DNA interaction, in vitro antibacterial and cytotoxic activities of Ru(III) heterochelates

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Abstract

Ruthenium(III) complexes [Ru(bphtpy)(PPh_3)$_2Cl_3$] (bphfpy = biphenyl furanyl pyridine derivatives) were synthesized and characterized by LCMS, IR spectroscopy, elemental analysis and magnetic measurements. All the complexes were screened for their anti- bacterial activity in terms of minimum inhibitory concentration against two gram$^{(+ve)}$ and three gram$^{(-ve)}$ bacterial species. DNA binding study by absorption titration and viscosity measurement shows that complexes binds in an intercalating mode, which is also confirmed by molecular docking. All the complexes were also screened for the DNA nuclease property of pUC19 plasmid DNA. The cytotoxicity study of the synthesized complexes was performed to elucidate the LC_{50} values to find out toxicity profile of the complexes.

Keywords: N,O –donor ligand; Ruthenium(III)complexes; DNA interaction; Cytotoxicity

1. Introduction

A great history of transition metal complexes is associated with their effectiveness in the numerous diseases cure,[1-3] including the major application in the field of novel anticancer drug discovery. The interaction of coordination compounds with various biomolecules is facilitate due to varying oxidation states of central metal ion, which can eventually results into surprising pharmacological and exceptional curative properties.[4-8] Metals can alter the physiological condition and the toxicity of metals can be reduced by their coordination with ligands. The biological properties of ligands increases with metal chelation, and causes synergistic effect on both ligand and metal ion.[9] Recently large interest has been drawn on the ruthenium based coordination compounds in anti–cancer drug development.[10-12]

Ruthenium can be seen as a hopeful metal after platinum due to its kinetics and timescales comparison to cellular division processes similar to platinum,[13] while lower toxicity due to its iron mimic ability.[14] Ruthenium complexes with N,N –donor ligand have

found significant application as metallo-intercalators.[15, 16] Changing substituent groups in the ligand can create electron density distribution and space configuration differences of complexes, resulting in a diverse spectral properties and biological activities.[17, 18] The N,O – donor ligand has been selected owing to its antifungal activity exhibited due to furan ring,[19] and the role of bulky co-ligand is to stabilize the complex which prevent quick dissociation of the complex. hence the compound can reach the pharmacological target such as DNA.[20]

Keeping these aspects in mind, we synthesized of ruthenium(III) complexes with PPh_3 and N,O –donor ligand, and studied the antimicrobial activity, DNA interaction study and cytotoxic activity.

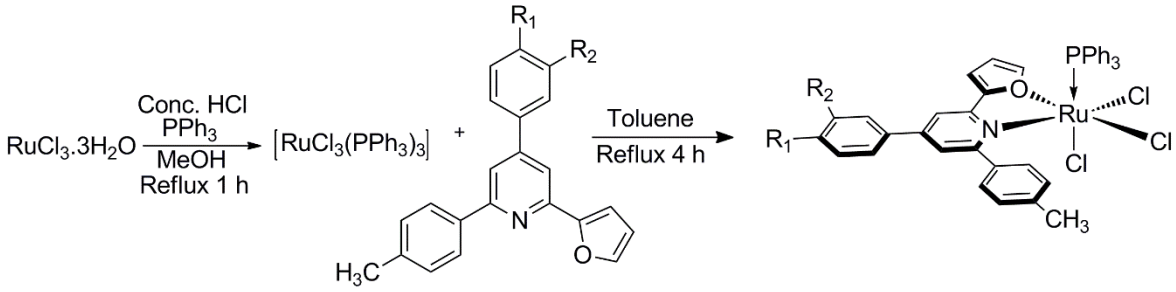
2. Experimental

Material and reagents: The analytical grade chemicals purchased were used as such without further purification. $RuCl_3.3H_2O$, 4-chlorobenzaldehyde, 2-acetylfuran, 4-fluorobenzaldehyde, 4-bromobenzaldehyde, 3-chlorobenzaldehye, 3-fluorobenzaldehyde, 3-bromobenzaldehyde and HS-DNA were purchased from Sigma Chemical Co., India. Bromophenol blue, Ethidium bromide (EB), Luria Broth and agarose were purchased from Himedia, India.

Synthesis of ligands: The ligands (L^1–L^6) were synthesized according to the reported method using modified Krohnke pyridine synthesis method.[21] The synthesis and characterization of ligands are provided in the supplementary data 1.

Synthesis of Ru(III) complexes (1-6): $[RuCl_3(PPh_3)_3]$ was prepared by refluxing $RuCl_3.3H_2O$ (1 mol) methanolic solution with PPh_3 (3 mol) and conc. HCl (60 mL) for 1 h. The obtained reddish brown precipitates were filtered, dried and recrystallized by using hot methanol.

Synthesis of $[Ru(L^1)(PPh_3)Cl_3]$ (1): It was synthesized by refluxing $[RuCl_3(PPh_3)_3]$ (0.1 mmol) solution in toluene and a methanolic solution of 4-(4-fluorophenyl)-2-(furan-2-yl)-6-*p*-tolylpyridine **$[L^1]$** (0.1 mmol) for 4 h. (Scheme 1). The blackish brown product obtained was washed with toluene to remove unreacted precursor and next washed with methanol to remove unreacted ligand and dried under vacuum. Yield: 22 %, m.p.: 287-290 ºC, μ_{eff}: 1.89 B.M. Anal. Calc. for: $C_{40}H_{31}Cl_3FNOPRu$ (799.08): Calc. (%):C, 60.12; H, 3.91;N, 1.75; Ru,12.65. Found (%): C, 60.03; H, 3.93; N, 1.70, $Ru_{(gravimetrically)}$, 12.60.UV–Vis λ_{max}(nm) (In DMSO): 560, 420, 260.



Complex	R_1	R_2
1	F	H
2	Cl	H
3	Br	H
4	H	F
5	H	Cl
6	H	Br

Scheme 1. Synthesis of ruthenium(III) complexes.

Synthesis of [Ru(L^2)(PPh_3)Cl_3] (2): It was synthesized using 4-(4-chlorophenyl)-2-(furan-2-yl)-6-*p*-tolylpyridine **[L^2]**. Yield: 19.9%, m.p.: 274-276 ºC, μ_{eff}: 1.81 B.M. Anal. Calc. for: $C_{40}H_{31}Cl_4NOPRu$ (815.54): Calc. (%):C, 58.91; H, 3.83; N, 1.72; Ru, 12.39. Found (%): C, 58.84; H, 3.89; N, 1.78, $Ru_{(gravimetrically)}$, 12.37. UV–Vis λ_{max}(nm) (In DMSO): 565, 425, 261.

Synthesis of [Ru(L^3)(PPh_3)Cl_3] (3): It was synthesized using 4-(4-bromophenyl)-2-(furan-2-yl)-6-*p*-tolylpyridine **[L^3]**. Yield: 20.1%, m.p. >300 ºC, μ_{eff}: 1.84 B.M. Anal. Calc. for: $C_{40}H_{31}Cl_3BrNOPRu$ (859.99): Calc. (%):C, 55.86; H, 3.63; N, 1.63; Ru,11.75. Found (%): C, 55.78; H, 3.64; N, 1.66, $Ru_{(gravimetrically)}$,11.79. UV–Vis λ_{max}(nm) (In DMSO): 575, 430, 263.

Synthesis of [Ru(L^4)(PPh_3)Cl_3] (4): It was synthesized using 4-(3-fluorophenyl)-2-(furan-2-yl)-6-*p*-tolylpyridine **[L^4]**. Yield: 18.4%, m.p.: 286-290 ºC, μ_{eff}: 1.86 B.M. Anal. Calc. for: $C_{40}H_{31}Cl_3FNOPRu$ (799.08): Calc. (%): C, 60.12; H, 3.91; N, 1.75; Ru,12.65. Found (%): C, 60.13; H, 3.97; N, 1.68, $Ru_{(gravimetrically)}$, 12.72. UV–Vis λ_{max}(nm) (In DMSO): 563, 420, 260.

Synthesis of [Ru(L^5)(PPh_3)Cl_3] (5): It was synthesized using 4-(3-chlorophenyl)-2-(furan-2-yl)-6-*p*-tolylpyridine **[L^5]**. Yield: 17%, m.p.: 276-278 ºC, μ_{eff}: 1.80 B.M. Anal. Calc. for: $C_{40}H_{31}Cl_4NOPRu$ (815.54): Calc. (%): C, 58.91; H, 3.83; N, 1.72; Ru, 12.39. Found (%): C, 58.89; H, 3.79; N, 1.68, $Ru_{(gravimetrically)}$, 12.39. UV–Vis λ_{max}(nm) (In DMSO): 570, 428, 262.

Synthesis of [Ru(PPh_3)(L^6)(Cl_3] (6): It was synthesized using 4-(3-bromophenyl)-2-(furan-2-yl)-6-*p*-tolylpyridine **[L^6]**. Yield: 19%, m.p.: >300 ºC, μ_{eff}: 1.89 B.M. Anal. Calc. for: $C_{40}H_{31}Cl_3BrNOPRu$ (859.99): Calc. (%):C, 55.86; H, 3.63; N, 1.63; Ru, 11.75. Found (%): C, 55.84; H, 3.60; N, 1.61, $Ru_{(gravimetrically)}$, 11.70. UV–Vis λ_{max}(nm) (In DMSO): 571, 424, 262.

***In vitro* antibacterial screening:** *In vitro* antibacterial study of all compounds was performed against three gram$^{(-ve)}$ and two gram$^{(+ve)}$ bacteria according to the literature method.[22]

DNA interaction study: Metal–DNA interactions was probed using electronic absorption titration and viscosity measurement, according to the literature method.[23] The molecular docking study was performed by HEX 8.0 software.[24]

Cytotoxicity study: The Brine shrimp lethality activity (BSLA) test was carried out referring the protocol of Mayer *et al*.[22]

Gel electrophoresis study: The DNA cleavage study for synthesized complexes was performed using reported procedure.[25]

3. Result and discussion

Spectral and analytical characterization: The electronic spectra showed three bands in the 260–575 nm region. The bands at 560–575 nm, 420–430 nm, 260 nm region corresponds to d–d transition, metal–to–ligand charge transfer and intra ligand charge transfer, respectively. The magnetic moment of Ru(III) complexes were measured using Gouy's magnetic balance at room temperature. The magnetic moment values were found in the range 1.80–1.89 BM. The theoretical spin–only value is 1.73 BM, which suggests that the metal ion in complexes possess one unpaired electron and possess s = ½ system.

The thermogravimetric curve of complex–**1** (Supplementary data 2) shows no mass loss upto 180ºC signifying the absence of water molecule or any volatile component. First mass loss (13.34%) during 190–260 ºC corresponds to loss of chlorine atoms. Second mass loss (32.72%) during 360–520 ºC corresponds to the loss of PPh_3 moiety. The third mass loss (41.14%) during 610–810 ºC corresponds to the loss of neutral bidentate ligand and leaving behind residual metal oxide.

The complex–**1** mass spectrum shows molecular ion peak at *m/z* = 800.06 (M), 802.06(M+2), 804.07 (M+4) and 806.06 (M+6) (Supplementary data 3), due to presence of covalently bonded three chlorine atoms (with metal ion). The peak observed at *m/z* = 763.09 corresponds to the one Cl atom loss. Other fragments observed are 728.09, 693.12, 470.91,

435.07, 431.07, 398.97, 364.07, 329.08 and 262.11 m/z, for which proposed fragmentation pattern is shown in supplementary data 4.

IR spectral data of ligands and complexes were compared (Supplementary data 5) to investigate the coordination of ligand with ruthenium ion. The ring stretching frequencies of ν(C=N) of ligands (1497–1497 cm^{-1}) were shifted to higher frequencies (1505–1512 cm^{-1}) in metal complex, suggests the metal ion coordination with the nitrogen atoms of heterocycles.[26] The $\nu(C=C)_{ar}$ and $\nu(C–H)_{ar.}$ bands were observed at 1541–1554 cm^{-1} and 3030–3063 cm^{-1}, respectively. Additional bands in metal complexes were observed at 543–563 cm^{-1} and 445–460 cm^{-1} corresponds to ν(Ru–N) and ν(Ru–O), respectively.

***In vitro* antibacterial activity:** The antibiotics resistance among bacteria has become a global problem, which has risen the need of novel antimicrobial agents. The results (Supplementary data 6) of antibacterial screening inferred higher efficiency of ruthenium complexes than the parent ligands and ruthenium salt against tested bacterial species under identical experimental conditions. However the synthesized complexes show lower antibacterial potency compared to standard antibiotic like ofloxacin (MIC = 1.24 - 2.0 μM, for different bacterial species under investigation). The increase in lipophilic nature due to chelation may be the reason for potentiation of antibacterial activity of complexes. The different molecular targets of antibacterial agents for exerting their mode of action are cell wall synthesis and cytoplasmic membrane. The chelation increase the ability of a complex to cross a cell membrane[27] according to the Tweedy's chelation theory,[28] by decreasing the polarity of metal ion through partial sharing of positive charge over chelating atoms.

DNA interaction study:

Absorption titration: The observed absorbance is plotted against wavelength and shift in absorbance and change in wavelength is calculated to investigate the binding mode. In the absorption spectra of $[Ru(L^1)(PPh_3)Cl_3]$ (Figure 1), it is found that upon increasing the DNA concentration, hypochromism is observed in MLCT(around 420 nm) and ILT bands (around 260 nm) with slightly red shift indicative of the intercalative mode of binding. The strength of binding is measured from K_b values obtained using the equation

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

where ε_a, ε_f, and ε_b correspond to A_{obsd}/[complex], extinction coefficient of free complex, and complex in the fully bound form, respectively. The K_b values for complexes 1-6 are found 5.18×10^5, 3.41×10^5, 1.69×10^5, 1.66×10^5, 1.46×10^5 and 1.78×10^5 M^{-1}, respectively. The obtained

K_b values of complexes are found higher than $[Ru(NH_3)_4(dip)]^{2+}$($1.50\times10^4 M^{-1}$),[29] comparable to $[Ru(phen)_2pzip]^{2+}$ (9.5×10^5 M^{-1})[30] and lower than $[Ru(bpy)_2(HBT)]^{2+}$ (5.71×10^7 M^{-1}).[31]

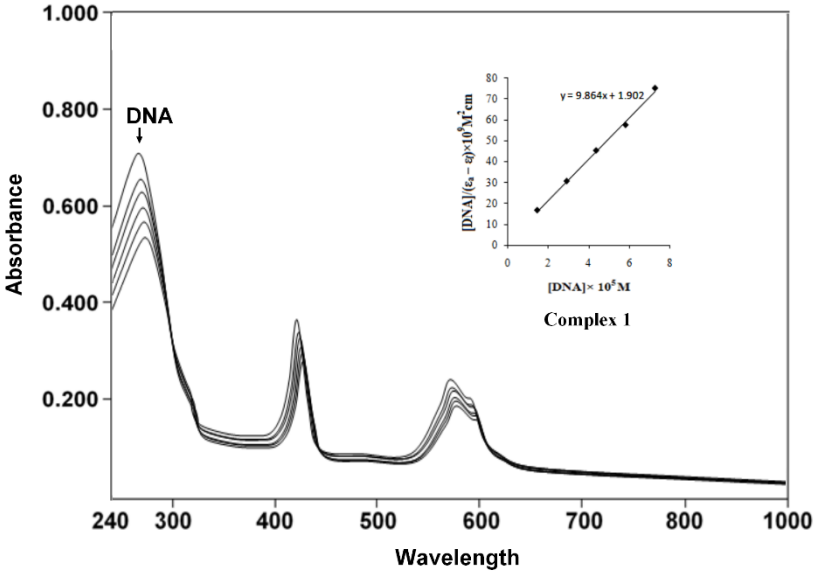


Figure 1. Electronic absorption spectra of complex–**1** with increasing concentration of Herring Sperm DNA (HS–DNA) in phosphate buffer Inset: Plots of [DNA]/($\mathscr{E}_a$–$\mathscr{E}_f$) versus [DNA] for the titration of DNA with ruthenium(III) complexes.

Viscosity measurement: The viscosity of HS–DNA was measured by varying the concentration of the complexes, to further explore the interaction between ruthenium(III) complexes and DNA. The relative viscosity of the HS–DNA increases with complex solution addition (Supplementary 7), suggesting intercalative mode of binding. The curve of complex-1 resembles very similar to EtBr and is much higher in magnitude than other complexes suggesting a strong interactive binding of complex-1 than other synthesized complexes.

Molecular docking study: The molecular docking study was used to discover a new drug in a minimum cost and at a less time by medicinal chemists. To explore the interaction mode and binding affinity, docking studies was performed. The binding interaction of Ru(III) complexes (Figure 2) with duplex DNA sequence $d(ACCGACGTCGGT)_2$ was performed to explore the DNA binding site and complex-DNA helix orientation. Molecular docking study suggests preferentially intercalative mode of complex to DNA interaction, involving stacking interaction. Docked structure showed the complexes fit well in between the stacks of rich A–T base pair region, which may be stabilized through hydrophobic or van der Waal's interaction. The binding energies of DNA-complexes interactions are -326.15, -326.58, -329.95, -325.63, -333.74 and -329.94 kJ mol^{-1} for complexes 1-6, respectively.

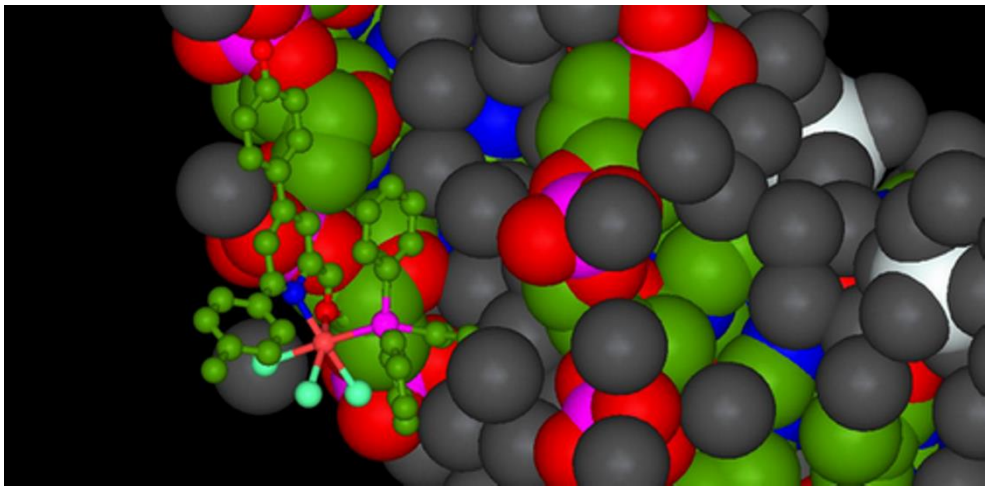


Figure 2. Molecular docking of the complexes 1 (ball and stick) with the DNA duplex.

Cytotoxicity: In this assay, the %mortality of brine shrimp nauplii was determined after 24 and 48 h of complexes treatment. The LC_{50} was evaluated from the plot of log[complex] against %mortality of nauplii. From the result, it is inferred that the complex–**1** shows higher toxicity than other synthesized complexes and toxicity value (LC_{50} = 9.72, 11.5, 11.8, 10.3, 17.3 and 15.9 µg/mL for complex 1-6, respectively) of synthesized complexes are comparable to standard anticancer agent *cis*-platin (LC_{50} < 4 µg/mL).

Gel electrophoresis study: Figure 3, shows the cleavage of DNA by the test compounds.

Table 1. Gel electrophoresis analysis of complexes.

Lanes	Complexes	% OC	% LC	% SC	% Cleavage
1	Control	4.90	-	95.1	-
2	$RuCl_3.3H_2O$	27.2	-	72.8	22.44
3	$[Ru(L^1)(PPh_3)Cl_3]$	68.2	24.0	7.80	91.79
4	$[Ru(L^2)(PPh_3)Cl_3]$	74.3	6.50	19.2	79.81
5	$[Ru(L^3)(PPh_3)Cl_3]$	73.3	9.00	17.7	81.39
6	$[Ru(L^4)(PPh_3)Cl_3]$	73.5	9.30	17.2	81.91
7	$[Ru(L^5)(PPh_3)Cl_3]$	58.7	5.20	36.0	62.14
8	$[Ru(L^6)(PPh_3)Cl_3]$	58.1	4.90	37.0	61.09

Lane 1 is a control representing DNA cleavage into only two forms, supercoiled (Form I) and open circular (Form III). Lane 2 with the reference compound $RuCl_3.2H_2O$ representing cleavage into only two forms similar to the control. Lane 3–8 contains synthesized ruthenium complexes 1–6 respectively, representing the cleavage of DNA into three forms Form I, Form III and Form II (linear) in between Form I and III generated by the scission of both the strands of DNA. The photographed image is quantified by AlphaDigiDoc software. The relative decrease in the supercoiled form of control after the addition of test compounds is a measure of percent cleavage. The results (Table 1) clearly indicate that percent cleavage value is highest for the complex–**1** indicating its strong binding efficiency to DNA.

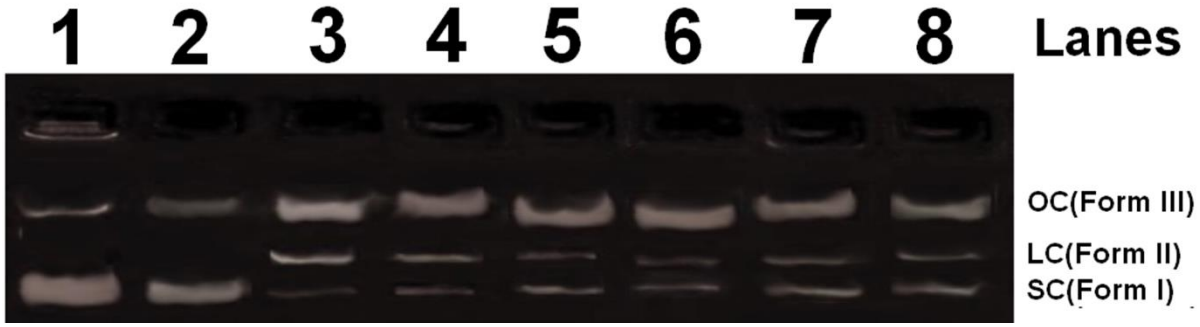


Figure 3. Cleavage of pUC19 plasmid DNA under the influence of ruthenium complexes. Lane 1, DNA control; Lane 2, $RuCl_3 \cdot 3H_2O$; Lane 3, $[Ru(L^1)(PPh_3)Cl_3]$; Lane 4, $[Ru(L^2)(PPh_3)Cl_3]$; Lane 5, $[Ru(L^3)(PPh_3)Cl_3]$; Lane 6, $[Ru(L^4)(PPh_3)Cl_3]$; Lane 7, $[Ru(L^5)(PPh_3)Cl_3]$; Lane 8, $[Ru(L^6)(PPh_3)Cl_3]$.

4. Conclusion

On the basis of various physico-chemical activities like gravimetry, magnetic moment measurement and electronic spectral measurement, it is deduced that the complexes possess octahedral geometry and are paramagnetic in nature. The formation of coordination compounds leads to increase in the antibacterial activity. Complex–**1** binds more efficiently to the DNA via classical intercalation mode. The cytotoxic study displays good potency of the complexes against brine shrimp and 100% mortality is observed after 48 h of incubation. The efficient cleavage of supercoiled pUC19 DNA by all the complexes was observed. The higher efficacy of complex-1 in the various activity performed may be attributed to strong electron withdrawing potency of F-atom at the *para*-position while and chlorine and bromine has less electron withdrawing capacity than bromine. This electron withdrawing capacity of fluorine

atom makes the complex more polar and hence easily permeable to liphophilic layers of the target species and onset its action readily.

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6. References

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