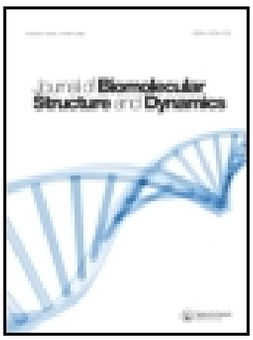

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LETTER TO THE EDITOR

Biological applications of pyrazoline-based half-sandwich ruthenium(III) coordination compounds

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1. Introduction

The inclusion of biologically active ligands into metal complexes deals much scope for the design of novel drugs with improved and targeted activity. Studies on such complexes indicate that new mechanisms of action are favorable when combining the bioactivity of the ligand with the properties inherent to the metal, leading to the possibility of overcoming current drug resistance pathways. It is well known that deoxyribonucleic acid (DNA) is an important target in the organism for some metal-based drugs or reagents. These reagents can interact with DNA thereby changing the replication of DNA and inhibiting the growth of the tumor cells. It has been reported that the metal complexes can interact with DNA non-covalently in the mode of intercalation, groove binding, and electrostatic effect.

Ruthenium complexes are also assumed to have a biological mode of action that is significantly dissimilar from those of platinum-based drugs. Additionally, the rich synthetic chemistry, redox accessible oxidation states, favorable ligand substitution reactions, and diverse coordination geometries of ruthenium complexes have been valuably considered for the design of new pharmaceutical agents. Ruthenium-based organometallic compounds are progressively gaining importance as promising aspirants for the design of novel and more effective metal-based drugs (Fricker, 2007). The antitumor activity of many organometallic ruthenium complexes is generally related to their enhanced DNA-binding affinity, which involves covalent coordination or simultaneous intercalation of extended aromatic groups and specific hydrogen bonding depending on the particular type of ligands used. In this regard, diverse ligand types are increasingly being developed and combined with the ruthenium(III)–pentamethylcyclopentadienyl ring moiety to enhance their DNA-binding properties, so as to achieve different biological functions and to maximize their effectiveness as therapeutic agents. The half-sandwich ruthenium(III) complexes with pyrazoline ligand show comparison and contrasts to many of the systems such as the actinomycins, porphyrins, Hoechst, and benzo[a]pyrene derivatives and others in forming complexes with the DNA or nucleic acids (Agarwal, Chadha, & Mehrotra, 2015; Basu & Suresh Kumar, 2016; Bathaie, Ajloo, Daraie, & Ghadamgahi, 2015; Jeon, Jin, Kim, & Lee, 2015; Monaselidze et al., 2015; Nagaraj, Ambika, & Arunachalam, 2015).

Heterocyclic compounds have so far been synthesized mainly due to the wide range of biological activities. Much attention has been paid to the synthesis of heterocyclic compounds bearing nitrogen containing ring system, like pyrazole mainly due to their higher pharmacological activity. Pyrazole and its derivatives are the important structural motifs in heterocyclic chemistry and occupy significant location in medicinal chemistry. They exhibited a broad spectrum of pharmacological activities (Mehta, Gajera, & Patel, 2016).

In the present study, a series of pyrazoline-based half-sandwich organometallic ruthenium complexes have been synthesized and well characterized. The synthesis and structural characterization of compounds were carried out with an aim to study their biological activity. A satisfactory property of this type of ruthenium complexes is the convenient piano-stool shape conferred by the metal center. Such a characteristic provides considerable opportunities for making new compounds with stimulating biological properties through functionalization and rational ligand design. All synthesized complexes were evaluated for their biological applications like DNA binding, DNA cleavage, antimalarial, antimicrobial, and cellular level cytotoxicity. These studies mainly focuses on exploring the trend in DNA-binding affinities of organometallic ruthenium complexes and the important differences in some related properties.

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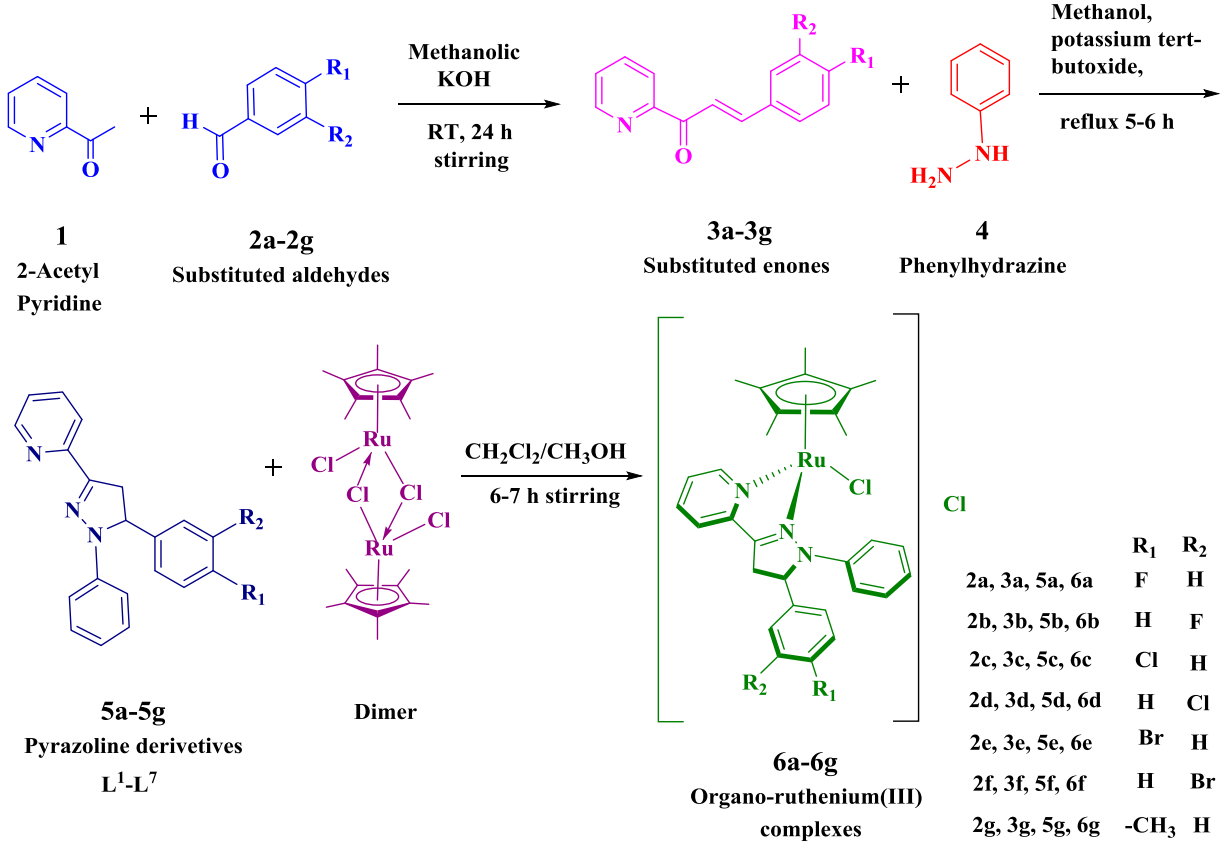
2. Materials and methods

2.1. Materials and reagents

All the chemicals and solvents were of reagent grade and used as purchased; double distilled water was used throughout the studies. Pentamethylcyclopentadienyl ruthenium(III) chloride [{(Cp*)Ru(μ-Cl)Cl}$_2$], 2-acetyl pyridine, 4-fluorobenzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 4-methylbenzaldehyde, 3-fluorobenzaldehyde, 3-chlorobenzaldehyde, 3-bromobenzaldehyde, phenyl hydrazine, potassium *tert*-butoxide, HS DNA, and ethylenediaminetetraacetic acid disodium salt (edta) were purchased from Sigma Aldrich Chemical Co. (India). Agarose, Luria Broth (LB), ethidium bromide (EtBr), tris-acetyl-edta (TAE), bromophenol blue, and xylene cyanol FF were purchased from Himedia (India). *Schizosaccharomyces Pombe* Var. Paul Linder 3360 was obtained from IMTECH, Chandigarh.

2.2. Physical measurements

Photo quantization of the gel after electrophoresis was done using AlphaDigiDoc™ RT. Version V.4.0.0 PC-Image software, California (USA). The liquid chromatography-mass spectroscopy (LC-MS) spectra were recorded using make waters model micromass quattro micro API mass spectrometer. The electronic spectra were recorded on a UV-160A UV–vis spectrophotometer, Shimadzu, Kyoto (Japan). The ^{1}H NMR and ^{13}C NMR were recorded with a Bruker Avance (400 MHz). IR spectra were recorded on a FT-IR Shimadzu spectrophotometer with sample prepared as KBr pellets in the range of 4000–400 cm^{-1}. C, H, and N elemental analyses were performed using thermofinnigan at CHN Lab, Panjab University, Chandigarh. Melting points (°C, uncorrected) were determined in open capillaries on ThermoCal$_{10}$ melting point apparatus (Analab Scientific Pvt. Ltd, India). Precoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin-layer chromatography. The magnetic moments were measured by Gouy's method using mercury tetrathiocyanatocobaltate(II) as the calibrant ($\chi_g = 16.44 \times 10^{-6}$ cgs units at 20°C), Citizen balance. Antibacterial study was carried out by means of laminar air flow cabinet Toshiba, Delhi (India). The thermogram of complexes was recorded with a Mettler Toledo TGA/DSC 1 thermo gravimetric analyzer.



Scheme 1. Synthesis of tri-substituted pyrazoline ligands (5a–5g) and its complexation with ruthenium metal ion.

2.3. Synthesis of the pyrazoline ligands and Ru(III) complexes

Detailed process for the synthesis and characterization of pyrazoline ligands as well as their Ru(III) complexes are shown in the Supplementary material 1. The proposed reaction for the synthesis of ligands (5a–5g) and Ru(III) complexes (6a–6g) is shown in scheme 1. ^{1}H NMR and ^{13}C NMR spectra of ligands are shown in Supplementary materials 2 and 3, respectively.

2.4. Biological study of synthesized compounds

2.4.1. In vitro *antibacterial study*

The *in vitro* antibacterial activities of pyrazoline ligands and organometallic ruthenium complexes were tested against two Gram positive: *Staphylococcus aureus*, *Bacillus subtilis* and three Gram negative: *Serratia marcescens*, *Escherichia coli*, *Pseudomonas aeruginosa* micro-organisms. The MIC value was determined by broth dilution technique. The detailed experimental process is reported in newly published literatures (Mehta, Gajera, & Patel, 2015).

2.4.2. *DNA-binding study*

2.4.2.1. By absorption titration. DNA-mediated hypochromic and bathochromic shifts under the influence of the organometallic ruthenium complexes were measured with the help of UV–vis absorbance spectra. UV–vis spectral titration of organometallic ruthenium complexes (in DMSO) with HS DNA in phosphate buffer was carried out to inspect the binding mode for the complexes. The concentration of HS DNA was determined by measuring absorbance at 260 nm and using 12,858 L mol^{-1}cm^{-1} as the molar extinction coefficient value. In this experiment, fixed amount of DNA solution (100 µL) in phosphate buffer was added to sample cell holding in definite concentration of complex solution (20 µM) and reference cell to nullify the effect of HS DNA, and allowed to incubate for 10 mins prior to the spectra being recorded. DMSO was also added into the reference cell as a control to nullify the effect of DMSO. The intrinsic binding constant, K_b, was determined by equation reported in published literatures (Mehta et al., 2015).

2.4.2.2. By viscosity measurements. An Ubbelohde viscometer maintained at a constant temperature of 27 ± 0.1°C in a thermostatic jacket, was used to measure the flow time of DNA in phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7.2) with accuracy of 0.01 s and precision of 0.1 s. DNA samples, approximately 200 base pairs in average length, were prepared by sonicating in order to minimize complexities arising from DNA flexibility. Flow time for buffer alone was measured and was termed as t_0. The detailed experimental process is reported in published literatures (Mehta et al., 2015).

2.4.2.3. By molecular docking with HS DNA. The rigid molecular docking study has been performed using HEX 8.0 software to determine the orientation of the organometallic Ru(III) complexes binding to DNA. Docking was performed and the most stable configuration was chosen as input for investigation. The coordinates of metal complexes were taken from their optimized structure as a .mol file and were changed to .pdb format. HS DNA used in the experimental work was too large for current computational resources to dock, therefore, the structure of the DNA of sequence d $(ACCGACGTCGGT)_2$ (PDB id: 423D, a familiar sequence used in oligodeoxynucleotide study) obtained from the *Protein Data Bank* (www.rcsb.org/pdb). All calculations were carried out on an Intel CORE i5-, 2.5-GHz-based machine running MS Windows 8, 64bit as the operating system. By default, parameters were used for the docking proposal with correlation type shape only, FFT mode at 3D level, grid dimension of 6 with receptor range 180 and ligand range 180 with twist range 360 and distance range 40.

2.4.3. *DNA cleavage study by agarose gel electrophoresis*

Gel electrophoresis of pUC19 DNA was carried out in TAE buffer (0.04 M tris-acetate, pH 8, .001 M edta). The 15 µL reaction mixture containing 300 mg/L plasmid DNA in TE buffer (10 mM tris, 1 mM edta, pH 8.0) and 200 µM complex solution. Reactions were allowed to proceed for 3 h at 37°C in dark and reactions were satiated by the addition of 5 µL loading buffer (0.25% bromophenol blue, 40% sucrose, 0.25% xylene cyanol, and 200 mM edta). The aliquots were loaded directly onto 1% agarose gel and electrophoresed at 50 V in 1× TAE buffer. Gel was stained with 0.5 mg/L ethidium bromide and was photographed on a UV illuminator. The percentage of each form of DNA was determined. After electrophoresis, the proportion of DNA in each fraction was estimated quantitatively from the intensity of the bands using AlphaDigiDoc™ RT. Version V.4.0.0 PC-Image software. The degree of DNA cleavage activity was expressed in terms of the percentage of conversion of the SC-DNA to OC-DNA and L-DNA according to the equation reported in published literatures (Mehta et al., 2015).

2.4.4. In vitro *cytotoxic study using brine shrimp lethality bioassay (BSLB)*

Brine shrimp (Artemia cysts) eggs were hatched in a shallow rectangular plastic dish (22×32 cm), filled with

artificial seawater, which was prepared with commercial salt mixture and double-distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately, 50 mg of eggs were sprinkled into the large compartment and was opened to ordinary light. After two days, nauplii were collected by a pipette from the lighted side. The detailed experimental process is reported in newly published literatures (Mehta et al., 2015).

2.4.5. *Cellular level bioassay using* S. pombe *cells*

Cellular level bioassay was done using *S. pombe* cells, which were grown in liquid yeast extract media in 150 mL Erlenmeyer flask containing 50 mL of yeast extract media. Flask was incubated at 30°C on shaker at 150 rpm till the exponential growth of *S. pombe* obtained (24–30 h). Then the cell culture was treated with the different concentrations (2, 4, 6, 8, 10 mg/L) of synthesized complexes, free ligands, and also with dimethylsulphoxide (DMSO) as a control and further allowed to grow for 16–18 h. Next day, by centrifugation at 10,000 rpm for 10 mins; treated cells were collected and dissolved in 500 µL of PBS. The 80 µL of yeast culture dissolved in PBS and 20 µL of 0.4% trypan blue prepared in PBS were mixed and cells were observed in a compound microscope (40X). The dye could enter the dead cell only so they appeared blue, whereas live cells resisted the entry of dye. The number of dead cells and number of live cells were counted in one field. Cell counting was repeated in two more microscopic fields and average percentage of cells which died due to synthesized compounds were calculated.

2.4.6. In vitro *antimalarial study using strain of* Plasmodium falciparum

All the synthesized organometallic ruthenium complexes and ligands were screened for their antimalarial study against the *P. falciparum* strain and was acquired from Shree R. B Shah Mahavir superspeciality hospital, Surat, Gujarat, India was used in *in vitro* tests. The detailed experimental process is reported in published literatures (Mehta et al., 2015).

3. Results and discussion

3.1. *Characterization of Ru(III) complexes*

Characterization of Ru(III) complexes by magnetic moments, electronic spectra, conductance measurements, LC–MS spectrum analysis, and thermal analysis techniques are shown in Supplementary material 4.

3.2. *Biological study of synthesized compounds*

3.2.1. In vitro *antibacterial screening*

The antimicrobial activity of free pyrazoline ligands (5a–5g) and synthesized complexes (6a–6g) has been tested against five different bacteria in terms of the minimum inhibitory concentration (MIC). Results shows that all the ruthenium complexes are active against all the micro-organisms than those of respective pyrazoline ligands (Supplementary material 5). The MIC values of all synthesized complexes ranges from 67 to 102 µM. The mechanism of toxicity of the complexes may be ascribed to the increase in the lipophilic nature of the complexes arising from chelation. Another mechanism of toxicity of these complexes to micro-organisms may be due to the inhibition of energy production or ATP production, by inhibiting respiration or by the uncoupling of oxidative phosphorylation.

The increase in antimicrobial activity of metal complexes can be the result of: (I) chelate effects, (II) nature of the ligands, (III) total charge of the complex, (IV) nature of the ion neutralizing the complex, and (V) nuclearity of the metal center in the compounds. The inhibition activity seems to be governed in certain degree by the facility of coordination at the metal center as well as electronic nature of the ligands. The lower MIC value of complex (6a) is probably due the greater lipophilic nature of the complex (6a) as well as might be due to presence of fluorine atom as a substituent. Presence of more electronegative environment in complex (6a) enhances its antimicrobial property. Such increased activity of the metal chelates and possible mode of increased toxicity of the Ru(III) complexes compared to that of the free pyrazoline and diverse biological activity due to coordination of the pyrazoline to the metal can be explained on the basis of Tweedy's chelation theory and Overtone's concept (Mehta et al., 2016).

3.2.2. *DNA-binding study*

Since DNA is the crucial target for many metal-based drugs, and DNA-binding is the critical step for the study of effective metal-based drugs, which is of prominence in illuminating the mechanisms involved in the site-specific recognition of DNA, and designing new types of pharmaceutical molecules, the binding behaviors of the synthesized compounds toward DNA are discovered both theoretically and experimentally with the aid of different procedures and techniques.

3.2.2.1. By absorption titration. Electronic absorption spectroscopy is one of the most useful techniques for the study of binding mode and binding strength of the metal complexes with DNA. The binding ability of the

complexes with HS DNA can be characterized by measuring their effects on absorption spectra. An absorption spectrum of synthesized complexes with HS DNA was recorded for a constant concentration of complexes i.e. 20 μM with varying concentration of DNA i.e. 100 μM to obtain different DNA/complex mixing ratio is shown in Figure 1.

The magnitude of the hypochromism and red shift is commonly found to depend on the strength of the intercalative interaction. The extent of spectral change is related to the strength of binding. The absorption spectral alterations are observed in the intraligand charge transfer (ILCT) band around 260–290 nm for the examination of DNA-binding mode and strength. As the DNA concentration is increased, the ILCT transition bands of synthesized complexes exhibit hypochromicity [hypochromicity, H% = $[(A_{free} - A_{bound})/A_{free}] \times 100\%$] of about 18.30 ± 0.74%−22.63 ± 0.86%, and bathochromicity of 2–4 nm. The complex (6a) has highest percentage of hypochromicity (22.63 ± 0.86%). These spectral characteristics may suggest a mode of binding that involves a stacking interaction between the aromatic chromophore and the DNA base pairs.

In order to elucidate the binding strength of the complexes, the DNA-binding constants K_b were determined by monitoring the changes of absorbance in the ILCT band with increasing concentration of HS DNA. The K_b values of ruthenium complexes are found in the range 1.04 (±0.08) × 10^5–7.28 (±0.25) × 10^5 M^{-1} and are higher than that of free pyrazoline ligand (Supplementary material 6).

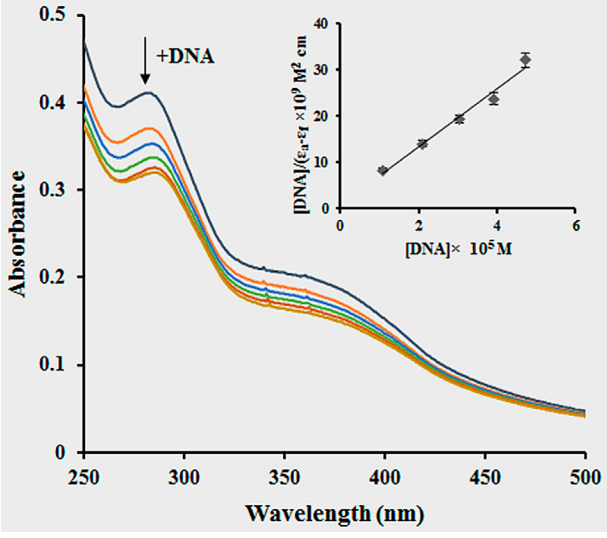


Figure 1. Absorption spectral changes on addition of HS DNA to the solution of complex 6a after incubating it for 10 mins at room temperature in phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7.2). Inset: plot of $[DNA]/((\varepsilon_a - \varepsilon_f))$ vs. [DNA]. Error bars represent standard deviation of three replicates. (Arrow shows the change in absorption with increase in concentration of DNA).

K_b values of complexes are comparable with classical intercalators ethidium bromide (7.16×10^7 M^{-1}), but higher than $[RuCl(AsPh_3)L^3]$ (3.2×10^4 M^{-1}) (Prakash, Manikandan, Viswanathamurthi, Velmurugan, & Nandhakumar, 2014) and from the K_b value and observed red shift, it is clear that the complexes bind to the DNA by intercalation mode and complex (6a) has the highest binding ability (Supplementary material 6) than the other complexes due to an existence of fluorine atom. Presence of fluorine atoms act as not only chemical isosteres for the oxygen atoms in the heterocyclic base of thymidine, but shows strong affinity of binding due to strong van der Waals force of interaction between fluorine and hydrogen atom compared to other substituents. As a result, it shows superior antimicrobial and DNA interaction activity than other compounds. The binding mode is further confirmed by viscosity measurement study.

3.2.2.2. By viscosity measurements. Viscosity measurement is regarded as the most critical means for studying the binding mode of metal complexes with DNA in solution and provides stronger fights for intercalative binding mode.

The effects of ruthenium complexes on the viscosity of HS DNA are shown in Figure 2. As illustrated in this figure, on increasing the amount of the complexes the relative viscosity of HS DNA increases progressively, which confirms that the complexes are bound to HS DNA by intercalation. This phenomenon may be explained by the insertion of the compounds between the DNA base pairs, leading to an increase in the separation of base pairs at intercalation sites and thus an increase in overall DNA length. It is clear from the figure that all these complexes show an increase in the relative viscosity of HS DNA. The viscosity results may reveal the tendency of each

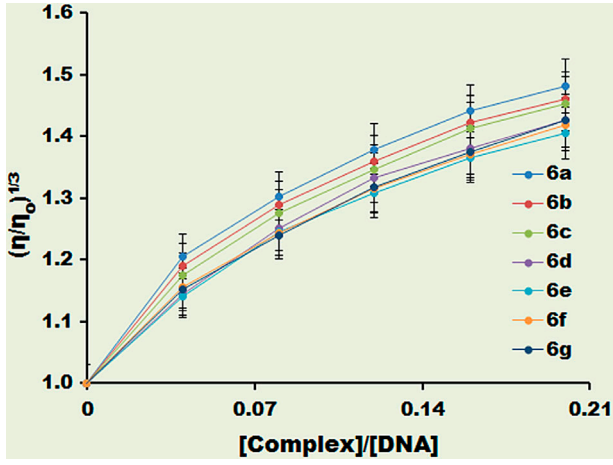


Figure 2. Effect on relative viscosity of HS DNA under the influence of increasing amounts of complexes at 27(±0.1)°C in phosphate buffer at pH 7.2. Error bars represent standard deviation of three replicates.

ligand to intercalate into DNA base pairs. The increase in DNA viscosity observed in the complexes suggests a classical intercalative mode. The increase in the degree of viscosity of all compounds depends on their affinity to DNA, with an order as follows: 6a > 6b > 6c > 6d = 6f > 6g > 6e > ligands (Supplementary material 7). This behavior is similar to that of ruthenium complexes reported by Haq et al. (1995).

3.2.2.3. By molecular docking with HS DNA. In order to test the intercalation binding mode and DNA intercalating potential of the studied compounds, we investigated their interactions with the double-stranded helical DNA. Molecular docking studies of the ruthenium complexes with the DNA duplex of sequence d $(ACCGACGTCGGT)_2$ were performed to predict the chosen binding site along with the preferred orientation of complex inside the DNA helix (Supplementary material 8).

The study shows that the complexes under investigation interact with DNA via an intercalation mode involving outside edge stacking interaction with oxygen atom of the phosphate backbone. From the ensuing docked structures, it is clear that the complexes 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. The resulting binding energies of docked complexes (6a−6g) are found to be −250.95, −249.33, −242.78, −259.73, −262.87, −261.64, and −254.93 kJ mol^{-1}, respectively. Also, resulting binding energies of docked pyrazoline ligands 5a–5g are found to be −237.34, −220.06, −238.09, −224.23, −238.05, −228.28, and −230.15 kJ mol^{-1}, respectively.

3.2.3. *DNA cleavage study by agarose gel electrophoresis*

The cleavage activity on supercoil form of pUC19 DNA by synthesized compounds has been monitored by agarose gel electrophoresis. To investigate the DNA-binding property of the synthesized pyrazoline and ruthenium complexes that are associated with further pharmacological activities, chemical nuclease activity assay has been performed. The DNA cleavage can occur by two major pathways, i.e. oxidative cleavage and hydrolytic cleavage: (Mehta et al., 2015) (I) oxidative DNA cleavage involves either oxidation of the deoxyribose moiety by abstraction of sugar hydrogen or oxidation of nucleobases. (II) Hydrolytic DNA cleavage involves cleavage of phosphodiester bond to generate fragments which can be subsequently relegated. Hydrolytic cleavage which started in a modest way of converting supercoil (SC) form of DNA to the open circular (OC) form and last in linear (L) form, is now being used for identifying the percentage of cleavage as a function of concentration of nuclease. The general accepted mechanism of the DNA hydrolysis reaction is a nucleophilic attack at the DNA phosphate backbone, to form a five-coordinate intermediate, which can be stabilized by the catalyst. Subsequent cleavage of either the 3′-PO or the 5′-PO results in a strand scission. After this nucleophillic attack, one group leaves as an alcohol (Sangeetha Gowda, Mathew, Sudhamani, & Naik, 2014).

The principle of this method is that molecules migrate in the gel as a function of their mass, charge, and shape, with supercoiled DNA migrating faster than open circular molecules of the same mass and charge. The native DNA remains in the supercoiled (SC) form, also known as covalently coiled coil DNA, here designated as Form I. Single strand cleavage results in so called nicked or open circular (OC) form of DNA (designated as Form II), whereas the double-strand cleavage results in linear (L) form of DNA (designated as Form III).

Figure 3 illustrates the cleavage of pUC19 DNA induced by the compounds under aerobic conditions. This clearly shows that the relative binding efficacy of complexes to DNA is much higher than the binding efficacy of ruthenium salt and free pyrazoline ligands (Supplementary material 9). The difference in DNA cleavage efficiency of complexes was due to the difference in binding affinity of complexes to DNA. The similar behavior of ruthenium complexes with plasmid DNA was shown by reported compounds of type cis,fac-[RuCl $(dmso\text{-}S)_3(L)$] (Gaur et al., 2011).

3.2.4. In vitro *cytotoxic study using BSLB*

The BSLB is considered as a useful method for preliminary calculation of toxicity of compounds and development in the assay procedure of bioactive compound, which indicates cytotoxicity as well as a wide range of pharmacological activities (such as antiviral, insecticidal, anticancer, pesticidal, etc.) of the compounds. All the synthesized compounds were studied for their cytotoxicity using the protocol of Meyer et al. (1982). The method is inexpensive, rapid, reliable, and economical.

Results for the lethality are noted in terms of deaths of larvae. The mortality rate of brine shrimp nauplii is found to increase with increasing concentration of complexes. A plot of the log of sample's concentration versus percentage of mortality showed a linear correlation. From the graph, the LC_{50} values of the compounds are calculated, and they were found in the range of 5.714−83.368 mg/L (Supplementary material 6). Complex (6a) is the most potent amongst all the compounds. From the data recorded, it is concluded that the synthesized complexes are good cytotoxic agent than that of respective ligands. The order of potency of compounds is 6a > 6b > 6d > 6c > 6g > 6e > 6f > pyrazole ligands.

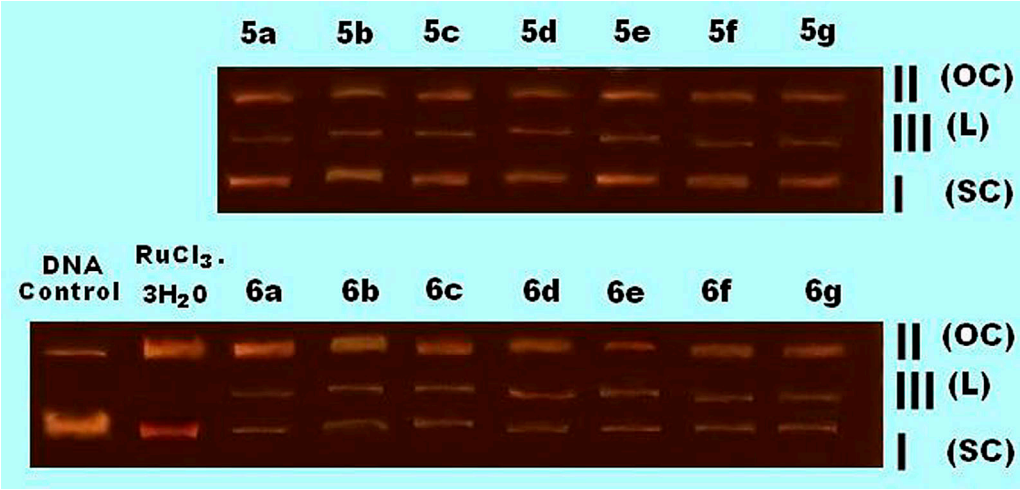


Figure 3. Photogenic view of cleavage of pUC19 DNA (300 μg/cm^3) with series of compounds using 1% agarose gel containing 0.5 μg/cm^3 EtBr. All reactions were incubated in TE buffer (pH 8) at a final volume of 15 mm^3 for 24 h at 37°C.

Table 1. Effect of compounds on viability of *S. Pombe* cells at different concentrations with error uncertainty in the value ±5%.

Concentration (mg/L)	2	4	6	8	10
Compounds			% Viability		
5a	72 ± 2	68 ± 2	62 ± 1	59 ± 1	56 ± 1
5b	73 ± 2	69 ± 2	65 ± 1	61 ± 1	58 ± 1
5c	74 ± 2	67 ± 2	64 ± 1	61 ± 1	59 ± 1
5d	76 ± 2	69 ± 2	66 ± 1	62 ± 1	60 ± 1
5e	79 ± 2	75 ± 2	72 ± 2	68 ± 1	63 ± 1
5f	82 ± 3	77 ± 2	74 ± 2	72 ± 2	70 ± 2
5g	85 ± 3	78 ± 3	72 ± 2	70 ± 2	67 ± 2
6a	62 ± 1	58 ± 1	55 ± 1	50 ± 1	46 ± 1
6b	64 ± 1	60 ± 1	57 ± 1	54 ± 1	47 ± 1
6c	67 ± 2	61 ± 1	56 ± 1	52 ± 1	48 ± 1
6d	70 ± 2	63 ± 1	56 ± 1	51 ± 1	48 ± 1
6e	72 ± 2	66 ± 2	60 ± 1	55 ± 1	49 ± 1
6f	73 ± 2	66 ± 2	61 ± 1	56 ± 1	50 ± 1
6g	75 ± 2	70 ± 2	64 ± 1	56 ± 1	50 ± 1
DMSO			96 ± 4		
Untreated cells			97 ± 4		

The degree of lethality is found to be directly proportional to the concentration of the compounds. The mortality rate of brine shrimp nauplii is found to increase with increasing concentration of complexes.

3.2.5. *Cellular level bioassay using* S. pombe *cells*

The cellular level cytotoxicity bioassay of free pyrazoline (5a–5g) and synthesized ruthenium complexes (6a–6g) has been tested against *S. pombe* in terms of the % viability. Table 1 shows that all the ruthenium complexes are active against *S. pombe* cells than those of respective pyrazoline ligands.

S. pombe cells have become an important tool to study cell biology due to its eukaryotic and fairly big size characteristics. Cell death caused by toxicity of the chemically synthesized compounds could be easily observed by vital staining (Supplementary material 10). The toxicity is found to vary with the type of substituent present and concentrations of the synthesized compounds. General observation is that as concentration of compounds increased, the cytotoxicity is also increased. After treatment, many of the *S. pombe* cells are killed due to toxic nature of the compound. Complex (6a) is the most active amongst all the compounds.

3.2.6. In vitro *antimalarial study using strain of* P. falciparum

Malaria is one of the parasitic infections that cause vast medical, financial, and emotional problem in the world. *P. falciparum* is the parasite responsible for most malaria

cases up to 80%, which often proves harmless. As part of this search for novel drugs against malaria, we report encouraging results for new compounds resulting from the modification of pyrazoline moiety by coordination to ruthenium metal centers; the new complexes are highly active against a chloroquine resistant strain of *P. falciparum*. The results of the pharmacological screening are expressed as the drug concentration resulting in 50% inhibition (IC_{50}) of parasite growth. The mean values of IC_{50} in mg/L for all complexes and respective ligands are given in Supplementary material 6.

Results of data show that all complexes exhibit good antimalarial activity than respective ligands (0.54–2.15 mg/L). The highest activity is observed for the complex (6a) which exhibits the lowest IC_{50} value (0.54 mg/L). It is thus clear that the combination of pyrazoline ligand and ruthenium metal in a single molecule does produce an enhancement of the activity against resistant strains of the parasite, demonstrating the validity of our concept in the search for novel antimalarial drugs capable of overcoming resistance. The IC_{50} values of all ruthenium complexes are comparable to $[Ru(A)_2(B)]Cl_2$ (10 mg/L) (Anchuri et al., 2013).

4. Conclusions

A series of substituted pyrazoline-based half-sandwich organometallic ruthenium complexes have been synthesized and well characterized. The synthesis and structural characterization of compounds were carried out with an aim to study their biological activity. Likewise, arene ring, pentamethylcyclopentadienyl ring derivatives occupy three coordination sites at the octahedral metal center and provide to the metal a lipophilic protecting face. On the other hand, the remaining three coordination sites allow the introduction of ligands with hydrophilic character. By analogy with their structure to a piano stool, these are known as piano stool complexes, with the metal center being coordinated by pentamethylcyclopentadienyl ring, a chlorido ligand and a chelating pyrazole ligand. The molar conductivities values of all organometallic Ru(III) complexes are indicates, one counter ion present outside the coordination sphere which accomplish all complexes are ionic in nature. The antimicrobial activity of the complexes has been tested on five different micro-organisms and the results show an enhanced biological activity in relation to the free ligands. All the ruthenium complexes show good *in vitro* cytotoxic as well as *in vitro* antimalarial activity. A comparative study of cellular level cytotoxicity values of the all compounds indicates that the metal complexes show better activity against *S. pombe* cells compared to the pyrazoline ligand. The viscosity data are in good agreement with bathochromicity and hypochromicity observed in UV–vis absorption curve and suggests classical intercalation for all the ruthenium complexes, where complex (6a) binds more strongly than rest of the complexes. Molecular docking studies of the complexes with the DNA duplex of sequence $d(ACCGACGTCGGT)_2$ were performed to predict the chosen binding site, which suggests intercalation between complex and DNA base pairs. The DNA cleavage study of pUC19 shows that all complexes have high cleavage ability than metal salt and ligands. From the biological activity, complex (6a) is found to be more potent than other due to the presence of fluorine atom at fourth position of aromatic ring as a substituent. Presence of more electronegative environment in complex (6a) improves its biological property. The preliminary studies encourage for carrying out further *in vivo* experiments. The results are of importance toward further designing and developing ruthenium-based complexes and systematic assessment of biological activity for their potential applications as therapeutic agents.

Supplementary material

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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