



# Binding studies of Ruthenium(II) polypyridyl complexes with DNA isolated from spinach extract

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**ABSTRACT:** Despite significant medical advancements, the need for a more effective and less toxic chemotherapeutic agent remains a pressing challenge in modern medicine. This study investigates the binding interactions of ruthenium polypyridyl complexes with spinach DNA samples, aiming to understand their potential as therapeutic agents and in other fields. The binding interactions between  $[\text{Ru}(\text{LL})_3]^{2+}$ , (L- bpy-bipyridyl and dmbpy-dimethyl bipyridyl) complexes, and the DNA from spinach (*Spinacia oleracea*) were analyzed by UV-visible spectroscopy and emission graphs. The binding constants were determined by the Benesi-Hildebrand method. Both complexes exhibit significant interaction with the spinach DNA, as indicated by the binding constants in a range of  $10^5 \text{ M}^{-1}$ . The results suggest potential applications of these complexes in biomedical and plant biotechnological fields. However, further in-depth mechanistic studies are warranted.

**KEYWORDS:** Ruthenium complex, Polypyridyl ligand, Spinach DNA, Benesi-Hildebrand plot.

## INTRODUCTION

Organo ruthenium complexes are a fascinating class of compounds due to their flexibility, remarkable biological activity, unique photophysical activity, catalytic properties, etc. These complexes exhibit the rules that govern the cellular uptake and cellular localisation of systems, and they are finding numerous applications ranging from imaging to therapeutics [1]. Organo ruthenium complexes are highlighted for their potential in photodynamic therapy, a treatment method for multiple cancers. [2]

Bipyridyl-based ruthenium complexes have been widely studied for their DNA binding capabilities, particularly with calf thymus DNA, where intercalative and groove binding modes have been observed. These interactions are largely influenced by ligand substitution and environmental conditions. The bipyridyl-based ruthenium complexes help in designing efficient dye-sensitized solar cells and efficient anticancer and antimalarial drugs. Ruthenium complexes exhibit diverse binding properties with nucleic acids, particularly DNA and RNA, showcasing their potential in biomedical applications, especially as an antitumor drug [3]. Studies reveal that ruthenium (II) polypyridyl complexes, such as  $[\text{Ru}(\text{bpy})_2(7\text{-F-dppz})]^{2+}$ , demonstrate strong intercalative binding to duplex RNA, with binding affinity influenced by ligand substituents and environmental conditions [4]. Similarly, ruthenium (III) hydroxamate

complexes show intercalative binding to CT-DNA, confirmed by hypochromism and redshifts in UV-visible spectra [5]. Furthermore, new organo-ruthenium(II) complexes exhibit significant binding efficiency with CT-DNA and human serum albumin, indicating their potential for drug delivery [6, 7].

DNA binding is one of the key points for which ruthenium bipyridyl complexes are studied. As these complexes can intercalate or bind through electrostatic interaction to the DNA [8] molecule and the binding mode is influenced by the sequence of the DNA, favoring purine-purine-pyrimidine-pyrimidine sequence [9]. They act as potential agents in photodynamic therapy, which is an innovative treatment modality that utilizes photosensitizers activated by specific wavelengths of light to target and destroy cancerous cells selectively and other pathological tissues. Ruthenium(II) polypyridyl complexes demonstrated high anti-proliferation activity against the SGC-7901 cancer cell line while showing low cytotoxicity against the normal NIH3T3 cells. This indicates a degree of selectivity in targeting cancer cells over normal cells [10].

While many studies utilize calf thymus DNA or synthetic oligonucleotides as model systems, this study employs spinach DNA to explore the feasibility of using plant-derived genetic material in binding studies. Spinach

(*Spinacia oleracea*) contains several vitamins and minerals, including potassium, magnesium, and vitamins B6, B9, and E. Spinach is an extremely nutrient-rich vegetable. It packs high amounts of carotenoids, vitamin C, vitamin K, folic acid, iron, and calcium. Phytochemicals in spinach may prevent cancer cell proliferation and support chemoprevention strategies. Carotenoids, also present in spinach, have been formulated to reduce toxicity in cancer treatments, suggesting a synergistic effect when combined with other therapies. While spinach and its components show potential in cancer treatment [11, 12], further clinical studies are necessary to establish definitive therapeutic protocols and understand the full scope of their efficacy. MGDG from spinach has shown cytotoxic effects on human cancer cells, prompting further investigation into its potential to enhance radiation therapy for pancreatic cancer [13]. Spinach packs high amounts of antioxidants, which may also Figureht cancer. The health benefits of spinach include skin care, improved eyesight, regulated blood pressure, stronger muscles, and prevention of age-related macular degeneration and hemophilia. It also helps with health conditions such as cataracts, atherosclerosis, heart attacks, and neurological disorders. It helps in bone mineralization and exerts anti-ulcerative and anti-cancerous benefits [14, 15].

Due to their potential applications in medicine and biotechnology, ruthenium complexes have been extensively studied for their interactions with DNA, including plant DNA. Despite promising claims regarding the therapeutic applications of these complexes, this study does not delve into the specific mechanism of interactions, such as intercalative versus groove binding. Therefore, while binding constants are calculated, future studies involving viscosity measurements, circular dichroism (CD), and electrophoresis are necessary to confirm this binding mode. Similarly, claims regarding ROS generation and photodynamic therapy potential are speculative without experimental validation.

## MATERIALS AND METHODS

Sigma Aldrich was the supplier of the ligands 2,2'-bipyridine and 4,4'-dimethyl-2,2'-bipyridine. The study's spinach leaves were acquired locally, and double-distilled deionized water was used for the binding assays. All of the other chemicals and solvents were of reagent grade and were employed precisely as directed.

### Synthesis of Tris (2,2'-bipyridine) Ruthenium (II) Chloride, $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$

The mixture was refluxed for 20 hours after 0.5g of  $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$  and 0.6g of 2, 2-bipyridine were dissolved in 25

mL of ethanol. The resulting orange-red complex was present in the ethanol solution. Using n-propanol as an eluent, the crude product was purified on a silica gel column. The pure complex was recovered after evaporation. The absorbance maximum ( $\lambda_{\text{abs}}^{\text{max}}$ ) of the chemical in  $\text{CH}_3\text{CN}$  is 448.

### Synthesis of Tris(4,4'-dimethyl-2,2'-bipyridine)ruthenium(II)tetrafluoroborate, $[\text{Ru}(\text{dmbpy})_3](\text{BF}_4)_2$

After dissolving  $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$  (1 mM) and 4,4'-dimethyl-2,2'-bipyridine (3 mM) in 20 mL of ethylene glycol, the mixture was refluxed for four hours. After allowing the solution to cool to ambient temperature, any insoluble contaminants were filtered out. Next, dropwise additions of a saturated sodium tetrafluoroborate solution were made to the filtrate until an orange precipitate appeared. A vacuum desiccator was used to dry the product further after it had been filtered and cleaned with cold water and diethyl ether. The product was recrystallized from water to further purify it. The compound in  $\text{CH}_3\text{CN}$  has an absorption maximum ( $\lambda_{\text{abs}}^{\text{max}}$ ) of 458 nm and an emission maximum ( $\lambda_{\text{em}}^{\text{max}}$ ) of 601 nm.

### Extraction of DNA from spinach leaf extract

About 20 g of spinach leaves were weighed and added to a cold blender with 150 ml cold saline citrate buffer, which was blended for 50-60 seconds. The homogenate was centrifuged for 15 mins at 4 C, and the supernatant was discarded. This step was repeated 3 times, and the pellet was then dissolved with 20 mL of 2.6 N NaOH and shaken vigorously. Then, it was centrifuged for 20 minutes to settle the insoluble protein. The supernatant is poured into a beaker, and 2-3 volumes of 95 % cold ethanol are added through the sides of the beaker. The genetic material floating on the surface was collected using a glass rod and washed with 70 % ethanol.

### Equipment

In addition to the binding analyses of the generated complexes with the DNA sample, the SYSTRONICS Double Beam Spectrophotometer 2203 was used to record the absorption spectrum for both the complexes  $[\text{Ru}(\text{bpy})_3]^{2+}$  and  $[\text{Ru}(\text{dmbpy})_3]^{2+}$ . The JASCO/FP 8200 spectrofluorometer was used to capture the emission spectrum. To guarantee that the volume of the sample solutions used for emission measurements did not vary, they were always stored in cold water. All measurements were performed at room temperature.

### Determination of Purity and Quantity of Isolated DNA

The purity of the DNA isolated from spinach extract was measured by spectrophotometric methods. The absorbance of the isolated DNA was measured at 260 and 280 nm using UV-

Visible spectrophotometer and its ratio ( $A_{260}/A_{280}$ ) was calculated. The DNA concentration was calculated as:

$$\text{Total DNA concentration (ng/}\mu\text{L)} = A_{260} \times 50 \text{ ng/}\mu\text{L} \times 100 \text{ eqn 1}$$

### Determination of association constants using absorption and emission techniques

The Benesi-Hildebrand method (eqn.1) was used to determine the association constants ( $K_a^{\text{abs}}$ ) of the  $[\text{Ru}(\text{NN})_3]^{2+}$  complexes with DNA isolated from spinach in a homogenous medium.[16]

$$\frac{1}{\Delta A} = \frac{1}{K_a^{\text{abs}}} \Delta \epsilon [\text{H}] + \frac{1}{\Delta \epsilon} [\text{Q}] \quad \text{Eqn.2}$$

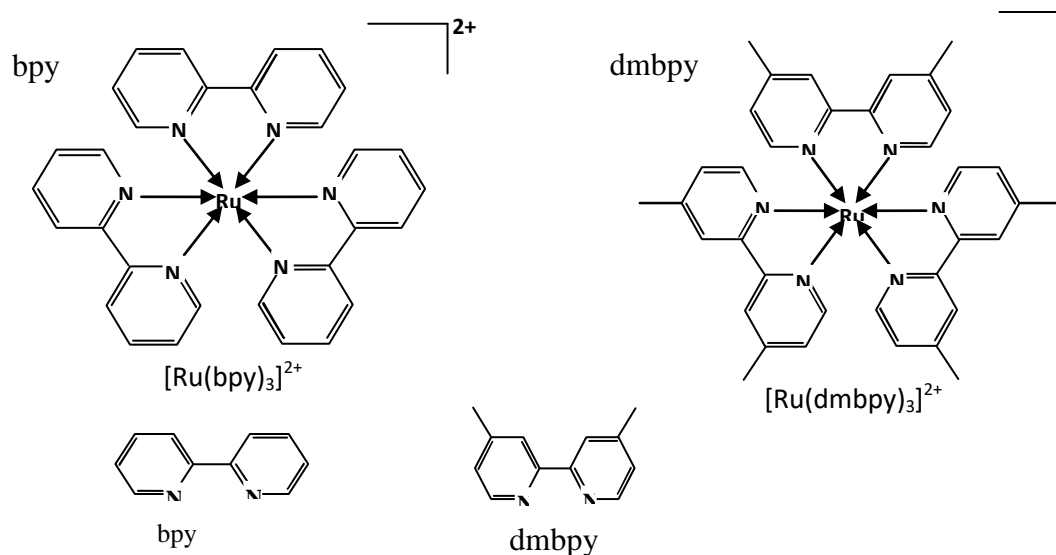
In this case, [H] represents the host's (sensitizer) concentration, [Q] represents the guest's (DNA) concentration, and  $\Delta A$  represents the change in [H]

absorbance upon the addition of [Q]. The molar extinction coefficient of the free [H] and [H]-[Q] complexes differs. The 1:1 complex formation is supported by the plot of  $1/\Delta A$  values as a function of  $1/[\text{Q}]$  values for each of the guest molecules that were studied. The ratio of the Y-intercept to the straight line's slope yields the association constant. The change in absorbance with DNA addition was plotted as  $1/\Delta A$  versus  $1/[\text{Q}]$ , and the binding constants were obtained from the slope-intercept ratio. Future studies will include correlation coefficients, residual plots, and error bars for enhanced statistical rigor. Analysis of the UV-visible absorbance spectra and fluorescence emission profile of indomethacin upon addition of Ct-DNA indicates the formation of a drug-DNA complex. UV-visible absorbance and steady state fluorescence experiments revealed a binding constant on the order of  $10^3 \text{ L mol}^{-1}$ , which is consistent with those of well-known groove binders[17].

## RESULT AND DISCUSSION

### Structure of the complexes

The ruthenium(II) complexes were synthesized and their molecular structures are shown below.



**Figure 1.** Structure of the chosen Ruthenium complexes and the ligands used.

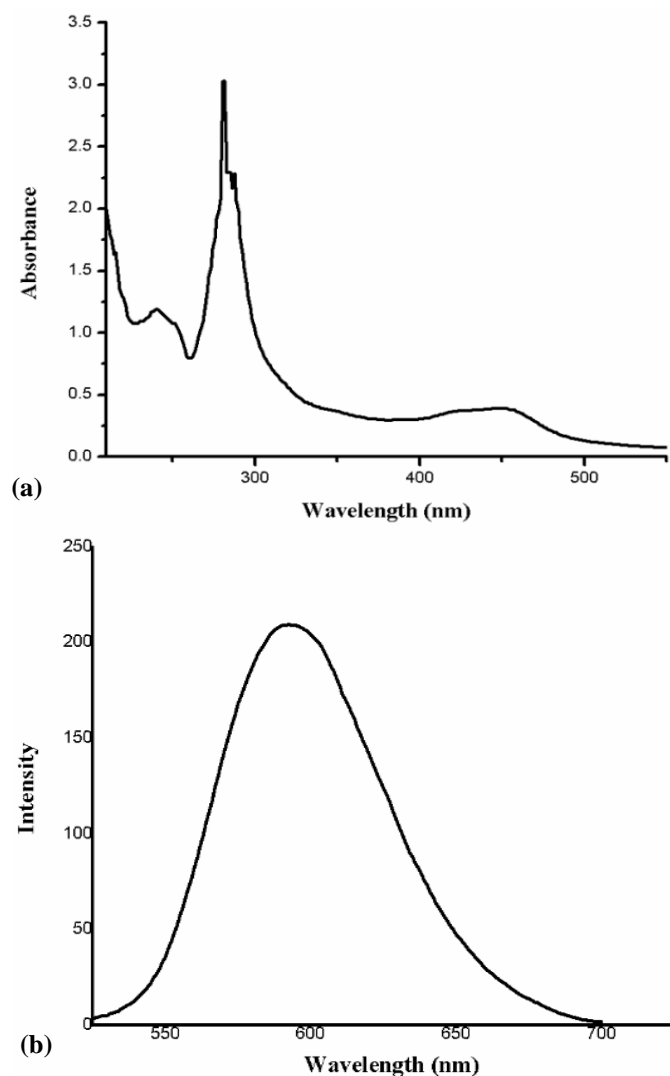
### Absorption and emission spectral measurement

Ruthenium complexes are the most researched complexes because of their photophysical and excited state characteristics. In an aqueous solution,  $[\text{Ru}(\text{bpy})_3]^{2+}$  exhibits an absorption maximum at 453 nm and an emission maximum at 596 nm. The triplet metal-to-ligand charge transfer state ( $^3\text{MLCT}$ ) is the lowest excited state of

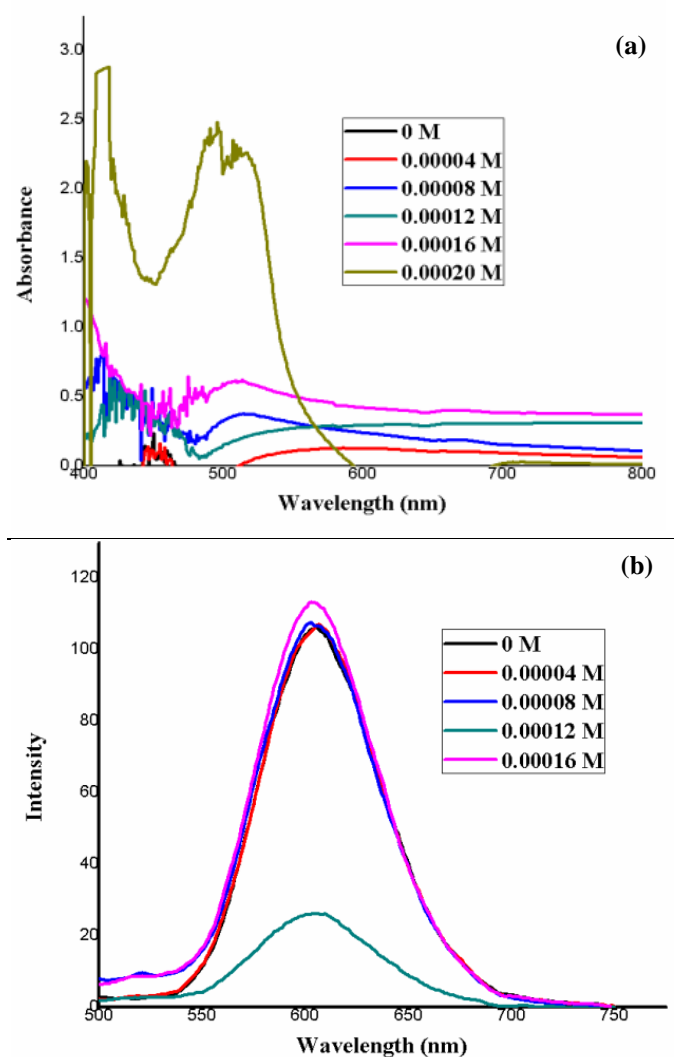
$[\text{Ru}(\text{bpy})_3]^{2+}$ . Three closely spaced, equilibrium-excited states that are discernible at 5K but in equilibrium at and above 77K combine to form the lowest  $^3\text{MLCT}$ . The emission maximum of Ru (II) complexes originates from the  $d\pi-\pi^*$   $^3\text{MLCT}$  transition.

Based on the absorption and emission spectral binding data, the binding constants for the samples for different

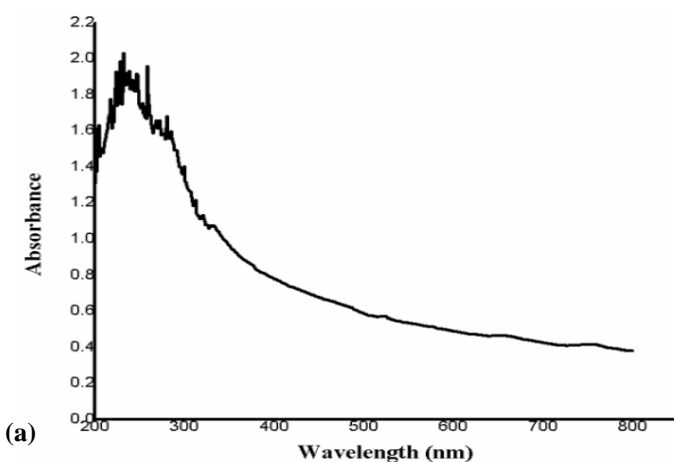
concentrations were calculated. The complex  $[\text{Ru}(\text{bpy})_3]^{2+}$  has a binding constant of  $2.305 \times 10^5$ , and  $[\text{Ru}(\text{dmbpy})_3]^{2+}$  has a binding constant of  $2.39 \times 10^5$ . These data show that both complexes show moderate binding to the spinach DNA. In the interaction of spinach DNA with ruthenium complex, the UV-visible spectroscopy interaction is indicated by a bathochromic shift with the increase in concentration of the DNA sample. This reflects strong interaction as the  $\pi-\pi^*$  transitions of the DNA bases are affected. Spinach DNA's specific base composition, particularly the guanine-cytosine (GC) ratio to adenine-thymine (AT), can influence how well the ruthenium complex binds. GC-rich regions tend to form stronger  $\pi-\pi$  stacking interactions with metal complexes, which enhances binding. However, specific intercalative binding remains hypothetical and needs confirmation via viscosity or CD studies.

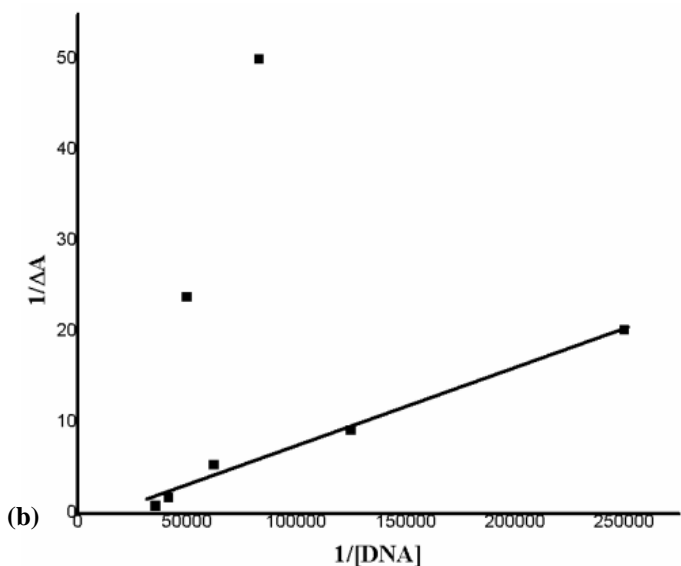


**Figure 2.** (a) Absorption; (b) emission spectrum of  $[\text{Ru}(\text{bpy})_3]^{2+}$  complexes in aqueous medium



**Figure 3.** (a) Absorption spectrum of the  $[\text{Ru}(\text{bpy})_3]^{2+}$  complex (2 mL) with incremental addition of Spinach Extract DNA (0- 2.8 mL) in aqueous medium and (b) Absorption spectrum of the  $[\text{Ru}(\text{dmbpy})_3]^{2+}$  complex with incremental addition of Spinach Extract DNA (S) in aqueous medium.





Equation	$y = a + b \cdot x$	
Adj. R-Square	0.14552	
	Value	Standard Error
Intercept	11.31736	11.69264
Slope	4.90918E-5	1.00671E-4

**Figure 4.** (a) UV absorption spectrum of Spinach extract DNA; (b) Benesi – Hildebrand plot from the absorption spectral data of  $[\text{Ru}(\text{bpy})_3]^{2+}$  with Spinach DNA extract in aqueous media.

## CONCLUSION

The complexes  $[\text{Ru}(\text{bpy})_2]^{2+}$  and  $[\text{Ru}(\text{dmbpy})_3]^{2+}$  demonstrate substantial binding affinity to spinach DNA, as evidenced by the binding constants and spectral shifts. The lift in the peak indicates the increase in the binding of the complex with the DNA sample. The complex  $[\text{Ru}(\text{dmbpy})_3]^{2+}$  exhibits significant binding affinity with spinach extract DNA with a binding constant of  $2.39 \times 10^5$ .

It may be possible to use this kind of ruthenium complex binding to plant DNA for precise gene editing, which would allow target genes to be added, removed, or altered in plant genomes. The Benesi-Hildebrand plot was used to assess binding constants. These experiments demonstrate that poorly soluble medications can be complexed with metapolyridyl complexes and delivered to increase their solubility significantly. Therefore, the current endeavor has investigated the interaction of spinach DNA extract with  $[\text{Ru}(\text{NN})_3]^{2+}$  (NN = 2,2'-bipyridine, 4,4'-dimethyl-2,2'-bipyridine). Our latest research indicates that the two ruthenium complexes attach to spinach DNA in nearly identical ways. Good interaction is demonstrated by this rise

in the binding constant, which is important since it implies that the complexes have the potential to attach to DNA efficiently, which is essential for their possible therapeutic uses.

## DECLARATION

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### Authorship Contributions

Concept and Writing: Prof. Dr.Thangadurai Sumitha Celin.; Data Collection and Interpretation: Lohidas Anitha Mabel Gaflin Shelty;.

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### Ethics approval and consent to participate

This study did not involve human participants or animals and therefore ethics approval is not required.

### Competing interests

The authors declared that there is no conflict of interest.

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