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New mixed ligand palladium(II) complexes based on the antiepileptic drug sodium valproate and bioactive nitrogen-donor ligands: Synthesis, structural characterization, binding interactions with DNA and BSA, in vitro cytotoxicity studies and DFT calculations

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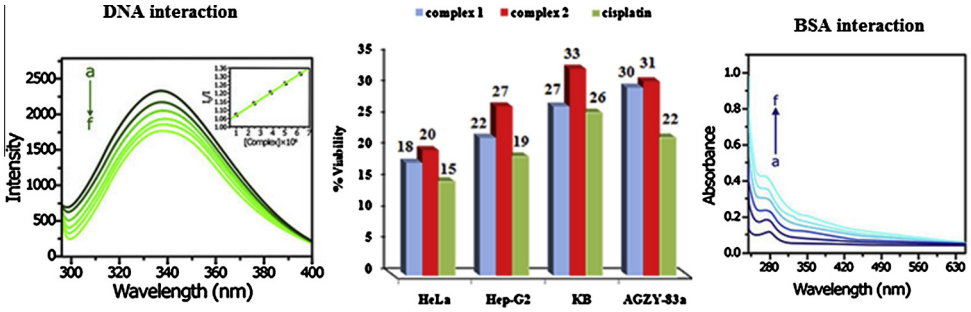
HIGHLIGHTS

- Two Pd(II) complexes have been synthesized and characterized.
- DFT calculations of Pd(II) complexes were studied.
- The interaction of the Pd(II) complexes with CT-DNA were investigated.
- The Pd(II) complexes can bind to bovine serum albumin proteins.
- Evaluation of cytotoxic activity of the Pd(II) complexes were carried out.

GRAPHICAL ABSTRACT



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ABSTRACT

The complexes [Pd(valp)$_2$(imidazole)$_2$] (**1**), [Pd(valp)$_2$(pyrazine)$_2$] (**2**) (valp is sodium valproate) have been synthesized and characterized using IR, ^{1}H NMR, ^{13}C{^{1}H} NMR and UV–Vis spectrometry. The interaction of complexes with CT-DNA has been investigated using spectroscopic tools and viscosity measurement. In each case, the association constant (K_b) was deduced from the absorption spectral study and the number of binding sites (n) and the binding constant (K) were calculated from relevant fluorescence quenching data. As a result, a non-covalent interaction between the metal complex and DNA was suggested, which could be assigned to an intercalative binding. In addition, the interaction of **1** and **2** was ventured with bovine serum albumin (BSA) with the help of absorption and fluorescence spectroscopy measurements. Through these techniques, the apparent association constant (K_{app}) and the binding constant (K) could be calculated for each complex. Evaluation of cytotoxic activity of the complexes against four different cancer cell lines proved that the complexes exhibited cytotoxic specificity and significant cancer cell inhibitory rate. Moreover, density functional theory (DFT) calculations were employed to provide more evidence about the observed data. The majority of trans isomers were supported not only by energies, but also by the similarity of its calculated IR frequencies, UV adsorptions and NMR chemical shifts to the experimental values.

Abbreviations: DNA, deoxyribonucleic acid; CT-DNA, calf thymus DNA; BSA, bovine serum albumin; HAS, human serum albumin; EB, ethidium bromide; IC_{50}, half maximal inhibitory concentration; SV, Stern–Volmer; Valp, sodium valproate; Pd, palladium; DFT, density functional theory; TD-DFT, time-dependent density functional theory; NBO, natural bond orbital; FT-IR, Fourier transform infrared spectroscopy; UV–Vis, ultraviolet visible; NMR, nuclear magnetic resonance; calc., calculated; DMSO, dimethyl sulfoxide; FMOs, frontier molecular orbitals; HOMO, highest occupied molecular orbitals; LUMO, lowest unoccupied molecular orbitals; RMS, root mean square; GIAO, Gauge-Independent Atomic Orbital.

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Introduction

Over the decades, there has been broad research in pharmaceutical chemistry to design effective anticancer drugs, potentially valuable in the treatment of diverse cancers [1–4]. Cisplatin is one of the leading chemotherapeutic drugs among actinomycin, anthracycline antibiotics and extensively used metal-based anticancer drugs for cancer therapy, but it possesses inherent limitations as serious side effects such as neurotoxicity, tissue toxicity, nausea, nephrotoxicity, emetogenesis, gastrointestinal, bone marrow toxicity and acquired drug resistance [5–8]. As a result, significant attempts are being made to replace this drug with appropriate alternatives and various transition metal complexes have been synthesized and tested for their anticancer activities [9–11].

Over the past decades, the investigations on the interaction of metal complexes with deoxyribonucleic acid (DNA) are of great interest of researchers due to their potential applications as anticancer medications and stereo selective probes of nucleic acid structures [12,11,13–25]. Bovine serum albumin (BSA) is soluble protein that has the ability to transport a multitude of endogenous and exogenous ligands such as fatty acids, amino acids, steroids, metal ions and drugs in blood stream. It is used to investigate the interaction of protein–drug complex in the circulatory system due to structural similarities with human serum albumin (HSA) [26].

Valproic acid (valp) is an extensive spectrum anti-epileptic drug which is efficient against all seizure varieties and that is increasingly used in the treatment of other diseases, including bipolar disorder, migraine, and neuropathic pain [27]. Furthermore, valproic acid was shown to improve the effect of chemotherapy on EBV-positive tumors, and to take a multitude of anti-tumor properties in vitro and in clinically appropriate animal models [28,29]. Esiobu and Hoosein [30], studied the effect of sodium valproate on the growth of a broad spectrum of microorganisms and they understood that it is selectively potent against yeast strains and *Mycobacterium smegmatis*. Though synthesis, characterization and biological activity of mixed ligands metal complexes of valproate with different nitrogen based ligands have been studied for copper [31–37], rhodium [38], zinc [39,40], and platinum [41], the biological application of palladium complexes with valproate is not well explored.

Herein, we are reporting the synthesis, characterization and potential DNA/protein binding abilities and in vitro cytotoxicity studies of new valproate complexes of palladium with two nitrogen based ligands of pyrazine and imidazole, $[Pd(valp)_2(imidazole)_2]$ (**1**) and $[Pd(valp)_2(pyrazine)_2]$ (**2**). The structure of ligand and its complexes were clarified by elemental analysis, FT-IR, ^1H NMR, ^{13}C NMR, UV/Vis spectroscopies. Theoretical calculations using density functional theory (DFT) were done in order to correlate between the theoretical and experimental results. In this line, natural bond orbital (NBO) analysis was performed to present details about the type of hybridization and the nature of bonding in the studied complexes.

Experimental section

General

Starting materials and solvents were purchased from Sigma–Aldrich or Alfa Aesar and used without further purification. CT-DNA and BSA were purchased from Sigma–Aldrich and were used as supplied. Cisplatin was gifted from Isfahan University of Medical Sciences. Infrared spectra were recorded on a FT-IR JASCO 680 spectrophotometer in the spectral range 4000–400 cm^{-1} using the KBr pellets technique. NMR spectra were recorded on Bruker spectrometer at 400.13 MHz for ^1H measurements and 75 MHz for the $^{13}C\{^1H\}$ measurements using TMS as an internal standard in DMSO-d_6 solvent at 298 K. Elemental analysis was performed on a Leco, CHNS-932 apparatus. Molar conductivity measurements were carried out with a Crison Basic 30 conductometer. UV–Vis spectra were recorded on a JASCO 7580 UV–Vis-NIR double-beam spectrophotometer using a quartz cell with a path length of 10 mm. The fluorescence spectra complex bound to DNA were obtained at an excitation wavelength of 522 nm on a Perkin–Elmer LS55 fluorescence spectrofluorometer. Viscosity experiments were conducted on an Ostwald's viscometer, immersed in a thermostated water-bath maintained at 25 °C.

*Synthesis of $[Pd(valp)_2(imidazole)_2]$ (**1**)*

An ethanolic solution (15 cm^3) of sodium valproate (0.33 g, 2 mmol) was added slowly and simultaneously with a ethanolic solution (15 cm^3) of imidazole (0.14 g, 2 mmol) to a stirred ethanolic solution (25 cm^3) of $Pd(OAc)_2$ (0.23 g, 1 mmol). The mixture was stirred for 4 h at room temperature and then filtered through a plug of $MgSO_4$. The filtrate was concentrated to ca. 5 cm^3 and to this concentrated solution; n-hexane (15 cm^3) was added to precipitate a white solid, which was collected and air-dried. The compound is soluble in water, methanol, ethanol, acetone, chloroform, dichloromethane, diethyl ether and ethyl acetate. Yield: 75%.

Elemental analysis results were in good agreement with the for $C_{22}H_{38}N_4O_4Pd$ stoichiometry. Found (calc.%): C, 49.86 (49.95); N, 10.41 (10.59); H, 7.14 (7.24).

^1H NMR (DMSO) (Fig. S1): δ (ppm) 0.84 (t, 12H, H-5), 1.29 (m, 8H, H-4), 1.40 (m, 8H, H-3), 2.33 (m, 2H, H-2), 7.09 (s, 2H, H-8), 7.37 (s, 2H, H-9), 8.15 (s, 2H, H-6), 9.53 (s, 2H, H-7). $^{13}C\{^1H\}$ NMR (DMSO) (Fig. S2): δ (ppm) 14.32 (C-5), 20.93 (C-4), 34.67 (C-3), 47.51 (C-2), 108.32 (C-9), 124.13 (C-8), 153.45 (C-6), 185.14 (C-1). IR (KBr, cm^{-1}) (Fig. S5): 3584vs, 3122vs, 2962s, 2973s, 1620s, 1520s, 1515m, 1489m, 1441s, 1346w, 1326m, 1268m, 1259m, 1221w, 1197w, 1115w, 1108w, 1091m, 1063vs, 1015w, 970w, 951w, 925w, 915w, 902w, 890w, 785vs 737w, 651m. Conductivity (Λ_M, mho cm^2 mol^{-1}) in water: 42. UV–Vis in water, λ_{max} (log ε): 282 (3.91), 302 (3.84).

*Synthesis of $[Pd(valp)_2(pyrazine)_2]$ (**2**)*

Complex **2** was prepared in a similar way to **1** with the use of pyrazine (0.16 g, 2 mmol) instead of imidazole. The compound is soluble in water, methanol, ethanol, acetone, chloroform, dichloromethane, diethyl ether and ethyl acetate. Yield: 72%.

Elemental analysis results were in good agreement with the for $C_{24}H_{38}N_4O_4Pd$ stoichiometry. Found (calc.%): C, 49.97 (54.08); N, 10.49 (10.51); H, 7.15 (7.18); ^1H NMR (DMSO) (Fig. S3): δ (ppm) 0.81 (t, 12H, H-5), 1.21 (m, 8H, H-4), 1.42 (m, 8H, H-3), 2.28 (m, 2H, H-2), 8.49 (d, 4H, H-11,12), 8.87 (d, 4H, H-10,13). $^{13}C\{^1H\}$ NMR (DMSO) (Fig. S4): δ (ppm) 14.28 (C-5), 20.83 (C-4), 33.87 (C-3), 47.34 (C-2), 143.42 (C-11, 12), 147.42 (C-10, 13), 184.91 (C-1). IR (KBr, cm^{-1}) (Fig. S6): 3115s, 3009m, 2960s, 2970s, 1631s, 1560vs, 1508s, 1489m, 1451s, 1341w, 1260m, 1220w, 1196w, 1181vs, 1110m, 1027w, 974w, 927w, 882m, 791m, 713w, 642w. Conductivity (Λ_M, mho cm^2 mol^{-1}) in water: 38. UV–Vis in water, λ_{max} (log ε): 275 (3.71), 310 (3.74).

DNA binding studies of palladium(II) complexes

The binding properties of the complex of CT-DNA have been studied using electronic absorption spectroscopy, competitive binding experiments, fluorescence spectroscopy and viscosity measurements. All the experiments involving the interaction of the