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Thermoresponsive carboplatin-releasing prodrugs

Aemilia D. McAdam, Lucinda K. Batchelor, Jan Romano-deGea, Dmitry Vasilyev, Paul J. Dyson *

Institute of Chemical Sciences and Engineering, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

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ABSTRACT

Platinum-based anticancer drugs, while potent, are associated with numerous and severe side effects. Hyperthermia therapy is an effective adjuvant in anticancer treatment, however, clinically used platinum drugs have not been optimised for combination with hyperthermia. The derivatisation of existing anticancer drugs with appropriately chosen thermoresponsive moieties results in drugs being activated only at the heated site. Perfluorinated chains of varying lengths were installed on carboplatin, a clinically approved drug, leading to the successful synthesis of a series of mono- and di- substituted platinum(IV) carboplatin prodrugs. Some of these complexes display relevant thermosensitivity on ovarian cancer cell lines, i.e., being inactive at 37 °C while having comparable activity to carboplatin under mild hyperthermia (42 °C). Nuclear magnetic resonance spectroscopy and mass spectrometry indicated that carboplatin is likely the active platinum(II) anticancer agent upon reduction and cyclic voltammetry revealed that the length of the fluorinated alkyl chain has a strong influence on the rate of carboplatin formation, regulating the subsequent cytotoxicity.

1. Introduction

Platinum-based complexes are extensively applied in anticancer chemotherapeutic regimes in the clinic [1,2]. Cisplatin, which was first introduced to the clinic in the 1960s [3], still remains extensively used despite a wide variety of side effects, including nephrotoxicity, neurotoxicity, myelosuppression, nausea and vomiting [4–8]. With the intention of reducing the side effects associated with cisplatin, carboplatin was developed and approved for clinical use in 1981 [9,10]. Other platinum(II) anticancer drugs have been approved for clinical use (Fig. 1) [2], although only cisplatin, carboplatin and oxaliplatin are approved for use worldwide, and thousands more platinum-based candidates have been proposed [11].

A common feature to all these drugs is that they have a similar mode of action to cisplatin, i.e. interaction with DNA [12], and hence they are typically applied in combination with adjuvant therapies [13–15]. To further improve efficacy, many drug delivery systems have been devised that use an external stimulus as a targeting, activation or release mechanisms [16–20]. An innovative strategy is the use platinum(IV) prodrugs that undergo reduction within cancer cell to release a platinum (II) drug and the functionalised axial ligands [1,21–25]. Platinum(IV) prodrugs allow bio-functional and targeting moieties to be appended (as axial ligands) [26] and some prominent examples are shown in Fig. 2. For example, platinum(IV) prodrugs of cisplatin containing functionalised arene carboxylate ligands display vastly improved uptake compared to cisplatin alone [23] or asymmetric platinum(IV) prodrugs of cisplatin containing two different functionalised axial ligands may even overcome cisplatin resistance [27].

Despite the potential of platinum(IV) prodrugs, none have gained clinical approval and, therefore, designing these complexes to operate with an effective adjuvant might provide further benefits and facilitate their translation to the clinic. In this context, platinum(IV) prodrugs with photoactivated groups have been reported [28–30]. An alternative adjuvant is hyperthermia, with which many existing chemotherapeutic agents show enhanced activity despite not specifically being designed for use in combination therapy [31]. Hyperthermia is already used as an

Abbreviations: A2780, ovarian cancer cell line; A2780cisR, cisplatin resistance ovarian cancer cell line; carboPt, carboplatin, *cis*-diammine(cyclobutane-1,1-dicarboxylate-O,O′)platinum(II); cisplatin, *cis*-diamminedichloroplatinum(II); CV, cyclic voltammetry; DMAP, 4-dimethylaminopyridine; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HEK-293, healthy human embryonic cell line; HRMS, high resolution mass spectrometry; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide; NaAsc, sodium ascorbate; NMR, nuclear magnetic resonance; RAPTA-C, [Ru(η^6-*p*-cymene)(1,3,5-triaza-7-phosphaadamantane)Cl_2]; UV–Vis, ultraviolet-visible spectroscopy.

* Corresponding author.
E-mail address: paul.dyson@epfl.ch (P.J. Dyson).

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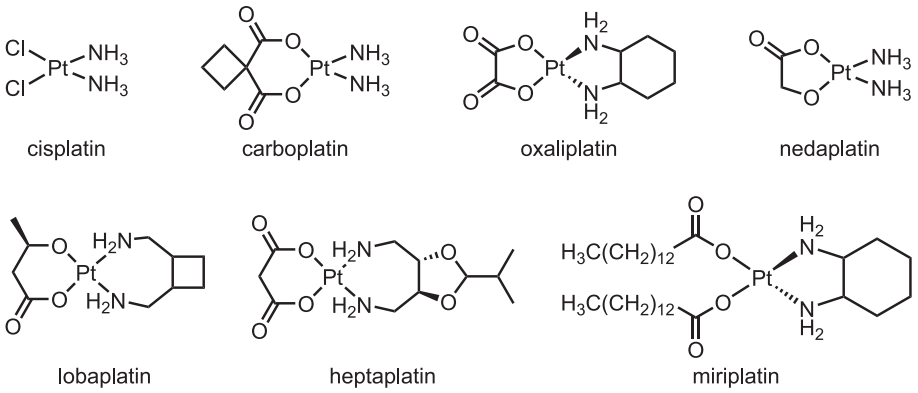


Fig. 1. Structures of clinically approved platinum anticancer agents.

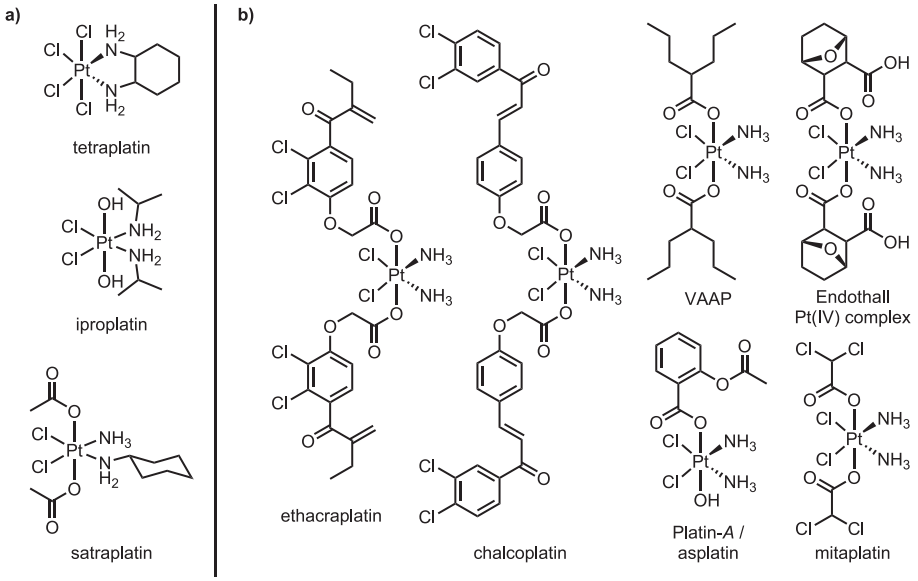


Fig. 2. Examples of platinum(IV) anticancer agents, **a**, that have undergone clinical trials, and **b**, bearing bio-functional axial ligands.

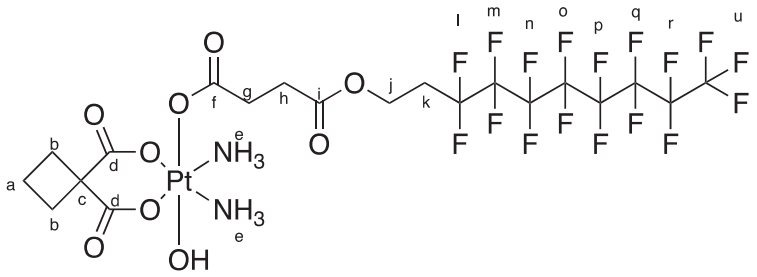
adjuvant therapy in the clinic, and is even applied in combination with carboplatin [32,33]. The design of chemotherapeutic agents that have built-in thermoresponsive properties that are selectively activated within the safe hyperthermic temperature region of 40 to 43 °C [34,35] would be advantageous [36]. In this respect, a number of compounds were developed that display thermoresponsive properties and are activated under hyperthermia [18,37–40], including a series of complexes based on a *trans*-platinum(II) core [41]. These thermoresponsive systems are designed to undergo chemical or structural changes under mild hyperthermia to change the size, shape, solubility, or biological behaviour of the compound in such a way as to augment their anticancer effect. Particularly, enhanced cell uptake under hyperthermic conditions is responsible for the higher anticancer effect of thermoresponsive ruthenium complexes [40]. Long alkyl and perfluorinated chains are common moieties in compounds exhibiting strong thermoresponsive properties [42–44], which were then translated to anticancer compounds [36]. The length of the perfluorinated chains has a considerable impact on the thermoresponsive character of the parent drug, with longer chains typically showing a greater effect [40], but the optimal length and their behaviour remains difficult to predict. Carboplatin, being currently used in combination with hyperthermia but not being specifically designed for this purpose, represents a promising candidate for functionalisation with thermoresponsive fragments. Therefore, redox-responsive platinum(IV) prodrugs with a carboplatin core and derivatised with perfluorinated chains of different length in the axial position(s) were developed. The complexes were evaluated for their cytotoxicity and for their thermoresponsive behaviour against ovarian cancer cells.

2. Experimental section

2.1. Materials and methods

All reagents and solvents were purchased from commercial suppliers and were used without further purification. $K_2[PtCl_4]$ was purchased from Precious Metals Online. Carboplatin was synthesised according to a literature procedure [45–47] or was purchased from TCI Chemicals. All reactions were performed under an inert N_2 atmosphere using Schlenk techniques with reactions carried out in dry solvents (stored over 4 Å molecular sieves) or in MilliQ water.

Nuclear magnetic resonance (NMR) spectroscopy measurements were recorded on a Bruker 400 MHz spectrometer (9.4 T) equipped with a console Avance II at 298 K at 400 MHz (^{1}H), 101 MHz (^{13}C), 188 MHz (^{19}F) and 86 MHz (^{195}Pt). Chemical shifts are reported in parts per million (ppm) and are referenced to residual peaks in the deuterated solvent. Coupling constants (*J*) are reported in Hertz (Hz). ^{19}F and ^{195}Pt chemical shifts were indirectly referenced with the ^{1}H signals of the residual protons of the deuterated solvent using the Ξ-scale (Ξ =



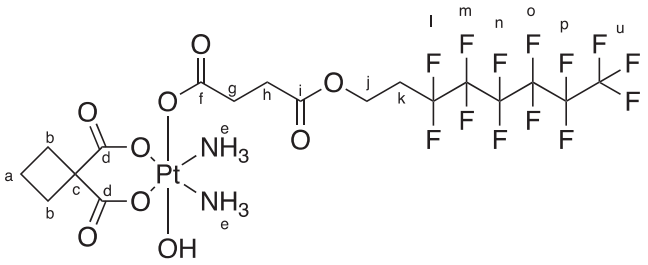
94.094011 MHz and 21.496784 MHz, respectively) with neat $CFCl_3$ ($\delta(^{19}F) = 0$ ppm) or 1.2 M K_2PtCl_6 in D_2O ($\delta(^{195}Pt) = 0$ ppm) as secondary references. The splitting pattern of proton resonances in ^1H NMR spectra are defined as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, and m = multiplet. High resolution mass spectrometry (HRMS) spectra were obtained on a Thermo-Finnigan LCQ Deca XP Plus quadrupole ion-trap instrument or a Thermo-Finnigan Orbitrap Elite instrument with an LTQ-Orbitrap analyser operated in both positive and negative mode. Elemental analysis was performed by the Mass spectrometry and Elemental Analysis platform at EPFL using a Thermo Scientific Flash 2000 Organic Elemental Analyser.

2.2. Synthesis and characterisation

Complexes **1** [46], **2** [48] and **3** [46] were prepared according to previously reported procedures (see Supporting Information).

2.2.1. General procedure for the synthesis of monosubstituted perfluorinated carboplatin prodrugs (*4, 5* and *6*)

A solution of **2** (100 mg, 0.198 mmol, 1 equiv.), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (49 mg, 0.257 mmol, 1.3 equiv.) and 4-dimethylaminopyridine (12 mg, 0.099 mmol, 0.5 equiv.) in dry DMSO (6 mL) was stirred for 1 h at room temperature and under N_2. This solution was added to the appropriate perfluorinated alcohol (1 equiv.), $HO\text{-}CH_2CH_2\text{-}(CF_2)_nCF_3$ (n = 5, 7 or 9), and washed with dry DMSO (4 mL). The mixture was stirred for 96 h at room temperature and protected from light. The reaction was concentrated and the residue was dried under vacuum. The crude was the suspended in DCM (14 mL), centrifuged, washed with MeCN (10 mL) and the obtained solid was isolated by centrifugation. The residue was dissolved in the minimum volume of DMSO, concentrated and dried under vacuum.



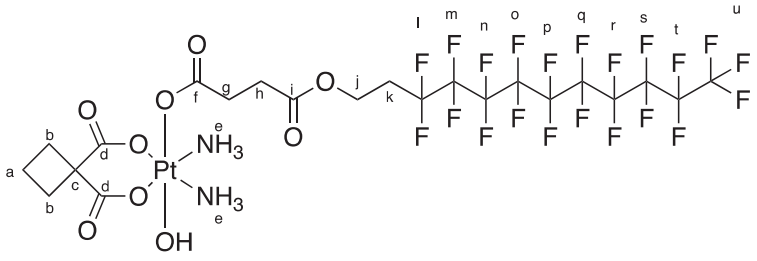
4: Pale yellow solid (127.7 mg, 0.150 mmol, 76%). ^1H NMR (400 MHz, DMSO-d_6) ppm: 7.21–6.67 (m, 6H, H_e), 3.03 (t, J = 6.2 Hz, 2H, H_j), 2.45–2.31 (m, 8H, H_b, H_g + H_h), 1.76–1.67 (m, 4H, H_a + H_k). ^{19}F NMR (188 MHz, DMSO-d_6) ppm: δ −80.31 (3F, F_u), −112.56 (2F, F_l), −121.74 (2F, F_n), −122.71 (2F, F_o), −123.33 (2F, F_m), −125.82 (2F, F_p). HRMS: $[M + H]^+$ requires m/z = 852.0800; found m/z = 852.0802.

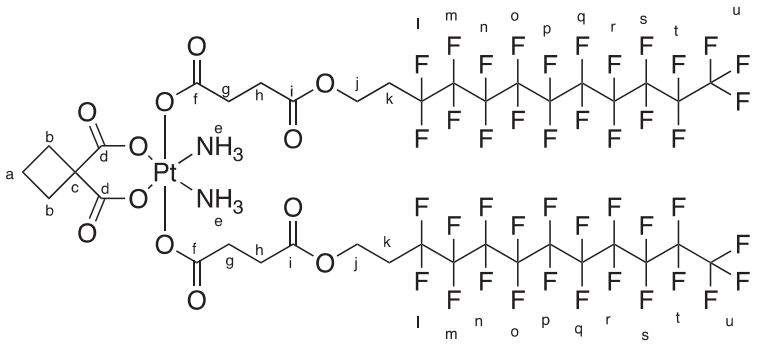
5: Orange solid (123.1 mg, 0.129 mmol, 64%). ^1H NMR (400 MHz, DMSO-d_6) ppm: 7.19–6.68 (m, 6H, H_e), 3.05–3.03 (m, 2H, H_j), 2.39–2.29 (m, 8H, H_b, H_g + H_h), 1.76–1.67 (m, 4H, H_a + H_k). ^{19}F NMR (188 MHz, DMSO-d_6) ppm: δ −80.94 (3F, F_u), −112.94 (2F, F_l), −121.96 (6F, $F_{n\text{-}p}$), −122.90 (2F, F_q), −123.61 (2F, F_m), −126.28 (2F, F_r). HRMS: $[M + Na]^+$ requires m/z = 974.0523; found m/z = 974.0515.

6: Yellow solid (130 mg, 0.124 mmol, 62%). ^1H NMR (400 MHz, DMSO-d_6) ppm: 7.27–6.72 (m, 6H, H_e), 3.01–3.00 (m, 2H, H_j), 2.30–2.26 (m, 8H, H_b, H_g + H_h), 1.95–1.89 (m, 4H, H_a + H_k). ^{19}F NMR (188 MHz, DMSo-d_6) ppm: δ −80.14z (3F, F_u), −112.48 (2F, F_l), −121.45 (10F, $F_{n\text{-}r}$), −122.38 (2F, F_s), −123.22 (2F, F_m), −125.66 (2F, F_t). HRMS: $[M + H]^+$ requires m/z = 1052.0600; found m/z = 1053.1331.

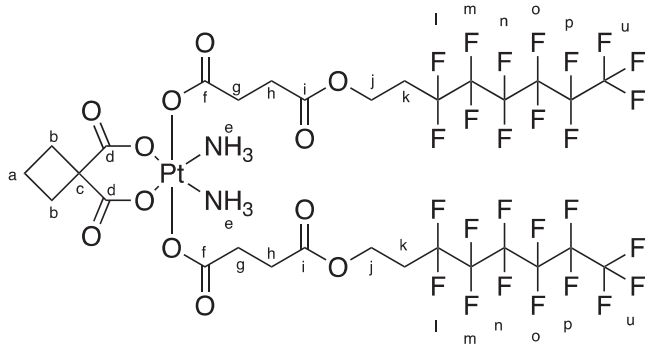
2.2.2. General procedure for the synthesis of disubstituted perfluorinated carboplatin prodrugs (*7, 8* and *9*)

A solution of **3** (110 mg, 0.182 mmol, 1 equiv.), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (76 mg, 0.396 mmol, 2.3 equiv.) and 4-dimethylaminopyridine (22.1 mg, 0.181 mmol, 1 equiv.) in dry DMF (6 mL) was stirred for 1 h at room temperature and under N_2. This solution was added to the appropriate perfluorinated alcohol (2 equiv.), $HO\text{-}CH_2CH_2\text{-}(CF_2)_nCF_3$ (n = 5, 7 or 9), and washed with dry DMF (4 mL). The mixture was stirred for 96 h at room temperature and protected

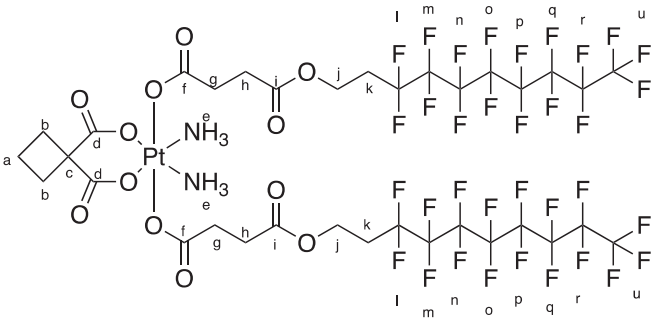




from light. The reaction was concentrated and the residue was dried under vacuum. The crude was the suspended in cold water (12 mL), centrifuged, washed with EtOH (10 mL) and MeCN (10 mL) and the obtained solid was isolated by centrifugation. The residue was dissolved in the minimum volume of DMF, concentrated and dried under vacuum.



7: Pale yellow solid (90 mg, 0.069 mmol, 38%). ^{1}H NMR (400 MHz, DMF-d_7) ppm: 6.99–6.46 (m, 6H, H_e), 3.88 (t, $J = 6.1$ Hz, 4H, H_j), 2.63 (t, $J = 2.0$ Hz, 4H, H_b), 2.55–2.51 (m, 12H, $H_g + H_h + H_k$), 1.91–1.85 (m, 2H, H_a). ^{19}F NMR (188 MHz, DMF-d_7) ppm: δ −81.20 (6F, F_u), −113.33 (4F, F_l), −122.12 (4F, F_n), −123.09 (4F, F_o), −123.85 (4F, F_m), −126.35 (4F, F_p). HRMS: $[M + Na]^+$ requires m/z = 1320.0775; found m/z = 1320.0763.



8: Orange solid (115 mg, 0.077 mmol, 46%). ^{1}H NMR (400 MHz, DMF-d_7) ppm: 7.04–6.52 (m, 6H, H_e), 3.88 (t, $J = 6.1$ Hz, 4H, H_j), 2.63 (t, $J = 1.9$ Hz, 4H, H_b), 2.52–2.50 (m, 12H, $H_g + H_h + H_k$), 1.94–1.89 (m, 2H, H_a). ^{19}F NMR (188 MHz, DMF-d_7) ppm: δ −81.05 (6F, F_u), −113.34 (4F, F_l), −121.94 (12F, $F_{n\text{-}p}$), −122.85 (4F, F_q), −123.68 (4F, F_m), −126.25 (4F, F_r). HRMS: $[M + Na]^+$ requires $m/z = 1520.0648$; found m/z = 1520.0654.

9: Dark yellow solid (278 mg, 0.164 mmol, 96%). ^{1}H NMR (400 MHz, DMF-d_7) ppm: 7.08–6.43 (m, 6H, H_e), 3.88 (t, $J = 6.5$ Hz, 4H, H_j), 2.67–2.58 (m, 4H, H_b), 2.56–2.41 (m, 12H, $H_g + H_h + H_k$), 1.96–1.80 (m, 2H, H_a). ^{19}F NMR (188 MHz, DMF-d_7) ppm: δ −81.15 (6F, F_u), −113.30 (4F, F_l), −122.08 (20F, $F_{n\text{-}r}$), −123.05 (4F, F_s), −123.81 (4F, F_m), −126.29 (4F, F_t). HRMS: $[M + Na]^+$ requires m/z = 1720.0520; found m/z = 1720.0583.

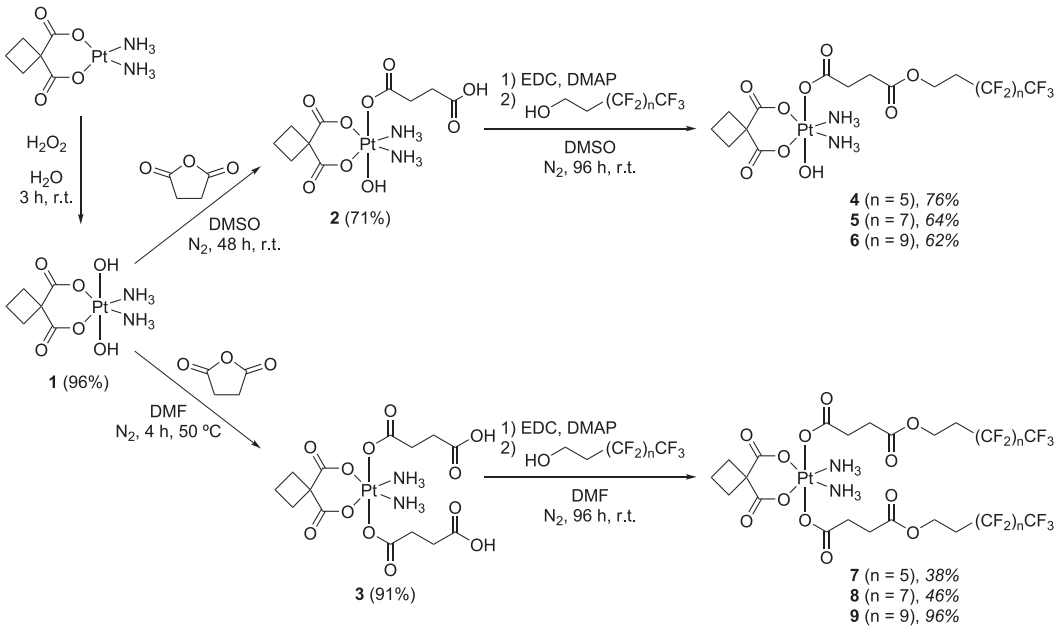
2.3. Cell culture and in vitro anticancer activity

The human ovarian carcinoma cell lines, A2780 and A2780-cisplatin-resistant (A2780cisR) were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK). The human embryonic kidney cell line, HEK-293, was obtained from the American Tissue Culture Collection (ATCC, Sigma, Switzerland). RPMI-1640 GlutaMAX, DMEM GlutaMAX and penicillin-streptomycin were obtained from Life Technologies and foetal bovine serum (FBS) was obtained from Sigma. A2780 and A2780cisR cells were cultured and grown routinely in RPMI-1640 GlutaMAX medium containing 10% FBS and 1% penicillin-streptomycin at 37 °C and 5% CO_2. HEK-293 cells were cultured and grown routinely in DMEM GlutaMAX medium containing 10% FBS and 1% penicillin-streptomycin at 37 °C and 5% CO_2. Cells were grown in 96-well plates in 100 μL of cell culture medium, to give approximately 10,000 cells per well, for 24 h. 40 mM stock solutions of **4**, **5** and **6** in DMSO and **7**, **8** and **9** in DMF were prepared. Serial dilutions were performed on these stock solutions in the appropriate cell culture medium, giving a concentration range of 0 μM to 200 μM for all complexes. Carboplatin (0 μM to 100 μM) was tested as a positive control on all cell lines, while RAPTA-C (200 μM) was tested on all cell lines as a negative control. The complexes were added to the pre-incubated 96-well plates in 100 μL aliquots. Thereafter, the plates were incubated for 72 h at 37 °C, or 4 h at 42 °C, followed by 68 h at 37 °C.

Cytotoxicity was determined using the MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl-2,5-diphenyltetrazolium bromide) [49]. Following the 72 h period of incubation, a solution of MTT (20 μL, 5 mg/mL) in Dulbecco's Phosphate Buffered Saline (DPBS) was added to the cells and the plates were incubated for a further 4 h. Thereafter, the culture medium was aspirated, and the purple formazan crystals, formed by the mitochondrial dehydrogenase activity of living cells, were dissolved in DMSO (100 μL per well). Since the optical density of the resulting solutions was directly proportional to the number of surviving cells, the absorbance was quantified at 590 nm using a SpectroMax M5e multimode microplate reader (using SoftMax Pro software, Version 6.2.2). The fraction of surviving cells was calculated based on the absorbance of untreated control cells. The IC_{50} values that are reported were evaluated based on two independent experiments, each comprising four microculture tests per concentration level.

2.4. Stability studies

The stability of **4**–**9** was studied in solution (0.1 mM in a DMF:H_2O mixture, 1:1) by UV–Vis spectroscopy under the mild hyperthermic conditions used in the cytotoxicity assay.: 4 h at 42 °C followed by 68 h



Scheme 1. Synthesis to the mono- and di-substituted perfluorinated carboplatin prodrugs **4**–**9** (n = 5, 7 and 9).

at 37 °C. Furthermore, the stability of **7** was studied by ^{1}H and ^{19}F NMR spectroscopy.

2.5. Cyclic voltammetry

The redox behaviour of **7**, **8** and **9** was investigated via electrochemical analysis according to known procedures [46]. Cyclic voltammetry (CV) experiments were measured using a custom-made glass electrochemical cell. The three-electrode cell consisted of a 5 mm diameter glassy carbon disk working electrode, a Pt wire counter electrode and a custom-made Ag/AgCl quasi-reference electrode. The Ag reference electrode was prepared by the polarisation of a Ag wire in 2 M HCl. The potential of the reference electrode was calibrated using an internal standard of ferrocenium/ferrocene. All measurements were performed at room temperature using a Bio-Logic SP200 potentiostat. Prior to each experiment, the working electrode was polished with an Al_2O_3 slurry (1 μm), washed with MilliQ H_2O via sonication and dried under a stream of air. Furthermore, before every measurement the experimental setup was flushed with Ar for 10 min, with stirring. A solution of Bu_4NPF_6 (0.1 M) in dry DMF (5 mL) was used as the supporting electrolyte. In each experiment, the concentration of the complexes being used was 0.01 M. In all experiments, all three electrodes were set at the same positions in the electrochemical cell in order to ensure ohmic losses remained constant. The potentials of **7**, **8** and **9** were measured at a scan rate of 100 mV s^{-1}.

3. Results and discussion

3.1. Synthesis of thermosensitive platinum(IV) carboplatin prodrugs

A series of redox-responsive carboplatin prodrugs, functionalised with thermoresponsive perfluorinated chains, were prepared (Scheme 1) and characterised (see Supplementary Information). In the first step, the dihydroxido platinum(IV) species, **1**, was prepared by the oxidation of carboplatin using a slightly adapted literature method in which the reaction was heated to 45 °C rather than conducting it at room temperature [46].

Next, derivatives with one, **2** [48], or two, **3** [50], axial succinato groups, were prepared using literature methods. After isolation, **2** was reacted with one equivalent of the appropriate perfluorinated alcohol to afford the mono-substituted carboplatin prodrugs, **4**, **5** and **6** in acceptable yields. The ^{1}H NMR spectra of **4**–**6** are very similar (see Supplementary Information), which is as expected since these complexes differ only in the length of the perfluorinated chain. The electron withdrawing effect of each fluorinated chain has minimal effect on deshielding the ^{1}H NMR signals, which may be attributed to the effectiveness of the succinato spacer, which insulates the parent platinum complex from the electron withdrawing effects of the perfluorinated chains. The formation of **4**–**6** is supported by their ^{19}F NMR spectra which show similarities to the spectra of their respective perfluorinated chain starting material, i.e. 1*H*,1*H*,2*H*,2*H*-perfluoro-1-octanol (**4** and **7**, n = 5), 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decanol (**5** and **8**, n = 7), and 1*H*,1*H*,2*H*,2*H*-perfluoro-1-dodecanol (**6** and **9**, n = 9) (Scheme 1): the number of peaks and their associated chemical shift range is the same within each spectrum. HRMS of **4**–**6** exhibit the distinctive platinum isotopic pattern with the most abundant peak observed for **4** and **6** corresponding to the $[M + H]^+$ ion at m/z = 852.0802 and 1053.1331, respectively. The most abundant peak in the spectrum of **5** was observed for the $[M + Na]^+$ ion at m/z = 974.0515.

Di-substituted carboplatin prodrugs, **7**, **8** and **9** were synthesised by the esterification of **3** with 2 equivalents of the appropriate perfluorinated chain following activation with EDC/DMAP in DMF. Yields ranged from 38% for **7** to 96% for **9** (Scheme 1). ^{1}H NMR spectra of **7**–**9** are very similar (see Supplementary Information), as observed for **4**–**6** (see above). The ^{19}F NMR spectra of **7**–**9** exhibit clear similarities to each other, to their respective mono-substituted analogues **4**–**6** and to the parent perfluorinated alcohol. HRMS spectra corroborate the expected structures of **7**–**9** with the distinctive platinum isotopic pattern for the $[M + Na]^+$ ion observed at m/z = 1320.0763 for **7**, 1520.0654 for **8** and m/z = 1720.0583 for **9**.

The stability of **4**–**9** in DMF:H_2O was studied by UV–Vis spectroscopy under the mild hyperthermic conditions used in the cytotoxicity assay: 4 h at 42 °C followed by 68 h at 37 °C. No major changes were observed in the intensity or position of the bands, highlighting the remarkable stability of the complexes, common in Pt(IV) complexes (Supplementary Fig. 1). Additionally, the stability of **7** was further confirmed by ^{1}H and ^{19}F NMR spectroscopy (Supplementary Fig. 2).

Table 1
IC_{50} values of **4**–**9** in A2780, A2780cisR and HEK-293 cell lines. Cells were incubated either at 37 °C for 72 h or under mild hyperthermia (42 °C for 4 h followed by 37 °C for 68 h – labelled as 42 °C in the table).

Complex	IC50 (μM) (average ± standard deviation) after 72 h					
	A2780 (μM)		A2780cisR (μM)		HEK-293 (μM)	
	37 °C	*42 °C*	*37 °C*	*42 °C*	*37 °C*	*42 °C*
4	>200	86 ± 20	>200	>200	>200	115 ± 10
5	>200	163 ± 39	>200	>200	>200	147 ± 22
6	>200	145 ± 25	>200	>200	>200	133 ± 17
7	>200	29 ± 6	>200	94 ± 22	44 ± 6	33 ± 3
8	>200	>200	>200	>200	>200	66 ± 11
9	>200	168 ± 23	>200	>200	>200	>200
CarboPt	52 ± 6	21 ± 2	>200	>200	95 ± 17	34 ± 3
RAPTA-C	>200	>200	>200	>200	>200	>200

3.2. Cytotoxicity studies

The cytotoxicity of the target complexes, i.e. **4**–**9**, was evaluated against the A2780 ovarian cancer cell line, A2780cisR cells which have acquired resistance to cisplatin and healthy human embryonic HEK-293 cells (Table 1), using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay [51]. The cytotoxicity was evaluated at 37 °C for 72 h and under mild hyperthermic conditions of 42 °C for 4 h followed by incubation at 37 °C for 68 h. Carboplatin was included as a positive control, as it is known to show a strong enhancement in activity when used in combination with thermotherapy [32,33], whereas RAPTA-C was used as a negative control given its limited cytotoxicity [52]. Note that under both sets of conditions, **4**–**6**, **8** and **9** are all inactive (IC_{50} > 200 μM) in the HEK-293 cell line.

As expected, the cytotoxicity of the positive control, carboplatin, increases under mild hyperthermia in both the A2780 and HEK-293 cell lines, e.g. changing from 52 ± 6 to 21 ± 2 μM upon the application of hyperthermia, but is inactive in the cisplatin resistant A2780cisR cells (Table 1). Note that the cytotoxicity of carboplatin also increases in the non-tumorigenic HEK-293 cell line. Similar thermoresponsive character is also observed in **4**–**9** with the level of cancer cell selectivity for some of these complexes being superior to that of carboplatin. The thermal enhancement of the cytotoxicity of complexes **4**–**6** and **9** is limited, with **4** having an IC_{50} value of 86 ± 20 μM in A2780 cells under hyperthermia. However, **7** shows a pronounced enhancement in cytotoxicity to A2789 cells under hyperthermia, changing from >200 μM under normal conditions to 29 ± 6 μM, i.e. approaching an order of magnitude increase in cytotoxicity compared to carboplatin which exhibits approximately twice the cytotoxicity under mild hyperthermia compared to normothermic conditions. **7** is also the only complex to show any activity, albeit rather limited, towards A2780cisR cells, with an IC_{50} value of 94 ± 22 μM when applied in combination with hyperthermia (note that all other complexes, particularly carboplatin, are inactive to this cell line under both sets of conditions).

Although the presence of fluorinated chains has an significant effect on the thermoresponsive properties of complexes **4**–**9**, a clear correlation between the length of the chain and the hyperthermia-induced cytotoxicity for this series of Pt(IV) complexes was not observed. This is not the case for other studies [37,40], where compounds bearing longer perfluorinated chains were more cytotoxic due to being internalised more efficiently under hyperthermic conditions. However, in these studies, the tested compounds were readily active (i.e., they were not prodrugs) and did not need to undergo activation. In the present study, the prodrugs with the shortest perfluorinated chains, i.e. **4** and **7**, show the highest increase in hyperthermia-induced cytotoxicity against the A2780 cell line. Partition coefficient ($logP_{OW}$) values of **4**–**9**, estimated using a previously developed model for Pt(II) and Pt(IV) complexes [53], indicate that the lipophilicity increases with the number of fluorinated chains and their length (Supporting Table S1). This would result in more efficient cellular uptake for **6** and **9**. Nevertheless, a clear correlation between the $logP_{OW}$ and the IC_{50} values of the complexes is not present, indicating that the cytotoxicity might be influenced by other properties, such as the reduction of the Pt(IV) prodrug. Cyclic voltammetry was used to determine the reduction potentials, i.e. Pt(IV) to Pt(II), of **7**–**9** (Supplementary Figs. 3–5), with **7** having the least negative reduction potential (−1.29 V). There is a direct correlation between the length of the fluorinated chain and a more negative reduction potential, with values of −1.35 V for **8** and −1.75 V for **9**. The reduction potential of Pt(IV) complexes can be roughly correlated with the rate of reduction of the complex [54], which relates to the higher presence of active Pt(II) species and, therefore, to a higher degree of cytotoxicity [55]. The product from the reduction of **7** were studied by ^{195}Pt and ^{19}F NMR spectroscopy and by HRMS. After reaction of **7** with sodium ascorbate, a new peak in the ^{195}Pt NMR spectrum with the same chemical shift as carboplatin (−1707 ppm) was observed (Fig. 3b), and a slight shift in the peaks of the ^{19}F NMR spectrum indicated the release of the axial ligands (Supplementary Fig. 6). Furthermore, ions arising from carboplatin (m/z = 370.03667 for [carboPt – H]$^-$) and oxidised ascorbate (m/z = 173.00898 for [oxAsc – H]$^-$) were observed in the mass spectrum after the reduction (Fig. 3c and Supplementary Fig. 7), confirming that carboplatin is formed upon reduction of the platinum(IV) prodrug. Hence, the higher cytotoxicity of **7** under hyperthermia may be attributed, at least in part, to the lower reduction potential and, in this case, to an easier and more efficient generation in situ of carboplatin.

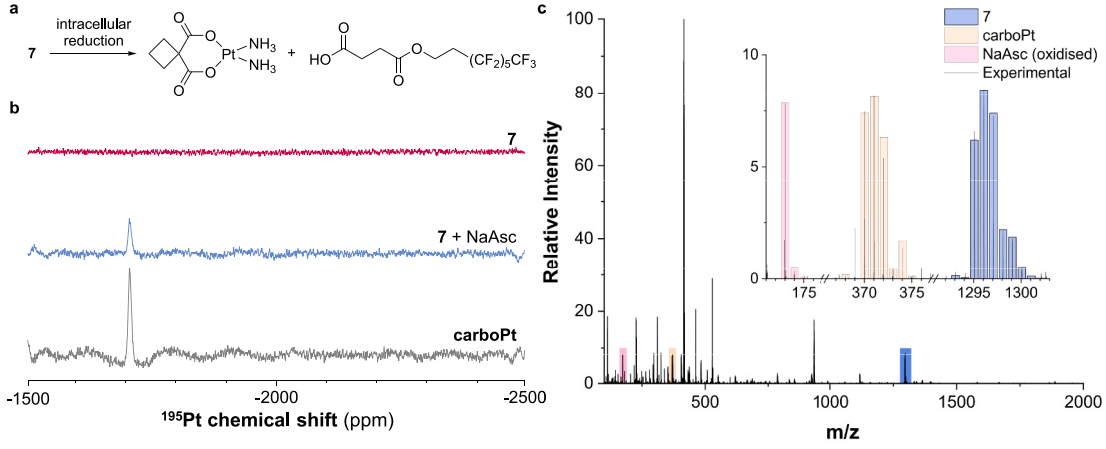


Fig. 3. **a**, Proposed reduction of **7**. **b**, ^{195}Pt NMR spectra of **7** (top), **7** after the addition of sodium ascorbate (middle), and carboplatin (bottom). **c**, Mass spectrum of **7** after reduction with sodium ascorbate.

4. Conclusions

A series of platinum(IV) complexes with fluorinated chains that potentially undergo reduction in vitro to release carboplatin were successfully synthesised. The expectation that these complexes would be endowed with thermoresponsive character, and show enhanced cytotoxicity when applied in combination with mild hyperthermia, was realised. In contrast to other studies, however, the complexes containing the shortest perfluorinated chains exhibited the strongest thermoresponsive behaviour, suggesting a more sophisticated relationship between the length of the fluorous chain and thermosensitivity is in operation, i.e. the reduction potential also appears to play a key role. Notably, **7** exhibits remarkable hyperthermia-induced cytotoxicity against all tested cell lines, even showing activity against cisplatin resistant A2780cisR cells. As such, **7** may be an alternative agent to carboplatin in certain chemothermotherapy regimens.

CRediT authorship contribution statement

Aemilia D. McAdam: Writing – original draft, Investigation. **Lucinda K. Batchelor:** Writing – original draft, Investigation, Conceptualization. **Jan Romano-deGea:** Writing – original draft, Visualization, Investigation. **Dmitry Vasilyev:** Investigation. **Paul J. Dyson:** Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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