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Article

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Glycosylated Platinum(IV) Complexes as Substrates for Glucose Transporters (GLUTs) and Organic Cation Transporters (OCTs) Exhibited Cancer Targeting and Human Serum Albumin Binding Properties for Drug Delivery

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KEYWORDS: antitumor, glycosylation, platinum, cancer

ABSTRACT Glycosylated platinum(IV) complexes were synthesized as substrates for GLUTs and OCTs for the first time, and the cytotoxicity, and detailed mechanism were determined *in*

vitro and *in vivo*. Galactoside Pt(IV), glucoside Pt(IV) and mannoside Pt(IV) were highly cytotoxic and showed specific cancer-targeting properties *in vitro* and *in vivo*. Glycosylated platinum(IV) complexes **5**, **6**, **7** and **8** (IC_{50} 0.24 μM-3.97 μM) had better antitumor activity of nearly 166-fold higher than the positive controls cisplatin (**1a**), oxaliplatin (**3a**) and satraplatin (**5a**). The presence of a hexadecanoic chain allowed binding with human serum albumin (HSA) for drug delivery, which not only enhanced the stability of the inert platinum(IV) prodrugs but also decreased their reduction by reductants present in human whole blood. Their preferential accumulation in cancer cells compared to noncancerous cells (293T and 3T3 cells) suggested that they were potentially safe for clinical therapeutic use.

INTRODUCTION

The FDA-approved platinum(II) drugs, such as cisplatin, carboplatin, and oxaliplatin, are widely used in all first-line clinical cancer treatments, including ovarian, lung, head, neck and colorectal cancer. They induce apoptosis in cancer cells primarily *via* DNA damage.[1a,1b] However, their drawbacks, such as toxicity, tumor recurrence and deactivation, remain deadly and problematic and originate from a lack of tumor selectivity and poor stability.[2,3a,3b] Recently, platinum anticancer drug candidates were designed as inert platinum(IV) prodrugs that can be activated by intracellular reduction following cellular uptake.[4] Reductants such as ascorbic acid and glutathione occur at higher concentrations in tumor cells than in the blood and normal tissues, which makes them good targets. Satraplatin, the most promising platinum(IV) prodrug, is orally absorbed *via* passive diffusion and has greater potential because of its better stability, lower toxicity, and higher blood-circulation time than cisplatin. The two functional “axial ligands” of platinum(IV) prodrugs can be used to tune the physical and chemical properties of the complex

and impart targeting and additional biological activity without altering the structure of the active pharmacophore.[5a,5b,5c,5d,5e,5f,5g,5h,5i]

The potential of glycoconjugation for diagnosis and therapy has been realized, and it has become an appealing strategy for the targeted delivery of anticancer drugs.[6a,6b,6c] Based on the "the Warburg effect", glucose transporters (GLUTs) and sodium-dependent glucose transporters (SGLTs) are widely overexpressed in many human cancers.[7a,7b,7c] Our group and others recently, developed glycoconjugation by platinum(II).[8a,8b,8c,8d,8e,8f,8g] Our group also first designed and synthesized a series of glycosylated Pt(IV) complexes protected by acetyl groups.[8h,8i] To explore whether the transformation of glycosylated Pt(IV) complexes was associated with GLUTs. Phlorizin was employed in the present study as a GLUT inhibitor to study its impact on their antitumor activities. Unfortunately, GLUT inhibition did not affect the IC_{50} values of the tested complexes, indicating that the tested glycosylated platinum(IV) complexes were not transferred by glucose transporters. However, the unprotected glycosylated platinum(II) conjugates not only exploited both GLUTs and OCTs, but compared with normal epithelial cells *in vitro,* the conjugates also accumulated in and killed the cancer cells, demonstrating the potential of glycoconjugation for the selective destruction of cancer cells by platinum compounds.[8f] In addition, the cellular uptake, cytotoxicity profile and glucose transporter 1 (GLUT1) specificity can be affected by variation in the position of D-glucose substitution in the unprotected glycosylated platinum(II) conjugates.[8g] However, the labile platinum(II) prodrugs would react with nucleophiles in human whole blood and never reach the tumor, which limits their bioavailability and precludes oral administration.[9a,9b] The reasons that the bioavailability is limited are its reactivity and lack of selectivity. The design of platinum(IV) prodrugs is predicated on the assumption that the low spin octahedral d^6 Pt(IV) complexes will not undergo

hydrolysis or ligand substitutions in the blood but will be activated in cells by reductive elimination, releasing the cytotoxic square-planar Pt(II) drug. Consequently, a series of cancer-targeting unprotected glycosylated Pt(IV) complexes were designed and synthesized.

Liposomes,[10a] polymers,[10b] inorganic nano-materials,[10c,10d,10e,10f] metal–organic frameworks,[10g] and calcium phosphate (CaP) nanoparticles[10h] have been recently used to synthesize platinum(IV) prodrugs for drug delivery. It was also reported that the platinum(IV) prodrugs that contain a hexadecyl chain can interact with HSA to form a platinum-protein complex for drug delivery and that the interaction was non-covalent. Extraction with octanol can completely remove the prodrug from an aqueous solution of HSA.[11] As an extension of our study on novel potent antitumor agents, it was of great interest for us to design and synthesize a series of glycosylated Pt(IV) analogues that contain a hexadecanoic chain to produce a dual-targeting mechanism. In addition to interaction with HSA during drug delivery, the hexadecanoic chain can also enhance the lipophilicity of the molecule, which can affect the ability of a platinum(IV) prodrug to enter a tumor cell.

We designed, synthesized and biologically evaluated a novel series of dual-functionalized glycosylated platinum(IV) complexes as antitumor agents for the first time. The details of the mechanism of glycosylated platinum(IV) complexes **1**-**8** (**Figure 1a**) were also studied. It was revealed that the targeting and activity can be improved by the incorporation of glycuronic acid into an antitumor agent.[12a] Therefore, we designed the Pt(IV) compounds **1**-**3**. Then, we also designed the glycoside compounds **4**-**8,** which incorporated variable aliphatic chains of different lengths, to determine whether varying the substitution position or the chain length could alter the cellular uptake, cytotoxicity profile and the specificity. We selected the clinically widely used oxaliplatin as the platinum core. These serial complexes contained different glycosyl moieties,

including glucose, galactose and mannose, and their impact on the antitumor potency could be evaluated *in vitro* and *in vivo*.

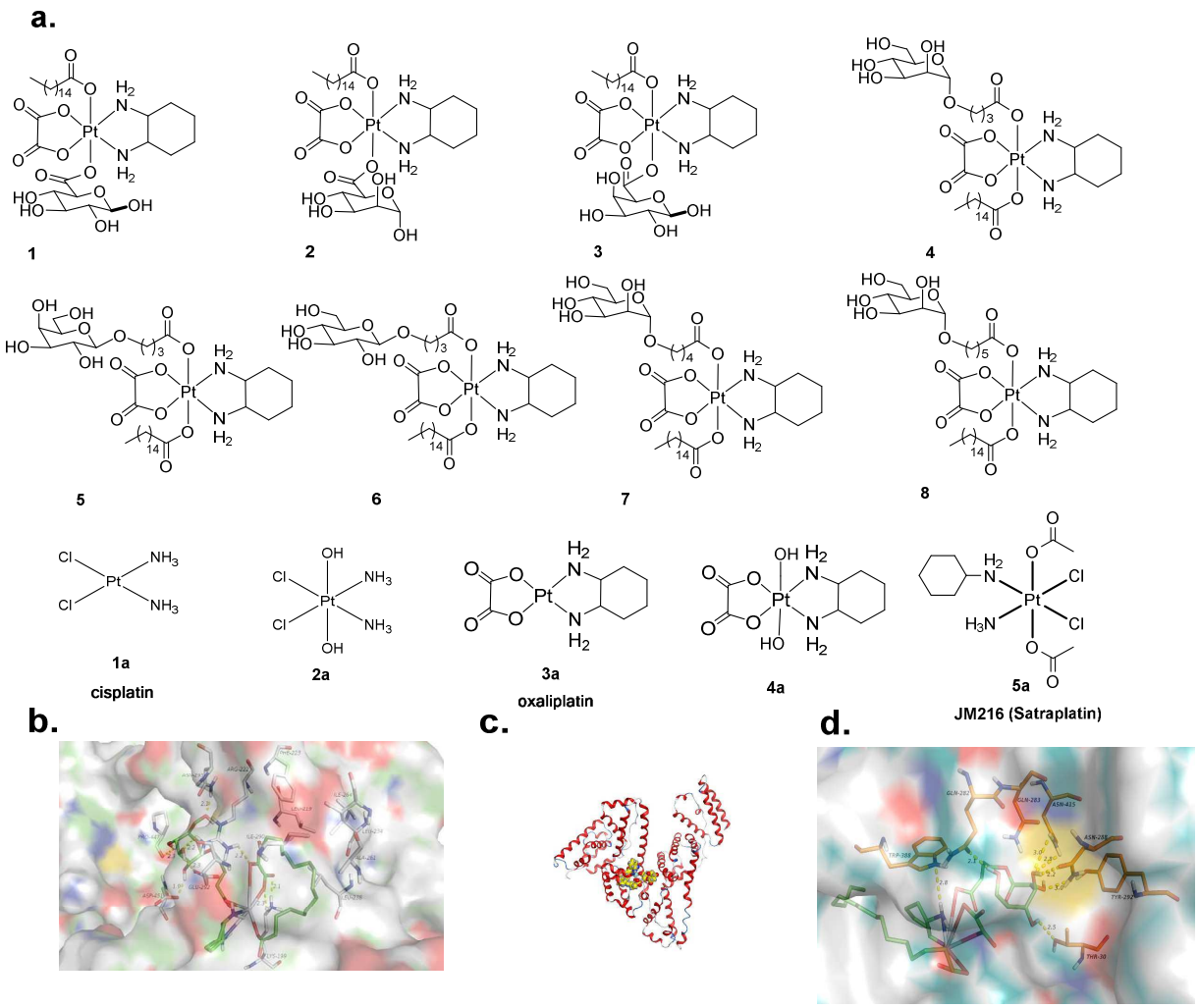


Figure 1. a). Structures of **1-8** and **1a-5a** as positive control. Docking studies of **6** with HSA and GLUT1. b). **6** interacting with a variety of amino acid residues of GLUT1 (pdb code 4PYP). c). The lowest energy structure and **6** buried beneath the protein surface (HSA pdb code 1E7H). d). **6** interacting with a variety of amino acid residues of HSA (pdb code 1E7H).

RESULTS AND DISCUSSION

Synthesis of target compounds

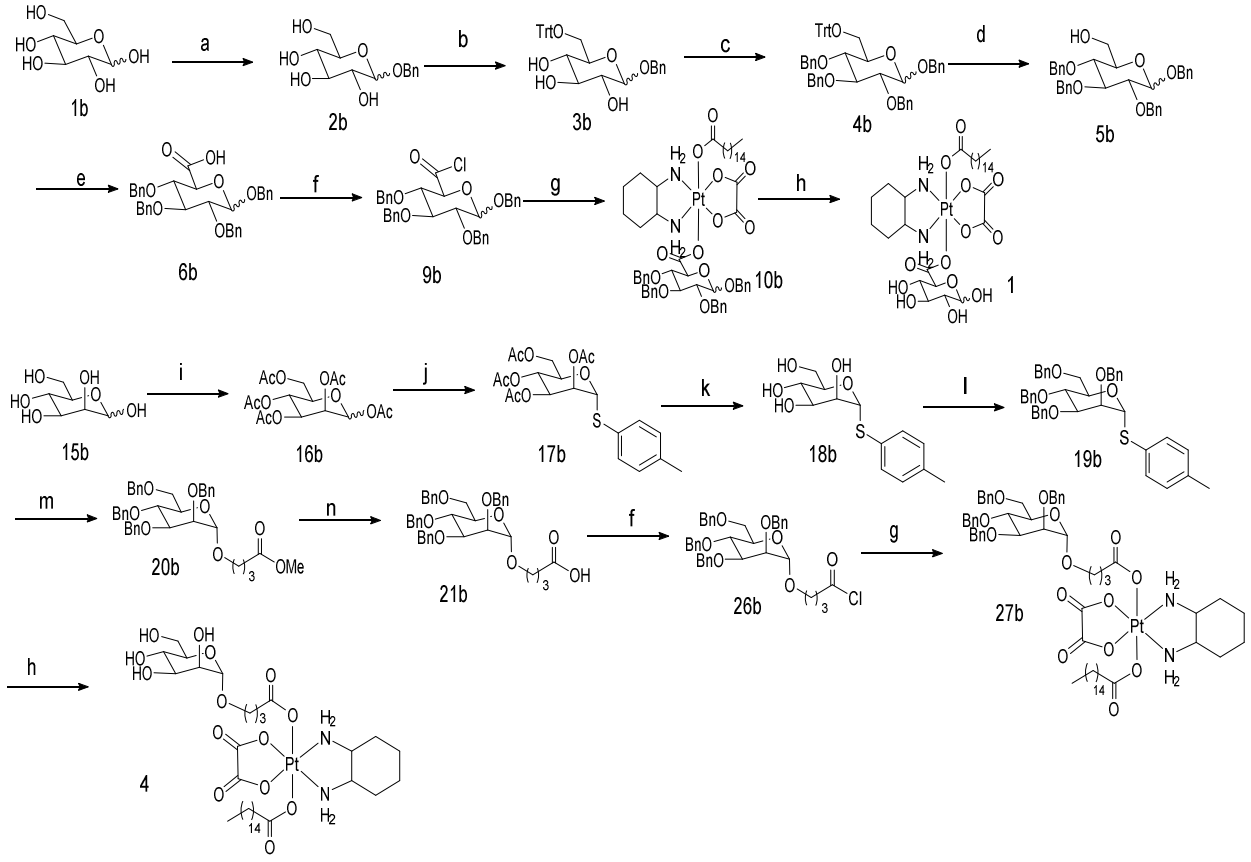
To the best of our knowledge, the synthetic route of **1**-**8** given in detail in the supporting information has not been previously reported. The oxidation of cisplatin and oxaliplatin by hydrogen peroxide in water afforded **2a** and **4a**. The synthetic route of the target compounds **1** and **4** is shown in **Scheme 1**. Compounds **2** and **3** were prepared according to the procedure described for compound **1** and **5**-**8** according to the procedure described for compound **4**. The asymmetrically functionalized Pt(IV) compounds **1**-**8** can be obtained by the reaction of a solution of **10b**, **12b**, **14b**, **27b**, **29b**, **31b**, **33b** and **35b** in anhydrous DCM. BCl_3 (1 M) was added at –78 □ for 2 h. Then, the DCM was removed and purified by column chromatography.

Characterization.

The Pt(IV) compounds **1**-**8** were fully characterized by (^{1}H and ^{13}C) NMR spectroscopy and electrospray ionization mass spectrometry (ESI-MS). The $[M+NH_4]^+$ parent ions of compounds **1**, **2** and **3** and the $[M+H]^+$ parent ions of compounds **4**, **5**, **6**, **7** and **8** were readily observed using ESI-MS. Characteristic resonances could be observed at approximately 1.18 ppm -1.30 ppm (CH_2) and 0.79 ppm – 0.92 ppm (CH_3) in ^{1}H NMR and were assigned to the hexadecanoic acid ligand. Resonances at 3.01 ppm - 2.65 ppm (**1**), 2.95 ppm and 2.79 ppm (**2**), 2.94 ppm - 2.65 (**3**), 2.78 ppm (**4**), 2.80 ppm (**5**), 2.89 ppm - 2.66 ppm (**6**), 2.71 ppm and 1.18 ppm (**7**) and 2.78 ppm (**8**) of $(CH)_2$ in ^{1}H NMR were assigned to the oxaliplatin core. We could observe characteristic resonances by ^{13}C NMR at approximately 183.11 ppm, 178.11 ppm, 176.63 ppm and 165.69 ppm (**1**), 182.78 ppm, 178.31 ppm, 166.07 ppm and 129.46 ppm (**2**), 183.17 ppm, 178.05 ppm and 165.50 ppm (**3**), 183.21 ppm, 182.66 ppm, 165.43 ppm and 128.02 ppm (**4**), 183.18 ppm, 182.61 ppm and 165.44 ppm (**5**), 183.19 ppm, 182.63 ppm and 165.40 ppm (**6**), 183.10 ppm, 182.81 ppm and 165.38 ppm (**7**) and 183.19 ppm, 183.06 ppm and 165.27 ppm (**8**) were assigned to the carbonyl group.

Docking studies of the dual targets of platinum(IV) pro-drugs.

First, we conducted docking studies of the dual targets of the platinum(IV) prodrugs to investigate the interaction between HSA-**6**[12b] (pdb code 1E7H) (**Figure 1b** and **1c**) and GLUT1-**6**[12c] (pdb code 4PYP) (**Figure 1d**). **6** can non-covalently interact with both HSA and GLUT1. Hydrogen-bonding interactions occurred with ASN-415, ASN-288, TYR-292, THR-30 and GLN-282 by ligand exchange to form glycosylated acid moieties because it occurred in the interaction between GLUT1 (pdb code 4PYP) and **6**. TRP-388 could non-covalently interact with the carboxylate platinum ligand center.



Scheme 1. Synthetic route of target compounds **1** and **4**. Conditions and reagents: (a) BnOH,CH_3COCl 50 °C, 2 h. (b) TrtCl, DMAP Py, 50 °C (c) BnBr, NaH, DMF, rt, overnight (d) HBr/AcOH 10 °C (e) $K_2Cr_2O_7$, H_2SO_4, acetone, 55 °C, 2 h (f) $C_2O_2Cl_2$ DMF(cat.) (g) Dry acetone rt, 2d (h) BCl_3, DCM -78 °C, 2 h (i) Ac_2O, AcONa 80 °C, 6 h (j) p-toluenethiol BF_3

Et_2O, DCM (k) MeONa, MeOH rt, overnight (l) BnBr, NaH, DMF, overnight (m) TMSOTf, NIS, CH_3CN, 2 h (n) NaOH (1 M), 24 h.

Hydrogen-bonding interactions of **6** with HSA (pdb code 1E7H) were observed in the pocket, located in the subdomain IIA (known as Sudlow's site I) with a model of the lowest energy structure. ARG-222 and LYS-199 of HSA were found to non-covalently interact with a platinum ligand. Hydrogen bonding interactions of glycosylated acid moieties occurred with PRO-447, ASP-451, GLU-292 and ASN-295 of HSA using non-covalent bonds. The lipophilic C16 tail of fatty acid moieties of **6** is coiled into a hydophobic channel lined by Tyr150, Leu238, Leu219, Leu234, Phe223, Ile264, Ala261, Ile290, and Tyr150 using non-covalent bonds.

The above results indicated stable and low-energy interactions of HSA-**6** and GLUT1-**6**, which contributed greatly to the dual target of a pyranoside-conjugated platinum(IV) complex. We can also deduce that once the target compound entered the human blood, it would bind with HSA during drug delivery, target the tumor tissue, and then preferentially accumulate in the cancer cells because of "the Warburg effect".

Antitumor activities in vitro

The cytotoxicity of compounds **1**–**8** and the positive controls **1a**-**5a** (**Figure 1**) were evaluated in seven human cancer cell lines using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The inhibitory effects (IC_{50} in μM) are summarized in **Table 1**.

The cytotoxicity of compounds **1**-**8** show a rather broad antitumor spectrum and are greater than that of cisplatin, oxaliplatin and satraplatin. Furthermore, the introduction of the pyranoside to Pt(IV) complexes prominently enhances the activity, in contrast to the oxoplatins **2a** and **4a** precursors. The HeLa (IC_{50} = 0.44–12.74 μM) and MCF-7 (IC_{50} = 0.22–15.62 μM) cell lines are

more sensitive to compounds **1**-**8** than the others. We can see that different structural carbohydrates, different linkers and different pyranoside positions also have a great effect on the bioactivity of the target compounds. Except for compound **4**, the C1-modified glycoside compounds (**5**-**8**) are more potent than the C6-modified pyranoside acids (**1**-**3**). In addition, the glycosylated Pt(IV) compounds almost fully overcome the drug resistance of A549R cells, especially the compounds **1** and **2** with RF 0.66 and 0.45, respectively.

Of the C6-modified pyranoside acids derivatives (**1**-**3**), compound **1** effectively inhibits the growth of all the tested cell lines with IC_{50} values of 1.07-8.81 μM. The introduction of glucose and mannose is more potent than of galactose (**1** > **2** > **3**). It is worth mentioning that galactoside derivative **3** shows better inhibition effects in HeLa cells than in the other cancer cell lines, indicating the targeting properties to HeLa. Additionally, prostatic carcinoma (LNCaP and PC3) shows more sensitivity to compounds **1** and **2**.

At the same time, for the C1-substituted glycoside compounds (**4**-**8**), when the lengths of the linkage change from 5 (**8**), 4 (**7**) to 3 (**4**), the antitumor activities dramatically decrease, which is opposite to the result with the glucoside platinum(II) prodrugs.[8f-8g] This may result from the significant different spatial structure of Pt(II) and Pt(IV) or the combination of transport and EPR effects. Compared with compound **4**, the glucoside (**6**) and galactoside (**5**) Pt(IV) complexes show better cytotoxicity to a panel of cancer cells, which is also different from the C6-substituted pyranoside acid derivatives (**1**-**3**).

Above all, compounds **5**, **6**, **7** and **8** show an especially better antitumor activity with an IC_{50} of 0.24 μM to 3.97 μM, which is almost 166-fold higher than cisplatin, oxaliplatin and satraplatin especially to A549R, LNCaP and PC3 cells, which have been reported to be resistant to cisplatin and oxaliplatin.

All of the above results indicate that Pt(IV) prodrugs containing different sugars such as glucose, mannose and galactose show significant cytotoxicity to cancer cells. It is also worth mentioning that they show different cytotoxicity profiles for different cancer cells. The chain length and positional isomers also have effects. These properties suggest that these compounds show great potential for further investigation.

Table 1. Cytotoxicity profiles of glycosylated Pt(IV) complexes (IC_{50} in μM) in seven human carcinoma cell lines. ([a] RF: Resistant factor = IC_{50}(A549R)/IC_{50}(A549).)

	HeLa	MCF-7	LNCaP	PC3	HepG-2	A549	A549R	RF[a]
1	1.09±1.89	6.36±1.44	8.81±0.38	6.33±1.45	1.22±1.58	1.63±0.39	1.07±1.90	0.66
2	2.27±1.87	0.88±1.87	12.87±0.89	2.77±0.49	3.47±0.49	24.51±1.78	10.91±0.48	0.45
3	1.59±0.67	13.33±1.67	38.10±0.78	>100	14.83±1.67	10.55±1.49	>100	/
4	12.74±0.78	15.62±0.98	>100	63.69±0.78	>100	12.05±0.48	48.45±1.76	4.02
5	0.89±0.56	0.25±0.44	3.03±0.12	0.43±1.56	0.72±0.79	0.98±0.89	3.52±0.67	3.59
6	0.44±0.45	0.19±0.47	2.31±1.67	0.24±0.56	0.79±1.79	0.44±0.78	1.10±1.69	2.5
7	1.57±0.78	0.31±0.45	3.97±0.45	0.53±1.89	1.70±1.46	0.40±1.31	0.76±1.69	1.9
8	0.74±0.65	0.22±0.78	2.94±0.45	0.24±1.56	1.68±1.79	0.31±1.98	0.65±0.69	2.1
1a	0.80±0.93	2.28±0.56	23.83±0.89	22.85±1.45	3.94±0.39	10.85±1.98	35.78±1.69	3.3
2a	11.36±1.56	27.01±0.34	50.78±0.79	61.42±0.67	50.00±1.79	>100	13.40±2.57	/
3a	3.96±0.75	5.22±2.78	16.78±0.34	39.74±0.76	6.64±1.79	10.06±1.20	39.67±0.69	3.94
4a	15.78±0.34	>100	22.97±0.89	>100	>100	>100	29.67±0.64	/
5a	3.10±0.89	8.01±0.56	5.23±1.77	16.77±1.56	6.70±1.89	2.17±1.68	9.96±0.57	4.59

The effect of incubation time of compounds 6, 7, 8 and 5a on the IC_{50} values for HeLa and MCF-7 cells.

Based on previous reports, if compounds **1-8** exhibit cancer-targeting abilities based on protein-mediated transport, which become saturated at longer time scales, the initial rate of accumulation of compounds **1-8** in cells would be more rapid than that for compound **5a** with passive uptake, which is slower but does not become saturated. We subsequently investigated the effect of the incubation time on the outcomes of these assays. We incubation the MTT assay for 8 h, 24 h, 30 h and 48 h with HeLa or MCF-7 cells.

The results revealed that the initial rate of accumulation of compounds **6**, **7** and **8**, was faster than that for compound **5a** (**Figures S1a** and **S1b**). Almost all of the compounds were observed intracellularly after 8 h incubation in the MTT assay for compounds **6**, **7** and **8**. The rapid rate of accumulation in the cell indicates the possibility that the glycosylated Pt(IV) compounds are transported by the targeted transporters.

Effects of EDG(1), phloretin(2), Ctd.(3), and the mixture of EDG and Ctd.(4) on the IC_{50} values of MCF-7 cells.

To understand whether the targeted transporters worked, we investigated the mechanism of compounds **1** and **6** in MCF-7 cells, which were co-incubated with the GLUT1 inhibitors 4,6-oethylidene-a-d-glucose (EDG) (1) and phloretin (2), the OCT2 inhibitor Ctd. (3), and a combination of EDG and Ctd. (4). Both the GLUT1 and OCT2 are overexpressed in MCF-7 cells [8f]. These results suggested that the cytotoxicity of **6** (IC_{50} = 0.57 μM and 0.68 μM) with EDG and phloretin were regulated *via* the glucose transporters because IC_{50} values were elevated compared with the 48 h IC_{50} of **6** (IC_{50} = 0.19 μM)) with no inhibitor. However, the glucuronic acid-modified compound **1** with an IC_{50} of 6.36 μM had respective cytotoxicity values of 5.98 μM and 5.89 μM with EDG and phloretin, but it showed no relationship with GLUT1. The magnitude of the cytotoxicity in the presence of EDG and phloretin was in the order **6** > **1** (**Figure 2a**), which was also observed for the inhibition of cellular uptake (**Figure 2c**) in a breast carcinoma cell line (MCF-7) that had a high level of GLUT1 expression. The results remind us that when we design sugar-platinum(IV) complexes, the C1-modified prodrugs can show a close relationship with the tranporters. EDG and phloretin do not affect compounds **1a** and **3a**, as evidenced by their IC_{50} values, which were not greatly different.

Furthermore, in the presence of Ctd. and a combination of EDG and Ctd., if IC_{50} and cellular uptake were greatly different, the energy-dependent organic cation transporters (OCTs) contributed. **Figure 2a** shows that the IC_{50} value of **6** (IC_{50} = 5.95 μM) in the presence of a combination of EDG and Ctd. was 31-fold higher than that in the 48 h incubation (IC_{50} = 0.19 μM) in the cytotoxicity assay and demonstrated greater sensitivity to the OCTs than the C6-substituted pyranoside acids compound (**1**). The same results in the cellular uptake inhibition also occurred with Ctd. (**Figure 2d**) It has been reported that the organic cation transporter 2 (OCT2), which is a primary factor responsible for the sensitivity of colorectal cancer to oxaliplatin, plays a considerable role in the cellular accumulation and consequent cytotoxicity of platinum complexes containing the ((1R, 2R)-cyclohexane-1,2-diamine (DACH) ligand.[13a,13b] In our study, the IC_{50} values for oxaliplatin with Ctd. (IC_{50} =10.89 μM) are 2.09-fold higher than that without Ctd.(IC_{50} = 5.22 μM) and cellular uptake are 1.38-fold higher than that with Ctd., indicating that OCT2 also affects the consequent cytotoxicity and cellular uptake of oxaliplatin (**Figure 2b** and **2d**). However, OCT2 did not contribute to compounds **5**, **7** and **8** (**Figure 2b**). The reason may be that OCT2 had a close relationship with the glucoside Pt(IV) prodrugs but not with the mannoside and galactoside complexes. As a result, compound **6** significantly exploits both glucose and organic cation transporters, both of which are widely overexpressed in cancer cells. Different sensitivities of Glc-Pt(IV), Gal-Pt(IV) and Man-Pt(IV) to the OCTs also occur.

The above observations indicate that the C1-modified glycoside compounds are superior to the C6-modified pyranoside acids derivatives for targeting transport. This phenomenon may be because of the differences in the spatial structure of the square-planar platinum(II) and the octahedral platinum(IV). The above results also suggest that the pyranoside conjugated

platinum(IV) complexes have important cancer-targeting properties and provide new insight into the potential of the glycosylated Pt(IV) anticancer prodrugs.

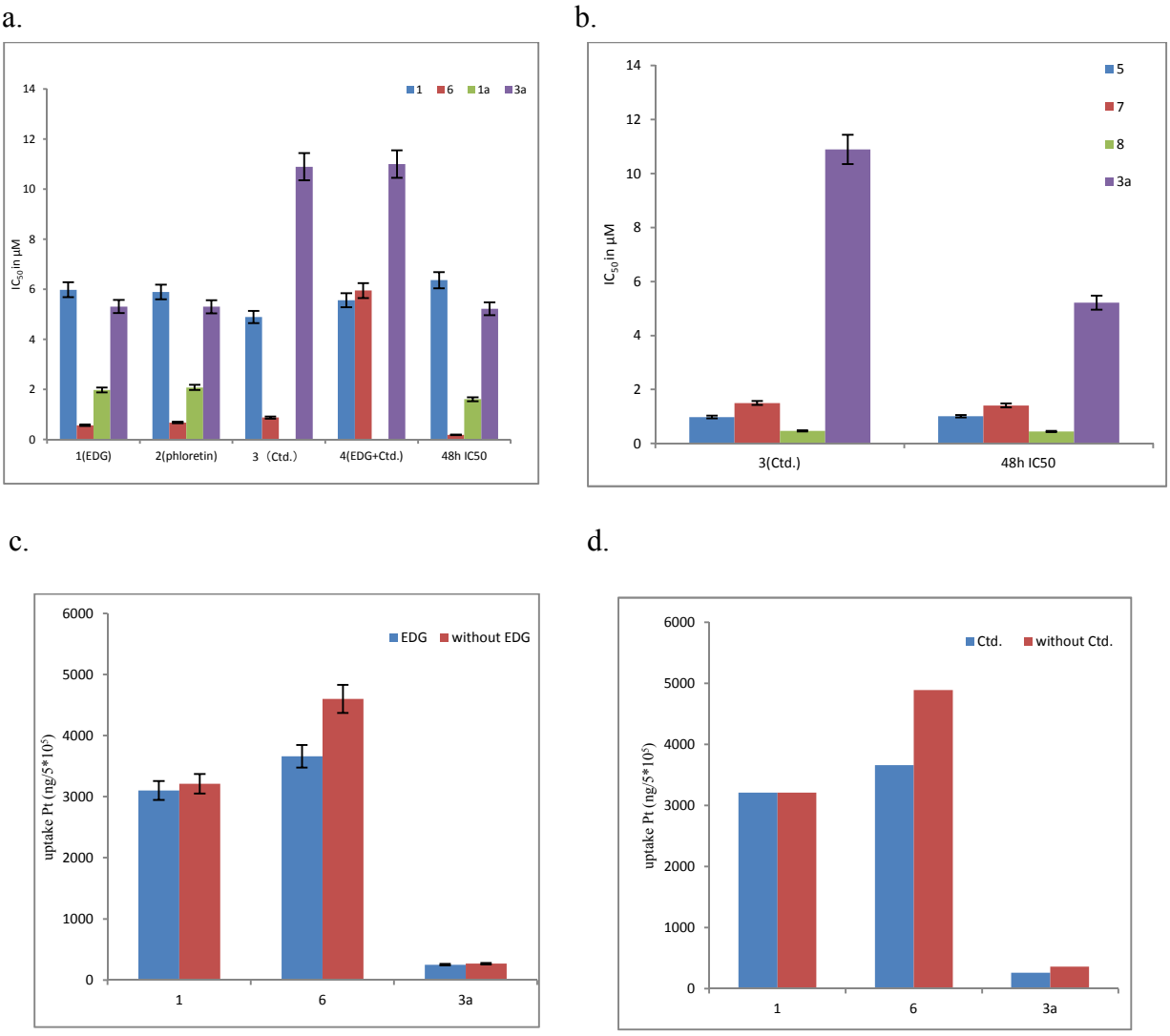


Figure 2. a). Effects of EDG(1), phloretin(2), Ctd.(3), and a mixture of EDG and Ctd.(4) on the IC_{50} values of compounds **1**, **6** and **1a** in MCF-7 cells. b). Effects of Ctd.(3) on the IC_{50} values of on the compounds **5**, **7**, **8** and **3a** in MCF-7 cells. c). Effects of EDG on the cellular uptake of compounds **1**, **6** and **3a** in MCF-7 cells. d). Effect of Ctd. on the cellular uptake of compounds **1**, **6** and **3a** in MCF-7 cells.

Whole cell uptake studies and DNA platination for HeLa and MCF-7 cells.

Subsequently, the intracellular distribution of compounds **5**, **6**, **8**, **3a** and **5a** that have potent cytotoxicity in HeLa and MCF-7 cells was quantified by inductively coupled plasma mass spectrometry (ICP-MS) using ^{196}Pt detection as shown in **Figure S2**.

After incubation with compounds **5**, **6** and **8**, the accumulation of platinum in MCF-7 cells was 5.11- to 17.39-fold and 1.41- to 4.79-fold higher than that of **3a** and **5a**, respectively. In HeLa cells, the accumulation was 7.25- to 30.96-fold and 1.41- to 6.07-fold higher than that of **3a** and **5a**. Platinum in the DNA isolated from HeLa and MCF-7 cells was also measured and was 1.91- to 8.34-fold and 1.99- to 8.69-fold higher than that with **3a** and **5a**, respectively.

Nuclear DNA was considered as a potential subcellular target and a correlation between the intracellular platinum accumulation and antitumor activity was confirmed. An annexin V/PI coupled flow cytometric analysis (Figures S4) clearly indicated that compounds **6** and **7** effectively induced cell damage in A549 cells.

Viability of the cancer cells such as breast cancer MCF-7, breast cancer HeLa, and normal cells such as 293T and 3T3 in a 48 h incubation assay

An ideal anticancer compound should be selective for cancer cells over normal healthy cells, thereby mitigating undesired toxic side effects associated with chemotherapy. Platinum(IV) itself always shows less toxicity to normal cells than platinum(II) drugs.

Therefore, we determined the viability of breast cancer MCF-7 cells, breast cancer HeLa cells, normal kidney epithelial 293Tcells and mouse embryo 3T3 fibroblasts in a 48 h incubation assay to evaluate their selectivity for cancer cells to mitigate undesired toxic side effects associated with chemotherapy. The pyranoside conjugated platinum(IV) complexes **5**-**8** show less cytotoxicity to 3T3 and 293T cells compared with the more sensitive HeLa and MCF-7 cells. (**Figures 3** and **S3**) For example, the cytotoxicity of **6** for 3T3 cells (IC_{50} = 2.54 μM) was 13.37-

fold higher than that for MCF-7 cells (IC_{50} = 0.19 μM). Compared to **1a** and **3a** in the clinic, they are also even safer for normal cells.

We can deduce that this phenomenon may contribute to the part of pyranoside with cancer-targeting properties and the platinum(IV) itself regaining its cytotoxicity *via* reductants such as ascorbic acid and glutathione presenting higher concentrations in the tumor cells than in the blood and normal tissues. It is worth mentioning that these properties cannot be realized by Pt(II) drugs.

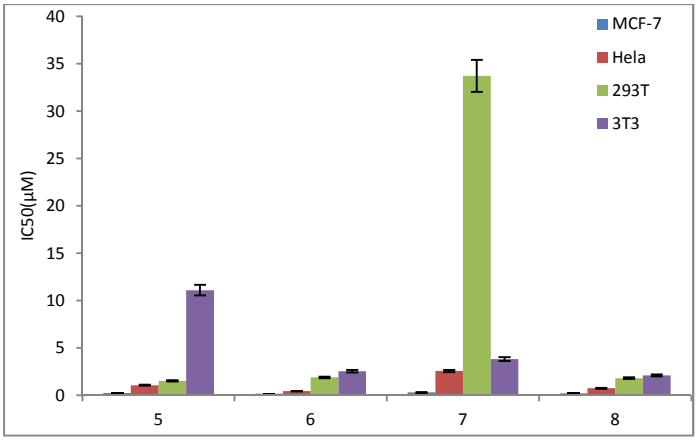


Figure 3. Viability of MCF-7 cells, HeLa cells, 293T cells and 3T3 cells in a 48 h incubation assay.

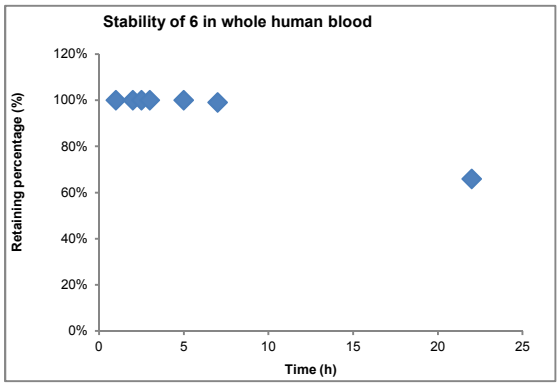
Stability of 6 in RPMI 1640 and the whole human blood

One of most important advantages of inert Pt(IV) complexes is that they are more stable than Pt(II) prodrugs. This is also a key point for the facilitated intravenous-to-oral switch of satraplatin. As previously reported for the activation of structurally similar platinum compounds by nucleophiles, the stability of **6** in RPMI 1640 medium and human whole blood was evaluated. The results are shown in **Figure 4**.

In RPMI medium, no significant decomposition of compound **6** was up to 12 h, and 66% of **6** remained unchanged even after 48 h, and a half-life ($t_{1/2}$) of up to 170 h in RPMI 1640 medium suggested that it was highly stable in biological media, which makes it superior to platinum(II) drugs. (**Figure 4a** and **S5**). Compound **6** was more stable in human whole blood, with a $t_{1/2}$ of nearly 20 h, compared with 21.6 min and 6.3 min for cisplatin and satraplatin respectively. This suggested a significant potential for a facilitated intravenous to oral switch of glycosylated platinum(IV) prodrugs with cancer-targeting properties.

The stability of glycosylated platinum(IV) complexes **6** is more advantageous than the platinum(II) prodrugs, which may contribute greatly to the development of platinum(IV) prodrugs for an intravenous to oral switch.

a.



b.

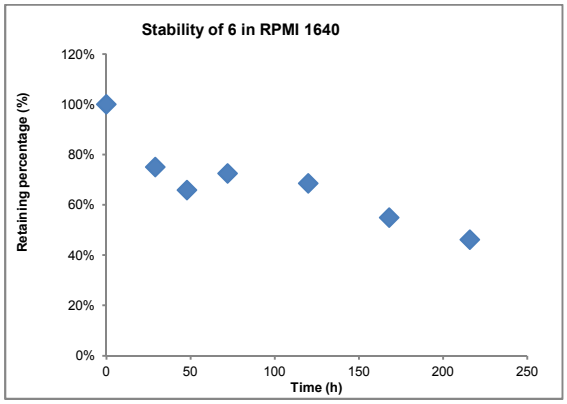


Figure 4. Stability of **6** in whole human blood (a) and RPMI 1640 (b).

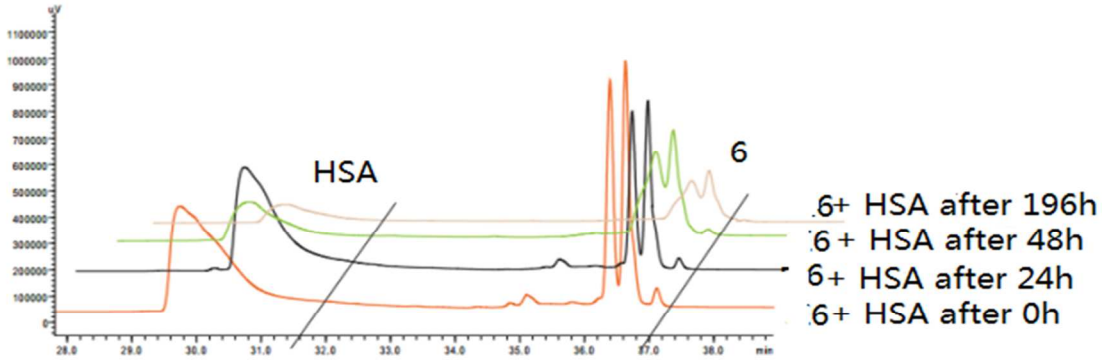
Binding studies of 6 with albumin using RP-HPLC

We previously assumed that the significant stability of compound **6** in human blood may have resulted from the HSA binding properties of the hexadecanoic chain. Based on these observations, we also determined the HSA binding properties of **6**. The results showed that the pyranoside conjugated platinum(IV) complex **6** containing a hexadecanoic acid chain as an axial ligand preferably interacted with HSA. (**Figure 5a**). Conversely, oxaliplatin did not bind HSA (**Figure S7**). Docking studies have shown that the interaction is the combination of non-covalent

interactions of the hexadecyl chain and hydrogen-bond interactions of the pyranoside, suggesting that the prodrug is buried below the surface of the protein.

We can also presume from the results that the rate of reduction of **6** by ascorbate can be reduced as a result of the interaction with HSA during drug delivery. Consequently, the reduction and DNA binding properties of **6** with and without HSA was studied as shown in **Fig. 5b**. At 24 h, 48 h, 96 h and 144 h, the remaining compound **6** for the groups that contained HSA was always less than those without HSA. In addition, the remaining HSA also gradually decreased. The smaller reduction of **6** by ascorbate in human blood as the result of the interaction with HSA during drug delivery can further enhance the stability of platinum(IV) prodrugs and their accumulation in cancer cells.

a.



b.

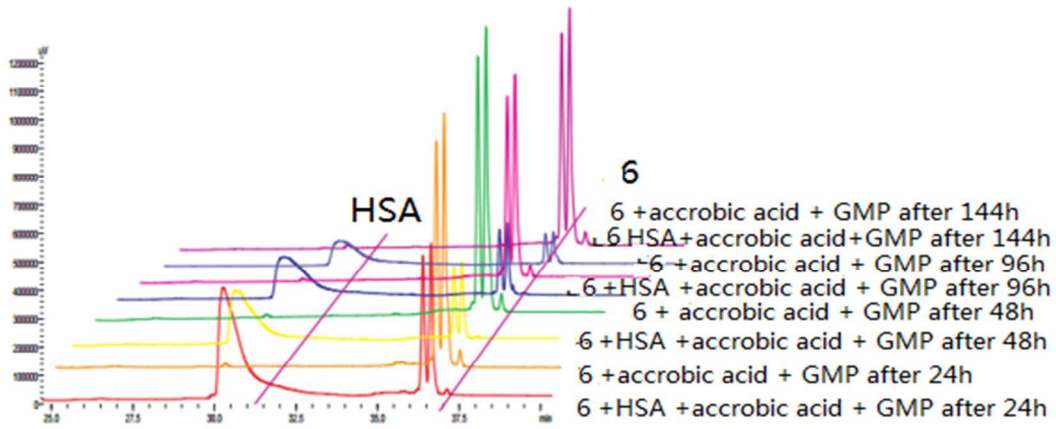


Figure 5. a) Binding studies of **6** with HSA after 0 h, 24 h, 48 h and 196 h. b) Reduction of **6** by ascorbate with and without HSA.

Reduction and DNA binding properties of 6 and oxaliplatin with and without ascorbic acid

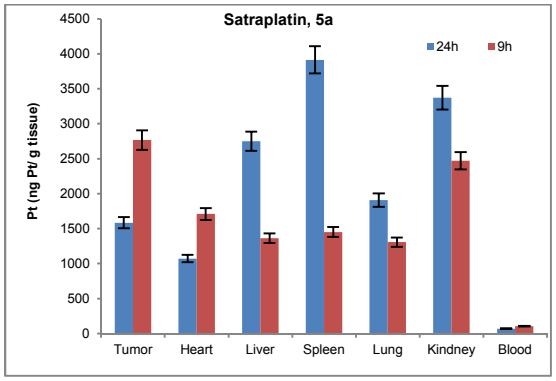
We also investigated the reduction and DNA binding properties of **6** and **3a** with and without ascorbic acid for the inherent proficiency of the Pt prodrugs to bind at the N7 position of guanine bases, thus leading to DNA damage and inducing cell death.[14a,14b,14c] 5'-dGMP was used as a model for DNA. Adducts can only be observed in the presence of the reductant ascorbic acid for RP-HPLC analysis at 37 °C in **Fig. S6**. The fractions that contained unknown peaks were separated and identified by ESI-MS analysis observing the same products with **3a**. The results showed that the bis-substituted products of **6** originating from the combination with 5'-dGMP of **3a**, were generated after the reduction by a reductant such as ascorbic acid.

Biodistribution of 5, 6, 8, and 5a in vivo

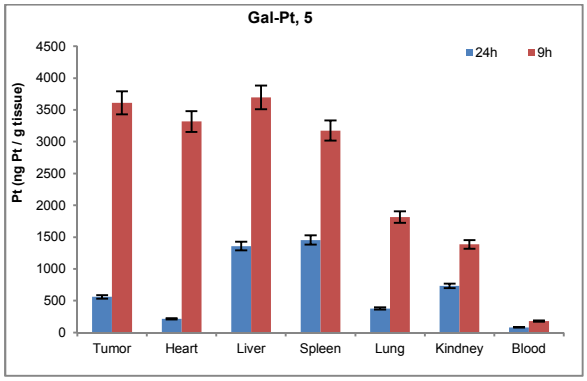
To determine whether pyranoside conjugated platinum(IV) complexes can exhibit cancer-targeting *in vivo*, the biodistribution of **5**, **6**, **8**, and **5a**, a non-targeted platinum complex used as the positive control in MCF-7 bearing animals was assessed by ICP-MS at 9 h, and 24 h post administration (*i.v.*).

The *in vitro* IC_{50} values of **5**, **6**, **8** and **5a** were respectively 0.25 μM, 0.19 μM, 0.22 μM and 8.01 μM, which were not greatly different. Surprisingly, the accumulation Pt of **6** in the tumor tissue after 24 h was approximately 6.2, 7.9 and 2.2-fold higher than **5**, **8** and **5a** as shown in **Figure 6A-D and 6F**, which were considerably different from the *in vitro* results. Additionally, the accumulation of **5** was higher in tumors and the heart, liver, spleen, and lungs than **5a** after 9 h. However, **5a** and **8** did not show *in vivo* cancer-targeting properties (**Figure 6A** and **6D**).

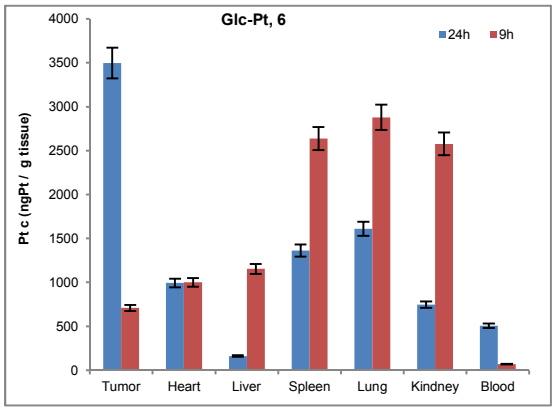
A.



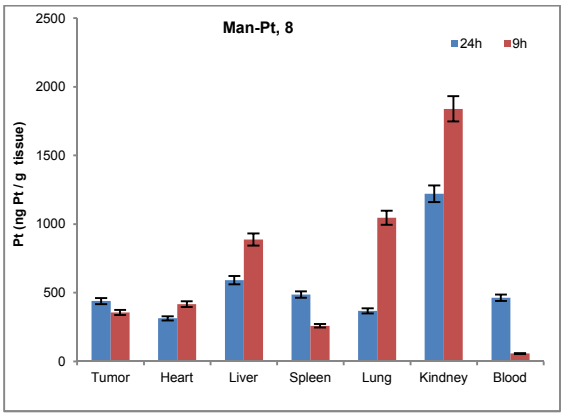
B.



C.



D.



E.

F.

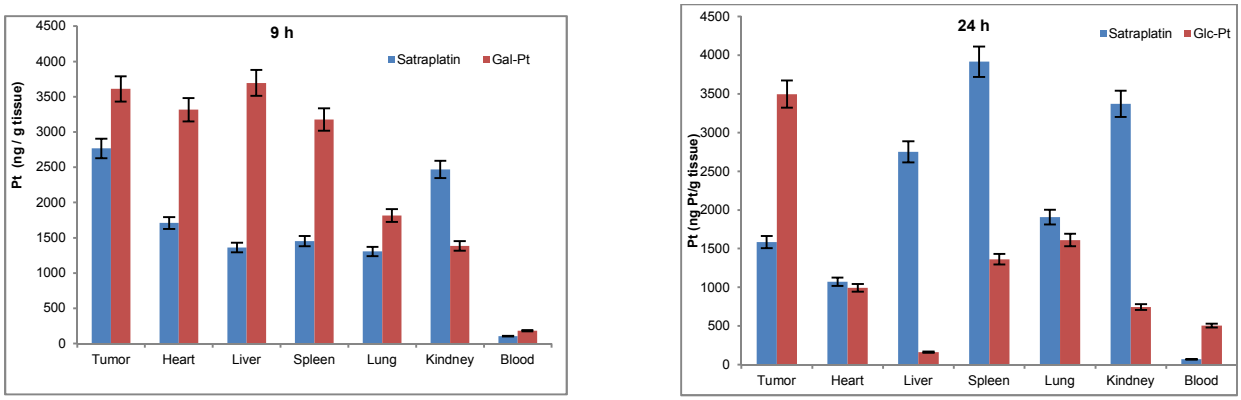


Figure 6. A-D. Biodistribution of **5**, **6**, **8**, and **5a**, as the positive control in MCF-7 bearing animals was assessed by ICP-MS at 9 h, and 24 h post administration (i.v.). E-F. Biodistribution of **5**, **6**, **8**, and **5a** at 9 h and 24 h.

The results *in vivo* indicated that **6** exhibited enhanced cancer-targeting abilities and decreased toxicity for MCF-7-bearing animals compared with **5**, **8** and the positive control **5a**. The encouraging selectivity of **6** to MCF-7 cells after 24 h *in vivo* prompted the adventurous assumption that different cancer cells might have different sensitivities to different glycosylated platinum(IV) prodrugs.

Effects of EDG(1), phloretin(2), Ctd.(3), and a mixture of EDG and Ctd.(4) on the IC_{50} values of the Madine-darby canine kidney (MDCK) cells.

It is well known that MDCK cells are widely used to establish an intestinal epithelial model for the evaluation of the oral administration of a drug, and can also be used to screen substrates and inhibitors *in vitro*. The most important goal regarding Pt(IV) prodrugs is to find a prodrug with an enhanced potential for oral administration. To determine whether targeted transport works in MDCK cells, we also investigated the mechanism of compounds **6** and **5a**, which were also co-incubated with the GLUT1 inhibitor 4, 6-o-ethylidene-a-d-glucose (EDG) and phloretin, the OCT2 inhibitor Ctd., and a combination of EDG and Ctd. as shown in **Figure 7**.

The results show that IC_{50} with inhibitors are higher than in the 48 h MTT assays, indicating a close relationships of **6** with the transporters in MDCK cells. As GLUT and OCT substrates in MDCK cells, compound **6** would contribute greatly to the intravenous to oral switch for Pt(IV) prodrugs.

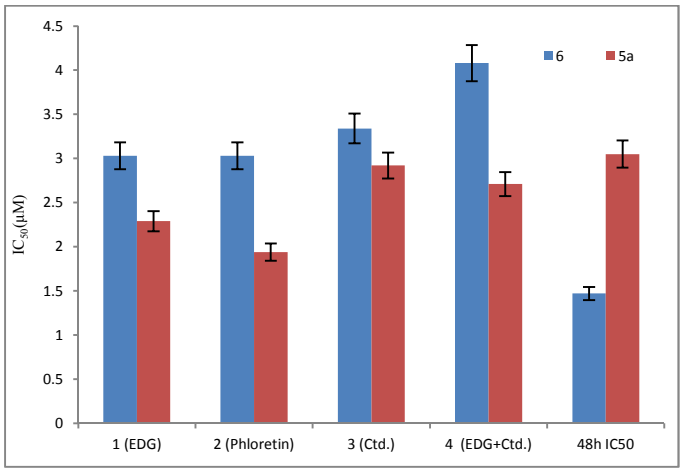


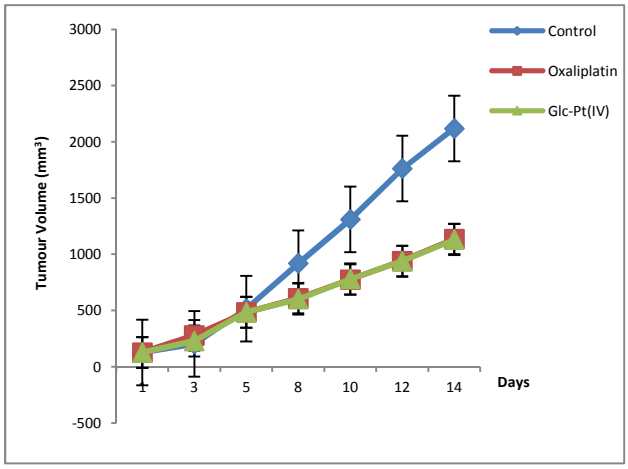
Figure 7. Effects of EDG(1), phloretin(2), Ctd.(3), and the mixture of EDG and Ctd.(4) on the IC_{50} values of compounds **6** and **5a** in MDCK cells.

In vivo efficacy of 6 and 3a.

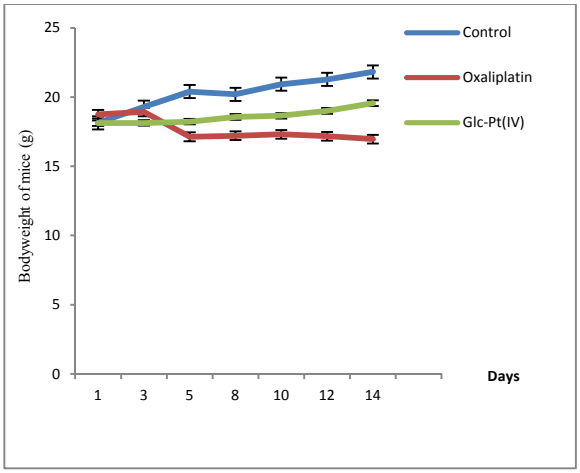
To further investigate the antitumor potential of the glycosylated Pt(IV) complexes, compounds with potent *in vitro* activity were selected for further evaluation *in vivo*, using a mouse model of GLUT1 expressing breast cancer (MCF-7) in Balb/c mice. Intraperitoneal injections of 5 mg Pt/kg of **6** or **3a** were begum when the tumors reached ~80-150 mm^3 for three successive treatments one week. Treatment groups included **6**, **3a**, and a control group. The antitumor efficacy was evaluated by measuring the tumor volume over a period of 14 days. The toxic side effects of the platinum agents under investigation were assessed daily by a thorough examination of physical condition of the animals, including body weight, lethargy, loss of appetite, and general behavior.

The tumor treatment revealed that **6** effectively reduced the disease burden by significantly delaying the tumor growth (**Figure 8A**). Moreover, **3a** treatment showed no statistically significant difference compared to the **6**-treated group. Notably, tumors treated with **6**, or **3a** were 2-fold smaller compared to control group tumors, indicating excellent efficacy. Importantly, the bodyweight of mice in **Figure 8B** indicates that the glycosylated Pt(IV) compounds **6** exhibited reduced toxicity compared with **3a**. Images of the tumors at the end of the experiment are shown in **Figure 8C**. The results reveal that the glycosylated Pt(IV) complexes had lower toxicity and higher dose tolerance *in vivo* with comparative tumor control rates to platinum(II) drugs such as **3a**.

A.



B.



C.

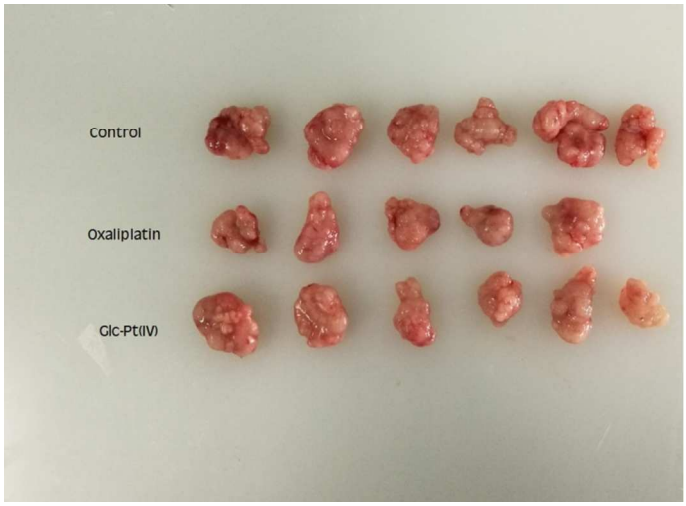


Figure 8. *In vivo* antitumor activities of compounds Glc-Pt(IV) **6** and oxaliplatin in MCF-7. (A) Tumor growth as a function of time. (B) The bodyweight of mice during the treatments. (C) The images of tumors at the end of the experiment.

CONCLUSIONS

Platinum anticancer drug candidates are designed as inert platinum(IV) prodrugs that can be activated following cellular uptake by intracellular reducing agents that are abundant in cancer cells. For the first time, we synthesized and determined the cytotoxicity, and the detailed mechanism of pyranoside conjugated platinum(IV) complexes *in vitro* and *in vivo*. Pyranoside-conjugated platinum(IV) complexes are GLUT and OCT substrates and contributed greatly to the targeted therapy. Previously reported glycosylated Pt(IV) complexes protected by acetyl groups [8h,8i] did not have such properties. The cellular uptake and target engagement among these glycosylated Pt(IV) complexes is variable. A major reason is off-target effects due to the presence of glucose.

Overall, compounds **4-8,** which were C1-substituted glycoside compounds, were more effective than compounds **1-3,** which were C6-substituted pyranoside acids. However, compounds **1-3** that contained different pyranosides such as glucose, galactose, mannose also

showed specific tumor targeting properties *in vitro*. Different chain lengths of between the sugar and the platinum also made a great difference, possibly because of a combination of effects of EPR and transporters. In a MTT assay after 8 h incubation, most of compounds **6**, **7** and **8** were intracellular, indicating that they would rapidly accumulate in cancer cells. They also demonstrated the potential to exploit the two transporters GLUT1 and OCT2, both of which are overexpressed on the surface of tumor cells. Their preferential accumulation kills more cancer cells than noncancerous cells (293T and 3T3 cells) *in vitro* and indicates a low toxicity for the antineoplastic chemotherapy of platinum drugs. Binding with HSA during drug delivery also enhanced the stability of the dual targeted Pt(IV) compounds in human whole blood. A half-life of **6** of up to 170 h in RPMI 1640 medium suggested that this compound was highly stable in biological media, more so than platinum(II) drugs. The biodistribution of **5**, **6** and **8** *in vivo* made a great difference with the *in vitro* results. The encouraging selectivity of **6** *in vivo* in MCF-7 tumor cells may contribute to the assumption that different cancer cells would have different sensitivities to different glycosylated platinum prodrugs *in vivo*. Compound **6** is a substrate of GLUT and OCT in MDCK cells, and it would contribute greatly to the intravenous to oral switch for Pt(IV) prodrugs. However, previously reported glycosylated Pt(IV) complexes protected by acetyl groups [8h,8i] also did not have similar properties. The *in vivo* antitumor activities revealed that the glycosylated Pt(IV) complexes exhibited important antitumor activities and reduced toxicity *in vivo* with a tumor control rate similar to that of the platinum(II) drug oxaliplatin.

All the results above show great differences with the glucose-platinum(II) prodrugs and enhanced of cancer-targeting effects, which will contribute to the design of platinum(IV) prodrugs that will facilitate an intravenous to oral switch. Eventually, the desirable biological

performance of the glycosylated Pt(IV) complexes mentioned above could prove to be of prime importance in the future development of platinum antitumor drugs.

Experimental section.

HPLC Studies.

The purities of all target compounds were determined by HPLC. The HPLC analyses were also performed as on a Waters E2695-2998 equipped with a Venusil MP C18 column (150×4.6 mm, 5 μM). The purity of the platinum complexes (**1**–**8**) was confirmed to be ≥95% by analytical HPLC (in the Supporting Information). The linear gradient was as follow. (Table 2)

Table 2. Methods of the HPLC analyses for the purities of all target compounds.

Time(min)	A (water)	B(Methol)
	90	10
5	90	10
35	0	100
45	0	100

General Procedure for the Synthesis of Compound 1.

Preparation of 6b.

To a stirred solution of **5b** (1.6 g) in 40 mL acetone, $K_2Cr_2O_7$ (1.49 g) dissolved in H_2SO_4 (18 mL, 6 mol/L) was added in a dropwise manner at 0 °C under an N_2 atmosphere and the resulting solution was stirred at 55 °C for an additional 2 h. After evaporation under reduced pressure, the precipitate was then washed with 100 mL (×3) of DCM and dried. The residue obtained was purified by flash column chromatography to give the desired compound **S6** as a white solid (yield: 1.2 g, 71%).

Preparation of 10b.

To **6b** (200 mg, 0.36 mmol) under N_2 atmosphere, $C_2O_2Cl_2$ (4 mL) was added with catalytic DMF at 0^0C. White smoke was observed and then the reaction was stirred for another 30 min at 0^0C. The resulting solution was stirred at room temperature for 2 h. The $C_2O_2Cl_2$ was removed to obtain **9b** as a slightly yellow solid. Pt(IV) compounds combined with **4a** and hexadecanoic acid as axial ligands (200 mg, 0.30 mmol) was added along with acetone (20 mL) under an N_2 atmosphere. After 2 days, the liquid was evaporated and purified by column chromatography to give the desired compound **10b** as a yellow solid (yield: 166 mg, 46%).

Preparation of 1.

To a solution of **10b** (120 mg, 0.10 mmol) in anhydrous DCM, BCl_3 (1 M)（0.80 mL，0.80 mmol）was added at –78 ℃ for 2 h. Then, the DCM was removed and purified by column chromatography to get the white solid **1** (41 mg, 48%). ^{1}H NMR (400 MHz, CD_3OD) δ 5.14 (s, 1H), 4.60 – 4.35 (m, 1H), 4.03 – 3.66 (m, 1H), 3.62 – 3.38 (m, 2H), 3.01 – 2.65 (m, 2H), 2.45 – 2.20 (m, 4H), 1.72 – 1.51 (m, 6H), 1.30 (s, 26H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 183.11, 178.11, 176.63, 165.69, 97.09, 92.95, 75.99, 75.89, 74.54, 73.09, 72.75, 72.43, 71.95, 71.50, 61.92, 61.29, 35.92, 31.71, 31.14, 29.46, 29.31, 29.18, 29.12, 28.92, 25.61, 23.75, 23.61, 22.37, 13.13. HRMS: Calcd. for $C_{30}H_{54}N_2O_{13}Pt$ $(M+NH_4)^+$: 863.3612, found: 863.3594.

General Procedure for the Synthesis of 2.

Preparation of 7b, 11b and 12b.

Compounds **7b, 11b** and **12b** were prepared according to the procedure described for compounds **6b**, **9b** and **10b**, respectively.

Preparation of 2.

To a solution of **12b** (120 mg, 0.10 mmol) in anhydrous DCM, BCl_3 (1 M) (0.80 mL, 0.80 mmol) was added at –78 ℃ for 2 h. The DCM was removed and purified by column chromatography to get the white solid **2** (46 mg, 54%). ^{1}H NMR (400 MHz, CD_3OD) δ 5.24 (s, 1H), 4.42 – 4.26 (m, 1H), 3.97 – 3.76 (m, 1H), 3.54 – 3.43 (m, 2H), 2.95 (s, 1H), 2.79 (s, 1H), 2.43 – 2.22 (d, *J* = 20.7 Hz, 4H), 1.70 – 1.52 (m, 6H), 1.30 (s, 26H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 182.78, 178.31, 166.07, 129.46, 96.77, 92.98, 81.72, 72.77, 71.62, 71.01, 70.50, 70.38, 69.11, 68.25, 61.78, 61.67, 35.78, 31.68, 31.15, 31.01, 29.41, 29.26, 29.12, 29.09, 28.88, 26.71, 25.49, 23.75, 23.57, 22.35, 22.27, 13.09. HRMS: Calcd. for $C_{30}H_{54}N_2O_{13}Pt$ $(M+NH_4)^+$: 863.3612, found: 863.3593.

General Procedure for the Synthesis of 3.

Preparation of 8b, 13b and 14b.

Compounds **8b, 13b and 14b** were prepared according to the procedure described for compounds **6b**, **9b** and **10b**, respectively.

Preparation of 3

To a solution of **14b** (120 mg, 0.10 mmol) in anhydrous DCM, BCl_3 (1 M) (0.80 mL, 0.80 mmol) was added at –78 ℃ for 2 h. The DCM was removed and purified by column chromatography to get the white solid **3** (87 mg, 48%). ^{1}H NMR (400 MHz, CD_3OD) δ 5.15 (s, 1H), 4.27 (d, *J* = 7.9 Hz, 1H), 3.92 (s, 1H), 3.85 – 3.69 (d, *J* = 14.3 Hz, 2H), 2.94 – 2.83 (m, 1H), 2.83 – 2.65 (m, 1H), 2.43 – 2.20 (m, 4H), 1.70 – 1.46 (m, 6H), 1.28 (s, 26H), 0.90 (t, *J* = 6.1 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 183.17, 178.05, 165.50, 94.55, 73.18, 70.95, 70.69, 69.73, 62.01, 61.23, 35.93, 31.71, 31.18, 29.45, 29.41, 29.30, 29.17, 29.12, 28.91, 25.62, 23.76, 23.57, 22.37, 13.11. HRMS: Calcd. for $C_{30}H_{54}N_2O_{13}Pt$ $(M+NH_4)^+$: 863.3612, found: 863.3606.

General Procedure for the Synthesis of 4.

Preparation of 21b.

NaOH (1 M) (17 mmol) was added to a solution of **20b** (1.1 g, 1.7 mmol) dissolved in dioxane 17 mL and stirred for 12 h at room temperature. The product **21b** was finally purified by column chromatography as a slightly yellow liquid with a yield of 84% (0.9 g).

Preparation of 26b and 27b.

To **21b** (113 mg, 0.18 mmol) under an N_2 atmosphere, $C_2O_2Cl_2$ (2 mL) was added with catalytic DMF at 0^0C. White smoke was observed and then the reaction was stirred for another 30 min at 0^0C. The resulting solution was stirred at room temperature for 2 h. The $C_2O_2Cl_2$ was removed to obtain **26b** as slightly yellow solid. Pt(IV) compounds combined with **4a** and a hexadecanoic acid as an axial ligand (100 mg, 0.15 mmol) was added along with acetone (10 mL) under an N_2 atmosphere. After 2 days, the reaction was evaporated and purified by column chromatography to give the desired compound **27b** as a yellow solid (yield: 120 mg, 63%).

Preparation of 4

To a solution of **27b** (120 mg, 0.10 mmol) in anhydrous DCM, BCl_3 (1 M) (0.80 mL, 0.80 mmol) was added at –78 ℃ for 2 h. The DCM was removed and purified by column chromatography to get the white solid **4** (36 mg, 41%). Data for compound **4**. ^{1}H NMR (400 MHz, MeOD) δ 5.01 (d, *J* = 3.6 Hz, 1H), 4.02 – 3.63 (m, 3H), 3.54 (t, *J* = 6.2 Hz, 2H), 2.91 (s, 1H), 2.78 (t, *J* = 9.3 Hz, 2H), 2.51 – 2.40 (m, 2H), 2.39 – 2.23 (m, 4H), 1.86 – 1.71 (m, 3H), 1.69 – 1.62 (d, *J* = 8.4 Hz, 2H), 1.60 – 1.46 (m, 5H), 1.28 (s, 26H), 0.89 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ 183.21, 182.66, 165.43, 128.02, 100.06, 73.23, 71.18, 70.74, 67.29, 66.11, 61.82, 61.49, 60.85, 35.98, 32.58, 31.72, 31.19, 29.46, 29.42, 29.31, 29.17, 29.13, 28.92, 28.28,

25.62, 23.73, 23.67, 22.38, 13.13. HRMS: Calcd. for $C_{34}H_{62}N_2O_{14}Pt$ $(M+H)^+$: 918.3922, found: 918.3879.

General Procedure for the Synthesis of 5.

Preparation of 22b.

Compound **22b** obtained as a slight yellow solid was prepared according to the procedure described for compound **21b**.

Preparation of 28b and 29b.

To **22b** (226 mg, 0.36 mmol) under an N_2 atmosphere, $C_2O_2Cl_2$ (4 mL) was added with catalytic DMF at 0^0C. White smoke was observed and then the reaction was stirred for another 30 min at 0^0C. The resulting solution was stirred at room temperature for 2 h. The $C_2O_2Cl_2$ was removed to obtain **28b** as slightly yellow solid. Pt(IV) compounds combined with **4a** and a hexadecanoic acid as an axial ligands (200 mg, 0.30 mmol) along with acetone (20 mL) was added under an N_2 atmosphere. After 2 days, the liquid was evaporated and purified by column chromatography to give the desired compound **29b** as a yellow solid (yield: 196 mg, 51%).

Preparation of 5.

To a solution of **29b** (120 mg, 0.10 mmol) in anhydrous DCM, BCl_3 (1 M) (0.80 mL, 0.80 mmol) was added at –78 ℃ for 2 h. The DCM was removed and purified by column chromatography to get the white solid **5a** (25 mg, 28%). Data for compound **5a**. ^{1}H NMR (400 MHz, MeOD) δ 4.02 – 3.82 (m, 1H), 3.80 – 3.60 (m, 2H), 3.58–3.42 (m, 2H), 2.80 (s, 2H), 2.58 – 2.40 (m, 2H), 2.38–2.22 (m, 4H), 1.99 – 1.73 (m, 2H), 1.72–1.48 (m, 6H), 1.30 (s, 28H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, MeOD) δ 183.18, 182.61, 165.44, 103.68, 75.23, 73.52, 71.18, 68.92, 68.56, 61.79, 61.46, 61.12, 60.84, 35.97, 32.56, 31.73, 31.18, 29.47, 29.43, 29.32, 29.19,

29.14, 28.92, 28.27, 25.62, 23.72, 22.39, 13.14. HRMS: Calcd. for $C_{34}H_{62}N_2O_{14}Pt$ $(M+H)^+$: 918.3922, found: 918.3854.

General Procedure for the Synthesis of 6.

Preparation of 23b.

Compound **23b** was obtained as a slightly yellow solid and was prepared according to the procedure described for compound **21b**, respectively.

Preparation of 30b and 31b.

To **23b** (226 mg, 0.36 mmol) under an N_2 atmosphere, $C_2O_2Cl_2$ (4 mL) added with catalytic DMF at 0^0C. White smoke was observed and then the reaction was stirred for another 30 min at 0^0C. The resulting solution was stirred at room temperature for 2 h. The $C_2O_2Cl_2$ was removed to obtain **30b** as a slightly yellow solid. The Pt(IV) compounds combined with **4a** and a hexadecanoic acid as an axial ligand (200 mg, 0.30 mmol) along with acetone (20 mL) was added under an N_2 atmosphere. After 2 days, the liquid was evaporated and purified by column chromatography to give the desired compound **31b** as a yellow solid (yield: 250 mg, 66%).

Preparation of 6.

To a solution of **31b** (120 mg, 0.10 mmol) in anhydrous DCM, BCl_3 (1 M) (0.80 mL, 0.80 mmol) was added at–78 ℃ for 2 h. The DCM was removed and purified by column chromatography to get the white solid **6** (31 mg, 35%). Data for compound **6**. ^{1}H NMR (400 MHz, MeOD) δ 4.24 – 3.83 (m, 1H), 3.75 – 3.50 (m, 2H), 3.39 – 3.32 (m, 1H), 3.28 – 3.20 (m, 1H), 2.89 – 2.66 (m, 2H), 2.57 – 2.38 (m, 2H), 2.39 – 2.21 (m, 4H), 1.97 – 1.70 (m, 2H), 1.68 – 1.48 (m, 6H), 1.28 (s, 28H), 0.89 (t, J = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ 183.19, 182.63, 165.40, 103.01, 76.61, 76.48, 73.69, 70.20, 68.52, 61.82, 61.49, 61.30, 60.85, 48.30,

35.98, 32.57, 31.73, 31.19, 29.48, 29.44, 29.33, 29.20, 29.15, 28.94, 28.28, 25.63, 23.73, 22.40, 13.16. HRMS: Calcd. for $C_{34}H_{62}N_2O_{14}Pt$ $(M+H)^+$: 918.3922, found: 918.3887.

General Procedure for the Synthesis of 7.

Preparation of 24b.

Compound **24b** was obtained as a slight yellow solid and was prepared according to the procedure described for compound **21b**.

Preparation of 32b and 33b.

To **24b** (232 mg, 0.36 mmol) under a N_2 atmosphere, $C_2O_2Cl_2$ (4 mL) added with catalytic DMF at 0^0C. White smoke was observed and the reaction was stirred for another 30 min at 0^0C. Then, the resulting solution was stirred at room temperature for 2 h. The $C_2O_2Cl_2$ was removed to obtain **32b** as a slightly yellow solid. The Pt(IV) compounds combined with **4a** and a hexadecanoic acid an axial ligand (200 mg, 0.30 mmol) along with acetone (20 mL) was added under an N_2 atmosphere. After 2 days, the liquid was evaporated and purified by column chromatography to give the desired compound **33b** as a yellow solid (yield: 220 mg, 57%).

Preparation of 7.

To a solution of **33b** (120 mg, 0.09 mmol) in anhydrous DCM, BCl_3 (1 M) (0.80 mL, 0.80 mmol) was added at –78 ℃ for 2 h. The DCM was removed and purified by column chromatography to get the white solid **7** (38 mg, 43%). Data for compound **7**. ^{1}H NMR (400 MHz, MeOD) δ 3.78 – 3.67 (m, 1H), 3.65–3.59 (m, 1H), 3.57 – 3.47 (m, 1H), 3.44 (s, 1H), 3.28–3.17 (m, 1H), 2.71 (s, 2H), 2.42–2.09 (m, 6H), 1.61–1.38 (m, 10H), 1.18 (s, 28H), 0.79 (t, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ 183.10, 182.81, 165.38, 100.07, 73.18, 71.21, 70.82, 67.31, 66.68, 61.78, 61.67, 61.52, 61.07, 36.00, 35.63, 31.76, 31.59, 31.20, 29.52, 29.47, 29.38,

29.25, 29.18, 28.98, 28.59, 25.65, 23.76, 22.43, 21.90, 13.23. HRMS: Calcd. for $C_{35}H_{64}N_2O_{14}Pt$ $(M+H)^+$: 932.4078, found: 932.4058.

General Procedure for the Synthesis of 8.

Preparation of 25b.

Compound **25b** was obtained as a slight yellow solid and was prepared according to the procedure described for compound **21b**.

Preparation of 34b and 35b.

To **25b** (232 mg, 0.36 mmol) under an N_2 atmosphere, $C_2O_2Cl_2$ (4 mL) added with catalytic DMF at 0^0C. White smoke was observed and the reaction was stirred for another 30 min at 0^0C. The resulting solution was stirred at room temperature for 2 h. The $C_2O_2Cl_2$ was removed to obtain **34b** as slightly yellow solid. The Pt(IV) compounds combined with **4a** and a hexadecanoic acid an axial ligands (200 mg, 0.30 mmol) along with acetone (20 mL) was added under an N_2 atmosphere. After 2 days, the liquid was evaporated and purified by column chromatography to give the desired compound **35b** as a yellow solid (yield: 180 mg, 46%).

Preparation of 8

To a solution of **35b** (120 mg, 0.09 mmol) in anhydrous DCM, BCl_3 (1 M) (0.80 mL, 0.80 mmol) was added at –78 ℃ for 2 h. The DCM was removed and purified by column chromatography to get the white solid **8** (29 mg, 33%). Data for compound **8**. ^{1}H NMR (400 MHz, MeOD) δ 4.74 (s, 1H), 3.99 – 3.21 (m, 5H), 2.78 (s, 2H), 2.31 (d, J = 29.4 Hz, 6H), 1.60 (d, J = 38.5 Hz, 10H), 1.28 (s, 26H), 0.90 (s, 3H). ^{13}C NMR (100 MHz, MeOD) δ 183.19, 183.06, 165.27, 100.12, 73.15, 71.23, 70.85, 67.29, 66.96, 61.74, 61.55, 61.37, 35.97, 35.93, 31.93, 31.72, 31.21, 29.47, 29.43, 29.32, 29.19, 29.14, 28.93, 28.85, 25.62, 25.47, 25.37, 25.32,

25.06, 23.72, 22.39, 13.15. HRMS: Calcd. for $C_{36}H_{66}N_2O_{14}Pt$ $(M+H)^+$: 946.4235, found: 946.4197.

DNA and HSA binding properties of oxaliplatin and 6 using RP-HPLC.

To investigate the binding properties of DNA with Pt(II) complexes, 5'-GMP was used as model of DNA. The DNA binding properties of oxaliplatin (1 mM) and 5'-GMP (1 mM) at 37 °C after 2 h, 24 h, 48 h, 96 h and 144 h were determined using RP-HPLC. (Fig. S6A) The results revealed new peaks of Oxp-Pt(II)-GMP (the conjugated complexes of oxaliplatin with 5'-GMP) generated by the mixture, and demonstrated the potency of 5'-GMP to combine with Pt(II) complexes.

Further experiments were designed to test the reduction potential of the Pt(IV) complexes. The reduction and DNA binding properties of **6** (1 mM) and 5'-GMP (3 mM) with and without ascorbic acid (5 mM) at 37 °C after 0 h, 24 h, 48 h, 96 h and 144 h were measured using RP-HPLC. (**Fig. S6B** and **S6C**) The results proved that the glycosylated platinum(IV) complexes could be reduced by ascorbic acid and release Pt(II) complexes. Then the Pt(II) compounds combined with 5'-GMP to form OxpPt(II)-GMP, which was confirmed by HRMS.

The reduction of compound **6** and its DNA binding properties by ascorbic acid with and without HSA were also studied at 37 °C after 24 h, 48 h, 96 h and 144 h as shown in **Fig. 5b**. The results showed that the rate of reduction of **6** by ascorbic acid can be reduced as the result of the interaction with HSA during drug delivery, which can further enhance its stability in human blood and its accumulation in cancer cells. Binding studies with compound **6** (Fig. 5A) and oxaliplatin (Fig. S7) with HSA at 37 °C after 0 h, 24 h, 48 h and 196 h indicated that compound **6** with a chain of hexadecanoic acid showed a preferable interaction with HSA. Oxaliplatin did not bind HSA. HPLC analyses were performed as on a Waters E2695-2998 equipped with a Venusil MP C18 column (150×4.6 mm, 5 μm).

ASSOCIATED CONTENT

Supporting Information

Full experimental details, NMR data and bioassay information. This material is available free of charge *via* the Internet at http://pubs.acs.org.

Molecular formula strings (CSV).

PDB ID Codes.

Docked model of **6** interacting with a variety of amino acid residues of GLUT1 (pdb code 4PYP) and HSA (pdb code 1E7H) was shown in Figure **1** (PDB). Authors will release the atomic coordinates and experimental data upon article publication.

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Notes

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ABBREVIATIONS

5'-GMP, 5'-Guanosine monophosphate; GLUTs, Glucose Transporters; OCTs, Organic Cation Transporters; HSA, Human Serum Albumin; SGLTs, sodium dependent glucose transporter; EDG, 4,6-Oethylidene-a-d-glucose; Ctd., Cimetidine; GLUT 1, Glucose Transporter 1; OCT2, Organic Cation Transporter 2; DACH, (1R, 2R)-cyclohexane-1,2-diamine; MDCK, Madine-darby canine kidney; DCM, dichloromethane; RPMI 1640, Roswell Park Memorial Institute 1640.

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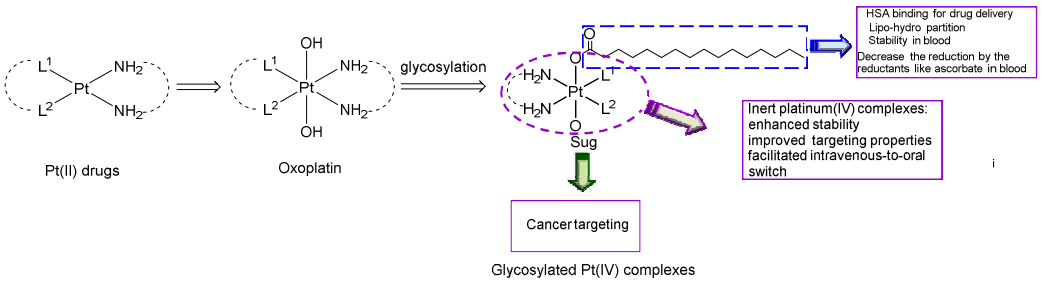
glycosylation
Pt(II) drugs
Oxoplatin
HSA binding for drug delivery
Lipo-hydro partition
Stability in blood
Decrease the reduction by the reductants like ascorbate in blood
Inert platinum(IV) complexes:
enhanced stability
improved targeting properties
facilitated intravenous-to-oral switch
Cancer targeting
Glycosylated Pt(IV) complexes