European Journal of Medicinal Chemistry 44 (2009) 4772–4777

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Laboratory note

Synthesis, cytotoxicity and DNA-binding levels of new type binuclear platinum(II) complexes

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ARTICLE INFO

Article history:
Received 9 July 2008
Received in revised form
29 April 2009
Accepted 12 June 2009
Available online 21 June 2009

Keywords:
Binuclear platinum(II) complexes
Synthesis
Cytotoxicity
Cell cycle
DNA-binding

ABSTRACT

Six new type binuclear platinum(II) complexes (**a**–**f**) have been synthesized and characterized by elemental analysis, conductivity, thermal analysis, IR, UV, ^{1}H NMR and mass spectra techniques. The cytotoxicity of the complexes was tested by MTT and SRB assays. The cell cycle analysis and the levels of total platinum bound to DNA were measured by flow cytometry and ICP-MS, respectively. The results indicate that the complex (**a**) has no cytotoxicity against HL-60, BGC-823, Bel-7402, KB and Hela, the complexes (**b**, **c**, **e** and **f**) have weaker cytotoxicity against some tested carcinoma cell lines, the complex (**d**) has better cytotoxicity against HL-60, BGC-823, Bel-7402, KB, MCF-7, HCT-8 and Hela with respect to the IC_{50} values obtained. The cytotoxicity of the complex (**d**) is equal to that of cisplatin against HL-60 and Bel-7402 ($P > 0.05$), but it has better cytotoxicity than that of cisplatin against BGC-823 and MCF-7 ($P < 0.05$). The complex (**d**) causes significant G_2/M arrest and a concomitant decrease of cell population in G_1 and S phases, and the total DNA platination levels of the complex (**d**) are higher than those of cisplatin under the same experimental conditions. It suggests that the bridging linker has important effect on their cytotoxicity. Indeed, when the bridging linker is dicarboxylic acid, their cytotoxicity is better than that of platinum complexes with an amino acid as bridging linker. The new type binuclear platinum(II) complexes represent a novel class of anticancer agents, which deserves further attention in search of anticancer lead compounds.

1. Introduction

By now, cisplatin has become one of the most commonly used compounds for the treatment of a wide spectrum of human malignancies. Unfortunately, cisplatin has several major drawbacks. Common problems 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 subpopulations. 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 [1–4]. Therefore, research work is still worthwhile.

The structure–activity relationships summarized by Cleare and Hoeschele dominated Pt drug design for over 20 years and remained valid until relatively recently. This is reflected in the fact that all Pt compounds that have entered clinical trials adhere to this set of guidelines. Although some progress had been made in reducing the toxic side effects and overcoming resistance, it has become quite evident that mere analogues of cisplatin or carboplatin will not probably offer any substantial clinical advantages over the existing

Abbreviations: MTT, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; SRB, sulforhodamine B; OD, optical density; FBS, fetal bovine serum; PBS, phosphate buffered saline; PI, propidium iodide; Tris, tris-(hydroxymethyl)-aminomethane; ICP-MS, inductively coupled plasma mass spectrometry.

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doi:10.1016/j.ejmech.2009.06.017

drugs. Evidence suggests that this is due to the cisplatin analogues forming a similar array of DNA adducts as cisplatin [5]. In recent years, several research efforts in the field of platinum anticancer drugs have been directed toward platinum complexes with innovative structural motifs that might exhibit a molecular mechanism substantially different from that of cisplatin and manifest a different pattern of cytotoxic and pharmacological effects. In this frame, various binuclear and multinuclear platinum complexes have been designed and synthesized that act as multidentate ligands toward DNA and produce adducts substantially different from those of cisplatin. The group of Nick Farrell has been very active in this field. Farrell et al. reported an innovative dinuclear complex in 1989, a simple complex consisting of two cisplatin platinum centers linked by a variable aliphatic diamine chain [6]. The series was extended to include complexes containing cis- and trans-platinum centers, and replacement of the chloro ligands with malonate in order to improve water solubility [7–9]. Many of the complexes showed activity in L1210 murine leukemia and its platinum resistant sublines at concentrations equal to or lower than cisplatin [7]. 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 [10–12]. Two of the complexes α-$[Cl_2Pt(dpzm)_2PtCl_2]\cdot 0.5dmf$ and β-$[Cl_2Pt(dpzm)_2PtCl_2]$ displayed significant activity against P388 lymphocytic leukemia in mice [10]. 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+ [11–18]. 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, cytotoxicity and DNA-binding levels of dicarboxylic acid and amino acid-bridged binuclear platinum complexes were not reported. In order to overcome the drawbacks of conventional platinum anticancer agents, the synthesis, cytotoxicity, cell cycle analysis and DNA-binding levels of six new type binuclear platinum complexes linked by dicarboxylic acid and amino acid are reported and discussed.

2. Chemistry

All reagents and solvents were of analytical grade.

Precursor complexes cis-[Pt(◯–NH_2)$_2$I$_2$] (**i**), [Pt(◯–NH_2)I$_2$]$_2$ (**ii**) and cis-[Pt(◯–NH_2)(NH_3)I$_2$] (**iii**) were synthesized according to the published procedures [19,20].

Six new type binuclear platinum(II) complexes [{Pt(H_2O)$_2$I}μ-OCO-[{Pt(H_2O)$_2$I}] (**a**–**c**) {R = –CH_3, –$CH(CH_3)_2$, -CH2–◯–OH} and {Pt(H_2O)$_2$I}μ–OOC–$(CH_2)_n$–COO–[{Pt(H_2O)$_2$I}] (**d**–**f**) ($n = 0, 1, 2$) were prepared by adding solid Ag_2CO_3 to a water solution of dicarboxylic acid or amino acid followed by cis-[Pt(◯–NH_2)(NH_3)I$_2$]. The mixture was stirred in the dark, filtered through celite, and the solution was evaporated to dryness under reduced pressure and washed a few times with a minimum quantity of very cold water. The final product was dried over P_2O_5 under vacuum condition.

3. Pharmacology

In the present study we investigated the cytotoxic effects of six new type binuclear platinum(II) complexes and cisplatin against eight different human carcinoma cell lines: HL-60 (immature granulocyte leukemia), BGC-823 (gastricarcinoma), KB (nasopharyngeal carcinoma), Hela (cervical carcinoma), EJ (bladder carcinoma), HCT-8 (colon carcinoma), MCF-7 (galactophore carcinoma) and Bel-7402 (liver carcinoma) by MTT and SRB assays. At the same time, the cell cycle analysis and the levels of total platinum bound to DNA in HL-60 cells were also measured by flow cytometry and ICP-MS.

4. Results and discussion

4.1. Physical properties of the complexes

As listed in Table 1, there is a good agreement between calculated and found values. Low molar conductances (A_M, 3.45–4.57 $\Omega^{-1}\,cm^2\,mol^{-1}$) for the complexes (**a**–**f**) in nitrobenzene correspond to non-electrolytes [21].

4.2. IR spectra

The IR spectra of the complexes (**a**–**f**) are similar, the main bands with tentative assignments are listed in Table 2. The bands of υ_{NH_2} in the precursor complexes (**i**–**iii**) shift to lower frequencies than those of free ammine and cyclohexylamine, and the bands of δ_{NH_2} shift to higher frequencies than those of free ammine and cyclohexylamine. Thus it indicates that ammine and cyclohexylamine are coordinated with platinum through nitrogen atoms. This contention is further confirmed by the presence of υ_{Pt-N} band at about 460 cm^{-1} in the far IR frequency region. The carboxylate group of the complexes (**a**–**c**) shows two bands, an intense asymmetric carboxylate stretching $\upsilon_{(as,COO^-)}$ and a symmetric carboxylate stretching $\upsilon_{(s,COO^-)}$, at about 1600 and 1450 cm^{-1}, respectively. The carboxylate group of the complexes (**d**–**f**) shows two bands, an intense asymmetric carboxylate stretching $\upsilon_{(as,COO^-)}$ and a symmetric carboxylate stretching $\upsilon_{(s,COO^-)}$ at about 1680 and 1380 cm^{-1}, respectively. Trends in the positions and separation between these bands are the most useful tools in assigning structures from infrared spectra. The values of $\Delta\upsilon_{(COO^-)}(\upsilon_{(as,COO^-)} - \upsilon_{(s,COO^-)})$ of the complexes (**a**–**c**) are in the range 136–166 cm^{-1} which are close to the corresponding ionic values. The values of $\Delta\upsilon_{(COO^-)}(\upsilon_{(as,COO^-)} - \upsilon_{(s,COO^-)})$ of the complexes (**d**–**f**) are in the range 221–272 cm^{-1} which are significantly less than the corresponding ionic values. This indicates the chelating coordination of COO^- group for the complexes (**a**–**c**) and unidentate coordination of COO^- group for complexes (**d**–**f**), which is in accordance with proposed structure [22]. This is further confirmed by the appearance of the peak of υ_{Pt-O}. At the same time, new bands appear. e.g., a broad υ_{OH} peak appears at about 3400 cm^{-1}, δ_{Pt-OH} peak appears at about 1100 cm^{-1}, $\rho r_{(H_2O)}$ and $\rho w_{(H_2O)}$ peaks appear at about 630 and 600 cm^{-1}, respectively. These results also prove the

Table 1
Physical properties of the complexes.

Complex	Colour	Yields (%)	Found (calculated) (%)			
			C	N	H	Pt
(**i**)	Yellow		22.20 (22.27)	4.30 (4.33)	4.03 (4.05)	–
(**ii**)	Yellow		13.09 (13.15)	2.50 (2.56)	2.34 (2.39)	–
(**iii**)	Yellow		12.70 (12.75)	4.80 (4.96)	2.70 (2.85)	–
a	Pale yellow	75	4.78 (4.49)	2.02 (1.74)	1.65 (1.63)	48.67 (48.58)
b	Pale yellow	72	7.50 (7.22)	1.49 (1.69)	2.48(2.06)	46.78(46.94)
c	Pale yellow	68	12.28 (12.07)	1.89 (1.57)	2.09 (1.91)	43.63 (43.58)
d	Pale yellow	70	3.14 (2.99)	–	0.95 (1.00)	48.35 (48.52)
e	Pale yellow	67	4.51 (4.40)	–	1.18 (1.23)	47.76(47.69)
f	Pale yellow	72	5.57(5.77)	–	1.34 (1.45)	46.78 (46.89)

Table 2
IR data (cm^{-1}) of the complexes.

Complex	υ_{OH}	υ_{NH_2}	δ_{NH_2}	$\upsilon_{(as,COO^-)}$	$\upsilon_{(s,COO^-)}$	$\Delta\upsilon_{(COO^-)}$	$\rho r(H_2O)$	$\rho w(H_2O)$	δ_{Pt-OH}	υ_{Pt-O}	υ_{Pt-N}
(**i**)		3195, 3109	1566								444
(**ii**)		3236, 3200	1570								450
(**iii**)		3270, 3200	1510								480
a	3456	3200, 3100	1500	1626	1460	166	615	602	1080	610	
b	3395	3100, 3150	1520	1583	1447	136	634	610	1134	622	
c	3436	3200, 3150	1510	1609	1447	162	632	607	1146	623	
d	3350			1672	1400	272	629	606	1155	580	
e	3471			1689	1468	221	638	608	1114	620	
f	3426			1683	1455	228	632	608	1125	620	

existence of coordinated water in the complexes and coincide with the thermal analysis.

4.3. Electronic spectra

As listed in Table 3, after formation of the complexes, one new absorption peak appears for complexes (**a**) and (**b**) at 205.0 and 193.0 nm. E_2 band red shifts by ca.1.6 nm, B band red shifts by ca. 7.2 nm for the complex (**c**) compared with the free ligands. One new absorption peak appears for complex (**d**) at 194.0 nm. No absorption peak appears for complexes (**e**) and (**f**).

4.4. ^1H NMR and mass spectra

As listed in Table 4, after formation of the complexes, the δ_H of the complexes shifts to lower field compared with those of free ligands. This is also further confirmed that the carboxylate is coordinated with platinum through oxygen atoms, which is in accordance with the proposed structure.

Mass spectra of complexes (**a**–**f**) were analyzed as follows: m/z: **a**: 843.4 $[M+K]^+$; **b**: 871.5$[M+K]^+$; **c**: 935.6$[M+K]^+$; **d**: 843.7$[M+K]^+$, **e**: 857.3$[M+K]^+$, **f**: 871.6$[M+K]^+$, there is a good agreement between calculated and found values.

4.5. Thermal stability of the complexes

As listed in Table 5, the thermal behaviour of the complexes (**a**–**f**) is similar. There is a small endothermic peak on the DTA curve at 90–235 °C, corresponding to 8.24–9.18% weight loss and coinciding with four water molecules or three water molecules and OH^-. According to the water loss temperature, it suggests that the complexes contain four coordinated water molecules, this is also confirmed by IR studies. In addition, there is a big endothermic peak on the DTA curve at 190–380 °C, corresponding to 29.08–32.58% weight loss and coinciding with $2I^-$. There is also a small endothermic peak on the DTA curve at 320–760 °C, corresponding to 9.21–18.69% weight loss and coinciding with organic ligand of the complexes. The total weight loss is 50.76–57.00%, this suggests that the residue may be platinum.

Based on the above studies and relative literature [22], we propose a tentative coordination structure for the complexes (**a**–**f**) (Fig. 1).

Table 3
UV spectral data of the complexes (**a**–**f**).

Complex	λ_{max} (nm)		
	$n \rightarrow \pi^*$	E_2 band	B band
DL-α-alanine	–	–	–
DL-α-valine	–	–	–
DL-α-tyrosine	196.8	222.6	274.2
Oxalic acid	212.0	–	–
Malonic acid	–	–	–
Succinic acid	–	–	–
a	205.0	–	–
b	193.0		
c	204.0	224.2	281.4
d	194.0	–	–
e	–	–	–
f	–	–	–

4.6. Cytotoxicity effect

As shown in Table 6, the complex (**a**) has no cytotoxicity against HL-60, BGC-823, Bel-7402, KB and Hela, the complexes (**b**, **c**, **e** and **f**) have weaker cytotoxicity against some tested carcinoma cell lines, the complex (**d**) has better cytotoxicity against HL-60, BGC-823, Bel-7402, KB, MCF-7, HCT-8 and Hela with respect to the IC_{50} values obtained. The cytotoxicity of the complex (**d**) is equal to that of cisplatin against HL-60 and Bel-7402 ($P > 0.05$), but it has better cytotoxicity than that of cisplatin against BGC-823 and MCF-7 ($P < 0.05$). In summary, the bridging linker has important effect on their cytotoxicity. Indeed, when the bridging linker is dicarboxylic acid, their cytotoxicity is better than that of platinum complexes with an amino acid as bridging linker. Thus new type binuclear platinum(II) complexes represent a novel class of anticancer agents, which deserves further attention in search of anticancer lead compounds.

4.7. Cell cycle analysis

As listed in Table 7, complex (**d**) causes significant G_2/M arrest and a concomitant decrease of cell population in G_1 and S phases.

Table 4
The ^1H NMR data of the ligands and the complexes.

Compound	Chemical shifts (δ, ppm)
DL-α-alanine	1.39 (d, 3H, $-CH_3$), 3.65 (m,1H, $-\underset{\vert}{C}H$)
DL-α-valine	0.99 (d, 6H, $-CH_3$), 2.22 (m, 1H, $-CH(CH_3)_2$), 3.55 (d, 1H, $-\underset{\vert}{C}H-NH_2$)
DL-α-tyrosine	6.58~7.00 (m, 4H, –⟨⟩–), 2.70 (d, 2H, $-CH_2-$), 3.40 (t,1H, $-\underset{\vert}{C}H$)
Oxalic acid	–
Malonic acid	3.37 (s, 2H, $-CH_2-$)
Succinic acid	2.43 (s, 4H, $-CH_2-CH_2-$)
a	1.47 (d, 3H, $-CH_3$), 3.85 (m,1H, $-\underset{\vert}{C}H$)
b	1.40 (d, 6H, $-CH_3$), 2.91 (m, 1H, $-CH(CH_3)_2$), 3.91 (d, 1H, $-\underset{\vert}{C}H-NH_2$)
c	6.77~7.12 (m, 4H, –⟨⟩–), 2.88 (d, 2H, $-CH_2-$), 3.50 (t,1H, $-\underset{\vert}{C}H$)
d	–
e	4.10 (s, 2H, $-CH_2-$)
f	2.89 (s, 4H, $-CH_2-CH_2-$)

Table 5
Thermal analytical data of the complexes (**a**–**f**).

Complex	H_2O (OH^-) loss temp. (°C)			I^- loss temp.(°C)			Organic part loss temp.(°C)			Total wt. loss (%)	Residue
	T_1	T_2	%	T_3	T_4	%	T_5	T_6	%		
a	90	205	9.02	205	350	32.58	350	720	11.32	52.92	Pt
b	86	190	8.79	190	330	29.08	330	740	12.89	50.76	Pt
c	93	206	8.24	206	320	30.07	320	760	18.69	57.00	Pt
d	100	220	9.14	220	370	32.36	370	750	9.21	50.71	Pt
e	110	235	9.18	235	360	29.87	360	740	13.09	52.14	Pt
f	120	210	9.04	210	380	31.89	380	730	15.42	56.35	Pt

4.8. DNA binding

As shown in Fig. 2, for complex (**d**), the levels of total platinum bound to DNA in HL-60 are increased with increasing concentrations, moreover, their total DNA platination levels are higher than those of cisplatin under the same experimental conditions. It is accepted that DNA is a major target of platinum-based anticancer drugs. So far, it is controversial if the degree of cytotoxicity of platinum complexes correlates with the amount of DNA platination. We previously reported that there was also no correlation between total DNA platination levels and cytotoxicity of tri-functional mononuclear platinum(II) complexes and ammine/propylamine(II) platinum complexes [23,24]. In the present work, the cytotoxicity of the complex (**d**) is equal to that of cisplatin against HL-60 ($P > 0.05$), but total DNA platination levels are higher than those of cisplatin under the same experimental conditions. So there was also no correlation between total DNA platination levels and cytotoxicity of new type binuclear platinum(II) complexes. The total DNA platination levels contain some kinds of Pt–DNA adducts formed by platinum complex. This suggests that it is probably the level of specific DNA adducts that is important in determining the cytotoxicity of platinum-based drugs [25]. In addition, although the mainstream of investigation has focused on DNA, platinum-based drugs might have other important targets apart from nuclear DNA [26]. Although the importance of these other targets in relation to cytotoxicity is unknown, they might have important effect on cytotoxicity and remained to be further studied.

5. Conclusion

The bridging linker has important effect on the cytotoxicity of new type binuclear platinum(II) complexes, when the bridging linker is dicarboxylic acid, their cytotoxicity is better than that of platinum complexes with an amino acid as bridging linker. The experimental results also indicate that new type binuclear platinum(II) complexes represent a novel class of anticancer agents, which deserves further attention in search of anticancer lead compounds.

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} M nitrobenzene using a DSS-11A type conductivity meter. The IR spectra were recorded in the 400–4000 cm^{-1} range using KBr pellets and a Magna-IR 560 spectrophotometer. The electronic spectra in H_2O were measured on a Shimadzu-UV-2401 PC spectrophotometer. The ^1H NMR spectra were recorded in D_2O on a Brucker AC 80 NMR spectrometer. ESIMS data were measured with Thermo Finnigan-LCQ. The OD was measured at 570 nm on a microplate spectrophotometer (Bio-rad Model 7550, USA). The cell cycle analysis was performed on a BECScan Flow cytometry (Becton–Dickinson). The levels of total platinum bound to DNA were measured by ICP-MS (PE Elan-5000).

6.1.2. Preparation of complexes

Precursor complexes cis-[Pt(◯–NH_2)$_2$I$_2$] (**i**), [Pt(◯–NH_2)I]$_2$ (**ii**) and cis-[Pt(◯–NH_2)(NH_3)I$_2$] (**iii**) were synthesized according to the literatures [19,20].

$[\{Pt(H_2O)_2I\}\mu\text{-}OCO\text{-}\{Pt(H_2O)_2I\}]$ with $H_2N\text{-}CH(CH_3)\text{-}$ (**a**): Solid Ag_2CO_3 (1 mmol) was added to a water solution of DL-α-alanine (2 mmol) followed by an equimolar amount of cis-[Pt(◯–NH_2)(NH_3)I$_2$]. The mixture was stirred in the dark for 35 h, filtered through celite, and the solution evaporated to dryness under reduced pressure. The pale yellow

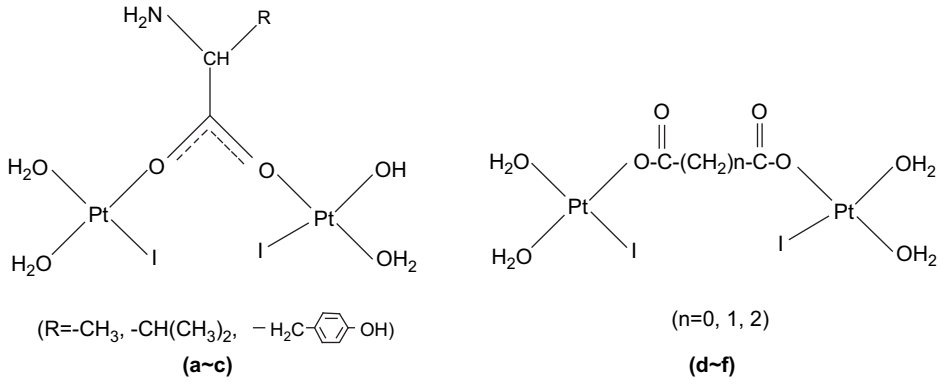

Fig. 1. Possible structures of the complexes (**a**–**f**).

Table 6
Cytotoxicity of the complexes against various human carcinomas.

Complex	IC_{50} (μM)							
	HL-60	BGC-823	Bel-7402	KB	Hela	EJ	MCF-7	HCT-8
Cisplatin	2.76 ± 0.12	6.18 ± 0.22	8.02 ± 1.01	2.54 ± 0.78	4.60 ± 0.34	4.24 ± 0.23	15.01 ± 1.02	7.24 ± 0.89
a	>50	>50	>50	>50	>50	–	–	–
b	12.24 ± 1.01	32.09 ± 3.45	40.87 ± 3.45	>50	20.74 ± 3.21	–	–	–
c	16.78 ± 2.09	>50	>50	43.67 ± 3.04	>50	–	–	–
d	2.87 ± 0.35	4.05 ± 0.89	8.98 ± 1.01	9.23 ± 1.20	12.38 ± 1.31	23.89 ± 3.56	11.45 ± 1.43	12.09 ± 1.34
e	34.98 ± 4.56	30.87 ± 3.47	>50	>50	32.58 ± 2.39	–	–	–
f	11.34 ± 1.23	15.78 ± 1.39	18.67 ± 2.03	13.76 ± 1.09	13.98 ± 1.87	–	–	–

precipitate was washed for a few times with a minimum quantity of very cold water (0–4 °C). The final product was dried over P_2O_5 under vacuum condition. Yield: 75%.

The synthetic procedure for $H_2N\text{-}CH(CH_3)\text{-}CO\text{-}[\{Pt(H_2O)_2I\}\mu\text{-}OCO\text{-}\{Pt(H_2O)_2I\}]$ (**b**), $H_2N\text{-}CH(CH_2\text{-}C_6H_4\text{-}OH)\text{-}CO\text{-}[\{Pt(H_2O)_2I\}\mu\text{-}OCO\text{-}\{Pt(H_2O)_2I\}]$ (**C**), $[\{Pt(H_2O)_2I\}\mu\text{–}OOC\text{–}COO\text{–}\{Pt(H_2O)_2I\}]$(**d**), $[\{Pt(H_2O)_2I\}\mu\text{-}OOC\text{–}CH_2\text{–}COO\text{–}\{Pt(H_2O)_2I\}]$ (**e**), $[\{Pt(H_2O)_2I\}\mu\text{-}OOC\text{–}CH_2\text{–}CH_2\text{–}COO\text{–}\{Pt(H_2O)_2I\}]$ (**f**) are in general the same.

6.2. Pharmacology

6.2.1. Cell culture

Eight different human carcinoma cell lines were used for cytotoxicity determination: HL-60 (immature granulocyte leukemia), BGC-823 (gastricarcinoma), KB (nasopharyngeal carcinoma), Hela (cervical carcinoma), EJ (bladder carcinoma), HCT-8 (colon carcinoma), MCF-7 (galactophore carcinoma) and Bel-7402 (liver carcinoma). They were obtained from the American Type Culture Collection (ATCC), and cultured in RPMI-1640 medium supplemented with 10% FBS, 100 units/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. Cytotoxicity analysis

The complexes were dissolved in PBS and diluted to the required concentration with culture medium when used. The cells harvested from exponential phase were plated equivalently into a 96-well plate, then complexes were added to the wells to achieve final concentrations ranging from 10^{-7} to 10^{-4} M. 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 [27]. Upon completion of the incubation, 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 measured on a microplate spectrophotometer at a wavelength of 570 nm. The SRB assay was performed as previously described [28]. 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 complexes added.

Table 7
The effect of the complex (**d**) on cell cycle of HL-60.

Complex	Concentration (μM)	Cell cycle (%)		
		G_1	S	$G_2 + M$
Control	0	31.25 ± 2.56	62.47 ± 4.58	6.28 ± 1.21
Cisplatin	0.625	18.76 ± 1.35[a]	58.89 ± 6.87	22.35 ± 3.58[a]
	1.25	16.87 ± 2.35[a]	49.87 ± 4.69[a]	33.26 ± 4.01[a]
	2.5	2.34 ± 0.12[a]	45.32 ± 5.87[a]	52.34 ± 6.89[a]
d	0.625	31.25 ± 4.56[a]	46.89 ± 7.89[a]	21.89 ± 4.02[a]
	1.25	12.58 ± 4.25[a]	41.68 ± 8.79[a]	45.74 ± 8.69[a]
	2.5	1.58 ± 0.23[a]	30.24 ± 8.56[a]	68.18 ± 9.68[a]

[a] $P < 0.05$ compared with the control group.

6.2.3. Cell cycle analysis

The cell cycle analysis was performed as described by Ferlini et al. [29]. HL-60 cells were treated with platinum complex (**d**) for the indicated times and harvested by centrifugation at 1200 rpm/min for 5 min at room temperature. Cell pellets were rinsed with PBS, suspended in a 1:1 (v/v) solution of PBS and 0.2 M Na_2HPO_4–0.1 M citric acid (pH = 7.5), and fixed with cold ethanol at 4 °C for 1 h. Fixed cells were washed with PBS and resuspended in a staining solution containing PI (10 μg/ml) and DNase-free RNase (100 μg/ml). The cell suspensions were incubated at 37 °C for 1 h in the dark and analyzed on a Flow cytometry. Data were collected by ModFit LT 2.0 for power software.

6.2.4. DNA binding

The levels of total platinum bound to DNA in HL-60 cells were performed as described by Mellish et al. [25]. Briefly, approximately 5×10^7 HL-60 cells were seeded in tissue-culture flasks, then the complex (**d**) was added in a concentration gradient, and the final concentrations were maintained at 10, 25, 50 and 100 μM, respectively. They were incubated at 37 °C in 5% CO_2 for 4 h, cells were harvested, and DNA was extracted according to the DNA extraction kit procedure. The purity and concentration of DNA were

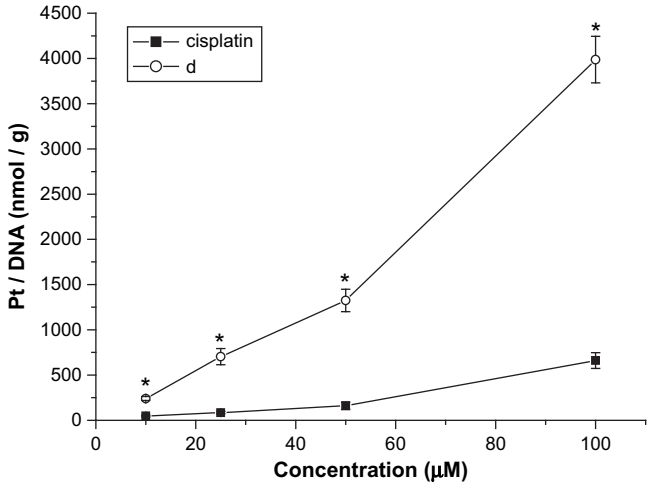

Fig. 2. Levels of total platinum bound to DNA in HL-60 after 4-h exposure to platinum complex (**d**).

measured by UV spectroscopy. An aliquot of the remaining sample was sonicated and subjected to platinum analysis by ICP-MS.

6.2.5. Statistical analysis

Data were collected from at least five separate experiments. The results are expressed as means ± standard deviation (SD). The statistical differences were analyzed using SPSS' *t*-test. *P* values less than 0.05 were considered to indicate statistical differences.

Acknowledgements

This work was supported by the Natural Science Foundation of Zhejiang Province (Grant No. 298068), the Research Project Foundation of Shijiazhuang Bureau of Science Technology (Grant No. 07120053A), the Research Project Foundation of Baoding Bureau of Science Technology (Grant No. 07F05), the Key Basic Research Special Foundation of Science Technology Ministry of Hebei Province (Grant No. 08966415D), the Research Project Foundation of Department of Hebei Education (Grant No. 2008311).

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