

2016

Synthesis of platinum(II) and palladium(II) complexes with
9,9-dihexyl-4,5-diazafluorene and their in vivo antitumour activity against Hep3B
xenografted mice

Q. W. Wang

Hong Kong Baptist University

P. L. Lam

Hong Kong Polytechnic University, tcstacey@polyu.edu.hk

R. S. M. Wong

The Chinese University of Hong Kong

G. Y. M. Cheng

University of Macau

K. H. Lam

Hong Kong Polytechnic University

See next page for additional authors

This document is the authors' final version of the published article.

Link to published article: <https://doi.org/10.1016/j.ejmech.2016.08.033>

APA Citation

Wang, Q., Lam, P., Wong, R., Cheng, G., Lam, K., Bian, Z., Ho, C., Feng, Y., Gambari, R., Lo, Y., Wong, W., & Chui, C. (2016). Synthesis of platinum(II) and palladium(II) complexes with 9,9-dihexyl-4,5-diazafluorene and their in vivo antitumour activity against Hep3B xenografted mice. *European Journal of Medicinal Chemistry*, 124, 537-543. <https://doi.org/10.1016/j.ejmech.2016.08.033>

Authors

Q. W. Wang, P. L. Lam, R. S. M. Wong, G. Y. M. Cheng, K. H. Lam, Z. X. Bian, C. L. Ho, Y. H. Feng, R. Gambari, Y. H. Lo, W. Y. Wong, and C. H. Chui

Synthesis of platinum(II) and palladium(II) complexes with 9,9-dihexyl-4,5-diazafluorene and their *in vivo* antitumour activity against Hep3B xenografted mice

Q.-W. Wang^{a,b}, P.-L. Lam^{c,*}, R. S.-M. Wong^d, G. Y.-M. Cheng^e, K.-H. Lam^c, Z.-X. Bian^{f,*}, C.-L. Ho^{a,*}, Y.-H. Feng^g, R. Gambari^h, Y.-H. Lo^{g,*}, W.-Y. Wong^{a,c,*}, C.-H. Chui^{c,d,f,*}

^aInstitute of Molecular Functional Materials, Department of Chemistry and Partner State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, P.R. China; HKBU Institute of Research and Continuing Education, Shenzhen Virtual University Park, Shenzhen, 518057, PR China

^bChengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu 610041, P.R. China

^cState Key Laboratory of Chirosciences, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, P.R. China

^dDepartment of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, P.R. China

^eFaculty of Health Sciences, University of Macau, Macau, P.R. China

^fClinical Division, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, P.R. China

^gDepartment of Applied Physics and Chemistry, University of Taipei, Taipei 100, Taiwan

^hCentre of Biotechnology, Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

Corresponding Author

* Fax: (+852) 2364-9932; E-mail: tcstacey@polyu.edu.hk

* Fax: (+852) 3411-7348; E-mail: clamho@hkbu.edu.hk, rwywong@hkbu.edu.hk

* Fax: (+852) 2637-5396; E-mail: bzxiang@hkbu.edu.hk, chchui@hkbu.edu.hk

* E-mail: yhlo@utaipi.edu.tw

ABSTRACT: Two complexes dichloro(9,9-dihexyl-4,5-diazafluorene)platinum(II) (Pt-DHF) and dichloro(9,9-dihexyl-4,5-diazafluorene)palladium(II) (Pd-DHF) were synthesized and their *in vivo* antitumour activity was investigated using an athymic nude mice model xenografted with human Hep3B carcinoma cells. Pt-DHF- and Pd-DHF-treated groups showed significant tumour growth inhibition (with about 9-fold and 3-fold tumour growth retardation) when compared with the vehicle control group. The liver toxicology effects on the animals of the two compounds were investigated. Pt-DHF and Pd-DHF-treated groups had a lower alanine transaminase and aspartate transaminase values than those of the vehicle treated group as the animals from the vehicle control group had very heavy hepatoma burden. We assume that both complexes could be further investigated as effective antitumour agents and it is worthwhile to study their underlying working mechanism.

KEYWORDS: *Platinum-diazafluorene complex; Palladium-diazafluorene complex; Antitumour; in vivo.*

1. Introduction

Metal complexes are important in the development of antitumour drugs because of the interaction between metal complexes with biomolecules in human body and the high stability of their final products under various conditions. Metal complexes have been employed in antitumour therapy since the discovery of the cytotoxicity of cisplatin (*cis*-Pt(NH₃)₂Cl₂) by Rosenberg and co-workers in the late 1960s [1,2]. Cisplatin was the first identified member of the platinum-based chemotherapeutic drugs. It has been applied clinically for more than three decades in oncology [2,3]. The introduction of cisplatin to the antitumour therapy has initiated the design and synthesis of metal complexes including platinum and palladium to improve the therapeutic activity of the antitumour drugs.

Pt(II) ion has a strong binding ability to sulfur. After taking the Pt(II)-based drug in the human body, there is a high potential for binding with sulfur-donor biomolecules, which can be found in peptides, proteins and enzymes. The interaction of Pt(II) complexes with S-containing biomolecules can lead to strong cytotoxicity [4,5]. The anti-tumour activity of Pt(II)-based drugs can be due to the interaction between the metal complex and genomic DNA [4,6]. Owing to the structural similarities and significant overlap of the coordination chemistry for palladium and platinum, these two metals are closely related [7]. Palladium related complexes have also been investigated intensively in the field of medicinal chemistry [8,9].

Utku et al. reported the *in vitro* anticancer activity of the synthesized benzimidazole-platinum(II) complexes on the human HeLa (ER⁻), MCF-7 (ER⁺) and MDA-MB 231 (ER⁻) cell lines. Pt(II) complex bearing oxalate

leaving ligand possess the most active anticancer activity, with about two or five-fold greater than those of the other benzimidazole-platinum complexes tested [10]. Kovala-Demertzi and co-workers studied the anticancer property of the palladium(II) complexes synthesized by the reaction of Pd(II) salt with 2-formylpyridine-4-*N*-ethyl-thiosemicarbazone, HFo4NEt. The complex [Pd(H₂Fo4NEt)(Fo4NEt)Cl₂] exhibited a better anticancer activity, with the IC₅₀ values against MCF-7 and T-24 cancer cell lines being 8.42 and 5.88 μM, respectively, when compared with the complexes [Pd(Fo4Nethyl)Cl] (IC₅₀ = 104.1 and 72.5 μM) and [Pd(Fo4Nethyl)₂] (IC₅₀ = 14.52 and 7.59 μM) [11]. Motswainyana and co-workers examined the anticancer property of the synthesized palladium(II) and platinum(II) complexes: dichloro[(2-diphenylphosphino-benzylidene)-2-methylphenyl-amine]palladium(II), dichloro[2-diphenylphosphino-benzylidene)-2,6-dimethylphenyl-amine]palladium(II), dichloro-[(2-diphenylphosphino-benzylidene)-2-methylphenyl-amine]platinum(II) and dichloro-[2-diphenylphosphino-benzylidene)-2,6-dimethylphenyl-amine]platinum(II). Both palladium(II) complexes possessed a stronger cytotoxicity towards MCF-7 and HT-29 cancer cell lines (mean IC₅₀ = 28.5 - 48 μM) when compared with the platinum(II) complexes (mean IC₅₀ = 50 - 87 μM) [12]. This team further reported that the synthesized bis(imino-quinolyl)platinum(II) complex displayed a slightly stronger anticancer activity (mean IC₅₀ = 55 and 41 μM against MCF-7 and HT-29 cancer cells, respectively) when compared with the bis(imino-quinolyl)palladium(II) complex (mean IC₅₀ = 60 and 46 μM) [13]. Oliveira et al. investigated the anticancer activity of complexes containing palladium(II) and platinum(II): [Pd(NH₃)₄][Pd(opba)] and [Pt(H₂opba)]·H₂O,

where opba = 1,2-phenylenebis(oxamate) against the chronic myelogenous leukemia cell line. The palladium(II) complex $[\text{Pd}(\text{NH}_3)_4][\text{Pd}(\text{opba})]$ showed a better growth inhibition against leukemia cells (mean $\text{IC}_{50} = 19.9 \mu\text{M}$) than the platinum(II) complex $[\text{Pt}(\text{H}_2\text{opba})] \cdot \text{H}_2\text{O}$ (mean $\text{IC}_{50} = 27.35 \mu\text{M}$) [14].

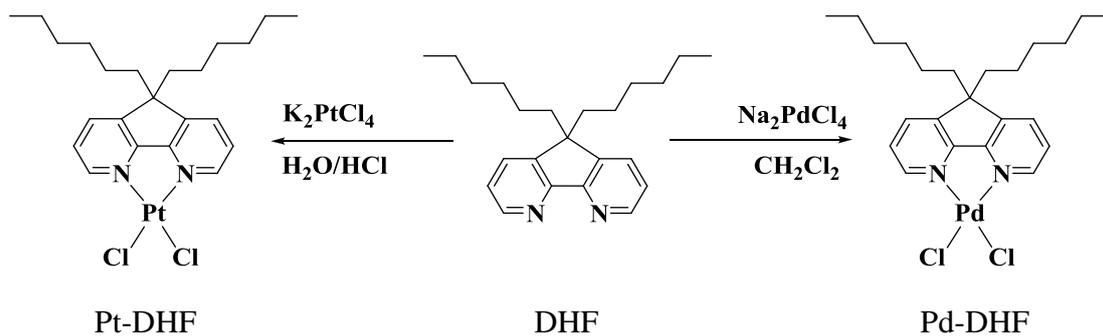
Barbara et al. reported the *in vitro* and *in vivo* biological properties of two bile acid-conjugated platinum(II) complexes: $(\text{NH}_3)_2\text{Pt}(\text{triacid})$ and $(\text{PPh}_3)_2\text{Pt}(\text{dehydrocholate})_2$. $(\text{NH}_3)_2\text{Pt}(\text{triacid})$ showed a stronger growth inhibition on rat hepatoma cells (mean $\text{IC}_{50} = 0.7 \mu\text{M}$) after 48 hours when compared with $(\text{PPh}_3)_2\text{Pt}(\text{dehydrocholate})_2$ (mean $\text{IC}_{50} = 3.8 \mu\text{M}$). In a syngeneic and orthotopic rat hepatoma model, the $(\text{NH}_3)_2\text{Pt}(\text{triacid})$ -treated group displayed more than 6-fold tumour weight reduction at the dose of 80 mg/kg as compared to the control group and the $(\text{PPh}_3)_2\text{Pt}(\text{dehydrocholate})_2$ -treated group [15].

It appears that most of these studies have focused on *in vitro* anticancer analysis while *in vivo* antitumour studies including tumour size reduction evaluation have been infrequently reported. In our present study, two platinum(II) and palladium(II) complexes with 9,9-dihexyl-4,5-diazafluorene (DHF) derivatives were synthesized and their *in vivo* antitumour activities were investigated using an athymic nude mice model xenografted with human Hep3B carcinoma cells.

2. Results and discussion

2.1. Chemistry

The chemical structures and the synthetic protocols of the novel diazafluorene-based organic compounds are shown in Scheme 1. 9,9-Dihexyl-4,5-diazafluorene (DHF) was prepared using the method reported in the previous paper [16]. Platinum(II)-9,9-dihexyl-4,5-diazafluorene (Pt-DHF) complex was obtained from DHF by treatment with K_2PtCl_4 in water solution in the presence of a catalytic amount of conc. HCl in a high yield of 80% [17], while palladium-9,9-dihexyl-4,5-diazafluorene (Pd-DHF) congener was successfully synthesized using Na_2PdCl_4 in CH_2Cl_2 solution in 86% yield [18]. These air-stable compounds were isolated in high purity as golden or brown solids by column chromatography on silica gel eluting with CH_2Cl_2 . These compounds were fully characterized by NMR (both 1H and ^{13}C) spectroscopy (see Supporting Information) and fast atom bombardment mass spectrometry (FAB-MS). Both of them can afford good single crystals for X-ray diffraction analysis from their solid samples (Fig. 1 and 2). Based on our prior results with 4,5-diazafluoren-9-one as the coordinated ligands which showed very poor solubility in common solvents [18], we have purposely substituted the oxo group with long alkyl chains at the 9-position of fluorene in the present study to tune the solubility and hydrophobicity of the metal complexes. The ligand DHF has been shown to possess a certain level of antitumour activity in athymic nude mice xenografted with human Hep3B carcinoma cells [16]. Since metal ions are promising candidates that have been successfully used for anticancer therapy [19], the potential antitumour potency was further investigated after the incorporation of metal ions (Pt and Pd) into the DHF.



Scheme 1. Synthetic profiles of Pt-DHF and Pd-DHF.

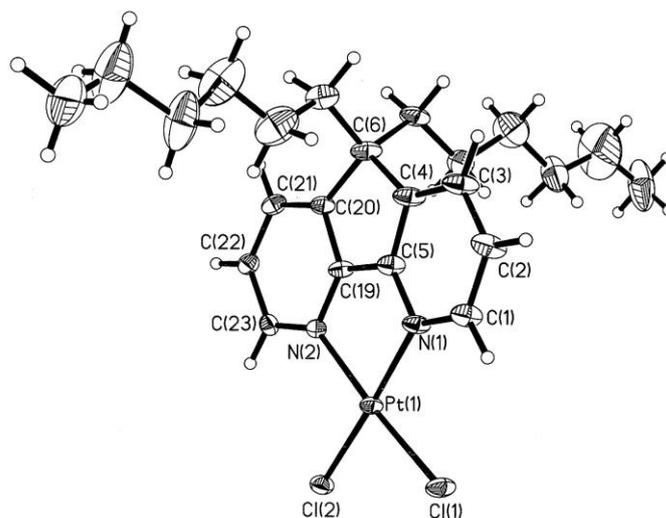


Figure 1. Solid-state structure of Pt-DHF. Selected bond distances (\AA) and angles ($^\circ$): Pt(1)-Cl(1) 2.271(1); Pt(1)-Cl(2) 2.281(1); Pt(1)-N(1) 2.047(4); Pt(1)-N(2) 2.073(3); N(1)-Pt(1)-N(2) 83.8(1); Cl(1)-Pt(1)-Cl(2) 92.89(4).

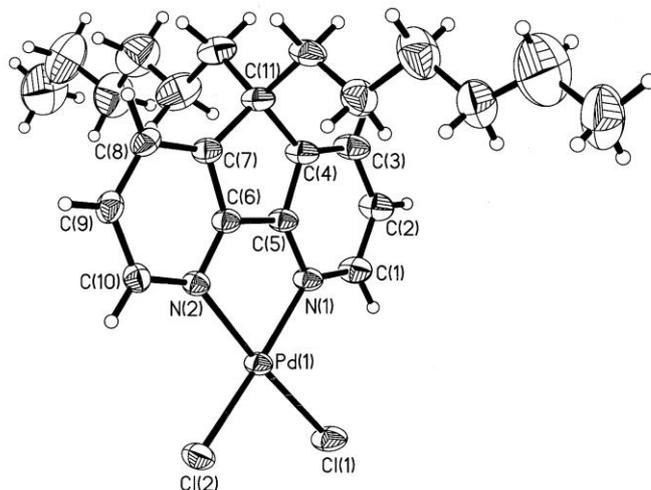


Figure 2. Solid-state structure of Pd-DHF Selected bond distances (\AA) and angles ($^\circ$): Pd(1)-Cl(1) 2.2611(7); Pd(1)-Cl(2) 2.2707(7); Pd(1)-N(1) 2.069(2); Pd(1)-N(2) 2.093(2); N(1)-Pd(1)-N(2) 84.31(8); Cl(1)-Pd(1)-Cl(2) 93.18(3).

Table 1 Crystallographic Data.

Compound	Pt-DHF	Pd-DHF
CCDC	1452122	1452123
Formula	$\text{C}_{23}\text{H}_{32}\text{Cl}_2\text{N}_2\text{Pt}$	$\text{C}_{23}\text{H}_{32}\text{Cl}_2\text{N}_2\text{Pd}$
Formula weight	602.5	513.81
Crystal system	Hexagonal	Hexagonal
space group	P3_22_1	P3_22_1
$a / \text{\AA}$	12.3554(3)	12.452(1)
$b / \text{\AA}$	12.3554(3)	12.452(1)
$c / \text{\AA}$	26.5988(1)	26.767(4)

$\alpha /$	90	90
$\beta /$	90	90
$\gamma /$	120	120
$V / \text{\AA}^3$	3516.5(2)	3594.2(7)
Z	6	6
Temperature/K	173(2)	293(2)
μ (Mo K_α)/mm ⁻¹	6.224	1.008
$D(\text{calcd})/\text{g}$ cm ⁻³	1.707	1.424
Collected refl.	21334	21967
Unique refl.	5756	5916
R_{int}	0.036	0.0203
$R_1^a [I > 2\sigma(I)]$	0.0262	0.0241
$wR_2^b [I >$ $2\sigma(I)]$	0.0526	0.0611
R_1 [all data]	0.0321	0.0281
wR_2 [all data]	0.0543	0.0637
GOF	1.07	1.039
$\Delta\rho / \text{e \AA}^{-3}$	0.725, -0.484	0.278, -0.291

^a $R_1 = \Sigma(|F_o| - |F_c|) / \Sigma|F_o|$, ^b $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2] / \Sigma[w(F_o^2)^2]\}^{1/2}$.

2.2. Preliminary animal experiments

Athymic nude mice xenografted with human Hep3B carcinoma cells were used as a model for this study. Mice with Hep3B cancer cells xenografted of

average tumour volume from 200 mm³ received intraperitoneal injection daily of either of buffer vehicle (saline for injection) or equal volume of Pt-DHF and Pd-DHF at 10 mg kg⁻¹ for 9 consecutive days, respectively. The treatment stopping time (day 10) for tumour appearances of mice are shown in Fig. 3. As shown in Figs. 3 and 4, both Pt-DHF- and Pd-DHF-treated groups showed significant tumour growth inhibition (with about 9-fold and 3-fold tumour growth retardation, respectively) when compared with the vehicle control group at the end of the study. Both Pt-DHF and Pd-DHF revealed a stronger tumour growth inhibition than the ligand DHF itself (which was only about 2-fold tumour growth retardation [16] at the same dose of 10 mg kg⁻¹ body weight). These results suggest the importance for the incorporation of Pt and Pd into the DHF as antitumour agents. Noticeably, extensively necrotic feature could be detected from the Pt-DHF-treated mice (Fig. 5B). For both the vehicle control group (Fig. 5A) and the Pd-DHF-treated group (Fig. 5C), tumour section displayed high cellular integrity.

In order to demonstrate whether the Pt-DHF and Pd-DHF at 10 mg kg⁻¹ body weight has any possible toxicology effects on the animals under the current therapeutic protocol, hematoxylin and eosin (H and E) staining of liver sections (Fig. 5A-C) and plasma liver functional enzyme analysis including ALT and AST (Fig. 6) were investigated. Neither necrotic nor damaged tissue could be observed from the histochemistry study of liver sections from sacrificed mice in all treated groups (Fig. 5D-F). Fig. 6 shows that both Pt-DHF and Pd-DHF-treated groups had a lower ALT and AST values than those of the vehicle treated group since the animals from the vehicle control group had very heavy hepatoma burden. Conclusively, no observable hepatic adverse effect

could be identified when Pt-DHF or Pd-DHF at a concentration of 10 mg kg^{-1} body weight per day was administrated intraperitoneally in athymic nude mice.

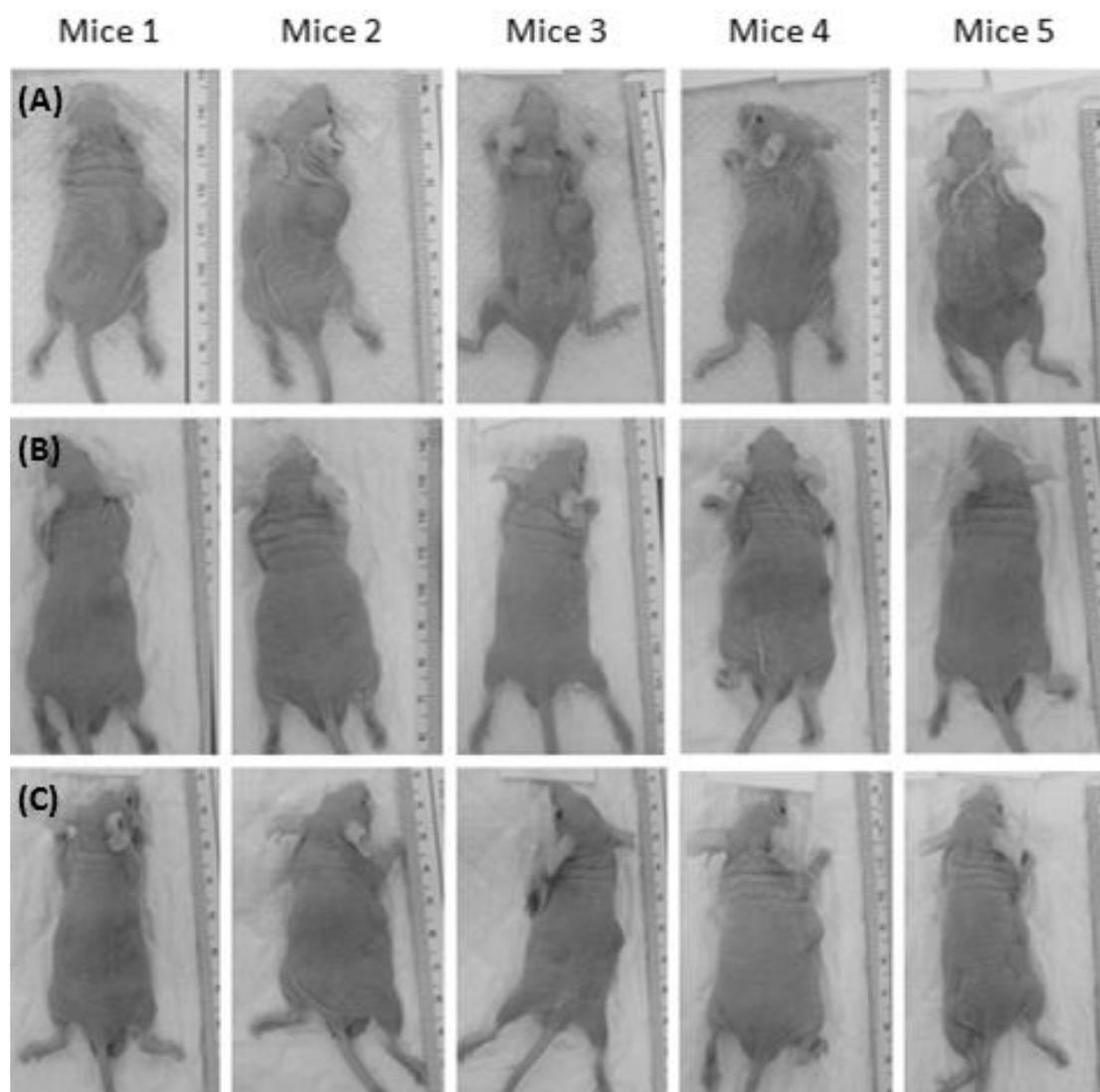


Figure 3. Hep3B xenografted nude mice treated with (A) vehicle, (B) Pt-DHF and (C) Pd-DHF at 10 mg kg^{-1} body weight. Tumour appearances are shown on the treatment stopping time (day 10).

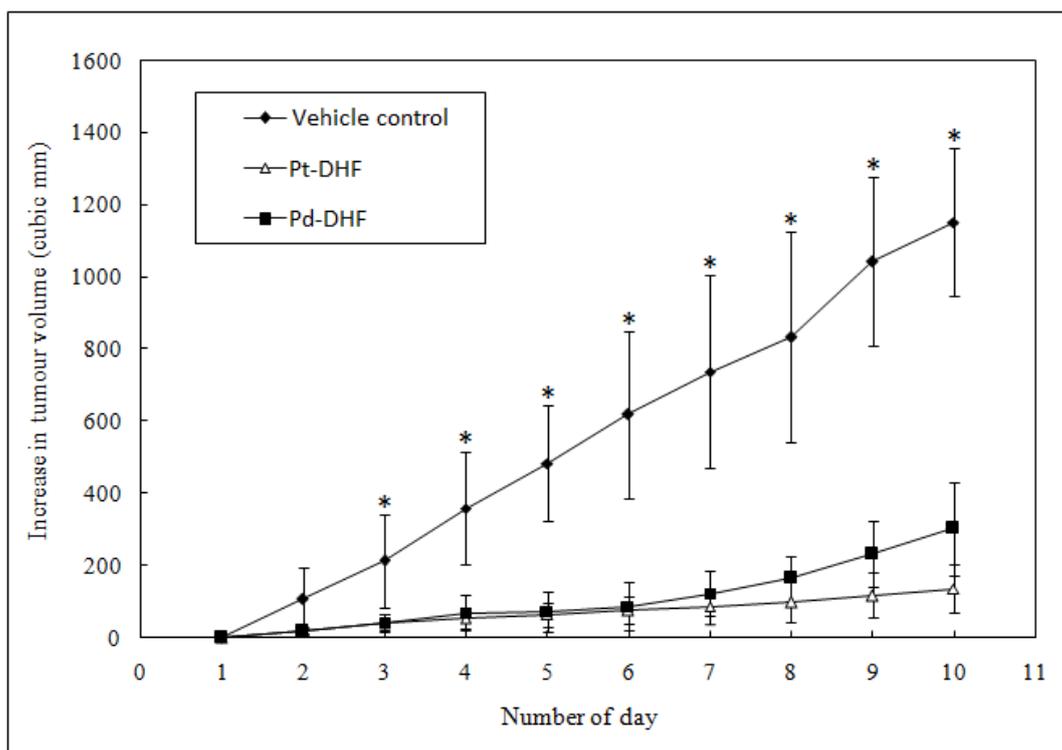


Figure 4. Representative results showing the changes of tumour volume (mm³) as a function of time for mice treated with vehicle, Pt-DHF and Pd-DHF. Intraperitoneal injection started for both groups when the mean tumour volume of mice reached ~200 mm³ on day 0. A total of 15 mice were randomly divided into three groups. On day 10, after measuring individual tumour volume, all mice were sacrificed for plasma collection. Results represent the mean±SD from 5 animals (each group); *p<0.05 relative to untreated controls.

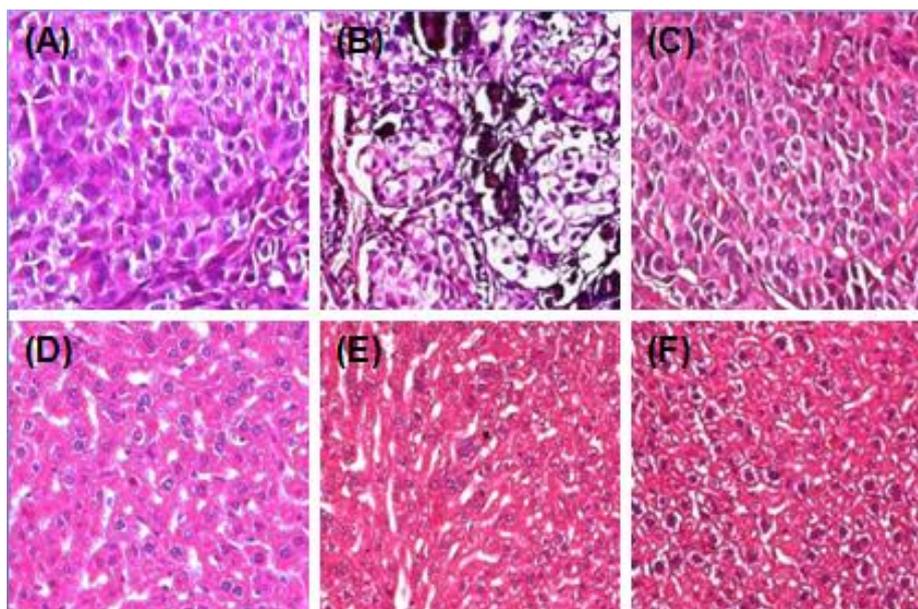


Figure 5. Representative histochemistry study of Hep3B tumour sections from sacrificed mice treated with (A) vehicle, (B) Pt-DHF and (C) Pd-DHF at 10 mg kg⁻¹ body weight to detect any possible xenograft tumour necrosis; and liver sections from sacrificed mice treated with (D) vehicle, (E) Pt-DHF and (F) Pd-DHF at 10 mg kg⁻¹ body weight to observe any possible liver toxicity.

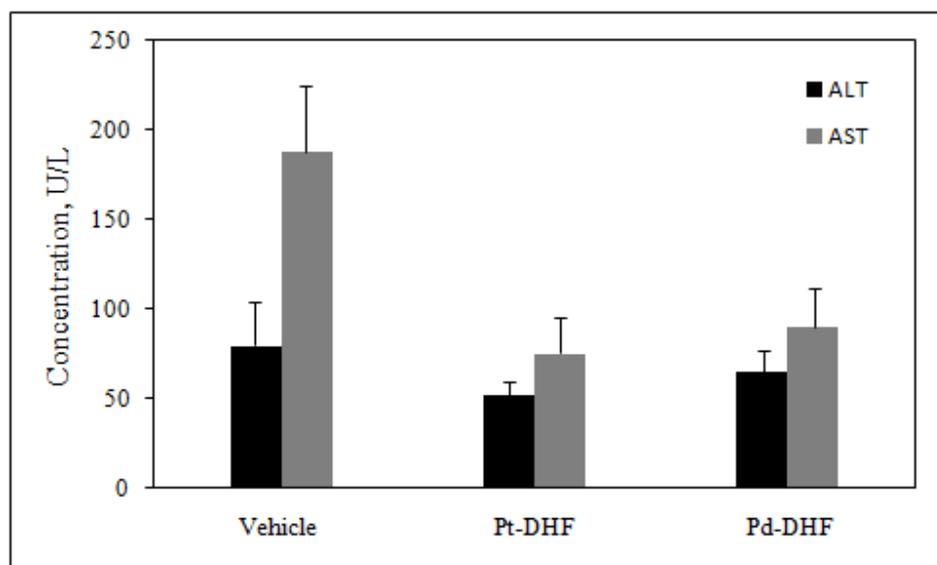


Figure 6. Plasma liver enzyme assays for vehicle control, Pt-DHF- and Pd-DHF-treated Hep3B xenografted athymic nude mice. N = 5 for all vehicle

control group, Pt-DHF- and Pd-DHF-treated groups (10 mg kg⁻¹ body weight). Enzymatic levels were determined by the IDEXX laboratories machine using its veterinary biochemistry assay kits and expressed as units per litre. Results represent the mean±SD.

3. Conclusions

We have demonstrated that the Pt-DHF and Pd-DHF complexes showed a much stronger *in vivo* antitumour activity than the DHF compound. The complexes-treated groups had a lower ALT and AST values than those of the vehicle treated group, suggesting that they exerted low liver toxicity under the therapeutic dosage (10 mg kg⁻¹). Our work here implies the importance of metal containing drugs for further antitumour regimen development. We assume that it is worthwhile to further study their underlying working mechanism and compare with those conventional metal containing antitumour regimens.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

All of the manipulations were performed under a dry nitrogen atmosphere by using Schlenk techniques. Solvents were dried by standard methods and distilled prior to use. All reagents and chemicals, unless otherwise stated, were purchased from commercial sources and used without further purification. Separation and purification of products were achieved by column chromatography on silica gel. The ligand precursor DHF was prepared according to the literature method [16].

4.1.2. Physical measurements

NMR spectra were measured in deuterated solvents as the lock and reference on a Bruker AV 400 instrument with ^1H and ^{13}C NMR chemical shifts quoted relative to tetramethylsilane standard. The positive-ion fast atom bombardment (FAB) mass spectra were obtained using Finnigan-MAT SSQ710 mass spectrometer. Crystals of our compounds suitable for X-ray diffraction studies were grown by slow evaporation of its solution in dichloromethane/hexane at room temperature and were mounted on a glass fibre. Geometric and intensity data were collected by using graphite-monochromated $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) on a Bruker Axs SMART 1000 CCD diffractometer. The collected frames were processed with the software SAINT [20] and an absorption correction (SADABS) [21] was applied to the collected reflections. The structures of all compounds were solved by the Direct methods (SHELXTLTM) [22] in conjunction with standard

difference Fourier techniques and subsequently refined by full matrix least-squares analyses on F^2 . All non-hydrogen atoms were assigned with anisotropic displacement parameters. Hydrogen atoms were generated in their idealized positions and allowed to ride on their respective parent carbon atoms.

4.1.3. Synthesis of Pt-DHF

A suspension of $K_2[PtCl_4]$ (27.2 mg, 0.066 mmol) in degassed water (20 mL), DHF (21.0 mg, 0.062 mmol), and 1 drop HCl was heated to boiling. Yellow precipitation was obtained, which was filtered off and washed with water. The yellow powder of pure Pt-DHF was obtained by column chromatography on silica gel eluting with CH_2Cl_2 (30.0 mg, 0.049 mmol, 80%). 1H NMR (400 MHz, $CDCl_3$): δ = 8.87 (m, 2H, Ar), 8.03 (m, 2H, Ar), 7.61-7.57 (m, 2H, Ar), 2.08-2.04 (m, 4H, C_6H_{13}), 1.18-1.12 (m, 12H, C_6H_{13}), 0.87-0.80 (m, 10H, C_6H_{13}); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.55, 146.06, 143.18, 134.44, 125.82 (Ar), 62.52 (quaternary C), 37.41, 31.20, 29.32, 24.82, 22.43, 13.92 (C_6H_{13}); FAB-MS: m/z 567.3 $[M-Cl]^+$; Anal. calcd for $C_{23}H_{32}Cl_2N_2Pt$ (%): C, 45.85; H, 5.35; N, 4.65. Found: C, 45.65; H, 5.40; N, 4.33.

4.1.4. Synthesis of Pd-DHF

A mixture of $PdCl_2$ (25.0 mg, 0.14 mmol) and NaCl (16.5 mg, 0.28 mmol) was stirred in MeOH (8 mL). The reaction mixture was warmed on a water bath at 40–50°C until all the brown solid was dissolved to give a clear solution. The solution was evaporated to a volume of 1 mL and was then added to CH_2Cl_2 (3 mL). To this resulting solution mixture consisting of $Na_2[PdCl_4]$, DHF (47.4 mg, 0.14 mmol) in CH_2Cl_2 (3 mL) was added dropwise to give a brown

suspension, which was then centrifuged to remove the liquid phase. The crude product was then purified by column chromatography on silica gel eluting with CH_2Cl_2 to give pure Pd-DHF (62.0 mg, 0.12 mmol, 86%) as a brown solid. ^1H NMR (400 MHz, CDCl_3): δ = 8.59 (m, 2H, Ar), 8.02 (m, 2H, Ar), 7.59 (m, 2H, Ar), 2.10-2.05 (m, 4H, C_6H_{13}), 1.19-1.09 (m, 12H, C_6H_{13}), 0.89-0.79 (m, 10H, C_6H_{13}); ^{13}C NMR (100 MHz, CDCl_3): δ = 162.18, 147.15, 143.41, 135.01, 125.75 (Ar), 61.75 (quaternary C), 37.43, 31.32, 29.32, 24.73, 22.44, 13.95 (C_6H_{13}); FAB-MS: m/z 479.1 $[\text{M}-\text{Cl}]^+$; Anal. calcd for $\text{C}_{23}\text{H}_{32}\text{Cl}_2\text{N}_2\text{Pd}$ (%): C, 53.76; H, 6.28; N, 5.45. Found: C, 53.66; H, 5.99; N, 5.32.

4.2. Preliminary animal experiments

4.2.1. Cell culture

Human hepatoma Hep3B cells were removed from the sterile cell culture flasks with trypsin and neutralized with fetal bovine serum. After washing with phosphate buffered saline and centrifugation, cells were re-suspended in complete cell culture medium [16,23-25].

4.2.2. Preliminary *in vivo* athymic nude mice experiment

Eight weeks old athymic nude mice, weighing approximately 15–20 g, were purchased from the animal unit of The Chinese University of Hong Kong and maintained in a sterile facility, in accordance with the guidelines from the institute and Department of Health, Hong Kong SAR, on animal care, with the required consistent temperature and relative humidity. Mice were housed under normal regular ambient light condition (a 12 h light and 12 h dark cycle) for the whole experimental period. All the procedures were approved by the Animal

Research Ethics Committee. Fifteen athymic nude mice were injected subcutaneously with the Hep3B cells. They were housed under a sterile condition. Tumour size was measured by the electronic calliper daily. When tumour size reached about 200 mm³ whereas the tumour volume was calculated by the formula (length×width×width)/2, they were randomly divided into three groups. Pt-DHF and Pd-DHF at 10 mg kg⁻¹ body weight per day were administered intraperitoneally for a continuous period of 9 days starting from day 1. Each group consisted of five mice. Body weight of each animal was also recorded. On day 10, mice were sacrificed and H and E staining of autopsy analysis including xenografted Hep3B tumour from all of the animals were investigated for any possible significant necrotic effects. Whole blood was also collected and plasma liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by the Vet biochemistry assay kit for the IDEXX laboratories machine to determine the hepatoma burden from the mice [16,25].

Acknowledgements

This work is supported by the Innovation Technology Commission to ABCT, HKPU, and the account codes: FRG1/14-15/021, FRG1/14-15/078 and 38-40-116 (C. H. Chui) as well as 03-16-176 (X. Z. Bian). R. Gambari is supported by AIRC (IG-13575). We thank the National Natural Science Foundation of China (project number: 51373145), Hong Kong Research Grants Council (HKBU12304715), Areas of Excellence Scheme of HKSAR (AoE/P-03/08), the Science, Technology and Innovation Committee of Shenzhen Municipality (JCYJ20140419130507116 and JCYJ20140818163041143) and Hong Kong Baptist University (FRG1/14-15/084) for financial support. The work was also supported by Partner State Key Laboratory of Environmental and Biological Analysis (SKLP-14-15-P011) and Strategic Development Fund of HKBU. Q. Wang and C.-L. Ho thank the International Science and Technology Cooperation Program of Chengdu (2014-GH02-00014-HZ) for financial support. Y. H. Lo gratefully acknowledges the financial support from the National Science Council of Taiwan (NSC 102-2113-M-845-001) and the project of the specific research fields in the University of Taipei, Taiwan.

References

- [1] B. Rosenberg, L. VanCamp, T. Krigas, Inhibition of cell division in escherichia coli by electrolysis products from a platinum electrode, *Nature* 205 (1965) 698–699.
- [2] B. Rosenberg, L. VanCamp, J.E. Trosko, V.H. Mansour, Platinum compounds: a new class of potent antitumour agents, *Nature* 222 (1969) 385–386.
- [3] C.A. Rabik, M.E. Dolan, Molecular mechanisms of resistance and toxicity associated with platinating agents, *Cancer Treat. Rev.* 33 (2007) 9–23.
- [4] Ž.D. Bugarčić, J. Bogojeski, B. Petrović, S. Hochreuther, R. van Eldik, Mechanistic studies on the reactions of platinum(II) complexes with nitrogen- and sulfur-donor biomolecules, *Dalton Trans.* 41 (2012) 12329–12345.
- [5] J. Reedijk, Why does cisplatin reach guanine-N7 with competing S-donor ligands available in the cell? *Chem. Rev.* 99 (1999) 2499–2510.
- [6] M.A. Fuertes, C. Alonso, J.M. Perez, Biochemical modulation of cisplatin mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance, *Chem. Rev.* 103 (2003) 645–662.
- [7] A. R. Kapdi, I. J. S. Fairlamb, Anti-cancer palladium complexes: a focus on PdX₂L₂, palladacycles and related complexes, *Chem. Soc. Rev.* 43 (2014) 4751–4777.
- [8] M.R. Maurya, B. Uprety, F. Avecilla, S. Tariq, A. Azam, Palladium(II) complexes of OS donor N-(di(butyl/phenyl)carbamothioyl)benzamide and their antiamebic activity, *Eur. J. Med. Chem.* 98 (2015) 54–60.
- [9] N. Bandyopadhyay, M. Zhu, L. Lu, D. Mitra, M. Das, P. Das, A. Samanta, J.P. Naskar, Synthesis, structure, spectral characterization, electrochemistry and evaluation of antibacterial potentiality of a novel oxime-based palladium(II) compound, *Eur. J. Med. Chem.* 89 (2015) 59–66.

- [10] S. Utku, A.B. Ozçelik, F. Gümüş, S. Yılmaz, T. Arsoy, L. Açık, A. Celebi Keskin, Synthesis, in-vitro cytotoxic activity and DNA interactions of new dicarboxylatoplatinum(II) complexes with 2-hydroxymethylbenzimidazole as carrier ligands, *J. Pharm. Pharmacol.* 66 (2014) 1593–1605.
- [11] D. Kovala-Demertzi, A. Galani, J.R. Miller, C.S. Frampton, M.A. Demertzis, Synthesis, structure, spectroscopic studies and cytotoxic effect of novel palladium(II) complexes with 2-formylpyridine-4-Nethyl-thiosemicarbazone: Potential antitumour agents, *Polyhedron* 52 (2013) 1096–1102.
- [12] W.M. Motswainyana, M.O. Onani, A.M. Madiehe, M. Saibu, N. Thovhogi, R.A. Lalancette, Imino-phosphine palladium(II) and platinum(II) complexes: Synthesis, molecular structures and evaluation as antitumor agents, *J. Inorg. Biochem.* 129 (2013) 112–118.
- [13] W.M. Motswainyana, M.O. Onani, A.M. Madiehe, M. Saibu, Antitumor activity of phenylene bridged binuclear bis(imino-quinolyl)palladium(II) and platinum(II) complexes, *Bioorg. Med. Chem. Lett.* 24 (2014) 1692–1694.
- [14] W.X.C. Oliveira, M.M. da Costa, A.P.S. Fontes, C.B. Pinheiro, F.C.S. de Paula, E.H.L. Jaimes, E.F. Pedroso, P.P. de Souza, E.C. Pereira-Maia, C.L.M. Pereira, Palladium(II) and platinum(II) oxamate complexes as potential anticancer agents: Structural characterization and cytotoxic activity, *Polyhedron* 76 (2014) 16–21.
- [15] C. Barbara, P. Orlandi, G. Bocci, A. Fioravanti, A. Di Paolo, G. Natale, M. Del Tacca, R. Danesi, In vitro and in vivo antitumour effects of novel, orally active bile acid-conjugated platinum complexes on rat hepatoma, *Eur. J. Pharmacol.* 549 (2006) 27–34.

- [16] Q. Wang, C.W.M. Yuen, C.L. Ho, K.H. Lam, R. Gambari, R.S.M. Wong, G.Y.M. Cheng, S.W. Tong, K.W. Chan, F.Y. Lau, F. Cheung, C.H. Chui, W.Y. Wong, Synthesis of 9,9-dialkyl-4,5-diazafluorene derivatives and their structure–activity relationships toward human carcinoma cell lines, *ChemMedChem* 5 (2010) 559–566.
- [17] J.A. Weinstein, M.T. Tierney, E.S. Davies, K. Base, A.A. Robeiro, M.W. Grinstaff, Probing the Electronic Structure of Platinum(II) Chromophores: Crystal Structures, NMR Structures, and Photophysical Properties of Six New Bis- and Di- Phenolate/Thiolate Pt(II)Diimine Chromophores, *Inorg. Chem.* 45 (2006) 4544–4555.
- [18] G.L. Lu, C.L. Ho, Q. Wang, W.Y. Wong, C.H. Chui, R.S.M. Wong, R. Gambari, F.Y. Lau, M.C.W. Yuen, C.S.W. Tong, A.K.W. Chan, J.C.O. Tang, K.P. Ho, G.Y.M. Cheng, Synthesis and characterization of some metal complexes of 4,5-diazafluoren-9-one and their biological effects on human carcinoma cells, *Aust. J. Chem.* 61 (2008) 975–980.
- [19] U. Jungwirth, C.R. Kowol, B.K. Keppler, C.G. Hartinger, W. Berger, P. Heffeter, Anticancer activity of metal complexes: involvement of redox processes, *Antioxid. Redox Signal.* 15 (2011) 1085–1127.
- [20] SAINT, Reference Manual, Siemens Energy and Automation, Madison, WI, 1994–1996.
- [21] G.M. Sheldrick, SADABS, Empirical Absorption Correction Program, University of Göttingen, 1997.
- [22] G.M. Sheldrick, SHELXTLTM, Reference Manual, version 5.1, Madison, WI, 1997.

- [23] P.L. Lam, G.L. Lu, K.H. Choi, Z. Lin, S.H.L. Kok, K.K.H. Lee, K.H. Lam, H. Li, R. Gambari, Z.X. Bian, W.Y. Wong, C.H. Chui, Antimicrobial and toxicological evaluations of the binuclear mercury(II) bis(alkynyl) complexes containing oligothiophenes and bithiazoles, *RSC Adv.* 6 (2016) 16736–16744.
- [24] P.L. Lam, G.L. Lu, K.M. Hon, K.W. Lee, C.L. Ho, X. Wang, J.C.O. Tang, K.H. Lam, R.S.M. Wong, S.H.L. Kok, Z.X. Bian, H. Li, K.K.H. Lee, R. Gambari, C.H. Chui, W.Y. Wong, Development of ruthenium(ii) complexes as topical antibiotics against methicillin resistant *Staphylococcus aureus*, *Dalton Trans.* 43 (2014) 3949–3957.
- [25] K.H. Lam, K.K.H. Lee, R. Gambari, S.H.L. Kok, T.W. Kok, A.S.C. Chan, Z.X. Bian, W.Y. Wong, R.S.M. Wong, F.Y. Lau, S.W. Tong, K.W. Chan, C.H. Cheng, C.H. Chui, J.C.O. Tang, Anti-tumour and pharmacokinetics study of 2-Formyl-8-hydroxy-quinolinium chloride as *Galipea longiflora* alkaloid analogue, *Phytomedicine* 21 (2014) 877–882.