TEMPLATE SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDY OF NEW METAL COMPLEXES FROM 2,6-DIAMINOPYRIDINE AND 1,4-DIHYDROQUINOXALIN-2,3-DIONE

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A new series of tetradentate N₂O₂ acyclic complexes of type [MLCl₂] (M: Zn(II), Cd(II), Hg(II); L: tetradentate acyclic ligand) and [ZrOL]Cl₂ have been prepared on the basis of condensation of 2,6-diaminopyridine and 1,4-dihydroquinoxaline-2,3-dione by template method. The ligand coordinates through the two imine nitrogen atoms formed up on condensation of -NH₂ of 2,6-diaminopyridine with -C=O group in 2-position of quinoxalin-2,3-dione, as well as the lactam form of metal complexes confirm the participation of oxygen atoms in coordination with the metal ions. However, the pyridine nitrogen atoms do not take part in coordination with the metal ions. These complexes have been characterized with the help of various spectral techniques ¹H and ¹³C NMR, FT-IR, electronic spectra, elemental analyses, and measurements of molar conductance in DMF solutions. Furthermore the determination of chloride content in the metal complexes assists in the investigation the concluded structures. The octahedral geometry was proposed for Zn(II), Cd(II) and Hg(II) while, Zr(IV) complex structure was proposed to be square pyramidal. The new metal complexes were investigated for antibacterial and antifungal properties of gram-positive bacteria (Staphylococcus aureus), gram-negative bacteria (Escherichia. Coli) and fungi Aspergillus fumigates and Candida were used in this study to assess their antimicrobial properties. The results showed that this skeletal framework exhibit marked potency as antimicrobial agents.

Introduction

The chemistry of macrocyclic complexes has attracted the interest of both inorganic and bioinorganic chemists in recent years.¹ The field of coordination chemistry of macrocyclic complexes has undergone remarkable growth during the past few decades and undergone remarkable growth during the past few decades and become a growing class of research. This enormous growth is due to synthesis of great number and variety of synthetic macro cycles which behave as coordinating agents for metal ions.² Template reactions lie at the heart of macro cyclic chemistry and are the best aids for the preparation of macro cyclic complexes.³,⁴ Generally transition metal ions have been used as templates.⁵ The importance of macro cyclic complexes in coordination chemistry is because of its various applications in biological processes such as photosynthesis and dioxygen transport catalytic properties, potential applications as metal extract ants and radio therapeutic agents.⁶ The importance of macrocyclic complexes is due to their resemblance with in-situ one pot template synthesis is the most widely adopted method for preparation of macrocyclic complexes.⁷ A number of nitrogen donor and oxygen macrocyclic and acyclic derivatives have been used for a long time in analytical, industrial and medical applications.⁸⁻¹⁰ The macrocyclic complexes have attracted attention because bacterial and fungal growth.¹⁰ Some macrocyclic complexes have been reported to have anti-inflammatory approach.¹¹ Several macrocyclic complexes with teta aza macrocyclic ligands, such as cyclam, or bicyclam have been reported to exhibit antitumor activity.¹² Macro cyclic metal complexes are of great importance due to their resemblances with many natural systems such as porphyrins and cobalamines.¹³

Satish and co-workers have investigated the binuclear Co(II), Ni(II), Cu(II) and Zn(II) complexes with tetradentate Schiff bases derived from 2,6-diformyl-4-methylphenol with 2-hydroxy-3-hydrazinoquinoxalin.¹⁴ In recently published papers some Mn(II), Cr(III), Fe(III), V(IV) and Zn(II) complexes with tetradentate N₂O₂ type quinoxaline organic moieties were isolated involving template synthesis and investigated them with using GC-MS, ¹H NMR and other analytical techniques.¹³,¹⁴ The template metal(II) complexes involving quinoxalin-2-one have been found to exhibit potential antibacterial activities and abnormal electronic characterizations.¹⁵ The continuous interesting with coordination chemistry of quinoxaline derivatives, in the present paper, synthesis and characterization of Zn(II), Cd(II), Hg(II) and Zr(IV) complexes derived from 2,6-diaminopyridine and 1,4-dihydroquinoxaline-2,3-dione have been prepared, characterized and biologically tested against some selected bacteria and fungi.

Experimental

The anhydrous metal chlorides ZnCl₂, CdCl₂, HgCl₂ and ZrOCl₂.8H₂O were purchased from Sigma-Aldrich company and used without purification. The oxalic acid, 2,6-diaminopyridine, 1,2-phenylenediamine and solvents were supplied from Fluka company in 99% purity. All other chemicals used were of Analy grade.

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Synthesis of 2,3-quinoxaline-dione

The 2,3-quinoxaline-dione was prepared according to the method published in literature, Scheme 1.

Scheme 1. Preparation of 1,4-dihydroquinoxalin-2,3-dione

Isolation of complexes

Our several attempts to isolate the free macrocyclic or acyclic ligand were unsuccessful. Hence, all the complexes were obtained by template synthesis. To a stirring methanol solution (~60 mL) of 2.6-diaminopyridine (10 mmol) was added a solution of divalent ZnCl₂, CdCl₂, HgCl₂ and zirconyl acetate (that obtained from direct reaction of ZrOCl₂.8H₂O with sodium acetate) (5 mmol) dissolved in minimum quantity of methanol (20 mL). The resulting solution was refluxed for 18–20 h, and after overnight cooling a dark colored precipitate formed which was filtered, washed with methanol, acetone, diethyl ether and dried in desiccators. The complexes were found soluble in DMF and DMSO, acetonitrile but were insoluble in water and other organic solvents like ethanol, methanol and chloroform. They were thermally stable up to ~350 °C and then decomposed. The template synthesis of the complexes may be represented by Scheme 2.

Scheme 2. Synthesis of Zn, Cd and Hg(II) complexes, n=2 for M=ZrO, n=0 for M(II)=Zn, Cd and Hg

Instrumentation

The elemental analyses of the new solid complexes were determined using Carlo-Erba 1106 Elemental analyzer. Electronic spectra were recorded for solutions of quinoxaline-2,3-dione and its metal template complexes using Shimadzu spectrometer in the range 200-800 nm in DMF solvent. The 1H and 13C NMR spectra were carried at Al-Bait University, Amman, Jordan on Bruker 300 MHz spectrometer in DMSO-d6 solvent. The Fourier transform infrared spectra of the prepared complexes were recorded in KBr and CsI discs on Shimadzu model FT-IR-8400 spectrometer. The molar conductance measurements were made on an ELICO conductivity bridge type CM-82 with a cell having a cell constant of 0.51 cm⁻¹ in DMF solutions. The percents of metal contents of the complexes were determined by flame atomic absorption on Shimadzu AA-670 spectrometer at instrumental analyses. Finally the estimation of ionic chloride content in the prepared complexes was done by conductometric titrations with standard solution of silver nitrate.

Antibacterial activity

Primary screening

The antibacterial activities of the newly synthesized complexes were evaluated by the Agar Well Diffusion Assay Technique against two gram-positive and negative bacteria, Staphylococcus aureus and, Escherichia coli, two gram-negative and positive fungi, Aspergillus fumigates and Candida albicans. The bacterial cultures were maintained on the nutrient agar media by sub-culturing them on fresh slants after every 4-6 weeks and incubating them at the appropriate temperature for 24 h. All stock cultures were stored at 5 °C. For the evaluation of antimicrobial activity of the synthesized complexes, a suspension of each test microorganism was prepared. The turbidity of each suspension was adjusted to 0.5 McFarland units by suspending the cultures in sterile distilled water. The size of final inoculums was adjusted to 6×120 CFU mL⁻¹. Volume of 20 mL of agar media was poured into each Petri plate and the plates were swabbed with broth cultures of the respective micro-organisms and kept for 15 min for adsorption to occur. Using a punch, ~8 mm diameter wells were bored in the seeded agar plates and a 100 μL volume of each test compound reconstituted in DMSO was added into the wells. DMSO was used as the control for all the test complexes. After holding the plates at room temperature for 2 h to allow diffusion of the compounds into the agar, the plates were incubated at 37 °C for 24 h. The antibacterial activity was determined by measuring the diameter of the inhibition zone. The entire tests were performed in triplicate and the mean of the diameter of inhibition was calculated. The antimicrobial activities of the complexes were compared against standard drugs.

Minimum inhibitory concentration (MIC)

Nutrient broth was adjusted to pH=7.0 for the determination of the MIC of synthesized Zn(II), Cd(II), Hg(II), and ZrO(IV) complexes. The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that prevents the development of visible growth of microorganism after overnight incubation. The inoculum of the test micro organisms were prepared using 20 h-old cultures adjusted by reference to the 0.8 McFarland standards (11 cells mL⁻¹). These cultures were further diluted up to 5-fold with nutrient broth to obtain an inoculum size of 1.5×10⁸ CFU mL⁻¹. A positive control (containing inoculum but no complex) and a negative control (containing complex but no inoculums) were also prepared. A stock solution of 5 mg mL⁻¹, of each compound was prepared in DMSO and further appropriately diluted to obtain final concentrations ranging from 100 to 3.0 μg mL⁻¹. The requisite quantity of antifungal drug (cyclohexamine) was added to the broth to obtain its desirable final concentration of 250 μg mL⁻¹. Separate flasks were taken for each test dilution. To each flask was added 100 μL of inoculum. Then the appropriately diluted test sample was added to each flask having broth and microbial inoculum. The contents of the flask were mixed and incubated for 36 h at 37 °C. The test bacterial cultures were spotted in a predefined pattern by aseptically transferring 10 μL of each bacterial culture onto the surface of solidified agar-agar plates and incubated at 37 °C for 24 h for determining the MIC value.
Table 1. The physical properties and elemental analysis of the prepared metal complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Color</th>
<th>M.p. oC*</th>
<th>Molecular Formula</th>
<th>C, % Calcd. (found)</th>
<th>H, % Calcd. (found)</th>
<th>N, % Calcd. (found)</th>
<th>Cl, % Calcd. (found)</th>
<th>M, % Calcd. (found)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ZrOL][Cl]</td>
<td>Pale</td>
<td>370</td>
<td>C₂H₃ClN₂O₂Zr</td>
<td>53.14</td>
<td>2.65</td>
<td>15.77</td>
<td>12.32</td>
<td>13.9</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[ZnLCl₂]</td>
<td>Red</td>
<td>329⁰</td>
<td>C₂H₃Cl₂N₂O₂Zn</td>
<td>47.62</td>
<td>2.67</td>
<td>18.37</td>
<td>13.29</td>
<td>12.25</td>
</tr>
<tr>
<td>[HgLCl₂]</td>
<td>Brown</td>
<td>356⁰</td>
<td>C₂H₃Cl₂N₂O₂Hg</td>
<td>37.11</td>
<td>2.26</td>
<td>14.66</td>
<td>10.60</td>
<td>29.29</td>
</tr>
<tr>
<td>[CdLCl₂]</td>
<td>Pale</td>
<td>360⁰</td>
<td>C₂H₃Cl₂N₂O₂Cd</td>
<td>43.83</td>
<td>2.63</td>
<td>17.04</td>
<td>12.21</td>
<td>15.15</td>
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<td>yellow</td>
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</tbody>
</table>

*D*=decomposed.

Antifungal study

Potato dextrose medium (PDA) was prepared in a flask and sterilized. To check the growth of bacterial culture in the medium, the requisite quantity of the standard antibiotic (ampicillin) was added to obtain the desired final concentration of 100 μg mL⁻¹ of the medium. Test samples were prepared in different concentrations (10, 50 and 100 μg mL⁻¹) in dimethyl sulphoxide and 200 μL of each sample was spread on the PDA media contained in sterilized Petri plates.

Mycelial discs taken from the standard cultures of fungi (Aspergillus fumigatus and Candida albicans) were grown on the PDA medium for 5-7 days. These cultures were used for the aseptic inoculation in the sterilized Petri dish. Standard cultures inoculated at 28±1 °C were also used as the control.

The efficacy of each sample was determined by measuring the radial mycelial growth. The radial growth of the colony was measured in two directions at right angle to each other and the average of two replicates was recorded in each case. The data are expressed as percent inhibition over the control obtained from the size of colonies. The percent inhibition (φ) was calculated using the formula:

\[
\varphi = 100 \frac{C-T}{C} \tag{1}
\]

where

C is the diameter of fungus colony in the control plate after incubation for 96 h and

T is the diameter of the fungus colony in the tested plate after the same incubation period.¹⁰

Results and discussion

The analytical and physical data of template metal complexes are listed in Table 1. The percent’s of C, H, N and M content obtained from elemental and analyse are in good agreement with the general molecular formula proposed for the complexes [MLCl₂] (M: Cd(II), Zn(II) and Hg(II)) and [ZrOL][Cl₂], where L = acyclic N₂O₂ organic segment formed by template route between 2,6-diaminopyridine and 1,4-dihydroquinolin-2,3-dione, as shown in Figure 3.

IR spectra

The IR spectra of acrylic metal template complexes has shown the absence of uncondensed functional groups –NH₂ and C=O of 2,6-diaminopyridine and quinoxalin-2,3-dione (i.e., stretching modes of starting materials) which confirm the formation of the proposed acyclic organic moiety involving imine –C=N- and –C=O groups¹³,¹⁴ (Table 2). The appearance of strong absorption band in the region 1600-1630 cm⁻¹ corresponds to νC=O stretching frequency. A medium absorption recorded in the regions 3300-3250 cm⁻¹ may be ascribed to νO=H in 1 and 4 positions of pyrazine ring, thus confirms the lactam form corresponding to quinoxaline moiety.¹⁴,¹⁵ Furthermore, no strong absorption band was observed near 1675-1660 cm⁻¹ indicating the absence of >C=O of quinoxalin-2,3-dione at 2-position, this confirms the condensation of carbonyl group of quinoxaline and amino groups of 2,6-diaminopyridine.¹²,¹⁴ These results provide strong evidence for the formation of acyclic frame.¹³ A strong absorption band in the region ~1585-1613 cm⁻¹ may be attributed to the C≡N group.¹⁴,¹⁵ The lower values in the wave numbers of ν(C≡N) may be explained on the basis of drift of lone pair density of imine nitrogen towards metal atoms.¹⁵,¹⁷ The bands present at ~3050-2966 cm⁻¹ may be assigned due to ν(C-H) vibrations of pyrazine and pyridine moieties. The bands present in the range ~1150-1130 cm⁻¹ are assigned due to ν(C-N) vibration. The IR spectra of the complexes do not show any change in the pyridine ring vibrations and interestingly enough, it appears that in these complexes pyridine nitrogen does not take part in coordination.¹⁷,¹⁸ Thus, in the presence of metal salts, a quadridentate acyclic system is formed which coordinates through imine nitrogen and two oxygen atoms of –C=O positionized in 3 position of lactam quinoxaline of while pyridine nitrogen does not take part in coordination. Moreover, the coordination through pyridine nitrogen does not take place, as it will result in the formation of unstable four member rings.¹⁴,¹⁵

The far IR spectra show bands in the region ~550-490 and 433-460 cm⁻¹ corresponding to νm-N and νm-O vibrations, respectively.¹⁸ As well as the tautomer’s of in position 2 of quinoxaline ring between C=O and C≡N- supports the coordination of organic moiety with appearance of strong bands at 1680-1640 cm⁻¹. Thus, the acyclic tetradentate ligand behaves as N₂O₂ system. Furthermore, the strong band at 880 cm⁻¹ in the spectrum of zirconium(IV) complex may be assigned to Zr=O vibration.¹⁹ The Zn(II), Cd(II) and Hg(II) displayed weak absorptions in the regions 260-380 cm⁻¹ which confirms the M-Cl bond.¹⁹

NMR spectra

The 1H NMR spectrum of mercury(II) complex shows a multiple signals observed at δ 6.6-8.5 ppm that may be attributed to Ar-H and quinoxaline protons (Fig. 1.) The multiple absorption in the region 9.1-12.5 ppm may be assigned to the spin resonance of the deshielded lactam-NH protons that are affected strongly by the electronic withdrawing of Hg(II) ion (Figure 2). The chemical shifts in the region 4.5-5.3 ppm confirms the tautomerism of –CN=N imine group with C=N of pyrazine ring, thus support the expected structure of the Hg(II) complex. Moreover, the 13C NMR spectrum of Hg(II) complex displays resonances at 40, 110, 130, 150 and 170-200 ppm that are attributed to CH3(DMSO), C=–C(Ar), C=N, and C=O groups, respectively.

The data obtained from 1H and 13C NMR spectra for Hg(II) complex in DMSO-d₆ solvent were shown as below: 13C NMR (300 MHz, DMSO-d₆, δ, ppm): 38-40(s, J = 7.2 Hz, DMSO), 155.0 (2C, C=O), 131.0 (1C, Ar–C), 128.1 (2C, N=C), 126.3 (2C, N-C), 128-126 (4C, Ar–C), 110-123(4C, C=N-Pyridine) 62.3 (1C, Py–C), 63.2 (1C, C=CH pyridine).

Electronic spectra and molar conductivity

The quinoxaline-2,3-dione solution in ethanol shows high intensity peaks at 31446 and 36100 cm⁻¹ that related to electronic transitions of C=N–, C=O and C=C– groups of the types (π→π* and n→π*). The electronic spectra of the complexes were recorded in DMF solutions. The pale yellow solution of Zn(II) complex in DMF exhibits two distinct peak in the region 34448 and 27777 cm⁻¹ that are consistent with ligand field and ligand to metal charge transfer transitions, respectively. The UV-visible spectra of Zn(II), Cd(II) and Hg(II) complexes show high intensity peaks at 22880-34448 due to M→L charge transfer transitions, respectively. The electronic spectrum of the zirconium(IV) complex exhibits bands at 20660 and 30288 cm⁻¹ which are assignable to charge transfer of Zr=O moiety and ligand field transitions, respectively. The geometry of the zirconium(IV) complex was proved to be square pyramidal based on the charge transfer bands of Zr=O in the UV region besides the 1:2 electrolyte behaviour.

The molar conductance of Zn(II), Cd(II) and Hg(II) complexes in DMF were recorded in the range 10-22 ohm⁻¹ cm² mol⁻¹ indicating that these complexes were neutral and agree well with the other spectral and elemental analyses. However, the zirconium(IV) complex solution in DMF shows electrolytic behavior in 1:2 ratio of molar conductance 175 ohm⁻¹ cm² mol⁻¹ due to the presence of two chloride ions in the outer sphere of the complex, Table 3.
Antimicrobial studies

In this study, all the chemically synthesized complexes were evaluated against gram-positive and gram-negative bacteria. The MIC values of the synthetic complexes were determined by the method given by Andrews. Standard antibiotic, namely streptomycin was used for comparison with the antibacterial activities exhibited by these complexes. All the complexes of the tested series possessed some antibacterial activity against gram-positive bacteria as well as Gram-negative bacteria, Table 4. In the whole series, complexes Zn(II) was found to be most effective against all the tested bacterial strains, showing zone of growth inhibition at 70 mm. The complexes of Cd(II) and Hg(II) exhibited good activity against all the tested bacterial strains with a zone of inhibition in the range from 33.2-49.44 mm and 23.03-29.96 mm, respectively. The ZrO(IV) complex showed activity in the region (22-33 mm) against E. coli and S. aurea, while its inhibition toward Candida fungus was 44 mm. The complexes of Zn(II) and Cd(II) showed the highest zone of inhibition 60-70 and 21-49.2 mm, respectively 36.70 mm against Staphylococcus aurea and Escherichia coli (Table 4). This would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedy’s chelation theory. The chelating will enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layers of the cell membrane and blocking the metal binding sites on enzymes of microorganisms.

Conclusion

According to the results obtained from elemental analyses, FT-IR, UV-Visible spectra, magnetic and molar conductivity measurements, the octahedral magnetic geometry was proposed for Zn(II), Cd(II) and Hg(II) complexes with formula [MLCl₂], while the zirconyl complex was suggested as square-pyramidal geometry of the formula [ZrOLCl₂]. The data obtained from infrared spectra revealed that nitrogen atoms of imine groups and two oxygen atoms of carbonyl participate in coordinating with the central metal ions. As well as, this study is a preliminary evaluation of antibacterial activity of acyclic metal complexes against two types of bacteria and fungi. The quinoxaline system is very active biologically. It indicates that the complexes have the potential to generate new antimicrobial metabolites. The acyclic metal complexes demonstrating antibacterial activity could result in the discovery of new chemical classes of antibiotics that could serve as selective agent for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases.

Table 4. Antimicrobial activity of the prepared metal complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>ZnLCl₂</td>
<td>70</td>
</tr>
<tr>
<td>CdLCl₂</td>
<td>49.2</td>
</tr>
<tr>
<td>HgLCl₂</td>
<td>29</td>
</tr>
<tr>
<td>ZrOLCl₂</td>
<td>22</td>
</tr>
<tr>
<td>Control DMSO</td>
<td>10</td>
</tr>
<tr>
<td>Streptomycine</td>
<td>25</td>
</tr>
</tbody>
</table>

Figure 3. Stereochemical structures of metal complexes.

References

Template synthesis of metal complexes from diaminopyridine and dihydroquinoxalin-2,3-dione

Section A - Research Paper


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