Supplementary Information

Multifunctional Pt(IV) pro-drug and its micellar platform: to kill two birds with one stone

Haiqin Song[a※], Haihua Xiao[b,c,※],Yu Zhang[d], Haidong Cai[e], Rui Wang[b,c], Yonghui Zheng[a], Yubin Huang[a], Yuxin Li[e], Zhigang Xie[b], Tongjun Liu[a*] Xiabin Jing[b*]

[a] Department of Colorectal and Anal Surgery, China-Japan Union Hospital of Jilin University, Changchun 130033, China

[b] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

[c] Graduate School of Chinese Academy of Sciences, Beijing 100049, China

[d] National Engineering Laboratory for Druggable Gene and Protein Screening, Northeast Normal University, Changchun 130024, People's Republic of China

[e] Department of Chemistry and Environmental Engineering, Changchun University of Science and Technology, Changchun 130022, China

[※]The two first authors of equal contribution

Experimental part

Materials and methods

Materials

Methoxyl-poly(ethylene glycol)-block-poly-(ε-caprolactone)-block-poly(L-lysine) (MPEG-b-PCL-b-PLL) was synthesized as previously described [1]. And its structure could be written as $MPEG_{114}$-b-PCL_{20}-b-PLL_{10}, where the subscript numbers denote degrees of polymerization of the blocks. N-Hydroxysuccinimide (NHS), 1-ethyl-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), 2′-Deoxy-guanosine 5′-monophosphate(5′-GMP), dichloroacetic

* Correspondence to: Xiabin Jing, State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, People's Republic of China. Tel & Fax: +86-431-85262775; E-mail: xbjing@ciac.jl.cn
* Correspondence to: Tongjun Liu, Department of Colorectal and Anal Surgery, China-Japan Union Hospital of Jilin University, Changchun 130033, China. E-mail: tjliu@jlu.edu.cn

acid (DCA) and dichloroacetic anhydride were purchased from Sigma-Aldrich. Cisplatin and carboplatin were purchased from Shangdong Boyuan Chemical Co. Ltd., China. Other chemicals and solvents were obtained commercially and used without further purification.

General measurements

Inductively coupled plasma optical emission spectrometer (ICP-OES, iCAP 6300, Thermo scientific, USA) was used to determine the total platinum contents in the polymer-Pt(IV) conjugate and samples obtained outside of the dialysis bags in drug release experiments. Inductively coupled plasma mass spectrometer (ICP-MS, Xseries II, Thermo scientific, USA) was used for quantitative determination of trace levels of platinum. The morphology of the polymer micelles was measured on a JEOL JEM-1011 electron microscope. Particle size and zeta potential measurements were conducted on a Malvern Zetasizer Nano ZS90. Fourier Transform Infrared (FT-IR) spectra were recorded on a Bruker Vertex 70 spectrometer. Mass Spectroscopy (ESI-MS) measurements were performed on a Quattro Premier XE system (Waters) equipped with an electrospray interface (ESI). Matrix-assisted laser-desorption ionization and time-of-flight mass spectroscopy(MALDI-TOF-MS, Waters, USA) was used to study the chelation products of P1 with 5'-GMP in the presence of sodium ascorbate.

Synthesis of DCA-Pt(II)

As shown in Scheme 1(A), cisplatin (1g) was suspended in 100ml pure water in a flask, to which 100uLconcentrated HNO_3 and 1.132g of $AgNO_3$ added. The reaction mixture was kept in dark and at room temperature for 24 hours, after which it was filtered to remove AgCl produced to obtain a clear solution. To this solution, 1.2g of sodium dichloroacetate was added to form precipitates of DCA-Pt(II) which can be obtained by filtration and dried under vacuum .

Synthesis of DCA-Pt(IV)-OH

As shown in Scheme 1(A), 0.5 g of DCA-Pt(II) was added into a mix solution of 20ml pure water and 10ml 30% H_2O_2 in a flask. The reaction mixture was kept under stirring for 24h at

room temperature until the solution becomes clear. After which water was removed by vacuum evaporation to gain a solid. Diethyl ether was added to the flask and the solid was grinded into white powders, which was collected by filtration. The obtained powder (DCA-Pt(IV)-OH) which was the oxidized form of DCA-Pt(II) with two hydroxyl group in axial positions was further dried under vacuum.

Synthesis of DCA-Pt(IV)-COOH

As shown in Scheme 1(A), 0.3g of DCA-Pt(IV)-OH was dissolved in dried 20ml DMSO in a flask, to which 1N succinic anhydride was added. The reaction mixture was kept at room temperature and under dark for 24h to form a clear yellow solution. Then the solution was poured in to a large excess of diethyl ether and cooled down to remove the DMSO. This was repeated for several times. Then the solid residues were dissolved in 5ml methanol and precipitated with diethyl ether to get a pale yellow powder. ESI-MS (negative mode): formula: $[C_8H_{13}Cl_4N_2O_9Pt]^-$, Calc. 619.09, Found 619.

Synthesis of carbo-Pt(IV)-OH

As shown Scheme 1(B), carboplatin (1 g) was suspended in 20 ml water in a flask at room temperature. Then, 5 ml 30% H_2O_2 was added to the flask and the mixture was stirred under dark for 24 h. After evaporation of the water, the reaction product was dissolved in methanol, and precipitated into ethyl ether. The final product carbo-Pt(IV)-OH was collected by filtration and dried under reduced pressure (0.9 g, yield 90%).

Synthesis of carbo-Pt(IV)-COOH

As shown Scheme 1(B), to a solution of carbo-Pt(IV)-OH (0.405 g, 1 mmol) in DMSO (10 ml) was added succinic anhydride (0.1 g, 1 mmol) and the reaction mixture was stirred at room temperature for 12 h. The solution was lyophilized to obtain the powder product carbo-Pt(IV)-COOH. It was purified by dissolution in 10 ml methanol, precipitation in ethyl ether, filtration, washing with diethyl ether, and vacuum-drying. Yield 85%. ESI-MS (negative mode): formula: $[C_{10}H_{17}N_2O_9Pt]^-$, Calc. 505.34, Found 505.3.

^{1}H NMR Spectra of DCA-Pt(II), DCA-Pt(IV)-OH and DCA-Pt(IV)-COOH

The ^{1}H NMR of DCA-Pt(II), DCA-Pt(IV)-OH and DCA-Pt(IV)-COOH in DMSO-d_6 are shown in Fig. S1. DCA-Pt(II) has a characteristic broad peak at 4.75ppm(Fig. S1, a), which can be attributed to proton of the $-NH_3$ ligand. It can be further inferred that the Pt atom in the DCA-Pt(II) is in +2 valence through the chemical shift of the $-NH_3$ ligand at 4.75ppm as extensively reported previously[2]. Moreover, two protons of DCA molecules (Cl_2-C**H**-COO-) showed two characteristic peaks at 5.8 and 6.5 ppm respectively. The difference in chemical shift of the two protons in the two DCA molecules may be attributed to difference in the spatial arrangement of the two molecules. After DCA-Pt(II) is reacted with H_2O_2 to its oxidized form DCA-Pt(IV)-OH, the Pt atom is in +4 valence, and two extra –OHs were introduced. The chemical shift of $-NH_3$ protons moved to 6.0ppm (broad), indicating that the Pt atom is in +4 valences according to change of ^{1}HNMR spectra after oxidation of similar compounds reported [3].However, the chemical shift of two protons in DCA didn't show great change (Fig. S1,b). Thereafter, DCA-Pt(IV)-OH was used to react with succinic anhydride to introduce a succinic linker to its axial position. In this way, DCA-Pt(IV)-COOH was prepared, in which the typical chemical shift of the protons in -OOC–C**H$_2$**-C**H$_2$**-COOH at 2.4ppm appeared as indicated in the spectrum.(Fig. S1,c).

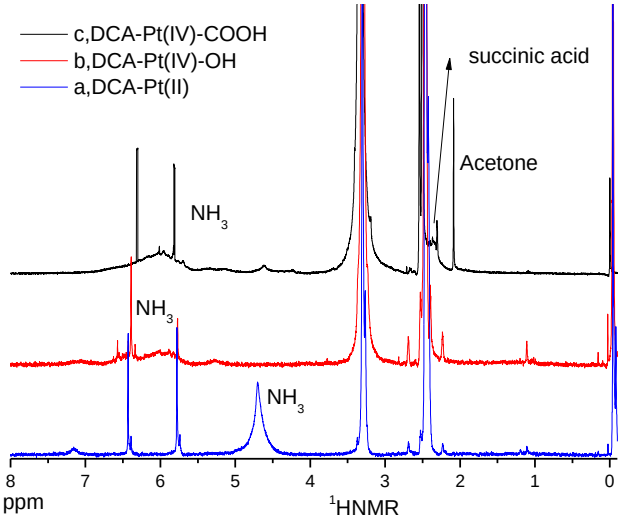


Fig. S1 ^{1}H NMR of DCA-Pt(II)(a), DCA-Pt(IV)-OH (b) and DCA-Pt(IV)-COOH (c) in DMSO-d_6.

IR spectra of carboplatin, carbo-Pt(IV)-OH and carbo-Pt(IV)-COOH

In Fig. S2 are collected FT-IR spectra of carboplatin, carbo-Pt(IV)-OH and carbo-Pt(IV)-COOH. Carbo-Pt(IV)-OH, which is obtained by oxidizing carboplatin with H_2O_2, displays a broad and intense peak at 3465 cm^{-1} (OH stretching) and a new Pt–OH stretch at 545 cm^{-1}, respectively, compared with carboplatin. After reacting with succinic anhydride, the broad and intense H-bonded OH absorption over 3600–2000 cm^{-1} remains because of the existence of the free COOH group derived from the succinic anhydride in place of the OH groups chelated with the Pt atom. Moreover, a new peak at 1724 cm^{-1} which can be attributed to the stretching of free C=O in succinic acid [4].Therefore successful synthesis of carbo-Pt(IV)-COOH can be confirmed.

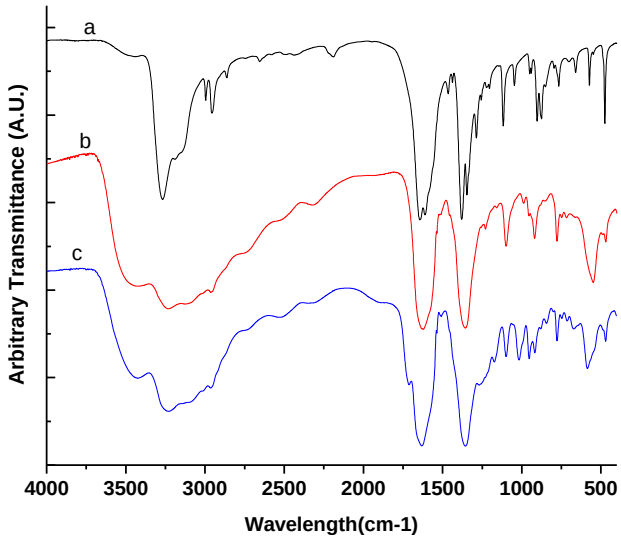


Fig. S2. IR spectrum of carboplatin(a), carbo-Pt(IV)-OH (b)，carbo-Pt(IV) –COOH (c)

^{1}H NMR Spectra of carboplatin, carbo-Pt(IV)-OH and carbo-Pt(IV)-COOH

The ^{1}H NMR of carboplatin, carbo-Pt(IV)-OH and carbo-Pt(IV)-COOH in DMSO-d_6 are shown in Fig. S3. According to the previous published results [2], carboplatin has one characteristic peak of NH_3 ligand at 4.07 ppm (br, 6H). Moreover, typical characteristic proton peaks of 4-membered ring of cyclobutane 2.8 ppm($C\mathbf{H}_2$-CH_2-$C\mathbf{H}_2$-) and 1.6ppm(CH_2-$C\mathbf{H}_2$-CH_2-). Then carboplatin was oxidized with H_2O_2 to form carbo-Pt(IV)-OH. The proton peak of NH_3 ligand moves to 5.8 ppm (br, 6H) and a slight shif of protons in $C\mathbf{H}_2$-CH_2-$C\mathbf{H}_2$- to 2.6ppm and CH_2-$C\mathbf{H}_2$-CH_2- to 1.7ppm. After carbo-Pt(IV)-OH is reacted with succinic anhydride to form carbo-Pt(IV)-COOH, the chemical shift peaks of NH_3 ligand

further moved to 6.0ppm (br, 6H), whereas the peaks of -C**H**$_2$-CH$_2$-C**H**$_2$- and -CH$_2$-C**H**$_2$-CH$_2$- kept unchanged. However, a new chemical shift of CH$_2$ of succinate appears at 2.45 ppm, supporting successful reaction with succinic anhydride.

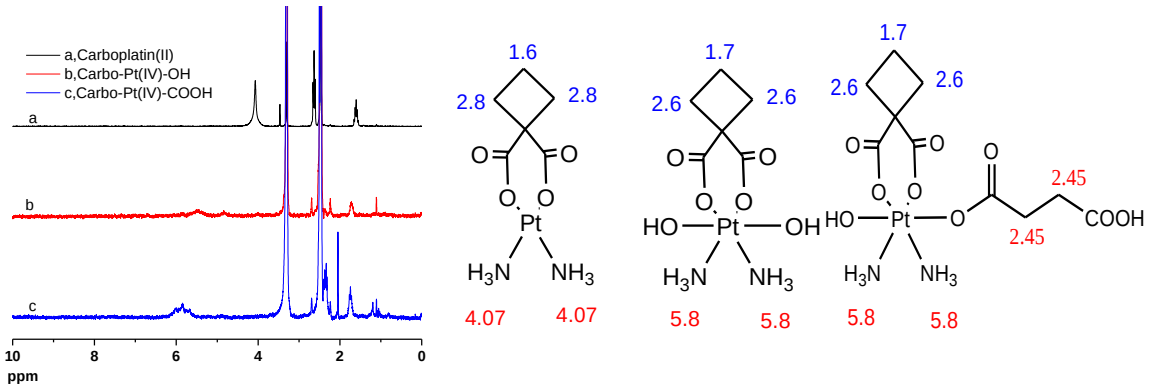


Fig. S3. ^{1}H NMR of carboplatin(a), carbo-Pt(IV)-OH (b), carbo-Pt(IV)-COOH (c)

Preparation of P1 and P2

DCA-Pt(IV)-COOH and carbo-Pt(IV)-COOH were conjugated to the carrier polymer MPEG-b-PCL-b-PLL using the EDC/NHS method in aqueous solution to prepare **P1** and **P2**, respectively. Take the synthesis of **P1** as an example, briefly, EDC·HCl (0.191 g, 1 mmol) and NHS (0.115 g, 1 mmol) were dissolved in de-ionized water under stirring. DCA-Pt(IV)-COOH (0.49 g, 0.79 mmol) was added into the aqueous solution. After the mixture (suspension) became clear, 0.5 g of MPEG-b-PCL-b-PLL in 100 ml water was added and the reaction mixture was kept stirring at room temperature for 24 hours, then it was dialyzed against water for 12 hours and lyophilized to obtain **P1** conjugates. **P2** was prepared in the similar way.

Preparation of M(P1) and M(P2)

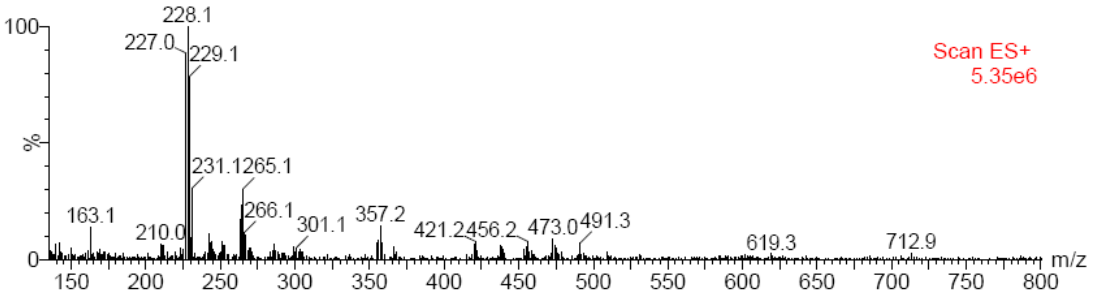
The micelles of **P1** and **P2** were defined as **M(P1)** and **M(P2)**, respectively. They were prepared by nano-precipitation method. Take the preparation of **M(P1)** as an example, In brief, 50 mg **P1** conjugate was dissolved in a flask with 5 ml DMF, and then 50 ml water was added dropwise into the flask under stirring to form a micellar solution. The solution was dialyzed against water to remove DMF and then freeze-dried. **M(P2)** was prepared in the similar way.

Drug Release from P1 and P2 conjugates

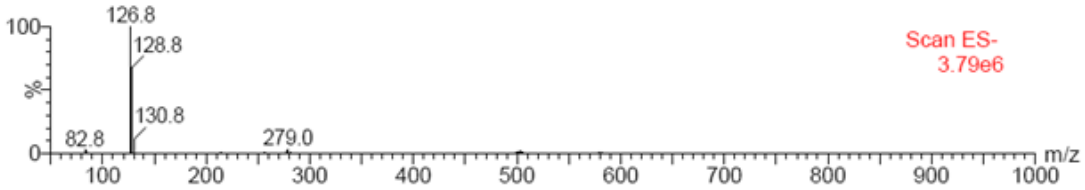
10 mg of **P1** (Pt content 10.2%) was dissolved in 5 ml of phosphate buffered saline (PBS, 0.1 M, pH=7.4). The solution was then placed into a pre-swelled dialysis bag (molecular weight cutoff of 3.5 kDa) and immersed into 140 ml of 0.1 mol/L PBS (pH=7.4). The dialysis was conducted at 37 °C in a shaking culture incubator. 1.5 milliliters of sample was withdrawn from the incubation medium at specified time intervals and measured for Pt concentration by ICP-OES. After sampling, equal volume of fresh PBS was immediately added into the incubation medium. The concentration of platinum released from the micelles was expressed as a percentage of the total platinum in the micelles. The same drug release procedure was performed in the presence of 5 mM sodium ascorbate. **P2** conjugates were tested in the same way as **P1.**

Hydrolysis of DCA-Pt(II) studied by ESI-MS

5 mg DCA-Pt(II) was incubated in 20 ml pure water at 37 °C for 12 h, then an aliquot of the solution was collected for ESI-MS study. Fig. S4 is the positive mode (A) and negative mode (B) spectra.



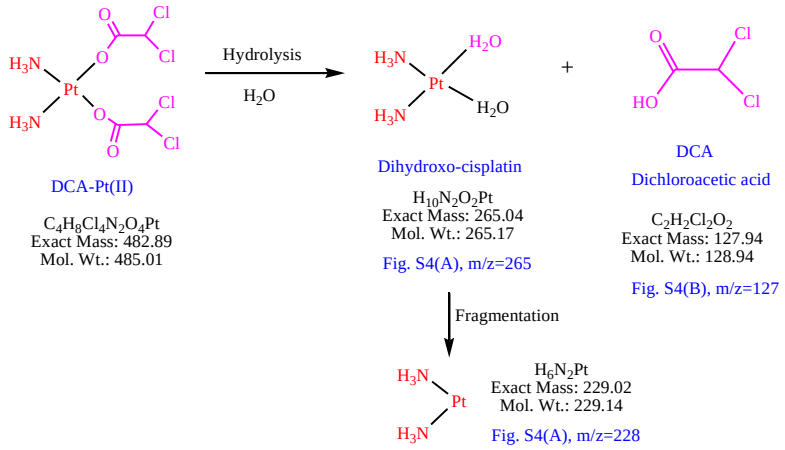
(A)



(B)

Fig. S4 ESI-MS spectra of hydrolyzed DCA-Pt(II) in positive mode (A) and in negative mode (B)

The main peaks in Fig. S4 can be assigned to the hydrolytic products:



Reduction of DCA-Pt(IV)-COOH studied by ESI-MS

DCA-Pt(IV)-COOH (1 mM, 5 mL) was incubated with (5 mM, 5 ml) sodium ascorbate solution for 12 h at room temperature. Thereafter, the mixed solution was collected for ESI-MS study.

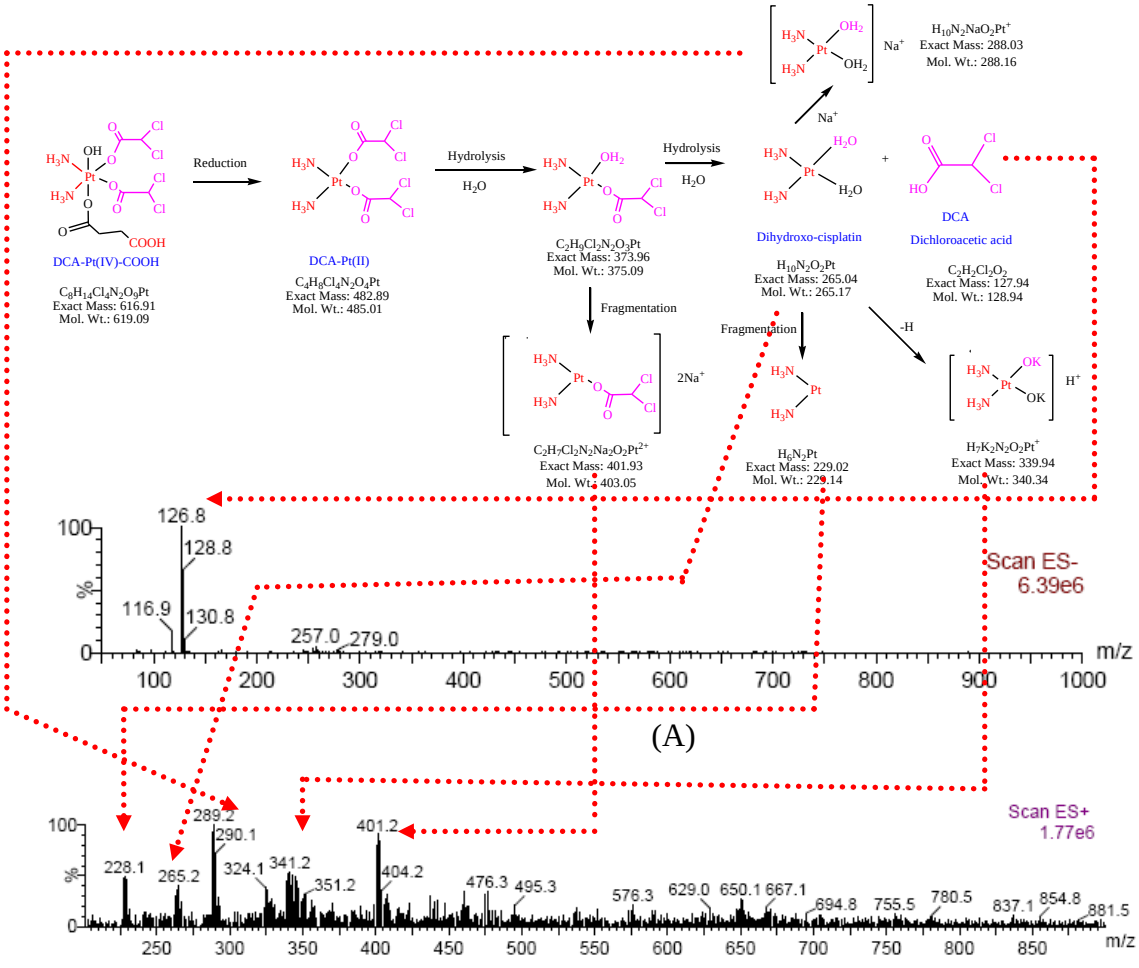
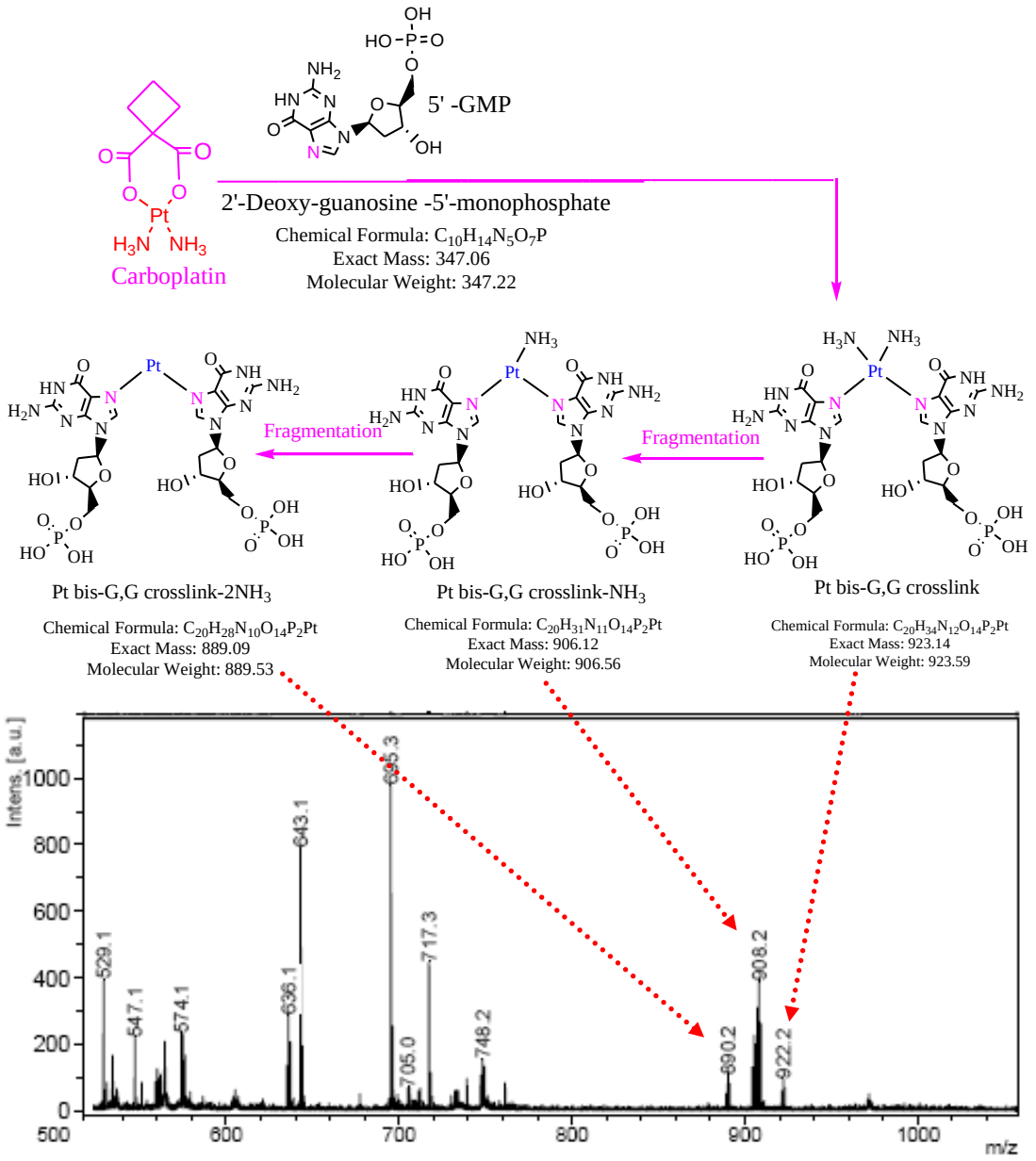


Fig. S5 ESI-MS spectrum of the reduction of DCA-Pt(IV)-COOH **i**n the presence of sodium ascorbate. (A) negative mode and (B) positive mode.

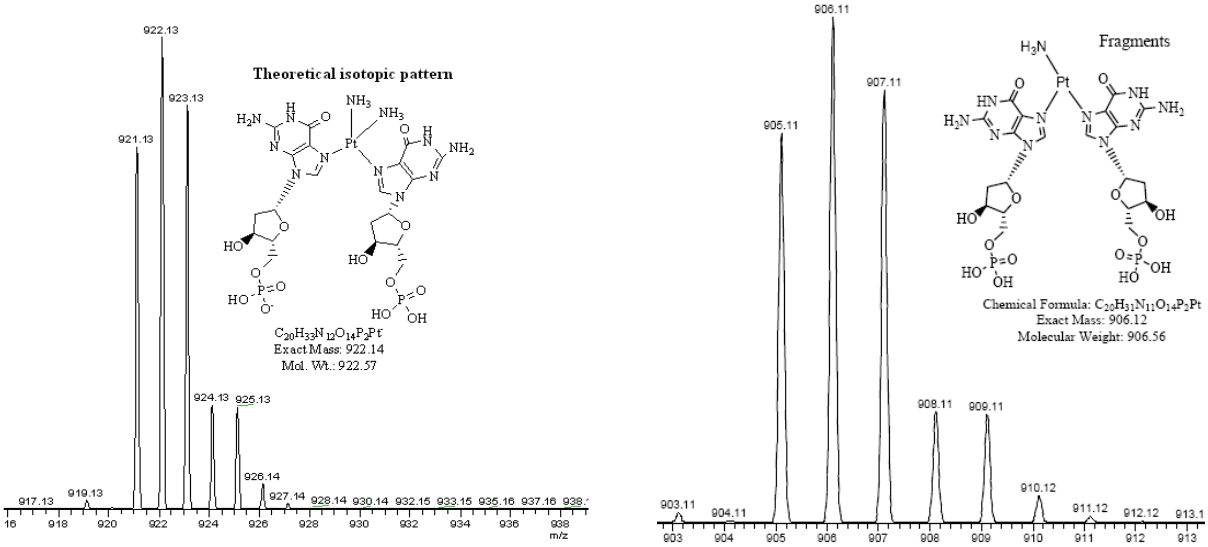
Possible released Pt species and its chelation with 5'-GMP studied by MALDI-TOF-MS

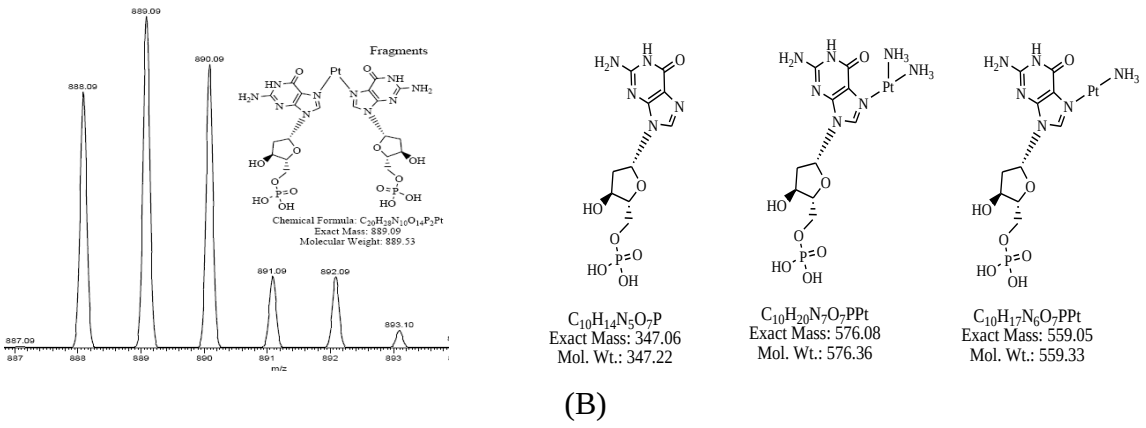
50 mg **P1** conjugate was dissolved in 20 ml of freshly prepared 5 mM sodium ascorbate aqueous solution by simply dissolving sodium ascorbate in de-ionized water. Then the solution (5 ml) was totally transferred to a dialysis bag (molecular cut-off, 1000), which was sealed firmly. The sealed dialysis bag was washed several times to remove any residual **P1** conjugates on the surface of dialysis bag and then the dialysis-bag-sealed **P1** was incubated in another 10 ml sodium ascorbate solution at 37 °C for 12 h. The dialysate was collected for MALDI-TOF-MS study.

As for the chelation of carboplatin and carbo-Pt(IV)-COOH with 5'-GMP, general procedures were below: 1) a solution of carboplatin plus 5'-GMP at final concentration of 1 mM and 5 mM, respectively, was prepared and incubated at 37 °C for 12 h. The solution was then collected directly and diluted to suitable concentration for MALDI-TOF-MS study; 2) a solution of carbo-Pt(IV)-COOH, 5'-GMP, and sodium ascorbate at final concentration of 1 mM, 5 mM and 5 mM, respectively, was prepared and incubated at 37 °C for 12 h. The solution was then collected directly and diluted to desired volume for MALDI-TOF-MS study.


5' -GMP
2'-Deoxy-guanosine -5'-monophosphate
Chemical Formula: C10H14N5O7P
Exact Mass: 347.06
Molecular Weight: 347.22
Carboplatin
Fragmentation
Fragmentation
Pt bis-G,G crosslink-2NH3
Chemical Formula: C20H28N10O14P2Pt
Exact Mass: 889.09
Molecular Weight: 889.53
Pt bis-G,G crosslink-NH3
Chemical Formula: C20H31N11O14P2Pt
Exact Mass: 906.12
Molecular Weight: 906.56
Pt bis-G,G crosslink
Chemical Formula: C20H34N12O14P2Pt
Exact Mass: 923.14
Molecular Weight: 923.59
Intens. [a.u.]
m/z

(A)


Theoretical isotopic pattern
C20H33N12O14P2Pt
Exact Mass: 922.14
Mol. Wt.: 922.57
Fragments
Chemical Formula: C20H31N11O14P2Pt
Exact Mass: 906.12
Molecular Weight: 906.56



(B)

Fig. S6 MALDI-TOF-MS spectrum of the reaction product between carboplatin and 5′-GMP (A) and possible Pt species and theoretical simulations (B).

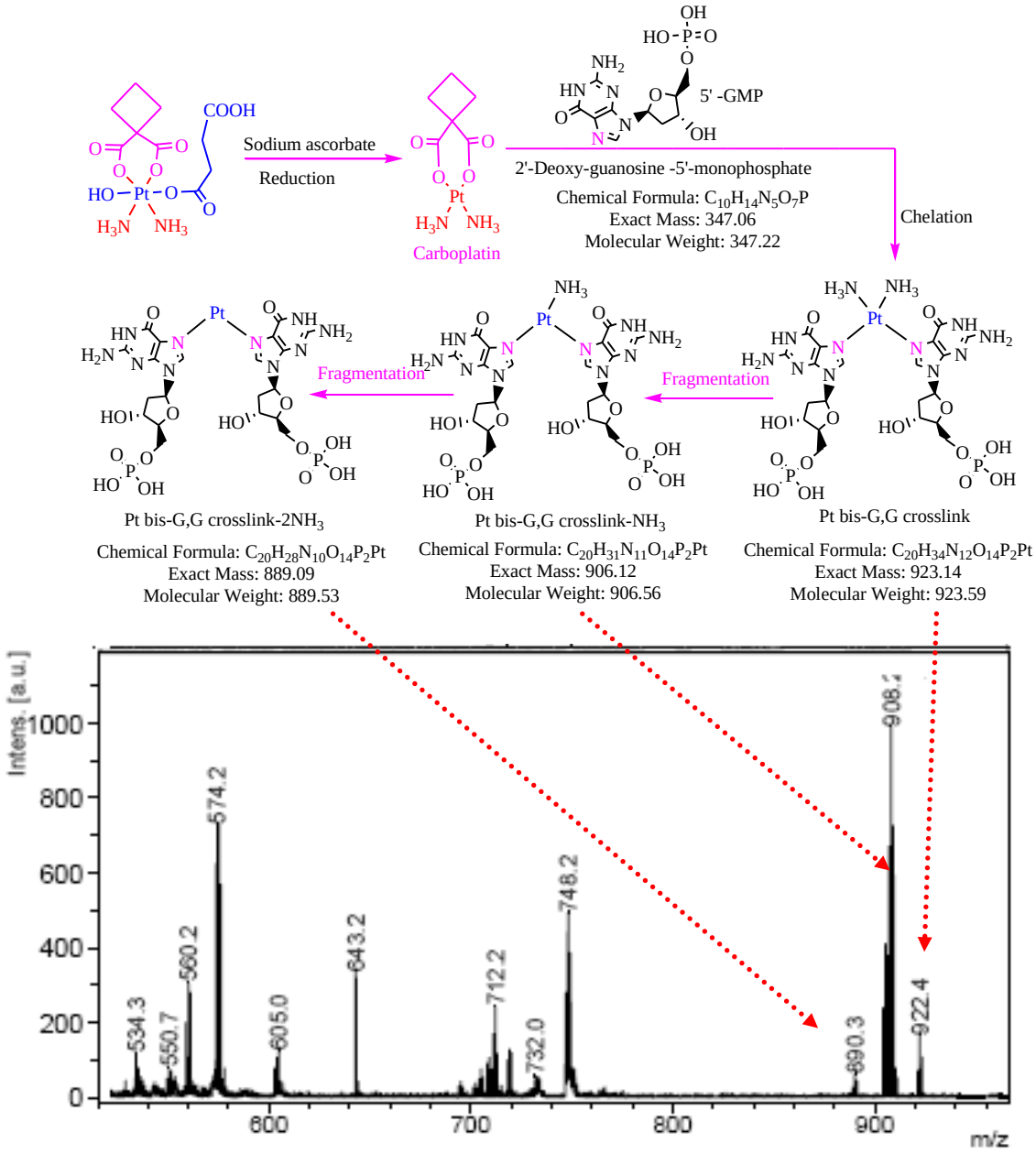


Fig. S7 MALDI-TOF-MS spectrum of the reaction product between carbo-Pt(IV)-COOH and 5’-GMP in the presence of sodium ascorbate

MTT (3-(4,5-dmethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Assay

SKOV-3 cells were purchased from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China, and grown in RPMI 1640 (Life Technologies) with 10% fetal bovine serum and supplemented with 0.03% L-glutamine and 1% penicillin/streptomycin in 5% CO_2 at 37 °C. SKOV-3 cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 10^5 cells/well and incubated in RPMI 1640 for 24 h. The medium was then replaced by DCA-Pt(II), DCA-Pt(IV)-COOH, M(P1), carboplatin, carbo-Pt(IV)-COOH and M(P2). Mixture of carrier polymer and DCA-Pt(IV)-COOH as well as carboplatin + 2DCA (molar ratio of carboplatin to DCA is equal to 1:2) and DCA was used as controls. All the drug formulations containing Pt were set at a final equivalent Pt concentration from 0.21 to 216 μM. The concentration gradient of DCA was set twice as much as Pt (range from 0.42 to 432 μM). As the free polymer MPEG-b-PCL-b-PLL was proved safe in our previous published works, there is no need to test its biocompatibility. The incubation time for all the drugs was 48 h. After incubation, then 20 μL of MTT solution in PBS with the concentration of 5 mg/mL was added and the plates were incubated for another 4 h at 37 °C, followed by removal of the culture medium containing MTT and addition of 150 μL of DMSO to each well to dissolve the formazan crystals formed. Finally, the plates were shaken for 10 minutes, and the absorbance of formazan product was measured at 492 nm by a micro plate reader.

Cellular Uptake

Confocal laser scanning microscopy (CLSM)

SKOV-3 cells were grown in RPMI 1640 with 10% fetal bovine serum and maintained at 37 °C in 5% CO_2. Cells were seeded in 6-well plates and grown for 24 h prior to incubation with 0.1 mg/mL RhB labeled composite micelles M(P1/RhB) and M(P2/RhB), respectively. Cells were imaged at 4 h post-treatment using a confocal laser scanning microscopy (CLSM, Olympus, FV-1000). 4',6-diamidino-2-phenylindole (DAPI, Sigma) was used to stain the nuclei.

Quantitative determination of platinum contents in the cancer cells. SKOV-3 cells were seeded in 6-well plates at a density of 10^6 cells per well. At their logarithmic phase of growth, the cells were treated with DCA-Pt(II), carboplatin, DCA-Pt(IV)-COOH,

carbo-Pt(IV)-COOH, M(P1) and M(P2) with the final platinum concentration in the culture medium regulated to 5 μM and incubated at 37 °C for 2 h or 4 h. As previously described [1,5,6], to remove surface-bound drugs, cells were washed three times with ice-cold PBS, incubated with 1.5 mL of 0.15 M sodium chloride (pH 3.0 was adjusted by acetic acid) for 3 min at 4 °C, then rinsed with 2 mL of cold PBS, harvested by scraping in ice-cold PBS, centrifuged. Thereafter, the cell pellet were lysed by adding 200 μL cell lysis buffer (Promega lysis buffer) and then the cell lysis solution was freezed at −20°C for 20 min and thawed at room temperature. 100 μL of the cell lysis solution for each sample was used directly to measure the Pt content by ICP-MS. The other 100 μL of the cell lysis solution was used to determine the protein content in each cell sample by using bicinchoninic acid (BCA) protein assay kit (Beyotime Institute of Biotechnology, Shanghai, China) according to previously published data[1,5,6,7]. The platinum content was expressed as nano-grams of Pt per milligram of total proteins.

Mitochondrial membrane potential (MMP) analysis

JC-1(5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide) (Beyotime Biotech, China) is cationic dye that exhibits potential-dependent accumulation in mitochondria by fluorescence emission shift from green (~520 nm) to red (~590 nm). Green fluorescence of JC-1 monomers is presented in the cell areas with high mitochondrial membrane potential, while red fluorescence of JC-1 aggregate is prevalent in the cell areas with low mitochondrial membrane potential. Consequently, mitochondrial depolarization is indicated by a decrease in the red to green fluorescence intensity ratio. SKOV-3 cells were seeded into black 96-well microplates. After 24 h, drugs (cisplatin, DCA, DCA-Pt(II) and DCA-Pt(IV)-COOH at 10uM of each drug) were added to the wells, and cells without treatment of drugs were used as controls. The cells were incubated with drugs (cisplatin, DCA, DCA-Pt(II) and DCA-Pt(IV)-COOH at 10uM of each drug) for 1 h, respectively. At the end of drug treatment, the medium was removed and the cells were incubated at 37℃ for 1 h with 5mg/L of JC-1, then washed twice with PBS and placed in fresh medium without serum. Then, images were viewed by an inverted fluorescence microscope (Olympus IX70) at an excitation wavelength of 490nm and an emission wavelength of 530nm for green, and at an excitation wavelength of 540nm and an emission wavelength of 590nm for red. The fluorescence of both green

fluorescence (JC-1 monomers) and red fluorescence (JC-1 aggregates) were measured with a FLUOstar optima plate reader (BMG LABTECH, Germany) with the filters set to 485 nm excitation/520 nm emission (green) and 544 nm excitation/590 nm emission (red), and data were presented as the ratio of green to red signals (520 nm/590 nm) with the ratio of green to red signals of non-treat cells as normalized unity of 1.

In vivo experiments

Animal use

Kunming mice (6 weeks old) were purchased from Jilin University (Changchun, China). All the mice used in this paper were maintained under required conditions (e.g. pathogen-free condition for nude mice) and they had free access to food and water throughout the experiments. Use of them for this study was approved by the Animal Ethics Committee of Changchun Institute of Applied Chemistry, Chinese academy of sciences

Tumor inoculation

H22 cells (murine hepato carcinoma cells) were purchased from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China, and cultured in RPMI 1640 (Life Technologies, Gaithersburg, MD) containing 10% fetal bovine serum (Life Technologies), 0.03% L-glutamine and 1% penicillin/streptomycin in 5% CO_2 at 37 °C.

Chinese KM mice (6–8 week old, female, 18–25 g) were purchased from Jilin University (Changchun, China). The mouse H22 xenograft tumor model was developed by injecting 1×10^6 cells in a 0.1 mL H22 cell suspension into the right flank of the animal. The tumor nodules were allowed to grow to desired volume before following treatment.

Biodistribution

To evaluate the biodistribution of DCA-Pt(II), M(P1), carboplatin and M(P2) at 5 mg Pt/kg in tumor-bearing mice, Kunming mice (6 weeks old) were assigned to four groups with 3 mice in each group. When the average tumor volume was ca. 250 mm^3 each drug was i.v. injected into the mice. 1 h and 12 h later, mice were sacrificed. Blood, organs (heart, liver, spleen, lung, kidneys, muscle), and tumor tissue were harvested, the organs were dissolved in 65% (v/v) nitric acid, and Pt concentrations were measured by inductively coupled plasma mass spectroscopy (ICP-MS).

Pt-DNA adducts analysis

To evaluate the Pt-DNA adducts concentration in the tumor site, mice with an average tumor volume of ca. 250 mm^3 were injected twice at day 1 and day 4 with the drug formulations and dosages listed in Table S1. After 24h post the second injection at day 4, the mice were sacrificed and the tumor of each mouse was collected. Genomic DNA was separated and purified from the collected solid tumor samples using DNAZOL (Life Technologies, Inc., Grand Island, NY). The tumor used for measurement of Pt content were thawed, homogenized with 1 mL DNAZOL for each 50 mg of tissue, and then incubated overnight at room temperature. The next day, a 0.75 mL aliquot was centrifuged at 13,000 rpm for 5 minutes. The supernatant was removed and 0.75 mL isopropyl alcohol was added to precipitate the DNA. This was then centrifuged at 13,000 rpm for 10 minutes to sediment the DNA into a pellet, and the residual supernatant was discarded. Additional DNAZOL (0.75 mL) was added to each DNA pellet, mixed, and centrifuged at 13,000 rpm for 10 minutes. These tumor samples contained a considerable quantity of melanin. During the isolation of DNA from these samples, it was necessary to re-extract the precipitated DNA samples with DNAXOL multiple times before all of the melanin was removed, as judged by visual inspection. The final DNA pellet was air dried and then dissolved in 0.1 mL distilled water overnight. The next day, the DNA concentration and purity was determined by measuring absorbance at 260/280 nm with a nanodrop UV spectrometer (NanoDrop Technologies, Inc., Wilmington, DE). An aliquot of DNA (60 μL) was digested with 70% nitric acid (64 μL) in a 65℃ water bath overnight. This was diluted with water (776 μL) containing indium and Triton X-100 to achieve a final concentration of 5% acid (final concentration of 1 ppb for indium and 0.05% Triton X-100). The Pt concentration was then determined by inductively coupled plasma mass spectroscopy (ICP-MS).

Table S1 Drug formulations and dosage for analysis of Pt-DNA adducts. Each mouse was injected the drug listed in the table twice at day 1 and day 4(with the first injection day as day 1). Right after 24h post second injection, the mice were sacrificed and tumors were harvested for Pt-DNA adducts measurement.

Code	Drug formulation	Dose (mg Pt/kg or mg DCA/kg)
a	Carboplatin	3.25
b	Carboplatin+2DCA	3.25+30
c	DCA-Pt(II)	3.25
d	DCA-Pt(IV)-COOH	3.25
e	carbo-Pt(IV)-COOH	3.25
f	M(P1)	3.25
g	M(P1)	6.5
h	M(P1)	13.0
i	M(P2)	3.25
j	M(P2)	6.5
k	M(P2)	13.0

Evaluation on systemic toxicity of various drugs

Healthy Kunming mice (6 weeks old) without tumors were randomly assigned to 11 groups. The drug formulations were listed as shown in Table S2. The drug solutions were administered once via the tail vein. The blood sample of each mouse was collected from the retro-orbital plexus into a coagulation-promoting tube (Shandong Nanyou Medical appliance, China) 7 days post administration of the drugs, and then the blood samples were centrifuged at 3000 rpm for 5 min to obtain plasma samples for measuring clinic parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatine kinase (CK) by an automatic biochemical analyzer (Beckman CX-5 Pro, USA)

Table S2. Evaluation on the systemic toxicity of various drugs after one i.v. injection

Group	Drug	Dose (mg Pt/kg)	No.	Death
a	DCA-Pt(II)	3.25	5	3(in one week)
b	DCA-Pt(II)	5.0	5	5(in 5 days)
c	DCA-Pt(IV)-COOH	3.25	3	0
d	M(P1)	3.25	3	0
e	M(P1)	6.5	3	0
f	M(P1)	13.0	3	0
g	carboplatin	20.0	3	0
h	carbo-Pt(IV)-COOH	20.0	3	0
i	M(P2)	20.0	3	0
j	DCA	30 mg DCA/kg	3	0
k	Saline	0.9% saline	5	0

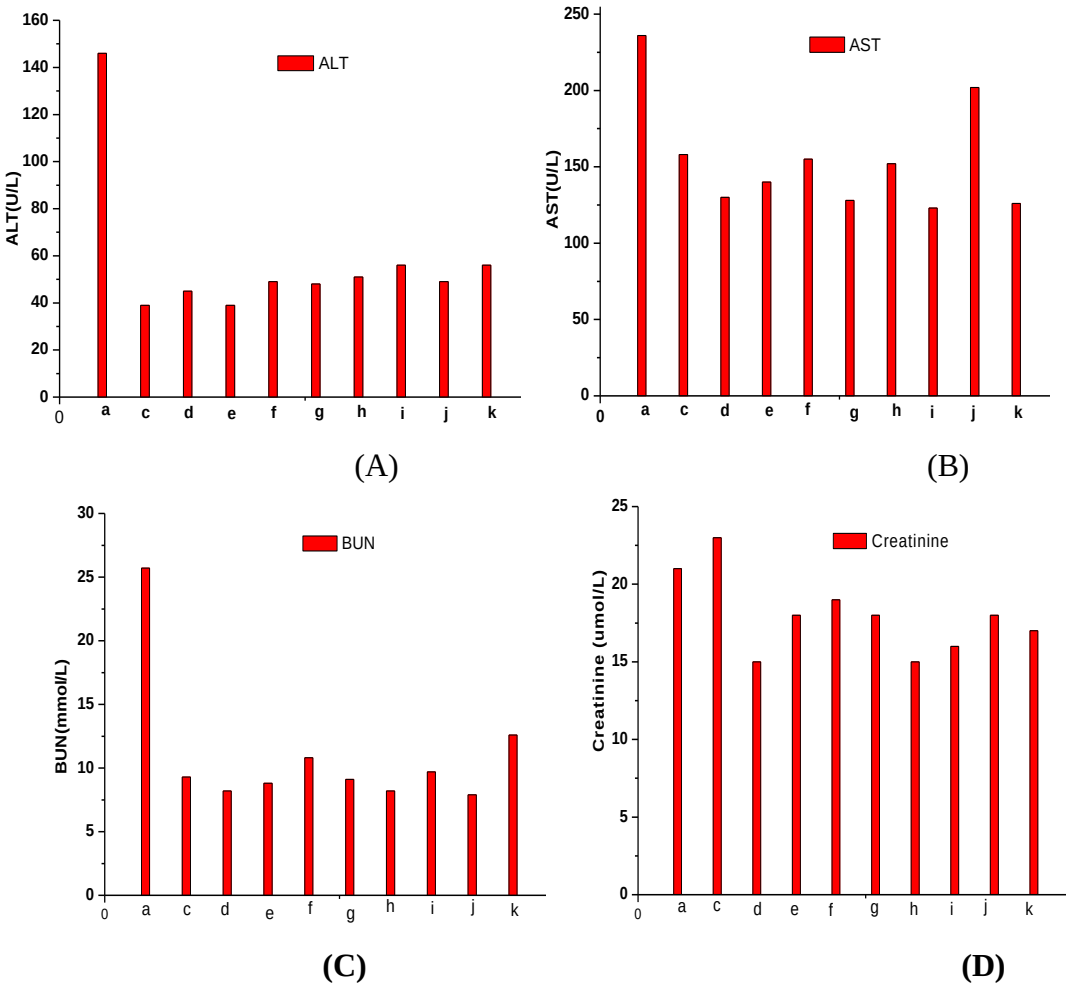


Fig. S8. Alteration of (A) ALT, (B) AST, (C) BUN and (D) creatinine levels at one week after one intravenous administration of various drugs with the doses given in the inset table. Group b(DCA-Pt(II) at 5mg Pt/kg)caused death of all mice in the beginning 5 days. Thus data of this group were not obtained.

In vivo anticancer efficacy evaluation

The tumor nodules for drug efficacy study were allowed to grow to ca. 50-100 mm^3 before initial drug injection. Tumor-bearing KM mice were randomly assigned to 8 groups (8 mice in each group). The tumor volume was calculated using the following equation: Tumor volume (V) = (length × $width^2$) / 2.

The weight and tumor volume of each mouse were measured every two days over a period of 17 d. Their weight and the initial tumor volume were measured and recorded. Test animals received three intravenous injections on day 1, 4, and 7 with the first drug injection day as day 1. Formulations for the 8 groups were listed in Table S3. Relative tumor volume and relative body weight curves were plotted using the average relative tumor volume and mean relative body weight in each experimental group at various time points. Relative tumor volume V_R is defined as $V_R=V_t/V_0$， where V_t and V_0 are the tumor volume of each mouse at day t and day 1, respectively. Similarly, Relative body weight is defined as $W_R=W_t/W_0$, where W_t and W_0 are the body weight of each mouse at day t and day 1, respectively.

Table S3. Drug formulations and dosages for KM mice in the tumor inhibition experiment

Code	Group	Dose[a)] (mg Pt/kg or mg DCA/kg)	No. of mice	Deaths in 15 days
a	DCA-Pt(II)	3.25	8	8
b	Carboplatin	20	8	0
c	Carboplatin+2DCA	20 +30	8	0
d	M(P1)	3.25	8	0
e	M(P1)	6.5	8	0
f	M(P2)	20	8	0
g	DCA	30	8	0
h	Control	Saline	8	0

[a)] Each mouse was i.v. injected three times on day 1, 4, and 7 at the dose indicated each time.

Statistical analysis

The data were expressed as mean± standard deviation (SD). Student's t-test was used to determine the statistical difference between various experimental and control groups. Differences were considered statistically significant at a level of $p < 0.05$.

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