The inhibitory effect of some dinuclear metal complex on clinical isolates of gram positive and gram negative bacteria

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Accepted 30 August, 2013

Dithiocarbamates (DTC) are well known compounds that bind strongly and selectively to many metal ions. In the past few years, self-assembly directed by metal-dithiocarbamate coordination have emerged as a useful supramolecular methodology for the preparation of macrocycles, cages, catenanes, and nanoparticles. A series of transition metals complexes of some new dithiocarbamates complex have been synthesized and characterized. The complexes conform to the general formula: [M(L)]Y and [M`(LX)]Y where (M = Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), M`= Fe(III) and Cr(III); y = 2, X= Cl), L= 1,2-N,N`Bis ammonium dithiocarbamoyl ethan [(NH4)2Endtc] (L1), Bis ammonium dithiocarbamoyl Hydrazine [(NH4)2Hdtc] (L2), 1,4-N,N` Bis ammonium dithiocarbamoyl benzene [(NH4)2PhAdtc] (L3), and Bis ammonium dithiocarbamoyl benzedine [(NH4)2Bedtc] (L4), and dtc = dithiocarbamate. The antibacterial activities of synthesized compounds were studied against Gram-negative pathogen bacterial species (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Enterococcus faecalis) and Gram-positive pathogen bacterial species (Staphylococcus aureus) using the disc diffusion method. The results showed that all complexes exhibited prominent antimicrobial activity against the tested isolates.

Key words: Syntheses, dithiocarbamates, ligands, biological activity.

INTRODUCTION

In recent years, much attention have been paid to the synthesis, characterization and biological activities of various dithiocarbamate derivatives with sulfur ligands such as thiene or dithiocarbamate (Pellerito and Nagy, 2002; Saxena, 1989; Xanthopoulou et al., 2003). The dithiocarbamates (R2NCS2-) are half-amides of dithiocarboxylic acid and sulphur analogues of carbamates (R2NCO2-). The strong metal binding properties analogues of carbamates were directly related to the two donor sulfur atoms.

Dithiocarbamate complexes have a wide range of applications and they were amongst the most widely used organometallic compounds. The biological activity of metal dithiocarbamate compounds was well known owing to their practical applications as fungicides, bactericides, biocides and pesticides (Sharma et al., 2002). Moreover, one of the areas that have been intensively studied was the activity against cancer (Crowe, 1994; Gielen et al., 2005; Nath et al., 2001). Those with biologically active ligands have attracted more attention towards the design of potential antitumor agents (Barbaric et al., 2005; Raymond, 1990).

The structures of the dithiocarbamate complexes showed that the coordination environments around the central atom ranging from square planer, tetrahedral and octahedral with the dithiocarbamate groups can act as bidentate (Sharma et al., 2000).

SYNTHESIS OF THE LIGAND

Ethylene diamine of 0.1 mol was added to a solution of ammonium hydroxide (NH4OH) (0.2 mol) and ethanol (CH3CH2OH) (0.1 mol) after which the solution was stirred. The reaction mixture was cooled in an ice bath at 0°C then drops of carbon disulfide (CS2) (0.2 mol) were added with continuous stirring for 2 h. A yellowish precipitate was formed after completing the addition. The

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Table 1. Antibacterial activity (inhibition zone/mm) of dithiocarbamate complex (concentration $10^3$) against Gram positive and Gram negative pathogen isolates.

<table>
<thead>
<tr>
<th>Dithiocarbamate complexes</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
<th>K. pneumonia</th>
<th>E. faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr L1</td>
<td>--------</td>
<td>14 mm</td>
<td>20 mm</td>
<td>17 mm</td>
<td>------------</td>
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<tr>
<td>Zn L1</td>
<td>12 mm</td>
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<td>18 mm</td>
<td>13 mm</td>
<td>14 mm</td>
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<tr>
<td>Co L2</td>
<td>--------</td>
<td>15 mm</td>
<td>14 mm</td>
<td>15 mm</td>
<td>17 mm</td>
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<tr>
<td>Co L4</td>
<td>17 mm</td>
<td>20 mm</td>
<td>15 mm</td>
<td>14 mm</td>
<td>------------</td>
</tr>
<tr>
<td>Cd L2</td>
<td>13 mm</td>
<td>17 mm</td>
<td>22 mm</td>
<td>14 mm</td>
<td>------------</td>
</tr>
<tr>
<td>Fe L3</td>
<td>14 mm</td>
<td>18 mm</td>
<td>19 mm</td>
<td>18 mm</td>
<td>15 mm</td>
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<tr>
<td>Ni L2</td>
<td>--------</td>
<td>14 mm</td>
<td>14 mm</td>
<td>18 mm</td>
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<tr>
<td>Ni L1</td>
<td>14 mm</td>
<td>17 mm</td>
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<tr>
<td>Cu L2</td>
<td>17 mm</td>
<td>14 mm</td>
<td>14 mm</td>
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<tr>
<td>Mn L2</td>
<td>18 mm</td>
<td>18 mm</td>
<td>15 mm</td>
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<tr>
<td>Co L1</td>
<td>18 mm</td>
<td>18 mm</td>
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<td>14 mm</td>
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<td>14 mm</td>
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<tr>
<td>Zn L2</td>
<td>13 mm</td>
<td>15 mm</td>
<td>11 mm</td>
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<tr>
<td>Ni L3</td>
<td>--------</td>
<td>18 mm</td>
<td>18 mm</td>
<td>14 mm</td>
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<tr>
<td>Cu L5</td>
<td>12 mm</td>
<td>12 mm</td>
<td>17 mm</td>
<td>12 mm</td>
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</tbody>
</table>

Pipette was washed with 30 ml of diethyl ether (three portions) and dried under vacuum for 2 h. Similarly, Bis ammonium dithiocarbamoyl hydrazine ([NH$_4$)$_2$Hdtc] (L2), 1,4-N,N’ Bis ammonium dithiocarbamoyl benzene [(NH$_4$)$_2$PhAdtc] (L3), and Bis ammonium dithiocarbamoyl benzidine [(NH$_4$)$_2$Bedtc] (L4), were prepared (Desai et al., 2006).

Synthesis of the transition metals dithiocarbamate complexes [M(L)$_2$] and [M'(L)X]$_2$:

The dithiocarbamate complexes [M(L)$_2$] and [M'(L)X]$_2$, where M = Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II); M’ = Fe(III), Cr(III); L= (ligands; L1,L2,L3, and L4); X= Cl. NiCl$_2.6$H$_2$O (0.01 mol), were dissolved in distilled water (5 ml), then added to a solution of L1 (0.01 mol) dissolved in 15 ml water:ethanol mixture (1:1). The resulting mixture was stirred for 2 h. The precipitated form was filtered off, washed with ethanol and water and then with 30 ml diethyl ether (three portions), after which it was dried under vacuum for 2 h. Similarly, complexes were prepared by the method of Desai et al. (2006) by using the stochometric quantities of all metal salts.

Antibacterial activity

Antibacterial activity of all prepared dithiocarbamate complexes were tested against five clinical isolates (isolated from Azadi hospital in Duhok city) of Gram-negative species (E. coli, P. aeruginosa, K. pneumonia and E. faecalis) and Gram-positive species (S. aureus) using National Committee for Clinical Laboratory Standards (Franklin et al., 2012) method with slight modifications:

1. A small portion of isolated single colony of each clinical isolate was inoculated into 5 ml of brain heart infusion broth and incubated over night (o/n) at 37°C.
2. Bacterial suspension was diluted with sterile normal saline up to $10^3$ (with optical density of 0.1 at wavelength of 450 nm using spectrophotometer) and this was used as inoculums.
3. Muller-Hinton agar plates were inoculated by dipping a sterile cotton swap into inoculums and streaked all over the surface of the Muller-Hinton agar plate three times, rotating the plates through an angle of 60°. Finally, the swap passed around the edges of the agar surface, and then the plates were left to dry for few minutes at room temperature.
4. Filter paper discs (diameter, 5 mm) were saturated with different concentrations of dithiocarbamate complex solution and then placed on the inoculated plates using sterile forceps. Discs were readily placed at 30-36 mm distance to avoid overlapping of inhibition zones. The plates were incubated (o/n) at 37°C.
5. After incubation, the diameter of each inhibition zone was measured.

RESULTS AND DISCUSSION

The antibacterial activity of a solution of synthesized compounds in dithiocarbamate complexes was tested against Gram positive and gram negative pathogen isolates species including E. coli, P. aeruginosa, S. aureus, K. pneumonia and E. faecalis. The activity of the compounds was evaluated by measuring the diameter of inhibition zone around the respective discs in the concentrations (con. $10^3$, $10^4$ ml). The results are presented in Tables 1 and 2. All dithiocarbamate
complexes have potent activity against Gram positive than Gram negative bacteria. In this study, these compounds were screened for their antimicrobial activity against various microbes in the hope of finding a new antimicrobial agent. Although antimicrobial activity was highly dependent on different compounds' structure, concentration and type of microbe, all synthesized compounds showed significance antimicrobial activity. Also, the best results between the dithiocarbamate complexes were obtained between Cu2 10 with con.10⁻³ and Cd L₂ with con.10⁻⁻. All of the synthesized compounds were found to have remarkable bacterial activity. However, Gram positive bacteria were remarkably more resistant than Gram negative bacteria as shown in Tables 1 and 2.

The minimum inhibition zone was 9 mm (Cu2 10 with con. 10⁻³) against E. faecalis bacteria while the maximum inhibition zone was 22 mm (Cd L₂ with con. 10⁻⁻) against S. aureus bacteria.

All dithiocarbamate complexes showed more potent activity against Gram positive than Gram negative bacteria. The reason could be ascribed to the presence of the outer phospholipidic membrane carrying the structural lipopolysaccaride in Gram negative bacteria. The Gram positive bacteria should be more susceptible having only one outer peptidoglycan layer which is not an effective permeability barrier (Nostro et al., 2000).

REFERENCES


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