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Review

Visible Tracking of Small Molecules of Gases with Fluorescent Donors

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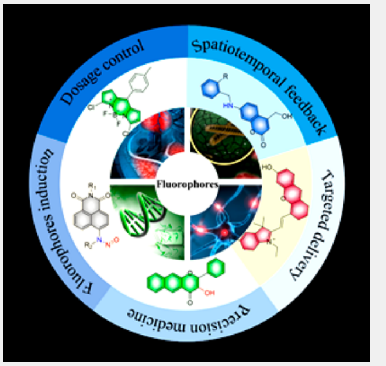
ABSTRACT: Biological gasotransmitters (small molecules of gases) play important roles in signal transduction mechanisms and disease treatments. Although a large number of small-molecule donors have been developed, visualizing the release of small molecules remains challenging. Owing to their unique optical properties, fluorophores have been widely applied in cellular imaging and tracking. Researchers have used various fluorophores to develop small-molecule donors with fluorescent activity for visualizing the release of small molecules and their related therapies. These include fluorophores and their derivatives such as boron-dipyrromethene (BODIPY), coumarin, 1,8-naphthalimide, hemicyanine, porphyrin, rhodamine, and fluorescein. In this review, we summarize the design concepts of functional fluorescent small-molecule donors in terms of different types of fluorophores. Then, we discuss how these donors release small molecules, and the imaging modalities and biomedical applications facilitated by their fluorescent properties. With the systematic discussion of these publications, we hope to provide useful references for the development of more practical, advanced fluorescent small-molecule donors in the future.



KEYWORDS: *biological gasotransmitters, fluorophores, small molecules, fluorescent donors, real-time monitoring, response mechanisms, synergistic therapy, disease models*

INTRODUCTION

Nitric oxide (NO),[1,2] carbon monoxide (CO),[3,4] hydrogen sulfide (H_2S),[5,6] and sulfur dioxide (SO_2)[7,8] play crucial roles as the endogenous gasotransmitters. Numerous studies have shown that these bioactive gases are closely linked to human health and are essential in treating various diseases. For instance, NO is a powerful active molecule that possesses a wide range of physiological functions, such as speeding up wound healing and reducing pulmonary vascular resistance.[9,10] CO acts as a signaling molecule in both the cardiovascular and central nervous systems, inducing vasodilation and facilitating neurotransmission.[11,12] H_2S exhibits strong neuroprotective properties in the cases of cerebral ischemia-reperfusion injury.[13,14] SO_2 has the ability to regulate blood pressure and inhibit the formation of atherosclerotic plaques in cardiovascular arteries.[15] Clearly, small-molecule donors offer a promising avenue for effectively treating severe diseases, leading to a wealth of literature on this topic. However, developing such donors presents challenges, including maintaining control over dosage, achieving targeted delivery, ensuring spatiotemporal feedback, and delivering precise therapy. Luckily, small-molecule donors with fluorescence capabilities can not only release the active molecules but also allow for the real-time monitoring of active small molecules in biological systems. This offers significant advantages in addressing these challenges. Furthermore, the integration of fluorescent small-molecule donors with chemotherapy, photodynamic therapy, or photothermal therapy can synergistically capitalize on the unique benefits of each mode to augment therapeutic efficacy, providing new insights into cancer treatment. In this review, we focus on the recent development of fluorescent small-molecule donors in recent years, especially the classification and summary of different fluorophores, aiming to provide strategies for the construction of fluorophores and functional materials.

BODIPY

BODIPY dyes have a rigid planar structure, high molar extinction coefficient, and fluorescence quantum yield in the visible region. They serve as a versatile platform for constructing fluorescent groups with unique properties. The covalent binding of BODIPY dyes with photoprotective groups enables the construction of light-controlled fluorescent compounds.[16−19] These fluorescent compounds can be activated under light exposure, allowing for noninvasive visual

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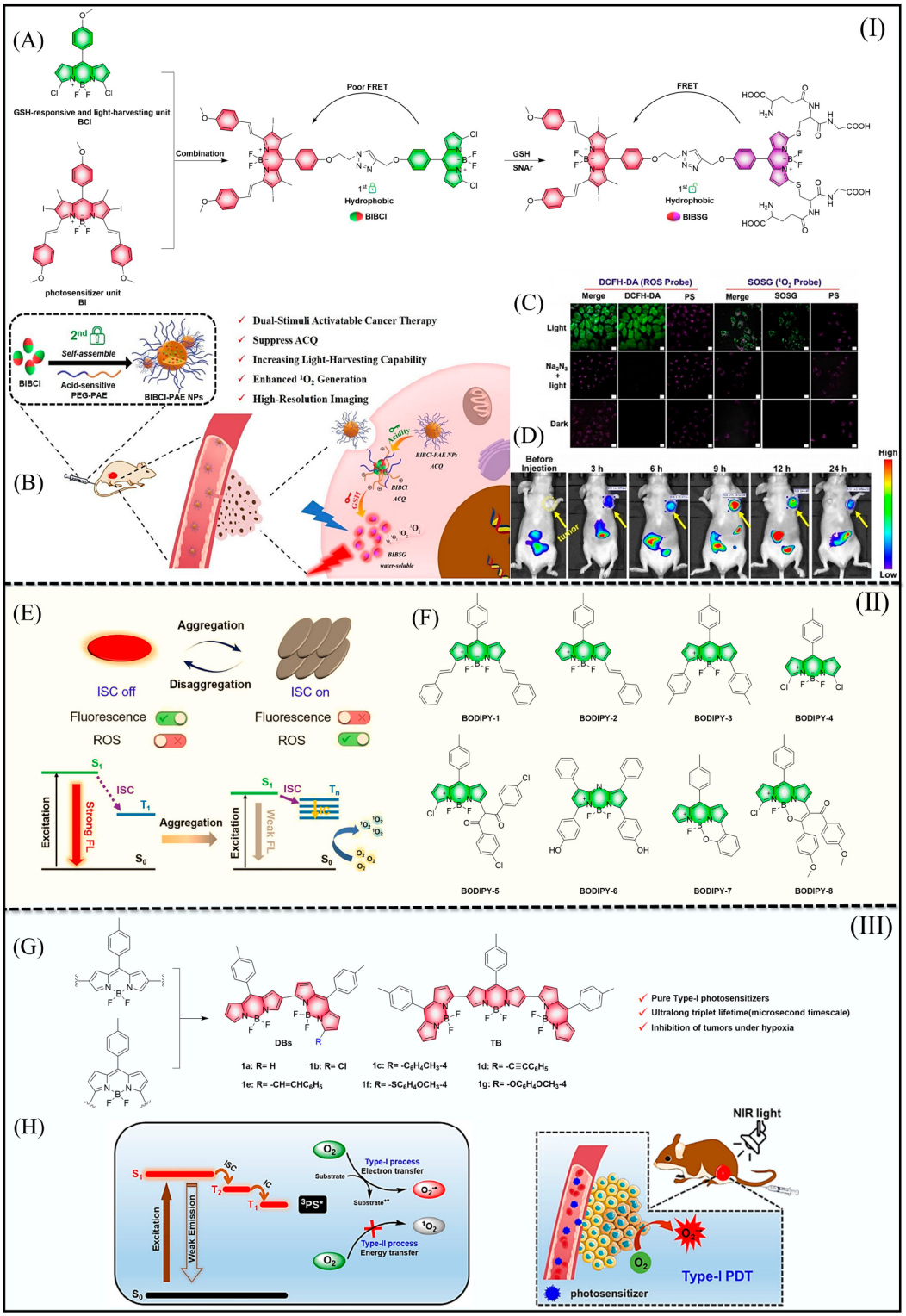

Figure 1. (I) (A,B) The structure characteristics of BIBCl-PAE NPs, the mechanism underlying the release of $^1O_2$, and the resulting fluorescence alterations. (C) The generation of $^1O_2$ by BIBCl-PAE NPs in HepG2 cells was assessed using DCFH-DA and SOSG. (D) Fluorescence imaging was performed in vivo on BALB/c mice bearing HepG2 tumors after intravenous administration of BIBCl-PAE NPs. Reproduced from ref 23. Copyright 2020 Royal Society of Chemistry. (II) (E) Upon aggregation, the fluorophore undergoes a transition to a PSs (ISC on), leading to fluorescence quenching and the generation of reactive ROS. Conversely, when the PSs are in a disaggregated state, they revert back to a fluorophore state (ISC off), resulting in fluorescence restoration without the production of ROS. (F) The structural composition of B ODIPYs derivatives. Reproduced from ref 26. Copyright 2021 Chinese Chemical Society. (III) (G) The structure characteristics of DBs and TB, the mechanism underlying the release of $O_2^{-\bullet}$. (H) The pathway of light-induced generation of $O_2^{-\bullet}$ by α,β-linked BODIPYs. Reproduced from ref 27. Copyright 2019 Wiley-VCH GmbH.

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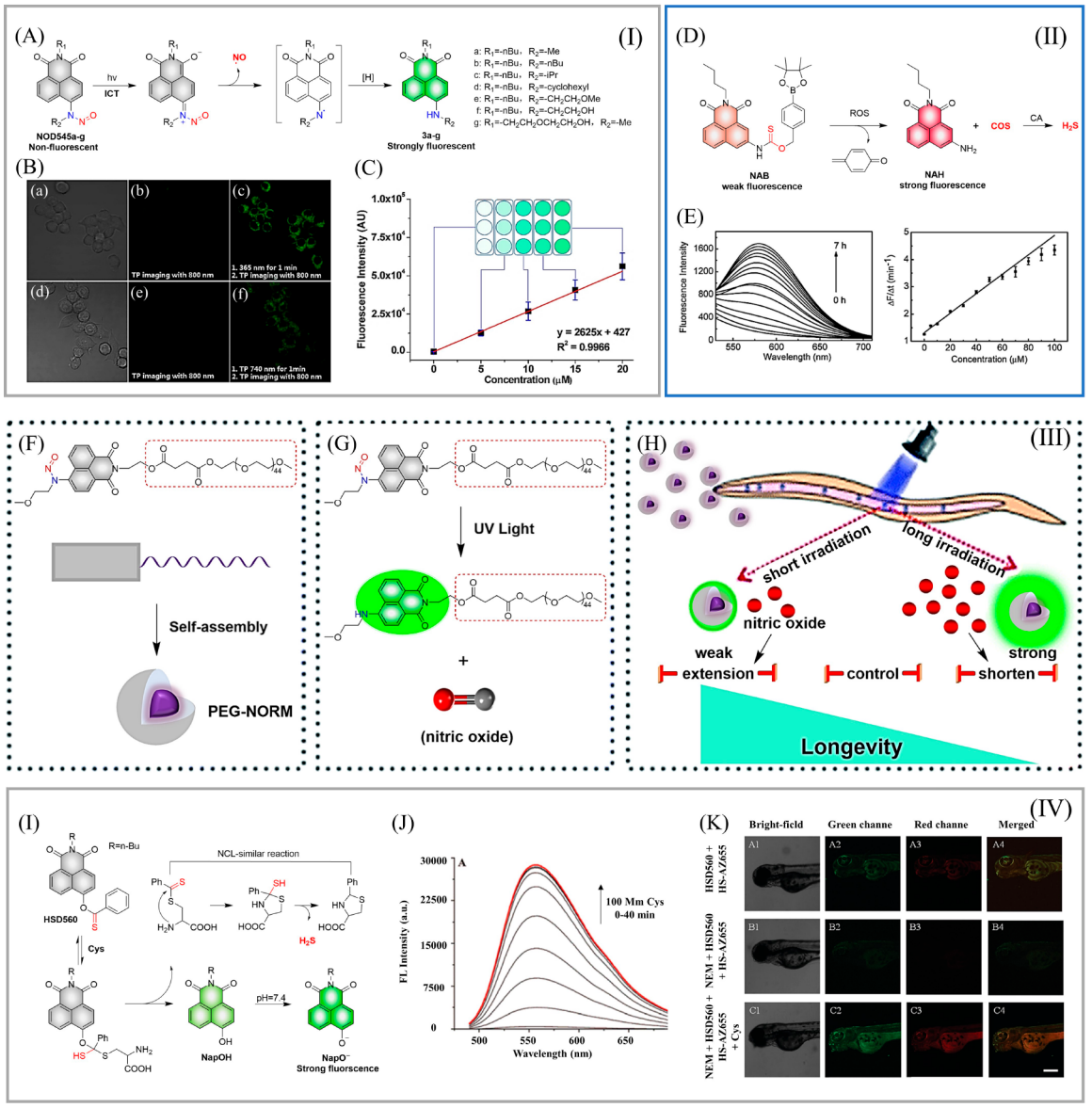

Figure 2. (I) (A) The structure characteristics of NOD545a−g, the mechanism underlying the release of NO, and the resulting fluorescence alterations. (B−C) Cell confocal imaging and NO quantification. Reproduced from ref 38. Copyright 2016 American Chemical Society. (II) (D−E) The structure characteristics of NAB, the mechanism underlying the release of NO, and the resulting fluorescence alterations. Reproduced from ref 42. Copyright 2019 Royal Society of Chemistry. (III) (F) Schematic representation of PEG-NORM nanoparticles self-assembly. (G) The structure characteristics of PEG-NORM, the mechanism underlying the release of NO, and the resulting fluorescence alterations. (H) Effect of PEG-NORM on longevity of *C. elegans*. Reproduced from ref 45. Copyright 2020 Royal Society of Chemistry. (IV) (I) The structure characteristics of HSD560, the mechanism underlying the release of H_2S, and the resulting fluorescence alterations. (G) The time-dependency of Cys-triggered HSD560. (K) Fluorescence imaging of H_2S release in vivo by HSD560. Reproduced from ref 46. Copyright 2021 Royal Society of Chemistry.

imaging. In addition, BODIPY is relatively insensitive to environmental polarity and pH, which can induce new photophysical processes such as intramolecular charge transfer (ICT) or photoinduced electron transfer (PET).[20] Due to the numerous excellent properties of BODIPY, it stands out among many fluorescent dyes.

Photodynamic therapy (PDT) is a novel cancer treatment approach that combines photosensitizers (PSs), light illumination, and O_2.[21,22] Compared to traditional treatment methods, PDT offers advantages such as noninvasiveness, minimal toxicity, and high selectivity. However, achieving precise PDT still faces a significant challenge, despite its widespread application in cancer treatment. The Yang research group reported an intelligent GSH (glutathione)/pH dual-activated supramolecular photosensitizer (BODIPY) (BIBCl-PAE NPs) (Figure 1IA).[23] By encapsulating the GSH-activatable photosensitizer BIBCl (the first lock-key) into a pH-responsive deblock copolymer poly(ethylene glycol)-poly-(*β*-amino ester) (PEG−PAE), the BIBCl-PAE NPs were created (the second lock-key).

In normal tissues, the hydrophobic nature of BIBCl and the amphiphilic polymer PEG−PAE act as "dual locks," keeping the photosensitizer in a tightly aggregated state that prevents efficient oxygenation to produce $^1O_2$. However, in the tumor microenvironment (TME), BIBCl is activated by low-pH and high-GSH levels. The decomposition of BIBCl promotes the reaction between BIBCl and GSH, leading to the release of BIBSG and achieving efficient PDT (Figure 1I,B−D). The

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development of this photosensitizer provides a feasible strategy for precision treatment.

Many traditional PSs still encounter inherent constraints, such as aggregation-caused quenching (ACQ), which significantly reduces ROS generation, leading to inefficient PDT.[24,25] To address this issue, the Kang research group developed a reversible conversion between the fluorescent molecule BODIPY and PSs through aggregation and disaggregation processes. This reversible switching holds significant importance for the development of intelligent PDT systems (Figure 1II,E).[26] In the aggregated state, the BODIPY1−8 exhibits fluorescence quenching characteristics and can generate a large amount of ROS with the addition of GSH. Theoretical research has indicated that aggregation reduces the energy gap between relevant singlet and triplet states, thereby enhancing the efficiency of the spin conversion process and promoting ROS generation. Interestingly, when the system undergoes disaggregation, the fluorescence can be recovered, and meanwhile the generation of ROS was inhibited (Figure 1II,F). This reversible switching mode between fluorescent groups and photosensitizers provides a new approach for the development of intelligent PDT systems.

BODIPY is one of the most commonly used photosensitizers in current PDT applications. In 2021, Yang research group developed a range of α,β-linked BODIPYs dimer DBs (**1a−1g**) and trimer TB (Figure 1III,G).[27] These compounds, when exposed to near-infrared (NIR) light, generate $O_2^{-\bullet}$ exclusively through Type-I processes, thereby avoiding the limitations associated with Type-II PDT (rapid O_2 consumption and accompanying vascular damage) (Figure 1III,H). This mechanism effectively induces cell apoptosis. In addition, TB displays high absorption at 740 nm and an exceptionally long lifetime for its triplet state. It also demonstrates minimal dependence on O_2 concentration, allowing it to generate ROS and inhibit tumor proliferation even under hypoxic conditions. These properties make it an excellent candidate for PDT. These results suggest that, in the near future, these BODIPYs have promising potential to be a new class of PDT drugs for clinical treatment.

1,8-Naphthalimide

1,8-Naphthalimide-based fluorescent groups are an early applied class of fluorescent groups that possess excellent photostability, large Stokes shift, high fluorescence quantum yield, simple structure, and easy modification.[28−30] Based on the easy modifiability of the 1,8-naphthalimide structure, a donor electron group is introduced at the 4-position, and an acceptor electron group is introduced at the N-position to form a "push−pull" electronic structure in the derivatives of 1,8-naphthalimide. This leads to an increased conjugation system, greatly enhancing the fluorescence intensity of this structure system, thus being widely used in the design of new fluorescent dyes.[31,32] Furthermore, structural modification of functional groups can improve the water solubility and targeting ability of 1,8-naphthalimide derivatives, laying the foundation for the development of fluorescent small-molecule donors with biocompatibility and targeted delivery.[33,34]

ICT mechanism significantly influences the fluorescent characteristics of chromophores. ICT is the process where conjugated charges move from an electron donor to an electron acceptor in the excited state, causing a reorganization of positive and negative charges within the molecule. The electron donor and acceptor also play key roles in the recognition group. When the analyte interacts with the system, the electronic properties of the recognition group change, leading to a change in the intramolecular dipole moment and subsequent modifications in the absorption and fluorescence spectra.[35,36]

In order to achieve desirable imaging characteristics, small-molecule donors need to provide excellent stability and negligible cytotoxicity.[37] The Yang group successfully reported a series of highly stable and low cytotoxic NO donors (NOD545a−g) (Figure 2I,A).[38] 1,8-Naphthalimide was chosen as the fluorophor due to its high fluorescence quantum yield and easy structural modification. Moreover, when exposed to 740 nm two-photon laser or 365 nm ultraviolet light, NOD545a−g experiences ICT, causing the N-nitroso moiety to convert into an electron-rich N−H bond, thereby emitting NO. NOD545a−g, based on a fluorescence self-calibration mechanism, allows for noninvasive imaging and visual tracking of NO release in complex environments (Figure 2I,B−C). While in the presence of physiological concentrations of biological thiols, it will not release NO, which ensured reliable quantitative NO release in complex environments.

As widely recognized, H_2S plays a crucial role in regulating numerous physiological and pathological processes.[39−41] Therefore, it is necessary to develop highly sensitive and selective H_2S donors with fluorescence activity to reveal their specific roles in the progression of certain diseases. In 2019, the Ma group successfully synthesized a ROS-responsive fluorescent H_2S donor (NAB) with 3-aminopropyl-1,8-naphthalimide (NAH) as the fluorescent moiety.[42] Upon ROS activation, NAB first releases a hydrolyzable COS, which is then rapidly converted to H_2S by the ubiquitous CA. The simultaneous release of the fluorophore along with COS provides an optical response for H_2S donors (Figure 2II,D−E). This strategy of using an intermediate to generate COS for H_2S release is a widely used method in the development of H_2S compounds.

Based on previous research reports, the biological effects of NO vary depending on its concentration.[43,44] Therefore, it is crucial to devise NO donors that can accurately control concentration and dosage. The Zhang research group prepared a novel NO donor nanoparticle (PEG-NORM) (Figure 2III,F).[45] Under light conditions, PEG-NORM can release NO and fluorescent reporters, with the released NO concentration depending entirely on the intensity and duration of light exposure (Figure 2III,G). This mechanism enables more effective regulation of biological processes. For example, low concentrations of NO release can promote the life span of *Caenorhabditis elegans*, while increasing light intensity and duration can induce apoptosis in the reproductive cells of *C. elegans*, thereby reducing their life span (Figure 2III,H). Therefore, the cellular manipulation and life span regulation mechanism of PEG-NORM hold promise for further applications of NO in biomedicine.

1,8-Naphthalimide has been widely applied in the development of H_2S donors and possesses a self-reporting capability for hydrogen H_2S. The Zhang group successfully synthesized HSD560, a H_2S donor that is activated in the presence of Cys.[46] HSD560 is composed of the classic fluorescent group 1,8-naphthalimide and benzothioate (the source of H_2S). In an enriched Cys environment, HSD560 undergoes a non-enzymatic native chemical ligation (NCL) reaction when the benzothioate moiety is exposed, releasing H_2S and NapOH.

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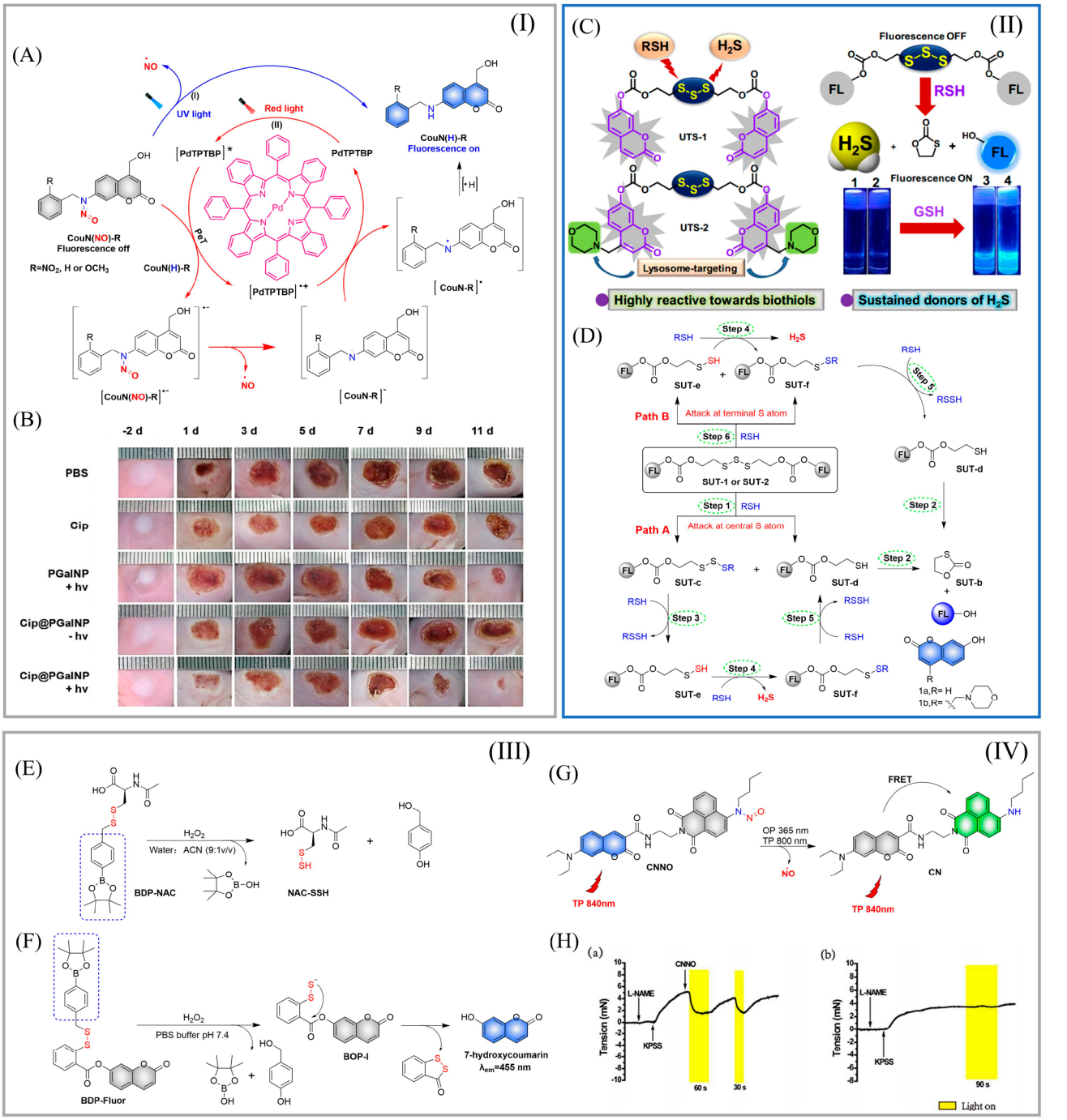

Figure 3. (I) (A–B) The structure characteristics of CouN(NO)-R, the mechanism underlying the release of NO, and the resulting fluorescence alterations. Reproduced from ref 58. Copyright 2021 Wiley-VCH GmbH. (II) (C–D) The structure characteristics of SUT-1 and SUT-2, the mechanism underlying the release of H_2S, and the resulting fluorescence alterations. Reproduced from ref 62. Copyright 2020 Royal Society of Chemistry. (III) (E) The structure characteristics of BDP-NAC, the mechanism underlying the release of RSSH. (F) The structure characteristics of BDP-fluor, the mechanism underlying the release of RSSR, and the resulting fluorescence alterations. (IV) (G) The structure characteristics of CNNO, the mechanism underlying the release of NO, and the resulting fluorescence alterations. (H) Light-triggered CNNO induces vasodilation in mice by releasing NO. Reproduced from ref 69. Copyright 2017 Royal Society of Chemistry.

Meanwhile, in neutral environments, NapOH further deprotonates to form $NapO^-$, restoring the ICT effect and exhibiting green fluorescence (Figure 2IV,I). The fluorescence of $NapO^-$ can be used to monitor the release of H_2S from HSD560 in real time (Figure 2IV,J–K). In addition, the study found that HSD560 can reduce levels of NO and prostaglandin E2 (PGE2) and has anti-inflammatory effects. Compared to traditional COS/H_2S donors, the H_2S release of HSD560 is nonenzymatic, which means it has the potential for precise H_2S release in vivo.

Coumarin

Coumarin compounds are lactone-type compounds formed by the fusion of a benzene ring and an α-pyrone ring. They have good biocompatibility.[47] Due to their simple structure and multiple modifiable reaction sites, they have been widely used

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in the design of small-molecule fluorescent donors.[48] From the molecular structure point of view, the fluorescence properties are related to the phenolic oxygen atom as the electron donor and the carbonyl group of α-pyrone as the electron acceptor, which undergoes an ICT mechanism and has a high fluorescence quantum yield. The presence of the lactone structure hinders the rotation of double bonds, thereby improving the photostability.[49] In addition, the diversity of structures can be achieved by modulating different substitutions on the coumarin framework, leading to a broad spectrum of pharmacological functions such as antithrombotic, anti-inflammatory, anticancer, and antitumor activities.[50−53] Both natural and synthetic coumarin compounds have attracted widespread attention for their applications in photodynamic therapy and cancer treatment.

The structural diversity resulting from different substitutions on the coumarin skeleton endows it with multiple pharmacological functions.[52,54,55] For example, coumarins containing small-molecule releasing groups exhibit excellent cancer therapeutic effects due to their ability to release biological gasotransmitters.[56,57] In 2021, the Hu research group bonded coumarin with N-nitrosoamine to prepare a red-light-mediated photocatalytic oxidation−reduction NO donor (CouN(NO)-R, R = NO_2/H/OCH_3) (Figure 3I,A).[58] CouN(NO)-R not only offers quantification of NO concentrations (due to absorbance changes resulting from the quantitative conversion from CouN(NO)-NO_2 to CouN(H)-NO_2) but also provides comprehensive reports on the release of NO (evidenced by significant fluorescence changes before and after the reaction). CouN(NO)-R exhibited two modes of NO release: (i) when exposed to 365 nm light, CouN(NO)-R was activated (this activation is characterized by the maximum absorbance of coumarin at 328 nm.), releasing NO and fluorescent reporters. (ii) With the presence of the photosensitizer tetraphenyltetrabenzoporphyrin (PdTPTBP), CouN(NO)-R can be effectively activated by red-light with wavelengths of 630/700 nm. This activation occurs through a triplet−triplet energy transfer (TTET) process, indirectly activating the acceptor molecule. As a result from NO release, CouN(NO)-R exhibits intense fluorescence emission, providing a reliable and sensitive detection method. Compared to process (i), the NIR light-exhibited excellent tissue penetration and lower phototoxicity, making it more suitable for biomedical applications. In addition, the carrier of this donor, norepinephrine, was capable of killing *Pseudomonas aeruginosa*, exhibiting antibacterial properties and promoting wound healing (Figure 3I,B), which provides a new approach for fluorescently active light-responsive NO donors in biomedical applications.

H_2S is another important gas signaling molecule following CO_2 and NO.[59−61] Given its involvement in numerous physiological and pathological processes, there is a growing need for extensive research and development of H_2S donors. In 2020, the Bhabak research group reported a biothiol triggered self-immolative organic trisulfide fluorescent H_2S donor (UTS-1 and UTS-2) (Figure 3II,C).[62] These donors exhibited good compatibility in both water and cell media. UTS-1 and UTS-2 are capable of controlled release of H_2S when activated by biothiol groups. This process is accompanied by the release of coumarin fluorescence groups, allowing for the real-time monitoring of intracellular H_2S release. (Figure 3II,D). In addition, UTS-2 was also organelle-specific, capable of delivering H_2S to lysosomes. This development provides a potential tool for studying the function of H_2S at the subcellular level.

RSSH are believed to play a similar role to H_2S as important factors and signal transducers in sulfur-mediated redox processes, participating in various physiological and pathological processes.[63−65] The Matson group successfully synthesized a ROS-triggered RSSH donor (BDP-NCA).[66] The authors selected N-acetylcysteine(NAC) and used it to react with a H_2O_2 bioorthogonal trigger, boronic acid ester, to obtain BDP-NAC. BDP-NAC can release NAC-SSH and nontoxic byproduct 4-hydroxybenzyl alcohol under the triggering of H_2O_2, breaking through the limitation of the previous 1,6-elimination reaction and avoiding the generation of highly electrophilic and harmful quinone compounds (Figure 3III,E). The resultant NAC-SSH can protect H9C2 cells under oxidative stress. Compared with conventional H_2S donors, such as Na_2S and GYY4137, BDP-NCA showed greater efficacy in maintaining redox homeostasis because there are nonbonding electron pairs on the sulfur atoms near the nucleophilic sulfur atom. This strategy provides a feasible approach for the authors to design fluorescent-active RSSSR donors. Based on the above inspiration, the authors replaced the terminal group of the disulfide bond to prepare a fluorescent-active RSSR donor (BDP-fluor). Upon exposure to H_2O_2, intermediate BOP-1 undergoes rapid cyclization to obtain the five-membered benzodithiolone and releases 7-hydroxycoumarin, which realizes the visual-monitoring of RSSR release (Figure 3III,F).

In comparison to single-photon excitation systems, two-photon excitation systems are more suitable for biological imaging due to their enhanced spatial resolution, lower autofluorescence background, and deeper penetration.[67,68] Therefore, small-molecule-based two-photon fluorescent donors have been widely developed for visualizing the release of biological gasotransmitters. In 2017, the Tang research group developed a two-photon excitable NO donor (CNNO).[69] The fluorescent scaffold of CNNO is mainly composed of coumarin and naphthalimide moieties. When exposed to 365/800 nm two-photon excitation, CNNO releases NO and triggers FRET through a red-shift in the absorption spectrum of the naphthalimide receptor, thus causing it to overlap with the emission spectrum of the coumarin donor. This enables the system to visualize NO release with the two-photon excitation-dependent fluorescence (Figure 3IV,G). In addition, the authors demonstrated that light-triggered NO release from CNNO can induce vasodilation in the mouse aorta, which could contribute to the study of NO-related cardiovascular diseases (Figure 3IV,H).

Flavonols

Flavonols are a unique class of dual-emissive fluorescent dyes with broad-spectrum pharmacological activities.[70] Due to the presence of intramolecular hydrogen bonds, flavonol compounds will have an excited-state intramolecular proton transfer (ESIPT) process upon irradiation. Due to the stronger electron-donating ability of the deprotonated form (O^-) compared to the protonated form (OH), they are sensitive to the microenvironment.[71,72] As a typical class of ESIPT molecules, structurally tunable 3-hydroxyflavone (3-HF) and 3-hydroxyquinolone derivatives can achieve visual tracking of CO release under visible light irradiation.[73,74] In addition, as natural products widely present in plants, flavonol compounds exhibit good biocompatibility and low cytotoxicity.[75] They are

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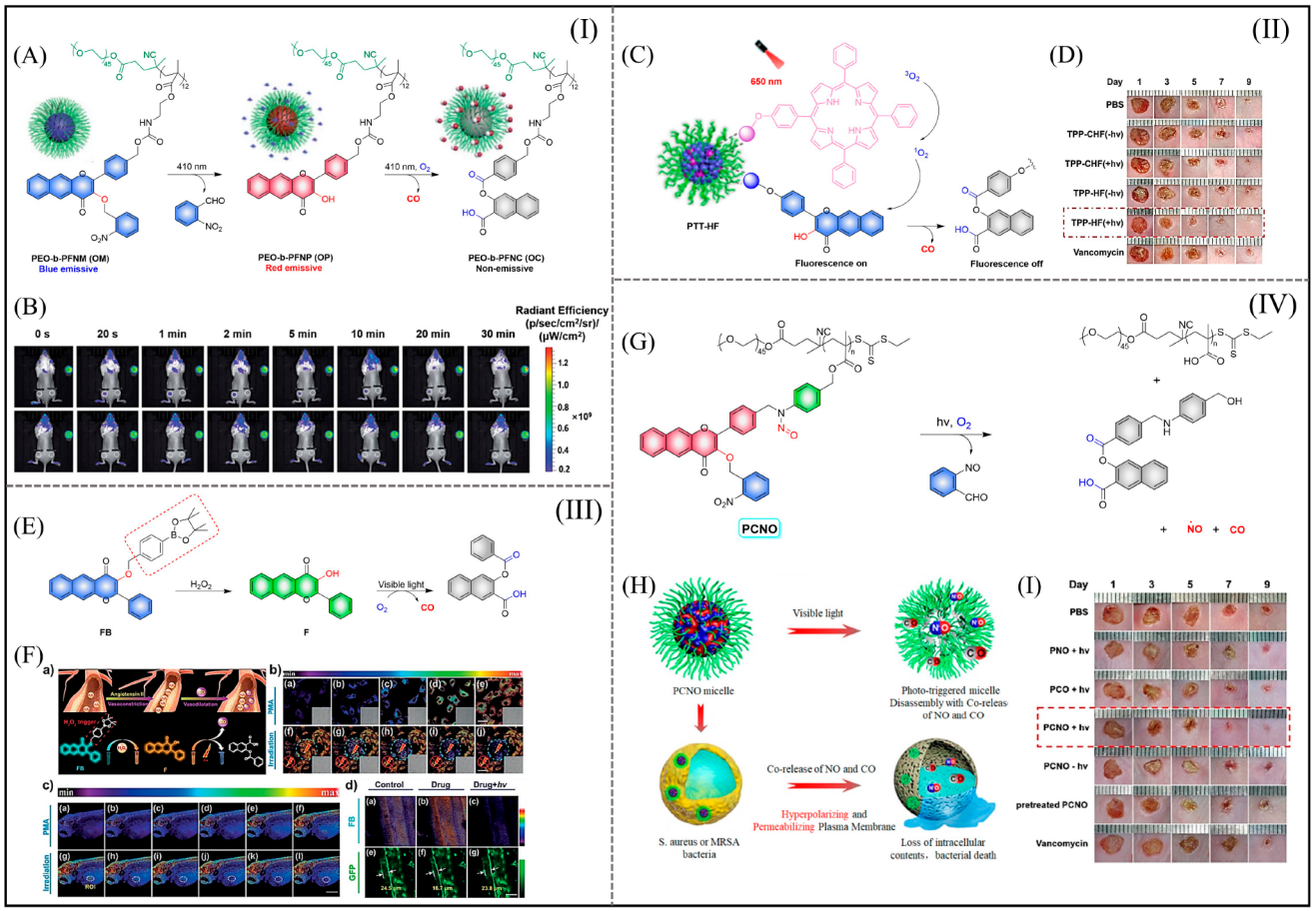

Figure 4. (I) (A) The structure characteristics of PEO-*b*-PFNM, the mechanism underlying the release of CO, and the resulting fluorescence alterations. (B) CO released by PEO-*b*-PFNM under light irradiation can promote wound healing in a full-thickness mouse skin wound model. Reproduced from ref 79. Copyright 2020 Royal Society of Chemistry. (II) (C) The structure characteristics of PTT-HF, the mechanism underlying the release of CO, and the resulting fluorescence alterations. (D) PTT-HF for the treatment of skin wounds. Reproduced from ref 80. Copyright 2021 Wiley-VCH GmbH. (III) (E) The structure characteristics of FB, the mechanism underlying the release of CO, and the resulting fluorescence alterations. (F) Fluorescence imaging of NO release in zebrafish by FB and its induction of vasodilation. Reproduced from ref 83. Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (IV) (G) The structure characteristics of PCNO, the mechanism underlying the release of CO, and the resulting fluorescence alterations. (H) Schematic illustration of visible light-mediated corelease of NO and CO from PCNO micelles. (I) Evaluation of therapeutic efficacy of PCNO against MRSA infection in mice. Reproduced from ref 84. Copyright 2021 Wiley-VCH GmbH.

safer than comprehensive fluorescent probes for cell diagnosis and treatment. Moreover, flavonol compounds also possess unique properties such as aggregation-induced emission (AIE), large Stokes shift, and high photostability, making them ideal fluorescent scaffolds for designing the CO donors.[76]

Structure-tunable 3-HF compounds have been widely applied in constructing nonmetallic CO donors.[77,78] In 2021, the Hu research group put forward a novel approach for CO donor synthesis utilizing 3-HF derivatives through direct polymerization.[79] The authors first combined a photo-responsive o-nitrobenzyl group with the 3-HF derivative. Subsequently, they utilized PEG to facilitate the self-assembly of CO-releasing molecules into micelles (PEO-*b*-PFNM), thereby enhancing the water solubility of 3-HF derivatives. Upon irradiation with 410 nm visible light, the o-nitrobenzyl moiety underwent cleavage, restoring the characteristics of 3-HF. Subsequently, in the presence of O_2, continued exposure to 410 nm light caused the ESIPT of the 3-HF segment of PEO-*b*-PFNP, resulting in the release of CO. The entire procedure was accompanied by fluorescence alterations, transitioning from blue to red and ultimately became colorless, thereby allowing facile real-time monitoring of CO-release in vitro and in vivo settings (Figure 4-I,A). In addition, the inclusion of the o-nitrobenzyl group protected the 3-HF segment from attack by biological thiols or ROS, ensuring the regulation of CO release by a single light source. Importantly, PEO-*b*-PFNM exhibited excellent therapeutic effects in antivascular injury and wound healing (Figure 4-I,B). This work achieved self-reporting of CO release behavior, and opened up new avenues for understanding and treating CO-related diseases.

In 2021, the same research group developed a transition-metal-free CO-releasing micelle that utilizes a photo-oxidation mechanism to activate flavonol derivatives for CO release (PTT-HF).[80] Upon exposure to 650 nm light, the photo-sensitizer PdTPTBP converts $^3O_2$ to $^1O_2$. This $^1O_2$ then spontaneously oxidizes the 3-HF derivatives, leading to the release of CO (Figure 4II,C). Furthermore, the micelle demonstrates antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) without impacting *Escherichia coli*, thus eradicating MRSA pathogens and promoting the healing process of MRSA-infected wounds (Figure 4II,D).

The elevation of intracellular reactive ROS and the antioxidant defense system has been recognized as one of

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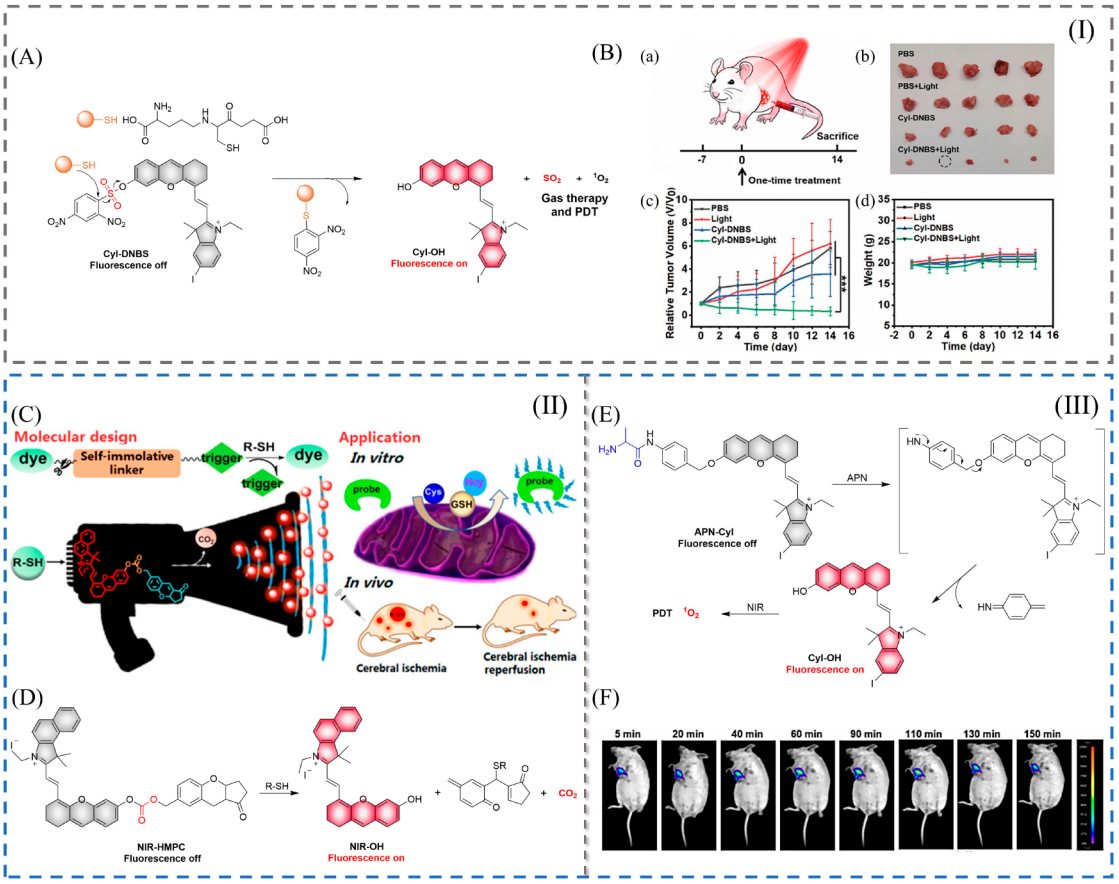

Figure 5. (I) (A) The structure characteristics of Cyl-DNBS, the mechanism underlying the release of SO_2 and $^1O_2$, and the resulting fluorescence alterations. (B) Schematic diagram of a mouse model for in vivo cancer therapy using Cyl-DNBS. Reproduced from ref 96. Copyright 2021 Wiley-VCH GmbH. (II) (C–D) The structure characteristics of NIR-HMPC, the mechanism underlying the release of CO_2, and the resulting fluorescence alterations. Reproduced from ref 97. Copyright 2020 American Chemical Society. (III) (E) The structure characteristics of APN-Cyl, the mechanism underlying the release of $^1O_2$, and the resulting fluorescence alterations (F) Fluorescence imaging of tumors in Balb/c mice using APN-Cyl was conducted to monitor endogenous APN levels over a 150 min. Reproduced from ref 98. Copyright 2020 Elsevier Ltd.

the hallmarks of cancer cells.[81,82] Exploiting this characteristic, a novel approach for cancer treatment can be developed by depleting excessive ROS within cells to maintain the redox balance in the human body. In 2018, the Tang research group reported a two-photon H_2O_2-activated CO donor (FB).[83] FB is synthesized through the condensation of a structurally extended 3-HF (F) with a borate ester moiety, which exhibits specific recognition toward ROS. In the presence of ROS, FB undergoes an ESIPT mechanism triggered by borate deprotection to generate F. This process effectively eliminates excessive ROS within cellular environments. Upon light exposure, F undergoes a photoinduced CO release process, accompanied by structural changes in the 3-HF moiety. This allows for the concurrent monitoring of H_2O_2 levels and CO release by monitoring fluorescence changes before and after the reaction. (Figure 4III,E). Moreover, FB provides evidence of H_2O_2-related oxidative stress after administration of angiotensin II (Figure 4III,F). Therefore, FB has potential applications in oxidative stress warning and light-controlled CO release.

The dual-molecule corelease phenomenon has ignited significant interest among researchers. In 2022, Hu research group reported a corelease nanoparticle (PCON) capable of releasing both NO and CO. The authors synthesized PCNO nanoparticles through reversible addition–fragmentation chain transfer (RAFT) polymerization.[84] Upon exposure to 410 nm light, the compound PCNO undergoes a photolytic process, leading to the liberation of NO. Simultaneously, the adjacent o-nitrophenyl group undergoes cleavage, restoring the properties of 3-HF and facilitating the release of CO. This reaction is accompanied by a remarkable enhancement in fluorescence at 603 nm, enabling the visual monitoring of the simultaneous corelease of NO and CO within biological systems (Figure 4IV,G). Moreover, the synergistic therapeutic effect of NO and CO demonstrated strong antibacterial activity against Gram-positive bacteria, and in a mouse skin wound model, PCNO showed better therapeutic efficacy than vancomycin against MRSA infection (Figure 4IV,H–I). The development of PCON provides a new approach for dual-molecule synergistic therapy.

Hemicyanine Fluorophore

The optical methods for visualizing target labeling and drug release processes have revolutionized modern biomedical research.[85,86] A persistent challenge is how to apply these powerful techniques to more complex physiological environments. Near-infrared (NIR) fluorescence (650–900 nm) is the optimal wavelength range for the application of fluorescent probes in biological systems. It possesses deep tissue penetration capability and ultralow tissue background interference, and can be used for noninvasive optical imaging. The NIR fluorescence probe shows great prospects in real-time

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monitoring and understanding disease mechanisms.[87−89] However, appropriate fluorescent groups are required to meet the above requirements. As a typical class of NIR dyes, hemicyanine fluorophore have advantages such as low background interference, deep tissue penetration, and easy structural modification. Therefore, an increasing number of hemicyanine fluorophore have been applied in small-molecule release and in vivo fluorescence imaging. In addition, positively charged semiphthalocyanine dyes can effectively target mitochondria, due to the attraction of mitochondrial negative charges, achieving precision therapy.[90−92] Interestingly, as NIR dyes, hemicyanine dyes can also generate photodynamic therapy effects under light conditions.[93,94] As a multifunctional fluorescent dye, it achieves visual imaging and small-molecule release, as well as synergistic treatment of photodynamic therapy, providing a new strategy for cancer treatment.

GSH is an important and abundant small-molecule thiol antioxidant in cells. Compared to normal cells, cancer cells have approximately 1000 times higher concentrations of GSH, making it an important biomarker for diagnosing cancer.[95] In 2022, the Sun research group bonded 2,4-dinitrobenzenesulfonate (DNBS) to an iodine-substituted hemicyanine scaffold (Cyl-OH) to synthesize a donor (Cyl-DNBS) for synergistic SO_2 and PDT therapy triggered by GSH under red-light mediation. (Figure 5I,A).[96] Due to the positive charge in the structure of the hemicyanine fluorophore, Cyl-DNBS can be quickly absorbed by tumor cells and efficiently targeted to mitochondria. Within cancer cells, the enriched GSH selectively activates the SO_2 generator (DNBS), leading to the generation of a large amount of SO_2. Simultaneously, when Cyl-DNBS is illuminated with 660 nm red-light in the presence of O_2, the photosensitizer (Cyl-OH) undergoes efficient ISC and populates the triplet state due to the heavy atom effect of iodine substitution, generating $^1O_2$ for PDT treatment. This multifunctional photosensitizer provides a new approach for the synergistic treatment of PDT and SO_2 gas, demonstrating excellent therapeutic effects for cancer treatment in vivo (Figure 5I,B).

Biological thiols mainly include cysteine, homocysteine, and glutathione, which play important roles in multiple physiological processes. Therefore, visualizing and monitoring the fluctuation of thiols inside the body provide an effective means for investigating the pathological processes of thiol-related diseases. In 2020, the research group headed by Yin reported a NIR fluorescent probe (NIR-HMPC) that can rapidly detect biological thiols.[97] The thiol-chromene "click" nucleophilic pyran ring-opening reaction allows the quick detection of thiol. The authors covalently bonded benzopyran molecules with hemicyanine fluorophore through carbonyl esters, achieving the specific detection of thiol compounds (Figure 5II,C). Therefore, this probe can be used to observe the changes in thiols during oxidative stress, cell apoptosis, and brain ischemia-reperfusion. In addition, NIR-HMPC releases CO_2 while detecting thiols, which can be used for subsequent treatment of related diseases (Figure 5II,D).

The overexpression of APN (Aminopeptidase N (APN/CD13)) on the surface of tumor cells enables APN-activated donors to effectively differentiate between tumor cells and normal cells. In 2020, the Peng research group developed an APN-activated photosensitizer (APN-Cyl) for PDT and tumor imaging.[98] In tumor cells, APN-Cyl is specifically activated by APN and hydrolyzed to fluorescent Cyl-OH (Cyl-OH has NIR characteristics, photostability, and good biocompatibility) (Figure 5III,E−F). The hydroxyl group in Cyl-OH and the hemicyanine dye have ICT effect, greatly improving NIR fluorescence signal. In addition, due to the positive charge in the chemical structure of Cyl-OH, it can be specifically targeted to the mitochondria. Particularly, the heavy atom effect of the iodine atom in the structure gives it photodynamic therapeutic properties. Under NIR irradiation, a large amount of $^1O_2$ is generated, which enhances the efficacy of PDT and induces cancer cell apoptosis. The fluorescence activation also enables NIR fluorescence imaging of endogenous APN during tumor progression and the PDT treatment process.

Though significant progress has been made in the research of fluorescent small-molecule donors, the fluorescence imaging still faces bottlenecks that limit the throughput of practical systems. Key issues that need to be addressed include eliminating background noise caused by excitation sources, improving absorption efficiency, and reducing light scattering in biological tissues. However, the preferred approach to overcoming these challenges is the development of a new generation of fluorescent moieties, such as modifying intrinsic fluorophores to extend excitation and emission wavelengths, enhance tissue penetration depth, improve water solubility and biodegradability, and optimize pharmacokinetic properties. This presents the new challenges in the development of next-generation prodrugs based on fluorescent small-molecule donors.

CONCLUSION

In this review, we have focused on summarizing the recent developments of gas-delivery donors with fluorescence activity that meet the aforementioned requirements in recent years. Specifically, we have classified different types of fluorescent groups and provided detailed explanations of the small-molecule release mechanism of the donors, enabling readers to easily understand the design strategies and delivery of these donors. In addition, fluorescent small-molecule donors have attractive advantages such as targeted delivery, real-time imaging, and minimal damage to biological samples; thus, in the future they can be used for precise drug release, in situ control of dosage, visible tracking and delivery, etc. Therefore, we have comprehensively described the biomedical applications of each donor, and highlighted the breakthroughs in cancer treatment, providing new ideas for human health development. This review covers a very wide range of disciplines and systematically explains the combined assembly of organic molecules, nanomaterials, and drugs, opening up new platforms for interdisciplinary cross-application.

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Notes

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■ VOCABULARY TERMS

Biological gasotransmitters: Biological gasotransmitters are small molecules of gases including NO, H_2S, and CO. These three gas transmitters can be endogenously produced and extensively participate in regulating pathophysiological processes within organisms; Fluorophores: Fluorophores can absorb light energy at specific wavelengths and re-emit light energy at longer wavelengths. The wavelengths at which fluorophores absorb and emit light respectively constitute the excitation and emission spectra of the fluorophores absorb; Fluorescent small-molecule donors: Under specific irradiation exhibit significant fluorescence changes while releasing small molecules; Aggregation-caused quenching: When the concentration of the solution is increased or the solution is solid the molecular aggregation will cause the luminescence to decrease or even disappear completely; Photodynamic therapy: After photosensitizers enter the body specific wavelengths of light are used to irradiate the cancerous site. At this time, the photosensitizer in the tissue generates a large amount of ROS, thereby inducing cancer cell death and achieving anticancer effects

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