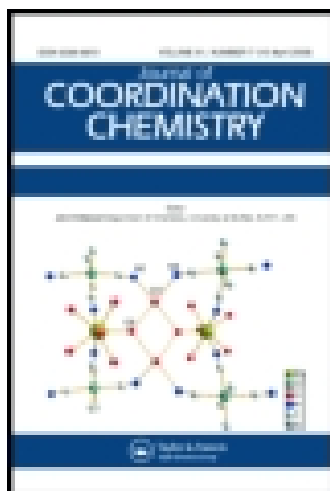


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Journal of Coordination Chemistry

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/gcoo20>

Synthesis, characterization, and in vitro antioxidant and anticancer studies of ruthenium(III) complexes of symmetric and asymmetric tetradentate Schiff bases

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Accepted author version posted online: 06 May 2015. Published online: 14 Jun 2015.



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To cite this article: Ikechukwu P. Ejidike & Peter A. Ajibade (2015): Synthesis, characterization, and in vitro antioxidant and anticancer studies of ruthenium(III) complexes of symmetric and asymmetric tetradentate Schiff bases, *Journal of Coordination Chemistry*, DOI: [10.1080/00958972.2015.1043127](https://doi.org/10.1080/00958972.2015.1043127)

To link to this article: <http://dx.doi.org/10.1080/00958972.2015.1043127>

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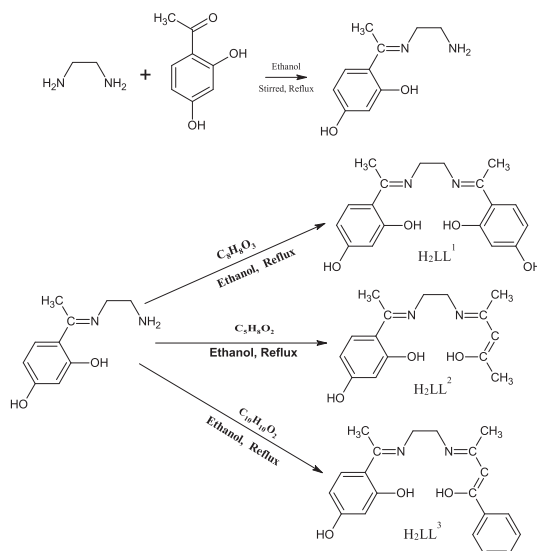
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Synthesis, characterization, and *in vitro* antioxidant and anticancer studies of ruthenium(III) complexes of symmetric and asymmetric tetradentate Schiff bases

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(Received 12 January 2015; accepted 26 March 2015)



Ruthenium(III) complexes of three tetradentate Schiff bases with N₂O₂ donors formulated as [RuCl(LL¹)(H₂O)], [RuCl(LL²)(H₂O)] and [RuCl(LL³)(H₂O)] were synthesized and characterized by elemental analyses, molar conductance, FTIR, and electronic spectral measurements. The FTIR data showed that the tetradentate Schiff base ligands coordinate to Ru ions through the azomethine nitrogen and enolic oxygen. The antioxidant activities of the complexes were investigated through scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals. The DPPH activity for [RuCl(LL²)(H₂O)] with IC₅₀ = 0.031 mg mL⁻¹ was higher than the values obtained for the other Ru(III) compounds. The study revealed that the synthesized Ru(III) complexes of the tetradentate Schiff base exhibited strong scavenging activities against DPPH and moderate against ABTS radicals. In addition, the antiproliferative studies of the complexes were also tested against human renal cancer cells (TK10), human melanoma cancer cells (UACC62), and human breast cancer cells (MCF7) using the SRB assay. The results indicated that the Ru(III) complexes showed low anticancer activities against the tested human cancer cell lines.

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Keywords: Ru(III) complexes; Tetradentate Schiff base; Spectral studies; Antioxidant; Anticancer

1. Introduction

Synthesis of biologically active molecules is a vigorous task and the variables affecting biological activity are diverse [1–4]. Many studies on the molecular structure of metal complexes and their bioactivity have created much awareness in the field of bio-inorganic chemistry [5, 6]. The interaction of DNA and transition metal complexes containing multi-dentate aromatic ligands with a prescribed N_4 or N_2O_2 coordination has been studied [7, 8]. Development of new therapeutic agents and DNA probes [9–11] stems from DNA binding studies, as it has inspired considerable interest in the study of the biochemical behavior of these metal compounds: interactions with DNA and serum proteins [12–15]. In the quest for small molecules that can efficiently bind to DNA and cleave it, Schiff bases and their metal complexes have gained recognition by various researchers and groups [16].

Schiff bases are important class of compounds widely studied for various applications [17–32]. Ruthenium Schiff base complexes have been widely studied, imperative as biochemical, analytical, and antimicrobial reagents [27, 28]. Metal complexes of Schiff bases have attracted considerable attention due to their antifungal, antibacterial, and antitumor activities [33, 34]. Physiological and biochemical processes are a pathway for generation of reactive oxygen species (ROS) through the living cells in the body [35–37]. Hence, antioxidants become important as they play vital roles toward protecting the human body against damage by ROS [38–40]. In view of growing interest in oxygenation and azomethination of Ru(III) complexes for development of new therapeutic agents and DNA probes for disease defense, we present the synthesis and characterization of some stable Ru(III) Schiff bases complexes of the type $[RuCl(LL')(H_2O)] \cdot 2H_2O$ ($LL' = H_2LL^1, H_2LL^2, H_2LL^3$) and their antioxidant and anticancer studies.

2. Experimental

2.1. Materials and methods

All reagents used were analytical grade and used as received, 2,4-pentanedione from Fluka, ethylenediamine and ascorbic acid from Merck, $RuCl_2 \cdot 3H_2O$, 2',4'-dihydroxyacetophenone and 1-phenylbutane-1,3-dione from Aldrich. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), rutin hydrate, and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Elemental analysis was obtained using a Perkin-Elmer elemental analyzer. Molar conductance of the Schiff base ligands and their Ru complexes were determined on freshly prepared 10^{-3} M solutions in CH_2Cl_2 at room temperature using a Crison EC-Meter Basic 30+ conductivity cell. IR spectra were recorded on an FT-IR spectrometer, Perkin-Elmer System (Spectrum 2000) from 4000 to 400 cm^{-1} using the KBr disk method. Electronic spectra were recorded on a Perkin-Elmer Lambda-25 spectrophotometer from 200 to 900 nm. Melting points were recorded with a Stuart melting point (SMP 11).

2.2. General procedure for the synthesis of Schiff base ligands ($H_2LL^1-H_2LL^3$)

2.2.1. $[OHC_6H_3OHC(CH_3):N(C_2H_4)N:C(CH_3)HOC_6H_3OH]$, H_2LL^1 . The ligand was prepared by modification of a literature method [41]. The tetradentate Schiff base was synthesized via the method: an ethanolic solution (20 mL) containing ethane-1,2-diamine (0.01 mol, 0.601 g) was added slowly to a stirring ethanolic solution (50 mL) containing 2',4'-dihydroxyacetophenone (0.02 mol, 3.043 g). The resulting light brown mixture was stirred and refluxed for 3 h. The obtained precipitate was filtered and washed with ethanol, followed by recrystallization in ethanol and air-drying to give a brownish yellow solid (Yield = 2.51 g, 76.52%).

2.2.2. $[OHC_6H_3OHC(CH_3):N(C_2H_4)N:C(CH_3)CH:C(CH_3)OH]$, H_2LL^2 . The ligand was prepared by modification of a literature method [42]. A typical procedure for the synthesis was as follows: ethylenediamine (0.015 mol, 0.902 g) in 30 mL ethanol was slowly added to an ethanolic solution (40 mL) containing 2',4'-dihydroxyacetophenone (0.015 mol, 2.282 g), followed by slow addition of acetylacetone (0.015 mol, 1.502 g) dissolved in 30 mL ethanol. The resulting colored mixture was refluxed with stirring for 4 h, cooled and the resultant precipitate was filtered, washed several times with ethanol, followed by recrystallization in ethanol (Yield = 2.53 g, 61.23%).

2.2.3. $[OHC_6H_3OHC(CH_3):N(C_2H_4)N:C(CH_3)CH:C(C_6H_5)OH]$, H_2LL^3 . The ligand was prepared by modification of a literature method [42]. A typical procedure for the synthesis was as follows: ethylenediamine (0.015 mol, 0.902 g) in 30 mL ethanol was slowly added to an ethanolic solution (40 mL) containing 2',4'-dihydroxyacetophenone (0.015 mol, 2.282 g), followed by slow addition of 1-phenylbutane-1,3-dione (0.015 mol, 2.4329 g), (H_2LL^3) dissolved in 40 mL ethanol. The resulting colored mixture was refluxed with stirring for 4 h, cooled and the resultant precipitate was filtered, washed several times with ethanol, followed by recrystallization in ethanol (Yield = 3.75 g, 74.17%).

2.3. General procedure for the preparation of the complexes

All operations were carried out under strictly anhydrous conditions. The various complexes were prepared by addition of $RuCl_3 \cdot 3H_2O$ (0.5 mmol) dissolved in 15 mL of absolute ethanol, into a hot ethanolic solution (20 mL) of $H_2LL^1-H_2LL^3$ ligands (0.5 mmol) in molar ratio (1 : 1). The color changed immediately. The resulting mixture was then refluxed for 6 h. The precipitated solids were allowed to cool and filtered off from the reaction mixture, thoroughly washed with absolute ethanol and then with diethyl ether (3×5 mL), and were dried over anhydrous calcium chloride.

2.4. Antioxidant assay

2.4.1. Scavenging activity of DPPH radical. The antioxidant activities for N_2O_2 Schiff base ligands and their synthesized Ru(III) complexes were studied spectrophotometrically by DPPH method. DPPH is known as a stable commercially available free radical, soluble in methanol to give a violet solution, which, upon reduction by an antioxidant, changes to a

corresponding light yellow to yellow. The free radical scavenging effects of the Ru(III) compounds and Schiff base ligands with the DPPH radical were evaluated as previously described with slight modification [43, 44]. A solution of 0.4 mM DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL DMF solutions of Schiff base ligands and Ru(III) complexes with various concentrations (8, 17, 25, 33, and 42 $\mu\text{g mL}^{-1}$). The reaction mixture was stirred thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Rutin and ascorbic acid (vitamin C) are used as standard drugs. The actual decrease in absorption was measured against that of the control. All tests and analyses were run in triplicate and the results obtained were averaged. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH radical + DMF, and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical + sample [test samples/standard].

2.4.2. ABTS radical scavenging assay. ABTS scavenging ability of the Ru(III) compounds and Schiff base ligands was evaluated by the previously described method of Adedapo and co-workers [45]. The working solution was prepared by mixing stock solutions of 7 mM ABTS solution and 2.4 mM potassium persulfate solution in equal amounts (1 : 1) and allowing the solution to react in the dark for 12 h at room temperature. The resulting solution was further diluted by mixing 1 mL ABTS^+ solution to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Test samples (1 mL) were allowed to react with 1 mL of the ABTS^+ solution, followed by the absorbance reading at 734 nm after 7 min using the spectrophotometer. The ABTS scavenging capacities of the Ru(III) compounds and Schiff base ligands were compared with that of rutin and BHT (standard drugs). All tests were run in triplicate, and the results obtained were averaged. The percentage inhibition was calculated as ABTS radical scavenging activity using the following equation:

$$(\%) \text{ Inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of ABTS radical + DMF, and $\text{Abs}_{\text{sample}}$ is the absorbance of ABTS radical + sample [test samples/standard].

2.5. Cell lines and culture conditions

Human renal cancer cell line (TK10), human melanoma cancer cell line (UACC62), and human breast cancer cell line (MCF7) were obtained from NCI in the framework a collaborative research program between CSIR and NCI. Cell lines were routinely maintained as a monolayer cell culture at 37.0 °C with 5% CO_2 , 95% air and 100% relative humidity in RPMI medium which is supplemented with 5% fetal bovine serum, 2 mM L glutamine and 50 $\mu\text{g mL}^{-1}$ gentamicin.

2.6. Cell viability assay

Cell viability was examined by Sulforhodamine B (SRB) assay as previously described [46, 47]. The cells (TK10, UACC62, and MCF7) (3–19 passages) were inoculated into 96-well microtiter plates at plating densities of 7–10,000 cells/well and were incubated for 24 h. After 24 h, the cells were treated with the experimental compounds which were previously dissolved in DMSO and diluted in medium to produce concentrations of 0.01, 0.1, 0, 10, and 100 μM . Cells without drug addition served as control. The blank contains complete medium without cells. Parthenolide was used as a standard. The plates were incubated for 48 h after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Thereafter, the unbound dye was removed, and protein-bound dye was extracted with 10 mM Tris base for optical density determination at 540 nm using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software; 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression. The Z' -factor coefficient was adapted to monitor the quality of immunocytochemical assays such as the SRB.

3. Results and discussion

3.1. Synthesis

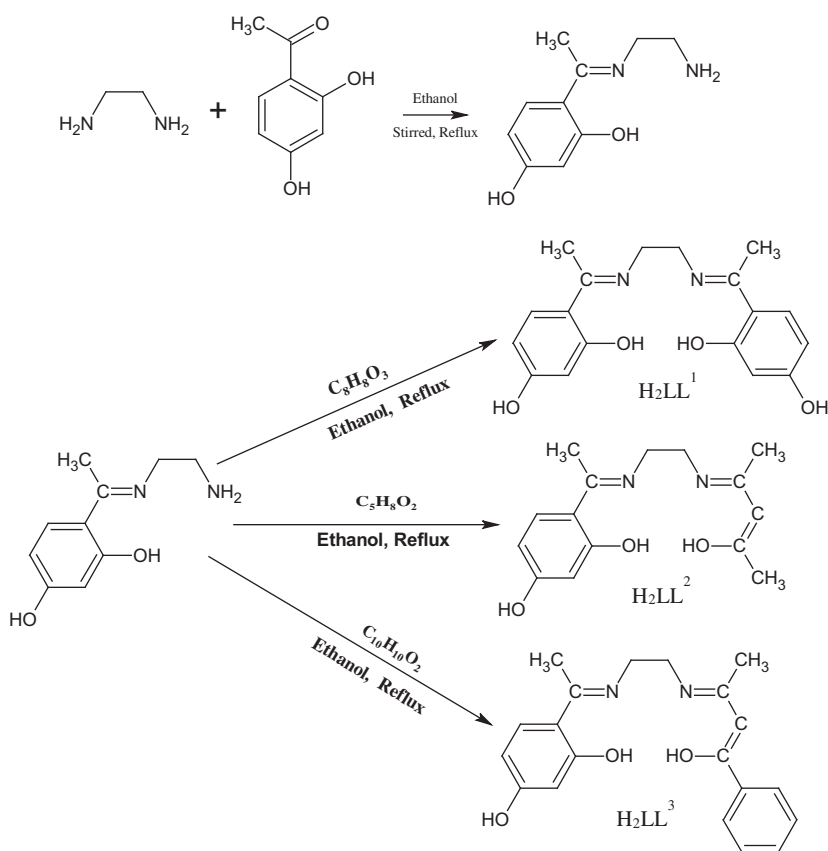
Mononuclear ruthenium(III) complexes, $[\text{RuCl}(\text{LL}')(\text{H}_2\text{O})]$ ($\text{LL}' = \text{H}_2\text{LL}^1, \text{H}_2\text{LL}^2, \text{H}_2\text{LL}^3$) ($\text{LL}' =$ dibasic tetradentate Schiff base ligand), were synthesized in good yields from reaction of $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ with Schiff base ligands in 1 : 1 M ratio in absolute EtOH to give six-coordinate ruthenium(III) Schiff base complexes according to the equation:



where $\text{H}_2\text{LL} = \text{H}_2\text{LL}^1 = [\text{OHC}_6\text{H}_3\text{OHC}(\text{CH}_3):\text{N}(\text{C}_2\text{H}_4)\text{N}:\text{C}(\text{CH}_3)\text{HOC}_6\text{H}_3\text{OH}]$, $\text{H}_2\text{LL}^2 = [\text{OHC}_6\text{H}_3\text{OHC}(\text{CH}_3):\text{N}(\text{C}_2\text{H}_4)\text{N}:\text{C}(\text{CH}_3)\text{CH}:\text{C}(\text{CH}_3)\text{OH}]$ and $\text{H}_2\text{LL}^3 = [\text{OHC}_6\text{H}_3\text{OHC}(\text{CH}_3):\text{N}(\text{C}_2\text{H}_4)\text{N}:\text{C}(\text{CH}_3)\text{CH}:\text{C}(\text{C}_6\text{H}_5)\text{OH}]$ (scheme 1). The synthesized mononuclear ruthenium(III) Schiff base complexes (figure 1) are stable in air at room temperature, non-hygroscopic and insoluble in water, partially soluble in common solvents such as dichloromethane, acetonitrile, chloroform, but easily soluble in polar solvents such as DMF and DMSO producing intense color in their solutions. The solubility of the complexes may be due to the presence of chlorides [48] and hydroxyl groups on the benzene ring [49]. The tetradentate N_2O_2 donor site of LL' ($\text{H}_2\text{LL}^1, \text{H}_2\text{LL}^2, \text{H}_2\text{LL}^3$) is capable of forming complexes with ruthenium. The analytical data are listed in table 1 and are in agreement with the proposed formulations for the complexes.

3.2. Infrared spectra

Important IR absorptions for the complexes are shown in table 2. The observed bands have been classified into those originating from the ligands and those arising from the bands formed between ruthenium(III) and the coordinating sites. IR spectra of the free ligands showed bands at 3475–3479, 2873–3076, 1605–1616, 1470–1588, and 1171–1288 cm^{-1}

Scheme 1. Synthesis of H_2LL^1 – H_2LL^3 .

assignable to $\nu(\text{OH})$, $\nu(\text{CH}_3/\text{CH}_2)$, $\nu(\text{C}=\text{N})$, $\nu(\text{C}=\text{C})$, and $\nu(\text{C}-\text{O})$, respectively [20, 49, 50]. H_2LL^1 , H_2LL^2 and H_2LL^3 show a very strong absorption at $1605\text{--}1616\text{ cm}^{-1}$ in their IR spectra, characteristic of the azomethine $\nu(\text{C}=\text{N})$ (table 2). In the Schiff base complexes, this absorption shifted to $1621\text{--}1623\text{ cm}^{-1}$ indicating coordination of the Schiff bases through nitrogen in accord with coordination of the azomethine function to the metal ion for all the complexes [28, 50, 51]; this shift of wavenumber is expected due to coordination of nitrogen of the azomethine group to ruthenium, thereby reducing electron density in the azomethine [50, 52].

A medium band corresponding to phenolic oxygen $\nu(\text{C}-\text{O})$ is observed at $1171\text{--}1288\text{ cm}^{-1}$ for the free ligands. Upon chelation, this band shifted to lower frequency ($1470\text{--}1543\text{ cm}^{-1}$) for all the ruthenium(III) Schiff base complexes [28, 53]. This indicates enolization of $>\text{C}=\text{O}$ followed by deprotonation and complexation with metal and the destruction of keto group presumably viz., enolization and ketolization bonding of the ligand through the resulting enolate and ketolate oxygen. This is further supported by the disappearance of $\nu(\text{OH})$ at $3475\text{--}3479\text{ cm}^{-1}$ in the complexes. The presence of coordinated water at $3419\text{--}3435$ and $846\text{--}861\text{ cm}^{-1}$, due to $\nu(\text{O}-\text{H})$ stretching and $\nu(\text{O}-\text{H})$ rocking vibrations, respectively, further confirmed the presence of water [49, 54, 55]. In the low frequency

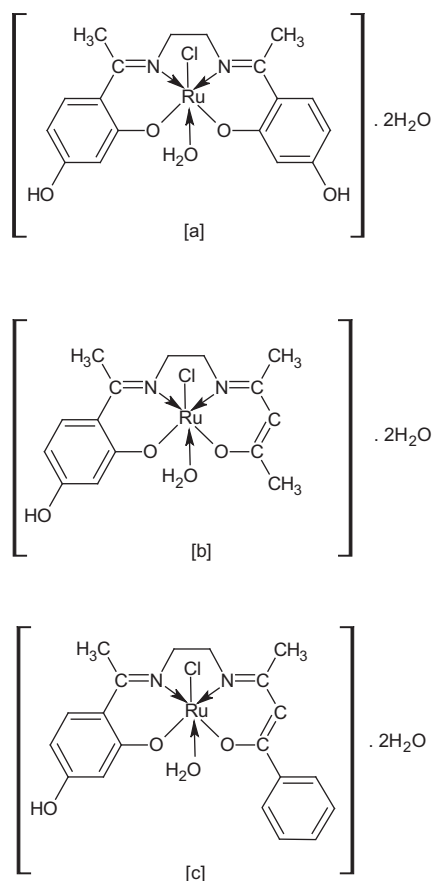


Figure 1. Proposed structure for the Ru(III) complexes (a) $[\text{RuCl}(\text{LL}^1)(\text{H}_2\text{O})]\cdot 2\text{H}_2\text{O}$, (b) $[\text{RuCl}(\text{LL}^2)(\text{H}_2\text{O})]\cdot 2\text{H}_2\text{O}$, and (c) $[\text{RuCl}(\text{LL}^3)(\text{H}_2\text{O})]\cdot 2\text{H}_2\text{O}$.

region, the observed bands at $519\text{--}535$ and $415\text{--}437\text{ cm}^{-1}$ are probably due to the formation of $\nu(\text{M}\text{--}\text{N})$ and $\nu(\text{M}\text{--}\text{O})$ vibrations, respectively [41, 56, 57].

3.3. Molar conductivity measurements

The molar conductivity (Λ_m) values of the Ru(III) complexes in 10^{-3} M DMF solution (table 1) at room temperature are $23.8\text{--}47.4\text{ }\mu\text{S cm}^{-1}$, indicating the essential non-electrolytic character of the compounds [1, 58].

3.4. The antioxidant assay

Oxidative reactions of biological molecules induce a variety of pathological events such as cellular injury and aging process, and these damaging events are caused by free radicals [59, 60]. Therefore, to prevent free radical damage in the body, it is important to administer drugs that may be rich in antioxidants. The antioxidant activity of ligands and their metal complexes have been investigated using the *in vitro* method [43, 61]. However, the antioxi-

Table 1. Analytical data and physical properties of the N₂O₂ Schiff bases and their Ru complexes.

Compound	Empirical formula	F.wt	Color	Yield (%)	% Found (Calcd)			Decomp. temp (°C)	Conductance (μS cm ⁻¹)
					C	H	N		
H ₂ LL ¹	C ₁₈ H ₂₀ N ₂ O ₄	328.36	Brownish-yellow	76.52	65.73 (65.84)	6.28 (6.14)	8.71 (8.53)	—	
[RuCl(LL ¹)(H ₂ O)]·2H ₂ O	C ₁₈ H ₂₄ N ₂ O ₇ RuCl	516.92	Dark-green	60.28	42.08 (41.82)	4.43 (4.68)	5.31 (5.42)	47.4	
H ₂ LL ²	C ₁₅ H ₁₀ N ₂ O ₃	275.32	Golden-yellow	61.23	65.26 (65.44)	7.13 (6.96)	9.98 (10.17)	—	
[RuCl(LL ²)(H ₂ O)]·2H ₂ O	C ₁₅ H ₂₃ N ₂ O ₆ RuCl	463.88	Darkish-green	76.20	38.75 (38.84)	4.81 (5.00)	5.83 (6.04)	30.6	
H ₂ LL ³	C ₂₀ H ₂₁ N ₂ O ₃	337.40	Orange-brown	74.17	70.96 (71.20)	5.13 (5.27)	8.09 (8.30)	—	
[RuCl(LL ³)(H ₂ O)]·2H ₂ O	C ₂₀ H ₂₅ N ₂ O ₆ RuCl	525.95	Darkish-green	53.67	45.88 (45.67)	4.56 (4.79)	5.18 (5.33)	23.8	

Table 2. FTIR spectral data of the N₂O₂ Schiff bases and their Ru complexes (cm⁻¹).

Compound	$\nu(\text{OH})$	$\nu(\text{CH}_3/\text{CH}_2)$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}=\text{C})$	$\nu(\text{C}-\text{O})$	$\nu(\text{Ru}-\text{N})$	$\nu(\text{Ru}-\text{O})$
H ₂ LL ¹	3475	2929, 2873	1616	1588, 1532	1267, 1173	–	–
[RuCl(LL ¹)(H ₂ O)]·2H ₂ O	3435	2977, 2845	1622	1530, 1479	1244, 1170	535	437
H ₂ LL ²	3475	2928, 2911	1612	1534, 1480	1243, 1171	–	–
[RuCl(LL ²)(H ₂ O)]·2H ₂ O	3419	2959, 2844	1621	1543, 1525	1243, 1168	522	428
H ₂ LL ³	3479	3076, 2981	1605	1543, 1470	1288, 1241	–	–
[RuCl(LL ³)(H ₂ O)]·2H ₂ O	3430	2997, 2901	1623	1543, 1508	1258, 1138	519	415

dant mechanism of the complexes has not been explained so far [62]. The antioxidant assay study was carried out using different concentrations of the test samples (Schiff base ligands and the Ru(III) complexes) with DPPH and ABTS radicals, while ascorbic acid, rutin, and BHT were used as standards in order to establish some structure antioxidant activity relationship.

3.4.1. DPPH radical scavenging assay. DPPH is a compound widely used to examine the ability of a given sample to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity of foods [63]. The results of the DPPH radical scavenging abilities of H₂LL¹, H₂LL² and H₂LL³ and the Ru complexes were studied and compared with the standard (ascorbic acid and rutin). The Ru(III) complexes exhibited significant DPPH radical scavenging ability in all the concentrations used, i.e. chelated Ru(III)-Schiff base complexes were more effective free radical scavengers than the corresponding free H₂LL¹, H₂LL², and H₂LL³ Schiff bases; this could be attributed to the acquisition of additional superoxide dismutating centers [64].

However, the Ru(III) complexes showed comparable or higher scavenging activity compared to the standards (ascorbic acid and rutin) with [RuCl(LL²)(H₂O)] showing significantly higher scavenging ability. The DPPH radical scavenging ability of the Ru(III) complexes can be ranked, [RuCl(LL²)(H₂O)] > [RuCl(LL³)(H₂O)] > [RuCl(LL¹)(H₂O)]. IC₅₀ values of Schiff bases H₂LL¹, H₂LL², and H₂LL³ on DPPH radical are 0.067 ± 0.006, 0.065 ± 0.001, and 0.055 ± 0.002 mg mL⁻¹, respectively, whereas [RuCl(LL¹)(H₂O)], [RuCl(LL²)(H₂O)], and [RuCl(LL³)(H₂O)] showed IC₅₀ values at 0.041 ± 0.003, 0.031 ± 0.006, and 0.036 ± 0.002 mg mL⁻¹, respectively (table 3). Therefore, the scavenging effect of the free ligand is lower compared to that of their corresponding Ru(III) complexes,

Table 3. DPPH and ABTS radical scavenging capacities (IC₅₀ ± SD, mg mL⁻¹) of standard drugs, N₂O₂ Schiff bases, and their Ru complexes.

Compound	DPPH	ABTS
H ₂ LL ¹	0.067 ± 0.006	0.008 ± 0.0003
[RuCl(LL ¹)(H ₂ O)]	0.041 ± 0.003	0.011 ± 0.0005
H ₂ LL ²	0.065 ± 0.001	0.009 ± 0.0004
[RuCl(LL ²)(H ₂ O)]	0.031 ± 0.006	0.036 ± 0.0027
H ₂ LL ³	0.055 ± 0.002	0.009 ± 0.0006
[RuCl(LL ³)(H ₂ O)]	0.036 ± 0.002	0.025 ± 0.0047
Vitamin C	0.045 ± 0.005	0.023 ± 0.0035
Rutin	0.037 ± 0.009	0.004 ± 0.0003

Note: (n = 3, X ± SEM), IC₅₀ – inhibitory concentration.

related to chelation of the organic molecules with the metal ions. Also, the violet color of DPPH radical changed to yellow upon addition of Ru(III) compounds because proton from the test samples were transferred to DPPH, converting it into the corresponding hydrazine form [40]. Thus, these compounds could be a promising therapeutic agents to treat stress-induced pathological conditions such aging, cancer, and cardiovascular and neurodegenerative diseases.

3.4.2. ABTS radical scavenging activity. ABTS cation radicals (ABTS^+) are produced by oxidation of ABTS with potassium persulfate and, thus, are reduced in the presence of hydrogen-donating antioxidants [65]. This has been the basis of one of the spectrophotometric methods applied to measurement of the total antioxidant activity of solutions of pure substances and aqueous extracts [66, 67]. The method described gives a measure of the antioxidant activity of test samples determined by the decolorization of ABTS^+ through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm [68]. H_2LL^1 , H_2LL^2 and H_2LL^3 and their Ru complexes exhibited low-to-moderate scavenging ability of the ABTS radical (figure S2) and showed comparable or slightly lower activity to that of rutin and BHT (standard drugs).

At a concentration of $4.35 \mu\text{g mL}^{-1}$, the percentage inhibition was 60.2, 66.4, 59.8, 54.8, and 50.6% for $[\text{RuCl}(\text{LL}^1)(\text{H}_2\text{O})]$, $[\text{RuCl}(\text{LL}^2)(\text{H}_2\text{O})]$, $[\text{RuCl}(\text{LL}^3)(\text{H}_2\text{O})]$, rutin, and BHT, respectively. Nevertheless, the ABTS activities of the Ru(III) complexes were significantly enhanced compared to their corresponding free Schiff base ligands. Lowest concentration of the H_2LL^1 , H_2LL^2 and H_2LL^3 and its Ru complexes were more effective in quenching ABTS radicals in the system. IC_{50} value of H_2LL^1 , H_2LL^2 , and H_2LL^3 on ABTS radical is 0.067 ± 0.006 , 0.065 ± 0.001 , and $0.055 \pm 0.002 \text{ mg mL}^{-1}$, respectively, while the IC_{50} values at 0.011 ± 0.0005 , 0.036 ± 0.0027 , and $0.025 \pm 0.0047 \text{ mg mL}^{-1}$ are for $[\text{RuCl}(\text{LL}^1)(\text{H}_2\text{O})]$, $[\text{RuCl}(\text{LL}^2)(\text{H}_2\text{O})]$, and $[\text{RuCl}(\text{LL}^3)(\text{H}_2\text{O})]$, respectively (table 3). Furthermore, the synthesized compounds scavenged the ABTS radical in a concentration-dependent pattern.

Generally, the scavenging activities of the DPPH radical by H_2LL^1 , H_2LL^2 , and H_2LL^3 and the Ru(III) complexes are higher than that of ABTS radical. Wang and co-workers [69] reported that some compounds which exhibited ABTS scavenging activity did not possess DPPH scavenging activity. Hence, this study revealed the synthesized tetradentate Schiff base Ru(III) complexes exhibited strong scavenging activities against DPPH and moderate activity against ABTS radicals. This result shows that the compounds in this study can scavenge different free radicals in different systems, indicating that they may be useful as therapeutic agents for treating pathological damage associated with radical generation.

3.5. Antiproliferative activity evaluation

The *in vitro* anticancer activities of Ru(III) complexes and parthenolide (at various concentrations ranging from 0.01 to 100 μM) were evaluated using three cancer cell lines: human renal cancer cell (TK10), human melanoma cancer cell (UACC62), and human breast cancer cell (MCF7) using the SRB assay and parthenolide was used as standard. Parthenolide demonstrated high levels of antiproliferative effect against all cell lines, in accord with previous reports [70]. The values of the concentration of the compounds for 50% inhibition (IC_{50}) were obtained from non-linear regression analysis of dose response data for the compounds tested and are presented in table 4. The Ru(III) complexes demonstrate low-to-moderate *in vitro* antiproliferative effect compared to parthenolide (standard agent)

Table 4. IC₅₀ values (μM) of Ru(III) complexes and parthenolide against human cell lines*.

Compound	Antiproliferative activity IC ₅₀ (μM) 48 h		
	TK-10	UACC-62	MCF-7
[RuCl(LL ¹)(H ₂ O)]·2H ₂ O	>100	>100	>100
[RuCl(LL ²)(H ₂ O)]·2H ₂ O	>100	>100	90 ± 8
[RuCl(LL ³)(H ₂ O)]·2H ₂ O	>100	>100	90 ± 5
Parthenolide	4.64 ± 1.43	11.37 ± 2.18	3.52 ± 2.02

*Cells were treated with various concentrations of tested compounds for 48 h. IC₅₀ values were calculated as described in Section 2. Each value represents the mean ± SD of three independent experiments (*Z'* factor > 0.5).

against selected tumor cell lines. [RuCl(LL¹)(H₂O)]·2H₂O displayed non-selective antiproliferative activity against all tumor cells tested, while [RuCl(LL²)(H₂O)]·2H₂O and [RuCl(LL³)(H₂O)]·2H₂O had a low antiproliferative effect with IC₅₀ at 90 μM against MCF-7 (*Z'* factor > 0.5). The inhibition effects were enhanced by increasing the concentration of the Ru(III) complexes. The results showed that [RuCl(LL²)(H₂O)]·2H₂O was more active against all the selected tumor cells than [RuCl(LL¹)(H₂O)]·2H₂O and [RuCl(LL³)(H₂O)]·2H₂O (*Z'* factor > 0.5), which is in agreement to their order of *in vitro* DPPH scavenging ability of the Ru(III) complexes. Binding of the three N₂O₂ Schiff base Ru(III) complexes to biological targets other than DNA could be responsible for the observed antiproliferative activity of the complexes.

4. Conclusion

Ru(III) complexes of symmetric and asymmetric Schiff base ligands derived from ethane-1,2-diamine, 4-acetylresorcinol, acetylacetone, and 1-phenylbutane-1,3-dione were synthesized and characterized. Conductance measurements revealed non-electrolytes. The ligands are dibasic, ONNO tetradentate, coordinated to the Ru(III) through the phenolic oxygen and imino nitrogen; octahedral geometry around the Ru(III) ions is completed by H₂O and a Cl⁻. *In vitro* anticancer studies of the Ru complexes show that they are inactive against human cancer cells (TK10) and human melanoma cancer cell (UACC62) but show mild activity against human breast cancer cell line (MCF7). The synthesized complexes exhibited low biological activities as potential anticancer agents. Derivatization of the Schiff base by substituting CH₃ on the C2 and C4 position of acetylacetone could increase the activity of the complexes. Nevertheless, antioxidant activities of the complexes exhibited moderate to strong free radical inhibitors or scavenger for treating pathological damage associated with radical generation.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by the Govan Mbeki Research and Development (GMRD), University of Fort Hare.

Supplemental data

Supplemental data for this article can be accessed here [<http://dx.doi.10.1080/00958972.2015.1043127>].

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