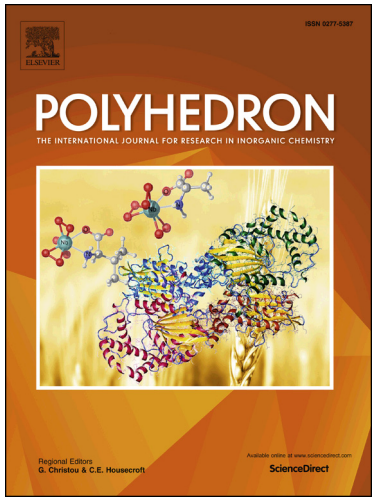


Synthesis, DNA binding, topoisomerase I inhibition and antiproliferation activities of three new binuclear terpyridine platinum(II) complexes

Keke Chai, Yihui Jiang, Tianzhi Han, Junlong Niu, Liushuang Yao, Haiyu Zhang, Min Zeng, Li Zhang, Xuemin Duan, Jintao Wang

PII: S0277-5387(18)30606-5
DOI: https://doi.org/10.1016/j.poly.2018.09.053
Reference: POLY 13452

To appear in: *Polyhedron*

Received Date: 29 July 2018
Accepted Date: 19 September 2018

Please cite this article as: K. Chai, Y. Jiang, T. Han, J. Niu, L. Yao, H. Zhang, M. Zeng, L. Zhang, X. Duan, J. Wang, Synthesis, DNA binding, topoisomerase I inhibition and antiproliferation activities of three new binuclear terpyridine platinum(II) complexes, *Polyhedron* (2018), doi: https://doi.org/10.1016/j.poly.2018.09.053

Synthesis, DNA binding, topoisomerase I inhibition and antiproliferation activities of three new binuclear terpyridine platinum(II) complexes

Keke Chai[a], Yihui Jiang[a], Tianzhi Han[a], Junlong Niu[a], Liushuang Yao[a], Haiyu Zhang[a], Min Zeng[a], Li Zhang[a], Xuemin Duan[a,*], Jintao Wang[a,*]

[a] Jiangxi Provincial Key Laboratory of Drug Design and Evaluation, School of Pharmacy, Jiangxi Science & Technology Normal University, Nanchang 330013, P.R. China

[*] Corresponding author: Fax: +86-791-83823320, Tel.: +86-791-88537967, E-mail: duanxuemin@126.com; jintaochem@163.com

ABSTRACT

Three binuclear platinum(II) complexes, **1** ($[Pt(L)Cl_2]Cl_2$), **2** ($[Pt(L)Br_2]Br_2$) and **3** ($[Pt(L)I_2]I_2$) (where L= 1,4-bi-[4'-(4-Hydroxyphenyl)-2,2',6',2''-terpyridine] butane) were synthesized and characterized by nuclear magnetic resonance, elemental analysis and mass spectrometry. The crystal structure of the new ligand L was determined by single crystal X-ray crystallography, and π-π stacking interactions were revealed in the geometry. Interactions of complexes with calf thymus DNA have been investigated by UV-vis spectroscopy and fluorescence spectroscopy. Due to the structural differences, binuclear complex **3** exhibited higher binding affinity for calf thymus DNA with a DNA-binding constant K_b of 8.72×10^6 M^{-1}. The competitive study indicated that the ethidium bromide (EB) can displaced by complexes **1**-**3** from the DNA-EB conjugation. The concentration-dependence unwinding of supercoiled

circular plasmid pBR322 DNA by complexes **1-3** was observed via agarose gel electrophoresis. Meanwhile, complexes displayed obvious activities for inhibition of topoisomerase I. Herein the halogen leaving groups $Cl^-/Br^-/I^-$ presented in **1-3** showed significant effect on DNA binding and topoisomerase I inhibition activities. An evaluation of in *vitro* cytotoxicity for three complexes was performed by MTT assay in two cancer cell lines and normal cells as the control, respectively.

Keywords: Platinum(II) complex; DNA binding; inhibitory activity; topoisomerase I

1. Introduction

Cancer is one of the significant factors which cause of death in recent years, accounting for nearly one-quarter deaths annually [1]. Medical treatment has a significant effect on reducing cancer mortality and morbidity, and rates should continue to improve with recent advances in new therapies. Following the discovered of antitumor activity of cisplatin by Barnett Rosenberg [2], the success of the classical structure-activity relationship platinum complexes in the treatment of cancer stimulated scientists research interests and the non-classical platinum complexes included binuclear or multinuclear platinum complexes were researched [3]. Terpyridine and its derivatives with plentiful potential application, such as DNA binding agents [4], topoisomerase I inhibitors [5] and anti-tumor agents [6], are of important ligands for constructing metal complexes. Specifically, Pt(II) terpyridine [7] complexes as planar intercalators, have been researched for interest in recent years due to their promising biological activity [8]. Besides the feature of the chelating ligands, the leaving groups in the metal complexes also have significant effects on the

rates of hydrolysis, binding to biomolecule and the cytotoxic activity [9, 10]. The cytotoxicity of some complexes in *vitro* can be improved when the chloride ligand was replaced by different halogenated ligand. For instance, the [Ru(η^6-*p*-cymene)(L^1)Cl]PF_6 and [Ru(η^6-*p*-cymene)(L^1)I]PF_6 (where L^1 = N,N-dimethyl-N'-(2-pyridinylmethylene)) displayed the IC_{50} values of 16.2 ± 0.9 and 3.0 ± 0.2 μM, against the A2780 human ovarian carcinoma cells [11]. The bromide complex ([Ru(η^6-*p*-cymene)(5,7-diiodo-8-quinoline)Br], IC_{50} = 2.92 ± 0.53 μM) was more potent than its chloride analog ([Ru(η^6-*p*-cymene)(5,7-diiodo-8-quinoline)Cl], IC_{50} = 4.60 ± 1.29 μM) against the NCI-H460 non-small cell lung carcinoma [12].

Topoisomerase I (Topo I) is a crucial nicking-closing enzyme, whose main function is deal with DNA super-helices in chromosomes during replication and transcription in biological cells [13, 14]. Topoisomerase I inhibition is one of the dominating means used in cancer therapy because it could lead to DNA damage that induce apoptosis and necrosis and stop DNA replication. It drew many researchers, who explored a drug targeting both Topo I and DNA using endogenous metals, as well as made efforts to reduce undesired side effects and improve the activity. Functionalized terpyridine derivatives [15-17] have a wide range of potential applications ranging from colorimetric metal determination to DNA binding agents, anticancer agents and topoisomerase inhibitors. Therefore, topoisomerases are the cellular targets of clinically important anticancer and antibacterial drugs, and inhibition of topoisomerases has been considered as an effective strategy for the design of many anticancer agents [18]. Inspired by the above considerations, we

designed and synthesized three binuclear Pt(II) complexes, with different leaving groups (Scheme 1). Their DNA binding abilities, in *vitro* topoisomerase I inhibition and cytotoxicity against cancer cell lines was also investigated.

2. Experimental

2.1. Reagents and instrumentation

All reagents and materials were purchased from commercial suppliers which for scientific laboratory. 2-Acetylpyridine, p-hydroxybenzaldehyde, K_2PtCl_4, KBr, KI and $AgNO_3$, were purchased from Energy-chemical. 4'-(4-hydroxyphenyl)-2,2',6',2''-terpyridine [19] and $Pt(DMSO)_2Cl_2$ [20] were prepared according to the published procedure. Calf thymus DNA (CT DNA) was purchased from Sigma-Aldrich. pBR322 DNA was obtained from BBI LIFE SCIENCES and GelRed was purchased from Biotium. Topoisomerase I (Topo I) was obtained from Takara Biomedical Technology (Beijing). Agarose gel, ethylenediaminetetraacetic acid (EDTA), and PBS (1×) buffer reagents was purchased from Sangon. A549 (human lung carcinoma cell lines), HepG2 (human liver hepatocellular carcinoma), and LO2 (human normal liver cell) were obtained from the Cell Resource Center, Peking Union Medical College (Beijing, China).

Nuclear magnetic spectra were recorded on a Bruker AVANCE 400 spectrometer at ambient temperature. Elemental analyses of C, H and N were measured on a Perkin-Elmer 240C elemental analyzer. High resolution mass spectrometric analysis was carried out on a Waters Xevo G2-XS Q-TOF instrument. A Shimadzu UV-2550 UV-vis spectrophotometer was used for UV scanning. Bio-Rad

Sub-Cell GT electrophoresis system was used for the electrophoresis experiment and JUNYI scanner (JY04s-3c) was used for gel imaging. Stock solutions (10 mM) of complexes **1-3** were prepared in DMSO, which were further diluted using buffer or cell culture medium until working concentrations were achieved.

2.2. Synthesis

Ligand L (1.4-bi-[4'-(4-hydroxyphenyl)-2,2',6',2''-terpyridine] butane)

The ligand was synthesized by a similar method as reported [21]. 2.16 g (36 mmol) of KOH was added into the round bottom flask with 150 mL DMF containing 3.90 g (12 mmol) dissolved 4'-(4-hydroxyphenyl)-2,2',6',2''-terpyridine. Then, 1,4-dibrombutane (0.597 mL, 5 mmol) was added dropwise to the mixture and it was heated at 80 °C for 24 hours. The resulting mixture was percolated and the residue was purified via flash chromatography (dichloromethane/methanol). Then the product was obtained by reduced pressure distillation. It was dried under vacuum to give 5.22 g the white powder. Yield: 74.0%. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.71 (m, 8H, 4, 7, 9, 12-tpyH), 8.68 (d, J=8 Hz, 4H, 1, 15-tpyH), 7.88 (m, 8H, 2, 3, 13,14-tpyH), 7.35 (m, 4H, 17, 21-tpyH), 7.06 (d, J=8 Hz, 4H, 18, 20-ArH), 4.15 (t, 4H, $OC\underline{H_2}$), 2.07 (m, 4H, $OCH_2C\underline{H_2}$). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 156.28, 155.67, 149.88, 148.95, 137.03, 130.64, 128.58, 123.78, 121.47, 118.43, 114.93, 67.62, 26.04. HR–MS (Acetonitrile) *m/z*: calcd 705.2978 for $C_{46}H_{37}N_6O_2$, found 705.2981 for $[L+H]^+$. Elemental analysis calcd (%) for $C_{46}H_{36}N_6O_2$: C 78.39, H 5.15, N 11.92; found: C 78.20, H 5.60, N 11.80.

Complex 1 ($[Pt(L)Cl_2]Cl_2$)

The complex **1** was synthesized as previously reported [22] with minor modifications. A 40 mL $CHCl_3$ solution containing $Pt(DMSO)_2Cl_2$ (1.06 g, 2.4 mmol) and 1 equiv of L (0.89 g, 1.2 mmol) was refluxed under argon for 24 h. The reaction mixture was cooled to ambient temperature, and the resulting yellow precipitate was collected by filtration, washed with 15 mL $CHCl_3$ and 15 mL diethyl ether. It was dried under vacuum to give 0.76 g the faint yellow powder. Yield: 65.5%.^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.90 (m, 4H, 7 and 9-tpyH), 8.83 (d, 8H, 1, 4, 12 and 15-tpyH), 8.51 (d, 4H, 17, 21-ArH), 8.19 (d, 4H, 3, 13-tpyH), 7. 91 (d, 4H, 2, 15-tpyH), 7.20 (t, 4H, 18, 20-ArH), 4.18 (m, 4H, $C\underline{H_2}OC\underline{H_2}$), 1.93 (m, 4H, $OCH_2\underline{CH_2}$). HR–MS (Acetonitrile) *m/z*: calcd 582.0786 for $C_{46}H_{36}N_6O_2Pt_2Cl_2$ (with two positive charges), found 582.0781 for $[\mathbf{1}\text{-}2Cl]^{2+}$. Elemental analysis calcd (%) for $C_{46}H_{36}N_6O_2Pt_2Cl_4$: C 44.67, H 2.93, N 6.80; found: C 44.42, H 3.26, N 6.66.

Complex 2 ([Pt(L)Br$_2$]Br$_2$)

Complex **2** was synthesized as previously reported [23] with modifications. Complex **1** (0.124 g, 0.1 mmol) and $AgNO_3$ (0.068 g, 0.4 mmol) were dissolved in a mixed solution containing H_2O (2 mL), CH_3CN (10 mL) and THF (20 ml). Then the mixture solution was stirred for 6 hours at 25 °C. After the solution was filtered to remove the generated AgCl precipitation, excess of KBr (0.120 g, 1.0 mmol) was added into the filtrate, which was stirred for another 6 hours. After filtration, the filtrate was reduced under vacuum, and the collected precipitate was washed with 10 mL KBr (1 M) aqueous solution, 10 mL H_2O and 10 mL CH_3OH respectively. It was dried under vacuum to give 0.07 g the yellow powder. Yield: 49.5%.^{1}H NMR (400

MHz, DMSO-d_6, δ, ppm): 9.05 (m, 4H, 7, 9-tpyH), 8.88 (d, 4H, 4, 12-tpyH), 8.81 (d, 4H, 1, 15-tpyH), 8.49 (d, 4H, 17, 21-ArH), 8.19 (d, 4H, 3, 13-tpyH), 7.88 (d, 4H, 2, 14-tpyH), 7.23 (t, 4H, 18, 20-ArH), 4.18 (m, 4H, $OC\underline{H_2}$), 1.92 (m, 4H, $OCH_2\underline{CH_2}$). HR–MS (Acetonitrile) *m/z*: calcd 626.0281 for $C_{46}H_{36}N_6O_2Pt_2Br_2$ (with two positive charges), found 626.0273 for [**2**-2Br]$^{2+}$. Elemental analysis calcd (%) for $C_{46}H_{36}N_6O_2Pt_2Br_4$: C 39.06, H 2.57, N 5.94; found: C 38.87, H 2.89, N 5.69.

Complex 3 ([Pt(L)I$_2$]I$_2$)

The complex **3** was synthesized as reported [23], with minor modifications. The methanol of complex **1** (0.248 g, 0.2 mmol) was added KI (0.166 g, 1.0 mmol) and it's stirred for 12 hours at room temperature. Then the color of mixture became more and more deep. After filtration, the precipitate ware collected, and washed with 10 mL KI (1 M) aqueous solution, 10 mL H_2O, and 10 mL CH_3OH. It was dried under vacuum to give 0.166 g the dark yellow powder. Yield: 51.9%.^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.82 (m, 8H, 4, 7, 9, 12 -tpyH), 8.65 (d, 4H, 1, 15-tpyH), 8.32 (d, 4H, 17, 21-ArH), 7.96 (d, 8H, 2,3, 13,14-tpyH), 7.01 (d, 4H, 18, 20-tpyH), 7.01 (d, 4H, 18, 20-ArH), 4.30 (t, 4H, $OC\underline{H_2}$), 2.00 (m, 4H, $OCH_2C\underline{H_2}$). HR–MS (Acetonitrile) *m/z*: calcd 674.0142 for $C_{46}H_{36}N_6O_2Pt_2I_2$ (with two positive charges), found 674.0135 for [**3**-2I]$^{2+}$. Elemental analysis calcd (%) for $C_{46}H_{36}N_6O_2Pt_2I_4$: C 34.48, H 2.26, N 5.24; found: C 34.20, H 2.49, N 5.03.

2.3. Single crystal X-ray data collection and structure refinement

The monocrystal data of the ligand L (0.3 × 0.08 × 0.07 mm) were collected using an Agilent Gemini EOS diffractometer with graphite-monochromated with Mo-

Kα radiation (λ = 1.54184) at 293 (2) K. An empiric absorption correction was applied. All the non-hydrogen atoms were refined anisotropically and the hydrogen atoms of organic molecule were refined in calculated positions, assigned isotropic thermal parameters, and allowed to ride their parent atoms. All calculations were performed using the *SHELX2014* program package [24]. Crystallographic data (excluding structure factors) for the structure of L reported in this paper have been deposited with the Cambridge Crystallographic Data Centre with the reference numbers 1853639. The crystal and refinement data are collected, and selective bone distances and angles are given in Table 1.

2.4. DNA binding experiment

2.4.1 ***UV/Vis Absorption Titration***

A pH 7.4 PBS (1×) buffer was used and UV/Vis spectra were recorded after each addition of concentrated DNA stock to 50 μM Pt(II) complex solutions in a quartz cuvette (path length = 1 cm) at 25 °C. The binding ability of complexes with CT DNA can be estimated through the binding constant K_b, which was obtained by monitoring the changes in the absorbance at the corresponding λ_{max} with increasing concentrations of CT DNA and is given by the ratio of slope to the y intercept in plots $\frac{[DNA]}{\varepsilon_A - \varepsilon_f}$ versus [DNA], according to the equation [25]:

$$[DNA]/(\varepsilon_A - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/(K_b(\varepsilon_b - \varepsilon_f))$$

Where [DNA] is the concentration of DNA in base pairs, $\varepsilon_A = A_{obsd}/[compound]$, ε_f = the extinction coefficient for the free compound and ε_b = the extinction coefficient for the compound in the fully bound form.

2.4.2 *Fluorescence DNA titration*

The competitive studies of complexes with EB have been investigated by means of fluorescence spectroscopy in order to prove EB can be displaced via complex from CT DNA-EB system. The CT DNA-EB mixture (5 μM EB and 10 μM CT DNA) was prepared which is incubated at least 8 hours at 37 °C. The competitive ability of complexes **1-3** have been obtained by recording the change of fluorescence emission spectra when adding solution of complex step by step into the DNA-EB conjugation solution.

The Stern–Volmer constant K_{sv} is used to evaluate the quenching efficiency for each complex according to the equation[26].

$$I_0/I = 1 + K_{sv}[Q]$$

Where I_0 and I are the emission intensities in the absence and the presence of the quencher. K_{sv} = the dynamic quenching constant, [Q] = the concentration of the quencher (complexes or ligand), the dynamic quenching constant (K_{sv}, M^{-1}) can be obtained by the slope of the diagram I_0/I versus [Q].

2.5. DNA unwinding experiment

A plasmid pBR322 DNA unwinding assay was used to investigate the effect of complexes **1-3** on the structural of plasmid by agarose gel electrophoresis. Typical reactions DNA-cleavage were carried out. DNA unwinding under various concentrations of **1-3** was determined in buffer (10 mM KH_2PO_4, 10 mM NaCl, 10 mM EDTA, pH 7.2). Briefly, after incubation of pBR322 DNA (19 μM base pair concentrations) with complex of different concentrations for 24 hours, loading buffer

(0.05% bromophenol blue, 50% glycerol, and 2 mM EDTA) was added to the mixture. Then the samples were loaded onto a 0.8% agarose gel and electrophoresed in TBE (1×) at a constant voltage of 120 mV for 80 mins. The gels were visualized in the electrophoresis gel documentation and analysis system.

2.6. Topoisomerase I inhibition assay

Inhibition assay was used to investigate the effect of complex on the activity of Topoisomerase I (Topo I) by agarose gel electrophoresis. In the experimental process, a reaction mixture containing 19 μM (base pair concentrations) pBR322 DNA in buffer (10 mM Tris–HCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM DTT, 0.1 mM EDTA, 150 μg/ml BSA, pH 7.5) was incubated with 1 unit Topo I in the absence or in the presence of complexes **1** (0-25 μM), **2** (0-70 μM) and **3** (0-150 μM), for 30 mins at 37 °C. Each sample was preincubated for 15 mins prior to the addition of pBR322 DNA. Loading buffer (0.2% bromophenol blue, 30% glycerol, 4.5% SDS, 0.2% xylene cyanol) was added to the reaction mixture. The image was visualized using analysis system.

2.7. Antitumor activity assays

A standard MTT assay was employed to evaluate the potential cytotoxicities of L and three complexes against A549, HepG2 and LO2 cell lines. The cells were maintained in DMEM supplemented with 10% FBS 100 μg mL^{-1} streptomycin, and 100 U mL^{-1} penicillin. The cells were cultured in a humidified incubator, which provided an atmosphere of 5% CO_2 and 95% air at a constant temperature of 37 °C. In a typical experiment, the cells were seeded in a 96-well

flat-bottomed microplate at 10,000 cell per well in growth medium solution. The microplate was incubated at 37 °C with 5% CO_2 in a humidified incubator for 24 hours. The medium was then changed to growth medium with or without complexes with serial concentrations. The microplate was then incubated at 37 °C with 5% CO_2 in a humidified incubator for 48 hours. After 48 hours, MTT (20 μL, 5 mg/mL) was added to each well. The microplate was reincubated at 37 °C in 5% CO_2 for another 4 hours. Then the medium was carried out and 150 μL DMSO was added into each well, the microplates were shaken for 10 mins. The absorbance at the wavelength of 570 nm was measured by a microplate reader (reference wavelength: 630 nm). The IC_{50} value of each complex (the concentration that is required to reduce the absorbance by 50% relatively to the controls) was analysed and calculated by SPSS.

3. Results and discussion

3.1. Synthesis and general characterization

The designed ligand L and complexes were prepared following the procedure shown in Scheme 1, and characterized by elemental analysis, ^{1}H NMR, ^{13}C NMR spectra along with HR-MS spectrometry. White crystals of L suitable for X-ray diffraction studies were obtained by slow volatilized from a trichloromethane and acetone mixture solution.

Information concerning X-ray data collection and crystal structure refinement is summarized in Table 1, with the selected bond lengths and bond angles listed in Table S1. The structure is shown in Fig. 1, that prove the synthetic ligand has the expected molecular, and the obtained bond lengths and bond angles are similar to a reported

related terpyridine derivative [27]. For instance, the determined bond angles among the three N atoms are (116.2(5) for Cl-N1-C5, 117.5(4) for C6-N2-C10 and 117.7(4) for Cl1-N3-C15) respectively. As shown, within one terpyridine unit, the dihedral angles between the middle pyridine ring (N2, C6-C10) and another two connected pyridine rings, ring1 (N1, C1-C5) and ring2 (N2, C6-C10) are only 3.26° and 3.47° respectively. This indicates that the terpyridine moieties are of good planar character. Whereas, the dihedral angle between pyridine ring (N2, C6-C10) and the adjacent benzene ring (C16-C21) is 25.82°. The most interesting feature of the crystal structure is the mode of intermolecular interactions. Two monomers stacked in a head to head fashion, with a distance of 3.703 Å between the two planes, suggesting that π-π stacking interactions are involved in stabilising the structure.

3.2. DNA binding

3.2.1 UV-Vis titration

DNA is one of the targets for the studies of biologically important small molecules, such as anticancer drugs. The complex interacted to calf thymus DNA (CT DNA) by different modes, as intercalation, electrostatic and major/minor groove binding [28]. Generally, intercalation was commonly detected between Pt-terpyridine complexes and DNA [4]. The interaction of between **1-3** and CT DNA was investigated in *vitro* by UV-vis spectroscopy, and the spectra of CT DNA in the presence of increasing concentrations of **1-3** are shown in Fig. S1. The low-energy absorption bands at 400 - 500 nm are tentatively assigned to the dπ (Pt) → π* (terpy) transition, that is, the MLCT (MLCT = metal to ligand charge transfer) bands [29].

Herein the MLCT bands of complex 1-3 are observed at 435 nm (**1**), 437 nm (**2**) and 430 nm (**3**). Obviously, the titration results revealed apparent hypochromism of the MLCT bands of complexes upon the addition of CT DNA, indicating strong π-π stacking interaction between an aromatic chromophore and the base pairs of DNA was involved. The DNA binding constants (K_b, Table 2) of complexes, were determined from the Wolfe-Shimer equation [25], and were found to be 1.83 × 10^6, 3.21 × 10^6 and 8.72 ×10^6 M^{-1} for complexes **1-3** respectively. The obtained K_b constants of complexes **1-3** were higher than that of the classical intercalator ethidium bromide (EB, 1.4 × 10^6 M^{-1}) [30], and another two mononuclear Pt-terpyridine complexes [Pt(4-hydroxylthiophenolato)(2,2′:6′,2″-terpyridine)]Cl (1.2 × 10^5 M^{-1}) [15] and [Pt (2,2′:6′,2″-terpyridine)(SCH_2CH_2OH)]NO_3 (2.0 × 10^5 M^{-1}) [4]. Complex **3**, with the coordinating group I^-, exhibited the highest K_b constant among the complexes. Besides, L was not involved here because the MLCT band was unobserved.

3.2.2 Competitive binding with EB

Competitive DNA-binding studies with EB were also used to reveal the binding abilities between compounds and CT DNA. The fluorescence emission spectra of the prepared EB-DNA system in the presence of increasing amounts of complexes **1-3** of different ratios of r (r = [compound]/[DNA]) were recorded. As shown in Fig. S2, the addition of L and complexes **1-3** at diverse concentrations induced a dramatic decrease of the fluorescence intensity of the emission band from the CT DNA-EB system, where the L and complexes **1-3** induced the reductions of up to 4%, 43%,

44% and 56.5% respectively. The Stern-Volmer plot of DNA-EB (Fig. S3) illustrate that the quenching of EB bound to DNA by these compounds, resulting in a decrease in the fluorescence intensity. Obviously, the K_{sv} (Table 3) value of L is lowest (K_{sv} value of 5.00×10^3 M^{-1}), and complex **3** bearing the highest binding constant (K_{sv} value of 7.60×10^4 M^{-1}). The calculated K_{sv} values are more potent than some reported partial intercalators, such as *cis*-[$PtCl_2$(liriodenine)(DMSO)] (5.85×10^4 M^{-1}) [31] and [Pt(MAMP)$(H_2O)_2$]$(ClO_4)_2$ (where MAMP = 2-[(N-methylamino)methyl]pyridine, 0.19×10^4 M^{-1}) [32]. Noteworthy, the results obtained here are consistent well with UV-vis titration data. The results from above DNA binding assays indicate strong binding affinities are occurred between complexes and CT DNA, and intercalation is probably the predominant binding mode between them [33].

3.3. DNA unwinding studies

The DNA unwinding property of complexes **1-3** was also explored by native agarose gel electrophoresis using pBR322 plasmid DNA. It is reported that the binding of an unwinding agent to the closed circular DNA (Form I) can reduce its superhelical density and hence decrease its rate of migration in the agarose gel [34]. As shown in Fig. 2, the mobility of the supercoiled DNA was decreased upon incubation with complexes **1-3**. With an increasing concentration of the Pt(II) complex, Form I gradually diminished accompanied by the increasing of Form II. When the concentration of complex **1** reached to 5 μM, Form I was converted into Form II completely. As a whole, the order of these complexes for DNA unwinding is

1 ≈ **2** > **3**. It's reported that the rate of hydrolysis of Cl^- from Pt was replaced with Br^- and I^- is fast, and the ability to leave increased order is shown to be $Cl^- < Br^- < I^-$ [35, 36]. The hydrolysis behavior of Pt(II) complexes in biological systems closely related to their anticancer activity and binding nature with DNA. Additionally, aqua adducts formation of Pt(II) complexes are of an essential step for the mode of action to bind DNA and form a monofunctional adduct.

3.4. Topoisomerase I inhibition

Topoisomerases are well-established targets in antitumor research, and compounds inhibiting these enzymes are potential antitumor agents. The inhibitory effect of the Pt(II) complexes on Topo I was investigated by unwinding plasmid pBR322 DNA. As shown in Fig. 3, with concentration of those complexes increased, the Form I is diminished accompany with Form I is increased. As the concentration of the complex **1** reached to 20 μM, the Form II was migrated to Form I completely, demonstrating that the Pt(II) complex was in possession of remarkable concentration-dependent inhibitory activity towards Topo I. While 40 μM of complex **2** or 100 μM of **3** could completely suppress the activity of enzyme, indicating the inhibitory effect of complex **1** was more potent than **2** and **3**. Thus, the Pt(II) complexes described herein possess potent concentration-dependent Topo I inhibitory activity and prevent enzyme-mediated unwinding of supercoiled pBR322 plasmid DNA, indicating the potential as anticancer agents.

3.5. Antitumor activity assays

The antiproliferative activity of three complexes against A549 and HepG2 cancer

cell lines and human normal liver LO2 cell lines were evaluated by MTT assays. Cisplatin was used as a positive control in these studies. The half maximal inhibitory concentrations (IC_{50}) of the complexes are summarized in Table 4. Based on these values, the in *vitro* antiproliferative activities of the complexes follow the order: **3** ≈ cisplatin > **2** > **1**. Complex **3**, with a coordination ion I^-, shows higher cytotoxicity than complexes **1** and **2** against two human cancer cell lines tested. Interestingly, a relatively high selectivity towards cancer cells (HepG2) over normal cells (LO2) is observed for complex **3**, showing approximately 2-fold higher cytotoxicity against cancer cell lines than that of LO2 cells.

4. Conclusions

A new ligand L and three binuclear complexes **1** ($[Pt(L)Cl_2]Cl_2$**]**), **2** ($[Pt(L)Br_2]Br_2$]) and **3** ($[Pt(L)I_2]I_2$) were synthesized and the structure of L was confirmed by X-ray crystallography. π-π stacking interaction was detected in the geometric structure of L. The binding abilities between those complexes and CT DNA were investigated and the result showed that the highest calculated DNA-binding constants K_b of complex **3** reach to 8.72×10^6 M^{-1}, which exhibited stronger DNA binding affinity than that of the classical DNA intercalator EB and complexes **1-2**. Agarose gel electrophoresis study revealed that the unwinding of plasmid DNA and Topo I inhibition activities of complex **1** was more potent compared to complexes **2-3**. Meanwhile, complex **3** achieved obvious anticancer activities against two cancer cell lines, which was comparable to the drug cisplatin. Overall, our results suggest that changing the leaving groups in the aromatic Pt(II) coordination center could be

promising strategies for developing Pt(II) polypyridyl complexes as important DNA binders and potential anticancer agents.

Acknowledgements

We thank the National Natural Science Foundation of China (21401105), the Research Fund for the Doctoral Program of Jiangxi Science & Technology Normal University (3000990404), Jiangxi Provincial Key Laboratory of Drug Design and Evaluation (20171BCD40015), Jiangxi Provincial Department of Education (GJJ170695), Natural Science Foundation of Jiangxi Province (20181BAB213001) and College Students' Science and Technology Innovation Project of Jiangxi Science & Technology Normal University (20171103057).

Appendix A. supplementary data

CCDC 1853639 contains the supplementary crystallographic data for this article. These data can be obtained free of charge via https://www.ccdc.cam.ac.uk/ , or from the Crystallographic data for the structures in this paper have been deposited with the Cambridge Crystallographic Database Center, CCDC, 12 Union Road, Cambridge CB21EZ, UK. (Fax: +44-1223-336-033; E-mail: deposit@ccdc.cam.ac.uk). Supplementary data associated with this article can be found online at https://doi.org/10.1xxxxxxxxxxx.

References

[1] J. Shelton, X. Lu, J.A. Hollenbaugh, J.H. Cho, F. Amblard, R.F. Schinazi, Chem. Rev. 116 (2016) 14379-14455. http://doi.org/10.1021/acs.chemrev.6b00209.

[2] B. Rosenberg, L. VanCamp, J.E. Trosko, V.H. Mansour, Nature. 222 (1969) 385-

386. http://doi.org/10.1038/222385a0.

[3] J.B. Mangrum, N.P. Farrell, Chem Commun (Camb). 46 (2010) 6640-6650. http://doi.org/10.1039/c0cc01254h.

[4] K.W. Jennette, S.J. Lippard, G.A. Vassiliades, W.R. Bauer, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 3839-3843. http://doi.org/10.1073/pnas.71.10.3839.

[5] L.X. Zhao, T.S. Kim, S.H. Ahn, T.H. Kim, E.K. Kim, W.J. Cho, H. Choi, C.S. Lee, J.A. Kim, T.C. Jeong, C.J. Chang, E.S. Lee, Bioorg. Med. Chem. Lett. 11 (2001) 2659-2662. http://doi.org/10.1016/s0960-894x(01)00531-5.

[6] M.J. MacLachlan, M. Ginzburg, N. Coombs, T.W. Coyle, N.P. Raju, J.E. Greedan, G.A. Ozin, I. Manners, Science. 287 (2000) 1460-1463. http://doi.org/10.1126/science.287.5457.1460.

[7] A.H. Wang, J. Nathans, G. van der Marel, J.H. van Boom, A. Rich, Nature. 276 (1978) 471-474. http://doi.org/10.1038/276471a0.

[8] G. Arena, L.M. Scolaro, R.F. Pasternack, R. Romeo, Inorg. Chem. 34 (1995) 2994-3002. http://doi.org/10.1021/ic00115a030.

[9] Y.K. Yan, M. Melchart, A. Habtemariam, P.J. Sadler, Chemical communications. (2005) 4764-4776. http://doi.org/10.1039/b508531b.

[10] R. Fernandez, M. Melchart, A. Habtemariam, S. Parsons, P.J. Sadler, Chem.-Eur. J. 10 (2004) 5173-5179. http://doi.org/10.1002/chem.200400640.

[11] I. Romero Canelon, L. Salassa, P.J. Sadler, J. Med. Chem. 56 (2013) 1291-1300. http://doi.org/10.1021/jm3017442.

[12] M. Kubanik, H. Holtkamp, T. Sohnel, S.M.F. Jamieson, C.G. Hartinger,

Organometallics. 34 (2015) 5658-5668. http://doi.org/10.1021/acs.organomet.5b00868.

[13] S.K. Lee, K.W. Tan, S.W. Ng, J Inorg Biochem. 159 (2016) 14-21. http://doi.org/10.1016/j.jinorgbio.2016.02.010.

[14] A.P. Neves, M.X. Pereira, E.J. Peterson, R. Kipping, M.D. Vargas, F.P. Silva, Jr., J.W. Carneiro, N.P. Farrell, J Inorg Biochem. 119 (2013) 54-64. http://doi.org/10.1016/j.jinorgbio.2012.10.007.

[15] Y. Lo, T. Ko, W. Su, T. Su, A. Wang, J. Inorg. Biochem. 103 (2009) 1082-1092. http://doi.org/10.1016/j.jinorgbio.2009.05.006.

[16] H. Kwon, C. Park, K. Jeon, E. Lee, S. Park, K. Jun, T. Kadaya, P. Thapa, R. Karki, Y. Na, M. Park, S. Rho, E. Lee, Y. Kwon, J. Med. Chem. 58 (2015) 1100-1122. http://doi.org/10.1021/jm501023q.

[17] J. Son, L. Zhao, A. Basnet, P. Thapa, R. Karki, Y. Na, Y. Jahng, T. Jeong, B. Jeong, C. Lee, E. Lee, Eur. J. Med. Chem. 43 (2008) 675-682. http://doi.org/10.1016/j.ejmech.2007.05.002.

[18] Y. Sun, J. Li, H. Zhao, L. Tan, J Inorg Biochem. 163 (2016) 88-94. http://doi.org/10.1016/j.jinorgbio.2016.04.028.

[19] O.A. Oyetade, V.O. Nyamori, B.S. Martincigh, S.B. Jonnalagadda, RSC Adv. 6 (2016) 2731-2745. http://doi.org/10.1039/c5ra23900a.

[20] J.H. Price, J.P. Birk, B.B. Wayland, Inorg. Chem. 17 (1978) 2245-2250. http://doi.org/10.1021/ic50186a046.

[21] N.K. Mitra, R. Meudom, H.H. Corzo, J.D. Gorden, B.L. Merner, J. Am. Chem.

Soc. 138 (2016) 3235-3240. http://doi.org/10.1021/jacs.6b00538.

[22] C.J. Adams, S.L. James, X. Liu, P.R. Raithby, L.J. Yellowlees, J. Chem. Soc.-Dalton Trans. (2000) 63-67. http://doi.org/10.1039/A907028A.

[23] L.C. HK Yip, KK Cheung and CM Che J. Chem. Soc.-Dalton Trans. (1993) 2933-2938 http://doi.org/10.1039/DT9930002933.

[24] G.M. Sheldrick, Acta Crystallogr. Sect. C-Struct. 71 (2015) 3-8. http://doi.org/10.1107/s2053229614024218.

[25] A.M. Pyle, Rehmann, J. P., Meshoyrer, R., Kumar, C. V., Turro, N. J., & Barton, J. K, J. Am. Chem. Soc. 111 (1989) 3051-3058. http://doi.org/10.1021/ja00190a046.

[26] R. Vikneswaran, N.E. Eltayeb, S. Ramesh, R. Yahya, Polyhedron. 105 (2016) 89-95. http://doi.org/10.1016/j.poly.2015.12.012.

[27] D.J. Mercer, S.J. Loeb, Dalton Trans. 40 (2011) 6385. http://doi.org/10.1039/c1dt10569h.

[28] L.S. Lerman, J. Mol. Biol. 3 (1961) 18-30. http://doi.org/10.1016/s0022-2836(61)80004-1.

[29] X. Liu, J. Feng, J. Meng, Q. Pan, A. Ren, X. Zhou, H. Zhang, Eur. J. Inorg. Chem. 2005 (2005) 1856-1866. http://doi.org/10.1002/ejic.200400533.

[30] J.B. LePecq, C. Paoletti, J. Mol. Biol. 27 (1967) 87-106. http://doi.org/10.1016/0022-2836(67)90353-1.

[31] Y.L. Li, Q.P. Qin, Y.C. Liu, Z.F. Chen, H. Liang, J Inorg Biochem. 137 (2014) 12-21. http://doi.org/10.1016/j.jinorgbio.2014.04.001.

[32] S. Mukherjee, I. Mitra, V.P.B. Reddy, S. Mahata, J.C.K. Bose, S. Dasgupta, W.

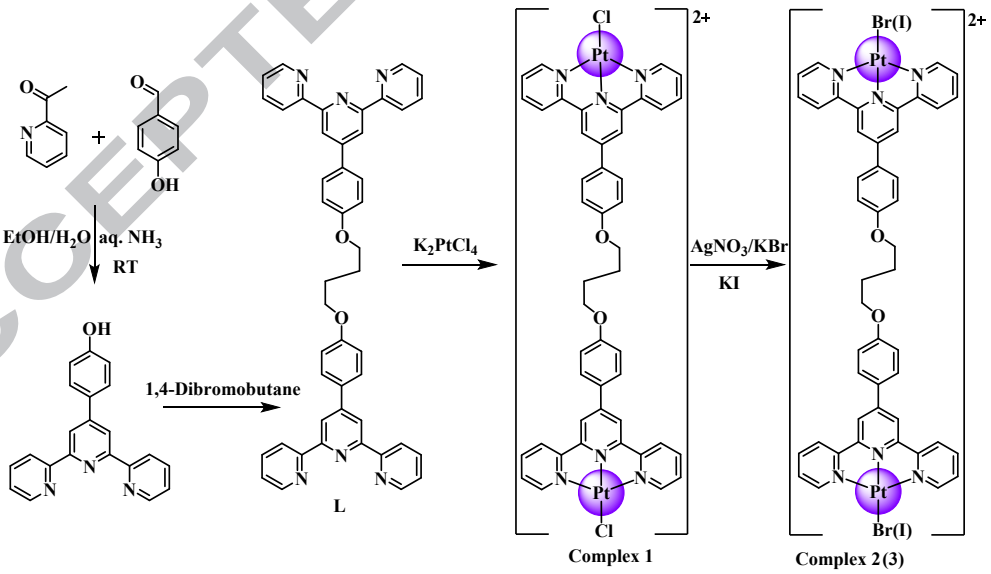
Linert, S.C. Moi, Polyhedron. 119 (2016) 84-97. http://doi.org/10.1016/j.poly.2016.08.024.

[33] A. Tarushi, C. Kakoulidou, C.P. Raptopoulou, V. Psycharis, D.P. Kessissoglou, I. Zoi, A.N. Papadopoulos, G. Psomas, J Inorg Biochem. 170 (2017) 85-97. http://doi.org/10.1016/j.jinorgbio.2017.02.010.

[34] M.V. Keck, S.J. Lippard, J. Am. Chem. Soc. 114 (1992) 3386-3390. http://doi.org/10.1021/ja00035a033.

[35] A. Hofmann, L. Dahlenburg, R. van Eldik, Inorg. Chem. 42 (2003) 6528-6538. http://doi.org/10.1021/ic034400±.

[36] S. Betanzos-Lara, O. Novakova, R.J. Deeth, A.M. Pizarro, G.J. Clarkson, B. Liskova, V. Brabec, P.J. Sadler, A. Habtemariam, J. Biol. Inorg. Chem. 17 (2012) 1033-1051. http://doi.org/10.1007/s00775-012-0917-9.



Scheme 1. Synthesis of the new ligand and complexes (**1-3**).

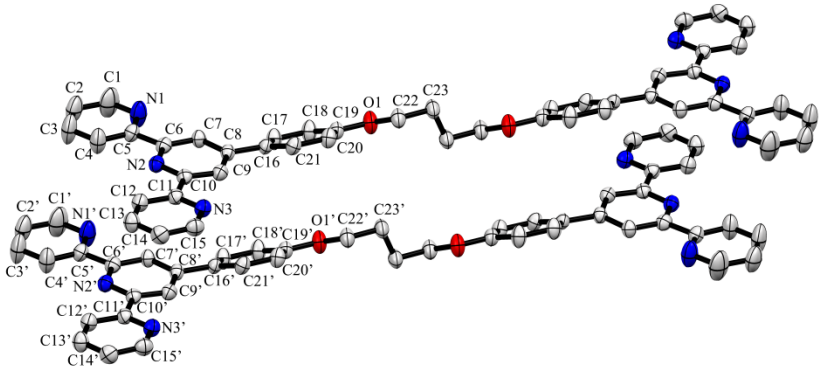


Fig. 1. Thermal ellipsoid plot of L in ORTEP view.

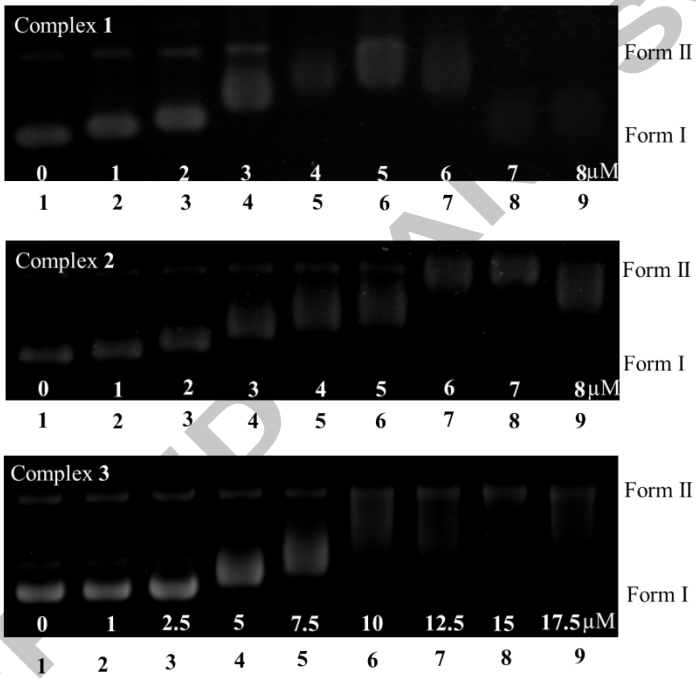


Fig. 2. Concentration-dependent of pBR322 DNA unwinding by Pt(II) complex **1** (0–8 μM), complex **2** (0–8 μM) and complex **3** (0–17.5 μM) in buffer (10 mM KH_2PO_4, 10 mM NaCl, 10 mM EDTA, pH 7.2) solutions at 37 °C for 24 h. Lane 1, DNA control, lane 2 - 9, with Pt(II) complexes of increasing concentrations.

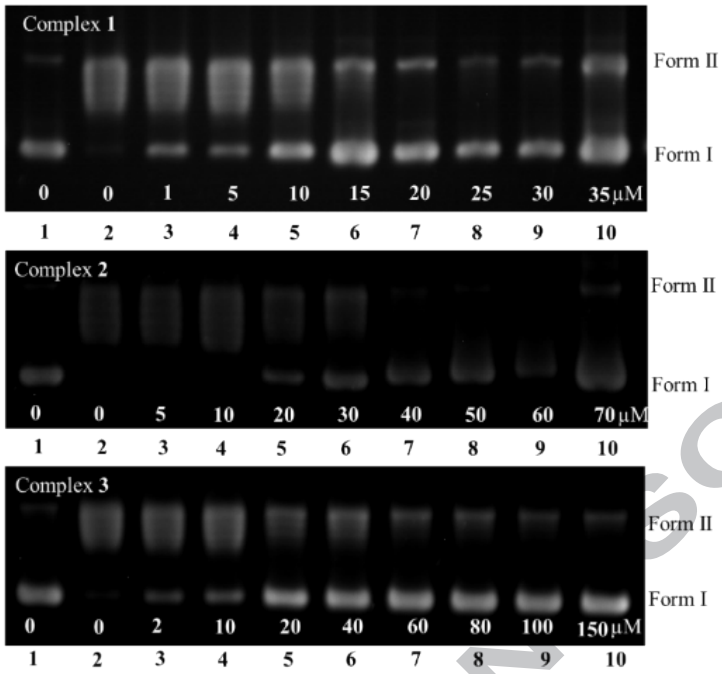


Fig. 3. Topo I inhibitory effect by Pt(II) complex **1** (0–35 μM), complex **2** (0–70 μM) and complex **3** (0–150 μM). Lane 1, DNA control, Lane 2 – 10, with Pt(II) complexes of various concentrations.

Table 1

Crystal and structure refinement data for L

Crystal data	L
Chemical formula	$C_{46}H_{42}N_6O_6$
Formular weight(g mol^{-1})	774.86
Crystal system	Monoclinic
Space group	*R -3*
a(Å)	46.504(5)
b(Å)	46.504(5)
c(Å)	4.8819(5)
α(°)	90
β(°)	90
γ(°)	120
V(Å^3)	9143(2)
Z	9
D_c(g cm^{-3})	1.445
θ range (°)	5.7030-51.4810
μ(mm^{-1})	1.54184

F(000)	3672.0
Crystal size(mm)	0.3 × 0.08 × 0.07
Temperature(K)	293(2)
Reflections collected	5518
Independent reflections	1523
Goodness-of-fit(GOF)	0.944
Largest difference in peak and hole(e Å $^{-3}$)	0.213, -0.156
R_1[a] wR_2[b] (I> 2σ (I))	0.1352, 0.1322
R_2[a] wR_2[b] (all data)	0.0602, 0.0921

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $wR_2 = |\Sigma w(|F_o|^2 - |F_c|^2)/\Sigma|w(F_o)^2|^{1/2}$,
Where $w=1/[s^2(Fo^2) + (0.0189P)^2]$ where $P=(Fo^2+2Fc^2)/3$.

Table 2
The DNA-binding constants (K_b) value of complexes **1-3**

Table 3 The percentage of fluorescence (%I/I_0) and the Stern-Volmer constants (K_{sv}) value of ligand L and complexes **1-3**.

Compound	$\Delta A/A_0$(%)	K_b(M^{-1})	Compound	$\Delta I/I_0$ (%)	K_{sv} (M^{-1})
1	26	1.83×10^6	L	4	5.00×10^3
2	32	3.21×10^6	**1**	43	5.85×10^4
3	29	8.72×10^6	**2**	44	6.56×10^4
			3	49	7.60×10^4

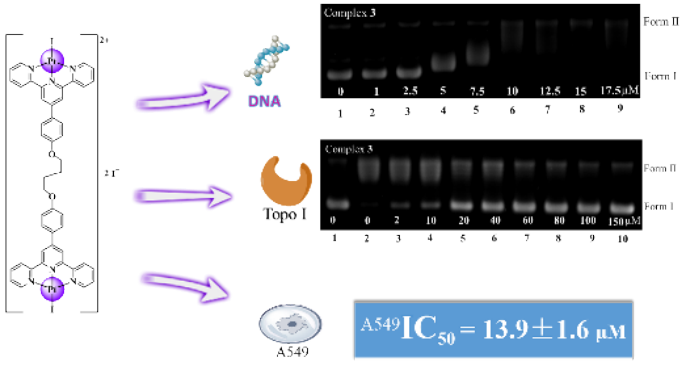
Table 4 IC_{50} values of complexes **1-3** towards different cell lines. [a]

	IC_{50} [μM]		
Compound	A549	HepG2	LO2
1	23.3 ± 1.5	25.9 ± 1.8	29.6 ± 5.1
2	21.9 ± 2.3	22.8 ± 2.1	26.7 ± 2.1
3	13.9 ± 1.6	15.2 ± 1.4	36.6 ± 8.1
cisplatin	14.6 ± 1.3	13.8 ± 1.5	16.6 ± 2.4

[a] IC_{50} values are drug concentrations necessary for 50% inhibition of cell viability. Data are presented as means ± standard deviations of at least three independent experiments and the drug treatment period was 48 h.

Graphical abstract:

Three new binuclear platinum(II) complexes were synthesized and characterized. Those complexes exhibited high binding affinities for calf thymus DNA and potent topoisomerase I inhibitory activities, and significant cytotoxicity activities.



Graphical abstract - synopsis

Three new binuclear platinum (II) complexes were synthesized and characterized. Those complexes exhibited high binding affinities for calf thymus DNA and potent topoisomerase I inhibitory activities, and significant cytotoxicity activities.

Graphical abstract – pictogram