



DNA-binding, cytotoxicity and apoptosis induction of Pt/Fe-based heterometallo-supramolecular polymer for anticancer drug application

Utpal Rana^a, Chanchal Chakraborty^{a,1}, Miki Kanao^a, Hiromi Morita^b, Takashi Minowa^b, Masayoshi Higuchi^{a,*}

^a Electronic Functional Macromolecules Group, National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba 305-0044, Japan

^b Nanotechnology Innovation Station, NIMS, 1-2-1 Sengen, Tsukuba 305-0047, Japan

ARTICLE INFO

Article history:

Received 24 November 2018

Received in revised form

17 March 2019

Accepted 30 March 2019

Available online 8 April 2019

Keywords:

Metallosupramolecular polymer

DNA binding

Cytotoxicity

Apoptosis

ABSTRACT

We synthesized two Pt/Fe-based metallo-supramolecular polymers with Pt (II) and Fe(II) introduced alternately (**polyFePtL1** and **polyFePtL2**) for anticancer drug application. They have octahedral Fe(II)-centers and square-planar Pt (II)-centers bound with acetylide TPy (4-ethynyl-(2,2':6',2''-terpyridine) and $-\text{PEt}_3$ (**polyFePtL1**) or $-\text{PPh}_3$ (**polyFePtL2**). We measured their binding affinity to calf-thymus DNA (ct-DNA) by the UV–vis spectral titration. The binding constant of **polyFePtL2** ($K_b = 6.0 \times 10^7 \text{ M}^{-1}$) is 15-fold higher than that of **polyFePtL1** ($K_b = 4.0 \times 10^6 \text{ M}^{-1}$). The high binding affinity of **polyFePtL2** indicates the intercalative binding of the $-\text{PPh}_3$ moieties to DNA in addition to the groove binding of the polymer to DNA. They showed high cytotoxicity against human lung cancer cell line (A549). **PolyFePtL2** has almost twice higher cytotoxicity (41% viable cell) than **polyFePtL1** in 10 μM concentration. We also inspected the cell apoptosis mechanism by FACS using Annexin-V FITC/PI double staining assay and found that **polyFePtL2** induced apoptosis selectively leading to cancer cell death with ~15% detectable apoptotic cell.

© 2019 Published by Elsevier B.V.

1. Introduction

Cancer is one of the major causes of death in the world and the development of anticancer drugs is still desired. Nearly 50% of present anticancer drugs are based on Pt complexes such as cisplatin, carboplatin and oxaliplatin [1–4]. Therapeutic activity of the anticancer drugs crucially depends on the DNA binding affinity. The binding of drugs with DNA over-stabilizes the DNA duplexes and inhibits the DNA replication, leading to the cellular apoptosis. There are two binding modes: intercalation and groove binding. In the intercalation, the planar part of drug stacks with DNA base pairs through the π – π interaction. In the groove binding, a drug binds to the minor or major groove of DNA duplexes based on the ionic interactions etc. The groove binding is normally stronger than the

intercalation [5]. So, we considered anticancer drugs driven by groove bindings are effective to enhance the binding affinity with DNA and reported anticancer activity of metallosupramolecular polymers. Metallosupramolecular polymers are composed of metal ions and organic ligands. Because metal ions have positive charge(s), the polymers are regarded as polycation, which can bind phosphate anions of DNA ionically. In our previous papers, we showed the strong groove binding activity of metallosupramolecular polymers to DNA and higher cytotoxicity to cancer cells [6–9]. It was also observed that metallosupramolecular polymer with a helical structure has higher binding affinity to DNA with the same helicity than the polymer with the opposite helicity [8]. Furthermore, we revealed that the DNA binding affinity and cytotoxicity of metallo-supramolecular polymer are controllable by changing the metal-metal distance of polymer [9].

Recently, heterometallic complexes with two different cytotoxic metals have received attention as a novel class of anticancer agents [10,11]. The two metals with different coordination geometries multiply interact with a biological target and enhance the anticancer activity. Higher cytotoxicity of heteropolynuclear Ru–Pt

* Corresponding author.

E-mail address: HIGUCHI.Masayoshi@nims.go.jp (M. Higuchi).

¹ Present Address: Department of Chemistry, Birla Institute of Technology & Science, Pilani, Hyderabad Campus, Shameerpet, Jawahar Nagar, Secunderabad, Telangana 500078, India.

anticancer complexes than monometallic complexes was reported by Reedijk et al. [12] Strong DNA binding and cytostatic behavior of a Ru–Pt bis(terpyridyl) complex was also reported by Smythe et al. [13] However, the bimetallic polymeric systems as anticancer drugs are relatively unexplored and only a few have been studied so far. Inspiring from the above-mentioned reports, we have designed heterometallo-supramolecular polymers with two metal species (Fe and Pt) introduced alternately through the polymer chain (Fig. 1) for the anticancer drug application. We chose Fe(II) due to the wide diversity of coordination, readily accessible oxidation states and good antitumor activity with fewer side effects [14]. We chose organometallic Pt (II) as the second metal for toxicity because organometallic Pt-compounds are also good anticancer candidates with their large structural variety and kinetic stability [15].

2. Experimental procedures

2.1. Materials

4-ethynylbenzaldehyde, 2-acetylpyridine, *trans*-[PtCl₂(PEt₃)₂], CuI and diisopropylamine, MgSO₄ and iron (II) tetrafluoroborate [Fe(BF₄)₂] were purchased from TCI (Tokyo, Japan) and Aldrich (St. Louis, MO, USA) and were directly used without further purification. **PolyFePtL2** was prepared according to our previously reported procedure. Dehydrated DMSO, 28% NH₃ solution were purchased from Aldrich. Dry DCM, CHCl₃, ethanol and acetonitrile solvents were purchased from Wako or Kanto Chemical Co. Inc. and used as received.

2.2. Instrumentation

UV–vis spectra of polymers and DNA binding titrations were recorded using a Shimadzu UV-2550 UV–visible spectrophotometer. IR spectra were recorded on a Shimadzu FTIR 8400S Fourier Transform Infrared Spectrophotometer with KBr pellets. ¹H and ¹³C NMR spectra were measured at 300 MHz and 75 MHz, respectively, on a JEOL AL 300/BZ instrument (Tokyo, Japan). Mass spectra (MS) were measured using a Shimadzu/Kratos TOF Mass spectrometer (Kyoto, Japan). High-resolution mass spectra (HRMS) were measured using a Shimadzu LCMS-IT-TOF spectrometer. Calf thymus DNA (ct-DNA) (10 mg/mL, 200–500 bp) was purchased from TREVIGEN (Gaithersburg, MD, USA) and the known molar extinction coefficient of 6600 M⁻¹ at 260 nm was used for ct-DNA concentration with respect to per nucleotide calculation. The

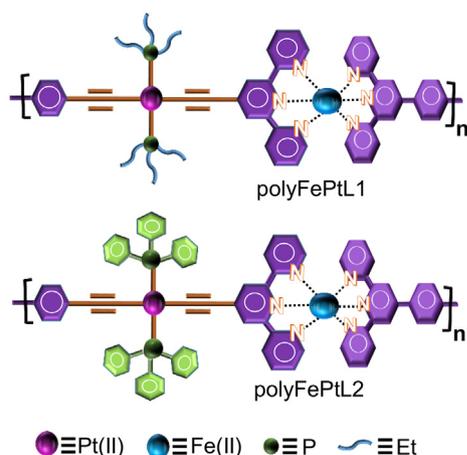


Fig. 1. Fe/Pt-based heterometallo-supramolecular polymers (**polyFePtL1** and **polyFePtL2**).

A549 human lung cancer cell line was purchased from the RIKEN Bio-Resource Center. A microplate reader (MTP-880, Corona Electric Co., Ltd., Hitachinaka, Japan) was used for the cytotoxicity assay with CCK-8. Cell-Counting Kit-8 (CCK-8), and calcein-AM and propidium iodide (PI) double staining kits were purchased from Dojindo (Kumamoto, Japan). Inverted light and fluorescence microscopes (DMIL, Leica Microsystems, Wetzlar, Germany) were used to observe cells stained with the calcein-AM and PI double staining kit. Annexin-V buffer and Annexin-V (10 μg/mL) were used for FACS measurements and purchased from SONY (Tokyo, Japan) and SONY SP6800 cell analyzer software was used for FACS analysis.

2.3. Synthesis of 1

Acetylide terpyridine 1 was prepared as reported previously by refluxing the 4-ethynylbenzaldehyde (8.92 g, 40.0 mmol), 2-acetylpyridine (9.85 mL, 88.00 mmol) and KOH (8.98 g, 160.00 mmol) in the mixture of 200 mL of EtOH and 40 mL of 28% NH₃ solution for 24 h. The mixture was cooled to room temperature and extracted with DCM. The combined organic layer was washed with water and brine and dried over MgSO₄. After removal of the solvent, the residue was purified by flash chromatography (1:1 hexane: DCM) to afford 1 as a white powder (9.4 g, 70%).

¹H NMR (300 MHz, CDCl₃, 298 K), δ (ppm) = 8.73 (s, 2H), 8.69 (d, 2H, *J* = 7.8 Hz), 7.95 (dd, 4H, *J* = 8.8 Hz), 7.28 (dd, 2H, *J* = 7.8 Hz), 7.65 (d, 2H, *J* = 8.4 Hz), 7.38 (t, 2H, *J* = 4.8, 6.9 Hz), 3.19 (s, 1H).

¹³C NMR (75 MHz, CDCl₃): 156.09, 156.06, 149.28, 149.13, 138.82, 136.88, 132.68, 127.23, 123.90, 122.80, 121.36, 118.71, 83.31, 78.51.

HRMS (*m/z*): [M + Na] found 356.126; calculated 356.116.

2.4. Synthesis of PtL1

To a solution of 1 (1.0 g, 3.00 mmol) in dry DCM (40 mL), *trans*-[Pt(PEt₃)₂Cl₂] (0.50 g, 1.00 mmol), CuI (0.03 g, 0.15 mmol) and diisopropylamine (20 mmol) were added. The mixture was stirred at room temperature under nitrogen for 48 h. The solvent was evaporated, residue was washed with water, methanol and hot ethanol and finally dried in vacuum for overnight to afford PtL1 as a light-yellow powder (1.02 g, 93%).

¹H NMR (300 MHz, CDCl₃, 298 K), δ (ppm) = 8.73 (s, 8H), 8.68 (d, 4H, *J* = 8.1 Hz), 7.90 (t, 4H, *J* = 7.2 Hz), 7.82 (d, 4H, *J* = 8.4 Hz), 7.42 (d, 4H, *J* = 7.8 Hz), 7.37 (t, 4H, *J* = 5.1, 7.2 Hz), 2.25 (m, 12H), 1.32 (m, 18H).

¹³C NMR (75 MHz, CDCl₃): 155.44, 155.88, 150.01, 149.07, 136.80, 134.70, 131.34, 129.85, 126.86, 123.72, 121.31, 118.40, 110.66, 109.62, 16.69, 8.38.

HRMS (*m/z*): [M + H] found 1119.293; calculated 1118.384.

2.5. Synthesis of polyFePtL1

The ligand Pt L1, (0.22 g, 0.20 mmol) and Fe(BF₄)₂·6H₂O (0.07 g, 0.20 mmol) were refluxed at 80 °C in argon-saturated dry CHCl₃-DMSO (20 mL, 1:1) mixture for 24 h. The reaction mixture was cooled to room temperature and added in CHCl₃. The residue was filtered and washed with MeOH and CHCl₃ several times and dried under vacuum for 24 h to get **polyFePtL1** with >95% yield.

¹H NMR (300 MHz, DMSO-*d*₆, 298 K), δ (ppm) = 9.66 (bs, 4H), 9.08 (s, 4H), 8.50 (d, 4H, *J* = 5.8 Hz), 8.06 (bs, 4H), 7.58 (bs, 4H), 7.29 (t, 8H), 2.30 (m, 12H), 1.34 (m, 18H).

HRMS (*m/z*): [M + H] found 576.103; calculated 576.653.

2.6. Cell viability

Cell viability of the cancel cells were measured by Cell Counting Kit-8 (CCK-8) assay. This assay kit provides an easy-to-use, non-

radioactive, and very good method for cell proliferation, cell viability, and cytotoxicity. CCK-8 assay Kit is a sensitive method for quantification of viable cells in proliferation and cytotoxicity assay. The method is colorimetric and based on the conversion of highly water soluble colorless Dojindo's tetrazolium salt to yellow color formazan. WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] produces a water-soluble formazan dye upon reduction. Viable cells with active metabolism convert WST-8 into formazan, however, dead cells lose this ability. Thus color formation serves as a useful and convenient marker of only the viable cells. The measured absorbance (450 nm) is proportional to the number of viable cells. For cell viability test, the lung cancer cell line, A549 was grown in DMEM medium (pH 7.4) supplemented with 10% (v/v) FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin (10% FBS-DMEM) and grown in the dark at 37 °C in a 5% CO₂ humidified environment. Cells were seeded with a density of approximately 15,000 cells/cm² into 96-well tissue culture plates. The old medium was removed from the cell culture medium after 24 h and medium containing different concentrations polymer was added and incubated for 24 h. For that polymers were dissolved in 20% acetonitrile aqueous solution and then diluted to 0, 2, 4, 6, 8 and 10 µM in 1% acetonitrile and 99% of 10% FBS-DMEM medium. After 24 h of incubating the cells with different concentrations polymer in 96-well plates, 10 µL of CCK-8 solution was added to each well. After an additional 2 h of incubation, the absorbance at 450 nm (reference 630 nm) was measured using a microplate reader. A higher absorbance value corresponded to higher cell proliferation, as the water-soluble formazan dye was formed via cellular respiration.

2.7. Calcein-AM and PI assay

Calcein-AM and PI double staining assay was performed into 24-well tissue culture plates at a density of approximately 15,000 cells/cm². After the cells were incubated for 24 h with 10 µM of **polyFePtL1** or **polyFePtL2** in 24-well plates, the cells were washed twice with sterile PBS and 500 µL of PBS containing calcein-AM (2 µM) and PI (4 µM) were added. Plates were incubated for 15 min before fluorescence imaging with a microscope.

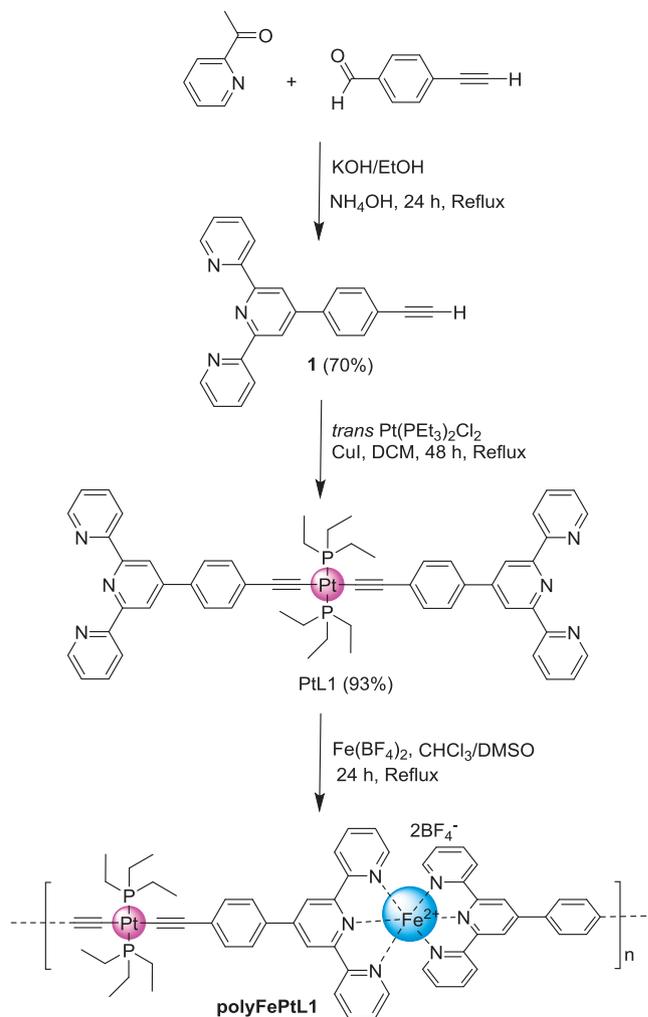
2.8. Apoptosis analysis

Apoptosis analysis of A549 cell was performed by double staining assay using TACS Annexin V-FITC Apoptosis Detection Kit and PI. After incubating the cells for 24 h with 10 µM of **polyFePtL1** or **polyFePtL2** in 4-well plates, the cells were washed twice with sterile PBS and then 100 µL of 2% BSA containing trypsin was added and incubated for 5 min. The cells were collected in 1.5 mL centrifuge tubes and centrifuged for 3 min (300×g). The medium was removed and 100 µL of staining solution was added to the cells and mixed gently. Next, the cell suspension was kept in the dark at room temperature for 15 min. Finally, 400 µL of 1X binding buffer was added and the cells were analyzed by FACS. The results were analyzed using SONY SP6800 Software.

3. Results and discussion

Pt/Fe-based heterometallo-supramolecular polymers, **polyFePtL1** and **polyFePtL2** were synthesized (Scheme 1), where the ditopic ligands of PtL1 and PtL2 with square planar center and TPY (TPY = 4-ethynyl-(2,2':6',2''-terpyridine)) allows an octahedral iron (II) terpyridyl center. The design and subsequent development of the bimetallic supramolecular polymer have been inspired by the cytotoxicity of Fe and Ru-based metallosupramolecular polymers, which can coordinate to DNA by groove binding [13,14].

Novel ligand, PtL1 was synthesized from *trans*-Pt [PEt₃]₂Cl₂



Scheme 1. Synthesis of **polyPtL1**.

(PEt₃ = triethylphosphine) and 4-ethynyl-phenyl-(2,2':6',2''-terpyridine) (**1**, Fig. S1–S4) using CuI as a catalyst according to Scheme 1, while the PtL2 was prepared from *trans*-[Pt (PPh₃)₂]Cl₂ (-PPh₃ = triphenylphosphine) treating with **1** according to our previous report.¹⁶ The ligand, PtL1 was characterized by ¹H, ¹³C NMR and HRMS analysis (Fig. S5–S8). The formation of a Pt–C≡C bond in PtL1 was confirmed by the ¹H NMR and ¹³C NMR spectra. No ethynyl proton signal (at 3.19 ppm) was detected in ¹H NMR of PtL1 (Fig. S5). The down-field shifting of the ethynyl carbon in ¹³C NMR (from 83.31 to 78.51 to 110.66 and 109.62) due to the π-back-donation from the Pt (II) to each ethynyl center of ligand indicated the formation of Pt–C≡C bond [17].

The complexation of PtL1 with Fe²⁺ was monitored by UV–vis spectra. Fig. 2a exhibits the UV–vis spectral change of ligand by simultaneous addition of Fe²⁺ in the chloroform–DMSO solvent mixture. A new metal-to-ligand charge transfer (MLCT) band appeared at ~570 nm, which was increased with metal concentration and became saturated. The complexation ratio of PtL1 to Fe²⁺ was determined from the absorbance change at the MLCT band with respect to the molar ratio of this solution, which indicates 1:1 between PtL1 and Fe²⁺ (Fig. 2b). The MLCT band intensity remain unchanged in presence of excess metal ions, indicated the stability of the polymer in solution [18,19].

Organometallic metallo-supramolecular polymer **polyFePtL1** was prepared in >90% yield by single-step complexation reaction of

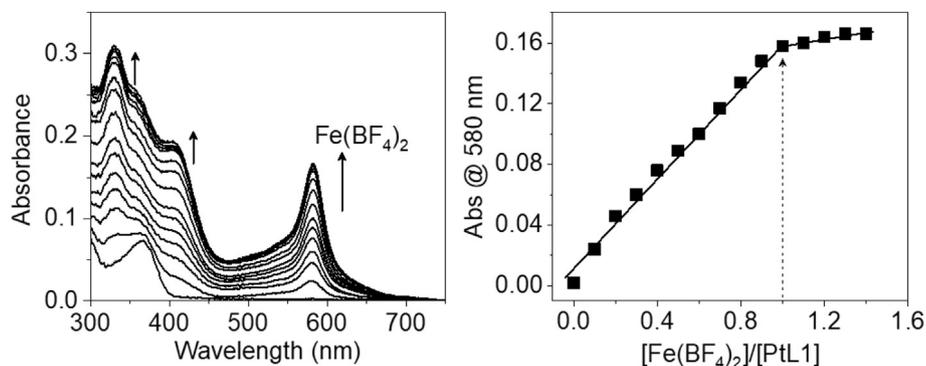


Fig. 2. (a) UV–vis spectral change of PtL1 by addition of Fe(II); (b) Absorption change at 580 nm as a function of the molar ratio of Fe(BF₄)₂/PtL1.

PtL1 with Fe²⁺. Typically, the PtL1 ligand and Fe(BF₄)₂ equimolar were refluxed in an argon-saturated dry DMSO-CHCl₃ mixture. The reaction mixture was cooled to room temperature and added to an excess CHCl₃ solution. The residue was washed with CHCl₃ to remove the impurities and dried under vacuum to get **polyFePtL1**. It was characterized by ¹H NMR and mass analysis. In the ¹H NMR spectrum of **polyFePtL1** at Fig. S9–10, all the characterized peaks of PtL1 were obtained with a downfield shifting of terpyridyl protons. The mass spectrometric studies of the polymer further support for the polymer formation. In the ESI-mass spectrum of FePt1, a peak at *m/z* 576.10 corresponding to the molecular ion of repeating unit [M+H]⁺ was observed. The preparation of **polyFePtL2** was reported in our previous paper [16].

In order to further characterize **polyFePtL1** and **polyFePtL2**, we recorded the UV–vis spectra in 1% aqueous-acetonitrile solution (5 μM) and representative spectra are shown in Fig. S11. The **polyFePtL1** showed characteristics MLCT absorption at 576 nm for Fe(II)-terpyridine octahedral complex and at 380 nm for Pt(II)-acetylacetonate square planar complex. Both the peaks were red-shifted to 580 and 390 nm in **polyFePtL2**. These significant red-shifting occurred due to presence of electron withdrawing –PPh₃ group (as lone pair of phosphorous conjugated with phenyl groups) at platinum center of PtL2, which considerably reduced the lowest unoccupied molecular orbital (LUMO) energy of Pt(II) containing ligand and facilitated the electron transfer from Fe(*dπ*) to π*(Pt-acetylacetonate) [17,20].

The single polymer chain of **polyFePtL1** or **polyFePtL2** contain multiple numbers of alternative Fe and Pt centers with octahedral and square planar geometry. So, it can interact strongly with the negative charges of phosphate groups present in the sugar backbone of DNA. As we know, the DNA is the pharmacological target of many of the useful drugs. So, interfering DNA replication by targeting is quite easy and conceptually straightforward [21,22]. Therefore, our prime intention is to stop DNA replication by enhancement of DNA binding properties of the metallo-supramolecular polymer.

To get insight about DNA binding of these polymers we have employed UV–vis spectrometric titration method. The binding constant of two polymers to ct-DNA were determined by UV–vis changes at MLCT band of polymers as a function of DNA concentration. For that, 5 μM of polymer in 1% aqueous-acetonitrile solution was taken in a UV–vis cuvette and simultaneously increased the DNA concentration up to [DNA]/[polymer] ~1.4. As the DNA concentration increased, changes in the MLCT (~580 nm) band of polymers were observed and plateau detected after certain concentration (Fig. 3). The binding constant (*K_b*) of the polymers were calculated using MvH equation (equation (1)) from the non-linear fitting plot of extinction coefficient change with respect to [DNA]/

[Polymer] [7,8]. The *K_b*(DNA–polyFePtL2) is 15 times higher than *K_b*(DNA–polyFePtL1). The reason can be explained by van der Waal's and electrostatic interaction of the DNA duplex with FePtL2. Presence of extra π-system (planar phenyl ring in –PPh₃ group) in the PtL2 ligand that extends sufficiently to permit the partial intercalation of the phenyl group to the DNA bases and enhances binding activity [23].

$$\frac{\epsilon_a - \epsilon_b}{\epsilon_b - \epsilon_f} = \frac{b - (b^2 - 2K_b^2 C_t [DNA]_t/s)^{1/2}}{2K_b C_t} \quad (1)$$

Here $b = 1 + K_b C_t + K_b [DNA]_t/2s$, *K_b* is binding constant between polymer and DNA, the total polymer concentration in repeat units is *C_t*, [DNA]_t is the DNA concentration per nucleotide, the size of the binding site per nucleotide is 's' and ϵ_a , ϵ_f , ϵ_b represent the apparent, free, and bound polymer molar extinction coefficients, respectively. The ϵ_b was determined from the plateau of DNA titration.

In addition, both polymers showed significant hypochromism and red shift of the MLCT band of Fe(II) upon sequential addition of ct-DNA. The bathochromic shift of **polyFePtL1** and **polyFePtL2** was 7 and 4 nm with hypochromicity 11.1% and 14.9% respectively at the plateau (see Table 1). The significant red-shift and maximum hypochromicity of **polyFePtL2** indicated the strong groove binding between the polymer chains and duplex DNA. As the PtL2 contains planar phenyl group, which can intercalate in the DNA duplex and stacked with DNA base pairs and favors the groove binding of **polyFePtL2** and ct-DNA, whereas no such intercalating groups were present in **polyFePtL1**. Interestingly, such partial intercalation was confirmed by the MLCT band change of Pt(II) in presence of DNA. Fig. 3a unveiled the Pt(II) MLCT of **polyFePtL1**, which was red shifted from 380 to 387 nm, whereas for **polyFePtL2**, blue shifting of Pt(II) MLCT from 390 to 385 nm (Fig. 3c) (at 1:1 DNA–Polymer). The blue shift of the Pt(II) MLCT band is the consequence of partial intercalation of ligand to the double strand DNA [24].

As our metallo-supramolecular polymers (**polyFePtL1** or **polyFePtL2**) contain two cytotoxic metals in the same polymer chain including very strong binding activity to DNA, it should be a good DNA-replication interfering agent and anticancer drug [25]. The

Table 1
DNA binding of polymers.

	Hypochromicity (%) ^a	bathochromic shift (nm) ^a	<i>K_b</i> × 10 ⁶ (M ⁻¹)
polyFePtL1	11.1	7	4.07
polyFePtL2	14.9	4	60.08

^a At 1:1 [DNA]/[Polymer].

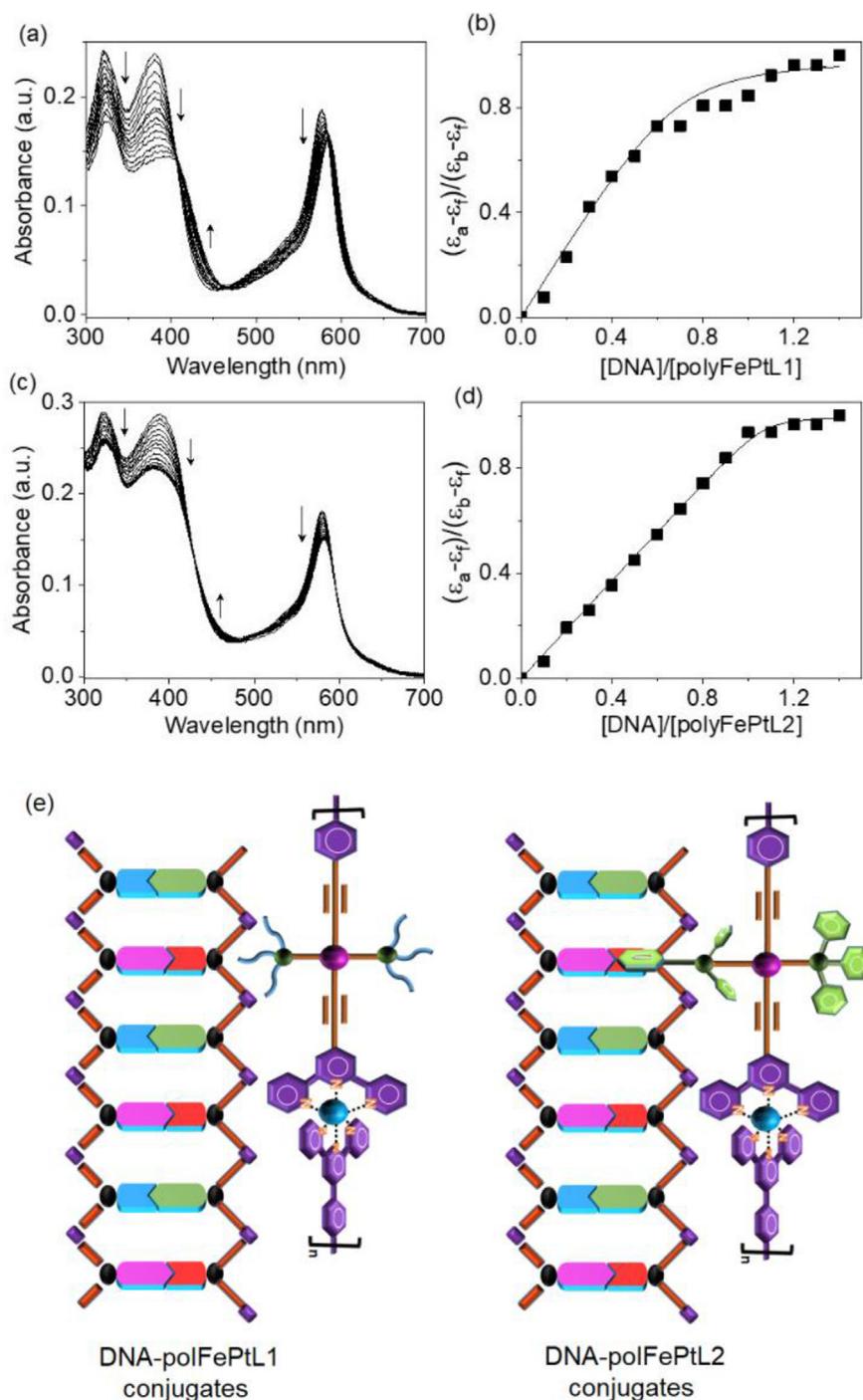


Fig. 3. UV–vis spectral changes of polymers with the addition of ct-DNA and non-linear fitting of the absorption change for polymer as a function of DNA concentration, (a) and (b) for **polyFePtL1**; (c) and (d) for **polyFePtL2**, respectively. (e) Possible conjugate structure of B-DNA with **polyFePtL1** and **polyFePtL2**.

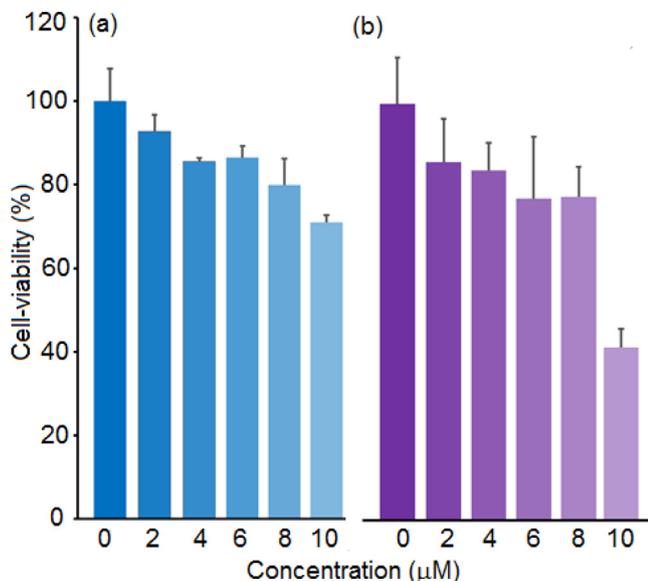
anticancer activity of **polyFePtL1** and **polyFePtL2** to human lung cancer cell (A549) were tested by established CCK-8 assay. For that, the A549 cells were incubated for 24 h and different concentrations dose of **polyFePtL1** or **polyFePtL2** was applied for the cell-viability test. Five different concentrations 2, 4, 6, 8 and 10 μM of polymers were used for cell viability test. Cell viability decreased with concentration for both polymers and survival rate reached 71% and 41% for **polyFePtL1** and **polyFePtL2** at 10 μM concentrations, respectively showing at Fig. 4. Finally, Calcein-AM and propidium iodide

(PI) double staining colorimetric assay was used for cellular uptake studies and to establish the cytotoxicity results with cellular levels. This method is fluorescence based for live (green) and/or dead cells (red) detection with two probes that reflect cellular activities and plasma membrane integrity [26]. The accumulation of **polyFePtL1** and **polyFePtL2** to A549 cell at 10 μM concentration are shown in Fig. 5. An average number of dead/total cells percentages were calculated from Fig. 5b and d and observed 12% and 58% for **polyFePtL1** and **polyFePtL2**, respectively (see Table 2). The result also a

Table 2

Live and dead cell assay of the polymers counted from Fig. 5.

	Average no. of live cells (L)	Average no. of dead cells (D)	D/Total no. of cells (L + D), %
polyFePt1	75	10	12
polyFePt2	32	45	58

**Fig. 4.** CCK-8 assay of A549 cells in the presence of polymers (a) **polyFePt1** and (b) **polyFePt2**.

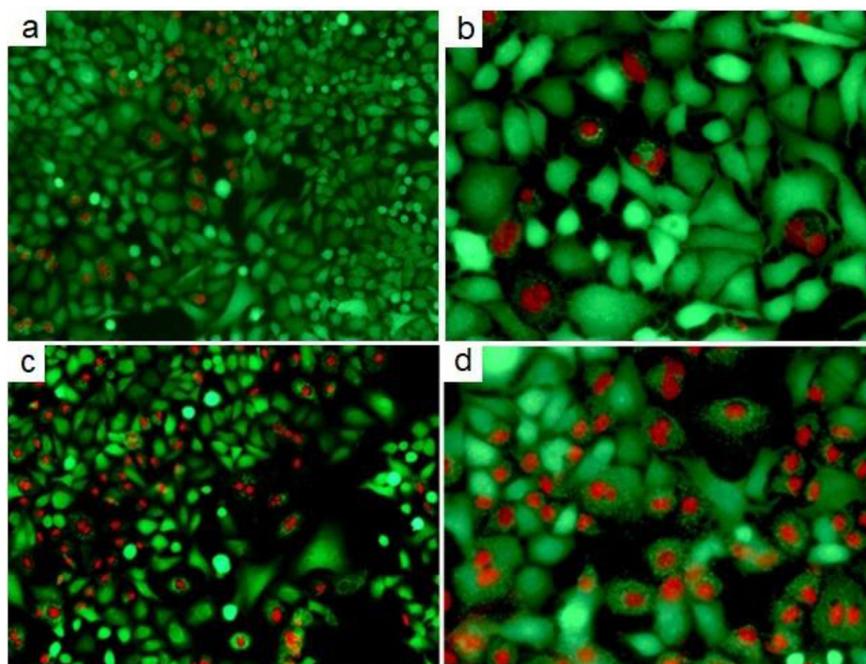
good agreement with the DNA-binding and cytotoxicity by CCK-8 assay results. So, from the above results, we conclude that the **polyFePt2** could effectively enter the cancer cell, and induces the apoptosis. Although, the cell death mechanism still not clear to us but we consider that passive diffusion through the cellular lipid

bilayer will be the dominant process involved in polymer uptake [27].

Considering the essential role of cytotoxicity of polymers are apoptosis induction, the cell apoptosis mechanism was studied by FACS using Annexin-V FITC/PI double staining assay. For that, A549 cells were treated with polymers for 24 h with 10 μM concentration and then cells were collected for staining. As shown in Fig. 6, the populations of apoptotic cells, including late apoptotic cells and early apoptotic cells for **polyFePt1** were 9.80% and 3.25% and for **polyFePt2** 14.47% and 4.49% respectively. The significant increases of the apoptotic cells for **polyFePt2** indicates that it effectively inhibits the cell proliferation and induced apoptosis.

4. Conclusion

In summary, the first organometallic based metallo-supramolecular polymer with alternative iron and platinum metals have synthesized and its binding to the DNA and cytotoxicity to cancer cell are presented. The results suggested that the platinum center in the metallo-supramolecular polymer is competent to interact to the DNA without being hindered to the iron unit, which simultaneously enhances the electrostatic binding to the DNA and results the strong cytotoxicity. The **polyFePt2** including planar phenyl group substituted platinum center showed ~15 times strong binding activity to DNA than **polyFePt1**, which contains ethyl substituted platinum. Strong DNA binding also reflected in the cytotoxicity properties of the polymers. Approximately double cytotoxicity was observed for **polyFePt2** than **polyFePt1** for A549 cell. Cell death mechanism was established by FACS and its showed apoptosis is the main reason for cytotoxicity

**Fig. 5.** Calcein-AM and propidium iodide (PI) double staining assay of A549 cells, (a) and (b) for **polyFePt1**; (c) and (d) for **polyFePt2**.

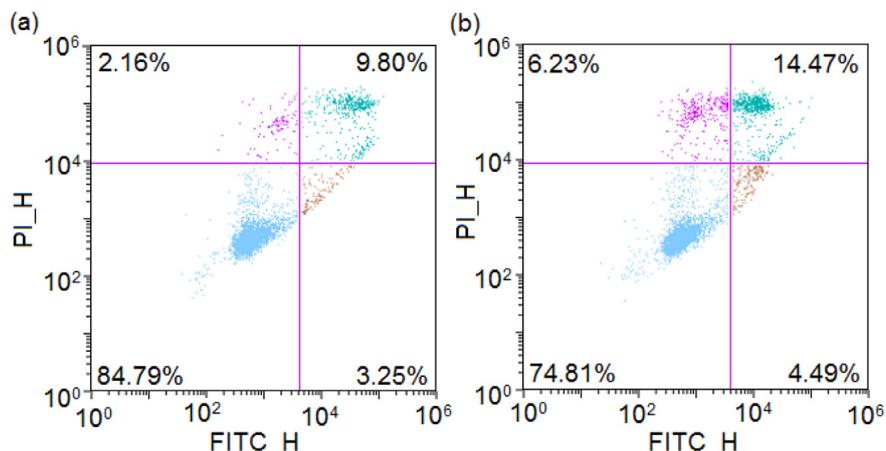


Fig. 6. FACS analysis of A549 cells in presence of 10 μM of (a) **polyFePtL1** and (b) **polyFePtL2** by Annexin-V FITC/PI double staining.

and a higher percentage of early and late apoptotic cells were observed for **polyFePtL2** than **polyFePtL1**. The Fe-Pt based heterometallo-supramolecular polymer is a unique example of a potentially antitumor-active polymer which offers stable and improves DNA binding with anticancer activities.

Acknowledgment

The authors would like to acknowledge JST CREST Grant Number JPMJCR1533, Japan for financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jorganchem.2019.03.021>.

6. References

- [1] E. Wong, C.M. Giandomenico, Current status of platinum-based antitumor drugs, *Chem. Rev.* 99 (1999) 2451–2466.
- [2] N.P. Farrell, Multi-platinum anti-cancer agents. Substitution-inert compounds for tumor selectivity and new targets, *Chem. Soc. Rev.* 44 (2015) 8773–8785.
- [3] D. Wang, S.J. Lippard, Cellular processing of platinum anticancer drugs, *Nat. Rev. Drug Discov.* 4 (2005) 307–320.
- [4] A. Florea, D. Büsselberg, Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects, *Cancers* 3 (2011) 1351–1371.
- [5] S. Saha, R. Majumdar, M. Roy, R.R. Dighe, A.R. Chakravarty, An iron complex of dipyrrophenazine as a potent photocytotoxic agent in visible light, *Inorg. Chem.* 48 (2009) 2652–2663.
- [6] J. Li, T. Murakami, M. Higuchi, Metallo-supramolecular polymers: versatile DNA binding and their cytotoxicity, *J. Inorg. Organomet. Polym. Mater.* 23 (2013) 119–125.
- [7] J. Li, Z. Futera, H. Li, Y. Tateyama, M. Higuchi, Conjugation of organic-metallic hybrid polymers and calf-thymus DNA, *Phys. Chem. Chem. Phys.* 13 (2011) 4839–4841.
- [8] U. Rana, C. Chakraborty, R.K. Pandey, M.D. Hossain, R. Nagano, M. Morita, S. Hattori, T. Minowa, M. Higuchi, Selective DNA Recognition and cytotoxicity of water-soluble helical metallosupramolecular polymers, *Bioconjug. Chem.* 27 (2016) 2307–2314.
- [9] M.D. Hossain, U. Rana, C. Chakraborty, R. Li, R. Nagano, T. Minowa, M. Higuchi, Effect of metal–metal distance in Ni (ii)-based metallo-supramolecular polymers: DNA binding and cytotoxicity, *RSC Adv.* 7 (2017) 38008–38013.
- [10] J.F. González-Pantoja, M. Stern, A.A. Jarzecki, E. Robles-Escajeda, A. Varela-Ramirez, R.J. Aguilera, M. Contel, Titanocene–phosphine derivatives as precursors to cytotoxic heterometallic TiAu2 and TiM (M = Pd, Pt) compounds. Studies of their interactions with DNA, *Inorg. Chem.* 50 (2011) 11099–11110.
- [11] C.G. Hartinger, A.D. Phillips, A.A. Nazarov, Ruthenium, osmium and gold complexes. The quest for innovative anticancer chemotherapeutics, *Curr. Top. Med. Chem.* 11 (2011) 2688–2702.
- [12] K. Schilden, F. Garcia, H. Kooijman, A.L. Spek, J.G. Haasnoot, J. Reedijk, A Highly flexible dinuclear ruthenium(II)–platinum(II) complex: crystal structure and binding to 9-ethylguanine, *Angew. Chem. Int. Ed.* 43 (2004) 5668–5670.
- [13] V. Ramu, M.R. Gill, P.J. Jarman, D. Turton, J.A. Thomas, A. Das, C. Smythe, A Cytostatic ruthenium(II)–platinum(II) bis(terpyridyl) anticancer complex that blocks entry into S Phase by up-regulating p27KIP1, *Chem. Eur. J.* 21 (2015) 9185–9197.
- [14] W.A. Wani, U. Baig, S. Shreaz, R.A. Shiekh, P.F. Iqbal, E. Jameel, A. Ahmad, S.H. Mohd-Setapar, M. Mushtaqueh, L.T. Hun, Recent advances in iron complexes as potential anticancer agents, *New J. Chem.* 40 (2016) 1063–1090.
- [15] C.G. Hartinger, N. Metzler-Nolte, P.J. Dyson, Challenges and opportunities in the development of organometallic anticancer drugs, *Organometallics* 31 (2012) 5677–5685.
- [16] C. Chakraborty, R.K. Pandey, U. Rana, M. Kanao, S. Moriyama, M. Higuchi, Geometrically isomeric Pt(II)/Fe(II)-based heterometallo-supramolecular polymers with organometallic ligands for electrochromism and the electrochemical switching of Raman scattering, *J. Mater. Chem. C* 4 (2016) 9428–9437.
- [17] M. Shiotsuka, N. Nishiko, K. Keyaki, K. Nozaki, Construction of a photoactive supramolecular system based on a platinum(II) bis-acetylide building block incorporated into a ruthenium(II) polypyridyl complex, *Dalton Trans.* 39 (2010) 1831–1835.
- [18] M. Hossain, T. Sato, M. Higuchi, A green copper based metallo-supramolecular polymer: synthesis, structure, and electrochromic properties, *Chem. Asia J.* 8 (2013) 76–81.
- [19] F.S. Han, M. Higuchi, D.G. Kurth, Metallo-supramolecular polyelectrolytes self-assembled from various pyridine ring-substituted bisterpyridines and metal ions: photophysical, electrochemical, and electrochromic properties, *J. Am. Chem. Soc.* 130 (2008) 2073–2081.
- [20] S. Zhao, S.M. Arachchige, C. Slebodnick, K.J. Brewer, Synthesis and study of the spectroscopic and redox properties of Ru^{II}, Pt^{II} mixed-metal complexes bridged by 2,3,5,6-tetrakis(2-pyridyl)pyrazine, *Inorg. Chem.* 47 (2008) 6144–6152.
- [21] L.H. Hurley, DNA and its associated processes as targets for cancer therapy, *Nat. Rev. Canc.* 2 (2002) 188–200.
- [22] C. Troiber, E. Wagner, Nucleic acid carriers based on precise polymer conjugates, *Bioconjug. Chem.* 22 (2011) 1737–1752.
- [23] P.K.L. Fu, P.M. Bradley, C. Turro, Stabilization of duplex DNA structure and suppression of transcription in vitro by bis(quinone diimine) complexes of rhodium(III) and ruthenium(II), *Inorg. Chem.* 42 (2003) 878–884.
- [24] J.M. Veal, R.L. Rill, Noncovalent DNA binding of bis(1,10-phenanthroline) copper(I) and related compounds, *Biochemistry* 30 (1991) 1132–1140.
- [25] M. Milkevitch, H. Storrie, E. Brauns, K.J. Brewer, B.W. Shirley, A new class of supramolecular, mixed-metal DNA-binding agents: the interaction of Ru^{II}, Pt^{II} and Os^{II}, Pt^{II} bimetallic complexes with DNA, *Inorg. Chem.* 36 (1997) 4534–4538.
- [26] P. Hu, R. Wang, L. Zhou, L. Chen, Q. Wu, M.Y. Han, A.M. El-Toni, D. Zhao, F. Zhang, Near-infrared-activated upconversion nanoprobe for sensitive endogenous Zn²⁺ detection and selective on-demand photodynamic therapy, *Anal. Chem.* 89 (2017) 3492–3500.
- [27] C.A. Puckett, J.K. Barton, Methods to explore cellular uptake of ruthenium complexes, *J. Am. Chem. Soc.* 129 (2007) 46–47.