Journal of Molecular Liquids 264 (2018) 386–397

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

# Multi experimental and computational studies for DNA and HSA interaction of new nano-scale ultrasound-assisted synthesized Pd(II) complex as a potent anticancer drug

Monireh Dehkhodaei [a], Mehdi Sahihi [a,*], Hadi Amiri Rudbari [a,*], Mahnaz Ariaeefar [a], Sajjad Gharaghani [b], Reza Azadbakht [c], Salman Taheri [d], Abolghasem Abbasi Kajani [a]

[a] Department of Chemistry, University of Isfahan, Isfahan 81746-73441, Iran
[b] Laboratory of Bioinformatics and Drug Design (LBD), Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[c] Department of Chemistry, Payame Noor University, P.O. Box 19395-3697, Iran
[d] Chemistry and Chemical Engineering Research Center of Iran, Tehran, Iran

ARTICLE INFO

*Article history:*
Received 29 December 2017
Received in revised form 25 April 2018
Accepted 18 May 2018
Available online 19 May 2018

*Keywords:*
Sonochemistry
Nano complex
Fluorescence enhancement
MD simulation
Molecular docking
QM/MM

ABSTRACT

As for daily increasing mortality rate in world due to the growth of cancer causing agents, design and synthesis of new compounds with anticancer potential benefits is one of the most important challenges for researchers. In the present work, we synthesized a new Schiff base Pd(II) complex in bulk-scale and also in nano-scales by Sonochemical method. The structure of synthesized complex was determined by single crystal X-ray diffraction technique. Then the cell viability percent of HeLa cancer cells was studied by MTT assay. The results confirmed that reducing the size has salient effect in annihilation of cancer cells. Also, nano-scale complex reached to IC50 in 10 μM of concentration. Binding ability of the nano- and bulk-scale Pd(II) Schiff base complex with calf thymus DNA and human serum albumin was investigated using combination of experimental (fluorescence, circular dichroism (CD) and viscosity) and computational (molecular docking, molecular dynamics simulation and QM/MM) methods. The estimated binding constants for the complex in both of bulk- and nano-scales showed that the nano-scale complex binds more tightly to DNA than its bulk-scale form. This finding is in good agreement with MTT assay results. Molecular docking studies revealed that Pd(II) complex binds to the minor groove and IB binding site of DNA and HSA, respectively. Also, MD simulation studies showed that complexation with the Pd(II) complex changes the structure of HSA with compared to free protein. Finally, the ONIOM results indicated that the structural parameters of the compound changed along with binding to DNA and HSA, indicating the strong interaction between the compound and these biomacromolecules. The values of binding constants depend on the extent of the resultant changes.



## 1. Introduction

Platinum-containing drugs such as cis-platin, carboplatin and oxaliplatin show their anti-cancer activity based on in-vivo interaction with the DNA of the cancerous cells. Studying on the interactions between DNA and the other transition metal complexes is key step to develop new metal based therapeutics [1]. Furthermore, some studies have investigated binding of the anti-cancer drugs to the Human Serum Albumin (HSA), as the most abundant plasma carrier protein in the blood stream [2,3]. The binding ability of a drug to the non-specific transporter protein HSA is an important research field in chemistry, biochemistry and clinical medicine [4–13]. Hence, investigation of the interaction between HSA and small drug molecules is very important for understanding the mystery of life and the interaction mechanisms of drugs. The results of investigation show that more than 90% plasma protein can be combined with metal drugs in form of covalent bonds. Studying on the metal base drugs is one of the basic area in bioinorganic chemistry field. The researchers in this field try to discover compounds with more impressive properties and less side effects from cis-platin that is known as appropriate anti-cancer drug. One of the approaches is using the synergic effect between metals and biological ligands. Schiff bases are the class of inorganic ligands that their biological activities have been proven, previously [14–16]. On the other hand, the simplistic synthesis and wide range of applications of Schiff base ligands have attracted a lot of interests. These compounds play an important role in development of coordination chemistry related to catalysis, organic synthesis [17,18], antitumor, antimicrobial and cytotoxic activity [19–22]. In recent years, remarkable attention

* Corresponding authors.
*E-mail addresses:* m.sahihi@chem.ui.ac.ir, (M. Sahihi), hamiri1358@gmail.com (H.A. Rudbari).

https://doi.org/10.1016/j.molliq.2018.05.077
0167-7322/

has been paid to biological applications of the metal complexes of Schiff bases due to their stability, biocompatibility and biological activity [23,24]. Their biological activity is due to the existence of imine group in their chemical structure [25–27]. Furthermore, complexation of Schiff base ligands with transition metal ions, enhances their biological activities [28–30]. Some palladium complexes had shown promising results as antimalarial and anti-cancer agents in previous investigations [31,32]. Also, due to remarkable similarity between Pd(II) and Pt(II) complexes [33]; and their less toxicity and higher activity in compared to platinum complexes; they are regarded as potential anticancer substances and have better anticancer activity than their Pt(II) analogues in some cases [34].

Due to the importance and significant growth of nanotechnology in all sciences, its role in the field of biology and drug design cannot be ignored. Previous studies showed that the interaction between nanoparticles and biomolecules can change the biomolecule conformation and perturb the normal protein function which can be lead to unexpected biological reactions and toxicity. Therefore, investigation of these types of interactions could not be ignored [35].

Herein, a new Schiff base Pd(II) complex ($PdL_2$) was synthesized and its molecular structure was determined by single crystal X-ray diffraction technique. The cell viability percent of HeLa cancer cells was first studied by MTT assay. In order to increase the colloidal stability and suitability for the biomedical applications, the nano-scale compound was also synthesized using ultrasound-assisted method and used for in vitro studies. Finally, binding ability of the nano- and bulk-scale $PdL_2$ with calf thymus DNA (CT-DNA) and HSA was investigated using combination of experimental (fluorescence, circular dichroism (CD) and viscosity) and computational (molecular docking, molecular dynamics simulation and qm/mm) methods.

## 2. Experimental section

### 2.1. Materials sources

All of the used chemicals for synthesis of the complex including 2-amino-propane (Isopropylamine), 2-hydroxybenzaldehyde (salicylaldehyde), triethylamine and Palladium chloride (PdCl2) were purchased from Merck Co. and were used without further purification. All of the used salts for buffer preparation were analytical grade and were dissolved in double distilled water. All of the solutions were used freshly after preparation. Also, HSA, CT-DNA, RPMI-1640 medium, Fetal bovine serum (FBS), Dimethyl sulfoxide (DMSO), antibiotics (penicillin-streptomycin) solution, and 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) were obtained from Sigma-Aldrich.

### 2.2. Synthesis of the bulk- and nano-scale Pd(II) Schiff base complex ($PdL_2$)

A methanolic solution (30 ml) of Isopropylamine (IUPAC name: 2-amino-propane) (5 mmol) was added slowly to 30 ml of a methanolic stirred solution of salicylaldehyde (IUPAC name: 2-hydroxybenzaldehyde) (5 mmol) in ambient temperature. The colour immediately changed to yellow and the mixture was then stirred for 2 h. Then a solution of triethylamine (7 mmol) in absolute methanol (10 ml) was added dropwise to ligand solution. The mixture was stirred for 15 min again. Then, a solution of appropriate $PdCl_2$ (2.5 mmol) in methanol (30 ml) was added to the mixture, gently, for synthesis of the complex in bulk-scale. The solution was refluxed overnight to proceed completely. The resulting yellow powder was isolated by filtration and washed by dry methanol several times. Appropriate single crystals for X-ray crystallography were obtained directly from the reaction mixture. In order to prepare the complex in nano scale, a saturated solution was prepared by adding excess powder to 10 ml of DMF at room temperature. The solution was pumped from a small orifice into 100 ml of the antisolvent (water), which was placed in an ultrasonic bath at 180 W for 30 min and was continuously stirred. The suspension was centrifuged at 5000 *g* for 15 min, and the supernatant was withdrawn and filtered through a 0.2 mm pore-size syringe filter [36].

### 2.3. Single crystal diffraction studies

X-ray data for $PdL_2$ complex was collected at room temperature with a Bruker APEX II CCD area detector diffractometer using Mo $K_\alpha$ radiation ($k = 0.71073$ Å). Data collections, cell refinements, data reductions and absorption corrections were performed using multiscan methods with Bruker software [37–39]. The structure was solved by direct methods using SIR2004 [40]. The non hydrogen atoms were refined anisotropically by the full matrix least squares method on $F^2$ using SHELXL [41]. All hydrogen atoms were added at ideal positions and constrained to ride on their parent atoms. Molecular graphics were prepared with the Olex$^2$ program [42]. Crystallographic data for complex are listed in Table S1. Selected bond distances and angles are summarized in Table S2.

### 2.4. Cell viability assay

MTT assay was used to investigate the anticancer potential of the bulk- and nano-scale $PdL_2$ on human cervical cancer cell line (HeLa) according to the previously reported procedure [43]. The cells were first cultured in RPMI-1640 medium supplemented with 10% FBS and 1% antibiotics solution and were maintained in a humidified 5% $CO_2$ incubator at 37 °C. When the cell confluence was reached to 70%, the cells were harvested and seeded on 96-well plates at a density of $10^4$ cells per well containing 200 μl medium and incubated overnight at the same conditions. After exposure to different concentrations of each compound for 48 h, the medium was removed and 100 μl MTT solution (0.5 $mg \cdot ml^{-1}$ in media) was added into each well and the plates were incubated again at 37 °C for 4 h. The medium was then completely removed and the formazan crystals were dissolved in 150 μl DMSO and the absorbance was measured at 570 nm. Each experiment was conducted in triplicate and the results were presented as the mean values obtained from three independent experiments. The cell viability was determined as ratio of absorbance values from each treatment and the control.

### 2.5. Preparation of DNA, HSA, bulk- and nano-scale $PdL_2$ stock solutions for binding experiments

The stock solution of CT-DNA was prepared in 50 mM Tris buffer at pH = 7.5 using double-distilled deionized water and was stored at 4 °C. The CT-DNA concentration per nucleotide was determined using absorption intensity at 260 nm after adequate dilution with the buffer and using the reported molar absorptivity of 6600 $M^{-1} \cdot cm^{-1}$ [44]. Purity of CT-DNA solution was confirmed by ratio of UV absorbance at 260 and 280 nm ($A_{260}/A_{280} = 1.9$), indicating that CT-DNA is free from protein impurity [45]. Also, a stock solution of HSA was prepared by dissolving the desired amount of HSA in 50 mM phosphate buffer (pH = 7). The HSA stock solution was stored at 4 °C in the dark and was used within 2 h. HSA concentration was determined by UV–Vis spectrophotometry using the molar absorption coefficient 35,700 $M^{-1} \cdot cm^{-1}$ at 278 nm [46]. For preparation of $PdL_2$ solution in bulk-scale, appropriate amounts of obtained complex powder was dissolved in DMSO as co-solvent, and then diluted with corresponding buffer to the required concentration for all experiments. The volume of co-solvent never exceeded 5% (v/v), so the effect of DMSO is negligible. Also, the appropriate amount of the nano-scale $PdL_2$ powder was dispersed in Tris and phosphate buffer for DNA and HSA binding experiments, respectively.