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A novel Schiff base derived from the gabapentin drug and copper (II) complex: Synthesis, characterization, interaction with DNA/protein and cytotoxic activity

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ABSTRACT

A novel Schiff base [$C_{20}H_{23}NO_3$], has been prepared and characterized using FT-IR, UV–vis, ^{1}H NMR spectroscopy, elemental analysis and X-ray crystallography. A copper (II) complex [$Cu(C_{20}H_{22}NO_3)_2]\cdot H_2O$ has also been synthesized and characterized. The new ligand and complex thus obtained were investigated by their interaction with calf thymus DNA and BSA using electronic absorption spectroscopy, fluorescence spectroscopy, and thermal denaturation. The intrinsic binding constants K_b of the ligand and Cu (II) complex, with CT-DNA obtained from UV–vis absorption studies were 1.53×10^4 M^{-1} and 3.71×10^5 M^{-1}, respectively. Moreover the addition of the two compounds to CT-DNA (1:2) led to an increase of the melting temperature of DNA up to around 2.61 °C for the ligand and 3.99 °C for the Cu (II) complex. The ligand and Cu (II) complex bind to CT-DNA via a partial intercalative, as shown by the experimental data. In addition, the albumin interactions of the two compounds were studied by fluorescence quenching spectra, the results indicating that the binding mechanism is a static quenching process. The in vitro cytotoxicity of the two compounds on three different cancer cell lines was evaluated by MTT assay. The results showed that the copper complex exerted enhanced cytotoxicity compared with the Schiff base ligand; thereby, this complex clearly implies a positive synergistic effect. Furthermore, the copper complex showed a high, selective, and dose-dependent cytotoxicity against cancer cell lines.

1. Introduction

Gabapentin, 1-(aminomethyl) cyclohexaneacetic acid, Neuronti (Gpn) structurally belongs to the neurotransmitter gamma-aminobutyric acid (GABA), widely studied for its significant inhibitory action in the central nervous system [1]. Gpn has been applied in the treatment of neuropathic pain. It is a new generation antiepileptic used as add-on therapy and monotherapy in patients with partial seizures [2]. A safe and effective seizure is a concern for an increasing number of adults suffering from epilepsy. Epilepsy also imposes a remarkable economic load on society [3]. The selection of an antiepileptic drug depends on how it functions with regard to the specific seizure type, tolerability, and safety. Furthermore, gabapentin can be considered as an emergent solution for the "pain riddle". Starting from this point, more randomized, double blind studies, which compare analgesic drugs with gabapentin, may be relevant to identifying the first choice therapy for acute and chronic pain relief [4]. Thus, the search for novel chemical entities for the cure of epilepsy is essential [5,6]. Usually formed by condensation of a primary amine with an active carbonyl compound, Schiff bases are compounds containing the azomethine group (R—C═N). The biological activities and decreased cytotoxicity of both metal ion and Schiff base ligand are due to the attachment of transition metals in to these compounds [7–9]. Considering their great flexibility and various structural aspects, a wide range of transition metal complexes of Schiff base ligands have been synthesized and the structure function relationships of the resulting complexes have been extensively focused on in recent years [10–14]. Piotr et al. have synthesized various compounds such as amino thiazoles, 2-hydroxy-1-napthalaniline, amino sugars, aromatic aldehydes, ketones, thiosemicarbazides, amino acids, pyrazolone, etc. [15]. Recently, some novel coordination compounds based on Schiff base ligands were found useful as potential pro drugs. Consequently, transition metal complexes of Schiff bases have been extensively studied as promising alternatives to traditional cisplatin for anticancer drugs. In addition, Schiff base complexes have attracted much attention given their important role in the improvement of novel therapeutic agents and novel nucleic acid structural probes [16]. DNA is an essential cellular receptor and is the primary target molecule for most anticancer and antiviral therapies. Many compounds apply their anticancer effects via binding

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to DNA, thereby changing DNA replication and inhibiting the growth of the tumor cells, which is the basis for designing new, more effective anticancer drugs, the efficacy of which depends on the mode and tendency of the binding [17]. Revealing the features leading to increased DNA binding capability by small ligands or metal complexes is important for designing efficient chemotherapeutic agents and better DNA targeted anticancer drugs [18]. Therefore, the DNA-binding studies of small metal complexes are very significant in the improvement of new anticancer drugs [19,20]. Moreover, naphthaldehyde Schiff base ligand, an aromatic planar ligand with carbonyl O and N donor atoms, can coordinate to metal ions to form metal intercalators, which show strong intercalation with DNA. The selection of a metal ion is the most significant factor in the design of a metal based chemotherapeutic agent [21]. Copper is a crucial cofactor in tumor angiogenesis processes and is a bioessential and bio-relevant element, which is an inseparable component of many enzymes, including superoxide dismutase, tyrosinase, ceruloplasmin, etc. [22,23]. Many copper (II) complexes with biological activities such as antibacterial, anti-cancer and cancer inhibiting properties have already been dealt with in the literature [24,25]. Palaniandavar and co-workers have reported that copper (II) complexes are the best alternatives to cisplatin because copper plays many significant parts in biological systems and is a biocompatible metal [26], considering the strong interactions of copper complexes with DNA through surface associations or intercalation [27]. Moreover, the investigation of the interactions of novel copper (II) Schiff base complexes with DNA is greatly important for disease defense, new medicine design and clinical application of drugs [28]. A copper complex has been synthesized from gabapentin drug Schiff base and the corresponding biological properties such as DNA binding, protein binding, and in vitro cytotoxicity have been studied. One of the important properties of a drug is its protein binding degree, affecting its effective solubility, biodistribution, and half life in the body [29]. Biomolecule proteins play an important role in transportation and deposition of endogenous and exogenous substances including fatty acids, hormones, and drugs. They also have many physiological functions. BSA, bovine serum albumin, is often selected as a target protein to study interactions with small molecules due to its low cost, ready accessibility and similarity to human serum albumin [30]. A stable protein drug complex with a dominant role in storage and drug disposition may be formed by a significant interaction of any drug with a protein. Therefore, understanding the mechanism of interaction of a bioactive compound with BSA, a well studied protein, is important [31]. A new Schiff base derived from the gabapentin drug and copper complex has been studied in this work from four aspects; (i) synthesis and characterization of the ligand and complex using spectroscopy and X-ray diffraction, (ii) study of the ability of the two compounds to interact with DNA and the interaction mechanism using UV–vis, fluorescence spectroscopy, and thermal denaturation, (iii) monitoring of the protein binding ability by UV absorption and tryptophan fluorescence quenching experiment in the presence of the two compounds using BSA as a model protein, and (iv) a comparative study of the in vitro cytotoxicity of the three compounds on three human carcinomas (JURKAT, SKOV3, and U87) and peripheral blood mononuclear cell (PBMC) by MTT assay.

2. Experimental

2.1. Materials

All chemicals and solvents used for synthesis were commercially available, reagent grade and were used without further purification. Solvents and starting materials were supplied by Sigma Aldrich or Alfa Aesar Chemical Companies and used without further purification. BSA and Calf thymus DNA (CT-DNA) were purchased from Sigma Aldrich Chemical Company and were used as supplied. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient ($\varepsilon = 6600\ M^{-1}\ cm^{-1}$ at 260 nm) [32]. The stock solutions were stored at 5 °C and used over a period of 4 days. All the experiments involving interactions of the compounds with DNA were performed using doubly distilled water buffer containing 5 Mm Tris–HCl [tris (hydroxymethyl)-aminomethane] and 50 mM NaCl, adjusted to pH 7.4 using hydrochloric acid. Ligand and Cu complex stock solutions were prepared by dissolving the two compounds in water and DMSO as the co-solvent, and then diluted with the corresponding buffer to the necessary concentrations for all the experiments. The final DMSO concentration did not exceed 0.5% v/v. JURKAT (human leukemic T cell line), SKOV3 (human ovarian cancer cell line), U87 (human glioblastoma cell line), and PBMC (peripheral blood mononuclear cell) were supplied by the National Cell Bank of Pasteur Institute, Tehran, Iran. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 5 mM L-glutamine. The cell lines were subsequently grown at 37 °C in a humidified atmosphere containing 5% CO_2. All reagents and cell culture media were purchased from Gibco Company (Germany).

2.2. Physical Measurements

Fourier transform infrared (FT-IR) spectra were recorded as KBr disks on an FT-IR JASCO-680 spectrophotometer in the 4000–400 cm^{-1} ranges using the KBr pellets technique. The elemental analyses were performed on a Leco, CHNS-932 apparatus. UV–vis spectra were recorded on a JASCO V-570 spectrophotometer in the 190–900 nm range. NMR spectra were measured on a Bruker spectrometer at 400.13 MHz. The T_m spectra were recorded on a Varian BioCary-100 UV–vis spectrophotometer using a 1 cm path length cell. Solutions were prepared by dissolving the complex in water buffer containing 5 mM Tris–HCl (pH 7.4) and 50 mM NaCl. Electrospray mass spectra were obtained using a Shimadzu LCMS-2010 EV liquid chromatography mass spectrometer.

2.3. Synthesis of the Schiff Base Ligand

The Schiff base ligand, was prepared by equimolar (1:1 mol ratio) condensation between gabapentin (0.160 g, 1 mmol) and 2-hydroxy1-naphthaldehyde (0.172 g, 1 mmol) by stirring for 3 h at room temperature in absolute ethanol and recrystallization using absolute methanol by slow evaporation. Ligand yield (86%) (0.75 mg); color: yellow; MP: 215 °C; anal., calc. for, [$C_{20}H_{23}NO_3$]; C, 73.82; H, 7.12; N, 4.30; O, 14.75%. Found; C, 73.50; H, 7.24; N, 4.35; O, 14.90%. FT-IR (KBr, cm^{-1}): 3436 ν (O—H), 2929 and 2855 ν (C—H), 1690 ν_{as} (COO^-), 1638 ν (C═N), 1545 ν (C═C), 1359 ν_s (COO^-) 0.1030 ν (C—O), 964 ν (C—N)·^{1}H NMR ($CDCl_3$, δ, ppm): 8.43 (s, 1H, HC═N), 7.65–7.67 (d, 1H^a, Ar—H, $^3J = 8.3$), 7.53–7.55 (d, 1H^b, Ar—H, $^3J = 7.1$), 7.47–7.49 (d, 1H^c, Ar—H, $^3J = 9.2$), 7.29–7.32 (t, 1H^d, Ar—H $^3J = 7.5$), 7.10–7.14 (t, 1H^e, Ar—H $^3J = 7.4$) 6.76–6.79 (d, 1H^f, Ar—H, $^3J = 9.2$) 3.50 (s, 2H, CH_2), 2.30 (s, 2H CH_2), 1.39–1.78 (m, 10H, cyclohexyl methylenes group). UV–vis (solvent methanol λ_{max}, nm (ε, $M^{-1}\ cm^{-1}$)): 232 (1.82×10^4), 310 (4.50×10^3), 394 (5.68×10^3).

2.4. Synthesis of $[Cu(C_{20}H_{22}NO_3)_2]\cdot H_2O$

The synthesis of copper complex is as follows: copper (II) acetate tetrahydrate (0.249 g, 1.0 mmol) and ligand (0.620 g, 2.0 mmol) were added to a 40 mL methanolic solution and stirred for 5 h. The formed Cu (II) complex was filtered and washed with diethyl ether.

Copper (II) complex $[Cu(C_{20}H_{22}NO_3)_2]\cdot H_2O$, yield (90%), color: light green; MP: 365 °C; anal., calc. for $C_{40}H_{46}CuN_2O_7$: C, 65.78; H, 6.35; N, 3.84; O, 13.48%. Found; C, 65.22; H, 6.53; N, 3.77%; O, 13.36%. FT-IR (KBr, cm^{-1}) 3382 ν (O—H), 2924 and 2856 ν (C—H), 1600 ν (C═N), 1549 ν_{as} (COO), 1509 ν (C═C), 1310 ν_s (COO), 1048 ν (C—OH), 961 ν (C—N),458 ν (Cu—N) and 541 ν (Cu—O). UV–vis (solvent methanol