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Synthesis, characterization and cytotoxicity of platinum(II) complexes containing reduced amino acid ester Schiff bases

Qin-Qin Yan | Zhen Yuan | Guo-Jun Liu | Zheng-Hua Lv | Bin Fu | Jian-Long Du | Li-Jun Li

College of Chemistry and Environmental Science, Chemical Biology Key Laboratory of Hebei Province, Hebei University, Baoding 071002, China

Correspondence
Li-Jun Li, College of Chemistry and Environmental Science, Chemical Biology Key Laboratory of Hebei Province, Hebei University, Baoding 071002, China.
Email: lljhbu@163.com

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Nine platinum(II) complexes containing reduced amino acid ester Schiff bases were synthesized and characterized using spectroscopy (^{1}H NMR, ^{13}C NMR, infrared), elemental analysis and molar conductivity. The interaction of these complexes with salmon sperm DNA was investigated by means of ultraviolet and circular dichroism spectroscopies. The potential antitumor activity of all compounds was tested *in vitro* on HeLa and A549 tumor cell lines. Almost all the complexes exhibited better cytotoxic activity than cisplatin against these cell lines.

KEYWORDS

cytotoxicity, DNA interaction, platinum(II) complexes, reduced amino acid ester Schiff bases

1 | INTRODUCTION

Cisplatin, *cis*-[$PtCl_2(NH_3)_2$], was first reported about 170 years ago,[1] but its medical application was discovered accidentally by Rosenberg and co-workers in 1969.[2] Several years later, the antitumor activity of cisplatin was revealed,[3] and in 1978 the US Food and Drug Administration approved this compound for clinical use. However, it has apparent clinical drawbacks including limited applicability, acquired resistance and serious side effects such as nausea, vomiting, ototoxicity, neurotoxicity and nephrotoxicity.[4–6] Therefore, great efforts have been dedicated to the development of improved platinum-based drugs to overcome these shortcomings.[7,8] Although thousands of platinum(II) complexes have been synthesized and screened in the last 35 years,[9,10] only about 35 entered clinical trials.[11–13] Up to now, besides cisplatin, only two further platinum-based anticancer drugs are used routinely in clinics, namely oxaliplatin and carboplatin.[14,15]

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 because of the possibility of tuning their redox potential through the choice of ligands.[16–18] Schiff bases are an important class of compounds in medicinal and pharmaceutical fields. They show biological applications including antibacterial,[19,20] antifungal[21,22] and antitumor[23,24] activities. Amino acids, as the basic unit of proteins and related enzymes, perform critical biological roles including as neurotransmitters and transport in the body, and are essential and indispensable nutrients *in vivo*. When they are introduced to drug molecules after being esterified, the drug lipophilicity, toxicity and bioavailability will be improved. Compared to Schiff bases, the corresponding reduced Schiff bases 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. Numerous papers on transition metal complexes of reduced Schiff bases derived from amino acids or amino acid esters have been published.[25,26] However, information on the corresponding derivatives of platinum(II) is still rare.

In the study reported here, nine platinum(II) complexes with reduced amino acid ester Schiff bases as ligands were synthesized and characterized. Their interaction with salmon sperm DNA was investigated using ultraviolet (UV) and circular dichroism (CD) spectroscopies, and their antitumor activity against a panel of human tumor cell lines, including HeLa and A549 cell lines, was also evaluated.

2 | EXPERIMENTAL

2.1 | Materials

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. Two different human carcinoma cell lines, HeLa (human cervix carcinoma) and A549 (human non-small-cell lung cancer), were obtained from American Type Culture Collection.

2.2 | Instrumentation and measurements

Elemental analysis was conducted using an Exeter Analytical CE-440 elemental analyzer. Infrared (IR) spectra were recorded using KBr pellets and a Thermo Nicolet 380 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in deuterated dimethylsulfoxide (DMSO-d_6) with a Bruker AVIII 600 NMR spectrometer. The interaction between DNA and complexes was measured using a UV-3400 Toshniwal spectrophotometer and a Bio-Logic MOS-450/SFM300 CD spectrometer. The molar conductivities were measured at room temperature in 1×10^{-3} M methanol using a DDS-12DW type conductivity meter.

2.3 | Synthesis of ligands

2.3.1 | Procedure for preparation of 5-chlorosalicylaldehyde

To a three-neck flask, equipped with a reflux condenser, thermometer, nitrogen source and magnetic stirrer, was added *para*-chlorophenol (16 mmol), sodium hydroxide (100 mmol), distilled water (2200 mmol) and chloroform (87.5 mmol). The mixture was heated to reflux for 8 h. After cooling, the reaction mixture was acidified to pH = 2–3 with aqueous 2 M hydrochloric acid, and extracted with ethyl acetate. The reaction was monitored by TLC. The ethyl acetate extract was treated with vacuum distillation and dried, affording crude 5-chlorosalicylaldehyde. The crude product was purified by column chromatography using petroleum ether giving a white solid product (2.158 g, 29.6%); m.p. 90–91 °C. IR (KBr pellet, ν, cm^{-1}): 1685, 1566, 1470, 1275, 1155, 885, 831, 650, 535. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 9.873 (s, 1H, —CHO), 7.354–7.365 (m, 2H, Ar-H), 6.919 (d, 1H, Ar-H).

2.3.2 | Procedure for preparation of 5-bromosalicylaldehyde

To a three-neck flask, equipped with a thermometer and magnetic stirrer, was added glacial acetic acid (140 mmol), aqueous salicylaldehyde (18.40 mmol) and aqueous hydrobromic acid (112 mmol). The mixture was heated to 35 °C for 90 min and $NaClO_3$ was slowly added dropwise, a milky precipitate then appearing. The reaction was monitored by TLC. The reaction mixture was then recrystallized from ethanol and the white crystalline solid was collected by filtration and washed with a small amount of ethanol. The product was dried to afford 5-bromosalicylaldehyde (1.53 g, 42.9%); m.p. 104–105 °C. IR (KBr pellet, ν, cm^{-1}): 1669, 1563, 1465, 1273, 1159, 888, 828, 696, 533. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 10.21 (s, 1H, —CHO), 6.98–7.73 (m, 3H, Ar-H).

2.3.3 | General procedure for synthesis of ligands 4a–4i

Amino acid esters were synthesized according to a published procedure.[27] The synthesis of reduced amino acid ester Schiff bases was according to a one-pot method. Amino acid ester (4.68 mmol) was added to a round-bottom flask containing dichloromethane (306 mmol). Then triethylamine (4.75 mmol) and a certain amount of anhydrous magnesium sulfate were also added to the round-bottom flask at room temperature, with stirring for 1 h. And then salicylaldehyde derivatives was added dropwise to the dichloromethane solution, with stirring for another 36 h at room temperature. Methanol solution (247 mmol) containing sodium borohydride (7.02 mmol) was then added to the solution, and stirred for 3 h. Rotary evaporation was followed by washing three times. The reduced amino acid ester Schiff bases were purified by column chromatography using petroleum ether–ethyl acetate (5:1 *v*/v) affording a pale yellow oil product.

2.3.3.1 | *Methyl 2-(2-hydroxybenzylamino)-3-phenylpropanoate* (4a)

Yellow oil; yield 50.3%. IR (cm^{-1}): 1728 (N—H), 1560 (C═O), 1574, 1266 (Ph—O), 1174, 1077, 865, 742. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.28 (t, J = 7.4 Hz, 2H, Ar-H), 7.21 (t, J = 7.4 Hz, 1H, Ar-H), 7.18 (d, J = 7.0 Hz, 2H, Ar-H), 7.03–7.06 (m, 2H, Ar-H), 6.70 (t, J = 7.8 Hz, 2H, Ar-H), 3.75 (d, J = 14.0 Hz, 1H, —CH_2—), 3.61 (d, J = 14.0 Hz, 1H, —CH_2—), 3.56 (s, 3H, —CH_3), 3.50 (t, J = 6.8 Hz, 1H, —CH—), 2.90 (d, J = 7.0 Hz, 1H, —CH_2—). ^{13}C NMR (DMSO-d_6, δ, ppm): 39.05 (C-7′), 48.20 (C-7), 51.84 (—OCH_3), 62.28 (C-8), 115.65 (C-3), 119.11 (C-5), 125.18 (C-1), 126.89 (C-4′), 128.35 (C-4), 128.67 (C-2′, C-6′), 129.20 (C-6), 129.59 (C-3′, C-5′), 137.97 (C-1′), 156.75 (C-2), 174.35(C-9). Anal. Calcd for $C_{17}H_{19}NO_3$ (%): C, 71.56; H, 6.71; N, 4.91. Found (%): C, 71.42; H, 6.84; N, 4.84.

2.3.3.2 | *Methyl 2-(5-chloro-2-hydroxybenzylamino)-3-phenylpropanoate* (4b)

Yellow oil; yield 59.5%. IR (cm^{-1}): 1741 (N—H), 1564 (C═O), 1573, 1256 (Ph—O), 1176, 1069, 868, 746. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.28 (t, J = 7.4 Hz, 1H, Ar-H), 7.22 (d, J = 7.2 Hz, 1H, Ar-H), 7.18 (d, J = 7.2 Hz, 1H, Ar-H), 7.11 (d, J = 2.5 Hz, 1H, Ar-H), 7.06 (dd, J = 8.6, 2.6 Hz, 1H, Ar-H), 6.72 (d, J = 8.6 Hz, 1H, Ar-H), 3.71 (d, J = 14.6 Hz, 1H, —CH_2—), 3.59 (d, J = 14.6 Hz, 1H, —CH_2—), 3.56 (s, 3H, —CH_3), 3.48 (t, J = 8.4 Hz, 1H, —CH—), 2.86–2.91 (m, 2H, —CH_2—). ^{13}C NMR (DMSO-d_6, δ, ppm): 39.27 (C-7′), 47.24 (C-7), 51.82 (—OCH_3), 62.42 (C-8), 117.10 (C-3), 122.78 (C-1), 126.90 (C-4′), 127.72 (C-5), 128.04 (C-6), 128.64 (C-2′, C-6′), 129.05 (C-4), 129.69 (C-3′, C-5′), 138.08 (C-1′),

155.36 (C-2), 174.12 (C-9). Anal. Calcd for $C_{17}H_{18}ClNO_3$ (%): C, 63.85; H, 5.67; N, 4.38. Found (%): C, 63.68; H, 5.78; N, 4.52.

2.3.3.3 | *Ethyl 2-(2-hydroxybenzylamino)-3-phenylpropanoate* (4c)

Yellow oil; yield 23.1%. IR (cm^{-1}): 1725 (N—H), 1552 (C=O), 1577, 1265 (Ph—O), 1173, 1068, 869, 751. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.34 (t, J = 7.4 Hz, 2H, Ar-H), 7.28–7.30 (m, 1H, Ar-H), 7.18–7.20 (m, 3H, Ar-H), 6.95 (d, J = 7.4 Hz, 1H, Ar-H), 6.85 (d, J = 8.2 Hz, 1H, Ar-H), 6.78 (t, J = 7.4 Hz, 1H, Ar-H), 4.04 (d, J = 13.6 Hz, 1H, —CH_2—), 3.73 (d, J = 13.6 Hz, 1H, —CH_2—), 3.76 (s, 3H, —CH_3), 3.64–3.66 (m, 1H, —CH—), 3.08–3.11 (m, 1H, —CH_2—), 2.96–3.00 (m, 1H, —CH_2—). ^{13}C NMR (DMSO-d_6, δ, ppm): 14.47 (—CH_3), 39.18 (C-7′), 48.14 (C-7), 60.64 (—OCH_2—), 62.19 (C-8), 115.60 (C-3), 119.20 (C-5), 125.20 (C-1), 126.85 (C-4′), 128.36 (C-4), 128.62 (C-2′, C-6′), 129.28 (C-6), 129.64 (C-3′, C-5′), 137.98 (C-1′), 156.86 (C-2), 174.05(C-9). Anal. Calcd for $C_{18}H_{21}NO_3$ (%): C, 72.22; H, 7.07; N, 4.68. Found (%): C, 72.36; H, 7.18; N, 4.49.

2.3.3.4 | *Ethyl 2-(5-chloro-2-hydroxybenzylamino)-3-phenylpropanoate* (4d)

Yellow oil; yield 62.9%. IR (cm^{-1}): 1739 (N—H), 1580 (C=O), 1575, 1261 (Ph—O), 1178, 1076, 867, 746. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.27 (t, 7.2 Hz, 2H, Ar-H), 7.19–7.22 (m, 3H, Ar-H), 7.13 (d, J = 2.4 Hz, 1H, Ar-H), 7.07 (dd, J = 8.6, 2.6 Hz, 1H, Ar-H), 6.73 (d, J = 8.6 Hz, 1H, Ar-H), 3.92–4.02 (m, 2H, Ar-H), 3.73 (d, J = 14.6 Hz, 1H, —CH_2—), 3.62 (d, J = 14.6 Hz, 1H, —CH_2—), 3.47 (t, J = 7.2 Hz, 1H, —CH—), 2.27–2.29 (m, 2H, —CH_2—), 1.08 (t, J = 7.2 Hz, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 14.44 (—CH_3), 39.20 (C-7′), 47.16 (C-7), 60.52 (—OCH_2—), 62.40 (C-8), 117.00 (C-3), 122.70 (C-1), 126.84 (C-4′), 127.70 (C-5), 127.91 (C-6), 128.59 (C-2′, C-6′), 128.64 (C-4), 129.63 (C-3′, C-5′), 138.00 (C-1′), 155.34 (C-2), 173.92 (C-9). Anal. Calcd for $C_{18}H_{20}ClNO_3$ (%): C, 64.77; H, 6.04; N, 4.20. Found (%): C, 64.62; H, 6.18; N, 4.32.

2.3.3.5 | *Ethyl 2-(5-bromo-2-hydroxybenzylamino)-3-phenylpropanoate* (4e)

Yellow oil; yield 30.3%. IR (cm^{-1}): 1736 (N—H), 1565 (C=O), 1580, 1265 (Ph—O), 1182, 1076, 869, 745. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.28 (t, J = 7.4 Hz, 2H, Ar-H), 7.18–7.24 (m, 5H, Ar-H), 6.67 (d, J = 8.4 Hz, 1H, Ar-H), 3.97–4.04 (m, 2H, —CH_2—), 3.70–3.73 (m, 1H, —CH_2—), 3.59–3.62 (m, 1H, —CH_2—), 3.44–3.46 (m, 1H, —CH—), 2.86–2.92 (m, 2H, —CH_2—), 1.06 (s, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 14.52 (—CH_3), 39.24 (C-7′), 47.40 (C-7), 60.48 (—OCH_2—), 62.35 (C-8), 110.78 (C-5), 117.76 (C-3), 126.80 (C-4′), 128.16 (C-1), 128.65 (C-2′, C-6′), 129.72 (C-3′, C-5′), 130.44 (C-4), 131.56 (C-6), 138.06 (C-1′), 155.36 (C-2), 174.50 (C-9). Anal. Calcd for $C_{18}H_{20}BrNO_3$ (%): C, 57.15; H, 5.33; N, 3.70. Found (%): C, 57.26; H, 5.42; N, 3.58.

2.3.3.6 | *Methyl 2-(2-hydroxybenzylamino)-3-methylbutanoate* (4f)

Yellow oil; yield 54.1%. IR (cm^{-1}): 1735 (N—H), 1558 (C=O), 1571, 1255 (Ph—O), 1172, 1045, 873, 756. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.19–7.22 (m, 1H, Ar-H), 6.99 (t, J = 7.4 Hz, 2H, Ar-H), 6.88 (t, J = 8.2 Hz, 2H, Ar-H), 6.81 (m, 2H, —CH_2—), 4.07 (d, J = 13.6 Hz, 1H, —CH_2—), 3.72 (d, J = 13.6 Hz, 1H, —CH_2—), 3.80 (s, 3H, —CH_3), 3.17 (d, J = 5.6 Hz, 1H, —CH—), 2.02–2.07 (m, 1H, —CH—), 1.01 (d, J = 6.8 Hz, 3H, —CH_3), 0.97 (d, J = 6.8 Hz, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 19.12, 19.54 (C-9′, C-10′), 31.34 (C-8′), 48.95 (C-7), 51.74 (—OCH_3), 66.50 (C-8), 115.66 (C-3), 119.09 (C-5), 125.10 (C-1), 128.35 (C-4), 129.20 (C-6), 156.98 (C-2), 174.67 (C-9). Anal. Calcd for $C_{13}H_{19}NO_3$ (%): C, 65.80; H, 8.07; N, 5.90. Found (%): C, 65.68; H, 8.18; N, 5.79.

2.3.3.7 | *Methyl 2-(5-bromo-2-hydroxybenzylamino)-3-methylbutanoate* (4 g)

Yellow oil; yield 58.6%. IR (cm^{-1}): 1736 (N—H), 1546 (C=O), 1567, 1259 (Ph—O), 1176, 1044, 871, 755. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.28 (s, 1H, Ar-H), 7.10 (d, J = 2.4 Hz, 1H, Ar-H), 6.75 (d, J = 8.6 Hz, 1H, Ar-H), 4.03 (d, J = 13.8 Hz, 1H, —CH_2—), 3.65 (d, J = 13.8 Hz, 1H, —CH_2—), 3.80 (s, 3H, —CH_3), 3.15 (d, J = 5.6 Hz, 1H, —CH—), 2.03–2.06 (m, 1H, —CH—), 1.00 (d, J = 6.8 Hz, 3H, —CH_3), 0.96 (d, J = 6.8 Hz, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 19.09, 19.54 (C-9′, C-10′), 31.39 (C-8′), 47.88 (C-7), 51.70 (—OCH_3), 66.64 (C-8), 110.28 (C-5), 117.62 (C-3), 128.38 (C-1), 130.63 (C-4), 131.46 (C-6), 156.02 (C-2), 174.72 (C-9). Anal. Calcd for $C_{13}H_{18}BrNO_3$ (%): C, 49.38; H, 5.74; N, 4.43. Found (%): C, 49.51; H, 5.62; N, 4.34.

2.3.3.8 | *Ethyl 2-(2-hydroxybenzylamino)-3-methylbutanoate* (4 h)

Yellow oil; yield 68.7%. IR (cm^{-1}): 1728 (N—H), 1572 (C=O), 1566, 1256 (Ph—O), 1176, 1045, 874, 758. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.05–7.10 (m, 2H, Ar-H), 6.72 (t, J = 7.5 Hz, 2H, Ar-H), 4.08–4.14 (m, 2H, —CH_2—), 3.79 (d, J = 13.8 Hz, 1H, —CH_2—), 3.59 (d, J = 13.8 Hz, 1H, —CH_2—), 2.97 (s, 1H, —CH—), 1.86–1.92 (m, 1H, —CH—), 1.20 (t, J = 7.2 Hz, 3H, —CH_3), 0.91 (d, J = 6.8 Hz, 3H, —CH_3), 0.88 (d, J = 6.8 Hz, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 14.68 (—CH_3), 19.09, 19.52 (C-9′,C-10′), 31.35 (C-8′), 48.95 (C-7), 60.45 (—OCH_2—), 66.45 (C-8), 115.66 (C-3), 119.09 (C-5), 125.11 (C-1), 128.36 (C-4), 129.21 (C-6), 157.00 (C-2), 174.11 (C-9). Anal. Calcd for $C_{14}H_{21}NO_3$ (%): C, 66.91; H, 8.42; N, 5.57. Found (%): C, 66.83; H, 8.58; N, 5.68.

2.3.3.9 | *Ethyl 2-(5-bromo-2-hydroxybenzylamino)-3-methylbutanoate* (4i)

Yellow oil; yield 49.6%. IR (cm^{-1}): 1739 (N—H), 1530 (C=O), 1572, 1260 (Ph—O), 1171, 1044, 873, 757. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.32 (d, J = 2.0 Hz,

1H, Ar-H), 7.21–7.21 (dd, J = 8.5, 2.2 Hz, 1H, Ar-H), 6.70 (d, J = 7.4 Hz, 1H, Ar-H), 4.05–4.12 (m, 2H, —CH_2—), 3.73 (d, J = 14.6 Hz, 1H, —CH_2—), 3.57 (d, J = 14.6 Hz, 1H, —CH_2—), 2.94 (d, J = 5.0 Hz, 1H, —CH—), 1.85–1.90 (m, 1H, —CH—), 1.18–1.25 (m, 3H, —CH_3), 0.92 (d, 6.8 Hz, 3H, —CH_3), 0.88 (d, 6.8 Hz, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 14.59 (—CH_3), 19.08, 19.56 (C-9′, C-10′), 31.36 (C-8′), 47.88 (C-7), 60.70 (—OCH_2—), 66.74 (C-8), 112.42 (C-5), 120.66 (C-3), 127.38 (C-1), 129.58 (C-4), 130.46 (C-6), 156.10 (C-2), 174.12 (C-9). Anal. Calcd for $C_{14}H_{20}BrNO_3$ (%): C, 50.92; H, 6.10; N, 4.24. Found (%): C, 50.81; H, 6.22; N, 4.33.

2.4 | Synthesis of complexes

2.4.1 | General procedure for synthesis of complexes 5a–5i

Platinum complexes **5a**–**5i** were prepared using the following method. K_2PtCl_4 (0.0482 mmol) was added to 3 ml of a CH_3OH–H_2O (2:1 *v*/v) solution of reduced Schiff bases **4a**–**4i** (0.0461 mmol) at the room temperature. The mixture was adjusted to pH = 8–9 at the beginning, then stirred for 24 h and the pH decreased to about 7. The solution was heated *in vacuo* and recrystallized from CH_3OH and CH_2Cl_2 under the protection of nitrogen.

2.4.1.1 | *Pt[methyl 2-(2-hydroxybenzylamino)-3-phenylpropanoate]Cl* (5a)

Yellow solid; yield 62.4%; m.p. 240–246 °C. IR (cm^{-1}): 1648 (N—H), 1541 (C═O), 1423, 1275 (Ph—O), 1174, 788, 644, 619 (Pt—OAr), 520 (Pt—N), 422 (Pt—O═C). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.25–7.28 (m, 2H, Ar-H), 7.21 (t, J = 7.4 Hz, 1H, Ar-H), 7.17 (d, J = 7.2 Hz, 2H, Ar-H), 7.03–7.05 (m, 2H, Ar-H), 6.69 (t, J = 7.6 Hz, 2H, Ar-H), 3.73 (d, J = 14.0 Hz, 1H, —CH_2—), 3.61 (d, J = 14.0 Hz, 1H, —CH_2—), 3.56 (s, 3H, —CH_3), 3.49–3.52 (m, 1H, —CH—), 2.90–2.91 (d, J = 7.2 Hz, 1H, —CH_2—). ^{13}C NMR (DMSO-d_6, δ, ppm): 39.16 (C-7′), 45.87 (C-7), 51.95 (—OCH_3), 60.04 (C-8), 115.87 (C-3), 119.19 (C-5), 125.32 (C-1), 126.91 (C-4′), 128.43 (C-4), 128.70 (C-2′, C-6′), 129.27 (C-6), 129.60 (C-3′, C-5′), 137.99 (C-1′), 155.89 (C-2), 177.21(C-9). Anal. Calcd for $C_{17}H_{18}ClNO_3Pt$ (%): C, 39.66; H, 3.52; N, 2.72. Found (%): C, 39.53; H, 3.41; N, 2.84. Λ_m = 46 S·cm^2·mol^{-1}.

2.4.1.2 | *Pt[methyl 2-(5-chloro-2-hydroxybenzylamino)-3-phenylpropanoate]Cl* (5b)

Yellow solid; yield 69.5%; m.p. 243–248 °C IR (cm^{-1}): 1648 (N—H), 1558 (C═O), 1421, 1270 (Ph—O), 1173, 786, 650, 611 (Pt—OAr), 520 (Pt—N), 420 (Pt—O═C). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.27–7.29 (m, 1H, Ar-H), 7.21 (d, J = 7.2 Hz, 1H, Ar-H), 7.17 (s, 1H, Ar-H), 7.10 (d, J = 7.2 Hz, 1H, Ar-H), 7.05–7.07 (m, 1H, Ar-H), 6.71 (d, J = 8.6 Hz, 1H, Ar-H), 3.70 (d, 14.6 Hz, 1H, —CH_2—), 3.58 (d, 14.6 Hz, 1H, —CH_2—), 3.56 (s, 3H, —CH_3), 3.47–3.49 (m, 1H, —CH—), 2.86–2.91 (m, 2H, —CH_2—). ^{13}C NMR (DMSO-d_6, δ, ppm): 39.13 (C-7′), 44.21 (C-7), 51.89 (—OCH_3), 59.65 (C-8), 116.97 (C-3), 122.64 (C-1), 126.94 (C-4′), 127.80 (C-5), 128.31 (C-6), 128.68 (C-2′, C-6′), 129.12 (C-4), 129.72 (C-3′, C-5′), 138.15 (C-1′), 154.45 (C-2), 176.52 (C-9). Anal. Calcd for $C_{17}H_{17}Cl_2NO_3Pt$ (%): C, 37.17; H, 3.12; N, 2.55. Found (%): C, 37.28; H, 3.25; N, 2.37. Λ_m = 59 S·cm^2·mol^{-1}.

2.4.1.3 | *Pt[ethyl 2-(2-hydroxybenzylamino)-3-phenylpropanoate]Cl* (5c)

Yellow solid; yield 64.2%; m.p. 241–244 °C. IR (cm^{-1}): 1656 (N—H), 1553 (C═O), 1421, 1270 (Ph—O), 1172, 786, 648, 612 (Pt—OAr), 522(Pt—N), 420 (Pt—O═C). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.08–7.10 (m, 3H, Ar-H), 7.28–7.30 (m, 1H, Ar-H), 7.19–7.21 (m, 3H, Ar-H), 6.94 (d, J = 7.2 Hz, 1H, Ar-H), 6.86 (d, J = 8.4 Hz, 1H, Ar-H), 6.77–6.79 (m, 1H, Ar-H), 4.03 (d, J = 13.6 Hz, 1H, —CH_2—), 3.72 (d, J = 13.6 Hz, 1H, —CH_2—), 3.76 (s, 3H, —CH_3), 3.65–3.67 (m, 1H, —CH—), 3.08–3.11 (m, 1H, —CH_2—), 2.96–3.00 (m, 1H, —CH_2—). ^{13}C NMR (DMSO-d_6, δ, ppm): 14.43 (—CH_3), 39.36 (C-7′), 45.11 (C-7), 60.81 (—OCH_2—), 59.07 (C-8), 115.45 (C-3), 118.94 (C-5), 125.07 (C-1), 126.84 (C-4′), 128.25 (C-4), 128.60 (C-2′, C-6′), 129.37 (C-6), 129.66 (C-3′, C-5′), 138.06 (C-1′), 155.68 (C-2), 176.91(C-9). Anal. Calcd for $C_{18}H_{20}ClNO_3Pt$ (%): C, 40.88; H, 3.81; N, 2.65. Found (%): C, 40.73; H, 3.98; N, 2.52. Λ_m = 51 S·cm^2·mol^{-1}.

2.4.1.4 | *Pt[ethyl 2-(5-chloro-2-hydroxybenzylamino)-3-phenylpropanoate]Cl* (5d)

Yellow solid; yield 72.1%; m.p. 244–246 °C. IR (cm^{-1}): 1645 (N—H), 1575 (C═O), 1421, 1272 (Ph—O), 1175, 786, 648, 619 (Pt—OAr), 519 (Pt—N), 428 (Pt—O═C). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.26–7.29 (m, 2H, Ar-H), 7.18–7.22 (m, 3H, Ar-H), 7.14 (d, J = 2.2 Hz, 1H, Ar-H), 7.06–7.08 (m, 1H, Ar-H), 6.72 (d, J = 8.4 Hz, 1H, Ar-H), 3.92–4.02 (m, 2H, Ar-H), 3.74 (d, J = 14.6 Hz, 1H, —CH_2—), 3.63 (d, J = 14.6 Hz, 1H, —CH_2—), 3.45–3.47 (m, 1H, —CH—), 2.27–2.29 (m, 2H, —CH_2—), 1.07–1.10 (t, J = 7.2 Hz, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 14.49 (—CH_3), 39.32 (C-7′), 44.27 (C-7), 60.75 (—OCH_2—), 59.62 (C-8), 116.87 (C-3), 122.78 (C-1), 126.86 (C-4′), 127.54 (C-5), 127.75 (C-6), 128.61 (C-2′, C-6′), 128.49 (C-4), 129.60 (C-3′, C-5′), 138.15 (C-1′), 153.53 (C-2), 177.13 (C-9). Anal. Calcd for $C_{18}H_{19}Cl_2NO_3Pt$ (%): C, 38.38; H, 3.40; N, 2.49. Found (%): C, 38.49; H, 3.22; N, 2.62. Λ_m = 58 S·$cm^{2\cdot}$·mol^{-1}.

2.4.1.5 | *Pt[ethyl 2-(5-bromo-2-hydroxybenzylamino)-3-phenylpropanoate]Cl* (5e)

Yellow solid; yield 68.1%; m.p. 243–247 °C. IR (cm^{-1}): 1656 (N—H), 1552 (C═O), 1420, 1278 (Ph—O), 1175, 785, 648, 615 (Pt—OAr), 519 (Pt—N), 419 (Pt—O═C). ^{1}H NMR

(600 MHz, DMSO-d_6, δ, ppm): 7.08–7.10 (m, 3H, Ar-H), 7.18–7.22 (m, 4H, Ar-H), 6.69 (d, J = 8.4 Hz, 1H, Ar-H), 3.97–4.04 (m, 2H, —CH_2—), 3.70–3.72 (m, 1H, —CH_2—), 3.59–3.61 (m, J = 7.4 Hz, 1H, —CH_2—), 3.44–3.47 (m, 1H, —CH—), 2.85–2.92 (m, 2H, —CH_2—), 1.06 (t, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 14.57 (—CH_3), 39.08 (C-7′), 44.69 (C-7), 60.63 (—OCH_2—), 60.13 (C-8), 110.64 (C-5), 117.51 (C-3), 126.82 (C-4′), 128.31 (C-1), 128.69 (C-2′, C-6′), 129.70 (C-3′, C-5′), 130.29 (C-4), 131.64 (C-6), 138.22 (C-1′), 153.39 (C-2), 177.89 (C-9). Anal. Calcd for $C_{18}H_{19}BrClNO_3Pt$ (%): C, 35.57; H, 3.15; N, 2.30. Found (%): C, 35.68; H, 3.25; N, 2.18. Λ_m = 55 S·cm^2·mol^{-1}.

2.4.1.6 | *Pt[methyl 2-(2-hydroxybenzylamino)-3-methylbutanoate]Cl* (5f)

Yellow solid, yield 64.8%; m.p. 240–245 °C. IR (cm^{-1}): 1650 (N—H), 1550 (C═O), 1421, 1275 (Ph—O), 1175, 786, 648, 615 (Pt—OAr), 522 (Pt—N), 420 (Pt—O═C). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.18–7.21 (m, 1H, Ar-H), 6.74 (t, J = 7.4 Hz, 2H, Ar-H), 4.08–4.14 (m, 2H, —CH_2—), 4.07 (d, J = 13.6 Hz, 1H, —CH_2—), 3.73 (d, J = 13.6 Hz, 1H, —CH_2—), 3.80 (s, 3H, —CH_3), 3.16–3.18 (m, 1H, —CH—), 2.02–2.08 (m, 1H, —CH—), 1.01 (d, J = 6.8 Hz, 3H, —CH_3), 0.98 (d, J = 6.8 Hz, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 19.10, 19.55 (C-9′, C-10′), 31.21 (C-8′), 45.67 (C-7), 51.86 (—OCH_3), 62.87 (C-8), 115.54 (C-3), 118.95 (C-5), 125.17 (C-1), 128.26 (C-4), 129.31 (C-6), 155.23 (C-2), 177.85 (C-9). Anal. Calcd for $C_{13}H_{18}ClNO_3Pt$ (%): C, 33.45; H, 3.89; N, 3.00. Found (%): C, 33.56; H, 3.78; N, 3.12. Λ_m = 53 S·cm^2·mol^{-1}.

2.4.1.7 | *Pt[methyl 2-(5-bromo-2-hydroxybenzylamino)-3-methylbutanoate]Cl* (5 g)

Yellow solid; yield 76.6%; m.p. 245–248 °C. IR (cm^{-1}): 1648 (N—H), 1568 (C═O), 1421, 1270 (Ph—O), 1174, 787, 647, 620 (Pt—OAr), 519 (Pt—N), 419 (Pt—O═C). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.28 (s, 1H, Ar-H), 7.11 (d, J = 2.2 Hz, 1H, Ar-H), 6.75 (s, 1H, Ar-H), 4.05 (d, J = 13.8 Hz, 1H, —CH_2—), 3.67 (d, J = 13.8 Hz, 1H, —CH_2—), 3.81 (s, 3H, —CH_3), 3.14–3.17 (m, 1H, —CH—), 2.03–2.07 (m, 1H, —CH—), 1.00 (d, J = 6.8 Hz, 3H, —CH_3), 0.98 (d, J = 6.8 Hz, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 19.10, 19.56 (C-9′, C-10′), 31.18 (C-8′), 44.96 (C-7), 51.82 (—OCH_3), 63.79 (C-8), 110.39 (C-5), 117.47 (C-3), 128.27 (C-1), 130.46 (C-4), 131.32 (C-6), 154.37 (C-2), 177.51 (C-9). Anal. Calcd for $C_{13}H_{17}BrClNO_3Pt$ (%): C, 28.61; H, 3.14; N, 2.57. Found (%): C, 28.52; H, 3.32; N, 2.68. Λ_m = 54 S·cm^2·mol^{-1}.

2.4.1.8 | *Pt[ethyl 2-(2-hydroxybenzylamino)-3-methylbutanoate]Cl* (5 h)

Yellow solid; yield 78.4%; m.p. 241–246 °C. IR (cm^{-1}): 1645 (N—H), 1542 (C═O), 1422, 1272 (Ph—O), 1173, 786, 649, 611 (Pt—OAr), 520 (Pt—N), 426 (Pt—O═C). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.06–7.10 (m, 2H, Ar-H), 6.71–6.74 (m, 2H, Ar-H), 4.08–4.14 (m, 2H, —CH_2—), 3.78–3.80 (d, J = 13.8 Hz, 1H, —CH_2—), 3.58–3.60 (d, J = 13.8 Hz, 1H, —CH_2—), 2.96 (s, 1H, —CH—), 1.86–1.92 (m, 1H, —CH—), 1.18 (t, J = 7.2 Hz, 3H, —CH_3), 0.92 (d, J = 6.8 Hz, 3H, —CH_3), 0.89 (d, J = 6.8 Hz, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 14.68 (—CH_3), 19.08, 19.54 (C-9′,C-10′), 31.20 (C-8′), 45.87 (C-7), 60.61 (—OCH_2—), 63.86 (C-8), 115.52 (C-3), 118.96 (C-5), 125.18 (C-1), 128.19 (C-4), 129.10 (C-6), 155.08 (C-2), 177.25 (C-9). Anal. Calcd for $C_{14}H_{20}ClNO_3Pt$ (%): C, 34.97; H, 4.19; N, 2.91. Found (%): C, 34.83; H, 4.35; N, 2.84. Λ_m = 57 S·cm^2·mol^{-1}.

2.4.1.9 | *Pt[ethyl 2-(5-bromo-2-hydroxybenzylamino)-3-methylbutanoate]Cl* (5i)

Yellow solid; yield 69.8%; m.p. 242–247 °C. IR (cm^{-1}): 1656 (N—H), 1528 (C═O), 1421, 1275 (Ph—O), 1175, 787, 649, 619 (Pt—OAr), 522 (Pt—N), 420 (Pt—O═C). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.28–7.31 (m, 1H, Ar-H), 7.20–7.22 (m, 1H, Ar-H), 6.69–6.70 (d, J = 7.4 Hz, 1H, Ar-H), 4.05–4.11 (m, 2H, —CH_2—), 3.75 (d, J = 14.6 Hz, 1H, —CH_2—), 3.57 (d, J = 14.6 Hz, 1H, —CH_2—), 2.93–2.95 (m, 1H, —CH—), 1.85–1.91 (m, 1H, —CH—), 1.18–1.24 (m, 3H, —CH_3), 0.92 (d, J = 6.8 Hz, 3H, —CH_3), 0.88 (d, J = 6.8 Hz, 3H, —CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 14.62 (—CH_3), 19.10, 19.55 (C-9′, C-10′), 31.21 (C-8′), 45.03 (C-7), 60.86 (—OCH_2—), 64.18 (C-8), 112.26 (C-5), 120.57 (C-3), 127.45 (C-1), 129.79 (C-4), 130.21 (C-6), 154.37 (C-2), 177.01 (C-9). Anal. Calcd for $C_{14}H_{19}BrClNO_3Pt$ (%): C, 30.04; H, 3.42; N, 2.50. Found (%): C, 30.15; H, 3.33; N, 2.38. Λ_m = 59 S·cm^2·mol^{-1}.

2.5 | UV absorption spectroscopy

A UV-3400 Toshniwal spectrophotometer emitting UV light was used mainly between 200 and 400 nm. Salmon sperm DNA (M = 208.8 g mol^{-1}) was dissolved in HCl–Tris (pH = 7.5) buffer solution, and rested for 24 h at 4 °C. Then complexes **5a** and **5i** were added in buffer solution in various concentrations (DNA: 20 μM; **5a/5i**: 2, 6, 10 μM), and rested for 24 h at 4 °C. The UV absorption spectra were recorded at room temperature (Δt = 1 s; n = 3).

2.6 | CD spectroscopy

The CD spectra of the drug–DNA complex (DNA concentration = 20 μmol l^{-1}, molar ratios =0.3) were recorded at room temperature with a Bio-Logic MOS-450/SFM300 CD spectrometer. Salmon sperm DNA was dissolved in HCl–Tris (pH = 7.5) buffer solution, and rested for 24 h at 4 °C. Then complex **5a** (6 μmol l^{-1}) was added in buffer solution and rested for 2 h at 4 °C. The sample was scanned twice in a range of wavelengths between 200 and 350 nm at

100 nm min^{-1} scan rate. The CD spectra obtained were the average of three independent scans.

2.7 | Cell culture

Two different human carcinoma cell lines, HeLa and A549, were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 mg ml^{-1} of penicillin and 100 mg ml^{-1} of streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air.

2.8 | Solutions

The complexes were dissolved in DMSO at a concentration of 5 mM as stock solution, and diluted in culture medium at concentrations of 1.0, 10 and 100 μM as working solutions. To avoid DMSO toxicity, the concentration of DMSO was less than 0.1% (*v*/v) in all experiments.

2.9 | Cytotoxicity analysis

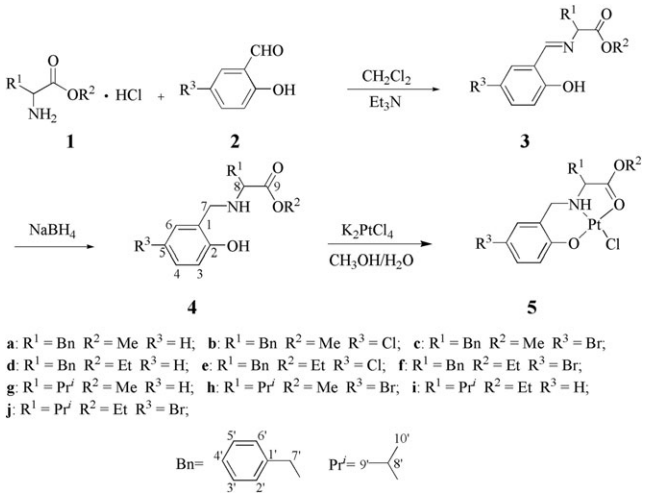
Cells harvested from exponential phase were seeded equivalently into a 96-well plate, and then the complexes were added to the wells to achieve final concentrations. Control wells were prepared by addition of culture medium. Wells containing culture medium without cells were used as blanks. All experiments were performed in quintuplicate. The cytotoxicity of the compounds under investigation against HeLa and A549 cell lines was examined using the microculture tetrazolium (MTT) assay.[28] Upon completion of incubation for 44 h, stock MTT dye solution (100 μl, 5 mg ml^{-1}) 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 measured with a microplate spectrophotometer at a wavelength of 570 nm. The IC_{50} value was determined from plot of % viability against dose of compounds added.

3 | RESULTS AND DISCUSSION

3.1 | Synthesis and characterization

Ligands **4a**–**4i** were synthesized from salicylaldehyde, 5-bromosalicylaldehyde and 5-chlorosalicyladehyde with amino acid esters. The platinum(II) complexes **5a**–**5i** were prepared by the reaction of K_2PtCl_4 with reduced amino acid ester Schiff bases **4a**–**4i** in a mixture of methanol and water at room temperature (Scheme 1).

The bands at about 1725–1739)cm^{-1} in the IR spectra of **4a**–**4i** can be assigned to the (N—H) stretching frequencies, which shift to lower wavenumber (1645–1656 cm^{-1}) upon complexation in **5a**–**5i**. A carbonyl (C═O) vibration band which appears in the IR spectra of ligands at 1530–1580 cm^{-1} similarly undergoes a shift to lower frequency (1575–1528 cm^{-1}) in the spectra of Pt(II) complexes. The



SCHEME 1 Synthetic pathway for preparation of compounds

bands for the phenolic group (C—O) stretching frequency observed in the region 1255–1266 cm^{-1} for free ligands shift to higher frequency (1267–1278 cm^{-1}) for their complexes. On the other hand, new bands appear at about 611–622, 519–524 and 419–428 cm^{-1} which can be assigned to νPt—OAr, νPt—N and νPt—O═C, respectively. The IR spectra of **4c** and **5c** are shown in Figure 1. The IR data reveal that nitrogen atoms of the imino groups, the oxygen atoms of the carbonyl groups and phenolic groups are coordinated to the metal ion (Table 1).

Although the overall pattern of the ^{1}H NMR spectra of **5a**–**5i** resembles very closely that of the free ligands, almost all the signals are shifted to lower field upon coordination. In the ^{13}C NMR spectra, the characteristic signals for the carbonyl carbon of ligands are observed in the range 173.92–174.72 ppm; however, coordination leads to a downfield shift. The signals for C-2 of ligands appear at around 156 ppm, which are shifted upfield in comparison with the corresponding complexes. What is more, elemental analysis further confirms the structures of the target compounds.

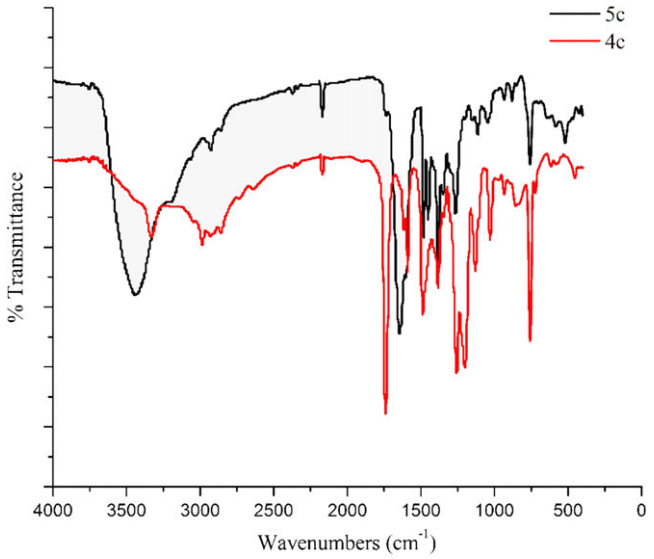


FIGURE 1 IR spectra of **4c** and **5c**

TABLE 1 Main IR absorption bands of ligands and complexes (cm^{-1})

	ν(N—H)	ν(C=O)	ν(Ph—O)	ν(Pt—OAr)	ν(Pt—N)	ν(Pt—O=C)
4a	1728	1560	1266			
4b	1741	1564	1256			
4c	1725	1552	1259			
4d	1739	1580	1265			
4e	1736	1565	1261			
4f	1735	1558	1265			
4 g	1736	1546	1255			
4 h	1728	1572	1259			
4i	1739	1530	1256			
5a	1648	1541	1275	619	520	422
5b	1648	1558	1270	622	520	420
5c	1656	1553	1267	620	524	422
5d	1645	1575	1270	612	522	420
5e	1656	1552	1272	619	519	428
5f	1650	1550	1278	615	519	419
5 g	1648	1568	1275	615	522	420
5 h	1645	1542	1270	620	519	419
5i	1656	1540	1272	611	520	426

The conductivity data of all complexes were also measured. When the molar conductance value is in the range 80–115 S cm^2 mol^{-1} in methanol, the electrolyte type is 1:1; when the value is in the range 160–220 S cm^2 mol^{-1}, the electrolyte type is 2:1.[29] The molar conductance values of the target compounds in deionized methanol are in the range 40–60 S cm^2 mol^{-1}. Therefore, all complexes are electroneutral.

3.2 | DNA binding study

3.2.1 | UV–visible titration with salmon sperm DNA

Electronic absorption spectroscopy in the UV–visible range is a useful technique for investigating the possible binding mode of metal compounds with biomolecules, including DNA. Therefore, in the spectral range 200–400 nm, DNA with various concentrations of complexes **5a** and **5i** was examined (Figures 2 and 3). According to Figure 2, one positive peak appears at 258 nm ($A = 0.9899$), which can be attributed to the absorption of DNA. As the concentration of complex **5a** ($C_{\mathbf{5a}}$:$C_{\mathrm{DNA}} = 0.1$, 0.3 and 0.5) increases, the absorbance values increase ($A = 1.2284$, 1.4781 and 1.7763). Meanwhile, the absorption at 258 nm shifts to lower wavelength (λ = 255, 253 and 251 nm). Similarly, in Figure 3, there is one positive peak at 258 nm ($A = 0.9806$). Addition of complex **5i** to a DNA solution results in hyperchromism and hypsochromic shift of the band at 258 nm compared to blank DNA. This might be due to the complex being inserted into the DNA double helix structure and then stacking with DNA base pairs where π-electron accumulation occurs; therefore the absorbance value increases and maximum absorption wavelength is blue-shifted. The results indicate that hyperchromicity occurs after coordination, and thus the binding mode of complexes with DNA may be of intercalation type.[30,31]

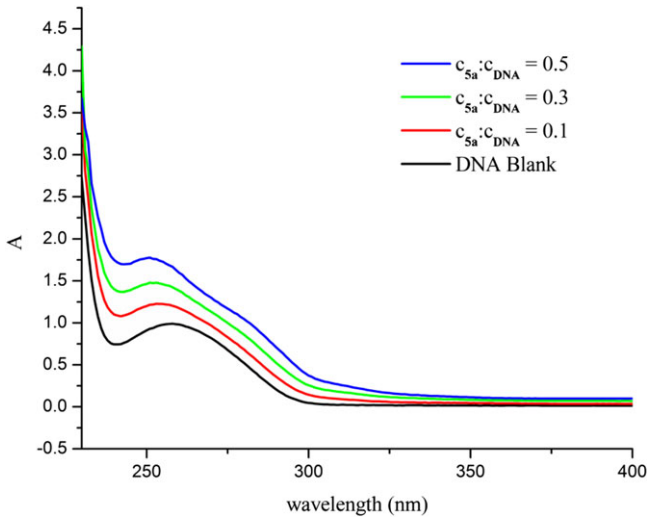


FIGURE 2 UV spectra for interaction between **5a** and salmon sperm DNA

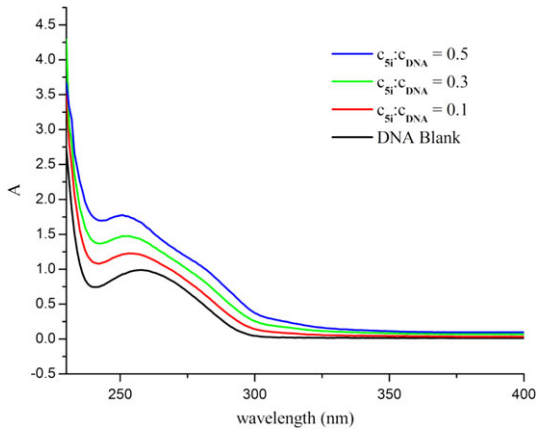


FIGURE 3 UV spectra for interaction between **5i** and salmon sperm DNA

3.2.2 | CD with salmon sperm DNA

CD spectroscopy is a common technique for probing the conformational changes of DNA double helix in solution. A solution of the most abundant B-form of DNA exhibits a positive band (277 nm) and a negative band (245 nm). The CD signals are quite sensitive to the mode of DNA interactions with small molecules, so the changes in CD spectra are commonly used to distinguish between different modes of coordinative binding of platinum complexes to DNA.[32,33] The CD spectra of DNA and **5a**–DNA were determined and are shown in Figure 4, where the 277 nm band is due to base stacking and the 245 nm band is due to right-handed helicity of DNA. With the addition of platinum complex, an increase in the positive band intensity of **5a** is observed, this specific change being a distinctive feature of intercalation into π-stacking of DNA. The results indicate that an intercalative binding consequently contributes to the weakening of the base stacking interactions.[34,35] Therefore, the results further confirm that the binding mode of the complexes with DNA may be of intercalation type.

3.3 | *In Vitro* antitumor activity

The cytotoxicities of platinum complexes **5a**–**5i** were tested *in vitro* against HeLa and A549 cell lines by MTT assay. Cisplatin was used as control. The corresponding IC_{50} values (the concentration that inhibits cellular proliferation by 50%) of the studied complexes are presented in Table 2 and Figure 5. Results show that the complexes demonstrate excellent cytotoxic activity, with IC_{50} values ranging from 5.94 to 27.08 μM against both the tested cell lines. All complexes show selective cytotoxicities against HeLa, which are superior to that of cisplatin. In particular, complex **5a** shows 4.7 times higher activity than cisplatin. For A549, compared with cisplatin, complexes **5a**, **5b**, **5d** and **5e** display fairly cytotoxic effects; the others demonstrate better cytotoxicity. In general, the presence of electron-withdrawing group (Cl, Br) at C-4 position of phenyl group of the Schiff bases would improve the activity. The inductive effect of electron-withdrawing group at the 4-position of phenyl group of amino acid-derived Schiff bases makes the phenolic hydroxyl group more acidic and the Pt—O bond is less stable than in the other compounds, thereby making it easier to hydrolyze and coordinate to DNA guanine residue. However, there are exceptions to the rule. Therefore, the inductive effect is not the only factor influencing the antitumor activities.

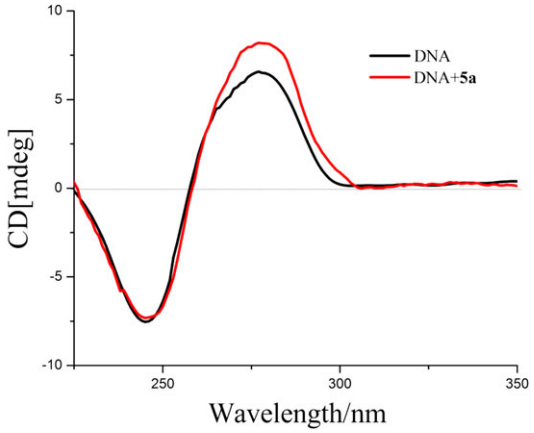


FIGURE 4 CD spectra for interaction between **5a** and salmon sperm DNA

TABLE 2 Cytotoxicity of complexes *in vitro*

Complex	IC_{50} (μM)	
	HeLa	A549
5a	5.94	26.73
5b	10.09	25.34
5c	10.72	16.71
5d	10.42	25.31
5e	10.17	26.57
5f	14.02	16.62
5 g	18.74	13.00
5 h	26.06	12.82
5i	27.08	15.30
Cisplatin	27.79	24.42

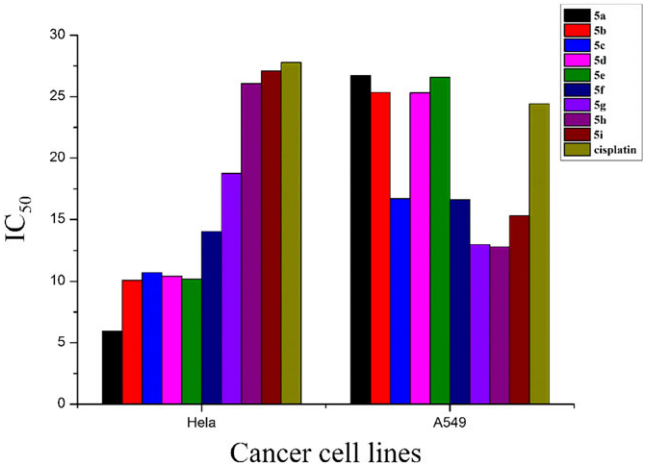


FIGURE 5 IC_{50} values of complexes

4 | CONCLUSIONS

In this study, nine platinum(II) complexes were synthesized and characterized. The interaction of these complexes with salmon sperm DNA was investigated by means of UV and CD spectroscopies, the results revealing that the binding mode of the complexes with DNA may be of intercalation type. The biological activities of all complexes were examined against two tumor cell lines, the results showing that complexes **5a**–**5i** have excellent cytotoxicity against the tested carcinoma cell lines. Most complexes exhibited higher cytotoxic activity than cisplatin. Furthermore, the structure–activity relationship has been analyzed, and the results of this study would be helpful in designing new platinum anticancer drugs.

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