CHAPTER I. INTRODUCTION

1.1 Backgound

Medical imaging plays a crucial role in healthcare, particularly in diagnosing and planning the treatment of various diseases, ranging from cardiovascular and neurodegenerative disorders to cancer and infections (Hussain et al., 2022). Chronic diseases are the leading cause of death globally, accounting for approximately 74% of all deaths worldwide, with cardiovascular diseases contributing to 17.9 million deaths and cancer to 9.3 million deaths annually (World Health Statistics, 2023). Therefore, the ability to detect diseases at an early stage and to obtain accurate visualizations of the structure and function of biological systems is crucial—not only to improve the chances of successful treatment but also to enable more targeted and effective therapies (Schlemmer et al., 2017).

Fluorescence imaging has emerged as a leading technique in the study of cellular structures and biomolecular dynamics due to its advantages of high sensitivity, detailed spatial resolution, and compatibility with biological systems without the need for additional contrast agents (Coogan & Fernández-Moreira, 2014). Technological advancements in this field have propelled the use of synthetic fluorophores as fluorescent probes for live-cell imaging, enabling the direct visualization of various biological processes such as protein trafficking, ion distribution, and intracellular pH fluctuations (Davies, Kasten, et al., 2014).

In this context, one of the primary targets in biological imaging is the mitochondrion, an organelle that plays a crucial role in energy production (ATP) through the process of oxidative phosphorylation (OXPHOS) (Srinivasan et al., 2017). Mitochondria possess a double-membrane system that generates a redox potential difference, resulting in a proton motive force (Δp), which comprises the mitochondrial membrane potential (ΔΨm) and the proton concentration gradient (ΔpH) (Yang et al., 2025). Mitochondrial dysfunction is often associated with various diseases such as cancer and neurodegenerative disorders, where an imbalance between ATP production and hydrolysis can disrupt the mitochondrial membrane potential (ΔΨm), elevate oxidative stress, and trigger further pathological conditions (Monsalves-Alvarez et al., 2020).

One of the most commonly employed strategies for visualizing dysfunctional mitochondria is the use of cationic fluorescent dyes such as MitoTrackers®, which possess a unique ability to covalently bind to mitochondrial proteins via reactive chloromethyl groups. The main advantage of MitoTrackers over other mitochondrial fluorophores lies in their capacity to remain within the mitochondria even after the loss of membrane potential (ΔΨm), making them

particularly valuable in experiments requiring dual labeling without compromising mitochondrial function (Neikirk et al., 2023). Therefore, the development of functional chemical probes capable of specifically targeting mitochondria has become a critical aspect of biomedical research

Fluorescent probes play a crucial role in detecting and labeling target biomolecules within complex biological systems by utilizing modifications of fluorescent dyes to generate specific changes in fluorescence signals (Byrne et al., 2022). This enables real-time monitoring of biomolecular localization and dynamics, which is crucial for various applications in biological imaging and sensing.

In recent years, emerging research has developed fluorescent phosphine metal complexes as dual probes for cellular imaging. This innovation combines the advantages of fluorescence with the unique properties of transition metals in interacting with biological targets, resulting in strong fluorescence signals while enhancing imaging specificity and sensitivity (Sun et al., 2024). This approach opens new avenues in bioimaging by enhancing the accuracy of biomolecular detection and expanding its potential applications in biomedical research and clinical diagnostics (Chen et al., 2020). However, it should be noted that in this approach, the incorporation of metals is typically intended to provide additional functionalities—such as radiolabeling—rather than solely to enhance fluorescence.

In recent years, research has focused on the development of fluorescent phosphine metal complexes as dual probes for cellular imaging, combining the advantages of fluorescence with the unique capabilities of transition metals in interacting with biological targets. For example, rhenium (Re) and technetium-99m (99mTc) complexes bearing $BodP_3$ ligands have demonstrated potential as multimodal probes for cancer imaging, with visualization possible via Positron Emission Tomography (PET)/Single-Photon Emission Computed Tomography (SPECT) and in vitro fluorescence reporting, respectively. The development of fluorescent phosphine metal complexes as dual probes offers an innovative approach to biological imaging, with the potential to make significant contributions to biomedical research and future clinical applications (Davies et al., 2014).

Moreover, the triphenylphosphonium cation ([TPP^+])-based approach has been widely employed in studies related to mitochondrial membrane potential ($\Delta\Psi m$) in tumors, given that cancer cells tend to exhibit a more polarized $\Delta\Psi m$ compared to healthy cells (Begum and Shen, 2023). [TPP^+]-based probes offer several advantages, including their lipophilic and cationic nature which facilitates

efficient penetration across biological membranes, high stability in physiological environments, and superior biocompatibility compared to other mitochondrial fluorophores such as Rhodamine 123 (Rh123) and Dequalinium (DQA) (Zielonka et al., 2017). The positive charge on the phosphorus center in [TPP^+] enables selective accumulation within dysfunctional mitochondria, with uptake levels hundreds of times higher than in the extracellular fluid. This makes [TPP^+]-based probes highly effective tools for imaging cancer and various other pathological conditions involving mitochondrial dysfunction (H. Wang et al., 2021).

Although [TPP^+]-based probes have proven highly effective in targeting mitochondria, their primary limitation lies in the absence of intrinsic fluorescence. To overcome this drawback, various structural modifications have been developed to enhance their functionality—one of which involves conjugating the probe with fluorophores such as boron dipyrromethene (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene), commonly known as BODIPY. BODIPY is one of the most widely used fluorophores in bioimaging due to its tunable properties, high fluorescence quantum yield, low toxicity, and remarkable photochemical stability (F.-Z. Li et al., 2022). The main advantage of BODIPY lies in its flexibility for structural modification through substitutions at various positions—such as the meso, β-, α-, and 4-F sites—which enables enhancement of fluorescence quantum yield and red-shifting of the emission spectrum via π-conjugation extension (Bumagina et al., 2022). For instance, a single substitution with an aryl group at the meso position does not significantly alter the absorption and emission profiles. However, further modification at the β-position has been shown to drastically enhance the quantum yield by restricting the free rotation of the aryl group, thereby minimizing energy loss through non-radiative relaxation processes (Orte et al., 2016).

One of the strategies employed in this study is the incorporation of a phosphonium group into the BODIPY core, resulting in BODIPY-phosphonium salts. The phosphonium group is both lipophilic and positively charged, enabling electrostatic accumulation within mitochondria due to the mitochondrial membrane potential ($\Delta\Psi m$). Therefore, BODIPY-phosphonium-based probes can be used to monitor changes in $\Delta\Psi m$, provide insight into mitochondrial conditions, and serve as carriers for bioactive molecules into cells (Zorova *et al.*,2018).

This study aims to utilize the BODIPY scaffold as a platform for the development of fluorescent tertiary phosphines. In addition, it contributes to the advancement of novel fluorescent compounds for applications in bioimaging, catalysis, and the investigation of molecular mechanisms. Thus, the results of this research are expected to make a significant contribution to the development of advanced diagnostic technologies and organophosphorus synthesis. Accordingly,

the author conducted this study under the title: **"SYNTHESIS OF FLUORESCENT PHOSPHONIUM SALTS FOR MITOCHONDRIAL IMAGING."**

1.2 Problem Identification and Formulation

Fluorescence microscopy has become a key technique in biological imaging, particularly in mitochondrial studies. However, current probes such as [TPP+] exhibit limitations, including the lack of intrinsic fluorescence and the need for dual-modal probes suitable for both in vitro and in vivo imaging. Based on the background outlined above, the research problems addressed in this study are:

1. How can a novel fluorescent tertiary phosphine based on BODIPY be synthesized?
2. How can the resulting fluorescent phosphonium salts be characterized, and what is their potential in biological imaging applications?

1.3 Research Objectives

The objectives of this research are as follows:

1. Synthesizing and characterizing fluorescent phosphonium salts based on BODIPY as dual-modal probes for cellular imaging.
2. To analyze the fluorescent properties of the synthesized phosponium salt and evaluate their potential for biological imaging applications.

1.4 Research Significance

The primary significance of this research includes:

1. Contributing to the development of novel fluorescent probes that enhance the effectiveness of biological imaging, particularly for mitochondrial targeting.
2. Providing characterization data that serve as a foundation for the optimized design of fluorescent probes based on phosponium salt for bioimaging applications.
3. Supporting further research in the development of more accurate and specific diagnostic technologies, particularly for early-stage disease detection through cellular imaging.