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# Platinum(II) complexes of reduced amino acid ester Schiff bases: synthesis, characterization, and antitumor activity

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

**Keywords** Platinum(II) complexes · Reduced amino acid esters Schiff-bases · DNA interaction · Antitumor activity

## Introduction

Cancer is a biggest health hazard for humanity [1]. Despite the dramatic development of antitumour drugs, the cancer death rate remains constant. Cisplatin is the important currently used platinum-based anticancer drugs [2]. Currently cisplatin is being used as an anticancer agent in several human cancers, particularly in ovarian, testicular, bladder, head, and neck cancers [3, 4]. However, structural analogues to cisplatin have not yet overcome cisplatin's clinical limitations, particularly with regard to a dose-limiting toxicity, such as nephrotoxicity, neurotoxicity, ototoxicity, nausea, etc., and the lower activity (acquired resistance) in some of the most common cancers [5]. In order to overcome severe side effects, numerous platinum-based complexes have been synthesized, like carboplatin and oxaliplatin [6, 7]. Furthermore, there are some

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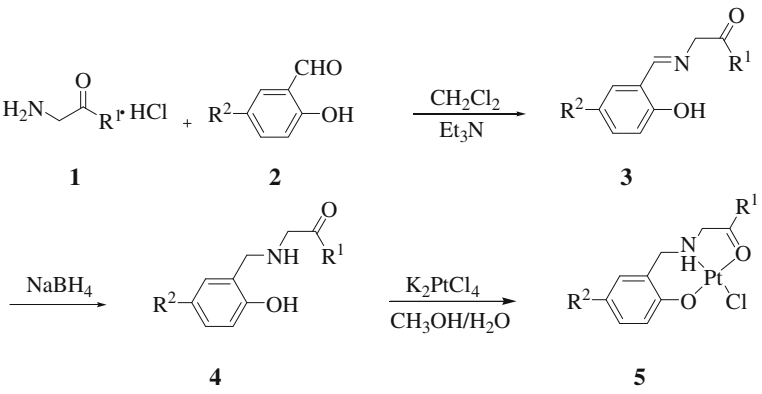
other platinum anticancer drugs like platinum(II)-containing oxaliplatin, carboplatin, picoplatin (JM473), nedaplatin (JM118), or redox-active platinum(IV)-based satraplatin, tetraplatin, and ormaplatin [8].

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 [10–12]. Transition metal complexes containing Schiff base ligands and their reduced products are often used as artificial chemical nucleases, and some of these complexes have proved to be efficient DNA cleavage reagents [13, 14]. Amino acids are the body's essential nutrients. They are the basic unit of body proteins and certain enzymes. Amino acids play an important physiological role in the body. When amino acids are esterified and introduced to the drug molecules, they can enhance the drug lipophilicity, ease drug toxicity, and improve drug bioavailability. Most of the model studies of the metal complexes of Schiff base ligands containing salicylaldehyde and amino acid esters have focused upon the binding mode of these ligands [15–17]. Structural studies on the metal complexes of reduced Schiff base ligands, derived from various amino acid esters and salicylaldehyde, are well documented [18–20]. Compared to the Schiff bases, the corresponding reduced Schiff bases are expected to be more stable and adaptable to form conformationally flexible 5- or 6-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 [21, 22], information on the corresponding derivatives of platinum(II) is still rare. We have prepared a series of reduced Schiff bases derived from amino acid esters coordinated to platinum(II), the interaction between salmon sperm DNA and these complexes was investigated by ultraviolet spectrum and CD 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**–**4f** were synthesized from salicylaldehyde and 5-bromsalicylaldehyde with glycine ester. The platinum(II) complexes **5a**–**5f** have been prepared by the reaction of $K_2PtCl_4$ with reduced Schiff bases derived from glycine esters in a mixture of $CH_3OH/H_2O$ in the 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 3,303–3,352 $cm^{-1}$, while in complexes they shifted to high wavenumbers at about 3,420–3,445 $cm^{-1}$. On the other hand, the (C=O)carbonyl stretching frequency was found at about 1,735–1,742 $cm^{-1}$ in the free ligands, whereas it shifted to about 1,601–1,648 $cm^{-1}$ in the complexes. In the spectra of metal complexes, the bands for the phenolic group (C–O) shifted from about 1,256–1,266 $cm^{-1}$ to 1,263–1,270 $cm^{-1}$, compared to the free ligands. New bands appeared at about 611–620, 519–524, and 412–429 $cm^{-1}$, and were assigned to $\upsilon_{Pt-OAr}$, $\upsilon_{Pt-N}$, and $\upsilon_{Pt-O=C}$, respectively. The IR spectra of **4b** and **5b** are shown in Fig. 1. All of these indicated that nitrogen atoms of




**a**: $R^1$=OMe $R^2$=H; **b**: $R^1$=OEt $R^2$=H; **c**: $R^1$=OMe $R^2$=Br
**d**: $R^1$=OEt $R^2$=Br; **e**: $R^1$=OMe $R^2$=Cl; **f**: $R^1$=OEt $R^2$=Cl

**Scheme 1** Synthetic pathway for the preparation of compounds

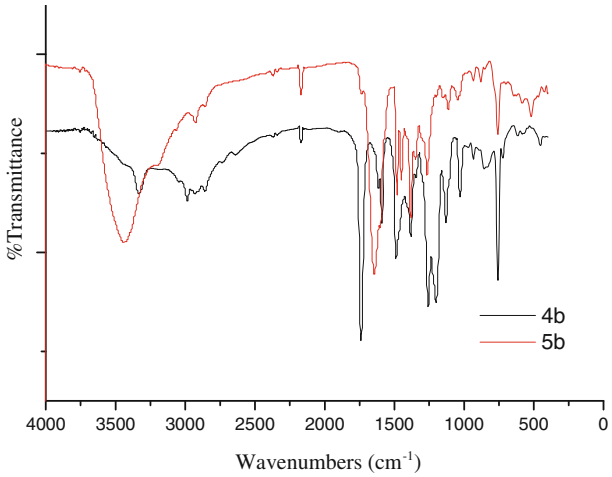



**Fig. 1** The IR spectrum of **4b** and **5b**

the imino groups and oxygen atoms of the carbonyl 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**–**5f** very closely resembled thoswe of the free ligands, the signals have been shifted to lower fields. The mass spectra of the complexes **5a**–**5f** have molecular peaks, and the elemental analysis data of the complexes **5a**–**5f** were in good agreement with the calculated values. The conductivity data of all complexes were also measured. The molar conductance values of platinum(II) complexes in deionized methanol are in the range of 40–60 S cm$^2$ mol$^{-1}$ [23]. All complexes are therefore electroneutral.

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

| | υ (N–H) | υ ($C=O^-$) | υ (ph–O) | υ (Pt–OAr) | υ (Pt–N) | υ (Pt–O=C) |
|---|---|---|---|---|---|---|
| **4a** | 3,352 | 1,735 | 1,256 | | | |
| **4b** | 3,332 | 1,739 | 1,256 | | | |
| **4c** | 3,309 | 1,742 | 1,266 | | | |
| **4d** | 3,303 | 1,737 | 1,266 | | | |
| **4e** | 3,309 | 1,741 | 1,265 | | | |
| **4f** | 3,303 | 1,736 | 1,265 | | | |
| **5a** | 3,420 | 1,631 | 1,270 | 412 | 520 | 619 |
| **5b** | 3,445 | 1,645 | 1,265 | 429 | 519 | 611 |
| **5c** | 3,445 | 1,645 | 1,268 | 420 | 520 | 620 |
| **5d** | 3,426 | 1,643 | 1,265 | 429 | 519 | 615 |
| **5e** | 3,440 | 1,648 | 1,264 | 420 | 524 | 615 |
| **5f** | 3,423 | 1,601 | 1,263 | 429 | 519 | 611 |

## Interaction between complexes and DNA

The interaction between DNA and the complexes was characterized in the ultraviolet spectrum. Some 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 [24]. In the wavelength range of 200–400 nm, DNA with different concentrations of complexes (**5c** and **5d**) was determined (see Figs. 2, 3). As can be seen from Fig. 2, there was one positive peak at 258 nm ($A = 1.4048$) due to the absorption of DNA. With the increased concentration of complexes **5c** ($c_{5c}$:$c_{DNA} = 0.1$, 0.3 and 0.5), the absorbance values were increased ($A = 1.4930$, 1.8788 and 2.3235), and the abosorption at 258 nm shifted to low wavelength ($\lambda = 256$, 255, and 251 nm). Also, in Fig. 3, there was one similar positive peak at 258 nm ($A = 1.4548$). When the concentration of complexes **5d** ($c_{5d}$:$c_{DNA} = 0.1$, 0.3, and 0.5) was increased, the absorbance values were enhanced ($A = 1.8461$, 2.1749, and 2.2781); meanwhile, the abosorption at 258 nm shifed to low wavelength ($\lambda = 257$, 255, and 254 nm). This might be because 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 was blue-shifted. The results confirmed that hyperchromicity occurred after coordination and the binding mode of complexes with DNA was right for intercalation [25–27].

CD (circular dichroism) is a very powerful technique to monitor the conformational state of the DNA double helix in solution. The CD spectrum of DNA displayed a canonical B-form conformation with a large positive band at 270–280 nm and a negative band at 248 nm. Later, the CD spectra of **5d** DNA was recorded and displayed in Fig. 4, where the 275 nm band was due to base stacking and 248 nm band to right-handed helicity. The decrease in the intensity of

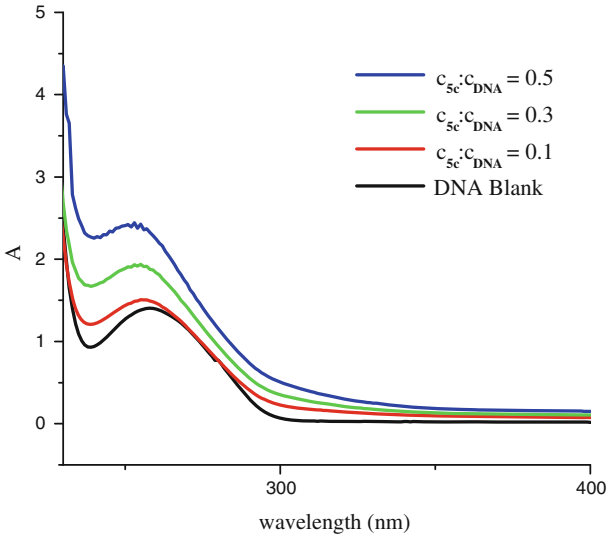



**Fig. 2** Ultraviolet spectrum of interaction between **5c** and salmon sperm DNA

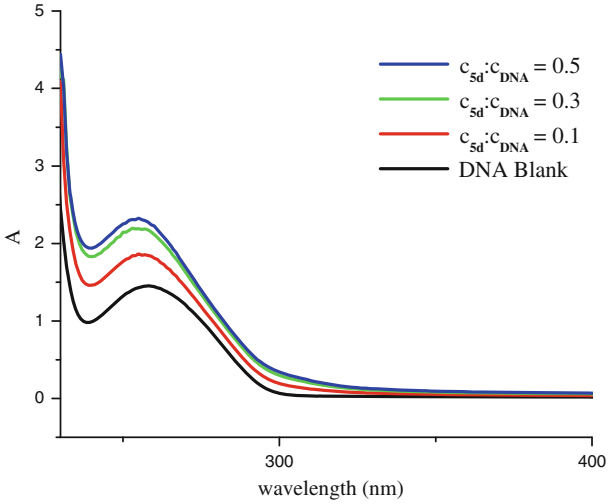



**Fig. 3** Ultraviolet spectrum of interaction between **5d** and salmon sperm DNA

the positive band and the increase in negative bands of **5d** were exhibited, which was typical of intercalation involving $\pi$-stacking and stabilization of the right-handed B form of DNA. This indicates a intercalative binding producing a consequent weakening of the base stacking interactions [28]. So the results confirmed that, after coordination, the binding mode of complexes with DNA might be intercalation.

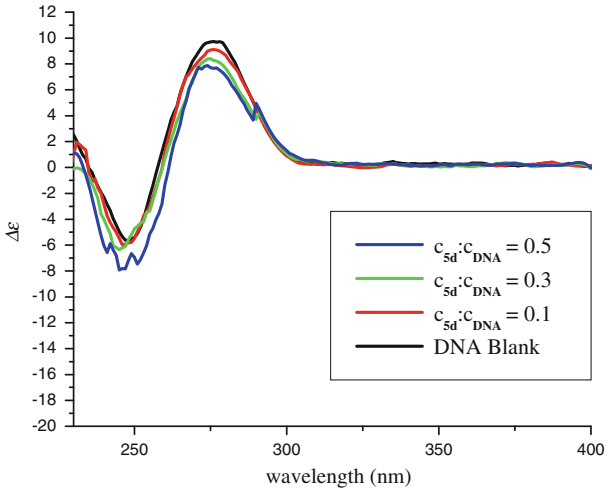



**Fig. 4** CD spectrum of interaction between **5d** and salmon sperm DNA

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

| Complex | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| | Bel-7402 | HL-60 | KB | BGC-823 |
| **5a** | 16.35 | 16.70 | 21.30 | 19.52 |
| **5b** | 18.98 | 19.52 | 24.10 | 24.94 |
| **5c** | 15.82 | 9.92 | 22.70 | 23.05 |
| **5d** | 4.91 | 3.01 | 13.26 | 21.49 |
| **5e** | 14.66 | 10.74 | 24.84 | 26.76 |
| **5f** | 7.70 | 6.87 | 19.68 | 24.77 |
| Cisplatin | 8.12 | 2.89 | 2.65 | 6.84 |

## In vitro cytotoxic activity

The cytotoxic activities of **5a**–**5f** 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 exerted cytotoxic effects against the tested carcinoma cell lines with a lower $IC_{50}$ value (<50 μM); moreover, they have selectivity against the tested carcinoma cell lines. Complex **5c** displayed the best cytotoxicity among the six complexes against the tested carcinoma cell lines. It can be seen that complexes **5d** and **5f** were more active against Bel-7402 cell lines than cisplatin, fairly active against HL-60, but less active than cisplatin against the BGC-823 cell line. The complexes **5a**, **5c**, and **5e** were fairly active against cisplatin and against the Bel-7402 cell line. The complexes **5a**–**5c** and **5e** demonstrated less active cytotoxicity than cisplatin against KB and the HL-60 cell line, while all the complexes had less active cytotoxicity against the BGC-823 cell line in compared with cisplatin.

## Conclusion

In this study, six platinum(II) complexes with reduced Schiff bases derived from glycine ester as ligands were synthesized and characterized. All complexes were tested for their DNA interaction ability with salmon sperm DNA using ultraviolet spectrum and CD spectrum, and the results showed that after coordination the binding mode of complexes with DNA might be intercalation. The in vitro anticancer activities of the platinum complexes have been validated against HL-60, KB, BGC-823, and Bel-7402 cell lines by MTT assay and the results show that the complexes **5a**–**5f** have selectivity against the tested carcinoma cell lines. The complexes **5d** and **5f** have the best cytotoxicity among the six complexes, and moreover their cytotoxicities are better than that of cisplatin against the Bel-7402 cell line, and display a fairly cytotoxic effect against the HL-60 cell line. Platinum(II) complexes with reduced Schiff bases derived from glycine ester 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, 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 the American Type Culture Collection.

### Instrumentation and measurement

Elemental analysis were determined on a Exeter Analytical CE-440 elemental analyzer. The IR spectra were recorded using KBr pellets and a Thermo Nicolet 380 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 an UV-3400 Toshniwal spectrophotometer. The CD spectrum was measured on an MOS-450 (BioLogic).The conductivity values were determined on a DDSJ-308A conductivity meter. Molar conductances at room temperature were measured in $1 \times 10^{-3}$ M methanol using a DDS-12DW-type conductivity meter.

### Synthesis of compounds

#### *Ligands synthesis*

5-Bromosalicylaldehyde was synthesized according to a published procedure [29]. Amino acid esters were synthesized according to a published procedure [30].

The synthesis of amino acid ester reduced Schiff bases were according to one-pot. Amino acid esters (4.68 mmol) were added to the round-bottom flask containing dichloromethane (20 mL), then triethylamine (4.75 mmol) and a certain

amount of anhydrous magnesium sulfate were added to the flask at room temperature, stirring for 1 h. Then salicylaldehyde (4.80 mmol) was added to the dichloromethane solution dropwise, stirring for another 36 h at room temperature. Methanol solution (10 mL) containing sodium borohydride (7.02 mmol) was then added to the above solution and stirred for 3 h, followed by rotary evaporation and washing three times. The amino acid ester-reduced Schiff bases were purified by column chromatography (ethyl acetate/petroleum ether) to give the white solid product.

*Complexes synthesis*

Platinum complexes **5a**–**5f** were synthesized by the following method: $K_2PtCl_4$ (0.0482 mmol) was added to a $CH_3OH/H_2O$ (4 mL, v/v = 1/1) solution of reduced Schiff bases **4a**–**4f** (0.0461 mmol) at room temperature, and the mixture was adjusted to pH 8–9 at the start, then stirred for 24 h, when the pH was down to about 7. The solution was heated in vacuo and recrystallized from $CH_3OH/CH_2Cl_2$ in the $N_2$ protection.

*Synthesis of methyl 2-(2-hydroxybenzylamino)acetate (**4a**)*

White solid. (34.6 %): $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.198–7.224 (t, 1H, Ar–H), 7.003–7.015 (d, 1H, Ar–H), 6.872–6.886 (d, 1H, Ar–H), 6.800–6.825(t, 1H, Ar–H), 4.003 (s, 2H, –$CH_2$–), 3.789 (s, 2H, –$CH_3$), 3.482 (s, 2H, –$CH_2$–); IR (KBr): 3,352 (N–H), 3,003, 1,735 (C=O), 1,587, 1,256 (ph–O), 1,130, 987, 858, 756 cm$^{-1}$. Anal. Calc. for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.18. Found: C, 61.12; H, 6.95; N, 7.31.

*Synthesis of ethyl 2-(2-hydroxybenzylamino)acetate (**4b**)*

White solid. (42.5 %): $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.062–7.100 (m, 2H, Ar–H), 6.720–6.748 (m, 2H, Ar–H), 4.087–4.122 (m, 2H, –$CH_2$–), 3.348 (s, 2H, –$CH_2$–), 3.789 (s, 2H, –$CH_2$–), 1.186–1.210 (t, 3H, –$CH_3$); IR (KBr): 3,332 (N–H), 2,984, 1,739 (C=O), 1,589, 1,256 (ph–O), 1,130, 1,029, 855, 757 cm$^{-1}$. Anal. Calc. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.45; H, 6.89; N, 6.91.

*Synthesis of methyl 2-(5-bromo-2-hydroxybenzylamino)acetate (**4c**)*

White solid. (33.4 %): $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.313–7.317 (d, 1H, Ar–H), 7.210–7.228 (dd, 1H, Ar–H), 6.703–6.717 (d, 1H, Ar–H), 3.722(s, 2H, –$CH_2$–), 3.635 (s, 3H, –$CH_3$), 2.509 (s, 2H, –$CH_2$–); IR (KBr): 3,309 (N–H), 2,921, 1,742 (C=O), 1,577, 1,266 (ph–O), 1,110, 990, 868, 763 cm$^{-1}$. Anal. Calc. for $C_{10}H_{12}BrNO_3$: C, 43.82; H, 4.41; N, 5.11. Found: C, 42.58; H, 4.18; N, 5.38.

*Synthesis of ethyl 2-(5-bromo-2-hydroxybenzylamino)acetate (**4d**)*

White solid. (47.7 %): $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.309–7.314 (d, 1H, Ar–H), 7.209–7.227 (dd, 1H, Ar–H), 6.703–6.717 (d, 1H, Ar–H), 4.079–4.114 (q,

2H, $-CH_2-$), 3.722 (s, 2H, $-CH_2-$), 2.505–2.511 (t, 2H, $-CH_2-$), 1.184–1.208 (s, 3H, $-CH_3$); IR (KBr): 3,303 (N–H), 2,971, 1,737 (C=O), 1,577, 1,266 (ph–O), 1,143, 992, 873, 763 $cm^{-1}$. Anal. Calc. for $C_{11}H_{14}BrNO_3$: C, 45.85; H, 4.90; N, 4.86. Found: C, 45.58; H, 5.31; N, 4.60.

*Synthesis of methyl 2-(5-chloro-2-hydroxybenzylamino)acetate (**4e**)*

White solid. (51.8 %): $^1$H NMR(600 MHz, DMSO-$d_6$): $\delta$ 7.191–7.195 (d, 1H, Ar–H), 7.088–7.106 (dd, 1H, Ar–H), 6.748–6.762 (d, 1H, Ar–H), 3.729 (s, 2H, $-CH_2-$), 3.638 (s, 3H, $-CH_3$), 3.369 (s, 2H, $-CH_2-$); IR (KBr): 3,309 (N–H), 2,924, 1,741 (C=O), 1,580, 1,265 (ph–O), 1,145, 992, 873, 762 $cm^{-1}$. Anal. Calc. for $C_{10}H_{12}ClNO_3$: C, 52.30; H, 5.27; N, 6.10. Found: C, 52.66; H, 5.61; N, 5.82.

*Synthesis of ethyl 2-(5-chloro-2-hydroxybenzylamino)acetate (**4f**)*

White solid. (51.5 %): $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.190–7.193 (d, 1H, Ar–H), 7.089–7.107 (dd, 1H, Ar–H), 6.745–6.759 (d, 1H, Ar–H), 4.079–4.115 (q, 2H, $-CH_2-$), 3.721 (s, 2H, $-CH_2-$), 3.366 (s, 2H, $-CH_2-$), 1.184–1.207 (s, 3H, $-CH_3$); IR (KBr): 3,303 (N–H), 2,973, 1,763 (C=O), 1,581, 1,264 (ph–O), 1,143, 983, 871, 765 $cm^{-1}$. Anal. Calc. for $C_{11}H_{14}ClNO_3$: C, 54.22; H, 5.79; N, 5.75. Found: C, 54.49; H, 5.41; N, 5.49.

*Synthesis of Pt[methyl 2-(2-hydroxybenzylamino)acetate]Cl (**5a**)*

Yellow Solid. (63.46 %): $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.088–7.169 (t, 1H, Ar–H), 6.993–7.005 (d, 1H, Ar–H), 6.812–6.876 (dd, 2H, Ar–H), 4.021 (s, 2H, $-CH_2-$), 3.769 (s, 3H, $-CH_3$), 3.463 (s, 2H, $-CH_2-$); IR (KBr): 3,420 (N–H), 3,170, 1,631 (C=O), 1,404, 1,270 (ph–O), 1,119, 757, 709, 619 (Pt-OAr), 520 (Pt–N), 412 (Pt–O=C) $cm^{-1}$. ESI–MS: 446.9964 $[M + Na]^+$. Anal. Calc. for $C_{10}H_{11}ClNO_3Pt$: C, 28.35; H, 2.62; N, 3.31; Found: C, 28.78; H, 2.26; N, 3.55. $\Lambda_m = 45$ S $cm^2$ $mol^{-1}$.

*Synthesis of Pt[ethyl 2-(2-hydroxybenzylamino)acetate]Cl (**5b**)*

Yellow Solid. (65.78 %): $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 6.972–7.011 (m, 2H, Ar–H), 6.702–6.726 (m, 2H, Ar–H), 4.108–4.151 (m, 2H, $-CH_2-$), 3.368 (s, 2H, $-CH_2-$), 3.769 (s, 2H, $-CH_2-$), 1.178–1.208 (t, 3H, $-CH_3$); IR (KBr): 3,442 (N–H), 2,929, 1,643 (C=O), 1,470, 1,265 (ph–O), 1,123, 823, 670, 644 (Pt-OAr), 519 (Pt–N), 429 (Pt–O=C) $cm^{-1}$. ESI–MS: 461.0121 $[M + Na]^+$. Anal. Calc. for $C_{11}H_{13}ClNO_3Pt$: C, 30.18; H, 2.99; N, 3.20; Found: C, 30.43; H, 2.67; N, 2.91. $\Lambda_m = 48$ S $cm^2$ $mol^{-1}$.

*Synthesis of Pt[methyl 2-(5-bromo-2-hydroxybenzylamino)acetate]Cl (**5c**)*

Yellow Solid. (66.41 %): $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.289–7.296 (d, 1H, Ar–H), 7.206–7.216 (dd, 1H, Ar–H), 6.710-6.726 (d, 1H, Ar–H), 3.730(s, 2H,

–$CH_2$–), 3.641 (s, 3H, –$CH_3$), 2.498 (s, 2H, –$CH_2$–); IR (KBr): 3,445 (N–H), 2,921, 1,645 (C=O), 1,471, 1,268 (ph–O), 1,121, 822, 664, 615 (Pt–OAr), 520 (Pt–N), 420 (Pt–O=C) $cm^{-1}$. ESI–MS: 524.9054 $[M + Na]^+$. Anal. Calc. for $C_{10}H_{10}BrClNO_3$ Pt: C, 23.90; H, 2.01; N, 2.79. Found: C, 23.68; H, 2.43; N, 2.51. $\Lambda_m = 57$ S $cm^2$ $mol^{-1}$.

*Synthesis of Pt[ethyl 2-(5-bromo-2-hydroxybenzylamino)acetate]Cl (**5d**)*

Yellow Solid. (70.32 %): $^1$H NMR (600 M Hz, DMSO-$d_6$): $\delta$ 7.294–7.310 (d, 1H, Ar–H), 7.198–7.216 (dd, 1H, Ar–H), 6.696–6.711 (d, 1H, Ar–H), 4.082–4.118 (m, 2H, –$CH_2$–), 3.728 (s, 2H, –$CH_2$–), 2.498–2.509 (t, 2H, –$CH_2$–), 1.188–1.213 (s, 3H, –$CH_3$); IR (KBr): 3,426 (N–H), 2,927, 1,643 (C=O), 1,481, 1,268 (ph–O), 1,113, 881, 636, 615 (Pt–OAr), 519 (Pt–N), 429 (Pt–O=C) $cm^{-1}$. ESI–MS: 538.9211 $[M + Na]^+$. Anal. Calc. for $C_{11}H_{12}BrClNO_3Pt$: C, 25.57; H, 2.34; N, 2.71. Found: C, 25.88; H, 2.61; N, 2.35. $\Lambda_m = 60$ S $cm^2$ $mol^{-1}$.

*Synthesis of Pt[methyl 2-(5-chloro-2-hydroxybenzylamino)acetate]Cl (**5e**)*

Yellow Solid. (68.44 %): $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.185–7.190 (d, 1H, Ar–H), 7.080–7.098 (dd, 1H, Ar–H), 6.741–6.756 (d, 1H, Ar–H), 3.732 (s, 2H, –$CH_2$–), 3.644 (s, 3H, –$CH_3$), 3.371 (s, 2H, –$CH_2$–); IR (KBr): 3,440 (N–H), 2,926, 1,648 (C=O), 1,473, 1,264 (ph–O), 1,121, 824, 680, 618 (Pt–OAr), 524 (Pt–N), 420 (Pt-O=C) $cm^{-1}$. ESI–MS: 480.9565 $[M + Na]^+$. Anal. Calc. for $C_{10}H_{10}Cl_2NO_3Pt$: C, 26.21; H, 2.20; N, 3.06. Found: C, 26.62; H, 2.44; N, 2.76. $\Lambda_m = 55$ S $cm^2$ $mol^{-1}$.

*Synthesis of Pt[ethyl 2-(5-chloro-2-hydroxybenzylamino)acetate]Cl (**5f**)*

Yellow Solid. (60.27 %): $^1$H NMR (600 MHz, DMSO-$d_6$): $\delta$ 7.182–7.186 (d, 1H, Ar–H), 7.081–7.098 (dd, 1H, Ar–H), 6.738–6.750 (d, 1H, Ar–H), 4.083–4.119 (q, 2H, –$CH_2$–), 3.728 (s, 2H, –$CH_2$–), 3.374(s, 2H, –$CH_2$–), 1.186–1.209 (s, 3H, –$CH_3$); IR (KBr): 3,423 (N–H), 2,912, 1,601 (C=O), 1,473, 1,263 (ph–O), 1,119, 825, 681, 611 (Pt–OAr), 519 (Pt–N), 429 (Pt–O=C) $cm^{-1}$. ESI–MS: 494.9722 $[M + Na]^+$. Anal. Calc. for $C_{11}H_{12}Cl_2NO_3Pt$: C, 27.98; H, 2.56; N, 2.97. Found: C, 27.72; H, 2.85; N, 2.66. $\Lambda_m = 58$ 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 **5c** and **5d** were added in buffer solution in different concentrations ($c_{complex}$:$c_{DNA} = 0.1$, 0.3 and 0.5), and rested for 24 h at 4 °C. 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 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.

### 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 the 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 [31]. 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 a plot of % viability against dose of compounds added.

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