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Combination of 7-hydroxycoumarin in a platinum(IV) complex derived from cisplatin enhanced cytotoxicity with multiple mechanisms of action

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ABSTRACT

A novel compound, Cou-platin, composed of 7-hydroxycoumarin and a platinum(IV) moiety derived from cisplatin was designed and synthesized. Significantly, Cou-platin exhibited more potent in vitro antitumor activity against all tested cancer cell lines than that of cisplatin, which was mainly attributed to the liberation of cisplatin and 7-hydroxycoumarin upon reduction with a biomolecular agent. Besides, cellular accumulation of Cou-platin was dramatically increased among several cancer cells in contrast to cisplatin. Flow cytometry study revealed that Cou-platin arrested cell cycle at G2 phase and induced cell apoptosis. Western blots results indicated that it not only activated cell apoptosis pathway, but also inhibited extracellular regulated protein kinases/mitogen-activated protein kinase pathway. In vivo tests showed that Cou-platin, at equimolar dose to cisplatin, could inhibit tumor growth in nude mouse HCT116 tumor xenograft models almost as cisplatin and oxaliplatin, but with less toxicity.

1. Introduction

Cisplatin has become one of the most popular agents in cancer chemotherapy since it was approved by FDA in 1978. After that, other cisplatin analogues, carboplatin and oxaliplatin have been successively approved (Fig. 1). Principal indications for cisplatin are metastatic testicular cancer, metastatic ovarian cancer, and transitional bladder cancer [1]. By cross-linking DNA and inhibiting DNA transcription [2], cisplatin can kill cancer cells in a relative high level and cure more patients compared with the drugs used before [3,4]. However cisplatin faces some embarrassments, such as drug resistance and side effects. Meanwhile, it has negligible effect on some certain kinds of cancer in vivo. Therefore, development of new platinum based anticancer agents with higher anticancer activity but less toxicity is very meaningful.

Platinum(IV) complexes with an octahedral geometry showed great promising due to their improved cytotoxicity and relatively lower toxicity [5–10]. Moreover, it has been reported that some platinum(IV) complexes were sensitive to some cisplatin-resistant cancer cell lines [11,12], indicating that platinum(IV) complexes can overcome cisplatin resistance. Usually, platinum(IV) complexes were generally designed as prodrugs by modification at the axial position with different functional groups, which may improve the anticancer efficacy, kinetic performance or selectivity of the platinum(IV) complexes against certain cancer cells [13–17].

The extracellular regulated protein kinases (ERK)/mitogen-activated protein kinase (MAPK) pathway plays a central role in regulating cell growth, proliferation and survival. Hence, ERK/MAPK pathway has emerged as a promising target for tumor therapy [18,19]. Coumarins, a wide class of natural compounds, are well known for their various biological functions, inclusive of antiviral [20], anti-inflammatory [21] and anticancer activities [22]. It has been found that some 7-hydroxycoumarin derivatives can induce cancer cell apoptosis through ERK/MAPK signaling pathway [23].

Based on the above, we herein report a novel dual-targeting platinum(IV) anticancer prodrug named Cou-platin, *cis*,*cis*,*trans*-{Pt$(NH_3)_2Cl_2(OH)$[2-((2-oxo-2H-chromen-7-yl)oxy)acetate]}, by introducing 7-hydroxycoumarin to the axial position of *cis*,*cis*,*trans*-[Pt$(NH_3)_2Cl_2(OH)_2$] derived from cisplatin, whose anticancer activities and underlying mechanisms were studied.

2. Results and discussion

2.1. Synthesis and characterization of Cou-platin

The preparation of Cou-platin is shown in Scheme 1. 7-Hydroxycoumarin was first treated with a linker, bromoacetic acid to produce

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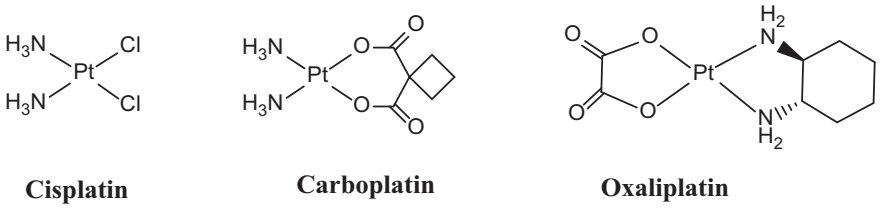
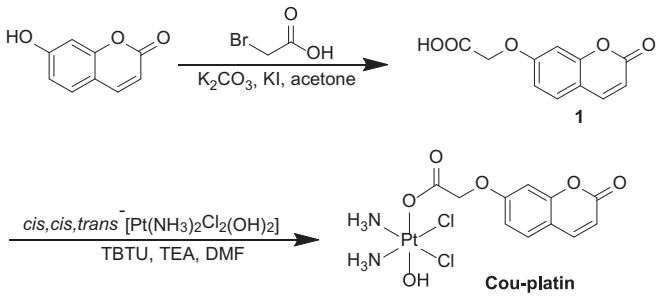


Fig. 1. FDA approved platinum(II) anticancer agents.



Scheme 1. Synthesis of the target compound, Cou-platin.

intermediate **1**. By taking the carboxylic acid of the linker, Cou-platin was then prepared by treatment of *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OH)_2]$ with equivalent molar amount of intermediate **1** in the presence of O-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) and triethylamine (TEA) in dried DMF. The final product of Cou-platin was characterized by microanalysis, ^{1}H NMR and ^{13}C NMR spectra together with ESI-MS mass spectrometry. The purity of Cou-platin was determined to be > 97% by reverse-phase HPLC (Fig. S1).

2.2. Reduction and stability study of Cou-platin

Ascorbic acid and glutathione are overexpressed in cancer cells [24], which gives platinum(IV) based anticancer drugs a target point so that they could be reduced by ascorbic acid or glutathione and release active free drugs. As Cou-platin was expected to be reduced by biomolecule such as ascorbic acid or glutathione, the reduction of Cou-platin was investigated in the presence of ascorbic acid by HPLC. As shown in Fig. 2, the reduction reaction of Cou-platin by ascorbic acid proceeded gradually. It was noted that the compound released from Cou-plaitn was 7-hydroxycoumarin instead of intermediate **1** as referring to the HPLC diagram of 7-hydroxycoumarin (Fig. S2), this might arise from the instability of phenolic ether bond in the presence of nucleophilic agents. Actually, the solution behavior of platinum(IV) complexes is very complicated because not only the reduction occurs at the axial position, but also the hydrolysis at the equatorial position also happens in the aqueous solution [16]. Despite cisplatin was not found due to its weak chromophore under the ultraviolet detecting condition in Fig. 2, those results indicated that Cou-platin as a platinum(IV) based anticancer prodrug could be reduced by ascorbic acid to release cytotoxic agents, which was consistent with our design. However, the specific reduction mechanism of Cou-platin needs further investigation in future.

The stability of Cou-platin was also studied by HPLC under both phosphate buffered saline (PBS, pH = 7.4) and biological conditions. As shown in Fig. S3, Cou-platin was stable in PBS solution for 12 h, and no disassociation was observed. However, no useful information was obtained in the solution of cell culture medium, because the peak of Cou-platin was overlapped by the constituent components of the medium, and we could not find a suitable condition to separate them for further analysis (Fig. S4).

2.3. In vitro cytotoxicity

The in vitro cytotoxicity of Cou-platin was evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against six human cancer cell lines: HepG2 (hepatoma), HCT116 (colon), A549 (lung), MCF7 (breast), SGC7901 (stomach), SGC7901/cis (cisplatin resistant) and HUVEC (human umbilical vein endothelial cells) with cisplatin and 7-hydroxycoumarin as positive controls. The corresponding half maximal inhibitory concentration (IC_{50}) values are given in Table 1. As predicted, the cytotoxicity of 7-hydroxycoumarin was negligible. It is noted that Cou-platin showed superior cytotoxicity to cisplatin against all tested cancer cell lines. Particularly, Cou-platin showed significantly enhanced inhibitory effect against HCT116 cells with a 30-fold higher cytotoxicity than cisplatin. Besides, Cou-platin also exhibited increased toxicity against HepG2, SGC7901 and SGC7901/cis cancer cells with 29-fold, 23-fold and 19-fold lower IC_{50} values than cisplatin, respectively. Despite Cou-platin showed considerable anti-proliferation activity against cisplatin resistant SGC7901/cis cells but it did not overcome the drug tolerance. The resistance index of Cou-platin was calculated as 5.37, which was higher than that of cisplatin (resistance index = 4.38). However, the IC_{50} value against HUVEC cells of Cou-platin was 4-fold higher than that of cisplatin, suggesting that, in contrast to cisplatin, Cou-platin may be selectively cytotoxic for human cancer cells. These in vitro results encouraged us to further explore the mechanism of inhibitory effect of Cou-platin upon cancer cells.

2.4. Cellular accumulation

To figure out the underlying mechanism of the considerable cytotoxicity of Cou-platin, inductively coupled plasma mass spectrum (ICP-MS) was used to evaluate the cellular accumulation of Cou-platin in cancer cells. As shown in Fig. 3 and Table 2, platinum amount in HCT116 cells treated with Cou-platin was 1232 ± 65 ng/(10^6 cells), which was 37-fold higher than that of cisplatin. In other two cancer

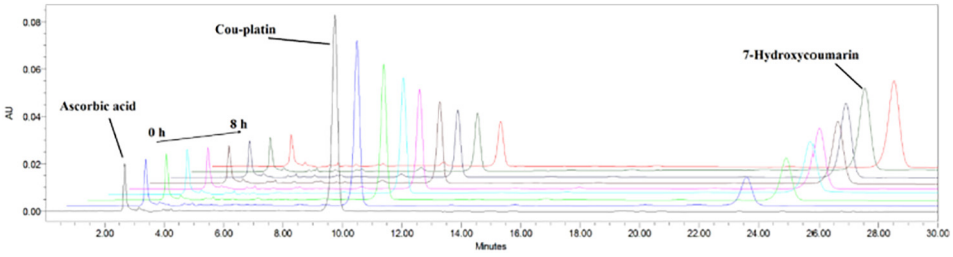


Fig. 2. HPLC analysis of Cou-platin incubated with ascorbic acid.