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Research paper

# Mono-functionalized glycosylated platinum(IV) complexes possessed both pH and redox dual-responsive properties: Exhibited enhanced safety and preferentially accumulated in cancer cells *in vitro* and *in vivo*

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ABSTRACT

A serious of carbohydrate-conjugated platinum(IV) complexes in the form Pt(L2)(A2)(OH)R based on the clinical drug cisplatin and oxaliplatin were designed, synthesized and evaluated as antitumor agents *in vitro* and *in vivo*. The conjugates possessing both pH and redox dual-responsive properties exhibited more potent cytotoxicity in seven different human cancer cell lines and lower toxicity to the normal 3T3 cells than cisplatin, oxaliplatin and even the reported bis-functionalized glycosylated platinum(IV) complexes indicating the enhanced safety of the sugar conjugates. Cellular drug uptake and DNA platination were also superior to cisplatin, oxaliplatin and the reported bis-functionalized ones. Peak current of **B7** and **B8** with the scan rate of 200mv/s at the concentration of 0.08 mM was 5-fold higher at pH 6.4 than the pH 7.4, indicating that carbohydrate-conjugated mono-functionalized platinum(IV) complexes possessed both pH and redox dual-responsive properties in the cancer cells. The *in vivo* assays demonstrated that the Pt(IV) compounds could inhibit the growth of MCF-7 tumour and exert more safety than oxaliplatin.



## 1. Introduction

Many of the limitations existing in the platinum(II)-based chemotherapies have been overcame by the platinum(IV)-based anticancer compounds. Due to the special inert of platinum(IV) complexes, the unwanted side reactions with biological nucleophiles are resisted to reduce the undesired side-effects and increase the lifetime in biological fluids [1]. The reductants like ascorbic acid and glutathione used to reduce the platinum(IV) complexes to regain their cytotoxicity present higher concentrations in the tumour cells than in blood and normal tissues. This characteristics afford unique opportu-nities for the design of platinum(IV) prodrugs, which are believed to be activated inside the cancer cells by reduction to cytotoxic platinum(II) complexes [2]. Many attempts have been done to tune the chemical properties of platinum(IV) complexes to enhance the anticancer efficacy of platinum agents [3]. The axial ligands play an important role in conferring the pharmacological properties and enhancing the target therapy [4,5].

Recent years, various cell-responsive prodrugs, such as redox active, pH dependent, and enzymes, have been intensively investigated. The nature of the axial ligands can influence the reduction potentials of the platinum(IV) prodrugs. Therefore, it is imperative to choose axial ligands that will minimize the chance of reduction in the blood stream before the prodrugs reach the cancer cells, but are amenable to reduction once they get inside these cells [6]. The intracellular pH (pHi) is about 7.2 and the extracellular pH (pHe) about 7.4 in normal cells. In cancer cells, the pHe value is lower than 7.1. Some cellular environments, such as endosomes (pH 5.0) and lysosomes (pH 4.5), are relatively acidic [7]. Due to tumour hypoxia,

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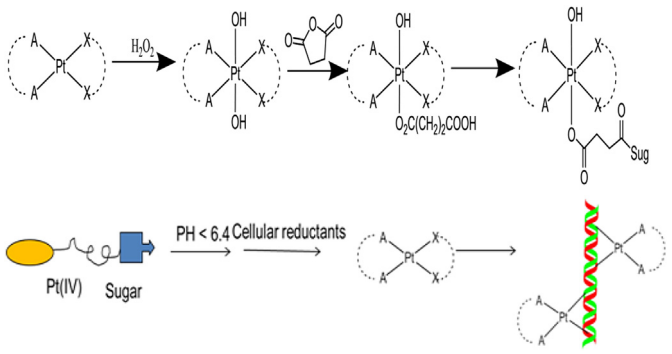
lower pH, elevated levels of GSH [8,9], tumours behave an altered redox state compared with normal tissues. Therefore, one of the important strategies to enhance drug efficacy and specificity is to improve the capacity of reduction at pH < 7.1, under which the platinum(IV) prodrugs possesses both pH and redox dual-responsive properties in the cellular environment.

Recently, the liposomes [10], polymers [11], inorganic nanomaterials [12–14], metal–organic frameworks [15,16], and calcium phosphate (CaP) nanoparticles [17] were used to synthesize the platinum(IV) prodrugs with pH or redox responsive properties. The platinum(IV) complexes platin-A with mono-functionalized ligands of aspirin were reported to have a positive shift of 42 mV at pH 6.4 and a negative shift of −563 mV at pH 7.4 [18,19a]. The reduction properties indicated that the reduced pHe in the cancer microenvironment would facilitate reduction of the platinum(IV) complexes with mono-functionalized ligands to release the active platinum(II) complexes. And also the mono-functionalized platinum(IV) complexes would behave a low toxicity to the normal cells as the result of the negative shift of reduction potential. And the use of the monohydroxido monocarboxylato framework was also reported to have advantages over the use of either the dihydroxido or the dicarboxylato framework due to its intermediate properties in the design of targeted Pt(IV) derivatives of oxaliplatin with reasonable rates of reduction [19b]. And thus we designed and synthesized a serious of carbohydrate-conjugated platinum(IV) complexes in the form Pt(L2)(A2)(OH)R based on the clinical drug cisplatin and oxaliplatin.

In the mitochondria of healthy non-cancerous cells, D-glucose is metabolized oxidatively and produces 36 mol ATP per mole of glucose. However, cancer cells metabolize glucose by aerobic glycolysis, which is energetically very inefficient and provides only 4 mol ATP from one mole of glucose. And therefore it is proved that carbohydrate uptake of cancer cells significantly increased with the unrestricted proliferation, which is explained by the “Warburg effect”. Therefore, the design of glycoconjugations can increase the specificity of the complex towards cancer cells whose receptors are over expressed in cancer cells [20]. Recently, many works have been devoted to the investigation of novel glycosylated Pt(II) derivatives, which indicated that the incorporation of sugar to platinum complex significantly reduced their toxicity and improved antitumor efficiency [21a–d]. And our group firstly synthesized the carbohydrate-conjugated platinum(IV) complexes and glycoconjugation is a promising strategy for specific targeting of cancer [21e,f]. The carbohydrate-conjugated platinum(IV) complexes in the form Pt(L2)(A2)(OH)R designed to possess both pH and redox dual-responsive properties are totally different from the reported carbohydrate-conjugated platinum complexes. In response to low pH and cellular reductants in cancer cells, it would behave an enhanced the cellular drug uptake and DNA platination (Scheme 1).




**Scheme 1.** Schematic illustration of the synthesis carbohydrate-conjugated with mono-functionalized platinum(IV) complexes and the release of platinum(II) in response to low pH and cellular reductants.

## 2. Results and discussion

Currently, functionalization is most commonly carried out using anhydrides and acyl chlorides, which undergo direct carboxylation reactions with platinum(IV) axial hydroxido ligands [22,23]. And therefore, succinic anhydride was introduced to the platinum(IV) axial hydroxido ligands. As we know, different glycosyl receptors are over expressed in different cancer cells. In order to evaluate their impact on antitumor potency, different glycosyl moieties including galactose, mannose and rhamnose with variable aliphatic chains in different lengths were introduced to these serial complexes. Two clinical Pt(II) drugs cisplatin and oxaliplatin were selected for construction of Pt(IV) complexes **B1**-**B10** to investigate the influence of different platinum cores on antitumor activities (Scheme 2).

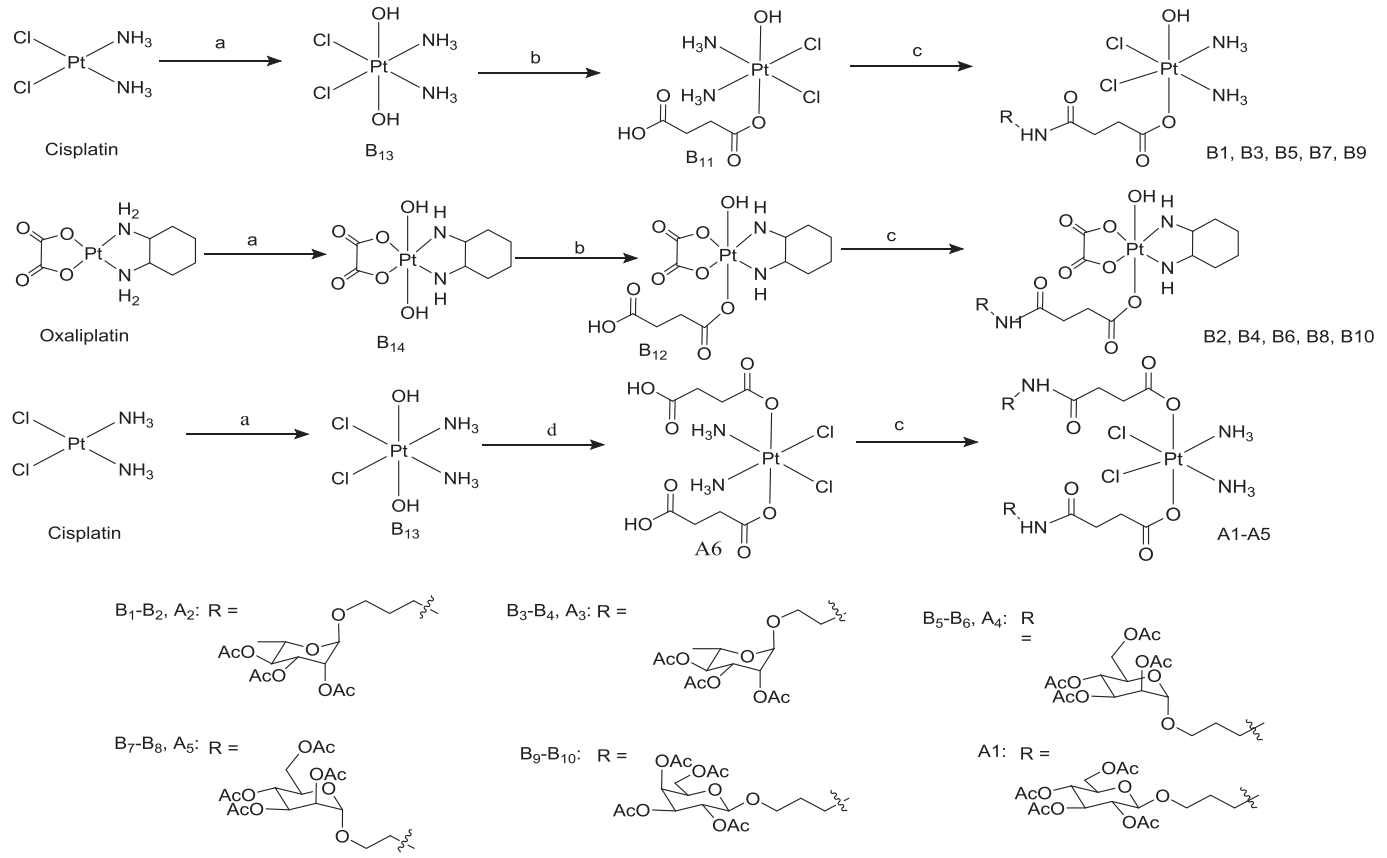
### 2.1. Antitumor activities in vitro

We evaluated the cytotoxicity of **B1**-**B10** against a panel of human cancer cells of different origin using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cytotoxicity of cisplatin, oxaliplatin, **B13** and **B14** were also determined as control. Inhibitory effect ($IC_{50}$ in μM) of carbohydrate platinum(IV) complexes on cancer cells are summarized in Table 1.

As shown in Table 1, the cytotoxicity of the carbohydrate platinum(IV) complexes are greater than that of oxoplatin **B13** and **B14** for MCF-7, HeLa and A549. It is worth mentioning that the cytotoxicity of mono-functionalized glycosylated platinum(IV) complexes **B1**-**B10** (RF 0.86 μM-3.22 μM) are generally superior to that of bis-functionalized glycosylated platinum(IV) complexes **A1**-**A5** ($IC_{50}$ > 100 μM) for A549R. And also for A549 and HepG-2, **B1** with $IC_{50}$ 9.38 μM and 8.96 μM, is more effective than **A1**-**A5** with $IC_{50}$ 11.71 μM–117.00 μM (A549) and 19.92 μM –51.12 μM (HepG-2), respectively. For MCF-7, **B1**, **B5**, **B6**, **B7**, **B8** and **B10** ($IC_{50}$ = 4.86 μM-9.34 μM), also show more prominent efficacy compared with **A1**-**A5**. ($IC_{50}$ = 18.18 μM –130.98 μM).

It is also observed that different glycosyl fragments behave different cytotoxicity to different cancer cells. And meanwhile the carbohydrate platinum(IV) complexes with cisplatin and oxaliplatin core display different antitumor efficacy. For the tested Pt(IV) compounds **B1**-**B10**, It is demonstrated that cisplatin derivatives **B1**, **B3**, **B5** and **B7** exhibit relatively more effective antitumor activities in comparison with corresponding oxaliplatin ones **B2**, **B4**, **B6** and **B8** for HeLa and A549. For PC3 and HepG-2, cisplatin derivatives **B1**, **B5**, **B7** and **B9** are also more effective than corresponding oxaliplatin ones **B2**, **B6**, **B8** and **B10**. And different structural carbohydrates and linkers also show great influences on the bioactivities of target compounds.

Noticeably, the rhamnose oxaliplatin derived compounds **B2** and **B4** show important cytotoxicity against the PC3 and HepG-2 than the mannosylated and galactosylated compounds **B6**, **B8** and **B10**. When the carbohydrate platinum(IV) complexes are replaced by mannose and galactose to yield **B6**, **B8** and **B10**, the antitumor activities dramatically decreased. Furthermore, the cisplatin derived compounds **B1**, **B3**, **B5**, **B7**, **B9**, exhibit relatively more effective antitumor activities against PC3 and HepG-2. The mannosylated and galactosylated compounds **B5** and **B9** are even more potent than cisplatin and oxaliplatin against PC3. Rhamnose containing propyl amino linker cisplatin derivative **B1** displays comparable $IC_{50}$ value to that of cisplatin, but rhamnosylated cisplatin derivative (**B3**) with ethyl amino linker has higher $IC_{50}$ value in




**Scheme 2.** Synthetic route of target compounds. Conditions and reagents: (a) $H_2O_2$ (30% w/v)/$H_2O$; (b) Succinic anhydride, DMSO; (c) HATU, DIPEA, DMF, r.t.; (d) Succinic anhydride, DMF.

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

| | HeLa | MCF-7 | LNCaP | PC3 | HepG-2 | A549 | A549R | RF[a] |
|---|---|---|---|---|---|---|---|---|
| **B1** | 1.32 ± 1.01 | 7.55 ± 1.44 | 5.52 ± 0.38 | 20.45 ± 1.45 | 8.96 ± 1.58 | 9.38 ± 1.39 | 16.83 ± 1.90 | 1.79 |
| **B2** | 4.59 ± 0.87 | 22.00 ± 1.87 | 8.23 ± 0.89 | 28.90 ± 1.49 | 28.75 ± 1.49 | 25.76 ± 1.47 | >100 | / |
| **B3** | 6.16 ± 0.54 | 22.61 ± 1.56 | 54.91 ± 0.78 | 44.34 ± 0.45 | 55.20 ± 1.67 | 23.53 ± 1.49 | 75.70 ± 1.78 | 3.22 |
| **B4** | 24.28 ± 0.53 | 18.99 ± 0.78 | 14.09 ± 1.34 | 40.73 ± 0.78 | 19.67 ± 1.49 | 30.9 ± 1.48 | 30.69 ± 1.79 | 0.99 |
| **B5** | 11.53 ± 0.34 | 9.34 ± 0.12 | 42.90 ± 1.45 | 15.23 ± 0.56 | 20.90 ± 1.79 | 26.39 ± 0.89 | 44.45 ± 1.67 | 1.68 |
| **B6** | 14.14 ± 0.45 | 4.86 ± 0.45 | 5.08 ± 1.78 | >100 | >100 | 31.44 ± 0.78 | 40.51 ± 1.69 | 1.29 |
| **B7** | 1.20 ± 0.78 | 9.20 ± 0.79 | 8.68 ± 0.45 | 33.69 ± 1.89 | 21.08 ± 1.46 | 38.17 ± 2.31 | 32.76 ± 1.69 | 0.86 |
| **B8** | 26.96 ± 0.65 | 8.60 ± 0.66 | 5.06 ± 0.89 | >100 | >100 | 40.59 ± 2.98 | >100 | / |
| **B9** | 24.99 ± 0.67 | 13.36 ± 0.77 | >100 | 13.76 ± 1.78 | 73.00 ± 1.38 | 38.93 ± 2.78 | 42.24 ± 1.23 | 1.09 |
| **B10** | 12.59 ± 0.78 | 7.40 ± 0.56 | 20.10 ± 1.66 | >100 | >100 | 18.44 ± 1.78 | >100 | / |
| **B13** | 35.98 ± 0.89 | 72.49 ± 1.34 | >100 | 58.08 ± 1.45 | 38.73 ± 1.39 | >100 | >100 | / |
| **B14** | 34.80 ± 1.12 | >100 | 18.90 ± 0.79 | >100 | >100 | >100 | 24.80 ± 2.57 | / |
| **A1** | 10.32 ± 1.34 | 94.64 ± 2.23 | 11.37 ± 2.24 | 19.92 ± 0.67 | >100 | 42.31 ± 1.21 | >100 | / |
| **A2** | 6.18 ± 1.70 | >100 | 65.76 ± 1.78 | 51.12 ± 1.12 | 80.89 ± 1.67 | >100 | >100 | / |
| **A3** | 21.23 ± 0.91 | 72.07 ± 2.95 | 30.07 ± 2.25 | 36.86 ± 0.69 | >100 | 35.13 ± 1.35 | >100 | / |
| **A4** | 2.81 ± 0.75 | 30.44 ± 2.56 | 4.82 ± 0.45 | 26.35 ± 0.76 | 46.85 ± 0.78 | 11.71 ± 1.20 | >100 | / |
| **A5** | 8.36 ± 0.91 | 18.18 ± 1.15 | 1.90 ± 0.67 | 41.53 ± 1.56 | 67.42 ± 1.03 | 24.71 ± 1.35 | >100 | / |
| cisplatin | 7.18 ± 0.89 | 10.00 ± 0.78 | 11.54 ± 1.77 | 28.00 ± 1.56 | 7.70 ± 1.89 | 10.47 ± 1.68 | 38.94 ± 1.57 | 3.72 |
| Oxaliplatin | 4.96 ± 0.96 | 11.60 ± 1.56 | 20.45 ± 1.55 | 42.93 ± 1.38 | 23.54 ± 1.38 | 16.07 ± 2.90 | 39.99 ± 2.67 | 2.49 |

[a] RF: Resistant factor = $IC_{50}$(A549R)/$IC_{50}$(A549).

comparison to that of cisplatin. In addition, glycosylated Pt(IV) compounds succeed to overcome the drug resistance of A549R cells with the RF from 0.86 to 1.79 (Table 1).

Furthermore, the rhamnose derived compounds **B1** show prominent efficacy against HepG-2, A549 and A549R than the galactosylated and mannosylated compounds. Compound **B1** exhibits competent activities with $IC_{50}$ values lower than 20.45 μM to all tested cell lines which are comparable or even more potent than cisplatin and oxali-platin. The results obtained enable these compounds to be of much potential for further investigations as new antitumor agents.

It is obvious that when the linkage of compound **B1** is replaced by shorter ones to yield **B3**, the antitumor activities decreased largely. We can also see the same phenomenon for **B2**-**B4** to HeLa, LNCap, PC3, A549 and **B6**-**B8** to HeLa, MCF-7, A549 and A549R. HeLa and MCF-7 were more sensitive to the carbohydrate

platinum(IV) complexes than the others. **B1** showed 5-fold and 4-fold cytotoxic increase compared with clinical drug cisplatin and oxaliplatin against HeLa with the $IC_{50}$ value of 1.32 μM.

In summary, to different cancer cells, different glycoside including rhamnoside, mannoside and galcatosde shows different antitumor abilities. This maybe enable these compounds to be of much potential for further investigations with target therapy.

### 2.2. Cell viability of 3T3

An ideal anticancer compounds should be selective for cancer cells over normal healthy cells, thereby mitigating undesired toxic side effects associated with chemotherapy. We therefore evaluated the selectivity of **B1**-**B10** using normal 3T3 cell (mouse embryo fibroblasts obtained from Prof. Yanming Wang, Nankai University). We evaluated the cytotoxicity of **B1**-**B10** against 3T3 cell using the MTT assay. Strikingly, as presented in Fig. 1, the cell viability of 3T3 was significantly higher after incubation with **B1**, **B6**, **B7**, **B8** as compared with cisplatin and oxaliplatin. Notably, at the concentration of 17.8 μM, cell viability of 3T3 is 100%, 100%, 70%, 72% for **B1**, **B6**, **B7**, **B8** and 25%, 65% for cisplatin and oxaliplatin, respectively. Compared with cisplatin ($IC_{50}$ = 8 μM), **B1** ($IC_{50}$ > 200 μM), **B6** ($IC_{50}$ > 200 μM), **B7** ($IC_{50}$ = 60 μM), **B8** ($IC_{50}$ > 100 μM) showed obvious lower toxicities. And compared with **A4** ($IC_{50}$ = 84 μM) and **A5** ($IC_{50}$ = 169 μM), **B1**, **B6**, **B8** also behaved a greater advantage indicating an enhanced safety of mono-carbohydrate platinum(IV) complexes. Compared with **A4** and **A5**, **B5** and **B7** with the same core, the same sugar and the same linker displaying comparable $IC_{50}$ values to 3T3, exhibited more effective to MCF-7.

### 2.3. Cell viability of MCF-7 cancer cells and the normal 3T3 cells

As shown in Fig. 2, cell viability of the normal 3T3 cells for **B1**, **B6**, **B7** and **B8** is better than that of MCF-7 cancer cells after incubation for 48 h at the concentration of 100 μM, 42.2 μM, 17.8 μM, 7.5 μM, 3.2 μM, 1.3 μM, 0.6 μM and 0.2 μM $IC_{50}$ values of **B1**, **B6**, **B7** and **B8** to the normal 3T3 cells are almost 50 folds higher than the most sensitive MCF-7 cells ($IC_{50}$ = 7.55 μM, 4.86 μM, 9.20 μM and 8.60 μM respectively) **(**Table 2**)**. This results indicate that **B1**-**B10** are much more potent in breast carcinoma MCF-7 cells compared with normal 3T3.

The inhibitory effects ($IC_{50}$ in μM) of **B1**, **B6**, **B7** and **B8** on MCF-7 cells are also almost 6-fold superior to the bis- functionalized prodrugs **A4** and **A5**. But to the normal cells, **B1**, **B6** and **B8** also behaved an enhanced safety, which indi-cates its more sensitive to low pH and cellular reductants in cancer cells than the normal cells performing totally different properties from the reported carbohydrate-conjugated platinum complexes.

### 2.4. Cellular uptake and DNA platination

Subsequently, the mechanism was explored to investigate the correlation between intracellular platinum accumulation and antitumor activity by inductively-coupled plasma mass spe trometry (ICP-MS) using $^{196}Pt$ detection after 10 h incubation (Fig. 3).

For HeLa, **A4** ($IC_{50}$ = 2.81 μM), **B1** ($IC_{50}$ = 1.32 μM) and **B7** ($IC_{50}$ = 1.20 μM) are more effective than the others. And meanwhile, **A5** ($IC_{50}$ = 1.90 μM), **B6** ($IC_{50}$ = 5.08 μM) and **B8** ($IC_{50}$ = 5.06 μM) exhibit enhanced cytotoxicity to LNCaP. So we compared cellular drug uptake for **A4**, **B1**, **B7** in HeLa cell line and **A5**, **B6** and **B8** in LNCaP cell line.

In contrast to the comparable activity of **B7** and **B8** observed in the 48 h incubation MTT assay, the accumulations of **B1**, **B6**, **B7** and **B8** in HeLa and LNCaP cells are higher than cisplatin and oxaliplatin. (2.5–18 times) And also the DNA was extracted and its platination was evaluated. **B7** exhibit 5.4 and 1.33 folds higher than oxaliplatin and cisplatin. Moreover, cellular drug uptake of **B7** in HeLa cells and DNA platination are 2.3 and 1.5 folds higher than **A4**. For LNCaP cells, **B6** and **B8** show 1.67 and 1.75 times higher than **A5**.

These results indicated that the carbohydrate-conjugated mono-functionalized platinum(IV) prodrugs are superior to the platinum(II) complexes and bis-functionalized platinum(IV) prodrugs in cell uptake and DNA platination. Simultaneously, cellular uptake studies by ICP-MS confirmed a good correlation between intracellular platinum accumulation and antitumor activity.

### 2.5. Flow cytometric analysis

In order to investigate the incidence of cell cycle arrest (LNCaP), the DNA-flow cytometric studies were conducted following treatment with **B6** and **B8**. A sum of 11.09% and 12.43% accumulated at the G2/M after 30 h incubation at 1 μM with **B6** and **B8**, indicating

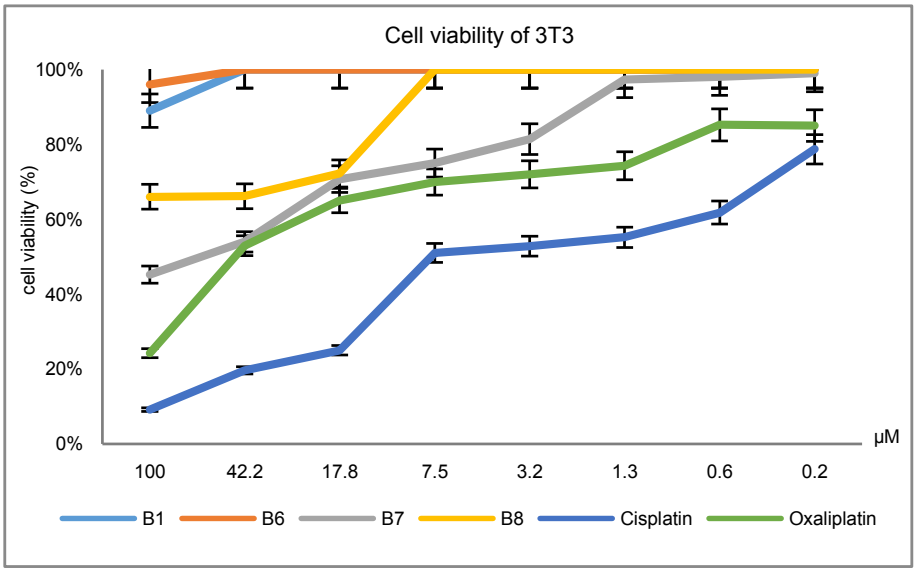



**Fig. 1.** Cell viability of 3T3.

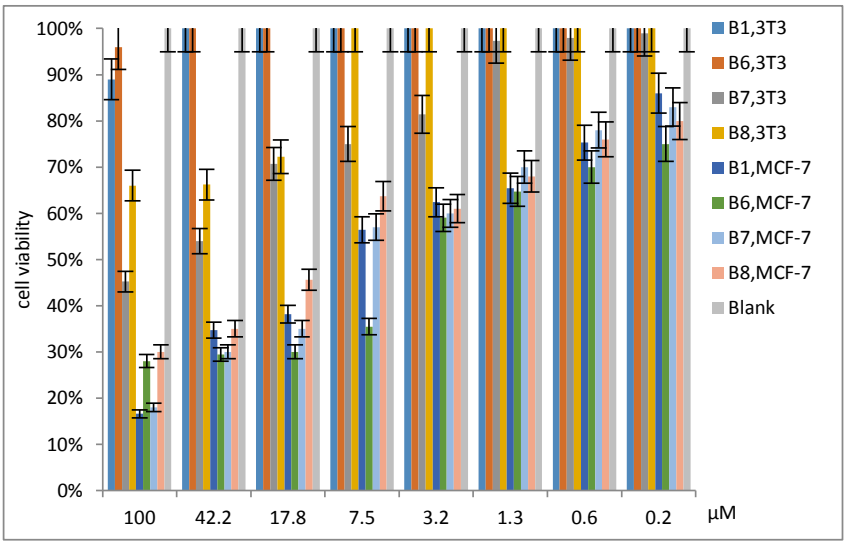



**Fig. 2.** Cell viability of MCF-7 cells and the normal 3T3 cells for **B1**, **B6**, **B7** and **B8** after incubation for 48 h at the concentration of 100 μM, 42.2 μM, 17.8 μM, 7.5 μM, 3.2 μM, 1.3 μM, 0.6 μM and 0.2 μM.

**Table 2**
Inhibitory effect ($IC_{50}$ in μM) of carbohydrate platinum(IV) complexes **B1**-**B10**, **A4**, **A5**, cisplatin and oxaliplatin on MCF-7 cells and 3T3 cells.

| | MCF-7 | 3T3 |
|---|---|---|
| **B1** | 7.55 | >200 |
| **B2** | 22.00 | >100 |
| **B3** | 22.61 | 88.16 |
| **B4** | 18.99 | >100 |
| **B5** | 9.34 | 85.06 |
| **B6** | 4.86 | >200 |
| **B7** | 9.20 | 60.00 |
| **B8** | 8.60 | >100 |
| **B9** | 13.36 | 55.21 |
| **B10** | 7.40 | >100 |
| **A4** | 30.44 | 84 |
| **A5** | 18.18 | 169 |
| cisplatin | 10.00 | 8 |
| Oxaliplatin | 11.60 | 71 |

that **B6** and **B8** arrested the cell cycle at the G2/M phases in a time-dependent manner. Meanwhile, quantification of apoptosis in HeLa cells using an annexin V/PI assay is showed in Table 3. Annexin V/PI coupled flow cytometric analysis at 10 μM after 30 h incubation in HeLa showed that B7 can efficiently induce apoptosis by prompting a larger population of cells to undergo early apoptosis (23.7%), late apoptosis (42.3%) and necrosis (12.8%) with the sum of 78.8% compared with cisplatin (35.5%) and A4 (59.8%) as shown in Table 3, respectively. And also the quantification of 51.4% was observed after incubation with **B1** in HeLa cells. As for the apoptosis-inducing properties, the carbohydrate platinum(IV) complexes are also superior to cisplatin at the same concentration for 30 h.

### 2.6. The reduction of Pt(IV) complexes

As far as we know, the Pt (IV) compounds are converted to Pt(II) in the existence of ascorbic acid (Vc) or GSH and then combined with DNA. The combination of DNA and Pt(II) complexes then induce the death of cancer cells. In order to investigate the mechanism of the glycosylated Pt(IV) compounds, B8 was incubated with and without Vc in the existence of 5'-dGMP (Fig. 4). After 24 h, a new peak was observed for both oxaliplatin and **B8** with Vc and the peak became clear at 48 h and 72 h. The adduct was isolated and identified by ESI-MS analysis. After reduction by Vc, oxaliplatin was formed and then combined with 5'-dGMP to generate the bis-substituted products. Simultaneously, in the absence of Vc we cannot observe the same peak, which indicate that the glycosylated Pt(IV) compounds exert their anticancer activity *via* the released Pt(II) complexes.

And we can see that in Fig. 4A and B, the adduct formation between oxaliplatin and 5'-dGMP is faster and more than **B8**. The reason is that firstly the platinum(IV) complexe (**B8**) need to be reduced by the reductants like ascorbic acid and glutathione and then bind with 5'-dGMP to form the adduct. But the Pt(II) drug oxaliplatin can bind with 5'-dGMP directly.

### 2.7. Antitumor activities in vivo

We also conducted the antitumor activities *in vivo* to know whether the target compounds sensitive to low pH and cellular reductants in cancer cells *in vitro* can also perform the properties of decreased toxicity. The acute toxicity study was done to evaluate the potential safety of the glycosylated Pt(IV) compounds. Kunming mice were administered intravenously (i.v.) and the experiment 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 4. The ratios of the cytotoxicity ($IC_{50}$) toward MCF-7 to animal lethal dosage values ($LD_{50}$) of the studied complexes were used as a measure for the therapeutic index.

The MTD and $LD_{50}$ for **B6**, **B7** and **B8** are nearly 5-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 results show that **B6** (TI = 37.04) **B7** (TI = 20.22) and **B8** (TI = 21.86) exhibit a therapeutic index over 5–11 folds higher than that of clinical drug oxaliplatin (TI = 6.51) and the reported bis-functionalized platinum(IV) prodrugs **A4** (TI = 3.02) and **A5** (TI = 5.37).

After the acute toxicity study, we also did the further evaluations *in vivo* with oxaliplatin as control to investigate the antitumor activity of the glycosylated Pt(IV) complexes. The *in vivo* activities were conducted on MCF-7 tumour mice models. NOD/SCID mice bearing MCF-7 tumours were treated with **B6**, **B7**, **B8** and oxaliplatin at the dose of 5 mg Pt/kg. PBS was also used as negative control. Tumour growth as a function of time and the tumour

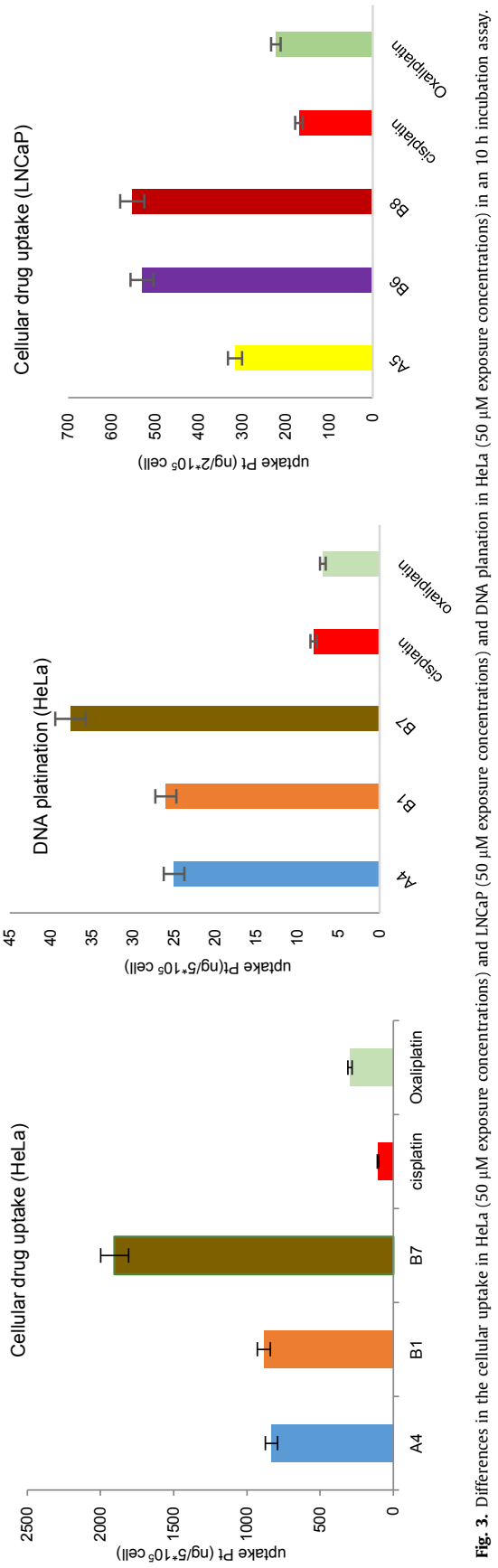



**Fig. 3.** Differences in the cellular uptake in HeLa (50 μM exposure concentrations) and LNCaP (50 μM exposure concentrations) and DNA planation in HeLa (50 μM exposure concentrations) in an 10 h incubation assay.

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

| Compd. | Early apoptosis | Late apoptosis | Necrosis | Sum |
|---|---|---|---|---|
| **B1** | 6.1 | 9.1 | 36.2 | 51.4 |
| **B7** | 23.7 | 42.3 | 12.8 | 78.8 |
| **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 |

weight in each group at the end of the experiment were recorded. The bodyweight during the treatments and the images of tumours at the end of the experiment were also shown in Fig. 5. The results indicated that the glycosylated Pt(IV) complexes **B6**, **B7** and **B8** show significant inhibition to the growth of tumour compared with the negative control and the tumour control rates of **B6**, **B7** and oxaliplatin are 41.2%, 52.9% and 64.7%. And it is important that the bodyweight of mice in Fig. 5 indicated that the glycosylated Pt(IV) compounds exhibited the deduced toxicity compared with oxaliplatin. The results reveal that the glycosylated Pt(IV) complexes **B7** exhibited the important antitumor activities *in vivo* with a higher MTD, $LD_{50}$, TI and comparative tumour control rates compared with oxaliplatin.

### 2.8. Cyclic voltammograms

Subsequently, we want to know whether the carbohydrate-conjugated platinum(IV) complexes with mono-functionalized ligands would possess both pH and redox dual-responsive properties in the cancer cells, the cyclic voltammograms of **B1**-**B10** in phosphate buffer-0.1 M KCl were done (Fig. 6). By mimicking the narrow pH range of cancer cells, it was found that the reduction potential of **B1**-**B10** behaved relative differences at pH 6.4 with the scan rate of 200 mv/s as shown in Fig. 6.

Importantly, the peak current and peak potential of **B1**, **B6**, **B7** and **B8** with the scan rate of 200mv/s at pH 6.4 for the microenvironment of cancer cells and 7.4 for microenvironment of the normal cells made great differences. As shown in Table 5, the peak current of **B1**, **B6**, **B7** and **B8** with the scan rate of 200mv/s at the concentration of 0.08 mM was 5-fold higher at pH 6.4 than the pH 7.4 and the peak potential also made differences. But the peak current of the bis-functionalized platinum(IV) prodrugs **A4** and **A5** at pH 6.4 is.

almost the same with that of 7.4. And also the peak current of mono-functionalized platinum(IV) prodrugs **B1**, **B6**, **B7** and **B8** at pH 6.4 is 2.3–3.2 times higher than that of the bis-functionalized platinum(IV) prodrugs **A4** and **A5**, indicating the pH and redox dual-responsive properties in the cancer cells. The results revealed that the glycosylated Pt(IV) complexes may be easier to be reduced at pH 6.4 than pH 7.4, which is consistent with the results that the carbohydrate-conjugated platinum(IV) complexes with mono-functionalized ligands exhibited more potent cytotoxicity in seven different human cancer cell lines and low toxicity to 3T3. All of the results indicate the potential safety of the mono-sugar conjugates.

## 3. Conclusions

Ten novel carbohydrate-conjugated platinum(IV) complexes with mono-functionalized ligands were designed, synthesized and evaluated the antitumor activity *in vitro* and *in vivo*. Pt(IV) complexes conjugated with different carbohydrate behaved different antitumor activities to different cancer cells. Importantly, the mono-functionalized carbohydrate-conjugated platinum(IV) complexes also showed more safety and more active to cancer cells than

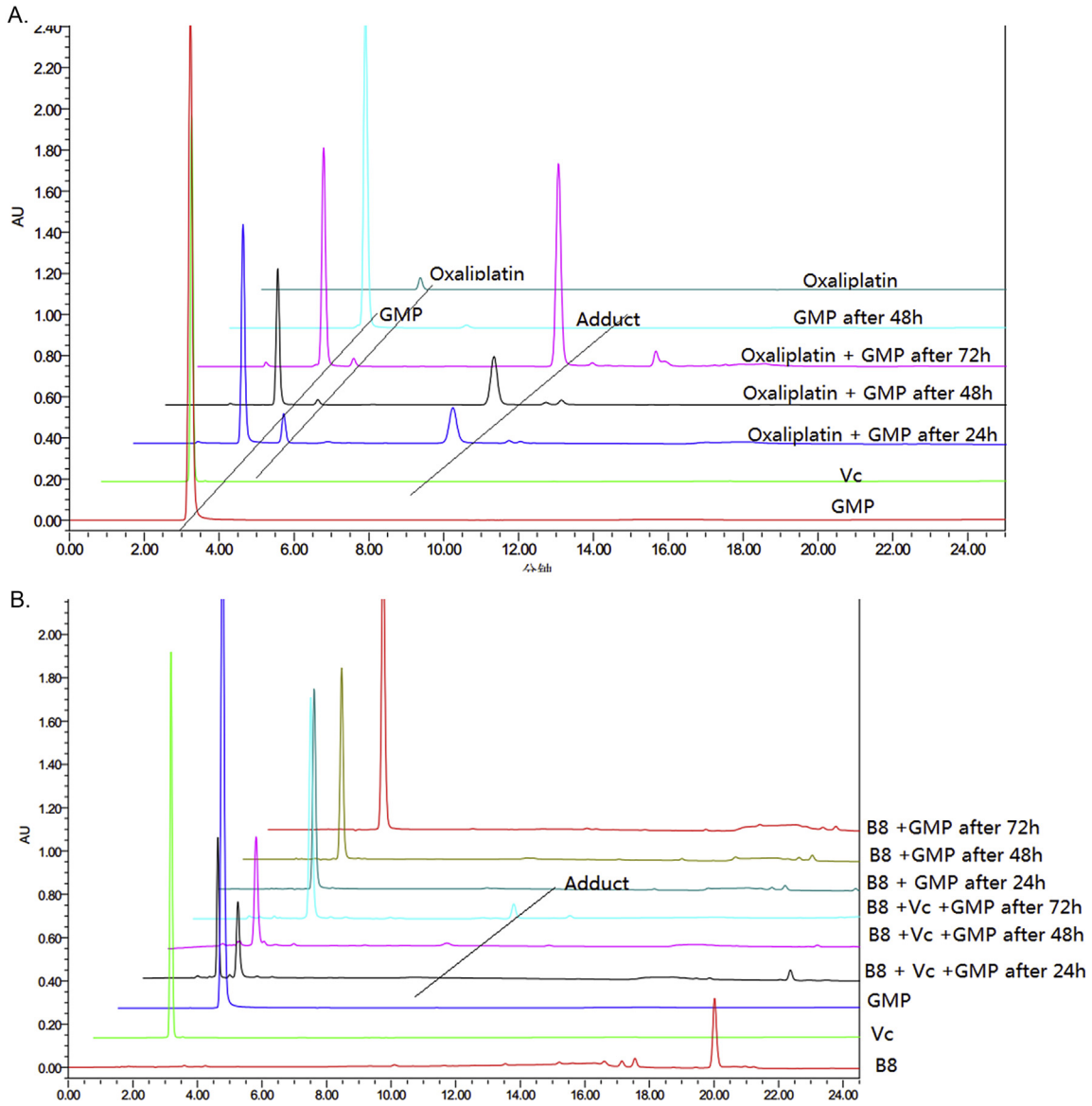



**Fig. 4.** A. Reaction of oxaliplatin with 5'-dGMP with and without ascorbic acid incubated at 370C after 24 h, 48 h and 72 h. B. Reaction of B8 with 5'-dGMP after 24 h, 48 h and 72 h.

**Table 4**
*In vivo* maximum tolerated dose and lethal dosage values, and calculated therapeutic indices ($LD_{50}/IC_{50}$) of complexes B6 and oxaliplatin.

| Complexes | MTD (mg/kg) | $LD_{50}$ (mg/kg) | $LD_{50}$ (μM/kg) | 48 h Avg $IC_{50}$ (μM) | $LD_{50}/IC_{50}$ |
|---|---|---|---|---|---|
| **B6** | 85 | 165 | 180 | 4.86 | 37.04 |
| **B7** | 75 | 150 | 186 | 9.20 | 20.22 |
| **B8** | 90 | 170 | 188 | 8.60 | 21.86 |
| oxaliplatin | 16 | 30 | 75.56 | 11.60 | 6.51 |

the bis-functionalized ones *in vitro* and *in vivo*, which supported its clinical development to become a new class of Pt(IV) antitumor agent.

The platinum(IV) complexes with mono-functionalized ligands also displayed a more positive shift of reduction properties at PH 6.4 than the bis-functionalized platinum(IV) prodrugs, which indicated that the reduced pHe in the cancer microenvironment would facilitate reduction of the platinum(IV) complexes to release the active platinum(II) complexes. Thus, when designing new Pt(IV) pro-drugs with axial ligands, the attractive biological performance of glycosylated Pt(IV) complexes with mono-functionalized ligands should be considered and could prove to be of much prime importance in future development of platinum antitumor drugs. The report of platinum(IV) anticancer prodrugs-hypotheses and facts pointed out that the bioactive axial ligands of the "dual action" prodrugs often performed the expected tasks inside the cells. More *in vivo* studies are needed in order to gain a better perspective on this approach [24].

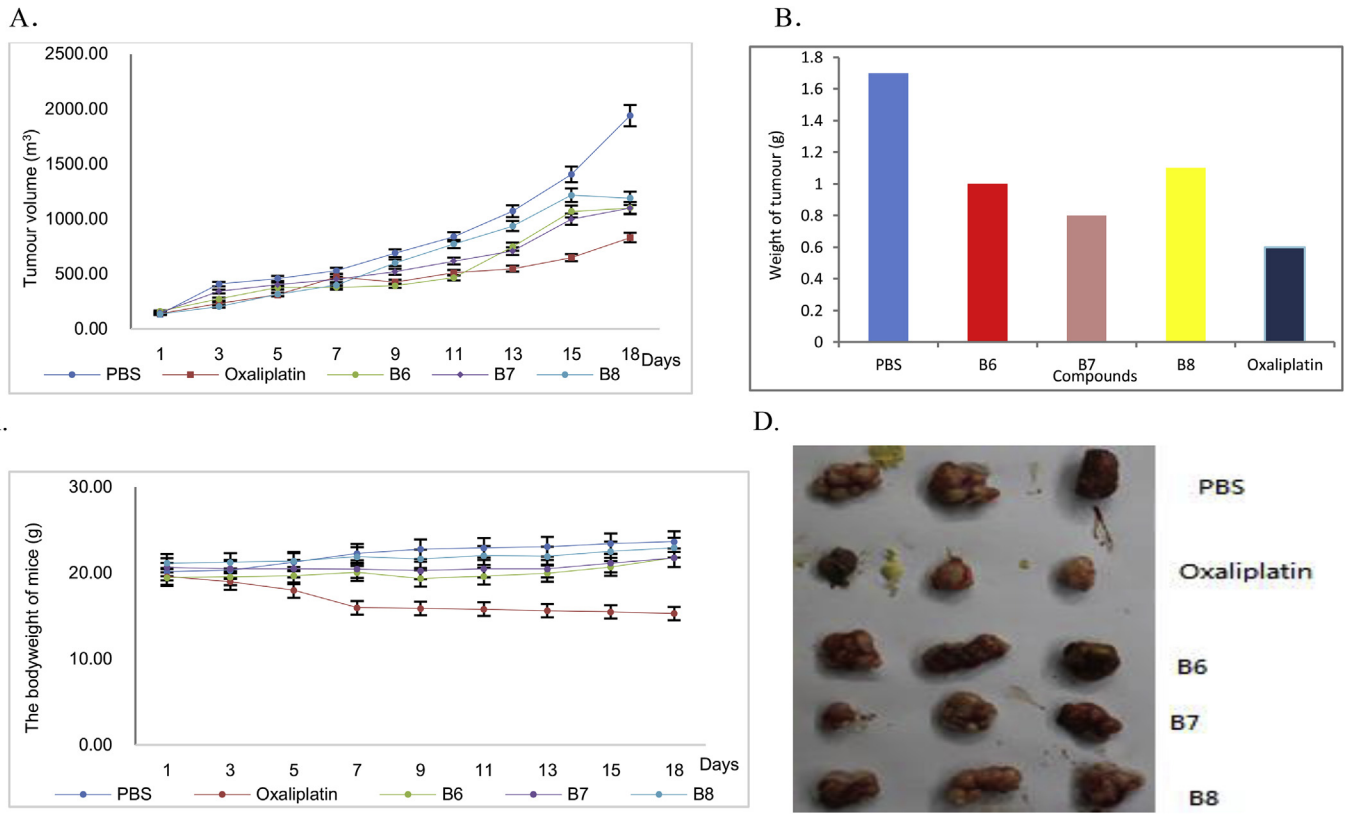



**Fig. 5.** *In vivo* antitumor activities of compounds **B6**, **B7**, **B8** and oxaliplatin in MCF-7. (A) Tumour growth as a function of time. (B) The tumour weight in each group at the end of the experiment. (C) The bodyweight of mice during the treatments. (D) The images of tumours at the end of the experiment.

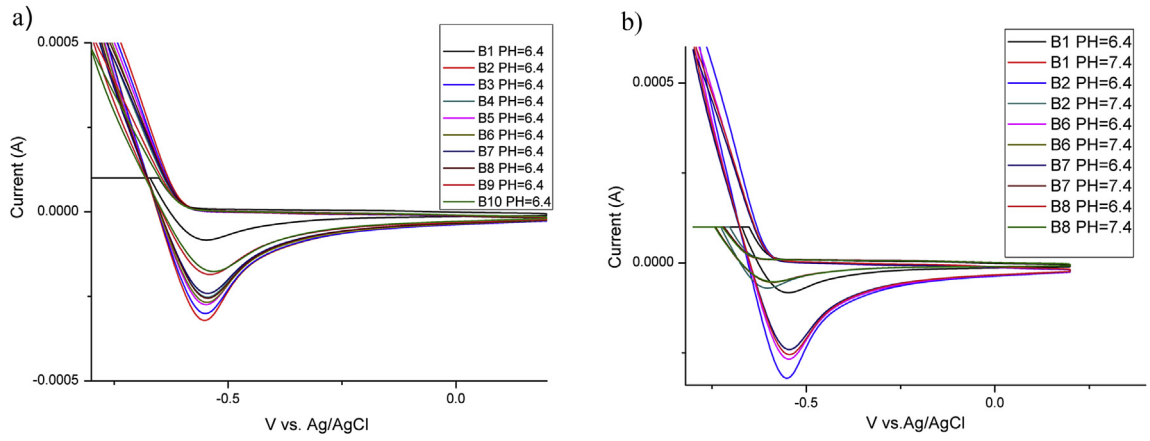



**Fig. 6.** Cyclic voltammograms of **B1**-**B10** in phosphate buffer-0.1 M KCl at two different pH values. (a). The cyclic voltammograms of **B1**-**B10** with the scan rate of 200mv/s at pH 6.4. (b).The cyclic voltammograms of **B1**, **B2**, **B6**, **B7** and **B8** with the scan rate of 200mv/s at pH 6.4 and 7.4.

**Table 5**
The peak current (1e-5A) and peak potential (mV) of **B1**, **B6**, **B7**, **B8**, **A4** and **A5** with the scan rate of 200 mv/s at the concentration of 0.08 mM.

| | B1 | B6 | B7 | B8 | A4 | A5 |
|---|---|---|---|---|---|---|
| Peak current pH = 7.4 (1e-5A) | −6.7 | −5.5 | −5.2 | −5.3 | −7.5 | −7.6 |
| Peak current pH = 6.4 (1e-5A) | −32.1 | −26.7 | −23.9 | −25.5 | −9.9 | −9.8 |
| Peak potential pH = 7.4 (mV) | −604 | −600 | −590 | −590 | −610 | −610 |
| Peak potential pH = 6.4 (mV) | −500 | −540 | −540 | −540 | −563 | −563 |

## 4. Experimental

### 4.1. Materials and methods

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 diphenyltetrazoliumbromide (MTT), ascorbic acid (AsA), 5′-GMP (purity > 98.0%), DMEM (for 3T3 cells and HepG-2 cells) and RPMI1640 (for A549, A549 R, 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 Na2HPO4 and 2 mM KH2PO4. 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), HepG-2 (hepatoma cell), LNCaP (Prostate), PC3 (Prostate) cells and 3T3 (mouse embryo fibroblasts) were obtained from Prof. Yanming Wang (College of Pharmacy, Nankai University, Tianjin, China). A549R cells were maintained with 2 μg/mL cisplatin.

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 $H_2O$ was used and the flow rate of 1 mL/min.

### 4.2. 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 °C for 24 h. Then 100 μL freshly prepared culture medium containing drugs at different concentrations were 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 (150 μL) 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.

### 4.3. In vivo antitumor assay

Kunming mice (28-42d) for the acute toxicity study 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. BALB/c-nu mice (28-42d) 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 MCF-7 single-cell suspension in PBS ($5 \times 10^6$ per mouse) was injected subcutaneously into the buttock of the mouse. When the tumour grew to a size of 80–150 $mm^3$ at 14 days after cell implantation, the mice were randomly divided into five groups (PBS, oxaliplatin 5 mg Pt/kg, B6 5 mg Pt/kg, B7 5 mg Pt/kg and B8 5 mg Pt/kg), with all drugs administered intravenously *via* tail vein. The drugs were given 5 times at 3-days intervals. Tumour growth was monitored by measuring the perpendicular diameter of the tumour using calipers every two days. Five days after the last treatment, animals were sacrificed and tumours were excised.

### 4.4. Synthesis of target compounds

The carbohydrate-conjugated mono-functionalized platinum(IV) prodrugs (**B1**-**B10**) were prepared by the reaction of an amide bond from peracetyl rhamnose, mannose and galactose with a propyl amino or ethyl amino linker at the reducing end and a carboxylic function in the Pt(IV) complexes (**B11** and **B12**) (Scheme 2). The key step involved the preparation of oxoplatin **B13** and **B14** which was reacted successively in water (12 mL) with cisplatin (0.2 g) or oxaliplatin (0.2 g) and H2O2 (20 mL) at 60 °C with the yield of 60% and 55% respectively. **B11** and **B12** were prepared from oxoplatin **B13** (1equiv) or **B14** (1equiv) and succinic anhydride (1equiv) in anhydrous DMSO at room temperature overnight. DMSO was removed under vacuum to afford a yellow oil and the product was washed with acetone and diethylether, and dried in vacuum. The target compounds **B11** and **B12** were obtained as a pale yellow solid after purification by recrystallization with the yield of 75% and 78% respectively. Finally, to a solution of **B11** or **B12** (1equiv) in DMF was added a DMF solution containing HATU. The mixture was stirred for 10 min at room temperature. A DMF solution containing amines and DIPEA was added to the resulting solution to obtain **B1** to **B10** in yields of 20–50%, respectively. The mixture was stirred at room temperature for 24 h in the dark. The product was characterized using $^1$H-NMR, $^{13}$C-NMR, ESI-MS spectrometry and elemental analysis (see ESI$^+$). And meanwhile, the synthesis methods of **A4** and **A5** which have been reported by our group recently with an increasing antitumor activities and a potential safety compared with cisplatin and oxaliplatin are also listed in Scheme 2.

#### 4.4.1. Preparation of *B1*

The complexes **B11** was synthesized as previously described. To a solution of **B11** (1equiv) in DMF was added a DMF solution containing HATU (1.5equiv). This mixture was stirred for 10 min at room temperature. To the resulting solution was added a DMF solution containing **S10** (1.2equiv) and DIPEA (2.4equiv). 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 **B1** was purified by silica gel column chromatography as yellow solid in yield of 20%. $^1$H NMR (400 MHz, MeOD) δ 5.30–5.13 (m, 1H), 5.00 (dd, $J$ = 13.8, 5.8 Hz, 1H), 4.76 (q, $J$ = 8.8 Hz, 1H), 3.93–3.68 (m, 2H), 3.62–3.19 (m, 4H), 2.73–2.40 (m, 4H), 2.27–1.91 (m, 9H), 1.82 (ddd, $J$ = 13.7, 10.7, 6.7 Hz, 2H), 1.42–1.02 (m, 3H). $^{13}$C NMR (100 MHz, MeOD) δ 183.04, 175.74, 171.92, 171.90, 171.82, 98.96, 72.33, 71.19, 71.00, 67.78, 67.00, 37.91, 37.86, 33.07, 30.31, 20.89, 20.81, 20.75, 17.92. HRMS: Calcd. for $C_{19}H_{35}Cl_2N_3O_{12}Pt$ ($M^+$): 762.1246, found: 0.762.1291. Elemental analysis, found C 29.67%; H 4.68%; N 5.62%, calcd for $C_{19}H_{35}Cl_2N_3O_{12}$ Pt C 29.89%; H 4.62%; N 5.50%.

#### 4.4.2. Preparation of *B2*

**B2** was synthesized according to **B1** with the yield of 35%. $^1$H NMR (400 MHz, MeOD) δ 5.17 (ddd, $J$ = 13.4, 6.7, 2.5 Hz, 1H), 4.97 (dd, $J$ = 18.6, 8.7 Hz, 1H), 3.97–3.36 (m, 5H), 3.00–2.15 (m, 8H), 1.98 (dd, $J$ = 56.4, 18.1 Hz, 9H), 1.83–1.00 (m, 13H). $^{13}$C NMR (100 MHz, MeOD) δ 182.17, 174.11, 170.36, 170.34, 170.26, 165.51, 165.43, 97.47, 70.80, 69.44, 66.26, 65.47, 61.65, 36.34, 31.72, 31.12, 30.91, 28.78, 23.77, 23.68, 19.31, 19.29, 19.23, 16.41. HRMS: Calcd. for $C_{27}H_{43}N_3O_{16}Pt$ ($M^+$): 860.2291, found: 0.860.2323. Elemental analysis, found C 37.63%; H 5.12%; N 4.83%, calcd for $C_{27}H_{43}N_3O_{16}$ Pt C 37.68%; H 5.04%; N 4.88%.

#### 4.4.3. Preparation of *B3*

**B3** was synthesized according to **B1** with the yield of 25%. $^1$H NMR (400 MHz, MeOD) δ 5.18 (dtd, $J$ = 12.4, 3.4, 1.7 Hz, 2H), 5.05–4.91 (m, 1H), 3.93–3.80 (m, 1H), 3.78–3.66 (m, 1H), 3.61–3.36 (m, 2H), 3.26 (dt, $J$ = 3.3, 1.6 Hz, 2H), 2.59–2.53 (m, 2H), 2.48–2.37 (m, 2H), 2.13–1.81 (m, 9H), 1.18–1.06 (m, 3H). $^{13}$C NMR (100 MHz, MeOD) δ 183.69, 176.07, 172.03, 171.92, 171.89, 98.89, 72.45, 71.08, 70.99, 67.82, 67.56, 40.36, 32.98, 27.23, 20.92, 20.87, 20.82, 17.96.

HRMS: Calcd. for $C_{18}H_{33}Cl_2N_3O_{12}Pt$ ($M^+$): 748.1089, found: 748.1138. Elemental analysis, found C 28.88%; H 4.57%; N 5.67%, calcd for $C_{18}H_{33}Cl_2N_3O_{12}$ Pt C 28.85%; H 4.44%; N 5.61%.

#### 4.4.4. Preparation of ***B4***

**B4** was synthesized according to **B1** with the yield of 35%. $^1$H NMR (400 MHz, MeOD) δ 5.55–5.22 (m, 4H), 4.36 (d, $J$ = 7.0 Hz, 1H), 4.24–4.11 (m, 2H), 3.87 (d, $J$ = 4.1 Hz, 2H), 3.71 (s, 4H), 3.57 (s, 2H), 2.90 (d, $J$ = 10.4 Hz, 2H), 2.44–2.13 (m, 9H), 1.89 (d, $J$ = 69.2 Hz, 5H), 1.49 (dd, $J$ = 27.8, 10.6 Hz, 3H), 1.37–1.02 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) δ 170.96, 170.60, 170.40, 162.64, 97.38, 70.41, 69.39, 69.28, 66.74, 66.20, 44.43, 38.87, 28.16, 19.27, 19.23, 16.40. HRMS: Calcd. for $C_{26}H_{41}N_3O_{16}Pt$ ($M + NH_4^+$): 864.2481, found: 864.2424. Elemental analysis, found C 36.86%; H 4.79%; N 4.93%, calcd for $C_{26}H_{41}N_3O_{16}$ Pt C 36.88%; H 4.88%; N 4.96%.

#### 4.4.5. Preparation of ***B5***

**B5** was synthesized according to **B1** with the yield of 40%. $^1$H NMR (400 MHz, MeOD) δ 5.25 (dd, $J$ = 7.9, 4.4 Hz, 2H), 4.46–3.97 (m, 4H), 3.87–3.42 (m, 6H), 3.01 (d, $J$ = 2.9 Hz, 1H), 2.82–2.32 (m, 3H), 2.24–1.90 (m, 12H), 1.82 (dd, $J$ = 12.0, 5.3 Hz, 2H). $^{13}$C NMR (100 MHz, MeOD) δ 183.69, 176.07, 172.03, 171.92, 171.89, 98.89, 72.45, 71.08, 70.99, 67.82, 67.56, 40.36, 32.98, 27.23, 20.92, 20.87, 20.82, 17.96. HRMS: Calcd. for $C_{21}H_{37}Cl_2N_3O_{14}Pt$ ($M^+$): 820.1300, found: 0.820.2892. Elemental analysis, found C 30.74%; H 4.63%; N 5.19%, calcd for $C_{21}H_{37}Cl_2N_3O_{14}$ Pt C 30.70%; H 4.54%; N 5.12%.

#### 4.4.6. Preparation of ***B6***

**B6** was synthesized according to **B1** with the yield of 48%. $^1$H NMR (400 MHz, MeOD) δ 5.27 (dd, $J$ = 9.8, 6.8 Hz, 3H), 4.59 (m, 1H), 4.29 (dd, $J$ = 12.3, 4.8 Hz, 1H), 4.20–4.03 (m, 2H), 3.90–3.77 (m, 2H), 3.56 (td, $J$ = 10.1, 6.1 Hz, 2H), 3.19–2.64 (m, 4H), 2.56–2.42 (m, 2H), 2.27 (d, $J$ = 11.3 Hz, 2H), 2.21–1.94 (m, 12H), 1.93–1.26 (m, 8H). $^{13}$C NMR (100 MHz, MeOD) δ 176.98, 175.32, 172.59, 171.91, 171.83, 171.78, 171.70, 99.17, 71.00, 70.90, 70.00, 67.47, 67.24, 63.81, 37.88, 37.82, 32.65, 32.45, 30.34, 25.31, 25.24, 20.83, 20.79, 20.76, 20.73. HRMS: Calcd. for $C_{29}H_{45}N_3O_{18}Pt$ ($M^+$): 918.2343, found: 918.2380. Elemental analysis, found C 37.94%; H 4.98%; N 4.64%, calcd for $C_{29}H_{45}N_3O_{18}$ Pt C 37.91%; H 4.94%; N 4.57%.

#### 4.4.7. Preparation of ***B7***

**B7** was synthesized according to **B1** with the yield of 37%. $^1$H NMR (400 MHz, MeOD) δ 5.44–5.12 (m, 2H), 4.39–3.95 (m, 3H), 3.95–3.71 (m, 2H), 3.55 (ddd, $J$ = 61.9, 21.5, 4.1 Hz, 2H), 3.27–2.78 (m, 2H), 2.75–2.37 (m, 3H), 2.05 (ddd, $J$ = 34.4, 29.6, 1.9 Hz, 12H), 1.31 (t, $J$ = 7.3 Hz, 1H). $^{13}$C NMR (100 MHz, MeOD) δ 172.65, 172.59, 171.84, 171.77, 171.73, 171.70, 99.08, 70.95, 70.83, 70.06, 67.94, 67.49, 63.78, 49.15, 40.37, 32.80, 32.56, 20.82, 20.76, 20.72, 9.38. HRMS: Calcd. for $C_{20}H_{35}Cl_2N_3O_{14}Pt$ ($M^+$): 806.1140, found: 806.1208. Elemental analysis, found C 29.79%; H 4.49%; N 5.26%, calcd for $C_{20}H_{35}Cl_2N_3O_{14}$ Pt C 29.75%; H 4.37%; N 5.20%.

#### 4.4.8. Preparation of ***B8***

**B8** was synthesized according to **B1** with the yield of 35%. $^1$H NMR (400 MHz, MeOD) δ 5.35–5.18 (m, 2H), 4.24 (dt, $J$ = 8.6, 4.3 Hz, 1H), 4.16–4.05 (m, 1H), 3.81–3.69 (m, 2H), 3.66–3.54 (m, 2H), 3.52–3.36 (m, 2H), 3.04 (d, $J$ = 21.8 Hz, 1H), 2.92–2.73 (m, 2H), 2.69–2.40 (m, 4H), 2.23 (d, $J$ = 8.9 Hz, 2H), 2.03 (dt, $J$ = 46.6, 22.3 Hz, 12H), 1.80–1.16 (m, 6H). $^{13}$C NMR (100 MHz, MeOD) δ 183.67, 181.75, 175.40, 172.59, 171.80, 171.77, 171.70, 99.09, 70.93, 70.03, 67.91, 67.46, 63.76, 63.20, 62.28, 40.34, 33.22, 32.58, 25.30, 25.21, 20.88, 20.85, 20.81, 20.75. HRMS: Calcd. for $C_{28}H_{43}N_3O_{18}Pt$ ($M^+$): 904.2187, found: 0.904.2205. Elemental analysis, found C 37.19%; H 4.81%; N 4.71%, calcd for $C_{28}H_{43}N_3O_{18}$ Pt C 37.17%; H 4.79%; N 4.64%.

#### 4.4.9. Preparation of ***B9***

**B9** was synthesized according to **B1** with the yield of 30%. $^1$H NMR (400 MHz, MeOD) δ 5.40–5.02 (m, 2H), 4.29–4.09 (m, 2H), 3.93 (d, $J$ = 14.9 Hz, 1H), 3.75 (d, $J$ = 11.7 Hz, 2H), 3.51 (d, $J$ = 4.7 Hz, 4H), 2.47 (s, 4H), 2.20–1.89 (m, 12H), 1.79 (d, $J$ = 6.0 Hz, 2H). $^{13}$C NMR (100 MHz, MeOD) δ 172.24, 172.19, 171.69, 171.63, 171.60, 171.52, 102.14, 72.53, 72.24, 70.55, 70.44, 69.02, 64.92, 62.73, 46.47, 40.70, 40.39, 20.94, 20.89, 20.74, 20.63. HRMS: Calcd. for $C_{21}H_{37}Cl_2N_3O_{14}Pt$ ($M^+$): 820.1297, found: 820.2892. Elemental analysis, found C 30.75%; H 4.61%; N 5.19%, calcd for $C_{21}H_{37}Cl_2N_3O_{14}$ Pt C 30.70%; H 4.54%; N 5.12%.

#### 4.4.10. Preparation of ***B10***

**B10** was synthesized according to **B1** with the yield of 38%. $^1$H NMR (400 MHz, MeOD) δ 5.41–4.96 (m, 3H), 4.34–4.02 (m, 3H), 3.93–3.36 (m, 4H), 2.98 (s, 3H), 2.66–2.20 (m, 4H), 2.02 (ddd, $J$ = 32.0, 25.6, 22.2 Hz, 12H), 1.80–1.02 (m, 8H). $^{13}$C NMR (100 MHz, MeOD) δ 183.67, 175.22, 172.27, 172.14, 171.68, 171.66, 167.02, 166.95, 102.33, 72.51, 71.95, 70.62, 69.05, 68.82, 62.73, 40.60, 37.59, 32.70, 30.64, 25.30, 25.22, 20.93, 20.75, 20.67, 20.65, 14.60. HRMS: Calcd. for $C_{29}H_{45}N_3O_{18}Pt$ ($M^+$): 918.2346, found: 918.2398. Elemental analysis, found C 37.95%; H 4.99%; N 4.64%, calcd for $C_{29}H_{45}N_3O_{18}$ Pt C 37.91%; H 4.94%; N 4.57%.

### 4.5. Cellular platinum uptake and DNA platination

The cellular uptake of cisplatin, oxaliplatin, the compounds were 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.

### 4.6. 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.

### 4.7. Reduction of Pt(IV) complexes

To investigate the binding properties of DNA with Pt(II) complexes, 5′-GMP was selected as a model of DNA. Oxaliplatin was incubated with 5′-GMP at 37 °C for 24 h, 48 h and 72 h. Further experiments were designed to test the reduction potential of Pt(IV) complexes **B8** with and without ascorbic acid after 24 h, 48 h and 72 h. Then, the Pt(II) compounds combined with 5′-GMP to form Oxp-Pt(II)-GMP, which were confirmed by HRMS.

HPLC analyses were performed on Waters E2695-2998 equipped with a Venusil MP C18 column (150 × 4.6 mm, 5 μm).

HPLC profiles were recorded by UV detector at 273 nm. Flow rate: 1 mL/min.

### 4.8. 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. **B1**-**B10** solutions were prepared in phosphate buffered saline (PBS) of pH 6.4. **B1**, **B2**, **B6**, **B7** and **B8** were also prepared for PH 7.4 and voltammograms were recorded at the scan rate of 200 mv/s.

## Notes

The authors declare no competing financial interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.01.032.

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