Study the Effect of Some New Synthesis Cysteine Complex on Clinical Bacterial and Fungal Isolates

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Abstract

Synthesis, characterization, and biological activity of a new (cysteine) complexes, the complex are consistent with the formula \([\text{M}_2\text{ClSn(C}_6\text{H}_{12}\text{N}_2\text{O}_4\text{S}_2)_2]\). The IR spectroscopy show coordination of the ligand to M(II) through four N atoms in a square planar geometry. The dinuclear complexes of Mn²⁺, Co²⁺, Ni²⁺, Cu²⁺ and Zn²⁺ were prepared by the reaction of the above ligands with the metal chloride in (1:2) metal to ligands ratio. The ligands and their complexes have been characterized by their analytical, spectral data, conductivity and magnetic measurements. Electronic spectra and magnetic measurements indicates that the dinuclear have square planar geometry. Final solutions of the complex against Bacterial and Fungicides showed a pronounced activity of the complex even at low concentrations at \(10^{-3}\) m in ethanol solvent. The antibacterial activities of synthesized compounds were studied against Gram-negative pathogen bacterial species (Gram-negative species *Pseudomonas aeregenosa*, *Escherichia coli* and *Klebsiella pneumonia*). Gram positive species *Staphylococcus aureus* and *Streptococcus pyogenes*. The antifungal activity of synthesized compounds were studied against some species of Candida *C. tropicalis*, *C. albicans*, *C. dubicans*, *C. krusei* and *C. glabrata* using the disc diffusion method. The results showed that all complexes exhibited prominent antimicrobial activity against the tested isolates measuring inhibition zones at several values in mm.

Keywords
Cysteine, Ligands, Antifungal, Antibacterial

1. Introduction

Interactions between transition metal and amino acids are very interesting in the biological applications. Complexes of some metals ions with amino acids can be used as models to study the pharmacodynamics effects of drugs or for increasing the biocompatibility and minimize toxic effects of some metal in. (Nagham S. Buttrus, et., al. 2014).

Cysteine residues are known to perform essential functions within proteins, including binding to various metal ions. In particular, cysteine residues can display high affinity toward zinc ions (Zn²⁺), and this resulting Zn²⁺-cysteine complexes are critical mediators of protein structure, catalysis and regulation. Recent advances in both experimental and theoretical platforms have accelerated the identification and functional characterization of Zn²⁺-bound cysteine. Zn²⁺-cysteine complexes have been observed across diverse protein classes and are known to facilitate a variety of cellular processes. Here, we highlight the structural characteristics and diverse functional roles of Zn²⁺-cysteine complexes in proteins and describe structural, computational and chemical proteomic (Nicholas J. Pace et.,al. 2014).

Zinc ions (Zn²⁺) have the ability to be chelated to cysteine residues within protein scaffolds. This resulting Zn²⁺-cysteine complexes...
complexes participate in a variety of functional roles, including structural, catalytic, regulatory and transport. Regulatory mechanisms consist of inhibitory, redox-switches, and protein-interface stabilization (Andreini et al., 2006; Lee and Lim, 2008).

Principle of Antimicrobial Activity:
The terms antimicrobial, antibiotic, and anti-infective encompass a wide variety of pharmaceutical agents that include antibacterial, antifungal, antiviral, and antiparasitic drugs. Of these, antibacterial agents are by far the most commonly used and thus are the focus of this article, although similar principles apply to the other agents as well. Evidence-based practice guidelines from the Infectious Diseases Society of America can help direct appropriate therapy for specific infectious disease syndromes as well as for infections caused by specific microorganisms. These guidelines should be applied in the context of host characteristics, response to therapy, and cost of therapy. It is critical to isolate the specific pathogen in many serious, life-threatening infections, especially for situations that are likely to require prolonged therapy (eg, endocarditis, septic arthritis, disk space infection, and meningitis). Similarly, when a patient does not benefit from antimicrobial therapy chosen on the basis of clinical presentation, additional investigations are needed to determine the etiologic agent or exclude noninfectious diagnoses (Leekha et al., 2011). Infectious disease diagnoses also frequently rely on a detailed exposure history, as in the case of a patient with nonresolving pneumonia who has resided in or traveled to the southwestern United States where coccidioidomycosis is endemic. Although the microbiological diagnosis is ideally based on data such as bacterial or fungal culture or serologic testing, frequently the “most likely” microbiologic etiology can be inferred from the clinical presentation. For example, cellulitis is most frequently assumed to be caused by streptococci or staphylococci, and antibacterial treatment can be administered in the absence of a positive culture. Similarly, community-acquired pneumonia that does not warrant hospitalization can also be treated empirically—with a macrolide or fluoroquinolone antibiotic—without performing specific diagnostic testing (Mandell et al., 2007). The frequent use of certain agents (or classes of antimicrobial agents) in a hospital or other health care setting can result in selection of organisms that are resistant to that particular antibiotic. For example, the increased use of fluoroquinolones during the past decade is thought to be, in part, responsible for the epidemic of a fluoroquinolone-resistant strain of C difficile (Pepin et al., 2005).

2. Material and Methods

2.1. Preparation of Ligand
In a 25 ml round bottom flask a solution of (0.002 mol) of Tin in Toluene solvent the mixture was refluxed for (24) h, till to dissolve all Tin. Then a clear solution of Cysteine in ethanol was added slowly (drop wise) to a solution in round bottom and the mixture was refluxed for (10-16) h, and the precipitate appeared, after cooling the precipitate filtered off, washed with ethanol and diethyl ether then dried under vacuum.

2.2. Preparation of the [M₂LCl₄] Complexes
M=Mn(II),Co(II),Ni(II), Cu(II) and Zn(II)
The ligand L was dissolved in 10 ml ethanol in a 100ml round bottom flask. A solution of (0.002 mol) of metal salt [MnCl₂.4H₂O (0.39g), CoCl₂.6H₂O (0.48g), NiCl₂.6H₂O (0.48g) or CuCl₂. 2H₂O(0.34g)] in 5 ml of ethanol continuous stirring at room temp. The mixture was refluxed for (4-5)h, and the precipitate appeared within 30 min, after cooling the precipitate filtered off, washed with ethanol and diethyl ether then dried under vacuum.

2.3. Antibacterial Activity
Gram-negative species Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumonia, Gram positive species Staphylococcus aureus and Streptococcus pyogenes were isolated from the clinical samples obtained from patients attending Government Hospital Azadi, Kurdistan region. The organisms were isolated in nutrient agar medium and selectively cultured at 37°C for 24 h. The bacterial strains were identified by biochemical and standard antibiogram tests 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 chemicals compound 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.

2.4. Antifungal Activity Against Some Species of Candida

Fungal strains used Fungi were obtained and an examination of its sensitivity was carried out in Mycology Laboratory in the Biology Department, college of Sciences, University of Dohuk, Kurdistan region of Iraq and the species were as follows: C. tropicalis, C. albicans, C. dublicans, C. krusei and C. glabrata.

2.4.1. Preparation of Inoculums

The suspension of fungus was prepared as per Mac-Farland Nephelometer Standard. A 24 h old culture was used for the preparation of fungus suspension. A suspension of fungus was made in a sterile isotonic solution of sodium chloride and the turbidity was adjusted such that it contained approximately 1.5 × 106 cells / ml. It was obtained by adjusting the optical density (650 nm) equal to 1.175% barium chloride in 100 ml of 1% sulphuric acid.

2.4.2. Antifungal Susceptibility Test

Stock fungi were maintained at room temperature on Potato Dextrose Agar. Active fungi for experiments were prepared by seeding a loopful of fungi into Potato dextrose broth and incubated without agitation for 48 h at 25°C. The broth was diluted with Potato dextrose broth to achieve optical densities corresponding to 2.0 × 105 spore/ml for the fungal strains. The disc diffusion method was also used to screen for antifungal properties. In vitro antifungal activity was screened by using Potato Dextrose Agar (PDA). The PDA plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 10 min and 1 ml of the test culture was introduced into agar and allowed to spread while the excess was drained off. The plate was incubated at room temperature for 10 min. A sterile cork borer of 5 mm diameter was used to make two ditches (wells) on each plate and filled with 1 ml (200 mg) of the plants extract. The same was repeated for each fungus strain using the extract. These were carried out in triplicate for each fungus. The plates were incubated at 25°C for 96 h and the resulting zone of inhibition around the ditches were measured to the nearest millimeter along two axes and the mean of the two measurements was calculated. Each set of seeded plates were compared for confirmation. Control test was carried out using 10 mg/ml of fluconazole.

3. Result and Discussion

The antibacterial activities of the ethanol extracts from the test samples in terms of minimum inhibitory concentrations (MIC) and diameters of inhibition zones are reported in Table 1. The activity of the compounds was evaluated by measuring the diameter of inhibition zone around the respective discs, these complexes 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 complexes structure, concentration and type of microbe, all synthesized complexes showed significance antimicrobial activity. The result obtained showed that all species of bacteria are sensitive for all cysteine complexes in different rate, high level of inhibition growth show with cysteine ligand C4 Zn against Streptococcus pyogenes (22mm) where was the low level of inhibition growth show with cysteine ligand C5 ligand against Escherishia coli (10mm) All cysteine 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). Also the reason of inhibition growth of bacteria because the cysteine complexes are bind with bacteria and Inhibit bacteria secretion of certain enzymes like protease (Saharkhiz et al., 2012; Loimaranta et al., 2005).

Table 1. Antibacterial activity of cysteine complexes against Gram positive and Gram negative bacterial pathogen isolates.

<table>
<thead>
<tr>
<th>cysteine Ligand complexes</th>
<th>Pseudomonas aeruginosa G-</th>
<th>Escherichia coli G-</th>
<th>Klebsiella pneumonia G-</th>
<th>Staphylococcus aureus G+</th>
<th>Streptococcus pyogenes G+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co+L5</td>
<td>14 mm</td>
<td>13mm</td>
<td>14 mm</td>
<td>15 mm</td>
<td>18 mm</td>
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<tr>
<td>C3 Cu</td>
<td>14 mm</td>
<td>13mm</td>
<td>12mm</td>
<td>19 mm</td>
<td>17 mm</td>
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<tr>
<td>C4 Zn</td>
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<td>13mm</td>
<td>15 mm</td>
<td>17 mm</td>
<td>22 mm</td>
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<tr>
<td>C1 Ni</td>
<td>16mm</td>
<td>19mm</td>
<td>17 mm</td>
<td>21 mm</td>
<td>18 mm</td>
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<tr>
<td>C2 Co</td>
<td>17 mm</td>
<td>16mm</td>
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<td>16 mm</td>
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<td>C5 ligand</td>
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<td>10mm</td>
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</table>

mm = millimeter, G- = gram negative, G+ = gram positive, = resistant
Antifungal susceptibility test showed that all the fungi were effective against cysteine complexes with different zones of inhibition (Table 2). The results obtained from this work showed that cysteine complexes exhibit antifungal effects against Candida spp. Even at low concentrations, these species showed antifungal activity nearly equal to that of the commercial fungicide used as a positive control, suggestive of antimicrobial activity, or previous studies that have demonstrated antifungal properties using different kinds of extracts (Wilson et al., 1997; Zhu et al., 2005).

Table 2. Antifungal activity of cysteine complexes against some species of Candida.

<table>
<thead>
<tr>
<th>Cysteine complexes</th>
<th>Candida tropicalis</th>
<th>Candida glabrata</th>
<th>Candida dubliicans</th>
<th>Candida albicans</th>
<th>Candida krusei</th>
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<tbody>
<tr>
<td>Co+L5</td>
<td>18mm</td>
<td>14mm</td>
<td>-----------------</td>
<td>12mm</td>
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<tr>
<td>C3 Cu</td>
<td>16mm</td>
<td>11mm</td>
<td>15mm</td>
<td>14mm</td>
<td>11mm</td>
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<tr>
<td>C4 Zn</td>
<td>19mm</td>
<td>20mm</td>
<td>17mm</td>
<td>10mm</td>
<td>18mm</td>
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<tr>
<td>C1 Ni</td>
<td>13mm</td>
<td>19mm</td>
<td>18mm</td>
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<td>C2 Co</td>
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<td>18mm</td>
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<tr>
<td>C1 ligand</td>
<td>13mm</td>
<td>12mm</td>
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<tr>
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<td>8mm</td>
<td>12mm</td>
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4. Conclusion

The results of this study indicated that the cysteine complexes have ability to inhibition growth of both kinds of microorganisms (Bacteria and Fungi) in different degree of inhibitor according to the species (fungi or bacteria), structure of the microorganisms cell wall, mode of resistance to the low condition growth and also show that all prepared cysteine complexes have ability to inhibitor compared with the control antibiotic (fluconazole).

References


