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Proceeding Paper

meso-Dimethylaminonaphthyl-BODIPY Derivatives as Fluorescent Probes for Lysosomes and Lipid Droplets Bioimaging †

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Abstract: Fluorescence-based probes are a powerful tool to observe living systems in real time with spatiotemporal precision. 3-Difluoroborodipyrromethene (BODIPY) derivatives have emerged as crucial tools for fluorescent labeling applications due to their advantageous qualities. In this context, we report two BODIPY-derived fluorescent probes for visualizing lysosomes and lipid droplets within living cells. In vitro studies by confocal microscopy in HeLa cells revealed that these compounds could effectively permeate the cell membrane and selectively label lysosomes and lipid droplets while causing no harm to the cell culture. In essence, these BODIPY derivatives show great potential for exploring the dynamics and functions of these organelles in living cells through fluorescence imaging.

Keywords: BODIPY; fluorescent probes; bioimaging; lysosomes; lipid droplets

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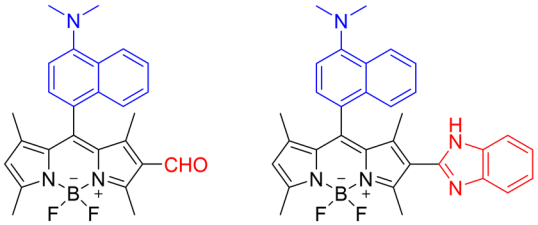
1. Introduction

Lipid droplets (LD) and lysosomes are important organelles in cellular metabolism and physiology, playing vital roles in maintaining intracellular homeostasis. Lipid droplets not only store and metabolize neutral lipids, but also contribute to energy balance, membrane dynamics, and lipid trafficking within cells. Dysfunctional LDs can lead to metabolic disorders and diseases like obesity, fatty liver diseases, diabetes, atherosclerosis, and certain cancers [1–3]. Likewise, lysosomes are acidic organelles that significantly contribute to degrading cellular waste and maintaining cellular health. Irregularities in lysosome function can lead, for example, to lysosomal storage disorders, neurodegenerative and metabolic diseases, as well as cancer [4–7]. Therefore, it is crucial to have reliable imaging tools for exploring these cellular structures.

Fluorescent probes represent a useful tool for studying specific structures or molecules within cells with spatiotemporal precision. These probes enable the visualization of cellular morphology, tracking of molecular processes, and studying biomolecules behaviour in real time [8–10]. 3-Difluoroborodipyrromethene (BODIPY) derivatives have stood out in this field due to their outstanding optical and physical properties, low phototoxicity and photobleaching, strong absorption, high quantum yield, and chemical stability, and ease of synthesis [11–14].

In this context, we report two BODIPY-based fluorescent probes (BODIPYs **2**–**3**) designed for live cell imaging of lysosomes and lipid droplets. BODIPY derivatives **2** and **3**

based on the tetramethyl-BODIPY core functionalized with an electron-donating *N*,*N*-dimethylaminonaphthyl group at 8-position (*meso* position), and formyl and benzimidazole electron-withdrawing groups at 2-position (Figure 1) have been reported previously by our research group [15,16]. In vitro experiments using confocal microscopy in HeLa cells demonstrated the probes' ability to permeate the cell membrane and selectively label lysosomes and lipid droplets without causing any adverse effects on the cultured cells. These BODIPY derivatives represent a promising tool for intracellular detection of lysosomes and lipid droplets through fluorescence imaging.



2 **3**

Figure 1. Structure of BODIPY derivatives **2**–**3**.

2. Methods and Materials

Fluorescence images in cell media were acquired using a confocal microscope LSM 780 (Zeiss). The synthesis and characterization of BODIPY derivatives **2** and **3** have been reported recently by our research group [15,16].

2.1. Cell Culture

Human cervical cancer (HeLa) cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco™, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS) and 1% of penicillin-streptomycin, at 37 °C in a humidified incubator with a 5% CO_2 atmosphere.

2.2. Cell Viability

HeLa cell viability was determined by resazurin assay [19,20]. Furthermore, 10^4 cells per well were seeded in 96-well plates and incubated overnight at 37 °C in a 5% CO_2 atmosphere. Then, cells were incubated with BODIPY derivatives **2** and **3** at concentrations ranging from 12.5 to 100 μM for 24 h. Afterward, cells were treated with resazurin reagent (1:100) and incubated for 4 h in the dark. The fluorescence intensity was read in the Biotek Synergy H1 microplate reader and the cell viability values were calculated considering the control (non-treated cells) as 100% viability using Equation (1). The blank represents the fluorescence mean value of the cell culture medium. Data were represented as mean ± SEM from triplicate samples of two independent experiments.

$$Cell\ viability\ \% = \frac{Fluo\ (treated\ cells)\ -\ Fluo\ (blank)}{Fluo\ (control)\ -\ Fluo\ (blank)} \times 100 \quad (1)$$

2.3. Sub-Cellular Localization

2.3.1. Lysosome Colocalization Assay

HeLa cells were seeded at a density of 2×10^4 cells per well in DMEM in glass bottom dishes and incubated overnight at 37 °C in a 5% CO_2 atmosphere. Then, cells were washed two times with PBS and treated with BODIPY **3** at a concentration of 40 μM (a stock solution of 10 mM in DMSO diluted with DMEM) and with a solution of LysoTracker Deep Red (85 nM, cat. no. L12492, Invitrogen) for 1 h to stain lysosomes in the cell cytoplasm. Afterward, cell nucleus was stained with Hoechst 33342 (1:1000, cat. no. ab139481, Abcam)

for 10 min. The fluorescence cell images were taken with confocal microscopy (LSM 780 Zeiss, Oberkochen, Germany) under a 63× oil-immersion objective lens, using the 405 nm laser for Hoechst 33342, a 488 nm laser for the BODIPY **3** and a 633 nm laser for LysoTracker Deep Red.

2.3.2. Lipid Droplet Colocalization Assay

To induce lipid droplet formation in cultured cells oleic acid complexed with BSA was used. HeLa cells were seeded at a density of 2×10^4 cells per well in DMEM in glass bottom dishes and incubated overnight at 37 °C in a 5% CO_2 atmosphere. Oleic acid and defatted BSA solutions were prepared for further complex formation. The oleic acid was diluted to 150 mM in 50% ethanol by mixing 47 μL oleic acid with 953 μL 50% ethanol. The mixture was vortexed and stored at 4 °C. A 100 mg/mL BSA solution was prepared by dissolving the defatted BSA in dH_2O and stored at −20 °C. Then, the oleic acid solution was resuspended by heating to 37 °C, and equal volumes (20 μL) of 150 mM oleic acid and 100 mg/mL defatted BSA in dH_2O were combined. The mixture was incubated at 37 °C for 1 h. This oleic acid/BSA complex was diluted 1:150 in growth medium for a final concentration of 500 μM oleic acid, and the cells were incubated with this solution overnight at 37 °C. Cells were incubated at 37 °C with a solution of LipidSpot™ (1:1000, cat. no. 70069-T, Biotium) for 30 min to label lipid droplets in the cell cytoplasm, with BODIPY **2** at a concentration of 40 μM (a stock solution of 10 mM in DMSO diluted with DMEM) for 20 min and, finally, with Hoechst 33342 (1:1000, cat. no. ab139481, Abcam) for 10 min to stain the cell nucleus. The cells were then imaged by confocal microscopy (LSM 780 Zeiss, Oberkochen, Germany) with 63× oil immersion objective lens, using a 405 nm laser for Hoechst 33342, a 488 nm laser for the BODIPY **2** and a 633 nm laser for LipidSpot™.

3. Results and Discussion

3.1. Cell Viability

Prior to cell imaging, BODIPYs **2–3** cytotoxicity was evaluated in HeLa cells using the resazurin assay. HeLa cells were treated with different concentrations of BODIPYs **2–3** (6.25, 12.5, 25, 50, and 100 μM), and, after 24 h of incubation, the cell viability was determined. As shown in Figure 2, cell viability remained greater than 95% with concentrations of 50 μM after 24 h. Thus, BODIPYs **2–3** proved to be biocompatible with this cell line and appropriate to perform bioimaging experiments.

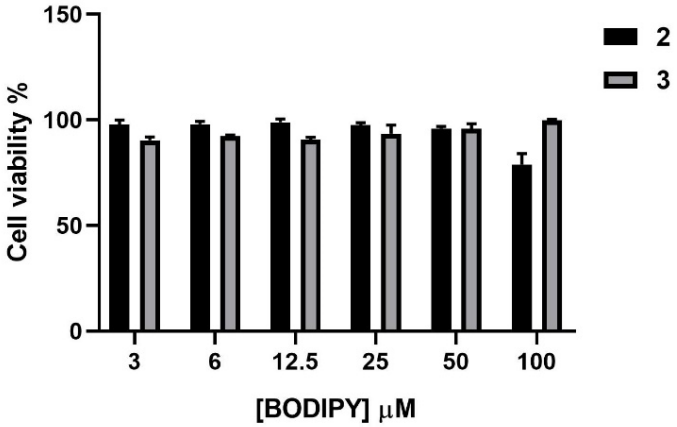


Figure 2. Viability assay in Hela cells. Cell viability was determined through the Resazurin method after 24 h of incubation with different concentrations of BODIPY derivatives **2–3**. Data are presented as mean ± SEM from triplicate samples of two independent experiments.

3.2. Sub-Cellular Localization

To confirm the internalization and determine the localization of BODIPYs **2**–**3** within HeLa cells, colocalization experiments by confocal microscopy were performed with commercial probes (LysoTracker Deep Red and LipidSpot™).

In Figure 3A,B, it is possible to observe the distribution of fluorescence signals emitted by BODIPY **3** and Lysotracker Deep Red in response to excitation at 488 nm (for the green channel) and 633 nm (for the red channel), respectively. When we combine the signals from both channels in the merged image (Figure 3C), we can clearly see an overlap between the green and red fluorescence, creating a yellow signal. This yellow signal is evidence of BODIPY **3** and Lysotracker Deep Red coexisting in the same cellular regions, indicating their colocalization. This finding demonstrates that BODIPY **3** can effectively penetrate HeLa cancer cells and accumulate within their lysosomes, where it serves as a staining agent.

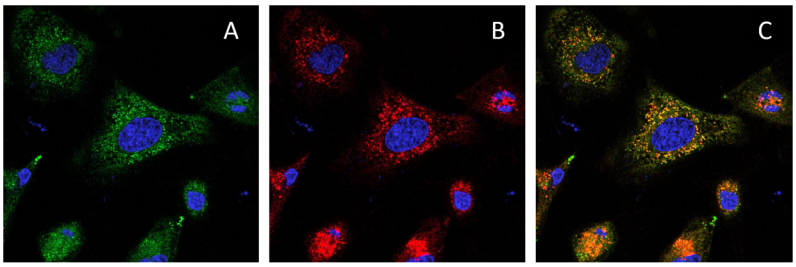


Figure 3. (**A**) Confocal fluorescence images of HeLa cells treated with BODIPY **3** and (**B**) Lysotracker Deep Red. (**C**) Merge of BODIPY fluorescence signal (green channel, λ_{ex} = 488 nm) and Lysotracker fluorescence signal (red channel, λ_{ex} = 633 nm).

Likewise, the internalization of BODIPY **2** in HeLa cells was explored. As can be seen in Figure 4A, BODIPY **2** was able to rapidly cross the cell membrane and exhibited bright and efficient fluorescence emission in the cytoplasm of cells. When cells were treated with oleic acid, prompting the formation of lipid vesicles, the accumulation of BODIPY **2** within these vesicles became evident (Figure 4B). Additionally, a similar fluorescence pattern emerged when comparing the staining of BODIPY **2** (Figure 4B) with the LipidSpot™ probe (Figure 4C). The resulting merging of these two signals created a yellow tone, signifying a precise co-localization between BODIPY **2** and lipid droplets (Figure 4D).

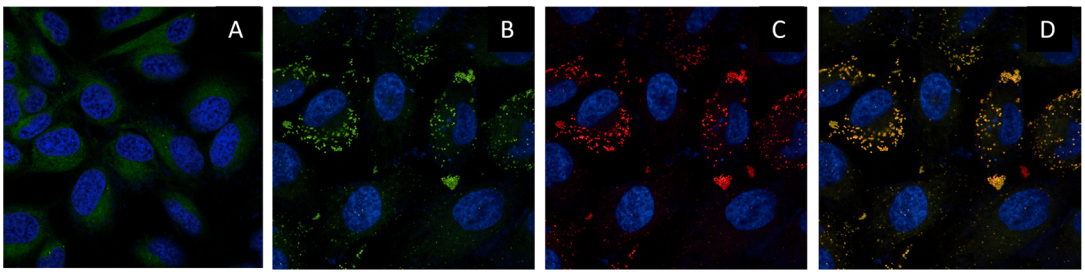


Figure 4. (**A**) Confocal fluorescence images of HeLa cells treated with BODIPY **2** in the absence of oleic acid. (**B**) Confocal fluorescence images of HeLa cells treated with BODIPY **2** and (**C**) LipidSpot™ in the presence of oleic acid. (**D**) Merge of BODIPY fluorescence signal (green channel, λ_{ex} = 488 nm) and LipidSpot™ fluorescence signal (red channel, λ_{ex} = 633 nm).

These studies demonstrated that BODIPY **3** has a high affinity towards lysosomes and BODIPY **2** towards lipid clusters. As a result, these compounds can be effectively employed as fluorescent markers for labeling these cellular structures within cells.

4. Conclusions

In summary, two *meso*-dimethylaminonaphthyl-BODIPY derivatives **2** and **3** were studied as fluorescent labels for lysosomes and lipid droplets. It was found that these BODIPY derivatives are compatible with cancer HeLa cells and can effectively penetrate their cell membranes. The subcellular localization studies demonstrated that BODIPY derivative **3** tends to accumulate in lysosomes, while BODIPY derivative **2** shows a strong affinity for lipid droplets. Consequently, these findings suggest that BODIPY derivatives **2** and **3** hold great promise as fluorescent probes for tracking lysosomes and lipid droplets within living cells.

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