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Synthesis, characterization and cytotoxicity studies of platinum(II) complexes with reduced amino acid ester Schiff-bases as ligands

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Abstract: A series of platinum(II) complexes with reduced amino acid esters Schiff-bases as ligands were prepared as potential anticancer agents and characterized by NMR, IR spectroscopy, elemental analysis and molar conductivity. These compounds were tested for their stability and DNA interaction with salmon sperm DNA by ultraviolet spectrum. The cytotoxicities of these complexes were validated against HL-60, BGC-823, Hela, and HepG2 cell lines by the MTT test. The complexes **5e** and **5f** exhibited better cytotoxic activity than cisplatin against HepG2 and BGC-823 cell lines, respectively.

Key words: platinum(II) complexes, synthesis, characterize, reduced amino acid esters Schiff-bases, DNA interaction, antitumor activity

1. Introduction

Much interest in developing metal based drugs appeared in the mid-1960s [1]. As frequently used anticancer agents, platinum containing compounds have a unique place in metal coordination chemistry [2,3]. Almost five decades after Rosenberg's accidental discovery of cisplatin [4,5], the antitumor activity of platinum based drugs still captures the attention of scientists worldwide, and new potential drugs are being synthesized and investigated [6-10]. Despite its wide application as a chemotherapeutic agent, cisplatin exhibits several main disadvantages: narrow range of activity, acquired resistance and toxicity. These side effects limit the use of cisplatin in some cancers. Recently, great efforts have been devoted to overcoming these main limits. Up to now, apart from cisplatin, metal-based anticancer agents that are in worldwide clinical use include carboplatin and oxaliplatin [11].

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 [12-14]. Transition metal complexes containing Schiff base ligands and their reduced products are often used as artificial chemical nucleases, these complexes have proved to be efficient DNA cleavage reagents [15,16]. Amino acids, as the basic unit of proteins and related enzymes, perform critical biological roles including neurotransmitters and transport in the body, are one of the essential and indispensable nutrients *in vivo*. When they are introduced to the drug molecules after esterified, the drug lipophilicity, toxicity and bioavailability will be improved. Most of the model studies of the metal complexes of Schiff-base ligands bearing salicylaldehyde and amino acid esters have focused on the binding mode of these ligands [17,18]. Structural studies on the metal complexes of reduced Schiff-base ligands derived from various amino acid esters and salicylaldehyde, are well documented [19,20]. 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. Numerous papers on transition metal complexes of reduced Schiff bases derived from amino acids or amino acid esters have been reported [21,22], however, information on the corresponding derivatives of platinum(II) is still rare. Herein, a series of platinum(II) complexes with reduced amino acid esters Schiff-bases as ligands were synthesized and characterized, their interaction with salmon sperm DNA was investigated by

ultraviolet spectrum, and their antiproliferative activity against a panel of human tumor cell lines from solid tumors including HL-60, BGC-823, Hela, and HepG2 cell lines has also been evaluated. It appears possible that the mechanism of activity is very similar to that of cisplatin since the ester ligand can be easily substituted by other ligands and thus there are two leaving groups.

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, MTT and salmon sperm DNA were from Sigma. Four different human carcinoma cell lines: HL-60 (immature granulocyte leukemia), Hela (human epithelial cancer), BGC-823 (human gastro carcinoma) and HepG2 (human hepatocellular carcinoma) were obtained from American Type Culture Collection.

2.2. 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 was measured by Bruker apex-ultra 7.0T. The interaction between DNA and complexes was measured on UV-3400 Toshniwal spectrophotometer. Molar conductances were measured at room temperature in 1×10^{-3} M methanol using a DDS-12DW type conductivity meter.

2.3. Synthesis of ligands

5-Chlorosalicylaldehyde

To a three-neck flask, equipped with a reflux condenser, thermometer, a nitrogen source and magnetic stirrer was added parachlorophenol (16 mmol), sodium hydroxide (100 mmol), distilled water (40 mL), chloroform (7.0 mL). The mixture was heated to reflux for 8 h. After cooling, the reaction mixture was acidified to pH 2~3 with 2M hydrochloric acid, and extracted with ethyl acetate. The ethyl acetate extract was treated with vacuum distillation, dried and obtained crude 5-chlorosalicylaldehyde. The crude product was purified by column chromatography gave white solid product (2.158 g, 29.6 %): mp: 90-91 ºC. IR (KBr, pellet) υ/cm^{-1}: 3225, 1685, 1566, 1470, 1275, 1155, 885, 831, 650, 535. ^{1}H NMR (600M Hz, DMSO-d_6), δ: 9.873 (s, 1 H, -CHO), 7.354-7.365 (m, 2H, Ar-H), 6.911-6.927 (d, 1H, Ar-H).

5-Bromosalicylaldehyde

5-Bromosalicylaldehyde was synthesized according to a published procedure [23]. To a three-neck flask, equipped with a thermometer and magnetic stirrer was added glacial acetic acid (8 mL), salicylaldehyde (18.40 mmol), hydrobromic acid (6 mL). The mixture was heated to 35 ºC for 90 minutes and $NaClO_3$ was slowly added in dropwise, milky precipitate was then appeared. The reaction mixture was then recrystallized from ethanol and the white crystalline solid was collected by filtration and washed with a bit of ethanol. The product was dried to obtain 5-bromosalicylaldehyde (1.53 g, 42.9%): mp: 104-105 ºC. IR (KBr, pellet) υ/cm^{-1}: 3445, 1669, 1563, 1465, 1273, 1159, 888, 828, 696, 533. ^{1}H NMR (600M Hz, DMSO-d_6), δ: 10.211 (s, 1 H,

-CHO), 6.986-7.732 (m, 3 H, Ar-H).

General procedure of ligands synthesis

Amino acid ester were synthesized according to a published procedure [24]. The synthesis of reduced amino acid ester Schiff bases were according to one-pot. Amino acid ester (4.68 mmol) was added to the round bottom flask containing dichloromethane (20 mL), then triethylamine (4.75 mmol) and a certain amount of anhydrous magnesium sulfate were also added to the round bottom flask at room temperature, stirring for 1 h. And then salicylaldehydethe (4.80 mmol) was added to the dichloromethane solution with 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, stirred for 3 h. Rotary evaporation, wash three times. The reduced amino acid ester Schiff bases were purified by column chromatography gave the white solid or colorless oil product.

Methyl 2-(2-hydroxybenzylamino)-4-methylpentanoate (**4a**)

Pale yellow oil. Yield 73.0%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.057-7.102 (m, 2H, Ar-H), 6.716-6.744 (m, 2H, Ar-H), 3.791 (d, 1H, N-CH_2), 3.636 (s, 3H, CH_3), 3.611 (d, 1H, N-CH_2), 3.259 (t, 1H, N-CH), 1.649-1.728 (m, 1H, CH), 1.391-1.497 (m, 2H, CH_2), 0.870 (d, 3H, CH_3), 0.826 (d, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 16.5 (C-3', C-4'), 27.4 (C-2'), 42.1 (C-7), 50.3 (-OCH_3), 65.4 (C-8), 115.6 (C-3), 120.7 (C-5), 123.9 (C-1), 128.1 (C-4), 129.0 (C-6), 156.8 (C-2), 173.1 (C-9). IR (KBr): 3316 (N-H), 2956, 1735 (C=O), 1586, 1254 (ph-O), 1150, 992, 840, 754 cm^{-1}. Anal. Calc. for $C_{14}H_{21}NO_3$: C, 66.91; H, 8.42; N, 5.57. Found: C, 66.78; H,8.56; N, 5.46.

Methyl 2-(2-hydroxybenzylamino)propanoate (**4b**)

Pale yellow oil. Yield 33.6%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.198 (t, 1H, Ar-H), 6.983 (d, 1H, Ar-H), 6.871 (d, 1H, Ar-H), 6.800 (t, 1H, Ar-H), 4.101 (d, 1H, N-CH_2), 3.813 (d, 1H, N-CH_2), 3.792 (s, 3H, CH_3) 3.467 (q, 1H, N-CH), 1.389 (d, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 16.3 (C-5'), 47.5 (C-7), 50.2 (-OCH_3), 57.8 (C-8), 116.3 (C-3), 121.2 (C-5), 124.6 (C-1), 129.2 (C-4), 130.6 (C-6), 157.4 (C-2), 172.8 (C-9). IR (KBr): 3430 (N-H), 2951, 1723 (C=O), 1569, 1256 (ph-O), 1160, 977, 864, 751 cm^{-1}. Anal. Calc. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.44; H, 7.51; N, 6.32.

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

Pale yellow oil. Yield 36.3%. 1H NMR (600 MHz, DMSO-*d6*): δ 7.104 (d, 1H, Ar-H), 7.071 (t, 1H, Ar-H), 6.720-6.745 (m, 2H, Ar-H), 3.826 (d, 1H, N-CH_2), 3.668 (d, 1H, N-CH_2), 3.635 (s, 3H, CH_3), 3.606 (d, 2H, CH_2), 3.324 (t, 1H, N-CH); ^{13}C NMR (DMSO-d_6) δ: 43.2 (C-7), 51.4 (-OCH_3), 64.5 (C-8), 65.8 (C-6'), 117.6 (C-3), 121.4 (C-5), 126.2 (C-1), 129.3 (C-4), 130.5 (C-6), 158.9 (C-2), 173.4 (C-9). IR (KBr): 3426 (N-H), 2955, 1735 (C=O), 1569, 1257 (ph-O), 1174, 1042, 871, 757 cm^{-1}. Anal. Calc. for $C_{11}H_{15}NO_4$: C, 58.66; H, 6.71; N, 6.22. Found: C,58.89; H, 6.45; N, 6.52.

Methyl 2-(2-hydroxybenzylamino)-3-(1H-indol-3-yl)propanoate (**4d**)

White solid. Yield 53.5%. mp 105-106 °C ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.852 (s, 1H, NH), 7.452 (d, 1H, Ar-H), 7.336 (d, 1H, Ar-H), 7.121 (s, 1H, Ar-H), 7.046-7.078 (m, 3H, Ar-H), 6.971 (t, 1H, Ar-H), 6.692-6.721 (m, 2H, Ar-H), 3.796 (d, 1H, N-CH_2), 3.654 (d, 1H, N-CH_2), 3.556 (s, 1H, N-CH), 3.545 (s, 3H, CH_3), 3.028-3.095 (m, 2H, CH_2), 2.836 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 31.5 (C-15'), 42.8 (C-7), 50.6 (-OCH_3), 64.7 (C-8), 111.6 (C-13'), 112.5 (C-8'), 115.7 (C-3), 119.8 (C-12'), 120.9 (C-5), 121.1 (C-10'), 121.9 (C-11'), 122.8 (C-7'),124.6 (C-1), 128.5 (C-4), 129.7 (C-6), 131.6 (C-9'), 136.4 (C-14'), 157.3 (C-2), 172.8 (C-9). IR (KBr): 3438 (N-H), 2955, 1739 (C=O), 1568, 1241 (ph-O), 1176, 1006, 868, 735 cm^{-1}. Anal. Calc. for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.64; H, 5.95; N, 8.91.

Methyl 2-(5-bromo-2-hydroxybenzylamino)-3-phenylpropanoate (**4e**)

Pale yellow oil. Yield 57.0%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.281 (t, 2H, Ar-H), 7.224 (dd, 2H, Ar-H), 7.186(d, 3H, Ar-H), 6.676 (d, 1H, Ar-H), 3.709 (d, 1H, N-CH_2), 3.584 (d, 1H, N-CH_2), 3.556 (s, 3H, CH_3), 3.463 (t, 1H, N-CH), 2.892 (dd, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 39.1 (C-21'), 47.7 (C-7), 51.7 (-OCH_3), 62.4 (C-8), 110.8 (C-5), 117.9 (C-3), 126.8 (C-18'), 127.8 (C-1), 128.7 (C-20', C-16'), 129.8 (C-19', C-17'), 130.3 (C-4), 131.6 (C-6), 137.9 (C-15'), 156.5 (C-2), 174.4 (C-9). IR (KBr): 3431 (N-H), 2953, 1725 (C=O), 1578, 1259 (ph-O), 1179, 1073, 867, 748 cm^{-1}. Anal. Calc. for $C_{17}H_{18}BrNO_3$: C, 56.06; H, 4.98; N, 3.85. Found: C, 56.35; H, 4.82; N, 3.97.

Methyl 2-(5-chloro-2-hydroxybenzylamino)-4-methylpentanoate (**4f**)

Pale yellow oil. Yield 87.6%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.203 (d, 1H, Ar-H), 7.087 (dd, 1H, Ar-H), 6.746 (d, 1H, Ar-H), 3.732 (d, 1H, N-CH_2), 3.625 (s, 3H, CH_3), 3.580 (d, 1H, N-CH_2), 3.237 (t, 1H, N-CH), 1.673-1.741 (m, 1H, CH), 1.383-1.490 (m, 2H, CH_2), 0.876 (d, 3H, CH_3), 0.835 (d, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 22.3 (C-3', C-4'), 23.6 (C-2'), 42.5 (C-1'), 42.0 (C-7), 50.2 (-OCH_3), 59.1 (C-8), 117.1 (C-3), 125.8 (C-1), 126.3 (C-5), 128.6 (C-4), 129.7 (C-6), 155.4 (C-2), 173.2 (C-9). IR (KBr): 3441 (N-H), 2957, 1735 (C=O), 1569, 1259 (ph-O), 1174, 999, 871, 719 cm^{-1}. Anal. Calc. for $C_{14}H_{20}ClNO_3$: C, 58.84; H, 7.05; N, 4.90. Found: C, 58.62; H, 6.75; N, 5.21.

2.4. Complexes synthesis

Platinum complexes **5a-5f** were synthesized with 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) in the room temperature, the mixture was adjusted to pH = 8~9 in 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_2Cl_2 under the protection of N_2.

Pt[methyl 2-(2-hydroxybenzylamino)-4-methylpentanoate]Cl (**5a**)

Yellow solid. Yield 70.2%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.061-7.106 (m, 2H, Ar-H), 6.715 (t, 2H, Ar-H), 3.801 (d, 1H, N-CH_2), 3.647 (d, 1H, N-CH_2), 3.604 (s, 3H, CH_3), 3.262 (t, 1H, N-CH), 1.642-1.720 (m, 1H, CH), 1.386-1.492 (m, 2H, CH_2), 0.873 (d, 3H, CH_3), 0.830 (d, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 16.7 (C-3', C-4'), 27.8 (C-2'), 38.9 (C-7), 50.5 (-OCH_3), 63.2 (C-8), 115.2 (C-3), 120.9 (C-5), 123.6 (C-1), 128.5 (C-4), 128.7 (C-6), 155.4 (C-2), 174.8 (C-9). IR (KBr): 3441 (N-H), 2957, 1649 (C=O), 1438, 1284 (ph-O), 1173, 786, 649, 620 (Pt-OAr), 520 (Pt-N), 412 (Pt-O=C) cm^{-1}. ESI-MS: 504.0671 [M + Na]$^+$.Anal. Calc. for $C_{14}H_{20}ClNO_3Pt$: C, 34.97; H, 4.19; N, 2.91. Found: C, 35.15; H, 4.04; N, 2.84. Λ_m = 48 S·cm^2·mol^{-1}.

Pt[methyl 2-(2-hydroxybenzylamino)propanoate]Cl (**5b**)

Yellow solid. Yield 78.9%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.201 (t, 1H, Ar-H), 6.989 (d, 1H, Ar-H), 6.8761 (d, 1H, Ar-H), 6.806 (t, 1H, Ar-H), 4.113 (d, 1H, N-CH_2), 3.820 (d, 1H, N-CH_2), 3.798 (s, 3H, CH_3), 3.472 (q, 1H, N-CH), 1.392 (d, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 16.4 (C-5'), 39.6 (C-7), 50.4 (-OCH_3), 55.2 (C-8), 116.1 (C-3), 122.3 (C-5), 124.3 (C-1), 129.4 (C-4), 130.5 (C-6), 156.1 (C-2), 175.3 (C-9). IR (KBr): 3440 (N-H), 2957, 1653 (C=O), 1439, 1267 (ph-O), 1117, 756, 649, 617 (Pt-OAr), 524 (Pt-N), 418 (Pt-O=C) cm^{-1}. ESI-MS: 462.0195 [M + Na]$^+$. Anal. Calc. for $C_{11}H_{14}ClNO_3Pt$: C, 30.11; H, 3.22; N, 3.19. Found: C, 30.39; H, 2.97; N, 3.02. Λ_m = 56 S·cm^2·mol^{-1}.

Pt[methyl 3-hydroxy-2-(2-hydroxybenzylamino)propanoate]Cl (**5c**)

Yellow solid. Yield 68.9%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.107 (d, 1H, Ar-H), 7.067 (t, 1H, Ar-H), 6.724-6.750 (m, 2H, Ar-H), 3.833 (d, 1H, N-CH_2), 3.676 (d, 1H, N-CH_2), 3.637 (s, 3H, CH_3), 3.611 (d, 2H, CH_2), 3.332 (t, 1H, N-CH); ^{13}C NMR (DMSO-d_6) δ: 40.5 (C-7), 51.9 (-OCH_3), 61.7 (C-8), 65.0 (C-6'), 117.2 (C-3), 120.8 (C-5), 125.6 (C-1), 129.5 (C-4), 129.9 (C-6), 156.7 (C-2), 176.6 (C-9)

IR (KBr): 3440 (N-H), 2957, 1638 (C=O), 1421, 1265 (ph-O), 1173, 787, 648, 620 (Pt-OAr), 520 (Pt-N), 420 (Pt-O=C) cm^{-1}. ESI-MS: 478.0145 $[M + Na]^+$. Anal. Calc. for $C_{11}H_{14}ClNO_4Pt$: C, 29.05; H, 3.10; N, 3.08. Found: C, 29.24; H, 2.95; N, 3.27. Λ_m = 58 $S \cdot cm^2 \cdot mol^{-1}$.

Pt[methyl 2-(2-hydroxybenzylamino)-3-(1H-indol-3-yl)propanoate]Cl (**5d**)

Yellow solid. Yield 78.5%. ^1H NMR (600 MHz, DMSO-d_6): δ 10.856 (s, 1H, NH), 7.457 (d, 1H, Ar-H), 7.340 (d, 1H, Ar-H), 7.126 (s, 1H, Ar-H), 7.054-7.086 (m, 3H, Ar-H), 6.977 (t, 1H, Ar-H), 6.699-6.730 (m, 2H, Ar-H), 3.810 (d, 1H, N-CH_2), 3.669 (d, 1H, N-CH_2), 3.566 (s, 1H, N-CH), 3.550 (s, 3H, CH_3), 3.034-3.102 (m, 2H, CH_2), 2.849 (s, 1H, NH) ; ^{13}C NMR (DMSO-d_6) δ: 31.2 (C-15'), 39.3 (C-7), 50.4 (-OCH_3), 60.2 (C-8), 110.9 (C-13'), 112.3 (C-8'), 115.4 (C-3), 119.5 (C-12'), 120.6 (C-5), 120.8 (C-10'), 121.7 (C-11'), 122.6 (C-7'),124.4 (C-1), 128.1 (C-4), 129.5 (C-6), 131.5 (C-9'), 136.4 (C-14'), 155.1 (C-2), 174.3 (C-9). IR (KBr): 3441 (N-H), 2957, 1645 (C=O), 1420, 1286 (ph-O), 1173, 786, 648, 620 (Pt-OAr), 520 (Pt-N), 418 (Pt-O=C) cm^{-1}. ESI-MS: 577.0630 $[M + Na]^+$. Anal. Calc. for $C_{19}H_{19}ClN_2O_3Pt$: C, 41.20; H, 3.46; N, 5.06. Found: C, 41.38; H, 3.20; N, 5.24. Λ_m = 55 $S \cdot cm^2 \cdot mol^{-1}$.

Pt[methyl 2-(5-bromo-2-hydroxybenzylamino)-3-phenylpropanoate]Cl (**5e**)

Yellow solid. Yield 68.8%. ^1H NMR (600 MHz, DMSO-d_6): δ 7.287 (t, 2H, Ar-H), 7.229 (dd, 2H, Ar-H), 7.192 (d, 3H, Ar-H), 6.680 (d, 1H, Ar-H), 3.720 (d, 1H, N-CH_2), 3.596 (d, 1H, N-CH_2), 3.560 (s, 3H, CH_3), 3.473 (t, 1H, N-CH), 2.895 (dd, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 39.2 (C-21'), 45.1 (C-7), 51.8 (-OCH_3), 60.1 (C-8), 111.2 (C-5), 117.6 (C-3), 126.8 (C-18'), 127.7 (C-1), 128.8 (C-20', C-16'), 129.8 (C-17', C-19'), 130.4 (C-4), 131.4 (C-6), 137.9 (C-15'), 155.4 (C-2), 177.5 (C-9). IR (KBr): 3442 (N-H), 2957, 1656 (C=O), 1420, 1267 (ph-O), 1173, 786, 649, 620 (Pt-OAr), 524 (Pt-N), 422 (Pt-O=C) cm^{-1}. ESI-MS: 615.9609 $[M + Na]^+$. Anal. Calc. for $C_{17}H_{17}BrClNO_3Pt$: C, 34.39; H, 2.89; N, 2.36. Found: C, 34.51; H, 2.68; N, 2.52. Λ_m = 53 $S \cdot cm^2 \cdot mol^{-1}$.

Pt[methyl 2-(5-chloro-2-hydroxybenzylamino)-4-methylpentanoate]Cl (**5f**)

Yellow solid. Yield 87.6%. ^1H NMR (600 MHz, DMSO-d_6): δ 7.207 (d, 1H, Ar-H), 7.092 (dd, 1H, Ar-H), 6.750 (d, 1H, Ar-H), 3.744 (d, 1H, N-CH_2), 3.629 (s, 3H, CH_3), 3.589 (d, 1H, N-CH_2), 3.245 (t, 1H, N-CH), 1.677-1.746 (m, 1H, -CH-), 1.386-1.492 (m, 2H, CH_2), 0.882 (d, 3H, CH_3), 0.839 (d, 3H, -CH_3) ; ^{13}C NMR (DMSO-d_6) δ: 22.3 (C-3', C-4'), 23.1 (C-2'), 41.3 (C-1'), 40.5 (C-7), 50.4 (-OCH_3), 57.2 (C-8), 116.7 (C-3), 125.8 (C-1), 126.2 (C-5), 128.5 (C-4), 129.7 (C-6), 153.2 (C-2), 174.8 (C-9). IR (KBr): 3442 (N-H), 2957, 1644 (C=O), 1438, 1270 (ph-O), 1173, 788, 649, 619 (Pt-OAr), 518 (Pt-N), 422 (Pt-O=C) cm^{-1}. ESI-MS: 538.0279 $[M + Na]^+$. Anal. Calc. for $C_{14}H_{19}Cl_2NO_3Pt$: C, 32.63; H, 3.72; N, 2.72. Found: C, 32.84; H, 3.50; N, 2.80. Λ_m = 50 $S \cdot cm^2 \cdot mol^{-1}$.

2.5. Determination of UV absorption spectra

An UV-3400 Toshniwal spectrophotometer emitting UV light was used mainly between 200~400 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 **5f**) 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. The UV absorption spectra was determined at room temperature (Δt = 1 s, n = 3).

2.6. Cell culture

Four different human carcinoma cell lines: HL-60, Hela, BGC-823 and HepG-2 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.

2.7. 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.

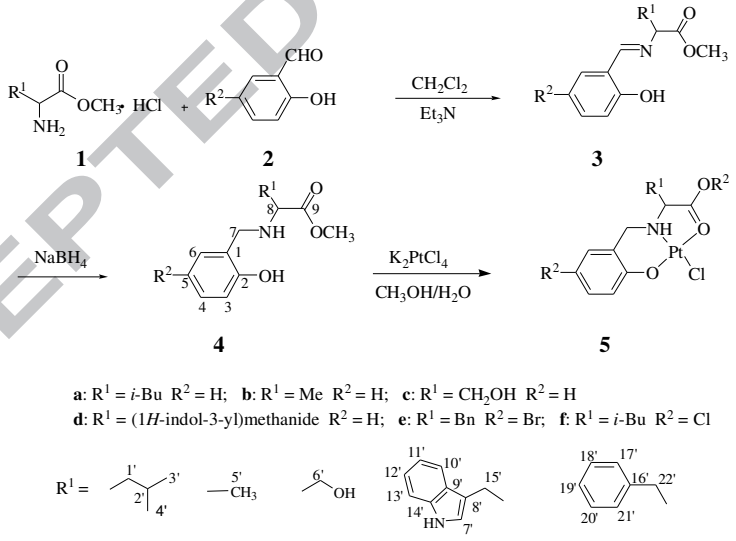
2.8. Cytotoxicity analysis

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 [25]. 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.

3. Results and discussion

3.1. Synthesis and characterization

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



a: R^1 = *i*-Bu R^2 = H; **b**: R^1 = Me R^2 = H; **c**: R^1 = CH_2OH R^2 = H
d: R^1 = (1*H*-indol-3-yl)methanide R^2 = H; **e**: R^1 = Bn R^2 = Br; **f**: R^1 = *i*-Bu R^2 = Cl

Scheme 1. Synthetic pathway for the preparation of compounds.

The bands at about 3316~3441 cm^{-1} in the IR spectra of **4a-4f** can be assigned to the (N-H) stretching frequencies, which shifted to the higher wave number (3440~3442 cm^{-1}) upon complexation in **5a-5f**. A carbonyl (C=O) vibration band which appeared in the IR spectra of ligands (1723~1739 cm^{-1}) similarly underwent a shift into lower frequency (1638~1656 cm^{-1}) in their Pt(II) complexes. The bands for the phenolic group (C-O) stretching frequency observed in the region 1241~1260 cm^{-1} of free

ligands shifted to higher frequency (1265~1286 cm^{-1}) in their complexes. On the other hand, new bands appeared at about 617~620, 518~524 and 412~422 cm^{-1} could be assigned to $\upsilon_{Pt-O=C}$, υ_{Pt-N}, and υ_{Pt-OAr}, respectively. The IR data revealed that nitrogen atoms of the imino groups and oxygen atoms of the carbonyl groups and phenolic groups were coordinated to the metal ion (**Table 1**).

Although the overall pattern of the ^{1}H NMR spectra of **5a-5f** resemble very closely to that of the free ligands, almost all the signals have been shifted to lower fields upon coordination. In the ^{13}C NMR analysis, most of the characteristic signals for the complexes changed slightly, however signals of 5a-5e observed at 38.9–45.1 ppm, 55.2–63.2 ppm and 153.2–156.7 ppm are assignable to the carbon atoms (C7, 8, 2) respectively, which have been shifted to higher fields upon coordination. What's more, the C9 signals for all the complexes have been shifted to lower fields which proved the -C=O coordination to the Pt(II) ion. All the platinum complexes were characterized by ESI-MS, the results showed the presence of $[M+Na]^{+}$ peaks were in good agreement with their formula weights and relative fragment peaks. Elemental analysis confirmed the structures of the target compounds further. 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 $S{\cdot}cm^{2}{\cdot}mol^{-1}$. Therefore, all complexes are electroneutral [30].

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	3316	1735	1254			
4b	3430	1723	1256			
4c	3426	1735	1257			
4d	3438	1739	1241			
4e	3431	1725	1260			
4f	3441	1735	1259			
5a	3441	1649	1284	412	520	620
5b	3440	1653	1267	418	524	617
5c	3440	1638	1265	420	520	620
5d	3441	1645	1286	418	520	620
5e	3442	1656	1267	422	524	620
5f	3442	1644	1270	422	518	619

3.2. DNA Binding Study

Interaction of the complexes with DNA has been studied with UV spectroscopy in order to investigate the possible binding modes [26]. Generally speaking, there are three main noncovalent binding modes between antitumor drugs with DNA: Electrostatic binding, intercalations between base pairs and binding into the groove of DNA. In the spectral range 200~400 nm, DNA with different concentration of complexes (**5c** and **5f**) was examined. As the concentration of complex increased, the absorbance value increased and maximum absorption wavelength occurred blue-shifted. It might be due to the complex was inserted into the DNA double helix structure and stacking with DNA base pairs where π-electron accumulation occurred. The results indicated that hyperchromicity occurred after coordination, thus the binding mode of complexes with DNA may be intercalation [27-29].

3.3. Stability of Complexes

Because the stability of platinum-based compounds is one of the key criteria for being an anticancer candidate, complex **5f** was evaluated by UV spectral analysis at different times in 1% DMSO/99% H_2O (**Figure 1**). According to the results in **Figure 1**, no detectable significant changes of the absorption bands of complex **5f** have been observed within 18 h, which demonstrates the excellent stability of the determined complex under the test conditions [31].

Figure 1

3.4. In Vitro Cytotoxicity Assay

In order to find possible structure-activity relationships, the *in vitro* cytotoxicity of complexes **5a-5f** was evaluated against four human cancer cell lines including HL-60, BGC-823, Hela, and HepG2. For comparison purposes, the cytotoxicity of cisplatin, a standard antitumor drug, was also evaluated under the same conditions. The IC_{50} values (the concentration that inhibited in 50% the cellular proliferation) of the studied complexes are presented in **Table 2**. It was noted that all complexes showed dose-dependent antiproliferative effect with a lower IC_{50} value (<50 μM) toward investigated cancer cell lines. Moreover, of all the studied complexes, complex **5f** displayed the best cytotoxicity against selected carcinoma cell lines, it showed better cytotoxicity against BGC-823 than cisplatin, but slightly less activity than cisplatin on HL-60, Hela and HepG2. When tested against HepG2 cell line, **5e** showed better activity than cisplatin, other complexes exhibited comparable cytotoxicity to **5e**, but a slightly less than cisplatin. Compared **5a** with **5f** , the antitumor activity increased with the introduce of electron-withdrawing groups at the C^4 position of the benzene ring. The complexes (**5a**, **5b**, **5c** and **5d**) demonstrated less active cytotoxicity than cisplatin against the tested cell lines, all the complexes had less active cytotoxicity against HL-60 and Hela cell lines in compared with cisplatin.

Table 2.

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

Complex	IC_{50} (μM)			
	HL-60	BGC-823	Hela	HepG2
5a	40.56	20.59	47.41	30.57
5b	17.32	15.62	38.91	23.80
5c	24.44	15.99	41.75	24.13
5d	21.68	26.96	43.12	28.00
5e	22.09	9.78	10.28	18.45
5f	4.08	6.36	9.92	21.46
Cisplain	2.29	6.48	4.41	20.60

4. Conclusion

In conclusion, some platinum(II) complexes with reduced Schiff bases derived from amino acid ester as ligands were synthesized and characterized. All complexes were tested for their DNA interaction ability with the salmon sperm DNA through ultraviolet spectrum, the results revealed that the binding mode of complexes with DNA may be intercalation after coordination. The *in vitro* antitumor activities of the platinum complexes have been validated against the HL-60, BGC-823, Hela, and HepG2 cell lines by the MTT assay, the results showed that the complexes **5a-5f** have selectivity against tested

carcinoma cell lines. The complex **5f** was the most effective agent among these complexes, furthermore its antitumor activity is better than that of cisplatin against BGC-823 cell line. The structure-activity relationship has been analyzed, and this study would be helpful in designing new platinum anticancer drugs.

Acknowledgements

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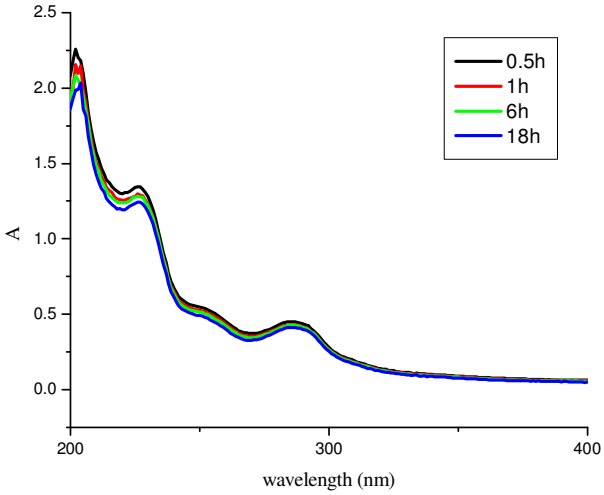

Figure 1. Ultraviolet spectrum of stability of complex **5f**

Synthesis, characterization and cytotoxicity studies of platinum(II) complexes with reduced amino acid ester Schiff-bases as ligands

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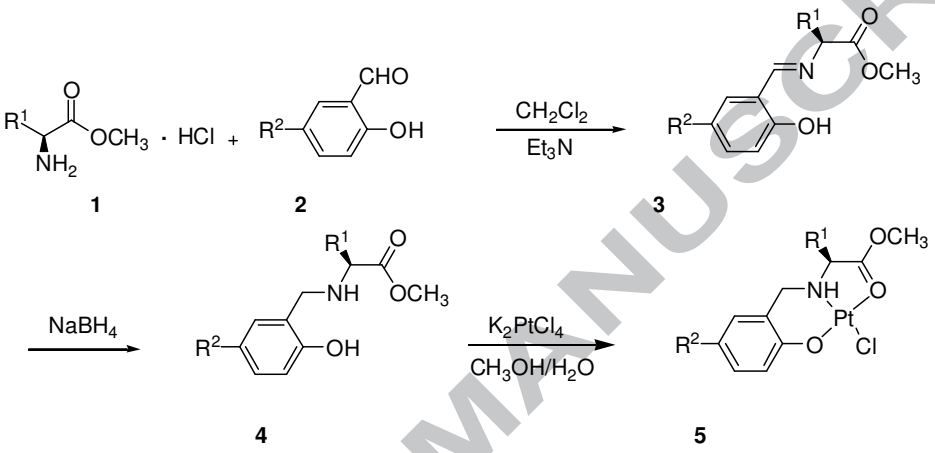

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- We synthesized six platinum(II) complexes which were rarely reported.
- All complexes were prepared with reduced amino acid esters Schiff-bases as ligands.
- Some complexes showed better activity than cisplatin.
- The complex we synthesized showed good stability.