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Authors: Chengken Chen, Chunmei Gao, Zigao Yuan, Yuyang Jiang

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Communication

Design, synthesis and biological evaluation of a novel platinum(II) complex possessing bioreductive groups for cancer therapy

Chengken Chen[a,b], Chunmei Gao[c], Zigao Yuan[b,e,*], Yuyang Jiang[b,d,e,**]

[a] *Department of Chemistry, Tsinghua University, Beijing 100084, China*

[b] *The Ministry-Province Jointly Constructed Base for State Key Lab-Shenzhen Key Laboratory of Chemical Biology, The Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China*

[c] *College of Chemistry and Chemical Engineering, Shenzhen University, Shenzhen 518060, China*

[d] *Department of Pharmacology and Pharmaceutical Sciences, School of Medicine, Tsinghua University, Beijing 100084, China*

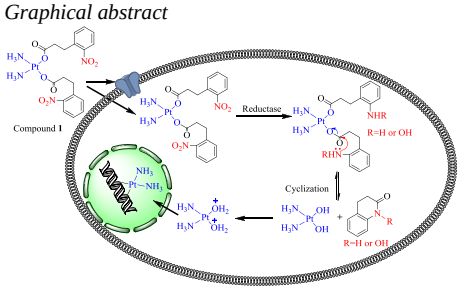
[e] *Shenzhen Kivita Innovative Drug Discovery Institute, Shenzhen 518055, China*

[*] Corresponding authors at *Shenzhen Kivita Innovative Drug Discovery Institute, Shenzhen 518055, China*.

[**] Corresponding authors at *the Ministry-Province Jointly Constructed Base for State Key Lab-Shenzhen Key Laboratory of Chemical Biology, The Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China.*

E-mail addresses: yzg12@tsinghua.org.cn (Z. Yuan), jiangyy@sz.tsinghua.edu.cn (Y. Jiang).

Graphical abstract



A Pt(II) complex possessing bioreductive groups was constructed and evaluated as a more potent antitumor agent than cisplatin.

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ABSTRACT

Cisplatin is one of the most successful antitumor agents, yet also restricted by its poor cellular uptake and low selectivity. Since 3-(2-nitrophenyl) propionic acid (NPPA) has been reported as a bioreductive prodrug moiety, herein we combined NPPA with cisplatin (compound **1**) to improve its lipophilicity and targetability and then to improve the antitumor outcomes. In addition, compound **2** possessing 3-phenyl propionic acid (PPA) was also synthesized as a comparison to test the influence of the NPPA to the cytotoxicity, since PPA was not a bioreductive moiety. Bioevaluations showed that **1** displayed more potent antitumor potency than cisplatin and **2**, suggesting Pt(II) complexes possessing NPPA groups may be a good strategy for future platinum drug discovery.

Platinum-based drugs, such as cisplatin, carboplatin and oxaliplatin, are used worldwide in clinical cancer therapy (Fig. 1A) [1,2]. As one of the most successful anticancer drugs, cisplatin can be taken up by cells and bond with DNA to form DNA adducts, causing DNA damage and resulting in cell death, so it is used to fight against a wide spectrum of solid neoplasms like ovarian, testicular, bladder, colorectal, lung, and head and neck cancers [3]. However, due to the high activity of chloride ions, platinum(II) complexes are instable and easily combined with nucleophiles such as glutathione, metallothioneins and other sulfur-containing amino acids, leading to drug resistance [4-6]. Moreover, cisplatin has low lipophilicity, which leads to its low uptake. In addition, cisplatin can cause serious adverse effects including dose-limiting nephrotoxicity, ototoxicity, as well as nausea and vomiting. Although some strategies have been explored to develop novel platinum drugs (*e.g.*, polynuclear Pt(II) complexes, Pt(IV) prodrugs) with reduced toxicities and improved stability [7-11], it is still necessary to develop new approaches for platinum drugs.

Due to the rapid growth of cancer cells and the abnormal angiogenesis within them, solid tumors usually cause a hypoxic microenvironment, which could be practically targeted by bioreductive prodrugs [12-15]. Up to now, several types of bioreductive prodrugs have been developed, including nitro groups, quinones, *N*-oxides and transition metals (Fig. 1B) [16-19]. Nitrophenylalkanoic acid has been recognized as a good bioreductive prodrug moiety [20], its derivatives are nontoxic or weakly toxic in normal tissues, but decompose under convertible reduction and release antitumor agents [21,22]. Complexes containing nitrophenylalkanoic groups show high selectivity to hypoxic cells [23,24]. For example, bioreductive paclitaxel prodrug (NPPA-PTX), which is stable in PBS and rat plasma as well as in the blood circulation, can be completely converted to the active PTX in hypoxic tumor tissues and then exhibits its antitumor activity [25]. Furthermore, researches have proposed that the activation process tends to be triggered in an acidic environment, which suggests that it is more likely to be reduced at a low pH in tumor tissues [26].

Considering the aforementioned information, and in continuation of our interest in the development of new anticancer compounds [27-31], herein we intend to combine nitrophenylalkanoic acid and cisplatin to develop novel bioreductive Pt(II) drug and compound **1** containing 3-(2-nitrophenyl) propionic acid (NPPA) was constructed. Besides, to assess the advantage of bioreductive reactivation for antitumor potency, compound **2** containing 3-phenyl propionic acid (PPA) was also synthesized as a control (Fig. 2A). We proposed that the nitrophenylalkanoic ester group in compound **1** would be helpful to improve lipophilicity and hypoxia-targeting ability, thereby enhancing the cellular uptake and improving antitumor potency compared to that of cisplatin.

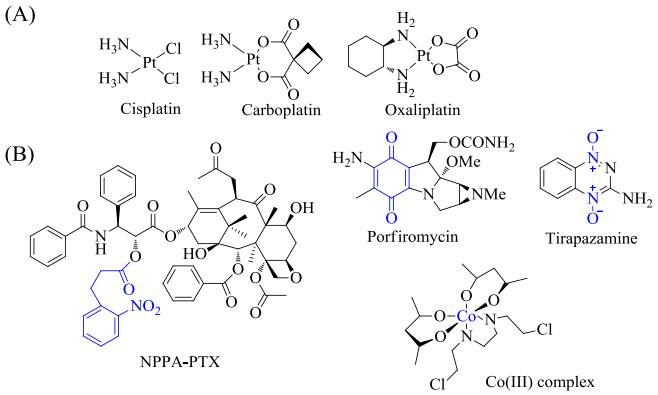


Fig. 1. (A) Chemical structures of the clinically used platinum drugs. (B) Chemical structures of bioreductive prodrugs.

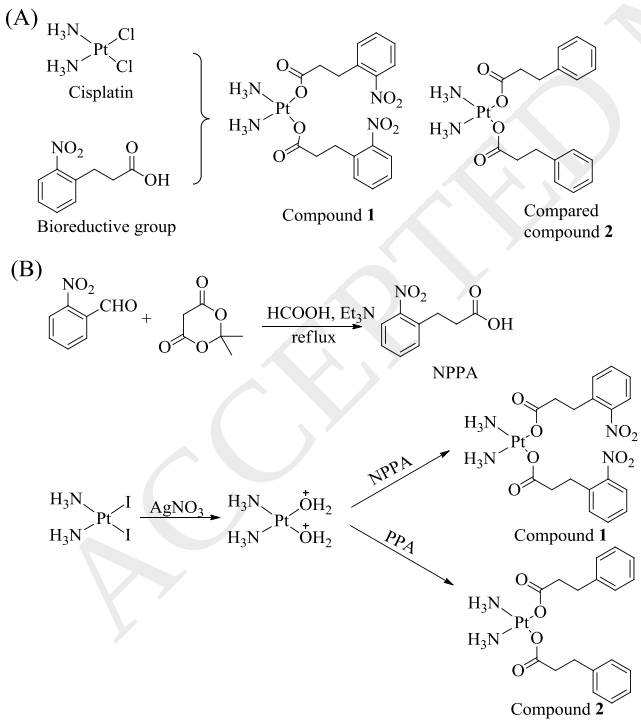


Fig. 2. (A) Chemical structures of target compounds **1** and **2**. (B) Synthesis route of compounds **1** and **2**.

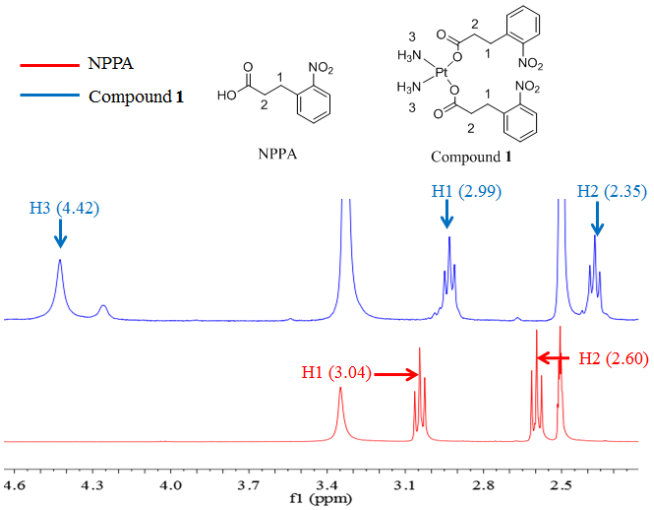


Fig. 3. ^{1}H NMR of complexes NPPA and its product **1.**

The synthesis of target compounds **1** and **2** was shown in Fig. 2B. Firstly, 3-(2-nitrophenyl) propionic acid (NPPA) was synthesized according to the reported method [32]. Secondly, **1** and **2** were synthesized *via* the combination of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with NPPA sodium or PPA sodium. NMR spectroscopy has been applied to characterize the structures of Pt(II) complexes **1** and **2**. As shown in Fig. 3, it is obvious that the chemical shifts of H1 and H2 on NPPA are 3.04 and 2.60 ppm respectively, but they are 2.99 and 2.35 ppm in complex **1**, respectively, which indicated that NPPA has been added to the Pt(II) anion [33]. Also, the appearance of NH_3 signal and the disappearance of OH signal on NPPA further confirmed the structure of complex **1**. HPLC was used to measure the purity of complexes **1** and **2** (Figs. S7 and S11 in Supporting information). All the electrospray ionization mass (ESI-MS) spectra of the complexes (Figs. S6 and S10 in Supporting information) gave main peaks corresponding to $[M+Na]^+$ or $[M-H]^-$ ions, which composed of a few isotopic peaks owing to the presence of platinum isotopes, suggesting the successful synthesis of **1** and **2**.

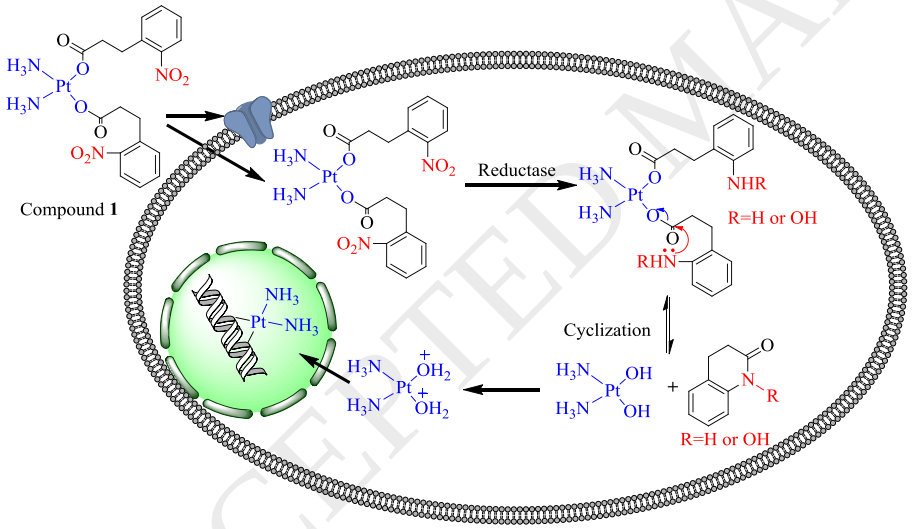


Fig. 4. Possible activation mechanism of compound **1** before and after it enters the cells.

ESI-MS is a highly sensitive method to investigate the binding ability of compounds and DNA. As we know, the preferred DNA binding site of cisplatin is the N7 position of guanine bases [34,35]. To investigate whether **1** could display the similar DNA binding activity as cisplatin, the binding of **1** to 2'-deoxyguanosine (2'-dG) as a DNA analogue was analyzed by ESI-MS, with cisplatin as a positive control. In the presence of nitroreductase (NTR) and NADPH only Pt/2'-dG-bisadduct could be found after 24 h (Fig. S12 in Supporting information). In comparison, both Pt/2'-dG-bisadduct and Pt/2'-dG-monoadduct were observed in 2'-deoxyguanosine with cisplatin or **1** after 2 days (Figs. S13 and S14 in Supporting information), which indicated the ability of cisplatin and **1** binding to DNA and supported that guanine bases were the target position of cisplatin and **1**, and also suggest that NTR can accelerate the decomposition of **1** and improve the binding ability with DNA.

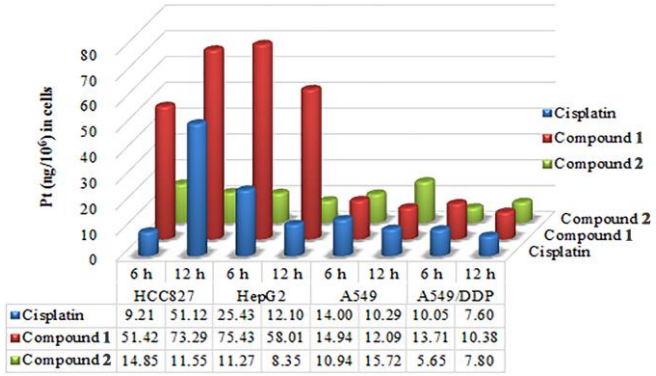
The proposed activation mechanism of compound **1** in cells was shown in Fig. 4. Compound **1** can be taken up by cells *via* active transport and passive permeability [36,37], then the nitro groups of **1** are reduced by reductase such as NTR and gradually generate hydroxylamine or amino derivatives [25,38,39]. The nitrogen atom of hydroxylamine or amino derivative can attack its carbonyl group, ultimately releasing *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ through intramolecular cyclization. To investigate whether the reductive products were generated, we dissolved compound **1** in water and then treated it with NTR and NADPH at 37 °C for 24 h. The resulting mass

spectrum from LC-MS (Figs. S15 and S16 in Supporting information) showed the appearance of reductive products (3,4-dihydro-2(1*H*)-quinolinone and its hydroxylamine derivative). Notably, we also found the signal of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, indicating the successful activation of compound **1** by NTR.

Table 1
Cytotoxicity of compound **1** and **2** against several cancer cell lines.

Complex	IC_{50} (μmol/L)[a]				RF[c]
	HepG2	Hcc827	A549	A549/CDDP	
Cisplatin	48.54 ± 2.27	28.10 ± 0.87	23.11 ± 0.67	67.02 ± 1.71	2.90
1	7.56 ± 0.58	9.15 ± 0.19	33.26 ± 0.65	30.22 ± 1.22	0.91
2	16.88 ± 0.84	25.53 ± 0.84	31.91 ± 0.95	42.39 ± 2.87	1.33
NPPA	>100	ND[b]	>200	ND[b]	ND[b]

[a] Compound concentration required to inhibit cell proliferation by 50%.
[b] Undetectable.
[c] The ratio between IC_{50} values calculated for the resistant cells and those obtained with the sensitive ones.



	6 h	12 h	6 h	12 h	6 h	12 h	6 h	12 h
	HCC827		HepG2		A549		A549/DDP	
Cisplatin	9.21	51.12	25.43	12.10	14.00	10.29	10.05	7.60
Compound 1	51.42	73.29	75.43	58.01	14.94	12.09	13.71	10.38
Compound 2	14.85	11.55	11.27	8.35	10.94	15.72	5.65	7.80

Fig. 5. Platinum contents of cisplatin, **1** and **2** in cancer cells after 6 h and 12 h.

The cytotoxicity of compounds **1** and **2** against several cancer cells was evaluated by the MTT method with cisplatin and NPPA as controls. The IC_{50} values (the concentration of compounds which causes death in 50% of cells) were shown in Table 1. After 72 h incubation, NPPA displayed hardly cytotoxicity against all tested cells, while **1** and **2** showed better or similar cytotoxicity against all tested cancer cells compared to cisplatin. In particular, **1** was about 6.4 and 3.1 times more effective than cisplatin against HepG2 and HCC827 cells, respectively. Although **1** and **2** had higher IC_{50} values compared to that of cisplatin against A549 cells, they showed better cytotoxicity against A549/DDP cells than cisplatin with RF values of 0.91 and 1.33 compared to 2.90, respectively. As a whole, the cytotoxicity of **1** is more potent than **2**, which indicated that the bioreductive activity may contribute much to the cytotoxicity, as **2** can only be hydrolyzed but not bioreducible. Compound **2** displayed better anti-proliferative activity than cisplatin, which might attribute to the improved lipophilicity compared to cisplatin by introducing phenylpropionic acid group. All these results indicated the success of our design.

In order to certify that our compounds had improved lipophilicity compared with cisplatin, the Pt contents of cisplatin, **1** and **2** in all tested cells were analyzed using ICP-MS after 6 h or 12 h of incubation. As shown in Fig. 5 and Table S1 (Supporting information), the Pt contents of **1** had a high value (51.42 ng/10^6 cells) in HCC827 cells after 6 h, and had a great increase after 12 h. In comparison, cisplatin showed a lower value in HCC827 cells after 6 h, and reach the value of over 50 ng/10^6 cells after 12 h. Also, the Pt content of **1** in HepG2 cells is 3.0 times higher than that of cisplatin after 6 h, and can keep in a high level after 12 h. It meant **1** can be quickly taken up by HCC827 and HepG2 cells than cisplatin. In addition, the platinum contents of all three complexes in A549 and A549/CDDP were kept in a low value, which indicated a low availability against these two cells.

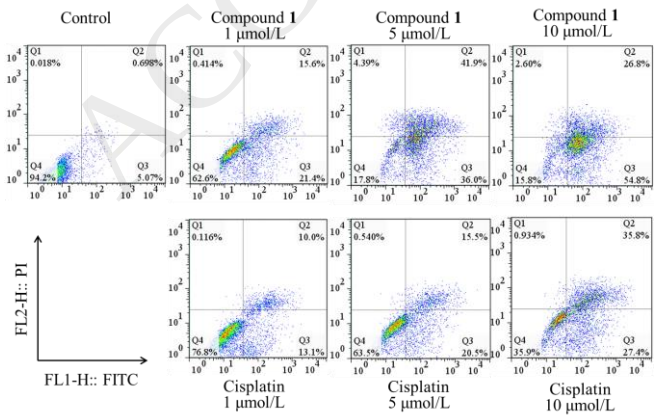


Fig. 6. Representative flow cytometric diagrams of apoptotic HepG2 cells after 72 h treatment with cisplatin and **1**.

In order to evaluate whether compound **1** could induce apoptosis like cisplatin does, we then conducted an Annexin-V/PI binding assay. HepG2 cells were treated with **1** for 72 h at different concentrations, with cisplatin as a positive control. As shown in Fig. 6, a few (5.07 %) apoptotic cells were present in the negative control group (DMSO). The apoptosis population rose to 37.0 %, 77.9 % and 81.6 % after treatment with **1** at 1 μmol/L, 5 μmol/L and 10 μmol/L, respectively. However, cisplatin only induced 23.1 %, 36.0 % and 63.2 % apoptosis populations at 1 μmol/L, 5 μmol/L and 10 μmol/L, respectively. It meant **1** is a more potent proapoptotic agent than cisplatin.

In this study, we proposed a novel strategy by introducing the bioreductive group NPPA into Pt(II) compounds, and then designed and synthesized two target compounds. Compound **1** showed better cytotoxicity, higher cellular uptake and more potent proapoptotic potency in HepG2 cells than that of cisplatin and **2**, indicating that Pt(II) complexes containing bioreductive groups can be an alternative strategy for novel platinum drug development, and compound **1** represents a good lead compound for further antitumor drug discovery.

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