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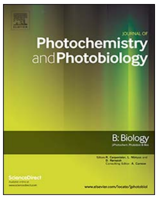

Microwave-assisted synthesis of mixed ligands organotin(IV) complexes of 1,10-phenanthroline and L-proline: Physicochemical characterization, DFT calculations, chemotherapeutic potential validation by *in vitro* DNA binding and nuclease activity

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ABSTRACT

Diorganotin(IV) and triphenyltin(IV) derivatives of L-proline (HPro) having general formula $R_2Sn(Pro)_2$ (R = *n*-Bu (**1**), Ph (**2**)) and $Ph_3Sn(Pro)$ (**3**), respectively, and the mixed ligands di-/triorganotin(IV) derivatives of L-proline and 1,10-phenanthroline (phen) with general formula $[R_2Sn(Pro)(Phen)Cl]$ and $[R_3Sn(Pro)(Phen)]$ (where R = Me (**4** and **7**), *n*-Bu (**5** and **8**), Ph (**6** and **9**)), respectively, have been synthesized by microwave-assisted method and characterized by elemental analysis, IR, NMR (^{1}H, ^{13}C and ^{119}Sn) and DART-mass spectral studies. The results suggest bicapped tetrahedron or a skew trapezoidal-bipyramid geometry for $R_2Sn(Pro)_2$, a distorted tetrahedral geometry for $Ph_3Sn(Pro)$ and a distorted octahedral geometry for $[R_2Sn(Pro)(Phen)Cl]$ and $[Ph_3Sn(Pro)(Phen)]$ around the Sn atom, and the same has been validated by density functional theory calculations (DFT). *In vitro* DNA binding studies of **1**–**9** have been investigated by UV–Vis, fluorescence and circular dichroism titrations, viscosity and DNA melting experiments. The observed hypochromic shift in UV–Vis and fluorescence studies evidenced a partial intercalative mode of binding of complexes to CT-DNA. The binding affinity and quenching ability have been quantified in terms of intrinsic binding constant (K_b) and Stern-Volmer quenching constant (*Ksv*). The determined values suggest that di- and triorganotin(IV) derivatives of L-proline possess lesser affinity to bind with CT-DNA in comparison to the mixed ligands di-/triorganotin(IV) derivatives of L-proline and 1,10-phenanthroline. The partial intercalative mode of binding of these complexes with CT DNA has also been supported by a change in the viscosity and melting point of DNA as well as a change in the intensity of positive and negative bands in circular dichroism spectra. The cleavage studies by agarose gel electrophoresis indicate effective cleavage of supercoiled plasmid DNA into its nicked form by all the complexes and even to its linear form in presence of **9**.

1. Introduction

The initial clinical success of *cis*-platin against several malignant tumours led to the discovery of various other platinum based chemotherapeutic metallopharmaceuticals such as oxaliplatin, carboplatin, picoplatin and nedaplatin [1–5]. However, their severe side effects and inefficiency against platinum resistant tumours stimulated the synthesis of non-platinum chemotherapeutics [6–13]. Among these, organotin (IV) complexes have shown potent *in vivo* and *in vitro* anti-tumour activities and appeared as biologically active metallopharmaceuticals [14–21]. An immense curiosity about the behaviour of these metallic species inside the biological systems, has led to the extended studies on their reactions with different biomolecules [22,23].

Amino acids are potentially bidentate ligands which can link to the metals, and thus, enable the exchange and transport mechanisms of trace metal ions in the human body [24]. Organotin(IV) complexes of amino acids are being extensively studied as possible biocides and as intermediates in peptide synthesis [23,25]. They have gained important pharmacological interest as several of them have shown a wide spectrum of effects including, antibacterial, antifungal, anti-inflammatory and antitumour activity [22,23,26–31]. Proline is the only naturally occurring amino acid with a secondary α-amino group fixed in a ring with its α-carbon atom, which explains its more basic character with respect to various other α-amino acids [32]. Some tri- and diorganotin (IV) derivatives of L-proline have been previously synthesized by our research group and they have shown low toxicity and moderate anti-

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inflammatory activity with no side effect on blood pressure [27]. 1,10-Phenanthroline is a rigid planar, hydrophobic, electron-poor heteroaromatic chelating bidentate ligand. The transition metal complexes of such chelating *N*-containing ligands have been used extensively as probe of DNA structure and conformation because of their photochemical properties [33–36]. It is effective for cancer treatment when used in combination with other antitumor drugs. Adducts of diorganotin dihalides with *N*-donor ligands, which were modelled on active platinum complexes, have exhibited reproducible therapeutic activity *in vivo* against P-388 lymphocytic leukemia in mice [37]. Organotin(IV) compounds were originally designed to emulate the platinum group of drugs, therefore, if it is assumed that they function through direct coordination to DNA, there must be a preferred mode of interaction with DNA which can be intercalative association, DNA groove binding or electrostatic interaction [38].

The eco-friendly and economical microwave assisted method has unparallel advantages over conventional thermal method in terms of shorter reaction time, lesser use of solvent, simple synthesis conditions, higher yield, more purity and easy optimization by rapid feedback [39].

In view of the above considerations and a quest to observe the effect of coordination of bidentate nitrogen ligand on the activity of amino acid-organotin(IV) complexes, encouraged us to synthesize di-/triorganotin(IV) complexes of L-proline (Fig. 1(a)) and their mixed ligand derivatives with 1,10-phenanthroline (Fig. 1(b)) by microwave-assisted method. In literature, there are some complexes of transition metals coordinating with these ligands being reported, such as [Pt(phen)(Pro)]Cl·$2H_2O$ and [Pd(phen)(Pro)]Cl·$2H_2O$ [40,41]. But to the best of our knowledge, there are no reports focusing on the *in vitro* DNA binding studies of the organotin(IV) derivatives with these ligands. Their structures have been proposed based on experimental findings *viz.* elemental analysis, I.R., NMR (^{1}H, ^{13}C and ^{119}Sn), DART-mass spectral studies and the same have been further validated by density functional theory calculations (DFT). Furthermore, to ascertain the mode of interaction of these mixed ligands complexes with DNA, and to know their chemotherapeutic aspects, *in vitro* interaction studies *viz.* absorption spectral titration, EB displacement assay, circular dichroism titration, viscosity and DNA melting measurements with CT-DNA, and cleavage studies with pBR322 plasmid DNA by agarose gel electrophoresis assay have also been carried out.

2. Materials and Methods

2.1. Reagents and Materials

Details of make, purity and other specifications of the reagents and materials used are described in supporting text file (S–I).

2.2. Methods and Instrumentation

The specifications of the instruments and details of methods used for characterization, computational studies and biological studies (DNA binding and gel electrophoresis) are presented in supporting file (S–I).

2.3. Microwave-assisted Synthesis

All the complexes have been synthesized by microwave assisted method. Microwave reactions were performed using a microwave oven model Magicook 20S (Galaxy) Whirlpool with a turntable. The microwave oven has ten power levels, *i.e.* P1 (10%)–P10 (100%) with a maximum output power of 800 W (P10). The syntheses were carried out in an open glass vessel utilizing 400 W (50% output power, P5) for 2 min. A beaker containing 400 mL of water was also kept inside the oven during the irradiation of reactants, and it was refilled after every 2 min. The temperature of reaction was monitored by measuring the temperature of water after 2 min, which was found to be ≈ 80 °C.

2.3.1. Synthesis of Dibutyltin/Diphenyltin(IV) Derivatives of L-Proline

Di-*n*-butyltin(IV) dichloride (0.304 g; 1.0 mmol)/diphenyltin(IV) dichloride (0.344 g; 1.0 mmol) and sodium salt of L-proline (0.274 g; 2.0 mmol) were mixed and grinded thoroughly in a mortar, in 1:2 (metal:ligand) ratio. To this homogeneous mixture, 1–2 mL of specially dried methanol was added. The reaction mixture was irradiated in a microwave oven for 2 min, between 80 and 85 °C, using irradiation power of 400 W. It was cooled to room temperature and then again exposed to the microwave radiations for 2 min. The optimized reaction time for the completion of reaction was 10 min. The products were scratched and recrystallized with methanol-hexane or methanol-petroleum ether (b.p. 40–60 °C) mixture (1:3 v/v), and dried under vacuum. Both of these complexes have also been previously synthesized by our research group [27] by conventional thermal method.

2.3.2. Synthesis of Triphenyltin(IV) Derivatives of L-Proline

Triphenyltin(IV) chloride (0.385 g, 1.0 mmol) and sodium salt of L-proline (0.137 g; 1.0 mmol) were mixed and grinded thoroughly in a mortar, in 1:1 (metal:ligand) ratio. Rest of the procedure is same as described in Section 2.3.1.

2.3.3. General Procedure for Synthesis of Diorganotin(IV) Derivatives of L-Proline and 1,10-Phenanthroline

Di-*n*-butyltin(IV) dichloride (0.304 g; 1.0 mmol)/diphenyltin(IV) dichloride (0.344 g; 1.0 mmol)/dimethyltin(IV) dichloride (0.219 g; 1.0 mmol) with sodium salt of L-proline (0.274 g; 2.0 mmol) and 1,10-phenanthroline monohydrate (0.198 g, 1.0 mmol) were mixed and grinded thoroughly in a mortar, in 1:2:1 (metal: Na salt of L-proline: 1,10-phenanthroline) ratio. Rest of the procedure is same as described in Section 2.3.1. The products were crystallized in 1:1:1 (metal: L-proline: 1,10-phenanthroline) stoichiometry instead of 1:2:1.

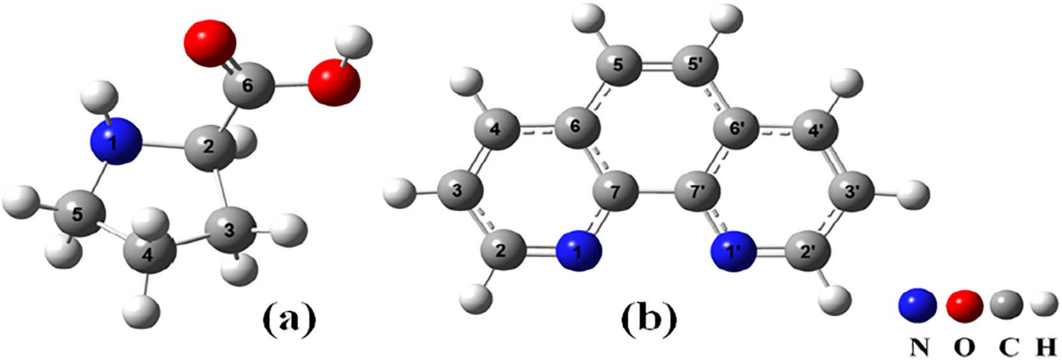

Fig. 1. Structure and numbering scheme of the ligands: (a) L-proline (b) 1,10-phenanthroline.