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# Towards Dual and Targeted Cancer Therapy with Novel Phthalocyanine-based Photosensitizers

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# Towards Dual and Targeted Cancer Therapy with Novel Phthalocyanine-based Photosensitizers

Doctoral Thesis accepted by
The Chinese University of Hong Kong, Shatin,
People's Republic of China

Springer

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"A Disulfide-Linked Conjugate of Ferrocenyl Chalcone and Silicon(IV) Phthalocyanine as an Activatable Photosenzitiser"

Janet T. F. Lau, Xiong-Jie Jiang, Dennis K. P. Ng,* and Pui-Chi Lo*

*Chemical Communications* **2013**, *49*, 4274–4276.

*This Thesis is Dedicated to*
*Prof. Dennis K. P. Ng, Prof. Gigi P. C. Lo,*
*My Family, George, and Lily*

*In Memory of My Grandfather*
*Mr. Ping Wu*
*1926–2012*

# Supervisor's Foreword

Light has long been used for medicinal purposes. By combining the action of this source of energy, a photosensitive drug and molecular oxygen, a new therapeutic modality, namely photodynamic therapy (PDT), has been developed. This can be used for the treatment of some localized and superficial cancers, as well as certain non-cancerous conditions. Compared with the traditional therapies in oncology, photodynamic therapy is relatively non-invasive and has fewer side effects, higher tolerance of repeated doses, and higher specificity that can be achieved through precise delivery of light with modern fiber optic systems and various types of endoscopy. Although positive therapeutic outcomes have been reported, only a few photosensitive drugs have been clinically approved so far and they still suffer from a number of deficiencies, such as weak absorption in the tissue-penetrating near-infrared region, sustained skin photosensitivity, low initial selectivity, and long drug-to-light intervals. As a result, considerable efforts have been expended in the development of more efficient photosensitizers toward targeted PDT and that can conjugate with chemotherapeutic agents to achieve dual cancer therapy. This multi-disciplinary field of study has received much current attention.

This thesis describes a series of novel phthalocyanine-based photosensitizers, including their molecular design, synthesis, spectroscopic and photophysical properties, in vitro photodynamic activity, and potential of being used for combined chemo- and photodynamic therapy and targeted PDT. After a general overview of the background and current status in this research area given in Chap. 1, the details of the studies are presented in the following chapters for conjugates with a chemotherapeutic oxaliplatin derivative, a polyamine ligand with a view to targeting the polyamine transporters over-expressed in tumor cells, and a ferrocene-based quencher that can inhibit their photodynamic activity, yet can be removed under a tumor-associated environment, such as low pH and high thiol concentration, thereby restoring their photocytotoxicity. A dual activatable photosensitizer has also been reported for the first time. The activation effects have been well demonstrated both in solution and at the cellular level. The studies

reported in the thesis are original and significant, and can stimulate further investigations in this important research field. We imagine that "smarter" photosensitizers that can respond selectively at tumors could be developed in the near future, which could advance further this promising treatment modality.

Hong Kong, May 2013 Dennis K. P. Ng

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Lastly, I offer my regards and blessings to all of those who supported me in any respect during my study.

# Contents

# Abbreviations

## General

| | |
|---|---|
| Ac | Acetyl |
| Anal. | Analytical |
| Ar | Aromatic |
| Boc | *tert*-Butoxycarbonyl |
| *ca.* | Coarse approximation |
| calcd | Calculated |
| conc. | Concentrated |
| equiv. | Equivalent |
| Et | Ethyl |
| Fc | Ferrocenyl |
| $IC_{50}$ | Dye concentration required to kill 50 % of the cells |
| Me | Methyl |
| Ms | Methanesulfonyl |
| n.d. | Not determined |
| NIR | Near-infrared |
| Pc(s) | Phthalocyanine(s) |
| PDT | Photodynamic therapy |
| PET | Photoinduced electron transfer |
| Ph | Phenyl |
| r.t. | Room temperature |
| $R_f$ | Retention factor |
| ROS | Reactive oxygen species |
| S.D. | Standard deviation |
| S.E.M. | Standard error of the mean |
| THP | Tetrahydropyranyl |
| TLC | Thin layer chromatography |
| Ts | Tosyl |
| UV | Ultraviolet |
| Vis | Visible |
| v/v | Volume-to-volume ratio |

| | |
|---|---|
| w/v | Weight per volume |
| w/w | Weight-to-weight ratio |

## Units of Measurement

| | |
|---|---|
| cm | Centimeter(s) |
| °C | Degree Celsius |
| g | Gram(s) |
| h | Hour(s) |
| Hz | Hertz |
| J | Joule |
| kJ | Kilojoule |
| M | Molarity |
| mg | Milligram(s) |
| MHz | Megahertz |
| min | Minute(s) |
| mL | Milliliter(s) |
| μm | Micromolar |
| μm | Micrometer(s) |
| μL | Microliter(s) |
| μs | Microsecond(s) |
| mm | Millimeter(s) |
| mM | Millimolar |
| mol | Mole(s) |
| mmol | Millimole(s) |
| mW | Milliwatt |
| nm | Nanometer(s) |
| nM | Nanomolar |
| rpm | Revolutions per minute |
| s | Second(s) |
| W | Watt |

## Chemicals and Solvents

| | |
|---|---|
| DBU | 1,8-Diazabicyclo[5.4.0]undec-7ene |
| DCC | *N*,*N*′-Dicyclohexylcarbodiimide |
| DCFDA | 2',7′-Dichlorodihydrofluorescein diacetate |
| DMAP | 4-(Dimethylamino)pyridine |
| DMEM | Dulbecco's modified Eagle's medium |
| DMF | *N*,*N*-Dimethylformamide |
| DMSO | Dimethylsulfoxide |
| DPBF | 1,3-Diphenylisobenzofuran |
| DTT | Dithiothreitol |
| EDTA | Ethylenediaminetetraacetic acid |

| | |
|---|---|
| GFP | Green fluorescent protein |
| HEPES | 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid |
| HOBt | 1-Hydroxybenzotriazole |
| MTT | 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide |
| PBS | Phosphate buffered saline |
| PI | Propidium iodide |
| RPMI | Roswell Park Memorial Institute |
| TFA | Trifluoroacetic acid |
| THF | Tetrahydrofuran |
| SDS | Sodium dodecyl sulfate |
| ZnPc | Unsubstituted zinc(II) phthalocyanine |

## Photophysical Data

| | |
|---|---|
| $\lambda$ | Wavelength in nm |
| $\lambda_{max}$ | Absorption maximum |
| $\lambda_{em}$ | Emission maximum |
| $\lambda_{ex}$ | Excitation wavelength |
| $\varepsilon$ | Molar extinction coefficient |
| $\Phi_F$ | Fluorescence quantum yield |
| $^1O_2$ | Singlet oxygen |
| $\Phi_\Delta$ | Singlet oxygen quantum yield |

## Nuclear Magnetic Resonance (NMR) Data

| | |
|---|---|
| COSY | Correlation spectroscopy |
| $\{^1H\}$ | Proton decouple |
| $\delta$ | Chemical shift in ppm |
| H | Proton |
| C | Carbon |
| $J$ | Coupling constant in Hz |
| ppm | Parts per million |
| s | Singlet |
| d | Doublet |
| t | Triplet |
| q | Quartet |
| p | Pentat |
| vt | Virtual triplet |
| dd | Doublet of doublet |
| dq | Doublet of quartet |
| m | Multiplet |
| br s | Broad signal |

## Mass Spectrometric (MS) Data

| | |
|---|---|
| $M^+$ | Molecular ion |
| *m/z* | Mass-to-charge ratio |
| ESI | Electrospray ionization |
| HRMS | High-resolution mass spectroscopy |

# Chapter 1
# Introduction

## 1.1 Introduction

Photodynamic therapy (PDT) involves the administration of a non-toxic drug known as photosensitizer systemically, locally, or topically to a patient bearing a lesion, which is frequently, but not always cancer [1, 2]. After an incubation period, the lesion is illuminated by red visible light (620–690 nm). In the presence of oxygen, it leads to the generation of cytotoxic reactive oxygen species (ROS) and consequently to cell death and tissue destruction [3, 4]. The use of PDT as a cancer therapy is particularly attractive because of its potential specificity. This is due to the fact that the photosensitizer can localize in the malignant tissue. When the light is directly focused on the lesion, the ROS that generated during photosensitization results in cellular destruction in that particular region. In recent years, PDT has become a subject of intense investigation as a possible treatment modality for various forms of cancer [4–6].

### *1.1.1 History of Photodynamic Therapy*

The use of light in the treatment of disease can be traced back over 4,000 years [7–9]. Ancient Egyptian, Indian, and Chinese civilizations used light to treat various diseases including psoriasis, rickets, vitiligo, and skin cancer [10]. Reports of contemporary PDT first appeared during the investigations led by Finsen in the late nineteenth century [11]. He found that exposure of red light can prevent the formation and discharge of smallpox pustules [11]. He also successfully demonstrated phototherapy by employing heat-filtered light from a carbon-arc lamp in the treatment of cutaneous tuberculosis, for which he won the Nobel Prize in Physiology and Medicine in 1903 [12]. In 1900, Raab, a German medical student discovered that a combination of acridine red and light can kill a species of *paramecium* [13]. He reported that the combined cytotoxic effect was greater than that of the individual components. In the same year, Prime, a French neurologist,

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