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Evaluation of novel N/S containing heterocyclic metal complexes as biologically potent agents

Mathur Neha* **and Bargotya Sonlata**
Department of Chemistry, Pt. N.K.S. Govt. P.G. College, Dausa, Rajasthan, India
nehavmathur@yahoo.co.in

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Abstract

Present co-ordination chemistry provides a valuable tool to design aromatics which are promising for future researches. Current era finds an interest in improving the versions of previously reported aromatics. Hence, prominent activities seem to be a result of Co-ordinating behaviour between ligands and the concerned metal ion. In the thematic issue we synthesized transition metal complexes with N/S donor ligands. The structures of the obtained complexes were characterized by FT-IR, NMR, elemental analysis, ESR spectral studies, conductometric and magnetic moment measurements. The magnetic moments and electronic spectral studies suggests that the complex has distorted octahedral geometry with unpaired electron lying in $d_x^2 - {}_y^2$ *orbital giving* ${}^2B_{1g}$ *as the ground state. The synthesized metal complexes were successfully investigated for biological activities namely antibacterial, antifungal, DNA binding and cleavage activity and plant growth regulatory activity. Based upon their widest applicability performa, we report herein the synthesis and characterization of metal complexes and the pronounced biocidal activities of the novel complexes.*

Keywords: Antibacterial, Antifungal, DNA Cleavage, Phenylthiourea.

Introduction

We know that metal complexes play a crucial role to existence, growth and maintenance of entire organism. Nitrogen, oxygen, sulphur containing organic molecules sets to be key components in biological systems and no doubt received a considerable attention by co-ordination chemists. In such co-ordination complexes central metal ion chelating to related ligands remark different characteristic property. Therefore, the total characteristics of a complex are based on the nature of metal as well as nature of ligands and their structural aspects. The continued interest of chemists to proliferate this area of chemistry is not only due to structural novelties of these compounds but also because of their varied applications in related concerned areas.

In the above regards, aromatics containing nitrogen and sulphur donor atoms have attracted increasing interest owing to their role in understanding of biological processes occurring in nature. Interaction of these ligands with transition metal has been the subject of range of studies. A number these investigations have been involved in synthetic[1], kinetic[2], thermodynamic[3] and structural aspects[4] of complex formation with multiple metal ions. These ligands are considerably important due to their relations to bio-mimetic and catalytic systems and related applications. Thus, these macrocyclic ligands have practical approach in modern clinical techniques[5] such as biochemistry, medicinal, biochemical, bioinorganic, environmental, industrial, catalytic processes[6], drugs and cosmetics[7], photochemical, photophysical, photoelectronic[8-9] etc.

No doubt, complexes of copper ion shares important application in above said areas of research. The chelation of metal ion with nitrogen and sulphur donor moieties is worth mentioning. Such copper compounds are applicable in various fields such as antitubuler, anticonvulsant, analgesic and anti-inflammatory anti-protozoal, anti-helmintics, anti-HIV, anti-hepatic, antiulcer activities, antibacterial, anti-malarial, anti-allergic, antibiotic agents[10-16].

Materials and methods

All the chemicals used were of LR/AR grade. Substituted aniline used purchased from Merck and were used as received. Solvents were purified according to standard procedures. Thin layer chromatography was used to access the purity of the synthesized compounds. The IR spectra of the complexes were obtained as KBr discs in the range 400-4000cm^{-1} on Perkin Elmer spectrophotometer and ^{1}H NMR spectra were recorded at Therachem laboratories, Jaipur using DMSO d6 as reference. ESR spectra of the complexes were recorded at liquid nitrogen temperature, at IIT, Mumbai.

The synthesis of complexes can be summarized in three steps as follows[17-18]:

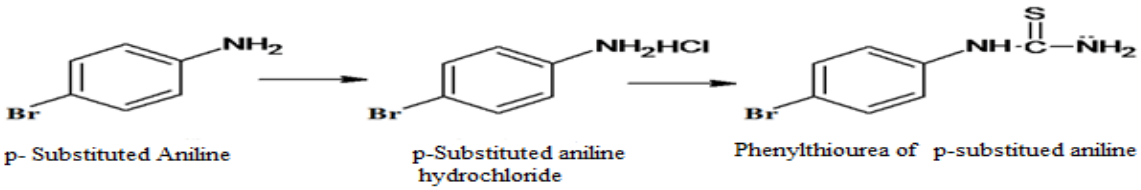
Synthesis of ligands: 0.1mole of p-bromo aniline was heated with a mixture of 9ml HCl and 25ml H_2O on water bath till aniline hydrochloride is formed. On cooling add NH_4SCN to it proceeding with reflux for four hours. Finally a solid separates out after cooling.

Synthesis of Copper Surfactants: Prepared the surfactants by mixing one gm of Palmitic acid/Caprylic acid into 25ml ethyl alcohol in hot water bath and add one drop of phenolphthalein. A saturated solution of KOH in another beaker was prepared then it was added into Palmitic acid/Caprylic acid solution drop by drop until the light pink color appears. Prepare a saturated solution of $CuSO_4$ (about 2-3gms in 5ml H_2O) and mix it into above solution with stirring till the blue colored soap is formed.

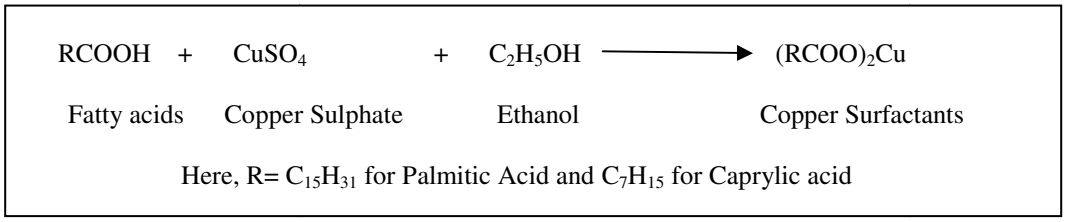
Preparation of Copper complexes: The complexes of soap surfactants were prepared by adding (0.001mole) copper palmitate/copper caprylate with (0.002mole) substituted benzothiazoles in 25-30ml ethyl alcohol and the mixtures were refluxed for about two hours with constant stirring.

Results and discussion

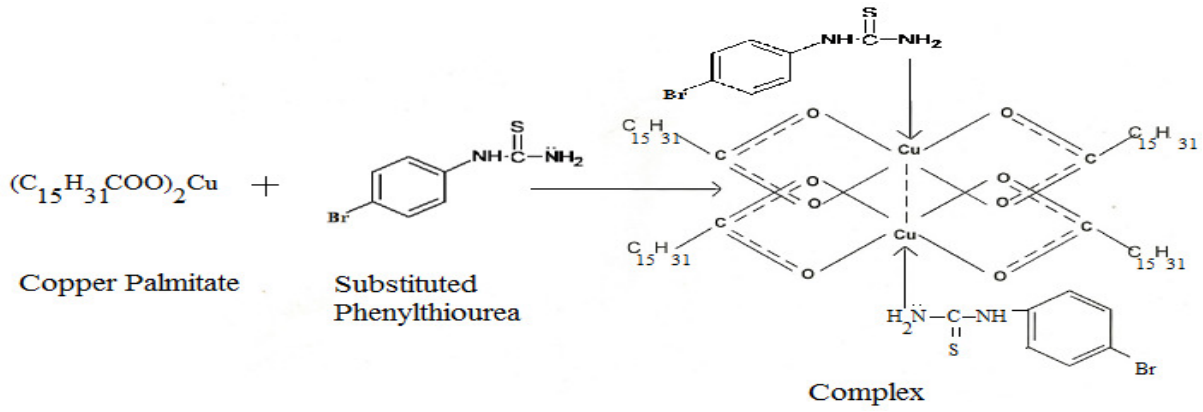
DNA cleavage analysis of synthesised compounds on bacterial DNA[19-20]: The study was conducted from 24h Old culture (primary culture) of *Lactobacillus acidophilus.* A nutrient broth was grown by Peptone-5g, Beef extract-3g, Sodium chloride-5g, distilled water-1000mL, pH-7.0 autoclaved at 1210˚C, 15 psi pressure and 15 min. ATCC 25922 culture of *Lactobacillus acidophilus* was used to inoculate the media. The primary culture was transferred to the fresh nutrient broth and a secondary culture was obtained by mixing 25mL of primary culture and 25mL of fresh medium. Cultures were incubated for 48h for DNA cleavage and after 24h of incubation the DNA was isolated. Agarose Gel Electrophoresis and visualization of cleavage pattern on UV transilluminator and the bands were visualized on UV transilluminator. After the cleavage of the DNA with the chemical treatment at 5mg/mL concentration the banding pattern was analyzed by agarose gel electrophoresis using 1.2% agrose gel. The cleavage pattern was detected after 48 h and 96 h of incubation; the bands were visualized on UV-transilluminator. DNA ladder was used 100-1000bp (100bp step up ladder, Merck).



Scheme-1: Synthesis of p–substituted phenylthiourea.



Scheme-2: Synthesis of Copper Surfactants.



Scheme-3: Synthesis of Complex.

Note: Complexes in the concerned studies are abbreviated as: $CP(PTU)_{Br}$: Complex of copper palmitate with p- bromo phenylthiorea, $CC(PTU)_{Br}$: Complex of copper caprylate with p- bromo phenylthiorea.

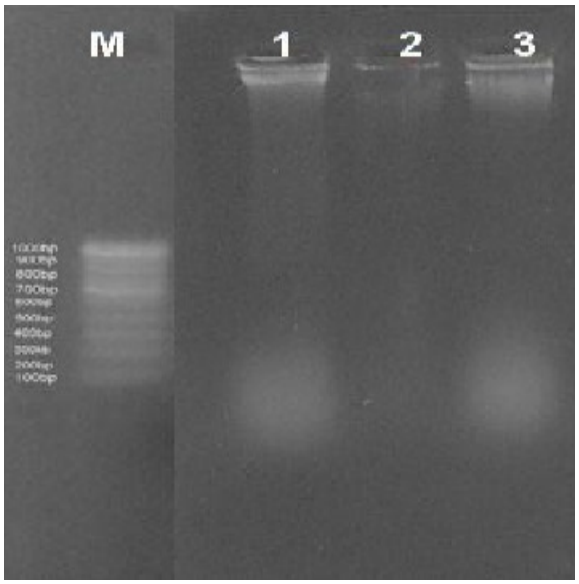


Figure-1: Image depicting the cleavage pattern of *Lactobacillus acidophilus* DNA.

In the present study, the DNA gel electrophoresis experiment was conducted using our synthesized complexes as oxidants. Control experiment using DNA alone does not show any significant cleavage of DNA even on longer exposure time. But on treatment of DNA with synthesised complexes, redox property of aforesaid compounds triggers oxidation of nucleic acids. Although the intermediates involved in the cleavage process are not clear. This possibly suggests that synthesized complexes inhibit the growth of pathogens by hindrance in genomic functioning. One of the affirmations that cannot be ignored is binding of DNA to complex molecules.

On comparing the DNA cleavage by both complexes we see that more effective cleavage is obtain by $CC(PTU)_{Br}$ than $CP(PTU)_{Br}$. Here, we see that Lane-I represents the untreated DNA. This DNA is not degraded throughout the lane. Lane-II consists of DNA treated with CC $(PTU)_{Br}$. Lane-III shows DNA cleavage pattern treated by $CP(PTU)_{Br}$. Visualizations predict a bit of smearing in DNA in Lane III. We see that here the cleavage is less effective than that of Lane-II. The gel electrophoresis images clearly reveal that the intensity of all the treated DNA samples has vanishes, possibly because of the disruption of the DNA. It shows that the Control DNA alone does not show any apparent cleavage whereas the synthesised complexes show a noticeable cleavage.

Antimicrobial Susceptibility Testing (Kirby-Bauer and Stokes' methods)[21-24]: i. Preparation of Mueller-Hinton agar medium (antibacterial screening) and Sabouraud Dextrose Agar medium (antifungal screening). ii. Preparation of positive control Streptomycin (5mg (w/v) for antibacterial and Itraconozole for antifungal, also negative control DMSO. iii. Antibacterial and Antifungal sensitivity testing by Kirby-Bauer and Stokes' methods.

Preparation of inoculum: Each culture to be tested should be streaked onto a non inhibitory agar medium to obtain isolated colonies. After incubation at 35°C overnight, select 4 or 5 well-isolated colonies with an inoculating needle or loop, and transfer the growth to a tube of sterile saline or non selective broth (Mueller-Hinton broth, Peptone water) and vortex thoroughly. The bacterial suspension should then be compared to the 0.5 McFarland standards. The turbidity standard should be agitated on a vortex mixer immediately prior to use. If the bacterial suspension does not appear to be the same density as the McFarland 0.5, the turbidity can be reduced by adding sterile saline or broth or increased by adding more bacterial growth.

Inoculation procedure: Within 15 minutes after adjusting the turbidity of the inoculums suspension, dip a sterile cotton swab into the suspension. Pressing firmly against the inside wall of the tube just above the fluid level, rotate the swab to remove excess liquid. Streak the swab over the entire surface of the medium three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculums. Finally, swab all around the edges of agar surface. The Mueller-Hinton plate should be swabbed over the entire surface of the medium three times, rotating the plate 60 degrees after each application.

Loading the plate with Positive, negative control and sample: i. 50µl of the antibiotic suspension was dispensed in the well labelled with C(control) to the plates as soon as possible, but no longer than 15minutes after inoculation. Diffusion of the drug in the well begins immediately. ii. 50µl of the sample (S) and 50µl of the reference (R, negative control) was dispensed in the well labelled with C (control) to the plates as soon as possible, but no longer than 15minutes after inoculation.

Recording and interpreting results: After the Loading of C, S, R on the plate, invert the plate and incubate at 35°C for 16 to 18 hours. After incubation, measure the diameter of the zones of complete inhibition (including the diameter of the well) and record it in millimetres. The distance from the colony(ies) closest to the well to the centre of the well should be measured and then doubled to obtain a diameter. The diameter of the outer clear zone should be recorded as well and an interpretation recorded for each diameter. The presence of colonies within a zone of inhibition may predict eventual resistance to that agent.

These vials were carefully sealed with cotton plug and sterilized by heating in oven. All the requirements used in dispensing were sterile. Sterile vials containing 100 disks, which absorbed all the solution. This dispensing was done in sterile hood/chamber that was already cleaned with methanol and exposed to U.V. light and blower; solutions were dispensed in the vials near the lighted sprit lamp kept in the hood. *Pseudomonas Aeruginosa* and *Lactobacillus Aciedophillus* are the two bacterial chosen for concerned study. Following figures depicts the chemical sensitivity of synthesized complexes against aforesaid bacteria.

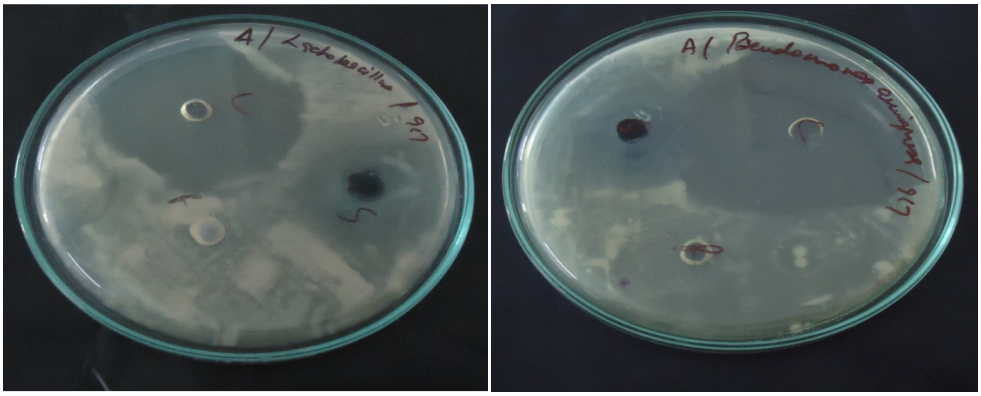


Figure-2: Image depicting Antibacterial sensitvity of $CP(PTU)_{Br}$ against bacteria under study.

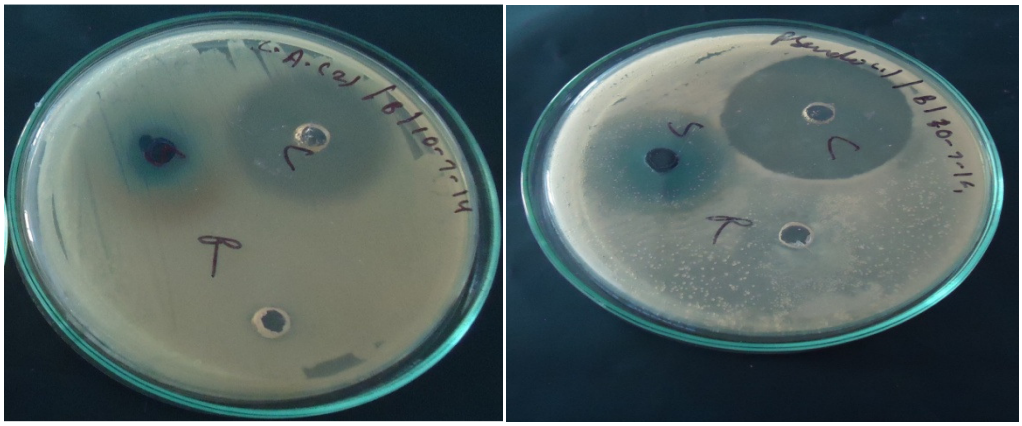


Figure-3: Image depicting Antibacterial sensitvity of $CC(PTU)_{Br}$ against bacteria under study.

Table-1: Antibacterial sensitivity of synthesized complexes against some bacteria.

Microorganism	Sample	Zone of Inhibition (in mm)	
		Sample	Streptomycin
Pseudomonas Aeuginosa	$CP(PTU)_{Br}$	48	28
	$CC(PTU)_{Br}$	51	31
Lactobacillus Aceidophillus	$CP(PTU)_{Br}$	33	25
	$CC(PTU)_{Br}$	39	25

The antimicrobial screening of the Complex $CP(PTU)_{Br}$ (namely Copper palmitate with p-bromo phenlythiourea) and $CC(PTU)_{Br}$ (namely Copper Caprylate with p-bromo phenylthiourea) were performed against two pathogenic bacteria *Pseudomonas aerugenosa* (gram negative), *lactobacillus acidophilus* (gram positive). As DMSO was used as a solvent, therefore it was necessary to screen the same for all organisms. The diameter of zone of inhibition induced by DMSO was reported 9 mm. The results of the investigated samples were summarized in Table-1.

The enhanced biocidal performance of the macrocyclic complexes can be evaluated on the basis of wide literature survey, encompassing Overtone's concept and Tweed's Chelation theory. Transition metal compounds bind multiple microbes or their metabolites through N O S donor. This may led to damage of cell wall or inhibit of cell wall synthesis alters alternation of permeability of cytoplasmic membrane, alternation of permeability of cytoplasmic membrane, alternation physical state of protein and nucleic acids and inhibition of enzyme action[20].

Conclusion

The newly synthesized copper (II) complexes have been tested on various biocidal fields namely DNA binding and Cleavage study and Antibacterial screening for pathogenic gram positive and gram negative bacteria such as Lactobacillus aciedophillus and Pseudomonas Aeruginosa. The results of these investigation show that the complexation increase the activity of the above metal complexes are more active than ligands. This complexation can be used for innovating promising prospective antibiotic agents against some known pathogenic organism and can be used as marketed drugs.

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