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Anticancer effect and mechanism of a Se-modified porphyrin Au(III) complex

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ABSTRACT

Au, Se and porphyrin are widely used components in the design of anticancer drugs, but their combination has never been referred to. In this work, a Se-modified porphyrin Au(III) complex, [AuTPP-Se]Cl, was designed and synthesized as a potential anticancer agent. This compound exhibits remarkable antiproliferative activity on all the six tested cancer cells. Its potency on HepG2 is even ten times higher than that of CDDP. The synergistic action among Au, Se and porphyrin components was validated. Mechanism study showed that both the induction of mitochondria-dependent apoptosis and the arrest of cell cycle contribute to the anticancer activity of [AuTPP-Se]Cl.

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The therapeutic value of Au has been recognized thousands of years ago, and its rational use in medicine began in early 1920s.[1] Because Au(III) is isoelectronic with Pt(II) and tetracoordinated Au(III) complexes are in the same square–planar geometries as cisplatin, the potential anticancer properties of Au(III) complexes have been investigated in the recent three decades.[2–4] A number of Au(III) complexes have been reported to exhibit inhibitory effect against a broad spectrum of tumor cells, and their potencies (IC_{50} values in low micromolar range) are even comparable with that of cisplatin.

As an essential micronutrient element, Se also plays an important role in anticarcinogenesis, antioxidation and immunoenhancement. It has been reported that many Se containing compounds exhibit anticancer activity in both in vivo and in vitro experiments.[5] The supplementation of Se is proven to be useful in the clinical treatment of different types of cancer.

Although Au and Se compounds possess anticancer activity, their high toxicity would restrict their clinical application. Combining these compounds with target components is highly possible to facilitate their accumulation in tumor tissues and thus enhance their performance and reduce their side effects. On the other hand, porphyrins and their complex with metal ions present character assembly in cancer cells. Taking advantage of this property, a series of porphyrin and metal porphyrin derivatives have been developed in the past decades as sensitizers in photodynamic therapy (PDT).[6–9] Therefore, in this work, a Se-modified porphyrin Au(III) complex, [AuTPP-Se]Cl (structure shown in Scheme 1), was designed and synthesized as a potential antitumor agent. This compound shows high antiproliferative activity on all the six tested human cancer cells. Additionally, the anticancer mechanism of [AuTPP-Se]Cl was investigated in detail.

The synthetic route of [AuTPP-Se]Cl is shown in Scheme 1. Firstly, monomethoxycarbonyl substituted tetraphenylporphyrin (intermediate **1**) was prepared by the reaction of stoichiometric methyl 2-formylbenzoate and benzaldehyde (1:3) with pyrrole.[10] By a demethylation process of this intermediate (obtaining intermediate **2**) and further amidation with methyl 2-amino-4-(methylseleno)butanoate, H_2TPP-Se was obtained. Finally, H_2TPP-Se reacted with $KAuCl_4$ to form the target compound [AuTPP-Se]Cl. The structures of [AuTPP-Se]Cl and the intermediates were confirmed by ^{1}H NMR spectroscopy and electrospray ionization mass (ESI-MS) spectroscopy.

The anticancer effect of [AuTPP-Se]Cl was evaluated by its antiproliferation on the cell lines of human pulmonary carcinoma (A549), human cervical epithelial carcinoma (HeLa), human breast carcinoma (MCF-7), human erythroleukemia (K562), human hepatocellular carcinoma (HepG2) and human glioblastoma multiforme (LN229). Experimental results showed that [AuTPP-Se]Cl could inhibit the proliferation of all the tested cancer cells to some extent, and the measured IC_{50}s of [AuTPP-Se]Cl are generally lower than those of the positive control *cis*-diaminedichloroplatinum

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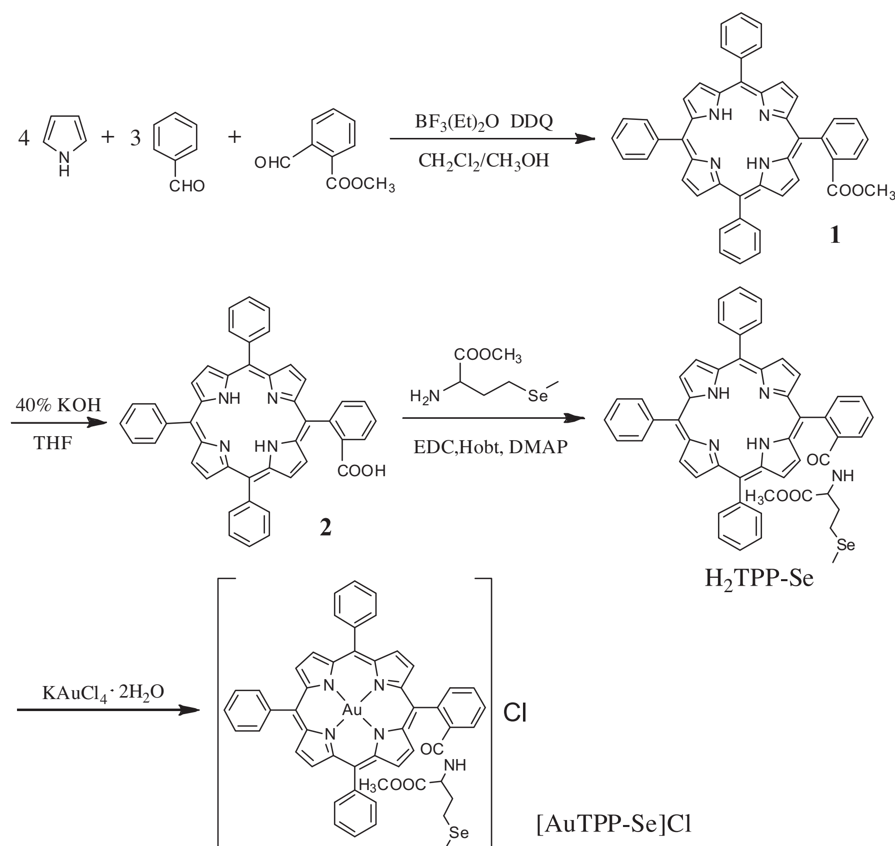

Scheme 1. Synthetic route of [AuTPP-Se]Cl.

(CDDP) (Table 1), which indicates that [AuTPP-Se]Cl could be used as an efficient broad spectrum anticancer drug. Among the tested cell lines, [AuTPP-Se]Cl exhibited the most remarkable inhibitory effect on HepG2 cells (with an IC_{50} of 1.42 ± 0.20 μg/ml), which is even ten folds higher than that of CDDP. The antiproliferative activity of [AuTPP-Se]Cl on HepG2 was further confirmed with colony formation experiments. It was observed that [AuTPP-Se]Cl at all the tested dosages could inhibit HepG2 cell colony formation remarkably and exhibits a dose-dependent manner (Fig. 1A).

We also tested the efficacies of tetraphenylporphyrin (H_2TPP), H_2TPP-Se and [AuTPP]Cl (also shown in Table 1), and compared them with that of [AuTPP-Se]Cl. The inhibitory effect of [AuTPP-Se]Cl is superior to those of H_2TPP, H_2TPP-Se and [AuTPP]Cl on all the six types of cancer cells, which proves the synergistic action among Au, Se and TPP components. It is noteworthy that H_2TPP exhibits the lowest antiproliferative effect. The IC_{50}s for K562 and LN229 cells are even above 100 μg/ml. These results validate the designing strategy of [AuTPP-Se]Cl, that is, the porphyrin ring only facilitates the accumulation of this compound in cancer cells but not as an effective cytotoxic component, while Au and Se contribute the main anticancer activity to this compound. This assumption is in accordance with that of Che et al.[11] In their work, n-$Bu_4N[Au(III)Cl_4]$ is observed to be much less cytotoxic than [Au(III)TPP]Cl to cancer cells, proving that the porphyrin ligand could carry the Au(III) center to the cellular target. The selective accumulation facilitated by porphyrin ring is expected to reduce the side effect of the formed agents. For example, in contrast to the moderate anticancer effect of [AuTPP]Cl observed in this work, it was reported that this compound exhibits no cytotoxicity to normal liver cells.[11] The combined action between Au and Se compartments was investigated with isobole method.[12] As shown in Figure S1, the IC_{50} value of [AuTPP-Se]Cl (considered as [AuTPP]Cl + H_2TPP-Se) located significantly left to the 95% confidence limits of theoretical additive line. This result confirms that Au and Se compartments work synergistically in [AuTPP-Se]Cl. It has been reported that both Au and Se exhibit remarkable inhibitory effect on HepG2 and MCF-7.[13–16] Since [AuTPP-Se]Cl contains Au and Se as the main anticancer components, and these two

Table 1
IC_{50}s of [AuTPP-Se]Cl, [AuTPP]Cl, H_2TPP-Se and H_2TPP for six types of human cancer cells (mean ± SD, n = 6)

Complex	IC_{50} (μg/ml)					
	Hela	HepG2	A549	K562	LN229	MCF-7
[AuTPP-Se]Cl	7.30 ± 0.68*	1.42 ± 0.20**	11.73 ± 1.16*	13.65 ± 0.98**	8.73 ± 0.78**	5.25 ± 0.55*
[AuTPP]Cl	17.76 ± 1.90**	13.08 ± 1.77	26.27 ± 1.70**	34.58 ± 3.34**	26.01 ± 1.96**	14.01 ± 1.32**
H_2TPP-Se	58.30 ± 3.45**	39.78 ± 2.67**	65.47 ± 2.99**	71.72 ± 5.89**	62.90 ± 5.08**	53.95 ± 3.75**
H_2TPP	69.81 ± 5.83**	64.50 ± 4.45**	84.35 ± 7.31**	>100	>100	76.31 ± 4.62**
CDDP	9.19 ± 0.59	13.73 ± 1.15	13.85 ± 1.17	16.94 ± 1.49	18.86 ± 1.61	7.15 ± 0.69

* $P<0.05$.
** $P<0.01$.

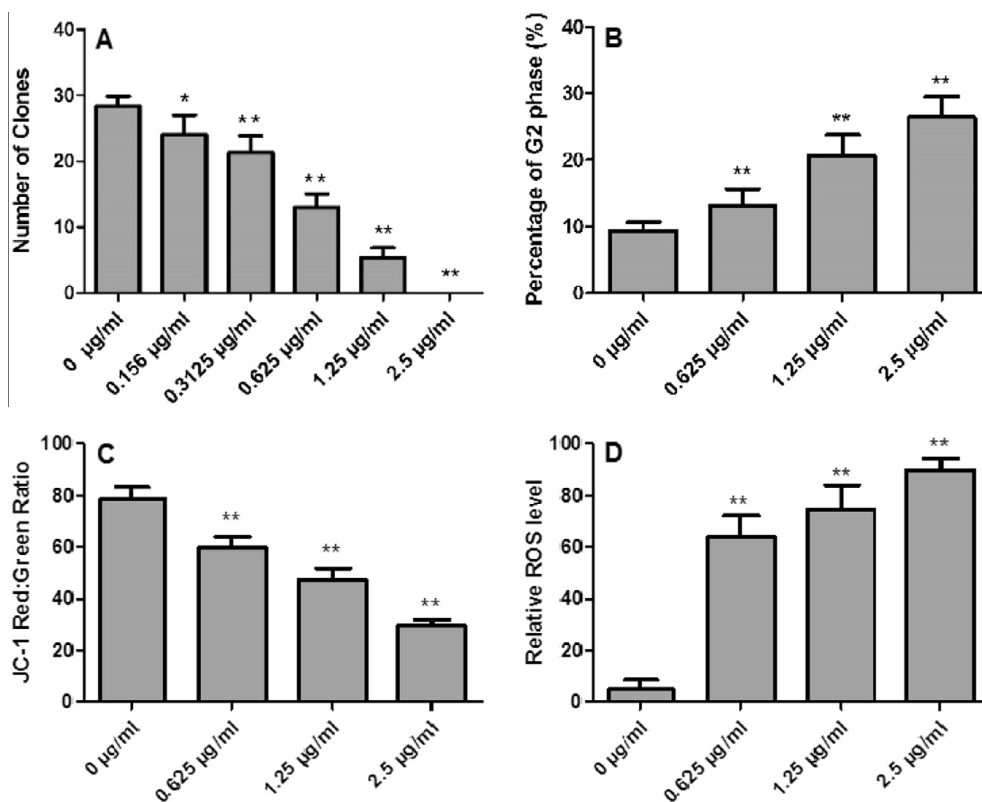

Figure 1. The effects of [AuTPP-Se]Cl on (A) colony formation, (B) G2 phase percentage, (C) MMP and (D) relative ROS level of HepG2 cells (mean ± SD, $n = 3$). $^{*}P<0.05$, $^{**}P<0.01$.

components work synergetically, the superior cytotoxicity of [AuTPP-Se]Cl to these two kinds of cell lines is reasonable.

Since [AuTPP-Se]Cl exhibits the highest inhibitory effect on HepG2, the following mechanism study was carried out mainly with this kind of cancer cell. Most of the anticancer agents inhibit the proliferation of cancer cells by the induction of apoptosis, the arrest of cell cycle, or both.[17] Therefore, we tested these two potential mechanisms respectively. The effect of [AuTPP-Se]Cl on the cell cycle was examined with flow cytometry assay.[18] As shown in Figures 1B and S2, after the treatment of 0.625, 1.25 and 2.5 μg/ml [AuTPP-Se]Cl for 24 h, the percentage of HepG2 cells in G2 phase was increased from 9.3% to 13.2%, 20.6% and 26.4%, respectively. It is well known that cell cycle checkpoints are important for the proper execution of cell events, and particularly, the transition from G2 to M phase is relevant to the proliferation of cell. These experimental results indicate that [AuTPP-Se]Cl could block HepG2 cell in G2 phase and thus inhibit its proliferation.

The apoptosis process was studied with fluorescent staining using Hoechst33342 and propidium iodide (PI) as fluorescent dyes.[19] Blue fluorescent Hoechst33342 could permeate slightly into normal cells and give them a weak emission upon the irradiation of UV. For apoptotic cells, due to the change of membrane permeability, the accumulative amount of Hoechst33342 would increase largely, which would enhance the fluorescence intensity of the stained cells. On the other hand, red fluorescent PI can only enter dead cells but is impermeable for normal and apoptotic cells. Therefore, with the staining of Hoechst33342 and PI, the normal, apoptotic and dead cells could be differentiated. Our experimental observation showed that, in the control group, most of the cells exhibited the weak blue fluorescence of normal cells (Fig. 2A). After the treatment of [AuTPP-Se]Cl, some cells emitted brilliant blue and red fluorescence, and their proportions increased with [AuTPP-Se]Cl concentration (Fig. 2B–D), showing that [AuTPP-Se]Cl promotes the apoptosis and death of HepG2 cells. These observations, as well as the results of flow cytometry assay, indicate that, with [AuTPP-Se]Cl, both the pro-apoptosis and cell cycle arrest contribute to the proliferation inhibition of HepG2.

Because the apoptosis has a profound effect on the progression of cancer, the elucidation of the pro-apoptosis mechanism is critical for the evaluation of an anticancer agent.

Since the apoptosis is an energy-dependent process and could be activated by the intracellular signal from the mitochondria,[20] we first examined the change of mitochondria after [AuTPP-Se]Cl treatment by the measurement of mitochondrial membrane potential (MMP) with JC-1 method.[21] The intensity ratio of the red fluorescence of JC-1 aggregation, which is formed due to the high MMP induced enrichment of this sensor in mitochondria, to the green fluorescence of its monomer was used to characterize the MMP. It was observed that the red:green ratio decreased with the increase of [AuTPP-Se]Cl concentration (Figs. 1C and S3). These results show that [AuTPP-Se]Cl leads to a significant decrease of MMP, indicating the dysfunction of mitochondria. The mitochondria dysfunction would promote the release of cytochrome C, which would activate caspase-9 and -3.[22] This process is regulated by Bcl-2, whose expression affects the apoptosis of the cell, that is, when Bcl-2 is hypoexpressed, free Bax would form death-inducing homodimers, different from the formation of protective Bcl-2/Bax heterodimer with the overexpression of Bcl-2.[23,24] Therefore, western blot analysis was carried out to assay the activities of these apoptosis relevant proteins.[25] We found that, after the treatment of [AuTPP-Se]Cl, the expression of Bcl-2 was down-regulated, while the expression of Bax was up-regulated (Fig. 3). These results corroborate that the pro-apoptosis of [AuTPP-Se]Cl is via a mitochondrial pathway.

Furthermore, to investigate the upstream mechanism of the pro-apoptosis of [AuTPP-Se]Cl, the intracellular reactive oxygen

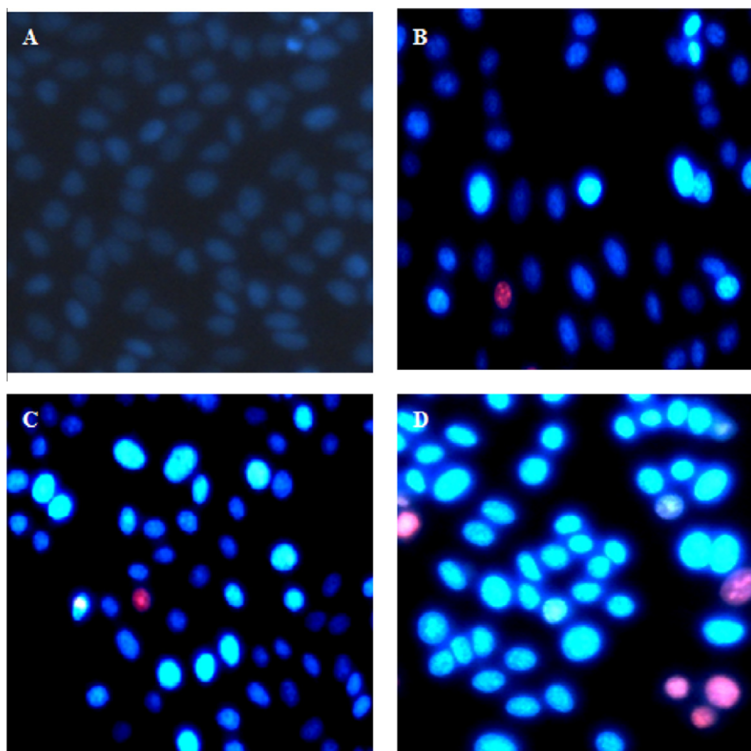

Figure 2. The morphological change of HepG2 cells induced by (A) 0, (B) 0.625, (C) 1.25, (D) 2.5 μg/ml [AuTPP-Se]Cl, observed with fluorescent microscope (×200).

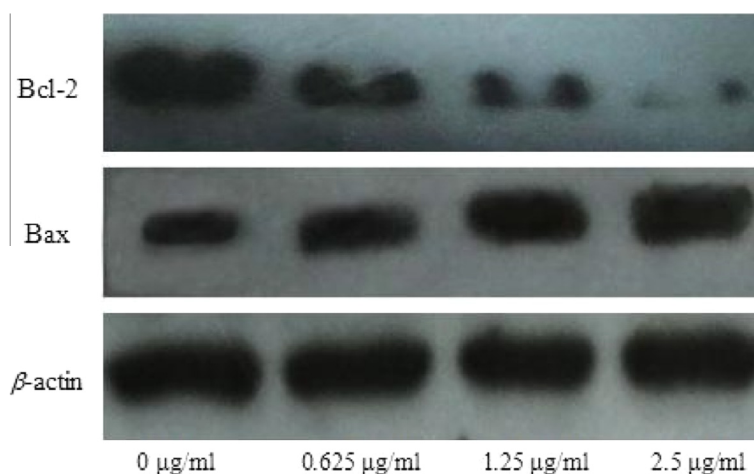

Figure 3. The effect of [AuTPP-Se]Cl on the expression of Bcl-2 and Bax in HepG2 cells.

species (ROS) was monitored using the fluorescence method with DCFH-DA as the probe.[26] As shown in Figure 1D, compared with the blank control, the fluorescence signal of trapped ROS increased remarkably by the treatment of [AuTPP-Se]Cl in a dose dependent manner, which implies that [AuTPP-Se]Cl promotes intracellular ROS generation to some extent. Excessive ROS would lead to the peroxidation of the lipid of mitochondria and impair its function, which initiates the apoptosis process.[27]

In summary, with the combination of the anticancer components of Au, Se, and porphyrin, a new compound, [AuTPP-Se]Cl, was designed and synthesized as an antitumor drug. This compound exhibited remarkable antiproliferative effect on all the tested cancer cells, especially for HepG2. The performance of [AuTPP-Se]Cl is superior to H_2TPP-Se, [AuTPP]Cl and H_2TPP, showing the synergistic action among its components. Mechanism study shows that [AuTPP-Se]Cl could enhance the intracellular ROS level, impair mitochondrial function and regulate the expression Bcl-2 and Bax, which induce the apoptosis of the cancer cell. Besides, [AuTPP-Se]Cl could also arrest cancer cell cycle in G2 phase. All of these processes contribute to the proliferation inhibition of HepG2. The results of this work indicate that [AuTPP-Se]Cl is promise to be developed as a chemotherapeutic and chemopreventive agent.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.06.075.

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