Overcoming tumor resistance to cisplatin through micelles mediated combination chemotherapy

Dongfang Zhou,[a] Yuwei Cong,[a,b] Yanxin Qi,[a] Shasha He,[a,b] Hejian Xiong,[a,b] Yanjuan Wu,[a,b] Zhigang Xie,[a] Xuesi Chen,[c] Xiabin Jing,[a] and Yubin Huang*[a]

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

[b] *University of Chinese Academy of Sciences, Beijing 100049, PR China*

[c] *Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China*

** Correspondence author. Tel & Fax: +86-431-85262769; E-mail: ybhuang@ciac.ac.cn*

CONTENTS

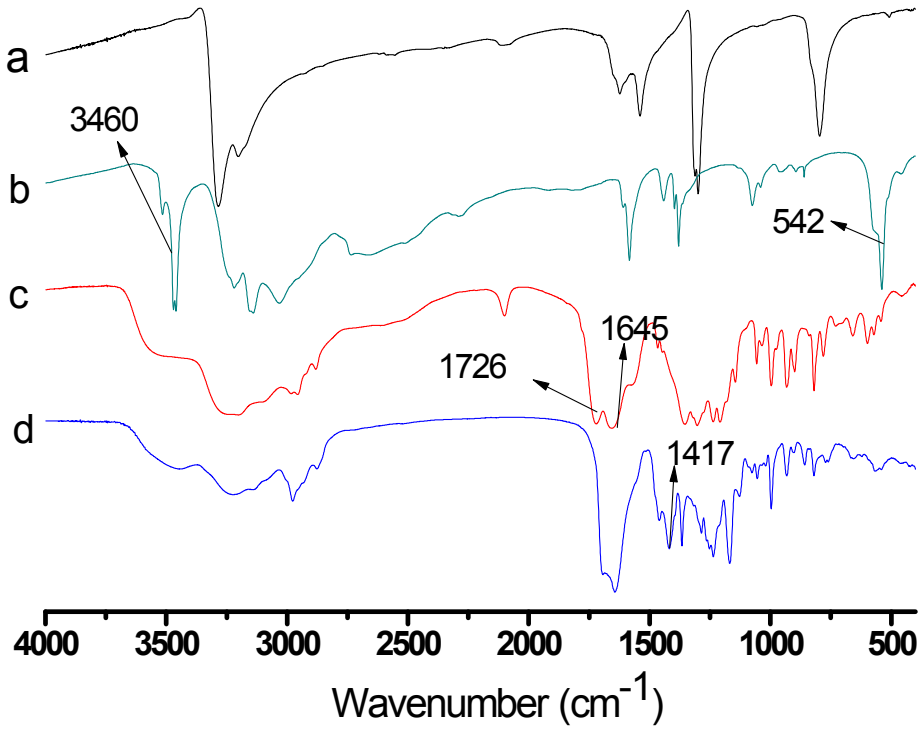

Fig. S1 FITR spectra of a) cisplatin, b) complex 1, c) DMC-Pt-DMC and d) canthaplatin.

In Fig. S1 are collected FTIR spectra of cisplatin, complex 1，DMC-Pt and canthaplatin. Complex 1, which is obtained by oxidizing cisplatin with H_2O_2, displays a sharp and intense peak at 3460 cm^{-1} (OH stretching) and a new Pt-OH stretch at 542 cm^{-1}, respectively, compared with cisplatin. After reacting with demethylcantharidin, the 3460 cm^{-1} band is weakened, and there appear two peaks (1726 cm^{-1} and 1645 cm^{-1}) characteristic of the carbonyl groups in coordinated carboxyl groups (1645 cm^{-1}) and free carboxyl groups (1726 cm^{-1}), respectively, confirming the structure of DMC-Pt. Compared with DMC-Pt, the enhanced absorption at 1645 cm^{-1} of the carbonyl groups and the absorption at 1417 cm^{-1} of the C-N stretching vibration indicates that DMC-Pt has condensed with Boc-piperazine to afford canthaplatin.

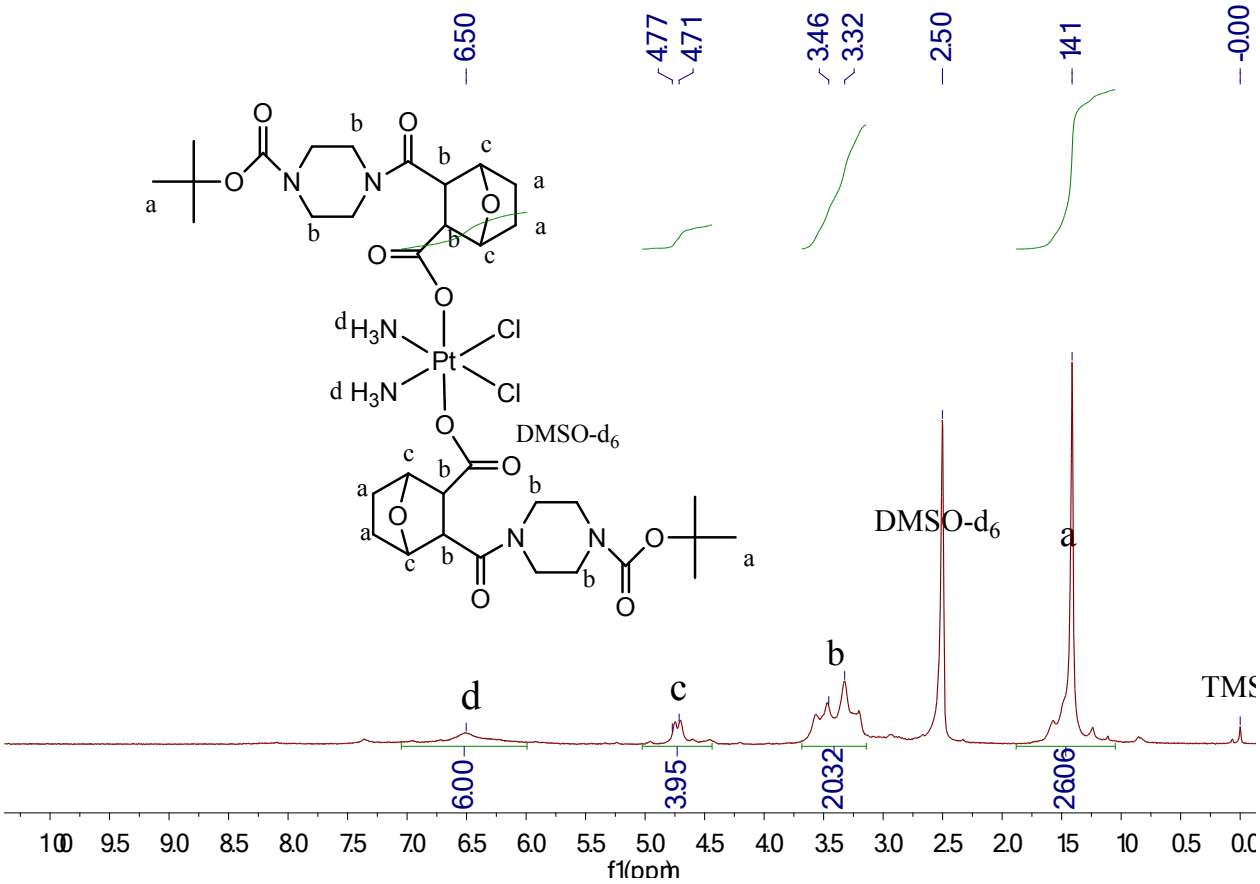

Fig. S2 ^{1}H NMR spectra of canthaplatin.

^{1}H NMR (400 MHz) of canthaplatin in DMSO-d_6 is shown in Fig. S2. Chemical shifts at 6.51 ppm (s, 6H; NH_3), 4.65-4.80 ppm (d, 4H; OCH), 3.15-3.75 ppm (m, 20H; NCH_2, CHCH) and 1.25-1.75 ppm (m, 26H; CH_3, CH_2CH_2) confirm the structure of canthaplatin.

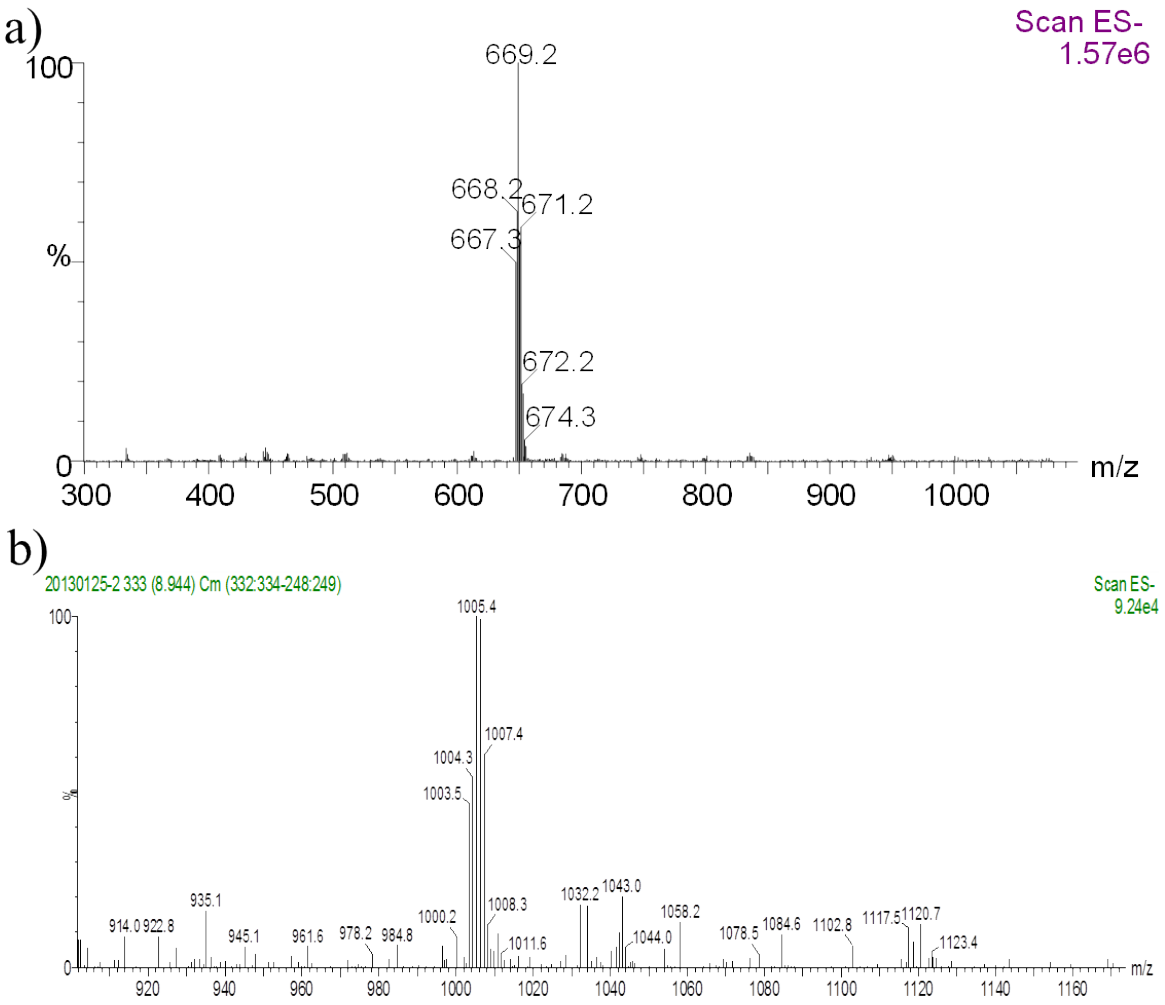

Fig. S3 ESI-MS spectra of a) DMC-Pt and b) canthaplatin.

The measuring ESI-MS spectra of DMC-Pt and canthaplatin are shown in Fig. S3. The major peak at m/z 669.2 in Figure S3a could be attributed to the deprotonated molecular ion of DMC-Pt (negative mode). Also, peak at m/z 1005.4 in Figure S3b corresponded to the deprotonated molecular ion of canthaplatin (negative mode).

Table S1. Elemental Analysis of Canthaplatin

$C_{34}H_{56}Cl_2N_6O_{12}Pt$	Anal. Calc.	Found [a]
C	40.56	40.73
H	5.61	5.57
N	8.35	8.40

[a] Detected by elemental analyzer.

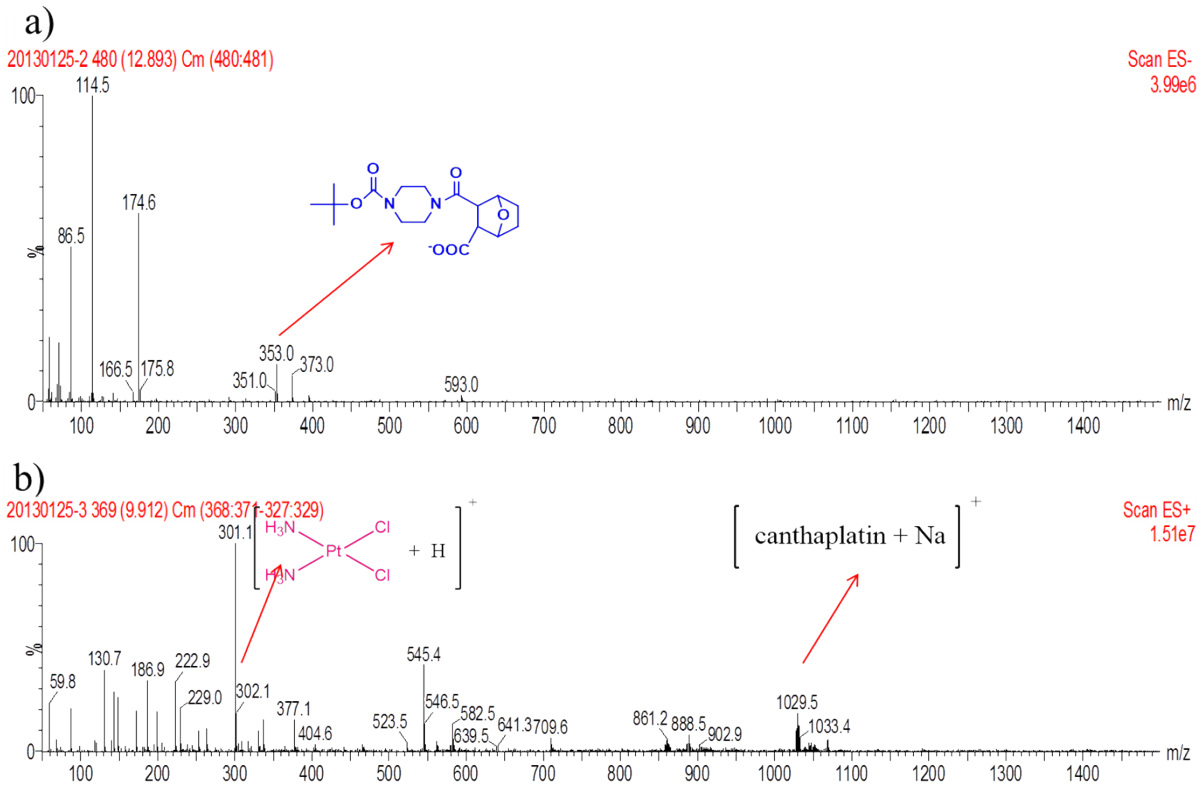

Fig. S4 Ligand Exchange Reactions of Canthaplatin.

To investigate the ligand exchange reaction of complex **2**, ESI-MS was used to detect the exchanging products. Canthaplatin was incubated with sodium ascorbate (5 mM) for 2 h, and the reacted solutions were detected by ESI-MS. Platinum(II) fragments at m/z 301.1 (positive mode, b) and LB at m/z 353.0 (negative mode, a) were found. According to ligand exchange products in reductive solution, canthaplatin should be reduced to cisplatin (II) and LB directly (Fig. S4).

Table S2. DNA-Pt adducts formation after 6 h incubation（ng Pt/mg DNA）

Formulation	MCF-7	A549	A549/DDP
Cisplatin	33.5 ± 1.2	346.1 ± 2.4	126.5 ± 3.0
Canthaplatin	20.6 ± 0.8	60.8 ± 2.2	36.6 ± 2.1
Polymer/canthaplatin	24.8 ± 0.3	114.9 ± 1.9	75.2 ± 0.8

Table S3. IC_{50} values of cispaltin, canthaplatin and polymer/canthaplatin micelles

	A549			A549/DDP		
	24h	48h	72h	24h	48h	72h
Cisplatin	20.1	6.3	3.6	65.2	87.3	61.2
Canthaplatin	77.4	26.4	14.4	61.6	51.8	31.9
Polymer/canthaplatin	56.5	22.7	12.1	43.8	36.2	17.4

`

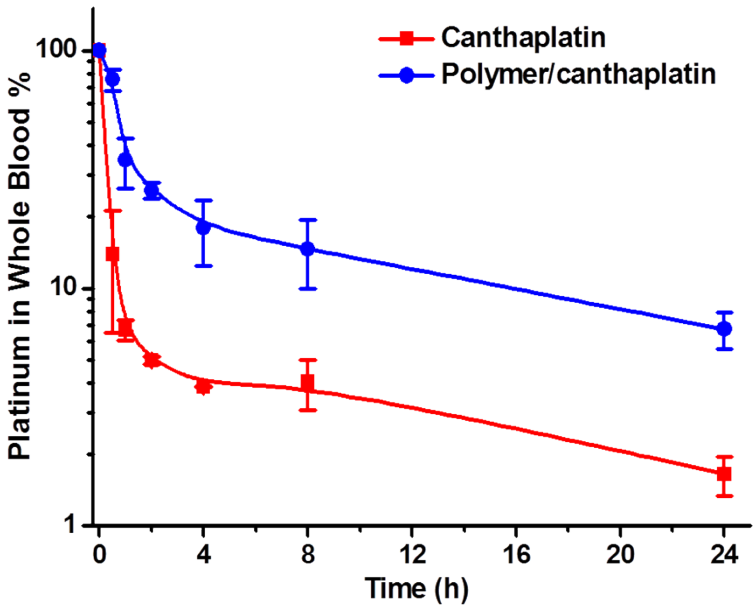

Fig. S5 Excretion study of canthaplatin and polymer/canthaplatin micelles in KM mice after a single intravenous injection (1 mg Pt/kg). Data are expressed as mean ± standard deviation (n = 3).

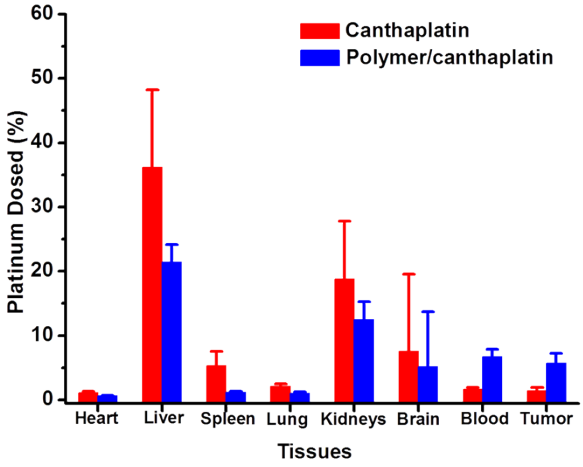

Fig. S6 Bio-distribution of Pt in KM mice for canthaplatin and polymer/canthaplatin micelles after 24 h single intravenous injection (1 mg Pt/kg). Data are expressed as mean ± standard deviation (n = 3).

Table S4. Dosing information for MTD studies in KM mice

Dose.	No. of mice	Death
Cisplatin (mg/kg)		
1	3	0
3	3	0
5	3	0
7.5	3	1
10	3	2
Canthaplatin (mg Pt/kg)		
1	3	0
3	3	0
5	3	0
10	3	2
15	3	3
Polymer/canthaplatin (mg Pt/kg)		
1	3	0
3	3	0
5	3	0
10	3	0
15	3	3
Polymer (mg/kg)		
5	3	0
10	3	0
25	3	0
50	3	0
75	3	0
100	3	0