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DNA Binding and Cleavage Study of Novel Ruthenium (II)-Polypyridine-5-(3-pyridyl)-4H-1,2,4-Triazole-3-Thiol Complex on *Escherichia Coli* Genomic DNA

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Abstract

Background: Transition metal complexes especially Ruthenium-Polypyridyl complexes interact with multidentate ligands considered as a new therapeutic agent to make the possible DNA probes and conformers due to several interests owing to their potential applications. The aim of the present work is to concentrate on the binding and cleavage activity of $[Ru(bpy)_2(pytrzSH)_2]^{2+}$ (complex 1) and $[Ru(phen)_2(pytrzSH)_2]^{2+}$ (complex 2) (bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline, pytrzSH = 5-(3-pyridyl)-4H-1,2,4-triazole-3-thiol) on *Escherichia coli* genomic DNA (gDNA). **Materials and Methods:** DNA binding and cleavage activity is carried out using ultraviolet-Visible spectral technique and Agarose gel electrophoresis method at three different concentrations against the standard genomic DNA isolated from *E. coli* bacteria. **Results:** The changes in the absorbance and wavelength upon incremental addition of the complexes on gDNA clearly depict the binding nature of complexes. The binding constant values for ligand centered and metal to ligand charge transfer transitions obtained from the Benesi Hildebrand plots are found to be 1.560×10^4 and 9.586×10^4 M^{-1} for complex 1 and 3.594×10^4 and 9.801×10^5 M^{-1} for complex 2. The results revealed that complex 2 shows better binding property than complex 1 on *E. coli* gDNA. The extent of DNA cleavage activity of the synthesized complexes on *E. coli* gDNA is determined from the band intensities, complex 2 shows full cleavage in all the three concentrations, whereas complex 1 exhibits full cleavage at 100 µg/mL. The cleaving ability depends on the nature of the ligands present in the complexes. **Conclusion:** The synthesized $[Ru(bpy)_2(pytrzSH)_2]^{2+}$ (complex 1) and $[Ru(phen)_2(pytrzSH)_2]^{2+}$ (complex 2) bind with the *E. coli* gDNA through electrostatic and intercalative modes. The $[Ru(phen)_2(pytrzSH)_2]^{2+}$ complex 2 shows better cleavage activity than $[Ru(bpy)_2(pytrzSH)_2]^{2+}$ complex 1.

Keywords: Binding constant, DNA binding, DNA cleavage, *E. coli* genomic DNA, ruthenium(II)-polypyridine complexes

Introduction

Bacterial genomic DNA (gDNA) is a highly condensed and functionally organized nucleus-like structure without a nuclear membrane resides inside the cells. The first important genome which gets completely sequenced and contributes major to recombinant DNA technology is *E. coli*.[1-3] It survives when released to the natural environment and gets colonized in the lower gut of animals allowing widespread dissemination to new hosts.[4] Therefore, *Escherichia coli* is frequently used as a model organism in microbiology and molecular biology studies.[5] The *E. coli* chromosome is composed of the gDNA, RNA and protein.[6,7]

Nowadays, research is focused toward the introduction of novel and biologically safe therapeutic agents. Metal complexes play a vital role in pharmaceutical and medicinal chemistry and are used as therapeutic agents.[8-10] Researchers have reported that the binding of a drug to a metal complex increases its activity.[11] Among the transition metal complexes, particularly ruthenium(II)-polypyridyl complexes have significant interest for developing new diagnostic and therapeutic agents that can

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recognize and cleave DNA. Ru(II)-ploypyridyl complexes binds with DNA, RNA, and proteins and act as therapeutic agents.[12-14] Ruthenium polypyridyl complexes have been widely tested in DNA binding studies and have become the ideal candidates for the design of DNA binding systems. There is a wide range of applications of ruthenium polypyridyl complexes in DNA molecular light switches, DNA structure probes, DNA-mediated charge transfer, and anticancer drugs, because of their unique properties.[15]

Ruthenium complexes with 2,2′-bipyridine (bpy) or 1,10-phenanthroline (phen) and their derivatives are also extensively studied due to their interesting physico-chemical and biological properties. The bpy ligand act as a precursor for helical assembly chiral molecular recognition luminescent devices and other applications in photonics and optoelectronics.[16] 1,10-Phenanthroline is also an important metal chelating agent. The planar nature of phen ligand can act as a DNA intercalator. Recently, nitrogen-containing heterocycles are commonly found in most of the medicinal compounds. Therefore, triazoles have received considerable attention owing to their synthetic and medicinal importance. Ruthenium(II) complexes with varying the nature of the substituent group in the intercalating ligands can create some differences in the spatial configuration and electron density distribution, resulting in differences in DNA binding behaviors and photocleavage properties.[17,18]

Based on this concept, the present investigation mainly focuses on the synthesis, characterization DNA binding and cleavage activity of novel $[Ru(bpy)_2(pytrzSH)_2]^{2+}$ (complex 1) and $[Ru(phen)_2(pytrzSH)_2]^{2+}$ (complex 2) (bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline, pytrzSH = 5-(3-pyridyl)-4H-1,2,4-triazole-3-thiol) on *E. coli* genomic DNA (gDNA).

Materials and Methods

$RuCl_3.3H_2O$, bpy, phen, and pytrzSH were procured from Sigma-Aldrich. All the solvents and NH_4PF_6 were procured from Merck. HPLC grade solvents and double-distilled deionized water were used for the synthesis, binding, and cleavage studies of the complex.

Synthesis of complex 1

The $[Ru(bpy)_2Cl_2]$ (0.5 mM) and pytrzSH (1 mM) were dissolved in 20 mL of methanol and refluxed for 4 h under a nitrogen atmosphere. The resulting solution was cooled and filtered to remove any insoluble impurities. A saturated solution of NH_4PF_6 was then added dropwise into the filtrate until a brown precipitate was formed. The product was filtered, washed with cold water and diethyl ether, and further dried in a vacuum desiccator. Further, the synthesized complex was purified by column chromatography using silica gel as the adsorbent and a mixture of methanol and dichloromethane in the ratio of 2:8 as the eluent and on subsequent evaporation to recover the complex. The yield obtained was found to be 0.4321 g.

Synthesis of complex 2

The $[Ru(phen)_2Cl_2]$ (0.5 mM) and pytrzSH (1 mM) were dissolved in 20 mL of methanol and refluxed for 4 h under nitrogen atmosphere. The resulting solution was cooled and filtered to remove any insoluble impurities. A saturated solution of NH_4PF_6 was then added dropwise into the filtrate until a dark brown precipitate was formed. The product was filtered, washed with cold water and diethyl ether, and further dried in a vacuum desiccator. The synthesized complex was purified by column chromatography using silica gel as the adsorbent and a mixture of methanol and dichloromethane as the eluent and on subsequent evaporation to recover the complex. The yield obtained was found to be 0.4472 g.

Equipment

The CHNS Elemental analysis of the synthesized complexes was measured using Truspec Micro Analyser. The absorption spectrum of the complexes was measured using SHIMADZU ultraviolet (UV) 1800 double beam spectrophotometer. The Fourier Transform Infrared (FTIR) spectral analysis was measured using SHIMADZU FTIR double beam spectrophotometer. The ^1H and ^{13}C NMR spectra were measured using BRUCKER spectrometer. The MALDI-TOF-MS analysis was carried out to determine the m/z peak using Bruker Daltonics Flex-PC microflex analyzer. The magnetic moment, molar conductance was measured using Guoy's balance and Systronics digital conductivity meter. The lipophilicity of the complexes was measured using shake-flask method.

DNA binding study

A fixed concentration of *E. coli* gDNA (1.5 µg/mL) in nuclease-free water was incubated individually with different concentrations (25, 50, 100 µg/mL) of the synthesized complexes in phosphate buffer at pH 7.4 and incubated at 37°C for 2 h. After incubation, UV-Visible spectra were recorded. The change in absorbance with wavelength in treated samples were analyzed and compared with that of untreated DNA control. The K_b of the synthesized complexes on *E. coli* gDNA was calculated from the Benesi Hildebrand equation:

$$1/\Delta A = 1/Kb\ \Delta\varepsilon\ [H] + 1/\Delta\varepsilon[G]$$

where, [H] = concentration of the host (DNA), (G) = concentration of the guest (complex), ΔA = change in the absorbance of the (DNA) on the addition of (complex) and $\Delta\varepsilon$ = difference in the molar extinction coefficient between the free (DNA) and (DNA)-(Ru) complex. The plot of $1/\Delta A$ *versus* 1/(complex) gives a straight line. The K_b value can be obtained from the ratio of Y-intercept to the slope of the straight line.

DNA cleavage study

DNA cleavage activity of the synthesized complexes was studied using gel electrophoresis method. The DNA was treated with 25, 50, and 100 µg of the respective complexes in a reaction volume of 15 µL and incubated at 37°C for 2 h. After incubation the treated as well as control DNA were subjected to agarose gel electrophoresis in 1% gel in 0.5% TAE buffer for 1 h. The cleavage property of the complexes on *E. coli* gDNA was determined from the respective photographs. The

band intensity observed in the lanes determined the cleavage activity of the complexes.

Results

Synthesis and characterization of complexes 1 and 2

The synthesized complexes 1 and 2 involve the coordination of Ru(II) atom individually with two bidentate bpy, phen, and two monodentate pytrzSH ligands *via* nitrogen atom of pyridine moiety, forming an octahedral complex as shown in schemes 1 and 2. The synthesized brown-colored complex 1 and dark brown colored complex 2 are stable solid soluble in water, acetonitrile, acetone, DMSO, and DMF.

Elemental analysis

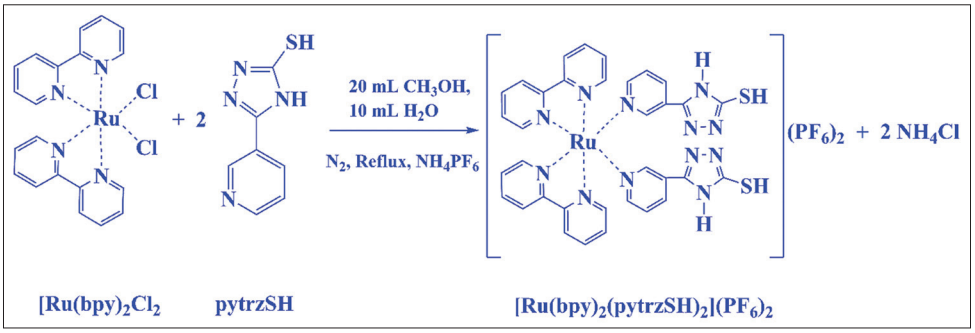
The percentage of C, H, N, and S is determined by elemental analysis. The experimental and theoretical values for the elemental analysis of complexes 1 and 2 are in good agreement with the proposed molecular formulas $C_{34}H_{28}N_{12}S_2Ru.\ 2PF_6$ and $C_{38}H_{28}N_{12}S_2Ru.\ 2PF_6$.

Ultraviolet-visible spectral analysis

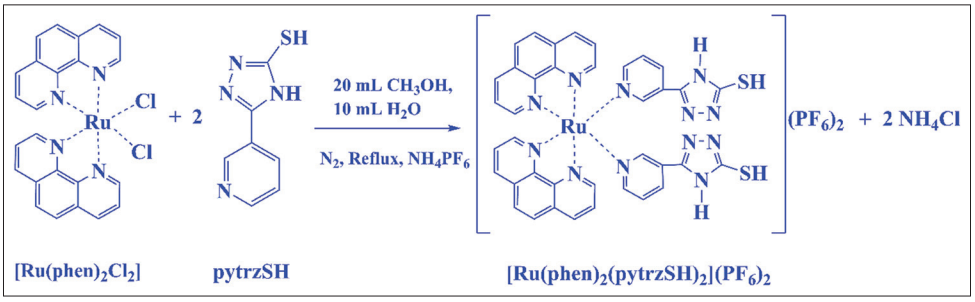
The absorption spectra of the synthesized complexes display two spin allowed π–π* transitions at 245 and 295 nm for complex 1 and 227.5 and 266 nm for complex 2. This is due to the presence of bpy, phen, and pytrzSH ligands present in the complexes. The weak absorption bands obtained at 365.5 nm for both the complexes 1 and 2 correspond to the metal centered transition and the absorption in the region 476 nm for complex 1 and 464 nm for complex 2 is assigned due to dπ (Ru)→L metal to ligand charge transfer (MLCT) transition [Figure 1].

FTIR spectral analysis

The FTIR spectrum of complex 1 shows absorption bands at 3455, 2941, 2876, 2729, 1653, 1456, 1411, 1301, 1290, 1033,



Scheme 1: Synthesis of complex 1



Scheme 2: Synthesis of complex 2

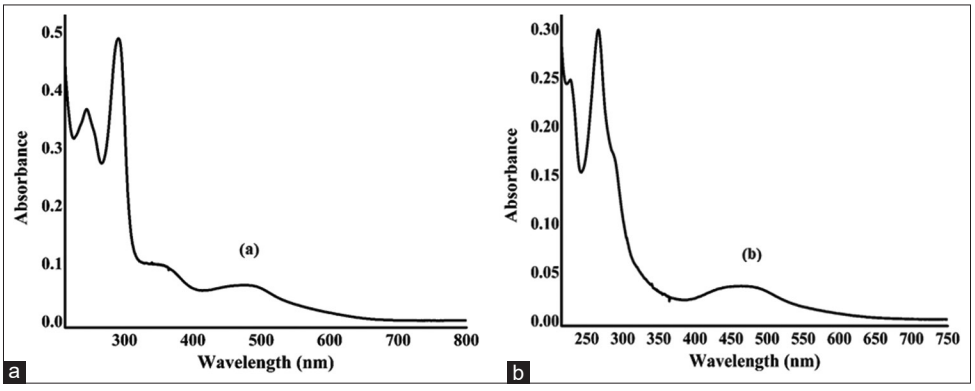


Figure 1: Absorption spectra of (a) complex 1 and (b) complex 2

1031, 842, 761, 729, 676, 559 and 435 cm^{-1} and for complex 2 at 3453, 2933, 2825, 2733, 1606, 1473, 1421, 1301, 1290, 837, 745, 715, 558 and 435 cm^{-1} respectively [Figure 2]. The absorption bands occur at 3455 cm^{-1} for complex 1 and 3453 cm^{-1} for complex 2 indicate the N-H stretching vibration of pytrzSH ligand. The existence of free–SH group in both the complexes are confirmed by the formation of weak bands at 2729 and 2733 cm^{-1}. The absorption bands obtained for complex 1 at 1653, 1456, 1411, 1301, and 761 cm^{-1} correspond to the bpy ligands. The complex 2 shows absorption peaks at 1606, 1473 and 1421 cm^{-1} which represents the presence of phen ligands. The peaks occur at 2941 and 2876 cm^{-1} for complex 1 and 2933 and 2825 cm^{-1} for complex 2 denotes the aromatic C − H stretching vibrations of bpy, phen, and pytrzSH ligands. The in-plane bending vibrations of pyridyl rings are obtained as a weak band at 1290 and 1031 cm^{-1} for complex 1 and 1290 cm^{-1} for complex 2. Bands obtained at 1033 cm^{-1} for complex 1 and 1346 cm^{-1} for complex 2 correspond to the N-N stretching vibration of 1,2,4-triazole ring present in the pytrzSH ligand. The C-S stretching band for pytrzSH ligand is obtained at 678 cm^{-1} for complex 1 and 715 cm^{-1} for complex 2. The bands obtained at 729, 559 and 435 cm^{-1} for complex 1 and 745, 558 and 435 cm^{-1} for complex 2 confirm the coordination of nitrogen atoms of bpy, phen and pytrzSH ligands to the central ruthenium metal. The weak absorption band obtained at 839-845 cm^{-1} for both the complexes confirm the presence of hexafluorophosphate counter ion.[19] Thus, the FTIR spectral data validate the formation of complexes 1 and 2 with PF_6^- counter ions.

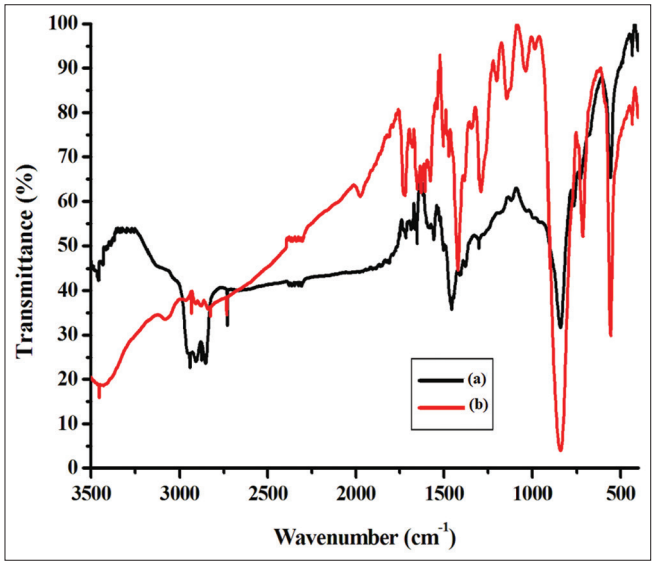


Figure 2: FTIR spectra of (a) complex 1 and (b) complex 2

^{1}H NMR spectral analysis

The ^{1}H NMR spectra of the synthesized complexes show the proton signals of bpy, pytrzSH, and phen ligands coordinated through the nitrogen atoms of pyridine rings to the Ru atom [Figure 3]. The chemical shift signals of bpy ligand range from 7.553 to 8.712 ppm to the Ru metal core. The pyridine ring attached to the 5th position of 1,2,4-triazole ring present in the ancillary ligand pytrzSH shows proton signals at 9.208 ppm for H^2 proton, 8.292 ppm for H^4, 7.582 ppm for H^5 and 9.096 ppm for H^6 proton. The free–SH group present in the 3rd position and the proton attached to the nitrogen atom at 4th position of 1,2,4-triazole unit shows signals at 10.035 and 6.5 ppm, respectively. The ^{1}H NMR spectral data confirms the formation of complex 1.

The complex 2 containing phen units exhibit the chemical shift signals ranging from 7.596 to 8.865 ppm. The pyridine ring attached to the 5th position of 1,2,4-triazole ring present in the pytrzSH ligand show signals at 9.134 ppm for H^2, 8.45 ppm for H^4, 7.57 ppm for H^5 and 9.139 ppm for H^6 protons. The–SH group present in the 3rd position and the proton attached to the nitrogen atom at 4th position of 1,2,4-triazole ring shows signals at 9.994 and 6.995 ppm respectively. The spectral data confirm the formation of complex 2.

^{13}C NMR spectral analysis

The ^{13}C NMR spectrum of complex 1 exhibits the carbon signals for bpy ligands is in the range 123–149 ppm. The carbon signals of phen ligands for complex 2 occur in the region 121–150 ppm. The ^{13}C NMR signals of pytrzSH ligand containing pyridine and 1,2,4-triazole rings occur in the region 121–170 ppm. In pytrzSH ligand, the C^3 atom of the pyridine ring is attached to the C^5 atom of the triazole ring. The C^3 atom of pyridine ring

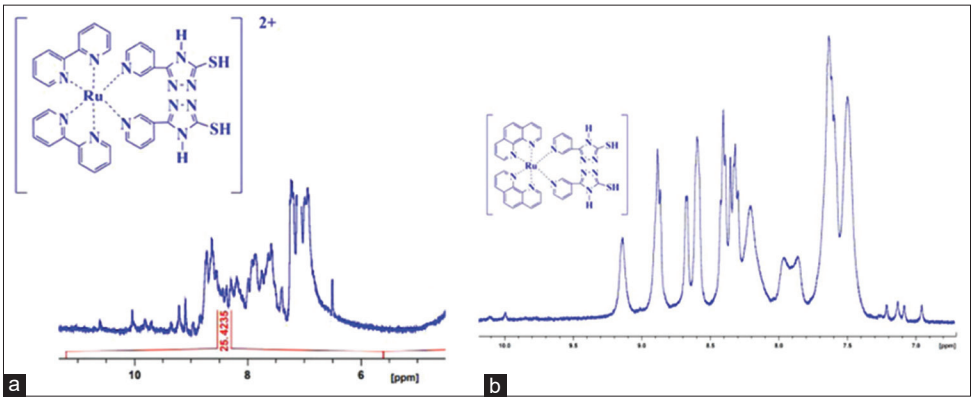


Figure 3: ^{1}H NMR spectrum of (a) complex 1 (b) complex 2

exhibits the carbon signal at 132.9 ppm for both the complexes. Similarly, the C^5 atom of the triazole ring of complex 1 and complex 2 displays at 158.6 and 156.1 ppm. The C^3 atom attached to the–SH group of triazole ring shows a chemical shift at 164.6 ppm for complex 1 and 169.5 ppm for complexes 2.

Mass spectral analysis

The MALDI-TOF mass spectra of the synthesized complexes depict the molecular ion, M + and the fragmentation peaks corresponds to the ruthenium metal coordinated with bpy, phen, and pytrzSH ligands along with $(PF_6)_2$ counter ions. The molecular ion peak with loss of one and two PF_6^- counter ions shows an m/z value corresponding to M^+-PF_6^- and M^+-$2PF_6^-$ respectively. The m/z values of molecular and fragmentation ions obtained from the mass spectral data of complexes 1 and 2 are in good agreement with the theoretical values calculated from the molecular formula of the corresponding complexes [Table 1]. Thus, the MALDI-TOF mass spectral data validate the assigned structure of the complexes [Figure 4].

Determination of magnetic susceptibility, molar conductance and lipophilicity of complexes

The synthesized complexes 1 and 2 when coordinated with strong field ligands such as bpy, phen, and pytrzSH possess low spin Ru^{2+} complexes of + 2 oxidation state. The μ_{eff} value for the both the synthesized complexes obtained from the magnetic susceptibility measurements are <0 BM and it is evident that the complexes 1 and 2 are said to be diamagnetic in nature.

The molar conductance value of the complexes 1 and 2 exhibits at 180 and 190 Ω^{-1} cm^2 mol^{-1} which clearly indicates the substitution of two monodentate ligands by the replacement of two chloride ions from the precursor complexes and it also point out the presence of two PF_6^- counter ions in the ratio 1:2. The molar conductivity results reveal that both the synthesized complexes are nonelectrolytic in nature and are suitable for biological studies. Molar conductance value of the synthesised complexes validate the octahedral geometry with low spin crystal field.[20]

The pharmacokinetic properties and their interaction with the macromolecular target of the synthesized complexes are determined by the lipophilicity measurement. The lipophilicity of complexes 1 and 2 are quantified by the measurement of the partition coefficient, *log P* in octanol/water mixture, and the values obtained is found to be 1.20 ± 0.00 for complex 1 and 1.86 ± 0.002 for complex 2. The lipophilicity values validate the lipophilic nature of the synthesized complexes. Hence, the synthesized complexes 1 and 2 are appropriate for biological studies.

Determination of DNA purity

The purity and the quantity of the diluted *E. coli* gDNA stock solution prepared from nuclease-free water in 0.1 M sodium phosphate buffer at pH 7.4 is determined by UV-visible spectrophotometric method. The absorbance value of *E. coli* gDNA at 260 and 280 nm is noted and the ratio of the two absorbance value is found to be 1.7 [Table 2]. This value indicates that the corresponding DNA is good for downstream studies. The obtained result suggests that the gDNA isolated from *E. coli* is of good quality and is used for DNA binding experiment.[21]

Binding and cleavage studies of complexes 1 and 2 on *E. coli* gDNA

The DNA binding nature of complexes 1 on *E. coli* gDNA is shown in Figure 5. As the concentration of the complex increases from 25 to 100 µg/mL, there is a slight increase in wavelength and considerable increase in absorbance corresponds to the LC and MLCT regions of the complex.

Table 1: Mass spectral data of complexes 1 and 2

Complex	M^+ (m/z)	$M^+ - PF_6^-$ (m/z)	$M^+ - 2PF_6^-$ (m/z)
Complex 1	1059.703	914.825	769.991
Complex 2	1107.885	963.089	817.696

Table 2: Purity and quantity determination of *Escherichia coli* genomic DNA

DNA source	A_{260}	A_{280}	$A_{260/A280}$	DNA concentration (ng/µL)
Escherichia coli	0.17	0.10	1.7	850

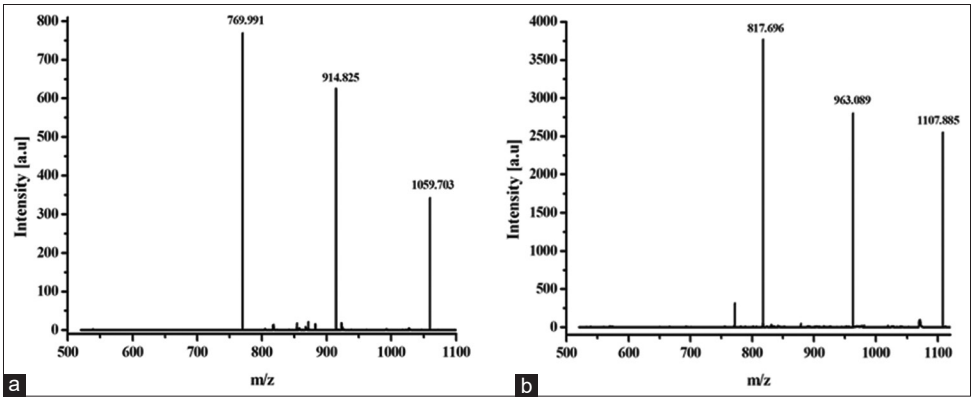


Figure 4: Mass spectrum of (a) complex 1 (b) complex 2

The interaction of complex 2 on *E. coli* gDNA shows an increase in absorbance and wavelength from 25 to 100 µg/mL in the LC and MLCT regions, which leads to hyperchromic and bathochromic shifts [Figure 6].

Complexes 1 and 2 cleave the *E. coli* gDNA at different concentrations and are shown in Figure 7. The extent of DNA cleavage activity of the synthesized complexes on *E. coli* gDNA is determined from the band intensities.

Discussion

The present investigation deals with the DNA binding and cleavage studies of novel complexes 1 and 2. Elemental analysis and spectral data confirm the formation of octahedral complexes 1 and 2. The molar conductance, magnetic susceptibility, and lipophilicity values suggest that these synthesized complexes are appropriate for biological studies.

DNA binding studies of complexes 1 and 2 on *E. coli* gDNA shows bathochromic and hyperchromic shifts in the LC and MLCT regions this clearly depicts that the DNA binding is taken *via* electrostatic as well as intercalative modes of binding. The bathochromic shift of complex 1 is due to the intercalative interaction of bpy and pytrzSH ligands. The 3-pyridyl group in the 5th position and the free thiol group in the 3rd position of the pytrzSH ligand bind with the DNA base pair. The hyperchromic shift of complex 1 on gDNA also exhibits the electrostatic binding of the cationic complex to the negative group of phosphate backbone, which leads to the overall damage to the secondary structure of the DNA. This result is in accordance with the DNA binding studies of various complexes with hyperchromic shifts.[22,23] The binding constant (K_b) values of complex 1 at LC and MLCT regions are 1.560×10^4 and 9.586×10^4 M^{-1} respectively. The K_b values clearly describe that the complex 1 bind with the gDNA of *E. coli*. The binding property of the complex is mainly due to the free-SH group and the pyridyl substituted triazole nucleus containing secondary amino group present in the pytrzSH ligand.

The presence of hyperchromism and the bathochromic shift in complex 2 indicate that the complex gets bind with the DNA through an intercalative mode of binding. The phen and pytrzSH ligand has a larger surface area which bind with the gDNA *via* partial insertion of the aromatic moiety between the base pair units of the DNA through groove binding. The bathochromic shift of the complex 2 in the LC and MLCT regions occurs because of the π-π stacking interactions between the π-conjugated chelator and the DNA base pairs. This is in accordance with the reported binding study of Ru(II)-benzimidazole complexes with ct-DNA.[24] The planar aromatic chromophore gets bind to the gDNA result in the structural damage in the phosphate backbone of the double helix and forms a cavity so that the complex gets accommodate into the gDNA leading to distortion in DNA binding.[25] The K_b values of complex 2 on gDNA at LC and MLCT regions are calculated to be 3.594×10^4 and 9.801×10^5 M^{-1} respectively. The LC region shows a lower binding effect than that of the MLCT region. This is due to the presence of different co-ligands present in the complex which gets bind to the gDNA.

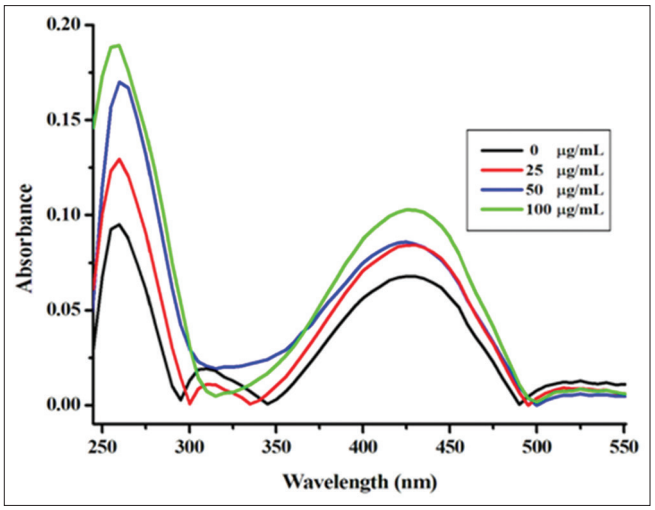


Figure 5: Absorption spectra of *Escherichia coli* genomic DNA with incremental addition of complex 1

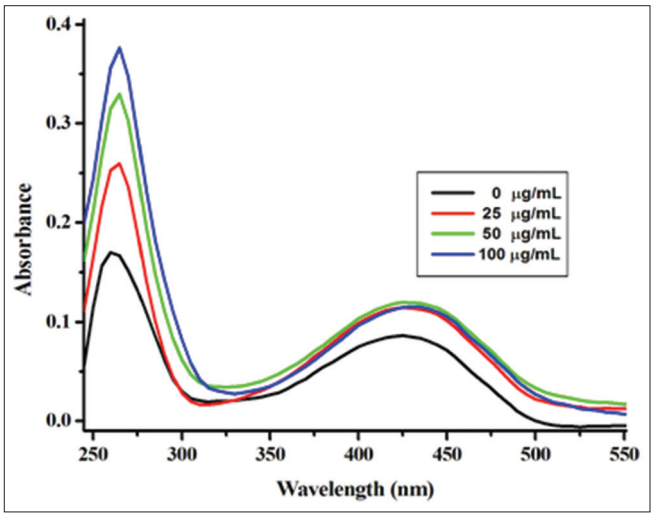


Figure 6: Absorption spectra of *Escherichia coli* genomic DNA with incremental addition of complex 2

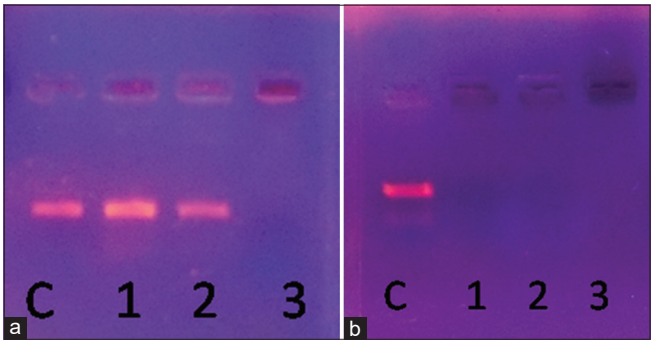


Figure 7: DNA cleavage studies of complexes on *Escherichia coli* genomic DNA at 25, 50 and 100 µg/mL (a) complex 1 and (b) complex 2

Complexes 1 and 2 cleave the *E. coli* gDNA at different concentrations and the extent of DNA cleavage activity of the synthesized complexes is determined from the band intensities. The obtained results revealed that complex 1 exhibit DNA cleavage only at 100 μg/mL and show no cleavage activity at 25 and 50 μg/mL, whereas complex 2 shows full cleavage activity at all the three concentrations. The pattern for the DNA cleavage of the synthesized complexes is analyzed by three-lane forms (1, 2, 3) and the extent of DNA cleavage for complex 1 is in the order 3 >1 = 2 and for complex 2 as 1 = 2 = 3. The auxiliary ligands present in the complexes cleave the DNA which result in the disappearance of supercoiled Form I and get converted into the relaxed DNA circular fragments of Form II. The Form II obtained due to the chemical nuclease activity leads the double helical strands get fragment to single strands resulting in the full cleavage of *E. coli* gDNA. The thiol groups present in the intercalating pytrzSH ligands are responsible for the DNA cleavage activity in both the complexes. The results indicate that the complex 2 shows better cleavage activity than complex 1. This is due to the π-π stacking interaction between the phen ligands present in the complex 2 with the DNA base pair units leads to better cleaving efficiency.

Conclusion

The DNA binding and cleavage activity of the complexes 1 and 2 on *E. coli* gDNA has been determined from UV-Visible spectral technique and Agarose gel electrophoresis method. Both the complexes bind with the *E. coli* gDNA through electrostatic and intercalative modes. The strong binding nature of the complex 2 on gDNA depends on the nature of the substituents present in the pytrzSH ligand and the π-π stacking interactions of the phen ligands, which gets stacked into the DNA base pair units. The DNA cleavage band intensities clearly depict that complex 2 shows full cleavage at all the three concentrations and complex 1 at 100 μg/mL. The results reveal that the synthesized complexes bind and cleave the *E. coli* genomic DNA to a different extent, the binding and cleaving ability depends on the nature of the ligands present in the complexes.

Ethical statement

This material is the authors' own original work, which has not been previously published elsewhere.

Human/animal rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors declare that none of the authors have any competing interest.

References

1. Khorasanizadeh S. The nucleosome: From genomic organization to genomic regulation. Cell 2004;116:259-72.
2. Malik S, Rana JS, Nehra K. Prevalence and antibiotic susceptibility pattern of uropathogenic *Escherichia coli* strains in Sonipat region of Haryana in India. Biomed Biotechnol Res J 2021;5:80-7.
3. Dwivedi VD, Bharadwaj S, Mohanty PS, Gupta UD. Bioinformatics tools for genomic and evolutionary analysis of infectious agents. Biomed Biotechnol Res J 2018;2:163-7.
4. Blattner FR, Plunkett G 3rd, Bloch CA, Perna NT, Burland V, Riley M, *et al.* The complete genome sequence of *Escherichia coli* K-12. Science 1997;277:1453-62.
5. Kurylo CM, Alexander N, Dass RA, Parks MM, Altman RA, Vincent CT, *et al.* Genome sequence and analysis of *Escherichia coli* mre600, a colicinogenic, nonmotile strain that lacks RNase I and the Type I methyltransferase, EcoKI. Genome Biol Evol 2016;8:742-52.
6. Verma SC, Qian Z, Adhya SL. Architecture of the *Escherichia coli* nucleoid. PLoS Genet 2019;15:e1008456.
7. Farhadi T. Advances in protein tertiary structure prediction. Biomed Biotechnol Res J 2018;2:20-5.
8. Sheikhshoaie I, Badiei A, Ghazizadeh M. Synthesis and characterization of a new poly(amidoamine) dendrimer-like iron (III) and molybdenum (VI) complexes. Der Chem Sin 2012;3:29-37.
9. Sabastiyan A, Suvaikin MY. Synthesis, characterization and antimicrobial activity of 2- (dimethylaminomethyl) isoindoline-1,3-dione and its cobalt(II) and nickel(II) complexes. Adv Appl Sci Res 2012;3:45-50.
10. Kostova I, Momekov G. New zirconium (IV) complexes of coumarins with cytotoxic activity. Eur J Med Chem 2006;41:717-26.
11. Rajeshirke M, Shah R, Yadav P, Purohit NV. Synthesis and antioxidant activity of metal (II) complexes of isocoumarin derivatives. Der Pharm Sin 2012;3:239-48.
12. Maksimoska J, Feng L, Harms K, Yi C, Kissil J, Marmorstein R, *et al.* Targeting large kinase active site with rigid, bulky octahedral ruthenium complexes. J Am Chem Soc 2008;130:15764-5.
13. Babu E, Muthu Mareeswaran P, Singaravadivel S, Bhuvaneswari J, Rajagopal S. A selective, long-lived deep-red emissive ruthenium(II) polypyridine complexes for the detection of BSA. Spectrochim Acta A Mol Biomol Spectrosc 2014;130:553-60.
14. Taghavi K, Fath PM, Hosseinkhani S, Mirzaei M, Behrooj H, Kiani A, *et al.* Construction and genetic improvement of copper bioreporter *Escherichia Coli*. Biomed Biotechnol Res J 2018;2:26-30.
15. Jia F, Wang S, Man Y, Kumar P, Liu B. Recent developments in the interactions of classic intercalated ruthenium compounds: $[Ru(bpy)_2dppz]^{2+}$and $[Ru(phen)_2dppz]^{2+}$with a DNA molecule. Molecules 2019;24:769.
16. Kaes C, Katz A, Hosseini MW. Bipyridine: The most widely used ligand. A review of molecules comprising at least two 2,2'-bipyridine units. Chem Rev 2000;100:3553-90.
17. Incesu Z, Bljnkl K, Akalin G, Karaburun NG. The effects of some phenanthroline ruthenium(II) complexes on A549 cell proliferation. Turk J Pharm Sci 2013;10:193-204.
18. Yu HJ, Huang SM, Li LY, Jia HN, Chao H, Mao ZW, *et al.* Synthesis, DNA-binding and photocleavage studies of ruthenium complexes [Ru(bpy) 2(mitatp)]2+ and [Ru(bpy)2(nitatp)]2+. J Inorg Biochem 2009;103:881-90.
19. Adeloye AO, Olomola TO, Adebayo AI, Ajibade PA. A high molar extinction coefficient bisterpyridyl homoleptic ru(II) complex with trans-2-methyl-2-butenoic acid functionality: Potential dye for dye-sensitized solar cells. Int J Mol Sci 2012;13:3511-26.
20. Matshwele JT, Nareetsile F, Mapolelo D, Matshameko P, Leteane M, Nkwe DO, *et al.* Synthesis of mixed ligand ruthenium (II/III) complexes and their antibacterial evaluation on drug-resistant bacterial organisms. J Chem 2020;1:1-10.
21. Bunu SJ, Otele D, Alade T, Dodoru RT. Determination of serum DNA purity among patients undergoing antiretroviral therapy using NanoDrop-1000 spectrophotometer and polymerase chain reaction. Biomed Biotechnol Res J 2020;4:214-9.
22. Dar AM, Khan MA, Mir S, Gatoo MA. DNA binding, cleavage activity, molecular docking, cytotoxicity and genotoxicity studies of newly synthesized copper based metal complexes. Pharm Anal Acta 2016;7:1-11.
23. Almarhoon ZM, Al-Onazi WA, Alothman AA, Al-Mohaimeed AM,

Al-Farraj ES. Synthesis, DNA binding, and molecular docking studies of dimethylaminobenzaldehyde-based bioactive Schiff bases. J Chem 2019;1:1-14.
24. Maikoo S, Chakraborty A, Vukea N, Dingle LM, Samson WJ, de la Mare JA, *et al.* Ruthenium complexes with mono- or bis-heterocyclic chelates: DNA/BSA binding, antioxidant and anticancer studies. J Biomol Struct Dyn 2020;1:1-23.
25. Gurudevaru C, Gopalakrishnan M, Senthilkumar K, Hemachandran H, Siva R, Srinivasan T, *et al.* Synthesis and structural and DNA binding studies of mono- and dinuclear copper(II) complexes constructed with -O and -N donor ligands: Potential anti-skin cancer drugs. Appl Organomet Chem 2017;32:1-15.