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Short communication

Synthesis of platinum(II) and palladium(II) complexes with 9,9-dihexyl-4,5-diazafluorene and their *in vivo* antitumour activity against Hep3B xenografted mice

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ABSTRACT

Two complexes dichloro(9,9-dihexyl-4,5-diazafluorene)platinum(II) (Pt-DHF) and dichloro(9,9-dihexyl-4,5-diazafluorene)palladium(II) (Pd-DHF) were synthesized and their *in vivo* antitumour activity was investigated using an athymic nude mice model xenografted with human Hep3B carcinoma cells. Pt-DHF- and Pd-DHF-treated groups showed significant tumour growth inhibition (with about 9-fold and 3-fold tumour growth retardation) when compared with the vehicle control group. The liver toxicology effects on the animals of the two compounds were investigated. Pt-DHF and Pd-DHF-treated groups had a lower alanine transaminase and aspartate transaminase values than those of the vehicle treated group as the animals from the vehicle control group had very heavy hepatoma burden. We assume that both complexes could be further investigated as effective antitumour agents and it is worthwhile to study their underlying working mechanism.

1. Introduction

Metal complexes are important in the development of antitumour drugs because of the interaction between metal complexes with biomolecules in human body and the high stability of their final products under various conditions. Metal complexes have been employed in antitumour therapy since the discovery of the cytotoxicity of cisplatin (*cis*-$Pt(NH_3)_2Cl_2$) by Rosenberg and co-workers in the late 1960s [1,2]. Cisplatin was the first identified member of the platinum-based chemotherapeutic drugs. It has been applied clinically for more than three decades in oncology [2,3]. The introduction of cisplatin to the antitumour therapy has initiated the design and synthesis of metal complexes including platinum and palladium to improve the therapeutic activity of the

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antitumour drugs.

Pt(II) ion has a strong binding ability to sulfur. After taking the Pt(II)-based drug in the human body, there is a high potential for binding with sulfur-donor biomolecules, which can be found in peptides, proteins and enzymes. The interaction of Pt(II) complexes with S-containing biomolecules can lead to strong cytotoxicity [4,5]. The anti-tumour activity of Pt(II)-based drugs can be due to the interaction between the metal complex and genomic DNA [4,6]. Owing to the structural similarities and significant overlap of the coordination chemistry for palladium and platinum, these two metals are closely related [7]. Palladium related complexes have also been investigated intensively in the field of medicinal chemistry [8,9].

Utku et al. reported the *in vitro* anticancer activity of the synthesized benzimidazole-platinum(II) complexes on the human HeLa (ER−), MCF-7 (ER+) and MDA-MB 231 (ER−) cell lines. Pt(II) complex bearing oxalate leaving ligand possessed the most active anticancer activity, with about two or five-fold greater than those of the other benzimidazole-platinum complexes tested [10]. Kovala-Demertzi and co-workers studied the anticancer property of the palladium(II) complexes synthesized by the reaction of Pd(II) salt with 2-formylpyridine-4-*N*-ethyl-thiosemicarbazone, HFo4NEt. The complex [Pd(H_2Fo4NEt)(Fo4NEt)Cl_2] exhibited a better anticancer activity, with the IC_{50} values against MCF-7 and T-24 cancer cell lines being 8.42 and 5.88 μM, respectively, when compared with the complexes [Pd(Fo4Nethyl)Cl] (IC_{50} = 104.1 and 72.5 μM) and [Pd(Fo4Nethyl)$_2$] (IC_{50} = 14.52 and 7.59 μM) [11]. Motswainyana and co-workers examined the anticancer property of the synthesized palladium(II) and platinum(II) complexes: dichloro[(2-diphenylphosphino-benzylidene)-2-methylphenylen-amine]palladium(II), dichloro[2-diphenylphosphino-benzylidene)-2,6-dimethylphenylen-amine]palladium(II), dichloro-[(2-diphenylphosphino-benzylidene)-2-methylphenylen-amine]platinum(II) and dichloro-[2-diphenylphosphino-benzylidene)-2,6-dimethylphenylen-amine]platinum(II). Both palladium(II) complexes possessed a stronger cytotoxicity towards MCF-7 and HT-29 cancer cell lines (mean IC_{50} = 28.5–48 μM) when compared with the platinum(II) complexes (mean IC_{50} = 50–87 μM) [12]. This team further reported that the synthesized bis(imino-quinolyl) platinum(II) complex displayed a slightly stronger anticancer activity (mean IC_{50} = 55 and 41 μM against MCF-7 and HT-29 cancer cells, respectively) when compared with the bis(imino-quinolyl) palladium(II) complex (mean IC_{50} = 60 and 46 μM) [13]. Oliveira et al. investigated the anticancer activity of complexes containing palladium(II) and platinum(II): [Pd(NH_3)$_4$][Pd(opba)] and [Pt(H_2opba)]·H_2O, where opba = 1,2-phenylenebis(oxamate) against the chronic myelogenous leukemia cell line. The palladium(II) complex [Pd(NH_3)$_4$][Pd(opba)] showed a better growth inhibition against leukemia cells (mean IC_{50} = 19.9 μM) than the platinum(II) complex [Pt(H_2opba)]·H_2O (mean IC_{50} = 27.35 μM) [14].

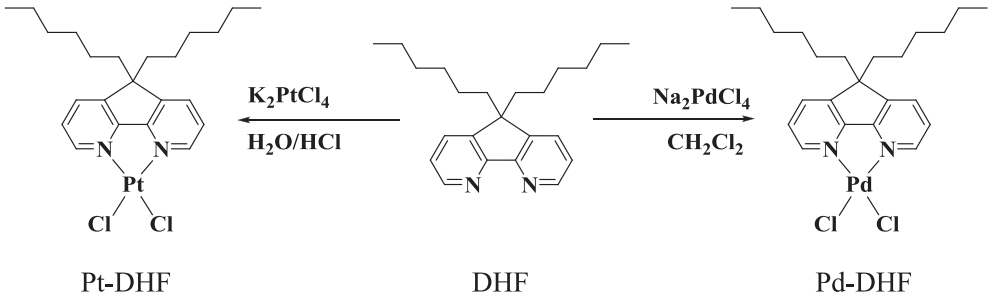
Barbara et al. reported the *in vitro* and *in vivo* biological properties of two bile acid-conjugated platinum(II) complexes: (NH_3)$_2$Pt(triacid) and (PPh_3)$_2$Pt(dehydrocholate)$_2$. (NH_3)$_2$Pt(triacid) showed a stronger growth inhibition on rat hepatoma cells (mean IC_{50} = 0.7 μM) after 48 h when compared with (PPh_3)$_2$Pt(dehydrocholate)$_2$ (mean IC_{50} = 3.8 μM). In a syngeneic and orthotopic rat hepatoma model, the (NH_3)$_2$Pt(triacid)-treated group displayed more than 6-fold tumour weight reduction at the dose of 80 mg/kg as compared to the control group and the (PPh_3)$_2$Pt(dehydrocholate)$_2$-treated group [15].

It appears that most of these studies have focused on *in vitro* anticancer analysis while *in vivo* antitumour studies including tumour size reduction evaluation have been infrequently reported. In our present study, two platinum(II) and palladium(II) complexes with 9,9-dihexyl-4,5-diazafluorene (DHF) derivatives were synthesized and their *in vivo* antitumour activities were investigated using an athymic nude mice model xenografted with human Hep3B carcinoma cells.

2. Results and discussion

2.1. Chemistry

The chemical structures and the synthetic protocols of the novel diazafluorene-based organic compounds are shown in Scheme 1. 9,9-Dihexyl-4,5-diazafluorene (DHF) was prepared using the method reported in the previous paper [16]. Platinum(II)-9,9-dihexyl-4,5-diazafluorene (Pt-DHF) complex was obtained from DHF by treatment with K_2PtCl_4 in water solution in the presence of a catalytic amount of conc. HCl in a high yield of 80% [17], while palladium-9,9-dihexyl-4,5-diazafluorene (Pd-DHF) congener was successfully synthesized using Na_2PdCl_4 in CH_2Cl_2 solution in 86% yield [18]. These air-stable compounds were isolated in high purity as golden or brown solids by column chromatography on silica gel eluting with CH_2Cl_2. These compounds were fully characterized by NMR (both ^1H and ^{13}C) spectroscopy (see Supporting Information) and fast atom bombardment mass spectrometry (FAB-MS). Both of them can afford good single crystals for X-ray diffraction analysis from their solid samples (Figs. 1 and 2). Based on our prior results with 4,5-diazafluoren-9-one as the coordinated ligands which showed very poor solubility in common solvents [18], we have purposely substituted the oxo group with long alkyl chains at the 9-position of fluorene in the present study to tune the solubility and hydrophobility of the metal complexes. The ligand DHF has been shown to possess a certain level of antitumour activity in athymic nude mice xenografted with human Hep3B carcinoma cells [16]. Since metal ions are promising candidates that have been successfully used for anticancer therapy [19], the potential antitumour potency was further investigated after the incorporation of metal ions (Pt and Pd) into the DHF (see Table 1).



Scheme 1. Synthetic profiles of Pt-DHF and Pd-DHF.