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Original article

Synthesis, characterization and cytotoxicity evaluation of some new platinum(II) complexes of tetrazolo[1,5-a]quinolines

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Abstract

Several new platinum(II) complexes of the general formulae *cis*-[$PtCl_2$(DMSO)**L**], where **L** is a Schiff base or hydrazone derived from tetrazolo[1,5-a]quinoline-4-carboxaldehyde as carrier ligands, have been synthesized and characterized physicochemically and spectroscopically. These platinum complexes which are structurally analogues to what so called cisplatin {*cis*-[$PtCl_2(NH_3)_2$]; the first generation anticancer agent} were evaluated for their cytotoxicity on HL-60 (human promyelocytic leukemia) cells. Two of the platinum complexes were almost similar in their activity to cisplatin, while the remaining three complexes have demonstrated higher efficacy than that of cisplatin. Based on our findings, these novel platinum complexes appear to be valuable leading compounds with high efficacy.

Keywords: Platinum complexes; Tetrazolo[1,5-a]quinolines derivatives; Cytotoxic agents; Anticancer agents

1. Introduction

The development of potent and effective antineoplastic drugs has become one of the most intensely persuaded goals of contemporary medicinal chemistry. Introduction of the square-planar complex *cis*-diaminodichloroplatinum(II) (cisplatin; CDDP) into clinical treatment of cancer has resulted in excellent response rates for some tumors, especially testicular and ovarian cancers [1]. CDDP is currently used as a first line chemotherapeutic agent for the treatment of testicular, bladder and other cancers. Chlorine atoms or other good leaving groups are displaced by water under physiologic conditions. The cytotoxicity of the hydrated molecule is thought to result from covalent bonds to DNA that form between these active groups and guanines or arginines. So, the antitumor activity of cisplatin arises from its binding to genomic DNA. After activation via intracellular hydration reactions, the cytotoxic effects of platinum compounds appear caused by the formation of stable bifunctional adducts on DNA. Different studies showed that monofunctional adducts occur only transiently, with complete rearrangement to bifunctional adducts within 2 h [2,3]. The majority of cisplatin-DNA adducts (>90%) are intrastrand 1,2-D(GpG) and -D(ApG) crosslinks in which the two chlorine atoms of the cisplatin molecule are replaced by the N_7-atoms of adjacent purine bases. These cisplatin-DNA complexes bend and unwind the duplex at the site of damage with its major groove being compressed and the minor groove widened [4]. Especially high-mobility group (HMG) domain proteins are specifically attracted by the hydrophobic surface created at the cisplatin-crosslinked site and might mediate the antitumor efficacy of cisplatin. Clinical success of cisplatin is associated with various mechanisms of resistance such as repair of cisplatin-modified DNA by enzymatic removal of the adducts [5]. This occurs primarily by the nucleotide excision-repair (NER) pathway [6] which is controlled by several damage-recognition proteins. The specific binding of HMG domain proteins to the widened hydrophobic minor groove can shield the major 1,2-intrastrand adducts from the access

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of the NER-complex, thus preventing resistance. However, the efficacy of CDDP is combined with its propensity to cause several types of dose-limiting toxicity, including nephrotoxicity, nausea, neurotoxicity and myelo-suppression. The nephrotoxicity is due to the fact that the kidney accumulates and retains platinum to a greater extent than other organs and is the prominent excretory organ for platinum complexes [7,8]. Platinum coordination complexes consist of a platinum leaving group and a carrier ligand. The advanced knowledge of structure–activity relationship of platinum(II) complexes has shed spot light on the carrier ligands and their leaving groups as being essential for their in vivo antitumor activities. The contribution of the carrier ligand may be related to the potency and spectrum of antitumor activity, and that of the leaving group may be related with the dissociation rate from the platinum complex. Moreover, it is essential to consider that the leaving group is an important factor influencing the activity of the platinum coordination complexes. Enlightened by the afore-mentioned facts and as a continuation of our on going program in the field of anticancer design and synthesis [9–12], it becomes of interest to prepare agents with lower toxicity and more favorable therapeutic indices. To accomplish this goal, some new platinum analogs containing Schiff bases or hydrazones of tetrazolo[1,5-a]quinoline-4-carboxaldehyde as a carrier ligand (**L**) were synthesized and the in vitro antitumor activities of the new platinum complexes were evaluated on HL-60 (human promyelocytic leukemia) cells.

2. Materials and methods

All chemicals were purchased from E. Merck, Fluka AG and Aldrich companies. Melting points were determined in open glass capillaries using Thomas capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded for KBr discs on a Pye-Unicam FTIR spectrophotometer or 470-Shimadzu infrared spectrophotometer. ^{1}H-NMR spectra were recorded on JEOL-400 MHz spectrometer ($CDCl_3$) and Varian Unity 500 (DMSO-d_6), and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. ^{13}C-NMR spectra were recorded on Gemini 2000 spectrometer. Elemental analyses were performed at the Institut für Anorganische Chemie, Martin-Luther-Universität Halle-Wittenberg, Germany and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254 nm for few seconds.

2.1. Preparation of Schiff bases (*L*^{1}–*L*^{3})

These were prepared according to the following general procedure [13].

Table 1
Physical and analytical data of compounds (**L**^{1}–**L**^{3}) [9] and (**L**^{4}–**L**^{5}) [9]

Compound numbers	Yield (%)	M.p. (°C)	Molecular formula (molecular weight)
L^1	93	170–172	$C_{16}H_{11}N_5$ (273.29)
L^2	96	209–211	$C_{16}H_{10}ClN_5$ (303.74)
L^3	94	182–184	$C_{17}H_{13}N_5O$ (287.32)
L^4	97	257–259	$C_{16}H_{12}N_6$ (288.31)
L^5	88	215–217	$C_{11}H_{10}N_6$ (226.24)

To a solution of tetrazolo[1,5-a]quinoline-4-carboxaldehyde (0.01 mol) in dioxane (20 ml), the appropriate aromatic amine (0.01 mol) was added. The reaction mixture was heated under reflux for 1 h, cooled and poured onto cold water. The formed precipitate was filtered, washed with water, dried and crystallized from *n*-butanol for compounds **L**^{1} and **L**^{3} or cyclohexane for compound **L**^{2}, Table 1.

IR (cm^{-1}) of **L**^{1}–**L**^{3}: 1630–1620 (C=N), 1550–1510 (C=C).

^{1}H-NMR of **L**^{1}: δ 7.34 (t, J = 7.6 Hz, 1H, phenyl-C_4–H), 7.40 (d, J = 8.4 Hz, 2H, phenyl-$C_{2,6}$–H), 7.50 (dd, J_1 = 7.6, J_2 = 8.4 Hz, 1H, phenyl-$C_{3,5}$–H), 7.86 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_8–H), 8.04 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_7–H), 8.38 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_6–H), 8.64 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_9–H), 8.88 (s, 1H, tetrazoloquin-C_5–H), 9.08 (s, 1H, C*H*=N).

^{1}H-NMR of **L**^{2}: δ 7.36 (d, J = 8.4 Hz, 2H, phenyl-$C_{2,6}$–H), 7.52 (d, J = 8.4 Hz, 2H, phenyl-$C_{3,5}$–H), 7.87 (dd, J_1 = 7.2, J_2 = 8.04 Hz, 1H, C_8–H), 8.07 (dd, J = 7.2, 8.4 Hz, 1H, C_7–H), 8.41 (d, J = 8.4 Hz, 1H, C_6–H), 8.58 (d, J = 8.4 Hz, 1H, C_9–H), 8.92 (s, 1H, C_5–H), 9.11 (s, 1H, CH=N).

^{1}H-NMR of **L**^{3}: δ 2.30 (s, 3H, CH_3), 7.29 (d, J = 8.4 Hz, 2H, phenyl-$C_{3,5}$–H), 7.34 (d, J = 8.4 Hz, 1H, phenyl-$C_{2,6}$–H), 7.87 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_8–H), 8.05 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_7–H), 8.40 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_6–H), 8.66 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_9–H), 8.89 (s, 1H, tetrazoloquin-C_5–H), 9.11 (s, 1H, C*H*=N).

2.2. Preparation of hydrazones (*L*^{4}–*L*^{5})

These were prepared according to the following general procedure [13].

To a solution of methylhydrazine or phenylhydrazine (0.01 mol) in dioxane (25 ml), tetrazolo[1,5-a]quinoline-4-carboxaldehyde (0.01 mol) was added portionwise and the reaction mixture was refluxed for 1 h. After cool, the mixture was diluted with water, the obtained product was filtered, washed with water, dried and crystallized from ethanol for compound **L**^{4} and acetic acid for compound **L**^{5}, Table 1.

IR (cm^{-1}) of **L**^{4}, **L**^{5}: 3275–3272 (NH), 135–16 333 (C=N).

^{1}H-NMR of **L**^{4}: 6.86 (t, J = 8.4, 1H, phenyl-C_4–H), 7.24 (d, J = 7.6 Hz, 2H, phenyl-$C_{2,6}$–H), 7.30 (dd, J_1 = 7.6, J_2 = 8.4 Hz, 2H, phenyl-$C_{3,5}$–H), 7.80 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_8–H), 7.91 (dd, J_1 = 7.2,

J_2 = 8.4 Hz, 1H, tetrazoloquin-C_7–H), 8.34 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_6–H), 8.42 (s, 1H, tetrazoloquin-C_5–H), 8.52 (s, 1H, CH=N), 8.58 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_9–H), 11.09 (s, 1H, NH, D_2O exchangeable).

^{1}H-NMR of **5**: δ 2.98 (s, 3H, CH_3), 7.74 (dd, J_1 = 7.6, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_8–H), 7.78 (s, 1H, tetrazoloquin-C_5–H), 7.86 (dd, J_1 = 7.6, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_7–H), 8.22 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_6–H), 8.28 (s, 1H, CH=N), 8.54 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_9–H), 10.36 (s, 1H, NH, D_2O-exchangeable).

2.3. Preparation of the platinum(II) complexes **1–5**

These were prepared according to the following general procedure.

The complex *cis*-[$PtCl_2(DMSO)_2$] {prepared according to our previous procedure [14] (0.221 g, 0.5 mmol) was suspended in absolute ethanol (20 ml) and the ligand $\mathbf{L^1}$ (0.170 g, 0.6 mmol) was added. The reaction mixture was heated gently for ca. 1 h until all the suspension had gone into solution. The clear yellow–orange solution was evaporated to until a solid started to deposit. It was then left in the refrigerator for several hours for complete precipitation. The colored solid thus formed was filtered off, washed with small portions of cold ethanol, ether and dried under vacuum to give complex **1**. In a similar manner, complexes **2–5** were prepared, Table 2.

IR (cm^{-1}) of complexes **1–3**: 1615–1610 (C=N), 1156–1150 (S=O). R (cm^{-1}) of complexes **4–5**: 3268–3265 (NH), 1610–1609 (C=N), 1155–1154 (S=O).

^{1}H-NMR of complex **1**: δ 7.31 (t, J = 7.6 Hz, 1H, phenyl-C_4–H), 7.37 (d, J = 8.4 Hz, 2H, phenyl-$C_{2,6}$–H), 7.46 (dd, J_1 = 7.6, J_2 = 8.4 Hz, 1H, phenyl-$C_{3,5}$–H), 7.84 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_8–H), 8.02 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_7–H), 8.40 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_6–H), 8.63 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_9–H), 8.89 (s, 1H, tetrazoloquin-C_5–H), 9.08 (s, 1H, C*H*=N).

^{13}C-NMR of **1**: δ 116.25 (phenyl-$C_{2,6}$), 120.90 (tetrazoloquin-C_4), 121.15 (tetrazoloquin-C_7), 123.49 (phenyl-C_4), 127.04 (tetrazoloquin-C_6), 128.47 (tetrazoloquin-C_{5a}), 129.42 (tetrazoloquin-C_8), 130.55 (tetrazoloquin-C_9), 130.81 (phenyl-$C_{3,5}$), 132.50 (tetrazoloquin-C_5), 132.72 (phenyl-C_1), 146.40 (tetrazoloquin-C_{9a}), 150.67 (tetrazoloquin-C_{3a}), 153.42 (C=N).

^{1}H-NMR of complex **2**: δ 6.56 (d, J = 8.4 Hz, 2H, phenyl-$C_{2,6}$–H), 7.12 (d, J = 8.4 Hz, 2H, phenyl-$C_{3,5}$–H), 7.82 (dd, J_1 = 7.2, J_2 = 8.04 Hz, 1H, C_8–H), 7.92 (dd, J = 7.2, 8.4 Hz, 1H, C_7–H), 8.38 (d, J = 8.4 Hz, 1H, C_6–H), 8.49 (d, J = 8.4 Hz, 1H, C_9–H), 8.89 (s, 1H, C_5–H), 9.17 (s, 1H, CH=N).

^{13}C-NMR of **2**: δ 113.09 (phenyl-$C_{3,5}$), 116.55 (tetrazoloquin-C_4), 120.42 (tetrazoloquin-C_7), 121.54 (phenyl-$C_{3,5}$), 124.79 (phenyl-C_1), 125.69 (tetrazoloquin-C_6), 128.69 (tetrazoloquin-C_{5a}), 129.05 (tetrazoloquin-C_8), 129.45 (tetrazoloquin-C_9), 129.63 (tetrazoloquin-C_5), 129.89 (phenyl-C_4), 136.95 (tetrazoloquin-C_{9a}), 144.82 (tetrazoloquin-C_{3a}), 146.60 (C=N).

^{1}H-NMR of complex **3**: δ 2.31 (s, 3H, CH_3), 7.30 (d, J = 8.4 Hz, 2H, phenyl-$C_{3,5}$–H), 7.34 (d, J = 8.4 Hz, 1H, phenyl-$C_{2,6}$–H), 7.87 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_8–H), 8.05 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_7–H), 8.42 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_6–H), 8.61 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_9–H), 8.92 (s, 1H, tetrazoloquin-C_5–H), 9.12 (s, 1H, C*H*=N).

^{13}C-NMR of complex **3**: δ 120.90 (tetrazoloquin-C_4), 121.15 (tetrazoloquin-C_7), 126.86 (phenyl-C_1), 127.14 (tetrazoloquin-C_6), 128.43 (tetrazoloquin-C_{5a}), 129.11 (phenyl-$C_{3,5}$), 129.46 (tetrazoloquin-C_8), 129.74 (phenyl-$C_{2,6}$), 130.28 (tetrazoloquin-C_9), 132.39 (tetrazoloquin-C_5), 133.68 (phenyl-C_4), 146.56 (tetrazoloquin-C_{9a}), 150.78 (tetrazoloquin-C_{3a}), 154.26 (C=N).

^{1}H-NMR of complex **4**: 6.84 (t, J = 8.4, 1H, phenyl-C_4–H), 7.22 (d, J = 7.6 Hz, 2H, phenyl-$C_{2,6}$–H), 7.26 (dd, J_1 = 7.6, J_2 = 8.4 Hz, 2H, phenyl-$C_{3,5}$–H), 7.77 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_8–H), 7.88 (dd, J_1 = 7.2, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_7–H), 8.25 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_6–H), 8.38 (s, 1H, tetrazoloquin-C_5–H), 8.52 (s, 1H, CH=N), 8.59 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_9–H), 11.05 (s, 1H, NH, D_2O exchangeable).

^{13}C-NMR of **4**: δ 116.45 (phenyl-$C_{2,6}$), 118.57 (tetrazoloquin-C_4), 121.36 (tetrazoloquin-C_7), 122.83 (phenyl-C_4), 122.98 (tetrazoloquin-C_6), 128.41 (tetrazoloquin-C_{5a}), 128.68 (tetrazoloquin-C_8), 129.33 (tetrazoloquin-C_9), 130.82 (phenyl-$C_{3,5}$), 131.61 (tetrazoloquin-C_5), 133.15 (phenyl-C_1), 134.39 (tetrazoloquin-C_{9a}), 135.34 (tetrazoloquin-C_{3a}), 139.70 (C=N).

^{1}H-NMR of complex **5**: δ 2.94 (s, 3H, CH_3), 7.69 (dd, J_1 = 7.6, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_8–H), 7.79 (s, 1H, tetrazoloquin-C_5–H), 7.85 (dd, J_1 = 7.6, J_2 = 8.4 Hz, 1H, tetrazoloquin-C_7–H), 8.17 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_6–H), 8.29 (s, 1H, CH=N), 8.55 (d, J = 8.4 Hz, 1H, tetrazoloquin-C_9–H), 10.39 (s, 1H, NH, D_2O exchangeable).

^{13}C-NMR of **5**: δ 33.73 (CH_3), 116.46 (tetrazoloquin-C_4), 121.90 (tetrazoloquin-C_7), 122.66 (tetrazoloquin-C_6), 123.18 (tetrazoloquin-C_{5a}), 124.97 (tetrazoloquin-C_8), 128.57 (tetrazoloquin-C_9), 129.05 (tetrazoloquin-C_5), 132.72 (phenyl-C_1), 129.59 (tetrazoloquin-C_{9a}), 130.29 (tetrazoloquin-C_{3a}), 146.74 (C=N).

Table 2
Physical and analytical data of complexes

Complex numbers	Yield (%)	M.p. (°C)	Molecular formula (molecular weight)
1	92	192 (decompose)	$C_{18}H_{17}Cl_2N_5OPtS$ (617.41)
2	94	176 (decompose)	$C_{18}H_{16}Cl_3N_5OPtS$ (651.85)
3	92	203 (decompose)	$C_{19}H_{19}Cl_2N_5OPtS$ (631.44)
4	93	216 (decompose)	$C_{18}H_{18}Cl_2N_6OPtS$ (632.42)
5	96	232 (decompose)	$C_{13}H_{16}Cl_2N_6OPtS$ (570.36)

2.4. Cytotoxicity of platinum(II) complexes

2.4.1. Cell line and treatment

HL-60 (human promyelocytic leukemia) cells were procured from ATCC (UK). The cells were grown in suspension

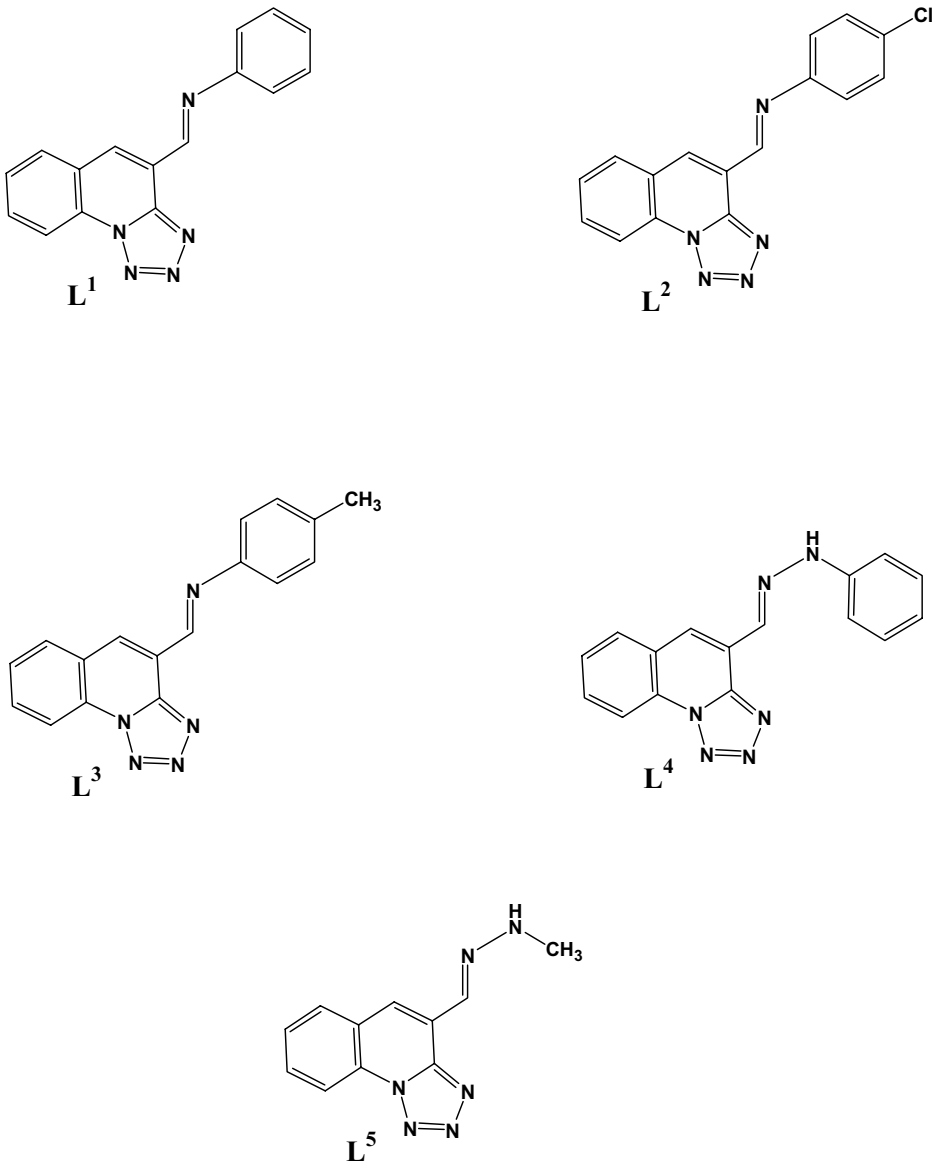

Fig. 1. Structure of the Schiff bases L^1–L^3 and hydrazones L^4–L^5 investigated in the present work.

culture using RPMI1640 media containing 20% fetal bovine serum and supplements. HL-60 cells were treated with 10 and 100 μM compounds **1–5** and cisplatin, as a standard reference, for 48 h at 37 °C incubator using 5% carbon dioxide. The platinum compounds were dissolved in DMSO/methanol (1:1). The vehicle received the mixture of DMSO and methanol. The cells were harvested by trypsinization for analysis of apoptosis as described below.

2.4.2. Induction of apoptosis

Apoptosis was observed with the platinum compounds (**1–5**) and cisplatin (10 and 100 μM) and measured by Flow cytometery using the Vibrant apoptosis assay kit #2 (Molecular Probes, Inc., Eugene, OR) according to the manufacturer's instructions. Cells were washed in cold phosphate-buffered saline and centrifuged. Cells were stained with annexin V and propidium iodide in annexin-binding buffer. After 15 min incubation at room temperature, the fluorescence was measured using the flow cytometer (FAC Scan, Becton Dickenson, USA). The results were analyzed using Cell Quest Pro software and represented as percentage of normal and apoptotic cells at various stages. Simultaneously, treated HL-60 cells were stained for nuclear DNA and sub-G_1 apoptotic population was analyzed [15].

3. Results and discussion

3.1. Chemistry

The Schiff bases and hydrazones, L^1–L^5 (Fig. 1) were prepared according to the reported methods, by reacting of tetrazolo[1,5-a]quinoline-4-carboxaldehyde with the appropriate amine or substituted hydrazine, respectively. The structures of these Schiff bases and hydrazones were assigned with the help of their IR, ^{1}H-NMR, ^{13}C-NMR spectra,

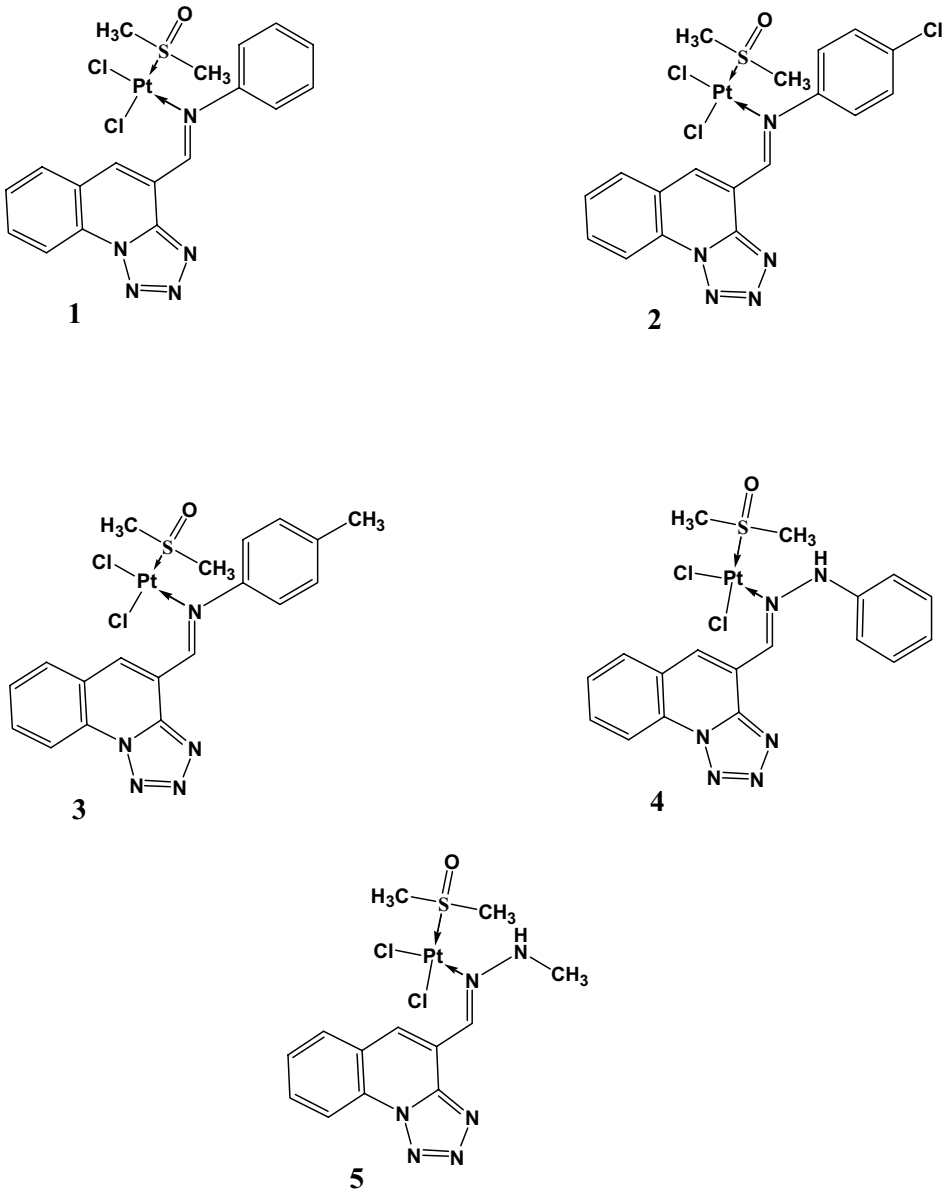

Fig. 2. Proposed structures of Pt(II) complexes (**1–5**) in the present study.

and analytical data. The metal complexes **1–5** (Table 1) of these Schiff bases and hydrazones were prepared by the stoichiometric reaction of *cis*-[$PtCl_2(DMSO)_2$] and the corresponding ligand $\mathbf{L^1}$–$\mathbf{L^5}$ in equimolar ratios with a slight excess of the ligand. The complexes are stable solids, which decompose without melting and are insoluble in the common organic solvents such as ethanol, methanol or acetone. However, DMSO or DMF dissolved all complexes.

Although the ligands $\mathbf{L^1}$–$\mathbf{L^5}$ (Fig. 1) possess several donating sites but for geometry reasons it plausible that the most active basic site is the azomethine (imine) one. Hence, the coordination of these ligands would occur via the lone pair of electrons available on the N atom of the imine group [16]. On the other hand, it was found that the reaction of these ligands with *cis*-[$PtCl_2(DMSO)_2$] was taken place via removal of one labile DMSO ligand. Therefore, the structures of the platinum complexes **1–5**, *cis*-[$PtCl_2(DMSO)\mathbf{L}$] listed in Fig. 2 are the most possible ones, i.e. the *cis*-configuration of the platinum starting material was retained.

Confirmation of these arguments comes from the analytical data (see Experimental part). Furthermore, the coordinated DMSO ligand in these complexes (Fig. 2) was approved by the appearance of a characteristic IR band at ca. 1155 cm^{-1} which definitely attributed to the band υ (S=O) and further indicating that DMSO is coordinated in the complex via sulfur atom [17,18]. Further support for this comes from the ^{1}H-NMR spectra of the complexes in $CDCl_3$ which showed a single signal of methyl groups of the coordinated DMSO appeared at ca. 3.5 ppm associated with platinum satellites with ^3J(Pt–S–CH) of ca. 22 Hz [8,12,13]. It worth to mention that trials to get crystal suitable for X-ray structure determination went in vain amorphous character of the complexes, but structures of similar compounds were determined by X-ray in our previous work [17].

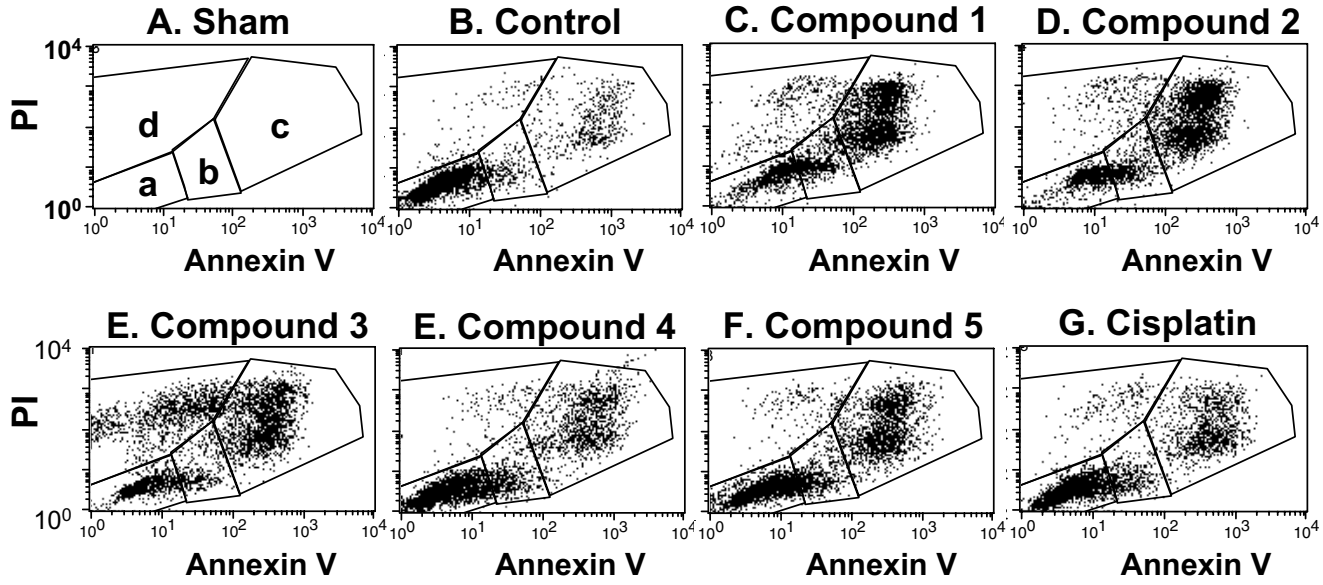

Fig. 3. Flow cytometric analysis showing the distribution of HL-60 cells treated with the test compounds and cisplatin. Briefly, cells were treated with the test compounds (100 μM) for 48 h followed by flow cytometric analysis using Cell Quest Pro software. Panel A (sham) shows the schematic representation of the cells distribution: a (normal), b (early apoptotic), c (middle and late apoptotic) and d (necrotic).

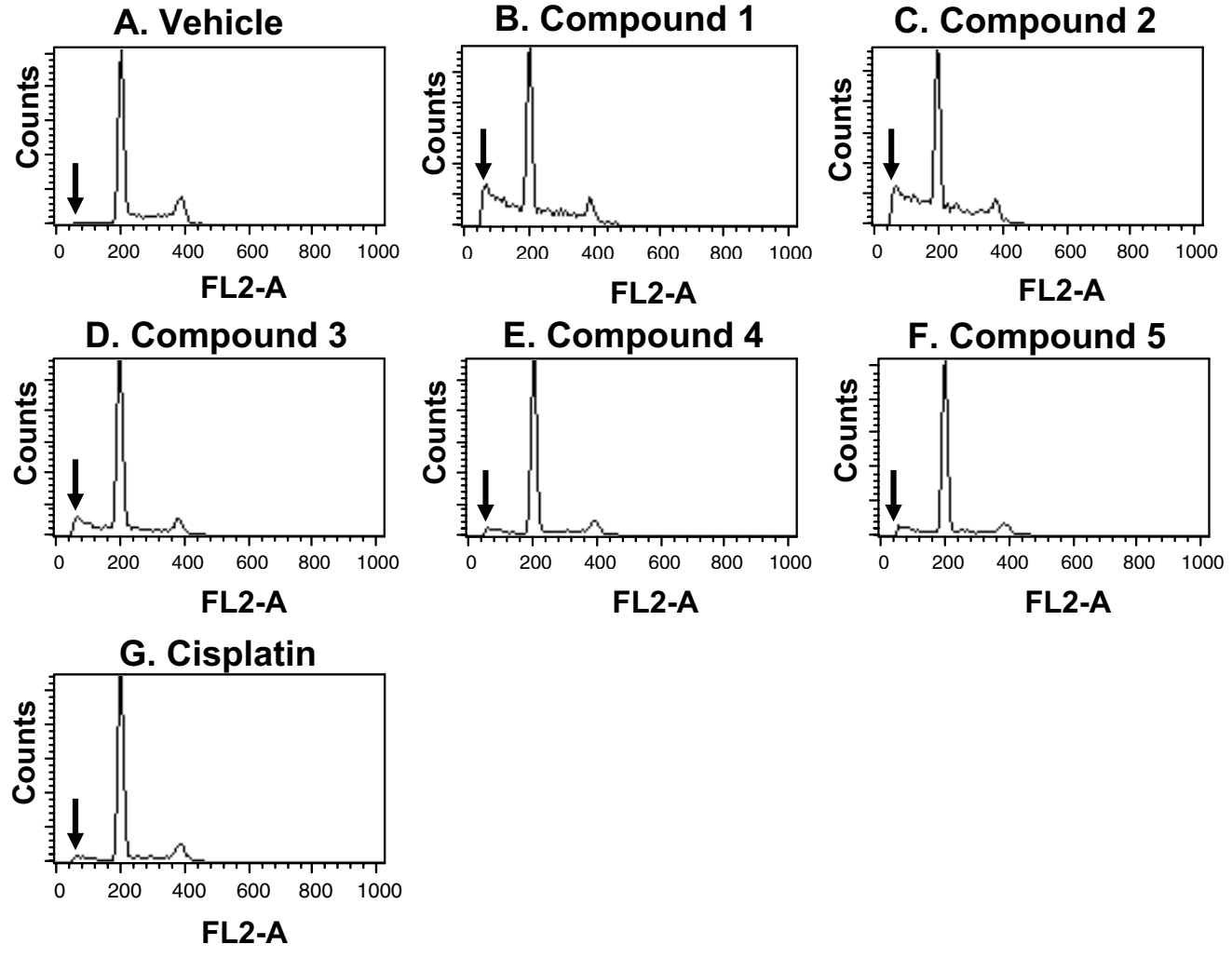

Fig. 4. DNA content analysis. HL-60 cells were treated with the test compounds as described in Fig. 1 legend. Arrow represents the sub-G_1 apoptotic population.

3.2. Cytotoxicity of platinum(II) complexes (*1–5*)

Interestingly, the newly synthesized platinum complexes **1–3** showed approximately 30% cells to be viable at 100 μM dose while at lower dose they showed 70% viability (Figs. 3 and 5A). The complexes **4** and **5** showed essentially similar pattern of cell viability (60–80%) as compared to the cisplatin. Exposure of HL-60 cells to the test compounds showed various degrees of apoptosis in a dose-dependent manner (Figs. 3, 4 and 5B). Complexes **1** and **2** at 100 μM dose induced apoptosis in more than 55% of cells while the remaining compounds **3–5** mildly induced the apoptosis (20–35%) (Fig. 5B). The lower dose of these compounds induced only 10–20% apoptosis. Cisplatin used as a positive control showed less than 20% apoptosis with both doses. Further, these complexes did not show any appreciable change in the G_0/G_1 and G_2/M population. Our preliminary results suggest that the complexes **1–3** are relatively more cytotoxic compared to the complexes **4** and **5**, which behaved like cisplatin. Generally, complexes having Schiff bases as ligands (**1–3**) are more active than complexes having hydrazone derivatives as ligands (**4** and **5**). Introducing of chlorine atom to the ligand $\mathbf{L}^2$, to give complex **2** increases its activity, while introducing methyl group to give complex **3** decreases its activity when both compared with complex **1**. On the other hand, the *N*-methyl hydrazone (complex **5**) is more active than the *N*-phenyl analogues (complex **4**). Therefore, such complexes would represent a fruitful matrix for the development of new platinum(II) complexes that would deserve further investigation and derivatization.

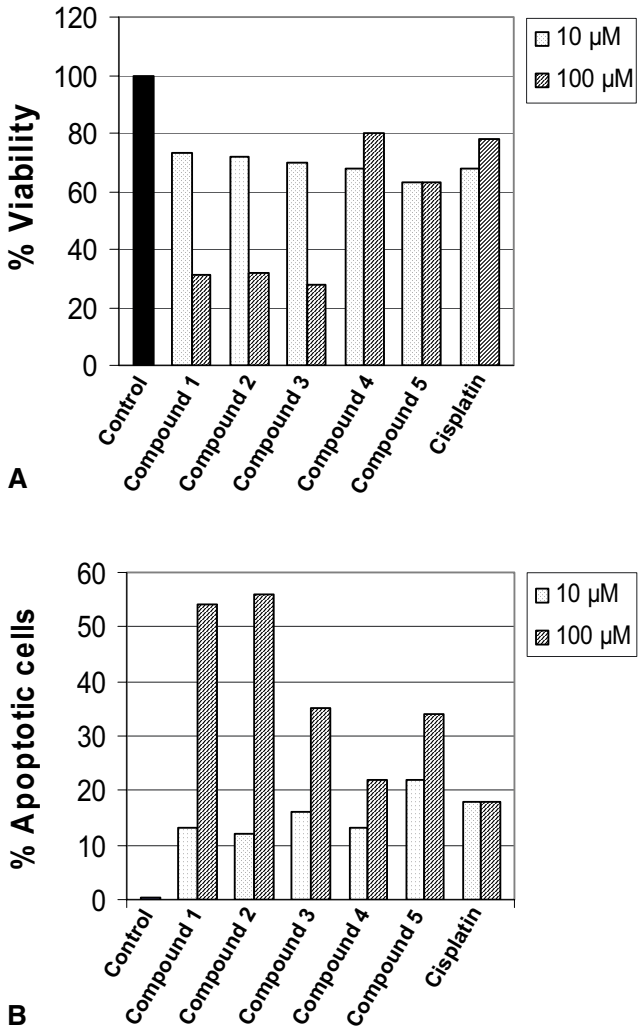

Fig. 5. Percentage of viable and apoptotic HL-60 cells following treatment with the test compounds at low (10 μM) and high (100 μM) doses. The data was normalized against the control (100% for viability or 0% for apoptosis).

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