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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF COPPER (II) COMPLEX WITH NSO-DONOR LIGAND

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ABSTRACT

A five coordinated Trigonal bipyramidal copper (II) complex formulated as [Cu(L) NClBrS(H_2O)] (1) (HL= 4-Bromo-2-[(2-thiophen-2-yl-ethylimino)-methyl]-phenol (BEP)) was synthesized and characterized by elemental, physico-chemical and spectroscopic methods. The interactions of copper (II) complex towards bovine serum albumin (BSA) were examined with the help of absorption and fluorescence spectroscopic tools. The ligand and copper complex-1 has been screened for antimicrobial activity by agar disk diffusion against two Gram-positive bacteria and one Gram-negative bacterium. The compound showed good antibacterial activity when compared with known antibiotic chloramphenicol.

Key words: Copper complex; NSO-donor ligand; BSA binding; antibacterial activity.

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INTRODUCTION

Investigations concerning the structural configuration and chemical properties of polynuclear transition metal compounds have aroused considerable interest mainly because of their implications for topics such as the nature of orbital interactions, electron transfer in redox processes, and biological electron transport chains *(*Saadeh, 2013). Transition metal complexes coordinated to tetradentate Schiff base ligands have been studied extensively(Solomon *et al.,* 2001), mainly because of their ease of preparation, flexibility and versatility in terms of chemical properties, geometry, coordination sites and ease of substitution. Among them, metal complexes of N,N-bis(salicylidene)-1,2-diiminoethane (salen) and derivatives have been widely investigated for their colour isomerism, conformational influences and oxidative catalysis *(Raman et al., 2001)*. Coordination chemistry of copper complexes of chelating ligands is of continuing interest in connection with their structures, spectral, and redox properties in general (Sarkar *et al.,* 2009), and from their relevance to copper-containing metalloproteinase (Nakao *et al.,* 1988). Copper (I) prefers tetrahedral geometry, while copper(II) complexes exhibit coordination number dependent structures, four-coordinate prefers square planar, five-coordinate is square pyramidal or trigonal bipyramidal and six-coordinate complexes are distorted octahedral.

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Redox nature of the complexes is important to control nuclease activity (Reichman *et al.,* 1954). In the present work, we simulated the interaction between copper complexes with BSA under physiological conditions by spectroscopic methods. The compound is strongly bound with protein, and then we investigate the antibacterial activity by agar disk diffusion method against two Gram-positive bacteria *(Bacillus cereus and Bacillus subtilis)* and one Gram-negative bacterium (*Vibrio parahaemolyticus)*.

MATERIALS AND METHODS

Materials and Physical measurements

All chemicals and reagents were obtained from commercial sources and used as received, unless otherwise stated. Solvents were distilled from an appropriate drying agent. The elemental (C, H, N) analyses were performed on a Perkin Elmer model 2400 elemental analyzer. Electronic absorption spectra were recorded on a SHIMADZU UV-1800 spectrophotometer. Copper analysis was carried out by Varian atomic absorption spectrophotometer (AAS) model-AA55B, GTA using graphite furnace. The fluorescence spectra were obtained in the Fluorimeter (Hitachi-2000). Electrochemical measurements were performed using computer-controlled CH-Instruments (Model No- CHI620D), All measurements were carried out under nitrogen environment at 298 K with reference to SCE electrode in dimethyl sulphoxide using [n-Bu_4N]ClO_4 as supporting electrolyte. The concentrations of BSA were determined from optical density measurements, using the

values of molar absorptivity of ε280 = 35 700 M^{-1} cm^{-1} for HSA. Stock solution of complex was prepared in DMF because of the lower solubility in water.

Preparation of the ligand (BEP)

The organic moiety, 4-Bromo-2-[(2-thiophen-2-yl-ethylimino)-methyl]-phenol (BEP) was synthesized by mixing an ethanolic solution of 5-bromo-2-hydroxy-benzaldehyde (0.82 g, 5.0 mmol) was added to thiophene 2-ethylamine (0.64 g, 5.0 mmol) in ethanol. The mixture was heated under reflux for 4 h when a deep yellow solution resulted. The mixture was cooled to room temperature and yellow precipitate was filtered off, washed with ethanol, and then dried in vacuum desiccators over P_4O_{10}. The purity was checked by FTIR, ^{1}HNMR and ^{13}CNMR study. $C_{13}H_{12}NOSBr$: Yield: 70 %, mp (^oC): 72 ± 2; Anal. Found: C, 55.32; H, 3.87; N, 4.51; Calc.: C, 55.16; H, 3.59; N, 4.73; IR (cm^{-1}): $\nu_{O-H,}$ 3448; $\nu_{C=C,}$ 2951; $\nu_{CH=N}$, 1628; $\nu_{C-S-C,}$ 696; ν_{C-Br}, 626; ^{1}H NMR (500 MHz, DMSO-d6): 13.46 (s, $1H_a$), 7.33 (dd, $1H_b$), 7.45 (dd, $1H_c$), 7.63 (d, $1H_d$), 8.49 (s, $1H_e$), 3.85 (t, $2H_f$), 3.21 (t, $2H_g$), 6.84 (d, $1H_h$), 6.95 (dd, $1H_i$), 6.94 (d, $1H_j$); ^{13}C NMR (125 MHz, DMSO-d6): 165.63, 160.55, 141.92, 135.24, 133.91, 127.41, 125.95, 124.73, 120.71, 119.54, 109.52, 60.00, 40.52, 40.36, 39.86, 39.52, 31.02.

Preparation of [Cu(L) NClBrS(H_2O)] (1)

To a 15 mL methanolic solution of HL (0.082 g), 10 mL methanolic solution of $CuCl_2{\cdot}2H_2O$ (0.05 g) was added drop wise under stirring condition. The stirring was continued for 1 h and then refluxed for 4 h. The product was collected by filtration and washing with cold methanol and water then dried in vacuo. The pure crystallized product was obtained from methanol.

[Cu(L) NClBr S(H_2O)]: Yield 80-85%; (**1**)

Complex **1**: $C_{13}H_{13}N_1S_1Cl_1Br_1H_2OCu$: Anal.Found; C,38.48; H, 2.96; N, 3.18; Cu, 14.46; Calc: C, 38.44; H, 2.94; N, 3.16; Cu, 14.38. IR (cm^{-1}): IR (cm^{-1}): $\nu_{C=C,}$ 2948; $\nu_{CH=N}$, 1626; $\nu_{C-S-C,}$ 692; ν_{C-Br}, 624; ν_{Cu-O}, 408, ν_{Cu-S}, 436; m.p. 248 ± 1^oC. Magnetic moment (μ, B.M.) : 1.78. Conductivity (Λo, ohm^{-1} cm^2 mol^{-1}) in acetonitrile: 145.

Antimicrobial Screening

The *in vitro* antibacterial activities of the test compounds were determined using a modified agar disc diffusion method *(Sheikh et al., 2004)*. The test compound and standard antibiotics are dissolved in DMSO solvent by using one gram negative bacterium *(Vibrio parahaemolyticus ATCC 17802)* and two gram positive pathogenic bacteria *Bacillus cereus (ATCC 14579)* and *Bacillus subtilis (ATCC 11774)*. The solution of BEP, copper complex-1 and standards were added to the agar plates and incubation of the plates was done at 37°C for 24 hours. Following which the zone of inhibition was measured, to compare the potency of test with that of standard (Senthil and Arunachalam, 2007).

RESULTS AND DISCUSSION

Synthesis and characterization

The Trigonal bipyramidal copper (II) complex was obtained in good yield by the reaction of NSO organic moiety with copper chloride in methanol medium at ambient temperature. The complex (Figure 1) is soluble in organic solvents including ethanol, methanol, acetonitrile, DMF, and DMSO. The conductivity in acetonitrile (Λo, ohm^{-1} cm^2 mol^{-1}) is 145 at 300 K, suggesting that the complex is a non-electrolyte in solution. At room temperature, the magnetic moment of complex is 1.78 B.M. corresponds to one unpaired electron, which indicates complex is distorted Trigonal bipyramidal geometry.

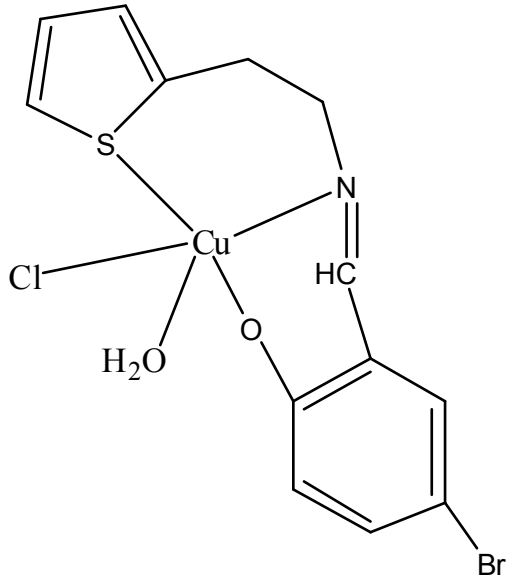

Figure 1. Probable structure of copper complex-1

Infrared and Electronic spectral studies

Infrared spectral data of the ligand, 4-Bromo-2-[(2-thiophen-2-yl-ethylimino)-methyl]-phenol (BEP) shows several bands at 3448, 1628 and 626 cm^{-1} due to O-H, CH=N and C-Br bond stretching vibrations in the solid state respectively (*Uneo et al., 1956*). These bands are shifted to lower frequency on complexation with $CuCl_2$, with 1:1 stoichiometry ratio. New vibrations at 436 cm^{-1} indicates Cu-S bond, which are not present in the free ligand. The appearance of these vibrations confirmed the Trigonal bipyramidal geometry of Copper ion (Summers *et al.,* 2009). The electronic spectra of BEP and its copper complex were recorded in acetonitrile at room temperature. The spectra of the BEP exhibit main peaks: at 298 nm, which was attributed to $\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ intra ligand transitions. The copper (II) complex absorption band observed at about 292, 318 nm is associated with $\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ and a band observed at the 410 nm is due to CT band, again 660 nm is well in agreement with the d–d transition for copper (II) in the Trigonal bipyramidal geometry.

Electrochemistry

The electrochemical studies of the copper (II) complex was examined by cyclic voltammetry using a Pt-disk working electrode and a Pt-wire auxiliary electrode in dimethylformamide using [*n*-Bu_4N]ClO_4 (0.1 M) as the supporting electrolyte shown in Figure 2. The complex exhibit a reduction peak at E_{pc} = 0.07 V with a corresponding oxidation peak at E_{pa} = 0.35 V at a scan rate interval 50–400 mV s^{-1} indicating quasi-reversible one-electron transfer process (*Mahadevan and Palaniandavar, 1998*). The ratio of cathodic to anodic peak height was less than one. However, the peak

current increases with the increase of the square root of scan rates.

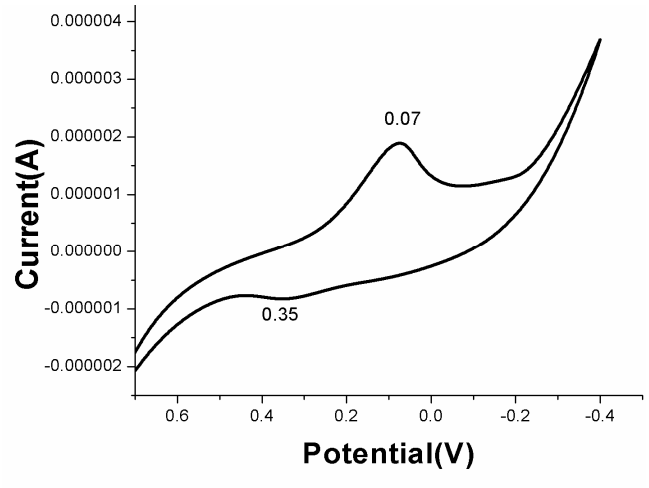

Figure 2. Cyclic voltammetry curve of Copper complex-1

BSA Protein binding experiments

Absorption characteristics of BSA–Cu(II) complex

The absorption spectra of BSA in the absence and presence of copper (II) complex was studied at different concentrations in phosphate buffer, pH 7.4 given in Figure 3.

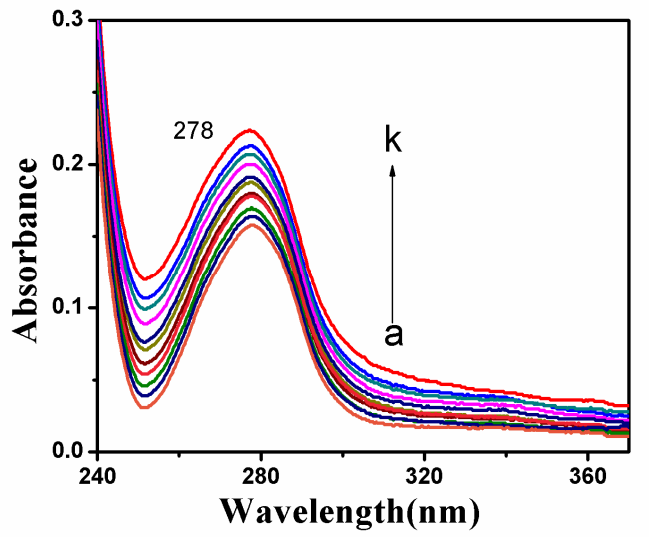

Figure 3. Electronic spectral titration of Cu complex with BSA at 278 nm in Phosphate buffer,

We observed that absorption of BSA increases frequently upon increasing the concentration of the complex may be due to the adsorption of BSA on the surface of the complex. From these data the apparent association constant (K_{app}) determined of the complexes with BSA has been determined using the Benesi-Hildebrand equation (Benesi *et al.,* 1949):

$$1/(A_{obs} – A_0) = 1/(A_c – A_0) + 1/ K_{app}(A_c – A_0)[comp]$$

Where, A_{obs} is the observed absorbance of the solution containing different concentrations of the complex at 280 nm, A_0 and Ac are the absorbances of BSA and the complex at 280 nm, respectively, with a concentration of complex and K_{app} represents the apparent association constant (Sulkowska *et al.,* 2002). The enhancement of absorbance at 280 nm was due to adsorption of the surface complex, based on the linear relationship between $1/(A_{obs} - A_0)$ *vs* reciprocal concentration of the complex with a slope equal to $1/K_{app}(A_c - A_0)$ and an intercept equal to $1/(A_c - A_0)$.The value of the apparent association constant (K_{app}) of BSA determined from this plot and the value is 3.62×10^4 M^{-1}(R = 0.9946). This result indicated that copper complex strongly bind to BSA protein.

Fluorescence quenching of BSA by the copper complex

The emission spectra of BSA in presence of different concentrations of complex were recorded in the wavelength range 300-550 nm by exciting the protein at 280 nm represented in Figure 4. As seen, with increasing the concentration of the copper complex the fluorescence intensities of the proteins are regularly decreased (*Peters, 1985*). Fluorescence quenching is described by the Stern–Volmer relation (Stern and Volmer., 1919).

$$F_0/F = 1 + K_{sv}[Q]$$

Where F_0 and F represent the fluorescence intensities in the absence and presence of quencher respectively. K_{sv} is a linear Stern–Volmer quenching constant, Q is the concentration of quencher. In case of fluorescence quenching of BSA a linear plot between I_0/I against [complex] was obtained (Figure 5) and from the slope we calculated the K_{SV} value is 4.82×10^5 M^{-1}(R = 0.99684).

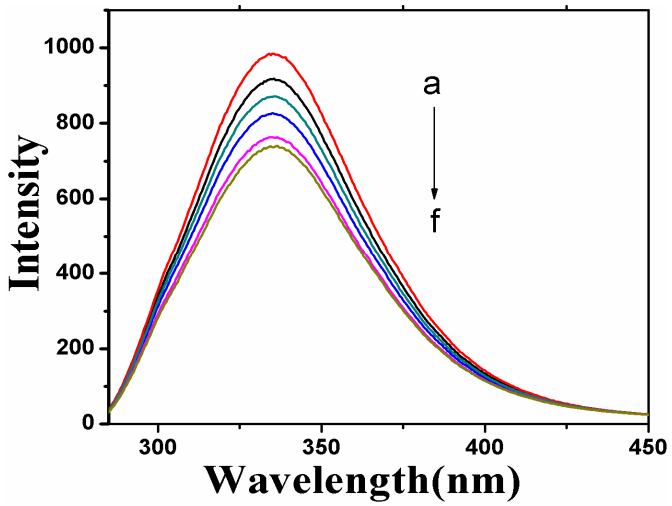

Figure 4. Change in fluorescence spectra of BSA through their titration with copper complex-1 in phosphate buffer. The concentration of complex varied from 0.0 to 4.8×10^{-6} M L^{-1}; λ_{ex} = 280 nm and pH 7.4.

Analysis of binding Sites

Number of binding sites can be calculated from fluorescence titration data using the following equation (Zhang *et al.,* 2010)

$$\log [(I_0 - I)/I] = \log K_b + n\log[Q]$$

According to the experimental results, the linear fitting plots of log $[(I_0 - I)/I]$ versus log[Q] can be observed. The number of binding sites (n) evaluated from the intercepts of the linear plots respectively, as seen, the value of n is nearly 1 for binding of both complexes to the proteins used, which indicates that, in the binding reactions the molar ratio of protein to drug is 1:1.

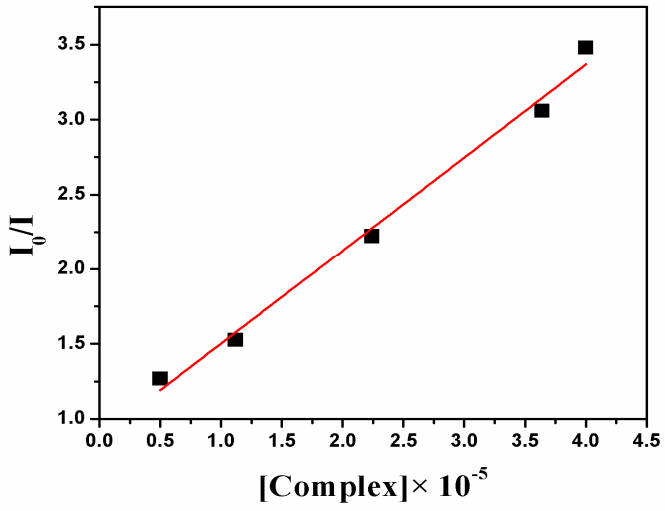

Figure 5. Plot of Io/I against [Complex] in fluorescence quenching of BSA in phosphate buffer

Antibacterial activity

Antibacterial activity of the BEP and its copper (II) complex was given in Figure 6. The *in vitro* studies result indicated that complex-1 compound exhibits highest activity than BEP ligand but lower activity than standard chloramphenicol. The increased activity of the copper complex can be explained by overtone concept and the Tweedy chelation theory (Raman *et al.,* 2010). The delocalization of π-electrons in complex is more than ligand and indicating the complex is more lipophilicity, which helps the penetration of the bacterial cell membranes (Patil *et al.,* 2010). For higher antibacterial activity, this copper compound also used in designing more potent antibacterial agents for therapeutic use.

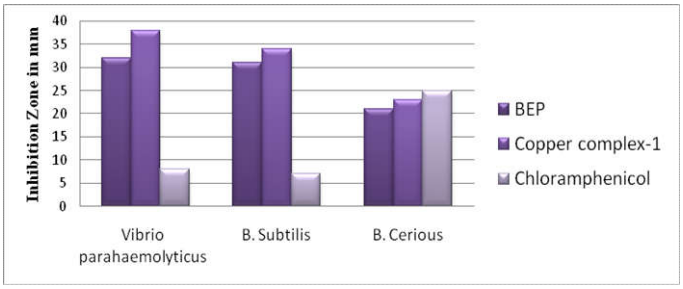

Figure 6. Comparison antibacterial studies of the investigated compound of BEP and copper complx-1 with standard antibiotics Chloramphenicol

Conclusion

Synthesis and characterization of mononuclear Trigonal bipyramidal copper (II) complex has been performed. The electrochemical study of the complex showed a quasi-reversible one-electron transfer process. BSA-binding properties of the copper (II) complex investigated by absorption and fluorescence spectroscopic tools. All the result indicated that complex is excellent binding with BSA protein. The antibacterial screening of ligand and its copper (II) complex displayed promising antibacterial activity compared to known antibiotic drug.

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