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单功能铂配合物的合成表征、DNA 结合和抗肿瘤活性

姜 琴[1] 施鹏飞*,[1] 朱建辉[2]

([1]淮海工学院化工系,连云港 222005)

([2]南京大学配位化学国家重点实验室,南京 210093)

摘要:本文合成了三种铂(Ⅱ)配合物 PtLCl(HL1=2-(3′,5′-二甲基-吡唑-1′-基)-*N*-(2″-吡啶甲基)乙基胺;HL2=2-(3′,5′-二甲基-吡唑-1′-基)-*N*-(2″-吡啶乙基)乙基胺;HL3=2-(3′,5′-二甲基-吡唑-1′-基)-*N*-(喹啉-8″-基)乙基胺,通过元素分析和质谱进行结构表征。利用荧光和圆二色光谱研究了 3 种配合物与小牛胸腺 DNA 的相互作用,结果发现配体结构对配合物与 DNA 的作用方式及强度产生极大影响。PtL3Cl 具有较大的共轭平面而易以嵌入模式与 DNA 结合,而 PtL1Cl 和 PtL2Cl 的空间位阻较小,易与 DNA 以共价模式结合。通过质谱跟踪发现,配合物 PtL1Cl 和 PtL2Cl 均能与 5′-鸟苷酸(5′-GMP)发生共价结合,但是没有发现 PtL3Cl 与 5′-GMP 的加合物。3 种配合物对人宫颈癌细胞的体外毒活性数据表明:PtL3Cl 的细胞毒活性最强。

关键词:吡唑铂配合物; DNA 结合; 细胞毒活性

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Synthesis, Characterization and DNA Binding Properties of Monofunctional Antitumor Pt(Ⅱ) Complexes

JIANG Qin[1] SHI Peng-Fei*,[1] ZHU Jian-Hui[2]

([1]*Chemistry Department, Huaihai Institute of Technology, Lianyungang, Jiangsu* 222005)

([2]*State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing* 210093)

Abstract: Three Pt(Ⅱ) complexes of pyrazolyl derivatives, PtLCl (HL1=2-(3′,5′-dimethyl-pyrazol-1′-yl)-*N*-(2″-pyridylmethyl)ethylamine; HL2=2-(3′,5′-dimethyl-pyrazol-1′-yl)-*N*-(2″-pyridylethyl)ethylamine and HL3=2-(3′,5′-dimethyl-pyrazol-1′-yl)-*N*-(quinolin-8″-yl)ethylamine have been synthesized and characterized. Fluorescence titration and circular dichroism analyses proved that coordination environment of the ligands remarkably influenced the interactions between their Pt(Ⅱ) complexes and DNA. The Pt(Ⅱ) complexes PtL1Cl and PtL2Cl could covalently combine to 5′-GMP (guanosine-5′-mono-phosphate), while the adduct of PtL3Cl with 5′-GMP could not be found in the ES-MS spectra due to the steric effect. The more DNA intercalative effect of PtL3Cl has been correlated with its larger planar coordination plan, while the covalent binding effect of PtL1Cl and PtL2Cl to the nucleotide prevented their intercalation into DNA base pairs. In vitro data demonstrated that PtL3Cl exhibited the highest cytotoxicity than the other two complexes against the Hela cell line.

Key words: pyrazolyl-Pt(Ⅱ); DNA-binding; cytotoxicity

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*通讯联系人。E-mail:shpf@hhit.edu.cn,0518-85895407

第一作者:姜 琴,女,30 岁,博士研究生,副教授;研究方向:生物无机化学,配位化学。

0 Introduction

Platinum-based drugs play an important role in clinical cancer chemotherapy, in which cisplatin is widely prescribed for a variety of tumors[1-4]. However, there are serious side effects associated with cisplatin, notably renal toxicity, emesis, neurotoxicity, bone marrow suppression and hearing loss[5,6]. To overcome these drawbacks, many platinum complexes with novel structural features and different biological properties have been designed and tested over the years [7,8]. Previous work found that monofunctional platinum complexes of nitrogen-containing heterocyclic amines can be cytotoxic[9~11]. For example, the trinuclear platinum complex BBR3464, composed of two monofunctional Pt moieties, is currently in clinical trial[12~14]. All these complexes appear to have different mechanisms of action compared to cisplatin and demonstrated distinct cytotoxicity profiles, which suggest that the monofunctional adducts alone may be sufficient to exhibit the cytotoxicity.

In this work, we synthesized and characterized three novel monofunctional platinum(Ⅱ) complexes to explore their potential antitumor activity.

1 Experimental section

1.1 Materials and characterizations

Reagents such as methanol, chloroform, tetrahydrofuran, K_2PtCl_4 were of analytical grade and were used without further purification. 5′-GMP and ct-DNA were purchased from the Aldrich Chemical Co.

Elemental analysis was performed on a Perkin-Elmer240C analytical instrument. The electrospray ionization (ESI) mass spectra were obtained using an LCQ electron spray mass spectrometer (ESMS, Finnigan). The circular dichroism (CD) spectra were acquired with a JASCO J-810 automatic recording spectropolarimeter. The fluorescence spectra were recorded using an AMINCO Bowman series 2 luminescence spectrometer. The ^{1}H NMR data were obtained with a 500 MHz Bruker DMX spectrometer.

1.2 Synthesis of the ligands and the complexes

The starting material *N*-(*p*-toluenylsulfonoxyeth-2′-yl)-3,5-dimethyl pyrazole (Tos-Nhed) was synthesized according to the literature[15].

1.2.1 General procedure for the syntheses of the ligands

The ligand was prepared by adding a solution of Tos-Nhed (1.5 g, 5 mmol) in THF (16 mL) dropwise into a stirred mixture of NaOH (1.0 g, 25 mmol) in 10 mL H_2O and 7 mmol organic amine (2-aminomethyl pyridine, 2-aminoethyl pyridine and 8-aminoquinoline, respectively) in 10 mL THF. The temperature was kept at about 50 ℃. After the addition of Tos-Nhed was completed in about 1.5 h, the temperature was raised to 70 ℃ and stirring was continued for another 48 h. Then the mixture was allowed to cool to 25 ℃. The ligand was extracted with three portions of 25 mL $CHCl_3$. The combined extracts were dried overnight with $MgSO_4$. The solvent was then removed at low pressure. The remained yellow oil was purified by column chromatography (silica gel). The eluting solution for 2-(3′,5′-dimethyl-pyrazol-1′-yl)-*N*-(2″-pyridylmethyl)ethylamine (HL1) and 2-(3′,5′-dimethyl-pyrazol-1′-yl)-*N*-(2″-pyridylethyl)ethylamine (HL2) was a mixture of $CHCl_3$ and CH_3OH (*V*/*V*=9:1), while for 2-(3′,5′-dimethyl-pyrazol-1′-yl)-*N*-(quinolin-8″-yl)ethyl amine (HL3) it was petroleum and acetidin (*V*/*V*=2:1).

ESI-MS (+p, *m*/*z*), three peaks located at 231, 245, 267 were attributed to the protonated species of the ligands, ($HL1+H^+$), ($HL2+H^+$), ($HL3+H^+$), respectively.

^{1}H NMR ($CDCl_3$, ppm) HL1, 2.09 (t, 6H, 3′,5′-met-H); 2.92 (t, 2H, 1-met-H); 3.78 (s, 2H, 2″-met-H); 3.88 (t, 2H, 2-met-H); 5.66 (s, 1H, pyrazol-H); 7.01 (m, 1H, pyridnie-H); 7.15 (d, 1H, pyridnie-H); 7.49 (m, 1H, pyridnie-H); 8.40 (d, 1H, pyridnie-H). HL2, 2.11 (s, 3H, 5′-met-H); 2.14 (s, 3H, 3′-met-H); 2.95 (t, 2H, met-H); 3.71 (t, 2H, met-H); 3.86 (t, 2H, met-H); 4.12 (t, 2H, met-H); 5.69 (s, 1H, pyrazol-H); 7.07 (t, 1H, pyridnie-H); 7.19 (d, 1H, pyridnie-H); 7.55 (t, 1H, pyridnie-H); 8.46 (d, 1H, pyridnie-H). HL3, 2.05 (s, 3H, 5′-met-H); 2.27 (s, 3H, 3′-met-H); 3.79 (t, 2H, 1-met-H); 4.27 (t, 2H, 2-met-H); 5.76 (s, 1H, pyrazol-H), 6.68 (d, 1H, quinolin-H); 7.07 (d, 1H, quinolin-H); 7.37 (m, 2H, quinolin-H); 8.06 (m, 1H, quinolin-H); 8.70 (m, 1H, quinolin-H).

1.2.2 General procedure for the syntheses of Pt(Ⅱ) complexes

The Pt(Ⅱ) complexes was prepared by added 0.2 mmol K_2PtCl_4 in 5 mL H_2O dropwise into the solution of 0.2 mmol ligand in 10 mL acetone, yellow precipate could be obtained shortly. The mixture was stirred at 60 ℃ for 20 min and then the yellow solid was filtered. The solid was washed with water and chlorfom, and then dried in vacuum. ESI-MS: (+p), $[Pt(HL1)Cl]^+$, *m/z*=461; $[Pt(HL2)Cl]^+$, *m/z*=475; $[Pt(NaL3)Cl]^+$, *m/z*=518. Elemental analysis for PtL1Cl ($C_{13}H_{17}ClN_4Pt$) calc.(%): C, 33.96; H, 3.72; N, 12.18. Found(%): C, 33.58; H, 3.59; N, 11.92. PtL2Cl ($C_{14}H_{19}ClN_4Pt$) calc.(%): C, 35.48; H, 4.04; N, 11.82. Found(%): C, 35.16; H, 3.96; N, 11.61. PtL3Cl ($C_{16}H_{17}ClN_4Pt$) calc.(%): C, 38.75; H, 3.45; N, 11.30; Found(%): C, 38.52; H, 3.29; N, 11.09.

1.3 Spectroscopic studies on DNA interaction

The concentration of calf thymus DNA (CT-DNA) was determined by recording the UV absorption at 260 nm using the molar absorption coefficient of 6 600 $mol^{-1}\cdot L\cdot cm^{-1}$. The UV absorbance at 260 and 280 nm of the CT-DNA solution in tris(hydroxymethyl)aminomethane-HCl buffer (pH=7.4) gives a ratio of 1.87, indicating that the DNA was sufficiently free of protein.

The fluorescence spectra were recorded at room temperature with an excitation wavelength at 525 nm and an emission wavelength of 600 nm. The experiment was carried out by titrating the complex (2.0×10^{-3} mol·L^{-1}) into 3 mL samples containing 5.0×10^{-5} mol·L^{-1} DNA and 5.0×10^{-5} mol·L^{-1} ethidium bromide (EB). The molar ratio of DNA to the complex increased from 0.0~1.0.

The CD spectra were recorded at 25 ℃ with increasing ratio of the complex to CT-DNA (*r*=0.0, 0.2, 0.4). Each sample solution was scanned in the range 220~320 nm with a speed of 10 nm·min^{-1}. The concentration of CT-DNA was 1.0×10^{-4} mol·L^{-1}.

1.4 Cytotoxicity assay

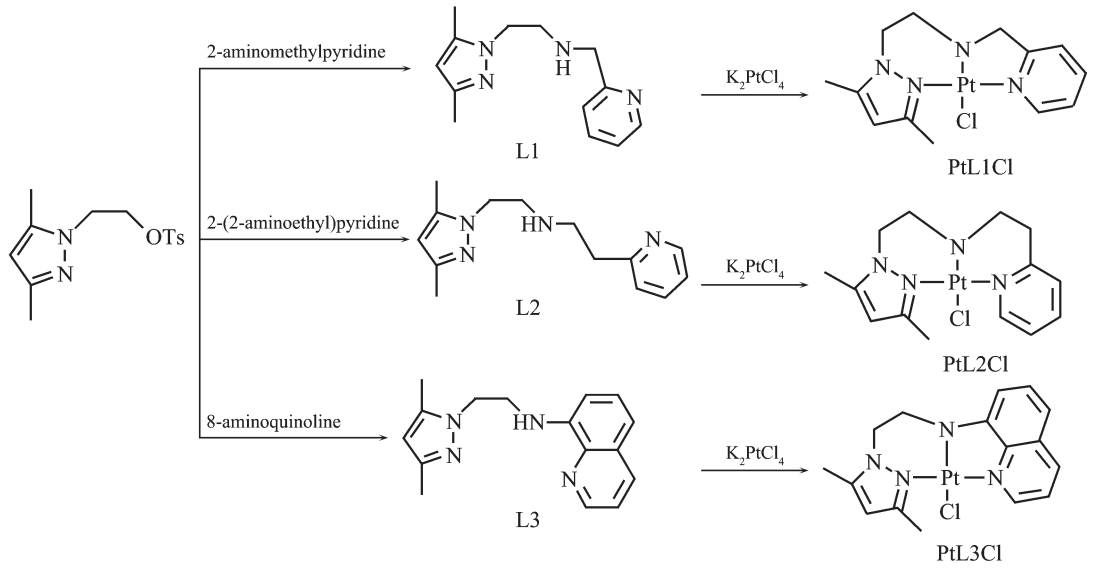
Tumor cells were grown in 96-well plates and allowed to attach for 24 h before drug addition under RPMI-1640 medium supplemented with 10% (*V*/*V*) fetal bovine serum, 2 mmol·L^{-1} glutamine, 100 U·mL^{-1} penicillin, and 100 μg·mL^{-1} streptomycin in a highly humidified atmosphere of 95% air with 5% CO_2 at 310 K. The cell densities were selected on the basis of preliminary tests to maintain control cells in an exponential phase of growth during the period of the experiment and to obtain a linear relationship between the OD and the number of viable cells. Each cell line was exposed to grade concentrations of the three complexes at desired final concentrations for 72 h, and each concentration was tested in triplicate wells. After exposure, cells were fixed by gentle addition of 100 μL of cold (4 ℃) 10% trichloroacetic acid to each well, followed by incubation at 4 ℃ for 1 h. Plates were washed with deionized water five times and allowed to air-dry. Cells were stained by addition of 100 μL of SRB solution (0.4% SRB (*W*/*V*) in 1% acetic acid (*V*/*V*) to wells for 15 min. Then the plates were quickly washed five times with 1% acetic acid to remove any unbound dye and allowed to air-dry. Bound dye was solubilized with 10 mmol·mL^{-1} Tris (pH 10.5) prior to reading plates. The OD value was read on a plate reader at a wavelength of 515 nm. Media and DMSO control wells, in which complexes were absent, were included in all the experiments. The percent growth inhibitory rate of treated cells was calculated by $(OD_{control}-OD_{test})/OD_{control}\times100$[11].

2 Results and discussion

2.1 Synthesis

The ligand contained a pyrazole nitrogen, an amine nitrogen and a pyridine/quinoline nitrogen as potential *N*-donor atoms. The introduction of pyridine or quinoline group created a tri-dentate ligand and enable the ligand to form a six-membered together with a five-membered chelate ring upon coordination to Pt(Ⅱ) ion (there were two six-membered chelate rings for PtL2Cl). As showed in Scheme 1.

In the ES-MS spectra of the Pt(Ⅱ) complexes, three major peaks at *m/z* 461, 475, 518 were observed, which could be attributed to the positively charged specie $[Pt(HL1)Cl]^+$, $[Pt(HL2)Cl]^+$, $[Pt(NaL3)Cl]^+$, respectively. The isotopic distribution patterns of the peaks match perfectly with the simulated one. These data suggested the formation of the Pt(Ⅱ) complexes.



Scheme 1　Synthesis of the ligands and Pt(Ⅱ) complexes

2.2　Interactions with 5′-GMP

Since cisplatin′s biological antitumor activity was suggested to arise from its coordination to N7 atoms of adenine and guanine bases in DNA[16~18], the reactions of the Pt(Ⅱ) complexes with guanosine-5′-monophosphate (5′-GMP) were firstly examined. The Pt(Ⅱ) complex and 5′-GMP were dissolved in CH_3CN/H_2O (V∶V=1∶1) with the molar ratio=1∶1, then the solution was monitored using ES-MS spectroscopy after incubation for 72 h. As showed in Fig.1, two positive peaks at m/z=787.1 and 801.1 could be assigned as $[PtL1(5′\text{-GMP})]^+$ and $[PtL2(5′\text{-GMP})]^+$, respectively. However, no change was found for PtL3Cl before and after the incubation. These data suggest that the coordinating Cl atom in PtL1Cl and PtL2Cl could be substituted by 5′-GMP.

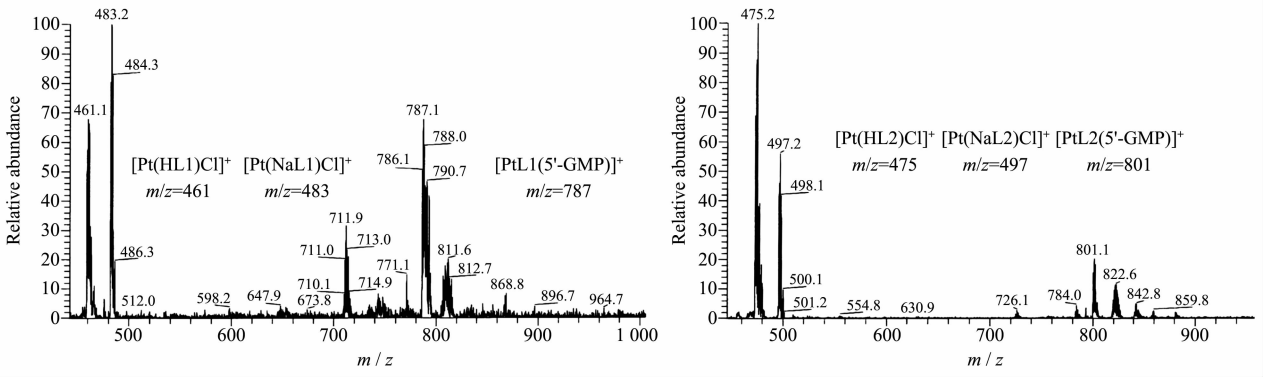


Fig.1　ES-MS spectra of PtL1Cl and PtL2Cl in the presence of 5′-GMP in 72 h

2.3　DNA binding properties.

The DNA-EB system was used to further probe the DNA binding mode of the Pt(Ⅱ) complexes. Ethidium bromide (EB) is an intercalator that gives a significant increase in fluorescence emission when bound to DNA and its displacement from DNA results in decrease in fluorescence intensity[19]. Fluorescence titration spectra were shown in Fig.2. The emission intensity decreased with the increase of the concentration of the Pt(Ⅱ) complexes, which suggests that they could replace EB from CT-DNA and intercalate into the DNA double helix. However, the largest hypochromicity was only 15% (r=1.0), which was suspected that the steric effect from the ligands prevented the Pt(Ⅱ) complexes intercalating deeply into the DNA base pairs. It is noticeable that the hypochromicities followed the order: PtL3Cl >PtL1Cl >PtL2Cl. The second ethyl group in PtL2Cl made it more flexible and easier to covalent

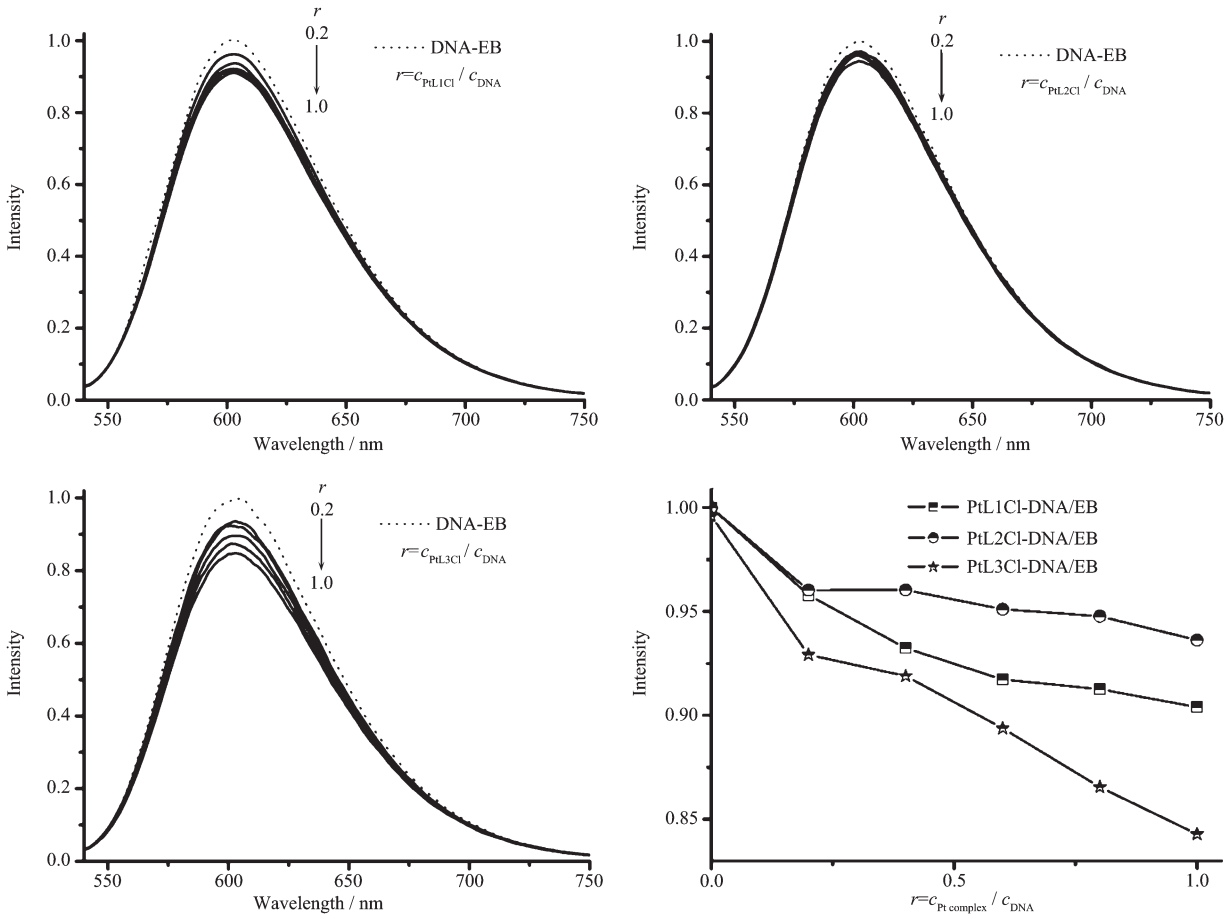


Fig.2 Fluorescence emission spectra of the EB-CT-DNA system in the absence and presence of Pt(Ⅱ) complex with r increase from 0.0, 0.2 to 1.0 ($r=c_{complex}/c_{DNA}$)

bind to the nucleotide, which results in the weaker intercalating effect. In the case of PtL3Cl, however, the coordination of Pt(Ⅱ) to the N atom in quinoline group made the conjugated systems extended, which made the PtL3Cl easier to intercalate into DNA by a larger plane.

The conformational changes of CT-DNA induced by Pt(Ⅱ) complexes were examined by circular dichroism (CD). The CD spectrum of CT-DNA contains a positive band at 275 nm due to base stacking and a negative band at 248 nm due to the right-handed helicity. Both bands are quite sensitive to the interaction mode with small molecules[20~22]. As shown in Fig.3, all the positive bands increased weakly when the ratio of $c_{compound}/c_{CT\text{-}DNA}$ changed from 0.0 to 0.4. The largest hyperchromicity was 5.1% for PtL3Cl, compared with that for the PtL1Cl (2.2%) and PtL2Cl (2.4%). which indicated that the

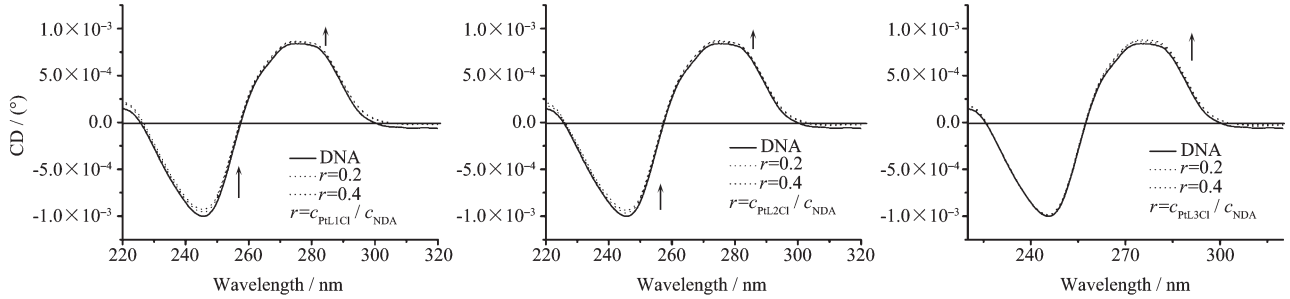


Fig.3 Circular dichroism spectra of CT-DNA in the absence and presence of Pt(Ⅱ) complexes with r increase from 0.0, 0.2 to 0.4 ($r=c_{complex}/c_{DNA}$)

PtL3Cl exert more disturbance on base stacking of DNA than others. Interestingly, the negative bands decreased evidently in intensity with the addition of the Pt(Ⅱ) complexes except the PtL3Cl, the hypochromicities were 7.1% and 6.6% for PtL1Cl and PtL2Cl, respectively. This suggested that the PtL1Cl and PtL2Cl could disturb more on polynucleotide helicity of DNA by covalently binding to nucleotides. These CD data accorded with the results of DNA-EB experiments.

2.4 *In vitro* cytotoxicity

Preliminary cytotoxicity data of the Pt (Ⅱ) complexes against the HeLa tumor cells were carried out by the sulforhodamine B (SRB) assay. As shown in Fig.4, At lower concentration ($\leqslant 4\times10^{-5}$ mol·L^{-1}), the cytotoxicity of these Pt (II) complexes follows the order: PtL3Cl >PtL1Cl >PtL2Cl, which is consistent with the order of DNA binding ability of the three complexes. For example, PtL3Cl showed an inhibition rate of 40% against the HeLa cell lines at a concentration of 1×10^{-5} mol·L^{-1}, whereas PtL1Cl and PtL2Cl had 37% and 18% inhibition rate at the same concentration. Compared with the in vitro cytotoxicity against HeLa tumor cells of the cisplatin at the same condition (45%), the cytotoxicity of all the three Pt(Ⅱ) complexes were slightly lower, however, their present activity indicate that to form bifunctional DNA adducts may not be a precondition for platinum(Ⅱ) complexes to exhibit cytotoxicity against tumor cells. To our surprised, at higher concentration of 8×10^{-5} and 1×10^{-4} mol·L^{-1}, the cytotoxicity of PtL2Cl were higher than that of PtL1Cl, indicating more complicated interactions other than DNA binding between the Pt(Ⅱ) complex and the tumor cells can influence their cytotoxicity.

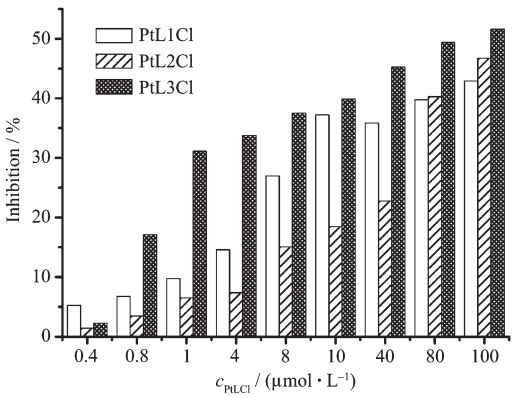


Fig.4　Cytotoxic activity of the Pt(Ⅱ) complexes against the Hela tumor cell

3　Conclusions

Three Pt(Ⅱ) complexes of pyrazolyl derivatives were obtained, characterized and their cytotoxicity against the Hela cell line was tested. Spectroscopic analyses demonstrated that the structure of the ligands remarkably influenced the interaction modes of their Pt(Ⅱ) complexes with DNA: PtL1Cl and PtL2Cl could covalently bind to the nucleotide which prevented their intercalation into DNA base pairs, while PtL3Cl exhibited more DNA intercalative effect which has been correlated with its larger planar coordination plan. The DNA binding mode of these complexes may somewhat response for their cytotoxicity.

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