Inorganic Chemistry Communications 96 (2018) 106–110

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

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

Short communication

High in vitro anticancer activity of a dinuclear palladium(II) complex with a 2-phenylpyridine ligand

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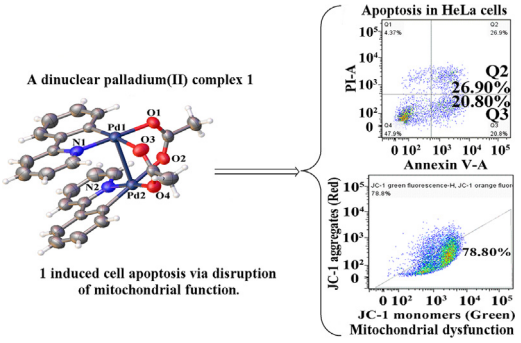
GRAPHICAL ABSTRACT

Apoptosis after treatment with the dinuclear palladium(II) complex **1** may be induced by the intrinsic mitochondrial dysfunction pathway.



ARTICLE INFO

Keywords:
2-Phenylpyridine
A dinuclear palladium(II) complex
Cell apoptosis
Mitochondrial dysfunction

ABSTRACT

A dinuclear palladium(II) complex, $[Pd_2(\mu\text{-OAc-}\kappa O,O)_2(phpy\text{-}\kappa N,C)_2]$ (**1**), with 2-phenylpyridine (H-phpy) as a ligand was synthesized and characterized by IR, ESI-MS, single-crystal X-ray diffraction, and elemental analysis. By the MTT assay, the dinuclear palladium(II) complex **1** showed lower cytotoxic activity toward normal HL-7702 cells (IC_{50} = 85.89 ± 0.89 μM). However, it displayed high cytotoxicity against SK-OV-3, NCI-H460, T-24, and HeLa cells, with IC_{50} values of 5.50 ± 1.08, 0.46 ± 0.28, 7.93 ± 1.16 and 0.35 ± 0.29 μM, respectively. Importantly, we found that this Pd(II) complex exhibited lower toxicity and better anticancer activity than that of cisplatin. Further cellular assays, including Western blotting and flow cytometry, gave evidence that apoptosis after treatment with the dinuclear palladium(II) complex **1** may be induced by the intrinsic mitochondrial dysfunction pathway.

Cisplatin, oxaliplatin, and carboplatin are widely used as anticancer drugs [1,2]. Unfortunately, the use of Pt-based drugs is restricted due to its side effect profile, which includes neurotoxicity, nephrotoxicity and ototoxicity [1–10]. Thus, there remain significant areas for

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https://doi.org/10.1016/j.inoche.2018.08.007
Received 17 June 2018; Received in revised form 2 August 2018; Accepted 6 August 2018
Available online 14 August 2018
1387-7003/

improvement in terms of increased clinical effectiveness, reduced toxicity, and a broader spectrum of action [3]. These opportunities for the development of novel Pt-based drugs have attracted a significant amount of attention in search of new non-platinum based drugs with various ligands [2,3]. Among these non-platinum complexes with potential for the clinical treatment of cancer, Pd(II) complexes have received considerable interest because of the structural similarity between the Pd(II) and Pt(II) complexes [4,5,11–22]. For example, Fong and Che found that [Pd(H-L)(nBu_2NHC)](CF_3SO_3) (**Pd1d**, H-L = 6-phenyl-2,2′-bipyridine, *N*,*N*′-nBu_2NHC = *N*,*N*′-di-*n*-butylimidazolylidene) significantly inhibited tumor growth in a nude mice model and displayed potent cytotoxic activity toward NCI-H1650, NCI-H460, MDA-MB-231, HeLa, A2780, and its cisplatin resistant clone, A2780cis, tumor cell lines (IC_{50} = 0.09–0.5 μM) [23]. Nagesh and co-workers reported that sugar-boronate ester scaffold tethered pyridylimine Pd(II) complexes have shown moderate to good cytotoxicity in both HT-29 and MDA-MB-231 tumor cell lines with IC_{50} values ranging from 4.27 to 34.76 μM [24]. Valentini suggested that a new ionic Pd(II) complex ([(bipy)Pd(Pcurc)][CF_3SO_3]) induces both inhibition of cell growth and apoptosis in human LnCaP, PC3, and DU145 tumor cells via JNK phosphorylation and the production of ROS [25].

A series of 2-phenylpyridine and its derivative metal complexes with good anticancer properties and potentially powerful luminophores have been reported, including luminescent cyclometalated Pt(II) complexes [26], 4,7-diphenyl-1,10-phenanthroline-bis(2-phenylpyridine) Ir(III) hexafluorophosphate [27], luminescent dialkynylgold (III) complexes [28], a cyclometalated iridium dinuclear complex [$(ppy)_2$Ir(μ-L)Ir$(ppy)_2]^{2+}$ [29], mixed 2-phenylpyridine and 5-substituted-8-hydroxyquinolines palladium(II) complexes [30], trifluoromethyl-substituted 2-phenylpyridine [31], binuclear and mononuclear ortho-palladated complexes [32], amphiphilic Ir complex–cationic peptide hybrids [33], and more. In addition, palladium-catalyzed C-H alkylation of 2-phenylpyridines with alkyl iodides has been successfully developed [34,35]. However, less is known about a dinuclear palladium(II) complex bearing a 2-phenylpyridine ligand, and detailed studies on their cellular mechanisms of action are lacking.

In the present study, a dinuclear palladium(II) complex, [Pd_2(μ-OAc-κO,O)$_2$(phpy-κN,C)$_2$] (**1**), with 2-phenylpyridine (H-phpy) as a ligand was synthesized and characterized by IR, ESI-MS, single-crystal X-ray diffraction analyses, and elemental analysis. The biological activity and mechanisms of action of the dinuclear palladium(II) complex **1** were studied in HeLa cells.

The reaction of 2-phenylpyridine (H-phpy) ligand (0.016 g, 0.10 mmol) with $Pd(OAc)_2$ (0.023 g, 0.15 mmol) in ethanol (3.0 mL) and $CHCl_3$ (2.0 mL) at 100 °C for 24 h afforded the dinuclear palladium (II) complex [Pd_2(μ-OAc-κO,O)$_2$(phpy-κN,C)$_2$] (**1**) in 98.3% yield (Scheme S1). In addition, the structure of the synthesized dinuclear palladium(II) complex **1** was determined by IR, ^{1}H NMR, elemental analysis, ESI-MS, and X-ray crystallography (Figs. 1 and S1–S3). Furthermore, the dinuclear palladium(II) complex **1** was stable for 48 h in 10 mM pH 7.35 Tris-HCl buffer solution by ESI-MS spectroscopy (Fig. S3).

As shown in Fig. 1, single-crystal diffraction analysis revealed that a dinuclear Pd(II) complex **1** consists of binuclear [Pd_2(μ-OAc-κO,O)$_2$(phpy-κN,C)$_2$] moieties. In addition, the crystal structure of the dinuclear Pd(II) complex **1** consists of discrete binuclear species. The two palladium(II) atoms are doubly bridged by two OAc ligands with a μ_2-O/O bridging mode via the negatively charged O and the carbonyl O atoms. The two phpy ligands demonstrate a μ_2-N/O bridging mode via the negatively charged C and one of the N atoms (Fig. 1). The bridging of two Pd(II) metal centers results in a significantly shortened Pd–Pd distance of 2.8784(3) Å (Tables S1–S3), which is similar to that of [Pd_2(μ-barb-κN,O)$_2$(ppy-κN,C)$_2$] (barb = 5,5-diethylbarbiturate, ppy = 2-phenylpyridine), [(bpy)Pd(l-MeT)$_2$Pd(bpy)]$^{2+}$ (l-MeT = l-methylthymine) and [{Pd(μ-sac)(ppy)}$_2$] (sac = saccharinate), respectively [36–38].

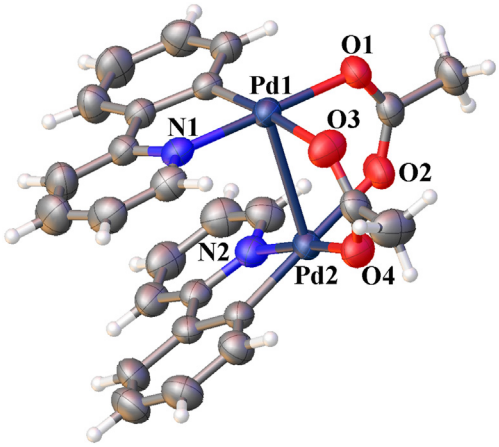

Fig. 1. The molecular structures of the dinuclear palladium(II) complex [Pd_2(μ-OAc-κO,O)$_2$(phpy-κN,C)$_2$] (**1**).

$Pd(OAc)_2$, cisplatin, 2-phenylpyridine (H-phpy), and its dinuclear Pd(II) complex **1** were tested for their cytotoxicity against human ovarian cancer cells (SK-OV-3 cells), NCI-H460, bladder cancer cells (T-24 cells), HeLa cells, and normal hepatocytes cells (HL-7702 cells) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT assay) [39–42]. The dinuclear Pd(II) complex **1** displayed higher cytotoxic activity toward SK-OV-3, NCI-H460, T-24, and HeLa cells, with IC_{50} values ranging from 0.35–7.93 μM (Tables S4 and S5). It showed a lower inhibitory effect on the cell growth of normal HL-7702 cells. Remarkably, the dinuclear Pd(II) complex **1** was more toxic than 2-phenylpyridine (H-phpy), $Pd(OAc)_2$, and cisplatin toward all of the selected human tumor cells. For instance, the dinuclear Pd(II) complex **1** was more effective against HeLa tumor cells, and exhibited IC_{50} values (0.35 ± 0.29 μM) in HeLa cells that were approximately 50.2, 42.9 and 42.9 fold less than that of the free 2-phenylpyridine (H-phpy), cisplatin and 6-amino-oxoisoaporphine Pd complex (15.22 ± 0.98 μM) [43], respectively.

To understand the uptake and distribution of cisplatin (15.02 μM) and the dinuclear Pd(II) complex **1** (0.35 μM) in HeLa cancer cells, an ICP-MS assay was performed to quantify the amount of Pd and Pt taken up by these cancer cells [43–47]. As shown in Fig. 2, treated of HeLa cells with the dinuclear Pd(II) complex **1** ((11.26 ± 0.25 nmol Pd)/10^6 cells) led to notably higher cellular accumulation of Pt than treated with cisplatin ((6.59 ± 0.56 nmol Pt)/10^6 cells) and 6-amino-oxoisoaporphine Pd complex ((3.48 ± 0.26 nmol Pd)/10^6 cells) [43], respectively. In addition, the dinuclear Pd(II) complex **1** (0.35 μM) was more accumulated in the mitochondrial fraction and the nuclear fraction than that of cisplatin (15.02 μM) and 6-amino-oxoisoaporphine Pd complex (15.00 μM) [43], which was likely related to the apoptotic pathways that they activated [43–47].

Because of the dinuclear Pd(II) complex **1** (0.35 μM) mainly accumulated in the mitochondrial fraction, thus cytotoxic compounds can induce cell apoptosis via mitochondrial disruption, which is associated with increased production of mitochondrial ROS [48,49]. Thus, flow cytometry analysis of ROS generated by the dinuclear Pd(II) complex **1** with HeLa cells was performed using the non-polar cell-permeable dye, 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) [50–52]. As shown in the Fig. 3A, the dinuclear Pd(II) complex **1** (0.18, 0.35 and 0.70 μM) produce elevated levels of ROS in HeLa cells as compared with the control group, which also demonstrated that the dinuclear Pd(II) complex **1** caused stronger accumulation of ROS level in HeLa cancer cells than 6-amino-oxoisoaporphine Pd complex (15.00 μM) [43], consistent with the results of in vitro cytotoxicity and cellular uptake assays.

It is well-known that Ca^{2+} fluctuations in cancer cells could disrupt