Dalton Transactions

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Dalton Transactions

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DOI: 10.1039/C6DT02207C

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The glycosylated platinum(IV) prodrugs demonstrated significant therapeutic efficacy in cancer cells and minimized side-effects

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Received 00th January 20xx,

Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Conjugates (**A1**-**A5**) of the Pt(IV) derivative (**A6**) with amino group from peracetyl glucose, rhamnose and mannose with a propyl amino or ethyl amino linker at the reducing end were synthesized and exhibited significant therapeutic efficacy in tumour cells, especially for prostate cancer (PCa). The antitumor activities are greatly affected by glycosyl groups. Cytotoxic experiments *in vitro* indicated that the antitumor activities were increased by 5-fold when its Pt(IV) derivative was conjugated to **S18** (IC_{50} = 4.82 ± 0.45 μM) and by 12-fold when conjugated to **S21** (IC_{50} = 1.9 ± 0.67 μM). The mannose substituted Pt(IV) complexes **A4** and **A5** were also over an order of magnitude more potent towards Hela, A549, MCF-7 and PC3 than cisplatin and oxaliplatin. Importantly, the glycosylated Pt(IV) derivatives **A4** and **A5** displayed a potential safety for clinical therapeutic exposure with the IC_{50} 84 μM and 169 μM compared with cisplatin (IC_{50} = 8 μM) to 3T3. Cellular uptake and DNA platination are higher than cisplatin and oxaliplatin. ESI-MS analysis of **A5** binding to 5'-dGMP revealed that the bifunctional DNA lesions were formed. The antitumor activities *in vivo* showed that the MTD and LD_{50} for **A4** and **A5** are nearly 4-fold higher than that of oxaliplatin indicating the potential safety for the glycosylated Pt(IV) complexes.

Introduction

The platinum-based drugs cisplatin, carboplatin and oxaliplatin are widely used in the clinical treatment of cancer.[1, 2] Nedaplatin, lobaplatin and heptaplatin are only approved in Asia. Cisplatin is one of the most favourable antitumor drugs for a variety of solid cancers,[3, 4] such as ovarian, lung, head and neck.[5] Despite great medical successes of platinum(II)-based cytostatics, severe dose-limiting side effects appeared due to the accumulation of platinum(II) in healthy tissues such as nephrotoxicity, myelosuppression, ototoxicity.[6, 7] The application of platinum(II) drugs is limited by the intrinsic or acquired drug resistance resulting from DNA repair/damage tolerance,[8] reduced cellular drug uptake and intensive way of administration. The cisplatin-based therapeutic strategy for hormone refractory disease failed[9] because of the resistance to apoptotic death of the advanced prostate cancer (PCa).[10] Therefore, we should devote more efforts to find novel platinum agents with high anti-tumour potency.

One of the most promising strategies is to develop a series of platinum(IV) complexes with bioactive ligands. Compared with Pt(II) complexes, Pt(IV) complexes have a low-spin d^6 electron configuration and exhibit octahedral geometry, which can improve the stability and facilitate the intravenous-to-oral switch in cancer chemotherapy. Recently, more and more favourable Pt(IV) prodrugs have been developed.[11] As shown in Figure 1, JM216 (Satraplatin) (1), Ormaplatin (2) and JM9 (3) (Iproplatin), have stepped into the clinical trials.[12] Satraplatin, designed to reduce toxicity and overcome acquired resistance of cisplatin, was effective against various cancer cells *in vitro*[13-16] and orally absorbed through intestinal membrane *via* passive diffusion[17] with a slower aquation rate, more potentials in improving stability, decreasing toxicity, increasing blood-circulation time than that of cisplatin.[18-23] It is also considered that Pt(IV) compounds regain antitumor activities *via* reduction to Pt(II) complexes by reductants like ascorbic acid and glutathione, which present higher concentrations in the tumour cells than in blood and normal tissues. Therefore, the Pt(IV) compounds will undergo fewer substitution reactions in blood and normal tissues than in cancer cells and thus improve its targeting properties. The activation order of platinum drugs by dissociation the leaving group ligands is dichloride (cisplatin) > oxalate (oxaliplatin) > malonate (carboplatin), indicating the high cytotoxicity for the Pt(IV) prodrugs modified by cisplatin. As shown in Figure 1, Mitaplatin (4) combining the orphan drug dichloroacetate (DCA) with cisplatin alters the mitochondrial membrane potential gradient in cancer cells. Moreover, the Pt(IV)

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Electronic Supplementary Information (ESI) available: Details characterization data, ^1H and ^{13}C NMR spectra of new compounds.

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DOI: 10.1039/C6DT02207C

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compounds (5) and asplatin (6) also show high cytotoxicity.[24, 25]

It is well known that carbohydrates play a key role in various biological processes.[26] It was proved that carbohydrate uptake of cancer cells significantly increased as the reason of their unrestricted proliferation, known as the "Warburg effect". Dysfunctional mutations may lead to GLUT1 deficiency syndrome and whereas the overexpression of GLUT1 is a prognostic indicator for cancer. Conjugating 2-deoxy glucose with therapeutic or diagnostic agents was successfully achieved as the GLUT-targeted tumour drug delivery.[27, 28] Sugar–platinum conjugates as enzyme activating compounds have been reported playing key roles in the antibody-directed enzyme prodrug therapy approach.[29, 30] As shown in Figure 1, our group firstly reported the synthesis, characterization and cytotoxicity of a novel carbohydrate Pt(II) analogue (compound 7),[31] but the glycosylated platinum(IV) cancer chemotherapy has remained virtually unexplored.[32]

In order to overcome the severe side-effects of cisplatin-, carboplatin- and oxaliplatin-based chemotherapy, the glycosylation of platinum(IV) prodrugs were developed. The following points should be taken into consideration during the development of more potent cisplatin analogues: (1) exploring a series of platinum(IV) prodrugs instead of the Pt(II) complexes to improve the stability, increase the lifetime in biological fluids and disfavour the reactions with biological nucleophiles, (2) adjusting the length and steric hindrance of axial ligands to accommodate the reduction potential with a positive shift in cancer cells but not the normal cells such as 3T3, (3) altering their lipophilicity which can affect the ability of entering tumour cells before being reduced to the active Pt(II) compound to explore the platinum(IV) prodrugs with a high tumour cellular uptake and DNA platination, (4) overcoming the failure of cisplatin-based therapeutic strategy for hormone refractory disease.

Based on these observations, we report, for the first time, a novel series of sugar conjugated platinum(IV) analogous (**A1**-**A5**) and evaluate their bioactivities *in vitro* and *in vivo*. (Figure 2). In order to evaluate the impacting potency of different glycosyl moieties to tumour cells, glucose, mannose and rhamnose were introduced to cisplatin, respectively. To the best of our knowledge, the length and steric hindrance of axial ligands play an important role in the cytotoxicity to cancer cells.[33-38] And therefore variable aliphatic chains in different lengths were designed with the aim of exploring suitable linker between sugar and platinum.

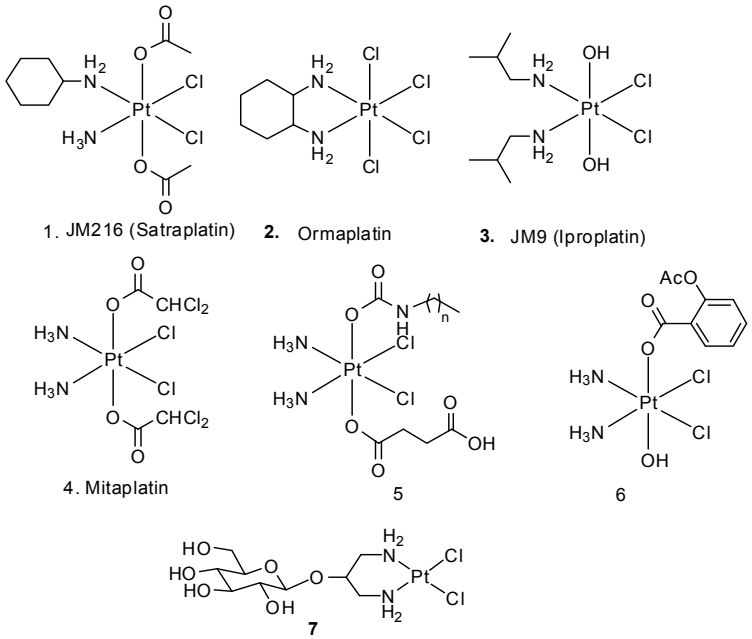

Figure 1. Pt(IV) compounds with bioactive ligands: (1) JM216 (Satraplatin), (2) Ormaplatin, (3) JM9 (Iproplatin), (4) Mitaplatin, (5) Pt(IV) compounds with variation of the length of the aliphatic tail, (6) Asplatin, (7) A carbohydrate-linked cisplatin analogue.

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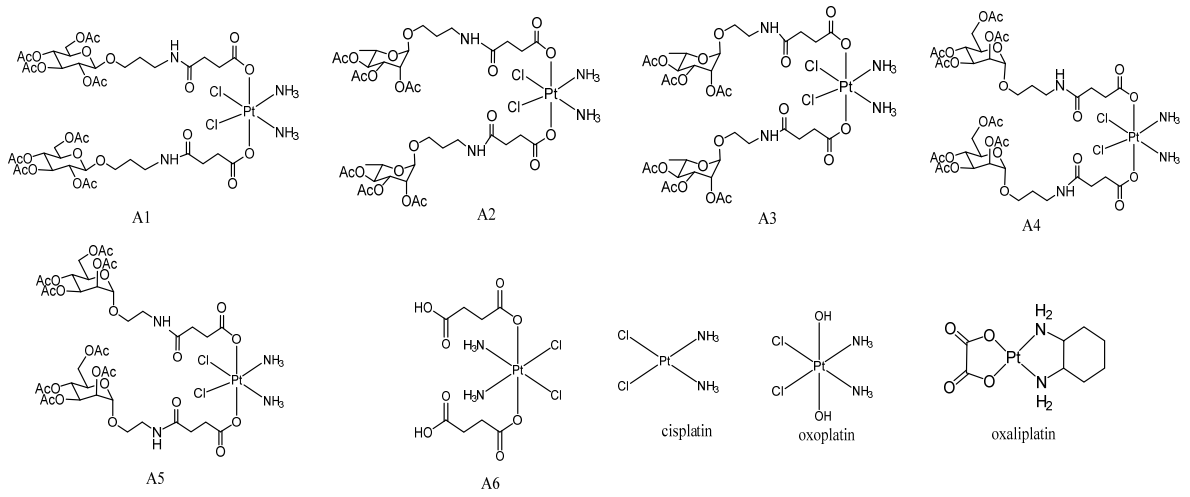
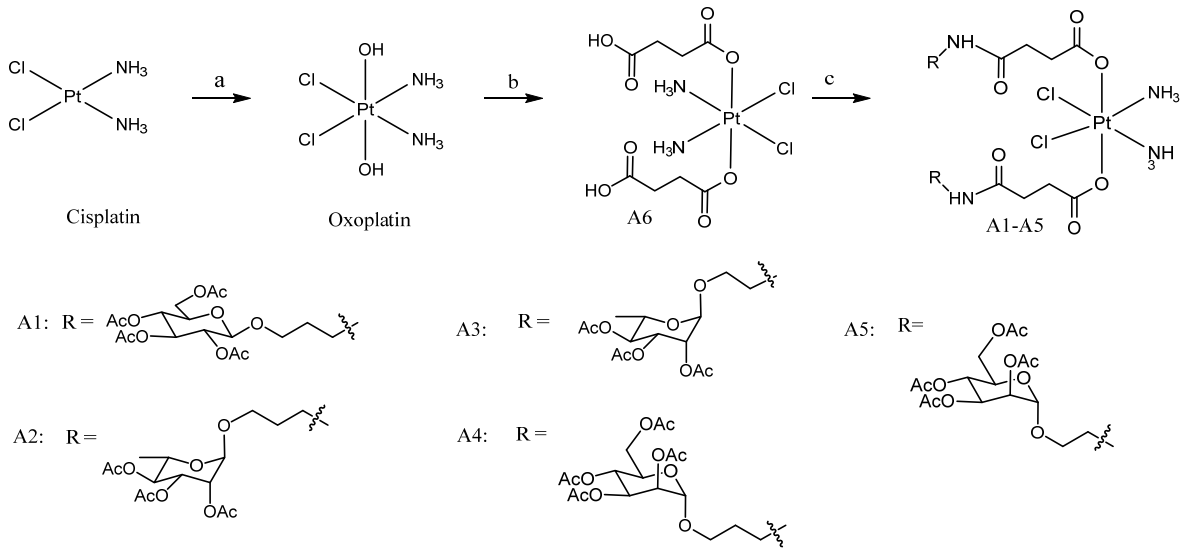


Figure 2. Chemical structures of the glycosylated platinum(IV) prodrugs (**A1**-**A6**), cisplatin, oxoplatin, and oxaliplatin.

Results and Discussion

Synthesis of target compounds



Scheme 1. Synthetic route of target compounds. Conditions and reagents: (a) H_2O_2 (30% w/v) /H_2O; (b) Succinic anhydride, DMF; (c) HATU, DIPEA, DMF.

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A novel series of sugar conjugated platinum(IV) analogous (**A1**-**A5**) were synthesized *via* the formation of an amide bond from peracetyl glucose, rhamnose and mannose with a propyl amino or ethyl amino linker at the reducing end and a carboxylic function in the Pt(IV) complex. In our case, we used cis,cis,trans-[$PtCl_2(NH_3)_2$ (succinate)] (**A6**) as the precursor for the synthesis of the conjugates. **A6** was prepared from oxoplatin (1equiv) and succinic anhydride (4equiv) in anhydrous DMF at 75℃. After 4h reaction, DMF was removed under vacuum to afford a yellow oil. And then the oil was washed with dichloromethane and diethylether, and dried in vacuum. The target complex **A6** was obtained as a pale yellow solid after purification by recrystallization. The key step involved the preparation of oxoplatin which was reacted successively in water (12 mL) with cisplatin (0.2 g) and H_2O_2 (20 mL) at 60℃. Finally, to a solution of **A6** (1equiv) in DMF was added a DMF solution containing HATU. The mixture was stirred for 10 min at room temperature. A DMF solution containing **S5**, **S10**, **S13**, **S18** or **S21** and DIPEA was added to the resulting solution to obtain **A1**, **A2**, **A3**, **A4** and **A5** in yields of 20-30%, respectively. The mixture was stirred at room temperature for 24 in the dark. The product was characterized using ^{1}H-NMR, ^{13}C-NMR, HRMS spectrometry and elemental analysis (see ESI).

Antitumor activities *in vitro*

Five human cancer cell lines, including lung carcinoma cells (A549), cervical cancer (HeLa), prostatic carcinoma (LNCaP and PC3) and breast carcinoma (MCF7) were used to evaluated the *in vitro* antitumor activities of glycosylated Pt(IV) compounds to determine their potential as anticancer agents using the MTT assay. The cytotoxicities of cisplatin, oxoplatin and oxaliplatin were also determined as control. As listed in Table 1, the cancer cells were exposured to the platinum(IV) (**A1–A5**) complexes for 48h. In contrast to their precursory oxoplatin, the introduction of glycosyl fragments to Pt(IV) complexes to yield compounds **A1**-**A5** leads rather broaden antitumor spectrum and enhanced activities. Especially, the introduction of mannoside fragments to Pt(IV) complexes to yield compounds **A4** and **A5** leads an more positive antitumor spectrum and activities of these target compounds in contrast to glucoside and rhamnoside (Table 1). These results manifest that different structural carbohydrates show great influence on the bioactivities of target compounds. Moreover, linkers between glycoside and platinum core also shade much effects on the antitumor potency of target compounds.

Noticeably, conjugate **A4** displayed significantly high cytotoxicity against the five cancer cells, especially for Hela and LNCaP. **A4** showed an IC_{50} value of 4.82±0.45 μM, which represents a 5-fold cytotoxic increase of the oxaliplatin and about 2-fold increase of the cisplatin to LNCaP. In addition, the cytotoxicity against Hela, A549 and PC3 cell lines was increased by 2-fold when compared with oxaliplatin. **A5** also showed obvious cytotoxicity to A549, MCF-7, LNCaP and PC3. Moreover, **A5** showed 12-fold and 6-fold cytotoxic increase compared with clinical drug oxaliplatin and cisplatin against LNCaP with the IC_{50} value of 1.9±0.67 μM.

In addition, glycosylated Pt(IV) compounds (**A1**, **A3**, **A4** and **A5**) almost fully overcame the failure of cisplatin-based therapeutic strategy for hormone refractory disease as the reason of resistance to apoptotic death to the advanced PCa with the IC_{50} from 1.9 μM to 30.07 μM, especially the compounds **A4** and **A5** with the IC_{50} 4.82 μM and 1.9 μM respectively. The results enable these compounds to be of much potential for further investigations as new antitumor agents.

Cell viability of 3T3

The antitumor activities *in vitro* indicated that the glycosylated Pt(IV) compounds **A4** and **A5** behaved enhanced cytotoxicity to a variety of cancer cells. To evaluate the potential safety of the sugar conjugates **A4** and **A5**, the inhibitory effect (IC_{50} in μM) of **A4** and **A5** on normal cell (3T3) was done. Compared with cisplatin (IC_{50} = 8 μM) and oxaliplatin (IC_{50} = 71 μM), A4 (IC_{50} = 84 μM) and A5 (IC_{50} = 169 μM) showed an obvious lower toxicities to 3T3. As to **A4** and **A5**, cell viability of 3T3 is superior to the control group cisplatin and oxaliplatin (Figure 3).

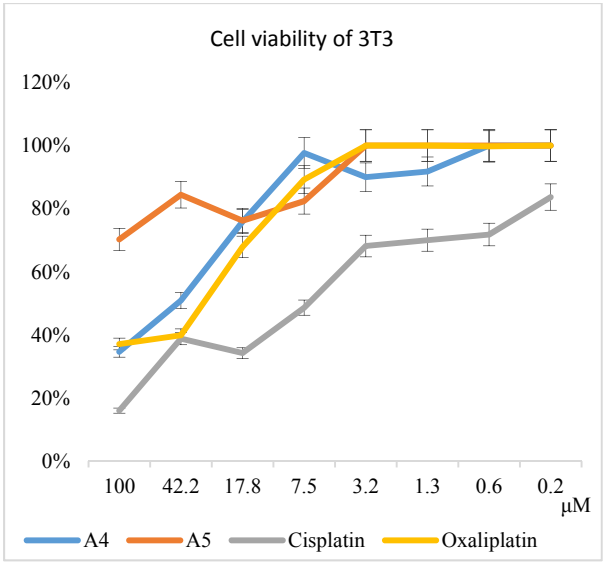

Figure 3. Cell viability of 3T3.

Antitumor activities *in vivo*

To evaluate the potential safety of **A4** and **A5**, we also conducted the antitumor activities *in vivo* (Table 2). An acute toxicity study using Kunming mice administered intravenously (i.v.) was proceeded for 2 weeks. The maximum tolerated dose (MTD) and the lethal dosage values (LD_{50}) were confirmed and the results were summarized in Table 2. The MTD and LD_{50} for A4 and A5 are nearly 4-fold higher than that of oxaliplatin indicating that the glycosylated platinum(IV) complexes may have significantly enhanced the feasibility and potential safety of high-dose treatment.

The ratios of the cytotoxicity (IC_{50}) toward LNCaP to animal lethal dosage values (LD_{50}) of the studied complexes were used as a measure for the therapeutic index. The results show that **A5** is the safest complex with a therapeutic index over 16-fold higher than that of clinical drug oxaliplatin

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Table 1. Inhibitory effect (IC_{50} in μM) of carbohydrate platinum(IV) complexes on cancer cells.

IC_{50} (μM)	Hela	A549	MCF-7	LNCaP	PC3
A1	10.32±1.34	42.31±1.21	94.64±2.23	11.37±2.24	19.92±0.67
A2	6.18±1.70	>100	>100	65.76±1.78	51.12±1.12
A3	21.23±0.91	35.13±1.35	72.07±2.95	30.07±2.25	36.86±0.69
A4	2.81±0.75	11.71±1.2	30.44±2.56	4.82±0.45	26.35±0.76
A5	8.36±0.91	24.71±1.35	18.18±1.15	1.90±0.67	41.53±1.56
cisplatin	1.83±0.14	15.23±1.14	28.02±1.01	10.72±1.65	28.00±1.45
oxaliplatin	4.96±1.56	26.07±1.83	11.60±0.89	22.76±1.78	42.93±1.55
oxoplatin	25.89±1.09	>100	>100	>100	50.08±1.61

indicating that it may potentially allow an adequate margin of safety over the *in vivo* and clinical therapeutic exposure.

Table 2. *In vivo* maximum tolerated dose and lethal dosage values, and calculated therapeutic indices (LD_{50} / IC_{50}) of complexes **A4**, **A5** and oxaliplatin.

Complexes	MTD (mg/kg)	LD_{50} (mg/kg)	LD_{50} (μM /kg)	48h Avg IC_{50} (μM)	LD_{50}/ IC_{50}
A4	60	120	91.79	4.82	19.04
A5	61	125	97.71	1.90	51.43
oxaliplatin	16	30	75.56	22.76	3.32

Annexin V/PI coupled flow cytometric analysis

The DNA-flow cytometric studies were conducted to identify the incidence of cell cycle arrest following treatment with **A4**. **A4** arrests the cycle in LNCaP cells in a time-dependent manner. A large population of cells (24.8%) accumulated at the G2/M after 30h incubation at 1μM. We found that the glycosylated Pt(IV) compounds **A4** effectively entered cancer cells and arrested the cell cycle at the G2/M phases, distinctive of that from cisplatin.

Annexin V/PI coupled flow cytometric analysis at 10 μM after 30 h incubation in Hela showed that **A4** can efficiently induce apoptosis by prompting a larger population of cells to undergo early apoptosis (8.3 %), late apoptosis (11.9 %) and necrosis (39.6 %) with the sum of 59.8 % compared with cisplatin (35.5 %) as show in Table 3, respectively. All these cell-based experiments clearly indicated that **A4** can effectively induced cell damage and thus led to cell cycle arrest and apoptosis in cancer cells.

Table 3. Quantification of apoptosis in Hela cells using an annexin V/PI assay.

Compd.	Early apoptosis	Late apoptosis	Necrosis	Sum
A4	8.3	11.9	39.6	59.8
Cisplatin	34.6	0.6	0.3	35.5
Untreated	0.2	0.2	0.1	0.5

Cellular uptake and DNA platination

For the cytotoxicity of metal-based anticancer drugs, the investigation of the cellular uptake efficiency is of high relevance to determine the potential activity of the compounds as well as to understand their mechanism of action. Subsequently, the mechanism was explored to investigate the correlation between intracellular platinum accumulation and antitumor activity. Intracellular levels of platinum in Hela and LNCaP cells and the platinum in DNA were quantified by inductively-coupled plasma mass spectrometry (ICP-MS) using ^{196}Pt detection after a 10 h exposure to the compounds at 50 μM as shown in Figure 4.

The accumulation of platinum in Hela cells after the incubation with **A4** was 7.9-fold and 2.8-fold higher than that of cisplatin and oxaliplatin, respectively. The platinum in DNA isolated from Hela cells were also measured, which showed 3.6-fold and 3.1-fold higher than oxaliplatin and cisplatin, respectively. The results indicated that the presence of sugar fragment in Pt(IV) complexes has a positive effect on the intracellular accumulation of the Pt(IV) pro-drug. Under the same incubation conditions, the evaluated compound **A5** also

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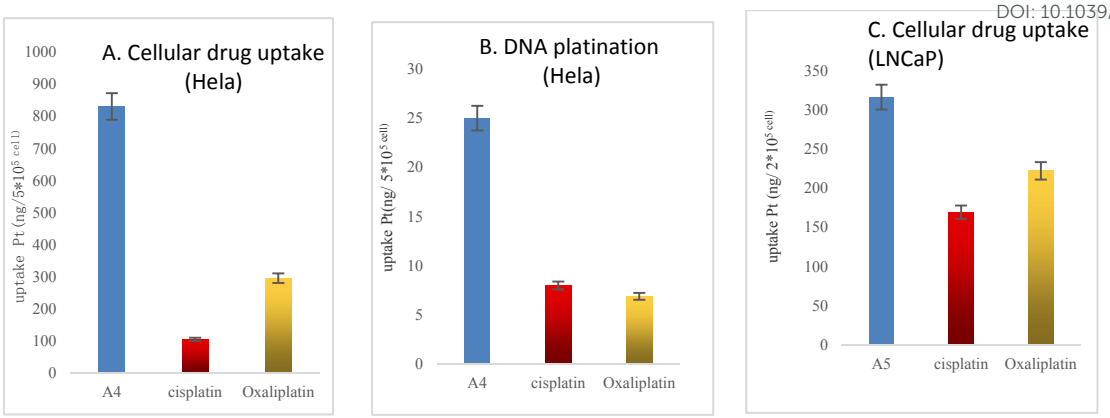


Figure 4. Cellular drug uptake and DNA platination. Platinum in HeLa and LNCaP cells were determined using ICP-MS at the 50 μM platinum complex. (A) Platinum in Hela cells, (B) Platinum in DNA (Hela), (C)Platinum in LNCaP cells.

showed 1.9-fold and 1.4-fold higher accumulation in LNCaP cells compared with cisplatin and oxaliplatin, respectively. The properties of better DNA targeting efficiency of **A4** and **A5** may account for the higher cytotoxicity. Simultaneously, cellular uptake studies by ICP-MS confirmed a good correlation between intracellular platinum accumulation and antitumor activity.

Cyclic voltammograms

To investigate the conceivable reasons why the introduction of mannoside fragments to Pt(IV) complexes to yield compounds A4 and A5 leads an more positive antitumor spectrum and activities in contrast to glucoside and rhamnoside, cyclic voltammograms were employed. At the same time, A4 and A5 also behaved lower toxicity to the normal cells such as 3T3 compared with the cisplatin and oxaliplatin. Generally, the intracellular pH is about 7.2, whereas the extracellular pH about 7.4 in normal cells. However, the extracellular pH is lower than 7.1 in cancer cells.[36] And therefore we studied the reduction properties of **A1** to **A5** at pH 6.4 to mimic the reduced extracellular pH of the cancer cells. Simultaneously, the cyclic voltammograms of **A4** and **A5** at pH 7.4 were studied to imitate the pH value of the normal cells.

At pH 6.4, a relative positive shift of the peak current for **A1**, **A2**, **A4** and **A5** was observed at the concentration of 0.08 mM (Figure 5). Compared with the reduction properties of **A4** and **A5** at pH 6.4, the relative negative shift of the peak current at pH 7.4 was observed. The results indicated that the peak current from **A1** to **A5** did not show much difference. The mechanism of the glycosylated platinum(IV) prodrugs (**A1**-**A5**) need to be further studied.

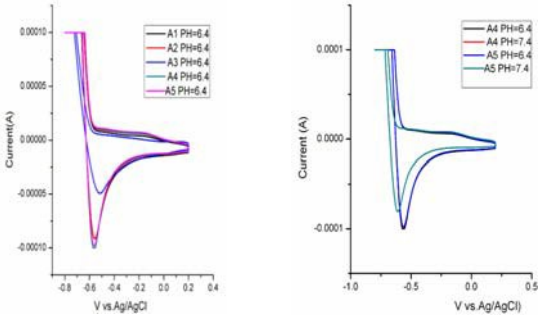
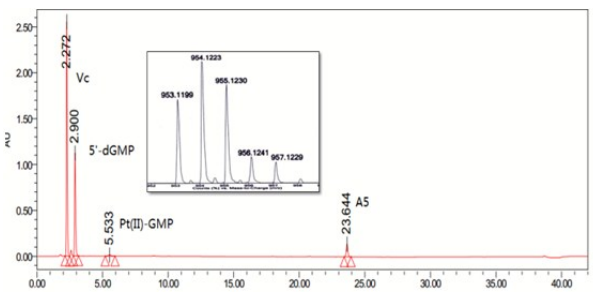


Figure 5. Cyclic voltammograms of **A1**-**A5** in phosphate buffer-0.1 M KCl at two different pH values. (a). The cyclic voltammograms of **A1**-**A5** with the scan rate of 200 mv/s at pH 6.4. (b).The cyclic voltammograms of **A4** and **A5** with the scan rate of 200 mv/s at pH 6.4 and 7.4.

The reduction of Pt(IV) complexes

The important anticancer activity of cisplatin resulted from the inherent proficiency to bind with the N7 position of guanine bases, thus leading to DNA damage and inducing cell death.[39] In these experiments 5'-dGMP was applied as a model of DNA. The reactivity of platinum(IV) pro-drugs **A5** towards 5'-dGMP was investigated by treatment with ascorbic acid for RP-HPLC analysis at 37 °C in presence of reducing ascorbic acid (Vc). The adducts (Figure 6) [40-41] were observed after the incubation of **A5**, Vc, and 5'-dGMP for 24h and 72h (other HPLC results are given in supporting information). The unknown peak fractions were identified by ESI-MS analysis. No visible reaction was observed in the absence of ascorbic acid, which indicated that the pro-drugs were activated upon the reduction by ascorbic acid and meanwhile glycosylated Pt(IV) compounds exerted their anticancer activity *via* the released Pt(II) complexes. Reaction of **A5** in the presence of ascorbic acid was also showed in Figure 6. After reduction by Vc, cisplatin was formed and then combined with 5'-dGMP to generate the bis-substituted products. Therefore, the glycosylated Pt(IV) complexes could be reduced by ascorbic acid. Following such reduction, glycosylated Pt(IV) compounds exert their anticancer activity *via* the released Pt(II) complexes.



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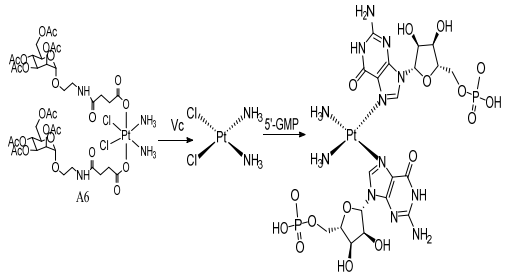

Figure 6. Reaction of **A5** with 5'-dGMP in the presence of ascorbic acid incubated at 37 °C after 24h.

Conclusions

In summary, five novel glycosylated platinum(IV) prodrugs were designed and synthesized. And the biological evaluations of the glycosylated platinum(IV) prodrugs indicated that the mannose conjugated platinum(IV) complexes A4 and A5 exhibited high potent cytotoxicity to cancer cells, especially to LNCaP. Furthermore, the high cytotoxic platinum(IV) complexes **A4** and **A5** showed low toxicities to the normal cell (3T3). It was worth mentioning that **A5** with a shorter chain compared with **A4**, exhibited a better cytotoxicity to LNCaP (IC_{50} = 1.9 μM). Compared with cisplatin and oxaliplatin, IC_{50} value of **A5** is higher than 100 μM against 3T3 indicated a low toxicity to normal cells. The cellular platinum in Hela and LNCaP cells and the DNA platinum of **A4** and **A5** indicated that the higher cytotoxicity could be correlated with its higher DNA targeting efficiency upon the reduction by ascorbic acid.

Interestingly, **A4** and **A5** overcame the failure of cisplatin-based therapeutic strategy for hormone refractory disease, which probably resulted from the different cellular responses of the mannose substituted platinum(IV) complexes relative to cisplatin. The favourable biological results of the mannose conjugated platinum(IV) complexes supports its clinical development to become a new class of Pt(IV) antitumor agent. These studies provide new insights into the potential of the glycosylated Pt(IV) anticancer prodrugs, particularly on how multivalency can improve both the selectivity and potency of such metallodrugs by increasing cellular accumulation in tumour tissues.

Experimental Details

Materials

Cisplatin and oxaliplatin was purchased from Yurui chemical Co. Ltd (Shanghai, China). All other chemicals obtained from commercial suppliers were used as received and were of analytical grade. If necessary, the reactions were carried out in dry solvents and under argon atmosphere. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE AV400 (400 MHz and 100 MHz). High resolution mass spectra (HRMS) were obtained on an IonSpec QFT mass spectrometer with ESI ionization. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ascorbic acid (AsA), 5'-GMP (purity ≥ 98.0%), DMEM (for 3T3 cells) and RPMI1640 (for A549, Hela, LNCaP, Mcf-7 and PC3 cells medium containing 10 % fetal bovine serums were purchased from GL Biochem Ltd. Genomic DNA Mini Preparation Kit from Beyotime, China was used for cellular drug uptake and DNA platination and Annexin V-FITC Apoptosis Detection Kit from KeyGEN Biothch, China was used for Annexin Ⅴ/PI coupled flow cytometric analysis. Phosphate buffered saline (PBS) contains 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4. Fetal bovine serum (FBS), 0.25% trypsin/EDTA solutions, and penicillin-streptomycin solutions were purchased from Invitrogen (Grand Island, NY, USA). Hela (Cervical), MCF-7 (breast cancer), A549 (lung carcinoma), LNCaP (Prostate), PC3 (Prostate) cells were obtained from Prof. Yanming Wang (College of Pharmacy, Nankai University, Tianjin, China).

HPLC analyses were performed as on an Waters E2695-2998 system equipped with a Venusil MP C18 column (150 × 4.6 mm, 5 μm). HPLC profiles were recorded by UV detector at 273 nm at room temperature. The mobile phase consisted of MeOH and H2O was used and the flow rate of 1 mL/min.

In Vitro Cellular Cytotoxicity Assays

Cells seeded in 96-well plates were incubated in a 5% CO_2 atmosphere in 100 μL of complete medium at 37 ℃ for 24 h. Then 100μL freshly prepared culture medium containing drugs at different concentrations was added and incubated for another 48 h. MTT (5 mg/mL, 20 μL) was added and incubated for 3 h. Finally, the medium was removed and DMSO (150μL) was added. The absorbance was measured at 570 nm using a Bio-Rad 680 microplate reader. The IC_{50} values were calculated using GraphPad Prism software, which were based on three parallel experiments.

In vivo antitumor assay

Kunming mice (28-42 d) were purchased from Laboratory Animal Center, Academy of Military Medical Science (Beijing, China). All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The maximum tolerated dose (MTD) evaluated by calculating body weight loss (mean weight loss < 15% and < 15% toxic deaths) and the lethal dosage values (LD_{50}) were determined. The value of TI was calculated according to the literature.[42]

Flow cytometric analysis

HeLa and LNCaP cells cultured in 6-well plates were treated with and without drugs at 37 °C. Cells were harvested from adherent cultures by trypsinization and centrifuged at 1000 rpm for 5 min. PBS was added to wash the cells. Fixed cells with 70% ethanol in PBS were collected by centrifugation at 2500 rpm for 3 min, washed with PBS, and centrifuged as before. Cellular pellets were resuspended in 50 μg/mL propidium iodide in PBS for nucleic acids staining and treated with 100 μg/mL RNaseA. Apoptotic cells were detected by flow cytometry after staining with Annexin V and Propidium Iodide (PI) using the AnnexinV-FITC apoptosis detection kit.

Cellular Platinum Uptake and DNA Platination

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DOI: 10.1039/C6DT02207C

The cellular uptake of cisplatin, oxaliplatin, **A4** and **A5** was measured on HeLa and LNCaP cells. HeLa and LNCaP cells were seeded in 6-well plates overnight and then incubated with 50 μM drugs in standard culture conditions for 10 h. Then the cells were washed with PBS buffer for three times, and harvested by trypsinization. The harvested cells were concentrated and digested by nitric acid for the ICP-MS. The cell numbers were counted before the digested. Genomic DNA Mini Preparation Kit was used for the isolation of DNA in Hela cells and Pt concentration in cellular DNA in HeLa cells digested by nitric acid was also measured by ICP-MS.

Cyclic Voltammetry

Electrochemical measurements were made at 25 °C on an analytical system model CHI 920c potentiostat from CH Instruments, Inc using a glassy carbon working electrode, platinum wire auxiliary electrode and an Ag/AgCl (3 M KCl used as a supporting electrolyte) reference electrode. **A1**, **A2**, **A3**, **A4** and **A5** solutions were prepared in phosphate buffered saline (PBS) of pH 6.4. **A4** and **A5** were also prepared for PH 7.4 and cyclic voltammograms were recorded at the scan rate of 200 mv/s.

Synthetic procedures

Preparation for A1

The complexe **A6** was synthesized as previously described.[43] To a solution of **A6** (0.38 mmol) in DMF (10 mL) was added a DMF solution (0.5 mL) containing HATU (1.52 mmol). This mixture was stirred for 10 min at room temperature. To the resulting solution was added a DMF solu tion containing S5 (3 mmol) and DIPEA (1.9 mmol). The mixture was stirred at room temperature for 24 h in the dark. The DMF was then removed under vacuum to afford a yellow oil. Compound **A1** was purified by silica gel column chromatography as yellow solid in yield of 20%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.41 (d, J = 146.8 Hz, 4H), 5.47 – 4.79 (m, 14H), 4.64 – 4.08 (m, 5H), 4.02 – 3.52 (m, 4H), 3.39 (d, J = 69.4 Hz, 3H), 2.55 (s, 5H), 1.96 (t, J = 61.2 Hz, 26H), 1.26 (dd, J = 167.1, 94.1 Hz, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.56, 173.52, 171.00, 170.38, 170.15, 169.59, 100.82, 90.43, 72.70, 72.00, 71.47, 71.25, 70.00, 68.56, 68.52, 67.37, 62.07, 61.95, 60.49, 37.40, 32.30, 29.85, 28.85, 21.01, 20.98, 20.94, 20.91, 20.88, 20.83, 20.75, 14.35. HRMS: Calcd. for $C_{42}H_{66}Cl_2N_4O_{26}Pt$ (M_+): 1307.2988, found: 1307.2979. Elemental analysis, found % C, 38.58; H, 5.06; N, 4.12% calcd. for $C_{42}H_{66}Cl_2N_4O_{26}$ Pt C,38.54 ; H, 5.08; N, 4.28.

Preparation for A2

Compound A2 was prepared according to the procedure described for compound A1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.28 (br, 6H), 5.22 – 4.89 (m, 5H), 4.72 (br, 1H), 3.78 (d, J = 34.5 Hz, 4H), 3.37 (d, J = 54.1 Hz, 8H), 2.63 (d, J = 73.3 Hz, 5H), 1.99 (dd, J = 77.6, 46.1 Hz, 21H), 1.21 (d, J = 11.5 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.83, 170.76, 170.20, 97.45, 70.91, 69.86, 69.63, 66.65, 66.23, 37.60, 32.47, 29.89, 28.85, 22.82, 21.14, 20.99, 17.49. Calcd. for $C_{38}H_{62}Cl_2N_4O_{22}Pt$ (M^+): 1191.2878, found: 1191.2777. Elemental analysis, found % C, 38.33; H, 5.30; N, 4.60. calcd. for $C_{38}H_{62}Cl_2N_4O_{22}Pt$ C, 38.26 ; H, 5.24; N, 4.70.

Preparation for A3

Compound A3 was prepared according to the procedure described for compound A1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.58 (d, J = 274.5 Hz, 6H), 5.37 – 4.64 (m, 6H), 3.62 (dd, J = 133.1, 48.2 Hz, 9H), 2.58 (d, J = 55.1 Hz, 6H), 2.33 – 1.50 (m, 18H), 1.02 (d, J = 136.3 Hz, 13H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.94, 173.50, 170.43, 170.29, 170.02, 97.32, 70.74, 69.51, 69.32, 66.46, 54.03, 53.53, 42.31, 39.09, 29.64, 20.94, 20.86, 20.80, 18.62, 17.42, 17.35, 12.17. Calcd. for $C_{36}H_{58}Cl_2N_4O_{22}Pt$ (M^+): 1163.2565, found: 1163.2526. Elemental analysis, found % C, 37.22; H, 5.08; N, 4.90. calcd. for $C_{36}H_{58}Cl_2N_4O_{22}Pt$ C, 37.12; H, 5.02; N, 4.81.

Preparation for A4

Compound A4 was prepared according to the procedure described for compound A1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.39 (d, J = 157.4 Hz, 6H), 5.39 – 5.03 (m, 5H), 4.81 (br, 2H), 4.25 (br, 2H), 4.10 (d, J = 11.7 Hz, 2H), 3.97 (br, 2H), 3.75 (br, 2H), 3.61 – 3.11 (m, 5H), 2.55 (d, J = 37.4 Hz, 8H), 2.23 – 1.49 (m, 27H), 1.19 (dd, J = 15.9, 9.0 Hz, 5H). ^{13}C NMR (100 MHz, CDCl3) δ 182.41, 173.59, 171.01, 170.49, 169.92, 97.70, 69.58, 69.45, 68.62, 66.49, 66.17, 62.72, 37.32, 32.35, 32.17, 29.07, 21.07, 20.95, 20.90. HRMS: Calcd. for $C_{42}H_{66}Cl_2N_4O_{26}Pt$ (M^+): 1307.2988, found: 1307.3002. Elemental analysis, found % C, 38.50; H, 5.10; N, 4.29. calcd. for $C_{42}H_{66}Cl_2N_4O_{26}Pt$ C, 38.54; H, 5.08; N, 4.28.

Preparation for A5

Compound A5 was prepared according to the procedure described for compound A1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.42 (d, J = 190.6 Hz, 6H), 5.28 (dd, J = 16.8, 9.1 Hz, 6H), 4.87 (br, 2H), 4.26 (dd, J = 12.2, 4.8 Hz, 2H), 4.14 (d, J = 10.4 Hz, 2H), 3.99 (br, 2H), 3.78 (br, 2H), 3.58 (d, J = 9.3 Hz, 3H), 3.41 (br, 2H), 2.58 (d, J = 38.5 Hz, 8H), 2.08 (dd, J = 39.8, 21.5 Hz, 24H), 0.88 (dd, J = 14.7, 8.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.95, 170.82, 170.49, 170.36, 169.77, 97.64, 69.37, 68.68, 67.08, 66.19, 66.02, 62.59, 39.42, 29.67, 29.33, 20.94, 20.88, 20.80, 20.75, 20.70, 20.64, 20.58, 20.53. HRMS: Calcd. for $C_{40}H_{62}Cl_2N_4O_{26}Pt$ (M^+): 1279.2675, found: 1279.2678. Elemental analysis, found % C, 37.66; H, 4.89; N, 4.40. calcd. for $C_{40}H_{62}Cl_2N_4O_{26}Pt$ C, 37.51; H, 4.88; N, 4.37.

Supporting Information

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DOI: 10.1039/C6DT02207C

Experimental details for the characterization of the compounds studied are provided in the Supporting Information.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grants 21102076, 91013013, and 31100587) and the Natural Science Foundation of Tianjin (Grant 10JCYBJC04100).

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DOI: 10.1039/C6DT02207C

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