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RESEARCH ARTICLE

Investigations on biomedical applications of some Schiff base metal(II) complexes

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Abstract

A Schiff base ligand have been prepared by reacting indole-3-carboxaldehyde and L-phenylalanine in 1:1 molar ratio and characterized by spectral studies. The representative Schiff base metal complexes have been characterized by elemental analysis, IR, ^{1}H NMR, electronic spectra, magnetic moment, and molar conductance. The analytical data confirms the stoichiometric ratio in the complexes. The IR results show that the ligand acts as a bidentate donor coordinating through the azomethine nitrogen and carboxyalto oxygen atoms. The conductance data reveal that the complexes are non-electrolytic in nature. Geometrical structures of the metal complexes were confirmed by the electronic spectra and magnetic moment measurements. The compounds were screened for their antibacterial, antifungal and DNA cleavage activities. The Ni(II) and Cu(II) complexes show effective DNA cleavage than other complexes. The anticancer activities of the complexes have been carried out towards HeLa and HCT116 cancer cells.

Keywords

Schiff base

IR

Electronic

Antibacterial

Antifungal

DNA cleavage

Anticancer

Introduction

Transition metal complexes have established a great consideration because of their biological activities. These are essentially due to their ability to form tetradentate chelate with heavy metal ions, bonding through sulfur and nitrogen atoms. The bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been documented that many of these complexes may assist as models for biologically important species [1, 2].

Schiff bases have been intensively considered due to their antibacterial and antitumor properties. Complexes formed from Schiff bases and metals such as cobalt, nickel, and copper have been studied as "oxygen carriers". Although no metal mixtures of this series had been tested for antitumor activity it appeared potential that some of them would show this property since their ability to act as "oxygen carriers" might give them a role in the metabolic

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processes of the body. The synthesis of Schiff bases and their cobalt complexes allow the provision of a large number of compounds with the same basic structure, but with different solubility's in water and other solvents which as an important factor in determining the usefulness of drugs [3, 4]. Copper is a cofactor essential for the tumor angiogenesis processes, whereas other transition metals are not.

Consistently, high serum or tissue levels of copper were found in many types of human cancer including breast, lungs, and brain, supporting the idea that copper could be used as a novel selective target for cancer therapies. Therefore, a novel concept was developed suggesting that the drugs targeting tumor cellular copper may be more effective and less toxic. This prompted researchers to develop copperbased compounds with potential use as anticancer drugs. Studies pertaining the metal ion-DNA intercalation have been utilized for developing novel chemother-apeutic agents, foot printing agents and for gene manipulation in biotechnology and medicine. In addition, new kind of chemotherapeutic Schiff bases are now attracting the attention of biochemists.

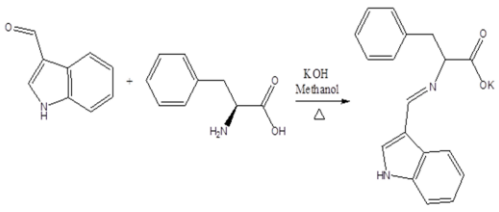
Experimental

Materials

Indole-3-carboxaldehyde and L-phenylalanine, metal chlorides were obtained from Merck, India of analytical grade.

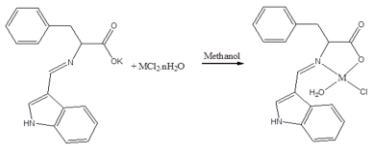
Preparation of Schiff base ligand

L-Phenylalanine (1 mmol) was dissolved in 20 ml of methanol containing KOH (1 mmol). A solution of indole-3-carboxaldehyde (1 mmol) in 10 ml of absolute methanol was added dropwise with stirring and refluxed for 3 h. The volume of the yellow solution was cooled at room temperature and then reduced in vacuo using a rotary evaporator. Anhydrous ether was added to deposit a yellowish precipitate, which was then recrystallized from ethanol.and dried *in vacuo* over anhydrous $CaCl_2$ (Yield: 78%).



Preparation of metal complexes

Schiff base ligand (1 mmol) was dissolved in 10 mL of methanol and Co(II)/Ni(II)/Cu(II)/Zn(II) chloride (1 mmol) was added dropwise into 20 mL of methanol. The resulting mixture obtained. was stirred for 2 h. After allowing it to stand in air at room temperature the precipitated complex was filtered off, washed several times with cold ethanol, ether and then dried in vacuo over anhydrous $CaCl_2$. (Yield: 68-73%).



Physical measurements

Elemental analysis (C, H, N) was carried out using a Perkin-Elmer elemental analyzer. Molar conductance was measured in DMSO (10^{-3} M) solution using a Coronation Digital Conductivity meter. ^{1}H NMR spectrum was recorded on a 300 MHz Varian spectrometer using $CDCl_3$ solvent system. IR spectrum was recorded on a JASCO FT/IR-410 spectrometer in the 4000-400 cm^{-1} region by KBr pellet method. The electronic spectra were recorded on a Perkin Elmer Lambda-25 UV-VIS spectrometer in the 200-1100 nm range. Room temperature magnetic measurements were performed on a Gouy balance by making diamagnetic corrections using Pascal's constant.

DNA cleavage activity

A solution of CT DNA in the buffer (5 mmol L^{-1} Tris–HCl/ 50 mmol L^{-1} NaCl buffer (pH 7.2)) gave a ratio of UV absorbance at 260 and 280 nm of about 1.89:1, demonstrating the DNA adequately free from protein contamination. The CT DNA concentration was investigated by UV using the molar absorption coefficient of 6600 M^{-1} cm^{-1} at 260 nm. Stock solutions were kept at 4 ^{0}C and used after not more than four days. The DNA cleavage activity was studied using agarose gel electrophoresis. CT DNA (0.3 µg) dissolved in NaCl buffer (pH 7.2), was treated with the complexes. The mixture was incubated at 37 ^{0}C for 2 h and then mixed with the load-ing buffer (2 µL) containing 25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol. Each sample (5 µL) was loaded into 0.8% (w/v) agarose gel. Electrophoresis was undertaken for 2 h at 50 V in Tris–acetate–EDTA (TAE) buffer (pH 8.0). The gel was stained with ethidium bromide for 5 min after electrophoresis and then photographed under UV light. To enhance the DNA cleaving activity of the complexes, hydrogen peroxide (100 µmol L^{-1}) was added to each sample.

Anticancer activity

The human cervical cancer cell line (HeLa) and colon

Cancer Cells (HCT116) were grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained at 37 ^{0}C, 5% CO_2, 95% air, and 100% relative humidity. The monolayer cells were isolated with trypsin–EDTA to make single cell suspension and viable cells were counted using a hemocytometer and diluted with a medium containing 5% FBS to give a final density of 1 x 10^5 cells/mL. One hundred microliters per well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells per well and incubated to allow for cell attachment at 37 ^{0}C, 5% CO_2, 95% air and 100% relative humidity. After 24 h, the cells were preserved with serial concentration of the test samples. They were originally dissolved in DMSO to prepare the stock (200 mM) and stored frozen prior to use. At the time of sample addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with serum free medium. Additional three, 2-fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µL of these different sample dilutions were added to the appropriate wells already containing 100 µL of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37 ^{0}C, 5% CO_2, 95% air, and 100% relative humidity. The medium without samples was served as control and a triplicate was maintained for all concentrations.

MTT assay

MTT is a yellow water soluble tetrazolium salt. Succinatedehydrogenase, a mitochondrial enzyme in living cells cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Thus, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15 µL of MTT (5 mg/mL) in phosphate buffered saline was added to each well and incubated at 37 ^{0}C for 4 h. The medium with MTT was then flicked off and formazan crystals obtained were solubilized in 100 µL of DMSO. The absorbance at 570 nm was measured using a micro plate reader [5].

Results and discussion

The ligand is pale yellow in colour and soluble in organic solvents. The metal complexes are coloured expect zinc complex and soluble in organic solvents. The elemental analysis data of ligand is in good agreement with those calculated for the suggested formula. Purity of the ligand and its complexes has been checked by TLC (silica gel). The ^{1}H NMR spectrum of the ligand shows a singlet signal at 9.08 ppm corresponds to azomethine proton. The aromatic ring protons signals appeared at 7.32-7.518 ppm respectively. The metal complexes dissolved in DMSO solution (10^{-3} M) at room temperature was measured to establish the charge of the metal complexes. The low molar conductance value (12-19 $\Omega^{-1}cm^2mol^{-1}$) of the metal complexes reveals their non-electrolytic nature.

IR spectra

The IR spectrum of the ligand exhibit a band at 1623 cm^{-1} assigned to the azomethine nitrogen. Sharp bands appeared in the region ~3200 and ~1510 cm^{-1} is due to N-H stretching frequency [6]. The stretching vibration band appeared at 1623 cm^{-1} for the azomethine group of free ligand was shifted to lower frequency range (~1612-1617 cm^{-1}) in the spectra of the complexes, indicating the coordination of azomethine nitrogen atom to the metal ion. The metal complexes also exhibit bands in the region (421– 454 cm^{-1}), corresponds to the formation of M-N stretching frequency [6]. The M-Cl stretching bands seemed below 400 cm^{-1} in the metal complexes [7]. From this, it is concluded that the Schiff base ligand binds the metal ion through the azomethine nitrogen and carboxylato oxygen atom. In addition to this two chlorine atoms are also coordinated to metal ion.

Electronic spectra

Co(II) complex shows an absorption bands at 615 nm, corresponds to [$^4A_2(F) \rightarrow {}^4T_1(P)$] transition suggesting the tetrahedral geometry. The magnetic moment value of 4.31 BM indicates the tetrahedral geometry for the complex [8]. The Ni(II) complex is diamagnetic and displays a medium intensity band at 450 nm due to the [$^3A_{1g} \rightarrow {}^3B_{1g}$] transition [8]. This suggests square planar geometry for this complex. The Cu(II) complex displays a broad band at 551 nm assignable to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition. The magnetic moment value of 1.81 BM also indicates the square planar geometry for Cu(II) complex. Zn(II) complex is diamagnetic and have tetrahedral geometry.

Antimicrobial activity

The Schiff base ligand and its metal complexes were tested *in vitro* against the bacterial species, *Escherichia coli (E. coli), Bacillus subtilis (B. subtilis),* and *Staphyl ococcus aureus (S. aureus);* fungal species, include *Aspergillus niger (A. niger), Aspergillus flavus (A. flavus),* and *Candida albicans* (C. albicans) by Kirby Bayer disc diffusion method [9]. DMSO was used as a negative control. Amikacin and Nystatin were used as standards. The copper complex shows greater antimicrobial activity than those of the free ligand and other complexes. This is explained on the basis of overtone's concept and chelation theory [10-12].

The copper complexes designs equal or better activity compared to the negative control amikacin. Copper complex shows equal activity against *E. coli* bacteria compared to the standard and moderate activity was found against other bacterial species. Other compounds are having moderate to low activity compared to the standards.

DNA cleavage activity

The complexes can interact with CT-DNA in the presence of H_2O_2. The metal complexes can catalyze the production of highly reactive hydroxyl radicals from H_2O_2. These hydroxyl radicals contribute in the oxidation of the deoxyribose moiety, followed by the hydrolytic cleavage of the sugarphosphate backbone. The general oxidative mechanisms proposed account for DNA cleavage by hydroxyl radicals via abstraction of a hydrogen from sugar units and predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed [13].

Anticancer activity

The Schiff base metal complexes on cancer cells, we used the compounds to treat with HeLa and HCT116 at the concentrations of 6.25, 12.5, 25, 50, and 100 μM for 48 h. The untreated cells were used as a control. Cell growth inhibition was analyzed by MTT assay and the results showed that the complexes and the ligand exhibited an inhibitory effect on the proliferation of HeLa and HCT116 cells in a dosedependent manner. Among them, Cu(II) complex shows the most potent inhibitory effect on the growth of both the cells compared to the other compounds. The IC_{50} values for the compounds against HCT116 cancer cells show moderate activity compared to the IC_{50} value of the clinicaly used drug such as etoposide (29.6 μM) [14, 15]. The IC_{50} value of the ligand and its complexes on the cancer cells is shown in **Fig 1**. From the IC_{50} value of Cu(II) complex on the cancer cells, it is assumed that the complex is more active on HCT116 cancer cells than on the HeLa cancer cells.

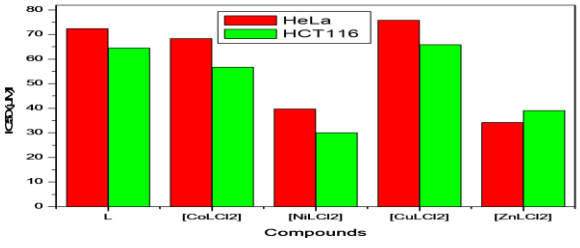


Fig 1 IC_{50} values of the compounds on the cancer cells.

Conclusions

The ligand and its metal complexes were synthesized and characterized by spectral methods. IR spectra indicate the coordination of the binding sites. By the electronic spectral data, tetrahedral and square planar geometry were assigned for the complexes. DNA cleavage studies demonstrates that the Cu(II) complex have more cleavage efficiency. The Cu(II) complex is more active than the other complexes on the HeLa and HCT116 cancer cells.

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