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RESEARCH ARTICLE

Synthesis, of structural elucidation of biochemical studies of transitions copper complex of biosensitive ligands

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Abstract

The ligand derived from 4-aminoantipyrine and benzophenone was synthesized and characterized by elemental analysis, IR, electronic spectrum, ^{1}H NMR and mass spectrum. Schiff base complex was synthesized and characterized by elemental analysis, molar conductance, IR, electronic spectra, magnetic measurements and electrochemical studies. Conductance measurements indicate that the complexes are non electrolytes. IR spectral data show that the ligand is bidentate and the binding sites are azomethine nitrogen and carbonyl oxygen atoms. Electronic spectral measurements indicate distorted square planar geometry for Cu(II) complex. Magnetic measurements showed mononuclear and paramagnetic behaviour for Cu(II) complex. The metal complexes are found to be stabilized in the unusual oxidation states of the metal ion during electrolysis.

Keywords

4-Aminoantipyrine
Benzophenone
IR
Electronic spectrum
Magnetic
Mass

Introduction

Coordination compounds, with bonds between a central metal atom and surrounding ligands play critical roles in biology biochemistry and medicine, controlling the structure and function of many enzymes and their metabolism. They play similarly vital roles in many industrial processes and in the development of new materials with specifically designed properties. The current research of active anticancer agents is broad in scope, and targets multiple cellular and biological properties across several tumor types. Over the last fifty years, the development of anticancer drugs moved away from conventional cytotoxicity and towards the rational design of selective agents that act on specific cellular targets [1, 2]. However, significant challenges remain, and the interface between structural biology and chemistry may provide the most productive means for discovering and improving upon novel anticancer agents [3]. In 1864, German chemist Hugo Schiff

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developed a new class of organic compounds. This group of compounds, imines are referred to as Schiff bases in his honour. The preparations of these compounds are simple and easier. They are prepared by condensing a carbonyl compound with an amine in refluxing alcohol medium. The active and well-designed Schiff base ligands are considered as ‘‘privileged ligands’’ by Cozzi. In fact, Schiff bases are able to stabilize many different metals in various oxidation states, controlling the performance of metals in a large variety of useful catalytic transformations. The presence of a lone pair of electrons in a sp^2 hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance. Because of the relative easiness of preparation, synthetic flexibility, and the special property of C=N group, Schiff bases are considered as excellent chelating agents, [4-8] especially when a functional group like –OH or –SH is present close to the azomethine group so as to form a five or six membered chelate ring with the metal ion. The formation of Schiff base is shown in **Fig 1.**

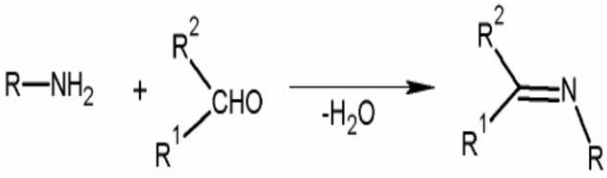


Fig 1 Formation of Schiff base by condensateion reaction (R groups may be variously substituted).

Almost all transition metal complexes of Schiff bases show good antibacterial, antiviral, antimalarial and antitubercular activity [9-12]. The potent biological activity can be related to the presence of nitrogen atom with a lone pair of electron in it so that the nitrogen can participate in hydrogen bonding with NH or OH groups present in biomolecules like amino acids, proteins, DNA or RNA. The copper is the active metal center involved in the catalysis process of the enzyme. The basic catalytic mechanism (**Fig 2**) of Cu/Zn-SOD takes place in two steps, first superoxide reduces the copper center of SODs to form di-oxygen and a second superoxide oxidizes the copper center to produce hydrogen peroxide. Cu(II) containing SOD enzyme, Cu^{II} Zn^{II} SOD is the most efficient catalytic species found in the mammalian cell plasma and extracellular spaces. It catalyzes the dismutation of superoxide radical ($O_2^{\bullet -}$) and converts it into molecular oxygen and hydrogen peroxide via one electron redox cycle involving its Cu(II) center.

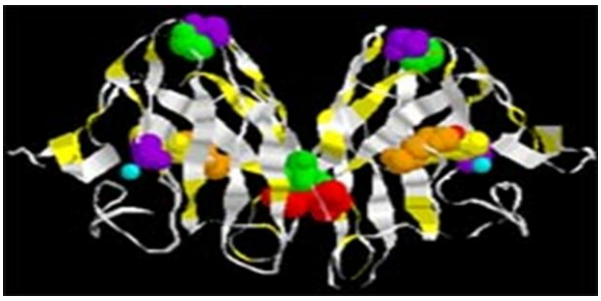


Fig 2 Copper-zinc superoxide dismutase enzyme.

Pyrazolone derivatives such as antipyrine, aminopyrine, and dipyrone are well known compounds used mainly as analgesic and antipyretic drugs. 4-Aminoantipyrine is used for the protection against oxidative stress as well as prophylactic of some diseases including cancer in medical applications.

Antipyrine derivatives are strong inhibitors of cycloxygenase isoenzymes, platelet tromboxane synthesis, and prostanoids synthesis, which catalyze the rate limiting step of prostaglandin synthesis. Pyrazolones are also a well-known elicitor of hypersensitivity.

Based on the above information and due to our interest in pyrazole as a biologically active pharmacophore, a new bioactive heterocycle incorporating antipyrine moiety starting from 4-aminoantipyrine to be evaluated for their SOD activity.

Experimental

Materials

All chemicals were obtained from Sigma and Aldrich. 1,2-Dichloromethane, acetonitrile, acetone, methanol, N, N-dimethylformamide and dimethylsulphoxide (DMSO) were used of spectral grade. Commercially available CT DNA was used for DNA binding studies.

Synthesis of ligand

The mixture of 4-aminoantipyrine (0.001 M) in ethanol and benzophenone (0.001 M) are allowed to stirr in 3 h. After cooling, the dark colour solution was filtered and washed with ethanol. A dark yellow precipitated was obtained.

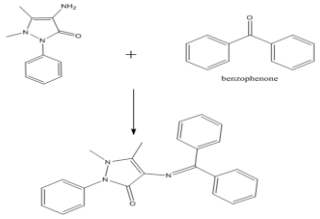
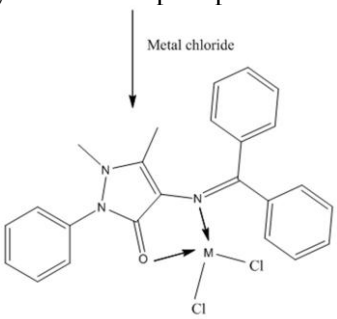


Table 1 Analytical and physical data of ligand and its copper complex.

Compounds	Formula	Colour	Analytical data (%)					
			C	H	N	O	Cl	Cu
Ligand	$C_{24}H_{21}N_3O$	Adark yellow	78.45	5.76	11.44	4.35	-	-
Cu(II) complex	$C_{25}H_{24}Cl_2CuN_3O$	Sky blue	58.09	4.68	8.13	3.10	13.72	12.29

Synthesis of metal complexes

Equimolar ethanolic solution of Schiff base and copper chloride was refluxed for 3 h. The brown colour solution was poured into crashed ice and refrigerated for 24 h. The green precipitate was washed with water and dried. The sky blue colour precipitate was obtained.



Analytical methods

Determination of CHN analysis was done by using Elementar Vario ELIII Carlo Erba. EDTA was used as calibrant. The amount of copper was estimated gravimetrically as its oxide [13] by fusion with Analar ammonium oxalate. Chloride ion content in the copper complexes was determined gravimetrically using silver nitrate test. Molar conductance were measured in DMSO solution using a coronation digital conductivity meter. The IR spectra were recorded on a Shimadzu FT-IR spectrometer using KBr disc with the range 4000-350 cm^{-1}. In the graph, the percentage transmission (%T) was recorded against wave number (cm^{-1}). The ^1H and ^{13}C-NMR spectram were recorded using Bruker Avance II 400 MHz spectrometer. TMS is used as standard The chemical shifts are expressed in units of parts per million relative to TMS. Electronic spectra were recorded in a Systronics 2201 Double beam UV-Vis., spectrometer with in the range of 200-1100 nm region. In the graph, absorbance values are plotted against wave length. The cyclic voltammogram of the copper complex was recorded by using CHI 604D electrochemical analyzer with three electrode system of gla-ssy carbon as the working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as the reference electrode. Tetrabutylammonium perchlorate was used as supporting electrolyte. Solution was deoxygenated by eradi-cation with N_2 previous to measurements.

SOD activity

In vitro SOD activity was measured using alkaline DMSO as a source of superoxide radical (O_2) and nitrobluetetrazolium (NBT) as O_2 scavenger. In general, 400 µl sample to be assayed was added to a solution containing 2.1 ml of 0.2 M potassium phosphate buffer (pH 8.6) and 1 ml of 56 µM NBT. The tubes were kept in ice for 15 min., and then 1.5 ml of alkaline DMSO solution was added while stirring. The absorbance was then monitored at 540 nm against a sample prepared under similar condition except that NaOH was absent in DMSO. A unit of SOD activity is the concentration of complex or enzyme, which causes 50% inhibition of alkaline DMSO mediated reduction of NBT.

Results and discussion

The analytical data for the ligand and its complex are summarized in **Table 1.** The analytical data of the complex corresponds well with the general formula $MLC1_2$, where M =Cu(II); L = $C_{24}H_{21}N_3O$.

Electronic spectra

The electronic absorption spectral data (**Fig 3)** were recorded in ethanol. In the metal complex, the wide range of bands appeared are due to transition of -HC=N-, charge transfer results from electrons interaction between the metal and the ligand which involves either a metal to ligand or ligand to metal electron transfer. The electronic spectrum of ligand shows a broad band at 236 and 353 nm, which is assigned to the $\sigma\rightarrow\sigma^*$ and $\pi\rightarrow\pi^*$ transition of the imine (>C=N-) chromophore. On complexation this band was shifted to lower wavelength, suggesting the coordination of azomethine nitrogen to the central metal ion. The copper complex exhibits band at 243 nm assigned to and $^2B_{1g}\rightarrow{}^2A_{1g}$ transitions characteristic of a square plannar geometry with $d_{x^2-y^2}$ ground state. These data and the magnetic moment value of 1.83 BM suggest square planar geometry around Cu(II). The magnetic susceptibility of the complex at room temperature is consistent with square planar geometry around the central metal ion.

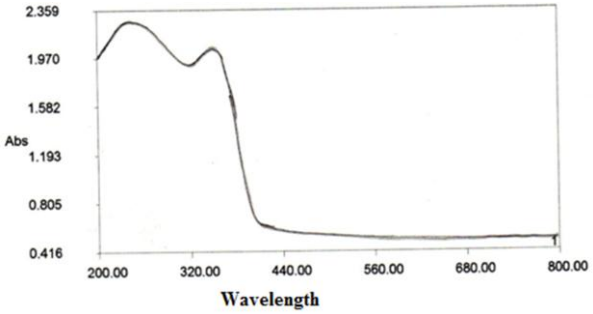


Fig 3 Electronic absorption spectrum of ligand.

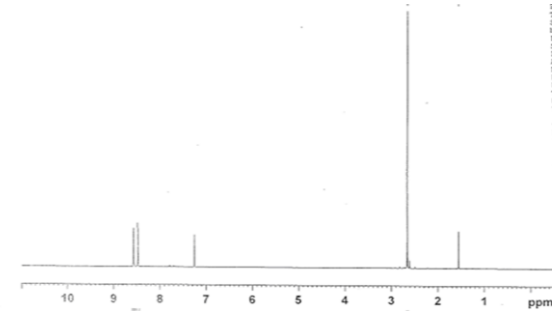


Fig 4 ^{1}H NMR spectrum of 4-aminoantipyrine derivative.

Molar conductance

The molar conductance of all the complexes was measured in ethanol using 10^{-3} M solutions at room temperature. The conductance values were occured in the range of 9.01 $\Omega^{-1}cm^{2}mol^{-1}$, which indicate that the non-electrolytic nature of complex [14] and confirmed by silver nitrate test.

IR spectra

The IR spectrum of the ligand shows a broad band in the region 3200-3600 cm^{-1} are assignable to intramolecular hydrogen bonded -OH groups. The appearance of this peak in the spectrum of the complex indicate that the -OH group is free from the complexation. The ligand shows two different -C=N bands in the region 1625-1680 cm^{1}, which are shifted to lower frequency in the spectrum of the complex (1590-1650 cm^{-1}) indicating the involvement of -C=N nitrogen in coordination to the metal ion. Accordingly, the ligand acts as a tetradentate chelating agent, bonded to the metal ion *via* the four nitrogen (-C=N) atoms of the Schiff base. This is further supported by the appearance of medium bands at 450-400 cm^{-1} which could be attributed to ν(M-N) vibration. The absorption band at 1719 cm^{-1} is assigned υ(C=O) in ligand. It is shifted to lower wave number 15 cm^{-1} in the spectrum of complex indicates the involvement of the pyrazolone oxygen in chelation. The absorption band at 1638 cm^{-1} in the spectrum of the free ligand is attributed to υ(-CH=N) vibration. This band is shifted by (~20 cm^{-1}) towards lower frequency in the spectrumof complex, which clearly indicates that complexation has taken place through the nitrogen atom of azomethine group.

^{1}H NMR spectrum

The ^{1}H NMR spectrum of ligand in $CDC1_3$ gives the following signals: phenyl as multiplet at 7.2-7.6 ppm, =C-CH_3 at 2.4 ppm, -N-CH_3 at 3.2 ppm and -CH=N protons at 9.8 ppm. The peak at 13.2 ppm is attrib-utable to the phenolic -OH group. The NMR spectrum of 4-aminoantipyrine derivative is shown in **Fig 4**.

Cyclic voltammetry

The electrochemical behaviour of copper complex was recorded in DMSO solution in the potential range +2 to –2V. The Cu(II) complex **(Fig 5)** showed an irreversible peaks observed at -0.4V (E_{pc}) and +0.4V (E_{pa}) which corresponds to the conversion of Cu(II)/(I) and Cu(II)/(III), respectively. The present ligand system was stabilized in the unusual oxidation states of Cu complex during electrolysis.

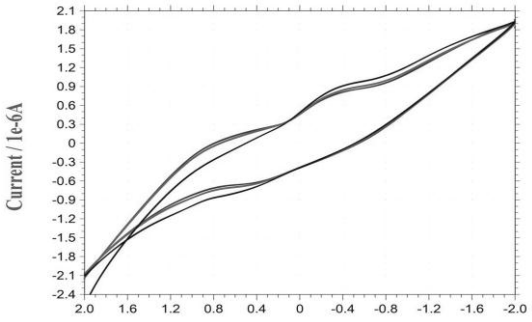


Fig 5 The cyclic voltammogram of $[CuLCl]_2$ complex.

SOD activity

Free radicals or reactive oxygen species are highly toxic and their damaging effects result in a variety of detrimental health issues such as neurodegenerative, cardiovascular and agerelated diseases [15-19]. Human body has evolved an effective defense system including SOD and catalase against the toxicity of these free radicals. SOD is a metalloenzyme and it acts as an excellent antioxidant to protect the body from superoxide radicals that are generated in the biological system. The SOD activities for the complexes were measured. Superoxide was enzymatically supplied from alkaline DMSO and SOD activity was evaluated by the NBT assay following the reduction of NBT to MF+ kinetically at 560 nm. These complexes exhibit significant catalytic activity towards the dismutation of superoxide anions. The concentration causing 50%

inhibition of NBT reduction is IC_{50}. The observed IC_{50} values of the Cu(II) complex (5 μmol dm^{-3}) are higher than the value exhibited by the native enzyme (IC_{50} = 0.04 μmol dm^{-3}) on a molar base (note that the smaller the IC_{50} value, the higher the SOD activity). The mechanism is shown in **Fig 4.**

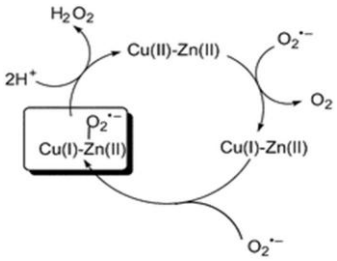


Fig 4 Schematic outline of SOD.

Conclusions

A novel biosensitive ligand have been synthesized and characterized by various physicochemical and spectroscopic techniques. The synthesized complex is stable powders, insoluble in common organic solvents and are non-electrolytes. The magnetic and spectroscopic data indicate a distorted square planar geometry. The prepared copper complex showed SOD activity may be explained in terms of 4-aminoantipyrine moiety, distorted square planar geometry and cathodic & anodic peak potentials of complex. The copper complex may be mimic natural SOD enzyme.

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