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Original Article

Synthesis and Antimicrobial Activities of Copper (I) Thiourea and Silver (I) Thiourea

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ABSTRACT

Complexes of copper (1) thiourea and silver (1) thiourea were synthesized from copper sulphate, silver nitrate and thiourea for the purpose of investigating its antimicrobial activities. The antimicrobial potentials of these complexes were tested with *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis and Candida albicans*. The minimum inhibitory concentration was determined for the metal complexes which ranges from 24.5-34mm. While that of the antibiotics ranges from 13.5-34.5mm. The complexes tested were effective against these groups of microorganism. The compounds showed a promising antimicrobial activity when compared with three antibiotics standards: Chloramphenicol, Amoxicillin and Nystatin used as positive control. These complexes have shown potential of being broad spectrum antibacterial and antifungal in nature.

Keywords: Copper (1) thiourea, Silver (1) thiourea, Antibiotics, Microbes.

INTRODUCTION

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection[13-15, 17]. In developing countries, synthetic drugs are not adequate even though they could be expensive for the treatment of diseases, therefore, there is need to search new infection-fighting strategies to control microbial infections.

The aim of this study was to evaluate the antimicrobial activity of copper (1) thiourea and silver (1) thiourea for treatment of manifestations caused by micro-organisms.

Transition metal complexes have shown a unique antimicrobial properties. They have been employed as antimalarial, anti-inflammatory, antitumor, antibiotic, antimicrobial agents etc[5]. More importantly, these metal complexes usually showed significant biological activities when compared to their non-complexed counterparts[1]. These suggest that the compounds with biological interests conjugated to transition metal might have some improved or different biological activities.

Saed *et al*, 200[12] has established that derivatives of thiourea complexes has the capabilities of forming a stable structural complexes. Several coordinated complexes in which the ligand is thiourea have been reported[11]. In the thiourea complexes, some are coordinated either through the sulphur or nitrogen atom of the thiourea molecules[11] while sulphur atom is exclusively involved in the coordination in the complex[11].

Thiourea and its derivatives is found to form a varieties of complexes of different symmetries with transition metal ions. These complexes assume tetrahedral, octahedral or tetragonal geometries with various degrees of distortion in some cases (Khan, 1999). Pd (II) and Pt (II) complexes has basically fourfold coordination, with a strong tendency to form penta-coordinated adducts[10].

Yuan *et al*, 2001, observed that oxygen, nitrogen and sulphur donor atoms of thiourea complexes provide a multitude of bonding sites. The ligands and their metal complexes also has a wide range of biological activities like, antibacterial, antifungal, anti-thyroid and plant growth regulator properties[18]. These thiourea complexes shows the ligand to metal charge transfer (LMCT) by an electron migrating from ligand to metal and metal to ligand (MLCT) in addition to π-π^* conjugation. Some metals with d^{10} configuration, such as zinc, cadmium and mercury readily combine with thiourea forming a stable compound with high optical nonlinearity and good physiochemical behavior[6].

Structure elucidation of Cu-thionein has shown Cu-thiourea a model for the Cu-thiolate chromophore. Ligands such as thiourea and thiocyanate with S and N donors are capable of combining with metal to form stable complexes via coordinated bonds.

Thiourea and substituted thioureas form complexes with most of the transition metal ions in the first period. These complexes are generally labile in solution and often partly solvolyzed. They therefore constitute a suitable series for systematic study of the kinetics and mechanisms of rapid ligand exchange reactions. These latter complexes are uniformly tetrahedral in non-aqueous solution[6].

Copper (I) complexes are usually obtained by direct interaction of ligands with a copper (II) compound. The stoichiometries of the compound give little clue to their structure which may be mononuclear, bridging, and poly-nuclear[9].

Cu (I) has no d-d transition, its complexes are usually colourless, but some were found to be red or orange because of charge transfer transitions[9]. The stereo-chemistry of Cu (I) thiourea and other substituted thioureas complexes are capable of interacting, not because of their structures but because of similarities of metallothioneines[2].

Silver (I) complexes

Silver is a white, lustrous, soft, and malleable metal with a melting point of 961^{o}C and has a high known electrical and thermal conductivity. It is chemically less reactive than copper, except towards sulphur and hydrogen sulphide, which rapidly blacken silver surfaces[3]. Silver has a single s electron outside a completed d shell, despite the similarity in electronic structure and ionization potentials there are few similarities between copper and silver and there is no explanation for the differences[3]. Apart from obviously similar stoichiometries of compounds in the same oxidation state (which do not always have the same structure), there are some resemblance within the group[3].

Studies on the thiourea complexes of silver (I) and copper (I) show they can both act as bridging and terminal ligands[12].

MATERIALS AND METHODS

Preparation of copper (I) thiourea

4.0g of thiourea was weighed and dissolved in 25ml of hot water in 250ml beaker. The solution was cooled to approx. 30^{0}C. To the solution was added 20ml of solution containing 4.0g of copper sulphate drop wise with constant stirring. The mixture was cooled in ice water until yellowish oil that adheres to the side of the beaker separated out. The mother liquor was separated out. The oil was cooled in ice water with constant stirring while adding a solution of 2.0g thiourea in 20ml of water until the oil crystallized completely.

The crystals were filtered and washed with 4ml of ice water[7]. The crude crystals were re-crystallized with 5% aqueous solution of thiourea in 0.02M H_2SO_4. The solution was filtered using filter paper, and the filtrate was cooled to room temperature for 2-3hrs. The crystals produced were again collected by filtering through a filter paper, washed with 6ml of ice water, and dried in a desiccators over silica gel[7].

$16\ Cu^{2+}(aq) + 8(H_2N)_2CS(aq) + 16H_2O(i) \longrightarrow 16Cu^{+}(aq) + S_8(s) + 16NH_4^{+}(aq) + 8CO_2(g)$

$Cu^{+}(aq) + 3(H_2N)_2CS(aq) \longrightarrow [Cu\{(H_2N)_2CS\}_3]^{+}(aq)$

Preparation of silver (I) thiourea

Silver nitrate (4.0) was weighed and dissolved in 40ml of distilled water. This solution was poured into a solution containing 4g of thiourea dissolved in 40ml methanol. Ash coloured precipitate formed was filtered and re-crystallized with equal volume of thiourea then dried in the oven at 45 ^{o}C overnight[4].

$AgNO_3 + 3(NH_2)_2CS \xrightarrow{\text{methanol}} [Ag(NH_2)_2CS)_3]NO_3$

Antimicrobial sensitivity test

Agar well diffusion method was used. The laminar flow station was swabbed with cotton wool soaked in 70 % ethanol, all glass wares, media and other materials pre-sterilised following manufacturer's instruction, after which the hood was switched on to ensure aseptic techniques was maintained throughout this work. The test organisms used were isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis and Candida albicans*.

Pure cultures of these test isolates were obtained from the Microbiology bank of the Department of Biotechnology Advanced Laboratory of Sheda Science and Technology Complex (SHESTCO).

Nutrient agar was used for the repeated sub culturing and preservation of the isolates, while Mueller Hinton agar was used for the antimicrobial assay.

The inoculums from these isolates were standardized at 1x10^{6} cfu/ml comparing with turbidity standard (0.5 McFarland tube).

Mueller Hinton agar was poured into each sterile Petri plates; the plates slightly opened to allow dry surfaces and allowed to gel in the hood.

The test bacteria were seeded onto the Mueller-Hilton agar plate surface by streaking using a sterile cotton bud for each organism. The swab was streaked on the surface of the medium three times, rotating the plate through an angle of 60^o after each application. Finally the swab was applied over the edge of the agar surface. The inoculation was allowed to dry for a few minutes with the lid closed.

The agar well diffusion method was used for the antimicrobial assay and this was done using a cork borer of 10mm diameter, to ditch the bore in plate, sterilized after each use by dipping in 70% ethanol and flaming with the Bunsen flame for each test organisms. Each hole was then sealed with 2 drops of molten medium to prevent the test solution sipping below the plate.

100ul of each organometallic complex (equivalent to 1 mg/well) were then applied to each of the holes using sterile tips attached to a micropipette. Antibiotics (Chloramphenicol, Amoxicillin and Nystatin) were used as the control. The set up was then incubated for 24-48 hours at 37^0C in the incubator. The zone of inhibition were then taken after the incubation period using a graduated ruler and recorded in millimeter (mm). All tests were carried out in duplicates[8].

RESULTS AND DISCCUSSIONS

See table 1.

The table above showed the complexes of copper (1) thiourea and silver (1) thiourea synthesized and the antimicrobial activity against all the tested organisms.

The minimum inhibitory zone for both copper (1) thiourea and silver (1) thiourea ranges between 22-34mm, while that of the know antibiotics ranges between 13.5 - 29mm. There was no inhibitory zone for chloramphenicol and amoxicillin against albican. Nystatine has no inhibitory zone for almost all the organisms used except albican with inhibitory zone of 25mm.

The agar well-diffusion method was used for the determination of inhibition zones and minimum inhibitory concentration (MIC). Broth culture (0.75 ml) containing ca. 10^6 colony forming units (cfu) per ml of the test strain was added to 75 ml of nutrient agar medium at 45 °C, mixed well, and then poured into a 14 cm diameter sterile Petri plate. The complexes formed at 1mg/ml showed a very significant antimicrobial activity against the microbes used. This result compared favourably with the antibiotics used: Chloramphenicol, Amoxicillin and Nystatin.

The nucleophilic character of surphur and nitrogen in between the complexes and the charge present in the molecules may have assisted in the penetration through the bacterial cell wall easily thereby arresting the growth[20]. It was also noted that increase in the polarity of the metal thiourea complexes can also help the molecules to interact or penetrate more easily through the cell membrane of microbes and thus deactivating them. It was also reported that some open chain thiourea derivatives have a good efficacy against microbial strains[20].

CONCLUSION

Copper (i) and silver (1) complexes of thiourea has shown potentials of being a broad spectrum of antibacterial and antifungal in nature, therefore the antimicrobial activity displayed is worth further investigation.

REFERENCES

1. Brownlee, J. M.; Johnson-Winters, K.; Harrison, D. H. T.; & Moran, G. R. (2004); *J. Biochemistry*, vol. 43, p 6370.
2. Buamger, R; (1995) *Thiourea Chemical Society (GDCh) Advisory Committee on Existing Chemicals of Environment Relevance* (Bua) Stuttgart, S. Hirzel Wisssenschaftliche Veragsgesellschaft (Bua Report 179).
3. Cotton, F. A. & Wilkinson, G (1980); *Advanced Inorganic Chemistry* Text Book. 4th Ed. p 799-804.
4. Dhandapani, M.; Kanhaswarny, & Srinivasan (2005); *Dept of Chemistry, Sri RamaKrishma Mission Vidyalaya, College of Arts and Science* 641, 020 tamil Nadu India.
5. Dimitrakopoulou, A.; Dendrinou-Samara, C.; Pantazaki, A. A.; Raptopoulou, C.; Terzis, A.; Samaras, E.; & Kessissoglou, D. (2007) ; *Inorg. Chim. Acta*, vol. 360, p 546.
6. Eaton, D. R. & Zaw, K; (2000); *Can J. Chemistry.* Vol. 49, p 3315.
7. Henrik, P. & Forlaget, k, (1999); Preparatory problems, 32nd *International Chemistry Olympiad*, LMFK – Sekretariate Slotsgade 2, 3 Dk-2200, Kobenhavn N.
8. Hugo, W.B. & Russell, A.B. (1987), Pharmaceutical Microbiology, 4th Ed., London: Blackwell scientific Publication; pp 265-70.
9. Jardine, F; (1975); *Advanced Inorganic Chemistry.* Vol. 17.
10. Narayana, B; & Gajendragad, M. R. (1997); *Tr. J. of Chemistry.* Vol. 21, pp 65-70.
11. Quagliano R; Teramoto, S; Kaneda, M; Aoyama, H; & Shiramu, Y. (1995); *J. Am. Chem. Soc*. Vol. 79 p 1575.
12. Saed Atimad *et al* (2001); *Transitional metal chemistry.* Vol. 27, pp 782-785, Kluwer Academic Publishers, Netherlands.
13. Gonzalez, C. E., Venzon, D., Lee, S., Mueller, B. U., Pizzo, P. A and Walsh, T. J. (1996). Risk factors for fungemia in children infected with human immunodeficiency virus: a case-control study. *Clin Infect Dis*. 23: 515-521.
14. Graybill, J. R. (1988). Systemic fungal infections: diagnosis and treatment. I. Therapeutic agents. *Infectious Disease Clinics of North America*. 805-825.
15. Ng, P. C. (1994). Systemic fungal infections in neonates. *Arch Dis of Childhood*. 71: F130-F135.
16. Sieradzki K, Wu SW and Tomasz A. (1999). Inactivation of the methicillin resistance gene mecA in vancomycin-resistant *Staphylococcus aureus*. *Micro. Drug Resist*. 5(4): 253-257.
17. Dean, D. A. and Burchard, K. W. (1996). Fungal infection in surgical patients. *Am J Surg*. 171: 374-382.
18. Yuan C, *et al*. (2001) Solution structures of two FHA1-phosphothreonine peptide complexes provide insight into the structural basis of the ligand specificity of FHA1 from yeast Rad53. *J Mol Biol.* 314(3):563-75.
19. Khan M. M. (1999), Ru (III), Pd (II), Pt (III), and Pt (IV) Complexes of a Novel 32- membered Unsymmetrical Dinu-cleating Macrocyclic Ligand, *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry*. 40:148–152.
20. Amita Mishra and Sanjay Batra, (2013) Thiourea and Guanidine derivatives antimalaria and antimicrobial agents. *Curr. Topics in Medicinal Chemistry*. 13(16)2011-25.

Table 1. Antimicrobial activity of the complexes

Complexes	Diameter of zone of inhibition (mm)				
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Candida albicans*
Copper (1) thiourea	34	33	25.5	32	24.5
Silver (1) thiourea	27	27	22	25	26
Chloramphenicol	28.5	34.5	19	29	-
Amoxicillin	14.5	32.5	24	13.5	-
Nystatin	-	-	-	-	25