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ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 286–291
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Laboratory note

Synthesis, cytotoxicity and DNA-binding levels of ammine/propylamine platinum(II) complexes with carboxylates

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Received 9 July 2006; received in revised form 19 September 2006; accepted 21 September 2006
Available online 3 November 2006

Abstract

Seven new mixed ammine/propylamine platinum(II) complexes with carboxylates (**a**–**g**) have been synthesized and characterized by elemental analysis, conductivity, IR, UV, and ^{1}H NMR spectra techniques. The cytotoxicity of these complexes was tested by MTT assay. The levels of total platinum bound to DNA were measured by ICP-MS. The results indicate that the complexes (**a**–**g**) have better cytotoxicity against EJ and HL-60 than other carcinoma cell lines. The cytotoxicity increases in the sequence: **g** < **f** < **e** < **d** < **b** < **c** < **a** against tested carcinoma cell lines. The cytotoxicity of complexes (**a**–**c**) is equal to that of cisplatin against HL-60. The cytotoxicity of complex **a** is also equal to that of cisplatin against EJ. However, the complexes (**a**–**g**) are significantly less potent than cisplatin against BGC-823, HCT-8 and MCF-7. The total DNA-platination levels increase in the sequence: cisplatin <**g** < **e** < **f** < **d** < **b** < **c** < **a** under the same experimental conditions. It suggests that there is no correlation between total DNA-platination levels in HL-60 cells and cytotoxicity of ammine/propylamine platinum complexes. When leaving groups are aromatic carboxylates, the complexes have better cytotoxicity, and moreover, the substituent in benzene ring also influences cytotoxicity.

Keywords: Ammine/propylamine platinum(II) complexes; Synthesis; Cytotoxicity; DNA binding

1. Introduction

By now, cisplatin has become one of the most successful antineoplastic drugs. On the one hand because it made metastatic testicular germ-cell cancer a curable disease in about 90% of cases, and on the other hand for its notable therapeutic efficacy in a broad range of other solid tumors. 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].

The mixed ammine/amine platinum complexes with chloride ions as leaving groups have been reported and have demonstrated better activity against cisplatin-resistant cells in vitro and much less toxicity than the parent cisplatin. For example, JM-216 has entered phase III studies and it is currently evaluated clinically as an orally active Pt drug [5–8]. The possible advantage of platinum anticancer drugs with decreased reactivity of leaving group is an established approach which commenced

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doi:10.1016/j.ejmech.2006.09.013

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. So carboxylate platinum complexes seem to be more promising than the corresponding chloro analogs [9]. So far, few mixed ammine/amine platinum(II) complexes with carboxylates are reported. We previously reported the synthesis and antitumor activity of platinum(II) complexes of mixed ammine/amine with bidentate carboxylates [10,11]. Here, the synthesis, cytotoxicity and DNA-binding levels of newly mixed ammine/propylamine platinum(II) complexes with carboxylates are reported and discussed.

2. Chemistry

All reagents and solvents were of analytical grade.

Precursor complexes *cis*-[Pt($CH_3CH_2CH_2NH_2$)$_2$I$_2$] (**i**), [Pt($CH_3CH_2CH_2NH_2$)I$_2$]$_2$ (**ii**) and *cis*-[Pt($CH_3CH_2CH_2NH_2$)(NH_3)I$_2$] (**iii**) were synthesized according to published procedures [5,11].

Seven new mixed ammine/propylamine platinum(II) complexes with carboxylates [Pt($CH_3CH_2CH_2NH_2$)(NH_3)X$_2$] (**a**–**g**) (X = 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^-, CH_3COO^-, CH_2ClCOO^- and $CHCl_2COO^-$) were prepared by adding *cis*-[Pt($CH_3CH_2CH_2NH_2$)(NH_3)I$_2$] to an aqueous $AgNO_3$ solution and the mixture was allowed to stir in the dark. The AgI precipitate 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 and 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 (gastricarcinoma), EJ (bladder carcinoma) and HCT-8 (colon carcinoma) using the standard MTT assay. At the same time, the levels of total platinum bound to DNA in HL-60 cells were measured by ICP-MS.

4. Results and discussion

4.1. Physical properties of the complexes

The physical properties of the complexes are presented in Table 1. There is a good agreement between calculated and found values. Low molar conductances (8.96–12.68 S cm^2 mol^{-1}) for the complexes (**a**–**g**) correspond to non-electrolytes [12].

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 propylamine. Thus it indicates that ammine and propylamine 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 of $\Delta\nu_{(coo^-)}(\nu_{(as,\ coo^-)} - \nu_{(s,\ coo^-)})$ of the complexes (**a**–**g**) are in the range 227–276 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 [13]. This contention is also further confirmed by the presence of ν_{Pt-O} band at about 580 cm^{-1} in the far IR frequency region.

4.3. Electronic spectra

UV spectral data of the complexes are listed in Table 3. After formation of the complexes, E_2 band blue shifts by ca. 6.0, 16.0, 31.0 and 16.0 nm, B band blue shifts by ca. 26.0, 30.0, 24.0 and 2.0 nm for the complexes **a**, **b**, **c** and **d** compared with the free ligands, respectively. Both acetic acid and complex **e** have no absorption peak. No absorption peak appears for chloroacetic acid, after formation of the complexes, and one new absorption peak appears at 199.0 nm for complex **f**. The absorption peak red shifts by ca. 4.0 nm for complex **g**.

4.4. ^{1}H NMR

The chemical shifts (δ, ppm) of complexes (**a**–**g**) are listed in Table 4. After formation of the complexes, the δH of the complexes shift to lower field compared with those of free ligands. This also further confirmed that the carboxylate and propylamine are coordinated with platinum through oxygen and nitrogen atoms.

Based on the above studies and relative literature [5], we propose a tentative coordination structure for the complexes (Fig. 1).

4.5. Cytotoxicity effect

As shown in Fig. 2, the complexes (**a**–**g**) have better cytotoxicity against EJ and HL-60 than other carcinoma cell lines. The cytotoxicity increases in the sequence: **g** < **f** < **e** < **d** < **b** < **c** < **a** against tested carcinoma cell lines. Moreover, the cytotoxicity of complexes (**a**–**c**) is equal to that of cisplatin against HL-60. The cytotoxicity of complex **a** is also equal to that of cisplatin against EJ. But the

Table 1
Elemental analysis data of the complexes

Complex	Yields (%)	Found (calculated) (%)			
		C	N	H	Pt
(i)		12.65 (12.71)	4.93 (4.94)	3.19 (3.20)	–
(ii)		7.08 (7.09)	2.75 (2.76)	1.77 (1.79)	–
(iii)		6.90 (6.86)	5.38 (5.34)	2.29 (2.30)	–
a	50	39.68 (39.76)	5.48 (5.46)	4.29 (4.32)	38.15 (38.00)
b	58	42.09 (42.14)	5.09 (5.17)	4.79 (4.84)	36.15 (36.03)
c	53	39.80 (39.79)	4.76 (4.89)	4.49 (4.57)	34.13 (34.02)
d	61	33.68 (33.83)	9.35 (9.29)	3.24 (3.34)	32.45 (32.33)
e	62	21.63 (21.59)	7.09 (7.20)	4.58 (4.66)	50.23 (50.11)
f	50	18.19 (18.35)	6.25 (6.11)	3.49 (3.52)	42.46 (42.58)
g	46	15.89 (15.95)	5.29 (5.32)	2.73 (2.68)	37.19 (37.01)

complexes (**a**–**g**) are significantly less potent than cisplatin against BGC-823, HCT-8 and MCF-7.

The mode of action of platinum-based anticancer drugs is still not completely understood but it is thought to depend on hydrolysis reactions when 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 and metallothioneins 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 ammine/propylamine platinum(II) complexes with carboxylates as leaving groups, when leaving groups are aromatic carboxylates, the complexes have better cytotoxicity, and moreover, the substituent in benzene ring also influences cytotoxicity.

4.6. DNA binding

As shown in Fig. 3, the total DNA-platination levels increase in the sequence: cisplatin $<$**g** $<$ **e** $<$ **f** $<$ **d** $<$ **b** $<$ **c** $<$ **a**.

It is accepted that DNA is a major target of platinum-based anticancer drugs. So far, it is controversial whether 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 [14]. However, a study on testicular cancer germ cell lines found no association between cisplatin DNA-platination and drug sensitivity and a similar observation has been made on a breast cancer cell line [15,16]. With respect to a correlation between the cytotoxicity of oxaliplatin and DNA-platination, no correlation could be found between DNA-platination and cytotoxicity. Mellish et al. reported no significant correlation between total DNA-platination levels and cytotoxicity of the seven-platinum based drugs in SKOV-3 or in CH1 cell lines [17]. In our work, we found that there was also no correlation between total DNA-platination levels and cytotoxicity of ammine/propylamine platinum 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 [17]. Although the mainstream of investigation has focused on DNA, platinum based drugs might have other important targets apart from nuclear DNA. It has been demonstrated that cisplatin may react with phospholipids, inhibit amino acid transport, protein synthesis, ATPases, uncouple oxidative phosphorylation [18]. Although the importance of these other targets in relation to cytotoxicity is unknown, they might have important effects on cytotoxicity and remained to be further studied.

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

Complex	ν_{NH}	δ_{NH}	$\nu_{(as,\ coo^-)}$	$\nu_{(s,\ coo^-)}$	$\Delta\nu_{(coo^-)}$	ν_{Pt-O}	ν_{Pt-N}
(i)	3258, 3218	1580					472
(ii)	3253, 3147	1575					473
(iii)	3281, 3186	1568					472
a	3225, 3150	1529	1632	1363	269	593	470
b	3225, 3149	1533	1634	1369	265	582	475
c	3220, 3115	1540	1668	1392	276	586	474
d	3246, 3128	1540	1646	1383	263	580	475
e	3225, 3147	1546	1625	1398	227	575	473
f	3247, 3149	1552	1658	1418	240	586	473
g	3246, 3135	1558	1649	1414	235	583	470

Table 3
UV spectral data of the complexes

Complex	λ (nm)		
	$n \rightarrow \pi^*$	E_2 band	B band
Benzoic acid		195.0	250.0
p-Toluic acid		210.0	265.0
p-Anisic acid		227.0	271.0
p-Nitrobenzoic acid		212.0	271.0
Acetic acid	–	–	–
Chloroacetic acid	–	–	–
Dichloroacetic acid	218.0		
a		189.0	224.0
b		194.0	235.0
c		196.0	247.0
d		196.0	269.0
e	–	–	–
f	199.0		
g	222.0		

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

Complex	Chemical shift (δ, ppm)
Propylamine	0.90 (t, 3H, $-CH_3$), 1.45 (m, 2H, $-CH_2-CH_3$), 2.65 (t, 2H, $-CH_2-NH_2$)
Benzoic acid	7.45–8.12 (m, 5H, $-C_6H_5$)
p-Toluic acid	2.34 (s, 3H, $-CH_3$), 7.30–7.87 (m, 4H, $-C_6H_4-$)
p-Anisic acid	3.84 (s, 3H, $-OCH_3$), 7.07–7.91 (m, 4H, $-C_6H_4-$)
p-Nitrobenzoic acid	8.24–8.38 (m, 4H, $-C_6H_4$)
Acetic acid	2.10 (s, 3H, $-CH_3$)
Chloroacetic acid	4.00 (s, 2H, $-CH_2-$)
Dichloroacetic acid	6.20 (s, 1H, $-CHCl_2$)
a	0.98 (t, 3H, $-CH_3$), 1.56 (m, 2H, $-CH_2-CH_3$), 2.89 (t, 2H, $-CH_2-NH_2$), 7.67–8.26 (m, 10H, $-C_6H_5$)
b	0.97 (t, 3H, $-CH_3$), 1.58 (m, 2H, $-CH_2-CH_3$), 2.56 (s, 6H, $-CH_3$), 2.86 (t, 2H, $-CH_2-NH_2$), 7.50–7.98 (m, 8H, $-C_6H_4-$)
c	0.96 (t, 3H, $-CH_3$), 1.54 (m, 2H, $-CH_2-CH_3$), 2.84 (t, 2H, $-CH_2-NH_2$), 4.05 (s, 6H, $-OCH_3$), 7.36–8.12 (m, 8H, $-C_6H_4-$)
d	0.95 (t, 3H, $-CH_3$), 1.60 (m, 2H, $-CH_2-CH_3$), 2.91 (t, 2H, $-CH_2-NH_2$), 8.37–8.56 (m, 8H, $-C_6H_4$)
e	0.99 (t, 3H, $-CH_3$), 1.62 (m, 2H, $-CH_2-CH_3$), 2.27 (s, 6H, $-CH_3$), 2.89 (t, 2H, $-CH_2-NH_2$)
f	1.00 (t, 3H, $-CH_3$), 1.63 (m, 2H, $-CH_2-CH_3$), 2.83 (t, 2H, $-CH_2-NH_2$), 4.19 (s, 4H, $Cl-CH_2-$)
g	0.99 (t, 3H, $-CH_3$), 1.59 (m, 2H, $-CH_2-CH_3$), 2.81 (t, 2H, $-CH_2-NH_2$), 6.49 (s, 2H, $-CHCl_2$)

5. Conclusion

The preliminary cytotoxicity screening program revealed that mixed ammine/propylamine 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. Moreover, they have better cytotoxicity against EJ and HL-60 than the other carcinoma cell lines; the cytotoxicity of complexes (**a**–**c**) is equal to that of cisplatin against HL-60. The cytotoxicity of complex **a** is also equal to that of cisplatin against EJ. 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. Thus mixed ammine/propylamine 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 a 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 ^{1}H NMR spectra were recorded on a Brucker AV 400 NMR spectrometer in D_2O using DSS as an internal standard. The optical density (OD) at 570 nm was measured on a microplate spectrophotometer (Bio-Rad Model 680, USA). The level of total platinum bound to DNA was measured by PE Elan-5000 ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

6.1.2. Preparation of complexes

Precursor complexes *cis*-$[Pt(CH_3CH_2CH_2NH_2)_2I_2]$ (**i**), $[Pt(CH_3CH_2CH_2NH_2)I_2]_2$ (**ii**) and *cis*-$[Pt(CH_3CH_2CH_2NH_2)(NH_3)I_2]$ (**iii**) were synthesized according to the literatures [5,11].

$[Pt(CH_3CH_2CH_2NH_2)(NH_3)(C_6H_5-COO)_2]$ (**a**): *cis*-$[Pt(CH_3CH_2CH_2NH_2)(NH_3)I_2]$ (0.525 g, 1 mmol) was mixed with $AgNO_3$ (0.336 g, 1.98 mmol) in water (20 ml). The mixture was allowed to stir in the dark. The AgI precipitate was removed; a slight excess of the sodium salt of benzoic acid was added to the filtrate. After 12 h, the mixture was evaporated to dryness under reduced pressure and washed a few times with a minimum quantity of very cold water (0–4 °C). The final product $[Pt(CH_3CH_2CH_2NH_2)(NH_3)(C_6H_5-COO)_2]$ was dried over P_2O_5 under vacuum. Yield: 50%.

The synthetic procedures for $[Pt(CH_3CH_2CH_2NH_2)(NH_3)(p\text{-}CH_3-C_6H_5-COO)_2]$ (**b**), $[Pt(CH_3CH_2CH_2NH_2)(NH_3)(p$-

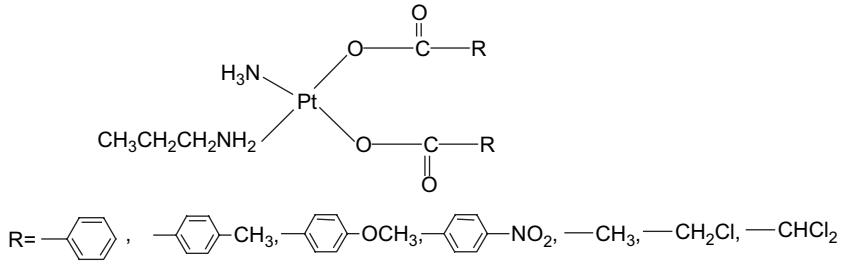

Fig. 1. Possible structures of the complexes (**a**–**g**).

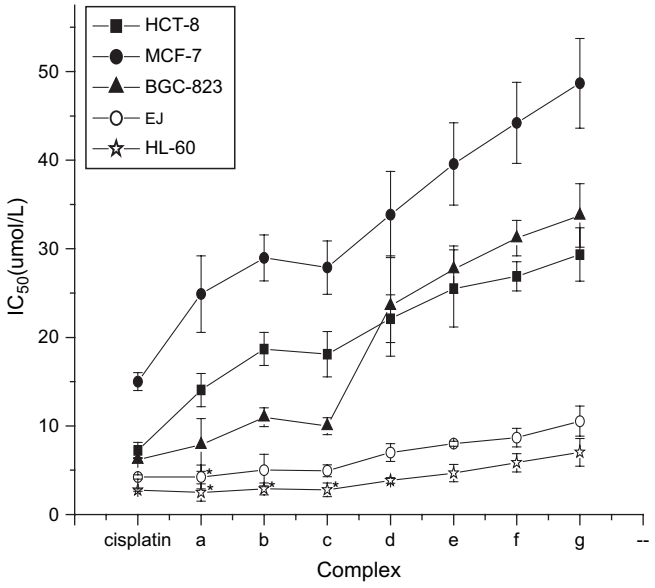

Fig. 2. Cytotoxicity of complexes against various human carcinomas (*$P > 0.05$ vs cisplatin, $n = 5$).

$CH_3O{-}C_6H_5{-}COO)_2]$ (**c**), $[Pt(CH_3CH_2CH_2NH_2)(NH_3)(p\text{-}NO_2{-}C_6H_5{-}COO)_2]$ (**d**), $[Pt(CH_3CH_2CH_2NH_2)(NH_3)(CH_3COO)_2]$ (**e**), $[Pt(CH_3CH_2CH_2NH_2)(NH_3)(CH_2ClCOO)_2]$ (**f**) and $[Pt(CH_3CH_2CH_2NH_2)(NH_3)(CHCl_2COO)_2]$ (**g**) were similar. The synthetic routines of the mixed ammine/propylamine 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 (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 (Gibco) supplemented with 10% fetal bovine serum (Gibco), 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.

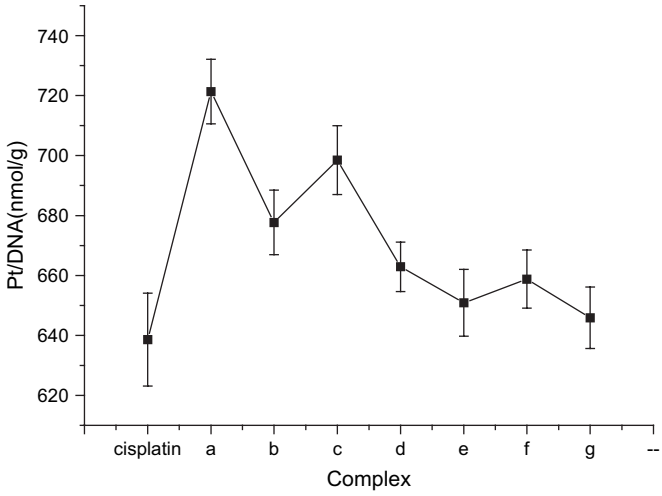

Fig. 3. Levels of total platinum bound to DNA in HL-60 cells ($n = 3$).

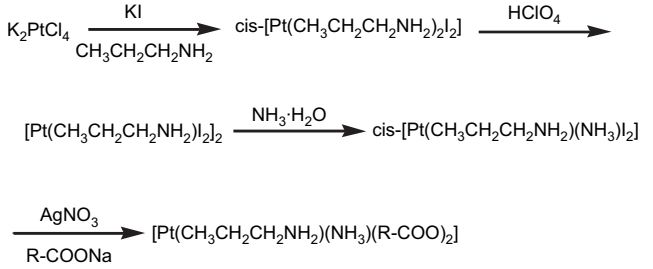

Fig. 4. The synthetic routines of the mixed ammine/propylamine platinum(II) complexes with carboxylates.

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 [19]. Briefly, cells were plated in 96-well microassay culture plates (10^4 cells per well) and grown overnight at 37 °C in a 5% CO_2 incubator. 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. At same time, the cisplatin (Qi Lu Pharmaceutical Factory, China) was used as positive control. The plates were incubated at 37 °C in a 5% CO_2 incubator for 48 h. Upon completion of the incubation, stock MTT (Sigma) 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 optical density of each well was then measured on a microplate spectrophotometer at a wavelength of 570 nm. The IC_{50} value was determined from plots of % viability against dose of complexes added.

6.2.3. DNA binding

The levels of total platinum bound to DNA in HL-60 cells were performed as described by Mellish et al. [17]. Briefly, approximately 5×10^7 HL-60 cells were seeded in tissue-culture flasks, then the complexes were added in a concentration gradient, each concentration in triplicate, and the final concentrations were maintained at 100 μM. 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 (Sigma) 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.

6.2.4. Statistical analysis

Data were collected from at least three separate experiments. The results are expressed as means ± sd.

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