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A Nonmetal-Containing Nitric Oxide Donor Activated with Single-Photon Green Light

Marco Blangetti^{+, [b]} Aurore Fraix^{+, [a]} Loretta Lazzarato,^[b] Elisabetta Marini,^[b] Barbara Rolando,^[b] Federica Sodano,^[b] Roberta Fruttero,^{*, [b]} Alberto Gasco,^[b] and Salvatore Sortino^{*, [a]}

Abstract: *Using a facile synthetic route, an organic NO release agent based on a BODIPY light-harvesting antenna was devised. This compound is stable in the dark and delivers NO under photoexcitation with biologically favorable green light. Temporally regulated vasodilation capability is demonstrated on rat aorta by green-light-induced NO release.*

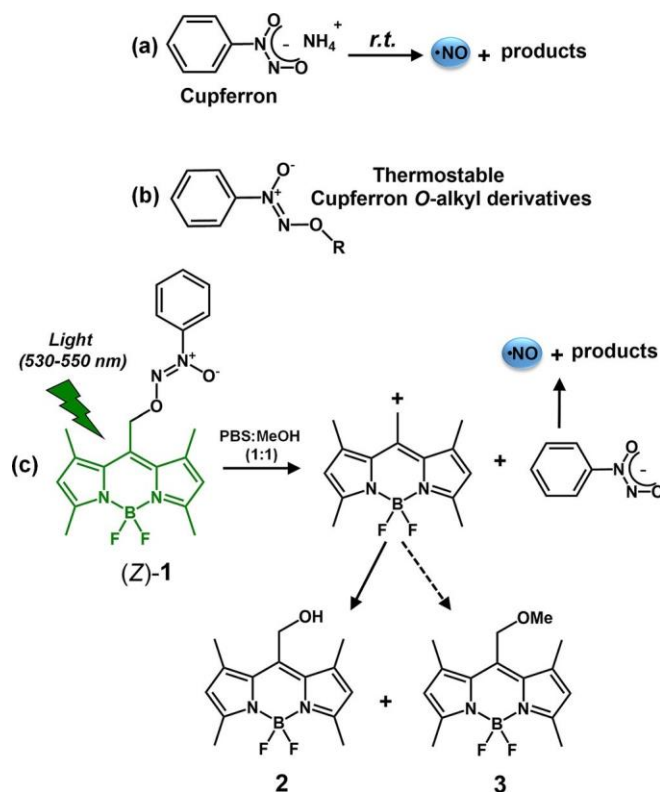
Nitric oxide (NO) is a small inorganic free radical, which is one of the most studied molecules in the fascinating realm of the biomedical sciences.[1] This ubiquitous gaseous messenger plays key roles in human physiology and pathophysiology, and is involved in a number of biological processes, including vasodilation, platelet aggregation, neurotransmission, hormone secretion, and macrophage-mediated immunity.[2] Further to these system-level effects, NO has proven to be a promising antioxidant,[3] antibacterial agent,[4] and anticancer agent.[5] The half-life in tissues of about 5 s, very small molecular size, lack of charge, and lipophilic character make NO capable of diffusing 40–200 nm in the cellular environment,[1] offering an advantage of confining its reactivity in the restricted region of space where it is produced. This scenario has stimulated an intense interest on compounds able to deliver NO to biological targets on demand as potential therapeutic agents to treat important diseases.[6]

Delivery of NO with precise spatiotemporal control is of fundamental importance, owing to the strict dependence of biological effects induced by NO in cells and organisms, along with the concentration and dosage.[7] These needs have made light-controlled NO release agents particularly appealing.[8] Light is the most elegant and finely tunable tool for the non-invasive introduction of therapeutic agents in a desired bio-environment, mimicking an “optical microsyringe” with a superb control in both space and time.[9] Light is also an ideal non-invasive stimulus, which does not affect the physiological parameters such as temperature, pH, and ionic strength, which are fundamentally important for practical biomedical applications. In this context, the design and development of NO photodonor triggered by visible light is, of course, highly desirable in view of its reduced biological impact and deeper optical penetration into tissue compared to UV light. Several metal complexes that are able to release NO with low-energy visible light have been developed relatively recently, especially in the groups of Ford and Mascharak.[8b–d] Although metal-complexbased NO releasers show excellent spectroscopic properties in the visible region and good photochemical performances, toxicity issues related to the transition metals may be a drawback for practical applications. On the other hand, the majority of nonmetal-containing NO photoreleasers developed require activation with one photon of relatively harmful UV light, or multiphoton excitation with the more biocompatible near-infrared light, with a drawback of requiring expensive laser sources. Only very limited examples of organic NO photoreleasers suitable for one-photon visible light activation are known.[10–13]

Therefore, new organic NO photoreleasers that can be activated with low energy visible light, preferably involving simple synthetic procedures, are needed for widespread use in biologically-oriented laboratories.

On the basis of these considerations, and motivated by our ongoing interest in NO photoreleasing systems,[8a,e, 14] we report herein the novel organic NO photoreleaser, (Z)-**1**, which integrates the spontaneous NO releaser Cupferron and a boron-dipyrromethene (BODIPY) derivative in the same molecular skeleton (Scheme 1). We demonstrate that photoexcitation of the BODIPY light-harvesting center with green light (530–550 nm) induces heterolytic cleavage of the C–O bond, unmasking Cupferron, which releases NO. We also demonstrate the capability of (Z)-**1** to trigger temporally regulated vasodilatation of rat aorta under the exclusive control of green light inputs.

The rationale underpinning the design of compound (Z)-**1** proceeds from the following key points: 1) Wang and coworkers showed that the room temperature-spontaneous NO donor Cupferron[15] (*Scheme 1a*) can be made thermostable upon O-alkylation[16] (*Scheme 1b*); 2) we demonstrated that O-alkylation of Cupferron with a methyl anthracene derivative offers the possibility to liberate Cupferron and, therefore, release NO by subsequently exciting the anthracene chromophore at 390 nm;[17] and 3) Winter and co-workers recently discovered that BODIPY derivatives are suitable photolabile protecting groups unmasked by green light.[18] Merging these discoveries inspired us to devise and synthesize compound (Z)-**g** (*Scheme 1c*) through an easy two-step synthesis from cheap and commercially available precursors (see Supporting Information). This compound was obtained with 100 % Z-stereoselectivity at the N=N bond.



Scheme 1. Molecular structures of the spontaneous NO releaser Cupferron (a), its thermostable O-alkyl derivatives (b), the BODIPY derivative **1** and the principle of NO release (c).

The BODIPY derivative (**Z**)-**1** is very soluble in phosphate buffer/methanol (PBS/MeOH; 1:1) solution, and the absorption features are dominated by the BODIPY chromophore in the visible region (Figure 1). (**Z**)-**1** is very stable under these conditions at room temperature in the dark for several days. In contrast, photodegradation is observed upon irradiation with green light at 532 nm (Figure 1).

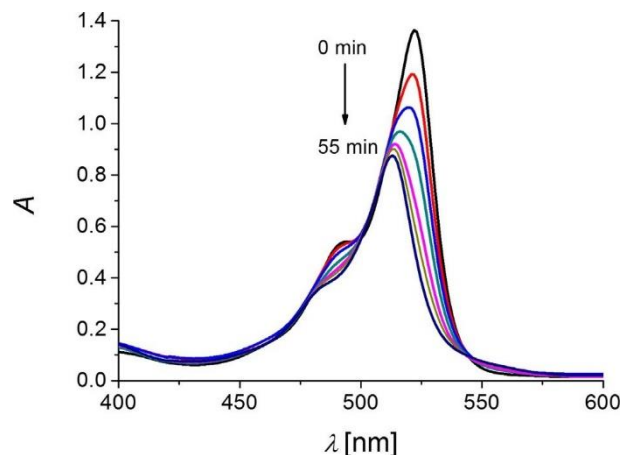


Figure 1. Absorption spectral changes observed upon exposure of a PBS:MeOH (1:1 v/v) solution of (**Z**)-**1** (20 μM) to $\lambda_{exc} = 532$ nm light for intervals of 0, 5, 10, 15, 25, 35, and 55 min. $T = 25$ °C.

LC-ESI-MS analysis (see Supporting Information) of the irradiated solution revealed the presence of the hydroxyl derivative **2** as a major photodegradation product alongside minor amounts of the methoxy derivative **3** (see *Scheme 1c*).^[19] This finding accounts very well for the photoinduced heterolytic rupture of the C-O bond in (**Z**)-**1**, with concomitant formation of a BODIPY-methyl carbocation that undergoes solvent substitution.

Steady-state and time-resolved fluorescence emission analyses suggest that photodecomposition of (**Z**)-**1** likely occurs from the lowest excited singlet state. As shown in *Figure 2A*, the fluorescence intensity of (**Z**)-**1** was significantly weaker than for model compound **2**, which was very stable under green light irradiation. In particular we obtained a value of fluorescence quantum yield, $\Phi_f = 0.36$ for (**Z**)-**1** versus $\Phi_f = 0.63$ for **2**. Accordingly, the fluorescence decay of (**Z**)-**1** was faster than that of **2** (*Figure 2B*). These results account for the occurrence of the photodecomposition process that competes with the fluorescence emission.

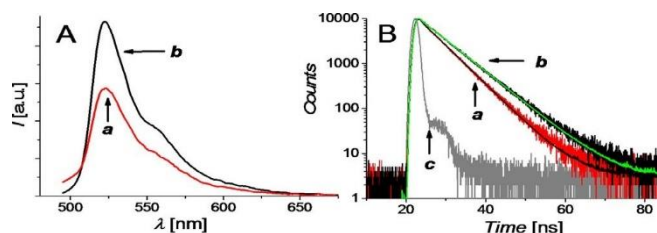


Figure 2. A) Fluorescence emission spectra of optically matched PBS:MeOH (1:1 v/v) solutions of (**Z**)-**1** (a) and **2** (b) at $\lambda_{exc} = 480$ nm. B) Fluorescence decay and related biexponential fitting of the same solutions as (A) recorded at $\lambda_{exc} = 455$ nm and $\lambda_{em} = 525$ nm. C) The instrumental response is also shown.

Photogeneration of the BODIPY-methyl carbocation implies the concomitant release of the Cupferron counterpart, which is expected to rapidly generate NO at room temperature.^[15] NO

release was monitored in real-time through an ultrasensitive NO electrode, which directly detects NO concentration by an amperometric technique. The results illustrated in Figure 3 provide clear evidence that compound (Z)-1 is stable in the dark, but generates NO under irradiation with green light.

The quantum yield for the photodecomposition of (Z)-1, Φ_P , determined at 532 nm, was 0.008 ± 0.001 . This value is larger than that observed for other NO releasers activated with visible light.[10–12] Furthermore, the efficiency of photoinduced reactions is generally expressed as the product of $\epsilon_{\max}\Phi_P$ (quantum efficiency). In our case, the large value of ϵ_{\max} ($69\,000\text{ Lmol}^{-1}$) gives a value of $\epsilon_{\max}\Phi_P \approx 550$, which is more than one order of magnitude larger than that found for a reported NO releaser based on a BODIPY antenna.[11] A pathway responsible for heterolytic C-O bond cleavage may follow an intramolecular photoinduced electron transfer from the BODIPY fragment to the Cupferron unit. This proposal is supported by the considerable change in free energy calculated for this process: $\Delta G \approx -0.3\text{ eV}$.[20]

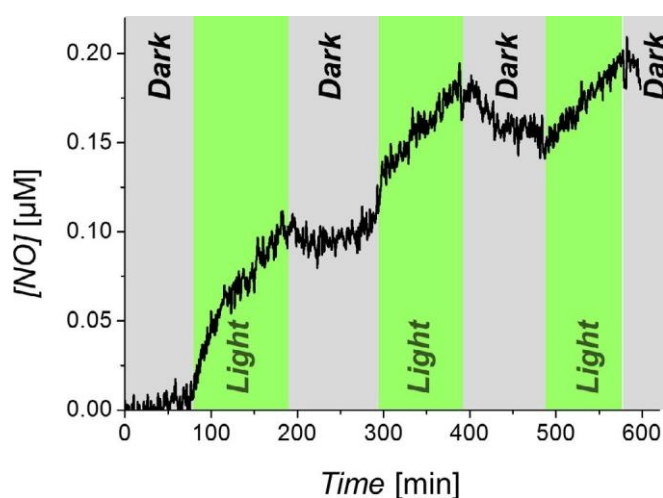


Figure 3. NO release profile observed upon 532 nm light irradiation of a PBS/MeOH (1:1 v/v) solution of (Z)-1 (20 mm).

As a proof of principle for the biological potential of (Z)-1, we tested its capability to induce NO-stimulated vasodilatation of rat aorta under green light irradiation. Denuded rat aorta were placed in a tube filled with physiological solution (see Supporting Information for details) and then contracted by treatment with l-phenylephrine (PE) 1 mM. After reaching equilibrium, a solution of compound (Z)-1 was added, and the tube was irradiated with a visible LED (515–565 nm, 6.5 mW cm^{-2}) at different time intervals. The results illustrated in Figure 4A provide unambiguous evidence for relaxation of the aorta occurring exclusively under irradiation with green light, and that the tension recovers instantaneously when the light is turned off. Note that the extent of the relaxation is strictly dependent on the irradiation time, according to the larger amount of NO produced, and on the concentration of (Z)-1, according to the larger number of photons absorbed. Taking into account that NO induces vasodilatation by activation of soluble guanylate cyclase (sGC),[22] we carried out a control experiment to further confirm that the vasodilatation observed is due to the photogenerated NO. To this end, the aorta was initially treated with 1H-1,2,4-oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a well-known sGC inhibitor,[23] before the addition of PE and (Z)-1. As shown in Figure 4B, light irradiation of the sample did not induce any significant aorta relaxation.

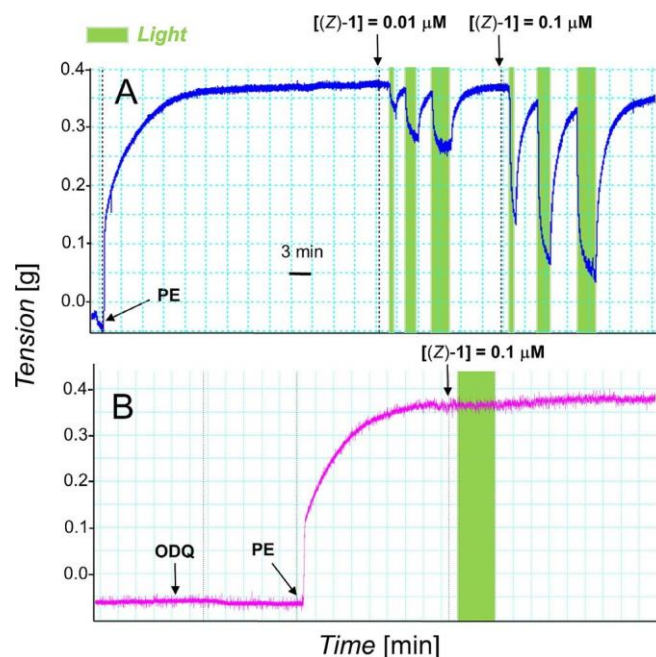


Figure 4. A) Changes in tension of rat aorta induced by NO release from (Z)-1 upon 1, 2, and 3 min irradiation with visible light (515–565 nm, 6.5 mW cm⁻²). B) Control experiment performed by pre-treating the aorta strips with ODQ (10 mm) before the addition of PE (1 mm) and (Z)-1, effectively inhibiting NO uptake.

In conclusion, a rare example of a nonmetal-containing NO photoreleaser that can be activated with single-photon excitation with the green light exploiting a BODIPY light harvesting antenna was reported. Compared with the other examples of NO photoreleasers activated by visible light,[10–12] the BODIPY derivative reported herein shows two key advantages: i) a very simple synthetic procedure, starting from cheap and commercially available precursors; ii) excellent photochemical performance, shown from the obtained values of Φ_P and $\epsilon_{\max}FP$.

These features, together with excellent light-regulated vasodilatation effects, make compound (Z)-1 an appealing candidate not only for biomedical research on the blood circulatory systems, but also for widespread use in biologically-oriented research fields requiring spatiotemporal delivery of NO. Some of these studies are currently underway.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

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[19] In the early stage of the photoreaction (within 15 % of transformation) we observed the formation of the E isomer of **1**, (E)-**1**, at the same time as products **2** and **3** (see Supporting Information). However, under continuous green-light excitation, this product does not accumulate, because it is selectively reconverted into (Z)-**1**.

[20] Estimated by the Rehm–Weller equation (Ref. [21a]) on the basis of the oxidation potential of BODIPY, the reduction potential of the azoxygroup, and the energy of the lowest excited singlet state of BODIPY (Ref. [21b]).

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