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Laboratory note

Synthesis, cytotoxicity and DNA binding levels of tri-functional mononuclear platinum(II) complexes

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Abstract

Seven new tri-functional mononuclear platinum(II) complexes (**a**–**g**) have been synthesized and characterized by elemental analysis, conductivity, thermal analysis, IR, UV and ^{1}H NMR spectral techniques. The cytotoxicity of these 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. The results indicate that the complexes (**a**–**g**) have selectivity against tested carcinoma cell lines; they have weaker cytotoxicity against HCT-8 and MCF-7. Complexes **a**, **b**, **d** and **g** also exert weaker cytotoxicity against BGC-823 and complexes **a**, **b**, **e** and **f** have better cytotoxicity against EJ, but their cytotoxicity is weaker than that of cisplatin. Complexes **c**, **e** and **f**, confer substantially greater cytotoxicity against HL-60 with an IC_{50} value of 7.68 ± 0.23, 3.87 ± 0.19 and 2.41 ± 0.18 μM, respectively, moreover, cytotoxicity of complex **f** is equal to that of cisplatin. Complexes **c**, **e** and **f** cause significant G_2/M arrest and a concomitant decrease of cell population in G_1 and S phases. The total DNA-platination levels of them are higher than that of cisplatin under the same experimental conditions. It suggests that there is no correlation between total DNA-platination levels in HL-60 cells and cytotoxicity of complexes. When leaving groups are aromatic carboxylates, the complexes have better cytotoxicity, moreover, the substituent in benzene ring also influences cytotoxicity. In addition, when leaving groups are dicarboxylates, dicarboxylates coordinating with platinum through oxygen atoms form different chelate cycle and cycle size also affects their cytotoxicity.

Keywords: Tri-functional mononuclear platinum(II) complexes; Synthesis; Cytotoxicity; Cell cycle; DNA binding

1. Introduction

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 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

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

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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 so far adhere to this set of guidelines. However, it has become quite evident that mere analogues of cisplatin or carboplatin will not offer any substantial clinical advantages over the existing drugs. Evidence suggests that this is due to the cisplatin analogues forming a similar array of DNA adducts as cisplatin. Consequently, attention turned to the synthesis of non-classical platinum complexes which were capable of forming a different range of DNA adducts [5,6].

In general, coventional mononuclear platinum(II) complexes are bi-functional mononuclear platinum(II) complexes which possess two anionic leaving groups. A few mononuclear platinum complexes which deviate from the traditional structure–activity relationships have been reported in 1989. These include cationic triamine complexes containing both ammonia and heterocyclic amine(*N*-het) ligands which, unlike conventional cisplatin analogues, possess only one anionic leaving group. In the past, it was believed that such complexes would be inactive because of their inability to form bidentate lesions on DNA as the classical neutral cisplatin analogues [7]. Related monofunctional complexes such as $[Pt(NH_3)_3Cl]Cl$ have been shown to be ineffective anti-tumor agents, while derivatives containing pyridine, purine and pyrimidine ligands show activity against S180 and L1210 in vivo [7–9]. Moreover, more evidences indicate that this group of monofunctional complexes has displayed better anti-tumor activity [10], although the mechanism of action for these complexes is not yet fully understood. It is expected that complexes bind to DNA in a monodentate fashion, although other targets cannot be ruled out yet. Complex $[PtA_2(N\text{-het})Cl]^+$ where *N*-het = 4-methyl- or brompyridine, binds monofunctionally to DNA in guanine residues, as shown by reaction with d(GpG) and monoclonal antibody and replication mapping studies [11,12]. So far, tri-functional mononuclear platinum(II) anticancer complexes including three leaving groups are not reported. In addition, the possible advantage of platinum anticancer drugs with decreased reactivity of leaving group is an established approach which commenced with the clinical success of carboplatin. It is reported that the decreased reactivity reduces the nephrotoxic and neurotoxic side effects of cisplatin. Moreover, decrease in reactivity may also lead to reduce detoxification reactions by intracellular thiols. This may increase the drug efficacy and help to circumvent resistance mechanisms [13]. So carboxylate platinum complexes seem to be more promising than the corresponding chloro analogues. In order to overcome drawbacks of cisplatin, seven new tri-functional mononuclear platinum(II) complexes were synthesized, cytotoxicity, cell cycle analysis and DNA binding levels were also studied in the present work.

2. Chemistry

All reagents and solvents were of analytical grade.

Precursor complexes *cis*-$[Pt(C_2H_5NH_2)_2I_2]$ (**i**), $[Pt(C_2H_5NH_2)I_2]_2$ (**ii**) and *cis*-$[Pt(C_2H_5NH_2)(NH_3)I_2]$ (**iii**) were synthesized according to the published procedures [14,15].

Seven new tri-functional mononuclear platinum(II) complexes $[Pt(II)(NH_3)(H_2O)X]$ (**a–g**) $\{X = (COO^-)_2$, $(CH_2)(COO^-)_2$, $(CH_2)_2(COO^-)_2$, $(CH{=}CH)(COO^-)_2$, $2p\text{-}CH_3O{-}C_6H_4{-}COO^-$, $2C_6H_5{-}COO^-$, $2CH_3COO^-\}$ were prepared by adding solid Ag_2CO_3 to a water solution of carboxylic acid and methanol followed by an equimolar amount of *cis*-$[Pt(C_2H_5NH_2)(NH_3)I_2]$. The mixture was stirred in the dark for 40 h, filtered through celite, and the solution was evaporated to dryness under reduced pressure and the product was washed 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.

The complexes were soluble in water, but almost insoluble in very cold water (0–4 °C).

3. Pharmacology

In the present study we investigated the cytotoxic effects of the seven newly synthesized platinum complexes and cisplatin against five different human carcinoma cell lines: HL-60 (immature granulocyte leukemia), MCF-7 (galactophore carcinoma), BGC-823 (gastrocarcinoma), EJ (bladder carcinoma) and HCT-8 (colon carcinoma) using the standard 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 good agreement between calculated and found values. Low molar conductances for the complexes in nitrobenzene correspond to non-electrolytes [16].

4.2. IR spectra

The IR spectra of the complexes (**a–g**) 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–iii**) and new complexes (**a–g**) shift to lower frequencies than those of free ammine and ethylamine. Thus it indicates that ammine and ethylamine are 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. The carboxylate group of the complexes (**a–g**) shows two bands, an intense asymmetric carboxylate stretching $\nu_{(as,COO^-)}$ and a symmetric stretching $\nu_{(s,COO^-)}$, at about 1650 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

Table 1
Physical properties of the complexes

Complex	Yield (%)	Found (calculated) (%)				Λ_M (Ω^{-1} cm^2 mol^{-1})
		C	N	H	Pt	
i	80	8.90 (8.91)	5.00 (5.20)	2.60 (2.62)	–	–
ii	90	4.80 (4.86)	2.80 (2.84)	1.40 (1.43)	–	–
iii	70	4.70 (4.70)	5.45 (5.48)	1.95 (1.97)	–	–
a	70	7.57 (7.55)	4.42 (4.40)	1.60 (1.58)	61.40 (61.32)	4.70
b	65	11.02 (10.85)	4.41 (4.22)	2.11 (2.12)	58.50 (58.73)	4.11
c	62	14.15 (13.88)	4.06 (4.05)	2.61 (2.62)	56.69 (56.36)	4.21
d	79	13.89 (13.96)	4.05 (4.07)	2.03 (2.05)	56.60 (56.68)	4.91
e	75	36.12 (36.09)	2.64 (2.63)	3.62 (3.60)	36.70 (36.64)	4.35
f	65	35.66 (35.59)	2.98 (2.97)	3.22 (3.20)	41.54 (41.30)	4.17
g	80	14.63 (14.46)	4.45 (4.22)	3.36 (3.34)	58.97 (58.72)	4.51

of $\Delta\nu_{(COO^-)}(\nu_{(as,COO^-)} - \nu_{(s,COO^-)})$ of the complexes (**a**–**g**) are in the range 238–318 cm^{-1}, which is greater than $\Delta\nu_{(COO^-)}$ of the corresponding sodium carboxylates, suggesting that the carboxylate group is a monodentate coordinated through oxygen atoms [17]. This is further confirmed by the appearance of the peaks of ν_{Pt-O}. At the same time, new bands appear, e.g., broad ν_{OH} peaks appear at about 3400 cm^{-1}, δ_{Pt-OH} peaks appear at about 1000 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 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, no absorption peak appears for complexes **c** and **g**, one new absorption peak appears for complex **b** at 206.0 nm, blue shifts by ca. 18.0 and 7.0 nm for complexes **a** and **d**, respectively, E_2 band blue shifts by ca. 30.0 and 5.0 nm, B band blue shifts by ca. 24.0 and 27.0 nm for complexes **e** and **f** compared with the free ligands, respectively.

4.4. ^1H NMR

The chemical shifts (δ, ppm) of the ligands and complexes are listed in Table 4. After formation of the complexes, the δ_H of the complexes shift to lower field compared with those of free ligands. This also further confirms that the carboxylates are coordinated with platinum through oxygen atoms. Moreover, the signals of hydrogen protons of ethylamine disappear, which further confirms that the ethylamine has been displaced by water molecule.

4.5. Thermal stability of the complexes

As listed in Table 5, the thermal behaviour of the complexes (**a**–**g**) is similar. There is a small endothermic peak on the DTA curve at 105–205 °C, corresponding to 3.56–5.81% weight loss and coinciding with one water molecule. The water loss temperature suggests that the complexes contain a coordinated water molecule, which is also confirmed by IR studies. In addition, there is a big endothermic peak on the DTA curve at 415–790 °C, corresponding to 39.68–64.53% weight loss, which suggests that the residue may be platinum.

Based on the above studies and relative literature [14], we propose tentative coordination structures for the complexes (Fig. 1).

4.6. Cytotoxicity effect

As shown in Fig. 2, the complexes (**a**–**g**) have selectivity against tested carcinoma cell lines; they have weaker cytotoxicity against HCT-8 and MCF-7. Complexes **a**, **b**, **d** and **g** also exert weaker cytotoxicity against BGC-823 with respect to the IC_{50} values obtained. Complexes **a**, **b**, **e** and **f** have better

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

Complex	ν_{OH}	ν_{NH}	δ_{NH}	$\nu_{(as,COO^-)}$	$\nu_{(s,COO^-)}$	$\Delta\nu_{(COO^-)}$	$\rho r_{(H_2O)}$	$\rho w_{(H_2O)}$	δ_{Pt-OH}	ν_{Pt-O}	ν_{Pt-N}
i		3220, 3198	1551								480
ii		3198, 3114	1568								500
iii		3262, 3189	1573								482
a	3400	3217, 3150	1500	1699	1381	318	635	600	1000	560	440
b	3443	3239, 3190	1585	1679	1411	268	631	607	1034	560	450
c	3424	3200, 3150	1560	1637	1399	238	641	603	1020	550	479
d	3420	3261, 3190	1571	1646	1403	243	630	609	1127	560	480
e	3400	3250, 3220	1440	1600	1340	260	634	601	1020	560	470
f	3447	3200, 3150	1551	1651	1361	290	636	602	1068	619	465
g	3416	3264, 3190	1540	1670	1414	246	632	605	1000	580	492

Table 3
UV spectral data of the ligands and complexes

Complex	λ (nm)			
	n → π*	E_2 band	B band	π → π*
Oxalic acid	212.0			
Malonic acid	–	–	–	–
Succinic acid	–	–	–	–
Maleic acid				212.0
p-Anisic acid		227.0	271.0	
Benzoic acid		195.0	250.0	
Acetic acid	–	–	–	–
a	194.0			
b	206.0			
c	–	–	–	–
d				205.0
e		197.0	247.0	
f		190.0	223.0	
g	–	–	–	–

cytotoxicity against EJ, but their cytotoxicity is weaker than that of cisplatin. Complexes **c**, **e** and **f** confer substantially greater cytotoxicity against HL-60 with an IC_{50} value of 7.68 ± 0.23, 3.87 ± 0.19 and 2.41 ± 0.18 μM, respectively, moreover, cytotoxicity of the complex **f** is equal to that of cisplatin.

The mode of action of platinum anticancer drugs is still not completely understood but it is thought to depend on hydrolysis reactions where the leaving group is replaced by a water molecule adding a positive charge on the molecule. The hydrolysis product is believed to be the active species reacting mainly with glutathione in the cytoplasm and the DNA in the nucleus, thereby inhibiting replication, transcription and other nuclear functions and arresting cancer cell proliferation and tumor growth. So the reactivity of leaving groups is an important factor which affects anticancer activity. For tri-functional mononuclear platinum(II) complexes, when leaving groups are aromatic carboxylates, the complexes have better cytotoxicity, moreover, the substituent in benzene ring also influences cytotoxicity. In addition, when leaving groups are dicarboxylates, dicarboxylates coordinating with platinum through oxygen atoms form different chelate cycle, cycle size also affects their cytotoxicity.

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

Complex	Chemical shift (δ, ppm)
Oxalic acid	–
Malonic acid	3.26 (s, 2H, $-CH_2-$)
Succinic acid	2.67 (s, 4H, $-CH_2-CH_2-$)
Maleic acid	6.28 (s, 2H, $-CH=CH-$)
p-Anisic acid	3.84(s, 3H, $-OCH_3$), 7.07–7.91(m, 4H, $-C_6H_4-$)
Benzoic acid	7.45–8.12(m, 5H, $-C_6H_5$)
Acetic acid	2.10 (s, 3H, $-CH_3$)
a	–
b	3.40 (s, 2H, $-CH_2-$)
c	2.80 (s, 4H, $-CH_2-CH_2-$)
d	6.37 (s, 2H, $-CH=CH-$)
e	3.95(s, 6H, $-OCH_3$), 7.11–8.01(m, 8H, $-C_6H_4-$)
f	7.71–8.31(m, 10H, $-C_6H_5$)
g	2.23 (s, 6H, $-CH_3$)

Table 5
Thermal analytical data of the complexes

Complex	H_2O loss temperature (°C)			Dec. temperature (°C)		Total weight loss (%)	Residue
	T_1	T_2	%	T_3	T_4		
a	120	200	5.36	448	790	39.68	Pt
b	120	200	5.21	415	728	42.05	Pt
c	110	180	5.45	426	756	43.21	Pt
d	105	185	5.41	435	789	44.04	Pt
e	125	205	3.56	420	785	64.53	Pt
f	110	190	3.56	435	750	59.19	Pt
g	110	170	5.81	425	786	41.97	Pt

4.7. Cell cycle analysis

As listed in Table 6, after treatment, the changes of cell cycle progression of HL-60 caused by different complexes are similar. All of them cause significant G_2/M arrest and a concomitant decrease of cell population in G_1 and S phases.

4.8. DNA binding

As shown in Fig. 3, for complexes **c**, **e** and **f**, 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 that 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. For cisplatin, in general the degree of cytotoxicity correlates with the amount of DNA-platination [18]. However, a study in testicular cancer germ cell lines found no association between cisplatin DNA-platination and drug sensitivity and a similar observation has been made in a breast cancer cell line [19,20]. No correlation could be found between DNA-platination and cytotoxicity for oxaliplatin. Mellish et al. reported that no significant correlation was also found between total DNA-platination levels and cytotoxicity of the seven platinum based drugs on SKOV-3 or on CH1 cell lines [21]. In the present work, we found that there was also no correlation between total DNA-platination levels and cytotoxicity of tri-functional mononuclear platinum(II) complexes. The total DNA-platination levels contain some kinds of Pt–DNA adducts formed by platinum complexes. This suggests that it is probably the level of specific DNA adducts that is important in determining the cytotoxicity of platinum based drugs [21]. Although the mainstream of investigation has focused on DNA, platinum based drugs might have other important targets apart from nuclear DNA. They may react with phospholipids, inhibit amino acid transport, protein synthesis, ATPases, uncoupled oxidative phosphorylation [22]. Although the importance of these other targets in relation to cytotoxicity

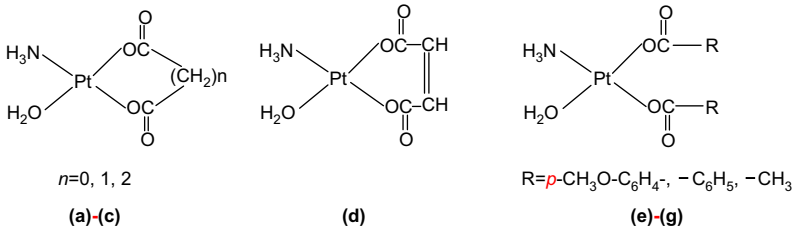

Fig. 1. Possible structures of the complexes (**a**–**g**).

is unknown, they might have important effect on cytotoxicity and remain to be further studied.

In addition, it is now generally agreed that the major adduct formed by cisplatin is via bidentate coordination through two sequential guanine bases on the same DNA strand, commonly referred to as a 1,2-GG intrastrand cross-link [2]. Muti-nuclear platinum DNA adducts are broadly defined as flexible, non-directional and mainly interstrand cross-links. These complexes are also able to induce conformational changes in DNA, particularly the conversion from B-type to Z- and A-type [6]. For monofunctional mononuclear platinum complexes, the mechanism of action is not yet fully understood. Complex $[PtA_2(N\text{-het})Cl]^+$, where *N*-het = 4-mehyl- or bromopyridine, binds monofunctionally to DNA in guanine residues, as shown by reaction with d(GpG) and bymonoclonal antibody and replication mapping studies [11,12]. In the present work, tri-functional mononuclear platinum complexes containing only one ammonia ligand as carrier group, possess three leaving groups. Moreover, their total DNA-platination levels are higher than that of cisplatin under the same experimental conditions. According to the characteristics of chemical structures, we deduced that the major adduct formed by tri-functional platinum complexes might be via tridentate coordination through bases on DNA. The mechanism of action for these complexes remains to be further studied.

5. Conclusion

The preliminary cytotoxicity screening program revealed that tri-functional mononuclear platinum(II) complexes induced 50% inhibition of the cell viability of BGC-823, HCT-8, MCF-7, EJ and HL-60 cells at micromolar concentrations and thus may be considered as biologically active. Complexes **a**, **b**, **e** and **f** have better cytotoxicity against EJ, but their cytotoxicity is weaker than that of cisplatin. Complexes **c**, **e** and **f** confer substantially greater cytotoxicity against HL-60 with an IC_{50} value of 7.68 ± 0.23, 3.87 ± 0.19 and 2.41 ± 0.18 μM, respectively, moreover, cytotoxicity of complex **f** is equal to that of cisplatin. When leaving groups are aromatic carboxylates, the complexes have better cytotoxicity, moreover, the substituent in benzene ring also influences cytotoxicity. In addition, when leaving groups are dicarboxylates, dicarboxylates coordinating with platinum through oxygen atoms form different chelate cycle, cycle size also affects their cytotoxicity. Thus tri-functional mononuclear platinum(II) complexes represent a novel class of anticancer agents, which

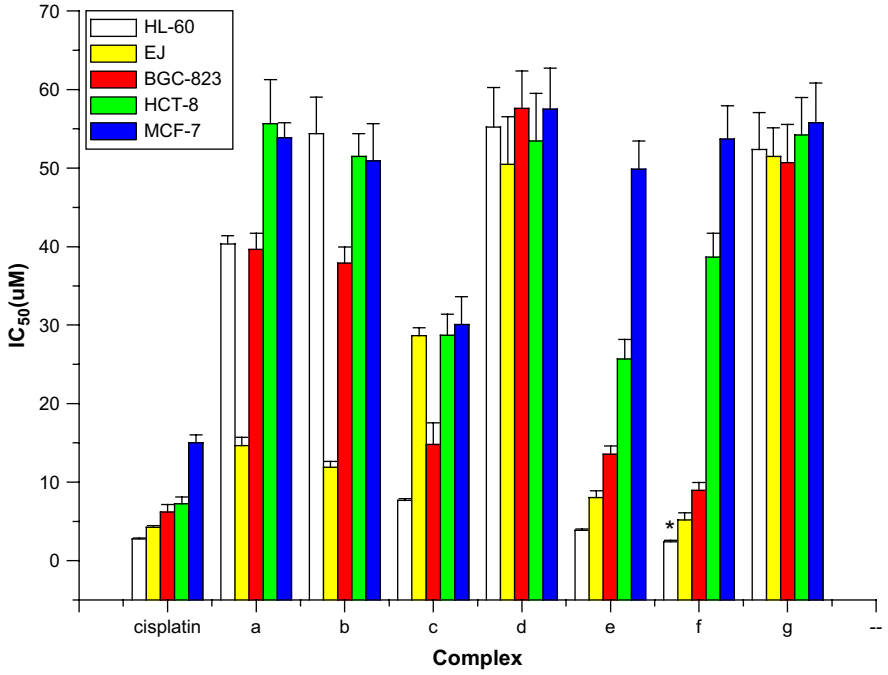

Fig. 2. Cytotoxicity of the complexes against various human carcinoma cell lines (*$P > 0.05$ vs. cisplatin, $n = 3$).

Table 6
The effects of the complexes on cell cycle ($n = 3$)

Complex	Concentration (μM)	Cell cycle (%) ($\bar{x} \pm s$)		
		G_1	S	G_2/M
Control	0.00	31.34 ± 1.06	60.85 ± 1.41	7.80 ± 1.18
Cisplatin	1.25	16.56 ± 0.89***	50.86 ± 2.18**	32.57 ± 1.73***
	2.50	2.15 ± 0.18***	48.60 ± 1.00***	49.25 ± 0.94***
c	1.25	26.47 ± 0.81**	58.45 ± 0.88*	15.08 ± 0.19***
	2.50	23.37 ± 1.00**	54.18 ± 1.59***	22.49 ± 0.79***
e	1.25	21.46 ± 0.86***	56.88 ± 1.65**	21.66 ± 1.46***
	2.50	18.40 ± 0.89***	44.03 ± 1.53***	37.60 ± 0.69***
f	1.25	18.64 ± 0.34***	53.53 ± 0.47***	27.58 ± 0.36***
	2.50	6.72 ± 0.22***	50.66 ± 0.62***	42.62 ± 0.84***

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

deserve 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 a EA-1110 elemental analyzer. Molar conductances at room temperature were measured in 10^{-3} M nitrobenzene using a DSS-11A type conductivitymeter. 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 on Brucker AC 80 NMR spectrometer in D_2O using DSS as an internal standard. The OD at 570 nm was measured on a microplate spectrophotometer (Bio-Rad Model 7550, USA). The cell cycle analysis was performed on a BEC Scan Flow cytometry (Becton–Dickinson). The levels of total platinum bound to DNA were measured by PE Elan-5000 ICP-MS.

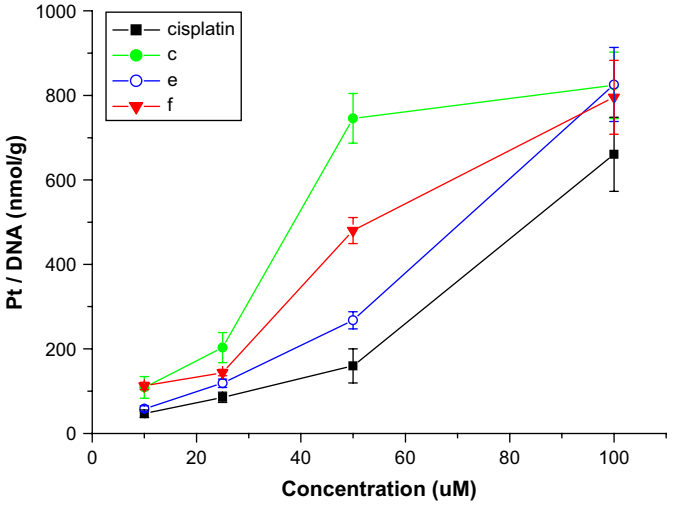

Fig. 3. Levels of total platinum bound to DNA in HL-60 cells ($n = 3$).

6.1.2. Preparation of complexes

Precursor complexes *cis*-$[Pt(C_2H_5NH_2)_2I_2]$ (**i**), $[Pt(C_2H_5NH_2)I_2]_2$ (**ii**) and *cis*-$[Pt(C_2H_5NH_2)(NH_3)I_2]$ (**iii**) were synthesized according to the literatures [14,15].

$[Pt(NH_3)(H_2O)(OOC)_2]$ (**a**): solid Ag_2CO_3 (0.001 mol) was added to a water solution of oxalic acid (0.001 mol) and methanol (5 ml) followed by an equimolar amount of *cis*-$[Pt(C_2H_5NH_2)(NH_3)I_2]$. The mixture was stirred in the dark for 40 h, filtered through celite, and the solution evaporated to dryness under reduced pressure. The pale yellow precipitate was washed 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; yield: 70%.

The synthetic procedure for $[Pt(NH_3)(H_2O)(OOC{-}CH_2{-}COO)]$ (**b**), $[Pt(NH_3)(H_2O)(OOC{-}CH_2CH_2COO)]$ (**c**), $[Pt(NH_3)(H_2O)(OOC{-}CH{=}CH{-}COO)]$ (**d**), $[Pt(NH_3)(H_2O)(p{-}OCH_3{-}C_6H_4{-}OOC)_2]$ (**e**) and $[Pt(NH_3)(H_2O)(OOC{-}C_6H_5)_2]$ (**f**), $[Pt(NH_3)(H_2O)(OOC{-}CH_3)_2]$ (**g**) 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 (gastrocarcinoma), EJ (bladder carcinoma) and HCT-8 (colon 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 seeded equivalently into a 96-well plate, complexes were then 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 [23]. 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 [24]. 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 plot of % viability against dose of compounds added.

6.2.3. Cell cycle analysis

The cell cycle analysis was performed as described by Ferlini et al. [25]. HL-60 cells were treated with platinum complexes for the times indicated 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 from 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. [21]. Briefly, approximately 5×10^7 HL-60 cells were seeded in tissue-culture flasks, then the complexes were added with a concentration gradient, each concentration in triplicate, 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 then harvested, and DNA was extracted according to DNA extraction kit procedure. The purity and concentration of DNA were 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 three separate experiments. The results are expressed as mean ± sd. The statistical differences were analyzed using SPSS' *t*-test. Values of *P* 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 Pat. Anticancer 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. Anticancer Agents 5 (2005) 267–279.
[7] L.S. Hollis, A.R. Amundsen, E.W. Stern, J. Med. Chem. 32 (1989) 128–136.
[8] A.L. Pinto, S.J. Lippard, Biochim. Biophys. Acta 780 (1985) 167–180.
[9] J.P. Macquet, J.L. Butour, J. Natl. Cancer Inst. 70 (1983) 899–905.
[10] C.L. Baird, A.E. Griffitts, S. Baffic, P. Bryant, B. Wolf, J. Lutton, M. Berardini, G.M. Arvanitis, Inorg. Chim. Acta 256 (1997) 253–262.
[11] E.L.M. Lempers, M.J. Bloemink, J. Brouwer, Y. Kidani, J. Reedijk, J. Inorg. Biochem. 40 (1990) 23–35.
[12] L.S. Hollis, W.I. Sundquist, J.N. Burstyn, W.J. Heiger-Bernays, S.F. Bellon, K.J. Ahmed, A.R. Amundsen, E.W. Stern, S.J. Lippard, Cancer Res. 51 (1991) 1866–1875.
[13] J. Zou, P.Y. Dou, K. Wang, J. Inorg. Biochem. 65 (1997) 145–149.
[14] A.R. Khokhar, Y.J. Deng, S. Al-Baker, M. Yoshida, Z.H. Siddik, J. Inorg. Biochem. 51 (1993) 677–687.
[15] J.C. Zhang, Y. Shen, Synth. React. Inorg. Met.-Org. Nano-Met. Chem. 36 (2006) 345–351.
[16] W.J. Geary, Coord. Chem. Rev. 7 (1971) 81–122.
[17] G.B. Deacon, R.J. Phillips, Coord. Chem. Rev. 33 (1980) 227–250.
[18] Z. Yang, P.J. Faustino, P.A. Andrews, R. Monastra, A.A. Rasmussen, C.D. Ellison, K.J. Cullen, Cancer Chemother. Pharmacol. 46 (2000) 255–262.
[19] X. Luo, I. Budihardjo, H. Zou, C. Slaughter, X. Wang, Cell 94 (1998) 481–490.
[20] T. Mueller, W. Voigt, H. Simon, A. Fruehauf, A. Bulankin, A. Grothey, H.J. Schmoll, Cancer Res. 63 (2003) 513–521.
[21] K.J. Mellish, C.F. Barnard, B.A. Murrer, L.R. Kelland, Int. J. Cancer 62 (1995) 717–723.
[22] K. Wang, J. Lu, R. Li, Coord. Chem. Rev. 151 (1996) 53–88.
[23] T.R. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[24] S.P. Fricker, Toxicol. In Vitro 8 (1994) 821–822.
[25] C. Ferlini, S.D. Cesare, G. Rainaldi, V. Malorni, P. Samoggia, R. Biselli, A. Fattorossi, Cytometry 24 (1996) 106–115.