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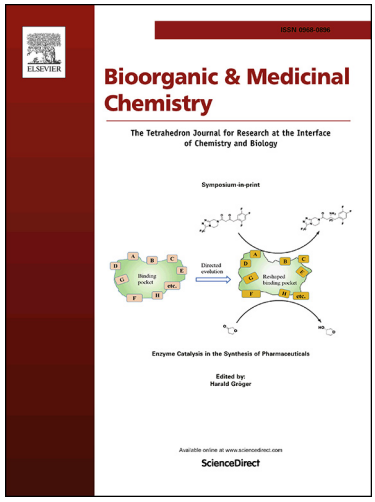

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Graphical Abstract

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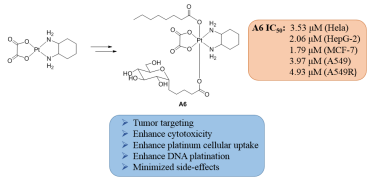

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Glucose-conjugated platinum(IV) complexes as tumor-targeting agents: design, synthesis and biological evaluation

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ABSTRACT

A new series of glucose-conjugated Pt(IV) complexes that target tumor-specific glucose transporters (GLUTs) was designed, synthesized, and evaluated for their anticancer activities. All six compounds, namely, A1-A6, exhibited increased cytotoxicity that were almost six fold higher than that of oxaliplatin to MCF-7 cells. These Pt(IV) complexes can be reduced to release Pt(II) complexes and cause the death of tumor cells. Simultaneously, the glycosylated Pt(IV) complexes (30.21–91.33 μM) showed lower cytotoxicity that normal LO2 cells compared with cisplatin (5.25 μM) and oxaliplatin (8.34 μM). The intervention of phlorizin as a GLUTs inhibitor increased the IC_{50} value of the glycosylated Pt(IV) complexes, thereby indicating the potential GLUT transportability. The introduction of glucose moiety to Pt(IV) complexes can effectively enhance the Pt cellular uptake and DNA platination. Results suggested glucose-conjugated Pt(IV) complexes had potential for further study as new anticancer agents.

1. Introduction

Chemotherapy can effectively control or eliminate metastasis focus. Hence, chemotherapy plays a significant role in the treatment of advanced malignant and refractory cancers. Since 1978, Pt-based antitumor drugs, which are represented by cisplatin, carboplatin, and oxaliplatin, have been the first choice of chemotherapy drugs for malignant tumors, such as testicular cancer, colorectal cancer, nonsmall cell lung cancer, ovarian cancer, breast cancer, head and neck cancer, and nasopharyngeal cancer[1]. Pt-based antitumor drugs are also one of the most common components in clinical treatment of multiple malignancies. However, due to the lack of selection of cancer cells and the structural characteristics of Pt(II) drugs in cancer treatment, serious problems still exist with Pt(II) anticancer drugs, which are accompanied by the toxic side effects, such as renal toxicity, ototoxicity, neurotoxicity, and alopecia, as well as the problems of drug and cross resistance[1a]. Therefore, the exploration of targeted drugs is important in improving curative effect and reducing toxicity.

Pt(IV) complexes have attracted the attention and favor of scientists [2]. Compared with Pt(II) complexes, Pt(IV) complexes have octahedral configuration, which is characterized by substitutional kinetics inertia and reduced reactivity. Pt(IV) is difficult to replace with biomolecules, such as glutathione and protein in cells. Thus, Pt(IV) can reach cancer cells completely. Subsequently, Pt(IV) prodrugs should be activated to Pt(II) complexes through reduction with intracellular reductants, such as ascorbic acid and glutathione, which are in excess amounts in tumor cells[3]. With attention given to Pt(IV) complexes, many Pt(IV) complexes with anticancer activity were synthesized. The most typical representatives are iproplatin, ormaplatin, and satraplatin (JM216)[4]. However, most researchers stopped further research because of their considerable toxicity or indifferent efficacy[5]. Through chemical modification, the introduction of functional molecules into the axial ligands of Pt complexes can increase the selectivity of Pt(IV) complexes for cancer cells and improve the pharmacological properties of the drugs[6]. Targeted and synergistic sensitization can be achieved by modifying the axial ligand of the Pt(IV) complexes. Therefore, Pt(IV) complexes with anticancer activity can overcome the toxic side effect and drug resistance of traditional Pt(II) anticancer drugs[3, 7].

Oncosis involves not only the removal of control over cell proliferation but also the corresponding regulation of energy metabolism to promote cell growth and division. Even under sufficient oxygen supply, tumor cells mainly obtain energy through glycolysis, which is called aerobic glycolysis or Warburg effect[6, 8]. The abnormal glycolysis of tumor cells can promote glucose uptake and lactic acid generation, thereby facilitating tumor occurrence and development[9]. Therefore, this abnormal uptake of glucose can be used as a target for cancer treatment. Many studies have found that Pt(II) glycan complexes have

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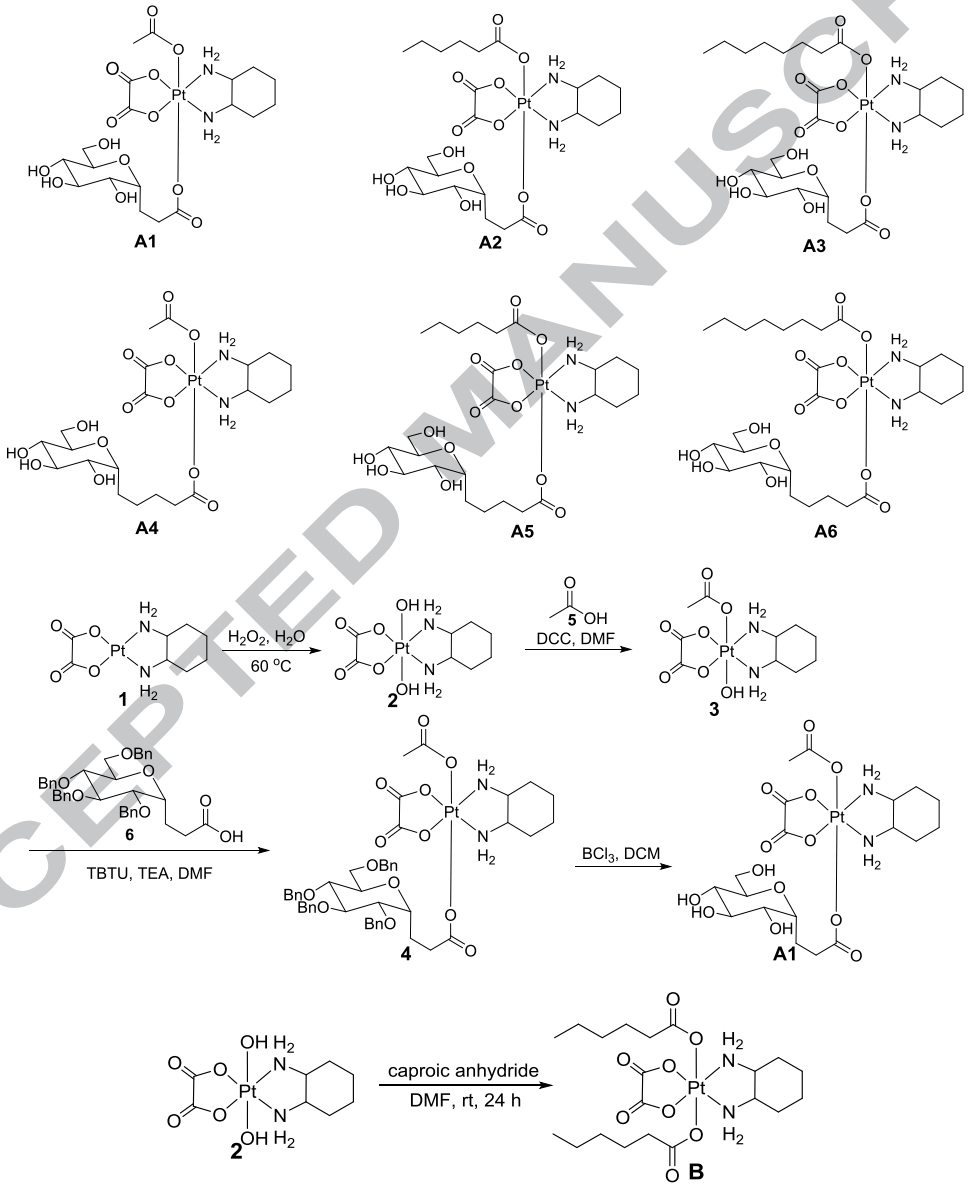
strong antitumor activity and low toxicity and have been proven to target glucose transporters (GLUTs)[8, 10]. For the first time, our group synthesized a series of acetyl protective glycosylated Pt(IV) complexes and tested whether they target GLUTs by using phlorizin as a GLUT inhibitor[11]. Unfortunately, the results showed that glycosylated Pt(IV) complexes do not target GLUTs. Subsequently, the unprotected glycosylated Pt(IV) complex is successfully synthesized and found to be transportable by GLUT[12]. Meanwhile, the antitumor activity of glucose-modified Pt(IV) is superior to that of other sugar types, such as galactose and mannose.

On the basis of the results above, we designed and synthesized a series of novel glycosylated Pt compounds and evaluated their biological activities. Glucose was selected as a glycoligand because its antitumor activity was superior to that of other sugar types[12]. To improve the stability of the glycosylated portion to the glucosidase, we selected the C-glycoside bond. C chains with different lengths were designed to explore the appropriate linker between the glycoligand and the Pt nucleus. Meanwhile, alkanes with different lengths were also designed to study their effects on the activity of glycosylated Pt(IV) complexes [7a, b, 13].

2. Results and Discussion

*2.1. Synthesis of glycosylated Pt(IV) compounds **A1-A6***

The synthesis of the target compounds is shown in Scheme 1. For example, in **A1**, oxaliplatin was oxidized in H_2O_2 at 60 °C to obtain oxoplatin, and monocarboxylic acid chain was introduced under dicyclohexylcarbodiimide condensation by adding 1.4 equivalent of carboxylic acids. The synthesis of glycoligands was



Scheme 1. Chemical structures of glycosylated Pt(IV) complexes and synthesis of the title compounds **A1** and **B**.

Table 1. Cytotoxicity profiles of the glycosylated Pt(IV) complexes in five human carcinoma cell lines expressed as IC_{50} (μM)

Compounds	Hela	HepG-2	MCF-7	A549	A549R	LO2	RF[a]	SI[b]
A1	7.36±0.05	8.13±0.07	3.91±0.06	8.23±0.16	15.63±0.17	30.21±0.21	1.89	3.72
A2	6.30±0.07	7.41±0.08	4.00±0.04	6.68±0.08	10.14±0.11	44.46±0.09	1.51	6.00
A3	2.06±0.11	2.75±0.06	3.00±0.08	3.12±0.28	4.55±0.20	25.56±0.16	1.45	9.29
A4	5.42±0.05	6.39±0.10	4.63±0.10	5.44±0.11	8.59±0.39	90.34±0.12	1.57	14.14
A5	3.68±0.08	3.79±0.07	2.05±0.07	4.74±0.16	7.29±0.14	91.33±0.07	1.53	24.10
A6	3.53±0.08	3.02±0.08	1.79±0.34	3.97±0.06	4.93±0.32	33.12±0.34	1.24	10.90
B	32.68±0.12	40.68±0.34	68.34±0.64	54.65±0.76	72.56±0.15	44.32±0.27	1.33	1.09
Cisplatin	4.60±0.58	6.31±0.07	8.71±0.15	11.90±0.08	33.40±0.32	5.25±0.23	2.80	0.83
Oxaliplatin	6.60±0.15	10.90±0.07	10.10±0.59	12.69±0.24	36.07±0.11	8.34±0.15	2.84	0.77

[a] RF: Resistant factor = IC_{50}(A549R)/IC_{50}(A549). [b]SI: selectivity index= IC_{50}(LO2)/IC_{50}(HepG-2). IC_{50} was evaluated by the MTT assay for 48 h. All compounds are tested in the same batch and the experiments were performed tree times in five replicates. Hela (human cervical cancer cell line), HepG-2 (human liver cancer cell line), MCF-7 (human breast cancer cell line)， A549 (human lung cancer cell line), LO2 (human normal liver cell line).

described in ESI.† The asymmetrically functionalized Pt(IV) compound can be prepared by **3** and **6** in dimethyl formamide in the presence of O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate and triethylamine. The removal of benzyl protective groups of **4** by using BCl3 in anhydrous dichloromethane afforded the corresponding target **A1**. **B** was prepared from **2** (1 equivalent) and caproic anhydride (6 equivalent) in anhydrous dimethyl formamide at 25 °C. **A2–A6** were prepared according to the procedure described above. The purity of **A1–A6** have been determined by HPLC, which was shown in ESI.†

2.2. Antitumor activities in vitro

The antitumor activity of **A1–A6** were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay by using five human cancer cell lines. The structure of the synthesized targets **A1–A6** contained comparable carboxyl ligands. Therefore, di-hexanoyl-Pt(IV)-derivative **B** was selected as the control. Cisplatin and oxaliplatin were also used as the control group. The IC_{50} values were calculated based on the dose dependence of surviving cells that incubation to the compounds for 48 h (Table 1).

A1–A6 showed enhanced activities and a relatively broad antitumor spectrum compared with cisplatin and oxaliplatin. Compared with **B**, the increased activity was attributed to the introduction of glycosylated modules, which targeted GLUT that was overexpressed in tumor cells[10c, d, 14]. In particular, for MCF-7 cells (IC_{50} = 1.79 to 4.63 μM), all six compounds exhibited the highest cytotoxicity, which was almost sixfold higher than that of oxaliplatin. This result may be caused by the high GLUT1 expression in MCF-7 cells[15].

The length of the C chain between glycoligand and the Pt core had a significant influence on the antitumor activity. **A4**, **A5,** and **A6** with longer C chains were generally more cytotoxic than **A1**, **A2**, and **A3**, which have the same alkyl ligands. At the same time, the cytotoxicity of **A1**, **A2**, **A3 A4**, **A5**, and **A6** enhanced with the increase in alkane chain. **A3** (IC_{50} = 2.05 to 5.55 μM) and **A6** (IC_{50} = 2.06–5.93 μM) with octanoic ligand showed better cytotoxic for five cell lines than those with cisplatin and oxaliplatin.

Drug-resistant A549R cells were also more sensitive to Pt(IV) glycoconjugates than cisplatin and oxaliplatin; even the resistance factors of **A3** and **A6** can be reduced to 1.45 and 1.24, respectively. The results above suggested that these compounds had the potential to be further studied as new anticancer agents.

2.3. LO2 cell viability

To assess the potential safety of glycosylated Pt(IV) complexes, we used MTT assay to evaluate the cytotoxicity of these complexes against normal LO2 cells. We compared the IC_{50} values of **A1–A6** with cisplatin and oxaliplatin in HepG-2 and LO2 cells (Table 1). The cytotoxicity of glycosylated Pt(IV) complexes on normal cells was significantly reduced compared with HepG-2 cells, and the selectivity index of **A5** even attained 24.1, which was significantly higher than those of cisplatin and oxaliplatin (0.83 and 0.77, respectively). When the concentration range was 0–100 μM, the cell viability of LO2 cells incubated with glycosylated Pt(IV) complexes was significantly higher than those of cisplatin and oxaliplatin (Fig. 1). At the concentration of 8.34 μM (oxaliplatin, IC_{50} = 8.34 μM), the cell viability of LO2 cells corresponding to the glycosylated Pt(IV) complexes was >70%, especially for **A4** and **A5**, as the corresponding cell viability of LO2 cells at this concentration was almost 100%. These results indicated that glycosylated Pt(IV) complexes may have enhanced the potential safety while maintaining the antitumor activity.

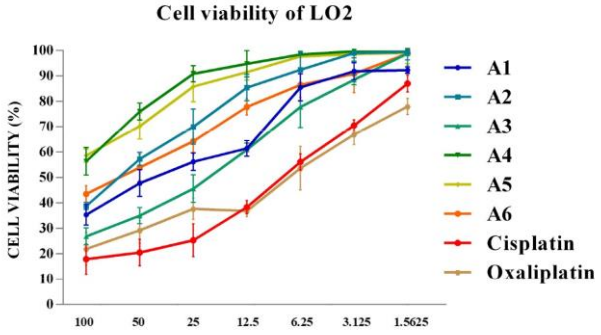


Fig. 1. Viability of LO2 cells in a 48 h incubation assay

2.4. Effects of GLUTs inhibitor phlorizin on antitumor activities

To investigate whether the Warburg effect affected the transport of glycosylated Pt(IV) complexes to cancer cells,

we studied the mechanism of **A3**, **A5**, **A6**, cisplatin, and oxaliplatin in MCF-7 cells by using phlorizin as GLUT inhibitor. As shown in Fig. 2, the IC_{50} values of **B**, cisplatin, and oxaliplatin showed insignificant difference with and without phlorizin, which indicated that GLUT inhibitor was unassociated with the cytotoxicity of **B,** cisplatin, and oxaliplatin. However, the IC_{50} values of **A3**, **A5,** and **A6** in the presence of phlorizin increased by approximately fourfold compared with those without phlorizin. Therefore, the transport of glycosylated Pt(IV) complexes is regulated by GLUTs.

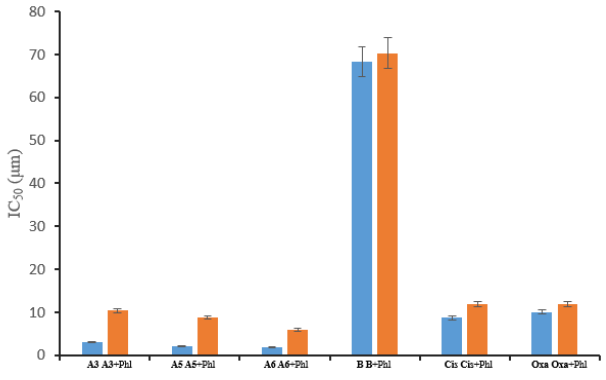


Fig. 2 *In vitro* antitumor activities of compounds **A3**, **A5**, **A6**, **B**, cisplatin and oxaliplatin to MCF-7 without and with GLUTs inhibitor phlorizin (phlorizin，100 μM； Phl: phlorizin; Cis: Cisplatin; Oxa: Oxaliplatin).

2.5. Flow cytometric analysis

To evaluate the apoptosis-inducing effect of glycosylated Pt(IV) complexes on MCF-7 cells, we performed annexin V/PI double staining via flow cytometry by using **A3**, **A5,** and **A6** (10 μM). Pt(IV)-derivative **B** without saccharide ligand, cisplatin, and oxaliplatin was used as the control (Fig. 4). As shown in Table 2, **A6** induced 51.83% of apoptosis of MCF-7 cells (9.89% of early apoptosis, 38.6% of late apoptosis, and 3.34% of necrosis), which was significantly higher than those of the positive control of **B** with a sum of 16.87% (3.54% of early apoptosis, 10.8% of late apoptosis, and 2.53% of necrosis). **A3** and **A5** also had higher apoptosis-inducing levels than **B**. All glycosylated Pt(IV) complexes showed higher or comparable apoptosis-inducing level than cisplatin and oxaliplatin. Hence, these results indicated that with the introduction of saccharide ligand, the apoptosis-inducing ability of Pt(IV) complexes was enhanced.

Table 2 Quantification of apoptosis in MCF-7 cells using an annexin V/PI assay

Compounds	Early apoptosis	Late apoptosis	Necrosis	Sum
A3	7.56	37.7	4.25	49.51
A5	5.53	34.6	3.77	43.9
A6	9.89	38.6	3.34	51.83
B	3.54	10.8	2.53	16.87
Cisplatin	13.9	22.4	2.92	39.22
Oxaliplatin	7.29	23.7	3.12	34.11
Untreated	0.58	4.31	1.21	6.1

2.6. Cellular uptake and DNA platination

The cellular uptake of Pt-based anticancer drugs and their DNA-binding capacity are directly related to the antitumor activity of the drugs. Therefore, we detected intracellular Pt accumulation and DNA-binding capacity by using inductively coupled plasma mass spectrometry (ICP-MS) after MCF-7 cells was incubated with **A4**, **A5**, **A6**, **B**, cisplatin, and oxaliplatin at 20 μM for 10 h. As shown in Fig. 3A, the intracellular Pt accumulation of the glycosylated Pt(IV) complex was 1.7-3.3 fold higher than that of di-hexanoyl-Pt(IV)-derivative **B**, which did not contain the glycosylated part. DNA was extracted from MCF-7 cells, and its Pt content was determined. The results (Fig. 3B) showed glycosylated Pt(IV) complexes exhibited 8.4- to 22.8-fold higher than **B**. Similarly, for cell uptake and DNA platination, the glycosylated Pt(IV) complex was higher than cisplatin and oxaliplatin (Fig. 3). These results indicated that the introduction of glycosylated components to Pt(IV) complexes can effectively increase the Pt cellular uptake and DNA platination. Meanwhile, the cytotoxicity of Pt-based complexes was correlated with Pt cellular uptake.

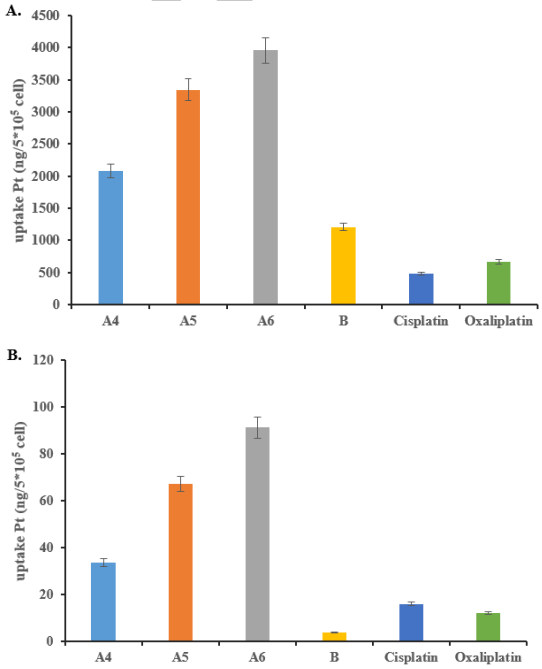


Fig. 3 Cellular uptake and DNA platination of compounds **A4**, **A5**, **A6**, **B**, cisplatin and oxaliplatin (20 μM, respectively) in MCF-7 cells. (A) Platinum in whole cells. (B) Platinum in DNA.

2.7. Reduction and DNA-binding properties

To the best of our knowledge, Pt(IV) prodrugs should be activated to Pt(II) complexes through the reduction by intracellular reductants, such as ascorbic acid and glutathione, which are in excess in tumor cells. The resulting divalent form, generally cisplatin or a related derivative, binds to DNA, inhibits transcription and replication, and induces apoptosis. To investigate the mechanism underlying the title glycosylated Pt(IV) prodrugs, we incubated **A6** with or without ascorbic acid at 37 °C (Fig. 5). The 5′-dGMP was added as a model of DNA. After 24 h, the peak of oxaliplatin 5’-dGMP adduct appeared for both oxaliplatin and **A6** with ascorbic acid. The peak increased after 48, 72, and 96 h. Meanwhile, when the reducing agent, that is, ascorbic acid, was absent, the peak of the oxaliplatin 5′-dGMP

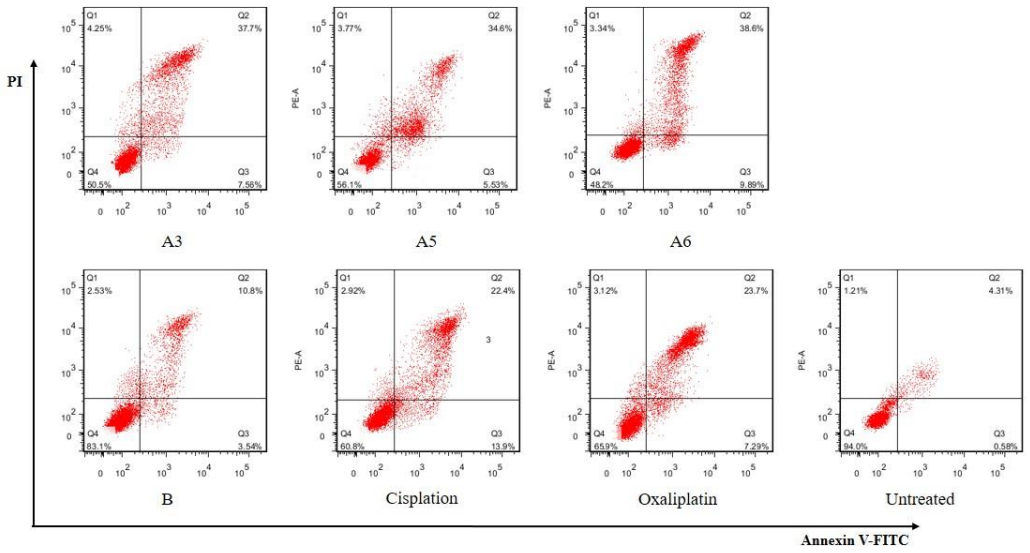


Fig. 4 Induction of apoptosis at 24 h by **A3**, **A5**, **A6**, **B**, cisplatin and Oxaliplatin in MCF-7 cells

adduct did not appear even after 96 h. Subsequently, the adduct was isolated and identified by HRMS analysis (Fig. S7). Following such a reductive experiment, the new peak of the oxaliplatin 5'-dGMP (confirmed by HRMS) proved the combination of Pt(II), which was converted from Pt(IV), with 5'-dGMP and DNA. Therefore, Pt(IV) precursors exert their anticancer activity by reducing to Pt(II) complexes.

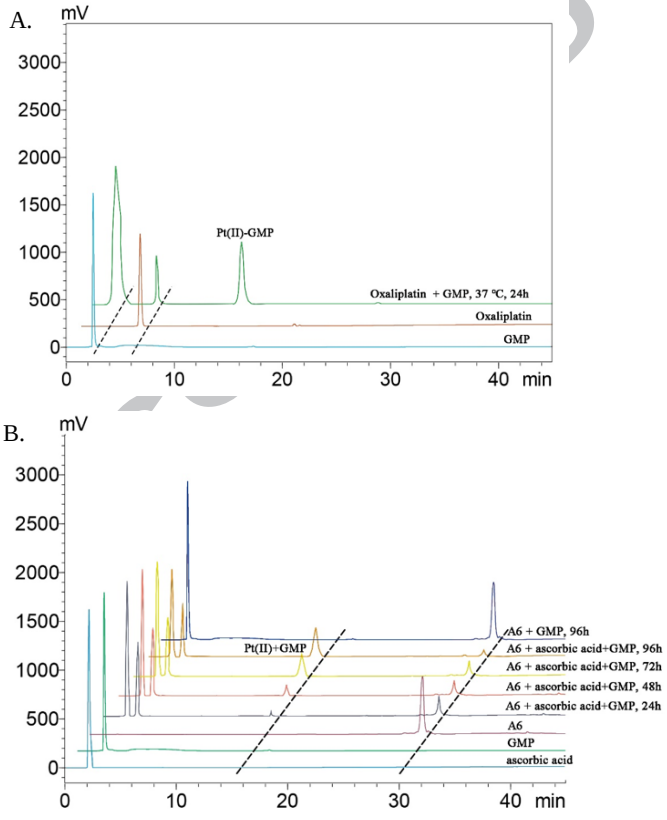


Fig. 5 A. Reaction of oxaliplatin with 5'-dGMP incubated at 37 ºC after 24 h. B. Reaction of **A6** with 5'-dGMP with and without ascorbic acid after 24 h, 48 h, 72 h and 96 h.

3. Conclusions

Six novel glucose-conjugated Pt(IV) complexes that target tumor-specific GLUTs were designed, synthesized, and evaluated for their anticancer activities. All the tests on Pt(IV) complexes showed enhanced cytotoxicity to five human cancer cell lines compared with cisplatin and oxaliplatin due to the transport-mediated effect of GLUTs. The effect of GLUTs on the transportation of glycosylated Pt(IV) complexes was confirmed by inhibitory experiment, in which phlorizin was used as an inhibitor. The effect of alkyl ligands on cytotoxicity was greater than that of the ligand between the glycoligand and Pt nucleus because **A3** exhibited higher cytotoxic effects than **A4**. These complexes showed higher apoptosis-inducing level than cisplatin and oxaliplatin. The transport properties of glucose transporters increased the Pt cellular uptake and DNA platination of the glycosylated Pt(IV) compounds. The glycosylated Pt(IV) complexes showed lower cytotoxicity to normal LO2 cells than cisplatin and oxaliplatin while maintaining the antitumor activity, which may increase the potential safety of their cancer treatment. Glycosylated Pt(IV) compounds can be reduced in the attendance of ascorbic acid and further combined with DNA to cause the death of tumor cells. All the results above suggested the possibility of the development of glycosylated Pt(IV) complexes as suitable and effective antitumor drugs and further provided a reference for our subsequent research.

4. Experimental Section

4.1. Materials and general methods

Oxaliplatin and cisplatin were purchased from Yurui chemical Co. Ltd (Shanghai, China). All other chemicals were obtained from Aldrich, Alfa Aesar and J&K as received and were of analytical grade. Platinum (IV) glycoconjugates were carried out under nitrogen atmosphere and in dry solvents. ^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker AVANCE AV400 MHz NMR spectrometers in $CDCl_3$ or CD_3OD using TMS as internal standard. Chemical shifts were reported as δ values (ppm). High resolution mass spectra (HRMS) were obtained on an IonSpec QFT mass spectrometer with ESI ionization. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide),

DMEM (for HeLa, MCF-7, HepG-2, A549 cells and 3T3 cells), RPMI1640 (for A549R cells), medium containing 10% fetal bovine serums, Fetal bovine serum (FBS), 0.25% trypsin/EDTA solutions, penicillin-streptomycin solutions and phloretin purchased from Invitrogen (Grand Island, NY, USA) were used for elementary cytotoxicity evaluation. Phosphate buffered saline (PBS) contains 2.7 mM KCl, 137 mM NaCl, 2 mM KH_2PO_4 and 10 mM Na_2HPO_4. Annexin V-FITC Apoptosis Detection Kit and Genomic DNA mini preparation kits were purchased from KeyGEN BioTech, China. A549 (human lung carcinoma), HeLa (Cervical), MCF-7 (breast cancer), HepG-2 (hepatoma cells), 3T3 (mouse embryo fibroblasts) and A549R cells were obtained from Prof. Jing Li (College of Pharmacy, Nankai University, Tianjin, China). A549R cells were maintained with 2 mg/mL cisplatin. ICP-MS was measured in the laboratory of Cuihong Chen (College of environmental science and engineering, Nankai University, Tianjin, China). Reactions involved in the preparation of platinum compounds were carried out in the dark. RP-HPLC analyses were performed as on a Shimadzu SPD-20A system equipped with a Venusil MP C18 column (150 ×4.6 mm, 5 μm). RP-HPLC profiles were recorded by UV detector at 273 nm at room temperature. The mobile phase consisted of MeOH and H_2O was used and the flow rate of 0.8 mL/min.

4.2. In vitro cellular cytotoxicity assays

Cells were seeded in a 96-well flat-bottomed microplate plates at 2000-3000 cells per well in 100 uL of growth complete medium in a 5% CO_2 atmosphere at 37 °C for 12 h. Next 100 uL freshly culture medium containing different concentrations drugs was added and the microplate was incubated at 37 °C , 5 % CO_2 for 48 h. After incubation, pipet directly MTT (5 mg/ mL , 20 μL) into each well of the 96-well assay plate and the plate was placed at 37 °C in the incubator for another 4 h. Finally, the medium was disposed completely and DMSO (150 μL) was added. The absorbance resulting was measured spectrophotometrically at the absorbance of 570 nm using a BioRad 680 microplate reader. The IC_{50} values based on three parallel experiments were counted using GraphPad Prism 6.

4.3. Apoptosis analysis by annexin V-FITC assay

The apoptosis analysis was performed using MCF-7 cells according to the manufacturers protocol (KeyGEN Annexin V-FITC/PI Apoptosis Detect-ion Kit). MCF-7 cells cultured in 6-well plates were handled with and without drugs at 37 °C. Then cells were collected with adherent cultures by trypsinization and centrifuged at 2000 rpm for 5 min. Following PBS was used to wash the cells and centrifuging as before. Then transfered segmental solution to a culture tube. Then annexin V-FITC 5 μL and propidium iodide 10 μL were added at different sample and incubated for 15 min at room temperature keep out of the sun. After staining, the samples were detected by flow cytometry.

4.4. In vitro antitumor assay with GLUTs inhibitor

MCF-7 cells were cultivated in complete medium with and without GLUTs inhibitor phlorizin (100 μM) at 37 °C incubator. Antitumor bioactivities of selected compounds **A3**, **A5** and **A6**, cisplatin and oxaliplatin were respectively measured according to the procedure of in vitro cellular cytotoxicity test mentioned above.

4.5. Cell uptake and DNA platination

MCF-7 cells were used to detect the cellular uptake of cisplatin, oxaliplatin, **A4**, **A5** and **A6**. MCF-7 cells were seeded in 6-well plates and were placed at the 37 °C incubator overnight. Next, different complexes (20 μM) were added to the cells and incubated for 10 h at the same standard atmosphere. Then, Cells were collected by trypsinization and PBS buffer was used to wash the cells three times. The harvested cells were calculated as well as concentrated and nitric acid was added. Pt concentration in cellular DNA in MCF-7 cells was detected by ICP-MS. The Genomic DNA Mini Preparation Kit was applied to the isolation of DNA in MCF-7 cells.

4.6. Synthetic procedures

Synthesis of **A1**

Preparation of **4.** To a solution of **3** (238.00 mg, 0.50 mmol, 1.0 equiv.) and **6** (0.30 g, 0.50 mmol, 1.0 equiv.) in anhydrous DMF (30 ml) was added TBTU (242.30 mg, 0.75 mmol, 1.5 equiv.), TEA (105.20 μL, 0.75 mmol, 1.5 equiv.). The reaction was stirred 48 h at room temperature under Argon atmosphere. Then the reaction was concentrated and purified by column chromatography on silica gel to give the compound **4** as a white solid (410.00 mg, 78.00%).

Preparation of **A1.** To a solution of **4** (235.00 mg, 0.22 mmol, 1.0 equiv.) in anhydrous DCM (47 ml), BCl_3 (1.79 mL, 1.79 mmol, 1.0 M in hexanes, 8.0 equiv.) was added dropwise at -78 °C under argon atmosphere. Then the reaction was concentrated and purified by column chromatography on silica gel to give the compound **A1** as a pale solid (38mg, 25%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 3.87 (m, 1H), 3.80 (d, J = 11.9 Hz, 1H), 3.58 (m, 3H), 3.39 (m, 1H), 3.23 (m, 1H), 2.81 (m, 1H), 2.54 (m, 1H), 2.41 (m, 1H), 2.28 (m, 2H), 2.05 (s, 3H), 1.95 (m, 3H), 1.61 (m, 4H), 1.31 (m, 3H). ^{13}C NMR (100 MHz, MeOD) δ 183.82, 181.79, 167.11, 76.29, 75.11, 74.66, 72.96, 72.33, 62.92, 33.35, 32.52, 31.05, 25.03, 23.03, 22.09, 21.23. HRMS: Calcd. for $C_{19}H_{32}N_2O_{13}Pt$ M^+: 691.1552, found: 691.1563.

Synthesis of **A2**

Compound **A2** was synthesis according to the procedure of **A1**. ^{1}H NMR (400 MHz, Methanol-d_4) δ 3.88 (m, 1H), 3.81 (dd, J = 11.8, 2.2 Hz, 1H), 3.57 (m, 3H), 3.40 (m, 1H), 3.22 (m, 1H), 2.79 (m, 2H), 2.32 (m, 4H), 1.94 (m, 2H), 1.59 (m, 7H), 1.28 (m, 7H), 0.89 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ 184.66, 183.79, 166.89, 166.86, 76.28, 75.09, 74.64, 72.95, 72.32, 63.10, 37.30, 33.34, 32.54, 32.39, 26.66, 25.06, 25.00, 23.43, 22.08, 14.30. HRMS: Calcd. for $C_{23}H_{40}N_2O_{13}Pt$ M^+: 747.2178, found: 747.2217.

Synthesis of **A3**

Compound **A3** was synthesis according to the procedure of **A1**. ^{1}H NMR (400 MHz, Methanol-d_4) δ 3.88 (m, 1H), 3.81 (dd, J = 11.7, 2.4 Hz, 1H), 3.57 (m, 3H), 3.39 (m, 1H), 3.21 (m, 1H), 2.79 (m, 2H), 2.35 (m, 4H), 1.92 (m, 2H), 1.59 (m, 6H), 1.29 (m, 10H), 0.89 (t, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ 184.68, 183.84, 166.90, 166.87, 76.30, 75.11, 74.68, 72.97, 72.34, 63.13, 37.34, 33.35, 32.85, 32.56, 30.18, 30.15, 26.98, 25.07, 25.01, 23.69, 23.67, 22.10, 14.44. HRMS: Calcd. for $C_{25}H_{44}N_2O_{13}Pt$ M^+: 775.2491, found: 775.2538.

Synthesis of **A4**

Compound **A4** was synthesis according to the procedure of **A1**. ^{1}H NMR (400 MHz, Methanol-d_4) δ 3.86 (m, 1H), 3.80 (dd, J = 11.9, 2.4 Hz, 1H), 3.58 (m, 3H), 3.39 (m, 1H), 3.22 (dd, J = 9.7, 8.2 Hz, 1H), 2.79 (m, 2H), 2.28 (m, 2H), 2.05 (s, 3H), 1.61 (m, 10H), 1.30 (m, 4H). ^{13}C NMR (100 MHz, MeOD) δ 184.45, 181.74, 166.99, 77.00, 75.22, 74.31, 73.13, 72.52, 63.18, 37.33, 32.51, 26.73, 26.07, 25.07, 25.05, 23.01. HRMS: Calcd. for $C_{21}H_{36}N_2O_{13}Pt$ M^+: 719.1865, found: 719.1882.

Synthesis of **A5**

Compound **A5** was synthesis according to the procedure of **A1**. ^{1}H NMR (400 MHz, Methanol-d_4) δ 3.86 (m, 1H), 3.80 (d, J = 11.8 Hz, 1H), 3.57 (m, 3H), 3.39 (m, 1H), 3.22 (t, J = 9.0 Hz, 1H), 2.76 (m, 2H), 2.33 (m, 5H), 1.59 (m, 12H), 1.28 (m, 8H), 0.89 (t, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ 184.68, 184.48, 166.79, 166.76, 77.01, 75.24, 74.34, 73.14, 72.54, 63.20, 37.34, 37.31, 32.58, 32.42, 26.72, 26.67, 26.10, 25.09, 25.06, 23.45, 14.29. HRMS: Calcd. for $C_{21}H_{36}N_2O_{13}Pt$ M^+: 775.2491, found: 775.2531.

Synthesis of **A6**

Compound **A6** was synthesis according to the procedure of **A1**. ^{1}H NMR (400 MHz, Methanol-d_4) δ 3.86 (m, 1H), 3.80 (dd, J = 11.9, 2.2 Hz, 1H), 3.58 (m, 3H), 3.39 (m, 1H), 3.22 (t, J = 8.9 Hz, 1H), 2.76 (m, 2H), 2.32 (m, 5H), 1.59 (m, 10H), 1.29 (m, 14H), 0.89 (t, J = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ 184.65, 184.47, 166.83, 166.80, 77.00, 75.23, 74.33, 73.13, 72.52, 63.18, 37.34, 32.85, 30.18, 30.15, 26.97, 26.72, 26.09, 25.09, 25.06, 23.70, 14.44. HRMS: Calcd. for $C_{27}H_{48}N_2O_{13}Pt$ M^+: 803.2804, found: 803.2848.

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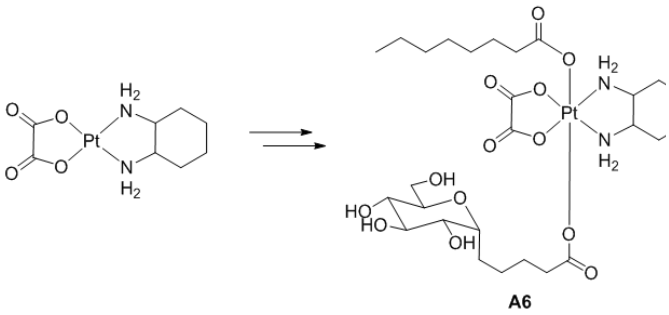
Graphical Abstract



- Tumor targeting
- Enhance cytotoxicity
- Enhance platinum cellular
- Enhance DNA platination
- Minimized side-effects