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SYNTHESIS, DNA BINDING, ANTICANCER AND CYTOTOXIC EVALUATION OF NOVEL RUTHENIUM(II) ISATIN BASED SCHIFF BASE COMPLEX

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ABSTRACT

Cancer is a leading cause of death worldwide, currently the cancer treatments are radiation therapy, surgery or chemotherapy with the well-known side effects. So far there is no curative therapy available for most of disseminated cancer and thereby the development and discovery of novel therapeutic agents are largely needed. From the development of cisplatin, many scientific groups are actively worked/working for the inorganic anticancer drugs. and have developed a number of Ru(II) complexes with lower systemic toxicity. Ruthenium complexes can penetrate effectively with the tumor cells and bind with DNA. In view of this, we have synthesized novel Ru(II) isatin complex cis-[Ru^{II} $(Phen)_2$ FPIMI] $ClO_4.2H_2O$ from the reactions between cis-[Ru $(Phen)_2$ Cl_2].$2H_2O$ and 4-fluoro phenyl imino methyl isatin(FPIMI). The new complex was analyzed by elemental analysis and spectral (UV-Vis, FTIR, and ^1H NMR) studies. Further, the complex was subjected to DNA intercalation studies, *in vitro* anticancer studies and cytotoxic activities. DNA intercalative assay was followed by UV-Vis spectral titration studies with relatively high DNA binding constant, K_b=4.1±2 10^5 M^{-1} at room temperature. The above studies showed intercalative mode of cleavage with Calf thymus(CT) DNA. The newly synthesized ruthenium(II) complex exhibited more effective *in vitro* cytotoxic activity against selected cell lines with IC_{50} value 26±0.5 µM. Also, the anticancer activity of the compound is apparently four times more potent than one of the presently used clinical drug Cisplatin against human tumor cell lines with less cytotoxicity.

Keywords: Isatin hydrazone, Schiff base, Ruthenium(II) Complex, DNA Binding, Anticancer, Cytotoxic Evaluation and Tumor Cell Lines.

INTRODUCTION

Chelating compounds have been used to probe DNA as agents for mediation of strand scission of duplex DNA and as chemotherapeutic agents.[1-7] Metal chelates of 1,10–phenanthroline (phen) or modified phenanthroline ligands are particularly attractive for developing a new class of therapeutic agents that can cleave DNA.[8-14] Ruthenium complexes having phenanthroline/bipyridyl along with other co–ligands such as hydride, halides and CO have been studied extensively.[15-17] Ruthenium phen and/or bpy complexes have been extensively investigated because of their interesting biological properties. The success of cisplatin and related platinum complexes as anticancer agents has created interest of search for more effective and curative transition metal complexes and ruthenium, in particular, has attracted the researchers due to its less toxicity. By comparing the general toxicity of ruthenium compounds with platinum drugs the ruthenium has low toxicity, that it has been attributed to the ability of ruthenium compounds to specific accumulation in cancer tissues. The specificity of ruthenium compounds for their targets is also be linked due to the selective absorption by the tumor cells compared to the healthy tissue.

EXPERIMENTAL

The solvents are used after purification. All solvents are used at different stages of this project were purified based on the standard procedures described either by Weiss Berger series or according to a

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qualitative analysis by Vogel.[18,19] The solvents used are AR grades, the reagents are obtained from E. Merk and Loba Chemie. All the reagents are used as received. UV-Visible spectral measurements were recorded using JASCO V-550 UV-vis spectrophotometer. Infrared spectra were recorded for the ligand and the complex on a JASCO FTIR 410 (4000-400) spectrophotometer. KBr disc was used for sample preparation. The instrument was standardized/calibrated against polystyrene film. ^{1}H NMR spectra were recorded in $CDCl_3$ with TMS as an internal standard on a Joel GSX-400/500 MHz, FT NMR spectrophotometer.

DNA Binding Experiment

Preparation of Tris-hydrochloride Buffer Solution

Tris-HCl (197 mg, 5 mM) and NaCl (730 mg, 50 mM) were weighed accurately and made up to 250 mL in a Standard Measuring Flask using double distilled water. The pH of this solution was adjusted to 7.2 using 1 mM NaOH solution with the help of pH meter (EUTECH INSTRUMENT, pH 510) before making up. This buffer pH 7.2 was used for all DNA studies.

UV-Visible Absorption Spectral Titration

DNA binding titration was carried out on a CARY 100 UV-Visible spectrometer at 25°C. The solution of Calf thymus DNA (SISCO) in the Tris-buffer (5 mM Tris and 50 mM NaCl, pH 7.2) gave the absorbance ratio at 260 and 280 nm is above 1.8, shows that the DNA is free of protein.[20] The concentration of DNA was determined using an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm and a stock of 100 μM is prepared.[21] All experiments were carried out in Tris buffer of pH 7.2 in milli Q triply deionized water.

Synthesis of Isatin Based Ligand (FPIMI)

The ligand FPIMI (4-fluro phenyl imino methyl isatin) was prepared by refluxing isatin with 4-fluoro aniline in alcohol for 2hr and kept overnight. The solid obtained is separated, dried and finally recrystallized.

Synthesis of Ruthenium Starting Material

Ruthenium trichloride and 1,10 phenonthroline were reflexed in dimethyl formamide, the dark green solid separated is Cis- $[Ru(Phen)_2Cl_2]\cdot 2H_2O$.

Synthesis of Cis-$[Ru^{II}(Phen)_2(FPIMI)]ClO_2 \bullet 2H_2O$

The starting complex Cis-$[Ru(Phen)_2Cl_2]\cdot 2H_2O$ (1.0 mM) in dry ethanol was added with $AgNO_3$. The AgCl formed is removed, the hydrazine ligand (1.0 mM) & sodium acetate was added, refluxed, the brownish solid of the compound cis-$[Ru^{II}(Phen)_2FPIMI]ClO_4\cdot 2H_2O$ formed was separated and dried in vacuo over $CaCl_2$. The product obtained was checked by TLC on silica plates and then purified using column chromatography. Silica gel as the stationary phase and chloroform and ethanol as mobile phase(Scheme-1).

RESULTS AND DISCUSSION

The complex formed is amorphous powder, completely soluble in dimethyl formamide(DMF) & dimethyl sulphoxide(DMSO), sparingly soluble in methyl cyanide & chloroform and insoluble in water. The electronic spectra of the compound showed metal to ligand charge transfer (MLCT) transition at 438 nm. The IR and NMR spectral data of the ligand and its Ruthenium (II) compound is more helpful to determine the mode of coordination to the central ruthenium atom and the structure formed is octahedral.

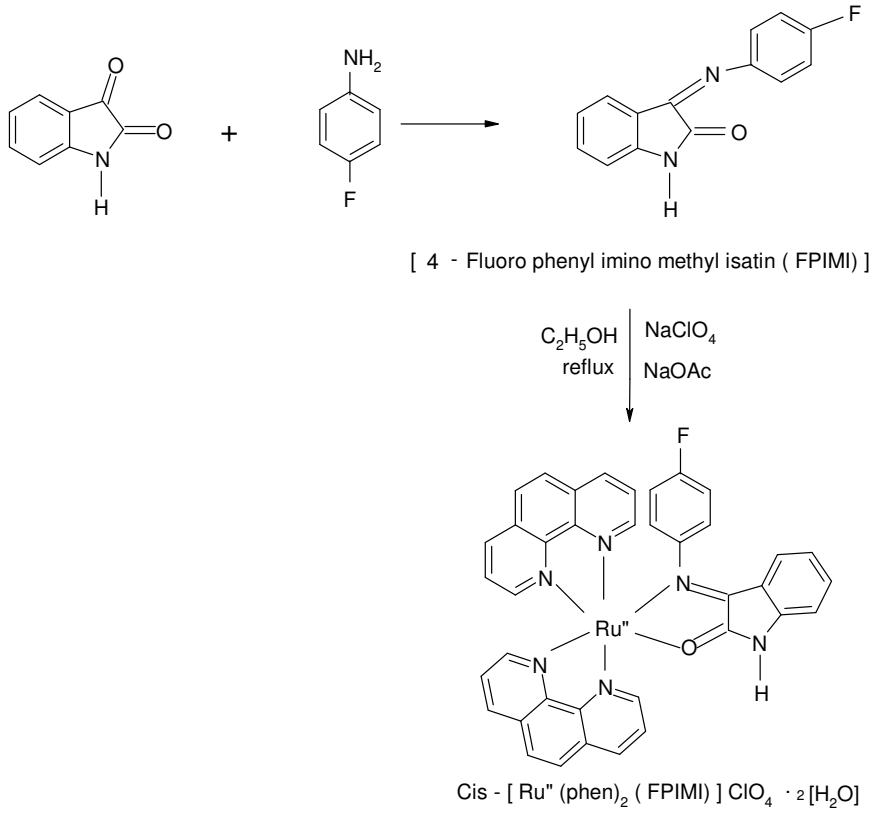
Biological Activity

Evaluation of Therapeutic Effect *in Vitro* Protocol for Testing

The complex is tested on 24-well trays. Cells are allowed in a flask, the grown-up cells are harvested just before they become confluent, counted by haemocytometer and diluted up to a concentration of l04 cells per mL. The cells are then seeded in the 24-well trays at a density of l04 cells per well in which 1 mL of the diluted cell suspension is added to each well. The cells are then grown for 72 hrs before adding the Ru compound. The ruthenium complex is weighed and made up in such a way that the 1 mg/mL with 1%

DMSO. The appropriate volume of the ruthenium complex solution is added to 5mL of media to make it up to a concentration of 100 µM for the drug. This 100 µM solution is again diluted as 10 µM, 1 uM and 0.1 µM solutions. The media is then removed from the cells and replaced with 1mL of the media dosed with the synthesized complex. Each plate, containing media and DMSO without adding drug is placed as control.

The cells are allowed to expose to the drugs for one day and then washed with phosphate buffer before adding fresh media and are allowed to grow further for 3 days before being counted by Coulter counter.



Scheme-1: Synthesis of Rutheniun(II) Isatin Based Schiff Base Complex

Preparation of Cells for Counting

1 mL of PBS is added to the cells after removing the media. 250 µL of trypsin is added and cells kept in an incubator for few minutes to allow the monolayers to detach and then trypsinised. 250 µL of media is added to neutralize the trypsin. 200 µL of this suspension is added to 10 mL of sodium chloride solution for counting.

Cytotoxic Evaluation

The *in vitro* cytotoxicity was investigated for the synthesized ligand and its ruthenium compound using human cell lines CEM. From the results of the determinations it is understood that the ruthenium compounds are more potent than its ligand. Also the Ruthenium (II) mononuclear bidentate compound cis-[$Ru^{II}(Phen)_2FPIMI]$ $ClO_4 \cdot 2H_2O$ have a significant cytotoxic effect (IC_{50} 26±0.5 µM).

DNA Interaction Studies

UV-Visible Absorption Spectral Titration

The binding of drug with DNA helix has been extensively studied using absorption spectral titration by observing the changes in absorbance.[22] In the present study, the interaction of cis-

$[Ru^{II}(Phen)_2FPIMI]ClO_4{\cdot}2H_2O$ complex in CH_3CN-Tris buffer solution with calf thymus (CT) DNA has been monitored. The gradual addition of calf thymus DNA to the complex shows hypochromism i.e a decrease in molar absorptivity with a slight red shift in their $\pi - \pi^*$ absorption band. This redshift indicates that the complex is binding with DNA. Also, it has been observed by large hypochromism, due to the interaction between the DNA base pairs and aromatic chromophore of the ligand.[23-25] Though there is a red-shift and hypochromism observed for the complex, which is lower than those observed for classical intercalators like ethidium bromide, which indicates that the binding of drug with DNA is purely electrostatic.

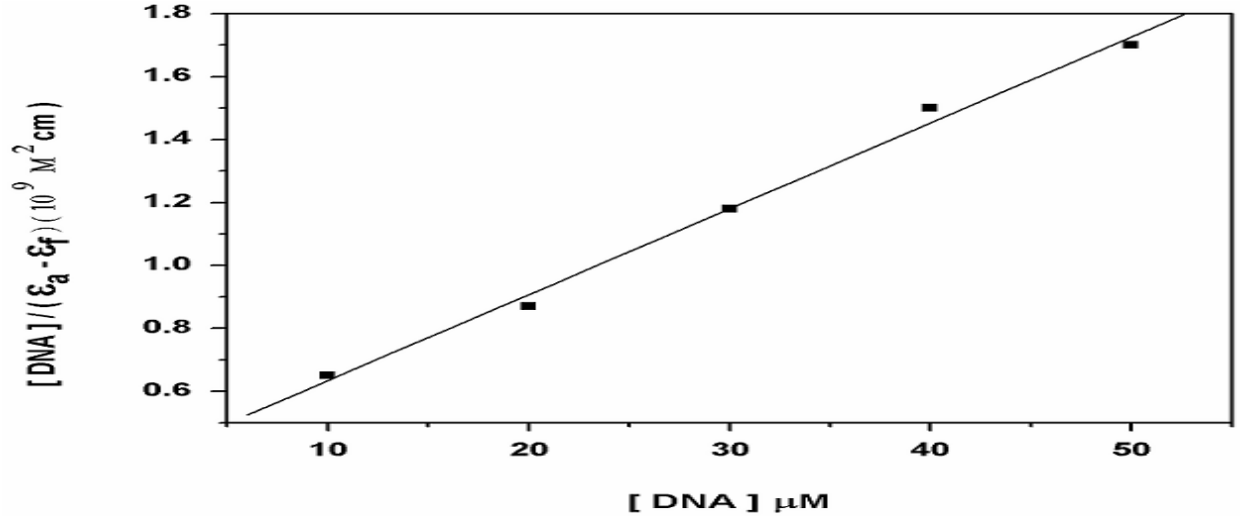


Fig.-1: Plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs [DNA] for the Absorption Spectral Titration of DNA (10, 20, 30, 40 & 50 µM) with $Cis[Ru^{II}(Phen)_2(FPIMI)]ClO_2{\bullet}2H_2O$ (5 µM)

The quantitative comparison of the DNA binding affinity of the complex, the intrinsic binding constants K_b of the complexes for binding with CT DNA were evaluated by using equation (1).[26]

$$[\ DNA\ ] / (\ \varepsilon_A - \varepsilon_f\ ) = [\ DNA\ ] / (\ \varepsilon_b - \varepsilon_f\ ) + 1 / K_b\ (\ \varepsilon_b - \varepsilon_f\ ) \qquad (1)$$

Where, ε_A, ε_f and ε_b correspond to A_{obsd} / [Ru], the extinction coefficient for the free ruthenium complex and the extinction co-efficient for the complex in the fully bound form respectively.

A plot of [DNA] Vs [DNA] / ($\varepsilon A - \varepsilon f$) gave a straight line (Fig. 1) with a slope of $1 / (\varepsilon_b - \varepsilon_f)$ and an intercept of $1 / K_b (\varepsilon_b - \varepsilon_f)$. The intrinsic binding constant K_b was determined using the ratio of the slope to intercept. The calculated K_b values are in the order of $4.1 \pm 2 \times 10^5\ M^{-1}$ which are found to be lower than those observed for classical intercalators namely ethidium-DNA with $K = 7 \times 10^7\ M^-$ indicates the complete insertion of the planar ethidium molecules between the base pairs of DNA. The K_b value indicates that the binding of the complex with DNA affinity is less than the classical intercalators, but it has an external binding involves both placements of the ligand in the grooves and electrostatic interaction with the phosphate backbone of DNA helix. Though the K_b values are lower than the typical chelators, the synthesized complex has less systemic cytotoxicity.

CONCLUSION

In conclusion, the ligand (FPIMI) and its isatin based ruthenium (II) complex have been synthesized and extensively characterized by (UV - Vis, FTIR and ^1H NMR) spectral studies. The results showed that the complex formed is octahedral in structure. The results revealed that the synthesized compound exhibited a remarkable inhibitory effect on human tumor cell lines at micromolar concentrations with low cytotoxicity. Also, the compound is more potent than its ligand.

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