



**UNIVERSITI PUTRA MALAYSIA**

***SYNTHESIS, CYTOTOXICITY AND DNA BINDING STUDIES OF  
RUTHENIUM(II) MIXED-POLYPYRIDYL COMPLEXES***

***SITI NORAIN HARUN***

**FS 2015 29**



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RUTHENIUM(II) MIXED-POLYPYRIDYL COMPLEXES**

By

**SITI NORAIN HARUN**

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

**March 2015**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**SYNTHESIS, CYTOTOXICITY AND DNA BINDING STUDIES OF RUTHENIUM(II) MIXED-POLYPYRIDYL COMPLEXES**

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**SITI NORAIN HARUN**

**March 2015**

**Chair: Haslina Ahmad, PhD**

**Faculty: Science**

A series of ruthenium(II) complexes, including two novel compounds  $[\text{Ru}(\text{dppz})_2\text{L}]^{2+}$  where  $\text{dppz}$  = dipyrido-[3,2-a:2',3'-c]phenazine, and  $\text{L}$  = 2-phenylimidazo[4,5-f][1,10]phenanthroline (PIP) or 2-(4-hydroxyphenyl)imidazo[4,5-f][1,10]phenanthroline (*p*-HPIP) have been synthesized and characterized. The previously reported complexes  $[\text{Ru}(\text{bpy})_2\text{L}]^{2+}$  and  $[\text{Ru}(\text{phen})_2\text{L}]^{2+}$  were also prepared. All complexes were characterized by elemental analysis,  $^1\text{H-NMR}$  spectroscopy, ESI-Mass spectroscopy and FT-IR spectroscopy. The photophysical properties were analyzed by UV-Visible spectroscopy and fluorescence spectroscopy.  $[\text{Ru}(\text{dppz})_2\text{PIP}]^{2+}$  and  $[\text{Ru}(\text{dppz})_2\text{p-HPIP}]^{2+}$  displayed 'molecular light-switch' effect as they have high emission in acetonitrile but no emission in water. The cytotoxicity of all complexes against cancer cell lines HeLa and MCF-7 were investigated through standard MTT assay.  $[\text{Ru}(\text{dppz})_2\text{PIP}]^{2+}$  showed moderate toxicity on both MCF-7 and HeLa with  $\text{IC}_{50}$  of 37.64  $\mu\text{M}$  and 28.02  $\mu\text{M}$ , respectively. Interestingly,  $[\text{Ru}(\text{dppz})_2\text{p-HPIP}]^{2+}$  exhibited remarkable cytotoxicity results with  $\text{IC}_{50}$  of 13.52  $\mu\text{M}$  on HeLa and 11.63  $\mu\text{M}$  on MCF-7 cell lines which are comparable to the infamous anti-cancer drug, cisplatin. The cytotoxicity of this complex series increased as the ligands size extended in order of  $[\text{Ru}(\text{bpy})_2\text{L}]^{2+} < [\text{Ru}(\text{phen})_2\text{L}]^{2+} < [\text{Ru}(\text{dppz})_2\text{L}]^{2+}$ . The interaction of both new complexes  $[\text{Ru}(\text{dppz})_2\text{PIP}]^{2+}$  and  $[\text{Ru}(\text{dppz})_2\text{p-HPIP}]^{2+}$  with CT-DNA were explored by using UV-Vis absorption titration, fluorescence quenching and viscosity measurement. These studies suggest that the two Ru(II) complexes bind to DNA *via* intercalation, which involves the insertion of ligands in between DNA base pairs. The absorption titration determined that,  $[\text{Ru}(\text{dppz})_2\text{p-HPIP}]^{2+}$  ( $K_b = 5.0 \times 10^7 \text{ M}^{-1}$ ) bind strongly to DNA strongly than  $[\text{Ru}(\text{dppz})_2\text{PIP}]^{2+}$  ( $K_b = 2.5 \times 10^6 \text{ M}^{-1}$ ). The intramolecular hydrogen bonding in HPIP complex increases the surface area of the intercalating diimines and enhances the DNA binding affinity substantially.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**SINTESIS, KAJIAN KESITOTOKSIKAN DAN PENGIKATAN DNA  
SEBATIAN RUTHENIUM(II) POLIPIRIDIL CAMPURAN**

Oleh

**SITI NORAIN BINTI HARUN**

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Suatu siri kompleks ruthenium(II) termasuk dua sebatian baharu  $[\text{Ru}(\text{dppz})_2\text{L}]^{2+}$  di mana  $\text{dppz} = \text{dipirido-[3,2-a:2',3'-c]fenazin}$ , dan  $\text{L} = 2\text{-fenilimidazo[4,5-f][1,10]fenantrolin}$  (PIP) atau  $2\text{-(4-hidroksifenil)imidazo[4,5-f][1,10]fenantrolin}$  ( $p\text{-HPIP}$ ) telah disintesis dan dianalisa. Empat lagi kompleks dari  $[\text{Ru}(\text{bpy})_2\text{L}]^{2+}$  dan  $[\text{Ru}(\text{phen})_2\text{L}]^{2+}$  yang telah dilaporkan sebelum ini juga turut disediakan. Semua kompleks dianalisa menggunakan Analisis Elemen, spektroskopi  $^1\text{H-NMR}$ , spektroskopi jisim dan spektroskopi FT-IR. Sifat fotofizikal kompleks dianalisa menggunakan UV-nampak dan pendaflour.  $[\text{Ru}(\text{dppz})_2\text{PIP}]^{2+}$  dan  $[\text{Ru}(\text{dppz})_2p\text{-HPIP}]^{2+}$  menunjukkan fenomena 'suis nyalaan molekul' apabila kompleks tersebut menunjukkan pancaran tinggi dalam asetonitril tetapi tiada pancaran dalam air. Kesitotoksikan kompleks terhadap sel kanser Hela dan MCF-7 disiasat melalui kaedah piawai cerakin MTT.  $[\text{Ru}(\text{dppz})_2\text{PIP}]^{2+}$  menunjukkan ketoksian sederhana terhadap kedua-dua sel kanser dengan nilai  $\text{IC}_{50}$   $37.64 \mu\text{M}$  untuk sel Hela dan  $28.02 \mu\text{M}$  untuk sel MCF-7. Lebih menarik lagi,  $[\text{Ru}(\text{dppz})_2p\text{-HPIP}]^{2+}$  menunjukkan kesitotoksikan yang sangat bagus dengan nilai  $\text{IC}_{50}$   $13.52 \mu\text{M}$  untuk sel Hela dan  $11.63 \mu\text{M}$  untuk sel MCF-7, setara dengan ubat anti-kanser yang terkenal iaitu cisplatin. Kesitotoksikan siri kompleks ini didapati meningkat seiring dengan pertambahan saiz ligan dalam kompleks, dengan turutan  $[\text{Ru}(\text{bpy})_2\text{L}]^{2+} < [\text{Ru}(\text{phen})_2\text{L}]^{2+} < [\text{Ru}(\text{dppz})_2\text{L}]^{2+}$ . Interaksi sebatian baharu dengan CT-DNA diteroka menggunakan penitratan UV-vis, padaman fluoresen dan ukuran kelikatan. Hasil kajian menunjukkan bahawa kedua-dua sebatian terikat dengan DNA melalui interkalasi, yang melibatkan sisipan ligan di antara pasangan bes DNA. Penitratan UV-vis menyimpulkan yang sebatian  $[\text{Ru}(\text{dppz})_2p\text{-HPIP}]^{2+}$  ( $K_b = 5.0 \times 10^7 \text{ M}^{-1}$ ) terikat lebih kuat terhadap DNA berbanding  $[\text{Ru}(\text{dppz})_2\text{PIP}]^{2+}$  ( $K_b = 2.5 \times 10^6 \text{ M}^{-1}$ ). Ikatan hidrogen intramolekul dalam sebatian HPIP meningkatkan luas permukaan diimine terinterkalasi dan menatar affiniti ikatan DNA dengan ketara.

## ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my supervisors Dr. Haslina Ahmad, Dr. Norazizah Shafee and Prof. Mohd Basyaruddin Abdul Rahman for their guidance, advice, encouragement and unlimited help throughout the duration of my studies.

I would like to extend my gratitude to UPM and the staffs at Chemistry Department for the facilities, instruments and chemicals provided to complete the research. A million thanks to Timothy and Nadia for always having my back. Special thanks to Aini, Sien Yei and Bon on their help and guidance on cell culture and MTT assay.

Last but not least, my sincere gratitude towards my family, especially my parents Harun and Arosmi, and also my grandmother, Uwan Fatimah for their unlimited support, love and faith in me. Thank you, always.

## APPROVAL

I certify that a Thesis Examination Committee has met on 20 March 2015 to conduct the final examination of Siti Norain Harun on her thesis “Synthesis, Cytotoxicity and DNA Binding Studies of Ru(II) Mixed-Polypyridyl Complexes” in accordance with the 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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## LIST OF ABBREVIATIONS

BH	[Ru(bpy) <sub>2</sub> <i>p</i> -HPIP] <sup>2+</sup>
BP	[Ru(bpy) <sub>2</sub> PIP] <sup>2+</sup>
bpy	2,2'-bipyridine
CT-DNA	Calf Thymus DNA
DH	[Ru(dppz) <sub>2</sub> <i>p</i> -HPIP] <sup>2+</sup>
DNA	Deoxyribonucleic Acid
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DP	[Ru(dppz) <sub>2</sub> PIP] <sup>2+</sup>
dpq	1,10-phenanthroline-5,6-dione
dpqc	Dipyrido[3,2- <i>a</i> :2',3'- <i>c</i> ](6,7,8,9-tetrahydro) phenazine
dpqo	Dipyridoquinoxaline
dppz	Dipyrido[3,2- <i>a</i> :2',3'- <i>c</i> ]phenazine
FBS	Fetal Bovine Serum
HeLa	cervical cancer cell line
IC <sub>50</sub>	half maximal inhibitory concentration
KPF <sub>6</sub>	Potassium hexafluorophosphate
MCF-7	Human breast cancer cell line
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaBr	Sodium bromide
<i>p</i> -HPIP	2-(4-hydroxyphenyl)imidazo[4,5- <i>f</i> ] [1,10]phenanthroline
PBS	Phosphate Buffer Saline
phen	1,10-phenanthroline
PH	[Ru(phen) <sub>2</sub> <i>p</i> -HPIP] <sup>2+</sup>
PIP	Phenylimidazo[4,5- <i>f</i> ][1,10]phenanthroline
PP	[Ru(phen) <sub>2</sub> PIP] <sup>2+</sup>
RuCl <sub>3</sub> ·3H <sub>2</sub> O	Ruthenium(III) chloride hydrated

## CHAPTER 1

### INTRODUCTION

#### 1.1 Cancer therapy

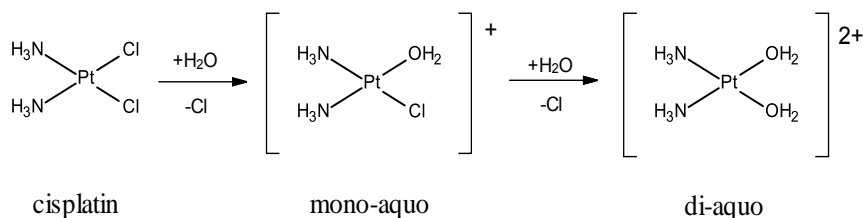
The objectives of drugs in cancer therapy are to inhibit, destroy or cure cancer cells. When the cancer is incurable, therapy drugs often aid to prolong life. Cancer drugs can function in different number of therapy manner to handle cancer cells such as:

- Chemotherapy drugs can inhibit the growth or multiplication of cancer cells (cytotoxic), and encourage the cells to die naturally (apoptosis). Chemotherapy is carry out when cancer has spread, or recurred. However, chemotherapy drugs can generally harm healthy cells in the same way, instigating side effects.
- Biological therapy drugs help immune system in the body to battle against cancer.
- Hormone therapy drugs commonly interfere with hormone production or hormone action from assisting cancer growth.
- Targeted drug therapy comprises drugs that are designed to attack specific area of the cancer cells. These type of therapy manage to lessen harm to healthy cells which greatly reduce the side effects.

#### 1.2 Platinum cancer drugs

In 1964, an observation made by Florea & Büsselberg (2011) on bacterial growth influenced by platinum electrodes brought scientists to realize a property of platinum; the ability to inhibit division of living cells. Based on this trait, researchers began to actively pursue developing platinum compound to treat cancer cells. The first platinum-based drug that entered the clinical trials was cisplatin in 1971 and towards 1977, it has demonstrated success in treating testicular, ovarian and a few other cancer and in late 80s became the most widely used drug in cancer therapy.

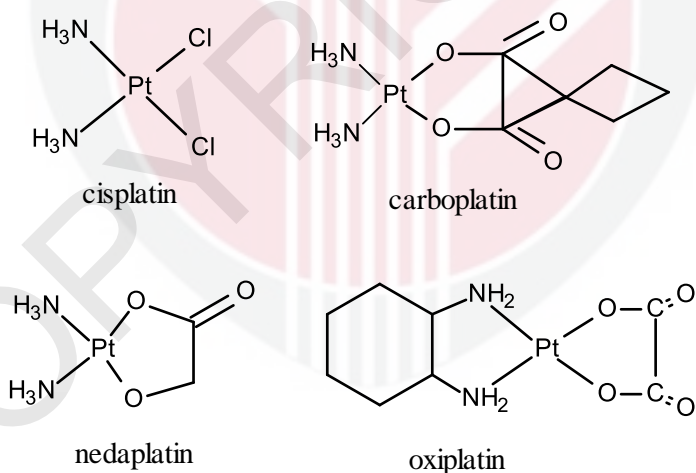
The cytotoxicity of cisplatin is generally triggered by DNA. In order for the neutral cisplatin diffuses into tissues and bind with DNA, a series of spontaneous aqation reaction involving sequential replacement of the chloride ligands with water molecules has to occur. The strong reactivity between platinum and sulfur of thiol groups in amino acids will subsequently form  $[\text{Pt}(\text{H}_2\text{O})\text{Cl}(\text{NH}_3)_2]^+$  and  $[\text{Pt}(\text{H}_2\text{O})_2(\text{NH}_3)_2]^{2+}$  as described in **Scheme 1.1** (Moreno-Gordaliza *et al.*, 2010).



**Scheme 1.1: Activation of cisplatin**

The displacement of aqua-ligand leads platinum to bind to DNA bases *via* crosslinking, thus hindering cell division by mitosis. This reaction induce biological effects of cisplatin which culminate either in the repair of DNA damage and cell survival or activation of irreversible apoptosis of the cells when the damage is extensive for repair to be completed (Siddik, 2003).

However, cisplatin was found to affect only a limited range of selective cancer and dose-dependent. Additionally, delivery of drugs in high dosage generates unpleasant side effects to the patients, namely nausea, vomiting, neurotoxic, nephrotoxic and ototoxic. In a few cases, the cancer cells was found to be intrinsically resistance against the drugs. In 1986, carboplatin has been introduced as another preferred option to cisplatin as it provided lesser severe side effects. Carboplatin was similarly active in treating ovarian cancer cells but was inferior to cisplatin in testicular, neck and head cancer treatment. A few of registered platinum cancer drugs are shown in **Figure 1.1** (Ott & Gust, 2007).



**Figure 1.1: Platinum-based drugs**

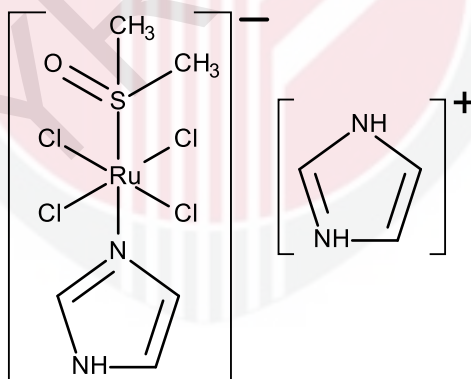
Since then, substantial amount of platinum compounds have been synthesized to overcome the problems in cisplatin but none has yet to surpass its superiority in standard cancer treatment. This led scientists to ventures for alternative compounds from other group of metals that can provide similar therapeutic value and reduced side effects.

### 1.3 Alternative metal-based complexes

Scientists have been exploring alternatives for platinum-based drugs from other range of available metals in the hopes of finding active compounds with improved clinical efficacy, reduced toxicity and wider range of activity towards different cancers. Non-platinum active compounds most likely to have different approach mechanism, bio-distributions and toxicity than platinum complexes and might likely be active against intrinsic or acquired resistant malignancies. The few chosen metals pursued to overcome cisplatin problems are ruthenium, rhodium, rhenium and others among the transition metal group (van Rijt & Sadler, 2009; Muhammad & Guo, 2014).

Among those transition metals, ruthenium complexes exhibit potential of outdoing cisplatin resistance with low general toxicity (Clarke, 1989). Other than that, ruthenium complexes may be one type of complexes exhibiting multi-target anti-cancer activity (Yu *et al.*, 2014). Ruthenium compounds that have successfully entered the clinical trial are NAMI-A and KP1019 (Jakupec *et al.*, 2008).

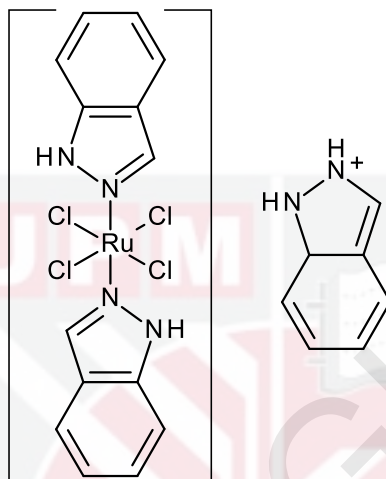
NAMI-A (imidazolium *trans*-imidazoledimethylsulfoxide tetrachloro-ruthenate) in **Figure 1.2**, is considerably the most stable ruthenium-based anti-cancer drugs. It is developed was the first ruthenium complex to enter and completed the phase I of clinical trial. The anti-cancer characteristic of NAMI-A is activated by the reduction of Ru(III) to Ru(II) upon reacting with cancer cells (Zhang & Lippard, 2003). Even though NAMI-A has a low *in vitro* cytotoxicity, it compensates in having a high *in vivo* ability to reduce metastases weight without distressing the primary tumour which makes it a good anti-metastatic agent (Sadler *et al.*, 2005).



**Figure 1.2: Structure of NAMI-A**

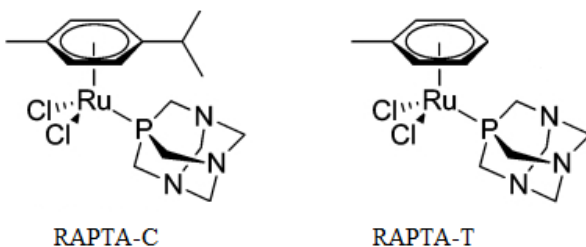
The *trans*-tetrachlorobis(indazole)ruthenate(III), commonly known as KP1019 (**Figure 1.3**) is another ruthenium-based compound that has entered clinical trial. KP1019 adapted octahedral structure with two *trans* *N*-donor indazole and four chloride ligands along the equatorial plane, which facilitate oxidation state changes without altering the geometry. In the clinical stage, KP1039 is used to prepare KP1019 as a sodium salt, providing better solubility to improve transportation in bloodstream. These *bis*-indazole-ruthenate(III) which often known as 'Keppler-type' complexes (Mura *et al.*, 2005)

affected the mechanism of action by “activation-by-reduction” process and the transferrin-mediated transport into cells (Clarke, 2003). KP1019 has the capability to form crosslinks with DNA and is a good binding partner to albumin which facilitate cellular uptake and induce apoptosis. The success of KP1019 is mainly observed in colorectal cell lines treatment but in the clinical stage, it also showed potential to influence other cancer cell lines (Hartinger *et al.*, 2008).



**Figure 1.3: Structure of KP1019**

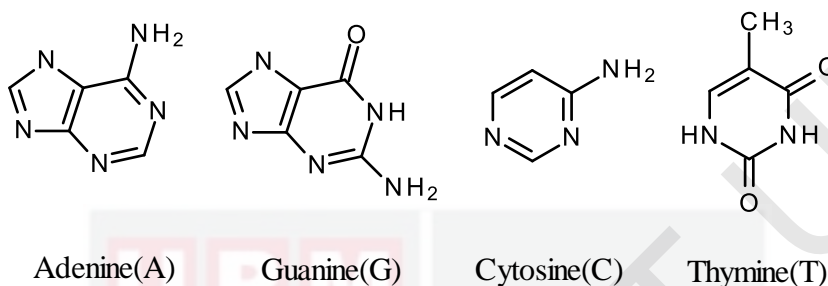
Another potential ruthenium active compound is RAPTA, known to adapt the ‘piano stool’ shape, bearing the 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane (pta) ligand. RAPTA has shown selectivity to cancer cells over non-tumor cells but is has a weak *in vitro* toxicity which increase its appeal as alternative metastazing agent. The most reputed antimetastatic agents in the RAPTA family are the RAPTA-T and RAPTA-C as shown in **Figure 1.4**. Although the specific target of RAPTA is unknown, it involves in disrupting the cell cycle regulation that leads to apoptosis of cancer cells. (Ang *et al.*, 2011)



**Figure 1.4: Structures of RAPTA**

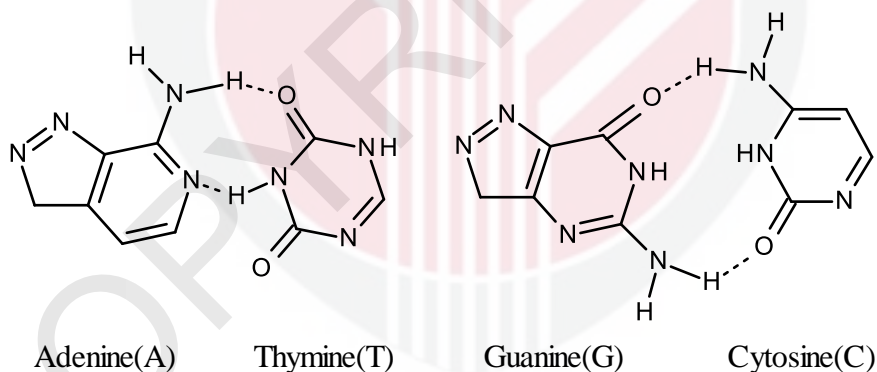
## 1.4 DNA

Deoxyribonucleic acid or DNA is a hereditary molecule found inside nucleus of cells in living organisms. The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T).



**Figure 1.5: Molecular structures of four deoxyribonucleotides**

Watson & Crick (2003) made a classical proposal for the secondary structure of DNA. According to the Watson-Crick model, the DNA under physiological conditions consists of two polynucleotide strands, running in opposite directions and coiled around each other in a double helix like the handrails on a spiral staircase. The two stands are complementary rather than identical and are held together by hydrogen bonds between specific pairs of bases, A with T and C with G. (**Figure 1.6**)



**Figure 1.6: Hydrogen bonding between base pairs in DNA double helix.**

DNA is suitable for biological information storage as it contains the instructions needed for an organism to develop, survive and reproduce, therefore, the integrity and stability of DNA are essential. DNA as a chemical entity is subjected to assault from the environment, and any resulting damage, if not repaired, will lead to mutation and disease.

An important property of DNA is that it can replicate, which is critical in cell division because each new cell needs to have an exact copy of the DNA present in the old cell. In fact, cells have evolved a number of mechanisms to detect and repair the various types of damage that can occur to DNA, even if the damage is caused by environment or

replication error. As DNA plays an active and critical role in cell division, control of DNA repair is closely tied to regulation of the cell cycle (Branzei & Foiani, 2008).

### 1.5 DNA binding of ruthenium complexes

Previously, the classical ruthenium anticancer therapy was based on the capability of ruthenium to bind with DNA *via* some of the nitrogen atoms of nucleic bases. The interaction between transition metal complex and nucleic acids were studied extensively in order to develop the drugs that can react and bind to nucleic bases.

For study of complexes ability to bind with CT-DNA, the intrinsic binding constant or binding affinity is determined. The DNA binding affinity is relative to the dimension of  $\pi$ -conjugated aromatic area of the intercalating ligand and the hydrophobic property of ancillary ligands. The extend  $\pi$ -system of the intercalating ligand together with the hydrophobic character of ancillary ligands might enlarge the binding strength of ruthenium complex to DNA. These features facilitate the complex to receive electron charges from the DNA base pairs, when inserted in between DNA's double helix. The metal complexes can bind to DNA through covalent and non-covalent interaction. The most important interaction is non-covalent where the mode of binding are divided into three main category: electrostatic binding, groove binding, and intercalation binding mode.



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