Biological investigations of ruthenium(III) 3-(Benzothiazol-2-yliminomethyl)-phenol Schiff base complexes bearing PPh3 / AsPh3 coligand

Sathiyaraj Subbaiyan\textsuperscript{a*} and Indhumathi Ponnusamy\textsuperscript{b}

\textsuperscript{a}Department of Chemistry, Dr. N.G.P. Arts and Science College, Coimbatore - 641048, India
\textsuperscript{b}Department of Chemistry, Shri Nehru Maha Vidyalya College of Arts & Science, Coimbatore - 641050, India

C H R O N I C L E

Article history:
Received September 23, 2018
Received in revised form April 18, 2019
Accepted April 18, 2019
Available online April 18, 2019

Keywords:
Ruthenium(III) complex
Schiff base
DNA-binding
Scavenging activity
In vitro cytotoxicity

A B S T R A C T

New ruthenium(III) complexes with 3-(Benzothiazol-2-yliminomethyl)-phenol (HL) ligand have been synthesized and characterized with the aid of elemental analysis, IR, electronic, and electron paramagnetic resonance spectroscopic techniques. The binding mode of the ligand and complexes with DNA and their ability to bind DNA have been investigated by UV-vis absorption titration. In addition, the ligand and complexes have been subjected to antioxidant activity tests which showed that HL and its ruthenium(III) complexes possess significant scavenging effect against DPPH and OH radicals. Cytotoxic activities of the ligand and ruthenium(III) complexes showed that the ruthenium(III) complexes exhibited more effective cytotoxic activity against HeLa and MCF-7 cells than the corresponding ligand.

1. Introduction

It is familiar that medicinal inorganic chemistry is a multidisciplinary field combining elements of chemistry, pharmacology, toxicology and biochemistry. Transition metal complexes that are able of cleave DNA under physiological environment are of attention in the development of metal-based anticancer agents.\textsuperscript{1-3} In this framework, platinum-based chemotherapy agents have been extensively used in the last 40 years in the treatment of various cancers.\textsuperscript{4,5} Owe to the firm side effects that platinum-based agents reveal, interest in chemotherapeutic agents has shifted to non-platinum metal-based drugs. This is a thrust to inorganic chemists to extend inventive strategies for the preparation of more successful, less toxic, target specific and preferably non-covalently bound anticancer drugs. Many studies put forward that DNA is the chiefly intracellular target of antitumor drugs, because the interface between small molecules and DNA can cause DNA damage in cancer cells.\textsuperscript{6,7} In the recent years, the delve into on ruthenium compounds in sight to their cytotoxic properties has augmented, motivated by the shows potential results previously obtained in both inorganic and organometallic fields where the cytotoxicity reported for some of the compounds is similar or even improved than that of cisplatin.\textsuperscript{8} In addition, it has been confirmed that free radicals can damage lipids, proteins and DNA of bio-tissues, foremost to greater than before rates of cancer and auspiciously antioxidants can avert this damage due to their free radical scavenging activity.\textsuperscript{9} Moreover, Schiff bases in concert with various metals have been widely used as building blocks to produce great diversity of topologies. Among them, 2-
aminobenzothiazole is generally found in bioorganic and medicinal chemistry with applications in treatment sighting and has concerned substantial thought to the researchers, seeing as the families have effective antitumor activity.

Based on the exceeding particulars, we here details on the synthesis, characterization of ruthenium(III) Schiff base complexes containing 2-(Benzothiazol-2-yliminomethyl)-phenol (HL) ligand. Single crystal X-ray structure of the ligand was resolute have been reported.10 DNA binding abilities of the ligand and ruthenium(III) complexes were carried out by means of calf-thymus (CT-DNA) proved their capability to bind and cleave the DNA. We at the present entered these studies to exemplify cytotoxicity of the new ruthenium(III) complexes to a array of cancer cell lines. Moreover, the antioxidant effects were evaluated for the complexes by DPPH and OH radicals.

2. Results and Discussion

The analytical data of the ligand and the ruthenium(III) Schiff base complexes were summarized in Table 1 agreed well with the theoretical values within the limit of experimental error and confirmed the formulae \([\text{RuX}_2(\text{EPh}_3)\text{L}]\) (where, \(X = \text{Cl or Br}; \ E = \text{P or As}; \ L = \text{monobasic tridenate Schiff base}\) proposed for new mononuclear octahedral ruthenium(III) Schiff base complexes. Ruthenium(III) Schiff base complex is quite stable in air and light and soluble in most of the common organic solvents. The reactions involved in the synthesis of Schiff base ligand and ruthenium(III) complexes are given in Scheme 1.

![Scheme 1](image)

where, \(X=\text{Cl or Br}; \ E=\text{P or As}\)

**Scheme 1.** Synthesis of ruthenium(III) Schiff base complexes

| Table 1. Analytical data of ligand and ruthenium(III) complexes. |
|-------------------|-------------|-----------------|-----------------|------------------|------------------|
| Ligand and Complexes | Colour | Yield % | Melting point °C | Elemental Analysis Calculated (found) |
| | | | | C % | H % | N % | S % |
| HL | Yellow | 74 | 152 | 66.12 (66.24) | 3.96 (3.75) | 11.02 (11.18) | 12.61 (12.48) |
| \([\text{RuCl}_2(\text{PPh}_3)\text{L}]\) | Brown | 64 | 188 | 55.83 (55.72) | 3.51 (3.69) | 4.07 (3.95) | 4.65 (4.49) |
| \([\text{RuCl}_2(\text{AsPh}_3)\text{L}]\) | Brown | 68 | 196 | 52.48 (52.29) | 3.30 (3.43) | 3.82 (3.89) | 4.37 (4.09) |
| \([\text{RuBr}_2(\text{PPh}_3)\text{L}]\) | Dark Brown | 62 | 182 | 50.62 (50.45) | 3.18 (3.38) | 3.69 (3.71) | 4.22 (4.13) |
2.1 Infrared spectra

The IR spectra afford helpful information concerning the nature of the functional group attached to the metal atom. The IR spectrum of the Schiff base ligand was compared with that of the ruthenium complexes to acquire the information regarding the binding mode of the ligand to ruthenium metal in the complexes (Table 2). A strong band is observed at 1654 cm\(^{-1}\) in the spectrum of the free Schiff base ligand which is the characteristic of the azomethine \((>\text{C}=\text{N}–)\) group. It is probable that coordination of the nitrogen to the metal atom would lessen the electron density in the azomethine link and thus lower the \((>\text{C}=\text{N}–)\) absorption. In the spectra of the complexes, this band is shifted to the region at 1629-1590 cm\(^{-1}\), representing that the coordination of the Schiff base ligand all the way through azomethine nitrogen.\(^{11}\) The band around 1260-1258 cm\(^{-1}\) were appeared for the complexes which show higher frequency range when compared to Schiff base ligand band obtained at 1251 cm\(^{-1}\) has been assigned to phenolic \(\nu(\text{C}-\text{O})\) absorption indicating that the other coordination of Schiff base through the phenolic oxygen atom.\(^{12}\) In the IR spectrum of all the complexes, the band is observed at 456-474 cm\(^{-1}\) which is attributed to the \(\nu(\text{M-N})\) stretching vibrations and the second band appeared at 668-690 cm\(^{-1}\) which is assigned to the phenolic oxygen to metal atom stretching vibrations \(\nu(\text{M-O})\).\(^{13}\) In addition, the other characteristic bands due to triphenylphosphine/arsine are also present in the expected region.\(^{14}\)

2.2 Electronic spectra

The electronic spectra of the free Schiff base ligand and its complexes were recorded in DMSO solvent, which shows four to six bands in the 261-596 nm regions (Table 2). The electronic spectrum of the complex \([\text{RuBr}_2(\text{PPh}_3)L]\) is shown in Fig. 1. The electronic spectra of Schiff base ligand showed two types of transitions, the first one appeared in the range 261-296 nm which can be assigned to \(\pi-\pi^*\) transition was due to transitions involving molecular orbitals located on the phenolic chromophore. These peaks have been shifted in the spectra of the complexes. This is may be owing to the contribution of a lone pair of electrons through the oxygen of the phenoxy group toward the central metal atom.\(^{15}\) The succeeding kind of transitions appears next to the range 366-412 nm which can be assigned to \(n-\pi^*\) transition, and this was due to the transition involving molecular orbitals of the \(\text{C}=\text{N}\) chromophore. These bands have moreover been shifted upon complexation indicated that, the imine group nitrogen atom appears in the way of coordinated to the metal ion.\(^{16}\)

The ground state of ruthenium(III) is \(^2\text{T}_{2g}\) and the first excited doublet levels in the sort of increasing energy are \(^2\text{A}_{2g}\) and \(^2\text{T}_{1g}\) arising as of \(t_{2g}^4e_{g}^1\) configuration.\(^{17}\) The spectral profiles below 400 nm are very comparable and are ligand-centered transitions. These bands encompass as \(\pi-\pi^*\) and \(n-\pi^*\) transitions arising from the ligand.\(^{18}\) In the majority of the ruthenium(III) complexes the charge transfer bands of the type \(\text{L}^\pi\rightarrow\text{T}_{2g}\) are prominent in the low energy region, which obscures the weaker bands owing to d-d transitions.\(^{19}\) It is so difficult to assign convincingly the bands that emerge in the visible region. Therefore, all the bands that appear in this region have been assigned to charge transfer transitions, which are in compliance with the assignments made for similar ruthenium(III) octahedral complexes.\(^{20}\)

| Ligand and Complexes | FT-IR cm\(^{-1}\) | UV-Vis |
|----------------------|----------------|--------|
| HL                   | 1654 1251 1605 | 261, 296, 366, 412 |
| [RuCl\(_2\)(PPh\(_3\))L] | 1590 1258 1578 | 288, 318, 368, 406, 534 |
| [RuCl\(_2\)(AsPh\(_3\))L] | 1629 1260 1588 | 298, 301, 392, 471, 531, 574 |
| [RuBr\(_2\)(PPh\(_3\))L] | 1598 1259 1582 | 298, 313, 370, 406, 596 |
2.3 Magnetic moment and EPR spectra

The room temperature magnetic susceptibility measurements of the ruthenium(III) complexes shows that they are paramagnetic ($\mu_{\text{eff}} = 1.82$-$1.94$ BM) corresponds to single unpaired electron in a low-spin 4$d^5$ configuration and confirms that ruthenium is in +3 oxidation state in all the complexes. All the complexes are consistently paramagnetic through magnetic moments analogous toward one unpaired electron at room temperature (low-spin ruthenium(III), $t_2g^5$). The solid state EPR spectra of the complexes were recorded in X-band frequencies at room temperature and the ‘g’ values are given in Table 3. The low spin $d^5$ configuration is a good seem into of molecular structure and bonding because the observed ‘g’ values are extremely receptive to small changes in structure and to metal ligand covalency. The EPR spectrum for the complex [RuCl$_2$(PPh$_3$)L] show a characteristic of an axially system with $g_\perp$ 2.52 and $g_\parallel$ around 2.36. For an octahedral field with tetragonal distortion ($g_x = g_y \neq g_z$) and hence two ‘g’ values point toward tetragonal distortion in these complexes. The complex [RuCl$_2$(AsPh$_3$)L] shows rhombic spectrum with three dissimilar ‘g’ values ($g_x \neq g_y \neq g_z$) $g_x = 2.54$, $g_y = 2.56$, $g_z = 2.44$. The rhombicity of the spectrum reflects the asymmetry of the electronic environment around the ruthenium in the complex. However, the EPR spectrum of the complex [RuBr$_2$(PPh$_3$)L] reveal distinct single isotropic lines with ‘g’ values at 2.37 (Fig. 2). Isotropic lines are usually the results of either intermolecular spin exchange, which is able to broaden the lines or tenure of the unpaired electron in a degenerate orbital. In addition, the nature and position of the lines in the spectra of the complexes are similar to those of the octahedral complexes.$^{19,21}$

### Table 3. EPR and magnetic moment data of ruthenium(III) complexes

| Complexes            | $g_x$ | $g_y$ | $g_z$ | $<g>^*$ | $\mu_{\text{eff}}$ (BM) |
|----------------------|-------|-------|-------|---------|--------------------------|
| [RuCl$_2$(PPh$_3$)L] | 2.52  | 2.52  | 2.04  | 2.36    | 1.82                     |
| [RuCl$_2$(AsPh$_3$)L]| 2.54  | 2.56  | 2.24  | 2.44    | 1.91                     |
| [RuBr$_2$(PPh$_3$)L] | 2.37  | 2.37  | 2.37  | 2.37    | 1.94                     |

$<g>^* = [1/3g_x^2 + 1/3g_y^2 + 1/3g_z^2]^{1/2}$
2.4 DNA Binding Study

The interactions of metal complex with DNA encompass the subject of interest for the expansion of efficient chemotherapeutic agents. Presently, spectrophotometric DNA titration appears towards the majority used method for determines DNA binding constants of ligand and metal complexes. Generally, hypochromism and hyperchromism are the two spectral features which are intimately linked with the double helix structure of DNA. The observation of hypochromism is indicative of electronic or intercalative mode of binding of DNA to the complexes along with the stabilization of the DNA double helix structure. On the other hand, the observation of hyperchromism is indicative of the breakage of the secondary structure of DNA. The absorption spectral titration of the complexes with CT-DNA was followed through the absorbance of intraligand bands (Fig. 3). Any interaction between the complex and the DNA is probable to disturb the ligand centered spectral transitions of the complexes. Intensity of the spectral band of the ligand and complexes at 256-278 nm were found to increase with the increasing concentration of the DNA. Significant hyperchromism with red shift was observed for all the ligand and the complexes. This can be attributed to a strong interaction between DNA and complexes. On the other hand, there were no appreciable wavelength shifts in the charge transfer band. Based on the results obtained from the spectral titration, it is inferred that the complexes underwent a non-intercalative mode of binding with DNA. Hence, the observation of hyperchromism with red shift for our compounds showed that the ligand and complexes interact with the secondary structure of CT-DNA by breaking its double helix structure.
In order to compare the DNA-binding affinity of these compounds quantitatively, their intrinsic binding constants were calculated with the changes monitored in absorption at the higher energy band with increasing concentration of DNA, plotting [DNA] versus [DNA]/(εₐ-εₐ) give slope 1/[εₐ-εₐ] and a Y intercept equal to 1/Kₐ [εₐ-εₐ], respectively (Fig. 4). The intrinsic binding constants Kₐ were calculated using the above plot and were found to be 3.56 × 10⁴ M⁻¹, 5.88 × 10⁴ M⁻¹, 4.37 × 10⁴ M⁻¹, 5.01 × 10⁴ M⁻¹ corresponding to the ligand HL and complexes [RuCl₂(PPh₃)L], [RuCl₂(AsPh₃)L], [RuBr₂(PPh₃)L], respectively. The extent of the binding constant value obviously showed that complex [RuCl₂(PPh₃)L] bound more strongly with CT-DNA than the rest of the compounds. Amusingly, the Kₐ values obtained for the over ruthenium(III) complexes are analogous than those for the other recognized Ruthenium(II)/(III) complexes of 4-hydroxy-pyridine-2,6-dicarboxylic acid with PPh₃/AsPh₃ as co-ligand.²⁴

Fig. 4. Plots of [DNA] / (εₐ-εₐ) versus [DNA] for the titration of compounds with CT-DNA

2.5 Antioxidant activity

The antioxidant activity of free Schiff base ligand HL and their corresponding ruthenium(III) complexes was evaluated by in vitro assay involving 2-2’- diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals along with the standard ascorbic acid and the determination of 50 % activity (IC₅₀) values. It was experiential that the compounds can certainly decrease the concentration of the initial DPPH radical in solution and this is taken as substantiation of their antioxidant capabilities and Hydroxyl radical is well-known to be able of abstracting hydrogen atoms as of membrane lipids and brings about peroxide reaction of lipids.²⁵ The scavenging activity of the tests compounds were shown in Fig. 5. IC₅₀ values of the ligand HL on DPPH and OH radicals are 136.21 and 116.52 µM respectively. Whereas, the ruthenium(III) complexes [RuCl₂(PPh₃)L], [RuCl₂(AsPh₃)L], [RuBr₂(PPh₃)L] showed their IC₅₀ values at 26.15, 95.14, 83.12 µM, respectively, for DPPH radical and 20.41, 85.37, 65.59 µM, correspondingly, for OH radical. As a result of comparing the antioxidant activity of the ligand with that of the ruthenium(III) complexes it turn out to be obvious that the ruthenium(III) complexes hold higher scavenging activity towards DPPH and OH radicals than the own parent ligand in the order of [RuCl₂(PPh₃)L] > [RuBr₂(PPh₃)L] > [RuCl₂(AsPh₃)L] > HL. This might exist due to the d⁵ low spin electronic configuration as well as the ease of use of an odd electron in ruthenium(III) complexes, which increases the ability to stabilize the unpaired electrons and thus scavenge the free radicals.²⁴ The lower IC₅₀ values experimental in antioxidant assays did show with the intention of these complexes contain a strong potential to be functional as scavengers to eradicate the radicals.
Fig. 5. Antioxidant activity of compounds ligand (HL), Complexes [RuCl₂(PPh₃)L] (1), [RuCl₂(AsPh₃)L] (2) and [RuBr₂(PPh₃)L] (3) with control ascorbic acid.

2.6 Cytotoxic activity evaluation

The positive results obtained from DNA binding and antioxidation studies of HL and ruthenium(III) complexes confident us to test their cytotoxicity against a pair of selected human cervical cancer cell line (HeLa) and the human breast cancer cell line (MCF-7). The results were analyzed by means of cell viability curves and expressed with IC₅₀ values in the studied concentration range from 1-100 μM (Fig.6, Table 4). Upon increasing the concentration of complexes, the results of MTT assays reveals that complex [RuCl₂(PPh₃)L] showed a higher antiproliferative effect followed by complexes [RuBr₂(PPh₃)L] and [RuCl₂(AsPh₃)L]. But the antiproliferative property of this ligand are fewer when compared to the complexes which inveterate that the chelation of the ligand by means of the ruthenium ion is the merely liable factor for the observed cytotoxic properties of the ruthenium(III) complex. Among the two different cell lines used in this study, the percentage cell inhibition of HeLa cells was found to be higher than MCF-7 cells (Fig. 7). In addition, the complexes containing triphenylphosphine as co-ligand showed enhanced cytotoxic effects than the complex containing triphenylarsine. This might exist due to the lipophilic effect of triphenylphosphine in the ruthenium(III) complexes which helps to cross the cytoplasmic membrane.²⁶

Table 4. The Cytotoxic activity of the compounds

| Compounds               | IC₅₀ Values (μM) |
|-------------------------|------------------|
|                         | HeLa             | MCF-7            |
| HL                      | 178.21           | 184.15           |
| [RuCl₂(PPh₃)L]          | 29.32            | 44.57            |
| [RuCl₂(AsPh₃)L]         | 67.54            | 93.18            |
| [RuBr₂(PPh₃)L]          | 53.12            | 79.08            |
3. Conclusion

The biological properties of ruthenium(III) Schiff base complexes were synthesized and characterized by elemental analyses, and various spectroscopic studies. Based on the characterization an octahedral geometry has been tentatively proposed for all the new ruthenium(III) complexes. Initially, the binding behaviors of the ligand and the complex with DNA investigated using absorption spectroscopy. The results supported the fact that the complex could bind to CT-DNA via electrostatically to DNA double helix surface. From the binding constant values, it is inferred that the triphenylphosphine complexes bind more with CT-DNA than the corresponding triphenylarsine complexes. In addition, the complex also exhibited excellent radical scavenging activities over the ligand. Furthermore, the complex [RuCl₂(PPh₃)L] and [RuBr₂(PPh₃)L] shows considerable cytotoxic activity against HeLa and MCF-7 cancer cell lines. The IC₅₀ value indicates that the cytotoxic activity of [RuCl₂(PPh₃)L] is greater than that of [RuBr₂(PPh₃)L]. Among the two different cell lines used in this study, the percentage cell inhibition of HeLa cells is found to be higher than MCF-7 cells.
4. Experimental

4.1 Materials and Instrumentation

Reagent grade chemicals were used without further purification in all the synthetic work. Salicylaldehyde, 2-amino benzothiazole were purchased from sigma-aldrich chemie. RuCl$_3$.3H$_2$O, triphenylphosphine / arsine were purchased from Himedia. The starting precursors [RuCl$_3$(PPh$_3$)$_3$], [RuCl$_3$(AsPh$_3$)$_3$], [RuBr$_3$(PPh$_3$)$_3$] were prepared by reported literature methods. Calf-thymus DNA (CT-DNA) was purchased from Bangalore Genei, Bangalore, India. The Human Cervical cancer cell lines HeLa and human breast cancer cell line (MCF-7) were obtained from National centre for cell science (NCCS), Pune, India.

Elemental analyses were performed with a model Vario ELIII CHNS at Sophisticated Test and Instrumentation Centre (STIC), Cochin University, Kerala. Magnetic susceptibility measurements of the complexes were recorded by means of Guoy balance at room temperature. Infrared spectra were recorded on a FT-IR Perkin Elmer spectrophotometer RXI model as KBR pellets in the range 4000-400 cm$^{-1}$. Electronic spectra were recorded in DMSO solution in a Systronics 2202 Double beam spectrophotometer in 800-200 nm range. The X-band EPR spectra of the complexes were recorded on a varian E-112 spectrometer using tetracyanoethylene (TCNE) as the standard, at the SAIF, Indian Institute of Technology Bombay, Mumbai, India. Antioxidant and cytotoxicity studies were carried out at the Kovai Medical Centre and Hospital Pharmacy College, Coimbatore, Tamil Nadu. Melting points were recorded with a Veego DS model apparatus and are uncorrected.

4.2 Synthesis of 3-(Benzothiazol-2-yliminomethyl)-phenol (HL)

The Schiff base ligand was prepared by the reported procedure$^{10}$ This solid was recrystallized from chloroform, yielding spine shaped yellow crystals, suitable for X-ray diffraction analysis.

4.3 Synthesis of ruthenium(III) Schiff base complexes

All the new ruthenium(III) complexes were synthesized by the following general procedure (Scheme 2). To a solution of [RuX$_3$(EPh$_3$)$_3$] (0.198 - 0.225 g, 0.2 mmol) in benzene (20 mL) (X= Cl/Br, E= PPh$_3$/AsPh$_3$) the Schiff base ligand, HL (0.05 g, 0.2 mmol) in chloroform (10 mL) was added and the mixture was refluxed for 8 h. The Solvent was then evaporated under reduced pressure and then solid mass was filtered, washed with petroleum ether. The purity was checked by TLC. This solid was recrystallized from CH$_2$Cl$_2$/ n-hexane mixture.

4.4 DNA interaction experiment

Experiments involving the interaction of free Schiff base ligand and the ruthenium(III) complexes with CT-DNA were carried out in double distilled water with tris(hydroxymethyl)-aminomethane (Tris, 5 mM) and sodium chloride (50 mM) and adjusted to pH 7.2 with hydrochloric acid$^{10}$ The data were then fit into the following equation and the intrinsic binding constant $K_b$ was calculated in each case.$^{30}$

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

where [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficient $\varepsilon_a$, $\varepsilon_f$ and $\varepsilon_b$ correspond to $A_{obsd}$/[complex], the extinction coefficient of the free compound and the extinction coefficient of the compound when fully bound to DNA respectively. In plots of [DNA]/ ($\varepsilon_a - \varepsilon_f$) versus [DNA], $K_b$ is given by the ratio of slope to the intercept.

4.5 Antioxidant studies

The free radical scavenging ability of the free Schiff base ligand and ruthenium(III) complexes were resolute against both DPPH and hydroxyl radicals. The DPPH radical scavenging activity of the
compounds was investigated using the technique described by Elizabeth, whereas hydroxyl radical scavenging activity of the compounds was evaluated by the adapted method of Yu. For each of the over assays, the tests were run in triplicate by changeable the concentration. The percentage activity was calculated by using the formula \[ \% \text{ activity} = \left( \frac{A_o - A_r}{A_o} \right) \times 100 \], where \( A_o \) and \( A_r \) represent the absorbance in the absence and presence of the test compounds, respectively. The 50 \% activity (IC\(_{50}\)) is calculated from the result of percentage activity.

4.6 Cytotoxicity studies

Cytotoxicity studies of the free Schiff base and complexes were carried out on human cervical cancer cells (HeLa) and human breast cancer cell line (MCF-7). Cell viability was carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method. The compounds were dissolved in DMSO and diluted in the respective medium containing 1 % FBS. Triplication was maintained, and the medium not containing the compounds served as the control. After 48 h, 10 \( \mu \)L of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The medium with MTT was then flicked off, and the formed formazan crystals were dissolved in 100 \( \mu \)L of DMSO. The absorbance was then measured at 570 nm using a micro plate reader. The % cell inhibition was determined using the following formula.

\[ \% \text{ Growth Inhibition} = 100 - \left( \frac{\text{Abs (sample)}}{\text{Abs (control)}} \right) \times 100. \]

Nonlinear regression graph was plotted between % Cell inhibition and Log\(_{10}\) concentration and IC\(_{50}\) values were calculated from the graph plotted between % cell inhibition and concentration.

References

1. Chifotides H.T., & Dunbar K.R. (2005). Interactions of Metal–Metal-Bonded Antitumor Active Complexes with DNA Fragments and DNA. Acc. Chem. Res., 38, 146-156.
2. Bednarski P.J., Mackay F.S., & Sadler P.J. (2007). Photoactivatable platinum complexes. Anticancer Agents Med. Chem., 7, 75-93.
3. Rose M.J., Fry N.I., Maelow R., Hinck L., & Manscharak P.K. (2008). Sensitization of Ruthenium Nitrosyls to Visible Light via Direct Coordination of the Dye Resorufin: Trackable NO Donors for Light-Triggered NO Delivery to Cellular Targets. J. A. Chem. Soc., 130, 8834-8846.
4. Lippert B., (1999). Cisplatin: Chemistry and Biochemistry of a leading Anticancer Drug. Zürich: Verlag Helvetica Chimica Acta, Weinheim, New York, Wiley-VCH,
5. Allardyce C.S., & Dyson P.J. (2001). Ruthenium in medicine: Current clinical uses and future prospects. Platinum Met. Rev., 45, 62-69
6. Nori A., & Kopeczek J. (2005). Intracellular targeting of polymer-bound drugs for cancer chemotherapy. Adv. Drug. Deliv. Rev., 57, 609-636.
7. Shi M., Ho K., Keating A., & Shoichet M.S. (2009). Doxorubicin-Conjugated Immuno-Nanoparticles for Intracellular Anticancer Drug Delivery. Adv. Funct. Mater., 19, 1689-1696.
8. Habtermamiam A., Melehart M., Erandez R., Parsons S., Oswald I.D.H, Parkin A., Fabbiani F.P.A., Davidson J.E, Dawson A., Aird R.E, Jodrell D.I., & Sadler P.J. (2006). Structure-activity relationships for cytotoxic ruthenium(II) arene complexes containing N,N-, N,O-, and O,O-chelating ligands. J. Med. Chem. 49, 6858-6868.
9. Chem W.J., Guo P., Song J., Cao W., & Bian J. (2006). The ortho hydroxy-amino group: another choice for synthesizing novel antioxidants. Bioorg. Med. Chem. Lett., 16, 3582-3585.
10. Sathiyaraj S., Sampath K., Butcher R.J., & Jayabalakrishnan C. (2013). Synthesis, DNA binding, antioxidant and cytotoxic activities of ruthenium (II) complexes of a Schiff base ligand. Transition Met. Chem. 38, 291-298.
11. Ramesh R., & Sivagamasundari M. (2003). Synthesis, spectral and antifungal activity of Ru (II) mixed-ligand complexes. Synth. React. Inorg. Met. Org. Chem., 33, 899-910.
12. Maurya R.C., Patel P., & Rajput S. (2003). Synthesis and Characterization of N-(o-Vanillinidene)-p-anisidine and N, N′-bis (o-Vanillinidene) ethylenediamine and Their Metal Complexes. Synth. React. Inorg. Met. Org. Chem., 23, 817-836.

13. Nakamoto K. (1971). Infrared and Raman spectra of Inorganic and Co-ordination compounds. Wiley Interscience, New York.

14. Sathiyaraj S., Butcher R.J., & Jayabalakrishnan C. (2012). Synthesis, characterization, DNA interaction and in vitro cytotoxicity activities of ruthenium(II) Schiff base complexes. J. Mol. Struct., 1030, 95-103.

15. Sharma R.K., Singh R.V., & Tandon J.P. (1980). Biscyclopentadienyl titanium(IV) complexes of monofunctional bidentate ketamines., J. Inorg. Nucl. Chem., 42, 95-103.

16. Cambell M.J.M. (1975). Transition metal complexes of thiosemicarbazide and thiosemicarbazones. Coord. Chem. Rev., 15, 279-319.

17. Ballhausen C.J. (1962). Ligand Field Theory. Mc Graw Hill, New York,

18. Chichak K., Jacquemard U., & Brande N.R. (2002). The Construction of (Salophen)ruthenium(II) Assemblies Using Axial Coordination. Eur. J. Inorg. Chem., 2002, 357-368.

19. Ramesh R., Maheswaran S. (2003) Synthesis, spectra, dioxygen affinity and antifungal activity of Ru (III) Schiff base complexes. J. Mol. Struct., 1030, 95-103.

20. Perz S., Lopez C., Caubet A., Solans X., Bardia M.F., Gich M., & Molins E. (2007). Versatility in the mode of coordination \{(N), (N,O)−, (C,N)−, or (C,N,O)2−\} of \{[η5C5H5]Fe{[η5-C5H4–CH=N–(C6H4-2OH)}\} to palladium(II). J. Organomet. Chem., 692, 2402-2414.

21. Long E.C., Barton K. (1990). On demonstrating DNA intercalation. Acc. Chem. Res., 23, 271-273.

22. Chatterji T.S., Chitrapiya N., Lee H., Fronczek C.F., Fronczek F.R., & Natarajan K. (2012). Ruthenium (II)/(III) complexes of 4-hydroxy-pyridine-2, 6-dicarboxylic acid with PPh3/AsPh3 as co-ligand: impact of oxidation state and co-ligands on anticancer activity in vitro. Dalton Trans., 41, 2066-2077.

23. Chatt J., Leigh G.J., Mingos D.M.P., & Paske R.J. (1968). Complexes of osmium, ruthenium, rhenium, and iridium halides with some tertiary monophosphines and monoarsines. J. Chem. Soc. (A), 2636-2641.

24. Poddar R.K., Khullar I.P., & Agarwala U. (1974) Some ruthenium(III) complexes with triphenylarsine. Inorg. Nucl. Chem. Lett., 10, 221-227.

25. Natarajan K., Poddar R.K., & Agarwala U. (1977). Mixed complexes of ruthenium(III) and ruthenium(II) with triphenylphosphine or triphenylarsine and other ligands. J. Inorg. Nucl. Chem., 39, 431-435.

26. Wolf A., Shimer G.H., & Meehan T. (1987). Polycyclic aromatic hydrocarbons physically intercalate into duplex regions of denatured DNA. Biochem. 26, 6392-6396.

27. Elizabeth K., & Rao M.N.A. (1990). Oxygen radical scavenging activity of curcumin. Int. J. Pharmaceut. 58, 237-240.
32. Yu W., Zhao Y., & Shu B. (2004). The radical scavenging activities of *Radix purariae* isoflavonoids: A chemiluminescence study. *Food chem.* 86, 525-529.
33. Blagosklonny M., & EL-Diery W.S. (1996). In vitro evaluation of a p53-expressing adenovirus as an anti-cancer drug. *Int. J. Cancer*, 67, 386-392.

© 2019 by the authors; licensee Growing Science, Canada. This is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).