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Water-soluble platinum(II) complexes of reduced amino acid Schiff bases: synthesis, characterization, and antitumor activity

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Abstract A series of water-soluble platinum(II) complexes of reduced amino acid Schiff bases were synthesized as potential anticancer agents and characterized by ^{1}H NMR, EA, MS, IR, and molar conductivity. These compounds were tested for their DNA interaction with salmon sperm DNA, and their in vitro anticancer activities have been validated against HL-60, KB, BGC-823, and Bel-7402 cell lines by the MTT assay. The cytotoxicity of one complex (**5g**) is better than that of cisplatin against BGC-823 and HL-60 cell lines, and show close cytotoxic effect against Bel-7402 cell line.

Keywords Water-soluble platinum(II) complexes · Reduced amino acid Schiff bases · DNA interaction · Antitumor activity

Introduction

The biological activity of cisplatin was discovered fortuitously in 1965 during studies of the effect of an electric current on *Escherichia coli* [1]. Currently, cisplatin is being used as an anticancer agent in several human cancers, particularly ovarian, testicular, bladder, and head and neck cancers [2–4]. However, structural analogues to cisplatin have not yet overcome cisplatin's clinical limitations, particularly with regard to a dose-limiting toxicity and the lower activity (acquired resistance) in some of the most common cancers, and therefore, many platinum complexes have been designed and synthesized to overcome such disadvantages of cisplatin [5–7]. A drug that could be effectively delivered orally is strongly desirable, as it would allow substantial flexibility in dosing and increase the potential for the use of platinum drugs. However, an effective oral formulation of

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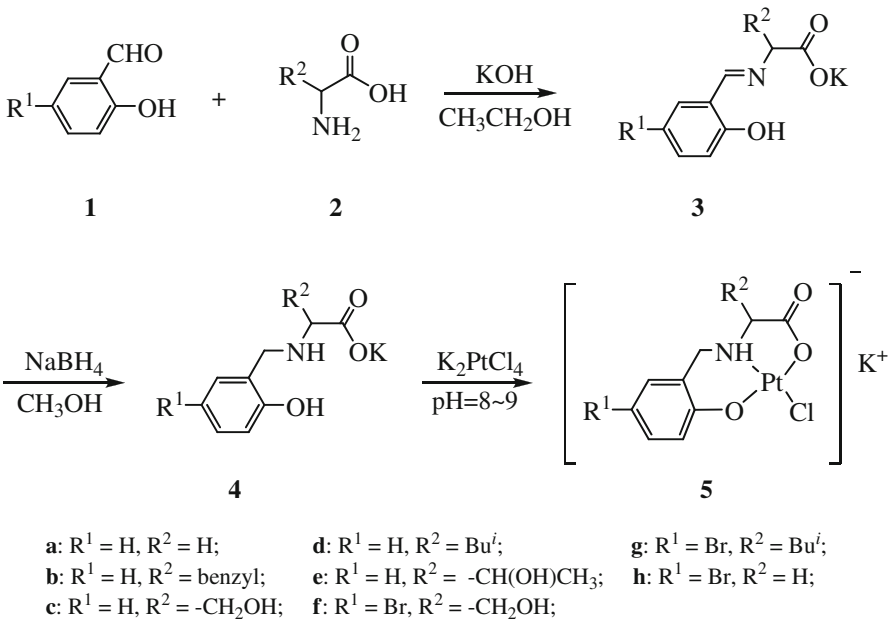
cisplatin is not achievable at the clinical level because of its poor water solubility and low level of bioavailability [8]. Overcoming the adverse effects and eventual resistance, the Pt(II) compounds should be tailored to meet the various requirements such as water solubility, chemical stability, biodegradability, tumor targeting, and so on.

Over the last few decades, transition metal complexes have been well studied for their application as artificial nucleases, because of their diverse structural features and the possibility to tune their redox potential through the choice of ligands [9–11]. Transition metal complexes containing Schiff-base ligands and their reduced products are often used as artificial chemical nucleases and some of such complexes have proved to be efficient DNA cleavage reagents [12, 13]. Most of the model studies of the metal complexes of Schiff-base ligands containing salicylaldehyde and amino acids have focused upon the binding mode of these ligands [14–16]. Structural studies on the metal complexes of reduced Schiff-base ligands, derived from various amino acids and salicylaldehyde, are well documented [17–19]. Compared to Schiff bases, the corresponding reduced ones are expected to be more stable and adaptable to form conformationally flexible five- or six-membered rings upon complexation as they are not constrained to be planar. Although there are numerous reports on transition metal complexes of reduced Schiff bases derived from amino acids [17, 20], information on the corresponding derivatives of platinum(II) is still very scanty. We have prepared a series of reduced amino acid Schiff bases coordinated to platinum(II), the interaction between salmon sperm DNA and these water-soluble complexes was investigated by ultraviolet spectrum and their antitumor activities have also been tested on HL-60, Bel-7402, BGC-823, and KB cell lines in vitro.

Results and discussion

Ligands **4a**–**h** are synthesized from salicylaldehyde and 5-bromsalicylaldehyde with *L*-amino acid: Gly, Phe, Ser, Thr, and Leu. The platinum(II) complexes **5a**–**h** are prepared by the reaction of K_2PtCl_4 with reduced amino acid Schiff bases in CH_3OH/H_2O at room temperature (See Scheme 1).

Comparison of the IR spectra of the free ligands with that of their Pt(II) complexes, the (N–H) stretching frequencies were found in free ligands at about 3,370–3,450 cm^{-1}, while in complexes shifted to high wavenumbers at about 3,410–3,480 cm^{-1}. On the other hand, the $(COO^-)_{carboxyl}$ asymmetric stretching frequency and symmetric stretching frequency was found at about 1,580–1,630 cm^{-1} and 1,450–1,480 cm^{-1} in the free ligands, whereas shifted to about 1,600–1,650 cm^{-1} and 1,380–1,480 cm^{-1} in the complexes. In the spectra of a metal complexes, the bands for the phenolic group (C–O) shifted from about 1,250–1,290 cm^{-1} to 1,260–1,340 cm^{-1}, comparing to the free ligands. New bands appeared at about 610–630 cm^{-1}, 510–550 cm^{-1} and 430–480 cm^{-1} and were may assigned to υ_{Pt-OAr} and υ_{Pt-N}, and $\upsilon_{Pt-OOC,}$ respectively. The IR spectra of **4g** and **5g** was shown in Fig. 1. All of these indicated that nitrogen atoms of the imino groups



a: R^1 = H, R^2 = H; **d**: R^1 = H, R^2 = Bu^i; **g**: R^1 = Br, R^2 = Bu^i;
b: R^1 = H, R^2 = benzyl; **e**: R^1 = H, R^2 = -CH(OH)CH_3; **h**: R^1 = Br, R^2 = H;
c: R^1 = H, R^2 = -CH_2OH; **f**: R^1 = Br, R^2 = -CH_2OH;

Scheme 1 Synthetic pathway for the preparation of compounds

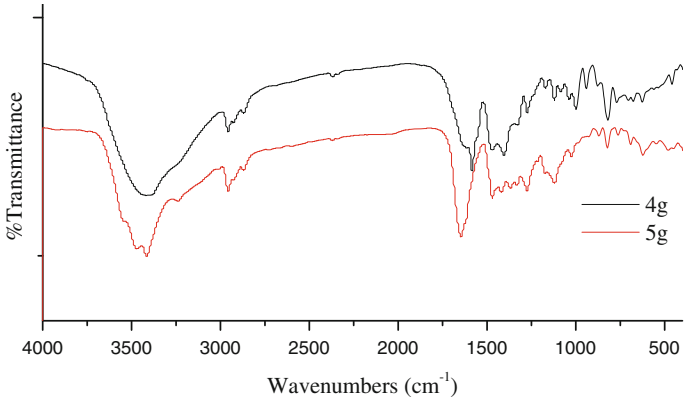


Fig. 1 The IR spectrum of **4g** and **5g**

and oxygen atoms of the carboxyl groups and phenolic groups are coordinated to the metal ion (see Table 1).

Although the overall pattern of the ^{1}H NMR spectra of the complexes (**5a–h**) was very closely to that of the free ligands, the signals have been shifted to lower fields. The mass spectra of the complexes (**5a–h**) have molecular peaks and the elemental analysis data were in good agreement with the calculated values. The conductivity data of all complexes were measured. The molar conductance values of platinum(II) complexes

Table 1 Main IR absorptions of ligands and complexes (cm^{-1})

	υ (N–H)	υ_{as} (COO^-)	υ_s (COO^-)	υ (ph-O)	υ (Pt–OAr)	υ (Pt–N)	υ (Pt-OOC)
4a	3381	1613	1455	1254			
4b	3420	1589	1454	1257			
4c	3414	1618	1478	1270			
4d	3378	1591	1459	1255			
4e	3417	1594	1450	1283			
4f	3445	1624	1473	1281			
4g	3418	1583	1470	1274			
4h	3371	1613	1464	1255			
5a	3417	1638	1474	1264	622	517	476
5b	3471	1642	1480	1269	616	533	478
5c	3421	1648	1384	1265	615	525	476
5d	3414	1643	1447	1270	615	544	477
5e	3438	1610	1453	1271	632	525	468
5f	3422	1604	1413	1269	627	512	434
5g	3471	1645	1418	1331	623	547	480
5h	3426	1650	1471	1263	617	516	476

in deionized water are in the range of 1.32–1.60 S cm^2 mol^{-1}. All complexes therefore formulated as 1:1 electrolytes having one non-bonded K^+ counter ion.

Interaction between complexes and DNA

The interactions between DNA and complexes were characterized by UV spectra. Some of DNA binding drugs produce direct DNA strand breaks: Some possess planar multiring chromophores and are also able to bind noncovalently to DNA by intercalation between base pairs; Other drugs bind into the minor groove of DNA [21]. In the wavelength range of 200–400 nm, DNA with different concentrations of complexes (**5b**, **5c**, and **5e**) were determined (See Figs. 2, 4). As can be seen from Fig. 2, there was one positive peak at 258 nm (A = 1.3293) due to the absorption of DNA. With the increased concentration of complexes **5b** ($c_{\mathbf{5b}}$:$c_{\mathbf{DNA}}$ = 0.1, 0.3 and 0.5), the absorbance values were increased (A = 1.3906, 1.4730, and 1.5168), and the abosorption at 258 nm shifted to low wavelength (λ = 257, 256 and 255 nm). Also, in Fig. 3, there was one similar positive peak at 258 nm (A = 1.3563). When the concentration of complexes **5c** ($c_{\mathbf{5c}}$:$c_{\mathbf{DNA}}$ = 0.1, 0.3 and 0.5) was increased, the absorbance values were enhanced (A = 1.3977, 1.4256 and 1.4773); meanwhile, the abosorption at 258 nm shifted to low wavelength (λ = 257, 256, and 256 nm). In addition, with the same increased concentration of complexes **5e** (Fig. 4), the absorbance values were improved (A = 2.0658, 2.1559, and 2.3337); the abosorption shifted to low wavelength (λ = 257, 256, and 256 nm). It might be due to that the complex was inserted into the DNA double-helix structure and stacking with DNA base pairs where π-electron accumulation occurred, thus the absorbance value increased and maximum absorption wavelength occurred blue-shifted. The results

confirmed that hyperchromicity occurred after coordination and the binding mode of complexes with DNA was might for intercalation [22–24].

In vitro cytotoxic activity

The cytotoxic activities of **5a**–**h** were evaluated against four human cancer cell lines consisting of HL-60, BGC-823, KB, and Bel-7402, and the results are listed in Table 2. As shown in Table 2, almost all the complexes (except **5f**) exerted cytotoxic effects against tested carcinoma cell lines with a lower IC_{50} value (<50 μM), moreover, they have selectivity against tested carcinoma cell lines. Complex **5g** displayed the best cytotoxicity among the eight complexes against tested carcinoma cell lines. It can be seen that complex **5g** was more active against BGC-823 and HL-60 cell lines than cisplatin, fairly active against Bel-7402, but less active than cisplatin against KB cell line. The complexes (**5c, 5d,** and **5g**) were more active than cisplatin against HL-60 cell line, and the **5g** had the best antitumor activity against HL-60 cell line. The complexes (**5b–5e**, **5g–5h**) demonstrated higher cytotoxicity than cisplatin against BGC-823 cell line and the **5c** had the best antitumor activity. Only **5g** displayed fairly anticancer activity against Bel-7402 compared to cisplain. However, **5a** and **5f** did not show any better activity than cisplain against all four cell lines, all the complexes had a little cytotoxicity against KB cell line in compared with cisplatin.

Conclusions

In this study, eight platinum(II) complexes with reduced amino acid Schiff bases were synthesized and characterized. All complexes were tested for their DNA

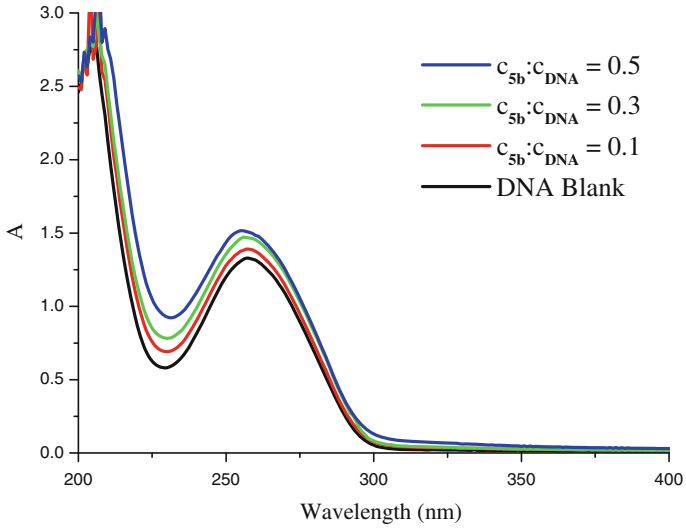


Fig. 2 Ultraviolet spectrum of interaction between **5b** and salmon sperm DNA

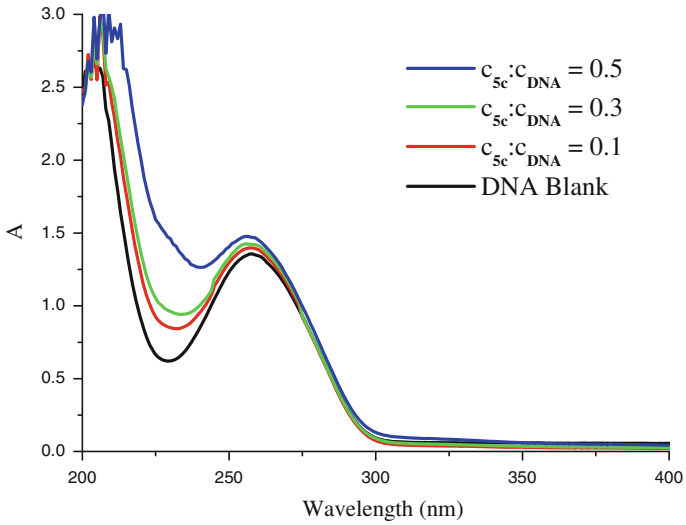


Fig. 3 Ultraviolet spectrum of interaction between **5c** and salmon sperm DNA

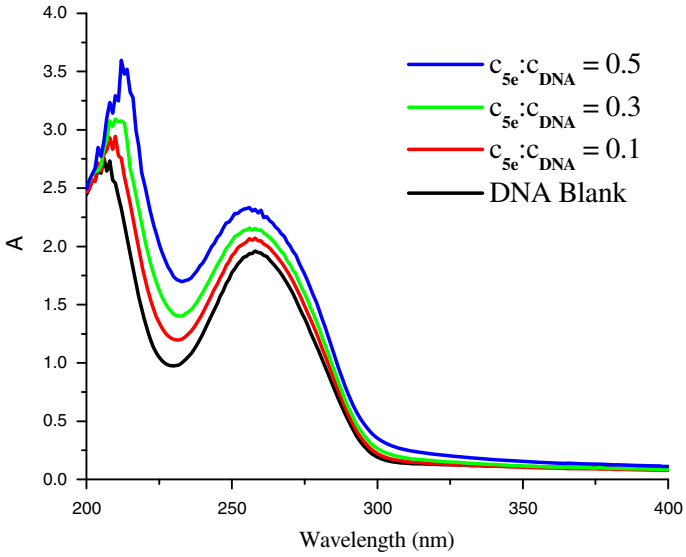


Fig. 4 Ultraviolet spectrum of interaction between **5e** and salmon sperm DNA

interaction ability with the salmon sperm DNA using UV. The results informed that hyperchromicity occurred after coordination and the binding mode of complexes with DNA was might for intercalation. The in vitro anticancer activities of the platinum compounds have been validated against the HL-60, KB, BGC-823, and the Bel-7402 cell lines by the MTT assay and the results show that the complexes (**5a–h**) have selectivity against tested carcinoma cell lines. Complex **5g** has the best

Table 2 The cytotoxicity of complexes in vitro (IC_{50})

Complex	IC_{50} (μM)			
	HL-60	BGC-823	KB	Bel-7402
5a	10.71	39.78	26.03	20.74
5b	4.80	6.36	9.98	20.21
5c	2.85	3.47	7.75	33.91
5d	2.24	5.30	7.35	19.25
5e	3.93	4.79	4.47	13.58
5f	34.71	84.16	91.10	253.45
5g	2.83	4.63	8.60	8.26
5h	4.00	5.68	10.61	22.98
Cisplatin	2.89	6.48	2.65	8.12

cytotoxicity among the eight complexes, moreover its cytotoxicity is better than that of cisplatin against BGC-823 and HL-60 cell lines, and displays close cytotoxic effect against the Bel-7402 cell line. Platinum(II) complexes with reduced amino acid Schiff bases might be a promising source of metal-based antitumor agents.

Experimental

All reagents and chemicals were purchased from commercial sources and used as received. Salicylaldehyde and K_2PtCl_4 were of chemical grade, L-amino acids were of analytical grade, and MTT and salmon sperm DNA were from Sigma. Four different human carcinoma cell lines: HL-60 (immature granulocyte leukemia), Bel-7402 (liver carcinoma), BGC-823 (gastrocarcinoma) and KB (nasopharyngeal carcinoma) were obtained from American Type Culture Collection.

Elemental analysis were determined on an Elemental Vario EL III elemental analyzer. The IR spectra were recorded using KBr pellets and a Perkin-Elmer Model-683 spectrophotometer. The ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker AVIII 600 NMR spectrometer. The mass spectra were measured by Bruker apex-ultra 7.0T. The interaction between DNA and complexes was measured on a UV-3400 Toshniwal spectrophotometer. The conductivity values were determined on a DDSJ-308A conductivity meter. The molar conductivity of 8×10^{-4} mol/L solutions of the complexes **5a–h** in deionized water (K = 0.785 μs/cm) was measured at 10 °C.

Ligand synthesis: general procedure

5-Bromosalicylaldehyde was synthesized according to a published procedure [25]. Amino acid Schiff bases (**3a**–**h**), reduced amino acid Schiff bases (**4a**–**h**) were synthesized according to published procedures [25] and [26], respectively.

Platinum complexes (**5a**–**h**) were synthesized in the following method: K_2PtCl_4 (20 mg, 0.0482 mmol) was added to a CH_3OH/H_2O (v/v = 1/1, 4 mL) solution of reduced Schiff bases (**4a**–**h**) (0.0461 mmol) at room temperature, the mixture was adjusted to pH = 8–9 at the beginning, then stirred for 24 h and the pH was down to about 7. The solution was heated in vacuo and recrystallized from $CH_3OH/CH_3COOC_2H_5$ with N_2 protection.

Potassium 2-(2-hydroxybenzylideneamino)acetate (**3a**)

Light yellow solid (62.21 %): ^{1}H NMR: δ 14.40 (s, 1H, Ar–OH), 8.285 (s, 1H, –CH=N–), 6.650–7.310 (m, 4H, Ar–H), 3.895 (s, 2H, $–CH_2–$); IR: 3411, 1644(C=N), 1604(COO^-)as, 1510, 1384(COO^-)s, 1308, 1218(ph-O), 1187, 734, 563, 523 cm^{-1}. Anal. Calc. for $C_9H_8NO_3K$: C, 49.75; H, 3.71; N, 6.45. Found: C, 49.42; H, 3.95; N, 6.71.

Potassium 2-(2-hydroxybenzylideneamino)-3-phenylpropanoate (**3b**)

Light yellow solid (80.30 %): ^{1}H NMR: δ 14.30 (s, 1H, Ar–OH), 8.09 (s, 1H, –CH=N–), 7.180–7.220 (m, 4H, Ar–H), 6.650–7.170 (m, 5H, Ar–H), 3.800–3.840 (dd, 1H, CH–H), 3.260–3.300 (dd, 1H, CH–H), 2.900–2.950 (dd, 1H, C–H); IR: 3406, 1630(C=N), 1605(COO^-)as, 1512, 1371(COO^-)s, 1215(ph-O), 1145, 1074, 743, 700, 477 cm^{-1}. Anal. Calc. for $C_{16}H_{14}NO_3K$: C, 62.52; H, 4.59; N, 4.56. Found: C, 62.35; H, 4.25; N, 4.80.

Potassium 3-hydroxy-2-(2-hydroxybenzylideneamino)propanoate (**3c**)

Light yellow solid (78.01 %): ^{1}H NMR: δ 14.20 (s, 1H, Ar–OH), 8.35 (s, 1H, –CH=N–), 6.69–7.35 (m, 4H, Ar–H), 3.681–3.702 (t, 1H, C–H), 3.544–3.572 (dd, 1H, CH–H), 3.727–3.754 (dd, 1H, CH–H); IR: 3175, 1633(C=N), 1609(COO^-)as, 1493, 1382(COO^-)s, 1233(ph-O), 1147, 1094, 758, 727, 672, 567 cm^{-1}. Anal. Calc. for $C_{10}H_{10}NO_4K$: C, 48.57; H, 4.08; N, 5.66. Found: C, 48.72; H, 4.42; N, 5.31.

Potassium 2-(2-hydroxybenzylideneamino)-4-methylpentanoate (**3d**)

Light yellow solid (70.14 %): ^{1}H NMR: δ 14.50 (s, 1H, Ar–OH), 8.364 (s, 1H, –CH=N–), 6.660–7.320 (m, 4H, Ar–H), 3.700–3.723 (dd, 1H, N–CH–), 1.692–1.737 (m, 1H, –CH–H), 1.571–1.618 (m, 1H, –CH–H), 1.485–1.551 (m, 1H, CH_3–C–H), 0.879–0.890 (d, 3H, $–CH_3$), 0.849–0.859 (d, 3H, $–CH_3$); IR: 3422, 1640(C=N), 1604(COO^-)as, 1516, 1374(COO^-)s, 1217(ph-O), 1142, 1018, 741, 578, 539 cm^{-1}. Anal. Calc. for $C_{13}H_{16}NO_3K$: C, 57.12; H, 5.90; N, 5.12. Found: C, 57.41; H, 5.47; N, 5.44.

Potassium 3-hydroxy-2-(2-hydroxybenzylideneamino)butanoate (**3e**)

Light yellow solid (58.33 %): ^{1}H NMR: δ 14.33 (s, 1H, Ar–OH), 8.37 (s, 1H, –CH=N–), 6.74–7.36 (m, 4H, Ar–H), 1.031–1.041 (d, 3H, CH3), 3.815–3.852 (m,

1H, O–CH–), 3.618–3.624 (d, 1H, N–CH–); IR: 3382, 1638(C=N), 1609(COO^-)as, 1513, 1375(COO^-)s, 1280, 1225(ph-O), 1128, 1005, 762, 697 cm^{-1}. Anal. Calc. for $C_{11}H_{12}NO_4K$: C, 50.56; H, 4.63; N, 5.36. Found: C, 50.22; H, 4.97; N, 5.14.

Potassium 2-(5-bromo-2-hydroxybenzylideneamino)-3-hydroxypropanoate (**3f**)

Light yellow solid (62.49 %): ^1H NMR: δ 8.299 (s, 1H, –CH=N–), 6.618–7.507 (m, 3H, Ar–H), 3.739–3.766 (dd, 1H, –CH–H), 3.521–3.550 (dd, 1H, –CH–H), 3.700–3.721 (t, 1H, N–CH–); IR: 3424, 1638(C=N), 1607(COO^-)as, 1485, 1378(COO^-)s, 1308, 1230(ph-O), 1164, 1025, 826, 775, 617 cm^{-1}. Anal. Calc. for $C_{10}H_9NO_4BrK$: C, 36.82; H, 2.78; N, 4.29. Found: C, 36.48; H, 2.54; N, 4.57.

Potassium 2-(5-bromo-2-hydroxybenzylideneamino)-4-methylpentanoate (**3g**)

Light yellow solid (74.52 %): ^1H NMR: δ 8.323 (s, 1H, –CH=N–), 6.585–7.479 (m, 3H, Ar–H), 3.706–3.728 (dd, 1H, N–CH–), 1.684–1.729 (m, 1H, –CH–H), 1.553–1.599 (m, 1H, –CH–H), 1.481–1.527 (m, 1H, –CH–), 0.872–0.883 (d, 3H, –CH_3), 0.849–0.860 (d, 3H, –CH_3); IR: 3432, 1638(C=N), 1613 (COO^-)as, 1493, 1378(COO^-)s, 1219(ph-O), 1062, 1018, 868, 827, 615, 560 cm^{-1}. Anal. Calc. for $C_{13}H_{15}NO_3BrK$: C, 44.32; H, 4.29; N, 3.98. Found: C, 44.06; H, 4.57; N, 3.64.

Potassium 2-(5-bromo-2-hydroxybenzylideneamino)acetate (**3h**)

Light yellow solid (81.02 %): ^1H NMR: δ 8.314 (s, 1H, –CH=N–), 6.712–7.603 (m, 3H, Ar–H), 4.290 (s, 2H, –CH_2–); IR: 3428, 1630(C=N), 1612(COO^-)as, 1483, 1374(COO^-)s, 1310, 1224(ph-O), 1056, 837, 725 cm^{-1}. Anal. Calc. for $C_9H_7BrNO_3K$: C, 36.50; H, 2.38; N, 4.73. Found: C, 36.31; H, 2.57; N, 4.45.

Potassium 2-(2-hydroxybenzylamino)acetate (**4a**)

White solid (82.01 %): ^1H NMR: δ 6.916–6.951 (m, 2H, Ar–H), 6.509–6.523 (d, 1H, Ar–H), 6.421–6.440 (t, 1H, Ar–H), 3.587 (s, 2H, Ar–CH_2), 2.895 (s, 2H, –CH_2–); IR: 3381(N–H), 2367, 1613(COO^-)as, 1455(COO^-)s, 1254(ph-O), 1163, 1071, 930, 756, 727 cm^{-1}. Anal. Calc. for $C_9H_{10}NO_3K$: C, 49.30; H, 4.60; N, 6.39. Found: C, 49.52; H, 3.92; N, 6.64.

Potassium 2-(2-hydroxybenzylamino)-3-phenylpropanoate (**4b**)

White solid (87.04 %): ^1H NMR: δ 7.131–7.243 (m, 5H, Ar–H), 6.494–6.985 (m, 4H, Ar–H), 3.712–3.733 (d, 1H, Ar–CH–H), 3.319–3.339 (d, 1H, Ar–CH–H), 3.016–3.037 (m, 2H, –CH_2–), 2.595–2.631 (dd, 1H, N–CH–); IR: 3420(N–H), 1589(COO^-)as, 1454(COO^-)s, 1398, 1257(ph-O), 1017, 866, 754, 700 cm^{-1}. Anal. Calc. for $C_{16}H_{16}NO_3K$: C, 62.11; H, 5.21; N, 4.53. Found: C, 62.53; H, 4.97; N, 5.61.

Potassium 3-hydroxy-2-(2-hydroxybenzylamino)propanoate (**4c**)

White solid (80.11 %): ^{1}H NMR: δ 6.705–7.173 (m, 4H, Ar–H), 3.969–3.991 (d, 1H, Ar–CH–H), 3.300–3.352 (d, 1H, Ar–CH–H), 1.693–1.772 (m, 1H, –CH–), 3.244–3.272 (dd, 1H, CH–H), 3.527–3.554 (dd, 1H, CH–H); IR: 3414(N–H), 1618(COO^-)as, 1478(COO^-)s, 1406, 1270(ph-O), 1129, 1037, 875, 759 cm^{-1}. Anal. Calc. for $C_{10}H_{12}NO_4K$: C, 48.18; H, 4.85; N, 5.62. Found: C, 47.89; H, 4.48; N, 5.81.

Potassium 2-(2-hydroxybenzylamino)-4-methylpentanoate (**4d**)

White solid (84.13 %): ^{1}H NMR: δ 6.614–7.048 (m, 4H, Ar–H), 3.773–3.794 (d, 1H, Ar–CH–H), 3.494–3.516 (d, 1H, Ar–CH–H), 2.761–2.785 (dd, 1H, N–CH–), 1.698–1.777 (m, 1H, –CH–), 1.367–1.412 (m, 1H, CH–H), 1.217–1.263 (m, 1H, CH–H), 0.807–0.818 (d, 3H, $-CH_3$), 0.844–0.855 (d, 3H, $-CH_3$); IR: 3378(N–H), 1591(COO^-)as, 1459(COO^-)s, 1336, 1255(ph-O), 1117, 1003, 941, 826, 753 cm^{-1}. Anal. Calc. for $C_{13}H_{18}NO_3K$: C, 56.70; H, 6.59; N, 5.09. Found: C, 56.43; H, 6.76; N, 5.46.

Potassium 3-hydroxy-2-(2-hydroxybenzylamino)butanoate (**4e**)

White solid (81.07 %): ^{1}H NMR: δ 6.670–7.290 (m, 4H, Ar–H), 3.935–3.957 (d, 1H, Ar–CH–H), 3.660–3.682 (d, 1H, Ar–CH–H), 2.978–2.990 (d, 1H, N–CH–), 3.804–3.837 (q, 1H, O–CH), 1.053–1.142 (m, 3H, $-CH_3$); IR: 3417(N–H), 1594(COO^-)as, 1450(COO^-)s, 1347, 1283(ph-O), 1114, 978, 878, 757 cm^{-1}. Anal. Calc. for $C_{11}H_{14}NO_4K$: C, 50.17; H, 5.36; N, 5.32. Found: C, 50.37; H, 5.52; N, 5.14.

Potassium 2-(5-bromo-2-hydroxybenzylamino)-3-hydroxypropanoate (**4f**)

White solid (78.17 %): ^{1}H NMR: δ 6.427–7.055 (m, 3H, Ar–H), 3.679–3.731 (m, 2H, Ar–CH_2–), 3.554–3.582 (t, 1H, CH–H), 3.350–3.379 (t, 1H, CH–H), 2.793–2.815 (t, 1H, –CH–); IR: 3445(N–H), 1624(COO^-)as, 1473(COO^-)s, 1407, 1281(ph-O), 1102, 873, 820 cm^{-1}. Anal. Calc. for $C_{10}H_{11}BrNO_4K$: C, 36.60; H, 3.38; N, 4.27. Found: C, 36.32; H, 3.51; N, 4.71.

Potassium 2-(5-bromo-2-hydroxybenzylamino)-4-methylpentanoate (**4g**)

White solid (83.12 %): ^{1}H NMR: δ 7.064–7.558 (m, 3H, Ar–H), 4.395–4.417 (d, 1H, Ar–CH–H), 3.946–3.968 (d, 1H, Ar–CH–H), 3.507–3.530 (dd, 1H, N–CH), 2.203–2.259 (m, 1H, –CH–), 1.981–2.025 (m, 1H, CH–H), 1.842–1.888 (m, 1H, CH–H), 1.310–1.321 (d, 3H, $-CH_3$), 1.284–1.295 (d, 3H, $-CH_3$); IR: 3418(N–H), 1583(COO^-)as, 1470(COO^-)s, 1404, 1274(ph-O), 1120, 1037, 999, 819, 770 cm^{-1}. Anal. Calc. for $C_{13}H_{17}BrNO_3K$: C, 44.07; H, 4.84; N, 3.95. Found: C, 44.31; H, 4.53; N, 3.66.

Potassium 2-(5-bromo-2-hydroxybenzylamino)acetate (**4h**)

White solid (81.02 %): ^{1}H NMR: δ 6.564–7.140 (m, 3H, Ar–H), 3.625 (s, 2H, Ar–CH_2), 2.835 (s, 2H, CO–CH_2), 1.608 (s, 1H, NH); IR: 3371(N–H), 1613(COO^-)as, 1464(COO^-)s, 1409, 1255(ph-O), 1007, 816 cm^{-1}. Anal. Calc. for $C_9H_9BrNO_3K$: C, 36.25; H, 3.04; N, 4.70. Found: C, 36.53; H, 3.41; N, 4.52.

K[Pt(2-(2-hydroxybenzylamino)acetoxyl)Cl] (**5a**)

Yellow Solid (63.46 %): ^{1}H NMR: δ 6.464–6.997 (m, 4H, Ar–H), 3.598 (s, 2H, Ar–CH_2), 2.889 (s, 2H, –CH_2–); IR: 3417(N–H), 1638(COO^-)as, 1474(COO^-)s, 1404, 1342, 1264(ph-O), 1026, 622(Pt–OAr), 517(Pt–N), 476(Pt–OOC) cm^{-1}. ESI–MS: 471.9433 $[M + Na]^+$. Anal. Calc. for $C_9H_9ClNO_3KPt$: C, 24.09; H, 2.02; N, 3.12. Found: C, 24.33; H, 2.45; N, 3.22. $\Lambda_m = 1.36$ S cm^2 mol^{-1}.

K[Pt(2-(2-hydroxybenzylamino)-3-phenylpropanyloxy)Cl] (**5b**)

Yellow Solid (65.78 %): ^{1}H NMR: δ 7.124–7.238 (m, 5H, Ar–H), 6.536–7.028 (m, 4H, Ar–H), 3.720–3.742 (d, 1H, Ar–CH–H), 3.326–3.348 (d, 1H, Ar–CH–H), 3.012–3.035 (m, 2H, –CH_2–), 2.598–2.636 (dd, 1H, N–CH–); IR: 3471(N–H), 1642(COO^-)as, 1480(COO^-)s, 1372, 1269(ph-O), 1151, 1030, 867, 754, 616(Pt–OAr), 533 (Pt–N), 478(Pt–OOC) cm^{-1}. ESI–MS: 561.9934 $[M + Na]^+$. Anal. Calc. for $C_{16}H_{15}ClNO_3KPt$: C, 35.66; H, 2.81; N, 2.60. Found: C, 35.24; H, 2.51; N, 2.33. $\Lambda_m = 1.41$ S cm^2 mol^{-1}.

K[Pt(3-hydroxy-2-(2-hydroxybenzylamino)propionyloxy)Cl] (**5c**)

Yellow Solid (66.41 %): ^{1}H NMR: δ 6.736–7.208 (m, 4H, Ar–H), 3.974–3.995 (d, 1H, Ar–CH–H), 3.306–3.359 (d, 1H, Ar–CH–H), 1.688–1.768 (m, 1H, –CH–), 3.242–3.268 (dd, 1H, CH–H), 3.524–3.552 (dd, 1H, CH–H); IR: 3421(N–H), 1648(COO–)as, 1384(COO–)s, 1265(ph-O), 615(Pt–OAr), 525(Pt–N), 476(Pt–OOC) cm^{-1}. ESI–MS: 501.9556 $[M + Na]^+$. Anal. Calc. for $C_{10}H_{11}ClNO_4KPt$: C, 25.08; H, 2.32; N, 2.93. Found: C, 25.31; H, 2.57; N, 2.58. $\Lambda_m = 1.32$ S cm^2 mol^{-1}.

K[Pt(2-(2-hydroxybenzylamino)-4-methylpentanyloxy)Cl] (**5d**)

Yellow Solid (70.32 %): ^{1}H NMR (600 M Hz, DMSO-d6): δ 6.636–7.081 (m, 4H, Ar–H), 3.760–3.795 (q, 1H, Ar–CH–H), 3.491–3.525 (q, 1H, Ar–CH–H), 2.524–2.530 (t, 1H, N–CH–), 1.603 (s, 1H, NH), 0.883–0.906 (m, 1H, –CH–), 1.220–1.254 (m, 1H, CH–H), 1.047–1.085 (m, 1H, CH–H), 0.655–0.859 (m, 6H, CH3); IR: 3414(N–H), 2957, 1643(COO^-)as, 1447(COO^-)s, 1270(ph-O), 1027, 758, 615(Pt–OAr), 544(Pt–N), 477(Pt–OOC) cm^{-1}. ESI–MS: 528.0074 $[M + Na]^+$. Anal. Calc. for $C_{13}H_{17}ClNO_3KPt$: C, 30.92; H, 3.39; N, 2.77. Found: C, 31.21; H, 3.52; N, 2.43. $\Lambda_m = 1.47$ S cm^2 mol^{-1}.

K[Pt(3-hydroxy-2-(2-hydroxybenzylamino)butanyloxy)Cl] (**5e**)

Yellow Solid (68.44 %): ^{1}H NMR: δ 6.712–7.336 (m, 4H, Ar–H), 3.944–3.968 (d, 1H, Ar–CH–H), 3.665–3.688 (d, 1H, Ar–CH–H), 2.974–2.985 (d, 1H, N–CH–), 3.802–3.838 (q, 1H, O–CH), 1.051–1.139 (m, 3H, –CH_3); IR: 3438(N–H), 1610(COO^-)as, 1453(COO^-)s, 1385, 1271(ph-O), 1117, 874, 759, 632(Pt–OAr), 525(Pt–N), 468(Pt–OOC) cm^{-1}. ESI–MS: 476.0235 $[M + Na]^+$. Anal. Calc. for $C_{11}H_{13}ClNO_4KPt$: C, 26.81; H, 2.66; N, 2.84. Found: C, 26.52; H, 2.84; N, 2.51. $\Lambda_m = 1.48$ S cm^2 mol^{-1}.

K[Pt(2-(5-bromo-2-hydroxybenzylamino)-3-hydroxypropanyloxy)Cl] (**5f**)

Yellow Solid (60.27 %): ^{1}H NMR: δ 6.468–7.096 (m, 3H, Ar–H), 3.689–3.748 (m, 2H, Ar–CH_2–), 3.551–3.579 (t, 1H, CH–H), 3.347–3.375 (t, 1H, CH–H), 2.795–2.818 (t, 1H, –CH–); IR: 3422(N–H), 2169, 1604(COO^-)as, 1413(COO^-)s, 1269(ph-O), 1161, 819, 627(Pt–OAr), 512(Pt–N), 434(Pt–OOC) cm^{-1}. ESI–MS: 579.8648 $[M + Na]^+$. Anal. Calc. for $C_{10}H_{10}BrClNO_4KPt$: C, 21.54; H, 1.81; N, 2.51. Found: C, 21.35; H, 1.64; N, 2.77. $\Lambda_m = 1.42$ S cm^2 mol^{-1}.

K[Pt(2-(5-bromo-2-hydroxybenzylamino)-4-methylpentanyloxy)Cl] (**5g**)

Yellow Solid (64.45 %): ^{1}H NMR: δ 7.106–7.613 (m, 3H, Ar–H), 4.408–4.431 (d, 1H, Ar–CH–H), 3.956–3.977 (d, 1H, Ar–CH–H), 3.504–3.525 (dd, 1H, N–CH), 2.205–2.263 (m, 1H, –CH–), 1.984–2.030 (m, 1H, CH–H), 1.838–1.882 (m, 1H, CH–H), 1.313–1.325 (d, 3H, –CH_3), 1.286–1.297 (d, 3H, –CH_3); IR: 3471(N–H), 2957, 1645(COO^-)as, 1418(COO^-)s, 1331(ph-O), 1120, 1024, 822, 623(Pt–OAr), 547(Pt–N), 480(Pt–OOC) cm^{-1}. ESI–MS: 605.9154 $[M + Na]^+$. Anal. Calc. for $C_{13}H_{16}BrClNO_3KPt$: C, 26.75; H, 2.76; N, 2.40. Found: C, 26.41; H, 2.89; N, 2.13. $\Lambda_m = 1.43$ S cm^2 mol^{-1}.

K[Pt(2-(5-bromo-2-hydroxybenzylamino)acetoxyl)Cl] (**5h**)

Yellow Solid (61.13 %): ^{1}H NMR: δ 6.610–7.188 (m, 3H, Ar–H), 3.637 (s, 2H, Ar–CH_2), 2.841 (s, 2H, CO–CH_2); IR: 3426(N–H), 2921, 1650(COO^-)as, 1471(COO^-)s, 1263(ph-O), 1122, 1027, 820, 617(Pt–OAr), 516(Pt–N), 476(Pt–OOC) cm^{-1}. ESI–MS: 549.8545 $[M + Na]^+$. Anal. Calc. for $C_9H_8BrClNO_3KPt$: C, 20.48; H, 1.53; N, 2.65. Found: C, 20.14; H, 1.82; N, 2.33. $\Lambda_m = 1.60$ S cm^2 mol^{-1}.

Determination of UV absorption spectra

An UV-3400 Toshniwal spectrophotometer emitting UV light was used mainly between 200 and 400 nm. The salmon sperm DNA (M = 208.8 g/mol) was dissolved in Tris–HCl (pH = 7.5) buffer solution, and rested in 24 h at 4 °C. Then complexes (**5b**, **5c**, and **5e**) were added in buffer solution in different concentrations

($c_{complex}$:c_{DNA}) = 0.1, 0.3, and 0.5), and rested in 24 h at 4 °C again. The UV absorption spectra was determined at room temperature ($\Delta t = 1$ s, $n = 3$.).

Cell culture

Four different human carcinoma cell lines: HL-60, Bel-7402, BGC-823, and KB were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5 % CO_2 in air.

Solutions

The complexes were dissolved in DMSO at a concentration of 5 mM as stock solution, and diluted in culture medium at concentrations of 0.1, 1.0, and 10 μM as working solution. To avoid DMSO toxicity, the concentration of DMSO was less than 0.1 % (v/v) in all experiments.

Cytotoxicity analysis

The cells harvested from exponential phase were seeded equivalently into a 96-well plate, and then the complexes were added to the wells to achieve final concentrations. Control wells were prepared by addition of culture medium. Wells containing culture medium without cells were used as blanks. All experiments were performed in quintuplicate. The MTT assay was performed as described by Mosmann for HL-60 [27]. Upon completion of the incubation for 44 h, stock MTT dye solution (20 mL, 5 mg/mL) was added to each well. After 4 h incubation, 2-propanol (100 mL) 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 IC_{50} value was determined from plot of % viability against dose of compounds added.

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References

1. B. Rosenberg, L. VanCamp, T. Krigas, Nature **205**, 698 (1965)
2. B. Rosenberg, Interdiscip. Sci. Rev. **3**, 134 (1978)
3. E. Wong, C.M. Giandomenico, Chem. Rev. **99**, 2451 (1999)
4. E.R. Jamieson, S.J. Lippard, Chem. Rev. **99**, 2467 (1999)
5. T.W. Hambley, Coord. Chem. Rev. **166**, 181 (1997)
6. Y.P. Ho, K.K.W. To, S.C.F. Au-Yeung, X. Wang, G. Lin, X. Han, J. Med. Chem. **44**, 2065 (2001)
7. Y.S. Kim, R. Song, D.H. Kim, M.J. Jun, Y.S. Sohn, Bioorg. Med. Chem. **11**, 1753 (2003)
8. Y.P. Ho, C.F. Steve, A. Yeung, K.K.W. To, Med. Res. Rev. **23**, 633 (2003)
9. P.U. Maheswari, S. Roy, S. Barends, B. Kozlevcar, P. Gamez, J. Reedijk, J. Am. Chem. Soc. **128**, 710 (2006)
10. J. Qian, W. Gu, H. Liu, F.X. Gao, L. Feng, S.P. Yan, D.Z. Liao, P. Cheng, Dalton Trans. **11**, 1060 (2007)

11. J.L. Tian, L. Feng, W. Gu, G.J. Xu, S.P. Yan, D.Z. Liao, Z.H. Jiang, P. Cheng, Inorg. Biochem. **101**, 96 (2007)
12. J.H. Li, J.T. Wang, Z.W. Mao, L.N. Ji, J. Coord. Chem. **62**, 446 (2009)
13. T.D. Tullius, J.A. Greenbaum, Curr. Opin. Chem. Biol. **9**, 127 (2005)
14. K. Tatsumoto, A.E. Martell, R.J. Motekaitis, J. Am. Chem. Soc. **103**, 6197 (1981)
15. K. Korhonen, R. Hamalainen, U. Turpeinen, Acta Crystallogr. Sect. C. **40**, 1175 (1984)
16. V. Kettmann, E. Fresova, Acta Crystallogr. Sect. C. **49**, 1932 (1993)
17. C.Y. Gao, X.F. Ma, J.L. Tian, D.D. Li, S.H. Yan, J. Coord. Chem. **63**, 115 (2010)
18. B. Sreenivasulu, M. Vetrichelvan, F. Zhao, S. Gao, J.J. Vittal, Eur. J. Inorg. Chem. **2005**, 4635–4645 (2005)
19. R. Ganguly, B. Sreenivasulu, J.J. Vittal, Coordin. Chem. Rev. **252**, 1027 (2008)
20. X.F. Ma, D.D. Li, J.L. Tian, Y.Y. Kou, S.H. Yan, Transit. Metal. Chem. **34**, 475 (2009)
21. A. Skladanowski, P. Bozko, M. Sabisz, Chem. Rev. **109**, 2951 (2009)
22. M. Wang, L.R. Hou, Z.N. Gao, T.L. Yang, Chin. J. Appl. Chem. **25**, 1186 (2008)
23. L.N. Ji, Q.L. Zhang, J.G. Liu, Sci. Chin. (Ser. B) **31**, 193 (2001)
24. P.R. Cao, M.M. McHugh, T. Melendy, T. Beerman, Mol. Cancer Ther. **2**, 651 (2003)
25. C. Elzbieta, E. Zygmunt, K. Romuald, Diss. Pharm. **15**, 369 (1964)
26. M.A. Alam, M. Nethaji, M. Ray, Angew. Chem. Int. Ed. **42**, 1940 (2003)
27. T. Mosmann, J. Immunol. Methods **65**, 55 (1983)