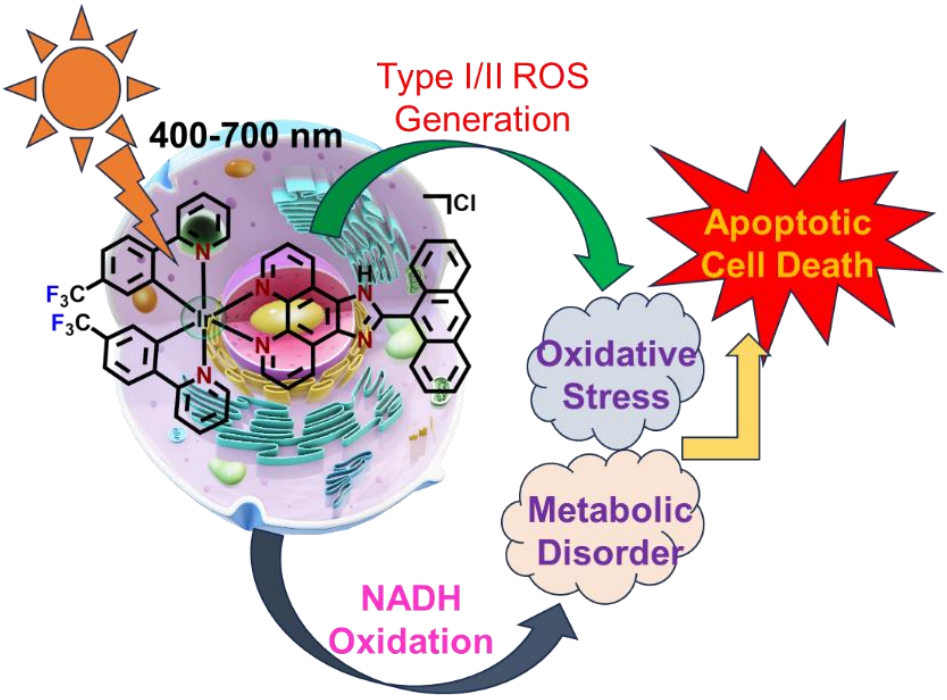
Chapter V: Summary, Conclusion, and Future Directions

This thesis aims to develop novel Ir(III)-based photocatalysts capable of efficiently oxidizing NADH under biologically relevant conditions, while addressing the limitations of light absorption and catalytic efficiency. A key strategy involves tuning the photophysical properties of the photocatalysts by rational ligand design to red-shift the absorption maxima into the green-light region, thereby enhancing tissue penetration. Coumarin 6, a fluorescent ligand with strong absorption in the green-light region, was employed to impart red-shifted absorption and imaging capabilities to the photocatalysts. Additional polypyridyl and phenanthroline-based ligands were incorporated in the photocatalysts to enhance photocatalytic activity and light responsiveness further. The research also explores the correlation between in-solution and intracellular NADH to NAD^+ photo-conversion, providing mechanistic insights into photocatalytic cytotoxicity. The fluorescent properties of the designed photocatalysts enable cellular imaging, allowing investigation of their uptake and subcellular localization. Overall, this work advances the field of photocatalytic cancer therapy by introducing Ir(III) photocatalysts with enhanced catalytic performance and improved tissue-penetrating ability. The findings contribute to developing oxygen-independent anticancer strategies with significant potential for treating hypoxic tumors.

Chapter I of this thesis provides a comprehensive overview of cancer as a global health crisis, highlighting its increasing incidence and mortality rates worldwide. The chapter introduces various existing treatment modalities, including surgery, radiotherapy, immunotherapy, and chemotherapy. Then it narrows its focus to the role of organic and transition metal-based chemotherapeutic agents in current clinical practice. Among these, platinum-based drugs have been pivotal in cancer therapy, primarily targeting DNA to induce

cancer cell death. A concise discussion on the modes of interaction between cisplatin and DNA and its mechanistic aspects is presented to elucidate how such agents function at the molecular level. Despite their clinical success, FDA-approved chemotherapeutic drugs, particularly platinum-based agents, suffer from significant drawbacks, including non-selectivity, severe systemic toxicity, and the development of acquired drug resistance. These limitations underscore the urgent need for new therapeutic strategies that are both effective and selective, with minimal side effects. The chapter then discusses photodynamic therapy (PDT), a non-invasive light-activated treatment modality. PDT requires the presence of a photosensitizer, molecular oxygen, and an appropriate light source to generate cytotoxic reactive oxygen species (ROS). The progress in developing metal-based PDT agents is outlined with examples from recent literature. However, PDT's effectiveness is hindered by its dependence on oxygen, making it less effective in hypoxic tumor environments. The chapter introduces photocatalytic therapy (PCT) as a promising alternative to address these limitations. Unlike PDT, PCT operates through oxygen-independent pathways, primarily via the photocatalytic oxidation of biologically relevant molecules like NADH. The fundamental mechanism of PCT, its therapeutic advantages, and its ability to overcome PDT-associated shortcomings are discussed in detail. The recent advancements in the development of metal-based PCT agents are also reviewed, specifically focusing on Ir(III) photocatalysts due to their favorable photophysical and photocatalytic properties. Since this thesis centers around Ir(III)-based photocatalysts, a focused discussion is presented on the evolution, design, and various cell death mechanisms of such photocatalysts. The chapter also highlighted how

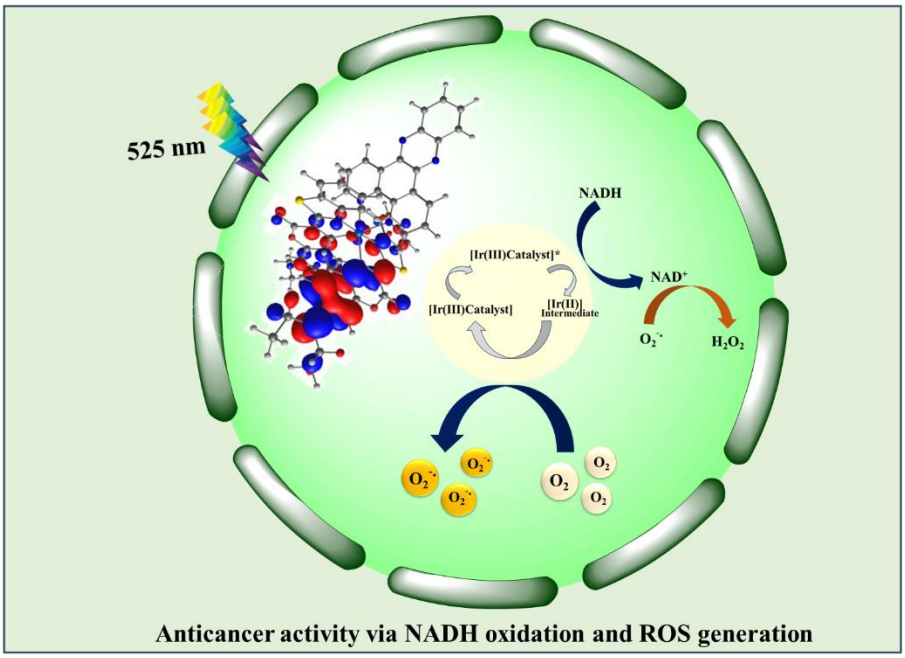
ligand tuning influences the photophysical behavior and catalytic efficiency of these Ir (III) based photocatalysts. The rationale behind choosing Ir(III) as the metal core is also explained, highlighting its long excited-state lifetimes, photostability, and high singlet oxygen quantum yields. Additionally, organic ligands such as coumarin derivatives, phenanthroline, and polypyridyl systems are discussed in the context of enhancing light absorption, catalytic activity, and cell-targeting capabilities. The chapter concludes by outlining the primary objectives of the research: to design and develop novel Ir(III) photocatalysts with red-shifted absorption, improved NADH oxidation activity, and effective intracellular performance, ultimately aimed at developing potent and selective agents for oxygen-independent cancer therapy.



Chapter II of this thesis presents the synthesis and characterization of three novel cyclometalated Ir(III) photocatalysts, (**1**-**3**), *viz.* [Ir(C^N)$_2$(ip)]Cl (**1**), [Ir(C^N)$_2$(ph-ip)]Cl (**2**) and [Ir(C^N)$_2$(aip)]Cl (**3**) (C^N= 2-(4-

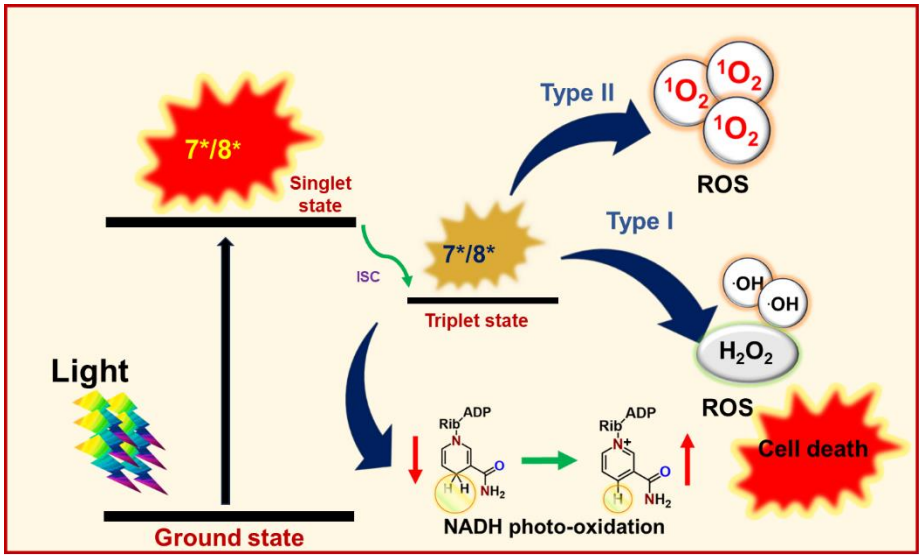
(Triflurомethyal)phenyl)pyridine; ip = 1H-imidazo[4,5-f][1,10]phenanthroline; ph-ip = 2-phenyl-1H-imidazo[4,5-f][1,10]phenanthroline; aip = 2-(anthracene-9-yl)-1H-imidazo[4,5-f][1,10]phenanthroline). These photocatalysts exhibited absorption in the 350-480 nm range, making them suitable candidates for visible-light-mediated photocatalytic cancer therapy. Under visible-light (400-700 nm, 5-10 J cm^{-2}) exposure in a DMSO:PBS (1:99 v/v) solvent system, all three photocatalysts demonstrated high efficiency in facilitating NADH oxidation, attaining turnover frequencies (TOFs) in the range of 499.1±26.5 to 697.4±26.4 h^{-1}, exceeding the performance of most of the previously reported Ir(III)-based photocatalysts. Mechanistic studies verified the involvement of type I and type II pathways in ROS generation. Cytotoxicity studies highlighted significant photocytotoxic effects of **1-3** in human lung adenocarcinoma cells (A549), with **3** emerging as the most potent under light exposure (400-700 nm, 10 J cm^{-2}). Additionally, the negligible dark and light cytotoxicity of **3** against human embryonic kidney cells (HEK-293) demonstrated the initial safety profile of **3**. Furthermore, the mechanistic studies in A549 cells revealed that **3** promoted mitochondrial membrane depolarization and activated caspase-3/7-dependent apoptotic pathways through light-triggered ROS generation and NADH oxidation. These findings highlight **3** as a potent dual-action cancer phototherapeutic, capable of synergistic type-I and type-II anticancer activities, and efficient NADH photo-oxidation. This work presents a promising platform for developing multifunctional photocatalytic agents in cancer therapy. This part of the work has appeared in *Chem. Asian J.* **2025**, *e00681*. DOI: https://doi.org/10.1002/asia.202500681.

Chapter III of this thesis present a series of three novel coumarin 6-based Ir(III) photo-catalysts *viz.*, [Ir(CO6)$_2$(phen)]Cl **(4),** [Ir(CO6)$_2$(dppz)]Cl **(5)**, and [Ir(CO6)$_2$(aip)]Cl **(6)**, where, CO6 = coumarin 6, phen = 1,10-phenanthroline, dppz = dipyrido[3,2-a:2',3'-c]phenazine, and aip = 2-(anthracen-9-yl)-1H-imidazo[4,5-f][1,10]phenanthroline. Coumarin 6 introduced a green light absorption property. When exposed to green light (525 nm, 50.2 J cm^{-2}), **4-6** demonstrated catalytic NADH photo-oxidation with TOF *ca.* 840-1003 h^{-1} in PBS, comparable or higher to the highest achieved TOF with previously reported Ir(III)-based photo-catalysts for NADH oxidation. **4-6** also generated $^1O_2$ on green light (525 nm, 50.2 J cm^{-2}) irradiation. These photocatalyst showed photo-toxicities against A549 and human cervical cancer cell line (HeLa) cells with the IC$_{50}$ and PIs (Dark IC$_{50}$/Light IC$_{50}$) in the range of 0.5-1.6 μM and > 28-71, respectively. Interestingly, these photo-catalysts showed no notable toxicities toward normal human bronchial epithelial (BEAS-2B) cell lines in the dark. The selectivity index (SI = Light IC$_{50}$



in normal cells /Light IC_{50} in cancer cells) of **4-6** for cancer cell killing was in the range of 7-19. Moreover, photocatalysts **5** and **6** showed good phototoxicity against A549 cancerous cells under hypoxic conditions (1% O_2), revealing their potential for hypoxic tumor treatment. The mechanistic investigation of the most potent photocatalyst **6** in A549 cells revealed that it localized in the mitochondria, generated ROS, and oxidized NADH during light exposure, ultimately causing cell apoptosis via mitochondrial depolarization. This strategy of killing cancer cells by combining type-I and type-II mechanisms, *i.e.*, via NADH oxidation, H_2O_2, $^{\bullet}OH$, and $^1O_2$ generation, could provide efficient photo-activated chemotherapy. This part of the work has appeared in *Inorg. Chem. Front.* ***2024****, 11, 5435-5448* (**Highlighted as journal Front Cover Page**).

Chapter IV of this thesis present synthesis, characterization and biological evaluation of two novel coumarin 6 conjugated Ir(III) photocatalysts, [Ir(CO6)(Ph-tpy)Cl]Cl (**7**) and [Ir(CO6)(An-tpy)Cl]Cl (**8**)



(CO6 = Coumarin 6, Ph-tpy = 4'-phenyl-2,2':6',2"-terpyridine, An-tpy = 4'-anthracenyl-2,2':6',2"-terpyridine), for application in cancer therapy. Upon green light irradiation (525

nm, 50.2 J cm^{-2}), **7** and **8** effectively catalyzed NADH oxidation with turnover frequencies (TOFs) ranging from 840 to 1100 h^{-1} in phosphate-buffered saline. These photo-catalysts also generated reactive oxygen species (ROS), including $^1O_2$ and $^{\bullet}OH$, through type I and II mechanisms. **7** and **8** exhibited significant toxicity against human breast (MCF-7) and cervical (HeLa) cancer cells, with **8** demonstrating enhanced anticancer activity upon light activation. Notably, both photo-catalysts showed minimal dark toxicity toward non-cancerous human embryonic kidney (HEK-293) cells. The selectivity index (SI) for **7** and **8** reached up to 22, highlighting their preferential activity in cancer cells. Mechanistic studies in MCF-7 cells with the most effective photocatalyst, **8**, revealed that light exposure increased ROS production and induced mitochondrial depolarization and apoptosis *via* caspase 3/7 activation. This part of the work has appeared in *Inorg. Chem. Commun.* **2025**, *175*, 114184.

This thesis presents a detailed study on novel cyclometalated Ir(III) photocatalysts with red-shifted absorption and enhanced photocatalytic properties for cancer phototherapy. The primary aim was to shift absorption into the green light region and use their photocatalytic efficiency for ROS generation (type I & II) and NADH oxidation under both normoxic and hypoxic conditions.

Conclusion and Future Direction

Photocatalysts **1**-**3** exhibited improved visible-light-driven (400-700 nm, 10 J cm^{-2}) catalytic NADH oxidation (TOF up to *ca*. 697 h^{-1}). Among **1**-**3**, **3** with higher conjugation showed superior photocytotoxicity against A549 cells (IC_{50} *ca*. 0.8 μM) with low toxicity toward

normal HEK-293 cells (IC_{50} = 23.4 μM and >25.0 μM under light and dark, respectively) (**Chapter II**). Incorporation of coumarin 6 in photocatalysts **4**-**6** shifted the absorption to the green region, and further improved the photocatalytic activity for NADH oxidation (TOFs up to *ca*. 1003.5±24.6 h^{-1}). Photocatalyst **6** exhibited photoinduced (400-700 nm, 10 J cm^{-2}) NADH oxidation potential inside the A549 cancerous cell with excellent photocytotoxicity (400-700 nm, 10 J cm^{-2}) in both normoxic (IC_{50} *ca*. 0.6 μM) and hypoxic conditions (1% O_2) (IC_{50} *ca*. 1.7 μM) while remained nontoxic against Beas2B healthy cells (IC_{50} = >50.0 μM) (**Chapter III**). The incorporation of terpyridine ligands in photocatalysts **7**/**8** further improved photocatalytic NADH oxidation performance, even achieving the highest TOF reported till now (TOFs up to 1084.6±39.4 h^{-1}). This incorporation not only improved NADH photocatalytic activity but also significantly improved the photocytotoxicity of **8** against the MCF-7 cancerous cell line (IC_{50} = 0.3 μM). **8** induced intracellular NADH oxidation and caspase 3/7 activation-mediated apoptotic cell death (**Chapter IV**). Overall, these Ir(III) based photocatalysts showed potential as promising candidates for oxygen-dependent as well as oxygen-independent cancer therapy.

Looking forward, in order to enhance the therapeutic potential for deeply buried tumors, future studies could focus on red-shifting the absorption profile toward the red and near-infrared light region through rational ligand framework design. Further, tumor-targeting moieties (such as glucose, biotin, folic acid, and vitamin B6) may be attached to enhance the cancer cell selectivity and cellular uptake of the photocatalysts. *In vivo* studies will be crucial to validate the therapeutic potential and biocompatibility of these photocatalysts.

Additionally, exploring alternative intracellular electron acceptors under hypoxic conditions other than Fe^{3+}–cytochrome c could help to increase the understanding of the effectiveness of photocatalytic cancer therapy in oxygen-deprived tumors. Exploration of combined treatment strategies (e.g., with ultrasound or immunotherapy) could advance these systems toward clinical application.