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Mixed ligand complex of monovalent copper with benzimidazole derivatives and alanine: Synthesis, characterization and antimicrobial studies

Poonam Yadav, Shobhana Sharma, Suman Kumari, Seema & Mamta Ranka*

Department of Chemistry, University of Rajasthan, Jaipur 302 004, Rajasthan, India

*E-mail: mmt31ran@gmail.com

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Mixed ligand complexes of transition metal ions exhibit broad range of biological activities. Schiff bases containing azomethine (C=N) group and amino acids are versatile compounds for design of ternary complexes. An attempt has been made to synthesize novel copper(I) complex by the reaction of two ligands, 2-N-(4-methyl phenyl ethanimine) benzimidazole [MPEIBI] as L_1 and an amino acid Alanine as L_2, and freshly prepared cuprous chloride solution in 1:1:1 molar ratio through conventional reflux method. The synthesized ligand and copper(I) complex are characterized by elemental analysis, molecular weight determination, magnetic moment measurement, spectroscopic techniques. These synthesized compounds were evaluated for antimicrobial studies against bacterial strains, *B. subtilis*, *E. coli* and fungal pathogens, *T. ressei*, *C. albicans*. The Cu(I) complex has shown remarkable antibacterial and antifungal activity.

Keywords: Mixed ligand complex, Azomethine, Benzimidazole, Alanine, Antibacterial, Antifungal

A novel concept of design of mixed ligand complexes of transition metal ions employing chelating ligands is far-reaching area of research as these are found to be biologically potent. Schiff based metal complexes are important and relevant compounds for current research in bioinorganic and medicinal chemistry[1]. Schiff bases are synthesized by condensation of primary amines with carbonyl compounds and form complexes with transition metals[2]. Benzimidazole based compounds are bioactive heterocyclic compounds[3]. Benzimidazole and its derivatives attracted more attention in the medical field due to their strong efficiency in biological and pharmacological areas[4]. Schiff bases of benzimidazole derivatives are also previously reported to possess a wide range of therapeutic and biological properties[5] such as; anticancer[6,7,8], anti-inflammatory[9], antiviral[10], antihypertensive[11], antioxidant[12], antifungal[13] activities etc. Amino acids are biologically significant compounds and have important biological applications[14]. Mixed ligand complexes play a significant role in biological process and exhibit higher antimicrobial activity than the free ligands because of chelation which decreases the polarity of the metal ions[15]. A brief literature summary on the medicinal and biological applications of Schiff based mixed ligand complexes have been recently reported[16]. Moreover, the azomethine functionality (-HC=N-) in the mentioned Schiff bases seems to be responsible for the various biological activities.

Materials and Methods

All the starting materials as well as solvents were of AR grade (purchased from Sigma Aldrich) and used without further purification. Elemental analysis of C, H, N and Cl were carried out by CHNX method. Molecular weights were estimated by Rast method. Cu metal was estimated gravimetrically. Melting points were recorded with open capillary tubes and are uncorrected. Reaction progress was monitored by thin layer chromatography (TLC) using silica gel-G plates and the purity of the synthesized compounds were checked by single spot TLC. Magnetic moment and molar conductance were measured by Gouy's Balance Model no: HO-ED-EM-08 and Systronics Direct Reading Conductivity Meter-304 using glass cell (cell constant =1.0 cm^{-1}) at room temperature, respectively. The spectra of ^{1}H-nuclear magnetic resonance (^{1}H NMR) was recorded on model Hitachi Perkin Elmer spectrometer using TMS as internal standard in DMSO-d_6 and FTIR spectra was recorded on SHIMADZU-JAPAN 8400 FTIR spectrophotometer in region 4000-400 cm^{-1} (using KBr pellets). Mass spectrum was recorded on TOF MS ES, mass spectrometer operating at an ionization potential of 70 eV.

Synthesis of Ligand and Complex

Synthesis of 2-N-(4-methyl phenyl ethanimine) benzimidazole [MPEIBI] (L_1)

Previously reported[4] 2-N-(4-methyl phenyl ethanimine) benzimidazole [MPEIBI] ligand was prepared using equimolar mixture of 10 mL of 4-methylacetophenone (1.34 g, 0.01 M) and 10 mL of 2-aminobenzimidazole (1.33 g, 0.01 M). This reaction mixture was refluxed for ~ 6 h on heating mental in presence of condensing agent and glacial acetic acid and monitored by TLC using Silica Gel-G plate. After completion of reaction, reaction mixture was cooled, dried and recrystallized with alcohol then dried in vacuum. We obtained pale yellow crystal with 55.4% yield (m.p. 247.3 ℃).

Elemental analysis: for $C_{16}H_{15}N_3$, calcd. (%): C 77, H 5.6, N 16.85; found (%): C 76.42, H 5.659, N 16.80. IR IR (υ, cm^{-1}): 1440 (C-C_{str}), 814 (C-C_{bend}), 1552 (C=N), 2960 (C-H_{str}).

Synthesis of complex [CuL_1L_2]

A fresh solution of CuCl (0.98 g, 0.01 M) was prepared in distilled water containing small amount of concentrated HCl. For complex formation 10 mL ethanolic solution of MPEIBI (2.49 g, 0.01 M) was mixed to 10 mL aqueous solution of L-alanine (0.89 g, 0.01 M) and then 10 mL aqueous acidic solution of CuCl is added slowly with constant stirring. In this reaction mixture, metal to ligands molar ratio M:L_1:L_2 is 1:1:1. This overall reaction mixture was stirred continuously but no precipitation was observed. The reaction mixture was refluxed for ~ 6 h and progress of reaction monitored by TLC. After completion of reaction, product was washed, recrystallized, dried and collected in vacuum. The reaction scheme is shown in Scheme 1. We obtained deep blue coloured product with 49.35% yield. (m.p. 180.4 ℃).

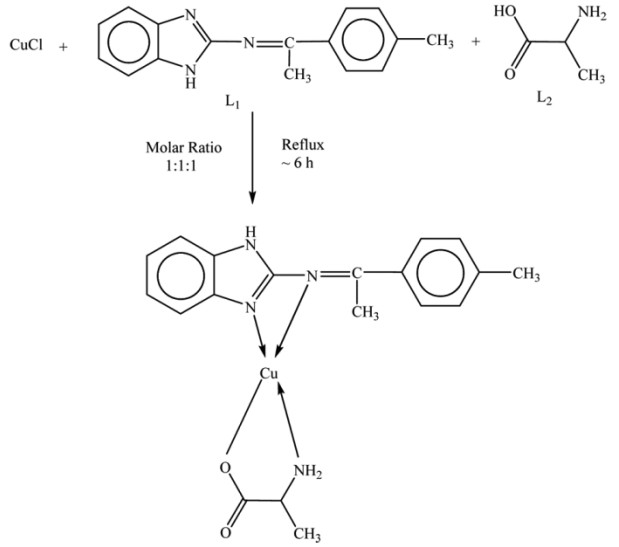
Elemental analysis: for $CuC_{19}H_{21}N_4O_2$, calcd. (%): C 56.91, H 5.27, N 13.97, Cu 15.85, , O 7.98; found (%): C 56.94, H 5.28, N 13.98, Cu 15.85, O 7.98. IR (υ, cm^{-1}): 1402 (C-C_{str}), 737 (C-C_{bend}), 1538 (C=N), 3076 (C-H_{str}), 1605 (C=O), 609 (M-O), 465 (M-N).

Antimicrobial studies

In antibacterial analysis, *in vitro* antibacterial activity of the test compounds was screened by agar well diffusion method against gram-positive bacterium *Bacillus subtilis* and gram-negative bacterium *Escherichia coli*. Mueller Hinton agar used as the bacteria growth medium. Ciprofloxacin was used as standard antibacterial drug. The test samples were diluted in dimethylsulphoxide (DMSO) at the concentrations of 1 mg/mL. The Mueller Hinton agar was melted and cooled to 48 – 50 ℃ and was poured on glass petriplates and was allowed to solidify. Standardized inoculum (1.5×108 CFU/mL, 0.5 Mc Farland) of the test organism was uniformly spread on the surface of these plates. Wells with diameter of 6 mm were prepared in the seeded agar plates. The test compound solution (20-80 µg/mL) was introduced in the well (6 mm). The agar plates were incubated overnight at 37 ℃ for 24 h. After incubation, clear zones were observed. The antibacterial activity was measured on the basis of diameter of bacterial growth inhibition zone (in mm). In antifungal analysis, *in vitro* antifungal activity of the synthesized compounds was investigated against two fungal species *Trichoderma reesei* and *Candida albicans* by agar well diffusion method. A sterile swab was used to distribute fungal culture evenly over the PDA agar medium and incubated at 37 ℃ for 24 h and 25 ℃ for 2-5 days, respectively. Ketoconazole used as standard antifungal drug. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106 cells/mL. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min before use in the test. The test compound solution (20-80 µg/mL) at desired concentration was added to the well. The plates were incubated at 37 ℃. After incubation of 2 days, the antifungal activities were determined by measuring the inhibition zones size (in mm).

Results and Discussion

Physicochemical analysis of complex showed that it is coloured and soluble in ethanol, DMF and



Scheme 1 — Proposed synthetic route for mixed ligand complex

DMSO. Molar conductance of complex measured in DMSO found to be ~11.37 Ω^{-1} cm^2 mol^{-1} indicates non-electrolytic nature of complex. Magnetic moment of complex found negligible which shows diamagnetic nature of complex.

In FTIR spectra, shifting of absorption band to lower frequencies confirms coordination of C=N to metal ion. Similarly, the characteristic carboxylate υ(C=O) absorption band at 1720 cm^{-1} for alanine shift towards lower frequency region in complex that appears at 1605 cm^{-1}. New absorption band appeared at 609 cm^{-1}, 647 cm^{-1} correspond to M-O band and 465 cm^{-1}, 424 cm^{-1} correspond to M-N band as shown in Fig. 1.

^{1}H NMR spectra of synthesized complex was recorded in solvent DMSO. We observed signals for Ar-H, CH_3 (azomethine), CH_3 (Ar-CH_3), NH_2 (amine), NH (cyclic), CH (alanine), CH_3 (alanine) at 6.5-7.0 (multiplet), 1.7, 2.5, 4.4, 8.3, 3.6, and 1.2 ppm, respectively, shows slightly lower field shift compare to ligand. (Fig. 2)

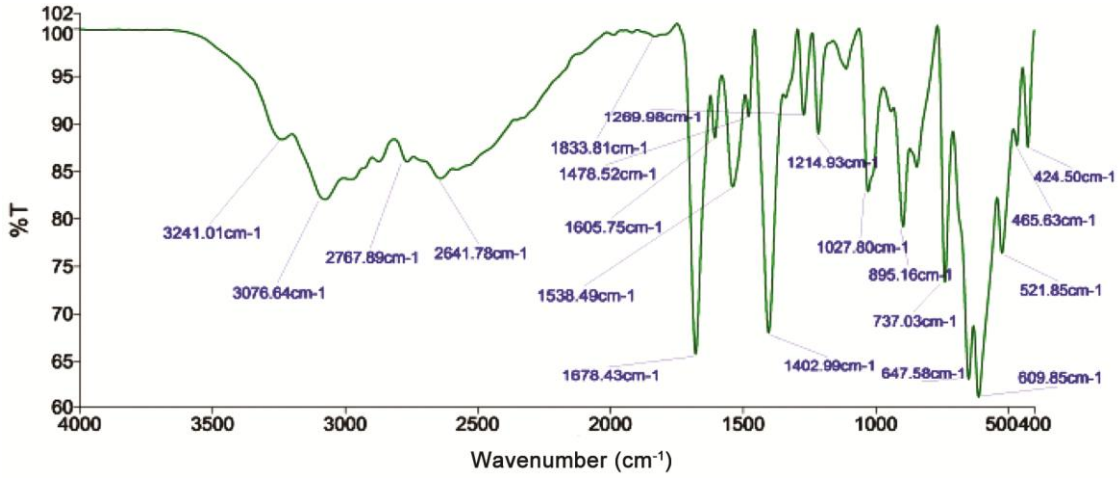


Fig. 1 — FTIR spectrum of complex

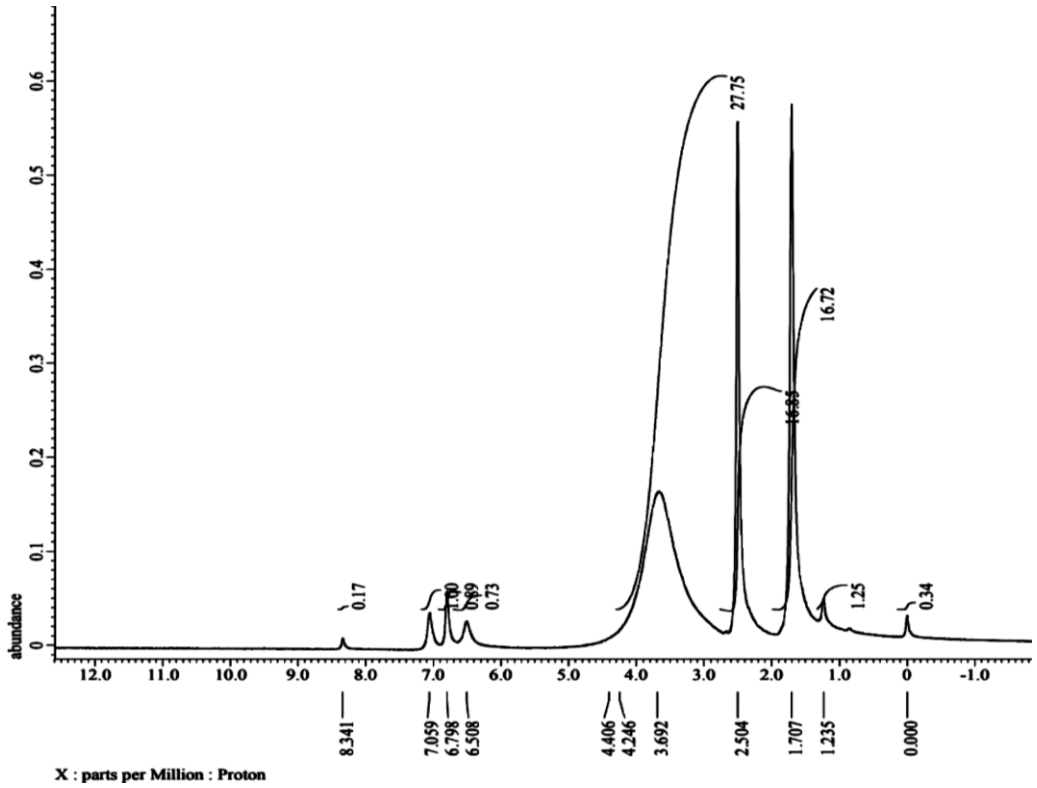


Fig. 2 — ^{1}H NMR spectrum of complex

Mass spectrum of synthesized complex shows the investigated metal chelates. The mass spectrum of the complex provided a good evidence for its molecular formula $CuC_{19}H_{21}N_4O_2$ (MW 401.94). The ESI-MS spectrum of synthesized mixed ligand complex ($CuC_{19}H_{21}N_4O_2$) shows the molecular ion peak at m/z 401.947 which is in good agreement with the calculated value for the molecular weight of the complex of 401.927 (Fig. 3). The found difference of about 0.02 between the observed and calculated molecular weight value is acceptable within the allowed experimental errors. In addition, other prominent peaks can also be observed in range m/z 85 to 402.

Antimicrobial activity results are tabulated in Table 1 and shown in Fig. 4. Antimicrobial evaluation of synthesized ligand and complex shows remarkable activity for selected bacterial and fungal strain, Results reveal that the complex is more effective against *E. coli* but lesser effective for *B. subtilis* as

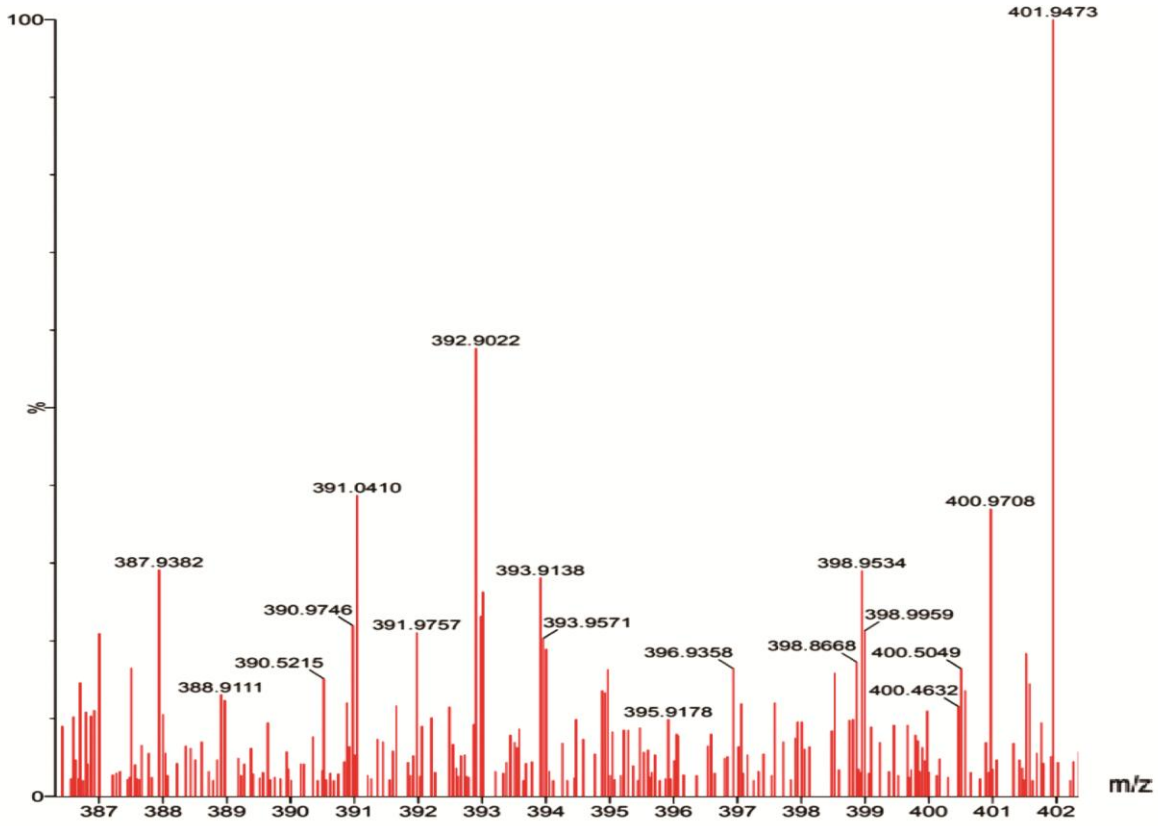


Fig. 3 — Mass spectrum of complex

Table 1 — Antimicrobial and antifungal activities of compounds

Compound	Conc. (µg/µL)	Zone of inhibition (mm)			
		Antibacterial activity		Antifungal activity	
		B. subtilis	*E.coli*	*T. reesei*	*C. albicans*
MPEIBI (L_1)	80	25	22	11	18
	60	19	16	9	15
	40	14	12	5	12
	20	12	10	4	11
CuL_1L_2	80	14	26	30	25
	60	12	24	28	22
	40	10	21	25	15
	20	9	18	23	12
Standard	40	52*	46*	39**	32**

*Ciprofloxacin, **Ketocanazole

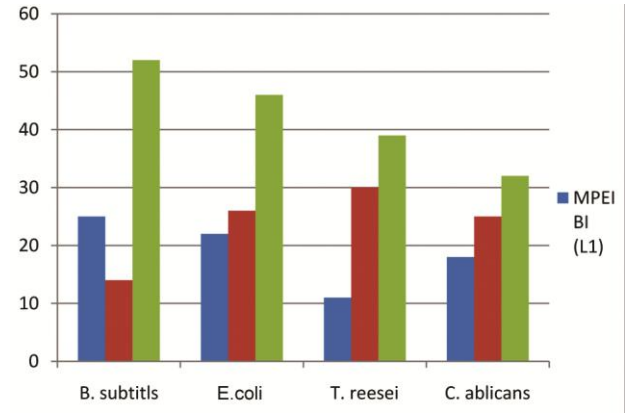


Fig. 4 — Bar diagram for antimicrobial studies of complex and ligand [L_1] showing maximum zone of inhibition

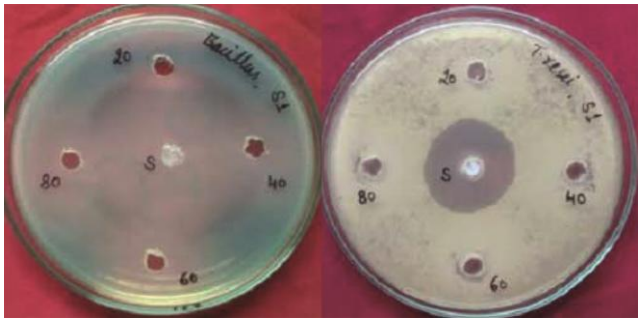


Fig. 5 — Antibacterial and antifungal study of ligand MPEIBI (L_1)

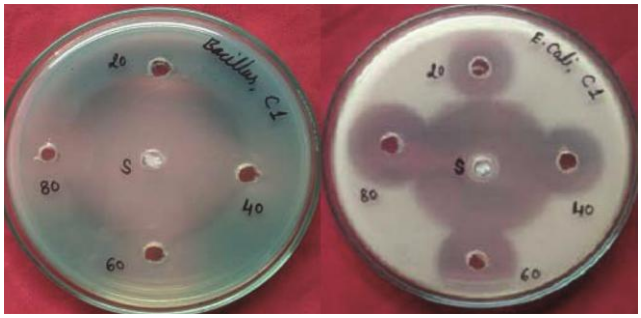


Fig. 6 — Antibacterial activity study of complex

compared to synthesised ligand. Moreover, the complex shows significant inhibition effect for fungal strain i.e., *T.reesei*, but less activity for *C. albicans.* The antimicrobial data shows that the complex has an excellent biological activity against *T. reesei* with 30 mm zone of inhibition at 80 µg/mL. The presence of clear zones shows that compounds are active. Overall, experimental results indicate that Cu(I) complex is more active towards most of the test microorganism than the ligand. Selected slides for antimicrobial studies of ligand and complex are shown in Fig. 5, 6 and 7.

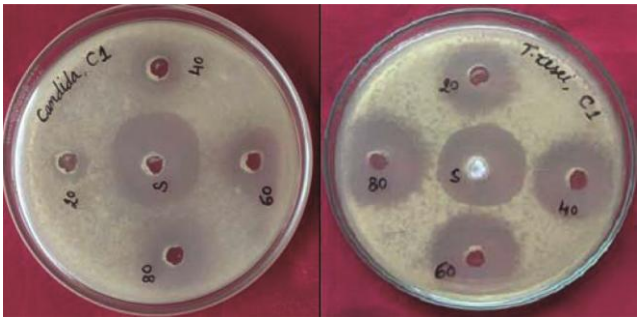


Fig. 7 — Antifungal activity study of complex

Conclusions

On the basis of physicochemical analysis and spectral studies, four coordinated geometry has been expected for synthesized mixed ligand complex where the synthesized ligand act as bidentate chelating agent and the amino acid as mono-ionic bidentate moiety. Tetrahedral geometry has been proposed for synthesized complex and low conductance supports the non-electrolytic nature of the complex with diamagnetic nature. On the basis of FTIR data, no water molecule coordinated to metal ion was observed. In addition, it is observed that mostly, the Cu(I) complex exhibit more distinct antimicrobial activity against selective the bacterial and fungal pathogens compared to the free ligand. The enhancement in the antimicrobial activity may be rationalized on the basis that Cu(I) complex possesses azomethine (C=N) bond and donor atoms (nitrogen and oxygen) exhibiting more penetration effect for microbes either by killing them or through blocking their active sites. Moreover, our research continues for recognising the pharmacophore responsible for efficacy of the compound/complex. We emphasize our present work to find more substituted analogues with remarkable biological activity.

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