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# A systematic investigation on biological activities of a novel double zwitterionic Schiff base Cu(II) complex

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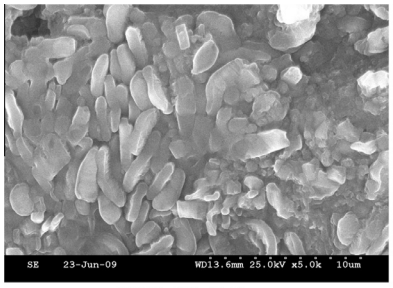
## HIGHLIGHTS

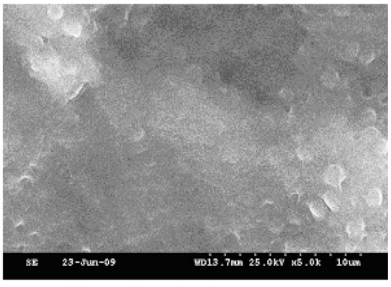
- Zwitterionic Schiff base and its copper complex were synthesized and characterized.
- Dissociation constants of the Schiff base were determined.
- Copper complex interact with DNA and can efficiently cleave pUC 19 DNA.
- Synthesized compounds were tested for their antimicrobial activity.
- Copper complex exhibits higher scavenging activity than free ligand.

## GRAPHICAL ABSTRACT

Double zwitterionic Schiff base and its copper complex have been synthesized and characterized. The p$K_a$ of the Schiff base was determined by potentiometric and spectrophotometric methods. The DNA binding and cleaving nature of the synthesized compounds was studied. The copper complex shows remarkable effect on morphology of *Escherichia coli* bacterium.




SEM of E. Coli




SEM of E. Coli with copper complex

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## ABSTRACT

Double zwitterionic amino acid Schiff base, o-vanillylidene-L-histidine (OVHIS) and its copper complex (CuOVHIS) have been synthesized and characterized. CuOVHIS has distorted octahedral geometry, and OVHIS coordinates the copper ion in a tetradentate manner ($N_2O_2$). The p$K_a$ of OVHIS in aqueous solution was studied by potentiometric and spectrophotometric methods. DNA binding behavior of the compounds was investigated using spectrophotometric, cyclic voltammetric, and viscosity methods. The efficacy of DNA cleaving nature was tested on pUC19 DNA. The *in vitro* biological activity was tested against various micro organisms. The effect of CuOVHIS on the surface feature of *Escherichia coli* was analyzed by SEM. DPPH assay studies revealed that CuOVHIS has higher antioxidant activity. OVHIS inhibits proliferation of HCT117 cells with half maximal inhibition ($IC_{50}$) of 71.15 ± 0.67. Chelation of OVHIS with Cu(II) ion enhances the inhibition of proliferation action ($IC_{50}$ = 53.14 ± 0.67).



## Introduction

Histidine, an essential amino acid in humans plays an important role in the growth and repair of tissues in the body. It helps the body in the detoxification process in presence of heavy metals [1,2]. Histidine contains ionizable imidazole ring as side chain with p$K_a$ ~ 7.0, so at physiological pH, both the acid and base forms are present [3]. Amino acid Schiff base complexes are considered to constitute new kinds of potential antibacterial and anticancer reagents [4,5]. Knowledge of the acid–base behavior of a ligand is essential in the estimation of ADME (absorption, distribution, metabolism and excretion) properties [6]. Dissociation constants can predict the behavior of the ligands in living organisms [7]. Considerable attention has been paid in the study of transition metal complexes as drugs due to their diverse biological activity [8,9].

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It is known that many antibiotics, carcinogens and so on, influence mainly through their direct interaction with DNA [10,11]. The study on interaction of drug and DNA plays a key role in designing and synthesizing the new drugs targeted to DNA and their effectiveness depends on the mode and affinity of the binding.

This aroused our interest to explore DNA binding of amino acid Schiff base complexes, which have interesting physical and spectroscopic properties [12,13]. A novel double zwitterionic amino acid Schiff base, o-vanillylidene-L-histidine (OVHIS) and its copper(II) complex were synthesized with a view towards evaluating their binding behavior with calf thymus DNA (CT-DNA). The DNA cleaving nature of the compounds was tested against pUC19 DNA in the absence and presence of hydrogen peroxide. Dissociation constants ($pK_a$) of OVHIS were determined by a combination of pH-metric and UV–Vis spectrophotometric methods. The *in vitro* biological activity of the compounds was assessed against various bacteria, fungi and yeast. The effect of the complex on the surface feature of the *Escherichia coli* bacterium has been analyzed by Scanning Electron Microscope (SEM). The antioxidant and anticancer activities of the compounds were also experimentally explored.

## Experimental

### *Materials and instrumentation*

L-histidine, o-vanillin and copper perchlorate were purchased from Aldrich and used without further purification. All other reagents were procured from commercial sources. The CT-DNA and pUC19 DNA were purchased from Genie, India. Agarose (molecular biology grade) and EB (ethidium bromide) were from Sigma. Solvents used for spectroscopic and electrochemical studies were purified and dried by standard procedures [14]. The elemental analysis was performed using Elementar Model Vario EL III analyzer. The IR spectra were recorded with samples as KBr pellets in a Shimadzu FTIR-8400S spectrophotometer. UV–Vis spectra were obtained on Shimadzu UV–Vis 2450 spectrophotometer. The fluorescence spectra were recorded on Shimadzu RF-5301PC spectrofluorophotometer. Cyclic voltammetric studies of copper complex in DMSO were carried out at room temperature with a CHI603C electrochemical analyzer. ESI mass spectra of OVHIS and the copper complex were taken in CDRI Lucknow.

Doubly distilled water was used in preparation of all the solutions. Alkali titer concentrations and the absence of carbonate were periodically checked by means of the appropriate Gran titration against potassium hydrogen phthalate [15]. The concentration of sodium perchlorate was checked by the estimation of perchloric acid obtained when a known volume of diluted sodium perchlorate was passed through Amberlite IR 120 ($H^+$) resin column. Grade A glassware was employed throughout the investigation.

### *Synthesis of o-vanillylidene-L-histidine (OVHIS)*

The preparation and single crystal structure of OVHIS was given in our earlier work [16]. A mixture of L-hisditine (0.1551 g 1.0 mmol) and o-vanillin (0.1522 g 1.0 mmol) dissolved in 50% ethanol:water (50 mL) was refluxed for 18 h. The resulting solution was reduced to one third of its original volume and on slow evaporation at room temperature yielded large yellow colored crystals, which were separated by filtration and washed with hot ethanol. The compound was further dried under vacuum before recrystallization (ethanol–water). Yield: (0.2657 g) 86.5%. M.pt. 165 °C, Anal. calcd. for $C_{14}H_{15}N_3O_4.H_2O$ %: C, 54.74, H, 5.53, N, 13.67. Found %: C, 55.01, H, 5.72, N, 13.94. IR (KBr pellet, $cm^{-1}$, s (strong), m (medium), b (broad)): 3300(b) [$\nu$(OH + NH)]; 1610 (s) [$\nu_{asymm}(COO^-)$]; 1404 (m) [$\nu_{symm}(COO^-)$]; 1640(s) [$\nu$(C=N)]; 1224 (s) [$\nu$(C—O)]. $^1$H NMR (400 MHz, DMSO-$d_6$, ppm): 10.28 (singlet (s), 1H , H1,), 7.62 (singlet, 1H, H2), 6.94 (doublet, 1H, H3), 3.02 and 3.20 (dd, 2H, H4a, Hb), 4.34 (dd, 1H, H5), 8.31 (singlet, 1H, H7), 7.03 (doublet, 1H, H8), 6.78 (multiplet, 2H, H9, H10), 3.77 (singlet, 3H, H11).

### *Synthesis of CuOVHIS complex*

Ethanolic solution (10 mL) of $Cu(ClO_4)_2.6H_2O$ (0.1705 g 1.0 mmol) was stirred well. To this well stirred solution, 50% ethanolic solution (10 mL) of OVHIS (0.3073 g 1.0 mmol) was added drop wise at room temperature. The initial pH of the reaction mixture was ~1.5. The pH of the solution was then adjusted to 6–7 using 0.01 M sodium hydroxide solution and it was refluxed for 2 h. The resulting solution was allowed to stand at room temperature for 24 h. The solid copper complex was separated and washed with hot ethanol. The compound was further dried under vacuum. The product was obtained as a green powder. Yield: (0.2631 g) 65%. M.pt. > 300 °C, Anal. calcd. for CuOVHIS complex ($[Cu(C_{14}H_{13}N_3O_4)(H_2O)_2]$) %: M, 16.43, C, 43.47, H, 4.43, N, 10.86. Found %: M, 16.52, C, 43.55, H, 4.49, N, 10.93. IR (KBr pellet, $cm^{-1}$, s (strong), m (medium), b (broad)): 3400(b) [$\nu$(OH + NH)]; 1595 (s), [$\nu_{asymm}(COO^-)$]; 1398(m) [$\nu_{symm}(COO^-)$]; 1635(s) [$\nu$(C=N)]; 1242(s) [$\nu$(C—O)]; 420 [$\nu$(M—O)]; 580 $\nu$(M—N).

### *pH metric measurements*

The pH-metric titrations were carried out at 310 ± 0.1 K in a digital pH meter (Systronics *μpH* System 361) with extension of a combined glass electrode (accuracy ± 0.01 pH unit) and a glass reaction flask of 50 mL capacity. The experimental procedure for the pH metric studies has already been described [17–19]. The ionic strength of each solution was adjusted to 0.15 M with $NaClO_4$ as supporting electrolyte. In both the acidic and alkaline regions, the electrode system was calibrated in terms of hydrogen ion concentration instead of activities. The calibration of the electrode system was done by direct titration of a dilute solution of perchloric acid using sodium perchlorate background and also by recognized buffer solutions [20], potassium hydrogen phthalate (0.05 M; pH = 4.02) and borax (0.05 M; pH = 9.08) at 310 ± 0.1 K. The ionic product of water ($pK_w$) is −13.62 at 310 K. All the titrations were carried out under magnetic stirring, and oxygen-free nitrogen gas was bubbled through the solution before and during titrations to exclude $O_2$ and $CO_2$ inside. The solutions were prepared in doubly distilled water and titrated pH-metrically against standard carbonate free NaOH (0.3 M) solution. The total volume of the solution was adjusted to 25 mL by adding water. The pH-metric data were measured in the range $1.20 \leqslant pH \leqslant 11.5$. Each set of titrations were repeated at least three times for the ligand concentrations. The dissociation constants for OVHIS were obtained from its solutions of concentration ranging from $3 \times 10^{-3}$ to $1.5 \times 10^{-2}$ M. The color changes indicate that there is some protic equilibrium takes place in the OVHIS during the course of titration. The dissociation constants of OVHIS were evaluated with the aid of the HYPERQUAD program [21]. The concentration distribution profiles were obtained with HySS [22].

### *UV measurements*

Spectrophotometric titrations were performed with Shimadzu UV–Vis 2450 Spectrophotometer with cell length 1 cm using water as the solvent at 310 K. The spectra of OVHIS (five to seven solutions) were recorded in the range 200–450 nm at various pH values ranging from 1.2 to 11.5 by the appropriate addition of dilute $HClO_4$ or NaOH solutions. The pH for a particular species has been obtained from the species distribution diagram.