window, thereby limiting their practical applications. As a result, curcumin-based theranostic agents have received less attention in recent years.

This review will summarize the recent advances in theranostic agents based on natural products categorized as porphyrins, perylenequinone, curcumin, and other natural products, and introduce some representative examples for the disease diagnosis in fluorescence/photoacoustic imaging and the disease treatment in PDT and SDT. In particular, hypecrellin, as an important category of perylenequinonoid pigment (PQP), will be elucidated in detail. The potential limitations and future perspectives of these natural products were also discussed.
3 | VARIOUS NATURAL PRODUCTS FOR THERANOSTIC AGENTS

3.1 | Porphyrin

Porphyrins are a class of conjugated skeleton macrocyclic compounds. In nature, porphyrin is the main component of hemoglobin, cytochrome, and chlorophyll and participates in a series of important processes in organisms. Hematoporphyrin derivative was the first photosensitizer used in PDT with many studies in the 1980s and early 1990s, and HpD was approved for clinical use in 1994 with a trademark “Photofrin.” Although HpD made great progress in the treatment of cancers at that time, it had some disadvantages of weak absorption in the red region, complex component, poor selectivity, and some side effects, such as skin phototoxicity, that required patients to avoid light up to 4–6 weeks after injection of drugs, which limited its practical use. Afterwards, a large number of porphyrin derivatives were developed as new theranostic agents. 29,30

Porphyrin has attracted considerable attention of many researchers because of its high 1O2 efficiency and low toxicity. Porphyrin-based theranostic agents can pass through the barrier of the plasma membrane in cells, either through passive diffusion or by receptor-mediated endocytosis, and they are incorporated into the cytoplasm as a photosensitizer. 32 These agents can preferentially accumulate in tumor tissues with a long retention time. Therefore, porphyrin analogs have been widely used as theranostic agents in PDT and SDT. However, most porphyrin derivatives display serious π–π stacking because of their large planar structure, which tends to induce aggregation and reduce their ROS efficiency accordingly. 32 Furthermore, porphyrin derivatives have low extinction coefficient in the PDT window (600–900 nm), limiting their clinical applications. Much effort has been dedicated to solving these defects through organic structural modification to extend the absorption wavelength, supramolecular assembly, or organic–inorganic nanocomposites as carriers. Given that these studies have been well documented, herein, only a couple of recent new examples for illustration of new strategies of porphyrin-based theranostic agents are listed. Other examples are described in reviews and literatures therein. 29,30,33–36

Metal-organic frame structure (MOFs) can be applied for the design of multifunctional theranostic agents because of their unique material properties. Zheng et al. synthesized MOFs with a photoactive porous–organic polymer (POP) nanocomposite (HUC–PEG) to improve the phototherapy effect of porphyrin. 37 As shown in Figure 2, nonphotosensitive MOFs as a nanocarrier and chlorin-based POPs as a photosensitizer were conveniently integrated through a self-template strategy to prepare nanocomposites as a light-triggered therapeutic agent. In this system, HUC–PEG displays a controllable particle size, good stability, and biocompatibility, and a high photothermal conversion efficiency of 41%. Interestingly, the generation of singlet oxygen for PDT and the local hyperthermia for PTT are enhanced in a synergy “0 + 1 > 1” interface effect, thereby realizing a favorable inhibition of tumor proliferation.

Aggregation of theranostic agents in tumor cells generally decreases efficiency of PDT. Wang et al. constructed a nanosystem (TPFcNP) by assembling a hydrophobic theranostic agent containing multiple alkenyls in the terminal of tetrakis(4-methacryloyloxyphenyl)porphyrin (TMPP) and an amphiphilic copolymer with ferrocene unit (PEG-b-PMAEFc) to overcome the hydrophobicity of porphyrin. 38 Figure 3 shows that the ferrocene unit in TPFcNP can catalyze H2O2 overexpressed in the acidic tumor microenvironment through a Fenton reaction to generate hydroxyl radicals. The produced hydroxyl radicals further promote the addition reaction of the alkenyl terminal of the hydrophobic TMPP with overexpressed glutathione (GSH), resulting in a TMPP–GSH adduct with enhanced hydrophilicity. As a result, the photosensitizer (TMPP) deaggregates in the tumor cells, which greatly increases the singlet oxygen production and ultimately achieves a high PDT efficacy.

Targeting delivery of the theranostic agent to tumor cells is important for PDT. Satrialdi et al. designed an extended π-conjugated porphyrin derivative (rTPA) containing two triarylaminemoieties (Figure 4). 39 Such a conjugate structure in rTPA increases the ability of porphyrin to absorb near-infrared (NIR) light without sacrificing 1O2 efficiency. After rTPA is assembled with the mitochondrial targeting liposome (MITO-Porter system) as a nanocarrier, the nanoparticles can absorb NIR light at 700 nm to generate ROS. As a result, this system effectively controls the production of active oxygen and induces an irreversible oxidative damage to mitochondria in tumor cells, eventually causing apoptosis.

Extensive research has been conducted on a precise tumor treatment based on a dual-modal imaging–guided theranostic agent. However, good interfacial compatibility in different functional components is difficult to achieve. Wang et al. used a simple co-assembly strategy to combine zinc porphyrin (ZnTPP) as a theranostic agent and Gd porphyrin (GdTTPP) as a contrast agent into uniform nanocomposites (GZNs, Figure 5). 40 As-prepared GZNs with homogeneous building blocks enhance the interface compatibility between GaTPP and ZnTPP and distinctly increase their hydrophilicity. GZNs also show excellent property in terms of fluorescence imaging, high relaxation rate, and singlet oxygen yield. Under the guidance of MR/FL imaging in
vivo, the antitumor targeting activity of GZNs to HeLa cells increases by 80.6%, achieving a visual diagnosis and effective treatment of cancer.

Additionally, a nano-emulsion system (NewPS) with superior colloidal stability with a porphyrin shell was prepared by self-assembling a porphyrin salt around the oil core. In addition to its own phototherapy effect, the as-prepared NewPS was loaded with a hydrophobic chemotherapeutic agent to achieve effective drug delivery.

3.2 Perylenequinone

Perylenequinones were found in the 1960s and widely distributed in ascomycetes and fungi imperfecti of plants. Perylenequinonoid pigments have been developed as a new generation of theranostic agent over the past few decades. PQPs have been studied as diagnostic agents in fluorescence imaging and photoacoustic imaging and as therapeutic agents in PDT and SDT in recent years. In general, 3,10-dihydroxy-4,9-perylenequinones include a couple of natural pigments with unique chemical and biological properties, which show considerable potential for clinic applications as diagnostic and therapeutic agents in PDT and SDT. The natural PQPs of this class include some pigments such as hypocrellins, elsinochrome, hypericin, cercosporin, and others (Figure 6).

3.2.1 Hypocrellin

Hypocrellin was first found in China in the early 1980s and studied as a photosensitizer for many years. It is a natural PQPs extracted and isolated from the Hypocrella bambusae, which is parasitic on Fargesia and distributed in Yunnan and Tibet, China. Natural hypocrellins include hypocrellin A (HA) and hypocrellin B (HB), and HA can be converted to HB in a dilute KOH solution. In traditional Chinese medicine, hypocrellin has been clinically applied for the treatment of some skin diseases under the exposure of light, that is, keloid, vitiligo, white lesion of the vulva, tinea capitis, and lichen amyloidosis.

Hypocrellin has several advantages as a theranostic agent in PDT and SDT compared with porphyrin. First, natural hypocrellin has a singlet oxygen yield as high as 0.70–0.80 and also generates a small amount of
hydroxyl and superoxide radicals. Although the active oxygen yield of the chemically modified hypocrellin is slightly decreased, it is still sufficient for PDT in hypoxic tumors. As a result, hypocrellin is especially suitable for PDT in hypoxic environment of tumors. Second, the metabolic time in vivo for hypocrellin is less than 1 week, which is important for clinic applications as theranostic drugs, whereas porphyrin-based theranostic agents generally need a metabolic time as long as 4–6 weeks. Moreover, hypocrellin has a low dark toxicity and is easy for a chemical tailing modification, resulting in a new generation of theranostic agent in PDT and SDT favored by many researchers. Hypocrellin still has some shortcomings that should be improved; for instance, natural hypocrellin has poor water solubility due to the lipophilic structure. After intravenous injection, hypocrellin tends to aggregate in the plasma and block the vascular network. Furthermore, hypocrellin lacks efficient absorption in the PDT window (600–900 nm), which considerably affects its clinic application as a theranostic agent.

In our continuous efforts to develop highly efficient theranostic agents, we aimed to address the inherent limitations of hypocrellin as follows: (i) a rational molecular modification to extend the absorbance wavelength in the PDT window and (ii) construction of various nanostructures as carriers to increase water solubility and biocompatibility. For instance, Zheng et al. modified HB with an ethylenediamine derivative to synthesize an amino-derived HB analog (DPAHB, Figure 7A) to improve the limitation of the molecular structure of hypocrellin. DPAHB covers an extensive and continuous absorption band from 400 to 800 nm (Figure 7B). Its maximum
FIGURE 4  Synergistic interaction between the NIR photosensitizer TPA and the MITO-Porter system for the mitochondria targeting cell apoptosis. Reprinted with permission. 39 Copyright 2020, Royal Society of Chemistry

FIGURE 5  Schematic of the co-assembly nanocomposites between GaTPP and ZnTPP and their MR/FL bimodal imaging guided PDT for HeLa cells. Reprinted with permission. 40 Copyright 2020, Elsevier
The absorption peak is at 630 nm with a molar coefficient of 36,300 M/cm, indicating a strong absorbance ability in the PDT window. It also has a NIR emission band that peaks at 720 nm, corresponding to an NIR light-driven theranostic agent that can reduce damage to normal cells and tissues. DPAHB shows a strong generation of ROS, and its $^1\text{O}_2$ quantum yield is 0.33. Biodegradable hypocrellin nanovesicles (DPAHB NVs) were prepared from a self-assembly of PEG–PLGA and DPAHB by a double emulsion method to improve the water solubility and biocompatibility. The formation of nanovesicles with a diameter of 150 nm leads to a small red shift in the absorption and fluorescence spectra due to the noncovalent interaction between DPAHB and PEG–PLGA. Moreover, DPAHB NVs show high photostability and suitable biodegradation rate. Given the excellent property of DPAHB NVs in absorption and NIR emission, it is especially suitable as a diagnostic agent in fluorescence imaging and photoacoustic imaging. In vitro fluorescence imaging shows that DPAHB NVs can easily enter the cytoplasm of cells, indicating that the tumor cells have a good uptake of the diagnostic agent. After intravenous (i.v.) injection of DPAHB NVs into mice (Figure 7D), the fluorescence intensity in tumor sites increases significantly with time and reaches the maximum at 12 h, indicating the diagnostic agent well enriches in tumors by the EPR effect. Similar results were found in photoacoustic imaging (Figure 7E). By comparing two different imaging modes, photoacoustic imaging has a sharper contrast and image quality than fluorescence imaging. Such a different diagnostic mode helps to realize the early disease diagnosis and evaluation of therapeutic effects.

On the other hand, DPAHB NVs display a good therapeutic effect because it has an efficient ROS generation and photothermal conversion efficiency of approximately 0.24 by using NIR light irradiation at 721 nm. In vitro study indicates that DPAHB NVs efficiently kill tumor cells by over 90% via a low concentration of theranostic agent, indicating a good PDT effect (Figure 7C). In vivo experiments indicate that the temperature in tumor regions rapidly reaches 58.3°C in the PDT/PTT group, implying a good photothermal therapy (Figure 7F). In the PDT/PTT group, the tumors were inhibited and completely disappeared after 14 days, whereas in the PDT group, the tumors were only effectively suppressed but did not completely disappear (Figure 7G). In other contrast groups, the tumor volume increased exponentially (Figure 7H). Hematoxylin and eosin (H&E) staining showed a significant separation of cytoplasm and nucleus, and mass cell death occurred in the PDT/PTT group. All results show that DPAHB NVs have a stronger PDT/PTT efficiency than a commercially available theranostic agent (chlorin e6). Biological evaluation shows that DPAHB NVs do not cause significant damage to any organs, indicating that the vesicles have good biocompatibility and low toxicity.

The triplet energy levels of theranostic agents can be changed by appropriate structural modification, which affects the singlet oxygen efficiency and the photothermal conversion efficiency. Zhang et al. modified HB with amino group in the 4-site and sulfhydryl group in the 5-site to synthesize a hypocrellin derivative (AETHB, Figure 8A). When strong nucleophilic groups (amino and sulfhydryl units) are modified in the mother ring of hypocrellin, AETHB displays an efficient singlet oxygen generation as high as 0.64. Natural HB has a maximum absorption wavelength at 470 nm, whereas AETHB has a wide and continuous absorption band that peaks at 660 nm because of the charge transfer from amino group to hypocrellin ring, extending its absorption in the PDT window (Figure 8B). HSA–AETHB NPs were prepared by self-assembly of HSA and AETHB to improve their water solubility. In this case, two natural-origin molecules are integrated together to form HSA–AETHB NPs as a theranostic agent. The maximum emission peak of HSA–AETHB NPs is at 710 nm (Figure 8C), and the fluorescence quantum yield is approximately 1%, indicating that HSA–AETHB NPS can be used as a NIR diagnostic agent in photoacoustic and fluorescence imaging for drug tracers in vivo. The concentration of nanoparticles in tumor sites increased gradually with time, implying that HSA–AETHB
NPs were passively enriched in tumor sites via the EPR effect (Figure 8E). Following the fluorescence intensity in tumors with different time intervals, HSA−AETHB NPs in tumor cells reach the maximum after 4 h of tail vein injection and decrease gradually. This result is consistent with that of photoacoustic imaging. The primary organs (heart, liver, spleen, lung, and kidneys) and tumors in mice were harvested at different intervals for fluorescence imaging to detect the pharmacokinetics of the theranostic agent in vivo. The fluorescence intensity in tumors was the maximum after 4 h and primarily metabolized in the kidney, liver, and lung. The half-life of retention in tumors was 8.85 h and almost completely metabolized out of tumors after 7 days. These results indicate that
HSA–AETHBNPs is an efficient diagnostic agent to trace drug distribution and metabolism in different organs.

HSA–AETHBNPs reserve a high ROS efficiency and have a high photothermal conversion efficiency of approximately 50%. NIR photo irradiation at 671 nm can reduce the damage to normal cells and tissues. ROS generation in tumor tissues were confirmed using DCFH–DA as a ROS probe, in which green fluorescence appeared in tumors injected with HSA–AETHBNPs. Cell experiments revealed that HSA–AETHBNPs killed 90% tumor cells with a low concentration of the theranostic agent under NIR irradiation (Figure 8D). For therapy, this material was
used for the therapeutic agent for synergistic PDT/PTT in hypoxic solid tumors. In the PDT/PTT group, the intra-tumor temperature rapidly increased to 23.1°C, whereas no obvious temperature changes were observed in other groups (Figure 8F). In the PDT/PTT group, the tumors in mice progressively vanished after treatment for 14 days (Figure 8G and 8H). These tumors could be almost 100% inhibited in the synergic therapy with high efficiency. The advantages of HB derivative make it promising in clinical use as a theranostic agent.

Diagnosis and therapy of malignant glioblastoma remain a considerable challenge because of the blocking of the blood–brain barrier to efficient drugs. Zhang et al. synthesized a 5- and 8-modified HB derivative (DCHB) as a theranostic agent with a high $1^O_2$ quantum yield of 0.51 (Figure 9A). DCHB and TMZ-C18 are encapsulated with DSPE–mPEG and DSPE–mPEG–cRGD to form a multifunctional phototheranostic agent (Figure 9B). As-prepared DTRGD NPs have a wide absorption spectrum at 703 nm, an NIR emission peak at 720 nm, and a photothermal conversion efficiency of 33% (Figure 9C). The encapsulated TMZ-C18 is effectively degraded to temozolomide for chemotherapy. In addition to the EPR effect, DTRGD NPs can actively target U87MG cells over-expressing $\alpha_v\beta_3$ integrin receptor. Compared with cRGD free nanoparticles, DTRGD NPs are the most effectively absorbed and enriched in U87MG tumors through a dual targeting direction. Fluorescence imaging in subcutaneous tumors indicates that enrichment of DTRGD NPs in tumors reaches the maximum concentration after 8 h
of tail vein injection (Figure 9D). The tumors and major organs of mice were harvested for fluorescence imaging after 24 h. The concentration of DTRGD NPs in tumors was 1.61 and 1.92 times higher than that of cRGD-free nanoparticles in tumors. Importantly, DTRGD NPs can break through the blood-brain barrier and accumulate in the orthotopic glioblastoma (Figures 9E and 9F). DTRGD NPs is 1.35 times more abundant in brain tumors than DT NPs due to the uptake of DTRGD NPs by receptor-oriented tumor cells. As-prepared DTRGD NPs present a high antitumor efficiency in U87MG tumor-bearing mice (Figures 9G and 9H). Accordingly, the synergistic therapy for the combination of PDT, PTT, and targeting chemotherapy could be achieved with a good diagnosis and glioblastoma treatment.

Hypocrellin was attempted to be loaded onto the clavate calcium phosphate to enhance tumor enrichment and to effectively deliver the theranostic agent to tumor sites. Wang et al. prepared a dopamine-substituted HB derivative (DAHB) and used with CaCl₂, NH₃·H₂O, and H₃PO₄ through a microwave reaction to form nanorods (DAHB@CaP NRs, Figure 10).⁴⁹ DaHB@CaP NRs show an extensive absorption band from 650 to 800 nm and its maximum emission peak at 735 nm; they could be used as an NIR light-driven theranostic agent. DAHB@CaP NRs also have an enhanced efficiency of cellular uptake, offering an efficient inhibition of tumor growth. This nanotheranostic platform provides an NIR fluorescence imaging-guided PDT, thereby providing a new prospect for clinical uses. Similarly, Krishnaswami et al. designed and synthesized PLGA–TPGS nanoparticles loaded with HB and silver to increase the production of singlet oxygen, thereby enhancing the photodynamic effect for the therapy of an age-related macular degeneration.⁴⁹b

In addition to the chemical tailing-modified hypocrellin derivatives above, natural HB is also used as an efficient theranostic agent. For instance, Jiang et al. designed an iRGD-based peptide amphiphile (PA) with an off-on mode to form spherical nanovesicles via self-assembly, in which HB as a photosensitizer is encapsulated into PA vesicles.⁵⁰ HB in PA vesicles is inactivated due to the aggregation quenching. When iRGD is selectively targeted and degraded in the tumor cells, HB will restore fluorescence activity to achieve the tumor-targeted fluorescence imaging. As a result, the assembled HB–PA nanovesicles show an enhanced efficiency for the photodynamic anticancer with negligible side effects, providing a new strategy for an effective tumor-targeting PDT.

Xuan et al. coated HB with magnetic mesoporous silica nanoparticles (MMSNs) and a red blood cell (RBC) membrane.⁵¹ HB-loaded RBC@MMSNs could extend the drug circulation time in vivo, prevent immune clearance, and realize a magnetic field-induced tumor accumulation. As a result, an efficient PDT with an enhanced inhibition of tumor growth and recurrence was achieved. Such RBC-assembled magnetic nanocarrier can effectively target photosensitizer delivery, an enhanced PDT and an immunological adjuvant, thereby providing a new method for cancer therapy.

Apoferitin (AFT) as a macromolecular protein was found to serve as an attractive nanocage for HB nanocarrier to enhance water solubility and tumor selectivity of HB. Jiang et al. integrated AFT nanocages with HB via a self-assembly strategy to form HB–AFT nanoparticles.⁵²
The as-prepared HB-AFT NPs have an average particle size of 12 nm and a high loading efficiency of HB to 85%. The encapsulation strategy of HB retains the structure of ferritin, so the tumor targeting ability of AFT remains unchanged. HB–AFT NPs also increase ROS generation, providing a distinct PDT activity on MDA-MB-231 cells.

Li et al. used HB and a biodegradable polymer, PEG-poly(lactic acid)-folate to form an assembly, HB/FA–PEG–PLA. Such a drug delivery system significantly increases biocompatibility, extends blood circulation of HB, and shows a good targeting antitumor activity for ovarian cancer. Moreover, existing paclitaxel-encapsulated hyaluronic acid ceramide nanoparticles contain HB to achieve a combined treatment of chemotherapy and PDT.

Lanthanide up-conversion nanoparticles (UCNP) can absorb NIR light for UV and visible emission due to their unique step-like energy level structures (Figure 11). UCNP is used as light modulators because NIR-driven PDT results in minimal light damage and high-tissue permeability. Zhang et al. used UCNP (NaYF4:Tm, Yb@NaYbF4) loaded with a mesoporous silica layer to develop a nanocapsule for the effective NIR regulation of siRNA delivery. This nanocapsule includes hyporcrellin A (HA) as a photosensitizer, a small interfering RNA (siRNA) against polo-like kinase 1, and a thin PEG film bound with polyethylene via a photo cleavable connector (PhL). With light irradiation at 980 nm, UCNP generates UV emission to destroy PhL and PEG film to release siRNA and emits blue emission to activate HA to generate ROS. The intracellular generation of ROS helps the endosome escape of siRNA to improve the efficiency of gene therapy and further destroys organelles to promote apoptosis.

SDT is noninvasive and low toxic method developed in recent years for cancer treatment. It utilizes the mechanism of ROS generated by ultrasound to realize the treatment of various tumors. Sonosensitizers that originate from natural products play important roles as theranostic agents, and hyporcrellin is used as a natural sonosensitizer for the therapy of cancers and other infectious diseases. For example, Zheng et al. modified HB with 1,2-diaminopropane to synthesize APHB, which further self-assembles with PEG-PLGA to fabricate APHB nanoparticles with a diameter of approximately 100 nm and surface potential of $-27.5$ mV (Figure 12A). Such a suitable diameter and negative surface property of the nanoparticles help to passively target to the tumor sites via the EPR effect. APHB NPs also have excellent water solubility and biocompatibility. The maximum absorption and fluorescence peaks of APHB NPs are approximately 630 and 690 nm, respectively (Figure 12B), offering an NIR fluorescence imaging reagent in vivo. Fluorescence imaging shows that APHB NPs can well enter the cytoplasm of Hela cells via an endocytosis interaction. In vivo fluorescence of tumor sites in mice increases gradually and reaches maximum after 7 h of tail vein injection (Figure 12E).
that APHB NPs in tumors are much higher than those in other organs through NIR fluorescence imaging, indicating a good enrichment in tumors (Figure 12F).

APHB NPs can effectively generate ROS under ultrasound stimulation both in solution and in cells (Figure 12C). For in vitro SDT, the ultrasound at the frequency of 1 MHz and the intensity of 0.6 W/cm² for 60 s will cause nearly 90% cell death. For in vivo SDT, the tumor growth and recurrence of mice are efficiently inhibited by the combination of APHB NPs and ultrasound stimulation, whereas it cannot be inhibited by the APHB NPs or ultrasound alone (Figure 12G). From the tissue section after treatment of SDT, cytoplasm and nucleus are separated and a large number of cells are died (Figure 12H). As a result, APHB NPs have excellent tumor accumulation, and they can also effectively generate ROS under ultrasound stimulation to achieve the treatment of deep tumors in vivo.

El-Sikhry et al. developed a 2-amino-substituted HB analog (SL-017) as a sonosensitizer to study its cytotoxic mechanism. A maximal uptake of SL017 for 30 min causes mitochondrial subcellular localization. SL017 activation via ultrasound produces significant ROS. A sonosensitizer could target mitochondria and control the loss of
mitochondrial membrane potential that would lead to ROS production and eventual mitochondrial fragmentation.\textsuperscript{58} Similarly, Wang et al. investigated the SDT-induced cell damage for subcellular localization of HB in HepG2 cells. HB-induced damage of mitochondrial structures and functions in SDT produces survival inhibition and apoptosis, eventually leading to cell death. Other subcellular organelles, that is, Golgi apparatus, lysosomes, and ER, also serve as the targets of HB-induced SDT.\textsuperscript{59}

Although hypocrellin has good antitumor activity, its exact antitumor molecular mechanism is still unclear. Qi et al. found that the oxidative damage regulated by HA is primarily caused by the anti-NF-κB signal in the apoptotic cascade, which leads to the dysfunction of mitochondria, the change of MMP, the release of cytochrome, the activation of apoptotic protease, and subsequent apoptosis (Figure 13).\textsuperscript{60}

3.2.2 Hypericin

Hypericin, as a natural anthraquinone derivative isolated from St. John’s Wort, has been extensively used in antitumor, antiviral, antibiotic, and antidepressant drugs.\textsuperscript{61} Given that hypericin is a hydrophobic molecule (Figure 6), some drug carriers, such as micelles, liposomes, and nanoparticles, are required to achieve good results for their practical applications as theranostic agents.\textsuperscript{62} Hypericin with light excitation produces both singlet oxygen and superoxide anion radical with high quantum yields. These reactive oxygen species produced in PDT may result in an oxidative damage to tumor cells and tissues. Liu et al. found that hypericin can effectively inhibit the growth of pancreatic cancer cells with irradiation in vitro and in vivo.\textsuperscript{63} Ritz et al. found that hypericin is effective in PDT for many types of tumor cells in the concentration as low as the nanomolar range and considerably better than ALA-induced PpIX against D283 myeloblastoma cell lines.\textsuperscript{64} Ocker et al. revealed that hypericin together with radioactive iodine can inhibit soft tissue sarcoma in children under a combinational therapy of PDT and radiotherapy.\textsuperscript{65}

3.3 Curcumin

Curcumin pigments are another class of natural products used as theranostic agents. Curcumin is a bright yellow natural product and is a polyphenolic pigment extracted from turmeric rhizomes of the traditional Chinese herb Curcuma longa. Curcumin usually exists in the form of ketones or enols, as shown in Figure 14. Curcumin absorbs light for ROS generation that can be used as a photosensitizer for anticancer therapy.\textsuperscript{66} Moreover, the antibacterial, antiviral, and anti-inflammatory pharmacological activities of curcumin have also attracted considerable attention in recent years.\textsuperscript{67} However, ingested curcumin mostly
cannot enter the blood, and the cell permeability of curcumin metabolites is poor. Low water solubility, poor absorption of the gastrointestinal tract, and rapid elimination hinder the practical applications of curcumin. To overcome the shortcomings of curcumin in PDT, researchers have exerted much effort to improve the bioavailability, antibacterial, and anticancer activity of curcumin through the preparation of curcumin nanoparticles, modification of curcumin with metal-bound complexes, or combination with other therapeutic agents.

Jiang et al. found that under light irradiation at 470 nm, a low concentration of curcumin (2.5 μM) significantly destroys the permeability of Staphylococcus aureus cell membranes, leading to cell death. The enhanced intracellular ROS in the presence of curcumin is an important factor behind the killing of S. aureus. Panhoca et al. found that curcumin reduces the number of living cells of the main pathogen causing caries, Streptococcus mutans without damaging the biofilm. Spaeth et al. used curcumin derivatives with cationic substituents to achieve good water solubility, improved photostability, and low aggregation. These derivatives have good photodynamic antibacterial activity against Escherichia coli and S. aureus under blue light irradiation. The PDT efficiency is 100 times higher than that of natural curcumin.

The anticancer activity of curcumin was demonstrated in many tumor cells. Jamali et al. found through in vitro experiments that curcumin (25 μM) can effectively kill human glioma cells under blue light irradiation. Xin et al. found that demethoxycurcumin as a photosensitizer can attack A431 and HaCaT cells. Under the PDT treatment of demethoxycurcumin, the p53 and the caspase pathways of cancer cells are activated, thereby upregulating Bax and p-P65 expression and downregulating the Bcl-2, Mcl-1, and nuclear factor-kB expression, which causes cell apoptosis.

3.4 Other natural photosensitizers

3.4.1 Pheophytin

Chlorophyll is natural and widely distributed in green plants for the photosynthesis of oxygen. Pheophytin is a magnesium-free chlorophyll derivative, which has a similar structure to porphyrin. Pheophytin, as a phototherapeutic agent, cannot be directly applied in PDT due to its hydrophobicity and instability. Wen et al. overcome this problem by using pheophytin as an original carbon source to synthesize carbon dots with a high singlet oxygen quantum yield and good stability. These carbon dots had the maximum absorption in the NIR region that peaked at 680 nm. After DSPE-mPEG2000 was self-assembled with pheophytin carbon dots, it shows good water solubility and biocompatibility. Further experiments proved that the carbon dots are efficient for fluorescent imaging and excellent for PDT and PTT. Moukheiber et al. used a surfactant stripping method to obtain pheophytin micelles (ss-Pheo). As an excellent photodynamic agent, ss-Pheo has a high singlet oxygen quantum yield, long circulation time in the blood, and strong enrichment ability in tumor sites.

3.4.2 Psoralens

Psoralens are a class of linear and umbelliferous cysts that are primarily found in plants of the Umbelliferae family. In terms of the use of phototherapy, psoralen analogs work in synergy with long-wave ultraviolet (300–400 nm) radiation to treat psoriasis, atopic dermatitis, seborrheic dermatitis, eczema, and other skin diseases (Figure 15). The first psoralen analog isolated in 1974 by Parrish et al. was 8-methoxy psoralen (8-MOP), and experiments showed that 8-MOP under UVA could treat psoriasis in PDT. Bagchi et al. used ribosomes to encapsulate psoralens, evaluated their light-induced kinetics, and confirmed the antibiofilm effects of psoralen-loaded ribosomes against Gram-negative and positive bacteria under UVA light excitation activity. However, light-activated psoralen cannot be used clinically to treat certain skin diseases by using 365 nm excitation light, which is harmful to human health; also, long-term use of PUVA treatment increases the risk of skin cancers.

4 CONCLUDING REMARKS

In this review, we summarized recent advances in natural products (porphyrins, perylenequinone, curcumin, and
others) as diagnostic agents in fluorescence and photoacoustic imaging, as well as therapeutic agents in PDT and PTT. Given the unique advantages of PDT and SDT compared with traditional therapies, photo- and/or sonotheranostic agents have been extensively developed in recent years. It is probably needed to pay attention to some issues.

(i) For disease diagnosis, in addition to fluorescence and photoacoustic imaging, a combination of multiple imaging, such as dual-mode imaging and multimode imaging, can provide clear information for early diagnosis, clinical treatment, and postoperative intervention. Diagnostic reagents that can function in multiple imaging should be developed.

(ii) For disease treatment, SDT lags behind PDT, and the mechanism of SDT remains unclear. In most cases, SDT can only inhibit the growth of solid tumors, whereas PDT can inhibit and also kill tumors completely. Therefore, a couple of photosensitizers have been used as photodynamic drugs in the clinical treatment of tumors and skin diseases, whereas no sonodynamic drugs have been developed for clinic uses. In the future, the action mechanism of SDT should be understood, and sonodynamic drugs that are close to clinical requirements should be developed.

(iii) Given the depth restriction of light penetration, most phototheranostic agents are suitable only for the treatment of superficial diseases. As a result, development of NIR-II phototheranostic agents is highly desirable because they can penetrate deeper depth compared with other agents. However, the compounds with NIR-II property are very limited, and all of them are artificially synthetic. The theranostic agents based on natural products with NIR-II have not appeared yet until now, which should be noted in future. Alternatively, researchers also use the two-photon treatment to achieve a deeper depth PDT, but the two-photon therapy is still in the basic research stage because of a high demand of materials and low two-photon efficiency.

(iv) Hypocrellin has the advantages of broad and continuous absorption at 400–800 nm and high ROS generation yield. It produces not only singlet oxygen but also superoxide free radicals, and is especially suitable for the treatment of tumors in hypoxia environment. Hypocrellin has less toxicity and fast metabolism in vivo and is much superior compared with porphyrin-based theranostic agents. Hypocrellin is expected to be a new generation of phototheranostic agent after porphyrin and will be developed as clinical drugs.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGMENTS
This research was supported by the National Natural Science Foundation of China (Grant Nos. 21873110, 21673265, and 61720106014), the External Cooperation Program of Chinese Academy of Sciences (No. GJHZ1723), the Beijing Municipal Science & Technology Commission (No. Z191100004819004), the Instrument Developing Project of the Chinese Academy of Sciences (No. YJKYYQ20170015), and the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDB17000000).

ORCID
Jiasheng Wu @ https://orcid.org/0000-0003-1002-6784

REFERENCES
1. E. K. Lim, T. Kim, S. Paik, S. Haam, Y. M. Huh, K. Lee, Chem. Rev. 2015, 115, 327.
2. K. K. Ng, G. Zheng, Chem. Rev. 2015, 115, 11012.
3. J. P. Celli, B. Q. Spring, I. Rizvi, C. L. Evans, K. S. Samkoe, S. Verma, B. W. Pogue, T. Hasan, Chem. Rev. 2010, 110, 2795.
4. C. Kim, C. Favazza, L. V. Wang, Chem. Rev. 2010, 110, 2756.
5. U. Chilikamarthi, L. Giribabu, Chem Rec 2017, 17, 775.
6. L. Cheng, C. Wang, L. Feng, K. Yang, Z. Liu, Chem. Rev. 2014, 114, 10869.
7. D. K. Chatterjee, L. S. Fong, Y. Zhang, Adv. Drug Deliv. Rev. 2008, 60, 1627.
8. X. Li, N. Kwon, T. Guo, Z. Liu, J. Yoon, Angew. Chem. Int. Ed. 2018, 57, 11522.
61. J. Barnes, L. A. Anderson, J. D. Phillipson, *J. Pharm. Pharmacol.* 2001, 53, 583.
62. a) X. Liu, C. Jiang, Y. Li, W. Liu, N. Yao, M. Gao, Y. Ji, D. Huang, Z. Yin, Z. Sun, Y. Ni, J. Zhang, *J. Pharm. Sci.* 2015, 104, 215; b) V. Paba, M. Quarto, L. Varriale, L. Crescenzzi, G. Palummo, *J. Photochem. Photobiol. B* 2001, 60, 87.
63. C. D. Liu, D. Kwan, R. E. Saxton, D. W. McFadden, *J. Surg. Res.* 2000, 93, 137.
64. R. Ritz, C. Scheidle, S. Noell, F. Roser, M. Schenk, K. Dietz, W. S. Strauss, *PLoS One* 2012, 7, 51974.
65. L. Ocker, A. Adamus, L. Hempfling, B. Wagner, R. Vahdad, F. A. Verburg, M. Luster, G. Seitz, *Photodiagnosis Photodyn. Ther.* 2020, 29, 101588.
66. Q.-Q. Yang, A. K. Farha, G. Kim, K. Gul, R.-Y. Gan, H. Corke, Trends Food Sci. Technol. 2020, 97, 341.
67. S. Ghosh, S. Banerjee, Ch. A. Verburg, M. Luster, T. Schurrat, D. Bier, M. Frank, J. Lisec, *Tissue Engineered* 2015, 53, 583.
68. M. N. Arshad, H. A. Vogel, T. Schurrat, D. Bier, M. Frank, J. Lisec, *Tissue Engineered* 2015, 53, 583.
69. Y. Jiang, A. W. Leung, H. Hua, X. Rao, C. Xu, *Int. J. Photoenergy* 2014, 2014, 673601.
70. V. H. Panhóca, F. Florez, B. D. F. N. Júnior, A. N. Rastelli, J. Panhóca, A. Graeler, T. Maisch, K. Plaetzer, *Eur. J. Med. Chem.* 2018, 159, 423.
71. J. Parrish, T. B. Fitzpatrick, L. Tanenbaum, M. A. Pathak, *N. Engl. J. Med.* 1974, 291, 1207.
72. D. Moukheiber, U. Chitgupi, K. A. Carter, D. Luo, B. Sun, S. Goel, C. A. Ferreira, J. W. Engle, D. Wang, J. Xia, W. Cai, J. F. Lovell, *ACS Appl. Bio. Mater.* 2019, 2, 544.
73. A. B. Ormond, H. S. Freeman, *Materials (Basel)* 2013, 6, 817.
74. J. A. Parrish, T. B. Fitzpatrick, L. Tanenbaum, M. A. Pathak, *N. Engl. J. Med.* 1974, 291, 1207.
75. J. A. Parrish, T. B. Fitzpatrick, L. Tanenbaum, M. A. Pathak, *N. Engl. J. Med.* 1974, 291, 1207.
76. D. Bagchi, S. Dutta, P. Singh, S. Chaudhuri, S. K. Pal, *ACS Omega* 2017, 2, 1850.
77. G. Xu, Q. Yan, X. Lv, Y. Zhu, K. Xin, B. Shi, R. Wang, J. Chen, W. Gao, P. Shi, C. Fan, C. Zhao, H. Tian, *Angew. Chem. Int. Ed.* 2018, 130, 3688.
78. B. Li, L. Lu, M. Zhao, Z. Lei, F. Zhang, *Angew. Chem. Int. Ed.* 2018, 57, 7483.

**AUTHOR BIOGRAPHIES**

**Jiasheng Wu** received his PhD in organic chemistry in 2006 from Technical Institute of Physics and Chemistry, Chinese Academy of Sciences (TIPC, CAS). After post-doctoral training in Dankook University (Korea) and City University of Hong Kong for 3 years, he joined TIPC in 2009 as an associate professor. His current research interests include design and synthesis of new phototheranostic agents and fluorescent sensors.

**Pengfei Wang** is a professor at TIPC. He received his PhD in organic chemistry from the Institute of Photochemical Research, CAS, in 1993. After completing professional experience at Laboratoire PPSM of CNRS (France) as a visiting researcher, at the National Institute of Materials and Chemical Research (NIMC), Tsukuba, Japan, as a COE fellow, and at the Department of Applied Physics and Materials Science, City University of Hong Kong as a senior research fellow, he joined TIPC in 2005 as a professor. His current research interests include phototheranocitcs, chemosensors/biosensors, and organic electroluminescent materials and devices.

**How to cite this article:** Wu J, Sha J, Zhang C, Liu W, Zheng X, Wang P. Recent advances in theranostic agents based on natural products for photodynamic and sonodynamic therapy. *VIEW.* 2020;1:20200090. [https://doi.org/10.1002/VIW.20200090](https://doi.org/10.1002/VIW.20200090)