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Synthesis, structural elucidation, biological, antioxidant and nuclease activities of some 5-Fluorouracil–amino acid mixed ligand complexes

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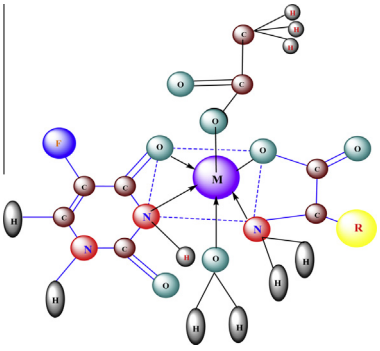
HIGHLIGHTS

- 5-FU and amino acids form distorted octahedral with M(II) ions via bidentation.
- XRD and SEM analyses show complexes are microcrystalline with uniform morphology.
- Complexes prevent bacterial and fungal growth as a result of specific interactions.
- Binding of complexes with CT DNA show hypochromism and red shift in absorption band.
- CT DNA cleavage by Cu and Ni complexes follow Fenton's radical oxidative mechanism.

GRAPHICAL ABSTRACT

Mixed ligand complexes (**1**–**9**) have been synthesized from 5-Fluorouracil (5-FU; A) and amino acids(B) such as glycine (gly), L-alanine (ala) and L-valine (val) with Ni(II), Cu(II) and Zn(II) ions and were characterized by various physico-chemical, spectral, TG, XRD and SEM studies. They have been tested for their *in vitro* biological and antioxidant activities by the agar well diffusion method and the DPPH assay method respectively. The results demonstrate that Cu(II) mixed ligand complexes (**4**–**6**) exhibit higher biological as well as antioxidant activities than 5-Fluorouracil and their counterparts, Ni(II) (**1**–**3**) and Zn(II) (**7**–**9**) mixed ligand complexes. The cleavage activity of CT DNA under aerobic condition reveals moderate activity with Cu(II) and Ni(II) mixed ligand complexes (**1**–**6**) while Zn(II) complexes (**7**–**9**) show no activity. Binding studies of CT DNA with these complexes show 5–15% of hypochromicity and a minor red-shift in the charge transfer band. The negative free energy change values ($\Delta^{\ddagger}G$) calculated for the binding indicate that the mixed ligand complexes can interact with DNA in a spontaneous manner.



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ABSTRACT

Some biologically active mixed ligand complexes (**1**–**9**) have been synthesized from 5-Fluorouracil (5-FU; A) and amino acids (B) such as glycine (gly), L-alanine (ala) and L-valine (val) with Ni(II), Cu(II) and Zn(II) ions. The synthesized mixed ligand complexes (**1**–**9**) were characterized by various physico-chemical, spectral, thermal and morphological studies. 5-Fluorouracil and its mixed ligand complexes have been tested for their *in vitro* biological activities against some pathogenic bacterial and fungal species by the

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agar well diffusion method. The *in vitro* antioxidant activities of 5-Fluorouracil and its complexes have also been investigated by using the DPPH assay method. The results demonstrate that Cu(II) mixed ligand complexes (**4**–**6**) exhibit potent biological as well as antioxidant activities compared to 5-Fluorouracil and Ni(II) (**1**–**3**) and Zn(II) (**7**–**9**) mixed ligand complexes. Further, the cleaving activities of CT DNA under aerobic conditions show moderate activity with the synthesized Cu(II) and Ni(II) mixed ligand complexes (**1**–**6**) while no activity is seen with Zn(II) complexes (**7**–**9**). Binding studies of CT DNA with these complexes show a decrease in intensity of the charge transfer band to the extent of 5–15% along with a minor red shift. The free energy change values ($\Delta^{\ddagger}G$) calculated from intrinsic binding constants indicate that the interaction between mixed ligand complex and DNA is spontaneous.

Introduction

In recent years, the formation of mixed ligand complexes between bioactive ligands having N, O/S donor binding sites and transition metal(II) ions have received much attention [1,2] because of their broad spectrum of biological, clinical, medicinal, agricultural, industrial, analytical and therapeutic applications. Pyrimidines and its N-base analogs like uracil, thymine and cytosine are biologically important classes of heterocycles which play a significant role in human cellular life, coenzymes, antibiotics and drugs. Their derivatives are the basic building units of nucleic acids, RNA and DNA [3–5]. Recently, the compounds containing pyrimidine moiety have been investigated widely for the design and synthesis of various drugs in clinical, biological, pharmacological, medicinal and therapeutical fields [6]. These compounds were found to have wide range of applications like antibacterial, fungicidal, antitumor, antiviral, anti-inflammatory, antipyretic, antimitotic, antimetabolites, antithyroidal and surface anaesthesia activities [7–9]. The mono fluorinated pyrimidine derivative, 5-Fluorouracil (5-FU; Adrucil) was found to act as chemical mutagens in human as well as in animals and was broadly tested as potent antibacterial and anticancer drugs. 5-Fluorouracil alone or in combination with other drugs/hormones is often used in the treatment of early stage cancers [10,11]. It is also used in topical delivery with minimum side effects [12]. Transition metal(II) ions are the essential elements for the healthy life in humans, plants and in higher animals. Amino acids are the fundamental components in living organisms [13] and they constitute the basic building blocks of proteins in plants and humans.

The noncovalent interactions between transition metal(II) complexes and DNA can occur via intercalation, groove binding or through external electrostatic binding [14]. DNA bound metal complexes are stabilized through π-stacking interactions between the base pairs of the aromatic heterocyclic groups, hydrogen bonding and Van der Wall's forces between functional groups inserted along the groove of the DNA helices. Gel electrophoresis [15], footprinting, X-ray crystallography [16], NMR, fluorescence [17], UV–vis spectroscopy and electrochemical [18] techniques have been used to investigate such interactions. As the interactions of transition metal(II) ions with DNA play a crucial role [19] they are used in the development of new therapeutic agents for bio-reactivity [20]. The binding studies of transition metal(II) complexes with DNA have been used as probes to find the DNA structure in solution [21], to understand the gene mutation and to know the action mechanism of some antitumor and antivirus drugs.

Great attention is being paid to the study of natural and safer antioxidants because of their vital role in food, cosmetics and pharmaceutical products [22] and in the prevention of diseases caused by oxidative stress. Moreover, these compounds are found to prolong the life expectancy of animals and plants and prevent age related diseases by retarding the process of lipid peroxidation and chain reactions [23]. By considering all these in mind, the authors have decided to investigate the in *vitro* biological, antioxidant and nuclease activities of 5-Fluorouracil and its nine mixed ligand complexes (**1**–**9**) with biologically active amino acids, glycine, L-alanine and L-valine and Ni(II), Cu(II) and Zn(II) ions. These mixed ligand complexes were synthesized and characterized by means of analytical, spectral, thermal and morphological techniques.

Experimental

Materials and physico-chemical measurements

All the ligands, metal(II) salts, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid used were extra pure AR grade (Sigma Aldrich/Fluka) and used without further purification. Solvents used for the physical measurements are of AR grade and purified by standard methods [24]. Triply distilled water (specific conductance = 1.81 ± 0.1 Λ^{-1} cm^{-1}) was stored in a CO_2 free atmosphere and was used for solution preparations. Melting points of all the mixed ligand complexes were determined on Gallenkamp apparatus in open glass capillaries and were uncorrected. The elemental analysis of C, H and N were performed on Elementar Vario EL III CHNS analyzer at STIC, CUSAT, India. Metal content of the complexes was estimated gravimetrically by standard procedures. Molar conductance of the complexes was measured in DMSO solvent using Elico CM 180 Conductivity Bridge by using 0.01 M KCl solutions as calibrant. Fast atomic bombardment mass spectra (FAB-MS) were recorded on a VGZAB-HS spectrometer in a 3-nitrobenzylalcohol matrix. Magnetic susceptibility measurement on powder samples was carried out by the Gouy method using $Hg[Co(SCN)_4]$ as calibrant and the diamagnetic corrections were applied in compliance with Pascal's constant. Electronic absorption spectra were recorded in DMSO medium using Hitachi U-2000 double beam spectrophotometer (path length, 1 cm) in the 200–1100 nm range. Infrared spectra were recorded using KBr pellets on a JASCO FT/IR-410 spectrophotometer in the 4000–400 cm^{-1} range. ^{1}H and ^{13}C NMR spectra of the diamagnetic zinc complexes were recorded in DMSO-d_6 at room temperature using TMS as internal standard on Perkin Elmer R-32 spectrometer at IIT, Chennai, India. Thermal analyses (TGA/DTA) were recorded on a Perkin Elmer (TGS-2 model) thermal analyzer with a heating rate of 10 K/min in dynamic N_2 atmosphere (flow rate 20 mL/min). Powder X-ray diffraction (PXRD) patterns were recorded on a Bruker AXS D8 advance powder X-ray diffractometer (Detector: Si(Li)PSD, X-ray source: Cu, Wavelength: 1.5406 Å). The SEM image of the complexes was recorded on a JSM-5610 scanning electron microscope. Calf thymus DNA was purchased from Gene (India). DNA binding was recorded in ethanol solution with a Perkin Elmer Lambda-20 UV–visible 2995S–2998S spectrophotometer.

*General procedure for the synthesis of mixed ligand complexes (**1**–**9**)*

5-Fluorouracil (0.013 g/10 mM) was dissolved in water (10 mL) containing few drops of concentrated ammonia and stirred to get a clear solution. Ten mL of 10 mM of an appropriate metal salt