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IMIDAZO[1,5-*a*]PYRIDINE BASED Ru(III) COMPLEXES AS BIOLOGICAL ACTIVE AGENT

DARSHANA N. KANTHECHA AND MOHAN N. PATEL*

*Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India

Corresponding Author tel.: +91 2692 226856

Email: jeenen@gmail.com

ABSTRACT

A series of imidazo[1,5-a]pyridine based Ru(III) complexes of type $[Ru(L^{1-5})_2Cl_2]PF_6$ has been synthesized and characterized by elemental analysis, conductance measurements, electronic spectroscopy and mass spectroscopy (MS). The interactions of the complexes with Herring Sperm (HS) DNA have been carried out by absorption titration and viscosity measurement. The studies suggest the intercalative mode of binding and also confirmed theoretically using a molecular docking study. The in vitro cytotoxicity of the complexes has been examined with a brine shrimp bioassay using Artemia cyst. In vivo cytotoxicity against S. pombe cells at a cellular level has been carried out for the synthesized compounds. Results indicate that the metal complexes show better activity against S. pombe cells compared to the ligands. The complexes have been screened for their antibacterial activity against two Gram$^{(+ve)}$ and three Gram$^{(-ve)}$ microorganisms. DNA extraction was carried out from S. pombe cells to study the DNA cleavage by agarose gel electrophoresis. Smearing of DNA in agarose gel suggests that complexes exhibit toxicity at the cellular level and break the DNA from nucleus to express their toxic effect.

Keywords: Ru(III) complexes, Imidazole, S. Pombe, Molecular modeling, Genotoxicity

INTRODUCTION

The chemistry of metallonuclease is a remarkable discovery for anticancer treatment. Therefore, metal based chemotherapeutic agents have received attention in the treatment of various types cancers or inflammatory disease. After the successful clinical use of anticancer agent cis-platin, a various platinum based chemotherapeutic agents, such as carboplatin, oxalinoplatin are also available in the market. Transition metal complexes including both platinum and non-platinum (Au, Cu, Os, Pd, Ru, Rh and Ir) with a larger variety of ligands utilized for the design and development of metal based drug. The alteration in the structure of ligand occurs when metal bind to the ligand and also changes the orientation of the complex, affects the mode of interaction, and binding stability of DNA and cytotoxicity. In order to understand the mechanisms of DNA binding and to design a bioactive agents, the study of various complexes having altered structural characteristics of intercalating ligands has been carried out. In this context, interactions of metal complexes with DNA are an interesting subject in relation to design the effective anticancer drugs [5,6]. Platinum based drugs exhibit side effects, severe toxicity, selectivity and resistance to living cells. The disadvantages of platinum based drugs have forced researcher and to expand the scope of non-platinum metallodrugs[7,8]. Ruthenium complexes are attractive alternates to platinum complexes because of their rich synthetic chemistry, variable oxidation states and photochemical activity towards DNA under physiological conditions [9,10]. DNA binding studies show that ruthenium(III) complexes can bind to DNA with different binding fashion such as intercalative binding, covalent binding, electrostatic binding and groove binding and to exhibit effective nuclease activities[11,12]. Ruthenium based drugs, such as imidazole based NAMI-A3 and indazolium based KP1019 are effective against lung metastasis and colon carcinomas respectively[4,13]. Further, investigation of biological active agents has been carried out on Ru(II) and Ru(III) complexes with N, N donor hetero moiety based ligands. It inspire us to design, biological activity ruthenium(III) complexes with imidazo[1,2-a]pyridine and evaluating biological properties such as DNA

binding, DNA cleavage, antibacterial and cytotoxicity.

EXPERIMENTAL SECTION

Chemicals and materials

All analytical grade chemicals and solvents were used as commercially received. Metal salt of $RuCl_3 \cdot XH_2O$ was purchased from Chemport (Mumbai, India). 2,2-Dipyridylketone, 2-benzoyl pyridine, benzaldehyde, 4-methyl benzaldehyde, 4-bromo benzaldehyde, 4-chloro benzaldehyde, 4-methoxy benzaldehyde, HS-DNA, KPF_6 and edta were purchased from Sigma Aldrich Chemical Co. (India). Agarose, Luria Broth (LB), ethidium bromide (EtBr), Tris–acetyl–edta (TAE) and bromophenol blue were purchased from Himedia (India). Culture for antibacterial activity *Bacillus subtilis (B. Subtilis-7193*), *Staphylococcus aureus (S. Aureus-3160), Pseudomonas aeruginosa (P. Aeruginosa-1688), Escherichia coli (E. Coli-433)* and *Serratia marcescens (S. Marcescens-7103)* were purchased from the Institute of Microbial Technology (Chandigarh, India). *S. Pombe* Var. Paul Linder 3360 was obtained from IMTECH, Chandigarh.

Physical measurements

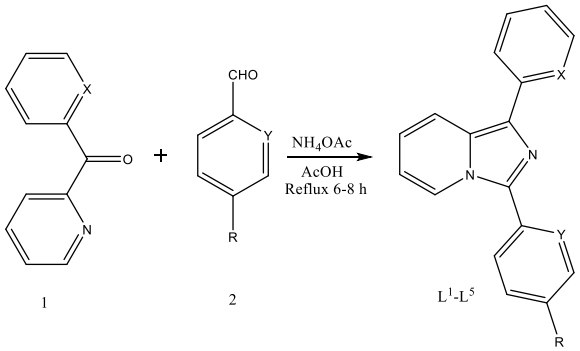
Elemental analysis of C, H, and N were performed with a model EuroVector EA3000 (for ligands) and Perkin-Elmer 240 (for complex) elemental analyzer. The LC-MS spectra were recorded using Thermo scientific mass spectrophotometer (USA). Bruker Avance spectrophotometer was recorded ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) using an appropriate solvent in which compound properly dissolve. Melting points were determined in open capillaries on hermocal10 melting point apparatus (Analab Scientific Pvt. Ltd, India). The Gouy's method used for magnetic measurement of metal complexes taking mercury tetrathiocyanatocobaltate(II) as the calibrant (χ_g=16. 44 x 10^{-6} cgs units at 20 °C), citizen balance. UV-160A UV-Vis spectrophotometer, Shimadzu, Kyoto (Japan), was used for electronic spectra of metal complexes in the range of 200-800 nm using quarts cell having path length 1 cm. Ubbelohde viscometer in viscosity bath having a controllable temperature (25.0 ± 0.5^{0}C) were used for the study of hydrodynamic chain length. Antibacterial study was carried out by means of laminar air flow cabinet Toshiba, Delhi (India). Alphadigidoc^{tm} RT. Version V.4.0.0 PC– Image software, CA (USA) used for the Photo quantization of the DNA cleavage activity.

General method for synthesis of ligands and complexes

Synthesis of imidazo[1,2-a]pyridine derivatives were carried out by taking a mixture of pyridylketone (5 mmol), substituted benzaldehyde (5 mmol), and NH_4OAC (15 mmol) in 15 mL of glacial acetic acid in a 100 mL round bottom flask equipped with a condenser. The reaction mixture was heated at 80-120 °C under N_2 gas for 6-8 h. Upon checking TLC in interval of 1 h, when all the reactants were consumed and spot of product appears the reaction mixture was cooled to room temperature and then kept the solution under temp 15-20 °C to slow evaporation overnight. The resulting yellow residue was filtered and purified using column chromatography on silica gel by ethyl acetate: hexane (2:8) system as a mobile phase.

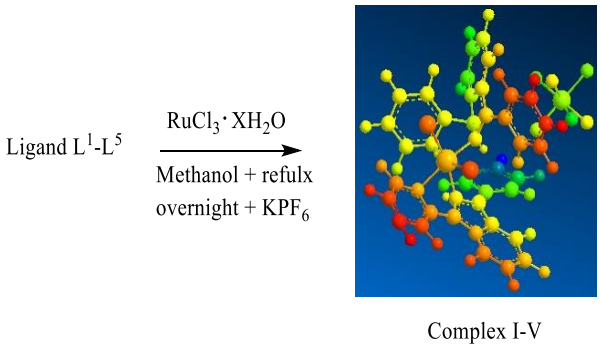
The methanolic solution of $RuCl_3 \cdot XH_2O$ was heated to reflux for 10 min to activate metal. Then dropwise a methanolic solution of ligand was added and the reaction mixture was heated to reflux overnight. Impurity was removed by filtering the hot solution. Then a saturated solution of KPF_6, was added. The solution was kept at 5-10 °C overnight. The obtained precipitate was washed with water and ether.

The proposed reaction mechanism for the general synthesis of imidazo[1,2-a]pyridine and their ruthenium(III) complexes are shown in scheme 1 and 2.



Scheme 1. General synthesis of the imidazo[1,2-a]pyridine ligands.

Ligand	1	2	3	4	5
X	CH	N	N	N	N
Y	N	CH	CH	CH	CH
R_1	H-	H-	CH_3-	Cl-	Br-



Scheme 2. General synthesis of the imidazo[1,2-a]pyridine based Ru(III) complexes.

1. 1-phenyl-3-(pyridin-2-yl)imidazo[1,5-a]pyridine (L^1)

Synthesized using 2-benzoyl pyridine with pyridine carboxaldehyde as per given procedure. Colour: yellow, Yield: 58%, mol. wt.: 271.32 g/mol, m.p.:124.3°C, Anal. Calc. (%) For $C_{18}H_{13}N_3$: C, 79.68; H, 4.83; N, 15.49 Found (%): C, 79.85; H, 4.72; N, 15.25. ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 6.793(1H, m), 6.963(1H, m), 7.236(1H, m), 7.351(1H, t, 7.6 Hz), 7.518(2H, t, 7.6 Hz), 7.823(1H, m), 7.930(1H, d, 9.2 Hz), 7.993(2H,dd, 0.8, 7.2 Hz), 8.510(1H, d, 8.0 Hz), 8.677(1H, dd, 0.8, 4.0 Hz), 10.057(1H, d, 7.6 Hz), ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 113.67(CH), 118.37(CH), 120.97(CH), 121.63(CH), 122.26(CH), 126.39(CH), 126.71(CH), 127.00(CH), 128.76(CH), 129.05(C_{qat}), 129.33(C_{qat}), 135.03(C_{qat}), 136.55(CH), 148.12(CH), 149.57(C_{qat}), 151.50(C_{qat}). Mass (m/z): 272

2. 3-phenyl-1-(pyridin-2-yl)imidazo[1,5-a]pyridine (L^2)

Synthesized using 2,2'-dipyridylketone with benzaldehyde as given procedure. Colour: yellow, Yield: 82%, mol. wt.: 271.32 g/mol, m.p.:108°C, Anal. Calc. (%) For $C_{18}H_{13}N_3$: C, 79.68; H, 4.83; N, 15.49 Found (%): C, 79.86; H, 4.74; N, 15.26. ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 6.668(1H, t, 6.4 Hz), 6.944(1H, dd, 2.8, 6.4 Hz), 7.120(1H, m), 7.486(1H, d, 7.6 Hz), 7.574(2H, t, 8.0 Hz), 7.741(1H, m), 7.868(2H, d, 7.2 Hz), 8.279(2H, d, 8.4 Hz), 8.658(1H, d, 5.2 Hz), 8.736(1H, d, 8.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 113.91(CH), 119.96(CH), 120.45(CH), 121.02(CH), 121.62(CH), 121.89(CH), 128.41(CH), 128.94(CH), 129.05(CH), 130.17(C_{qat}), 130.23(C_{qat}), 130.65(CH), 136.27(CH), 138.06(C_{qat}), 149.01(CH), 155.09(C_{qat}). Mass (m/z): 272

3. 1-(pyridin-2-yl)-3-(p-tolyl)imidazo[1,5-a]pyridine (L^3)

Synthesized using 2,2'-dipyridylketone with p-tolylbenzaldehyde as given procedure. Colour: yellow, Yield: 79%, mol. wt.: 285.35 g/mol, m.p.:154.7°C, Anal. Calc. (%) For $C_{19}H_{15}N_3$: C, 79.98; H, 5.30; N, 14.73 Found (%): C, 80.08; H, 5.19; N, 14.73. ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 2.472(3H, s), 6.654(1H, t, 6.4 Hz), 6.930 (1H, dd, 2.8, 6.4 Hz), 7.113(1H, m), 7.378(2H, d, 8.0 Hz), 7.741(3H, m), 8.260(2H, t, 7.2 Hz), 8.650(1H, d, 4.8 Hz), 8.711(1H, d, 9.2 Hz). 13C NMR (100 MHz, DMSO-d6) δ/ppm: 21.43(CH_3), 113.74(CH), 119.94(CH), 120.36(CH), 120.86(CH), 121.68(CH), 121.82(CH), 127.27(Cqat), 128.32(CH), 129.72(CH), 130.09(Cqat), 130.57(Cqat), 136.20(CH), 138.26(Cqat), 138.95(Cqat), 148.98(CH), 155.15(Cqat). Mass (m/z): 286

4. 3-(4-chlorophenyl)-1-(pyridin-2-yl)imidazo[1,5-a]pyridine *(L^4)*

Synthesized using 2,2'-dipyridylketone with p-chlorobenzaldehyde as given procedure. Colour: yellow, Yield: 89%, mol. wt.: 305.77 g/mol, m.p.:165.01°C, Anal. Calc. (%) For $C_{18}H_{12}ClN_3$: C, 70.71; H, 3.96; N, 13.74 Found (%): C, 70.71; H, 3.63; N, 13.74. ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 6.709(1H, t, 6.0 Hz), 6.967(1H, dd, 3.6, 6.4 Hz), 7.131(1H, dd, 2.0, 4.4 Hz), 7.551(2H, d, 8.8 Hz), 7.748(1H, m), 7.825(2H, d, 8.4 Hz), 7.242(2H, t, 7.2 Hz), 8.657(1H, d, 4.0 Hz), 8.744(1H, d, 9.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 114.28(CH), 116.99(C_{qat}), 119.96(CH), 120.63(CH), 121.13(CH), 121.39(CH), 122.04(CH), 122.72(C_{qat}), 128.73(C_{qat}), 129.33(CH), 129.57(CH), 134.87(C_{qat}), 136.41(CH), 136.80(C_{qat}), 149.16(CH), 154.92(C_{qat}). Mass (m/z): 306

5. *3-(4-bromophenyl)-1-(pyridin-2-yl)imidazo[1,5-a]pyridine (L^5)*

Synthesized using 2,2'-dipyridylketone with p-bromobenzaldehyde as given procedure. Colour: yellow, Yield: 85%, mol. wt.: 350.22 g/mol, m.p.:172.03°C, Anal. Calc. (%) For $C_{18}H_{12}BrN_3$: C, 61.73; H, 3.45; N, 12.00 Found (%): C, 61.62; H, 3.33; N, 11.55. ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm: 6.708(1H, t, 6.0 Hz), 6.966(1H, m), 7.133(1H, m), 7.718(2H, m), 7.751(1H, t, 2.4 Hz), 7.771(1H, d, 1.6 Hz), 8.248(2H, m), 8.655(1H, m), 8.730(1H, t, 0.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 114.30(CH), 119.95(CH) 120.60(CH), 121.12(CH), 122.02(CH), 122.92(C_{qat}), 129.13(C_{qat}), 129.81(CH), 130.44(C_{qat}), 132.18(CH), 136.29(CH), 136.97(CH), 139.58(C_{qat}), 144.19(C_{qat}), 149.04(CH), 154.89(C_{qat}). Mass (m/z): 350

I. $Ru(L^1{}_2Cl_2)PF_6$

Colour: brown, Yield: 89%, mol. wt.: 859.58 g/mol, m.p.: >300 °C, Anal. Calc. (%) For $C_{36}H_{26}Cl_2F_6N_6PRu$: C, 50.30; H, 3.05; N, 9.78 Found (%): C, 50.24; H, 3.02; N, 9.81. Mass (m/z): 714

II. $Ru(L^2{}_2Cl_2)PF_6$

Colour: brown, Yield: 92%, mol. wt.: 859.58 g/mol, m.p.: >300 °C, Anal. Calc. (%) For $C_{36}H_{26}Cl_2F_6N_6PRu$: C, 50.30; H, 3.05; N, 9.78 Found (%): C, 50.24; H, 3.01; N, 9.81. Mass (m/z): 714

III. $Ru(L^3{}_2Cl_2)PF_6$

Colour: brown, Yield: 93%, mol. wt.: 887.63 g/mol, m.p.: >300 °C, Anal. Calc. (%) For $C_{38}H_{30}Cl_2F_6N_6PRu$: C, 51.42; H, 3.41; N, 9.47 Found (%): C, 51.54; H, 3.35; N, 9.38. Mass (m/z): 742

IV. $Ru(L^4{}_2Cl_2)PF_6$

Colour: brown, Yield: 90%, mol. wt.: 928.46 g/mol, m.p.: >300 °C, Anal. Calc. (%) $C_{36}H_{24}Cl_4F_6N_6PRu$: C, 46.57; H, 2.61; N, 9.05 Found (%): C, 46.60; H, 2.67; N, 9.09. Mass (m/z): 783

V. $Ru(L^5{}_2Cl_2)PF_6$

Colour: brown, Yield: 91%, mol. wt.: 1017.37 g/mol, m.p.: >300 °C, Anal. Calc. (%) For $C_{36}H_{24}Br_2Cl_2F_6N_6PRu$: C, 42.50; H, 2.38; N, 8.26 Found (%): C, 42.47; H, 2.42; N, 8.30. Mass (m/z): 872

BIOLOGICAL SCREENING OF SYNTHESIZED COMPOUNDS

UV–Vis absorbance titration

Binding mode and interaction strength of DNA with metal complexes have been examined effectively by electronic absorption spectra (UV–Vis absorbance titration) using Herring Sperm DNA (HS-DNA)[14].

Viscosity measurement

An Ubbelohde viscometer was used to measure the flow time of HS-DNA in phosphate buffer (pH 7.2) with a digital stopwatch and each compound were carried out three times to calculate average flow time[15].

Molecular docking study

Interaction between DNA and complexes at the molecular level were studied by advanced computational typical technique like molecular docking. The rigid molecular docking study was executed using HEX 8.0 software to conclude the orientation of the complexes binding to DNA. The structure of the DNA of sequence (5'-d(CGCGAATTCGCG)-3')$_2$ (PDB id: 1BNA, a familiar sequence used in oligodeoxynucleotide study) obtained from the Protein Data Bank (http://www.rcsb.org/pdb)[16].

Cytotoxicity

For the determination of *in vitro* cytotoxicity of compounds, brine shrimp (Artemia cysts) lethality bioassay method given by Meyer et al. [17] accomplished with brine shrimp nauplii in artificial seawater. After treatment counted the number of viability of nauplii (observing several seconds if nauplii did not exhibit any internal or external movement considered nauplii dead). Data were analyzed by the log concentration of sample vs percentage mortality of nauplii that gives LC_{50} values.

Cellular level cytotoxicity assay

Eukaryotic *Schizosaccharomyces pombe cells* were an important organism for the study of the effects of the metal complexes at the cellular level (cytotoxicity) to the DNA damage. Percentage viability were counted in triplicate where the number of dead cells and number of live cells were counted in three microscopic fields (microscope (40X)) and calculated the average percentage of live cells [18].

Antibacterial activity

All of the newly synthesized compounds were screened for their antibacterial activity using *Staphylococcus aureus, Bacillus subtilis, Serratia marcescens, Pseudomonas aeruginosa and Escherichia coli* micro-organisms. The broth dilution technique was used to determine the bactericidal effect by minimum inhibitory concentration (MIC) in terms of μM. MIC, lowest concentration that prevents the microbial growth. Antibacterial activity carried out according to reported method[19].

DNA cleavage study

Effect of compounds on the integrity of *S. pombe* cell's DNA was studied by agarose gel electrophoresis. The *S. pombe* cells were grown and treated by same manner in the cellular level cytotoxicity assay. The image was captured by a CCD camera and the Alpha Digi Doc system was used for analyzing gel[20].

RESULTS AND DISCUSSION

Magnetic moments, electronic spectra and conductance measurements

Magnetic moments measurement of Ru(III) complexes was carried out at room temperature. The obtained μ_{eff} values are in the range of 1.78–1.93 BM, which corresponds to a single unpaired electron in low-spin t_{2g}^5 configuration in an octahedral environment and confirms the +3 oxidation state of ruthenium complexes. The electronic spectra of Ru(III) complexes in DMSO showed three bands in the region 285–670 nm. Ru(III) complexes have relatively high oxidation properties, and the d–d transition band appeared at 645–670 nm. The spectral data in the UV region (below 400 nm) are designated as π–π* [350–380 nm] and n–π* [285–305 nm] transitions of non-bonding electrons present on the nitrogen of the imidazol group in Ru(III) complexes. The pattern of the electronic spectra of all the Ru(III) complexes indicate the presence of an octahedral environment around the ruthenium(III) metal ion. The molar conductivities values of the Ru(III)

complexes are in the range of 39-70 $cm^2\ \Omega^{-1}\ mol^{-1}$ at room temperature, representing electrolytic nature and one counter ion existing outside the coordination sphere of Ru(III) complexes. So, we conclude that all Ru(III) complexes exhibit ionic in nature.

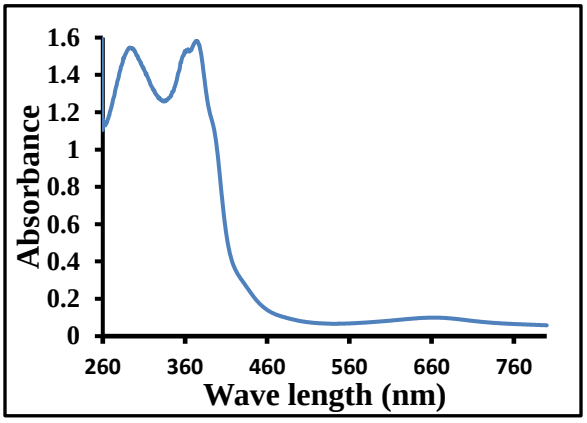


Figure 1. UV- Absorption spectra of representative complex-I in DMSO

Binding behaviour of complex with HS DNA

To examine the binding mode of metal complexes with DNA electronic absorption titration technique has been used in phosphate buffer solution (pH 7.2). Strong stacking interaction among chromophore of complex and DNA base pair results in hypochromic shift and red shift (bathochromic shift) in absorption spectra which generally indicate that intercalation binding[5,21]. An absorption titration graph for complex-II is represented in Figure 2. elucidate hypochromism with bathochromic shift with increasing in DNA concentration indicates intercalation mode of binding. The K_b value is determine from the ratio of the slope to intercept from the plot of [DNA]/(ε_a - ε_f) versus [DNA] and values for ligands and complexes are shown in figure 3.

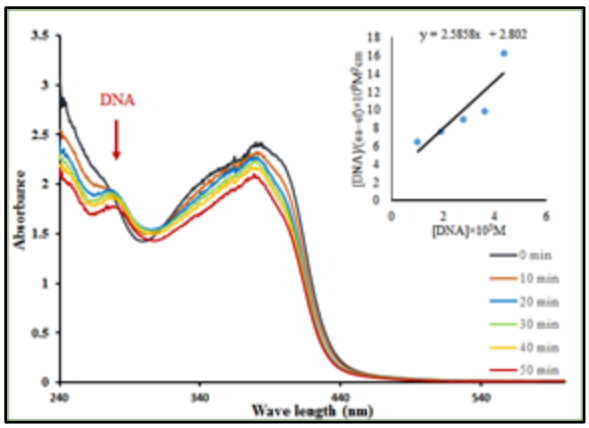


Figure 2. Absorption spectra of complex-**I** with increasing concentration of HS–DNA after incubation 10 min of each addition at 37 °C in phosphate buffer (pH 7.2) Inset: Plots of [DNA]/($\mathcal{E}_a$–$\mathcal{E}_f$) versus [DNA] for the titration of DNA with compounds.

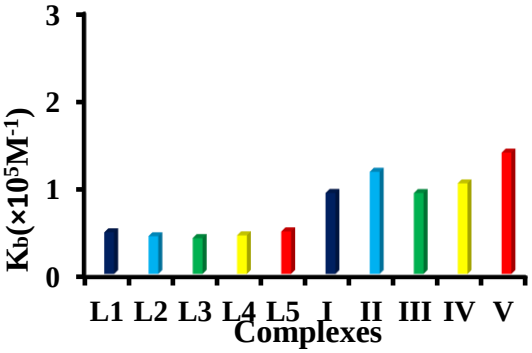


Figure 3. Plot of *Kb* values of synthesized ligands and complexes in L mol−1 using HS-DNA. Error bars represent the standard deviation of three replicates

Hydrodynamic volume measurement

The intrinsic viscosity depending on the mode of interaction with increasing concentration of complexes. Increasing in viscosity explain as separation of base pair occur when complex Insert in DNA base pair. Which leads to the DNA helix lengthens and proposed that the intercalation type of binding present [5][20]. Plot of relative specific viscosity $(\eta/\eta_0)^{1/3}$ versus [complex]/[DNA] (Figure 4) shows increasing in viscosity of the DNA with the addition of complexes. Which suggests that the intercalation binding mode of complexes with HS-DNA that cumulative observed in electronic absorption spectra.

Orientation of docked structure

The molecular docking study was accomplished to determine the orientation of the Ru(III) complexes with DNA and its binding affinity using HEX 8.0 software.

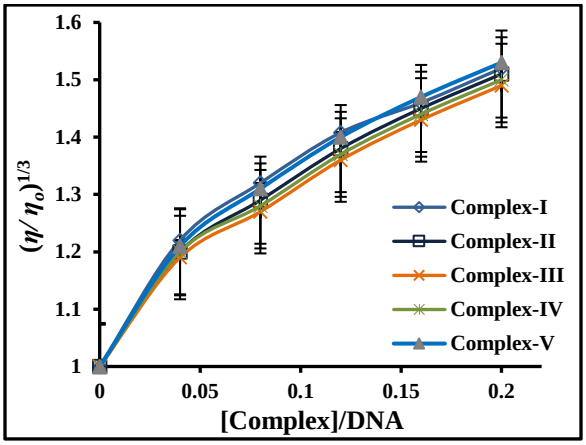
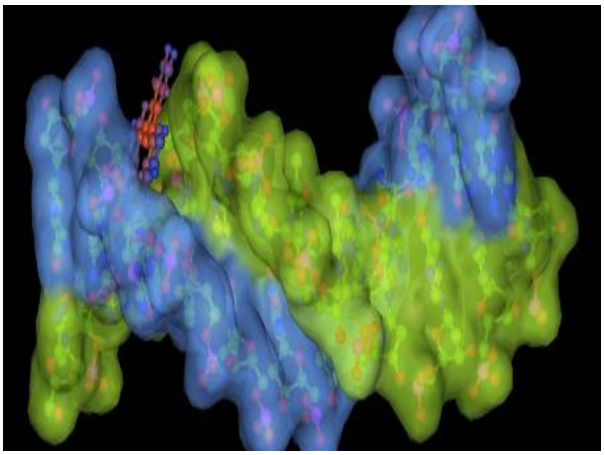


Figure 4. Effect on relative viscosity of HS DNA under the influence of increasing amount of complexes at 37±0.1 °C in phosphate buffer (pH 7.2) with standard deviation.

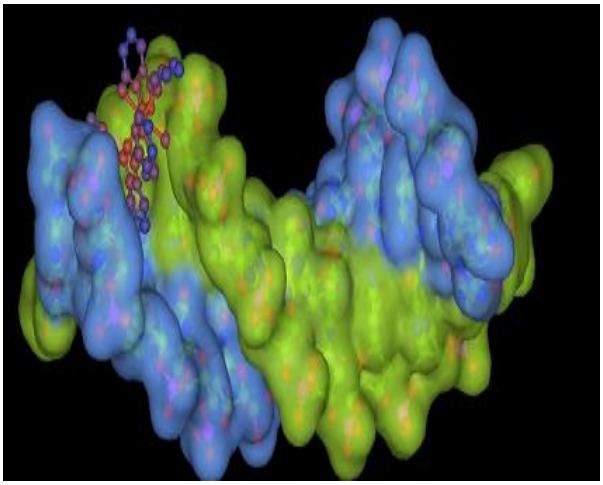
The resulting binding energies of docked ligands L^1-L^5 and complexes I-V are measurable as kJ mol^{-1} and values of energy are represent in Table 1. The results indicate the docked structure of complex (Figure 5) fit well into the intercalative mode of the targeted DNA and A–T rich region stabilized by van der Waal's interaction and hydrophobic contacts [19]. The docking study shows preferential intercalation binding when complexes interact with DNA. The more negative values of docked energy, the more effective binding between DNA and target molecules. Result arising from molecular docking promote binding similar to that of viscosity and spectroscopic absorption titration.

Docking energy			
Ligand	kJ\mol	Complex	kJ\mol
L1	-232.8	I	-289.98
L2	-225.9	II	-303.17
L3	-233.7	III	-309.57
L4	-241.9	IV	-306.54
L5	-240.8	V	-310.10

Table 1. Docked energy values of ligands and complexes in kJ/mol



(a)



(b)

Figure 5. Docked structure of (a) Ligand-1 and (b) complex-I with the HS-DNA duplex of sequence $(5'\text{-d(CGCGAATTCGCG)-}3')_2$

Brine shrimp lethality bioassay

Simple and cheap methodology employed for the cytotoxicity study was brine shrimp (Artemia cysts) lethality bioassay[22]. The LC_{50} values obtained from the log concentration of sample vs. percentage mortality of nauplii. The comparative result of toxicity is represent in Figure 6 (LC_{50} = 5.63 – 27.82). The data suggest that tested compounds have strong ability to interact with the biological model system. The complexes show more toxicity than corresponding ligands. It is strongly supported that *in vitro* cytotoxicity study against brine shrimp assay is useful for the further study and preparation of effective medicine against various diseases. The order of

toxicity of compounds is V>IV>III>I>II>L^5>L^4>L^3>L^1>L^2. The result shows that the electron withdrawing group increases the toxicity of compounds.

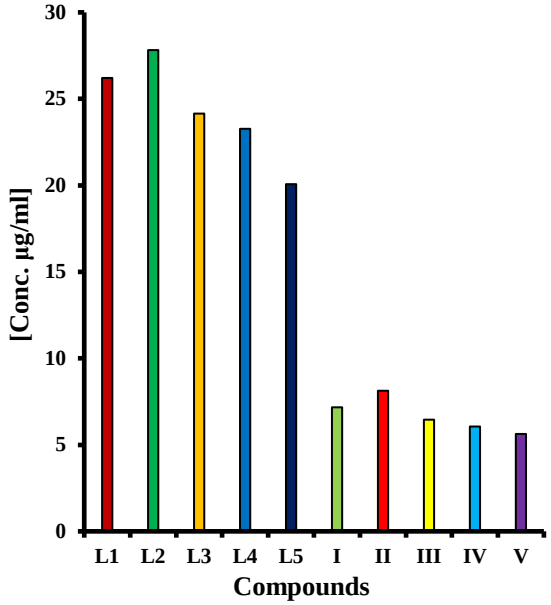


Figure 6. Cytotoxicity of synthesized ligand and complexes by brine shrimp assay.

In vivo cytotoxicity

Cytotoxicity data represented graphically in Figure 7, which shows % viability of cells treated by complexes and their ligand after 17 h treatment. From the results, it is observed that the % viability of ligands is higher than complexes, in another word toxicity of complexes is higher than their corresponding ligands.

Antibacterial activity

Antibacterial activity of the synthesized compound has been carried out against two Gram(+ve) (B. *subtilis and S. aureus)* and three Gram(–ve) *(P. aeruginosa, E. coli and S. marcescens)* bacteria.

Increasing in antibacterial activity may be considered in the light of an Overtone's concept [19] and chelation theory [23]. The result of *in vitro* antibacterial activity values of the ligands and corresponding complexes indicates that the metal complexes show relatively higher activity against bacteria. The MIC values for ligands and complexes are in the range of 230-320 µM and 75-120 µM, respectively (Figure 8). The complexes are comparatively more potent than ligands against all bacteria and they more effective on *S. aureus, B. subtilis and E. coli.*

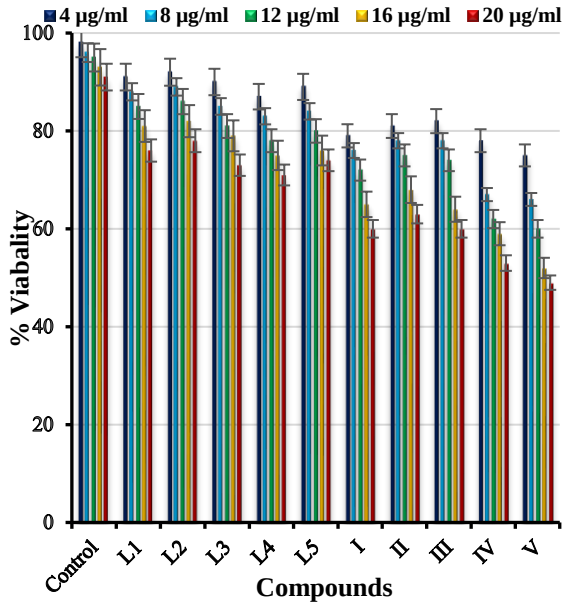


Figure 7. *S. pombe* cell viability represents as a percentage with a standard deviation of three independent experiments of synthesized compounds.

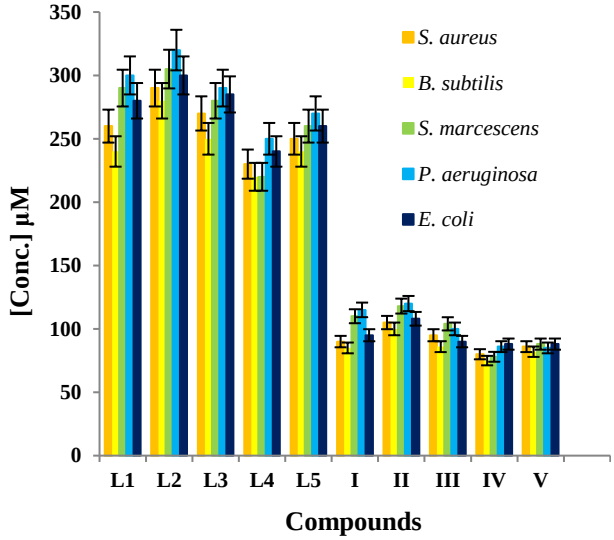


Figure 8. Minimum inhibition concentration (MIC) values of synthesized compounds given in µM.

Effect of compound on integrity of S. pombe cell's DNA

Agarose gel electrophoresis used for the study of DNA cleavage. The DNA extraction was carried out from *S. pombe* cells as reported method[20]. All the treated compound were incubated in TE buffer (pH 8) at a final volume of 10 μgL^{-1} for 24 h at 37 °C. Effects of compound on the integrity of DNA in agarose gel are shown in Figure 9. Smearing of DNA in complexes suggests that the damage occurs due to the toxic nature of the compound. Whereas control cell DNA and complex salt treated DNA appeared in intact band indicate that non-toxic or less toxicity compared to complexes. So, the result of complexes expresses their toxic effect at the cellular level and break the DNA from the nucleus.

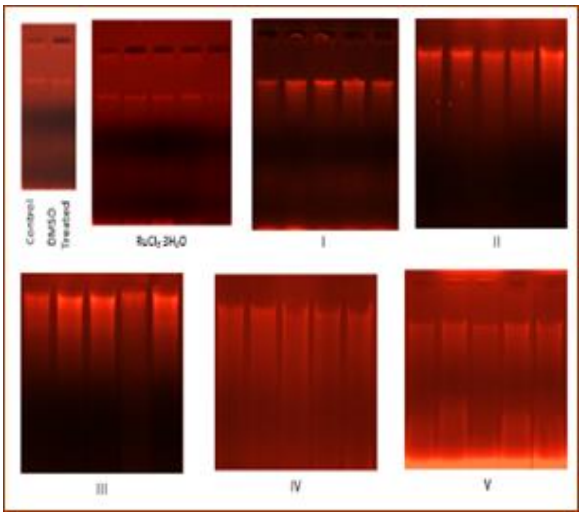


Fig. 9. Photogenic view of cleavage of *S. pombe* DNA (1 μgL^{-1}) with series of compounds using 1% agarose gel containing 0.5 (μgL^{-1}) EtBr.

CONCLUSION

The present study describes the synthesis of imidazo[1,5-a]pyridine based Ru(III) complexes of type $[Ru(L^{1-5})_2Cl_2]PF_6$ and characterized by elemental analysis, conductance measurements, electronic spectra, infrared (FT-IR) spectroscopy and mass spectroscopy. The characterization confirms the +3 oxidation of ruthenium complexes. The DNA interaction study shows the intercalation type of binding, which confirms through spectroscopic titration, viscosity measurements and molecular docking study. The result of antibacterial activity shows that complexes exhibit higher antibacterial activity than corresponding ligand. *In vitro* and *in vivo* cytotoxicity against brine shrimp and *S. pombe* indicate that toxicity increases when ligand coordinates to metal ion due to, alteration of structure of complexes. The DNA cleavage study of complexes shows the integrity of complexes with DNA and breaking of DNA from nuclease suggest that the toxic effect of the complexes at the cellular level. From all the results, we conclude that the synthesized Ru(III) complexes are biologically active and further study of the complexes may be leads to the alternate for anticancer drugs.

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