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Laboratory note

Synthesis, characterization and antitumor activity of new type binuclear platinum(II) complexes

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Abstract

Five new type binuclear platinum(II) complexes (**a**–**e**) have been synthesized and characterized by elemental analysis, conductivity, thermal analysis, IR, UV, ^{1}H NMR and mass spectral techniques. The cytotoxicity of the complexes was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and sulforhodamine B (SRB) assays. The acute toxicity and antitumor activity of complex **e** *in vivo* were also studied. The results indicate that complexes **a**–**d** have no activity against HL-60, MCF-7, BGC-823, EJ and HCT-8 cell lines, with a higher IC_{50} value (>50 μM). Complex **e** confers substantially greater cytotoxicity against HL-60, MCF-7, BGC-823, EJ and HCT-8 cell lines with an IC_{50} value of 0.02 ± 0.009, 1.70 ± 0.21, 4.00 ± 0.35, 0.98 ± 0.02 and 1.02 ± 0.21 μM, respectively. LD_{50} of complex **e** is 815.3 mg/kg, it was significantly higher than that of cisplatin and carboplatin. Complex **e** at dose of 4, 12 and 20 mg/kg has no activity against mouse hepatocarcinoma H22 and Lewis lung carcinoma in mice, but displays significant activity against human ovarian carcinoma A2780 and human colon carcinoma HCT-116 in nude mice at dose of 12 mg/kg, and activity is similar to that of cisplatin at dose of 4 mg/kg. Complex **e** at dose of 20 mg/kg has no activity against human lung adenocarcinoma A549 in nude mice ($P > 0.05$). The results suggest that the species of amine for the new type binuclear platinum complexes have important effect on their cytotoxicity, and they may be a new class platinum anticancer drugs.

Keywords: Binuclear platinum(II) complexes; Synthesis; Cytotoxicity; Acute toxicity

1. Introduction

By now, cisplatin has become one of the most commonly used compounds for the treatment of a wide spectrum of human malignancies. As a single agent or in combination, cisplatin is the mainstay of treatment for testicular, ovarian, bladder, cervical, small-cell and non-small-cell lung cancers. Unfortunately, cisplatin has several major drawbacks. Common problems associated with the clinical use of cisplatin include cumulative toxicities of nephrotoxicity, ototoxicity and peripheral neuropathy. In addition to the serious side effects, the therapeutic efficacy of cisplatin is also limited by inherent or treatment-induced resistant tumor cell sub-populations. Driven by the impressive impact of cisplatin on cancer chemotherapy, great efforts have been made to develop new derivatives with improved pharmacological properties. Among the over 30 platinum agents which have entered clinical trials after the onset of clinical studies with cisplatin in the early 1970s, only carboplatin has received worldwide approval so far, oxaliplatin, nedaplatin, lobaplatin and SKI2053R have gained regionally limited approval, and a few drugs continue to be evaluated in clinical studies. Therefore, research work is still worthwhile [1–4].

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Although some progress had been made in reducing the toxic side effects and overcoming resistance, little improvement in cytotoxicity or spectrum of activity had been observed for the many analogues of cisplatin. Evidence suggests that this is due to the cisplatin analogues forming a similar array of DNA adducts as cisplatin [5]. Consequently, attention turned to the synthesis of non-classical platinum complexes which were capable of forming a different range of DNA adducts which could therefore display a different spectrum of anticancer activity compared to cisplatin. Non-classical platinum complexes include *trans*-platinum complexes, mono-functional platinum complexes, bi- and multi-nuclear platinum complexes, etc. [6,7]. Farrell et al. reported an innovative dinuclear complex in 1989, a simple complex consisting of two cisplatin platinum centres linked by a variable aliphatic diamine chain [8]. The series was extended to include complexes containing *cis*- and *trans*-platinum centres, and replacement of the chloro ligands with malonate, to improve water solubility [9–11]. Many of the complexes showed activity in L1210 murine leukemia and its platinum resistant sub-lines at concentration equal to or lower than cisplatin [9]. Around the same time, Broomhead et al. reported a similar series of dinuclear platinum complexes, but instead linked with the 4,4′-dipyrazolylmethane (dpzm) ligand [10,11]. Two of the complexes: α-$[Cl_2Pt(dpzm)_2PtCl_2]\cdot 0.5$ dmf and β-$[Cl_2Pt(dpzm)_2PtCl_2]$ displayed significant activity against P388 lymphocytic leukemia in mice [12]. Farrell and co-workers then expanded their series to include complexes that broke two of the structure–activity rules for cisplatin. These new complexes contain just one leaving chloro group on each reactive platinum centre and have charges ranging from 1+ to 3+ [13–20]. These complexes represent a completely new paradigm for platinum based anticancer complexes, and appear to offer great potential as new anticancer agents. So far many diamine-bridged binuclear platinum complexes have been reported, but the synthesis and antitumor activity of double bridged binuclear platinum complexes linked by iodo ligand were not reported. In order to overcome drawbacks of conventional platinum anticancer agents, five new double bridged binuclear platinum complexes linked by iodo ligand were synthesized, anticancer activity *in vitro* and *in vivo* were also studied in the present work.

2. Chemistry

All reagents and solvents were of analytical grade.

Precursor complexes *cis*-$[Pt(CH_3NH_2)_2I_2]$ (**i**), *cis*-$[Pt(C_2H_5NH_2)_2I_2]$ (**ii**), *cis*-$\{Pt[(CH_3)_2NH]_2I_2\}$(**iii**), *cis*-$[Pt(CH_3CH_2CH_2NH_2)_2I_2]$ (**iv**) and cis-$[Pt(\bigcirc\!\!-NH_2)_2I_2]$ (**v**) were synthesized according to published procedures [21,22].

Five new type binuclear platinum(II) complexes $[Pt(CH_3NH_2)I_2]_2$ (**a**), $[Pt(C_2H_5NH_2)I_2]_2$ (**b**), $\{Pt[(CH_3)_2NH]I_2\}_2$ (**c**), $[Pt(CH_3CH_2CH_2NH_2)I_2]_2$ (**d**) and $[Pt(\bigcirc\!\!-NH_2)I_2]_2$ (**e**) were prepared by adding corresponding precursor complexes (1 mmol) to a solution of perchloric acid (1.0 ml) and ethanol (30 ml), then the mixture was stirred at room temperature for 25 h. The precipitate was filtered off, washed 10 times with 1:1 mixture of water–ethanol, and dried under vacuum condition.

3. Pharmacology

In the present study we investigated the cytotoxic effects of the five newly synthesized platinum complexes and cisplatin against five different human carcinoma cell lines: HL-60 (immature granulocyte leukemia), HCT-8 (colon carcinoma), MCF-7 (galactophore carcinoma), BGC-823 (gastricarcinoma) and EJ (bladder carcinoma) using the standard MTT and SRB assays. At the same time, the acute toxicity and antitumor activity of complex **e** *in vivo* were further studied.

4. Results and discussion

4.1. Physical properties of the complexes

As listed in Table 1, there is good agreement between calculated and found values. Low molar conductances (A_M, 6.24–7.92 Ω/cm^2/mol) for the complexes **a**–**e** in DMF correspond to non-electrolytes [23].

4.2. IR spectra and electronic spectra

The IR spectra of the complexes **a**–**e** are similar, the main bands with tentative assignments are listed in Table 2. The bands of ν_{NH} and δ_{NH} in the precursor complexes **i**–**v** and

Table 1
Physical properties of the complexes

Complex	Colour	Yield (%)	Found (calculated) (%)			
			C	N	H	Pt
i	Yellow		4.68 (4.70)	5.40(5.48)	2.00 (1.97)	–
ii	Yellow		8.90 (8.91)	5.00 (5.20)	2.60 (2.62)	–
iii	Yellow		8.71 (8.91)	5.00 (5.20)	2.60 (2.62)	–
iv	Yellow		12.75 (12.71)	4.90 (4.94)	3.20 (3.20)	
v	Yellow		22.20 (22.27)	4.30 (4.33)	4.01 (4.05)	
a	Red brown	95.4	2.48 (2.50)	2.89 (2.92)	1.10 (1.05)	40.85 (40.65)
b	Red brown	92.6	4.79 (4.86)	2.80 (2.84)	1.40 (1.43)	39.61 (39.50)
c	Red brown	90.6	4.80 (4.86)	2.80 (2.84)	1.41 (1.43)	39.56 (39.50)
d	Red brown	91.5	6.89 (7.09)	2.74 (2.76)	1.70 (1.79)	38.42 (38.40)
e	Red brown	98.3	13.14 (13.15)	2.50 (2.56)	2.40 (2.39)	35.58 (35.60)

Table 2
Main IR spectral data (cm^{-1}) of the precursor and new complexes

Complex	ν_{NH}		δ_{NH}	$\nu_{Pt-I(t)}$	$\nu_{Pt-I(b)}$		ν_{Pt-N}
i	3270	3240	1580				470
ii	3220	3198	1551				480
iii	3250	3177	1550				490
iv	3204	3118	1580				512
v	3195	3109	1566				450
a	3220	3140	1580	172	146	164	480
b	3198	3114	1568	176	145	163	500
c	3200	3150	1580	174	142	161	499
d	3218	3118	1550	173	143	158	492
e	3236	3200	1570	175	140	160	480

t, terminal mode; b, bridge mode.

new complexes **a**–**e** shift to lower frequencies than those of free alkylamine. Thus it indicates that the alkylamine is coordinated with platinum through nitrogen atoms. This contention is further confirmed by the presence of ν_{Pt-N} band at about 470 cm^{-1} in the far IR frequency region. New bands appear at about 175 and 140 cm^{-1}, and are assigned to terminal and bridge mode of Pt–I stretching, indicating that binuclear platinum complexes have been synthesized [24].

The electronic spectra of the complexes **a**–**e** are also similar, they all have two absorption peaks as follows: (**a**) (λ_{max}/nm): 229.0, 289.5; (**b**) (λ_{max}/nm): 233.5, 288.0; (**c**) (λ_{max}/nm): 232.5, 268.0; (**d**) (λ_{max}/nm): 232.0, 275.0; (**e**) (λ_{max}/nm): 236.5, 278.5.

4.3. ^1H NMR and mass spectra

The chemical shift (δ, ppm) of the complexes **a**–**e** was listed as follows: **a**: 2.32 (s, 6H, $-CH_3$), 4.75 (br, 4H, $-NH_2$); **b**: 1.12 (t, 6H, $-CH_3$), 2.80 (m, 4H, $-CH_2-$), 4.94 (br, 4H, $-NH_2$); **c**: 2.50 (s, 12H, $-CH_3$), 5.42 (br, 2H, –NH); **d**: 0.84 (t, 6H, $-CH_3$), 1.55 (m, 4H, $-CH_2-CH_3$), 2.70 (t, 4H, $-CH_2-NH_2$), 4.90 (br, 4H, $-CH_2-NH_2$); **e**: 1.10–2.40 (m, 20H, CH(alkyl)), 3.10 (br, 2H, CH(methine)), 3.30 (br, 4H, $-NH_2$). After formation of the complexes, the δ_H of the complexes shifts to lower field compared with that of free ligands. This is further confirmed that the alkylamine is coordinated with platinum through nitrogen atoms.

Mass spectra of complexes **a**, **b**, **d** and **e** were analyzed as follows: *m/z* **a**: 998.6 $[M+K]^+$; **b**: 1026.7 $[M+K]^+$; **d**: 1055.6 $[M+K]^+$; **e**: 1135.8 $[M+K]^+$, there is good agreement between calculated and found values, but complex **c** has no molecular ion peak.

4.4. Thermal stability of the complex

As listed in Table 3, the thermal behaviour of the complexes **a**–**e** is similar. There is a big endothermic peak on the DTA curve at 140–750 °C, corresponding to 58.20–63.50% weight loss, this suggests that the residue may be platinum.

According to the literature [25], the new type binuclear platinum complexes were synthesized from the reaction of *cis*-$[Pt(L)_2I_2]$ (L = methylamine, ethylamine, dimethylamine, propylamine and cyclohexylamine) with perchloric acid. In these conditions, one amine is protonated. The formation of aquo species is limited because of the strength of the Pt–I bonds and the equilibrium is shifted towards the formation of the binuclear platinum complexes since the dimer is insoluble in the reaction medium. In addition, the binuclear platinum complexes can be cleaved with a second nitrogen ligand (L′) in aqueous media to give bright yellow *cis*-$[Pt(L)(L')I_2]$. Under these conditions, no isomerisation occurs, since *cis*-$[Pt(L)(L')I_2]$ is very insoluble in water. Since only *cis*-$[Pt(L)(L')I_2]$ is obtained from the bridge splitting of the dimer, we can come to the conclusion that the dimer was the *trans* isomer as in the chloro-bridged dimer $[Pt(2,6\text{-lutidine})Cl_2]_2$, since the cleavage of a *cis* isomer could produce a mixture of isomers $[Pt(L)(L')I_2]$. The cleaved Pt–I bonds are those in *trans* position to the iodide ligands, as predicted by the *trans* effect ($I^- > RNH_2$) (see Fig. 1).

Table 3
Thermal analytical data of the complexes

Complex	Dec. temp. (°C)		Total wt. loss (%)	Residue
	T_1	T_2		
a	150	390	58.73	Pt
b	160	600	60.48	Pt
c	140	510	61.28	Pt
d	150	575	58.20	Pt
e	150	750	63.50	Pt

Based on the above analysis, we propose a tentative coordination structure for the complexes (Fig. 2).

4.5. Cytotoxicity effect

As listed in Table 4, complexes **a**–**d** have no cytotoxicity against HL-60, MCF-7, BGC-823, EJ and HCT-8 cell lines, with a higher IC_{50} value (>50 μM). Complex **e** confers substantially greater cytotoxicity against HL-60, MCF-7, BGC-823, EJ and HCT-8 cell lines, moreover it has better cytotoxicity than cisplatin. The results indicated that the species of amine for new type binuclear platinum complex have important effect on their cytotoxicity.

4.6. Acute toxicity

Complex **e** causes a slight body weight loss and death began to appear after a 1-day treatment. The body weight of the survival increases after two weeks. No significant change was observed in viscera by naked eye. As listed in Table 5,

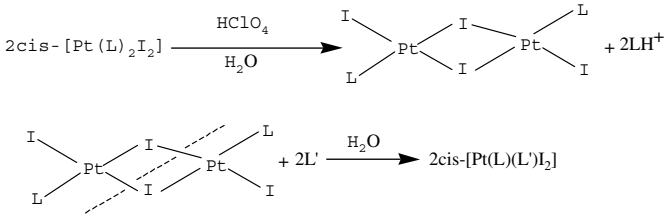
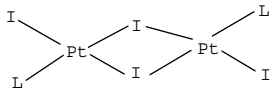


Fig. 1. The synthetic routines of the new type binuclear platinum complexes and *cis*-$[Pt(L)(L')I_2]$.



(L=methylamine, ethylamine, dimethylamine, propylamine and cyclohexylamine)

Fig. 2. Possible structures of the complexes **a**–**e**.

LD_{50} of complex **e** is 815.3 mg/kg, it was significantly higher than that of cisplatin and carboplatin. This means that it may improve curative effect by higher dose or increasing administering frequency.

4.7. Antitumor activity in vivo

As listed in Tables 6–10, complex **e** at dose of 4, 12 and 20 mg/kg has no activity against mouse hepatocarcinoma H22 and Lewis lung carcinoma in mice, but displays significant activity against human ovarian carcinoma A2780 and human colon carcinoma HCT-116 in nude mice at dose of 12 mg/kg, and activity is similar to that of cisplatin at dose of 4 mg/kg. Complex **e** at dose of 20 mg/kg has no activity against human lung adenocarcinoma A549 in nude mice ($P > 0.05$). The results suggest that complex **e** has selectivity against mouse or human carcinomas in mice or nude mice. They represent a novel class of anticancer agents, which deserve further attention in search of anticancer lead compounds.

5. Conclusion

The preliminary cytotoxicity screening program revealed that complexes **a**–**d** have no activity against HL-60, MCF-7, BGC-823, EJ and HCT-8 cell lines, with a higher IC_{50} value (>50 μM). Complex **e** confers substantially greater cytotoxicity against HL-60, MCF-7, BGC-823, EJ and HCT-8 cell lines with an IC_{50} value of 0.02 ± 0.009, 1.70 ± 0.21, 4.00 ± 0.35, 0.98 ± 0.02 and 1.02 ± 0.21 μM, respectively. LD_{50} of complex **e** is 815.3 mg/kg, it was significantly higher than that of cisplatin and carboplatin. Complex **e** at dose of 4, 12 and 20 mg/kg has no activity against mouse hepatocarcinoma H22 and Lewis lung carcinoma in mice, but displays significant activity against human ovarian carcinoma A2780 and human colon carcinoma HCT-116 in nude mice at dose of 12 mg/kg, and activity is similar to that of cisplatin at dose of 4 mg/kg. Complex **e** at dose of 20 mg/kg has no activity against human lung adenocarcinoma A549 in nude mice ($P > 0.05$). The results suggest that the species of amine for the new type binuclear platinum complexes have important effect on their cytotoxicity, and they may be a new class platinum anticancer drugs.

Table 4
Cytotoxicity of the complexes against various human carcinomas ($n = 5$)

Complex	IC_{50} (μm) ($\bar{x} \pm s$)				
	HCT-8	BGC-823	EJ	HL-60	MCF-7
Cisplatin	7.07 ± 0.69	6.12 ± 0.56	4.15 ± 0.35	2.45 ± 0.35	14.56 ± 0.36
a	>50	>50	>50	>50	>50
b	>50	>50	>50	>50	>50
c	>50	>50	>50	>50	>50
d	>50	>50	>50	>50	>50
e	1.02 ± 0.21	4.00 ± 0.35	0.98 ± 0.02	0.02 ± 0.009	1.70 ± 0.21

Table 5
The data of acute toxicity of complex **e**

Dose (mg/kg)	Animal number	Death number	LD_{50}[a] (95% confidence limit) (mg/kg)
1000	10	8	815.3 (745.6–891.6)
850.0	10	6	
722.5	10	4	
614.1	10	0	

[a] LD_{50} of cisplatin and carboplatin is 14 and 150 mg/kg, respectively.

6. Experimental protocols

6.1. Chemistry

6.1.1. Instrumentation and measurement

Elemental analyses were determined on an EA-1110 elemental analyzer. Pt was determined by Jarrell-ISH 110 + 2000 inductively coupled spectrometry. Molar conductances at room temperature were measured in 10^{-3} mol/L DMF solutions using a DFS-1 type conductivity meter. The IR spectra were recorded using KBr pellets and a Perkin–Elmer Model-683 spectrophotometer. The electronic spectra in DMSO were measured on an UV-3400 Toshniwal spectrophotometer. The ^{1}H NMR spectra were measured on a Bruker AV-400 NMR spectrometer in dimethyl sulfoxide-d_6 with solvent peaks as references, and in chloroform with TMS as internal standard. ESIMS data were measured with Thermo Finnigan-LCQ. The thermal analysis was conducted using RI-GAKU 8150 meter (Ar, 10 °C min^{-1}, Al_2O_3). The optical density (OD) at 570 nm was measured on a microplate spectrophotometer (Bio-Rad Model 680, USA).

6.1.2. Preparation of complexes

Precursor complexes *cis*-$[Pt(CH_3NH_2)_2I_2]$ (**i**), *cis*-$[Pt(C_2H_5NH_2)_2I_2]$ (**ii**), *cis*-$\{Pt[(CH_3)_2NH]_2I_2\}$ (**iii**), *cis*-$[Pt(CH_3CH_2CH_2NH_2)_2I_2]$ (**iv**) and cis-$[Pt(\bigcirc\!\!-NH_2)_2I_2]$ (**v**) were synthesized according to the literatures [21,22].

$[Pt(CH_3NH_2)I_2]_2$(**a**): *cis*-$[Pt(CH_3NH_2)_2I_2]$ (1 mmol) was mixed with 1.0 ml of perchloric acid. Ethanol (30 ml) was

Table 6
The antitumor activity of complex **e** against mouse hepatocarcinoma H22 in mice by administering intraperitoneally

Group	Dose (mg/kg/day)	Animal number begin/end	Body weight (g) begin/end		Carcinoma weight (g)	Inhibition rate (%)
Control	–	10/10	21.5	+7.6	4.10 ± 0.969	–
Cisplatin	4×4	6/6	21.8	−0.5	1.0 ± 0.206	73.2**
e	4×4	6/6	22.5	+8.2	4.03 ± 0.934	−4.67
e	12×4	6/6	21.2	+6.3	3.47 ± 1.094	9.87
e	20×4	6/6	21.7	+6.5	3.54 ± 0.822	8.05

**$P < 0.01$ compared with the control group.

Table 7
The antitumor activity of complex **e** against mouse Lewis lung carcinoma in mice by administering intraperitoneally

Group	Dose (mg/kg/day)	Animal number begin/end	Body weight (g) begin/end		Carcinoma weight (g)	Inhibition rate (%)
Control	–	10/10	21.5	+7.6	4.10 ± 0.969	–
Cisplatin	4 × 4	6/6	19.7	−1.1	1.61 ± 0.204	47.4**
e	4 × 4	6/5	19.8	+5.6	2.96 ± 0.539	3.27
e	12 × 4	6/4	20.2	+3.6	2.92 ± 0.241	4.58
e	20 × 4	6/2	19.7	+2.8	3.51 ± 0.481	−14.7

**$P < 0.01$ compared with the control group.

added to the mixture which was stirred at room temperature for 25 h. The precipitate was filtered off, washed 10 times with 1:1 mixture of water–ethanol, and dried under vacuum condition. Yield: 95.4%.

The synthetic procedure for $[Pt(C_2H_5NH_2)I_2]_2$ (**b**), $\{Pt[(CH_3)_2NH]I_2\}_2$ (**c**), $[Pt(CH_3CH_2CH_2NH_2)I_2]_2$ (**d**) and $[Pt(\bigcirc\!\!-NH_2)I_2]_2$ (**e**) are in general the same.

6.2. Pharmacology

6.2.1. Cell culture

Five different human carcinoma cell lines were used for cytotoxicity determination: HL-60 (immature granulocyte leukemia), MCF-7 (galactophore carcinoma), BGC-823 (gastricarcinoma), EJ (bladder carcinoma) and HCT-8 (colon carcinoma). They were obtained from the American Type Culture Collection (ATCC) and were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air.

6.2.2. Animals

Kunming (KM) mice (Clean, SCXK 11-00-0006) were purchased from Experimental Animal Institute of Chinese Academy of Medical Sciences. Balb/c/neu nude mice (SPF, SCXK(jing) 2002.0003) were purchased from Beijing Vital River Laboratory Animal Technology Limited Corporation. All experimental animals were bred at Laboratory Animal Service Center at Chinese Academy of Medical Sciences. Kunming (KM) mice (aged 6–8weeks, weighing 18–22 g) were housed in groups of four in metal cages, and had free access to standard rat chow and water. Male Balb/c/neu nude mice at 6–8 weeks of age were housed in sterile microisolator cages with access to autoclaved laboratory animal diet and tap water. Animals were exposed to 12 h cycles of light and dark. All experiments involving animals were approved by the Chinese Academy of Medical Sciences Animal Ethics Committee and were carried out under the supervision of Laboratory Animal Service Center at Chinese Academy of Medical Sciences.

Table 8
The antitumor activity of complex **e** against human ovarian carcinoma A2780 in nude mice by administering intraperitoneally

Group	Dose (mg/kg/day)	Animal number begin/end	Body weight (g) begin/end		Carcinoma weight (g)	Inhibition rate (%)
Control	–	6/6	18.9	+3.0	1.72 ± 0.44	–
Cisplatin	4 × 4	6/4	20.4	+0.3	0.08 ± 0.13	95.2***
e	4 × 4	6/3	18.9	+5.7	2.34 ± 1.48	−36.4
e	12 × 4	6/4	19.8	+0.1	0.19 ± 0.25	89.2***

***$P < 0.001$ compared with the control group.

Table 9
The antitumor activity of complex **e** against human colon carcinoma HCT-116 by administering intraperitoneally

Group	Dose (mg/kg/day)	Animal number begin/end	Body weight (g) begin/end		Carcinoma weight (g)	Inhibition rate (%)
Control	–	7/7	22.4	+1.5	1.13 ± 0.37	–
Cisplatin	4 × 3	6/6	22.7	−0.3	0.60 ± 0.51	46.5**
e	4 × 3	6/6	22.9	−0.8	0.84 ± 0.20	25.5*
e	12 × 3	6/6	22.4	−0.5	0.68 ± 0.40	39.4*

*$P < 0.05$, **$P < 0.01$ compared with the control group.

6.2.3. Cytotoxicity analysis

The cells harvested from exponential phase were plated equivalently into a 96-well plate, complexes were then added to the wells to achieve final concentrations. Control wells were prepared by addition of culture medium. Wells containing culture medium without cells were used as blanks. The plates were incubated at 37 °C in a 5% CO_2 incubator for 44 h. The MTT assay was performed as described by Mosmann [26]. Upon completion of the incubation, stock MTT dye solution (20 μl, 5 mg/ml) was added to each well. After 4 h incubation, 2-propanol (100 μl) was added to solubilize the MTT formazan. The OD of each well was then measured on a microplate spectrophotometer at a wavelength of 570 nm. The SRB assay was performed as previously described [27]. Upon completion of the incubation, the cells were fixed in 10% trichloroacetic acid (100 μl) for 30 min at 4 °C, washed five times in tap water and stained with 0.1% SRB in 1% acetic acid (100 μl) for 15 min. The cells were washed four times in 1% acetic acid and air-dried. The stain was solubilized in 10 mM unbuffered Tris base (100 μl) and OD was measured at 540 nm as above. The IC_{50} value was determined from plots of % viability against dose of compounds added.

6.2.4. Acute toxicity study

Acute toxicity was studied using Kunming mice. The animals were divided into four groups, 10 mice per group.

Table 10
The antitumor activity of complex **e** against human lung adenocarcinoma A549 by administering intraperitoneally

Group	Dose (mg/kg/day)	Animal number begin/end	Body weight (g) begin/end		Carcinoma weight (g)	Inhibition rate (%)
Control	–	6/4	17.8	−1.4	0.38 ± 0.35	–
Cisplatin	4 × 6	6/3	17.5	+4.5	0.10 ± 0.00	73.3*
e	12 × 6	6/2	17.7	−0.3	0.40 ± 0.28	−6.70
e	20 × 6	6/3	19.1	+2.4	0.23 ± 0.06	37.8

*$P < 0.05$ compared with the control group.

They were injected intraperitoneally with Pt complexes by 614.1, 722.5, 850 and 1000 mg/kg, respectively. Toxicity was observed over a period of 14 days. The amount of a drug, given all at once, which causes the death of 50% (one half) of a group of tested animals (LD_{50}) and 95% confidence limit were calculated according to the Bliss method.

6.2.5. Antitumor activity in vivo

The antitumor activity *in vivo* was studied by Kunming (KM) mice and Balb/c/neu nude mice as follows:

Kunming (KM) mice underwent axilla subcutaneous injection with the mouse carcinoma tissue to generate carcinoma. After 24 h, the animals were randomly divided into blank control group (10 animals), vehicle control group, the cisplatin positive control group and the complex **e** groups with two different dosages, with each group having six mice. The animals were administered intraperitoneally with cisplatin and complex **e** by 0.2 ml/10 g, once a day and for four times. After the last drug administration for 24 h, the animals were sacrificed by decapitation, the body weight and carcinoma tissue weight were measured and the carcinoma inhibition rate (%) was calculated according to the formula:

$$\text{The carcinoma inhibition rate } (\%) = \left(\text{tumor weight}_{\text{control}} - \text{tumor weight}_{\text{drug}}\right) / \text{tumor weight}_{\text{control}} \times 100\%$$

Balb/c/neu nude mice underwent axilla subcutaneous injection with the human carcinoma tissue to generate carcinoma. The animals were randomly divided into blank control group, the cisplatin positive control group and the complex **e** groups with two different dosages, with each group having six mice. When the tumor volumes reached 60 mm^3, the animals were administered intraperitoneally with cisplatin and complex **e** by 0.2 ml/10 g, once a week and for four times. The diameter of the carcinoma was measured for twice every week, the relative carcinoma volume was calculated and the body weight was measured. After the last drug administration for 24 h, the animals were sacrificed by decapitation, the body weight and carcinoma tissue weight were measured and the carcinoma inhibition rate (%) was calculated to evaluate the therapeutic effect.

6.2.6. Statistical analysis

Data were collected from at least three separate experiments. The results are expressed as means ± SD. The statistical differences were analyzed using SPSS *t*-test. p Values less than 0.05 were considered to indicate statistical differences.

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References

[1] T. Boulikas, M. Vougiouka, Oncol. Rep. 10 (2003) 1663–1682.
[2] Y.P. Ho, S.C.F. Au-Yeung, K.K.W. To, Med. Res. Rev. 23 (2003) 633–655.
[3] I. Kostova, Recent Patents Anti-Cancer Drug Discov. 1 (2006) 1–22.
[4] M.A. Jakupec, M. Galanski, B.K. Keppler, Rev. Physiol. Biochem. Pharmacol. 146 (2003) 1–53.
[5] E. Wong, C.M. Giandomenico, Chem. Rev. 99 (1999) 2451–2466.
[6] N.J. Wheate, J.G. Collins, Curr. Med. Chem. Anti-Cancer Agents 5 (2005) 267–279.
[7] N.J. Wheate, J.G. Collins, Coord. Chem. Rev. 241 (2003) 133–145.
[8] N.P. Farrell, S.G. de Almeida, K.A. Skov, J. Am. Chem. Soc. 110 (1988) 5018–5019.
[9] N.P. Farrell, Y. Qu, M.P. Hacker, J. Med. Chem. 33 (1990) 2179–2184.
[10] N.P. Farrell, Y. Qu, Inorg. Chem. 28 (1989) 3416–3420.
[11] K.A. Skov, H. Adomat, N.P. Farrell, J.B. Matthews, Anti-Cancer Drug Des. 13 (1998) 207–220.
[12] J.A. Broomhead, M.J. Lynch, Inorg. Chim. Acta 240 (1995) 13–17.
[13] J.A. Broomhead, L.M. Rendina, M. Sterns, Inorg. Chem. 31 (1992) 1880–1889.
[14] J.A. Broomhead, L.M. Rendina, L.K. Webster, J. Inorg. Biochem. 49 (1993) 221–234.
[15] Y. Qu, T.G. Appleton, J.D. Hoeschele, N.P. Farrell, Inorg. Chem. 32 (1993) 2591–2593.
[16] H. Rauter, R. Di Domenico, E. Menta, A. Oliva, Y. Qu, N. Farrell, Inorg. Chem. 36 (1997) 3919–3927.
[17] Y. Qu, H. Rauter, A.P. Soares Fontes, R. Bandarage, L.R. Kelland, N. Farrell, J. Med. Chem. 43 (2000) 3189–3192.
[18] A. Hegmans, Y. Qu, L.R. Kelland, J.D. Roberts, N. Farrell, Inorg. Chem. 40 (2001) 6108–6114.
[19] Y. Qu, J.A. Fitzgerald, H. Rauter, N. Farrell, Inorg. Chem. 40 (2001) 6324–6327.
[20] B.A.J. Jansen, J. van der Zwan, H. den Dulk, J. Brouwer, J. Reedijk, J. Med. Chem. 44 (2001) 245–249.
[21] A.R. Khokhar, Y.J. Deng, S. Al-Baker, M. Yoshida, Z.H. Siddik, J. Inorg. Biochem. 51 (1993) 677–687.
[22] J.C. Zhang, Y. Shen, Synth. React. Inorg. Met-Org. Nano-Met. Chem. 36 (2006) 345–351.
[23] W.J. Geary, Coord. Chem. Rev. 7 (1971) 81–122.
[24] D.M. Adams, P.J. Chandler, Chem. Commun. 3 (1966) 69–70.
[25] F.D. Rochon, P.C. Kong, Can. J. Chem. 64 (1986) 1894–1896.
[26] T.R. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[27] S.P. Fricker, Toxicol. In Vitro 8 (1994) 821–822.