

# Facile Synthesis of a Pt(IV) Prodrug of Cisplatin and Its Intrinsically $^{195m}\text{Pt}$ Labeled Analog: A Step Closer to Cancer Theranostic

## Abstract

**Background, Aims and Objectives:** Cisplatin is extensively used in chemotherapy for treatment of a broad range of cancers. But its undesired side reactions with biomolecules that lead to severe side effects especially on kidney and nervous system, are limiting its clinical utility. To reduce its side effects, the kinetically inert Pt(IV) prodrug was recognized as an alternative approach from satisfactory results of preliminary experiments. But, its approval as anticancer drug for clinical use requires detailed investigations of its anticancer action and pharmacological pathways by employing its analogue which can be traced by a suitable technique. As a step closer towards translation of Pt(IV)-based prodrug from research to clinical level, a protocol for efficient synthesis of  $^{195m}\text{Pt}$ -radiolabeled Pt(IV) prodrug was devised. **Materials and Methods:** In order to achieve the aim, we started synthesis from elemental platinum avoiding lengthy steps. The synthesis protocol was standardized on its cold analogue, as  $[\text{PtCl}_2(\text{NH}_3)_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2]$  which has been characterized with nuclear magnetic resonance ( $^1\text{H}$ ,  $^{13}\text{C}\{^1\text{H}\}$  and  $^{195}\text{Pt}\{^1\text{H}\}$ ) spectroscopy, microanalyses and cyclic voltammetry. Also, cytotoxicity of  $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$  was evaluated against MCF-7 human breast cancer cell lines using cisplatin as test control. **Results:** Intrinsically,  $^{195m}\text{Pt}$ -labeled analogue of prodrug was obtained with high radionuclidic and radiochemical purity. It was confirmed by chromatography and  $\gamma$ -ray spectrometry. **Conclusion:** The  $^{195m}\text{Pt}$ -radiolabeled prodrug was synthesized in a facile manner. It can be utilized in evaluating the mechanism of anticancer action and pharmacokinetics by enabling synergistic use of molecular imaging and targeted drug delivery.

**Keywords:**  $^{195m}\text{Pt}$ , 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay, cisplatin, cytotoxicity, image guided delivery, prodrug

## Introduction

Among the metal based anticancer drugs, platinum based anticancer drugs especially cisplatin, are globally used in chemotherapy for clinical treatment of various types of cancers.<sup>[1]</sup> But due to their severe toxic side effects,<sup>[2]</sup> their therapeutic index is limited. Hence research was focused on understanding the chemical basis of cisplatin to avoid toxicity and its consequent side effects on normal tissues with incorporation of novel designing and developments of its analogue in a rational manner. Eventually, a promising approach of converting the platinum based anticancer compounds to their kinetically inert Pt(IV) prodrug form was identified. In course of research, it has been established that the Pt(IV) prodrugs get reduced to their cytotoxic Pt (II) forms with the loss of two axial ligands through intracellular 2

electrons ( $2e^-$ ) reduction process by cellular reductants.<sup>[3]</sup> Subsequently, these insights led to the development and pharmacological studies of many Pt(IV)-based prodrugs.<sup>[4]</sup> The structural features of Pt(IV)-based prodrugs of having two axial ligands enable them to link with appropriate molecules to enhance lipophilicity and better cellular accumulation.<sup>[5]</sup> Additionally, the axial ligands play a vital role by acting as sites for bonding to antigens, fluorescent moieties or drug delivery vehicles which can direct towards tumor tissue or another drug which acts synergistically (adjunct) during cancer treatment.<sup>[6]</sup> However, the aliphatic side chains with bulkier and more electron withdrawing functional groups are found to be more susceptible to get reduced<sup>[7]</sup> or hydrolysed<sup>[8]</sup> easily at cancer site owing to its acidic environment. Even though the Pt(IV) prodrug possess salient features for approval as an anticancer

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drug, the transfer of Pt(IV)-based prodrug from research modality to clinical level is hindered by the lack of proper understanding the mechanism of its anticancer actions and its pharmacology.<sup>[9]</sup> To achieve this, the synergistic use of molecular imaging and targeted drug delivery approaches provides unique opportunities in a relatively new area called “image-guided drug delivery” (IGDD), which holds tremendous potential towards clinical translation of novel cancer drugs.<sup>[10]</sup> When an image-guided approach is not used, there is neither any means to track or image the *in-vivo* fate nor the ability to measure the delivery efficiency of drugs. Also, the bioavailability, therapeutic efficacy, and dose response of drug treatment has to be estimated based on separate sets of procedures. In case of Pt(IV)-based prodrug, this has been attempted by conjugating fluorescent probes.<sup>[11]</sup> However, the development of alternative probes for more potent IGDD system are still warranted to overcome the properties like non-photostability or auto fluorescence associated with endogenous biomolecules during use of these fluorescence probes.<sup>[12]</sup>

One of the most promising options would be the synthesis of suitable radiolabeled analogues of Pt(IV) prodrugs. This could be achieved by incorporation of the radionuclide  $^{195m}\text{Pt}$  during the synthesis of Pt(IV)-based prodrug of cisplatin.<sup>[13]</sup> The radionuclide  $^{195m}\text{Pt}$  decays to stable  $^{195}\text{Pt}$  by isomeric transition with a half-life of 4.02 day and emits gamma photons of 66.8 keV (39%), 65.1 keV (22.5%), 75.7 keV (16.8%) and 98.9 keV (11.4%),<sup>[14]</sup> which would allow the visualization of the  $^{195m}\text{Pt(IV)}$ -prodrug after it is administered into the patient. This can help to achieve multiple goals, real-time assessment of efficiency of targeting of the drug in the diseased site, its accumulation in healthy organ/tissue, modes of excretion and monitoring the progress of drug treatment.

For the efficient synthesis of  $^{195m}\text{Pt-Pt(IV)}$  prodrug, it is required to devise a protocol which avoids the lengthy steps and unreliable processes that are not suitable for handling radioactivity in order to synthesize within a time frame. The synthesis of Pt(IV) prodrug from cisplatin involves tricky and longer steps as compared to the synthesis of a parent cisplatin which can be easily synthesized within 10 h starting from platinum metal as evidenced from literature.<sup>[15]</sup> Usually after oxidation of Pt (II) of cisplatin to Pt(IV) of prodrug, functionalization of the axial hydroxyl groups with succinic anhydride is performed. This step converts the axial hydroxyl groups to the ester groups with the generation of two carboxyl groups. Through these introduced carboxyl groups, it can be conjugated to any biological molecules by forming amide bond.<sup>[16]</sup> Likewise, conjugation of the  $^{195m}\text{Pt}$ -radiolabeled Pt(IV) prodrug with any desired biomolecule helps on conducting their pharmacological studies.

In view of these facts, Pt(IV) based prodrug was prepared by the modified procedure after considering

the suitability for synthesizing the corresponding radioactive analogue,  $^{195m}\text{Pt}$ -radiolabeled Pt(IV) prodrug. The characterization of the synthesized cold Pt(IV) based prodrug was done by nuclear magnetic resonance (NMR) ( $^1\text{H}$ ,  $^{13}\text{C}$  { $^1\text{H}$ },  $^{195}\text{Pt}$  { $^1\text{H}$ }) spectroscopy and cyclic voltammetric studies. The *in-vitro* studies were performed on human breast carcinoma (MCF-7) cell lines by employing the synthesized normal Pt(IV) based prodrug. Following the same procedure,  $^{195m}\text{Pt}$ -radiolabeled Pt(IV) prodrug was synthesized and characterized by gamma ray spectrometry and paper chromatography (PC) techniques. In this paper, we are reporting a novel strategy for synthesis of a cold Pt(IV) prodrug of cisplatin and its  $^{195m}\text{Pt}$ -labeled analog.

## Experimental

### Materials and equipments

The following chemicals and solvents were used for performing this work: hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), succinic anhydride, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), ammonium hydroxide, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), hydrazine hydrochloride ( $\text{H}_2\text{N-NH}_2\cdot\text{H}_2\text{O}$ ), silver nitrate ( $\text{AgNO}_3$ ), potassium chloride (KCl), potassium iodide (KI) *etc.*, All these chemicals and solvents were purchased from commercial sources (Sigma-Aldrich). Isotopically enriched platinum metal wire (98.5% in  $^{194}\text{Pt}$ , >99.9% chemical purity) used for the production of platinum radiotracer was procured from Ms Isoflex, Russia. The solvents were purified and dried by following the standard procedure.<sup>[17]</sup>

The Pt(IV) compound was purified by recrystallization in ethanol-water mixture at room temperature. NMR spectra were recorded on the Agilent/Varian 600 MHz NMR spectrometer with a Unity Inova console operating at 599.88 ( $^1\text{H}$ ), 150.84 ( $^{13}\text{C}$  { $^1\text{H}$ }) and 128.95 ( $^{195}\text{Pt}$  { $^1\text{H}$ }) MHz. The  $^1\text{H}$  and  $^{13}\text{C}$  { $^1\text{H}$ } NMR chemical shifts were relative to internal DMSO peak. The  $^{195}\text{Pt}$  { $^1\text{H}$ } NMR chemical shifts were relative to external  $\text{Na}_2\text{PtCl}_6$  in  $\text{D}_2\text{O}$  ( $\delta$  0 ppm). The cyclic voltammetric studies were performed with potentiostatic control using Eco Chemie Potentiostat, AUTOLAB-100 with the VA663 stand. Electrochemical impedance measurements were carried out using the frequency response analyzer module attached with Autolab-100 potentiostat.

The cytotoxicity of Pt(IV) prodrug was evaluated against human breast adenocarcinoma cell line (MCF-7) by MTT assay. The cancer cell lines, MCF-7 were obtained from National Centre for Cell Sciences, Pune, India. Cells were cultured in Dulbecco's Modified Eagle Medium (GIBCO, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum (Himedia Laboratories, Mumbai, India).

## 2.2. Synthesis of Pt(IV) prodrug of cisplatin $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$ (1)

Initially cisplatin,  $[\text{PtCl}_2(\text{NH}_3)_2]$  was prepared by following the literature.<sup>[18]</sup> Starting from it, the Pt(IV) prodrug was synthesized as described below:

The cisplatin,  $[\text{PtCl}_2(\text{NH}_3)_2]$  (0.205 g, 0.6832 mmol) was dissolved in distilled water (10 ml) followed by treatment with aqueous solution of hydrogen peroxide (30%, 1 ml). The reaction mixture was warmed to  $60^\circ\text{C}$  for 2 h and cooled to room temperature. After concentrating the mixture by heating on a hot plate and cooling to room temperature, acetone was added to the reaction mixture yielding Pt(IV) prodrug (0.221 g, 97%) as the precipitate which was followed by decanting the supernatant and drying the compound *in vacuo*. Later, this Pt(IV) prodrug was dispersed in DMF (DMF, 2 ml) followed by addition of succinic anhydride (0.144 g, 1.439 mmol) and then the mixture was warmed at  $60^\circ\text{C}$  for 6 h. The reaction mixture was allowed to cool to room temperature, followed by addition of dried acetone (10 ml) which afforded a yellow precipitated. It was washed with acetone thrice and then dried *in vacuo* to yield a solid compound (0.31 g, 85%). It was characterized by NMR spectroscopy.  $^1\text{H}$  NMR in  $\text{DMSO-}D_6$   $\delta$ : 2.36 (t, 8 H), 2.49 (t, 9 H), 6.44 (br, s, 13 H);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR in  $\text{DMSO-}D_6$   $\delta$ : 30.2, 30.8, 174.2, 180.0 ppm;  $^{195}\text{Pt}$   $\{^1\text{H}\}$  NMR in  $\text{DMSO-}D_6$   $\delta$ : 1220 ppm.

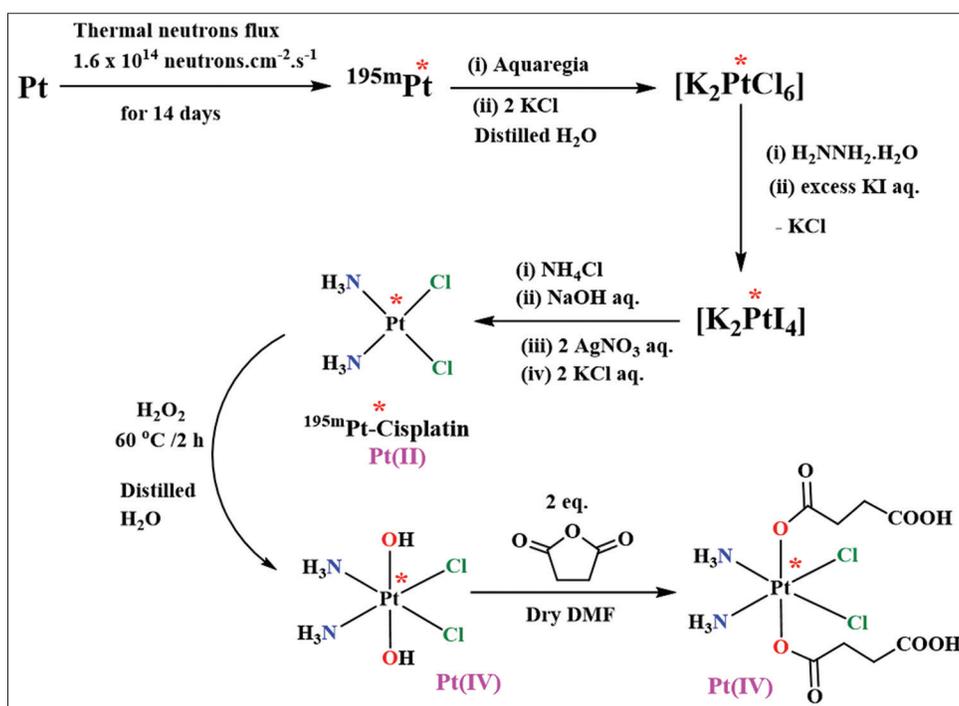
## Cytotoxicity study of $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$ against MCF-7 cell lines *in vitro* by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay<sup>[19]</sup>

The MTT assay was performed in cell culture media. The cytotoxicity of cisplatin, Pt(IV) prodrug of cisplatin,  $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$  was evaluated *in vitro* against MCF-7 human cancer cell line using MTT assay. The 10000 cells were plated in a 96 well plate and incubated overnight in humidified incubator with 5%  $\text{CO}_2$ . The stock solutions (10 mM) were prepared in a minimum amount of DMSO (0.1 ml) followed by dilution to desired volume using either phosphate-buffered saline or saline solutions (0.9 M). The cells were treated with various concentrations of Pt(IV) compound (5, 10, 20 and 40  $\mu\text{M}$ ) for 48 h. In each well, a solution (10  $\mu\text{l}$ ) of MTT (20 mM) was added and further incubated for 4 h. The formazan crystals formed during this process were dissolved in DMSO and absorbance was measured at 590 nm. The toxicity of Pt(IV) prodrug was expressed as percentage cytotoxicity which was calculated by following formula:

$$\text{Percentage cytotoxicity} = \frac{\text{Control}_{\text{abs}} - \text{Test}_{\text{abs}}}{\text{Test}_{\text{abs}}} \times 100$$

## Synthesis of intrinsically $^{195}\text{Pt}$ -labeled prodrug of cisplatin: $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$

The radio-labeled analogue of  $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$  was synthesized as depicted in Scheme 1 and it is described in the following steps:



Scheme 1: Synthesis of  $^{195m}\text{Pt(IV)}$  prodrug of cisplatin (\*Pt indicates  $^{195m}\text{Pt}$ )

### Preparation of $^{195m}\text{Pt}$ $\text{K}_2\text{PtCl}_4$

The isotopically enriched (98.5% in  $^{194}\text{Pt}$ ) Pt metal (10 mg) was encapsulated in quartz ampoule and irradiated with a thermal neutron flux of  $1.6 \times 10^{14}$  n/cm<sup>2</sup>/s for 14 days in the Dhruva reactor of Bhabha Atomic Research Centre. After completion of irradiation, the irradiated target was allowed to cool for ~ 6 h for the decay, if any, and subsequently removed from the quartz ampoule into a clean 100 mL glass beaker. The obtained Pt target was dissolved in 10 mL of boiling aqua regia and the resulting solution was evaporated to near dryness. Nitric acid and nitrates were removed by repeated (3 times) dissolution of the residue in concentrated HCl (5 mL) and evaporation of the resulting solution. Finally, the residue was dissolved in 5 mL of double distilled water to obtain a clear colorless solution. To this solution, potassium carbonate (0.33 mmol/mL) was added dropwise until the solution of pH 6.5 was obtained which was measured using universal pH scale. The resulted solution of  $^{195m}\text{Pt}$ -labeled potassium hexachloroplatinate(IV) ( $^{195m}\text{Pt}$   $\text{K}_2\text{PtCl}_6$ ) was cooled to about  $-5^\circ\text{C}$ – $0^\circ\text{C}$  followed by dropwise addition of a stoichiometric quantity of hydrazine hydrochloride (95%) (0.1 mmol/mL) over a period of 10 min. Then the reaction mixture was allowed to attain a room temperature within 5–10 min. The obtained acidic solution of  $^{195m}\text{Pt}$ -labeled potassium tetrachloroplatinate (II) potassium ( $^{195m}\text{Pt}$   $\text{K}_2\text{PtCl}_4$ ) was neutralized with potassium carbonate (0.33 mmol/mL) as in the previous case.

### Preparation of $^{195m}\text{Pt}$ $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$

To an aqueous solution (10 mL) of  $\text{K}_2\text{PtCl}_4$  (0.475 g, 1.14 mmol) spiked with ~20 MBq of  $^{195m}\text{Pt}$   $\text{K}_2\text{PtCl}_4$  as radiotracer (prepared as described in the section 2.3.1), a solution of potassium iodide (1.52 g, 9.15 mmol) (in excess) in distilled water (5 mL) was added with stirring at room temperature. To this, aqueous  $\text{NH}_4\text{OH}$  (1.5 mL) was added whereupon a yellow precipitate ( $^{195m}\text{Pt}$ ) $\text{Pt}(\text{NH}_3)_2\text{I}_2$  appeared within few minutes. The solution was warmed slightly upto  $50^\circ\text{C}$ – $60^\circ\text{C}$ . The yellow precipitate was filtered through a sintered disc and washed with distilled water followed by ethanol. The yellow precipitate was dried *in vacuo* (0.44 g, 80%). Further, to an aqueous suspension of  $^{195m}\text{Pt}$ ) $\text{Pt}(\text{NH}_3)_2\text{I}_2$  (0.44 g, 0.91 mmol), an aqueous solution of  $\text{AgNO}_3$  (0.309 g, 1.82 mmol) was added with stirring which continued for 2 h. Precipitated  $\text{AgI}$  was filtered off and the clear yellowish solution was concentrated *in vacuo* to afford ( $^{195m}\text{Pt}$ )  $\text{Pt}(\text{NH}_3)_2(\text{ONO}_2)_2$  (0.27 g, 84%). This ( $^{195m}\text{Pt}$ ) $\text{Pt}(\text{NH}_3)_2(\text{ONO}_2)_2$  (0.27 g, 0.764 mmol) was dissolved in distilled water (30 ml) followed by addition of two molar equivalents of  $\text{KCl}$  (0.12 g, 1.6 mmol). The solvent water was evaporated on water bath till it reduced to 10 ml solution and then allowed to cool to room temperature which gave the yellow precipitate of  $^{195m}\text{Pt}$  labeled cisplatin, *cis*- $^{195m}\text{Pt}$   $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$  (0.14 g, 61%).

### Preparation of radiolabeled prodrug $^{195m}\text{Pt}$ $\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2$

The radiolabeled cisplatin, *cis*- $^{195m}\text{Pt}$ ) $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$  (0.11 g, 0.3666 mmol) was suspended in distilled water (1 ml) and then treated with aqueous hydrogen peroxide (30%) (1 ml) with stirring. Then the reaction mixture was warmed at  $60^\circ\text{C}$  for 2 h and then stirring continued for next 10 h. The clear solution of *cis*- $^{195m}\text{Pt}$ ) $\text{PtCl}_2(\text{OH})_2(\text{NH}_3)_2$  was obtained. The solvent from reaction mixture was evaporated to dryness *in vacuo* which afforded the pale green colored powder (0.12 g, 98%). This powder of *cis*- $^{195m}\text{Pt}$ ) $\text{PtCl}_2(\text{OH})_2(\text{NH}_3)_2$  was suspended in dry DMF (500  $\mu\text{L}$ ) at room temperature followed by addition of two molar equivalents of succinic anhydride (0.077 g, 0.063 ml, 0.7635 mmol). Then the reaction mixture was stirred for 5 h. The evaporation of solvent afforded the pale greenish compound,  $^{195m}\text{Pt}$   $\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2$  (0.17 g, 87%). This radio-active prodrug spiked with ~ 5.6 MBq of  $^{195m}\text{Pt}$   $\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2$  as radiotracer (radiochemical yield ~ 28%).

This strategy of synthesis of  $^{195m}\text{Pt}$   $\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2$  is clearly depicted in the Scheme 1 as follows.

### Radiochemical characterization of $^{195m}\text{Pt}$ $\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2$

#### Radionuclidic purity

Radionuclidic purity of  $^{195m}\text{Pt}$   $\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2$  was determined by recording  $\gamma$ -ray spectrum of the appropriately diluted sample solutions using a high purity germanium radiation detector coupled with a multichannel analyzer. A  $^{152}\text{Eu}$  reference source (Amersham Inc.) was used for both energy and efficiency calibration. The gamma ray spectra of processed products were recorded for each batch at regular time intervals. The total radioactivity of  $^{195m}\text{Pt}$ -labeled product was also determined using gamma ray spectrometry from the data of total counts acquired under 129.6 keV gamma photopeak attributable to  $^{195m}\text{Pt}$ .

#### Radiochemical purity

Radiochemical purity of  $^{195m}\text{Pt}$   $\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2$  was determined by PC. For this purpose, 5  $\mu\text{L}$  of radioactive formulation was spotted on a 12 cm  $\times$  1 cm Whatman 3 MM chromatography paper strip at 1.5 cm from the bottom. The chromatogram was developed in 50% methanol in distilled water (v/v). Subsequently, the PC plate was dried and cut into 1 cm pieces and activity of each piece was determined using a  $\text{NaI}$  (Tl) counter. As a control, radio-chromatogram of  $^{195m}\text{Pt}$ ) $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$  was also developed in 50% methanol in distilled water (v/v) medium. The radiochemical purity of the desired radiolabeled product was expressed as the percentage

of the total activity which remained at the point of application ( $R_f = 0-0.1$ ) in the chromatogram developed.

## Results and Discussion

The synthesis of Pt(IV) prodrug was optimized using normal platinum wire and following the same procedure,  $^{195\text{m}}\text{Pt}$  labeled Pt(IV) prodrug was prepared. The Pt metal (500 mg) was digested using a mixture of concentrated nitric acid and hydrochloric acid to form an aqueous solution of  $\text{H}_2\text{PtCl}_6$ . The formed  $\text{H}_2\text{PtCl}_6$  when was treated with the saturated aqueous solution of KCl which afforded  $\text{K}_2\text{PtCl}_6$ . The  $\text{K}_2\text{PtCl}_6$  (+4 oxidation state) was reduced to  $\text{K}_2\text{PtCl}_4$  (+2 oxidation state) by using stoichiometric amount of hydrazine hydrochloride. It was followed by the treatment of  $\text{K}_2\text{PtCl}_4$  with saturated aqueous solution of KI which yielded  $\text{K}_2\text{PtI}_4$  after replacing chlorides of the former. The replacement of the chloride groups by more labile iodide groups, gives easy approach for the introduction of the ammine groups maintaining cis-position. Then  $\text{K}_2\text{PtI}_4$  was treated with ammonium chloride and the pH of the reaction mixture was adjusted to obtain a basic mixture (pH = 12) using 1 mM sodium hydroxide solution, there is a formation of  $[(\text{NH}_3)_2\text{PtI}_2]$  due to the trans-effect shown by the square planar nature of  $\text{K}_2\text{PtI}_4$ . The iodide group of  $[(\text{NH}_3)_2\text{PtI}_2]$  was replaced with more labile  $\text{NO}_3^-$  group by treating with aqueous silver nitrate to obtain  $[(\text{NH}_3)_2\text{Pt}(\text{NO}_3)_2]$  which after treatment with two molar equivalents of aqueous solution of KCl led to exchange of  $\text{NO}_3^-$  group with chloride group affording the cisplatin, *cis*- $[(\text{NH}_3)_2\text{PtCl}_2]$ . The square planar *cis*- $[(\text{NH}_3)_2\text{PtCl}_2]$  having +2 oxidation state of Pt centre was oxidized forming an octahedral compound, *cis*- $[\text{PtCl}_2(\text{NH}_3)_2(\text{OH})_2]$  having +4 oxidation state. Here the oxidation of Pt (II) to Pt(IV) leads to the introduction of two axial hydroxyl groups which enable the prodrug for conjugating various hydrophilic organic frames for enhancing water solubility and various biocompatible groups or biomolecules, or luminescent groups for diverse applications in cancer therapy.

Functional groups attached to these two axial hydroxyl groups decide the easiness of the reduction of the Pt(IV) prodrug to the cytotoxic drug at the physiological condition. It has been reported that carboxyl groups attached to prodrugs are prone to reduce faster to the corresponding Pt (II) based drug as compared to the prodrugs bearing free hydroxyl groups. In order to achieve axial carboxyl groups bearing prodrugs, the commonly practiced method is the conjugation of the axial hydroxyl groups of  $[(\text{NH}_3)_2\text{PtCl}_2(\text{OH})_2]$  with succinic anhydride. In the literature, the coupling of the succinic anhydride with the hydroxyl groups of the oxidized cisplatin was reported using DMSO as the solvent.<sup>[20]</sup> Usually, removal of DMSO is carried out by deep freezing technique which has to be operated manually in most of cases. As the handling of the  $^{195\text{m}}\text{Pt}$  has to be performed in a lead shielded area and

in this situation the removal of DMSO is a challenging task, DMF was chosen as the solvent instead of DMSO. The advantages of the using DMF are non-solubility of the oxidized cisplatin,  $[(\text{NH}_3)_2\text{PtCl}_2(\text{OH})_2]$  and the solubility of the final product,  $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$  formed after treating the former with succinic anhydride. In this reaction, as the suspension of oxidized cisplatin was treated with succinic anhydride in DMF, slowly the final product  $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$  was formed turning the earlier suspension to clear solution. The purification of the final product was done by addition of an excess of dried acetone to the reaction mixture followed by cooling at  $0^\circ\text{C}$  for overnight which led to formation of precipitate which was filtered out. The obtained precipitate was characterized with NMR ( $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{195}\text{Pt}$ ) spectroscopy [Supplementary Materials: Figures S1-S3].

The stability of the synthesized Pt(IV) prodrug of cisplatin was studied using cyclic voltammetry at pH of 6.5 and 7.4. A stock solution of 10 mM was prepared in distilled water. Phosphate buffers of pH 6.5 and 7.4 were prepared. The solutions of prodrug (1 mM) in each pH were prepared and then the reduction and oxidation potentials of prodrug at both the pH were measured. In these studies, reduction of Pt(IV) prodrug to cisplatin was observed but there was no further re-oxidation of the generated cisplatin to Pt(IV) prodrug. This revealed that the reactivity of Pt(IV) prodrug in conditions of cyclic voltammetric reaction mixture ceases after its reduction and there is no further oxidation, which demonstrated the suitability of Pt(IV) prodrug for treatment of acidic tumor tissues. Additionally, it was found that the amount of Pt(IV) prodrug reduced to cisplatin at acidic pH (pH = 6.5) was about 10 times of the amount of Pt(IV) prodrug reduced at physiological pH (7.4) as shown in Figure 1. This revealed that the synthesized Pt(IV) prodrug of cisplatin is fairly stable at physiological pH but susceptible to reduction at the acidic

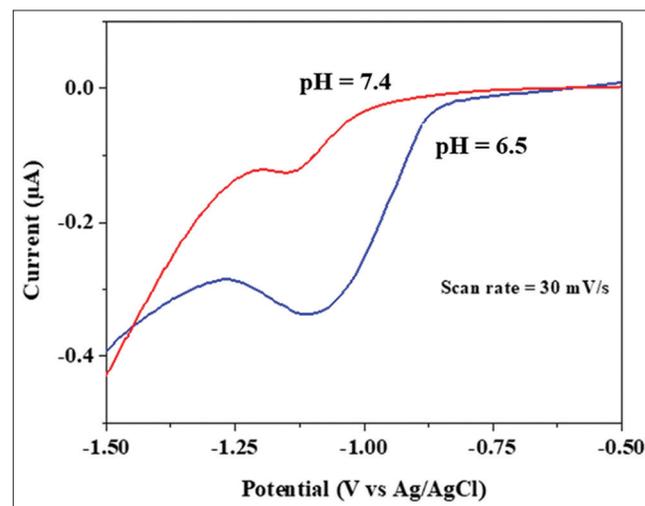


Figure 1: Cyclic voltammetry of 1.0 mM Pt(IV) prodrug of cisplatin in phosphate-buffered saline buffer solution using Ag/AgCl with scan rates of  $30 \text{ mV}\cdot\text{S}^{-1}$  indicating variation of peak current of the first oxidation peak with the square root of the scan rates

pH 6.5. Since the extracellular pH of the tumoral region is acidic as compared to that of the normal tissue, the reduction of Pt(IV) prodrug to cisplatin must be more at tumor site, as compared to the normal tissues. This phenomenon will be helpful to overcome the unwanted toxicity associated with the cisplatin against the normal tissues.

The cytotoxicity of the Pt(IV) prodrug of cisplatin was studied along with cisplatin as control for 24 h and 48 h against MCF-7 cell lines. There was no significant cell death of Pt(IV) prodrug treated cells up to 40  $\mu\text{M}$  as compared to  $\text{IC}_{50}$  of 15  $\mu\text{M}$  for cisplatin [Figure 2]. This is possibly due to the inertness in the reduction of the Pt(IV) prodrug to cisplatin at the physiological conditions (pH 7.4) maintained for cell culture condition, lack of biological reducing agents in sufficient quantities or inability to internalize inside the cells. Though, the pH of medium effects on drug release kinetics<sup>[21]</sup> or reduction to active drugs, the MTT assay was performed at pH 7.4. It is reported that the pH of the culture media usually changes due to the metabolites released from cells, e.g., change of pH from 7.4 to 6.5 in 72 h was reported.<sup>[22]</sup> As a result, it is difficult to maintain the constant acidic pH during any assay. Furthermore, the reduction of Pt(IV) prodrug to cisplatin is higher at acidic regions by biological reducing agents like glutathione and ascorbic acid; which is possible only during *in vivo* studies at tumor regions.<sup>[23]</sup> In regard to the present study, no significant viability of cancer cells was observed from MTT assay which is probably due to lack of reducing environment or less internalization of Pt(IV) prodrugs inside cancer cells.<sup>[24]</sup>

For potential use in IGDD, intrinsically  $^{195m}\text{Pt}$ -labeled Pt(IV) prodrug was synthesized and characterized for its radionuclidic as well as radiochemical purity.

Radionuclidic purity of [ $^{195m}\text{Pt}$ ]  $\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2$  was ascertained by gamma ray spectrometry. The total radioactivity of the radiotracer synthesized was also determined by same technique. A typical gamma ray spectrum of intrinsically  $^{195m}\text{Pt}$ -labeled Pt(IV)-cisplatin prodrug [ $^{195m}\text{Pt}$ ]  $\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2$  is shown in Figure 3. Gamma ray photopeaks were found at energies of 66.8, 75.8, 98.9, 129.6 and 174.2 keV; all of which correspond to the characteristic photopeaks of  $^{195m}\text{Pt}$ . No other peak corresponding to any other radioisotope could be found in the gamma ray spectrum. Hence, the synthesized radiotracer was radionuclidically pure with respect to  $^{195m}\text{Pt}$  radioisotope.

A typical radiochromatogram of the synthesized radiotracer obtained using Whatman 3 MM chromatography paper as the stationary phase and 50% methanol in water (v/v) as the mobile phase (HPLC grade solvents) is shown in Figure 4. In this system, the synthesized radiotracer, [ $^{195m}\text{Pt}$ ]  $\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2$  exhibited  $R_f$  of 0.0–0.2, while the starting material for synthesis of the same i.e., [ $^{195m}\text{Pt}$ ]  $\text{K}_2\text{PtCl}_4$  exhibited  $R_f$  of 0.7–0.8 (confirmed from blank experiment). The percentage of total radioactivity

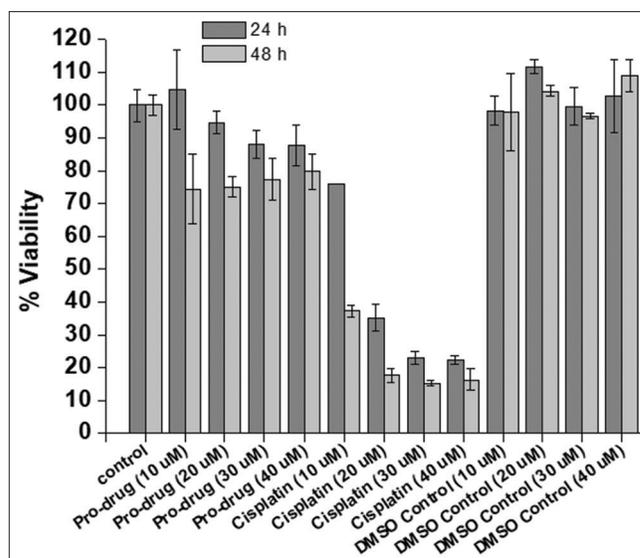


Figure 2: Viability of cancer cell lines after treatment with cisplatin and Pt(IV) prodrug

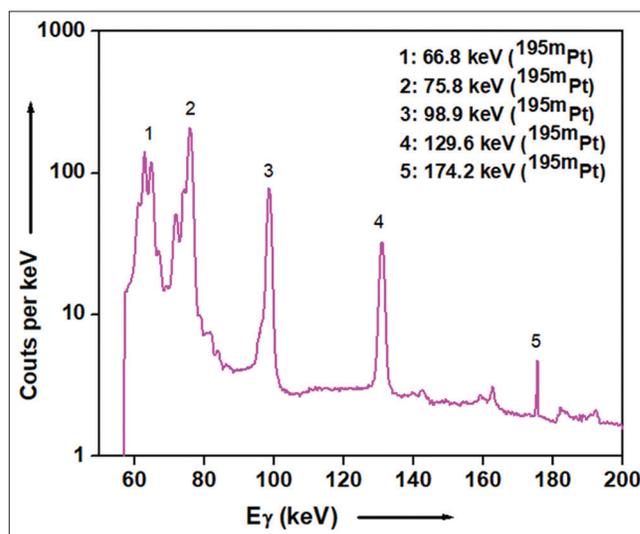


Figure 3: A typical gamma ray spectrum of intrinsically  $^{195m}\text{Pt}$ -labeled Pt(IV)-cisplatin prodrug

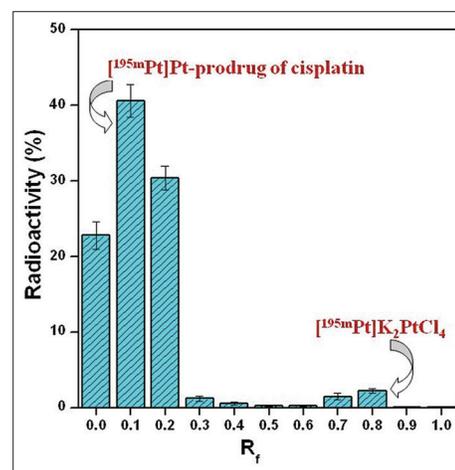


Figure 4: A typical paper chromatography pattern of intrinsically  $^{195m}\text{Pt}$ -labeled Pt(IV)-cisplatin prodrug

associated with the desired radiotracer was calculated and found to be 97.8%.

## Conclusions

We have synthesized the cold (elemental) and intrinsically  $^{195m}\text{Pt(IV)}$ -labeled analog  $^{195m}\text{Pt(IV)}$  prodrug of cisplatin,  $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$ . The cyclic voltammetry studies have exhibited the maximum reduction of Pt(IV) to cisplatin at acidic pH (pH 6.5) which reveals that the prodrug is anticipated to get reduced to cisplatin at tumor site. The  $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$  exhibited less cytotoxicity against MCF-7 human breast cancer cell lines for lack of reducing agents or less internalization inside cancer cells. In order to develop a potent molecular probe for IGDD suitable for tumor model studies,  $^{195m}\text{Pt(IV)}$ -labeled analogue of Pt(IV) prodrug was synthesized with high radionuclidic as well as radiochemical purity required for its clinical translation.

## Supplementary Materials

The characterization data of  $[\text{PtCl}_2(\text{OCOCH}_2\text{CH}_2\text{COOH})_2(\text{NH}_3)_2]$  by NMR ( $^1\text{H}$ ,  $^{13}\text{C}$   $\{^1\text{H}\}$  and  $^{195}\text{Pt}$   $\{^1\text{H}\}$ ) spectroscopy is given in supplementary materials.

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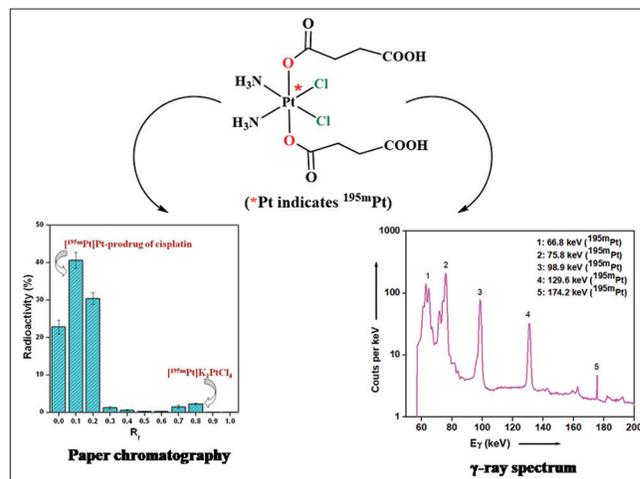
## Highlights

1. Synthesis of Pt(IV) prodrug of cisplatin and its cytotoxicity against MCF-7 cancer cells.
2. Its reduction at acidic pH 6.5 to active cisplatin
3. Synthesis of  $^{195m}\text{Pt}$ -radiolabeled Pt(IV) prodrug avoiding the lengthy steps
4. High radionuclidic and radiochemical purity
5. Anticipate application of  $^{195m}\text{Pt(IV)}$  prodrug for image-guided drug delivery (IGDD)

(Graphical abstract)

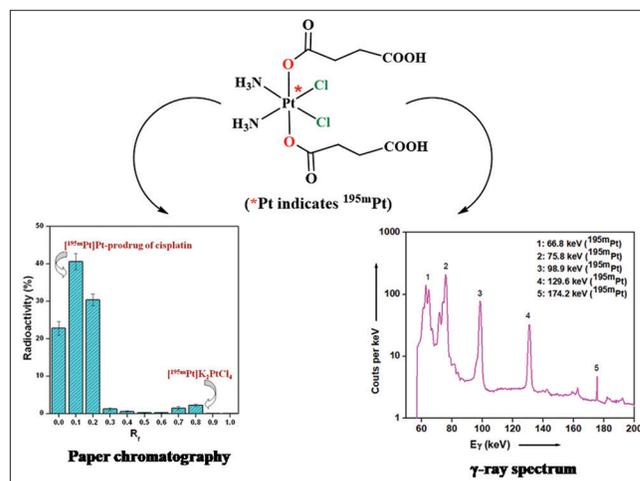
Facile synthesis of a Pt(IV) prodrug of cisplatin and its intrinsically  $^{195m}\text{Pt}$  labeled analog: A step closer to cancer theranostic.

## Graphical Abstract



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Facile synthesis of a Pt(IV) prodrug of cisplatin and its intrinsically  $^{195m}\text{Pt}$  labeled analog: A step closer to cancer theranostic.



Stable Pt(IV) prodrug of cisplatin was reduced to active cisplatin [Pt (II)] only at acidic conditions (pH 6.5) present similarly at cancer cells. But for its approval as anticancer drug, understanding of its mechanism is of utmost importance. Hence,  $^{195m}\text{Pt}$ -radiolabeled prodrug was synthesized anticipating its synergistic use for molecular imaging and targeted drug delivery known as “image-guided drug delivery.”

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Nil.

## Conflicts of interest

There are no conflicts of interest.

## References

1. Rosenberg B, VanCamp L, Trosko JE, Mansour VH. Platinum compounds: A new class of potent antitumour agents. *Nature*

- 1969;222:385-6.
- Sastry J, Kellie SJ. Severe neurotoxicity, ototoxicity and nephrotoxicity following high-dose cisplatin and amifostine. *Pediatr Hematol Oncol* 2005;22:441-5.
  - a) Hall MD, Mellor HR, Callaghan R, Hambley TW. Basis for design and development of platinum (IV) anticancer complexes. *J Med Chem* 2007;50:3403-11. b) Chin CF, Tian Q, Setyawati MI, Fang W, Tan ES, *et al.* Tuning the activity of platinum (IV) anticancer complexes through asymmetric acylation. *J Med Chem* 2012;55:7571-82. c) Hall MD, Hambley TW. Platinum (IV) Antitumour Compounds: Their bioinorganic chemistry. *Coord Chem Rev* 2002;232:49-67.
  - Nemirovski A, Kasherman Y, Tzaraf Y, Gibson D. Reduction of cis, trans, cis-[PtCl<sub>2</sub>(OCOCH<sub>3</sub>)<sub>2</sub>(NH<sub>3</sub>)<sub>2</sub>] by aqueous extracts of cancer cells. *J Med Chem* 2007;50:5554-6.
  - Ravera M, Gabano E, Zanellato I, Bonarrigo I, Alessio M, Arnesano F, *et al.* Cellular trafficking, accumulation and DNA platination of a series of cisplatin-based dicarboxylato Pt(IV) prodrugs. *J Inorg Biochem* 2015;150:1-8.
  - Gabano E, Ravera M, Osella D. Pros and cons of bifunctional platinum (IV) antitumor prodrugs: Two are (not always) better than one. *Dalton Trans* 2014;43:9813-20.
  - Choi S, Filotto C, Bisanzo M, Delaney S, Lagasee D, Whitworth JL, *et al.* Reduction and anticancer activity of platinum (IV) complexes. *Inorg Chem* 1998;37:2500-04.
  - Wexselblatt E, Yavin E, Gibson D. Platinum (IV) prodrugs with haloacetato ligands in the axial positions can undergo hydrolysis under biologically relevant conditions. *Angew Chem Int Ed Engl* 2013;52:6059-62.
  - a) Ammar AA, Raveendran R, Gibson D, Nassar T, Benita S. A Lipophilic Pt(IV) Oxaliplatin derivative enhances antitumor activity. *J Med Chem* 2016;59:9035-46. b) Zanellato I, Bonarrigo I, Colangelo D, Gabano E, Ravera M, Alessio M, *et al.* Biological Activity of a Series of Cisplatin-Based Aliphatic Bis (carboxylato) Pt(IV) Prodrugs: How long the organic chain should be?. *J Inorg Biochem* 2014;140:219-27. c) Basu U, Banik B, Wen R, Pathak RK, Dhar S. The Platin-X series: Activation, targeting, and delivery. *Dalton Trans* 2016;45:12992-3004.
  - Chakravarty R, Hong H, Cai W. Positron emission tomography image-guided drug delivery: Current status and future perspectives. *Mol Pharm* 2014;11:3777-97.
  - Song Y, Suntharalingam K, Yeung JS, Royzen M, Lippard SJ. Synthesis and characterization of Pt(IV) fluorescein conjugates to investigate Pt(IV) intracellular transformations. *Bioconjug Chem* 2013;24:1733-40.
  - Leblond F, Davis SC, Valdés PA, Pogue BW. Pre-clinical whole-body fluorescence imaging: Review of instruments, methods and applications. *J Photochem Photobiol B* 2010;98:77-94.
  - Sathekge M, Wagener J, Smith SV, Soni N, Marjanovic-Painter B, Zinn C, *et al.* Biodistribution and dosimetry of <sup>195m</sup>Pt-cisplatin in normal volunteers. Imaging agent for single photon emission computed tomography. *Nuklearmedizin* 2013;52:222-7.
  - Firestone RB. Table of Isotopes. Amsterdam, Netherlands: John Wiley & Sons Inc.; 1998. Reus U, Westmeier W. Catalog of gamma rays from radioactive decay. *Atomic Data Nuclear Data Tables* 1983;29:1-192.
  - a) Jackson H, McAuliffe CA, Perera A, Sharma HL, Tinkar NJ. An improved synthetic procedure for the preparation of <sup>195m</sup>Pt labelled antitumour complexes. *J Label Compd Radiopharm* 1991;29:1121-30. b) Dhar S, Gu FX, Langer R, Farokhzad OC, Lippard SJ. Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt(IV) prodrug-PLGA-PEG nanoparticles. *Proc Natl Acad Sci USA* 2008;105:17356-61. c) Shi Y, Liu SA, Kerwood DJ, Goodisman J, Dabrowiak JC. Pt(IV) complexes as prodrugs for cisplatin. *J Inorg Biochem* 2012;107:6-14.
  - Zheng YR, Suntharalingam K, Johnstone TC, Yoo H, Lin W, Brooks JG, *et al.* Pt(IV) prodrugs designed to bind non-covalently to human serum albumin for drug delivery. *J Am Chem Soc* 2014;136:8790-8.
  - Armarego WL, Perrin DD. Purification of Laboratory Chemicals. 4<sup>th</sup> ed. Pergamon, Oxford: Butterworth-Heinemann; 1996.
  - Dhara SC. A rapid method for the synthesis of cis-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>]. *Ind J Chem* 1970;8:193-94.
  - Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55-63.
  - Shanmugam V, Chien YH, Cheng YS, Liu TY, Huang CC, Su CH, *et al.* Oligonucleotides-assembled Au nanorod-assisted cancer photothermal ablation and combination chemotherapy with targeted dual-drug delivery of Doxorubicin and Cisplatin prodrug. *ACS Appl Mater Interfaces* 2014;6:4382-93.
  - Trebinska-Stryjewska A, Swiech O, Opuchlik LJ, Grzybowska EA, Bilewicz R. Impact of medium pH on DOX toxicity toward HeLa and A498 cell lines. *ACS Omega* 2020;5:7979-86.
  - Arora M. Cell culture media: A review. *Mater Methods* 2013;3:175.
  - Bruijninx PC, Sadler PJ. New trends for metal complexes with anticancer activity. *Curr Opin Chem Biol* 2008;12:197-206.
  - Sharma KS, Dubey AK, Kojam AS, Kumar C, Ballal A, Mukherjee S, *et al.* Synthesis of 2-deoxy-d-glucose coated Fe<sub>3</sub>O<sub>4</sub> nanoparticles for application in targeted delivery of the Pt(IV) prodrug of cisplatin A novel approach in chemotherapy. *New J Chem* 2020;44:13863-74.