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Laboratory note

Synthesis, characterization and cytotoxicity of ammine/ethylamine platinum(II) complexes with carboxylates

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ABSTRACT

Six new mixed ammine/ethylamine platinum(II) complexes with carboxylates [Pt(II)(NH_3)($C_2H_5NH_2$)X_2] (**a**–**f**) (X = CH_3COO^-, CH_2ClCOO^-, C_6H_5–COO^-, *p*-CH_3–C_6H_4–COO^-, *p*-CH_3O–C_6H_4–COO^-, *p*-NO_2–C_6H_4–COO^-) (**a**–**f**) have been synthesized and characterized by elemental analysis, conductivity, spectra techniques (IR, UV and ^1H NMR). The cytotoxicity of the complexes was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The relative reactivity of leaving groups of complex (**c**) with G-actin was determined spectrophotometrically at 412 nm. The results show that the complexes (**a**–**f**) confer substantially greater cytotoxicity against EJ and HL-60 than the other carcinoma cell lines, moreover, the cytotoxicity of complexes (**c**–**e**) is equal to that of cisplatin against HL-60, and the cytotoxicity of complex (**c**) is also equal to that of cisplatin against EJ. However the complexes (**a**–**f**) are significantly less potent than cisplatin against BGC-823, HCT-8 and MCF-7. The reactivity of leaving groups decreases in the sequence: cisplatin > **c** > carboplatin. The results suggest that ammine/ethylamine platinum(II) complexes with carboxylate anion as leaving group have selectivity against carcinoma cell lines. When leaving group is aromatic carboxylate ion, the complexes have better cytotoxicity, moreover, the substitution radical in benzene ring also influences cytotoxicity.

1. Introduction

By now, cisplatin has become one of the most successful antineoplastic drugs. Despite its success, cisplatin has serious side effects that 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. So far only carboplatin and oxaliplatin have received worldwide approval, nedaplatin, lobaplatin and heptaplatin have gained regionally limited approval and a few platinum drugs continue to be evaluated in clinical studies. Therefore, research work is still worthwhile [1–4].

The mixed ammine/amine platinum complexes with chloro ions as leaving groups have been reported and demonstrated better cytotoxicity against cisplatin-resistant cells *in vitro* and more less toxicity than cisplatin [5–8]. 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 of carboplatin reduces the nephrotoxic and neurotoxic side effects of cisplatin, increases the efficacy of the drug and helps to circumvent drug resistance [9]. So platinum complexes with carboxylate ions as leaving groups may be quite promising than the corresponding chloro analogs. Previously we reported the synthesis, characterization and cytotoxicity of some mixed ammine/amine platinum(II) complexes with carboxylate as leaving groups [10–12]. In the present work, the synthesis and cytotoxicity of six new mixed ammine/ethylamine platinum(II) complexes with carboxylates as leaving groups are reported and discussed.

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 literatures [8,12].

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Six new mixed ammine/ethylamine platinum(II) complexes with carboxylate ions as leaving groups $[Pt(C_2H_5NH_2)(NH_3)(CH_3COO)_2]$ (**a**), $[Pt(C_2H_5NH_2)(NH_3)(CH_2ClCOO)_2]$ (**b**), $[Pt(C_2H_5NH_2)(NH_3)(C_6H_5{-}COO)_2]$ (**c**), $[Pt(C_2H_5NH_2)(NH_3)(p\text{-}CH_3{-}C_6H_5{-}COO)_2]$ (**d**), $[Pt(C_2H_5NH_2)(NH_3)(p\text{-}CH_3O{-}C_6H_5{-}COO)_2]$ (**e**) and $[Pt(C_2H_5NH_2)(NH_3)(p\text{-}NO_2{-}C_6H_5{-}COO)_2]$ (**f**) were prepared by adding *cis*-$[Pt(CH_3CH_2NH_2)(NH_3)I_2]$ to an aqueous solution of $AgNO_3$, and the mixture was allowed to stir in the dark. AgI was removed and a slight excess of the sodium salt of the carboxylic acid was added to the filtrate. After 12 h, the mixture was evaporated to dryness under reduced pressure condition and washed a few times with a minimum quantity of cold water (0–4 °C). The final product was dried over P_2O_5 under vacuum condition.

3. Pharmacology

In the present study we investigated the cytotoxicity of the six 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 (gastrocarcinoma) and EJ (bladder carcinoma) by MTT assay. The relative reactivity of complex (**c**) with G-actin was determined spectrophotometrically at 412 nm.

4. Results and discussion

4.1. Physical properties of the complexes

The physical properties of the complexes are presented in Table 1. There is good agreement between the calculated and the found values. Low molar conductances (A_M, 6.32–7.68 $\Omega^{-1}\,cm^2\,mol^{-1}$) for the complexes (**a**–**f**) correspond to non-electrolytes [13].

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} and δ_{NH} in the precursor complexes (**i**–**iii**) and new complexes (**a**–**f**) shift to lower frequencies than those of free ammine and ethylamine. New band appears at about 470 cm^{-1} and is assigned to Pt–N stretching. Thus it indicates that they are coordinated with platinum through nitrogen atoms. The carboxylate group of the complexes (**a**–**f**) shows two bands, an intense antisymmetric carboxylate stretching $\upsilon_{(as,coo^-)}$ and a symmetric carboxylate stretching $\upsilon_{(s,coo^-)}$, at about 1650 and 1380 cm^{-1}, respectively. The values of $\Delta\upsilon_{(coo^-)}(\upsilon_{(as,coo^-)} - \upsilon_{(s,coo^-)})$ of the complexes (**a**–**f**) are in the range 229–271 cm^{-1}, which is greater than $\Delta\upsilon_{(coo^-)}$ of the corresponding sodium carboxylates, so the carboxylate group may be monodentate coordinated through oxygen atoms [14]. This is further confirmed by the appearance of the peaks of υ_{Pt-O}.

Table 1
Physical properties of the complexes.

Complex	Colour	Yields (%)	Found (Calcd.) (%)			
			C	N	H	Pt
(**i**)	Yellow		8.78 (8.91)	5.18 (5.20)	2.49 (2.62)	–
(**ii**)	Red brown		4.91 (4.86)	2.85 (2.84)	1.44 (1.43)	–
(**iii**)	Yellow		4.65 (4.70)	5.38 (5.48)	1.89 (1.97)	–
a	Pale yellow	51	19.15 (19.20)	7.23 (7.47)	4.15 (4.30)	51.86 (51.98)
b	Pale yellow	45	16.09 (16.22)	6.16 (6.31)	3.12 (3.18)	43.89 (43.92)
c	Pale yellow	42	38.32 (38.48)	5.70 (5.61)	4.13 (4.04)	39.25 (39.06)
d	Pale yellow	46	40.86 (40.98)	5.21 (5.31)	4.60 (4.59)	36.78 (36.99)
e	Pale yellow	39	38.48 (38.64)	5.08 (5.01)	4.40 (4.32)	34.68 (34.87)
f	Pale yellow	48	32.48 (32.60)	9.38 (9.51)	3.05 (3.08)	33.25 (33.10)

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

Complex	υ_{NH}	δ_{NH}	$\upsilon_{(as,coo^-)}$	$\upsilon_{(s,coo^-)}$	$\Delta\upsilon_{(coo^-)}$	υ_{Pt-O}	υ_{Pt-N}
(**i**)	3268, 3220	1575					470
(**ii**)	3225, 3130	1570					475
(**iii**)	3270, 3190	1560					470
a	3230, 3150	1550	1630	1401	229	578	475
b	3258, 3156	1549	1650	1406	244	590	475
c	3229, 3156	1535	1630	1360	270	595	470
d	3230, 3158	1535	1635	1368	267	585	476
e	3219, 3110	1536	1660	1389	271	590	475
f	3250, 3135	1536	1649	1381	268	585	476

4.3. Electronic spectra

As listed in Table 3, after formation of the complexes, no absorption peak appears for complex (**a**), one new absorption peak appears for complex (**b**) at 199.0 nm, E_2 band blue shifts by ca. 9.0, 17.0, 33.0 and 16.0 nm, B band blue shifts by ca. 25.0, 31.0, 24.0 and 3.0 nm for the complex (**c**)–(**f**) compared with the free ligands, respectively.

4.4. ^1H NMR

The chemical shift (δ, ppm) of the complexes (**a**–**f**) was listed as follows: (**a**): 2.89 (m, 2H, $-CH_2$), 1.10 (t, 3H, $-CH_3$), 2.25 (s, 6H, $-CH_3$); (**b**): 2.90 (m, 2H, $-CH_2$), 1.15 (t, 3H, $-CH_3$), 4.13(s, 4H, $-CH_2-$); (**c**): 2.85 (m, 2H, $-CH_2$), 1.16 (t, 3H, $-CH_3$), 7.65–8.28 (m, 10H, $-C_6H_5$); (**d**): 2.79 (m, 2H, $-CH_2$), 1.18 (t, 3H, $-CH_3$), 7.51–7.96 (m, 8H, $-C_6H_4-$), 2.57 (s, 6H, $-CH_3$); (**e**): 2.83 (m, 2H, $-CH_2$), 1.19 (t, 3H, $-CH_3$), 7.38–8.10 (m, 8H, $-C_6H_4-$), 4.00 (s, 6H, $-OCH_3$); (**f**): 2.80 (m, 2H, $-CH_2$), 1.21 (t, 3H, $-CH_3$), 8.39–8.58 (m, 8H, $-C_6H_4$). After formation of the complexes, the hydrogen protons of the complexes shift to low field compared with those of free ligands. This is also further confirmed that the carboxylate and ethylamine are coordinated with platinum through oxygen and nitrogen atoms.

According to the literature [15], the binuclear platinum complexes $[PtLI_2]_2$ were synthesized from the reaction of *cis*-$[Pt(L)_2I_2]$ (L = amine) 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. Moreover the dimer was the *trans* isomer. 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 highly insoluble in water. The mixed ammine/amine platinum(II) complexes with carboxylate ions as leaving groups can be prepared by *cis*-$[Pt(L)(L')I_2]$. (See Fig. 1).

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

4.5. Cytotoxicity effect

As listed in Table 4, the complexes (**a**–**f**) confer substantially greater cytotoxicity against EJ and HL-60 than the other carcinoma

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

Complex	λ/nm		
	$n \rightarrow \pi^*$	E_2 band	B band
a	–	–	–
b	199.0		
c		186.0	225.0
d		193.0	234.0
e		194.0	247.0
f		196.0	268.0

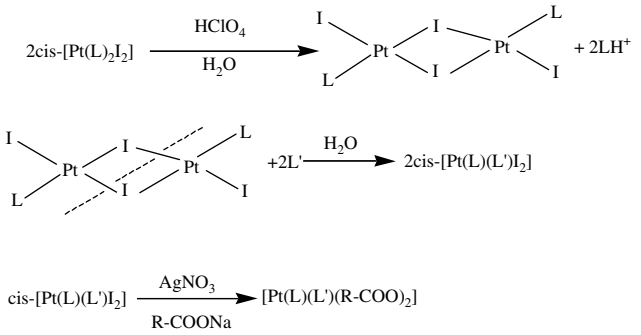

Fig. 1. The synthetic routines of $[Pt(L)(L')(RCOO)_2]$.

cell lines, moreover, the cytotoxicity of complexes (**c**–**e**) is equal to that of cisplatin against HL-60, and the cytotoxicity of complex (**c**) is also equal to that of cisplatin against EJ. However the complexes (**a**–**f**) are significantly less potent than cisplatin against BGC-823, HCT-8 and MCF-7.

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 by 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 [2]. So the reactivity of leaving groups is an important factor to affect anticancer activity. For ammine/ethylamine platinum(II) complexes with carboxylate anions as leaving groups, they have selectivity against carcinoma cell lines. When leaving groups are aromatic carboxylate ions, the complexes have better cytotoxicity, moreover, the substitution radical in benzene ring also influences cytotoxicity. In addition, ethylamine has also effect on the cytotoxicity of complexes.

4.6. Relative reactivity of the platinum complexes with thiol groups of G-actin

As shown in Fig. 3, the reactivity of leaving groups decreases in the sequence: cisplatin > **c** > carboplatin. The ligand exchange reactions of leaving groups of platinum complexes with biological nucleophiles are likely governing their antitumor and toxic properties. The nephrotoxicity, gastrointestinal toxicity, and possible bone marrow suppression induced by platinum-based antitumor agents might be attributed to the ligand exchange reactions of platinum complexes with the sulfhydryl groups and the subsequent inactivation of essential enzymes and other proteins. The platinum complexes have been shown to react with a series of peptide and proteins, from glutathione, metallothionein to plasma proteins by binding to sulfhydryl groups [16]. In this paper, G-actin was selected to determine the change in sulfhydryl groups in order to evaluate the relative reactivity of the leaving groups with proteins, since it has been shown as one of the critical targets in the cells. Our

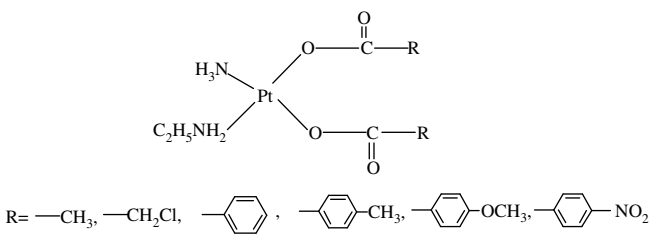

Fig. 2. Possible structures of the complexes (**a**–**f**).

Table 4
Cytotoxicity of complexes (**a**–**f**) against various human carcinomas[a].

Complex	IC_{50} (μM)				
	HCT-8	MCF-7	BGC-823	EJ	HL-60
Cisplatin	7.24 ± 0.89	15.01 ± 1.02	6.18 ± 0.22	4.24 ± 0.23	2.76 ± 0.12
1	25.52 ± 4.36	39.56 ± 4.65	27.68 ± 2.65	8.00 ± 0.28	4.67 ± 0.98
2	26.87 ± 1.65	44.2 ± 4.58	31.2 ± 2.01	8.68 ± 1.03	5.84 ± 1.04
3	14.06 ± 1.87	24.89 ± 4.32	7.86 ± 2.98	4.25 ± 1.35	2.48 ± 0.98
4	18.68 ± 1.87	28.97 ± 2.58	10.98 ± 1.05	5.01 ± 1.78	2.89 ± 0.68
5	18.1 ± 2.56	27.88 ± 3.02	9.98 ± 0.96	4.95 ± 0.68	2.8 ± 0.78
6	22.1 ± 2.68	33.86 ± 4.86	23.54 ± 5.68	7.00 ± 1.02	3.85 ± 0.32
a	25.68 ± 1.78	40.68 ± 3.05	28.68 ± 1.89	8.36 ± 1.02	4.86 ± 0.98
b	26.98 ± 2.65	44.68 ± 4.56	31.87 ± 2.68	8.97 ± 1.89	5.98 ± 1.03
c	14.36 ± 1.89	25.68 ± 1.87	8.03 ± 1.09	4.43 ± 0.86	2.68 ± 0.38
d	19.35 ± 2.03	30.65 ± 4.26	11.23 ± 2.01	5.08 ± 1.03	3.02 ± 0.98
e	18.25 ± 2.65	28.68 ± 1.38	10.02 ± 1.8	4.98 ± 0.38	2.89 ± 0.68
f	22.36 ± 2.08	34.89 ± 3.89	23.68 ± 0.89	7.06 ± 1.85	4.00 ± 0.45

[a] The IC_{50} (μM) of $[Pt(CH_3CH_2CH_2NH_2)(NH_3)X_2]$ (**1**–**6**) (X = CH_3COO^-, CH_2ClCOO^-, $C_6H_5COO^-$, *p*-CH_3–C_6H_4–COO^-, *p*-CH_3O–C_6H_4–COO^- and *p*-NO_2–C_6H_4–COO^-) was cited from Ref. [12].

results suggest that platinum complexes with bidentate dicarboxylate or monodentate carboxylate as leaving groups appear to be less reactive with sulfhydryl groups than cisplatin, but platinum complexes with bidentate dicarboxylate as leaving group appear to be less reactive with sulfhydryl groups than platinum complexes with monodentate carboxylate as leaving group.

5. Conclusion

The preliminary cytotoxicity screening program revealed that mixed ammine/ethylamine platinum(II) complexes with carboxylate anions as leaving groups induced 50% inhibition of the cell viability of BGC-823, HCT-8, MCF-7, EJ and HL-60 cells at micromolar concentrations and may be considered as biologically active. Moreover, they have better cytotoxicity against EJ and HL-60 than the other carcinoma cell lines, the cytotoxicity of complexes (**c**–**e**) is equal to that of cisplatin against HL-60, and the cytotoxicity of complex (**c**) is also equal to that of cisplatin against EJ. However the complexes (**a**–**f**) are significantly less potent than cisplatin against BGC-823, HCT-8 and MCF-7. The reactivity of leaving groups decreases in the sequence: cisplatin > **c** > carboplatin. The results suggest that ammine/ethylamine platinum(II) complexes with carboxylate anions as leaving groups have selectivity against

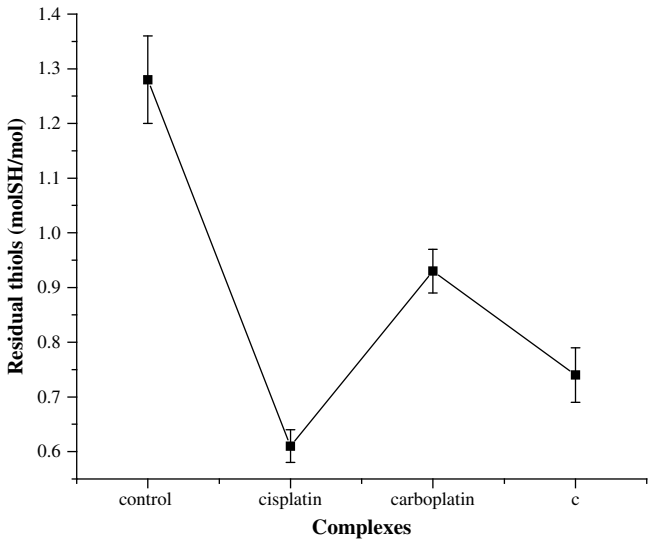

Fig. 3. Relative reactivity of the platinum complexes with thiol groups of G-actin.

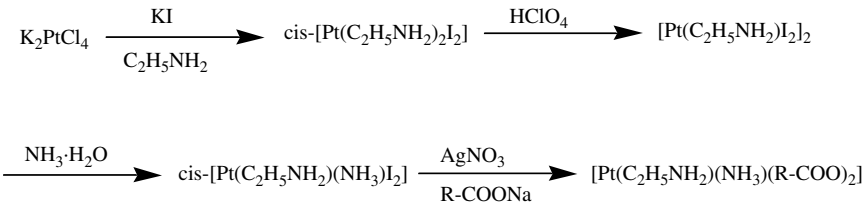

Fig. 4. The synthetic routines of the mixed ammine/ethylamine platinum(II) complexes with carboxylates.

carcinoma cell lines. The leaving groups have effects on their cytotoxicity, when leaving groups are aromatic carboxylates, the complexes have better cytotoxicity, and moreover, the substituent in benzene ring also influences cytotoxicity. The platinum complexes with bidentate dicarboxylate or monodentate carboxylate as leaving groups appear to be less reactive with sulfhydryl groups than cisplatin, but platinum complexes with bidentate dicarboxylate as leaving group appear to be less reactive with sulfhydryl groups than platinum complexes with monodentate carboxylate as leaving group. In addition, ethylamine has also effect on the cytotoxicity of complexes. Thus mixed ammine/ethylamine platinum(II) complexes represent a novel class of anticancer agents, which 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 an EA-1110 elemental analyzer. Molar conductances at room temperature were measured in 10^{-3} M aqueous solutions using a DSS-11A type conductivity meter. The IR spectra were recorded in the 400–4000 cm^{-1} range using KBr pellets and a Perkin–Elmer Model-683 spectrophotometer. The electronic spectra in H_2O were measured on an UV-3400 Toshniwal spectrophotometer. The ^1H NMR spectra in D_2O was recorded on a Brucker AV 400 NMR spectrometer. 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*-$[Pt(C_2H_5NH_2)_2I_2]$ (**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 [8,12].

$[Pt(C_2H_5NH_2)(NH_3)(CH_3COO)_2]$ (**a**): *cis*-$[Pt(C_2H_5NH_2)(NH_3)I_2]$ (0.511 g, 1 mmol) was mixed with $AgNO_3$ (0.336 g, 1.98 mmol) in 15 mL of water. The mixture was allowed to stir overnight in the dark. AgI was removed. When all the silver ions have been removed, a slight excess of the sodium salt of the acetic acid was added to the filtrate. After 8 h, the mixture was evaporated to dryness under reduced pressure condition and washed a few times with a minimum quantity of cold water (0–4 °C). The final product was dried over P_2O_5 under vacuum condition.

The synthetic procedure for $[Pt(C_2H_5NH_2)(NH_3)(CH_2ClCOO)_2]$ (**b**), $[Pt(C_2H_5NH_2)(NH_3)(C_6H_5{-}COO)_2]$ (**c**), $[Pt(C_2H_5NH_2)(NH_3)(p\text{-}CH_3{-}C_6H_5{-}COO)_2]$ (**d**), $[Pt(C_2H_5NH_2)(NH_3)(p\text{-}CH_3O{-}C_6H_5{-}COO)_2]$ (**e**) and $[Pt(C_2H_5NH_2)(NH_3)(p\text{-}NO_2{-}C_6H_5{-}COO)_2]$ (**f**) is similar. The synthetic routines of the mixed ammine/ethylamine platinum(II) complexes with carboxylates are given below (Fig. 4).

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% fetal bovine serum (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 phosphate buffered saline (PBS) and diluted to the required concentration with culture medium when used. The cytotoxicity was evaluated by MTT assay [17]. Briefly, cells were plated in 96-well culture plates (10^4 cells per well) and incubated overnight at 37 °C in a 5% CO_2 incubator. 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. 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 was measured at a wavelength of 570 nm by a microplate spectrophotometer. The IC_{50} value was determined from plots of % viability against dose of complexes added.

6.2.3. Determination of residual sulfhydryl of G-actin

The residual sulfhydryl of G-actin was determined by a modification of the method previously reported [9]. Briefly, the G-actin was incubated with cisplatin, carboplatin and complex (**c**) for 24 h at 4 °C, and then allowed to react with 0.01 mol/L 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) for 15 min at room temperature. The experiment was conducted in a buffer solution of pH 8.0 which is composed of 2 mmol/L Tris, 0.2 mmol/L $CaCl_2$, and 0.005% NaN_3. The concentration of residual thiols of G-actin was determined spectrophotometrically at 412 nm.

6.3. Statistical analysis

Data were collected from at least three separate experiments. The results are expressed as means ± standard deviation (SD).

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