SYNTHESIS, CHARACTERIZATION AND DNA BINDING STUDIES OF LUMINESCENT RUTHENIUM(II) IMIDAZO COMPLEXES

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By

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Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia
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Feb 2014

Chairman : Haslina Ahmad, PhD
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Ruthenium complexes have attracted as alternative drugs to cisplatin in cancer chemotherapy. Ruthenium polypyridyl complexes show lower toxicity than cisplatin and one of the application usages of ruthenium polypyridyl complexes with imidazole is in pharmaceutical chemistry. Long carbon chain length imidazoles have not been discussed in literature. Therefore, this study focuses on long carbon chain length imidazoles as ligands for ruthenium polypyridyl complexes and the studies with DNA. Four long carbon chain 2-(3,4-dialkoxy)-imidazo-[4,5-f][1,10]phenanthroline ligands were synthesized by condensation of [1,10]phenanthroline-5,6-dione and ammonium acetate first and then addition of 3,4-dialkoxybenzaldehydes into it. ^{1}H NMR, mass spectrometry and FT-IR were used to identify the structure of 2-(3,4-dialkoxy)-imidazo-[4,5-f][1,10]phenanthroline. Three novel ruthenium(II) complexes, $[Ru(II)(bpy)_2L]^{2+}$where L = 1,10-phenanthroline derivatives of imidazole having 3,4-didecyloxy-phenyl (L10), 3,4-ditetradecyloxyphenyl (L14) and 3,4-dihexadecyloxyphenyl (L16) at position 2 were prepared by refluxing 2-(3,4-dialkoxy)-imidazo-[4,5-f][1,10]phenanthroline and $Ru(bpy)_2Cl_2$in a mixture of hot ethanol and water (7:3) for 24 hours under nitrogen which gives red complex. The previously reported ligand 3,4-dioctadecyloxyphenyl (L18) was also synthesized by reacting with $Ru(bpy)_2Cl_2$. All complexes were fully characterized by elemental analysis, ^{1}H-NMR spectroscopy, ESI-MS and FT-IR. Their photophysical properties have also been studied by UV-visible spectroscopy and fluorescence spectroscopy. The complexes exhibit Ru(II) centered emissions at approximately 610 nm in acetonitrile solution at room temperature. In addition, the binding of these complexes to CT-DNA was studied using UV-Vis titration, luminescence titration and also viscosity measurements. The results showed that $[Ru(bpy)_2L10]^{2+}$ binds to CT-DNA by partial intercalation, while $[Ru(bpy)_2L14]^{2+}$, $[Ru(bpy)_2L16]^{2+}$and $[Ru(bpy)_2L18]^{2+}$were indicated to bind intercalatively *via* extended ligands. The affinities of the binding constants of these complexes were found to be in the order of 10^5- $10^6 M^{-1}$.

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Sebagai memenuhi keperluan untuk ijazah Master Sains

SINTESIS, PENCIRIAN DAN KAJIAN IKATAN PENGIKATAN DNA DENGAN KOMPLEKS RUTHENIUM(II) IMIDAZOLA

Oleh

TIMOTHY TING TZE CHUN

Feb 2014

Pengerusi: Haslina Ahmad, PhD
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Kompleks ruthenium telah dijadikan sebagai ubat alternatif untuk cisplatin dalam kemoterapi kanser. Kompleks polypyridyl ruthenium menunjukkan keracunan rendah daripada cisplatin dan salah satu penggunaan kompleks polypyridyl ruthenium dengan imidazola adalah inovasi dalam kimia farmasi. Rantaian karbon imidazola panjang belum dibincangkan dalam kesusasteraan. Oleh itu, kajian ini memberi tumpuan kepada rantai karbon panjang imidazola sebagai ligan untuk kompleks polypyridyl ruthenium dan kajiannya dengan DNA. Empat rantai karbon panjang 2 - (3,4-dialkoxy)-imidazo-[4,5-f] [1,10] phenanthroline ligan telah disintesis oleh kondensasi[1,10]phenanthroline-5,6-dion dan ammonium asetat dahulu sebelum penambahan 3,4-dialkoxybenzaldehid.Spektroskopi resonans magnet nukleus, spektrometri jisim dan FT-IR telahdigunakan untuk mengenal pasti struktur 2 - (3,4-dialkoxy)-imidazola-[4,5-f] [1,10] phenanthroline. Tiga ruthenium novel (II) kompleks, $[Ru(II)(bpy)_2L]^{2+}$ di mana L = 1,10 derivatif-phenanthroline daripada imidazola mempunyai 3,4-didecyloxyphenyl (L10), 3,4-ditetradecyloxy-phenyl (L14) dan 3,4-dihexadecyloxy-phenyl (L16) di kedudukan 2 telah disediakan oleh refluks 2-(3,4-dialkoxy)-imidazola-[4,5-f] [1,10] phenanthroline dan $Ru(bpy)_2Cl_2$ dalam campuran etanol panas dan air (7:3) selama 24 jam di bawah nitrogen yang memberi kompleks berwarna merah. Ligan 3,4-dioctadecyloxyphenyl (L18) yang pernah dilaporkan juga disintesis dengan bertindak balas dengan Ru $(bpy)_2Cl_2$. Semua kompleks telah dicirikan oleh analisis unsur, spektroskopi resonans magnet nukleus, ESI-MS dan FT-IR. Sifat-sifat fotofizikal juga dikaji spektroskopi UV dan spektroskopi pendarfluor. Kompleks Ruthenium(II) mempunyai pusat pelepasan kira-kira 610 nm dalam larutan asetonitril pada suhu bilik. Di samping itu, pengikatan kompleks ini dengan CT-DNA juga dikaji menggunakan pentitratan UV-Vis, pentitratan luminesens dan xjugaukurankelikatan.Hasil kajian menunjukkan bahawa$[Ru(bpy)_2L10]^{2+}$mengikat kepadaCT-DNA olehinterkalasisepara manakala ikatan interkalasi terjadi apabila $[Ru(bpy)_2L14]^{2+}$,$[Ru(bpy)_2L16]^{2+}$dan $[Ru(bpy)_2L18]^{2+}$mengikat kepada DNA melalui ligan.Afiniti pemalar mengikat untuk kompleks didapati dalam 10^5 -$10^6 M^{-1}$.

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APPROVAL

I certify that a Thesis Examination Committee has met on 24 February 2014 to conduct the final examination of Timothy Ting Tze Chun on his thesis entitled "Synthesis, Characterization and DNA binding studies of luminescent ruthenium(II) imidazo complexes" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

A10	3,4-didecyloxybenzaldehyde
A14	3,4-ditetradecyloxybenzaldehyde
A16	3,4-dihexadecyloxybenzaldehyde
A18	3,4-dioctadecyloxybenzaldehyde
bpy	2,2'-bipyridine
CIP	2-(2-chlorophenyl)-imidazo-[4,5-f][1,10]phenanthroline
CT-DNA	calf thymus DNA
dppz	dipyrido[3,2-a:2′,3′-c]phenazine
HNAIP	2-(2-hydroxy-1-naphthyl)-imidazo-[4,5-f][1,10]phenanthroline
HPIP	2-(2-hydroxyphenyl)-imidazo-[4,5-f][1,10]phenanthroline
L10	2-(3,4-didecyloxy)-imidazo-[4,5-f][1,10]phenanthroline
L14	2-(3,4-ditetradecyloxy)-imidazo-[4,5-f][1,10]phenanthroline
L16	2-(3,4-dihexadecyloxy)-imidazo-[4,5-f][1,10]phenanthroline
L18	2-(3,4-dioctadecyloxy)-imidazo-[4,5-f][1,10]phenanthroline
MC10	$[Ru(bpy)_2L10]^{2+}$
MC14	$[Ru(bpy)_2L14]^{2+}$
MC16	$[Ru(bpy)_2L16]^{2+}$
MC18	$[Ru(bpy)_2L18]^{2+}$
MLCT	metal ligand charge transfer
MOPIP	2-(2-methoxyphenyl)-imidazo-[4,5-f][1,10]phenanthroline
NPIP	2-(2-nitrophenyl)-imidazo-[4,5-f][1,10]phenanthroline
phen	phenanthroline
pip	2-phenylimidazo[4,5-*f*][1,10]phenanthroline
UV	ultraviolet
s	singlet
d	doublet
dd	double doublet
m	multiplet
t	triplet

CHAPTER 1

INTRODUCTION

The studies of deoxyribonucleic acid (DNA) as a potential drug target have been carried out since the discovery of the role of DNA as template for protein synthesis (Latchman, 1997). Small molecules that bind to DNA are extremely useful as biochemical tools for the visualization of DNA both *in vitro* and inside the cell. In the last few decades, coordination complexes were selected to be ideal template for designing new DNA-interactive systems.

1.1 Cisplatin

Medicinal chemistry was boosted since the discovery of anticancer properties of cisplatin. *cis*-Diamminedichloridoplatinum(II) was first discovered by Peyrone (1844). However, its activity against cancer was unknown until noted in 1969 by Rosenberg *et al*. They noticed that the platinum electrodes used in experiments influenced bacterial growth and determined the main species accountable to be *cis*-$Pt(NH_3)_2Cl_2$(Rosenberg *et al.*, 1969).

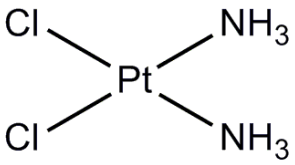

Figure 1.1: Cisplatin

The drug entered clinical trials in 1971 and by 1987 it had become the most widely used anticancer drug. Unfortunately, there are some limitations. Firstly, the drug did not bring a definitive end to cancer as it only showed anticancer activity against some types of tumours. In addition, cisplatin therapy produces severe side effects such as nephrotoxicity, neurotoxicity, ototoxicity, nausea and vomiting. Since cisplatin is effective against certain tumours, studies continue to be done on retaining the therapeutic value of the drug while avoiding its side effects.

Cisplatin diffuses into tissues and tightly binds to proteins. There is strong reactivity between platinum and sulfur of thiol groups of amino acids such as cysteine. Loss of chloride ions from cisplatin is required for binding to occur. Consequently, water molecules replace one or both chloride ions to form $[Pt(H_2O)Cl(NH_3)_2]^+$ and $[Pt(H_2O)_2(NH_3)_2]^{2+}$ cations.

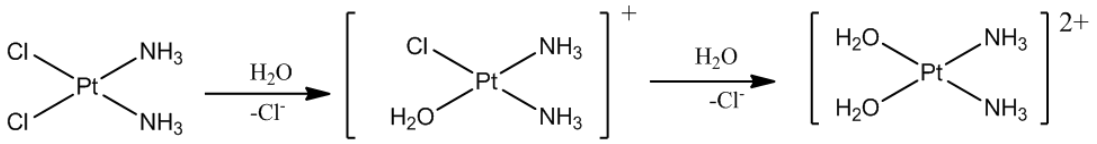

Figure 1.2: Formation of *cis*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$

The aqua ligand is itself easily displaced, leading the platinum atom to bind to DNA bases. Therefore, crosslinking occurs interfering with cell division by mitosis. The damaged DNA elicits DNA repair mechanisms, which activate apoptosis when repair proves impossible.

Cisplatin-DNA lesions are repaired in cells primarily through the nucleotide excision repair (NER) pathway, which consists on a group of proteins with enzymatic functions.

1.2 Alternatives to cisplatin

In the search for drugs with improved clinical effectiveness, other metals besides platinum have been considered. Non-platinum active compounds are likely to have different mechanisms of action, toxicities and DNA binding modes. The d^6 transition metal centres and polypyridyl ligands as structure and site specific reversible DNA binding agents are promising due to the inertness and stability of complexes under physiological conditions. In particular, complexes based on ruthenium(II) centres have caught the attention of scientists due to their attractive photophysical properties (Juris *et al.*, 1988; Gao *et al.*, 2012; Yang *et al.*, 2010 and Gill *et al.*, 2012). The most studied group of metal complexes that reversibly interact with DNA are metallointercallators (Gill *et al*., 2012).

1.3 Ruthenium complexes with alkyl chain

The synthesis of ruthenium complexes with alkyl chain is not new in research. However, most ruthenium complexes synthesized were short chain ligand until recently some short to medium length fluorous or alkyl derivatived pyridine chain have been reported and show considerable chemothermal selectivity towards cancer cells (Clavel *et al*., 2014) especially for longer chains. This shows that different chain length on ligands do play a role in affecting the properties of the complexes activity.

The objectives of this study are

i. To synthesize a series of imidazole ligands with different alkyl chain lengths
ii. To synthesize and characterize a series of ruthenium(II) imidazo complexes using NMR spectroscopy, MS and elemental analysis
iii. To determine the DNA binding constant, K_b of the complexes by studying the effect of changing the alkyl chains of the intercalating ligand of the Ru(II) complexes

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APPENDICES

Spectroscopy data for all compounds are contained in the attached CD.

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