Bisursodeoxycholate(ethylenediamine)platinum(II): a new autofluorescent compound. Cytotoxic activity and cell cycle analysis in ovarian and hematological cell lines

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The present paper describes for the first time an intrinsic fluorescent square-planar platinum(II) complex carrying two ursodeoxycholate ligands ([Pt(UDC)$_2$(en)], where UDC$^-$ = ursodeoxycholate), that emits at room temperature once free in solution. Kinetic studies were carried out in aqueous solution and in the presence of different NaCl concentrations: 4 mM (similar to cytoplasmic concentration) and 150 mM (similar to plasmatic concentration). This novel compound was synthesized from a [PtCl$_2$(en)] complex and shows increased cytotoxic activity against both resting and cycling HeLa cells, with no toxicity for cell lines derived from neoplastic haematopoietic cells.

Introduction

The oncogenic transformation of neoplastic cells is frequently characterized by uncontrolled cell proliferation. Accordingly, DNA is the biological target with which many anti-tumour drugs and potential anti-neoplastic agents interact. Cytotoxic platinum-derived drugs[1–3] are believed to inhibit DNA synthesis in rapidly growing cells, such as cancer cells, by binding to their DNA. Formation of these drug–DNA adducts alters the structure of DNA in such a way that replication either cannot proceed, leading to a blockade of cell cycle progression and/or apoptosis, or results in non-viable daughter cells.[4,5] Thus, advances in the design and synthesis of new anti-tumour agents require exhaustive knowledge about the different DNA-binding mechanisms in order to obtain new alternative and more selective agents. However, major secondary effects, such as severe nephro- and neurotoxicity and the development of resistance, frequently limit the use of such compounds.[6] In addition, the design of new metallo–organic anticancer drugs with broader activity spectra than those of already existing drugs, and with fewer side effects for patients, also requires a deep knowledge of the mechanisms of drug transport to biological targets and their interactions with them.

For several decades attempts have been made to develop methods for monitoring the metabolic pathways and intracellular accumulation of active cytotoxic metallo–organic compounds. Thus, many different biochemical and biophysical methods have been applied to elucidate the critical aspects of the mechanisms of action of cytotoxic platinum complexes; in particular, those of cisplatin.[7] Platinum(II) complexes are considered to be non-fluorescent in solution at room temperature (except those carrying additional fluorophores) and, to the best of our knowledge, no intrinsically fluorescent cisplatin analogue with cytotoxic activity has yet been described, with the exception of the platinum compounds containing bile acids previously synthesized and characterized by our group.[8–11] Recently, approaches using platinum complexes with appended fluorophores have been only partially successful in this regard, since once inside the cell fluorescence is not long-lasting.[12–13] In order to monitor the biological effects of cisplatin analogues, it would be of great interest to have intrinsically fluorescent compounds showing cytotoxic activity, especially those able to act against drug-resistant cell lines.[7,14] Over the past few years, bile acid-cisplatin derivatives with potential cytotoxic activity and reduced toxicity owing to their lower lability, their amphipathic character, and their possible use in drug targeting have been described by our group.[8,11,14] In the present paper, we describe for the first time an intrinsic fluorescent square-planar platinum(II) complex bearing two ursodeoxycholate ligands that emits at room temperature once free in solution. This new platinum complex has different DNA-binding properties from those of cisplatin and shows intrinsic fluorescence[10] this may contribute to a better understanding of its activity. Synthesized from [PtCl$_2$(en)], this novel complex shows increased cytotoxic activity against human cervix cancer cell lines, leading to the induction of apoptosis in a way different from that of cisplatin.

Experimental

Chemicals

Dichloro(ethylenediamine)platinum(II), cisplatin, HUDC (ursodeoxycholic acid) and salmon sperm DNA were purchased from Sigma-Aldrich (St Louis, MO). Penicillin/streptomycin, sucrose, spermine tetrahydrochloride, trypsin, trypsin inhibitor, RNAse, Nonidet Np40, and propidium iodide were obtained from Sigma (St Louis, MO). HBSS (Hank's balanced salt solution), DMEM cell culture medium, L-glutamine, fetal calf serum and sodium citrate were all purchased from Gibco BRL (Carlsbad,

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† Electronic supplementary information (ESI) available: ^{1}H, ^{13}C and ^{195}Pt NMR spectra, Fig. A–E. See DOI: 10.1039/b807965j

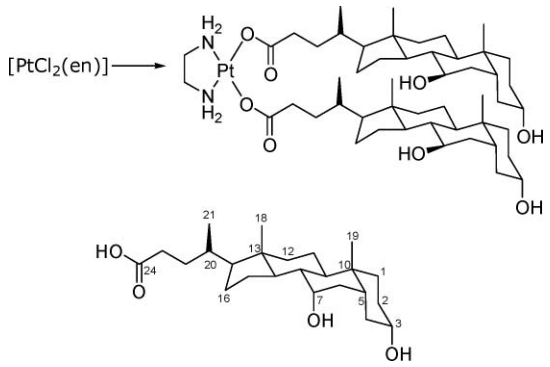
CA). Trisodium citrate and dimethylsulfoxide were obtained from Merck (Darmstadt, Germany). All other reagents were of high purity and were used as purchased without any further purification.

Analytical methods

Chemical analyses for C, H and N were performed on a Perkin-Elmer 2400 elemental analyzer (Applied Biosystems, Foster City, CA). Platinum was determined by atomic absorption on a Hitachi Z-8100 flameless graphite furnace spectrophotometer (Hitachi, Tokyo, Japan) set at a wavelength of 265.9 nm, using the following temperature sequence: 90 °C (20 s), 100 °C (20 s), 800 °C (20 s), 1600 °C (30 s), 2800 °C (5 s) and 3000 °C (4 s). Infrared (IR) spectra were recorded in the 4000–400 cm^{-1} range on a Perkin-Elmer FT-IR 1730 instrument coupled to a Perkin-Elmer Data Station (Applied Biosystems), with KBr pellets and spectrophotometric grade Nujol (Fluka, St. Louis, MO). Electrospray ionization mass spectra (ESI-MS) were recorded on a Waters ZQ 4000 (Waters S.A., Milford, MA); positive and negative ion mass spectra were obtained by signal averaging of 25 consecutive shots. ^{1}H (400 MHz), ^{13}C (102.6 MHz) and ^{195}Pt (64.5 MHz) NMR spectra were obtained in D_2O, methanol-d_4 or DMSO-d_6 solutions on a Bruker DX400 instrument (Bruker, Germany) (Supplementary figures A to E). Carbon resonances were distinguished by DEPT-90 and DEPT-135 experiments. TMS was used as internal standard for the ^{1}H and ^{13}C spectra. In order to obtain the ^{195}Pt chemical shifts were referenced to an external standard of 0.1M K_2PtCl_4 in D_2O (–1624 ppm). During acquisition no broadband ^{1}H decoupling was used. Acquisition time is 0.095 s per scan, relaxation delay is 2 s. Electrical conductivity in solution was measured using a CDM2e conductimetry radiometer with a CDC-104 immersion cell (Radiometer, Copenhagen, Denmark). All kinetic measurements were carried out at 37 °C, temperature being controlled in a Unitherm water bath (Selecta, Barcelona, Spain) with a precision of ±0.01 °C. Fluorescence measurements were performed in both solid-phase compounds using an Olympus BX51 microscope fitted with a 330–385 nm band-pass excitation filter and a DP70 Olympus colour camera, in solution (room temperature), using a Shimadzu RF 5000 spectrofluorometer system (Shimadzu, Tokyo, Japan) equipped with a 150 W Xenon lamp and a sensitivity unit. The optimal excitation wavelength was obtained from fluorescence excitation spectra acquired at the optimum emission wavelength. Quantum yield, Φ, was measured at room temperature using quinine sulfate as a standard.

Synthesis and characterization of bisursodeoxycholate(ethylenediamine)platinum(II), [Pt(UDC)$_2$(en)]

The bisursodeoxycholate(ethylenediamine)platinum(II), [Pt-(UDC)$_2$(en)] complex was obtained in a series of sequential steps, as described below (Scheme 1). First, the AgUDC silver salt was prepared by adding 200 mg of HUDC (0.51 mmol) in 200 cm^3 of ultra-pure water, to which 28 mg of Na_2CO_3 (0.26 mmol) was added, until a clear solution was obtained. The resulting solution was then mixed with a solution of $AgNO_3$ (86 mg, 0.51 mmol) in water (10 cm^3). During this step, all processes were carried out in the darkness at room temperature under constant stirring. During the reaction, a precipitate of AgUDC was formed, which



Scheme 1 Synthesis of the complex (see text).

was separated by filtering. Then, several washes in water were performed to eliminate traces. In the next step, [PtI$_2$(en)] was prepared. For this purpose, 100 mg of [PtCl$_2$(en)] (0.31 mmol) was dissolved in ultra-pure water by heating at 50 °C under continuous stirring. Once the platinum complex had dissolved, a solution of 108 mg of KI (0.65 mmol) in water (2 cm^3) was added drop-wise to the platinum solution. The resulting solution was then heated and stirred for 2 hours at 50 °C in the darkness. The precipitate of [PtI$_2$(en)] obtained was filtered and washed with water to eliminate soluble traces of the compounds. Immediately after this, the complex was synthesized. To accomplish this, the two previously obtained compounds (AgUDC and [PtI$_2$(en)]) were mixed at an AgUDC/[PtI$_2$(en)] ratio of 2 : 1. The AgUDC was dissolved in 60 cm^3 of water by heating at 65 °C under continuous stirring in the darkness. The platinum complex was dissolved in 40 cm^3 of water by heating at 65 °C under stirring. Once both compounds were in solution, the [PtI$_2$(en)] solution was slowly added to the AgUDC solution. Then, the resulting solution was kept for three days at room temperature. Once the reaction had been completed, the solution obtained was filtered and purified as follows. Liquid–liquid extraction through C18 cartridges afforded (after final methanol extraction) the newly formed platinum complex (60% from the starting NaUDC) free of excess unreacted [PtI$_2$(en)]. Preparative TLC chromatography using butyl acetate/methanol at 35/65 (v/v) afforded the desired complex as a yellow solid after evaporation to dryness in P_4O_{10}.

Complex [Pt(UDC)$_2$(en)]

R_f (butylacetate–methanol, 35/65, v/v): 0.37. Yield: 15%, mp (with dec.) = 183 ± 2 °C. Elemental Analyses for $C_{50}H_{86}N_2O_8Pt$, (Found: C 58.17; H 8.23; N 2.51; Pt 19.36% Calc. C, 57.94; H, 8.56; N, 2.70; Pt, 18.82%. IR (KBr) (ν, cm^{-1}): 3429, 1560 (ν_{as}COO), 1413 (ν_sCOO). ESI-MS: 1038.5 [M + 1]$^+$, 1060.7 [M + Na]$^+$, 668.3 [M – UDC + Na]$^+$, 646.4 [M – UDC]$^+$. ^{1}H NMR (CD_3OD), δ (ppm): 0.69 (3H, s; Me-18); 0.95 (3H, s; Me-19); 0.95 (3H, d, J = 6.5 Hz; Me-21); 1.88 (4H, bs; en); 3.46 (1H, m; H-3); 3.46 (1H, bs; H-7); 3.59 (1H, bs; 3α-OH); 3.65 (1H, bs; 7β-OH). 13C-NMR (CD_3OD), δ (ppm): 184.2 (C-24); 71.9 (C-3); 71.7 (C-7); 57.3 (C-14); 56.5 (C-17); 49.4 (en); 49.0(en); 44.6 (C-13); 44.5 (C-8); 44.2 (C-5); 40.5 (C-9); 38.4 (C-6); 37.8 (C-4); 36.8 (C-20); 35.9 (C-1); 33.8 (C-23); 34.9 (C-10); 32.7 (C-22); 30.8 (C-2); 28.7 (C-16); 27.8 (C-15); 23.8 (C-19); 22.2 (C-11); 18.8 (C-21); 12.4 (C-18). 195Pt-NMR (DMSO-d_6) δ = –1966 ppm.

Cell lines and cell culture assays

The cytotoxic activity of [Pt(UDC)$_2$(en)] was evaluated in comparison with that of the most widely used platinum compound: cisplatin (cisDDP). To this end, HeLa (human cervix cancer cells), MV4-11 (human acute leukaemia cells) and HMC-1 (human mast cell leukaemia cells) cell lines were cultured in parallel in the presence of different concentrations of [Pt(UDC)$_2$(en)] and cisplatin (0, 1, 2.5, 5 and 10 μM) dissolved in an identical volume of methanol; adjustment of the final concentration of the Pt compound was performed with DMEM medium supplemented with 10% foetal calf serum, 1% L-glutamine and 1% penicillin/streptomycin, to assure the same volume of methanol was added to the different culture conditions. To analyse the cytotoxic activity, cells were harvested either prior to the addition of the platinum compounds or at 24 h and 48 h after the cells had been placed in culture in the presence of each of the two platinum compounds evaluated. Prior to HeLa cell harvesting, these cells were incubated (60 s at 37 °C) with a solution containing 0.25% (w/v) of trypsin (Gibco, Invitrogen, Rockville, MD).

Evaluation of the cytotoxic activity of the platinum complexes

For the specific evaluation of the cytotoxic activity of the platinum compounds, harvested cells were centrifuged (5 min at 540 g), washed once (5 min at 540 g) in phosphate buffered saline (PBS; pH = 7.4), and resuspended in PBS at a concentration of 10^5 cells/100 μL. Then, cells were stained with the Annexin V-FITC Apoptosis Detection Kit (Immunostep, Salamanca, Spain) according to the manufacture's instructions. Information about a minimum of 5×10^4 cells was acquired in a FACSCalibur flow cytometer (BDB) within one hour after sample preparation was completed. To calculate the percentage of cells stained with annexin-V alone and both annexin-V and propidium iodide, from all cells in the sample, the Infinicyte software program (Cytognos SL, Salamanca, Spain) was used. All experiments were repeated three times.

Evaluation of cell proliferation

In order to evaluate the effects of the platinum compounds on the cell cycle, cells harvested from the different cell lines were centrifuged (5 min at 540 g) and fixed in 200 μL of 70% ethanol in water. After an incubation period of at least 30 min at 4 °C, cells were washed twice in sodium citrate buffer containing sucrose (250 mM), trisodium citrate (40 mM), dimethylsulfoxide 5% (v/v), Nonidet P40 0.1% (v/v), trishydroxymethylaminomethane 0.5% (v/v) and spermine tetrahydrochloride (1.5 mM) in distilled water, and resuspended in the same citrate buffer at a concentration of 10^4 cells μL^{-1}. A volume of 750 μL of a solution containing 0.5 g L^{-1} of RNAse and 0.1 g L^{-1} of trypsin inhibitor was then added; after gently mixing, cells were incubated for 10 min at room temperature. After this incubation, 750 μL of a solution containing 50 mg L^{-1} of propidium iodide in sodium citrate buffer was added, and other incubation was performed in the darkness for 15 min at room temperature. Analysis of cell DNA contents was accomplished by measuring the propidium iodide-cell-associated fluorescence on a FACSCalibur flow cytometer and recording information about at least 10^4 cells for each cell line and experimental condition assayed. For the specific calculation of the distribution of surviving cells in the different cell cycle phases, the ModFit software program was used (Verity Software, Thopsham, MA) after cell debris and dead cells had been excluded.

Statistical analysis

The equations for kinetic studies were adjusted to the experimental data by means of a nonlinear regression program available in the SIMFIT statistical package (Bardsley 1992 SIMFIT package 3.2. Department of Obstetrics and Gynecology, Manchester, UK).

For the calculation of the distribution of surviving cells in the different cell cycle phases, the ModFIT software program was used (Verity Software, Topsham, MA). The Mann–Whitney U test was used to estimate the statistical significance of the differences observed between groups at experiments. P values ≤ 0.05 were considered to be associated with statistical significance.

Results and discussion

In the present paper, we describe for the first time a new square-planar platinum(II) complex carrying ethylenediamine and ursodeoxycholate ligands. The platinum complex, [Pt(UDC)$_2$(en)], was characterized with its ^{1}H, ^{13}C, and ^{195}Pt NMR, IR and MS spectra and elemental analysis. In MS, the peaks corresponding to the platinum compound were readily recognized owing to six different isotopic masses for the metal (190, 192, 194, 195, 196 and 198) (Fig. 1). The observed molecular ion ESI-MS (m/z) and other remarkable peaks were: [M + Na]$^+$ = 1060.7, [M – UDC + Na]$^+$ = 668.3, [M – UDC]$^+$ = 646.4.

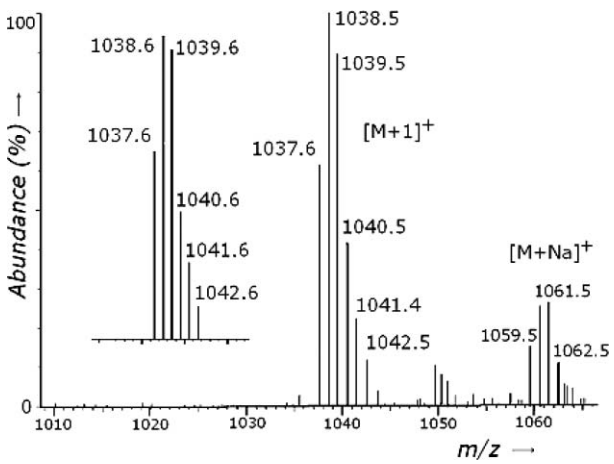


Fig. 1 Isotopic simulation and mass spectrum of the [Pt(UDC)$_2$(en)] compound and the [M + Na]$^+$ fragment.

The ^{1}H NMR and ^{13}C NMR spectra of the newly synthesized platinum complex (supplementary Fig. A–E†) displayed peaks corresponding to two ursodeoxycholate ligands and to ethylenediamine. A single set of resonances may be observed for the two ursodeoxycholate ligands and the two ethylenediamine carbons of the complex are isochronous, (see values in the experimental section). The ^{195}Pt NMR resonance found for the synthesised complex is centred at $\delta = -1966$ ppm, indicating a square-planar complex with a PtN_2O_2 environment.[15] The carboxylate ν_{as} and ν_s absorption bands in the IR spectra of the complex - 1560 cm^{-1} (ν_{as}COO) and 1413 cm^{-1} (ν_sCOO) - also support the occurrence of monodentate binding of the ursodeoxycholate ligands.[16]

The platinum complex synthesised was soluble in methanol and DMSO, and dissolved in water over time, with a molar conductivity at 25 °C of 161 S cm^2 mol^{-1}, supporting a 1 : 1 electrolyte ratio. The electronic absorption and emission spectra of the platinum complex dissolved in water, at room temperature, are shown in Fig. 2.

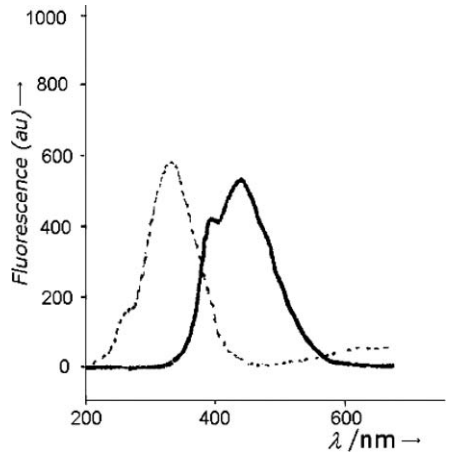


Fig. 2 Excitation (···) λ_{ex} = 350 nm and emission (---) spectra of the [Pt(UDC)$_2$(en)] complex. Results for a concentration of 10^{-4} M in water at room temperature are displayed; au: arbitrary units.

The absorption spectrum of this platinum compound typically comprised very intense charge-transference bands in the UV zone. These bands hid weaker d–d transitions located at higher wavelengths, which did not show up in the absorption spectra at the concentrations used (10^{-4} M). The emission spectrum of the compound exhibited a concentration-dependent, broad and unstructured fluorescence emission band, which persisted for weeks.

Kinetic studies

The molar conductivities at 37 °C of the complex (10^{-3} M solutions) was 161 S cm^2 mol^{-1}, in agreement with a 1 : 1 electrolyte ratio.

$$[Pt(UDC)_2(en)] + H_2O \rightarrow [Pt(UDC)(en)H_2O]^+ + UDC^-$$

The hydrolysis kinetics at 37 °C in water and in solutions of increasing NaCl concentrations (4 and 150 mM) were followed by the overtime variation in absorbance at 245 nm. The decay curve was adjusted to a pseudo first-order rate equation, $A = A_0e^{-kt} + C$, where A is absorbance at time t; A_0 is absorbance at time zero; k_{obs} is the rate constant (h^{-1}), and C is a constant term introduced to take into account the baseline variations observed over time and the absorption of other non-reacting species (Fig. 3). The calculated values for the rate constants (k_{obs}) and the half-lives are: kinetic parameters for hydrolysis reactions ($C = 10^{-4}$ M, 37 °C, and pH 6.8). H_2O k_{obs} (h^{-1}) 7.30 ± 0.01 × 10^{-2}, $t_{1/2}$ (h) 9.5; 4 mM NaCl (cytoplasmatic) k_{obs} (h^{-1}) 7.80 ± 0.01 × 10^{-2}, $t_{1/2}$ (h) 8.9; 150 mM NaCl (plasmatic) k_{obs} (h^{-1}) 7.80 ± 0.01 × 10^{-2}, $t_{1/2}$ (h) 8.9. These results suggest that there is only one replaceable ligand in these complexes, as opposed to cisplatin and its analogues, where two substitutions are seen from the kinetics of hydrolysis reactions.[17,18] In the aquation process, there might be a second order kinetic in

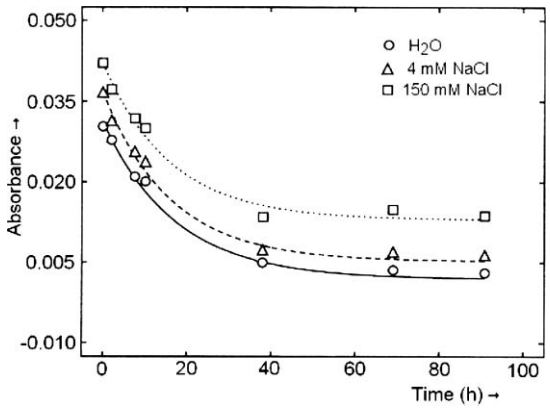


Fig. 3 Absorbance *vs.* time curves for complex [Pt(UDC)$_2$(en)]. The hydrolysis reactions for the complex were followed by monitoring the decay of the 245 nm absorption band at 37 °C. Increasing concentrations of sodium chloride (4 and 150 mM) were selected to mimic the effect of different environments on the hydrolysis kinetics. The traces connecting the experimental data-points were drawn by SIMFIT and correspond to first order decays.

a period of time over 80 h, which is represented in Fig. 3. The remaining ursodeoxycholate ligand might be lost there, although the study has not been carried out because this paper is aimed at measuring the activity of the compound for periods of between 24 and 48 h.

One important physical property of this compound is its fluorescence characteristics in both the solid state and in aqueous solution at room temperature. In the solid state, the complex shows orange fluorescence emission, as illustrated in Fig. 4.

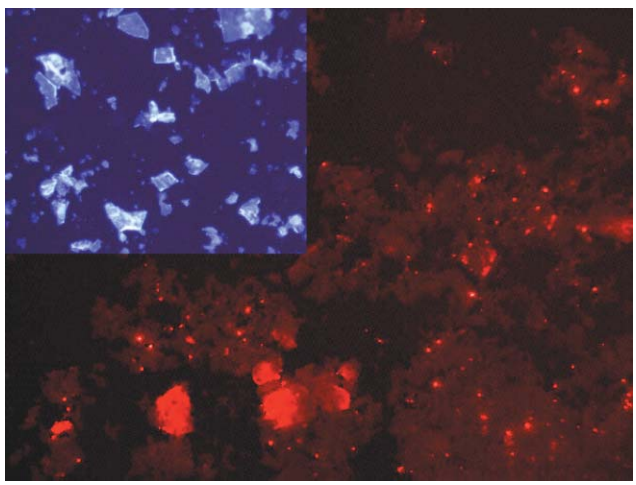


Fig. 4 Fluorescence emission at room temperature of the solid-state [Pt(UDC)$_2$(en)] compound (orange particles). The insert in the upper left quadrant shows the fluorescence emission of quinine sulfate (blue particles). Both solids were excited at λ_{ex} = 330–380 nm.

Interestingly, the intensity of fluorescence emission in aqueous solution did not change in the presence or absence of Tris buffer or increasing salt concentrations (4 and 150 mM of NaCl). Maintenance of the fluorescence properties of the Pt compound in different media would support detection of the compound in both the extracellular (150 mM NaCl) and intracellular (4 mM NaCl) compartment for relatively long periods of time after

administered. However, it should be noted that the optimum excitation wavelength shifted to the red with the change of solvent: $\lambda_{exc}(H_2O) = 325$ nm; $\lambda_{exc}(CH_3OH) = 350$ nm; $\lambda_{exc}(DMSO) = 363$ nm. The quantum yields, Φ_F, in water were calculated with the Williams method[19] and values of $\Phi_{QS} = 0.54$ and $\Phi_{complex} = 0.11$ were obtained.

Neither free ursodeoxycholate nor the $PtCl_2(en)$ compound showed fluorescence emission in the 200 nm to 700 nm region of the excitation, at room temperature.

Cytotoxic activity of the [Pt(UDC)$_2$(en)] platinum complex on HeLa cells

As shown in Fig. 5, [Pt(UDC)$_2$(en)] exerted an important cytotoxic activity against HeLa cervical carcinoma cells, this effect already being clearly detectable after 24 h. Moreover, the amount of apoptotic cells detected after incubation with this newly synthesized platinum complex was significantly higher ($p \leq 0.05$) at doses of 2.5 μM and 5 μM in comparison with cisDDP, the later requiring longer incubation periods to reach similar apoptotic rates at identical concentrations of the drug (Fig. 5).

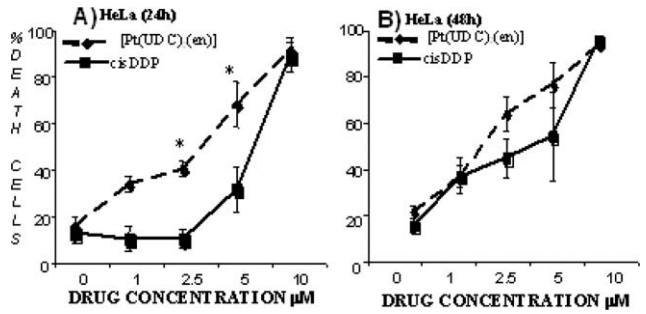


Fig. 5 Cytotoxic activity of the newly synthesized [Pt(UDC)$_2$(en)] complex *versus* cisplatin (cisDDP) against HeLa cervical carcinoma cells after culturing of these cells in the presence of different amounts of both compounds for 24 h (panel A) and 48 h (panel B). Results are expressed as percentages of dead (apoptotic) HeLa cells. *$p \leq 0.05$.

This increased cytotoxicity of the [Pt(UDC)$_2$(en)] complex was also reflected by the IC_{50}, since this for the newly synthesized Pt complex was around half of that found for cisplatin at both 24 h and 48 h (see Table 1).

At present there is limited information about the cytotoxic activity of cisplatin analogues bearing a bile acid complex. However, in comparison with cisplatin previous results have reported increased cytotoxicity against other ovarian cancer cell lines for this group of compounds,[11] as also found in the present study for the new platinum complex. Because of their amphipathic character, bile acids afford these analogues easier diffusion, providing them with an advantage over cisplatin, whose uptake depends on the availability of high-affinity copper transporters.[20] This property could help to explain why the cytotoxic effects of these platinum derivatives appear earlier than those of cisplatin.

Table 1 *In vitro* growth inhibition due to cisplatin and the newly synthesised [Pt(UDC)$_2$(en)] complex

Compound	Time of incubation/h	IC_{50} (on HeLa cells)[a]/μM
[Pt(UDC)$_2$(en)]	24	5.2
	48	3.7
cisDDP	24	8.0
	48	6.2

[a] IC_{50} results, expressed in μM units at different periods (24 h and 48 h) of drug exposure.

Analysis of the cell cycle distribution of surviving HeLa cells at 48 h and with the highest drug concentration (10 μM), revealed differences between [Pt(UDC)$_2$(en)] and cisplatin, as illustrated in Fig. 6. In this sense, whereas cisplatin induced an accumulation of surviving cells at the G_0/G_1 cell cycle phases, no major changes were observed in the cell cycle distribution of HeLa cancer cells cultured with [Pt(UDC)$_2$(en)] *versus* untreated cells. The absence of alterations in cell cycle distribution, even at higher rates of cell death, has been previously observed in CH1 and CH1cisR ovarian cell lines treated with other bile acid cisplatin analogues.[8] All these observations, support the notion that these derivatives exert their cytotoxicity not only on cycling cells but also in resting cells, since no cell accumulation was observed in any of the cell cycle phases in association with the reported increased cell death rate.

Currently it is well established that the cytotoxic activity of cisDDP is directly linked to the formation of DNA bis-adducts. In contrast, we have previously observed that the cytotoxic activity of cisplatin analogues bearing a bile acid complex is mediated by the formation of monoadducts.[21] The formation of DNA-monoadducts is faster than that of DNA bis-adducts, which could also help to explain earlier apoptotic effects observed for the new platinum complex. Moreover, this different mode of drug targeting would explain, at least to a certain extent, the cytotoxicity elicited

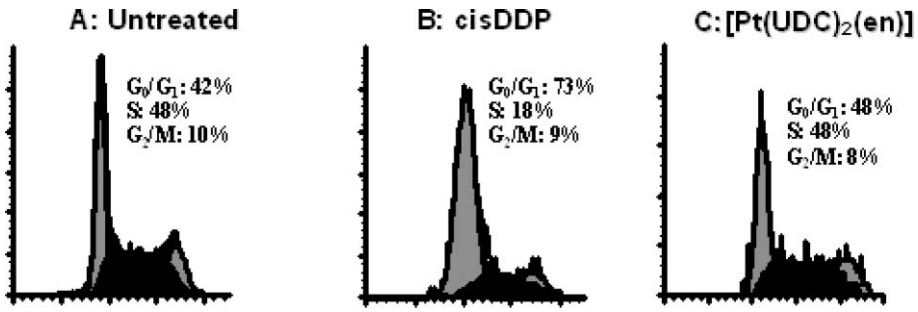


Fig. 6 Cell cycle distribution of HeLa cervical carcinoma cells cultured in the absence (panel A) or the presence of either cisplatin (panel B) or the newly synthesized [Pt(UDC)$_2$(en)] platinum complex (panel C), both at 10 μM concentration.

by the [Pt(UDC)$_2$(en)] complex against S + G$_2$/M- as well as G$_0$G$_1$-phase tumour cells, and not only against cycling HeLa cells, as found for cisplatin. In keeping with this, other authors have recently reported that bile acids alone induce apoptosis in ovarian cell lines.[22] This cytotoxicity seems to overcome resistance to cisplatin owing to the use of different mechanisms of action by bile acids and cisDDP. Unfortunately, the doses of bile acids required to significantly decrease cell viability is much higher than desirable (200 μM). In contrast, our study shows that combination of these bile acids with a conventional chemotherapeutic drug induces cytotoxicity after shorter periods of time and at low doses of the drug; even below those of cisDDP.

In contrast to the reported cytotoxic effect of the new platinum complex on HeLa cancer cells, this novel analogue did not show any clear cytotoxic activity against the HMCL-1 and MV4-11 hematological cell lines, in which cisplatin was clearly more active (Fig. 7).

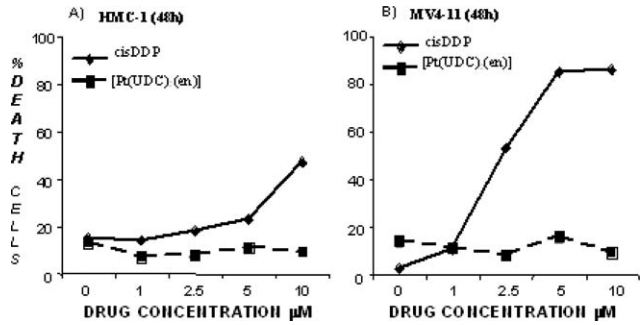


Fig. 7 Cytotoxic activity at 48 h of different doses of the newly synthesised [Pt(UDC)$_2$(en)] complex *versus* cisplatin against the HMC-1 (panel A) and MV4-11 (panel B) hematological cell lines. Results are expressed as percentages of dead cells.

Conclusion

In the present study we describe a novel cisplatin analogue with increased cytotoxic activity against both cycling and non-cycling HeLa cervical carcinoma cells *versus* cisplatin, but no clear cytotoxic effects against different hematological cell lines.

Acknowledgements

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