European Journal of Medicinal Chemistry 73 (2014) 8–17

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

In vitro cytotoxicity studies of palladacyclic complexes containing the symmetric diphosphine bridging ligand. Studies of their interactions with DNA and BSA

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ARTICLE INFO

Article history:
Received 16 October 2013
Received in revised form
18 November 2013
Accepted 24 November 2013
Available online 12 December 2013

Keywords:
Palladacyclic complex
FS-DNA binding
BSA binding
Cytotoxicity
Crystal structure

ABSTRACT

The reactions between $[Pd_2\{(C,N)-C_6H_4CH_2NH(Et)\}_2(\mu\text{-}X)_2]$ (X = Cl or Br) and 1,2-bis(diphenylphosphino)ethane (dppe) in the 1:1 molar ratio resulted in the dppe-bridged Pd(II) complexes, $[Pd_2\{(C,N)-C_6H_4CH_2NH(Et)\}_2(\mu\text{-dppe})(Cl)_2]$ (**1**) and $[Pd_2\{(C,N)-C_6H_4CH_2NH(Et)\}_2(\mu\text{-dppe})(Br)_2]$ (**2**), respectively, which were characterized by elemental analyses, infrared (IR), ^{1}H- and $^{31}P\{^1H\}$ NMR spectroscopy. The molecular structure of **1** was determined by single-crystal X-ray diffraction. *In vitro* cytotoxicity of **1**, **2**, dppe, $PhCH_2NH(Et)$ and cisplatin were carried out against four human tumor cell lines. The interactions of complexes towards DNA and protein are investigated. The results suggested that both complexes could interact with *FS*-DNA through the intercalation mode. Moreover, the reactivity towards BSA revealed that the microenvironment and the secondary structure of BSA were changed in the presence of Pd(II) complexes.

1. Introduction

To date, cisplatin and its analogs are some of the most effective chemotherapeutic agents in clinical use as the first line of treatment in testicular and ovarian cancers [1–6]. Furthermore, these analogs are increasingly used against other tumors, such as cervical, bladder and head/neck tumors. Unfortunately, they have several major side effects. Cumulative toxicities of nephrotoxicity, ototoxicity and tumor resistance related to them have stimulated the search for other antitumor-active metal complexes with improved pharmacological properties [7–12].

Mechanistic investigations of the mechanism of action of Pt(II) anticancer drugs, represent that their Pd(II) analogs are suitable model compounds since they exhibit ca. 10^4–10^5 times higher reactivity, whereas their structural and equilibrium behavior are very similar [13]. Among palladium(II) complexes, special attention has been paid to metallacycle complexes with nitrogen donor ligands, such as various alkyl and aryl substituted amines and imines, azo, hydrazo and heterocyclic compounds. The chelate ring generally possesses three to seven members, with the five-membered ring being most favored. These compounds are used successfully in organic synthesis [14–16], homogeneous and heterogeneous catalysis [17–19], asymmetric synthesis [20], photochemistry [21], optical resolution [22,23], and are rather promising as liquid crystals [24,25] and potential biologically active materials [26–30].

In the development of new such metal-based therapeutics, detailed studies on the interactions between DNA and transition-metal complexes is needed [31]. Depending on the exact nature of the metal and ligand, the complexes can bind with nucleic acid covalently or non-covalently [32,33]. Non-covalent interactions between transition-metal complexes and DNA can occur by intercalation, groove binding, or external electrostatic binding. Therefore, the study on the interaction of the transition metal complexes with DNA is of great significance for the design of new drugs and their application.

It has been found that some ortho-metalated species may bind to DNA by means of intercalative or monofunctional covalent

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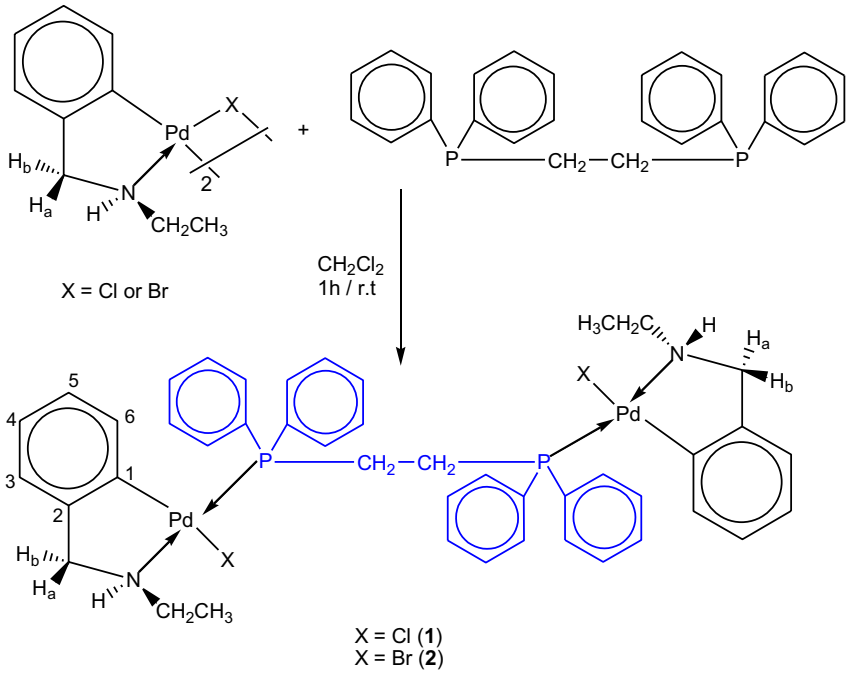
http://dx.doi.org/10.1016/j.ejmech.2013.11.042



Scheme 1. Synthesis of the dppe-bridged palladacyclic complexes **1** and **2**.

interactions [34–36]. In case of palladacycles, proving that their intercalative mode of cytotoxic action is strictly related to the presence of a planar and highly stable aromatic metallacycle [37,38]. It was also reported that some cyclopalladated complexes containing planar structures such as aromatic and aliphatic amines exhibit cytotoxic effects against some tumor cells producing intercalative lesion on DNA [39,40], which drew our attention to study the biological activity of amine palladacycles in the form of biphosphinic complexes **1** and **2** (Scheme 1).

On the other hand, investigation on the effect of metal ions on drug–protein binding is useful to understand the transport and mechanism of the drug in the body [41,42]. Bovine serum albumin (BSA) is a protein with hydrophobic patches that could be the initial targets of their association to biomolecules [43]. Formation of a stable drug–protein complex can exert important effect on the distribution, free concentration and metabolism of the drug in the bloodstream. Thus the drug–albumin complex may be considered as a model for gaining fundamental insights into the drug–protein interactions [44,45].

Enlightened by the above-mentioned facts, it is necessary to design and synthesize new palladium complexes to evaluate their cytotoxicity and reactivity towards DNA and protein. Recently, much efforts have been devoted to the design and synthesis of new Pd(II) complexes and investigate their biological properties. Nevertheless, the studies on the interaction of cyclopalladated complexes with DNA and BSA are very limited.

Regarding to these facts and as a continuation of our ongoing program in the field of design and synthesis of new cyclopoalladated complexes, in this report, two new cyclopalladate–dppe complexes **1** and **2**, were synthesized and fully characterized. The detailed structure of **1** was also determined by X-ray single crystal analysis. We have evaluated the *in vitro* cytotoxic potential (IC_{50}) of the compounds **1** and **2** against the human cervix carcinoma (Hela), colon cancer (HT-29), leukemia cancer (K562) and human breast carcinoma (MCF-7) tumor cell lines. The interactions of the palladacyclic complexes with fish sperm DNA (*FS*-DNA) are investigated by UV absorption and fluorescence spectra. Furthermore, the protein binding ability has been monitored by UV absorption and tryptophan fluorescence quenching experiment in the presence of the complexes using BSA as a model protein.

2. Results and discussion

2.1. Synthesis and spectroscopic characterization

The reactions of the starting material $[Pd_2\{(C,N)-C_6H_4CH_2NH(Et)\}_2(\mu\text{-}X)_2]$ (X = Cl or Br) [46,47] with $Ph_2PCH_2CH_2PPh_2$ (dppe) in a 1:1 molar ratio give the complexes **1** and **2** in good yields. The preparation process of the complexes is very simple, and only one type of compound could be detected by the occurrence of only one precipitate, after the mixture of reactants was stirred at room temperature. This compound was determined as μ-dppe dinuclear palladium complex of the type *trans-**N**,**P***-$[Pd_2\{(C,N)-C_6H_4CH_2NH(Et)\}_2(\mu\text{-dppe})(X)_2]$ (X = Cl (**1**)) or (X = Br (**2**)), in which the symmetric bidentate ligand dppe bridges two identical cyclopalladated units. Both complexes were isolated as white solids, stable at room temperature, soluble in chlorinated solvents such as CH_2Cl_2, $CHCl_3$ and polar, aprotic solvent like DMSO (dimethylsulfoxide). Formation of isolated complexes has been confirmed on the basis of characteristic bands in the IR spectra, elemental analysis, resonance signals in the ^1H-, and $^{31}P\{^1H\}$ NMR, and single crystal X-ray crystallography.

The IR spectra of the complexes showed typical bands at 3193, 3205 cm^{-1}, 3048, 3049 cm^{-1}, 2962, 2966 cm^{-1}, 1100, 1101 $cm^{-1}$and 521, 522 cm^{-1}assigned to ν(N–H), ν(C–H Ph), ν(C–H alph), ν(C–P) and ν(Pd–P) for **1** and **2**, respectively.

The ^1H NMR spectra of **1** and **2** showed that these compounds represent a molecular structure with an apparent center of inversion, which divided the molecules into two symmetrical parts. This is in agreement with the structures proposed for these compounds with symmetric diphosphine ligand i) bridging the two palladium(II) centers and ii) coordinated to the cyclopalladated units with *trans-**N**,**P*** stereochemistry.