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Synthesis, Characterization and DNA Binding Study of Ruthentum(II) Thiosemicarbazone Complexes

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Abstract

Four ruthenium(II) complexes of the type [RuCl(CO)(py)(PPh_3)L] (L= thiosemicarbazone ligand) have been synthesized and the structural features were determined by various physico-chemical and spectral techniques. The thiosemicarbazone ligands act as bidentate, monobasic chelating ligands with S and N as the donor sites and are preferably found in thiol form in all the complexes studied. In order to ascertain the potential of the above synthesized complexes towards biomolecular interactions, titration experiments involving the interaction with CT-DNA were carried out by absorption spectroscopy which revealed that the ruthenium(II) complexes bind to DNA via intercalation.

***Keywords**: DNA binding; pyridine complexes; Ruthenium(II) complexes.*

1. INTRODUCTION

DNA binding metal complexes have been extensively studied as DNA structural probes, DNA-dependent electron probes and so on during the past decade. Numerous biological experiments have also demonstrated that DNA is the primary intracellular target of anticancer drugs due to the interaction between small molecules and DNA, which can cause DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death (Xu *et al*. 2008). Several ruthenium compounds have been shown to inhibit DNA replication, possess mutagenic activity, induce SOS repair, bind to nuclear DNA and reduce RNA synthesis, which are all consistent with DNA binding of these compounds *in vivo*. Moreover, the ruthenium based drugs, NAMI-A and KP1019 have entered into clinical trials for the treatment of metastatic tumors. Thus, by analogy to platinum antitumor drugs, DNA interactions of antitumor ruthenium agents are of a great interest.

Thiosemicarbazones are versatile ligands and the chemical interest arises from the ability of thiosemicarbazones to adopt various coordination modes, leading to enormous structural diversity. Also, the thiosemicarbazones possess a variety of biological properties including antiproliferative activity. Studies have demonstrated that thiosemicarbazones are potent inhibitors of the enzyme ribonucleotide reductase and are capable of interrupting DNA synthesis and repair. Incorporation of metals onto these thiosemicarbazone ligands can result in alteration or enhancement of their biological activity. In the case of pharmaceuticals, the

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binding capacity of thiosemicarbazones is further increased by condensation of the thiosemicarbazide with an aldehyde containing heteroatom (Prabhu *et al.* 2009). Hence, in view of the aforementioned facts, the present article embodies the various spectroscopic characterization of ruthenium(II) complexes containing 2-choloro/nitro benzaldehyde 4-methyl/ phenyl-3-thiosemicarbazone ligands. Further, the biological experiments on DNA binding study of all the synthesized complexes were also carried out.

2. EXPERIMENTAL

2.1 Materials & Methods

All the chemicals used were chemically pure and AR grade. Solvents were purified and dried according to the standard procedure. Calf-thymus (CT-DNA) was purchased from Bangalore Genei, Bangalore, India. The metal precursor, $[RuHCl(CO)py(PPh_3)_3]$ and thiosemicarbazone ligands, HL^1-HL^4, were prepared by literature methods (Sampath *et al* 2013). Micro analyses (C, H, N & S) were performed on a Vario EL III CHNS analyser at STIC, Cochin University of Science and Technology, Kerala, India. IR spectra were recorded as KBr pellets in the 400-4000 cm^{-1} region using a Perkin Elmer FT-IR 8000 spectrophotometer. Electronic spectra were recorded in DMSO solution with a Systronics double beam UV-vis spectrophotometer 2202 in the range 200-800nm. ^1H and ^{31}P NMR spectra were recorded on a Bruker AV III 500 MHZ instrument using TMS and ortho phosphoric acid as an internal references respectively. Melting points were recorded with Veego VMP-DS heating table and are uncorrected.

2.2 Synthesis of ruthenium (II) thiosemicarbazone complexes

A methanolic solution (20 ml) containing thiosemicarbazone ligands (0.110-0.150 g, 0.5 mmol) was added to $[RuHCl(CO)py(PPh_3)_2]$ (0.380 g, 0.5 mmol) in benzene (20ml). The resulting solution was refluxed for 8 h. The reaction mixture was then cooled to room temperature, which results in the formation of precipitate. It was filtered off and the purity of the complexes was checked by TLC. This solid was recrystallized from CH_2Cl_2/Hexane mixture. Our sincere effort to obtain single crystal of the complexes went unsuccessful.

$[RuCl(CO)py(PPh_3)L^1]$ ***(1)***

Color: red. Yield: 54%. M.P: 262°C. Anal. calcd. For $C_{33}H_{29}Cl_2N_4OPRuS$ (%): C, 54.10; H, 3.99; N, 7.65; S, 4.38. Found (%): C, 54.67; H, 4.08; N, 7.55; S, 4.23. IR (KBr, cm^{-1}): 1501 ν(C=N); 743 ν(C–S); 1948 ν(C≡O). UV-vis (DMSO), λmax (nm): 310, 366 (ILCT), 411 (MLCT). ^1H NMR (DMSO-d_6): δ 8.62 (s, 1H, methyl NH); δ 12.11 (s, 1H, H–C=N); δ 6.75-8.56 (m, 24H, aromatic). ^{31}P NMR (DMSO-d_6): δ 36.1 (PPh_3).

$[RuCl(CO)py(PPh_3)L^2]$ ***(2)***

Color: Brown. Yield: 56%. M.P: 271°C. Anal. calcd. For $C_{33}H_{29}ClN_5O_3PRuS$ (%): C, 53.33; H, 3.93; N, 9.42; S, 4.31. Found (%): C, 53.35; H, 3.82; N, 9.92; S, 4.43. IR (KBr, cm^{-1}): 1551 ν(C=N); 752 ν(C–S); 1923 ν(C≡O). UV-vis (DMSO), λmax (nm): 312, 368, 408 (ILCT), 431 (MLCT). ^1H NMR (DMSO-d_6): δ 8.56 (s, 1H, methyl NH); δ 12.08 (s, 1H, H–C=N); δ 6.99-8.77 (m, 24H, aromatic). ^{31}P NMR (DMSO-d_6): δ 37.5 (PPh_3).

$[RuCl(CO)py(PPh_3)L^3]$ ***(3)***

Color: Orange. Yield: 52%. M. P: 284 °C. Anal. calcd. for $C_{38}H_{31}Cl_2N_4OPRuS$ (%): C, 57.43; H, 3.93; N, 7.05; S, 4.03. Found (%): C, 57.12; H, 4.20; N, 7.82; S, 4.58. IR (KBr, cm^{-1}): 1556 ν(C=N); 735 ν(C–S); 1956 ν(C≡O). UV-vis (DMSO), λmax (nm): 315, 369, (ILCT), 402 (MLCT). ^1H NMR (DMSO-d_6):

δ 8.57 (s, 1H, phenyl NH); δ12.16 (s, 1H, H–C=N); δ 6.85-8.66 (m, 29H, aromatic). ^{31}P NMR (DMSO-d_6): δ 37.8 (PPh_3).

[RuCl(CO)py(PPh_3)L^4] ***(4)***

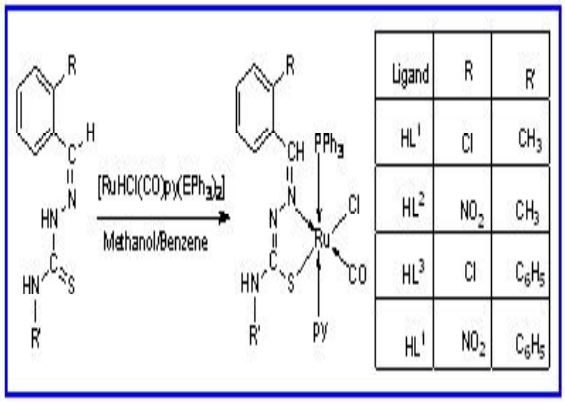
Color: Brown. Yield: 55%. M.P: 255 °C. Anal. calcd. for $C_{38}H_{31}ClN_5O_3PRuS$ (%): C, 56.68; H, 3.88; N, 8.70; S, 3.98. Found (%): C, 56.71; H, 3.46; N, 8.65; S, 3.49. IR (KBr, cm^{-1}): 1568 ν(C=N); 743 ν(C–S); 1935 ν(C≡O). UV-vis (DMSO), λmax (nm): 312, 368, 405 (ILCT), 432 (MLCT). ^{1}H NMR (DMSO-d_6): δ 8.66 (s, 1H, phenyl NH); δ 12.25 (s, 1H, H–C=N); δ 6.71-8.88 (m, 29H, aromatic). ^{31}P NMR (DMSO-d_6): δ 36.6 (PPh_3).

2.3 DNA binding - Titration experiments

All the experiments involving the binding of complexes with CT-DNA were carried out in a doubly distilled water buffer with tris(hydroxymethyl)-aminomethane (Tris, 5 mM) and sodium chloride (50 mM) and adjusted to pH 7.2 with hydrochloric acid. A solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.8-1.9 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. The CT-DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 $M^{-1}cm^{-1}$ at 260 nm. The complexes were dissolved in a mixed solvent of 5% DMSO and 95% Tris-HCl buffer. Stock solutions were stored at 4 °C and used within 4 days. Absorption titration experiments were performed with fixed concentrations of the complexes (25 µM) with varying concentration of DNA (0-50 µM). While measuring the absorption spectra, an equal amount of DNA was added to both the test solution and the reference solution to eliminate the absorbance of DNA itself.

3. RESULTS & DISCUSSION

Analytical and spectroscopic data for the ligands and its complexes indicate a 1:1 metal-ligand stoichiometry for all the complexes. The synthetic route of the complexes and the proposed structure of the complexes are shown in Scheme 1. The complexes are soluble in most common organic solvents like CH_2Cl_2, $CHCl_3$, DMF, DMSO, etc.



Scheme 1 Synthetic route of the ruthenium (II) thiosemicarbazone complexes, where PPh_3 = triphenylphosphine; py = pyridine.

3.1 Infrared spectra

The IR bands for the metal complexes derived from the benzaldehyde thiosemicarbazone ligands are most useful in attempting to determine the mode of coordination. A medium sharp band at 1530-1599 cm^{-1} due to the azomethine C=N stretching frequency of the free ligands was shifted to lower frequency in the spectra of the complexes at 1501-1568 cm^{-1} indicating that the coordination through N atom. A band appeared at 846-951 cm^{-1} for free ligands due to vibration of the C=S double bond which disappeared in the spectra of the complexes and a new band, C–S appeared at 735-752 cm^{-1} indicating that the other coordination is through thiolate sulphur after

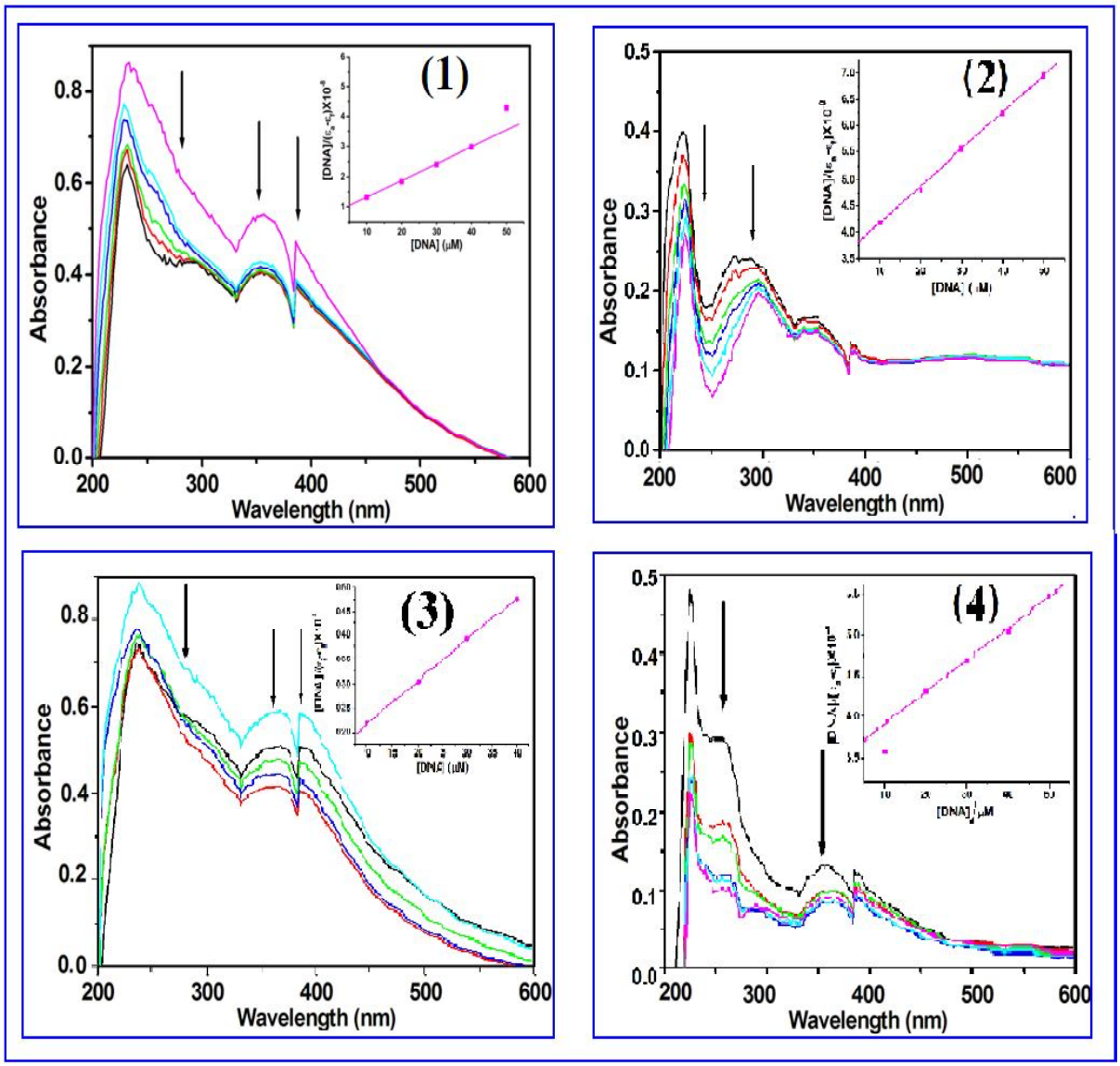


Fig. 1: Electronic spectra of complexes 1, 2, 3 and 4 in Tris-HCl buffer upon addition of CT-DNA. [Complex] = 25 μM, [DNA] = 0-50 μM. Arrow shows the absorption intensities decrease upon increasing DNA concentrations (Inset: Plot between [DNA] and [DNA]/ [$å_a$-$å_f$] X 10^{-8}).

enolization followed by deprotonation on sulphur (Mahalingam *et al.* 2010). In all the complexes, the strong band in the region 1923-1956 cm^{-1} and 1119-1126 cm^{-1} are due to coordinated carbonyl group and nitrogen base respectively. Overall, the complexes contain monobasic NS coordinated thiosemicarbazones. In addition, the characteristic absorption bands due to triphenylphosphine are also observed for all the complexes in their expected regions.

3.2 Electronic spectra

The electronic spectra of the complexes showed three to four bands in the region 310-432nm. The lower wavelength bands (310-315 and 366-408 nm) are characterized as ligand centered transitions occurring within the ligand orbitals. These bands are shifted when compared to ligands indicating the involvement of imine nitrogen and thionyl sulphur in coordination with ruthenium atom (Mahalingam *et al.* 2010). The bands appearing in the region 402-432 nm are assigned to charge transfer transitions arising from the excitation of an electron from metal t_{2g} level to an unfilled molecular orbital derived from the π* level of the ligands. The pattern of the electronic spectra for the complexes are similar to other ruthenium(II) octahedral complexes (Sathiyaraj *et al.* 2011).

3.3 ^{1}H and ^{31}P NMR spectra

The NMR spectra of the complexes were recorded in DMSO-d_6 solution for confirming the binding mode of the ligands to the ruthenium. In the ^{1}H NMR spectra, the hydrazine NH proton of the free ligands (δ 8.47-10.20) was absent indicating the enolization and deprotonation of the –NH–C=S group prior to coordination of ligand to metal through thiolate sulphur (Mahalingam *et al.* 2010). The –CH=N proton at δ 11.67-12.12 for the ligands, was shifted slightly downfield in the spectra of the complexes in the region δ 12.08-12.25. This observation supports the involvement of C=N chromophore in coordination. The aromatic protons, as multiplets in the region δ 7.21-8.47, of the ligands, are remain more or less unchanged in the complexes in the region δ 6.71-8.88, due to the delocalisation of electron density in the system and these signals in the complexes cannot be distinguished from the aromatic signals of PPh_3/py due to their extensive overlap. The chemical shifts were observed at δ 8.56-8.62 and δ 8.57-8.66 due to methyl and phenyl NH protons respectively.

In order to confirm the presence of triphenylphosphine group, ^{31}P NMR spectra were recorded. A sharp singlet for all the complexes was observed at δ 36.1-37.8 confirms the presence of only one phosphine group.

3.4 DNA binding - Titration experiments

Electronic absorption spectroscopy is one of the most useful techniques for DNA-binding studies of metal complexes. A complex bound to DNA through intercalation is characterized by the change in absorbance (hypochromism) and red shift in wavelength, due to the intercalative mode involving a stacking interaction between the aromatic chromophore and the DNA base pairs (Jadeja *et al.* 2012). The results of absorption spectra of the complexes in the absence and presence of CT-DNA are given in Fig. 1. Upon increasing the concentration of DNA to the test complexes, the absorption bands of the complexes, 1 and 2 exhibited hypochromism of 22.15% and 18.72% with red shifts of 2 nm at 351 and 298nm, respectively, whereas the absorption bands of complexes 3 and 4 exhibited a hypochromism of about 30.10 % at 352 nm and 26.64% at 353 nm with red shifts of 6 nm, respectively. These results suggested an intimate association of the complexes with CT-DNA, and it is also likely that these complexes bind to the DNA helix *via* intercalation. After the complexes intercalate to the base pairs of DNA, the π* orbital of the intercalated complexes could couple with π orbitals of the base pairs, thus decreasing the $\pi \rightarrow \pi^*$ transition energies, hence

resulting in hypochromism (Raja *et al.* 2011). In order to compare quantitatively the binding strength of the complexes, the intrinsic binding constants (K_b) of them with CT-DNA were determined from the following equation.

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b\,(\varepsilon_b - \varepsilon_f)$$

Where [DNA] is the concentration of DNA in base pairs and the apparent absorption coefficient ε_a, ε_f and ε_b correspond to A_{obs}/[complex], the extinction coefficient of the free complex and the extinction coefficient of the complex when fully bound to DNA, respectively. The plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA] gave a slope and the intercept which are equal to 1/($\varepsilon_b - \varepsilon_f$) and 1/$K_b$ ($\varepsilon_b - \varepsilon_f$), respectively; K_b is the ratio of the slope to the intercept. The magnitudes of intrinsic binding constants(K_b) were calculated to be $1.5x10^5$ M^{-1}, $1.0x10^5 M^{-1}$, $3.2x10^5$ M^{-1} and $2.8x10^5$ M^{-1} for complexes 1, 2, 3 and 4 respectively. The observed values of K_b revealed that the ruthenium(II) complexes (1-4) bind strongly than the respective ligands to DNA *via* intercalative mode. From the results obtained, it has been found that complex 3 strongly binds with CT-DNA in comparison to that with 1, 2 and 4, and binding affinity was in the order 3 > 4 > 1 > 2. The K_b values obtained for the above ruthenium(II) complexes are comparable to those of the other known ruthenium(II) complexes (Sampath *et al.* 2013 and Sathiyaraj *et al.* 2011).

4. CONCLUSION

In this work, a systematic approach to the synthesis of mononuclear ruthenium(II) complexes containing benzaldehyde N(4)-substituted thiosemicarbazones were carried out and, were characterized by various spectroscopic techniques. An octahedral geometry has been tentatively assigned for all the complexes. The DNA binding ability of the complexes was assessed by absorption spectra which inferred an intercalative mode of binding with binding constants ranging from 1.0-3.2 x 10^5 M^{-1}. The experimental results suggested that the complex 3 can bind to DNA more strongly than the other three complexes.

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