Inorganic Chemistry Communications 94 (2018) 92–97

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

journal homepage: www.elsevier.com/locate/inoche

Short communication

Novel fluorinated platinum(II) complexes with pyridine-2-carboxylate ligand as potent radiosensitizer and antiviral agent

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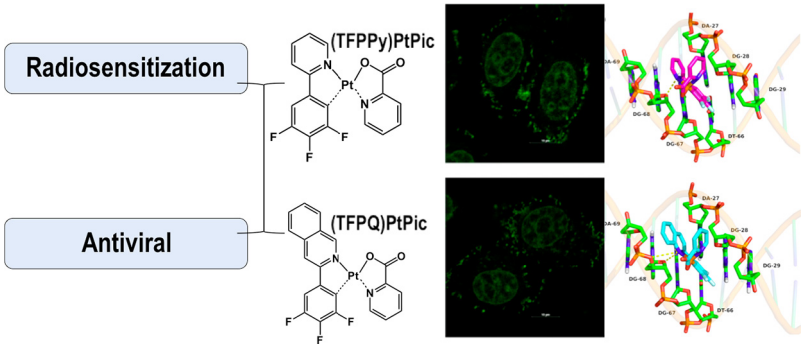
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Pt(II) complex
Radiosensitizer
Antiviral agent
Radiochemotherapy (RT-CT)

ABSTRACT

Novel Pt(II) complexes, (TFPPy)PtPic and (TFPQ)PtPic, containing fluorinated phenylpyridine or phenylquinoline with pyridine-2-carboxylate as chelating ligands were synthesized according to rational design, their therapeutical activities examined. Remarkable radiosensitization SER values were observed even in radio-resistant cell line after treated with these complexes. Furthermore, the two complexes have shown antiviral activity against DNA virus HSV-1. Comply with live cell imaging results, molecular docking have revealed the complexes forms stable hydrogen bond and hydrophobic interactions to the minor groove of target DNA double helix. These findings demonstrate the non-classical structure complexes are promising for further evaluation as a new type of prodrug radiochemotherapy and antiviral treatment.

Rational design and synthesis of platinum based drugs has been constantly drawing research attention, since over 70% combination therapy and radiochemotherapy (RT-CT) in today's clinical practice of cancer treatment involves platinum drug regiments. However, the existing platinum drugs involve the issue of resistance and side effects. Besides cancer treatments, the use of platinum drugs as antiviral agents

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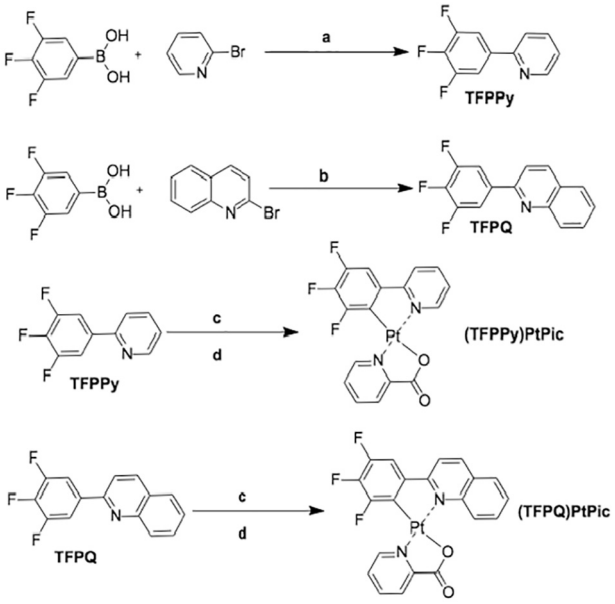
https://doi.org/10.1016/j.inoche.2018.06.013
Received 7 April 2018; Received in revised form 13 June 2018; Accepted 15 June 2018
Available online 21 June 2018
1387-7003/



Scheme 1. Synthetic routes of (**TFPPy**)**PtPic** and (**TFPQ**)**PtPic**.

hasn't been fully explored. Therefore, there is still an unmet need for examine novel structure-activity relationship and innovation mechanisms for platinum prodrugs [1].

Classical mechanism of clinically used $[Pt(A)_2X_2]$ type of platinum drugs is based on the production of inter-/intra-strand cross-links (CLs) between Pt and DNA [2]. These complex leads to DNA rupture and interferes with the native conformation, which result in error coding, prohibition of enzyme (*e.g.*, DNA glycosylase) and/or DNA from binding to nuclear and cytoplasmic proteins (*e.g.*, as mismatch repair proteins) to regulate replication and transcription process [3]. In first and second generation of $[Pt(A)_2X_2]$ drug, the reactivity of X moiety as leaving group has been found relevant to the drug's resistance and side effects. Therefore, the relatively stable leaving group of butane carboxylic acid (in Carboplatin) and glycolic acid (in Nedaplatin) have replaced the reactive chlorines (in Cisplatin) as X group. More stable oxalic acid (in Oxaliplatin) and lactic acid (in Laboplatin) has been chosen as X group in third generation. However, the classical structure of Pt drugs still associated with severe side effects and cross drug

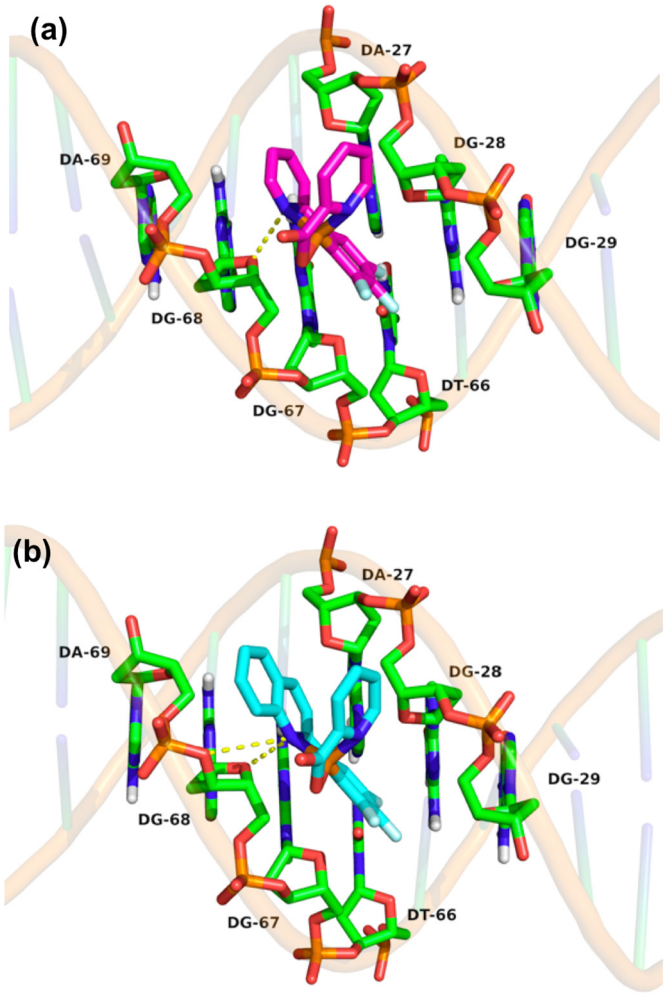


Fig. 2. Detailed view of the interaction between the HSV-1 DNA (X14112: 65866-65957) and (a) (**TFPPy**)**PtPic**; (b) (**TFPQ**)**PtPic**.

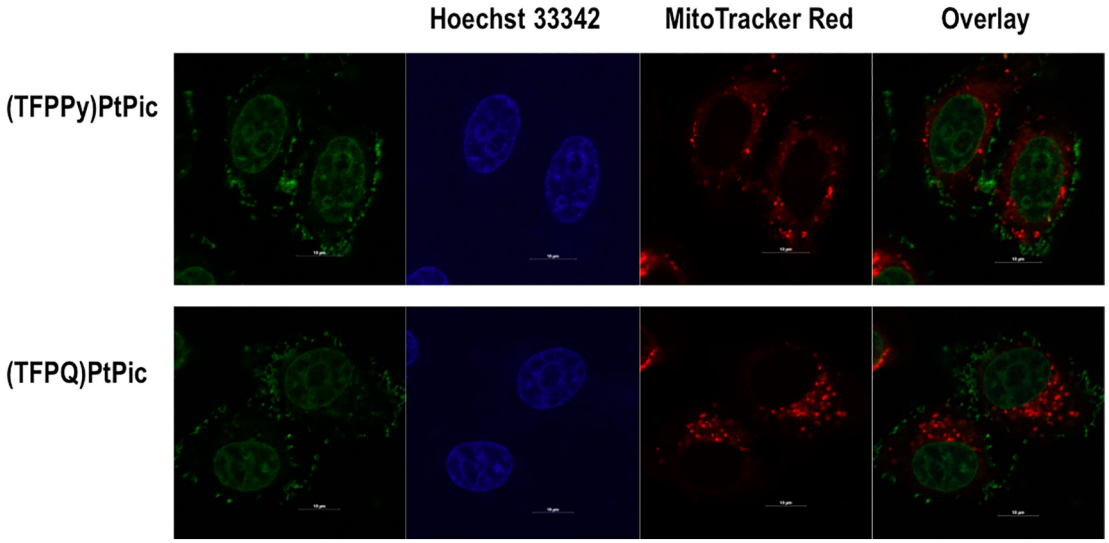


Fig. 1. CLSM images of Hep-G2 cells after treatment of (**TFPPy**)**PtPic** (2.0 μM) and (**TFPQ**)**PtPic** (2.0 μM) (0.1% DMSO in medium, green) for 2 h. Cells were co-stained with commercial nucleus tracker (Hoechst 33,342, blue) and lysosome tracker (MitoTracker, red). Scale bar: 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
IC50 (μM) values of (**TFPPy**)**PtPic** and (**TFPQ**)complexes against selected cancer cell lines after 24 h of treatment, calculated as mean ± SD from six independent experiments.

Cell line	HEK293	Eca-109	Eca-109R	OS-RC-2	TE-1	TE-1R	HCR116	Vero
(**TFPPy**)**PtPic**	24.90 ± 1.56	3.78 ± 0.13	2.79 ± 0.10	0.50 ± 0.07	4.29 ± 0.11	3.07 ± 0.14	7.10 ± 0.12	29.22 ± 3.20
(**TFPQ**)**PtPic**	24.75 + 1.07	3.14 ± 0.11	2.75 ± 0.66	0.81 ± 0.16	3.13 ± 0.08	2.57 ± 0.11	6.94 ± 0.14	28.54 ± 1.70
Cisplatin	25.58 + 1.29	18.45 ± 1.32	20.46 ± 1.04	26.77 ± 1.05	25.49 ± 1.22	32.35 ± 1.06	106.18 ± 5.20	–
Fluorouracil	10.33 ± 1.05	2.65 ± 0.61	2.42 ± 0.48	117.78 ± 4.10	2.46 ± 0.61	3.55 ± 0.19	5.15 ± 0.19	–
Acyclovir	–							120.00 ± 6.54

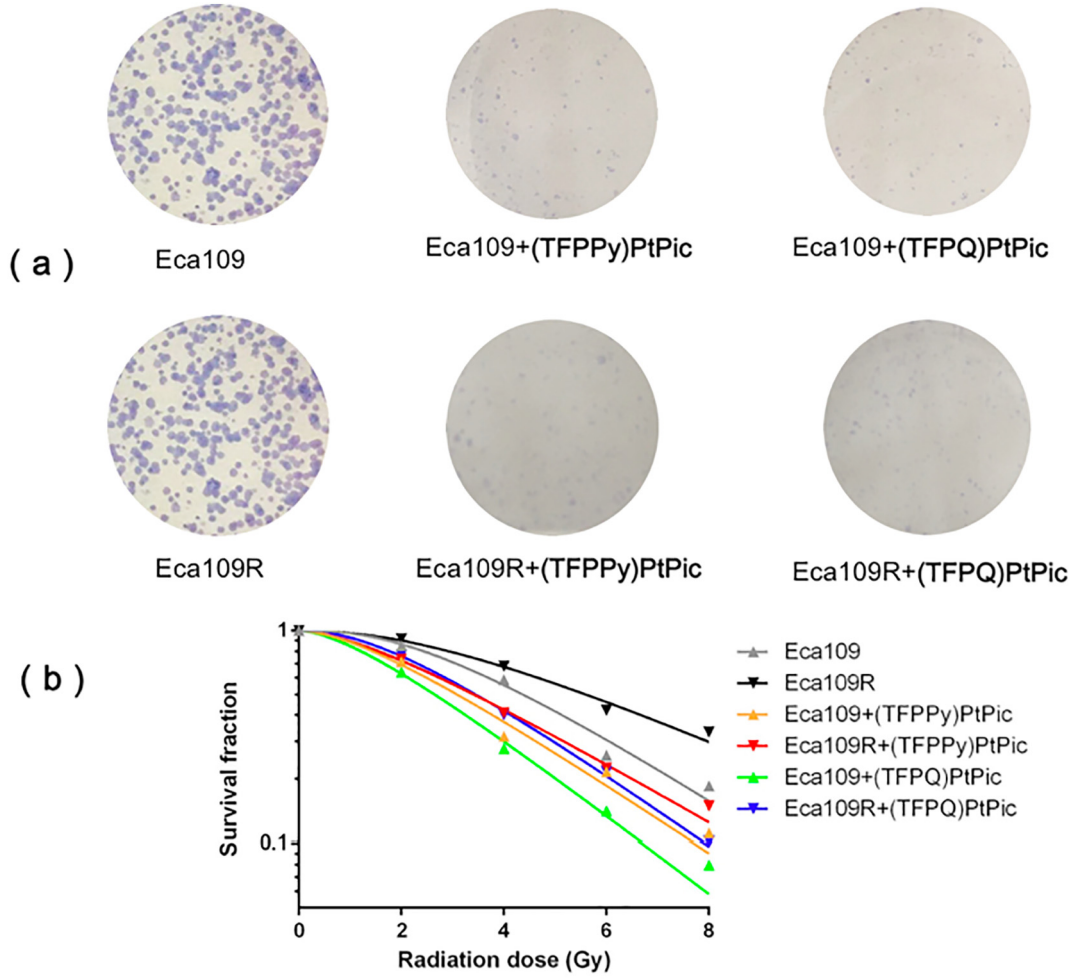


Fig. 3. (a) Clonogenic survival of Eca-109 cells and radioresistant Eca-109R cells treated with (**TFPPy**)**PtPic** and (**TFPQ**)**PtPic** combined with X-ray radiation (8 Gy). (b) Survival fractions of Eca109 cells and Eca109R cells after respective treatment.

resistance (CDR) in clinical practice due to their hydrolysis mechanism and covalent DNA binding mode [4].

Non-classical structural alternations in recent reports include the $[PtCl_2(NH_3)L]$ and $[PtCl_2L_2]$ type platinum complexes. NCI60 panel screening has shown that when L group is planar heteroaromatic ligands, the prodrugs display unique therapeutic activities in cell lines resistant to Cisplatin and Oxaliplatin [5]. It has been well accepted the steric hindrance induced by bulky heteroaromatic ligands is crucial on the metal complex's interaction and binding with DNA. Improved bioactivity was observed, attributed to the non-covalent binding between DNA helix and the L ligands (L = pyridine, quinoline, isoquinoline, thiazole, or benzothiazole) [6]. Besides, fluorination of ancillary L ligands has been a well known strategy in prodrug design, for the short van der Waals radius of fluorine atom [7], the entropically favourable hydrophobic interactions between fluorine atom and hydrocarbons [8]. The number of substituted fluorine atoms in prodrugs has also been proven to be correlated to the binding interactions, enhanced pharmacokinetic properties, improved metabolic stability and increased efficiency of cancer cell proliferation inhibition [9].

Inspired by the proven structure-activity relationship and mechanism of platinum drugs and prodrugs, we herein report the rational design, synthesis and bioactivity investigation of two novel Pt[II] complexes, *i.e.*, (**TFPPy**)**PtPic** and (**TFPQ**)**PtPic**. This [PtXL] (X = pyridine-2-carboxylate, L = 2-(3,4,5-trifluoro-phenyl) pyridine or 2-(3,4,5-trifluorophenyl) quinoline) type of complex was designed to explore the synergistic functionalities of the Pt(II) complexes with fluorinated heteroaromatic chelating ligands as a non-classical type prodrug. Bioactivity studies have been undertaken to demonstrate the potency of these two complexes as cancer cell proliferation inhibitor, radiosensitizer and antiviral agents. According the design rationale, the saturated trifluorinated pyridine/quinoline ligands would create kinetically inert coordination sphere, and the picolinic acid group on

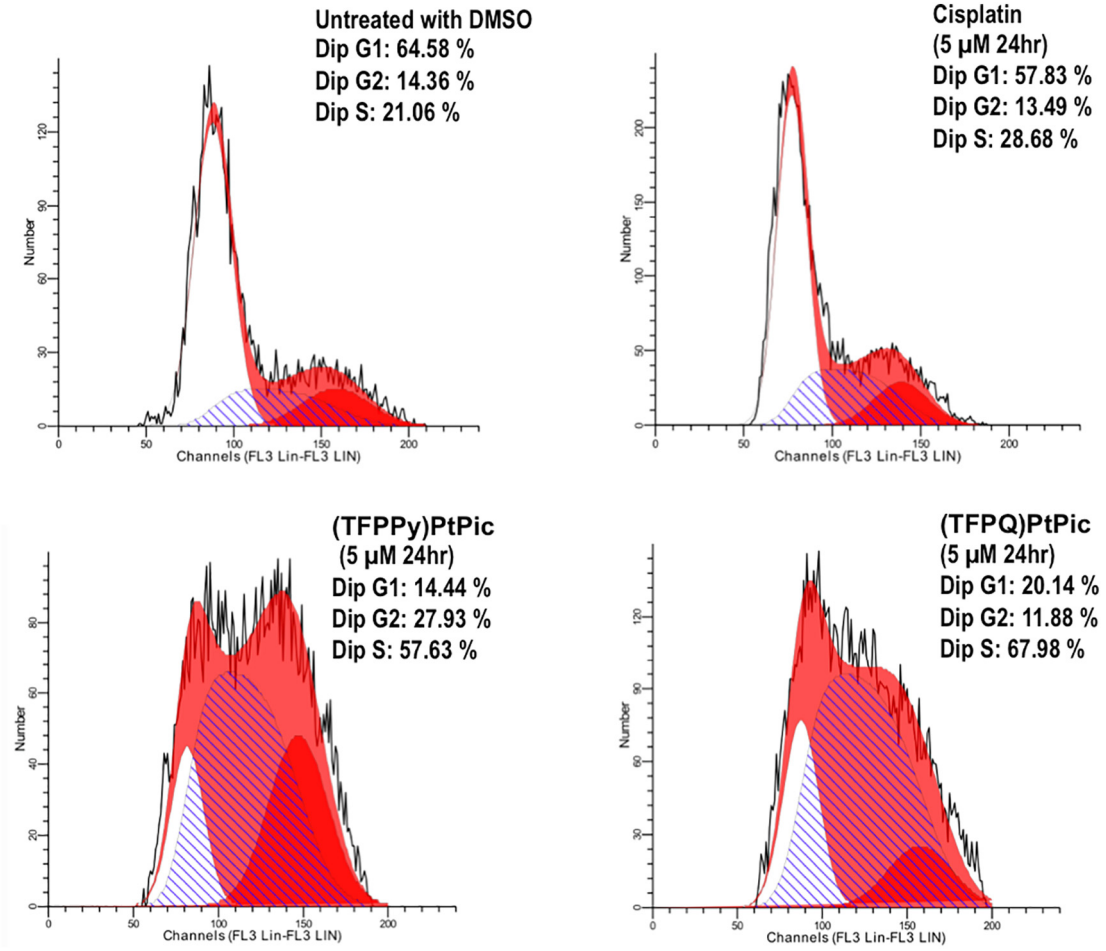


Fig. 4. Effects of (**TFPPy**)**PtPic** (5 μM 24 h) and (**TFPQ**)**PtPic** (5 μM 24 h) on S-phase and G2-phase profiles by flow cytometry. Cells were treated with DMSO (0.1%, 24 h) as blank, with Cisplatin (5 μM 24 h) as control. Data presented were from one of two experiments with similar results.

pyridine-2-carboxylate was chosen rather than more active leaving group. Thus the two complexes were expected more resistant to ligand substitution reactions entering physiological environments, side reactions such as binding to macro biomolecules prior to DNA binding will be minimized to reduce unwanted side effects (Scheme 1).

The synthesis procedure of the two complexes are described in the ESI[2]. The complexes are characterized using ^{1}H,^{19}F NMR and mass spectroscopy. The spectra of (**TFPPY**)**PtPic** and (**TFPQ**)**PtPic** in DMSO show phosphorescent emission bands in the range of 565–595 nm at room temperature, the peak wavelength of 580 nm and 582 nm respectively (Fig. S1). Electron withdrawing fluorine atoms into ancillary ligand lower the HOMO and LUMO level of the complexes, enlarge the energy gap (Fig. S2).

CLSM imaging results (Fig. 1) show that (**TFPPY**)**PtPic** and (**TFPQ**)**PtPic** are partially held in the membrane and cell plasma, possibly due to the hydrophobic interactions between the aromatic ligands binding to the phospholipid bilayer and cytoplasm proteins. After entering cell membrane, the two complexes are found both enriched at cell nucleus, co-localize with the commercial nuclear DNA marker Hoechst 33,342 (2′-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1*H*-benzimidazole trihydrochloride).

Agree with the imaging results, molecular docking calculations has shown that (**TFPPy**)**PtPic** and (**TFPQ**)**PtPic** adopted compact conformation to bind in the minor groove of HSV-1 DNA (Fig. 2(a–b)). The 2-phenylpyridine and 2-phenylquinoline ligands were positioned at the bottom of the minor groove, forming stable hydrophobic bindings, surrounded by the nucleotides DA-27, DT-66, DG-67 and DG-68, aided by the electrostatic interactions on the molecule surface (Fig. S3). Importantly, two hydrogen bond interactions are shown between the (**TFPQ**)**PtPic** and the nucleotide DG-68 of the DNA, which was the main binding affinity between the (**TFPQ**)**PtPic** and the DNA (Fig. 2b). In addition, the estimated binding energies were $-7.8\ \mathrm{kcal\ mol^{-1}}$ for (**TFPPy**)**PtPic** and $-8.6\ \mathrm{kcal\ mol^{-1}}$ for (**TFPQ**)**PtPic**, respectively, indicating that (**TFPQ**)**PtPic** was more active than (**TFPPy**)**PtPic** against DNA duplex.

Improved IC50 values against various cell lines are found, with positive control of Cisplatin and Fluorouracil. The anti-proliferation effects in cancer cell lines should attribute to the DNA-binding cross links formed by these complexes, interfere with DNA replication. Hek293 (human embryonic kidney) and Vero (African green monkey) cell lines were used as normal cell control. Both Pt agents have shown selectivity against various cancer cell lines in 1–2 order of magnitude (Table 1).

Effects of (**TFPPy**)**PtPic** and (**TFPQ**)**PtPic** treatment after irradiation were exmained with Eca109 and Eca109R (radiation-resistant subline) cells, where survival fraction was detected by colony formation assay after treatment. Reduction in colony formation exhibited in both complexes treated cells after irradiation, indicating that treatment of these two complexes promotes the radio-sensitivities. Sensitization enhancement ratio (SER) was determined by multi target single-hit model (Fig. 3). Significant radio-sensitizing effects were observed at increased radiation doses in each group. The calculated SER values of two complexes against Eca109 and Eca109R cell lines were in the range

[2] Electronic Supplementary Information (ESI) available: Experimental details see DOI: 10.1039/c000000x/.

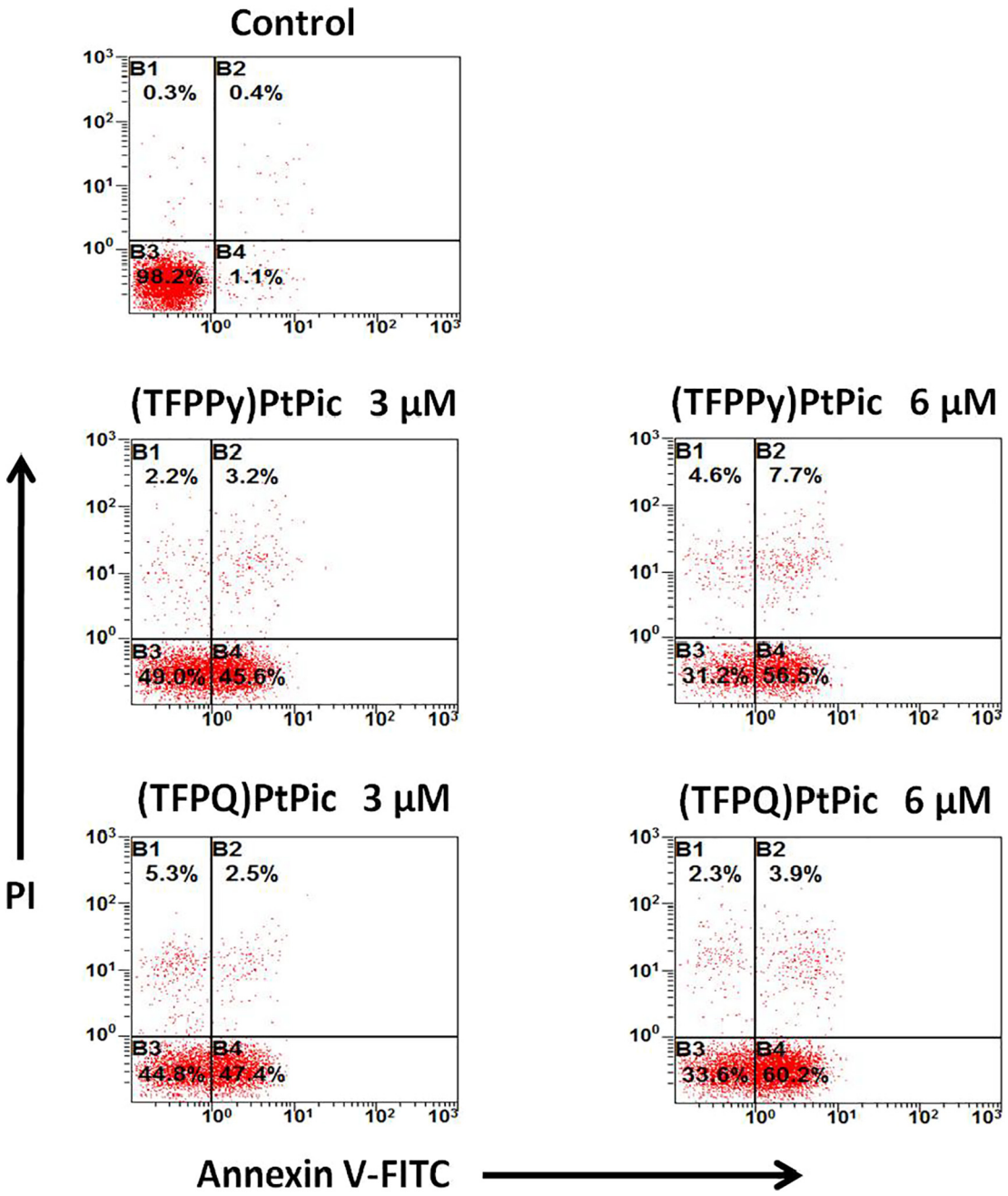


Fig. 5. Induced apoptotic cell death as examined by the Annexin V-FITC/PI assay. Eca109 cells were treated with (TFPPy)PtPic or (TFPQ)PtPic in different dosage of for 24 h respectively.

of 1.67–2.12. Noteworthy, (**TFPPy**)**PtPic** has a remarkably high SER value of 2.12 even in radio resistant Eca109R subline. The two complexes achieved improved SER value than the clinical used radiotherapy sensitizers such as Resveratrol (SER1.28) [10], Paclitaxel (SER1.32) [11], also surpassed the reported gold nanoparticles (SER1.19) and α-cyanostilbene (SER10 = 1.62) [12].

To further examine the mechanism of cancer cell proliferation and radiosensitization, cell flow assay was conducted. Results has shown the characteristic S phase arrest from both (**TFPPy**)**PtPic** and (**TFPQ**)**PtPic** treatment (Fig. 4).

It is well accepted that cells in G2-M phase are more sensitive to radiotherapy. (**TFPPy**)**PtPic** was found arresting cells in S and G2 phase (27.93% *versus* 14.36% in blank control), indicating that a higher number of Eca109 cells were blocked in a more radio-sensitive phase of the cell cycle. (**TFPQ**)**PtPic** treated cells were arrested in mainly S phase but little effects on G2 arresting, thus less potent as radiosensitizer.

To explore the nature of the cell death induced by the two complexes, apoptotic cells was determined by Annexin V and PI double labeling, measured by flow cytometry (Fig. 5). After treatment of Eca109 cells with each complex, the percentages of the early apoptotic cells (Annexin V-positive; PI-negative) was discovered 45.6% and 56.5% with (**TFPPy**)**PtPic**; 47.4% and 60.2% with (**TFPQ**)**PtPic** in 3 μM or 6 μM dosage for 24 h treatment respectively. These results indicate that the two complexes both induced dose-dependent apoptotic cell death, the effect of structural alternation between the two compounds agreed with cell cycle and molecular docking calculation results.

Considering the above effects and proposed mechanism, further investigate carry on the (**TFPPY**)**PtPic** or (**TFPQ**)**PtPic** treatment of the DNA virus HSV-1 infection. Effective suppressing of HSV-1 replication

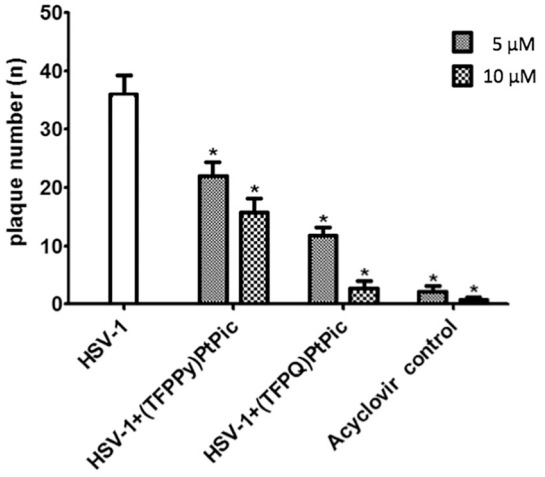


Fig. 6. (**TFPPY**)**PtPic** and (**TFPQ**)**PtPic** treatment suppresses HSV-1 replication.

by treatment of the two complexes were demonstrated *via* the plaque assay (Fig. 6).

Though not as effective as clinical used drug acyclovir (ACV), (**TFPPY**)**PtPic** and (**TFPQ**)**PtPic** can both inhibit HSV-1 replication at the concentrations of 5 μM–10 μM in a dosage dependence manner. The compound (**TFPQ**)**PtPic** seems more dependent on treatment concentration, as the more significant suppression difference. IC50 of the two complexes are on healthy Vero cells are above 28 μm, means the two complexes have antiviral selectivity of inhibition against HSV-1 infection rather than the un-infected hosting cells. Meanwhile the concentration used for treatment are within the maximum tolerated dosage if they were used as antiviral drug. Proposed inhibitory mechanism is the binding of complexes and interference directly to the virus genome DNA.

In conclusion, we have developed novel Pt agents targeting DNA with synergistic therapeutical properties against cancer (prohibit proliferation, radiosensitization) and DNA virus. The chemical structure basis of observed functionalities would attribute to the fluorinated pyridine/quinoline combined with pyridine-2-carboxylate as Pt(II) chelating ligands. The mechanism of action proposed complied with the imaging of cell localization and cell cycle results alongside the molecular docking calculations. The new [PtXL] (X = inactive leaving group, L = fluorinated heteroaromatic ligands) type of Pt complexes are worthy considering as alternative candidate when $[Pt(NH_3)_2X_2]$ type drug resistance occur. Although it is implicated that Acyclovir (ACV) have higher efficiency on anti-viral activities than these complexes, this type of platinum agent could be promising for drug mechanism studies and test in a broader range of DNA virus, furthermore for the treatment for disease complication scenario such as Epstein-Barr virus (EBV) caused lymphoma. Improved IC50 values and remarkable radiosensitizing SER values maketh them potent candidates for radiochemotherapy (RT-CT) and combination therapy.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (81601553, 21702158, 81001015, 31470890); the Fundamental Research Funds for the Central Universities (XJJ2015075); China Postdoctoral Science Foundation (2014M562390; 2017M613103). We gratefully thank Prof. Wenjing Luo at the Department of Preventive Medicine, the Fourth Military Medical University for her generous facilitates support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.inoche.2018.06.013.

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