Development of Ir(III)-Based Photocatalysts for NADH Oxidation and ROS-Mediated Cancer Phototherapy

Thesis submitted in partial fulfilment for the

Award of Degree

Doctor of Philosophy

By

Ashish Kumar Yadav

DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI-221005
INDIA

Roll No. 21051007

2025

Dedicated to my

loving

Parents…

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I, ***Mr. Ashish Kumar Yadav***, certify that the work embodied in this thesis is my own bona fide work and carried out by me under the supervision of **Dr. Samya Banerjee** from July 2021 to June 2025, at the ***Department of Chemistry***, ***Indian Institute of Technology (BHU), Varanasi***. The matter embodied in this thesis has not been submitted for any other degree/diploma award. I declare that I have faithfully acknowledged and given credit to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not willfully copied any other's work, paragraphs, text, data, results, etc., reported in journals, books, magazines, reports, dissertations, theses, etc., or available at websites and have not included them in this thesis and have not cited those as my work.

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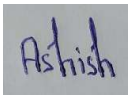

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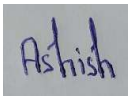

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(Mr. Ashish Kumar Yadav)
Research Scholar

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List of Abbreviations

μM	Micro Molar
$^1O_2$	Singlet Oxygen
2D	2-Dimensional
3D	3-Dimensional
$^3O_2$	Triplet Oxygen
5-ALA	5-Aminolevulinic acid
A431	Epidermoid Carcinoma Cell line
A549	Lung Cancer Cell
Abs	Absorbance
aip	2-(Anthracene-9-yl)-1H-imidazo[4,5-f][1,10]phenanthroline
An-tpy	4'-Anthracenyl-2,2':6',2''-terpyridine
AO	Acridine Orange
aq.	Aqueous
ATP	Adenosine Triphosphate
BEAS-2B	Normal Human Bronchial Epithelial
BN	Bombesin
ca.	Approximately
Calcd.	Calculated

CCCP	Carbonyl Cyanide m-chlorophenyl Hydrazone
$CHCl_3$	Chloroform
CNE-2Z	Human Nasopharyngeal Carcinoma
CO6	Coumarin 6
CPCM	Conductor-like Polarizable Continuum Model
DCF	2′,7′-Dichlorofluorescein
DCFH-DA	2′,7′-Dichlorodihydrofluorescein diacetate
DCM	Dichloromethane
DFT	Density Functional Theory
DMEM	Dulbecco's Modified Eagle Medium
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DPBF	Diphenylisobenzofuran
DPBS	Dulbecco's Phosphate Buffered Saline
dppz	Dipyrido[3,2-a:2',3'-c]phenazine
EB	Ethidium Bromide
EDTA	Ethylenediaminetetraacetic Acid
EPR	Electron Paramagnetic Resonance

Equiv.	Equivalent
ESI-MS	Electrospray ionization mass spectrometry
et al.	And Others
Etc.	Et cetera
ETC	Electron Transport Chain
FACS	Fluorescence Activated Cell Sorting
FDA	Food and Drug Administration
Fe^{3+}–cyt c	Fe^{3+}–cytochrome c
FBS	Fetal Bovine Serum
FMO	Frontier Molecular Orbitol
GRPR	Gastrin-Releasing Peptide Receptor
GSH	Glutathione
H_2O	Water
H_2O_2	Hydrogen Peroxide
HEK-293	Human Embryonic Kidney Cell Line
HeLa	Cervical Cancer Cell Line
HepG2	Liver Cancer Cell Line
HMGB1	High Mobility Group Box 1 Protein
HOMO	Highest Occupied Molecular Orbital
HPLC	High-Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry

HT-1197	Bladder Cancer Cell
ip	1H-imidazo[4,5-f][1,10]phenanthroline
$IrCl_3 \cdot xH_2O$	Iridium(III) Chloride Hydrate
ISC	Inter System Crossing
J	Coupling Constant
J/cm^2	Joule/square centimeter
JC-1	5,5',6,6'-Tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide
LO2	Human Hepatocyte Cell Line
log*P*	lipophilicity
LUMO	Lowest Unoccupied Molecular Orbital
MB	Methylene Blue
MCF-7	Breast Cancer Cell Line
MDR	Multidrug Resistance
MeOH	Methanol
MHz	Mega Hertz
MLCT	Metal-to-Ligand Charge Transfer
MMP	Mitochondrial Membrane Potential
MRC-5	Human Lung Fibroblasts
MTR	Mitotracker Red

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Molecular Weight
NADH	Nicotinamide Adenine Dinucleotide
NCI-H460	Lung Cancer Cell Line
NER	Nucleotide Excision Repair
NMR	Nuclear Magnetic Resonance
ns	Nanosecond
$O_2^{-\bullet}$	Super Oxide Radical anion
$^{\cdot}OH$	Hydroxyl Radical
Obtd.	Obtained
PACT	Photoactivated Chemotherapy
PBS	Phosphate Buffer Saline
PC3	Prostate Cancer Cell Line
PCC	Pearson Correlation Coefficient
PCET	Proton-Coupled Electron Transfer
PCT	Photocatalytic Cancer Therapy
PDT	Photodynamic Therapy
phen	1,10-Phenanthroline
phip	2-Phenyl-1H-imidazo[4,5 f][1,10]phenanthroline

List of Abbreviations

Ph-tpy	4'-Phenyl-2,2':6',2"-terpyridine
PI	Photo Therapeutic Index
PI	Propidium Iodide
PS	Photosensitizer
Pt	Platinum
Pvt. Ltd	Private Limited
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
rpm	Revolutions Per Minute
S_0	Ground Singlet State
S_1	Excited Singlet State
SAR	Structure Activity Relationship
SCE	Saturated Calomel Reference Electrode
SC-ICP-MS	Single-Cell Inductively Coupled Plasma Mass Spectrometry
SD	Standard Deviation
SGC-7901	Gastric Cancer Cell
SI	Selectivity Index
SK-OV-3	Human Ovarian Cancer Cell
SRB	Sulforhodamine B
T_1	Triplet Excited State

TCA	Trichloroacetic Acid
TD-DFT	Time-Dependent Density Functional Theory
tert-butanol	Tertiary Butanol
TLC	Thin-Layer Chromatography
TOF	Turnover Frequency
TON	Turnover Number
UV-Vis.	UV-Visible
viz.	Namely
WHO	World Health Organization
WST09	Padoporfin
γ-rays	Gama Rays
δ	Chemical Shift
$\Delta\Psi_m$	Mitochondrial Membrane Potential
ε	Molar Absorptivity Coefficient
λ_{em}	Emission Wavelength
λ_{ex}	Excitation Wavelength
τ	Excited State Lifetime

Preface

This thesis advances the photocatalytic cancer therapy field by introducing the different series of Ir(III) photocatalysts with enhanced NADH-oxidation photocatalytic 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. The chapter introduces various existing treatment modalities with their limitations. Finally, this chapter introduces the concept of photocatalytic cancer therapy (PCT) as a promising alternative to address these limitations. As this thesis is mainly based on Ir(III) based photocatalysts, this chapter gives a comprehensive overview of previously reported Ir(III) based photocatalysts, with a main focus on the reason behind their design and a detailed explanation of the anticancer mechanism. 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, characterization, in-solution light-induced NADH oxidation and ROS generation, photocytotoxicity in cancer cells, with a detailed study of the mechanism of cell death of three novel 2-phenyl pyridine appended phenanthroline-based Ir(III) photocatalysts.

Chapter III presents the synthesis, characterization, in-solution as well as in-cancer-cell photoinduced NADH oxidation, visible light assisted toxicity under both normoxic and

hypoxic conditions in different cancer cells, mode of photo-induced cell death, and cellular localization of three novel coumarin 6 appended phenanthroline based Ir(III) photocatalysts. The main objectives of this work are (i) to red shift the absorption wavelength of Ir(III) photocatalyst mainly in the green region, (ii) to enhance the photocatalytic performance, and (iii) to translate the light-induced in-solution NADH oxidation chemistry to inside the cancerous cell.

Chapter IV presents the synthesis, characterization, in-solution as well as in-cell photoinduced NADH oxidation, visible light assisted toxicity in different cancer cells and normal cells, mode of photo-induced cell death, and cellular localization of two novel coumarin 6 appended terpyridine based Ir(III) photocatalysts. The main objectives of this work are (i) to further enhance the photocatalytic performance, and (iii) to generalize the light-induced in-solution NADH oxidation chemistry in the presence of GSH.

Finally, **Chapter V** concludes the progress made in each chapter and provides future directions for the development of potential photocatalysts for photocatalytic cancer therapy. The references in the text have been indicated as superscript numbers and assembled at the end of each chapter. The photocatalysts presented in this thesis are represented by bold-faced numbers. Crystallographic data of the photocatalyst, which is characterized structurally by single-crystal X-ray crystallography, are provided in CIF format in the enclosed CD (Appendix I). Due acknowledgements have been made wherever the work described is based on the findings of other investigators. Any omission that might have happened due to oversight or mistake is regretted.

INDEX WORDS: Ir(III) photocatalyst · Crystal structure · High Turnover Frequency· In-cell NADH oxidation · Hypoxia active · Cellular Imaging. Apoptotic photocytotoxicity.

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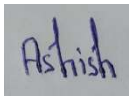

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