A Novel Scaffold for Nitric Oxide Photo-Releasers based on Meso-Aminomethyl-BODIPY


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Abstract: Nitric oxide (NO) is a unique biochemical mediator involved in the regulation of vital processes. Its short half-life and local action impede direct application in medicine because highly targeted NO-delivery systems are required. Light-controllable NO releasers are promising for the development of smart therapies. Here we present novel simply prepared meso-aminomethyl BODIPY dyes containing N-Nitroso moiety and show the photoinduced generation of NO in a solution. As an example of NO-mediated effects, we demonstrate efficient light-dependent inhibition of platelet activation in vitro. We also show that some compounds could additionally generate singlet oxygen, which is promising for the photodynamic therapy. The presented compounds could serve as the basis for the development of novel hybrid therapeutic methods.

Keywords: nitric oxide, light-controllable NO releasers, photoremoveable protecting group, photolabile “caged” nitric oxide, BODIPY, singlet oxygen, platelet activation.
1. Introduction

Nitric oxide (NO) is an important endogenously produced signaling molecule [1]. This gaseous messenger is critical for the regulation of vascular and muscle tone, neurotransmission, wound healing, platelet aggregation and many other physiological processes. Furthermore, nitric oxide plays a significant role in tumor biology participating in cell death, angiogenesis, and antitumoral immune response [2–7]. Dysregulation of NO production leads to various pathological processes. For instance, impaired NO production causes diabetic vascular complications [8–10]. Therefore, precise control of NO concentration opens promising possibilities for target therapy including anticancer therapy. For controlled delivery of nitric oxide, extensive efforts are being made to develop novel NO-releasing biomaterials [11] such as liposomes, nanoparticles, macromolecules. Short lifetime (~5s) in living tissues and limited diffusion range of nitric oxide imply the use of methodologies which rely on the triggering of NO release using external stimulus, for example, light radiation [12], X-ray radiation [13], or ultrasound [14]. Visible and NIR light represents a smart fine-tunable and non-invasive tool for practical usage due to deeper penetration in biological tissues. Therefore, design and development of such photoactivatable NO-donors are highly desirable.

In recent years, several general approaches toward such molecules were established. Briefly, one of the common methodologies is based on photolysis of metal nitrosyl complexes [15–17]. The main restrictions of this approach are potential difficulties of structure modifications and possible metal ions toxicity. Metal-free photoactivatable NO-donors often utilize homolysis of N-NO fragment or nitro-nitrite photorearrangement in sterically hindered nitroarenes followed by homolysis of O-NO bond. Typically, visible light absorbance of this molecules is reached by conjugation of NO-releasing moiety with chromophores such as rhodamine/roamine [18–21], BODIPY [22,23], aza-BODIPY [24] dyes. Often, the antenna (chromophore) with high fluorescence quantum yield can be used for the optical calibration of the NO release dosage [25].

In the present work, we search for simply synthesizable small-molecule NO photodonors with intense absorption in visible region. BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-S-indacene) chromophore was chosen due to its unique photochemical properties, such as narrow absorption bands with tunable wavelength, which provides successful usage of these dyes in various scientific areas, from optoelectronics to life sciences (see [26] and references therein). Several BODIPY-based NO photodonors have been already reported by Nakagawa [27] and Sortino [28] groups. We have also studied several hindered nitrobenzenes, linked to the BODIPY core in meso-position [29], but the NO release was undetectable. Meso-methylene BODIPYs have especially remarkable properties and have been used in various applications, including photolabile protecting groups
[30]. We hypothesized that this scaffold could be superior as light-harvesting moiety for N-Nitroso NO photodonors.

Herein, we present several simply-synthesizable NO photodonors based on meso-methyl-BODIPY core (BODIPY-NODs). We report the efficiency of NO photorelease, photoinduced $^1$O$_2$ generation and fluorescence quantum yields. The proportion of NO and $^1$O$_2$ can be easily tuned by introduction of substituents into the chromophore core, from the sole photorelease of NO without detectable $^1$O$_2$ to the opposite (no NO / high $^1$O$_2$). These compounds and their future derivatives are promising for the development of light-controllable smart therapies, including the photodynamic therapy of cancer.
2. Materials and Methods

2.1. Synthesis of the compounds
Synthesis is briefly outlined in Section 3.1 and described in detail in Supplementary information, together with NMR data.

2.2. Measurement of properties
Absorption spectra were measured with Shimadzu UV-1900 spectrophotometer, whereas fluorescence emission spectra were measured with Shimadzu RF-6000 fluorometer. Fluorescence quantum yield was determined using Rhodamine 6G in EtOH as a standard. For measurements in other solvents we accounted for the difference in refractive index using the factor \((n_{\text{solvent}}/n_{\text{EtOH}})^2\).

Photolysis was done in a quartz cuvette using high-power green LEDs (500 nm and 520 nm for different compounds). LEDs were fixed directly near the cuvette and were fed with the current of 0.25 A.

Photoinduced generation of nitric oxide was evaluated using fluorescent probe DAR-2 [31]. The procedure of determination of NO concentration from the measurement of fluorescence is described in the supplementary materials.

Singlet oxygen luminescence was measured using previously described setup [32].

2.3. Calculations
Quantum chemical calculations of the adiabatic potential energy surface (PES) profiles for the studied molecules were carried out in the DFT approach using CAM-B3LYP functional, implemented in the GAMESS program package. As shown in [33], energy levels and related parameters can be estimated more accurately with M06-2X functional, so we tested all the obtained parameters using this functional. PES calculations included the localization of stationary structures and the determination of their type based on the analysis of normal vibrations. The 6-31 + G* basis set was used for BODIPY-NODs which do not contain iodine atoms, whereas SPK-DZP was used for others. The influence of “environment” was considered by the polarizable continuum model (PCM). Excited state calculations were carried out using TD-DFT method.

2.4. Activation of blood platelets
The fasting blood samples were obtained from the cubital veins of healthy volunteer with informed consent. The sample were collected in the vacuum tube containing sodium citrate as anticoagulant (9:1). After collection, the sample was kept at room temperature for an hour to obtain a layer of plasma containing platelets. In the next step, the sample was labeled with fluorescent calcium probe Fluo-4-AM (Thermo Fisher Scientific, USA). The stock solution (1 mM) of Fluo-
4-AM was diluted 62.5 times in phosphate buffered saline (PBS) and mixed 1:1 with blood plasma. After incubation for 30 minutes in the dark, the sample was 10 times diluted in PBS with or without compound 2a or 2b, placed in a well of 96-well plate and allowed to rest for another 30 minutes before experiments. Calcium signaling in individual platelets was recorded using CarlZeiss AxioVert.A1 fluorescence microscope with 20x objective.

3. Results and Discussion:

3.1. Synthesis of NO photodonors
Generally, there are no approaches to predict the efficiency of NO photorelease, and its relation to the molecular structure is not fully understood. Moreover, different mechanisms of NO release were reported, including intramolecular charge transfer (ICT) [34], photoinduced electron transfer (PET) [35], or homolythic fission with the formation of aminium radicals [36]. BODIPY-based N-NO photodonors reported previously [27,28] contain extended linker connecting BODIPY and NO-releasing moiety. On the other hand, N-NO group in several cases was directly attached to a chromophore [24,35]. We decided to use short methylene linkage between BODIPY and N-NO. Such compounds are easily available starting from meso-CH₂Cl-BODIPY 1a (Scheme 1). Substitution of Cl with I followed with reaction with primary amine (i-propylamine or aniline, respectively) gives amines 2a,b, whose nitrosation leads to N-nitroso donors BODIPY-NOD-1 and BODIPY-NOD-2.

Interesting feature of some BODIPY NO-photodonors is the ability to generate ¹O₂ under light, which could be enhanced by introduction of iodine atoms in the BODIPY core [37]. Simultaneous generation of NO and ¹O₂ demonstrates synergetic effect and results in increased photocytotoxicity. In this purposes, we also decided to prepare iodinated BODIPY-NOD-3. Diiodo BODIPY 1b was prepared by iodinating 1a with N-iodosuccinimide in methylene chloride. Potassium carbonate and catalytic amount (10 mol%) of sodium iodide in acetonitrile were used to obtain compound 3. Further nitrosation allowed us to obtain target compound BODIPY-NOD-3 (Scheme 1B).

As it has been shown, replacement of fluorine atoms in BF₂-fragment with CH₃-groups in meso-CH₂-BODIPY photoremovable group led to increase of photorelease efficiency [30]. Analogously, we prepared BODIPY-NOD-4 and BODIPY-NOD-5 to study the effect of boron methylation (Scheme 1C). Compounds 2b and 3 reacted with freshly prepared MeMgI, giving products 4 and 5, respectively. After that, obtained compounds were nitrosated, using sodium nitrite and CH₂Cl₂/THF/AcOH mixture, to get final N-nitroso compounds BODIPY-NOD-4 and BODIPY-NOD-5. Working with iodinated and/or boron methylated compounds, we had to use milder conditions, due to starting and target compounds tendency to decompose under heating.
3.2. Photoinduced release of NO
In this section we compare the obtained molecules in terms of photophysical properties and NO generation efficiency. Figure 1 shows the normalized absorption spectra in ethanol. All compounds have high molar extinction coefficient at ~510-520 nm (Table 1). A slight hypochromic shift is observed upon boron methylation.

![Scheme 1. Synthesis of the compounds.](image)

![Figure 1. Normalized absorption spectra of BODIPY-NODs in ethanol.](image)
Table 1. Extinction coefficient and maximal absorption wavelengths in ethanol.

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<thead>
<tr>
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<th>BODIPY-</th>
<th>BODIPY-</th>
<th>BODIPY-</th>
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<tr>
<td></td>
<td>NOD-1</td>
<td>NOD-2</td>
<td>NOD-4</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}, \text{nm}$</td>
<td>515</td>
<td>517</td>
<td>511</td>
</tr>
<tr>
<td>$\varepsilon(\lambda_{\text{max}})$, M$^{-1}$cm$^{-1}$</td>
<td>7.75×10$^4$</td>
<td>7.2×10$^4$</td>
<td>8.2×10$^4$</td>
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</table>

We performed the stationary photolysis of EtOH solution in a 1 cm quartz cuvette using single 500 nm high-power LED. The absorption spectrum changed rapidly during first minutes of photolysis and then reached stationary shape. An example for BODIPY-NOD-2 is shown in Figure 2A. The final spectrum has the same shape as the spectrum of 2b, which is shown in Figure 2B. Therefore, the initial and final states differ by the presence and absence of NO in the molecule, respectively. This was additionally confirmed with HPLC measurements (Figure S2 in Supplementary). The same conversion was observed for compound BODIPY-NOD-4. It implies that NO is released during photolysis. In contrast, the spectrum of the photoproduction of BODIPY-NOD-1 does not coincide with its counterpart 2a: it has an additional red-shifted peak at about 550 nm. However, the formation of 2a during photolysis is visible with HPLC, whereas the unknown photoproduct absorbing at 500 and 550 nm has much lower retention time (Figure S3).

![Figure 2](image-url)

Figure 2. A: Changes in the absorption spectra of BODIPY-NOD-2 in the course of illumination with 500 nm high-power LED. B: Absorption spectrum of 2a, the same as the spectrum of the final product of 2b. C-E: The photoinduced release of NO monitored with DAR-2 fluorescence probe, given as the ratio of [NO] to the initial concentration of the photodonor (%).
To quantify the photorelease of NO, we used the DAR-2 fluorescent probe which acts as a cumulative counter of NO. Figure 2C shows the kinetics obtained by measuring the probe fluorescence and using the equation described in Supplementary materials. The result is shown as the ratio of NO concentration to the concentration of photodonor (%). The fastest and the most efficient NO photorelease is observed for BODIPY-NOD-2 (~20% total for 2 min), whereas BODIPY-NOD-4 shows the same speed but lower total yield (~4% total for 2 min). Compound 2b (the photoproduct of BODIPY-NOD-2) was photostable in EtOH, and the fluorescence of DAR-2 probe did not change in the same experiment. Measurements for the compound BODIPY-NOD-1 are obstructed because the absorption spectrum of the photoproduct overlaps with that of DAR-2 probe. This results in the background fluorescence increase during photolysis; however, the fluorescence of DAR-2 probe did not significantly exceed the background level, therefore we conclude that BODIPY-NOD-1 is probably capable of NO photorelease with relatively low efficiency. Obviously, the non-100% NO yield is related with some irreversible photomodification of the molecular structure which is not coupled with NO release.

In several papers, the photorelease of NO was associated with the fluorescence turn-on [35,38]. It is explained by the fact that the presence of NO in the molecule leads to the photoinduced electron transfer (PET), which eventually leads to the N-N bond dissociation. However, we did not observe the increase of the intrinsic fluorescence in the present study. For instance, the fluorescence quantum yield (QY) in EtOH is 0.20 for BODIPY-NOD-2 in and 0.12 for its counterpart without NO, 2b. Generally, the QYs are quite high (Table 2), resulting in bright fluorescence due to the high absorption coefficient of BODIPYs. The spectra of fluorescence can be found in supplementary materials.

Table 2. Fluorescence quantum yields and maximal emission wavelength. Excitation: 500 nm.

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<tr>
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<th>BODIPY-NOD-1</th>
<th>BODIPY-NOD-2</th>
<th>BODIPY-NOD-4</th>
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<tr>
<td>$\lambda_{\text{em}}$, nm</td>
<td>530</td>
<td>526</td>
<td>524</td>
</tr>
<tr>
<td>QY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO $\varepsilon = 46.7$</td>
<td>0.44</td>
<td>0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>EtOH $\varepsilon = 24.6$</td>
<td>0.30</td>
<td>0.20</td>
<td>0.07</td>
</tr>
<tr>
<td>CHCl$_2$ $\varepsilon = 8.93$</td>
<td>0.31</td>
<td>0.17</td>
<td>0.08</td>
</tr>
<tr>
<td>CHCl$_3$ $\varepsilon = 4.81$</td>
<td>0.45</td>
<td>0.18</td>
<td>0.10</td>
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</table>
The absence of fluorescence turn-on behavior may lead to the conclusion that PET probability is low in the molecules under study. To confirm this fact, we measured the fluorescence QY in a series of solvents having different polarity (Table 2). A library of meso-substituted BODIPYs was studied in [39], and it was showed that their fluorescence, if quenched in polar solvents due to PET, turns on in non-polar solvents. Our results confirms that the probability of PET is indeed low. This is additionally confirmed by the quantum chemical calculation of molecular orbitals (see Section 3.4): there is no profound relocation of charge density from BODIPY core to the substituent. However, some charge is transferred to the nitrogen atom, possibly weakening the N-NO bond.

The absence of PET is especially surprising because NO releaser described in [27], based on the same dye and N-nitroso bond as well, acts via PET mechanism. According to the authors, NO release process includes PET from the fragment containing N-NO to the BODIPY core and the formation of a radical pair. Most likely, this is also the case for our compounds (Scheme 2A), which seems to contradict the experimental data and calculations. However, they indicate only major photoprocesses, whereas PET occurs rarely. To confirm this assumption, we estimated the quantum yield of NO release by BODIPY-NOD-2 using 488 nm laser. We measured the absorbed power and the amount of released NO and obtained the value of QY $5.5 \times 10^{-4}$. This value is indeed much smaller than the QY of fluorescence and other photoprocesses as well. Interestingly, similar value of NO release QY ($1.9 \times 10^{-3}$) was reported for NOBL-1 [27], which was successfully used for the photomanipulation of vasodilation. We also note that, in principle, the release of NO without formation of a radical pair is also possible, according to Scheme 2B.

Scheme 2. Possible mechanisms of NO photorelease.
3.3. Singlet oxygen generation

Photoinduced singlet oxygen ($^{1}$O$_{2}$) generation is an intrinsic property of many dyes, including BODIPY. Simultaneous production of $^{1}$O$_{2}$ and NO is of interest for the development of hybrid photodynamic therapy [23]. We synthesized diiodine derivatives BODIPY-NOD-3 and BODIPY-NOD-5 for enhanced generation singlet oxygen due to heavy atom effect.

The normalized absorption spectra and photophysical properties of iodinated compounds are shown in the Figure 3A. Absorption spectra are red-shifted shifted by ~30 nm for the diiodine-substituted BODIPY compounds due to its electron-acceptor nature, in accordance with previously published data on similar compounds. Fluorescence for these compounds are weak (QYs are 0.015 and 0.003, respectively), probably due to the enhanced conversion into triplet state. Interestingly, BODIPY-NOD-3 is also capable of NO photorelease, as shown in Figure 3B. Despite relatively low speed and total yield (cf. Figure 2C), it could greatly enhance the action of $^{1}$O$_{2}$ as photodynamic agent. In contrast, the release of NO is not detected for BODIPY-NOD-5.

![Absorption spectra and photophysical properties of diiodine-substituted BODIPY-NOD-3, 5. B: NO photorelease by BODIPY-NOD-3. C,D: Luminescence of singlet oxygen measured during photoexcitation of compounds (excitation wavelength 500 nm).](image)

To evaluate the efficiency of $^{1}$O$_{2}$ generation, we measured its luminescence spectrum around 1270 nm. Figure 3C,D shows the luminescence intensity relative to the absorbance at the excitation
wavelength (500 nm). As expected, relative luminescence intensity is approximately 200-times higher for BODIPY-NOD-3 than for BODIPY-NOD-2 which does not contain heavy atoms. Surprisingly, its boron-alkylated analogue BODIPY-NOD-5 exhibits ~100-times lower relative luminescence compared to BODIPY-NOD-3. We conclude that exchange of fluorine atoms for methyl groups result in significant reduction of $^1$O$_2$ generation efficiency. Accordingly, the signal for compound BODIPY-NOD-4 is not detected. The QYs of $^1$O$_2$ generation was determined by comparison with 2I-meso-phenyl-BODIPY for which the QY was reported to be 0.81 (compound I2-BDP in [40]). Results are shown in the Table 3.


<table>
<thead>
<tr>
<th></th>
<th>BODIPY-NOD-2</th>
<th>BODIPY-NOD-3</th>
<th>BODIPY-NOD-4</th>
<th>BODIPY-NOD-5</th>
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<tr>
<td>QY, %</td>
<td>0.5</td>
<td>90</td>
<td>n.d.</td>
<td>1.4</td>
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To sum up, we present a family of compounds with NO-releasing ability and singlet oxygen formation. The maximal NO yield and the release speed was observed for BODIPY-NOD-2, whereas maximal $^1$O$_2$ generation was detected for BODIPY-NOD-3. Compound BODIPY-NOD-4 releases NO but does not generate $^1$O$_2$, whereas BODIPY-NOD-5 does the opposite. Observed low efficiency of NO photorelease in the case of significant $^1$O$_2$ generation could be also explained by the reaction with oxygen with the formation of peroxynitrite [41]. This process is an alternative to the reaction with DAR-2 probe, which reduces its apparent fluorescence. Still, these experimental results show that the introduction of heavy atoms into the BODIPY core does not enhance the photorelease of NO, indicating that the latter proceeds from the singlet state. This additionally proves that PET is not inherent for the NO photorelease.

3.4. Quantum chemical calculations

The geometry and molecular orbitals of the singlet, triplet, and excited states of all compounds were calculated in various solvents. The calculations were carried out in two functionals (CAMB3LYP, M06-2X) using different basis sets (6-31+G*, 6-311+G*, SPK-DZP). For the compounds under study, there were no significant differences from the choice of calculation parameters.

Figure 4 shows molecular orbitals for BODIPY-NOD-1,2,4 with computational parameters corresponding to H$_2$O. The same for diiodinated compounds BODIPY-NOD-3,5 is shown in Figure 5. It is worth noting that solvent does not affect the singlet-state orbitals (HOMO and LUMO), whereas those for a triplet state can be different in a gas phase, especially for BODIPY-NOD-2 (cf. TableS1, S2 in supplementary).
Figure 4. HOMO and LUMO for molecular singlet state and SOMO 1 and SOMO 2 for molecular triplet state of BODIPY-NOD-1,2,4 in H$_2$O calculated at the level of DFT CAMB3LYP/6-31+G* (see supplementary information for the results in gas).

To analyze the absorption spectra, we performed calculations for the excited states using TD-DFT approach with CAMB3LYP and M06-2X functionals and 6-31+G*, 6-311+G*, and SPK-DZP basis sets. First, the calculations were done for the ground-state geometry (i.e., all atoms were fixed). The results are shown in Table 4. Note that all the functionals and basis sets give close results. The obtained results give the main absorption band at 430-440 nm, which is quite far from experimental values (e.g., 505-515 nm in Table 1). To account for this difference, we performed the calculations of optimized excited-state geometry. Only the CAMB3LYP functional was used in these calculations. The obtained results are also shown in Table 4.
Figure 5. HOMO and LUMO for molecular singlet state and SOMO 1 and SOMO 2 for molecular triplet state of BODIPY-NOD-3,5 in H$_2$O calculated at the level of DFT CAMB3LYP/SPK-DZP (see supplementary info for the results in gas).

Table 4. Energy of excited singlet state in ground-state geometry and optimized geometry of exited state (eV) calculated at the level of TD DFT CAMB3LYP/6-311+G* and SPK-DZP, M06-2X/6-311+G* and SPK-DZP for BODIPY-NODs in H$_2$O.

<table>
<thead>
<tr>
<th></th>
<th>CAMB3LYP</th>
<th>M06-2X</th>
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<tr>
<td></td>
<td>6-311+G*</td>
<td>SPK-DZP</td>
</tr>
<tr>
<td></td>
<td>ground-state geometry</td>
<td>optimized geometry</td>
</tr>
<tr>
<td>BODIPY-NOD-1</td>
<td>2.79</td>
<td>2.458</td>
</tr>
<tr>
<td>BODIPY-NOD-2</td>
<td>2.78</td>
<td>2.436</td>
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<tr>
<td>BODIPY-NOD-3</td>
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<tr>
<td>BODIPY-NOD-4</td>
<td>2.83</td>
<td>2.477</td>
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<tr>
<td>BODIPY-NOD-5</td>
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The resulting energies of the excited state for the case of optimized geometry correspond to 490-510 nm, which is in good agreement with experimental data for compounds BODIPY-NOD-1,2,4.
3.5. Application to the study of blood platelets

Blood platelets are the foundation of hemostasis and also contribute to a variety of other normal and pathological processes, including thrombosis, inflammation, and tumor development [42,43]. The hemostatic function of platelets is closely related to their ability to change physical properties in response to vessel wall injury [44]. The first step of this process is platelet activation, which comprises a series of prothrombotic events, triggered by the increase of intracellular calcium [45]. Abnormal platelet activation is considered as a cornerstone in pathogenesis of atherosclerotic cardiovascular diseases [46], which are the principal cause of mortality globally. Therefore, it is highly anticipated that in-depth study of platelet activation and development of advanced methods for its assessment could contribute to further progress in cardiovascular medicine.

Despite novel methods are being developed [47], they usually rely on *in vitro* measurements, which is far from physiological conditions: in normal vessels, platelet activation is constantly inhibited, primarily by NO constantly released by endothelial cells. Concentration of NO as low as 3-90 nM effectively inhibits platelet activation [48], and attempts to study platelet activation using exogenous NO donors were made [49]. Light-activatable NO donors are promising in this respect because they provide steady and controllable release. In this study, we used compound BODIPY-NOD-2 as NO photodonor.

Platelets were isolated from blood as described in methods and loaded with Fluo-4 fluorescent calcium probe. This probe accumulates in cells, and the activation becomes visible under the fluorescent microscope as sharp flashing in the fluorescence intensity due to the spiking of intracellular calcium. The excitation spectrum was 450-490 nm, emission >515 nm. Measurements were done in a 96-well plate for adherent cells. In these conditions platelets activate spontaneously due to the interaction with the bottom surface of wells, so the cells were quite active (Figure 6A,B). The same is observed for the sample incubated with 150 nM of 2b as a control (Figure 6C,D). In contrast, platelets incubated with BODIPY-NOD-2 are much dimmer in the image and do not show significant calcium spikes (Figure 6E,F). We observed the same effect in experiments with higher concentration of the compounds. However, in samples with the concentration of BODIPY-NOD-2 ≥ 600 nM the observation of Fluo-4 signal is hindered because of the intrinsic fluorescence of photodonor, which decreases rapidly during NO photorelease and thereby change the background level. On the other hand, this fluorescence decrease shows that the photolysis indeed occurs in the sample.
Figure 6. Images of platelets in control sample (A), incubated with 2a (C) and 2b (E). Calcium dynamics in representative cells from each sample is shown in B,D,F.

This result demonstrates that the photoinduced NO release indeed can be used to mimic physiological conditions and inhibit spontaneous activation of platelets in the sample. It could be used to develop novel, more precise methods for *in vitro* testing of platelet function. Especially convenient in the described experiment is the fact that the same excitation source is used to monitor Fluo-4 fluorescence and to induce the NO photorelease – no dedicated light source is needed.

**Conclusion**

In this paper, we presented novel BODIPY dyes containing N-Nitroso moiety and showed the photoinduced generation of NO. We showed that for photodonors containing the phenyl ring (BODIPY-NOD-2,3,4,5) the maximal NO yield is observed for unsubstituted BODIPY, whereas boron alkylation and the introduction of the iodine atoms reduces the efficiency, although influence the singlet oxygen generation. For the isopropyl-containing photodonor BODIPY-NOD-1 the photorelease of NO was not detected. We measured the fluorescence QYs for all compounds in solvents with different polarity and did not observe significant differences inherent for PET behavior. It is most likely that for the described compounds the NO photorelease occurs
without PET. This fact is also confirmed by the fact that enhanced triplet state formation for diiodine-substituted compounds does not increase the yield of NO. Finally, using the developed NO photodonors, we demonstrated efficient light-dependent inhibition of platelet activation in vitro. We also show that some compounds could additionally generate singlet oxygen, which is promising for the photodynamic therapy. The presented compounds could be used in biological research and serve as the basis for the development of novel hybrid therapeutic methods.

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Author contributions
M.P.: chemical synthesis; T.K.: experiments (photochemistry), data treatment; A.S.: experiments (QY determination), data treatment; I.T.: calculations; A.V. conceptualization, chemical synthesis; A. M.: experiments (platelets), writing – original draft. All authors contributed to the data analysis and provided feedback on the manuscript.

Conflict of interest
The authors declare no conflicts of interest.

References


