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



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Janet T. F. Lau

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

Doctoral Thesis accepted by
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ISSN 2190-5053

ISBN 978-3-319-00707-6

DOI 10.1007/978-3-319-00708-3

Springer Cham Heidelberg New York Dordrecht London

ISSN 2190-5061 (electronic)

ISBN 978-3-319-00708-3 (eBook)

Library of Congress Control Number: 2013940440

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Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Parts of this thesis have been published in the following journal articles:

“A Zinc(II) Phthalocyanine Conjugated with an Oxaliplatin Derivative for Dual Chemo- and Photodynamic Therapy.”

Janet T. F. Lau, Pui-Chi Lo,^{*} Wing-Ping Fong, and Dennis K. P. Ng

Journal of Medicinal Chemistry **2012**, 55, 5446–5454.

“A Disulfide-Linked Conjugate of Ferrocenyl Chalcone and Silicon(IV) Phthalocyanine as an Activatable Photosensitizer”

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

Acknowledgments

Professor Dennis K. P. Ng, my supervisor, deserves the utmost gratitude for admitting me as one of his postgraduate students, which is the greatest honor in my life. Throughout my postgraduate studies, not only does he impart a host of chemistry knowledge to me, he also spends lots of effort on nourishing and cultivating me with the ability of critical thinking, a proactive and responsible attitude toward my work, and the qualities to be a good scientific researcher. His genuine and refined teaching has paved a concrete foundation toward my future development. I am extremely thankful to him for giving me ample opportunities to participate in international conferences, seminars, and manuscripts preparation. Without his continuous tolerance, trust, and inspiration, I may not be confident enough to overcome obstacles encountered during my research. To him, I owe my heartfelt thanks.

Sincere thanks should also go to Prof. Gigi P. C. Lo, my co-supervisor, for her willingness to entrust so much on my judgment toward my research. She is certainly a heavyweight throughout my five-year postgraduate research life. I probably would not be writing my doctoral dissertation if she did not introduce me to Prof. Ng's lab seven years ago. I would like to express my deepest gratitude to her for her patience, selfless teaching, and guidance throughout my study. Apart from sharing her valuable experience in her field of expertise with me, she has also established a harmonious atmosphere in our lab through frequent communication and participating in different gatherings and functions with us. She is a fine and delicate supervisor who always tries her very best to take care and support every one of us in every way she can.

Being in Prof. Ng's lab for five years, I am delighted to be able to befriend with many colleagues with various cultural and research background. Every one of them has played a very important role during my research. I am indebted to Dr. Xue-Bing Leng, Dr. Ming Bai, Dr. Bill C. F. Choi, Dr. Hu Xu, Dr. Jian-Yong Liu, Dr. Xiong-Jie Jiang, Dr. Hui He, Dr. Qun-Ling Fang, Venus Y. S. Huang, Mei-Rong Ke, Wen-Jing Shi, Liang Qu, and Esther S. L. Yeung. It is also my great honor to be able to associate with four summer research students, Cheok-Lam Wong, Chi-Him Lai, Kin-Lung Chui, and Yun-Sang Chow. I am grateful to them for bringing lots of fun and cutie little gadgets to our lab.

Particular acknowledgements are to be addressed to Prof. Wing-Ping Fong of the School of Life Sciences for allowing me to access his apparatuses, instruments, and other necessary reagents for all my *in vitro* studies. My research would not have been possible without his persistent and generous support. I would also like to thank Pui-Wah Choi, Chi-Lung Chan, and Victor H. Y. Yeung for teaching and sharing their experience with me in performing cell culture and various kinds of biochemical assays. It is my pleasure to be able to work with these excellent biochemists!

I would also like to express my deepest gratitude to Prof. Chi Wu for giving me his greatest support in my *in vitro* studies especially when the cell culture facilities with the School of Life Sciences are temporarily suspended. I would have never completed my biochemical assays without his understanding and kind offer. I would also like to thank Zhuo-Jun Dai, Jin-Ge Cai, Qian-Jin Chen, Shu Diao, Xiang-Jun Gong, Yong-Zheng Ma, Jian-Qi Wang, Yanan Yue, and Hong Zhao for their patience, assistance, and teachings.

I am heartily thankful to Prof. Hung-Kay Lee for giving me lots of advice toward my future career. I am exceptionally obliged to him for granting me the opportunity to work in the Hong Kong Government Laboratory. Not only do I gain hands-on experience in operating a vast number of analytical instruments, he has also indirectly brought me to Dr. Boris Y. T. Wong, Miss Twinnie S. C. Tso, and Mr. Hubert P. O. Tang who are three excellent government chemists, bosses, and friends. I really appreciate their unfailing support, enlightenment, and guidance throughout my work and study.

Special thanks are also due to Miss Sara H. Y. Ng and Mr. Chun-Wah Lin for performing all the mass spectroscopic measurements and tackling all unexpected NMR problems. I am particularly thankful to Mr. Ka-Fai Woo and Dr. Chui-Man Lo for their continuous encouragement and affable support during my postgraduate studies.

I would also like to thank my family (especially to my grandfather who has passed away during the preparation of this thesis) who have always stood by me and dealt with all of my absence from morning tea, dinner, and family gatherings with a smile. In addition, I have to extend my gratefulness to my fiancé, George, for his unconditional love, support, and encouragement.

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

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Abbreviations

General

Ac	Acetyl
Anal.	Analytical
Ar	Aromatic
Boc	<i>tert</i> -Butoxycarbonyl
<i>ca.</i>	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	<i>N,N'</i> -Dicyclohexylcarbodiimide
DCFDA	2',7'-Dichlorodihydrofluorescein diacetate
DMAP	4-(Dimethylamino)pyridine
DMEM	Dulbecco's modified Eagle's medium
DMF	<i>N,N</i> -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 <i>H</i> -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

λ	Wavelength in nm
λ_{\max}	Absorption maximum
λ_{em}	Emission maximum
λ_{ex}	Excitation wavelength
ε	Molar extinction coefficient
Φ_{F}	Fluorescence quantum yield
$^1\text{O}_2$	Singlet oxygen
Φ_{Δ}	Singlet oxygen quantum yield

Nuclear Magnetic Resonance (NMR) Data

COSY	Correlation spectroscopy
{ ^1H }	Proton decouple
δ	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
<i>m/z</i>	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,