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Synthesis, molecular docking and DNA binding studies of phthalimide-based copper(II) complex: *In vitro* antibacterial, hemolytic and antioxidant assessment

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ABSTRACT

In the present research work, we prepared N-substituted phthalimide, 2-(-(2-(2-(2-(1,3-dioxoisoindoline-2-yl-ethylamino)ethylamino)ethyl)isoindoline-1,3-dione (DEEI) and its copper(II) complex. The ligand (DEEI) and its Cu(II) complex were structurally identified using absorption, FTIR, NMR, electron spin resonance, X-ray diffraction spectral studies, thermogravimetric and elemental analyses. The electronic spectrum and magnetic moment value proposed that Cu(II) complex has square planar geometry. The DNA interaction ability of the ligand (DEEI) and Cu(II) complex was studied by means of absorption and fluorescence spectrophotometer, viscosity measurements, cyclic voltammetery, and circular dichroism methods. The extent of DNA binding (K_b) with Calf thymus (Ct-DNA) follows the order of Cu(II) complex ($1.11 \times 10^6\ M^{-1}$) > DEEI ($1.0 \times 10^5\ M^{-1}$), indicating that Cu(II) complex interact with Ct-DNA through groove binding mode and more sturdily than ligand (DEEI). Interestingly, *in silico* predictions were corroborated with *in vitro* DNA binding studies. The antibacterial evaluation of these compounds was screened against a panel of bacterial strains *Pseudomonas aeruginosa* (MTCC 2453), *Salmonella enterica* (MTCC 3224), *Streptococcus pneumoniae* (MTCC 655), *Enterococcus faecalis* (MTCC 439), *Klebsiella pneumonia* and *Escherichia coli* (ATCC 25922). The results showed that the copper(II) complex has significant antibacterial potential ($\mathbf{IC_{50} = 0.0019\ \mu g/mL}$) against *Salmonella enteric* comparable with ligand (DEEI) and standard drug ciprofloxacin. Growth curve study of Cu(II) complex against only three bacterial strains *S. enterica*, *E. faecalis* and *S. pneumoniae* showed its bactericidal nature. Cu(II) complex showed less than 2% hemolysis on human RBCs indicating its non toxic nature. The results of antioxidant assay demonstrated that scavenging activity of Cu(II) complex is higher as compared to ligand and ascorbic acid as standard.

1. Introduction

In past decades, coordination compounds have attracted much interest because of their diverse application in the field of bio-inorganic, biomedical and pharmaceuticals [1]. Transition metal complexes of *N-N*-tetracarboxydiethyloxamide have been reported in the literature as antimicrobial agents [2]. Nickel (II), copper(II) and zinc(II) complexes of ligand possessing phthalimide moiety have been synthesized and characterized [3]. A number phthalimide derivatives have been reported to possess antioxidant activities [4,5]. Several transition metal complexes with phthalimide ligands have been investigated for antioxidant activity. Beside their importance in the medicinal, various compounds containing phthalimide group have also been used potentially in the field of catalysis and material science [6]. Phthalimides possess planar aromatic ring and hydrophobicity, therefore interaction of these drugs with different biologically active targets constitutes the bottom for the evaluation of their biological activity. Phthalimide moiety is present in various chemotherapeutic agents include thalidomide and *N*-(3,5-dimethyl-4-isoxazolylmethyl)phthalimide

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(DIMP) [7]. It is noteworthy that the glutarimide ring of the thalidomide is not essential for bioactivity. In addition, various *N*-phenyl phthalimides reported to possess enhanced activity over thalidomide and due to this reason various thalidomide derivatives have been synthesized after the modification in glutarimide ring [8]. Numerous chemotherapeutic agents possessing thalidomide moiety, have been studied for their potent anticancer [9], antimicrobial [10,11], anti-inflammatory [12,13], antimalarial [14], antiproliferative, analgesic, antidepressant and insecticidal activities.

Several types of biological drugs such as antiviral, anticancer, and antitumor can easily target nucleic acids and can bind to DNA by intercalative and non-intercalative mode of interaction [15]. A great number of DNA-targeting drugs have been reported as approved anticancer drugs. The efficacy of these drugs could be credited to their DNA-interacting capabilities. Hence, investigation of DNA-drug interaction paid more consideration in cancer chemotherapy [16–22]. Reactive oxygen species such as free radical that can attack on biological polymers like DNA, RNA, proteins, lipids and cell membranes, leading to the development of oxidative stress which inturn causes some degenerative and chronic diseases [23].

Molecular docking study is a computational technique fascinated significant interest to researchers in the field of medicinal chemistry [24]. This method of approach is most frequently used in drug design and discovery to predict binding pattern and strength of small molecules with their targets. Biofilms are assemblies of microorganisms on biotic or abiotic surface surrounded by a polymer matrix. Since they offer protection for bacteria from antibiotics, disinfectants, an extensive research have been devoted recently for the discovery of new biofilm inhibitors.

Herein, we describe the synthesis and characterization of copper(II) complex of *N*-substituted phthalimide ligand. The DNA binding capabilities of synthesized compounds were investigated by absorption, fluorescence, electrochemical, circular dichroism and hydrodynamic studies. The antibacterial activity of the ligand (DEEI) and its Cu(II) complex was investigated against a panel of bacterial strains. The toxicity of Cu(II) complex was determined on human red blood cells (hRBCs) by hemolytic assay. Furthermore scavenging capacity against 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) and hydrogen peroxide were also carried out and the results were compared with the standard ascorbic acid.

2. Experimental

2.1. Materials and methods

All the reagents were purchased from Sigma Aldrich Chemicals Pvt. Ltd and solvents were of spectroscopic grade used as such without further purification. Yield percent was of purified product and was not optimized. Melting points were recorded in open capillary tubes and which are uncorrected. The thermogravimetric analysis was carried out using a TGA Exstar instrument in the temperature range of 25–800 °C with 10 °C/min heating rate and 50 mL/min air flow rate. The elemental analyses were obtained on a Vario MICRO Elementar analyzer. Magnetic moment was measured by magnetic susceptibility balance, Sherwood scientific Cambridge U. K. at room temperature. Electronic spectra of the compounds were recorded by using Perkin Elmer Lamda 40 UV–Visible spectrophotometer and Perkin Elmer Spectrum RXI IR Instrument was used to record the IR spectra of the compound using KBr pellets in the range of 4000–400 cm^{-1}. To record the ^{1}H NMR spectra, Bruker DPX-300 NMR spectrometer was used operating at 300 MHz and DMSO-d_6 used as solvent and TMS as internal standard and ^{13}C NMR spectra were recorded on Agilent-NMR-vnmrs 500 spectrophotometer. Cyclic voltammetric measurements were performed by using DY2312 potentiostat and CD spectra were accomplished on a Jasco J-815 spectropolarimeter. Fluorescence spectra were recorded on Agilent Technologies, Cary Eclipse Fluorescence Spectrophotometer using 450 W xenon lamp and R928P PMT as the excitation source and detector, respectively, [Instrument Parameters: Ex. Slit (nm): 10, Em. Slit (nm): 10, Scan rate (nm/min): 600.00, Data interval (nm): 1.0000, Averaging Time (s): 0.1000].

The antibacterial experiment was performed against *Pseudomonas aeruginosa* (MTCC 2453), *Salmonella enterica* (MTCC 3224), *Streptococcus pneumoniae* (MTCC 655), *Enterococcus faecalis* (MTCC 439), *Klebsiella pneumonia* and *Escherichia coli* (ATCC 25922) and selective bacterial starins were used for growth study against *S. enterica* (MTCC 3224), *E. faecalis* (MTCC 439) and *S. pneumonia* (MTCC 655). Human red blood cells (hRBCs) were choosen for the hemolytic assay of the test compound. Anti-biofilm formation activity of Cu(II) complex was performed by XTT (2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium-hydroxide) reduction assay.

To perform molecular docking study HEX 8.0 software was used. The antioxidant potential of the compounds was estimated using DPPPH free radical method and hydrogen peroxide assay.

2.2. General procedure for synthesis of ligand (DEEI) and its copper(II) complex

The *N*-substituted phthalimide ligand (DEEI) and its Cu(II) complex were synthesized and well characterized by physicochemical analysis. The compounds were stable in solid state. The progress of the reaction was monitored by thin layer chromatography in methanol: dichloromethane (1:4).

2.2.1. Synthesis of 2-(-(2-(2-(2-(1,3-dioxoisoindolin-2-yl) ethylamino)ethylamino)ethyl) isoindoline-1,3-dione (DEEI)

To a suspension of triethylenetetramine (0.73 g, 5 mmol) in 5 mL ethanol, phthalimide (1.47 g, 10 mmol) in 15 mL toluene was added. The reaction mixture was refluxed for 4 h with vigorous stirring. The compound was extracted in ethanol, filtered off and washed thoroughly with dry diethyl ether and ethyl acetate. After drying, crude product was recrystallized in ethanol to obtain pure product (Scheme 1).

Yield 58%; (1.17 g); white solid; m.p.: 240 °C. IR υ_{max} cm^{-1} (KBr): 3318 (NH), 1770, 1706 (C=O), 2940, 2826 (C–H), 721 υ(Ar). ^{1}H NMR (DMSO, δ ppm) 8.13–7.83 (m, Ar-H, 4H), 3.84–3.41 (t, CH_2, 8H), 2.79–2.66 (t, CH_2, 4H), 2.35 (s, NH, 2H). ^{13}C NMR (DMSO, δ ppm) 168.27–163.68 (C, C=O), 134.80–123.65 (C, Ar), 44.93 (C, N–CH_2–), 44.39 (C, CH_2–CH_2–), 43.38 (C, NH–CH_2–). Anal. Calc. for $C_{22}H_{22}N_4O_4$ (406.43): C, 65.01; H, 5.46; N, 13.78. Found: C, 64.97; H, 5.45; N, 13.74. UV–vis: λ_{max} (ACN/nm): 227, 254, 332. EI-MS (70 eV) *m/z* (%): 406 $[M]^+$ (100%) (Fig. S3).

2.2.2. Synthesis of Cu(II) complex with ligand (DEEI)

To a solution of ligand (DEEI) (0.40 g, 1.0 mmol) in 15 mL ethanol, the solution of copper(II) chloride (0.135 g, 1.0 mmol) in 10 mL ethanol, was added drop wise with constant string. The reaction mixture was stirred for 6 h. The coloured precipitate was filtered, washed with ethanol and dry diethyl ether and dried in vacuum desiccator on fused $CaCl_2$ (Scheme 1).

Yield 65%; (0.345 g); green solid; m.p.: >300 °C. IR υ_{max} cm^{-1} (KBr): 3300 (NH), 1771, 1706 (C=O), 2941, 2825 (C–H), 721 (Ar). Anal. Calc. for $C_{22}H_{22}N_4O_4Cl_2Cu$ (540.61): C, 48.85; H, 4.10; N, 13.11. Found: C, 48.90; H, 4.12; N, 13.14. UV–vis: λ_{max} (DMF/nm): 230, 266, 330, 725.