Research Article

Synthesis and Assessment of Antibacterial Activities of Ruthenium(III) Mixed Ligand Complexes Containing 1,10-Phenanthroline and Guanide

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In this work, two complexes of ruthenium(III) ([Ru(phen)_2Cl_2]Cl⋅2H_2O and [Ru(phen)_2(G)Cl]_2Cl⋅H_2O) were synthesized from 1,10-phenanthroline alone as well as from both 1,10-phenanthroline and guanide. The synthesis was checked using halide test, conductance measurement, and spectroscopic (ICP-OES, FTIR, and UV/Vis) analysis. Their in vitro antibacterial activities were also investigated on two Gram-positive (Staphylococcus aureus (S. aureus) and methicillin resistant Staphylococcus aureus (MRSA)) and two Gram-negative (Escherichia coli (E. coli) and Klebsiella pneumoniae (K. pneumoniae)) bacteria. These complexes showed wide-range better activities than the commercially available controls (Chloramphenicol and Ciprofloxacin) against even the most drug resistant K. pneumoniae. [Ru(phen)_2(G)Cl]_2Cl⋅H_2O inhibited S. aureus, MRSA, E. coli, and K. pneumoniae by 17.5%, 27.4%, 16%, and 52%, respectively, better than Chloramphenicol. It also inhibited these pathogens by 5.9%, 5.1%, 2.3%, and 17.2%, respectively, better than Ciprofloxacin. Similarly, [Ru(phen)_2(Cl)]_2Cl⋅H_2O inhibited these pathogens by 11%, 8.7%, 0.1%, and 31.2%, respectively, better than Chloramphenicol. Therefore, after in vivo cytotoxicity investigations, these compounds can be considered as potential antibiotic drugs.

1. Introduction

Coordination chemistry is about tuning properties of metal ions using different ligands [1–3]. This includes stabilization of different oxidation states and modulation of the solvophilicity and electrophilic and nucleophilic properties of the metal ion [4–6]. While coordinating, the properties of the ligands themselves are also modified. For instance, the pharmacological activities and their crucial role in DNA/RNA base pairing through several hydrogen-bonding patterns of free oxypurines such as guanine can significantly change after complex formation [7, 8]. Based on this, synthesis of different coordination compounds with desired properties by ligand tailoring has become a fascinating research field. Designing new coordination compounds with therapeutic abilities has been part of this activity [9–17]. From this perspective, there has been a growing interest in the chemistry community to examine the biological activities of ruthenium complexes [18, 19]. Ruthenium (5s^24d^7) frequently accesses +2 and +3 oxidation states at physiological conditions and can interact with nucleic acids, proteins, sulfur, or oxygen containing compounds and water in the cells [20–24]. Its interaction kinetics with the latter can be controlled using advantages of the unique properties of each kind of ligand. This enables the ligand exchange rates of ruthenium complexes to be close to those of cellular processes which make them suitable for therapeutic applications.

In this respect, numerous investigations in the properties and applications of ruthenium(II) complexes with 1,10-phenanthroline (phen) as a ligand or mixed with other ligands are reported [25–27]. Nevertheless, there is no report on the chemistry of ruthenium(III) complex containing 1,10-phenanthroline alone or 1,10-phenanthroline and guanidine mixed.

The ideally placed nitrogen atoms along with their rigid planar structure and hydrophobic, electron-poor heteroaromatic, and π-acidic properties cooperatively made 1,10-phenanthroline a classic chelating bidentate ligand. These properties enable it to have stacking interaction ability with DNA...
base pairs [28–31]. Guanine is a chemically inert oxypurine heteroaromatic molecule. Its inertness is changed by complex formation. The latter is favored in its guanide (G−) form which is derived by deprotonation of guanidine.

The purpose of this study is to examine the effects of 1,10-phenanthroline alone and mixed with guanidine on the biological activity of Ru(III). The complex would orchestrate the binding ability of ruthenium with a range of biomolecules, the unique stacking interaction ability of 1,10-phenanthroline on cell genetic material, and the interaction of guanidine through hydrogen bonding with cytosine residue of the genetic material.

2. Experimental

2.1. Chemicals. All chemicals used in the present work are as follows: 1,10-phenanthroline monohydrate (BDH Chemical Ltd., Poole, England), guanine (99%, ACROS), RuCl3, silver nitrate, sodium hydroxide, acetone, chloroform, sulfuric acid (Sigma Aldrich), methanol (Hi Media Laboratories Ltd., India), KBr, dichloromethane, Mueller Hinton agar, and barium chloride (BLULUX Laboratories Ltd., India), and nitric acid (TV. Industrial Estate, India).

2.2. Instruments and Methods. The electronic conductance was measured using 10−3 M solution of each complex in deionized water with JENWAY 4200 conductivity meter at room temperature. The electronic spectra were recorded in the 200–800 nm region on Sanyo SP650 UV/Vis spectrophotometer. IR spectra were recorded using KBr discs in the 4000–400 cm−1 region on AVATAR 330 FTIR, Thermo Nicolet spectrophotometer. Ruthenium content was determined by PerkinElmer, Optima 7300 V ICP-OES spectrometer. The complexes were maintained in the appropriate blood agar base at 4°C. Antibiotic discs (Ciprofloxacin 5 μg and Chloramphenicol 30 μg) were used as reference. The minimum inhibitory concentration (MIC) against each bacterium was determined by preparing aqueous solutions of different concentrations of the complexes by serial dilution (200 μg/mL, 300 μg/mL, 400 μg/mL, 500 μg/mL, 600 μg/mL, 800 μg/mL, and 1000 μg/mL). The experiments were repeated three times to obtain consistent results. The antibacterial tests were carried out at the Amhara Regional Health Research Microbiology Laboratory Center, Bahir Dar, Ethiopia.

3. Results and Discussion

The analytical data of the complexes are indicated in Table 1.

3.1. Molar Conductance of the Metal Complexes. The conductance measurements, recorded for 10−3 M solutions of the metal complexes in deionized water, are listed in Table 1. The data shows that both complexes are 1:1 electrolytes [32]. The lower conductance of [Ru(phen)2(G)(Cl)]Cl·2H2O compared to [Ru(phen)2(Cl)2]Cl·2H2O is a consequence of increase in molar mass and the surface area. Hence the speed of mobility of the cation decreases as a result of the decrease in the kinetic energy imparted by the electric field from measurement instrument [33].

3.2. Electronic Spectra. The electronic spectra of the complexes are displayed in Figure 1 and Table 2.

The complexes exhibited simple characteristic d-d transitions. The difference in the band position for d-d transition absorption of RuCl3 and the complexes may be explained by assuming different environments around the metal ion following the coordination [34, 35]. The coordination of 1,10-phenanthroline to Ru(III) results in a distorted octahedral geometry. Consequently, the eg orbitals split to d2 and d2g resulting in two transitions (t2g → eg(d2g) and t2g → eg(d2g)). [Ru(phen)2(G)(Cl)]Cl·2H2O demonstrated absorption at longer wavelength (Figure 1(c)) than [Ru(phen)2(Cl)2]Cl·2H2O (Figure 1(b)). This is probably
Table 1: Analytical data of the complexes.

| Complex (color)                          | Melting point/°C | Yield (%) | Elemental estimation     | Molar conductivity $\Lambda_M$ (S cm$^{-2}$ mol$^{-1}$) |
|-----------------------------------------|------------------|-----------|--------------------------|--------------------------------------------------------|
| [Ru(phen)$_2$(Cl)$_2$]Cl·2H$_2$O (reddish brown) | >300             | 80        | 16.74 (16.62)             | 5.88 (5.66) | 121.50                                                |
| [Ru(phen)$_2$(G)(Cl)]Cl·H$_2$O (orange red)       | >300             | 87        | 14.47 (14.23)             | 5.07 (4.89) | 87.26                                                 |

Scheme 1: Synthesis of [Ru(phen)$_2$(Cl)$_2$]Cl·2H$_2$O and [Ru(phen)$_2$(G)(Cl)]Cl·H$_2$O.
Table 2: Electronic spectral data of the salt and complexes.

| Complex                                      | Band position (nm) | Assignment                              |
|----------------------------------------------|--------------------|-----------------------------------------|
| RuCl₃                                        | 295, 573           | *LMCT, d-d(T₂g → ²T₁g)                  |
| [Ru(phen)$_2$(Cl)$_2$]Cl·2H₂O                | 643                | d-d(T₂g → ²T₁g)                         |
| [Ru(phen)$_2$(G)(Cl)]Cl·H₂O                 | 657                | d-d(T₂g → ²T₁g)                         |

*LMCT: ligand to metal charge transfer.

because guanide (G⁻) formed a shorter and stronger bond that narrowed the transition ($t_{2g} → e_g$) gap (Figure 1 and Table 2).

3.3. IR Spectroscopy. The infrared spectra of the ligands and the complexes are indicated in Figure 2 and selected characteristic frequencies are indicated in Table 3.

The bands at 1623 cm$^{-1}$ (s) and 1587 cm$^{-1}$ (s), characteristic for ν$_{C=C}$ and ν$_{C=N}$ stretching in the free 1,10-phenanthroline monohydrate (Figure 2(a)), appear at 1670 cm$^{-1}$ (w) and 1429 cm$^{-1}$ (w), respectively, in [Ru(phen)$_2$(G)(Cl)]Cl·H₂O (Figure 2(d)). They also appeared at 1633 cm$^{-1}$ (w) and 1540 cm$^{-1}$ (w), respectively, in [Ru(phen)$_2$(Cl)$_2$]Cl·2H₂O (Figure 2(c)). Similarly, the characteristic bands of guanide at 3335 cm$^{-1}$ (s), 3112 cm$^{-1}$ (s) ν$_{N-H(NH_2)}$, and 1710 cm$^{-1}$ (s) (ν$_{C=O}$) (Figure 2(b)) are displaced towards 3340 cm$^{-1}$ (w) and 1697 cm$^{-1}$ (w) (Figure 2(d)), respectively. The changes in absorption frequencies and strength suggest that 1,10-phenanthroline and guanide are coordinated. The strong and broad bands at 3439 cm$^{-1}$ and 3416 cm$^{-1}$ characteristic for
Table 3: Important characteristic IR bands of the ligands and complexes, cm$^{-1}$.

| Compound                        | $\gamma$(O-H) | $\gamma$(N-H) | $\gamma$(C-H) | $\gamma$(C=N) | $\gamma$(C=C) | $\gamma$(C=O) |
|---------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 1,10-Phenanthrolinemonohydrate   | 3439 (s, b)   | —             | 3045 (w)      | 1290 (w)      | 1623 (s)      | 1587 (s)      |
| Guanine                         | —             | 3335, 3112 (d)| 2992 (w)      | 1256 (w)      | 1563 (w)      | 1710 (s)      |
| [Ru(Phen)$_2$(Cl)$_2$]Cl$_2$H$_2$O | 3416 (s, b)   | —             | 2925 (w)      | 1208 (w)      | 1633 (w)      | 1540 (w)      |
| [Ru(Phen)$_2$(G)(Cl)]ClH$_2$O   | 3431          | 3340 (s)      | 2912 (w)      | 1270 (w)      | 1670 (w)      | 1429          | 1697          |

$s$: strong, $b$: broad, $w$: weak, and $d$: doublet.

$\gamma$(O-H) in the free 1,10-phenanthroline monohydrate and [Ru(phen)$_2$(Cl)$_2$]Cl$_2$H$_2$O, respectively (Figures 2(a) and 2(b)), appear at 3431 cm$^{-1}$ obscured in the band characteristic for $\gamma$(NH$_2$) in [Ru(phen)$_2$(G)(Cl)]Cl$_2$H$_2$O (Figure 2(d)). This change in the absorption frequency of water explains the change in the nature of its interaction. Moreover, the change in the intensity may explain the change in the relative amount of water molecules in 1,10-phenanthroline monohydrate and the complexes. The latter argument supports the proposed formula of the complexes.

3.4. Antibacterial Activity Testing. This observation shows that the complexes demonstrated biological activities against all the tested strains (Figure 3 and Table 4). The observed
increase in antibacterial activity can be explained on the basis of Overtone's concept [36] and Tweedy's chelation theory [37]. The lipid membrane that surrounds the cell favors the passage of only lipid soluble materials which is an important condition for antimicrobial activity. On coordination, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with the donor groups. Further, it increases the delocalization of $\pi$ electrons over the whole chelate ring and hence enhances the liposolubility
The percent activity index data of the complexes against the tested bacteria compared to (a) Chloramphenicol and (b) Ciprofloxacin.

(a)

| Compound                       | S. aureus | MRSA | E. coli | K. pneumoniae |
|--------------------------------|-----------|------|---------|---------------|
| [Ru(Phen)$_2$(Cl)$_2$]Cl$_2$H$_2$O | 11.00%    | 8.70%| 0.10%   | 31.20%        |
| [Ru(Phen)$_2$(G)(Cl)]Cl$_2$H$_2$O | 17.50%    | 27.40%| 15.85%  | 52.00%        |

(b)

| Compound                       | S. aureus | MRSA | E. coli | K. pneumoniae |
|--------------------------------|-----------|------|---------|---------------|
| [Ru(Phen)$_2$(Cl)$_2$]Cl$_2$H$_2$O | −8.80%    | −10.00%| −12.00%| 1.01%        |
| [Ru(Phen)$_2$(G)(Cl)]Cl$_2$H$_2$O | 5.90%     | 5.10%| 2.30%   | 17.17%        |

MRSA: methicillin resistant S. aureus.

Table 6: MIC assay of [Ru(Phen)$_2$(G)(Cl)]Cl$_2$H$_2$O against four bacterial pathogens.

| Microorganism | Minimum concentration of microorganism growth |
|---------------|---------------------------------------------|
|               | 200 μg/mL | 300 μg/mL | 400 μg/mL | 500 μg/mL | 600 μg/mL | 800 μg/mL | 1000 μg/mL |
| S. aureus     | +         | +         | −         | −         | −         | −         | −         |
| MRSA          | +         | +         | +         | +         | −         | −         | −         |
| K. pneumoniae | +         | +         | −         | −         | −         | −         | −         |
| E. coli       | +         | −         | −         | −         | −         | −         | −         |

Note: +: bacteria growth and −: no bacteria growth.

of the complexes. This increased liposolubility enhances the penetration of the complexes into the lipid membrane and interferes in the normal activities of the bacteria [38].

The percent activity indexes of the complexes against the reference antibiotics demonstrated significant comparative inhibitions (Table 5). [Ru(phen)$_2$(G)(Cl)]Cl$_2$H$_2$O showed better activity than the two commercial antibiotics (Ciprofloxacin and Chloramphenicol) against all the strains studied including the most drug resistant Gram-negative K. pneumoniae. [Ru(phen)$_2$(Cl)$_2$]Cl$_2$H$_2$O also showed nearly equal activities as Chloramphenicol against S. aureus and E. coli and better activities against MARSA and K. pneumoniae (Table 5). The better activities demonstrated by [Ru(phen)$_2$(G)(Cl)]ClH$_2$O compared to [Ru(phen)$_2$(Cl)$_2$]Cl$_2$H$_2$O are due to its additional interaction with the genetic material of the cell by guanide.

3.5. Minimum Inhibitory Concentration (MIC) Determination. MIC is the lowest concentration that completely inhibited the growth of microorganisms for 24 hours.

Around 300 μg/mL [Ru(Phen)$_2$(G)(Cl)]Cl$_2$H$_2$O is sufficient to inhibit the growth of E. coli while around 400 μg/mL is necessary to start inhibiting S. aureus and K. pneumonia (Table 6).

4. Conclusions

In this synthesis, Ru(III) and the ligands are brought together with rigid configuration. This resulted in delocalization of π electrons over the whole cationic unit and hence the reduction of the polarity of the complexes which increased the liposolubility. This has enhanced the penetration of the complexes into the lipid membrane and inhibited the growth of the tested Gram-positive and Gram-negative bacteria. The latter phenomenon demonstrates the wide-range activities of the complexes.

Competing Interests

There is no conflict of interests among the authors and the funding institution.

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